# Deliverable of WG2

## Deliverable 10

# Determination of the daily intake of organic microcontaminants and ARB and ARGs from irrigated crops with reclaimed waters of different quality

**April 2017**

COST is supported by the EU Framework Programme Horizon 2020

This article is based upon work from the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS), supported by COST (European Cooperation in Science and Technology).

## Contents





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## ACRONYMS

| | |
|---|---|
| ADI | Accepted Daily Intake |
| ARB | Antibiotic-Resistant Bacteria |
| ARGs | Antibiotic Resistance Genes |
| bw | Body weight |
| CBZ | Carbamazepine |
| CECs | Contaminants of Emerging Concern |
| DCF | Diclofenac |
| ECB | European Chemicals Bureau |
| EDI | Estimated Daily Intake |
| EFSA | European Food Safety Agency |
| EMA | European Medicine Agency |
| FAO | Food and Agriculture Organization |
| fw | Fresh weight |
| HQ | Hazard Quotient |
| IR | Intake Rate |
| MIC | Minimum Inhibitory Concentration |
| MTD | Minimum Therapeutic Dose (mg/d) |
| NOAEL | No Observable Effect Level |
| PNEC | Predicted Non Effect Concentration |
| RH | Relative Humidity |
| RWW | Reclaimed Wastewater |
| TPs | Transformation Products |
| TTC | Threshold of Toxicological Concern |



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**Keywords**:

carbamazepine; contaminants of emerging concern; diclofenac; estimated daily intake; hazard quotient; in vivo exposure; minimum therapeutic dose; non observable adverse effect level; threshold of toxicity concern; acceptable daily intake; hazard quotient.

## Executive summary

Plants growing in contaminated soil can take up the chemical contaminants from soil pore water through their roots, and depending on their physicochemical properties, the uptaken chemicals can then be translocated into plant tissues including the edible parts. Meat and dairy products from animals used as sources of food can become contaminated if grazing or foraging animals consume contaminated soil, water, or feed crops and bioaccumulate the contaminants in their tissues. In this report, the different methodologies used to estimate the risk associated with the consumption of crops contaminated with organic microcontaminants, are evaluated and discussed. The main limitations of every approach are discussed and the risk assessment associated to the consumption of vegetables contaminated with carbamazepine and diclofenac is presented. In addition, an approach to estimate the predicted non effect concentration to estimate the selective pressure of antibiotics on bacterial ecosystems from the minimum inhibitory concentration is presented.



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## 1. Introduction

Contaminated media to which humans might be exposed include air, water and sediment, soil and dust, food, aquatic biota, and consumer products. Food products (e.g., grains, fruits, vegetables) can become contaminated as a result of ambient pollutants in the air being deposited on plants, adsorbed onto or absorbed by the plants, or dissolved in rainfall or irrigation waters that contact the plants. In this respect, food products are considered one of the major exposure pathways of chemical contaminants to human beings and consequently targeted in any risk assessment.

Over the last decade, a number of papers have reported the occurrence of organic microcontaminants in agriculture irrigation waters at concentrations from ng/L to low μg/L (Calderón-Preciado et al., 2011a,b; Margenat et al., 2017) and some of them of particular environmental and health concern known as contaminants of emerging concern (CECs). In fact, the partial removal of CECs during wastewater treatment processes is one of the main sources in irrigation waters (Michael et al., 2013; Luo et al., 2014). In addition, the application of biosolids and manure in agriculture as soil amendment constitute another source of CECs (Walters et al., 2010; Clarke and Smith, 2011). Moreover, the increased use of reclaimed wastewater (RWW) in agriculture in arid and semiarid countries worldwide deserves a special concern. Basically, the soil contamination and plant uptake by roots or leaves leads to the entrance of CECs in the terrestrial food web. Accordingly, an increasing concern in the occurrence of CECs in irrigated crops is growing worldwide associated with human health implications.

The uptake of CECs by crops depends on the environmental and climatic factors such as soil characteristics, ambient temperature and relative humidity (RH) but also on the agronomical practices (irrigation technology, organic versus conventional farming), the plant physiology (leafy vegetable, root, trees), plant genotype and physical-chemical properties of contaminant (Miller et al., 2016). In addition, different cultivars of the same genus can exhibit different bioaccumulation patterns due to different metabolic pathways (Eggen et al., 2011). Therefore, due to the large number of factors that might affect to the bioaccumulation patterns of crops, predictive models to assess the uptake of contaminants by crops are challenging (Trapp, 2004). In addition, the existing experimental data that could be useful for their validation is of limited applicability since key experimental parameters are usually not reported in the published results. As a consequence, a large uncertainty exists in the current predictive models for the uptake



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of contaminants by crops and as a consequence risk assessment of CECs should be based on experimental field data obtained from crops irrigated with RWW.

The number of CECs whose uptake by crops have been evaluated is ca. 100 (Miller et al., 2016). Nevertheless, most of these assessments have been carried out in hydroponics, spiked irrigation water or soil and very few under field-scale studies using RWW that are so far the most valuable to estimate the contaminant intake. Hydroponic culture can be considered as the worst-case scenario since there is no soil interaction leading to the highest bioconcentration factors by crops (Wu et al., 2013).

Another important aspect is to consider the concentration of CECs in the edible part of crop in fresh weight since the concentration might be different from root, shoot leaves and fruits (Hurtado et al., 2016). Transformation products and conjugates of contaminants usually are not measured and can lead to an underestimate of the real exposure (Wu et al., 2016). In fact, some transformation products (TPs) might retain the toxicity of the parent compound or the toxicity can be enhanced. Accordingly, all of these aspects should be considered in the CECs exposure and risk assessment studies.

The aims of this report are (i) to give an overview of the approaches for human health risk assessment based on the consumption of crops cultivated in soil irrigated with the reclaimed wastewater, (ii) to present the range of the estimated intakes and corresponding risks by human taking into account different approaches for the potential exposure estimation associated with the consumption of RWW irrigated crops, and iii) to identify the research needs.

## 2. Risk assessment methodologies

There are different approaches to the estimation of the health risks as a consequence of dietary exposure. Both probabilistic and deterministic risk assessments have been used to evaluate the risk of contaminants in crops and they have been recently reviewed (Prosser and Sibley, 2015). Some of the most common approaches are reported below.

### 2.1 No Observable Adverse Effect Level (NOAEL)

This methodology compares the concentrations of the studied CECs present in the consumed quantity of crops to the single medical dose (Figure 1).



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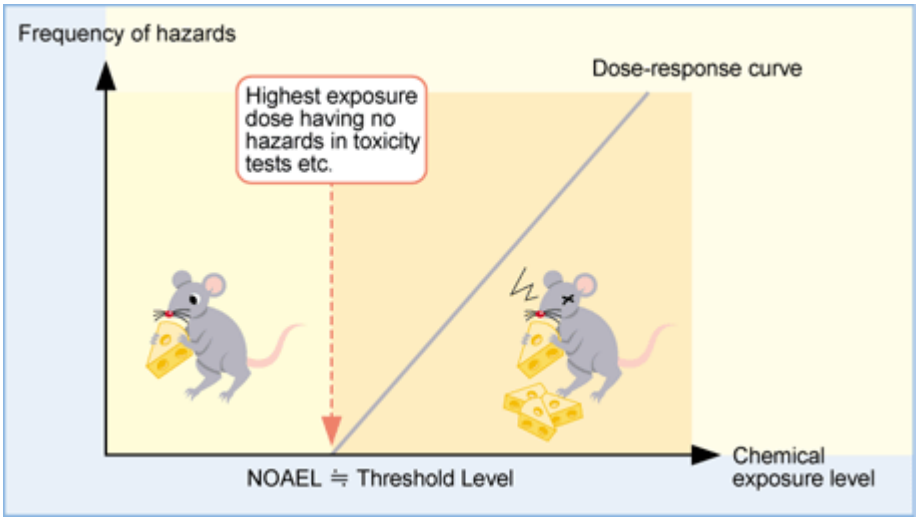


**Figure 1** - Schematic representation of the relationship between the chemical exposure and the frequency of hazards.

The risk can be calculated from the comparison of the **Estimated Daily Intake** (EDI) of a chemical to the **Accepted Daily Intake** (ADI) levels. The ADI value of a substance is the amount of a contaminant that can be consumed daily over a person's lifespan without evocating adverse effect. The ADI values for pharmaceuticals used for treatment of humans are determined by dividing the lowest daily therapeutic dose for an adult (mg/d) by a safety factor of 1000 and a body weight of 70 kg (WHO, 2011).

**The estimated daily intake (EDI)** for each of the pharmaceuticals and plant species under the investigation in a particular study can be calculated as follows:

$$\mathbf{EDI} = (C_{food} \times IR) / m$$

where $C_{food}$ is the concentration of the pharmaceutical in the analyzed plant tissue ($ng/g_{fw}$) in fresh weight,

*IR* is intake rate of vegetables (g/d),

*m* is body weight, usually averaged for the population group under investigation (e.g. infant, toddler, adult).

The comparison between EDI and ADI points out whether for a specific population group at specified consumption rate of food products leads to an intake of the investigated contaminant exceeding the ADI and then posing or not a health risk.



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It is worth mentioning that the IR depends on the specific diet and the food concentration. Indeed, many factors can affect the concentration of contaminants in food such as, (1) quality of irrigation water (reclaimed wastewater vs groundwater), (2) type of culture (hydroponics vs soil or substrate), (3) plant genotype (cultivars) used in a specific region, (4) environmental factors (T, pH, RH), (5) soil characteristics, and (6) agronomical practices (e.g. manure or biosolid amendment).

The IR is country dependent since the dietary habits might be quite different depending on the regions, type of diet (i.e. flexitarian, vegetarian, vegan) and even seasonality (high irrigation vs low irrigation season). Even cooking can affect the CECs intake so the assessments are usually based on the foods that are consumed raw.

In case that ADI is not available, the **Minimum Therapeutic Dose** (MTD) in (mg/d) that induces the desired therapeutic effect among target populations is used by default

In order to estimate the ADI of a pharmaceutical, the **No Observable Effect Level** (NOAEL) or **Minimum Therapeutic Dose** (MTD) is divided by an uncertainty factor (e.g. 1000 for most of compounds) and the body weight. The ADI is given in μg per kg of body weight per d ($\mu g/kg_{bw}$ /d). The ADI can be calculated from the following equation:

**ADI = NOAEL / Safety Factor**

The NOAEL values have been reported by the regulatory agencies such as Food and Agriculture Organization (FAO), European Medicine Agency (EMA), European Food Safety Agency (EFSA).

The safety factor is of 1000 for all compounds except carcinogenic compounds.

## 2.2 The Hazard Quotient (HQ) approach

The hazard quotient can be estimated from *EDI* and *ADI* as follows:

**HQ = EDI / ADI**

where *EDI* is the estimated daily intake and *ADI* the accepted daily intake.

In case of pharmaceuticals, taking into account the precautionary principle, the HQ value has been selected to be 0.1 in order to pose a risk, since many exposure sources of pharmaceuticals are expected.



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In case of genotoxic or potentially genotoxic compounds, the ADI approach needs to include an additional safety factor taking into account the chronic exposure to chemical contaminants. According with the Cramer's classification (Cramer et al., 1978) some pharmaceuticals have been described as genotoxic or potentially genotoxic such as lamotrigine, meprobamate, norfloxacin, progesterone (pregn-4-en-3,20-dione), streptomycin, sulfamethazine, sulfamethoxazole, sulfapyridine, testosterone, tetracycline, triamterene, tylosin and virginiamycin).

The formation of toxic metabolites should be also considered when ADI is calculated and not only the parent compound as in the case of 10,11-epoxycarbamazepine that can occur at higher concentrations than parent compound carbamazepine in some crops (Malchi et al., 2014). In addition, a large variety of conjugates can be originated during the Phase II plant metabolism. Since some of these conjugates can release the parent compound during the human tract transit, the conjugated fraction needs to be considered in the risk assessment.

## 2.3 Threshold of Toxicological Concern (TTC)

The **Threshold of Toxicological Concern** (TTC) provides a preliminary risk assessment evaluation for a large number of chemical contaminants occurring in food and environmental matrices. It provides a sound risk estimate of microcontaminants even in case of no available toxicity data. The threshold of toxicological concentration (TTC) has been developed to assess the risk of low-level substances present in the diet. It can be used for an initial assessment of a substance to determine whether a comprehensive risk assessment is required.

TTC is based on a conservative estimate of 5% of NOAEL with an additional $10^{-6}$ uncertainty factor.

It is estimated on the structural properties of chemicals and toxicological data according with the Cramer's et al. (1978) classification tree (Toxtree) in three categories (I, II and III) by increasing the suspected toxicity. The Toxtree software has been encoded specifically commissioned by the European Chemicals Bureau (ECB). This software determines the TTC values and the compound classification (Table 1).

- **Class I** contains substances with simple chemical structures. They are easily metabolized, thus presenting little oral toxicity concern.



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- **Class II** contains substances that may be more harmful than those of Class I but they do not contain structural features that are suggestive of toxicity.
- **Class III** contains substances with reactive functional groups and present greater toxic concern.
- **Potentially genotoxic compounds** may interact with DNA and cause mutation to genetic code (i.e. 10,11-epoxycarbamazepine, lamotrigine, sildenafil, sulfamethoxazole, and sulfapyridine).

**Table 1** - Thresholds of Toxicity Concern (TTC) according with the Cramer's structural classes (Cramer et al., 1978)

| Cramer's structural classes | TTC levels per day |
|---|---|
| I | 30 $\mu g/kg_{bw}$ |
| II | 9.1 $\mu g/kg_{bw}$ |
| III | 1.5 $\mu g/kg_{bw}$ |
| Potentially genotoxic compounds | 2.5 $ng/kg_{bw}$ |

## 2.4 Predicted Non Effect Concentration (PNEC) for Antibiotics

Selective pressure of ABs in the environment may accelerate the evolution and dissemination of antibiotic-resistant bacteria. These phenomena cannot be evaluated by the former risk assessment approaches. However, there is a limited knowledge of environmental concentrations that might exert selection for resistant bacteria. Although to experimentally determine minimal selective concentrations in complex microbial ecosystems demands a big effort, the upper boundaries for selective concentrations for common antibiotics have been estimated on the assumption that selective concentrations a priori need to be lower than those inhibiting growth. From the EUCAST database, the 1% lowest observed Minimum Inhibitory Concentrations (MICs) for 111 antibiotics can be identified. In order to compensate for the limited species coverage,



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predicted lowest MICs adjusted for the number of tested species can extrapoled through modelling (Bengtsson-Palme and Larsson, 2016). Predicted No Effect Concentrations (PNECs) for resistance selection were then assessed using a factor of 10 to account for differences between MICs and minimal selective concentrations. The resultant PNECs for selection resistance ranged from 8 ng $L^{-1}$ to 64 μg $L^{-1}$ which are below most of the reported PNECs for ecotoxicological effects.

## 3. General discussion

The ADI approach is a single compound approach while in common practice RWW usually contains a large number of contaminants and some of them can be uptaken by crops.

On the other hand, the HQ evaluation usually additive approach is applied.

$$\mathbf{HQ_T = \Sigma_i\ HQ_i}$$

where $HQ_T$ is the overall hazard quotient and $HQ_i$ is the one of compound *i*.

However, the additive approach cannot be realistic estimate since synergistic or antagonistic interactions between contaminants are not taken into account.

### 3.1 Reported Health Risk Values

According with the published reports, the estimated health risk associated with crops irrigated with RWWs span from "*de minimis*" to a substantial risk to children from minimum consumption. In the Prosser et al. (2015) assessment, the HQs of 29 pharmaceuticals derived from RWW were calculated and all the HQ values were lower than 0.1 for adults (76.6 kg) but several compounds exceeded that values for toddlers (15.4 kg bw; 1-4 years old) namely, flunixin (0.4), ketoprofen (0.1), lamotrigine (0.2), metoprolol (0.3), and sildenafil (0.3). In case of manure or biosolids, the HQ values can be different since concentrations and soil application can be different.

In the following section several examples of risk evaluation will be presented.

#### *3.1.1 Carbamazepine (CBZ)*

It is used primarily in the treatment of epilepsy and neuropathic pain. It is used in schizophrenia along with other medications and as a second line agent in bipolar



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disorder. It is recalcitrant to its elimination in conventional wastewater treatment and it is frequently detected in surface waters (Löffler et al., 2005)

The ADI value for adults is of 2.9 μg/kg/d and the TTC (Class III) is of 1.5 μg /kg/d. The lowest therapeutic dose is of 200 mg/d (i.e. 2.86 mg/kg/d for a 70 kg individual) (Tables 1 and 2).

Taking into account the CBZ intake even in the worst-case-scenario (hydroponic culture), the intake is very low (0.007-0.026 μg/$kg_{bw}$/d) in comparison to ADI (2.9 μg/$kg_{bw}$/d) and TTC (1.5 μg/$kg_{bw}$/d). Therefore, the **risk** associated with crops irrigated with RWW **is negligible** (Table 2).

However, in case of the **potentially genotoxic compounds** such as the carbamazepine's metabolite, **10,11-epoxycarbamazepine,** the corresponding rather low TTC level (2.5 ng/$kg_{bw}$/d, i.e 175 ng/d for adults of 70 kg wt, and 62.5 ng/d for child of 25 kg wt) **might be easily surpassed** even if its low levels were found in the vegetables, demanding for **specific toxicity analysis** for these metabolites (Table 3).

### *3.1.2 Diclofenac (DCF)*

Diclofenac (DCF) is a widely used non-steroidal anti-inflammatory drug. It is rather recalcitrant to wastewater treatment, and is hence commonly detected in wastewater effluents. The ADI value for adults is of 1.4 μg/kg/d and the TTC (Class III) is of 1.5 μg /kg/d.

In most studies assessing the uptake of DCF in reclaimed wastewater-irrigated crops, DCF concentrations in edible plant parts were below limit of detection or quantification limits. To date, DCF has been quantified in lettuce leaves irrigated with secondary wastewater effluent (Calderon-Preciado et al., 2013) and in tomato fruit irrigated with tertiary treated wastewater (3-year irrigation) (Christou et al., 2017).

The highest daily DCF intake, i.e. in the worst-case-scenario (lettuce irrigated with secondary effluent), was estimated to be 0.010 μg/$kg_{bw}$/d, being significant lower than ADI and TTC values. Even lower intake was estimated considering consumption of tomato (≤ 0.0005 μg/$kg_{bw}$/d) irrigated with tertiary-treated wastewater (Table 4). Therefore, the **risk** associated with crops irrigated with RWW is **negligible**, although calculations presented in Table 4 refer only to dietary intake of a single edible crop type.



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**Table 2** - Evaluation of risk (ADI and TTC) associated with the intake of carbamazepine from different crops

| Plant (edible part) | Type of irrigation water | Concentration in plant, ng/g fw | Consumption rate | Intake*, $\mu g/kg_{bw}/d$ | ADI (%) (TTC) (%) | Literature source |
|---|---|---|---|---|---|---|
| Lettuce | Hydroponic solution spiked at 0.5 µg/L | 2.9-67 in all plant leaves and stems | 0.54 $g_{fw}/kg_{bw}/d$ | 0.0007 | 0.02 (0.05) | Wu et al. 2013 |
| Spinach | Hydroponic solution spiked at 0.5 µg/L | | 0.54 $g_{fw}/kg_{bw}/d$ | 0.0001 | 0.003 (0.007) | Wu et al. 2013 |
| Lettuce | Hydroponic solution spiked at 5 µg/L | 23-520 in all plant leaves and stems | 0.54 $g_{fw}/kg_{bw}/d$ | 0.006 | 0.21 (0.40) | Wu et al. 2013 |
| Spinach | Hydroponic solution spiked at 5 µg/L | | 0.54 $g_{fw}/kg_{bw}/d$ | 0.001 | 0.03 (0.07) | Wu et al. 2013 |
| Green bean | RWW | 61.05 ($_{dw}$)-the maximum level | 2.8 cup/d | 0.026 | 0.90 (1.73) | Prosser and Sibley, 2015 |
| Sweet potato root | RWW | 0.116 | 5 g/d (Israel) | $8 \times 10^{-6}$ (Israel) | 0.0003 (0.0005) (Israel) | Malchi et al. 2014 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | | | 0.07 kg/d (China) | 0.00012(China) | 0.004 (0.008) (China) | |
| Carrot roots | RWW | 0.799 | 13-18 g/d (Europe) | 0.00018-0.00025 | 0.006 (0.012) – 0.009 (0.037) | Malchi et al. 2014) |

**adult 70 kg body wt*



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**Table 3** - Evaluation of risk (ADI and TTC) associated with the intake of carbamazepine from different crops

| Plant (edible part) | Type of irrigation water | Level in plant, ng/g fw | Consumption rate | Intake, ng/kg$_{bw}$/d | % of ADI ** (% of TTC) | Daily consumption in kg by an adult to reach TTC* or ADI** | Daily consumption in kg by a child to reach TTC* or ADI** |
|---|---|---|---|---|---|---|---|
| Sweet potato root* | RWW | 0.013 | 5 g/d (Israel)<br>70 g/d (China) | 0.0009 (Israel)<br>0.013 (China) | 3 x $10^{-5}$ (0.036) (Israel)<br>4.5 x $10^{-4}$ (0.52) (China) | 13.58* | 6.25* |
| Carrots Roots* | RWW | 0.244 | 13-18 g/d (Europe) | 0.045-0.063 | 0.001-0.002 (1.8-2.52) | **0.71*** | **0.25*** (~ 2-3 carrots) |
| Carrots** | RWW | 0.244 ($_{dw}$)** | 2.8 cup/d** | 1.2-adult<br>(6-child) | 0.04 (48) –adult<br>0.21 (240) - child | 910** | 183** |

** Malchi et al. Envrion.Sci.Technol. 48 (2014) 9325*

*** Prosser and Sibley, Environ. Int. 75 (2015) 223: the used ADI of 2900 ng/kg/d is calculated with safety factor of 1000, but it should be 10000; the used occurrence level is expressed on dry weight basis; the same consumption rate is used for adults and children*



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**Table 4** - Evaluation of risk (ADI and TTC) associated with the intake of diclofenac from different crops in field studies

| Plant (edible part) | Type of irrigation water | Concentration in plant, ng/g fw | Consumption rate | Intake, $\mu g/kg_{bw}/d$ (adult 70 kg) | ADI % TTC (%) | Literature source |
|---|---|---|---|---|---|---|
| Lettuce (leaf) | Secondary wastewater effluent | 19 (the maximum level) | 0.54 $g_{fw}/kg_{bw}/d$ | 0.010 | 0.7 (0.7) | Calderón-Preciado et al. (2013) |
| Tomato (fruit) | Tertiary wastewater effluent (WWTP 1, year 2) | 0.68 | 0.67 $g_{fw}/kg_{bw}/d$* | 0.0005 | 0.03 (0.03) | Christou et al. (2017) |
| Tomato (fruit) | Tertiary wastewater effluent (WWTP 1, year 3) | 0.23 | 0.67 $g_{fw}/kg_{bw}/d$* | 0.0002 | 0.01 (0.01) | Christou et al. (2017) |
| Tomato (fruit) | Tertiary wastewater effluent (WWTP 2, year 3) | 0.68 | 0.67 $g_{fw}/kg_{bw}/d$* | 0.0005 | 0.03 (0.01) | Christou et al. (2017) |

**Daily intake of tomato in Cyprus, 2013 based on FAOSTAT (http://www.fao.org/faostat/en/#home), assuming 70 $kg_{bw}$ for adult*



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## 4. Research needs

Several methodologies for risk assessment have been developed and applied to evaluate the risk associated with the irrigation of vegetables with RWW. These risk assessment methodologies usually do not estimate the risk associated with mixtures of compounds and usually only an additive hazard quotient is considered. However, synergic and antagonistic interactions might occur among contaminants and consequently a more realistic risk should be redefined.

In most of risk assessments, only parent compounds are considered. However, some transformation products (Phase I and II metabolites) can occur at higher concentration than the parent compound and they should be taken into account. In this regard, analytical techniques need to be developed for their determination including quantification or synthesis of standards since most of them are not commercially available.

Predictive models to accurately estimate the CECs concentration in crops grown in different environmental and agronomical practices would be extremely useful for risk assessment but they need to be consolidated. In fact, the number of CECs and the related metabolites is extremely high and cannot be accomplished experimentally. In addition, since the quality of RWWs and irrigation practices depend very much of the European regions, the concentration levels of CECs in food can be different.

The risk associated with the occurrence of antibiotics in the soil-plant environment and selective pressure on the endophytic bacteria needs to be addressed. The PNEC has been estimated from the MIC values for every class of antibiotics in aquatic ecosystems but similar approach has not been developed in the rizosphere where a specific microbiome exists. Further research needs to be addressed to develop and accurate model to predict ARB and ARGs in the root-soil interphase and in the endophytic bacteria.

Finally, the human exposure to CECs needs to be explored including not only vegetables but also other food products that might contain the same contaminants. In this regard, the proof of concept of *in vivo* exposure by analyzing CECs in urine samples of healthy individuals appears to be a successful approach to get a more realistic risk assessment to evaluate the human exposure to CECs. This approach has been successfully applied only for carbamazepine (Paltiel et al., 2016) but it would be of interest to extend it to other CECs with multiple exposure pathways such as organophosphate flame retardants, plasticizers and polymer monomers (bisphenol A).

## 5. Acknowledgments

**Biljana Škrbić**, University of Novi Sad. Faculty of Technology, Serbia.
**Fabio Poselel**, Technical University of Denmark, Denmark
**Marlen Vasquez**, Cyprus University of Technology, Cyprus.
**Anastasis Christou**, Agricultural Research Institute, Cyprus
**Josep M Bayona**, Institute of Environmental Assessment and Water Research IDAEA-CSIC, Spain
**Despo Fatta-Kassinos**, Nireas-IWRC, University of Cyprus, Cyprus
**Irene Michael-Kordatou**, Nireas-IWRC, University of Cyprus, Cyprus

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