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Deliverable of WG5

Deliverable 20

A list of parameters to be taken into account for a qualitative risk assessment framework

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Executive summary

The aim of this Deliverable is to develop a framework to support a qualitative assessment of risks to protection targets (exposed humans, fauna and/or flora) and receiving compartments (surface water, groundwater and/or soil) due to the reuse of treated wastewater (TWW) containing CECs in a range of applications. This risk assessment framework is proposed as a contribution to ensure the safe reuse TWW as identified in Article 1 of the recent proposal to establish minimum requirements for water reuse that (EU, 2018). It is also a contribution to achieving UN SDG Goal 6 (Ensure availability and sustainable management of water and sanitation for all, particularly indicator 6.3 which identifies the need to substantially increase current levels of water reuse). In stating that the risk assessment framework is qualitative, there is an implicit understanding that currently available data sets pertaining to this field are insufficient to support a fully quantitative approach. However, irrespective of this, decision-makers are required to develop policy which will impact on water use within a range of, if not all, sectors. It is within this context that NEREUS Working Group 5 (WG5; Risk Assessment and Policy Development) is actively liaising with and reviewing knowledge delivered by NEREUS WGs 1-4 with regard to identifying and extracting key information for use by those currently involved in TWW risk assessment and policy development.

A risk assessment (RA) methodology is used within various sets of national and international TWW guidelines (e.g. NWQMS, 2008; US EPA, 2012; WHO, 2006) to consider the impacts associated with a range of conventional parameters. However, the extension of this same approach to assess risks associated with the use of TWW containing CECs in relation to both human and environmental protection targets and receiving compartments is identified to pose a series of methodological and practical challenges. These are identified and discussed within this report and relate not only to limited data availability, but to more fundamental aspects such as:

- a conceptual understanding of the approach by lay users,
- the point of application within the reuse chain,
- a focus on single substances from a single source (as opposed to real world ‘chemical cocktails’ from multiple sources)
- the need to address chronic as well as acute impacts.

Despite these identified limitations and concerns, the development of a qualitative RA framework with respect to CECs (described herein) is recognised as a useful exercise towards identifying and clarifying research gaps and methodological short-comings. The NEREUS RA

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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, we argue that the output of the application is a ranking of hazards to protection targets and/or receiving compartments due to the use of TWW containing CECs during selected examples of reuse. The approach has supported the systematic co-identification of a list of parameters to be taken into account within a risk assessment process but questions the utility of a qualitative risk assessment process with regard to current knowledge. Both the time and cost of developing the conventional types of CECs data sets required to underpin a quantitative (or even semi-quantitative approach) is in no way underestimated and hence the utility of alternative methodologies (e.g. ecotoxicological assessments; see WG3 outputs) to generate new forms of data to enable a more quantitative (or semi-quantitative) approach to be undertaken is strongly supported.

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1. Introduction

It is estimated that since 1900, 11 million people have died from drought and the livelihoods of over 2 billion people have been affected by water shortages (UNISDR, 2011). By 2025, 2.4 billion people are predicted to be living in regions of physical or economic water scarcity (UNCCD, 2014), with half of the world's population expected to be living under conditions of high water stress by 2030 (UN Water, 2013). Water scarcity is a growing concern globally; no longer a feature limited to the arid North African and Mediterranean countries only (WBCSD, 2006), water scarcity is increasingly identified as an area of concern in the relatively wetter north western hemisphere. For example, in 2015 regions of Germany and the Benelux countries were affected by droughts (EDO, 2015), Latvia, Lithuania, Romania, Slovakia, Slovenia and Hungary suffered severe water stress (Bio by Deloitte, 2015) and a recent UK climate change study predicted national water deficits of 8–29% of total demand by the 2080s (CCC, 2016). Forecasts such as these highlight the urgent need to identify and routinely utilise alternative water resources.

At a strategic and policy level, water reuse including use of treated wastewater (TWW) is a priority objective towards the achievement of long term sustainable water resources within the EU. For example, the EU Water Framework Directive (EU WFD, 2000) identifies water reuse as a key supplementary measure to be considered within the development of river basin management plans and maximisation of water reuse is identified as a specific action within the EU's 'A Blueprint to Safeguard Europe's Water Resources' (EC, 2012). Water reuse is also a key component of the EU's Action plan for delivering a circular economy (EU, 2015). At a global level, UN SDG 6 includes a substantial increase in current levels of water reuse' within one of the support indicators under Goal 6 (water and sanitation). A water reuse policy instrument focussed on enhancing TWW reuse in agricultural irrigation and aquifer recharge is currently under development at an EU level (JRC, 2016, EU, 2018) but at present TWW remains an under-exploited water resource. Whilst, current levels of TWW reuse vary between and within Member States (MS) - from 100% in Cyprus and 95% in some areas of Spain to no evidence of reuse in e.g. Ireland - it is estimated that, on average only 2.4% of Europe's TWW is reused (Bio by Deloitte, 2015). A similar trend is apparent internationally. For example, despite wide recognition for its advanced and integrated stormwater and TWW reuse policy, on average Australia meets only 3% of its total supply output with reclaimed waters (Fletcher et al., 2008). The low uptake of TWW reuse, together with scientifically-validated information regarding a range of current TWW reuse practices, represents a genuine opportunity for

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transformative action: a generally under-exploited resource with 'pockets' of considerable expertise, experience and accrued knowledge in its use at MS level. In view of increasing water scarcity as a result of growing demands associated with an expanding population and changing climatic conditions, there is a need for MS to adopt a multi-disciplinary approach to robustly characterise the risks and benefits of TWW reuse. This robust characterisation, together with alternative strategies to mitigate risks and promote the benefits of TWW reuse, is a priority to ensure the delivery of sustainable water resource management plans from the local to European scale.

This report builds directly on Deliverable 19 (List of quality criteria concerning ARB&ARGs and biological risks related to contaminants of emerging concern) to identify a list of parameters for consideration within a qualitative risk assessment framework. In stating that the risk assessment framework is to be qualitative (as opposed to quantitative), there is an implicit understanding that, whilst the use of a quantitative (evidence-based) approach is always preferable, currently available data sets pertaining to this field (e.g. following searches of the peer review literature and the ToxPlanet database which draws on data entered within REACH registrations, Environmental Chemistry Information System and Toxic Substance Control Act Test Submissions (TSCATS) databases amongst several others; for full info see Deliverable 19) are insufficient to support a fully quantitative approach. Hence it is recognised from the outset that the conventional four stage approach to human health or environmental risk assessment (i.e. hazard identification, dose-response, exposure assessment and risk characterisation) cannot be systematically applied. However, irrespective of this, decision-makers are required to develop policy (e.g. the on-going development of the EU Water Reuse Directive) which will impact on water use within a range of, if not all, sectors. It is within this context that NEREUS Working Group 5 (WG5; Risk Assessment and Policy Development) is actively liaising with and reviewing knowledge delivered by NEREUS WGs 1-4 with regard to identifying and extracting key information for use by those currently involved in TWW risk assessment (RA) and policy development. The collective expertise of the NEREUS project (a global network of >350 researchers working in the field of TWW reuse from a number of perspectives), supports the development of advice which can be offered to TWW policy-makers and users, together with assessments of the level of confidence associated with the recommendations made.

Deliverable 19 drew on Deliverables from WG1-4 to present a long-list of contaminants of emerging concern (CECs) identified to date within the WGs from a range of perspectives including:

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- ARB&ARGs prevalence and persistence in raw and treated wastewater (WG1)
- potential for CECs bioaccumulation by crops (WG2)
- results of human- and environmental - endpoint ecotoxicity studies (WG3)
- consideration of the fate of CECs within a range of treatment technologies (WG4)

Within WG5, CECs are defined as substances which are not regulated under existing EU water quality regulations but which have been identified as having the potential to impact negatively on human health and/or environmental endpoints. Following the compilation of data on a range of biological and physico-chemical characteristics of identified substances by the NEREUS Blue Circle Society (BCS) in the form of a matrix, a short-list of seven substances were selected for consideration within WG5 (see D19 for the short-listing methodology).

The aim of the present Deliverable is to develop a framework to support the qualitative assessment of risks to humans, plants and/or animals (collectively referred to as protection targets) and/or receiving soils, surface water and/or groundwater (collectively referred to as receiving compartments exposed to TWW containing CECs during a range of TWW reuse applications. Risk assessments are usually performed using exposure scenarios related to specific identified uses. The OECD has developed a range of exposure scenarios as part its approach to chemical assessment (OECD, 2018). Whilst these cover specific uses within several sectors, the reuse of TWW has yet to be considered. It is currently recommended that water reuse risk assessment procedures be undertaken on a site-specific basis (NWQMS, 2008). However, it is argued that current understanding is sufficient to support the development of a systematic process for screening of TWW uses to:

- identify complete source-pathway-receptor chains
- qualitatively rank on a per use and per receptor basis:
  - the likelihood of occurrence of CECs
  - their magnitude of impact

It is noted that this approach aligns well with the Hazard Analysis and Critical Control Point (HACPP) system used to manage hazards within the food industry, as opposed to a conventional four-stage risk assessment procedure (US National Research Council 1983; Ball, 2006). The implications of this are considered within this report.

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1.1 Scope

As a starting point, this report focuses on developing a framework to support the assessment of TWW within agricultural irrigation and aquifer recharge in relation to practices undertaken within EU Member States (i.e. the EU Urban Waste Water Treatment Directive (1991) requires at least secondary treatment of municipal wastewater effluents). The decision to initially focus on these two applications was taken at the NEREUS Luxembourg meeting (October 2015) to:

- address the need for alignment among several WG activities
- complement on-going activities related to the development of an EU Water Use Directive

However, following development of a workable framework for these two applications, there is the potential for this approach to be applied to further TWW reuses including the following:

- urban uses - irrigation, street cleaning, fire protection systems, dust control;
- recreational uses - golf course irrigation, recreational impoundments;
- environmental uses - stream augmentation, wildlife habitat; and,
- potable uses - augmentation of aquifer and surface water used for potable supplies, treatment until potable water quality.

1.2 Definition of key terms

Please refer to the glossary presented in Annex I

1.3 Overview of a conventional risk assessment (RA) process

The conventional approach to undertaking a RA generally involves three key stages:

- **Step 1**: Identification of hazards
- **Step 2**: Consideration of the likelihood of the identified hazards impacting on receptors or identification of level of exposure
- **Step 3**: Consideration of the magnitude of impact if hazards impact on receptors

Once hazards are identified, data on the likelihood of occurrence and magnitude of impact of each hazard on a case-by-case basis must be sourced. This can involve accessing existing / historic data sets on the frequency and impact of identified hazards or by generating new data e.g. dose response curves and exposure models etc. Where available, combining these data provides an evidence base for understanding the conditions specified within the assessment. This RA process has been widely promoted as an approach to facilitate the consistent analysis

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of hazards in a range of contexts. It is used as a basis for policy development and implementation at local-to-global scales, and to support decision-making in a range of sectors.

A major challenge within this process is the sourcing of data which is 'fit for purpose' in relation to supporting an assessment of the risks to human health and/or environmental organisms and compartments. For example, an assessment of risks to exposed populations or environmental compartments ideally requires the availability of dose-response models (human health RA), an identified no observable effects level (NOEL) data set with regard to identified specific species or a relevant environmental quality standard developed to protect e.g. surface water (Deliverable 19). However, even where such data is available, this is typically on a substance specific basis which may – or may not – be a true reflection of a particular substance's field behaviour i.e. its impact when present as a component within a pollutant mixture or cocktail. Considerable uncertainty remains regarding the extrapolation of data from single-contaminant, single-exposure ecotoxicity tests to real-life scenarios of lifetime exposure to a wide range of multiple contaminant mixtures at sub-lethal concentrations from multiple sources (e.g. Peterson et al., 2003; Kwok et al., 2014; Pablos et al., 2015; van der Sluijs et al., 2015). Under such potentially dynamic and multi-variable conditions, transparency in data selection and caution in data interpretation in terms of implications for other scenarios, e.g. under differing soils types and crop species, is critical.

Whilst non-exhaustive in its nature, the BCS matrix (see D19) summarises the data available for the 42 CECs (identified by WGs 1-4 as being of potential concern from their specific WG perspectives) in relation to a range of bio-physico-chemical parameters commonly referred to within chemical risk assessment processes. It can be seen from this matrix that data availability is limited for many of the identified CECs and that, where data does exist, this is often from a single study. Furthermore, models to assess alternative TWW exposure scenarios are in the early stages of development, if available at all. Hence, the focus of this report is to develop an initial exposure scenario and a systematic framework for its qualitative assessment to assess risks to exposed humans and ecosystems.

Figure 1.1 presents a broad overview of the potential factors, processes, exposure pathways and endpoints to be considered within an assessment of the risks of reusing TWW. Commencing with a consideration of the wastewater characteristics (e.g. sources, treatment and residence time during storage), the schematic identifies key direct and indirect exposure pathways via which TWW containing CECs may come into contact with protection targets and/or receiving compartments. Major biological, chemical and physical parameters of both CECs and the receiving environmental compartment which influence CECs fate are then

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summarised, followed by indicative management practices. Finally, the human and environmental protection targets and receiving compartments which may need to be considered are identified. In providing a holistic overview of key aspects for consideration with TWW reuse, Figure 1.1 identifies the information necessary to support the generation of more detailed source-pathway-receptor (SPR) scenario required to support a RA process on a case-by-case basis (e.g. see Figure 3.1).

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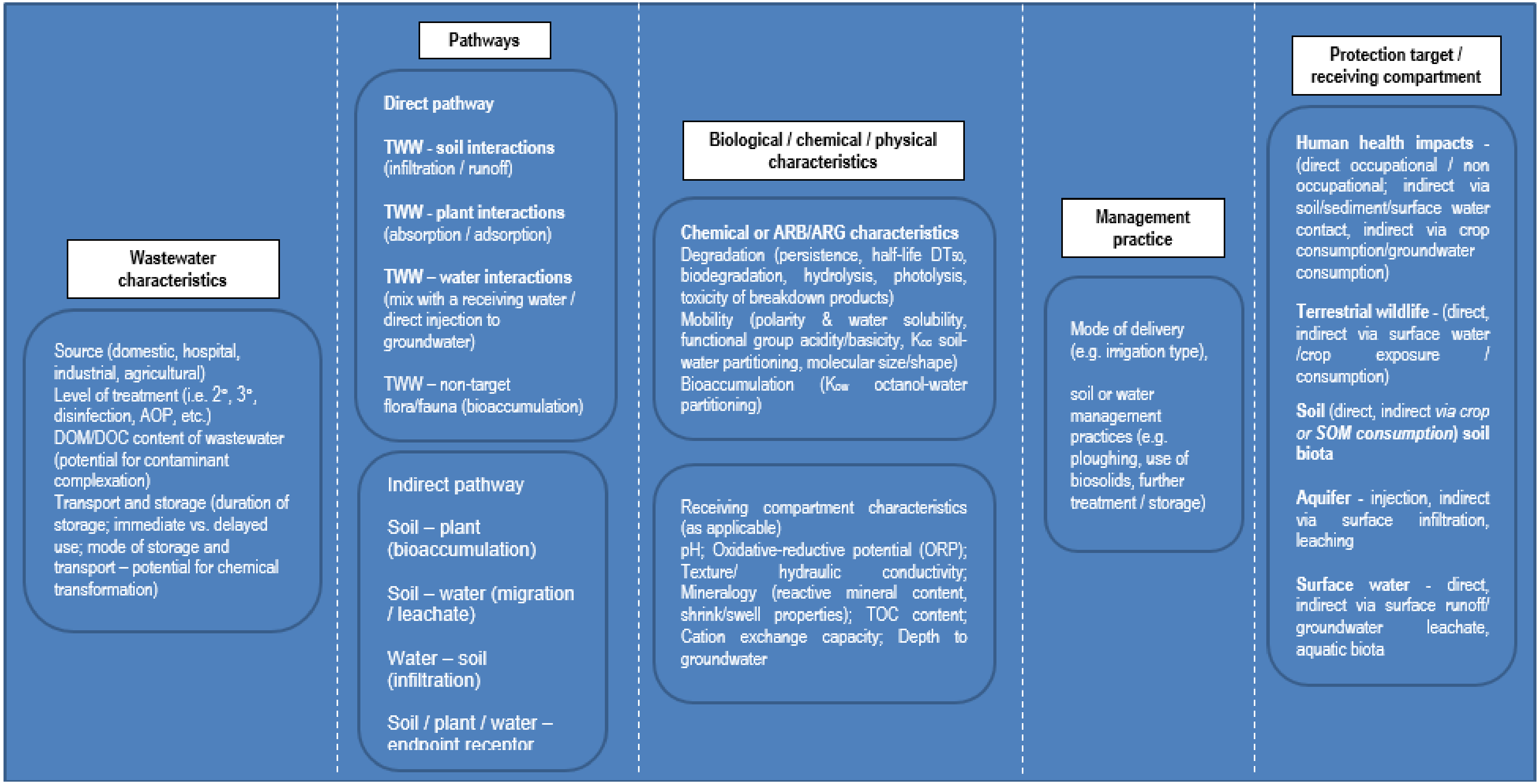

Figure 1.1 - Overview of the potential factors, processes, pathways and endpoints to be considered within an assessment of the risks of reusing TWW.

2. Challenges associated with a conventional risk assessment approach

2.1 Identification of hazards

The first step of a risk assessment process is the identification of hazards. Within NEREUS Deliverable 20, the hazards associated with TWW reuse (irrespective of type of application) are contaminants of concern (CECs). Activities within WGs 1-4 involved developing short-lists of priority substances from differing perspectives e.g. most hazardous ARBs and ARGs (see NEREUS D2), greatest potential to bioaccumulate within crops (NEREUS D7), availability of pertinent biotests (NEREUS D14) and fate within WWTPs (NEREUS D16). 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 2.1).

Table 2.1 – 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

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2.2 Development of a procedure for evaluating likelihood of occurrence

Following the identification of hazards, the next stage of a conventional RA procedure is exposure assessment. In the absence of required datasets, dose-response models, NOEL or EQS to support a quantitative assessment, a qualitative approach can be taken involving development of, for example, a scheme to identify and then rank the probability of a specific CECs being discharged within a TWW flow coming into contact with a protected target or receiving compartment i.e. evaluate the likelihood of occurrence. This probability is often assessed using a relative scale where numeric values are pre-defined to represent a comparatively escalating likelihood of occurrence (e.g. see Table 2.2). Such a qualitative risk assessment approach is well recognised and globally accepted (e.g. DEFRA, 2004; USDA, 2003; Elgallel et al., 2016, Standards Australia 2004; ISO31000). However, its use is not without its critics as, by definition, the approach is subjective and the results dependent on the experience of the team undertaking the assessment (Ramona, 2011). Whilst widely used in the occupational health and safety arena, its use within human health and environmental chemical risk assessment is a more recent step; presumably a response to incomplete data sets. Whilst arguably a pragmatic approach, its limitations should not be forgotten. The use of a matrix approach, by necessity, requires the simplification of a complex scenario (DoE, 1995), with some arguing that the outcome of applying a qualitative approach in relation to chemical assessments is, in effect, a hazard ranking process as opposed to an assessment of risks (*pers comm* David Ball).

Table 2.2 - Guide to how the likelihood of a specific CECs occurring in TWW at point of use could be graded.

Possible descriptors for relative grading	Ordinal value associated with likelihood
Likely (expected to occur)	4
Possible (may occur sometimes)	3
Unlikely (uncommon but known to occur)	2
Rare (lack of evidence but not impossible	1

It should be noted that the values given in Table 2.2 are ordinal in nature, not numeric, and therefore represent only the order of likelihood of specific CECs occurring in TWW at point of use relative to other CECs and do not have an exact quantitative meaning. With these *caveats* in mind and with regard to the need to apply a qualitative approach within the NEREUS context, the following theoretical and practical challenges were identified during NEREUS WG5 discussions with regard the use of TWW in reuse applications:

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Challenge 1. Lack of data: as noted above, whilst the use of quantitative data is preferred to support evidence-based policy making, a qualitative approach recognises that neither the likelihood of an identified CECs occurring in TWW nor its specific impact (see Section 2.3) can always be readily quantified, if at all. Hence, the methodology supports the use of more qualitative data and the use of 'expert judgement' which, in the absence of field or literature data, is a pragmatic approach to managing the need to make decisions in the face of uncertainty. The main weakness of this approach is that expert judgement will depend on the experiences/expertise of the expert(s) involved, and this should be kept in mind by risk assessors.

Challenge 2. Conceptual understanding by lay persons: example descriptors generically illustrate how a range of 'likelihood of occurrence' data might be comparatively graded and associated with the allocation of a numeric value. Numeric values do not necessarily need to reflect a linear escalating scale of occurrence (such that a value of 4 is twice as severe as that allocated a value of 2) but linear, positive or negative exponential scales may also be utilised. The key challenge is to ensure that users of the methodology are aware of the general relationship between the numeric values allocated to specific gradings and to provide sufficient justification for the scaling used. Bespoke guidance should be developed to support users in understanding and communicating the relationship between numeric values and gradings selected.

Challenge 3. Identification of the TWW reuse process stage at which the scheme should be applied: risk assessment is typically undertaken at the point where the protection target (whether organisms or receiving compartment) come into contact with TWW, However, there are a number of stakeholders involved in the 'distribution chain' (e.g. wastewater treatment plant operator, TWW distributor, TWW irrigator, picker, packer, seller and consumer) who are may be exposed to TWW under differing circumstances. This indicates the need for multiple risk assessments with the associated workload suggesting the need to prioritise certain exposure scenarios to focus data collection efforts. If this is accepted, what criteria should be used to prioritise use as the number of feasible exposure scenarios would appear to be almost infinite. For example, is it most pertinent to consider TWW at its point of discharge from the wastewater treatment plant or following TWW transportation (and possible storage) to its point of use? Once the TWW has reached its point of use, the way in which the TWW is then used (i.e. the reuse application) can also affect the likelihood of contact between a CECs and an identified target. For example, in agricultural irrigation under a scenario where a human is the protection goal, factors such as type of irrigation, type of crop and its subsequent processing can all impact on the likelihood of a specific CECs coming into contact with a human via an ingestion pathway. In relation to aquifer recharge, factors such as the mode of recharge, substrate type and retention time can all impact on the likelihood of a specific CECs within

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TWW coming into contact with a receiving compartment (e.g. a surface water body). Such considerations indicate the need for development of an integrated 'likelihood of occurrence' scheme which can incorporate several discrete aspects of CECs fate and behaviour into a single grading with associated numeric value. This is recognised as a complex challenge, and an approach to addressing this is explored further in Section 4.

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2.3 Development of a procedure for evaluating magnitude of impact

A qualitative approach, similar to that used to identify and rank the likelihood of occurrence (i.e. contact between a CEC and an identified receptor, see Section 2.2), can also be applied to considering the magnitude of impact of a specific CEC discharged within a TWW flow which has come into contact with an identified protected target. Table 2.3 sets out a series of possible descriptors to represent a comparatively escalating magnitude of impact over a four-point scale.

Table 2.3 - Example of descriptors to benchmark progressively increasing magnitude of impact.

Possible descriptors for relative grading	Ordinal value associated with magnitude of impact
High (irreversible effects)	4
Medium (reversible effects)	3
Low (effect detected but not at concentration thought to cause harm)	2
Very low (no obvious and direct impact)	1

Whilst generic in nature, Table 2.3 provides guidance which practitioners can use to support a consistent assessment of the consequences of a CECs discharged within a TWW flow coming into contact with a target organism or receiving compartment. As in Table 2.2, the example descriptions given in Table 2.3 are generic and only describe how a range of 'magnitude of impact' data might be comparatively graded.

With regard to the application of such an approach, the following theoretical and practical challenges were highlighted during NEREUS WG5 discussions with regard the use of TWW in reuse applications:

Challenge 1. Lack of data: as described in relation to 'likelihood of occurrence' (Section 2.2), the use of a qualitative scheme is an approach to supporting policy-makers and practitioners in their decision making. This is particularly true in light of the scarcity – or complete absence of - data sets relating to CECs concentrations at which protected targets are exposed to and/or the concentration at which there is no effect. Whilst the provision of such data is required under various legislative frameworks (e.g. REACH), progress to towards developing these data sets is slow and is as yet not available for many substances of interest as either parent compounds or the often multiple break-down products.

Challenge 2. Available data relates to the effect of single substances: where CECs data is available on its impact on a protection target, it is typically available in relation to the behaviour of a single substance. This is unlikely to be a true reflection of the field behaviour of a substance where a pollutant cocktail will exist, with potential for antagonistic or synergistic

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effects to occur over space and time in relation to both parent compounds and their breakdown products. Approaches to addressing this challenge include bioassays and the potential for the use of these within policy development and practice are considered and discussed within NEREUS WG3 (e.g. see Deliverable 14).

Challenge 3. Address acute and/or chronic CECs impacts? Exposure to microbial parameters (e.g. pathogens) are characteristically considered to have an acute impact, whereas CECs are deemed to have a chronic impact. Specifying a time period, together with an identified consequence, is an important component of a risk assessment procedure. The inclusion of a time frame enables the development of a risk score which reflects the probability of an identified CECs causing a specified impact during e.g. the next decade. As TWW may contain CECs of both a microbial and chemical nature, there is a need to develop an approach which has the flexibility to address both acute and chronic impacts e.g. through the use of risk assessment factors. Further consideration of this is aspect is included within Section 4.

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3. Overview of aspects likely to affect the impact of TWW-derived CECs on specific protection targets and receiving compartments

To inform the development of schemes to rank the 'likelihood of occurrence' and 'magnitude of impact' of CECs on specific receptors as a result of TWW reuse, NEREUS WG5 led the compilation of a series of tables which identify key variables with the potential to influence the fate of CECs pre-, during and post- TWW reuse during agricultural irrigation and aquifer recharge which represent the various key points at which different protection targets may be exposed.

For both applications, information collected was presented around five themes, as follows:

- Operational and maintenance aspects - for example, characteristics of TWW (sources, treatment type etc.), length and type of storage prior to use, soil characteristics and management, volume applied etc.
- TWW application method - e.g. type of agricultural irrigation system (subsurface, drip, sprinkler etc.), mode of aquifer recharge (surface spreading, direct injection)
- Protection targets - including human health (occupational and non-occupational exposures), crops, soil (and their fauna and flora), water bodies (and their fauna and flora) etc.
- Categories and types of crops irrigated by reclaimed water / types of aquifer irrigated
- Methods to categorise risks to health from using treated water in agricultural irrigation practices / aquifer recharge schemes

Draft agricultural and aquifer recharge tables were circulated to all NEREUS COST Action members for comment and received feedback was used to revise and update the tables (see Annex II and III for collated versions).

Tables set out in Annex II and III to act as a framework (or list of parameters) for the collection of data on CECs within TWW identified for use within agricultural and recharge applications. Inputs from NEREUS COST Action members to each of the parameters identified within the five tables contributes to the development of a knowledge database which – if /when data availability facilitates its completion - can be directly utilised to underpin a conventional risk assessment approach and subsequent policy development recommendations.

3.1 Overview of aspects likely to impact on the fate of CECs in agricultural irrigation

NEREUS WG5 discussions in Sarajevo (September, 2016) focussed on the agricultural irrigation tables (Annex II), with an output of these discussions being the source-pathway-receptor (SPR) model presented in Figure 3.1. The SPR model highlights key aspects for consideration within a risk assessment framework, highlighting a range of possible linkages between CECs (hazards) present in wastewater (see 'Sources' in Figure 3.1) and a wide range of protection targets (see 'Receptors' in Figure 3.1) via a range of possible exposure routes (see 'Pathways' in Figure 3.1). Having established that there are several complete SPR chains, the next step is to interrogate each of these identified complete chains in turn to assess the likelihood that identified protection targets come into contact with short-listed CECs (see NEREUS Deliverable 19), and if so the magnitude of impact.

Section 4 sets out possible approaches to identifying and ranking the likelihood of occurrence of CECs in TWW and their magnitude of impact on identified receptors following TWW use within an agricultural irrigation application.

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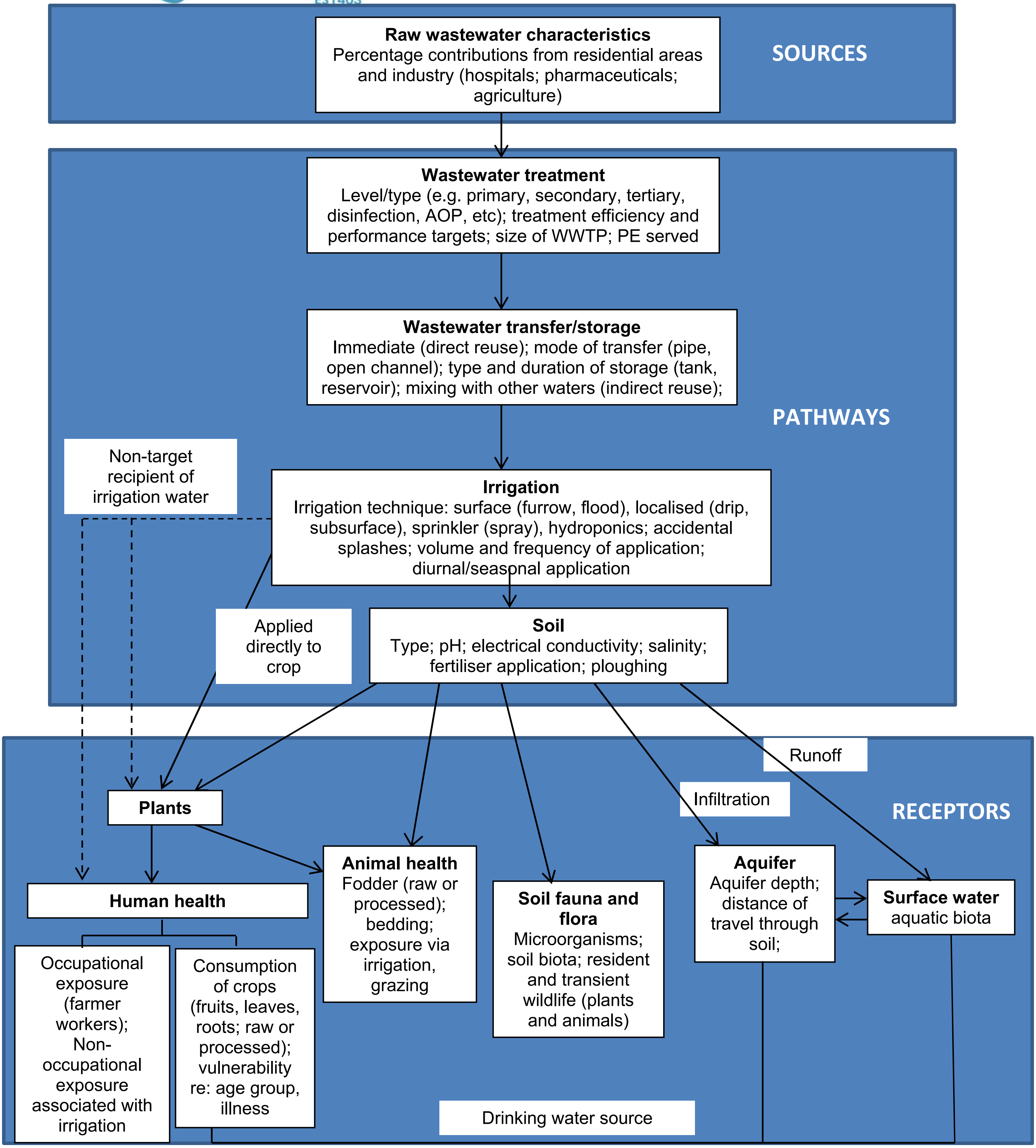

Key: AOP = advanced oxidation processes' PE = person equivalent

Figure 3.1 - Conceptual source-pathway-receptor model: overview of aspects with the potential to impact on the fate of CECs during agricultural irrigation with treated wastewater.

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4. NEREUS risk assessment framework

As a first step in developing structured approaches to assessing the 'likelihood of occurrence' and 'magnitude of impact' of CECs coming into contact with soil (the identified receiving compartment), the following assumptions are made:

- CECs are identified as the hazard
- the likelihood of occurrence is considered in terms of the likelihood of CECs from TWW reaching the soil
- the magnitude of impact is considered in terms of the impact of soil on potential CECs bioavailability

The framework provides a structured basis for assessing all is shortlisted CECs identified in NEREUS Deliverable 19.

4.1 Likelihood of CECs reaching the soil environment (occurrence)

4.1.1 Sources of wastewater

Contaminants of emerging concern (CECs) may be discharged into a wastewater treatment system from a predominantly rural, mixed urban/rural and/or predominantly urban catchments. Increasing levels of urbanisation / population density, increases the likelihood (and probably scale of) CECs sources with a catchment. Such sources include residential areas, hospitals and industry which are widely identified in the literature as major CECs sources, with their relative importance varying in relation to a range of catchment-specific characteristics such as population size and demographic, industry type, location of onsite hospital treatment systems, etc. (US EPA, 2009; Vidal-Dorsch et al., 2012; Petrie et al., 2015; Fairbairn et al., 2016). A working assumption in this report is that the greater the variety of sources, the greater the likelihood that CECs will be present in the untreated wastewater directed to the WWTP. Wastewaters from residential areas contain CECs as a consequence of a diversity of everyday activities, from the washing of textiles to the disposal of unused/out-of-date medicines and, in the case of pharmaceuticals, due to their excretion in an unchanged state. Additional specific sources entering wastewaters are industrial effluents and hospitals, particularly from those without any on-site treatment. It is likely that larger industries/hospitals will treat effluents prior to discharge to the sewer system whereas smaller units may lack treatment facilities (Pauwels and Verstraete, 2006). The proposed rank scoring for 'sources of wastewater' (Table 4.1) is dependent on the different combinations of sources which may contribute CECs into wastewater being directed to the WWTP. The identified sources include the following:

- Rural sources entirely composed of residential inputs with no commercial (i.e. retail), industrial or hospital contributions
- Urban sources which in addition to residential/commercial inputs may also include industrial and hospital inputs. If the latter two sources do not possess any on-site treatment, there will be a greater probability of CECs being present in the wastewater.

With specific regard to pharmaceuticals, these can enter the wastewater stream arriving at WWTPs from residential sources (private residences, hotels and residential care facilities) as well as from commercial facilities (including hospitals). The likelihood of the occurrence of pharmaceuticals is greatest when the WWTP influent contains effluent from pharmaceutical industries followed by hospitals, industry and residential areas (Guardabassi et al., 1998; Hartmann et al., 1998; Giger et al., 2003; Brown et al., 2006; Santos et al., 2013). The result is a decrease in wastewater pharmaceutical *concentration* in the order pharmaceutical manufacturers > hospitals > residential sources > non-pharmaceutical industrial sources. However, there is a decreasing order of pharmaceutical *load* in wastewater of municipal > pharmaceutical manufacturers > hospital > non-pharmaceutical industrial sources.

The rank scoring grid outlined in Table 4.1 is proposed to incorporate catchments comprised of different combinations of the previously identified sources.

Table 4.1 - 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>

4.1.2 Level of wastewater treatment

Untreated wastewater is not permitted to be used directly for irrigation in the EU. Additionally, current proposals on the development of water quality indicators for an EU Water Reuse Directive (JRC, 2017) state that secondary treated wastewater, where the treated effluent complies with the requirements of the Urban Waste Water Treatment Directive (UWWTD; EU, 1991), should not be used for this purpose. However, in the rank scoring system developed to identify the likely presence of CECs in TWW, the highest rank score (4) has been allocated to secondary treated wastewater (e.g. using activated sludge aeration) based on the premise that there may still be circumstances under which this could be used (see Table 4.2). These conventional systems are typically not designed to treat CECs with the result that a high proportion of the parent compounds and their metabolites can escape elimination in the WWTP and be discharged into the aquatic environment. This is particularly true of surfactants, pharmaceuticals and personal care products (PPCPs) and polar pesticides (Petrovic et al., 2003). Chlorinated phenoxy herbicides are generally resistant to activated sludge treatment and where biodegradability exists (e.g. in the case of mecoprop) there is a problem associated with a long adaptation time (Nitscheke et al., 1999). Many PPCPs are not fully degraded, with carbamazepine demonstrating particularly low removal (Ternes, 1998). In highly antibiotic-contaminated wastewater streams, there can be strong selection for ARB and therefore activated sludge treatment is not recommended in these situations unless bacteria from the treatment process can be eliminated before discharge (Pruden et al., 2013).

The increased efficiency achieved in microbiological wastewater treatment through the use of membrane bioreactors (MBRs) is indicated by allocating a rank score of 3 (see Table 4.2). MBR technology facilitates a low sludge load in terms of biological oxygen demand (BOD) which encourages bacteria to mineralise poorly-degradable organic compounds and supports a high sludge age, which gives the bacteria time to adapt to these substances (Peters et al., 2000; Cote et al., 1997). Gonzalez et al. (2016) report that MBR systems enhance the removal of many CECs compared to activated sludge systems, particularly in the case of hydrophobic compounds which have lengthy residence times. Although high levels of elimination (>90%) have been observed for many compounds there are some PPCPs, for example amitriptyline, carbamazepine, diazepam, diclofenac, fluoxetine, gemfibrozil, omeprazole, sulfamethoxazole, and trimethoprim, for which removal is less efficient (24-68%) (Trinh et al., 2012). Similar limited removal of PPCPs erythromycin, trimethoprim, naproxen, diclofenac, carbamazepine and the flame retardant TCEP in MBR systems was observed by Kim et al. (2007), although the hormones estriol, testosterone, and androstenedione were efficiently removed (99%).

Tertiary and advanced treatments include adsorption, ozonation and advanced oxidation processes. Ozonation and advanced oxidation processes can involve the formation of unwanted toxic by-products which must be accounted for in assessments of treatment utility for the production of irrigation water. This is recognised in the scoring system by allocating a lower rank score (1) to those oxidation treatment processes where there is the possibility of producing toxic by-products compared to a treatment scenario where this is known not to be the case (rank score 2).

Adsorption techniques using powdered and/or granulated activated carbon are widely practised, with removal efficiency depending on contact time and the physico-chemical properties of both the adsorbate and the adsorbent. Positively charged substances are generally removed efficiently, with increased removal efficiency as the substance's octonal-water partitioning coefficient (pK_{ow}) increases. A pK_{ow} value greater than 4 indicates a high potential for sorption to activated carbon (Margot et al., 2013). Granulated activated carbon has been shown to be capable of removing a range of different PPCPs and flame retardants to levels below detection limits (Kim et al., 2007).

Ozonation is a well-established technique in which compounds depending on the operational pH, are broken down either by direct reaction with ozone or with the generated hydroxyl radical. However, there are some recalcitrant compounds with low removal efficiencies including mecoprop, benzotrizole, gabapentin and sucralose, as well as microcontaminants containing amide groups (Margot et al., 2013; Reungoat et al., 2012). Nakada et al. (2007) monitored the behaviour of 24 different pharmaceutical compounds using a combination of ozonation and sand filtration with activated sludge treatment and found efficient removal (>80%) of most of the target compounds, primarily due to the ozonation step.

Common unintended and potentially toxic by-products associated with ozonation of wastewater include nitrosamine and N-Nitrosodimethylamine (NDMA) (Hollender et al., 2009). The treatment of secondary effluents with high doses of ozone has demonstrated both increased toxic potential (Microtox® test) or mutagenic activity (Ames test) due to the formation of toxic by-products (Petala et al., 2008). Similar problems are associated with advanced oxidation processes (AOPs) such as UV/H_2O_2, photo-Fenton, heterogeneous photocatalysis, or O_3/H_2O_2, with sand filtration or activated carbon filtration recommended for the removal of unwanted oxidation by-products. The non-selectivity of these AOPs is a substantial advantage and although well proven to efficiently remove organic contaminants at the laboratory scale, more pilot and full scale studies are needed to determine the optimal operational conditions for CECs including ARB&ARGs. Pilot scale studies using O_3/H_2O_2 showed considerable removal efficiency (>90%) for several steroid hormones and PPCPs but TCEP remained resistant with only 13% removal (Gerrity et al., 2011). The overall rank scoring grid postulated for wastewater effluents produced by different levels of treatment system is shown in Table 4.2. In addition to the below treatment process (grouped by treatment level), disinfection is an important and common WWTP process. However, because it can be used in combination with any of the below levels of treatment it is not specifically included in Table 4.2. The impact of disinfection (primarily by chlorination) is well characterised in terms of impact on a range of pathogens, as well as its role in the formation of toxic breakdown compounds e.g. tri-halomethanes. Hence, as an indicative approach, it is currently recommended that the use of disinfection as part of a WWTP reduces the score by 1 during conventional or enhanced secondary treatment if the priority CECs is of a microbial nature. If the CECs is not of a microbial nature, then scores should not be amended.

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Table 4.2 - 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.

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

4.1.3 Effect of storage prior to use

The critical factor regarding storage of treated wastewater prior to use for irrigation will be whether the CECs is retained or whether it is degraded to daughter products which in turn are either less toxic, or more toxic, than the original CECs. The extent to which these processes are possible will depend upon:

- The time between the TWW discharge from the treatment plant and use for irrigation purposes. This will include both the transfer within the distribution system and the storage time within either an open or closed system
- The susceptibility of the CECs to physical processes (e.g. adsorption to suspended solids followed by deposition), chemical processes (e.g. hydrolysis, photodegradation [reduced in closed systems]) and biological processes (e.g. biodegradation).

The efficiency of biotic/abiotic degradation processes varies widely between different CECs. However, differences have been reported between the results obtained from laboratory scale experiments and observations in the field. Ryan et al. (2011) found that allowing photolysis in wastewater stabilisation ponds led to enhanced PPCP removal, although direct UV radiation has been reported to be ineffective for the removal of antibiotics (Adams et al., 2002). There is also disagreement regarding the role of hydrolysis reactions with controlled tests showing limited evidence of ciprofloxacin, sulfamethoxazole and trimethoprim removal by hydrolysis (Al-Ahmed et al., 1999; Alexy et al., 2004). Where degradation does occur, the occurrence of increased toxicity of the resultant daughter products needs to be considered alongside the potential for changes in antibiotic resistance. The presumption herein is that if the daughter products are more toxic then they are relatively less likely to contribute to antibiotic resistance (Chait et al., 2012).

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The worst scenario would be where there is either no degradation of the original CECs or degradation results in the formation of a toxic daughter product. This is allocated a rank score of 2 compared to a rank score of 1 where the degradation of the original CECs results in the formation of a non-toxic daughter product (see Table 4.3). In the case of antibiotics, the existence of an increased toxicity has to be balanced against a reduction in antibiotic resistance.

Table 4.3 - Example of an approach for allocating rank scores in relation to the influence of storage on the likelihood of CECs occurring in soil.

Storage prior to use	
During the storage/distribution process there is either no breakdown of the original CECs or the breakdown results in a toxic daughter product	During the storage/distribution process breakdown of the original CECs results in a non-toxic daughter product
2	1
2	1
2	1
2	1

4.1.4 Irrigation techniques

The efficiency with which treated wastewater, and hence the CECs contained within it, reach the receiving soil and subsequently plants growing in the soil via the roots and the vascular system is dependent on the irrigation method employed. For the purposes of developing a scoring system for this process, four different categories of irrigation have been identified (Doneen and Westcot, 1988):

- Surface irrigation - this can be sub-divided into flood irrigation (TWW is applied over the entire area to be irrigated to directly infiltrate into the soil) and furrow irrigation (TWW is applied between ridges and reaches the plant roots in the ridge by capillary action). Both systems can result in contamination of the soil followed by entry into plants through the roots and translocation into the stem, leaves and fruits via the vascular system. Moderate direct contact between TWW and aboveground plant parts can be expected during surface irrigation. Surface irrigation processes operate by gravity and also present a risk to groundwater due to seepage.
- Spray/sprinkler irrigation - TWW is applied in the form of a spray simulating the action of rain reaching the soil. It is important that the rate of application avoids the ponding of water on the surface. In addition to permitting root uptake, this form of irrigation will lead to direct contact between the TWW and all plant surfaces, including edible plant parts.

- Drip irrigation - TWW is applied directly to the root zone of each plant or group of plants. Ideally, the application rate is controlled to meet evapotranspiration requirements in order to minimise percolation losses. The deposition of CECs in plant rhizosphere soil will be followed by entry into plants through the root system followed by translocation into the stem, leaves and fruits via the vascular system. There will be minimal direct contact between aboveground plant tissues and TWW.
- Sub-surface irrigation - TWW is supplied via e.g. sub-surface pipes below the root zone which is reached through capillary rise. Entry into the plants is through root uptake followed by translocation to aboveground plant parts via the vascular system. In sub-surface irrigation there is no direct contact between aboveground plant parts and the TWW.

All the identified irrigation systems have the potential to contaminate the soils and ultimately the plants through the presence of CECs in treated wastewater assuming no barriers to root uptake and internal translocation of the CECs. However, drip irrigation and sub-surface irrigation are more controlled means of TWW delivery in that direct contact between TWW and aboveground plant parts is limited, and the supply of TWW to the soil is regulated to crop requirements. This limits TWW percolation to groundwater and/or CECs build-up in the soil and enables the establishment of an equilibrium between uptake (e.g. by plants) and possible breakdown of CECs within the soil. Therefore, both these irrigation procedures have been given a rank score of 1. This level of control is not possible with surface irrigation where gravity systems are employed to effectively flood the irrigated area. The increased potential for soil contamination merits a rank score of 2. In spray/sprinkler irrigation, which is correctly adjusted to avoid surface ponding, the level soil contamination will be less severe but the potential for direct contamination of the plant surfaces also merits a rank score of 2.

Table 4.4 - Example of an approach for allocating rank scores in relation to the influence of irrigation type on the likelihood of CECs occurring in soil.

Soil irrigation technique			
Surface irrigation	Spray/sprinkler irrigation	Drip irrigation	Sub-surface irrigation
2		1	
2		1	
2		1	
2		1	

4.1.5. Development of an approach for developing a single score indicating the likelihood of CECs occurring in soil following irrigation with TWW

Table 4.5 gives an overview of the range of approaches to multiplying together the factors which are able to influence the likelihood of CECs reaching the soil. A multiplicative approach has been used to produce the combined values to highlight the ‘extremes’ (i.e. the best and worst values) and provide greater discriminatory power than would be achieved by addition. As noted in Section 2.1, users must be aware that ranked scores are ordinal and not numeric. Multiplying ordinal values together enables the development of a prioritised list of CECs in terms of (in this example) likelihood of occurrence; from most likely (highest combined score) to least likely (lowest combined score). It does not provide any information on what ‘most likely’ means nor can it be used to determine how important the difference is between, for example, a CECs ranked 1st as opposed to that rank 3rd or 8th. However, it can be used to identify a specific CECs or a short-list of CECs which are of most concern from the perspective of occurrence.

Multiplying the individual ranking scores allocated in Table 4.5 together generates combined scores ranging from 1-64 (see Table 4.6). These are grouped into ranges representative of ranking scores identifying the overall likelihood of a CECs occurring in soil following irrigation with TWW.

Table 4.5 - Example of an approach for developing a single combined score which represents the likelihood of CECs occurring in soil following irrigation with TWW.

Sources of wastewater				Characteristics of WW treatment				Storage prior to use		Soil irrigation			
Rural WW	Urban/Municipal WW			Secondary treatment (employing filter beds/ activated sludge aeration)	Enhanced secondary treatment (e.g. membrane bioreactors)	Tertiary/ advanced treatment; where oxidation processes can lead to toxic by-products	Tertiary/ advanced treatment; where there is NO possibility of toxic by-products	During the storage/ distribution process there is no breakdown of the original CECs or breakdown results in a toxic daughter product	During the storage/ distribution process breakdown of the original CECs results in a non-toxic daughter product	Surface irrigation	Spray/ sprinkler irrigation	Drip irrigate ion	Sub-surface irrigation
	Residential sources	Industrial/ hospital sources with on-site treatment	Industrial/ hospital sources with NO on-site treatment										
	4			4				2	1	2		1	
	3			3				2	1	2		1	
	2			2				2	1	2		1	
1				1				2	1	2		1	

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Table 4.6 - Quantitative approach to linking likelihood of occurrence scores to descriptor ranges.

Score	Likelihood of occurrence	Range
4	Likely to occur	37-64
3	Possible (may occur sometimes)	17-36
2	Unlikely (uncommon but know to occur	7-16
1	Rare (lack of evidence but possible)	1-6

4.2 Magnitude of impact of receiving soils on CECs bioavailability

4.2.1 CECs load in treated wastewater

In assessing the likelihood of CECs occurrence in soils as a consequence of irrigation with TWW, the different sources contributing to the raw wastewater were considered (Section 4.1.1). However, when considering the potential impact to the receiving soil it is important to include the influence posed by the quantity of CECs delivered to the soil, which, in turn, will be dependent on the contaminant load delivered during the irrigation. In practice, the irrigation flow rate is unlikely to be readily available and therefore, assuming that in most instances constant irrigation flow rates will be used, it is proposed that concentrations should be used as a surrogate for this purpose. However, the relationship between loadings and concentrations can also be influenced by the contaminant sources. For example, in the case of antibiotics it has been shown that whereas a decrease in wastewater pharmaceutical concentration exists in the order pharmaceutical manufacturers > hospitals > residential sources > non-pharmaceutical industrial sources the pharmaceutical load increases in the order non-pharmaceutical < hospital < pharmaceutical manufacturers < municipal (Brown et al., 2006; Guardabassi et al., 1998; Larsson et al., 2007; Lin and Tsai, 2009; Phillips et al., 2010; Pruden et al., 2013; Rodriguez-Mozaz et al., 2015; Santos et al., 2013; Sim et al., 2011)

Du et al. (2014) have monitored the influent to a traditional activated sludge municipal treatment plant (MTP) with a mean daily load of ~25 million gallons per day as well as the effluent following both conventional activated sludge treatment and advanced aerobic treatment. The reported results (Table 4.7) show the variability which can exist for the concentrations of six different pharmaceuticals arriving at the WWTP. The monitored removal performances vary from extremely low for carbamazepine (~5%) to a highest efficiency of 84.5% for diclofenac by conventional activated sludge treatment. There is also inconsistency between the treatments with the expected improved performance of the advanced aerobic treatment not always producing the lowest effluent concentration (e.g. erythromycin, sulfamethoxazole and trimethoprim).

Table 4.7 - Reported CECs concentrations in raw influent and treated effluents for a municipal wastewater treatment plant (Du et al., 2014).

CECs	Influent concentration (ng/L)	Effluent concentration (ng/L)	
		MTP*	ATS**
Carbamazepine	150	143.3	141.2
Diclofenac	104	16.1	57.2
Erythromycin	140	42.0	70.7
Sucralose	47,500	38475	39425
Sulfamethoxazole	2,600	806.0	1976
Trimethoprim	420	86.1	142.8

* MTP: traditional activated sludge municipal treatment plant (MTP)
**ATS: advanced aerobic treatment systems

Based on the assumption that CECs concentrations can be used as a surrogate for CECs loads for predicting the impacts on the receiving soil, the values reported in Table 4.7 can be used as a guide to developing an appropriate scoring system. Considering the span of reported effluent concentrations, the following scoring system is suggested for assessing the impact of contaminants on the receiving soil:

Treated wastewater individual CECs concentration range (ng/L)	**Allocated rank score**
>10,000	4
1,000-10,000	3
100-1000	2
<100	1

Table 4.8 - Example of an approach for allocating rank scores in relation to the influence of loads of CECs in treated wastewater reaching the receiving soil environment.

CECs load (concentration) in treated wastewater			
CECs concentration in TWW exceeds 10,000 ng/L	CECs concentration range in TWW is 1,000 to 10,000 ng/L	CECs concentration range in TWW is 100 to 1,000 ng/L	CECs concentration in TWW is less than 100 ng/L
4			
	3		
		2	
			1

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4.2.2 CECs bioavailability/bioaccessibility in the soil

Following their introduction into soils as a result of irrigation with TWW, CECs can be subjected to sorption/desorption and transformation processes, which influence their bioavailability and bioaccessibility in the soil pore water and hence their uptake by plants. The relevant factors controlling these processes are the soil properties and the chemical form of the CECs in the plant root environment (NEREUS Deliverable 2).

The flow of solutes and contaminants within the soil is dependent on the physical nature of the soil (particularly in terms of grain size distribution (e.g. gravel, sand, silt, clay) as well as the existence of voids and channels. Agricultural soils are highly heterogeneous in nature in terms of both organic content (e.g. humin, humic and fulvic acids, polysaccharides, lignin) and mineral fractions (e.g. SiO_2, oxides and hydrous oxides of iron, aluminium and manganese, silicate and aluminosilicate minerals, carbonates, sulphates, phosphates). This results in highly variable properties such as pH, electrical conductivity, cation exchange capacity. Soil water-holding capacity and porosity may affect the movement of CECs to the vicinity of plant roots and the level of soil porosity and permeability can influence the uptake of CECs by plants. A major mechanism controlling CECs availability in soils is partitioning due to mass transfer between the aqueous phase and solid surfaces as a result of physico-chemical interactions between dissolved contaminants in the pore water and soil solids (SOM and reactive mineral surfaces).

An important parameter in contaminant movement is the distribution, or partitioning, coefficient (K_d), which describes the relative potential for dissolved contaminants in soil pore water to be sorbed to bulk soil solids. The K_d is defined as the ratio of the contaminant concentration associated with the solid phase to the contaminant concentration of the surrounding solution when the system is at equilibrium, and thus is a property of a specific soil-solution combination. Note that the K_d value does not provide information about the mechanism of contaminant removal from solution, and reflects only the equilibrium ratio between sorbed and dissolved species. The previously-mentioned K_{ow} of a contaminant is particularly important when considering the movement of non-polar organic contaminants in soil. The K_{ow} denotes the degree of hydrophobicity (or lipophilicity) of a compound and is empirically related to the K_d value of a soil by the soil's organic carbon content. Thus, characteristics of both the soil (mineral surface reactivity, SOM content) and the contaminant (K_d and K_{ow}) are critical parameters for assessment of contaminant behaviour in soil.

The extent and degree of sorption is dependent on both the nature of the contaminant and the nature of the soil. The impact of differing soil properties on CECs sorption has been evaluated by Vasudevan et al. (2009). Soil pH was found to have a statistically significant effect on the

K_d values of ciprofloxacin, and cation exchange capacity was a key soil factor influencing the extent of sorption at all pH values (3-8). Colloids can play an important role in sorption processes with CECs strongly associated with colloidal particles having limited bioaccessibility/bioavailability to soil microbiota and plants. Although there is limited information on colloid sorption of CECs in agricultural soils, Zhou et al. (2007) have observed the relevance of colloid properties leading to interactions of differing strengths due H-bonding, van der Waals forces and charge transfer to the polar functional groups.

Soil factors which influence the sorption of CECs to the soil solid phase include the relative quantity of organic matter, soil pH, mineral composition and soil temperature. There is clear evidence that SOM is an important factor influencing the sorption of pharmaceuticals and thus, their ultimate fate in the environment (e.g. Park and Huwe, 2016). Because of the influence of soil organic matter, K_d values are often normalised to the percentage organic carbon present in the soil producing K_{oc} values. Generally, compounds with log K_{oc} values <2 are considered to be capable of only weak sorption. Where experimentally derived K_{oc} values are not available they can be estimated from octanol-water coefficients (K_{ow}) (e.g using EPI Suite). Other hydrophobicity-independent mechanisms which contribute to reduced CECs availability include cation exchange, cation bridging at clay surfaces, surface complexation and hydrogen bonding may contribute to reduced mobility (Yamamoto et al., 2009).

In terms of chemical characteristics, CECs may vary from highly hydrophilic (log K_{ow} <1; e.g. sucralose) to hydrophobic in nature (log K_{ow} >4; e.g. ciprofloxacin) affecting the relative affinity of CECs for the soil-water phase. They can also occur as neutral or ionic forms (e.g. cationic, anionic and zwitterionic). The ionisable/neutral distribution of a CECs is determined the value of its acid dissociation constant (pK_a) relative to the soil pH. The pK_a is the negative logarithmic expression of the equilibrium constant describing the reaction of acid in water, e.g. $HA + H_2O \leftrightarrow H^+ + A^-$. The smaller the value of the pK_a for a compound, the stronger the acid, or the more likely the compound is to dissociate. At a soil pH higher than the pK_a of an acidic CEC (pK_a <4; e.g. diclofenac), the anionic form of the CECs predominates. This anion sorbs less strongly to soil than its neutral counterparts as a result of the net negative surface charge exhibited by most soil solids. Acid compounds tend to be neutrally charged in acid soils and negatively charged (in anionic form) in alkaline soils.

In contrast, chemically basic compounds tend to be in cationic form (positively charged) in acidic soils and chemically neutral in alkaline soils. The behaviour of chemically basic compounds may either be described by a base dissociation constant, pK_b (the negative logarithmic expression of the equilibrium constant describing the the generalised reaction $B + H_2O \leftrightarrow BH^+ + OH^-$ or, more commonly, by a pK_a. Compounds with strong basic character exhibit a large pK_a value, or a small pK_b. Strongly basic compounds (e.g. pK_a >9) occur

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primarily in the cationic form in neutral to acid pH soils which supports increased adsorption of chemically basic compounds to negatively charged soil particles. Sulphonamides have been shown to exhibit increasing sorption potential with decreasing pH as positive species became more predominant (Park and Huwe, 2016). For very weak acids (pK_a >8; e.g. triclosan) and weak bases (pK_a <6; e.g. sulfamethoxazole, carbamazepine), the neutral form predominates in most soil environments (Wu et al., 2013). However, many CECs are complex molecules containing several functional groups including both ionisable acid and basic moieties leading to the formation of zwitterions. This is characteristic of most of the fluoroquinolones (e.g. ciprofloxacin) which can exist as both anionic and cationic forms and, depending on the soil pH, these can coexist in zwitterionic form (Chen et al., 2011).

The binding of CECs to soils can result in the formation of non-extractable residues (NER). This is often a controlling factor in relation to the fate and persistence of pesticides and may also apply to pharmaceuticals. Acetaminophen has been shown to be rapidly converted to bound residue (73.4-93.3%), compared to carbamazepine which was retained at <4.2% in the same soil (Li et al., 2013). Sulfadiazine and triclosan have also exhibited irreversible formation of NER. In contrast, it is the exchangeable or reversibly sorbed fractions together with dissolved species which are readily available for migration and biological uptake.

The availability of CECs introduced into soils for biological uptake can be reduced by biodegradation, volatilisation and photodegradation. Microorganisms have been shown to biodegrade diclofenac, with 57% removal over a 45-day laboratory incubation period (Xu et al., 2009). However, in tests using different soils, Dodgen et al. (2014) observed that only a small fraction of carbamazepine (<1.2%) was mineralised. The volatility of CECs from topsoil, as determined by the octanol-air (K_{oa}) and air-water (K_{aw}) partitioning coefficients, is limited (Undeman et al., 2009). Photodegradation of pharmaceuticals dissolved in water is a significant removal pathway (e.g. Fatta-Kassinos et al., 2011); however, photodegradation is limited in the case of soils due to limited UV penetration, and confined to only the top few millimetres of soil.

The overall availability of CECs for biological uptake is determined by their ease of movement through the soil (affected by CECs solubility in pore water and soil structure), the established sorption/desorption equilibria (determined by SOM content, soil complexation/chelation capacity, soil pH, and the pK_a of the CECs) and the existence of transformation processes (biodegradation, volatilisation, photolysis). The latter are either of minimal importance or, as in the case of biodegradation, occur relatively slowly. Therefore, a scoring scheme (Table 4.9) has been developed based on the balance between the ease of movement of the CECs within the soil and its bioavailability/bioaccessibility based on soil properties and the chemical characteristics of the CECs.

Table 4.9 - Example of an approach for allocating rank scores in relation to the influence of CECs bioavailability/bioaccessibility within the receiving soil environment.

CECs' bioavailability/bioaccessibility in the soil			
Ready movement of CECs within soil and ready availability for uptake	Limited movement of CECs within soil and ready availability for uptake	Ready movement of CECs within soil and limited availability for uptake	Limited movement of CECs within soil and limited availability for uptake
4			
	3		
		2	
			1

4.2.3 Biosolid/fertiliser addition to soils and ploughing

Both the addition of biosolids/animal manures and the action of ploughing can influence the availability of CECs in agricultural soils. Conventional cultivation is used to improve drainage and aeration, typically by breaking up undisturbed soil and reducing the size of soil aggregates. Ploughing can facilitate more rapid transport of contaminants from the soil surface to the root zone area (Dominguez et al., 2014). Application of biosolids or animal manures leads to an increase in SOM content as well as an elevated cation exchange capacity. Biosolids can modify the resistance of organic matter to decomposition more than the fertilizer treatments such that long-term biosolid application is able to significantly increase the organic matter stability. The increase in SOM leads to enhanced sorption and hence reduced mobility of many CECs, particularly those with hydrophobic characteristics.

In addition to increasing SOM content and providing reactive surfaces for CECs complexation, biosolids may introduce CECs from municipal wastewater. The occurrence of organic contaminants in biosolids has long been a concern as the chemical characteristics of many organic contaminants present in wastewater favour partitioning into the solid (sludge) phase, e.g. through hydrophobic interactions. One particular study analysed 87 organic compounds in nine different biosolids produced by WWTPs using different production methods. The study detected a minimum of 30 and a maximum of 45 different organic contaminants in any single biosolid sample, and noted that neither the percent composition of organic contaminants nor the identity of the most common contaminants varied substantially between the biosolids examined (Kinney et al., 2006). The sum total of contaminants in the biosolids investigated ranged from 64 to 1811 mg/kg dry weight (Kinney et al., 2006), indicating substantial potential for land-applied biosolids to contribute to CECs loading to agricultural soils.

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Animal manures have similarly been shown to contribute CECs to soil. A portion of the antibiotics added to animal feed are excreted and end up in animal manure. Food crops including corn (*Zea mays*), green onion (*Allium cepa* L.) and cabbage (*Brassica oleraceca* L. Capitata) have been shown to take up chlortetracycline from soils amended with animal manures (Kumar et al. 2005). In addition, organic contaminants associated with biosolids or animal manures may exhibit increased lability upon mixing with soil. Several studies have documented the transfer of veterinary antibiotics from soil-applied animal manures to local surface waters through desorption, surface runoff and soil erosion (e.g. Kim et al., 2011; Song et al., 2009). Composting has been shown to largely eliminate (>90%) veterinary antibiotics present in livestock manures, largely through abiotic processes (Kim et al., 2011; Ramaswamy et al., 2010). Thus, the possible introduction of an additional CECs load to soil should be considered along with the advantages conferred by increased SOM as a result of non-composted biosolids or animal manure application.

In the case of PPCPs, enhanced sorption to soil solids as a result of increased SOM content must also be balanced against the role of dissolved organic matter, which may either assist or retard movement depending on chemical and environmental properties (Navon et al., 2011; Haham et al., 2012). Increases in cation exchange capacity occurs as the organic matter associated with biosolids is an amorphous substance consisting of a large number of organic molecules with variable chemical composition containing 40-60% C, 30-50% O, 3-7% H and 1-5% N. The presence of a complex series of carbon chains and ring structures with numerous chemical functional groups (ligands) is responsible for the high cation exchange capacity. Qian and Mecham (2005) have reported that long-term wastewater application for crop irrigation increased the organic matter content of a soil from 0.9% to 1.3% and the cation exchange capacity 48.1 to 55.5 meq 100 g^{-1} soil via the addition of dissolved organic matter.

Although sorption of contaminants to SOM can retard contaminant movement thereby buffering impacts to the local environment, soil properties such as organic carbon content can also inhibit PPCP biodegradation by reducing contaminant bioavailability and hence inhibiting contaminant availability to microbial populations (Stumpe and Marschner, 2010; Xu et al., 2009). Therefore, biosolid amendment of soils reduces biodegradation (Li et al., 2014) and prolongs PPCP persistence in soil due to increased sorption. In addition, biosolids may serve as a more readily available nutrient or carbon source for microorganisms compared to PPCP, further contributing to a reduced biodegradation. This is in contrast to wastewater irrigation, which has been observed to have no discernible effect on the biodegradation of PPCP in soil (Grossberger et al., 2014).

Although biosolids encourage the persistence of contaminants in soils by limiting biodegradation, their major impact is on CECs mobility and availability in the soil environment. The soil structural changes brought about by ploughing activity to some degree counteract the effects of biosolids or manure amendment by assisting CECs movement within the soil but at a considerably reduced level. Therefore, in developing a scoring system relating to the combined effect of biosolid/animal manure application and ploughing on CECs availability in soils the following order is proposed: no biosolids/manure + ploughing > manure (only) + ploughing or no ploughing > biosolds + ploughing > biosolids + no ploughing. Note that composting of animal manures, which has been demonstrated to degrade veterinary pharmaceuticals, reduces CECs load associated with manure application and hence lowers the assigned score. The rank scores are allocated as shown in Table 4.10.

Table 4.10 - Example of an approach for allocating rank scores in relation to the influence of biosolids/fertiliser application and ploughing on CECs availability within the receiving soil environment.

CECs availability in the soil			
No biosolids/ animal manure application + ploughing	Animal manure (fresh or slurry) only application + ploughing or no ploughing	Biosolids/ composted animal manure application + ploughing	Biosolids/ composted animal manure application + no ploughing
4			
	3		
		2	
			1

4.2.4 Development of an approach for establishing a single combined score indicating the impact of CECs bioavailability within the soil environment

Table 4.11 - Example of an approach for developing a single score which represents the impact of CECs bioavailability within the soil environment.

CECs load (concentration) in treated wastewater				CECs bioavailability/bioaccessibility in the soil				CECs availability in the soil due to biosolids/fertiliser application and ploughing			
CECs concentration in TWW exceeds 10,000 ng/L	CECs concentration range in TWW is 1,000 to 10,000 ng/L	CECs concentration range in TWW is 100 to 1,000 ng/L	CECs concentration in TWW is less than 100 ng/L	Ready movement of CECs within soil and ready availability for uptake	Limited movement of CECs within soil and ready availability for uptake	Ready movement of CECs within soil and limited availability for uptake	Limited movement of CECs within soil and limited availability for uptake	No biosolids/ animal manure application + ploughing	Animal manure (fresh or slurry) only application + ploughing or no ploughing	Biosolids/ composted animal manure application + ploughing	Biosolids/ composted animal manure application + no ploughing
4				4				4			
	3				3				3		
		2				2				2	
			1				1				1

Table 4.12 gives an overview of the products of multiplying each of these factors which are able to influence the impact of CEC bioavailability within the soil environment. Possible scores range from 1-64.

Table 4.12 - Quantitative approach for linking impact of CECs' bioavailability scores and descriptors ranges.

Score	Likelihood of impact	Range
4	Likely to exert an impact; Major: Potentially lethal to local ecosystem; predominantly local, but potential for off-site impacts	37-64
3	Possible (may create an impact sometimes); Moderate: Potentially harmful to regional ecosystem with local impacts primarily contained to on-site	17-36
2	Unlikely (uncommon but impact may occur); Minor: Potentially harmful to local ecosystem with local impacts contained to site	9-16
1	Rare (little chance of impact); insignificant: Insignificant impact or not detectable	1-8

4.3 Overall hazard rating matrix

An overall assessment of the likelihood of a CEC occurring in soil in a mobile and bioavailable form with potential to have a detrimental impact can be deduced by multiplying the ranked scores described in Tables 4.6 and 4.12 together. As discussed earlier (see Section 4.1.5), ranked scores are ordinal values. Multiplying ordinal values together supports the development of a ranked list of CECs with regard to their potential to occur in soil in a bioavailable form resulting in uptake by an endpoint receptor (human or environmental). It does not provide any information on what e.g. a 'high probability' means, nor can it be used to determine how important the difference is between, for example, a CEC ranked 1st as opposed to one identified as being 2nd. However, the resulting score can be used to identify – or short-list -which CECs are relatively of most concern and should be prioritised for further research. Whilst the score itself has no quantitative meaning, such scores are often interpreted using a matrix (or heat map) such as that provided in Table 4.13. Despite their widespread use, there are currently no clear guidelines on how:

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- scores are segregated into discrete ranges
- different colours (e.g. the traditional red, amber, green) are allocated to identified ranges of numbers
- discrete ranges of values should be interpreted

Table 4.13 - Hazard rank score interpretation matrix.

		Likelihood of occurrence			
		Likely (4)	Possible (3)	Unlikely (2)	Rare (1)
Magnitude of impact	High (4)	16	12	8	4
	Medium (3)	12	9	6	3
	Low (2)	8	6	4	2
	Very low (1)	4	3	2	1

Examples in the literature vary greatly in relation to these three aspects, indicating this is generally a value judgement (Cox, 2008; Ball and Watt, 2013). In the absence of specific guidelines, the approach shown in Table 4.13 is proposed with an example of how the score ranges can be interpreted is provided below:

- A score of 12-16 indicates a high probability of the occurrence and bioavailability of a CEC in soil resulting in uptake by a receptor;
- A score of 9-11 indicates the possibility of the occurrence and bioavailability of a CECs in soil resulting in uptake by a receptor;
- A score of 5-8 indicates the unlikely (or limited possibility of) the occurrence and bioavailability of a CEC in soil resulting in uptake by a receptor;
- A score of 1-4 indicates that only on very rare occasions would the occurrence and bioavailability of a CEC in soil result in uptake by a receptor;

The assessment process outlined above provides an initial screening tool to evaluate potential CEC hazard. Users can combine this screening tool with available data / expert judgement for the transparent, auditable development of a prioritised list of CECs in relation to their availability for uptake from soil by receptors. Depending on the users 'appetite for risk' a predefined cut-off score can be selected, with CECs achieving a score of, for example ≥12, identified as requiring further analysis (e.g. quantitative risk

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assessment) in relation to the specific receptors of concern, either protection goals or receiving compartments.

4.4 Example of the application of the developed NEREUS risk ranking framework

The first step in trialling the developed approach is to construct a hypothetical scenario which provides the bio-physico-chemical conditions in which the framework will operate. The scenario presented here can be readily adapted by users to reflect any number / combination of site specific factors on a case-by-case basis that fall within the broad set of parameters identified in Sections 2-4. However, as always, the output of any tool is only as good as the data available / expertise of those implementing the system, and consideration must be given to robustness of the underlying data sets utilised.

The hypothetical scenario developed here relates to the use of TWW for agricultural irrigation, and the CECs selected is the antibiotic clarithromycin. Under the adopted scenario, TWW is piped directly from the WWTP following secondary treatment with membrane bioreactors to a closed tank, and is used for spray irrigation within 24 hours. The WWTP receives municipal wastewater from a mainly residential area with no major industrial or hospital contributions. The irrigated crops are growing in a neutral (pH 7) sandy soil. There is no soil amendment with biosolids or animal manure but the land is subjected to ploughing.

4.4.1 Assessment of likelihood of CECs reaching the soil environment (occurrence)

a) Dependence on sources of wastewater

As the incoming wastewater derives from a mainly residential area with no major industrial or hospital sources, a rank score of 2 is allocated (see Table 4.1).

b) Assessment of dependence on level of wastewater treatment

The scoring proposed for the different possible levels of wastewater treatment is shown in Table 4.2. For a treatment system employing enhanced secondary treatment with membrane bioreactors a rank score of 3 is allocated.

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c) Assessment of the dependence on effect of storage prior to use

The TWW is transferred directly to the irrigation site in a closed system and used within 24 hours allowing limited time for breakdown of the clarithromycin to occur by either hydrolysis or biodegradation. The absence of any exposure to natural light eliminates any degradation by photolysis. This situation identifies with the descriptor 'during the storage/distribution process there is no breakdown of the original CECs' category in Table 4.3 and earns a rank score of 2.

d) Assessment of dependence on technique used for soil irrigation

The different methods of irrigation have been divided into two categories for scoring purposes (Table 4.4). Spray irrigation is considered to pose an increased CECs risk due to both soil and plant contamination and is allocated a rank score of 2.

Therefore, the overall score relating to the likelihood of CECs reaching the soil environment is 2 x 3 x 2 x 2 = 24. This falls within the '16-36' range indicating an overall score of 3 in terms of 'likelihood of occurrence' (Table 4.6). Under the identified scenario there is the possibility of clarithromycin being found in the soil, i.e. it may occur sometimes.

4.4.2 Assessment of the magnitude of impact of CECs bioavailability within the receiving soil environment

a) Assessment of the dependence on CECs load in treated wastewater

As explained in Section 4.2.1, it is proposed that CECs concentrations are used as a surrogate for CECs loads in TWW and this is supported by the availability of relevant data. Tuckwell (2014) has reviewed available monitoring data for clarithromycin in TWW and reports a range of 103-996 ng/L. However, there is no indication of the level / types of wastewater treatment relating to these data. Following conventional activated sludge treatment, effluent clarithromycin concentrations in the range 57-598 ng/L have been reported (Tuckwell, 2014). After more sophisticated treatment (primary and secondary clarifiers followed by sand filtration), McArdell et al. (2003) found clarithromycin concentrations of between 57 ng/L and 135 ng/L in the treated effluent from a WWTP receiving wastewater from an urban catchment without major industrial or hospital inputs. The level of treatment and the nature of the catchment are considered similar to the

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previously described hypothetical scenario, leading to the allocation of a rank score between 2 and 1 based upon the score allocations proposed in Table 4.8. A rank score of 2 is selected as a ‘worst case’ scenario within the stated context.

b) Assessment of the dependence on the CECs bioavailability/bioaccessibility in the soil

There are a number of physical, chemical and biological factors that need to be balanced against one another to provide an overall assessment of the bioavailability/bioaccessibility of a given CECs in a particular soil. These factors are briefly summarised in Table 4.14:

Table 4.14 - Factors influencing the bioavailability/bioaccessibility of clarithromycin in soil in the hypothetical example.

Influencing factor	Situation for hypothetical scenario	Impact for soil bioavailability/bioaccessibility
Soil structure	Sandy soil	No inhibition of movement
log K_{ow} for clarithromycin	3.16; indicative of moderate hydrophobicity	Some tendency for clarithromycin to associate with solid as opposed to aqueous phase
log K_{oc} for clarithromycin	2.17; 1.37 (calculated values from EPI suite); indicative of fairly weak sorption to organic soil particles	Limited tendency for clarithromycin to sorb to organic matter associated with soil particles
pK_a for clarithromycin	8.99; compared to soil pH of 7 indicates a tendency for clarithromycin to exist in cationic form	Cationic form of clarithromycin will promote sorption to predominantly negatively charged soil particles.
Biodegradation / volatilisation / photodegradation	Not expected to readily occur in the soil environment.	Introduced clarithromycin levels in soil expected to be maintained.

The aforementioned factors indicate that clarithromycin movement in the sandy soil will be limited due to electrostatic attraction to negatively-charged soil minerals. However, clarithromycin is likely to exhibit only moderate interaction with soil organic material. Therefore, the category in Table 4.9 which best fits the behaviour of clarithromycin in the

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hypothetical example is 'ready movement of CECs within soil and limited availability for plant uptake' and is allocated a rank score of 2.

c) Assessment of dependence on biosolid/animal manure addition to soils and ploughing

The description of the hypothetical scenario identifies that land is subjected to ploughing but there is no application of either biosolids or animal manure in addition to the irrigated TWW. Comparison with the categories identified in Table 4.10 indicates the award of a score of 4.

Therefore, the overall score relating to the impact of CECs bioavailability within the receiving soil environment is 2/1 x 2 x 4 = 16/8. This falls within the '8-16' range indicating an overall score of 2 in terms of 'impact of CEC bioavailability' (Table 4.12). Under the identified scenario, the presence of clarithromycin in the soil is considered unlikely to exert an impact. An impact may occur but it is uncommon, and where it does occur the impact would be relatively minor and contained within the local site.

4.4.3 Overall risk rating matrix for hypothetical example

Combination of the 'likelihood of occurrence' and 'likelihood of impact' scores yields a value of 6 (3 x2). From the score interpretation matrix represented in Table 4.13 (Section 4.3) this falls within the '5-8' range corresponding to a scenario in which there is unlikely (or limited possibility of) the occurrence and bioavailability of clarithromycin in the soil resulting in crop contamination.

4.4.4 Sensitivity analysis

When using a model, it is important the user is aware of any uncertainties associated with the outputs it generates. The values associated with model input parameters are often a major source of uncertainty, and those used within the hypothetical worked example (Section 4.3) are no exception. Sensitivity analysis (SA) is an approach widely used to identify the variability of results generated due to any assumptions that may have been made about the input data (or methods used to derive them) (Scholes et al., 2007). In the absence of complete data sets, several assumptions are made in the worked example, and it is appropriate to test the sensitivity of the risk scores generated (i.e. the approach

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output) to changes in the input data (i.e. variations in the scores allocated to likelihood of occurrence and magnitude of impact).

To achieve this, a simple factorial approach was applied to testing the influence of likelihood of occurrence and magnitude of impact scores on the final risk calculation. Section 4.1 describes how likelihood of occurrence scores are a composite of four aspects with Section 4.2 presenting discussion on three aspects which inform the magnitude of impact. Within each category one parameter was selected for sensitivity testing for the following reasons:

- Likelihood of occurrence: dependence on the effect of storage – selected for sensitivity analysis as knowledge of the occurrence and impact of metabolites of many CECs, including clarithromycin, is incomplete.
- Magnitude of impact: dependence on CECs load in TWW – only limited data on the concentration of clarithromycin in TWW effluents is available.

To determine the impact of the assumptions underpinning the scores allocated to the above two parameters, two types of SA were undertaken. Firstly, the sequential increasing of allocated scores to a maximum of 4 and recalculating the overall risk score and secondly, not allocating any score to each criterion identified above and again recalculating the risk score. Increasing the value allocated to the effect of storage did not increase the risk score category associated with clarithromycin (remaining 'unlikely') whereas increasing the value allocated to dependence on CECs load in TWW resulted in a combined risk score one category higher (a change from 'unlikely' to 'possible'). This suggests that clarithromycin may be more susceptible to assumptions associated with magnitude of impact as opposed to its likelihood of occurrence. When the same two parameters were effectively removed from the equation, the combined risk score decreased in both cases, from a category of 'unlikely' to 'rare'.

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5. Extending the approach from soil to selected protection targets and further receiving compartments – feasibility assessment

Figure 3.1 provides an overview of those aspects which have the potential to impact on the fate of CECs during agricultural irrigation with TWW. Within this Figure, soil is identified as an exposure route to a number of further protection targets and receiving compartments (e.g. plants, humans, aquatic and terrestrial biota, surface- and ground- waters). Section 4 describes a structured approach to assessing the 'likelihood of occurrence' and 'magnitude of impact' of CECs coming into contact with soil through the identification, scoring and subsequent multiplication of scores into a single CECs-specific ordinal value. Repeating the developed approach for a number of CECs supports the development of a ranked list of CECs with regard to their potential to occur in soil in a bioavailable form. The resulting scores can then be used to identify – or short-list - which CECs are relatively of most concern and should be prioritised for further research.

5.1 Factors influencing the uptake of CECs by protection targets and further receiving compartments – what do we know?

Having developed an approach for systematically assessing the relative potential for CECs to occur in soil in a bioavailable form, a logical next step is to consider whether this approach can be extended to consider the likelihood that these CECs identified as having a high probability of being present in soils in a bioavailable form (i.e. a score of 12-16; see section 4.3) have the potential to accumulate within an identified receptor and – if so – at what level. Recent reviews (e.g. Piña et al., 2018; Christou et al., 2017) provide comprehensive overviews of current knowledge on the fate of a range of CECs within TWW irrigation schemes, receiving soils, micro- and macro-biota (including edible crops). Using antibiotics (ABs) as an example of CECs, Table 5.1 provides an overview of current state of knowledge with regard to their occurrence and impact following agricultural irrigation using TWW. ARB&ARGs are also included.

Data is only available on a few ARB&ARGs and these studies address varying genes and bacteria under only a limited number of soil conditions/plant genotypes/climates and irrigation methods. For example, only a few full-scale field experiments have focused on identifying the uptake of antibiotics by crops irrigated with TWW (e.g. Wu et al., 2015; Christou et al. 2017), and even fewer have considered the uptake of ARB&ARGs (Franklin

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et al., 2016). Results to-date are not conclusive with studies showing that irrigation with TWW containing CECs increases the potential for ARB&ARGs to occur in soils (Fahrenfeld et al., 2013) and disturb native microbial consortia, but do not necessarily increase their loadings in soils (Negreanu et al., 2012) and that ARB&ARGs are also present in agricultural soils that have not been irrigated with TWW (D'Costa et al., 2006). The presence of naturally occurring ARB&ARGs has been reported in other environments and the need to develop a method to differentiate between naturally occurring and TWW-derived ARB&ARGs is identified as a research priority.

With regard to the uptake of specific substances, antibiotics detected in crops irrigated with TWW include sulfonamides (sulfamethoxazole, sulfapyridine), trimethoprim, fluoroquinolones (ciprofloxacin, ofloxacin, enrofloxacin), tetracyclines (tetracycline, oxytetracycline) and macrolides (erythromycin) (Trapp, 2004; Goldstein et al., 2014). Whilst the bioaccumulation of antibiotics has been demonstrated (Christou et al., 2017), accumulation rates are lower than those for other organic CECs e.g. the anti-epileptic drug carbamazepine as a consequence of their reduced mobility in soil (e.g. fluoroquinolones, tetracyclines (Tolls, 2001)), their relatively large molecular size and or their ionisable nature (Trapp, 2004). Further, although the exact interplay of mechanistic processes governing uptake are poorly understood (Miller et al., 2016), factors reported to influence uptake potential are identified as the physico-chemical properties of the CEC (e.g. polarity, hydrophobicity, water solubility), soil and soil pore water chemistry (e.g. pH, mineral concentration, cation exchange capacity, dissolved organic matter), soil organic matter content and soil structure and plant genotype (including its physiological state) (Vasudevan et al., 2009; Miller et al., 2016; Park and Huwe, 2016). In a recent NEREUS COST ACTION position paper, Piña et al., (2018) conclude both that ARB&ARGs generated by human / veterinary use of ABs have the potential to reach the agricultural soil environment via TWW irrigation, but the survival time of such ARB&ARGs and their ability to compete with the resident soil microbiome is unclear.

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Table 5.1 - What we know about the impact of antibiotics (ABs), ARB and ARGs in soil on protection targets and further receiving compartments?

Key questions	Data reported in the peer-review literature
Do ABs occur in soils irrigated with TWW?	Reported in soils (Fatta-Kassinos et al., 2011; Fahrenfeld et al., 2013), soil runoff (Pederson et al., 2003) and soil pore water (REF). TWW irrigated soils contain concentrations of ABs several times higher than that in TWW (Kinney et al., 2006).
Do ARB&ARGs occur in soils irrigated with TWW?	TWW irrigation reported to alter the soil microbiome (Han et al.,20XX); ARGs reported in irrigation systems and soils (Fahrenfeld et al., 2013), resident soil microbial communities and plant-associated bacteria. No occurrence /absence data on many ARB&ARGs Studies indicate that TWW does not increase ARB&ARGs soil loads (Negreanu et al., 2012). Studies reported the high values of LOQ of ARGs in soil (Fortunato et al., 2018)
Do ABs occur in plants following irrigation with TWW? (Results based on a limited number of field studies)	Presence of pharmaceuticals/ABs documented in edible plant parts; Wu et al., 2015; Christou et al. 2017); but mechanistic understanding limited (Miller et al., 2016); ABs enter root tip through epidermis, pass through cortex to reach vascular tissues where they can be transported above ground by xylem; several studies show no accumulation. Other studies report ABs adsorbed to plant surfaces and accumulated internally (Franklin et al., 2016)
Have ARB&ARGs been documented to occur in plants following irrigation with TWW?	Presence of ARB&ARGs in TWW irrigated crops not yet systematically explored but potential for uptake demonstrated in geenhouses (Ye et al., 2016).
Have bio-accumulated ABs been documented within the food chain?	Christou et al., 2017; increasing concentration in tomatoes with increasing TWW irrigation time – reported BCF of 5-6.
Is there evidence that ARB&ARGs can be bioaccumulated?	Chitarra et al., 2014 report *E. coli* bio-accumulated; ARGs reported in endophytic microbiome (Megoni et al., 2015)

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Can ARB&ARGs consumed by humans /animals colonise the gut?	Unknown (Piña et al., 2018); the need to evaluate the contribution of TWW-derived ABs to the total ABs gut load in humans/animals is highlighted.
Have ecological impacts on plants and soil micro-organisms exposed to ABs been documented? (Results based on a limited number of field studies)	Crop plants exposed to environmentally-relevant concentrations of ABs show phytotoxic effects[1] (Carvalho et al., 2014). Impacts following exposure to ABs reported for: aquatic organisms (Christou et al., 2017); nematodes (Yu et al., 2016); collembolan (Pike and Kingcombe 2009); microbial community (Ma et al., 2016)
Have ecological impacts on plants and soil micro-organisms exposed to ARB&ARGs been documented?	Predicted to reach soils via TWW irrigation – impact on resident soil community unclear (Piña et al., 2018)
Have risks from ABs&ARB&ARGs via TWW irrigated crop consumption been assessed?	Yes; using DALYs (Marsoni et al., 2014), TTC (Malchi et al., 2014) and hazard quotient (Prosser and Sibley (2014). All indicate level of risk is negligible – but new knowledge / knowledge gaps yet to be integrated / addressed, respectively. For example, estimated daily/annual ABs exposure from crops and compared with therapeutic dose equivalent of TCC – studies indicate 10-200 times lower than medical dose = negligible level of risk.

Key: [1] phytotoxic effects identified include lower rates of germination, inhibition of growth, tissue deformation, reduced photosynthetic rate and chlorophyll content and other stress-related phenomena (Carvalho et al., 2014); DALY = disability adjusted life years; TTC = threshold of toxicological concern

5.2 Implications for extending the developed approach to specific protection targets and further receiving compartments

Collection of data pertaining to the physico-chemical characteristics of the CECs and soil are central components of the systematic approach to identifying the relative potential for CECs to occur in soil in a bioavailable form (see Section 4.2.2). Discussion of the potential to extend this approach to address human and environmental endpoints therefore focuses on identifying additional parameters (and supporting data sets) not considered in Sections 3 and 4 of this report (above). Based on work undertaken within NEREUS WGs 1 and 2

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and a review of the literature, the following parameters are identified as being necessary to achieve this objective.

Table 5.2 - List of parameters to be considered within a qualitative risk assessment framework to support an assessment of risks to protection targets and receiving compartments from CECs within TWW used in agricultural irrigation.

Protection goals	Parameters	Data availability
Plants (crops or non-target vegetation)	Species and genotype	Limited number of species tested for a limited range of CECs
	Plant physiological status	Limited understanding of its role
Soil organisms	Species	
	Species specific PNEC vs PEC per receiving compartment	Limited data on PNECs (no data for many species); limited data on PECs (no data for many CECs in many receiving compartments)
Humans (crop consumption and occupational and non-occupational exposure pathways)	Mass of CECs consumed (per year or day) in comparison to therapeutic dose; CECs load within TWW re: occupational and non-occupational (accidental exposure)	Available for a limited number of CECs in only a few crops; source-pathway-receptor linkage identified for carbamazepine; lack of dose-response models for many CECs
Animals (consumption of crops)	Mass of CECs consumed (per year or day) in comparison to therapeutic dose per species/ animal type e.g. sheep, cattle	Data could not be sourced

Complete source-pathway-receptor linkages have been identified for certain CECs within TWW used in agricultural irrigation applications (e.g. Paltiel et al., 2016). On this basis, it is plausible that complete source-pathway-receptor linkages also exist for CEC transformation products (of which it is likely only a subset have been identified to-date). The outputs of NEREUS WGs 1-4 (which encompass reviews of the recent literature), suggest that insufficient field data is available to inform even a qualitative assessment of the risks to human and environmental end-points from CECs in TWW used in agricultural irrigation. Further, validated exposure scenarios and uptake / impact models are not yet available for all parent CECs in relation to all crop species of interest, let alone for the

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numerous transformation products which can occur. A further challenge is the need to consider cumulative exposure of both CECs and multiple dietary sources. For example, it is reported that TWW-irrigated carrots accumulate carbamazepine and therefore it is plausible that firstly, a number of other CECs may also have been taken up and, secondly, that the carrot is consumed as part of a diet containing several other TWW-irrigated fruit and vegetables on a daily basis. These limitations also - currently - prevent the establishment of evidence-based TWW quality standards or maximum admissible threshold values for crops or receiving environments. Use of the precautionary principle is therefore strongly supported whilst a twin approach of further field studies and modelling is undertaken to enable the key questions and data sets (identified in Table 5.2 above) to be addressed and developed, respectively.

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6. Conclusions and recommendations for further NEREUS activities

Common risk assessment practice presumes that the level of risk associated with an identified hazard can be calculated by multiplying together discrete scores allocated to the likelihood of occurrence and the magnitude of impact. This combined risk score is frequently interpreted through the use of a three- or four-level interpretation matrix where, for example, a score of 1-3 indicates a low level of risk (a level of risk deemed acceptable), a score of 4-6 indicates a medium level of risk, and a score of 8-16 denotes a high level risk (identified as being unacceptable and thus requiring to be managed). This type of methodology is used within various sets of national and international TWW guidelines (e.g. NWQMS (2008); US EPA (2012); WHO, 2006) to consider the impacts associated with a range of conventional parameters, of which the impact of pathogens on human health is the central focus. The evidence base underpinning such assessments is extensive, with dose-response relationships having been developed and validated for a variety of pathogens e.g. (*Escherichia coli; Clostridium perfringens*). Hence assessments of both likelihoods of occurrence and magnitude of impact of pathogens within a range of TWW uses can be undertaken on a quantitative basis i.e. quantitative microbial risk assessment (QMRA). Where specific data sets are scarce (or lacking), the availability of pertinent associated data sets can, in some cases, act as 'surrogate' data sets, reducing the type and level of uncertainty associated with the use of expert judgement required when decision-makers need to proceed in the absence of comprehensive data.

However, the extension of this same approach to assess risks associated with the use of TWW containing CECs in relation to both human and environmental protection targets and receiving compartments poses a series of methodological and practical challenges. Key challenges to applying a conventional RA approach are identified in Sections 2.2 and 2.3, and relate not only to data availability (scarce or absent), but also to more fundamental aspects such as:

- the conceptual understanding of the approach by its users,
- the multiple points of application within the reuse chain,
- a focus on single substances (as opposed to real world 'chemical cocktails') and
- the need to develop risk assessment factors to enable both chronic and acute impacts to be addressed.

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Such issues raise questions as to the 'fitness for purpose' of a qualitative approach to meaningfully assess the levels of risk associated with the use of TWW containing CECs in selected applications in relation to protection targets and/or receiving compartments.

Despite these identified limitations and concerns, the development of a qualitative RA approach with respect to CECs (as set-out within this report) has been a useful exercise with regard to identifying research gaps and methodological short-comings. However, rather than identifying the level of the risks, we argue that the output of its application is a ranking of hazards to protection targets and receiving compartments exposed to TWW containing CECs in selected reuse applications. The process has also supported the systematic co-identification of a list of parameters to be taken into account within a risk assessment but questions the utility of a qualitative risk assessment process with regard to current knowledge. Both the time and cost of developing the conventional types of CECs data sets required to underpin a quantitative (or even semi-quantitative approach) is in no way underestimated and hence the utility of alternative methodologies (e.g. ecotoxicological assessments; see WG3 outputs) to generate new forms of data to enable a more quantitative (or semi-quantitative) approach to be undertaken is strongly supported.

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APPENDIX I NEREUS glossary

Advanced or tertiary treatment - Treatment steps added after the secondary treatment stage to remove specific constituents, such as nutrients, suspended solids, organics, heavy metals or dissolved solids (e.g. salts).

Aquifer - A geological area that produces a quantity of water from permeable rock.

Benchmark - a point of reference against which process or behaviour may be assessed (adapted from NWQMS, 2008).

Chronic toxicity - Toxicity that acts over a long period of time and that typically affects a life stage (e.g. reproductive capacity); it can also refer to toxicity resulting from a long-term exposure.

Contaminants of emerging concern (CECs) - Substances which are not regulated under existing water quality EU regulations but have been identified as having the potential to impact negatively on human health and/or environmental endpoints.

Critical control point - A point, step or procedure at which control can be applied and that is essential for preventing or eliminating a hazard, or reducing it to an acceptable level (adapted from Codex Alimentarius).

Critical limit - A prescribed tolerance that must be met to ensure that a critical control point effectively controls a potential health hazard; a criterion that separates acceptability from unacceptability (adapted from Codex Alimentarius).

Disinfectant - A chemical, typically an oxidising agent (eg chlorine, chlorine dioxide, chloramines and ozone), that is added to water and is intended to kill or inactivate pathogenic (disease-causing) microorganisms.

Disinfection - The inactivation of pathogenic organisms using chemicals, radiation, heat or physical separation processes (e.g. membranes). The process designed to kill most microorganisms in water, including essentially all pathogenic (disease-causing) bacteria.

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There are several ways to disinfect, with chlorine being most frequently used in water treatment.

Disinfection by-product - Products of reactions between disinfectants, particularly chlorine, and naturally occurring organic material.

Distribution system - A network of pipes leading from a treatment plant to customers' plumbing systems.

Direct reuse - Introduction of treated wastewater via pipelines, storage tanks, and other necessary infrastructure directly from a water treatment plant to a distribution system. An example would be the distribution of treated wastewater to be used directly in agricultural irrigation.

Drip irrigation - Irrigation delivery systems that deliver drips of water directly to plants through pipes. Small holes or emitters control the amount of water that is released to the plant. Drip irrigation does not contaminate aboveground plant surfaces.

Effluent - Liquid (e.g. treated or untreated wastewater) that flows out of a process or confined space). The out-flow water or wastewater from any water processing system or device.

Exposure - Contact of a chemical, physical or biological agent with the outer boundary of an organism (e.g. through inhalation, ingestion or dermal contact). Contact of a chemical, physical or biological agent with the outer boundary of an organism (eg through inhalation, ingestion or dermal contact).

Exposure assessment - The estimation (qualitative or quantitative) of the magnitude, frequency, duration, route and extent of exposure to one or more contaminated media. The estimation (qualitative or quantitative) of the magnitude, frequency, duration, route and extent of exposure to one or more contaminated media

Groundwater - Water contained in rocks or subsoil.

Groundwater recharge - Replenishing of groundwater naturally by precipitation or runoff, or artificially by spreading or injection.

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Hazard - A biological, chemical, physical or radiological agent that has the potential to cause harm

Hazard analysis critical control point (HACCP) system - A systematic method to control safety hazards in a process by applying a two-part technique:
• an analysis that identifies hazards and their severity and likelihood of occurrence
• an identification of critical control points and their monitoring criteria to establish controls that will reduce, prevent, or eliminate the identified hazards.

Hazard control - The application or implementation of preventive measures that can be used to control identified hazards.

Hazard identification - The process of recognising that a hazard exists and defining its characteristics (Standards Australia/Standards New Zealand 1998).

Hazardous event - An incident or situation that can lead to the presence of a hazard (ie 'What can happen and how').

Health-based target - A defined level of health protection for a given exposure. This can be based on a measure of disease, e.g. 10-6 DALY per person per year, or the absence of a specific disease related to that exposure.

Health impact assessment - The estimation of the effects of any specific action (plans, policies or programmes) in any given environment on the health of a defined population.

High-growing crops - Crops that grow above the ground and do not normally touch it (e.g. fruit trees).

Impact - An effect on end points, such as people, plants, soil, biota, water or a part of the environment.

Indicator - Measurement parameter or combination of parameters that can be used to assess the quality of water; a specific contaminant, group of contaminants or constituent that signals the presence of something else (eg the presence of *Escherichia coli* indicates the presence of pathogenic bacteria).

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Indirect reuse - The reuse of treated wastewater which is placed into a water body source such as a lake, river, or aquifer and then some of it retrieved for later use.

Insignificant - Not valuable or large enough to be considered important.

Irrigation - Provision of sufficient water for the growth of crops, lawns, parks and gardens by flood, furrow, drip, sprinkler or subsurface water application to soil.

Localized irrigation - Irrigation application technologies that apply the water directly to the crop, through either drip irrigation or bubbler irrigation. Generally, use less water and result in less crop contamination and reduce human contact with the wastewater.

Low-growing crops - Crops that grow below, on or near the soil surface (e.g. carrots, lettuce).

Major impact - Event that is potentially lethal to the local ecosystem.

Maximum risk - Risk in the absence of preventive measures.

Minor impact - Event that is potentially harmful to the local ecosystem.

Moderate impact - Event that is potentially harmful to the regional ecosystem.

Multiple barriers - Use of more than one preventive measure as a barrier against hazards. Use of more than one preventive measure as a barrier against hazards.

Quantitative microbial risk assessment (QMRA) - Method for assessing risk from specific hazards through different exposure pathways. QMRA has four components: hazard identification; exposure assessment; dose–response assessment; and risk characterization.

Protection target - Collective term for humans, micro- and macro- flora and fauna

Receiving compartment - Collective term for soil, surface water and groundwater

Reclaimed water - Treated wastewater effluent; Alternative but less accurate term for treated sewage

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Recycled water - Water generated from sewage, greywater or stormwater systems and treated to a standard that is appropriate for its intended use.

Reservoir - Any natural or artificial holding area used to store, regulate or control water.

Residual risk - The risk remaining after consideration of existing preventive measures.

Risk - The likelihood of a hazard causing harm in exposed populations in a specified time frame, including the magnitude of that harm. The likelihood of a hazard causing harm in exposed populations in a specified time frame, including the magnitude of that harm.

Risk assessment - The overall process of using available information to predict how often hazards or specified events may occur (likelihood) and the magnitude of their consequences. The overall process of using information to predict how often hazards or specified events may occur (likelihood) and the magnitude of their consequences (adapted from Standards Australia/Standards New Zealand 1999).

Risk management - The systematic evaluation of the wastewater, excreta or greywater use system, the identification of hazards and hazardous events, the assessment of risks and the development and implementation of preventive strategies to manage the risks. The systematic evaluation of a system (in this document, the water supply system), the identification of hazards and hazardous events, the assessment of risks, and the development and implementation of preventive strategies to manage the risks.

Standard (e.g. water quality standard) - An objective that is recognised in environmental control laws enforceable by a level of government.

Subsurface irrigation - Irrigation below the soil surface; prevents contamination of aboveground parts of crops

Surface water - All water naturally open to the atmosphere (e.g. rivers, streams, lakes and reservoirs).

Target criteria - Quantitative or qualitative parameters established for preventive measures to indicate performance; performance goals.

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Treated wastewater - Term used but not defined by WHO

Tolerable daily intake (TDI) - Amount of toxic substance that can be ingested on a daily basis over a lifetime without exceeding a certain level of risk

Tolerable health risk - Defined level of health risk from a specific exposure or disease that is tolerated by society, used to set health-based targets.

Unrestricted irrigation - The use of treated wastewater to grow crops that are normally eaten raw.

Untreated wastewater - term used but not defined by WHO

Water recycling - A generic term for water reclamation and reuse. It can also be used to describe a specific type of 'reuse' where water is recycled and used again for the same purpose (e.g. recirculating systems for washing and cooling), with or without treatment in between.

Wastewater - Liquid waste discharged from homes, commercial premises and similar sources to individual disposal systems or to municipal sewer pipes, and which contains mainly human excreta and used water. When produced mainly by household and commercial activities, it is called domestic or municipal wastewater or domestic sewage. In this context, domestic sewage does not contain industrial effluents at levels that could pose threats to the functioning of the sewerage system, treatment plant, public health or the environment.

Water reuse - The use of water which is generated from wastewater and that achieves, after treatment as necessary, a quality that is appropriate (taking account the health and environment risks and local and EU legislation) for its intended use.

Water reuse - Practice of using reclaimed water.

cost
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Annex II Co-development a 'long list' of irrigation aspects to inform development of NEREUS water reuse scenarios

Table 1 - Treated water – operational and maintenance aspects.

Aspects to consider	Descriptors	Comments	Potential microbiological risks	Potential CEC risks	Other risks
Treated WW characteristics	Source of raw wastewater (residential, industrial, hospital, agricultural) Temporally dynamic Level/type of treatment Size of WWTP, person equivalent treated	e.g. Temporal variation in quality (daily, seasonal etc); % volume and quality from different sources e.g. city with little, non-harmful industry etc	Occurrence / proliferation of ARB/ARG; Treatment-induced selection pressure for resistant and or pathogenic strains (human, animal); Treatment-induced increase of virulence of pathogenic strains	Development of transformation products	
Distance from WWTP to point of use "Typical operational scheme": WWTP – transport - local storage – application.	Km	Leakage and presence of source of pollution; Gene transfer and die off time should be considered both.	Microbial persistence and re-growth of potential pathogens and ARG-harbouring bacteria Horizontal gene transfer - Biofilm formation in pipelines, tank cars etc. may lead to an increased resistance of growing pathogens	Bio-physico-chemical transformations increases or decreases bioavailability and toxicity	
Duration and type of storage prior to use and type of transfer conduit (e.g. pipe or open channel)	Days	Leakage and presence of source of pollution	Microbial growth Horizontal gene transfer Biofilm and other resilience microbial structures may accumulate in the reservoirs	Bio-physico-chemical transformations increases or decreases bioavailability and toxicity	Remote but possible: (bacterial) toxin production?
Disinfection (when/where)	May be implemented at the WWTP, just before application or both.				
Nutrient load of TWW	mg N/l; mg P/l	Pollution of aquifer with nutrient prior to storage			
Soil characteristics	Type (e.g. clay, loam, sand, silt); pH; humus %, TOC %, N %, PO_4 mg/100g, K mg/kg		Protection of free ARG encoding DNA by adsorption to clay; Increase of persistence of free ARGs due to soil characteristics		
Soil management	Fertiliser applied: (manure; biosolid; synthetic)		Source of biosolids and its antibiotic load. Presence of potential resistance selectors	Pollutant load re: enhancement of ARB/ARG loads	

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	Ploughed; rotation; irrigation		(e.g. antibiotics, metals etc); horizontal gene transfer between human/animal bacteria from "organic" manure, soil and WW borne bacteria; Agricultural soils as gene exchange platforms - provide matrix for closing cycle of bi-directional antibiotic resistance gene transfer between human-, animal-, soil and plant-associated bacteria.		
Frequency of application	Daily, weekly, etc.				
Volume applied	m^3/ha	Calculate loads per crop type, per irrigation technique			
Time of irrigation	Day/night; season	e.g. for areas visited by people, irrigation is allowed only during the night	Microbial persistence and re-growth of potential pathogens and ARG-harbouring bacteria Horizontal gene transfer	Accumulation in our environment when continuously recycled	Need more information on the persistence of specific ARB/Gs to determine how long before harvest irrigation should be stopped. Also dependent on crop/irrigation technique
		Ceasing irrigation before collection of crops; Damaging for most crops - should demand better effluent quality. Changing water source during crop growing can cause engineering problems and health risks			See above- should be decided on a case-by-case decision

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All pipes transferring treated wastewater are coloured in a recognized manner					Reduces risk of 'wrong connections'
Presence of signs indicating the type of water, i.e. TWW and banning the use					
Weather conditions during application	Km/hr; the distance of an irrigated field from inhabited areas re: a strong wind in that area at that time of year	Wind direction / strength when sprinklers are used	Enhanced resuspension of particles and spread of irrigation water aerosols with adsorbed microbial load; regional climate conditions important e.g. high vs low temp; dry vs rainy/humid weather etc)	Enhanced resuspension of particles with adsorbed CEC load; enhanced runoff (rainfall)	
Treatment performance	*health outcome targets (e.g. tolerable burdens of disease) *water quality targets (e.g. guideline values for chemical hazards; Reduction of antibiotic/ metal/biocide to below minimal selective concentrations *performance targets (e.g. log reduction of specific pathogens) *specific technology targets (e.g. defined treatment processes)	Cyprus treatment train example: CAS, sand filtration and chlorination. Stored in an open lagoon (with birds)	Enrichment of clinically-relevant ARB and ARG including recognized and emerging pathogens and ARGs (see WG1 list) Selection of bacteria prone to acquire ARG	Risk of oxidation/disinfection by-products and CEC transformation products (and CEC itself)	
Mixing of treated water with other waters	*health outcome targets (e.g. tolerable burdens of disease) *water quality targets (e.g. guideline values for chemical hazards)	Not permitted in Cyprus Impact on monitoring costs to ensure compliance; Occasionally practiced in Tunisia when the amount of TWW is not sufficient;	Cross contamination of freshwater pipes with treated wastewater Mixing treated wastewater into aquifers		

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	*performance targets (e.g. log reduction of specific pathogens)	compliance. Permitted in Israel in reservoirs (not pipes) - mix product defined as the lower source water (e.g. rain water + secondary effluent = secondary effluent)			
Identification of areas as not suited for irrigation with treated water'		Lands grazed by cattle			
		Crops that are leafy, have bulbs or tubers Crops eaten raw			
		Distance to aquifers			

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Table 2 - Types of treated water application.

Irrigation technique	Irrigation method	Delivery system	Application rates	Potential microbiological risks	Potential CEC risks	Other risks
Surface irrigation	Furrow (also known as ditch irrigation)	Gravity via open channels	Variable (7-15 days usually)	Microbial contamination of leaves, fruits etc; movement from site e.g. leaching	CEC contamination of leaves, fruits etc	Greater risk to groundwater
	Flood (border strip and basin)	Gravity via open water body		Microbial contamination of soil and rhizosphere/ vascular system of the plants	CEC contamination of soil and rhizosphere/ vascular system of the plants	Greater risk to groundwater
Localised irrigation	Surface drip irrigation (also known as micro irrigation or trickle irrigation)	Distance to fruit etc		Microbial contamination of leaves, fruits etc	CEC contamination of leaves, fruits etc Probably lowest risk of leaf contamination	
	Mini sprinkler					
	Sub-surface irrigation	Subsurface textile irrigation or drip feeder			Microbial contamination of soil and rhizosphere/ vascular system of the plants	CEC contamination of soil and rhizosphere/ vascular system of the plants
Hydroponics				Microbial contamination of vascular system of the plants	CEC contamination of vascular system of the plants	
Incidental runoff		Consider by determining distance from an effluent watered field to a fresh watered field or a sensitive building (homes?)				Distance to open water reservoirs
Splashes				Microbial contamination of leafy crops and fruits	CEC contamination of leaves and fruits	

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Table 3 - Potential receptors following irrigation with TWW.

Potential receptors	Potential pathways	Concentration of microbial parameters the receptor is exposed to by identified pathway	Concentrations of CEC the receptor is exposed to by identified pathway
Target crops for human consumption (TWW-->leaves --> human; TWW--> roots --> human etc.	From TWW via leaves	Considered in WG1 deliverables	Considered in WG2 Deliverables
	From TWW via roots (directly or via soil)		
Target crops for animal consumption	From TWW → leaves→ animal		
	From TWW via roots (directly or via soil)		
Non-target vegetation (e.g. grass verges, recreational areas)	Splashes from TWW via leaves		
	From TWW via roots (directly or via soil)		
Microorganisms	Direct application of TWW to soil, target and non-target vegetation		
Terrestrial organisms and aquatic macro-organisms (e.g. insects, birds, mammals)	Direct application of TWW		
	Consumption of target/non-target vegetation and soil ingestion	Cross-contamination between soil and fruit and soil ingestion	
Synergistic detrimental effects of TWW and manure/sludge application	All previous pathways applies		
Soil	Surface runoff		
	Infiltration		
Groundwater	Vertical and lateral infiltration		
Air	Volatilisation		
	Resuspension		
Surface water bodies	Groundwater recharge		
	Surface runoff		
Humans (e.g. general public vs vulnerable groups e.g. old, young and immuno-depressed people)	Occupational exposure (e.g. hand labour, farmers and families) to TWW and splashes and volatilisation from soils; inhalation of particles in the field or in the treatment plant		
	Consumption of irrigated crops		
	Drinking contaminated groundwater		
	Ingestion of contaminated soils (e.g. pica)	Soil ingestion by children is a major non-crop-related exposure pathway	

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Table 4 - Categories and types of crops irrigated by reclaimed water.

Crop categories	Crop type	Examples	Concentration of microbial parameters in identified crop type	Concentrations of CECs in identified crop type
Below ground	Root crops – raw	Peeled e.g. Carrots, potatoes; not peeled e.g. radishes		
	Root crops – cooked*	Potatoes		
On the ground	leafy food crops - raw	Lettuce, herbs, aromatic crops, bioenergy crops (clover, millet, etc.)		
	leafy crops – cooked	Cabbage		
	Fruit/vegetables - raw	Melons, tomatoes, peppers, squash		
	Fruit / vegetables - cooked	Cauliflower		
Above ground	Fruit – raw	Apples (including fallen fruits)		
	Fruit – cooked	Quince		
	Processed before eating	Wheat		
	Animal fodder - raw	Grass (as grazing and silage)		
	Animal fodder - processed	Silage		
	Animal bedding	Straw		

Key: * Israeli law differs between veg requiring cooking and non-cooking veg. But the new law will not do so because e.g. microorganisms contaminated potatoes, put on a domestic kitchen table can cause cross contamination to other foods

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Table 5 - Methods to categorising risks to health from using TWW in agricultural irrigation.

Health based targets	Descriptors	Comments	Values for microbial parameters	Values for CECs
Health outcome targets	Tolerable burdens of disease	Combines expert judgment on safety and risk assessments of waterborne hazards.		
	DALYs			
	Tolerable daily intake	Applies to food/beverage (water) that are frequently consumed		
	Reference doses			
	Food quality standards	European Food Safety Agency	No food safety standards yet for ARB; only for pathogen content	Food safety standards only for a limited number of CEC
	EDI/ADI ratio	Estimated daily intake / acceptable daily intake), a threshold of 0.01 defines potential risk for human health		
	Threshold of toxicological concern (TTC) concept	For antibiotic loads / ARG		
Water quality (CM: This is at the same level as food quality and safety and not here…)	Guideline values for microbial and CEC hazards	Long-term hazards from chemicals vs Microbial short-term hazards (use application of quantitative microbiological risk assessment (QMRA)) EU WFD EQS	ARBs- E. coli, faecal coliforms, Pseudomonas aeruginosa, *Acenitobacter baumannii* (both these questioned), Currently used local and international public health (WHO) criteria only exist for coliforms. Alternative view: enterococci are also used as indicator bacteria for water quality. Enterococci are used as drinking water indicators contamination (EU, 1998). Enterococci are not permitted in a 100 mL sample of tested drinking water that flows from a tap, and they are not permitted in a 250 mL sample of bottled water. Enterococci are also used as indicators of fecal contamination of recreational waters throughout the world. Values for the other bacteria do not currently exist. ARGs- (see results of the WG1 deliverable). We currently cannot give actual values for ARGs	
Performance objectives (specify	e.g. log reduction of specific microbial			

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water for e.g. water, technology etc)	indicators / threshold values for targeted compounds			
Specific technology targets	application of defined treatment process to comply with standards			
Economic implications	Reduced crop yield			

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Annex III Co-development a 'long list' of aquifer recharge aspects to inform development of NEREUS water reuse scenarios

Table 1 - Treated water – operational and maintenance aspects pre aquifer recharge.

Aspects to consider	Descriptors	Comments	Potential microbiological risks	Potential CEC risks	Other risks
Treated WW characteristics	Source of WW (residential, industrial, hospital, agricultural) Level/type of treatment	e.g. Temporal variation in quality (daily, seasonal etc) may influence monitoring schemes; % volume and quality from different sources e.g. city with little, non-harmful industry etc	Occurrence / proliferation of ARB/ARG; Treatment-induced (increased) selection pressure for resistant, pathogenic strains (human, animal); Treatment-induced increase of virulence of pathogenic strains	Development of transformation products	
Distance from WWTP to point of use	Km	Gene transfer and die off occurring at the time between treatment and aquifer recharge should be considered.	Microbial persistence and re-growth of potential pathogens and ARG-harbouring bacteria Horizontal gene transfer – the role of Biofilm formed in pipelines, tank cars etc. may lead to an increased ARG-mediated resistance of growing pathogens	Bio-physico-chemical transformations increases or decreases bioavailability and toxicity	
Duration and type of storage prior to use and type of transfer conduit (e.g. pipe or open channel)	Days		Microbial growth Horizontal gene transfer Biofilm may accumulate in storage systems	Bio-physico-chemical transformations of CECs leading to increases or decreases in bioavailability and toxicity with regard to identified receptors	Remote but possible: (bacterial) toxin production?
Disinfection (when/where)	May be implemented at the WWTP or just before application or both.				
Nutrient load of TWW	mg N/l; mg P/l	Pollution of aquifer with nutrient prior to storage – clarify impact of this re: CECs/ARB/ARGs			

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Soil characteristics	Type (e.g. clay, loam, sand, silt); pH; humus %, TOC %, N %, PO4 mg/100g, K mg/kg; infiltration rate		Protection of free ARG encoding DNA by adsorption to clay; Increase of persistence of free ARGs due to soil characteristics	'Protection' of CECs by adsorption to clay; Increase of persistence of CECs due to soil characteristics	
Aquifer characteristics	Size; Depth; Geology Redox conditions; pH Temperature; Flow regime Residence time; Nutrient loading	Pollution of aquifer	Microbial persistence and re-growth of potential pathogens and ARG-harbouring bacteria Horizontal gene transfer - Biofilm formation upon/within the aquifer substrate may lead to increased resistance of pathogens	Bio-physico-chemical transformations of CECs leading to increases or decreases in bioavailability and toxicity with regard to identified receptors	
Frequency of application	Daily, weekly, etc.				
Volume applied	m^3/ha	Calculate loads per crop type, per irrigation technique			
Time of surface spreading	Day/night; season	e.g. for areas visited by people	Microbial persistence and re-growth of potential pathogens and ARG-harbouring bacteria Horizontal gene transfer	Accumulation in our environment when continuously applied	See above- should be decided on a case-by-case decision
Weather conditions during application (for surface spreading applications only)	Km/hr; the wind speed, UV sun light intensity, distance of an infiltration pond from inhabited areas re: a strong wind in that area at that time of year	Wind direction / strength when sprinklers are used	Enhanced resuspension of particles and spread of infiltration water aerosols with adsorbed microbial load; regional climate conditions important e.g. high vs low temp; dry vs rainy/humid weather etc)	Enhanced resuspension of particles with adsorbed CEC load;	
Treatment performance of the aquifer re-charge (i.e. change in pollution load at inlet to re-charge (treated WW) compared to water abstracted from recovery well - or native groundwater	*health outcome targets (e.g. tolerable burdens of disease) *water quality targets (e.g. guideline values for chemical hazards; Reduction of antibiotic/ metal/biocide to below minimal selective concentrations		Enrichment of clinically-relevant ARB and ARG including recognized and emerging pathogens and ARGs (see WG1 list) Selection of bacteria prone to acquire ARG	Risk of oxidation/disinfection by-products and CEC transformation products (and CEC itself)	

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beyond attenuation zone?)	*performance targets (e.g. log reduction of specific pathogens) *specific technology targets (e.g. defined treatment processes)				
Mixing of treated water with other waters (including contamination of aquifer by TWW as mixing with native groundwater)	*health outcome targets (e.g. tolerable burdens of disease) *water quality targets (e.g. guideline values for chemical hazards) *performance targets (e.g. log reduction of specific pathogens)		Mixing treated wastewater into aquifers		
Areas as not suited for TWW aquifer recharge					

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Table 2 - Types of aquifer recharge.

Recharge technique	Potential microbiological risks	Potential CEC risks	Other risks
Surface spreading	Microbial contamination of soils. Microbial contamination of water in the aquifer and the effluent recovered by transport of ARB/ ARGs/ pathogens via migration, especially at short detention time or via underground channelling	CEC contamination of soil and aquifer Low CEC removals due to underground conditions and short detention time	Splashes and aerosol formation carrying microorganisms to nearby public
Direct aquifer injection			

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Table 3 - Potential receptors following aquifer recharge with TWW.

Potential receptors	**Potential pathways**	**Concentration of microbial parameters the receptor is exposed to by identified pathway**	**Concentrations of CEC the receptor is exposed to by identified pathway**
Target crops for human consumption (TWW-->leaves --> human; TWW--> roots --> human etc	From TWW via leaves. Crops can potentially take in CECs via effluent from aquifer recharge but probably much lower than from irrigation with TWW		Refer to processes like plant uptake, phytoremoval, phytodegradation by certain crops as concentration of CEC in crops is little addressed??
	From TWW via roots (directly or via soil)		
Target crops for animal consumption	From TWW → leaves→ animal		
	From TWW via roots (directly or via soil)		
Non-target vegetation (e.g. grassed areas neighbouring recharge areas)	Migration within soil and/or aquifer		Review the DEMEAU database
Microorganisms (soil and aquifer)	Infiltration of TWW	Considered in WG1 deliverable	
Terrestrial organisms (e.g. insects, birds, mammals)	Infiltration of TWW	Considered in WG1 deliverable	
	Consumption of contaminated insects / soil ingestion		
Soil	Infiltration		
Groundwater	Vertical and lateral infiltration		
Air (surface applications only)	Volatilisation		
	Resuspension		
Surface water bodies	Groundwater recharge		
Humans (e.g. general public vs vulnerable groups e.g. old, young and immuno-depressed people)	Occupational exposure (e.g. labourers,) to TWW and splashes and volatilisation from soils; inhalation of particles in the field or in the treatment plant		
	If you consider general public you can consider farmers and families using recharged effluent.		
	Drinking contaminated groundwater		
	Ingestion of contaminated soils (e.g. pica)	Soil ingestion by children is a major non-crop-related exposure pathway	

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Table 4 - Categories of aquifer irrigated by TWW.

Aquifer categories	Water type	Examples	Concentration of microbial parameters in the aquifer?	Concentrations of CECs in the aquifer
Potable water aquifer	Potential drinking water source			
	Active drinking water zone			
Non-potable water	Source of non-potable freshwater			
	Saline			

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Table 5 - Methods to categorising risks to health from using TWW for aquifer recharge.

Health based targets	Descriptors	Comments	Values for microbial parameters	Values for CECs
Health outcome targets	Tolerable burdens of disease	Combines expert judgment on safety and risk assessments of waterborne hazards.		
	DALYs			
	Tolerable daily intake	Applied to drinking water that are frequently consumed		
	Reference doses			
	Water quality standards	Standards for raw drinking water? GW D or WFD?	No water quality standards yet for ARB; only for pathogen content	No water quality standards CECs (by definition)
	EDI/ADI ratio	Estimated daily intake / acceptable daily intake), a threshold of 0.01 defines potential risk for human health (why 0.01?)		
	Threshold of toxicological concern (TTC) concept	For antibiotic loads / ARG copy numbers?		
Water quality (environmental impact as oppose to human health which is addressed above)	Guideline values for microbial and CEC hazards	Long-term hazards from chemicals vs Microbial short-term hazards (use application of quantitative microbiological risk assessment (QMRA)) EU WFD EQS EU GW directive Guidelines for aquifer recharge include the California 22 (TC), US EPA (TC) and draft EU reuse water quality standards (*E. coli*)	ARBs- *E. coli*, faecal coliforms, *Pseudomonas aeruginosa*, *Acenitobacter baumannii* (both questioned)	
Performance objectives (specify water for e.g. water, technology etc)	e.g. log reduction of specific microbial indicators / threshold values for targeted compounds			
Specific technology targets	application of defined treatment process to comply with standards			

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Economic implications	Reduced / increased energy / treatment costs			

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