



# Od odpadka do pridelka: Priložnosti in izzivi ponovne uporabe odpadne vode v kmetijstvu

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**At least 11%**

of Europeans are affected  
by water scarcity

**1 billion m3**

of treated urban  
wastewater is reused  
annually

**6 times more**

treated water could be  
reused than current  
levels

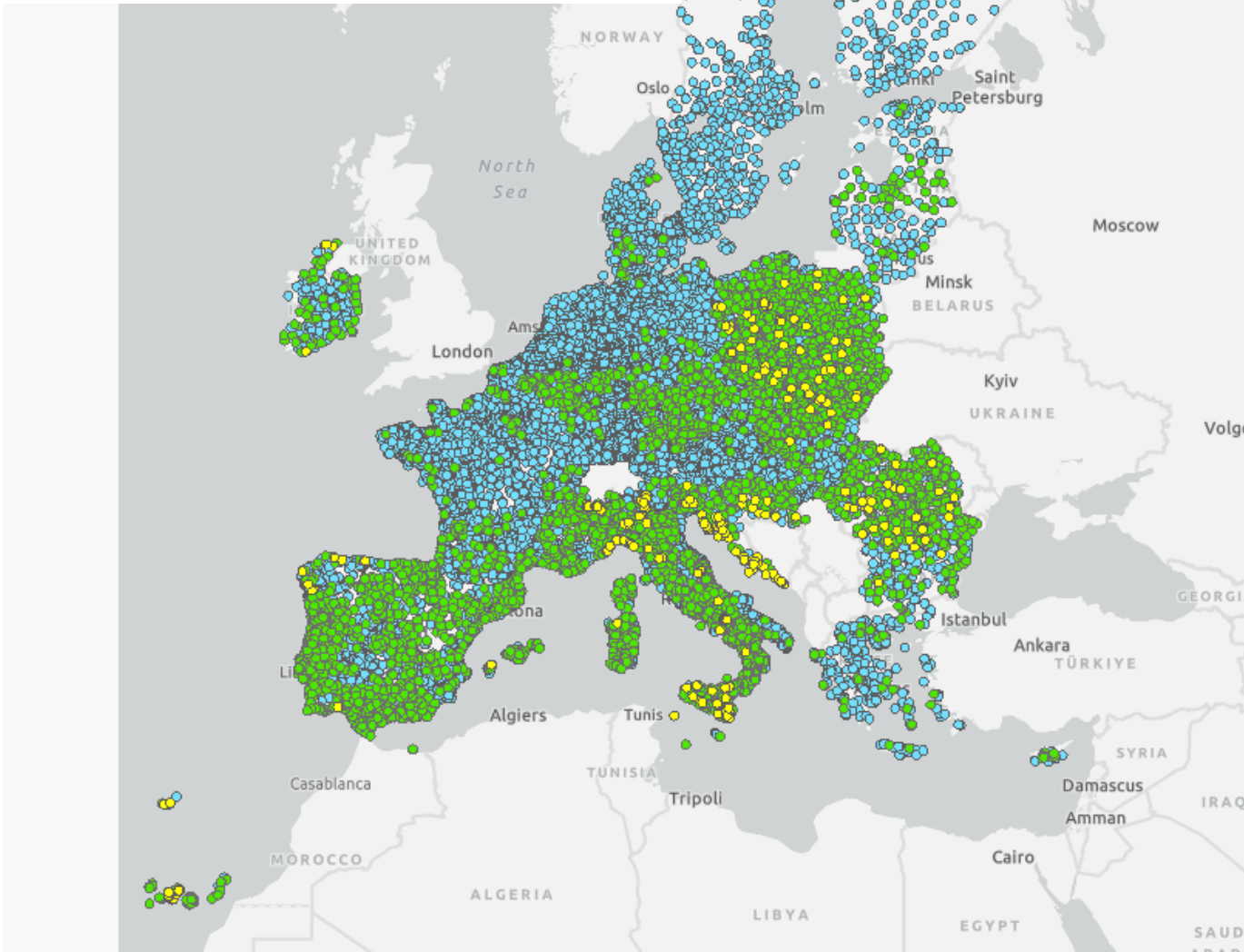


Effective management of water resources crucial for global food security and sustainable development!

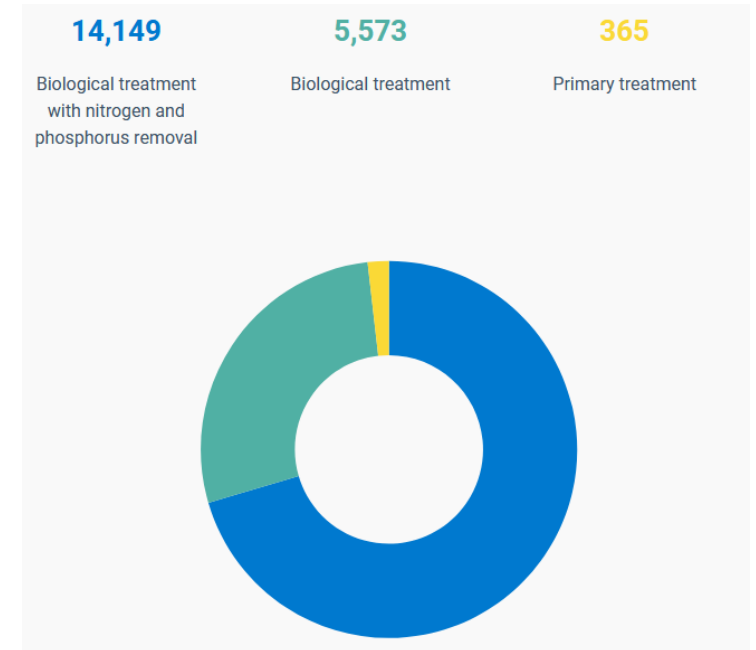
## Plants by treatment type

Urban waste water treatment plants by treatment type

- Biological treatment with nitrogen and/or phosphorus removal
- Biological treatment
- Primary treatment



>80% (>95%) of global WW untreated!  
82% of Europe's UWW is treated!



The limitations of TWW....

## Do EU countries reuse treated urban waste water?

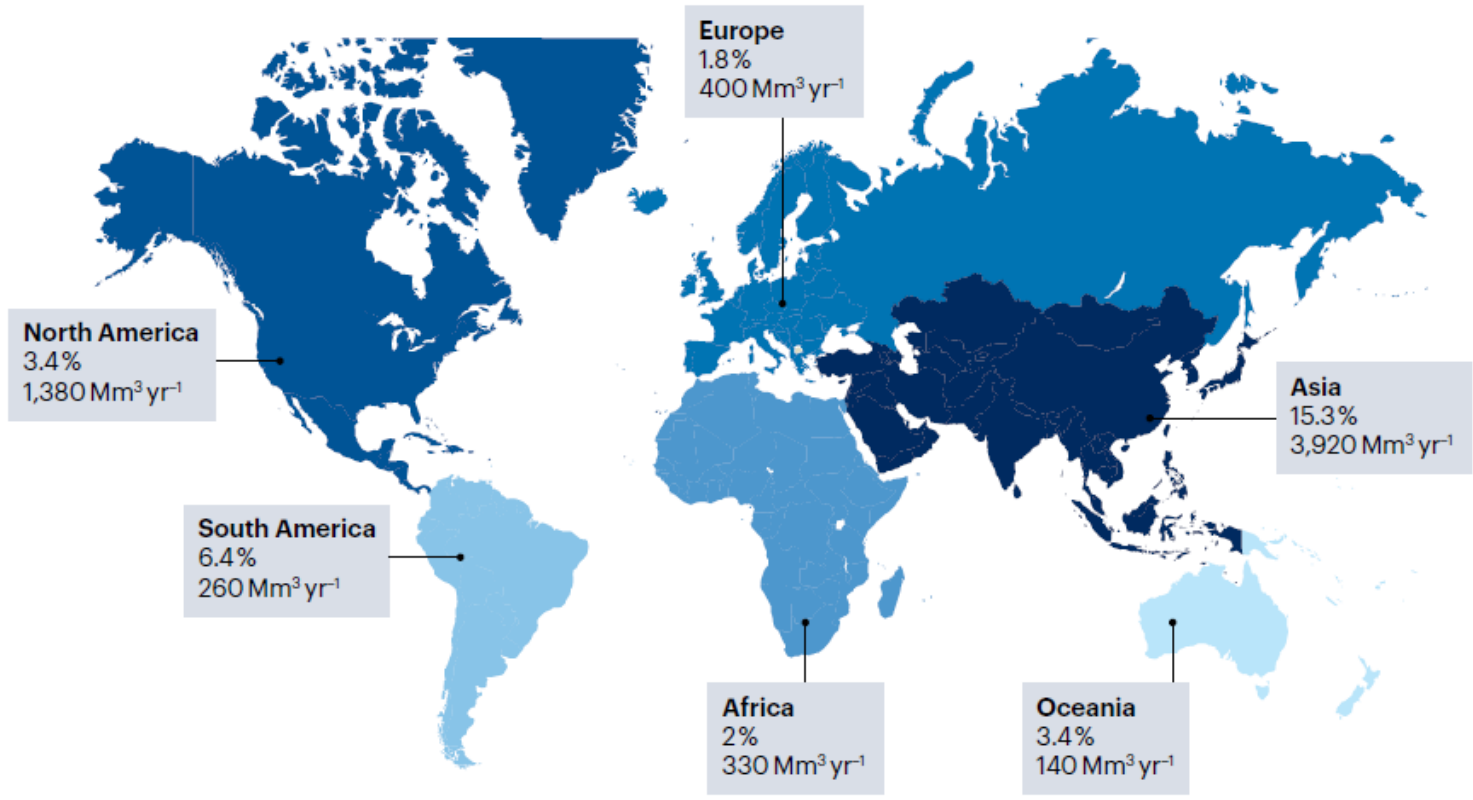
Water reuse has become a key part of water resources management in countries suffering from high water stress. The primary use of reused water is in irrigation for agriculture. Other uses are in irrigation of urban space, such as parks and sports fields, groundwater recharge and river flow improvement. Where water resources are less stressed, waste water reuse is usually driven by other factors, such as conservation of groundwater resources.



# Do EU countries reuse treated urban waste water?

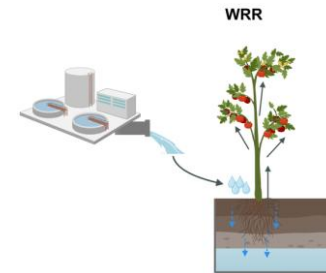
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## Annual volume and percentage of TWW reused directly for irrigation



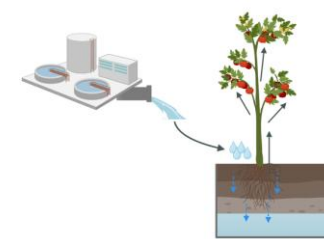
# Legislation: Water Reuse Regulation - WRR

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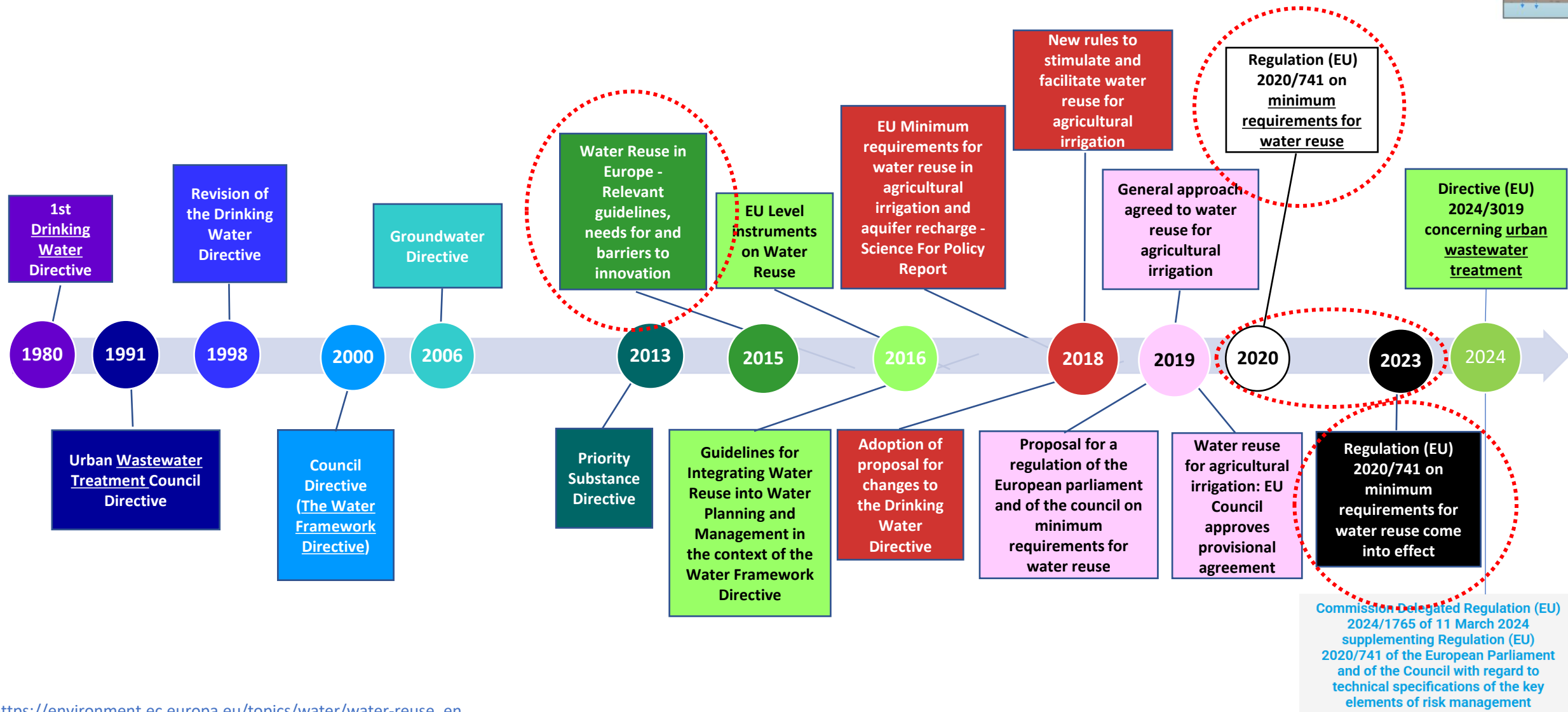


# Legislation: WRR

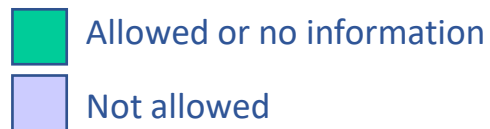
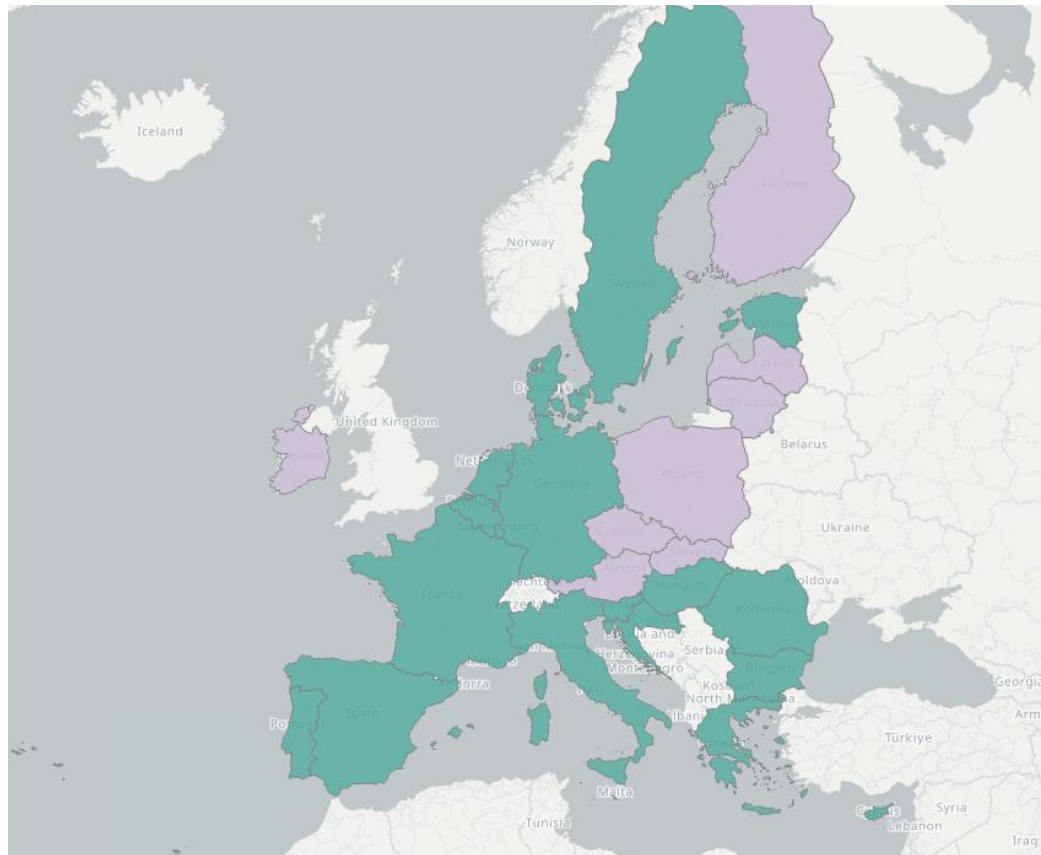
WRR



## Timeline of Regulatory Initiatives Relating to Water Reuse at EU Level



## Member States Where Water Reuse for Agricultural Irrigation is Allowed (October, 2024)

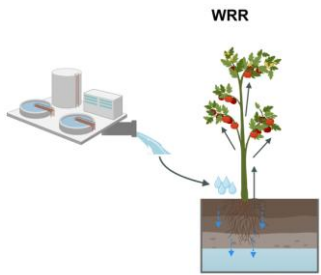


Member State can decide not to practice/limit in certain areas water reuse

Some Member states:

- Do not allow water reuse (where freshwater resources are abundant)
- Yet to take final decision





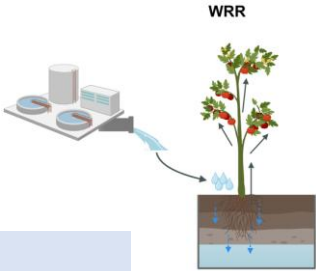
**REGULATION (EU) 2020/741 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL**  
**of 25 May 2020**  
**on minimum requirements for water reuse**  
(Text with EEA relevance)

- Aim:**
- Guarantee that reclaimed water is safe for agricultural irrigation
  - Ensure a high level of protection of the environment and of human and animal health
  - Promote the circular economy

(a) Minimum requirements for water quality

Table 2 – Reclaimed water quality requirements for agricultural irrigation

Reclaimed water quality class	Indicative technology target	Quality requirements				
		<i>E. coli</i> (number/100 ml)	BOD <sub>5</sub> (mg/l)	TSS (mg/l)	Turbidity (NTU)	Other
A	Secondary treatment, filtration, and disinfection	≤ 10	≤ 10	≤ 10	≤ 5	<i>Legionella</i> spp.: < 1 000 cfu/l where there is a risk of aerosolisation Intestinal nematodes (helminth eggs): ≤ 1 egg/l for irrigation of pastures or forage
B	Secondary treatment, and disinfection	≤ 100	In accordance with Directive 91/271/EEC (Annex I, Table 1)	In accordance with Directive 91/271/EEC (Annex I, Table 1)	-	
C	Secondary treatment, and disinfection	≤ 1 000			-	
D	Secondary treatment, and disinfection	≤ 10 000			-	



Aim:

- Guarantee that reclaimed water is safe for agricultural irrigation
- Ensure a high level of protection of the environment and of human and animal health
- Promote the circular economy

L 177/32

EN

Official Journal of the European Union

5.6.2020

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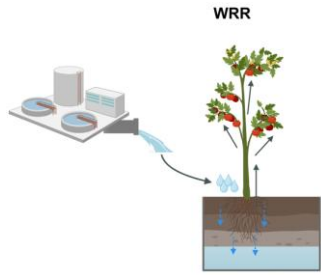
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C	Secondary treatment, and disinfection	≤ 1 000			-	
D	Secondary treatment, and disinfection	≤ 10 000			-	

**REQUIREMENTS: *E. Coli*, BOD5, TSS, Turbidity (and *Legionella* spp., Intestinal nematodes)**

# Legislation: WRR



Recycled Water Class	Crop category <sup>a</sup>	Allowable irrigation methods
A	All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw	All
B	Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals	All
C	Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals	Drip irrigation <sup>b</sup> or other irrigation method that avoids direct contact with the edible part of the crop
D	Industrial, energy and seeded crops	All <sup>c</sup>

Source: EU (2020)

<sup>a</sup> If the same type of irrigated crop falls under multiple classes, the requirements of the most stringent category apply.

<sup>b</sup> Drip irrigation is a micro-irrigation system capable of delivering water at low flowrates to plants and involves dripping water onto the soil or directly under its surface from a system of small-diameter plastic pipes fitted with outlets.

<sup>c</sup> In the case of irrigation methods which imitate rain, special attention should be paid to the protection of the health of people nearby.

## Water reuse for agriculture approved for use in the EU

The EU regulation (2020) defines the following approved crops for agricultural irrigation with treated municipal wastewater:

- Food crops consumed raw, i.e., intended for human consumption in an unprocessed state (Class A, B and C)
  - Root crops (e.g., carrots, onions)
  - Above-ground low-growing crops (e.g., lettuce, tomatoes)
  - Above-ground high-growing crops (e.g., fruit trees)
- Processed food crops intended for human consumption after being treated (Class B and C)
  - Cooked or industrially processed food crops (e.g., rice and wheat)
- Non-food crops (Class B, C and D)
  - Processed food crops not intended for human consumption:
    - Pastures and forage (Class B and C)
    - Industrial, energy and seeded crops (Class D)

# Legislation: WRR

## Recycled Water Class/Crop category/Irrigation method:

- A: all food crops ...edible part in contact/All
- B: food crops... edible part not in direct contact/All
- C: food crops... edible part above the ground, not in contact/Drip irrigation
- D: industrial, energy and seeded crops/All

Recycled Water Class	Crop category <sup>a</sup>	Allowable irrigation methods
A	All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw	All
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Recycled Water Class/Category (Approved Uses)	Source Water Type	Water Quality Parameter <sup>a</sup>	Specification	Sampling/Monitoring Requirements (Frequency of monitoring; site/ location of sample; quantification methods)
A, B, C and D	Treated municipal wastewater (secondary treatment and disinfection)	Legionella spp	<1000 CFU/L when there is risk of aerosol generation	Twice a month
		Helminth eggs	≤ 1 egg/L for pasture or forage irrigation	Twice a month or as determined by the wastewater treatment operator based on influent concentration
A	Treated municipal wastewater (secondary treatment, filtration and disinfection)	E. coli	≤ 10 units/100 mL; ≥ 5 Log Reduction Values (LRV)	Once a week
		Total coliphages/F-specific coliphages/somatic coliphages/coliphages <sup>b</sup>	≥ 6 LRV	Used for validation monitoring when a new treatment plant producing Class A effluent is set up. Not for routine monitoring.
		Clostridium perfringens spores/spore-forming sulfate reducing bacteria <sup>c</sup>	≥ 4 LRV for Clostridium perfringens spores, ≥ 5 for spore-forming sulfate-reducing bacteria	
		BOD <sub>5</sub>	≤ 10 mg/L	
		TSS	≤ 10 mg/L	Once a week
		Turbidity	≤ 5 NTU	Continuous
B, C and D <sup>d</sup>	Treated municipal wastewater (secondary treatment and disinfection)	BOD (BOD <sub>5</sub> at 20 °C) without nitrification <sup>d,e</sup>	≤ 25 mg/L Minimum reduction of 70-90%	Homogenized, unfiltered, undecanted sample. Determination of dissolved oxygen before and after five-day incubation at 20 °C ± 1 °C, in complete darkness. Addition of a nitrification inhibitor.
		TSS <sup>d</sup>	35 mg/L Minimum reduction of 90%	Filtering of a representative sample through a 0.45 µm filter membrane. Drying at 105 °C and weighing
B	Treated municipal wastewater (secondary treatment and disinfection)	E. coli	≤ 100 units/100 mL	Once a week
C			≤ 1000 units/100 mL	
D			≤ 10000 units/100 mL	



# Legislation: WRR

Recycled Water Class: A,B,C,D  
Source Water Type Water Quality Parameter  
Specification/Limit  
Frequency of Monitoring

Recycled Water Class	Crop category <sup>a</sup>	Allowable irrigation methods
A	All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw	All
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C			≤ 1000 units/100 mL	
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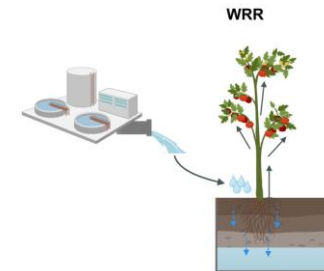
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		C. perfringens		Once a week
		BOD (BOD <sub>5</sub> at 20 °C) without nitrification <sup>d,e</sup>	≤ 25 mg/L Minimum reduction of 70-90%	Homogenized, unfiltered, undecanted sample. Determination of dissolved oxygen before and after five-day incubation at 20 °C ± 1 °C, in complete darkness. Addition of a nitrification inhibitor.
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B		E. coli	≤ 100 units/100 mL	Once a week
C	Treated municipal wastewater (secondary treatment and disinfection)	E. coli	≤ 1000 units/100 mL	
D		E. coli	≤ 10000 units/100 mL	

Source Water Type:  
TMWW  
(secondary treatment + disinfection)

## ANNEX II: Key elements of risk management

### ANNEX II



#### (A) Key elements of risk management

Risk management shall comprise identifying and managing risks in a proactive way to ensure that reclaimed water is safely used and managed and that there is no risk to the environment or to human or animal health. For those purposes, a water reuse risk management plan shall be established on the basis of the following elements:

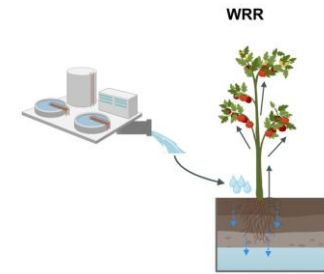
1. Description of the entire water reuse system, from the entry of waste water into the urban waste water treatment plant to the point of use, including the sources of waste water, the treatment steps and the technologies used at the reclamation facility, the supply, distribution and storage infrastructure, the intended use, the place and period of use (e.g. temporary or ad-hoc use), the irrigation method, the crop type, other water sources if a mix is intended to be used and the volume of reclaimed water to be supplied.
2. Identification of all parties involved in the water reuse system and a clear description of their roles and responsibilities.
3. Identification of potential hazards, in particular the presence of pollutants and pathogens, and the potential for hazardous events such as treatment failures or accidental leakages or contamination of the water reuse system.
4. Identification of the environments and populations at risk, and the exposure routes to the identified potential hazards, taking into account specific environmental factors, such as local hydrogeology, topology, soil type and ecology, and factors related to the type of crops and farming and irrigation practices. Consideration of possible irreversible or long-term negative environmental and health effects of the water reclamation operation, supported by scientific evidence.
5. Assessment of risks to the environment and to human and animal health, taking into account the nature of the identified potential hazards, the duration of the intended uses, the identified environments and populations at risk of exposure to those hazards and the severity of possible effects of the hazards considering the precautionary principle, as well as all relevant Union and national legislation, guidance documents and minimum requirements in relation to food and feed and worker safety. The risk assessment could be based on a review of available scientific studies and data.

#### (B) Conditions relating to the additional requirements

6. Consideration of requirements for water quality and monitoring that are additional to or stricter than those specified in Section 2 of Annex I, or both, when necessary and appropriate to ensure adequate protection of the environment and of human and animal health, in particular when there is clear scientific evidence that the risk originates from reclaimed water and not from other sources.

Depending on the outcome of the risk assessment referred to in point 5, such additional requirements may in particular concern:

- (a) heavy metals;
- (b) pesticides;
- (c) disinfection by-products;
- (d) pharmaceuticals;
- (e) other substances of emerging concern, including micro pollutants and micro plastics;
- (f) anti-microbial resistance.



## ANNEX II

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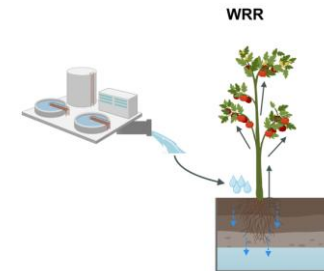
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**ANNEX II: Key elements of risk management:**  
identifying and managing risks in a protective way  
ensuring reclaimed water safety (no H/ERA)





## ANNEX II

### (A) Key elements of risk management

#### Additional parameters:

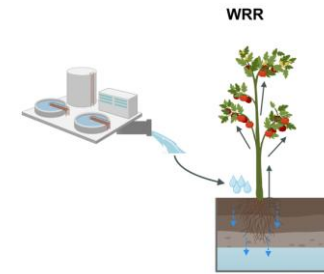
- when necessary and appropriate to ensure adequate protection of env. and human & animal health
- when there is a clear scientific evidence that the risk originates from reclaimed water
- Additional requirements:
  - Heavy metals
  - Pesticides
  - Disinfection by-products
  - Pharmaceuticals
  - Other (micropollutants and microplastics)
  - Anti-microbial resistance

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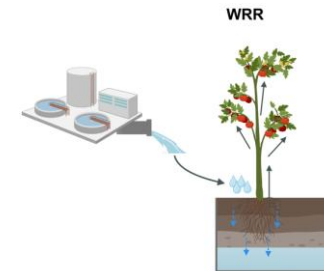
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- (f) anti-microbial resistance.

„... in the next year more data will be gathered and based on scientific evidence and also in hand with WW Directive, these parameters will be defined and **regulation reshaped in 2028.**“

# Scientific literature review: UPTAKE of CECs

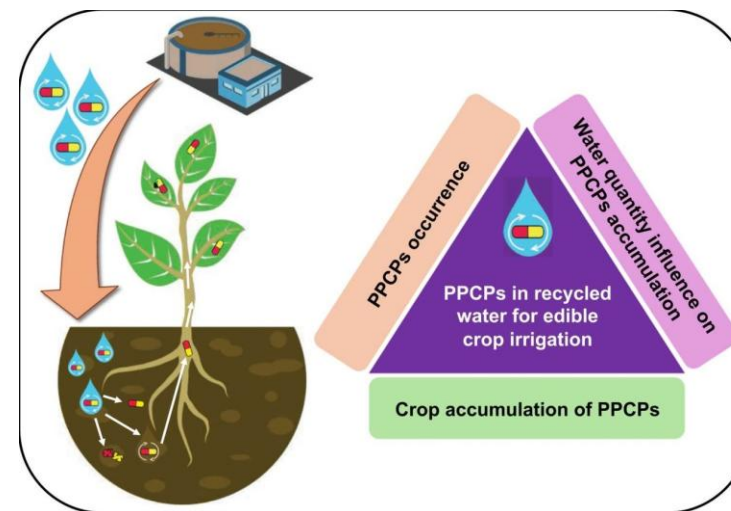


## Uptake:

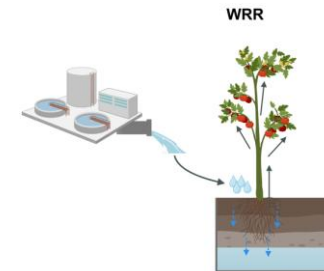
- Overall low
- Plants possess metabolic pathway that might transform and degrade CEC (decreasing potential risk?)
- Pollutants can induce transcriptomic and metabolomic rearrangements (impact plant physiology and morphology – indicating stress)
- Quality attributes affected?
- Agricultural use of biosolids >> CEC plant uptake than irrigation with TWW
- The risk related to the use of pesticides applied to crops >>

## Depends on:

- CEC physicochemical properties
- Plant species
- Soil physicochemical properties/irrigation mode and media
- Env. / Exp. conditions (evapotranspiration etc.)



# Scientific literature review: UPTAKE of CECs

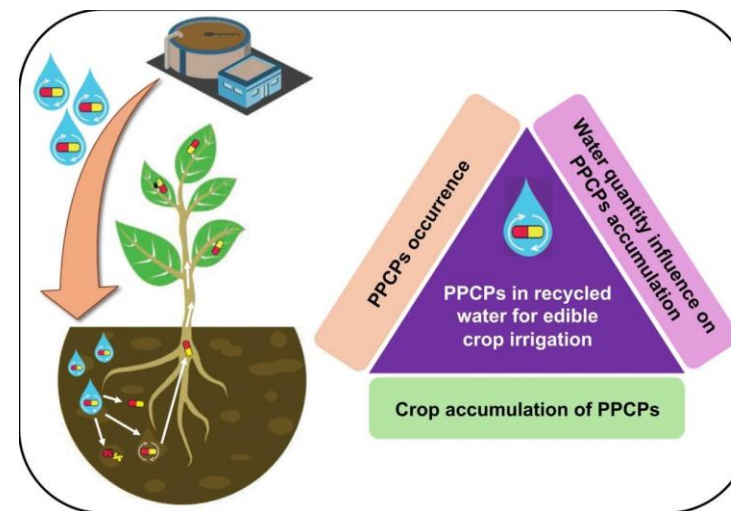


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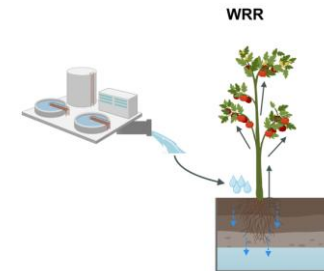
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# Scientific literature review: UPTAKE of CECs



## Uptake:

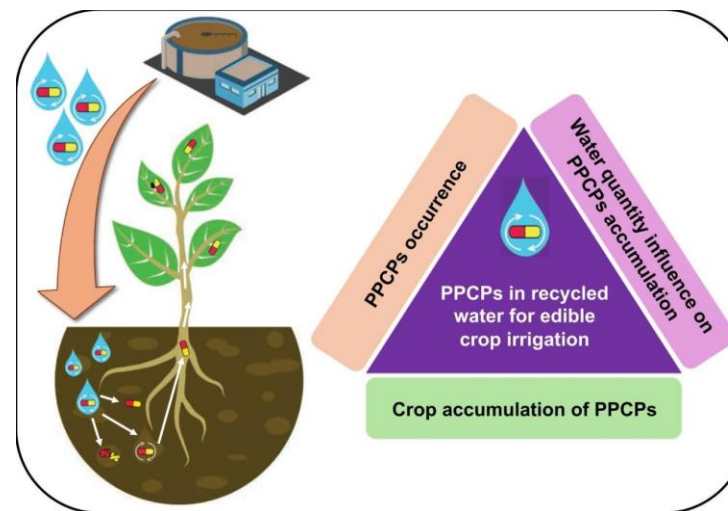
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## Depends on:

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- Plant species
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## Limitations:

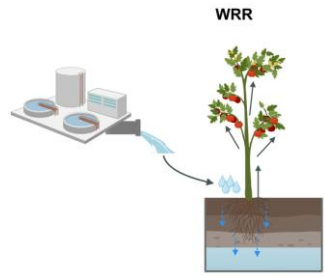
- Lack of reliable/comparable data
- Scientific literature: controlled conditions (artificially amended media: soil, water)
- **Real-world field experiments lacking**
- **Lack of long-term exposure data**



# Scientific literature review: Conclusion on RISks (CECs)

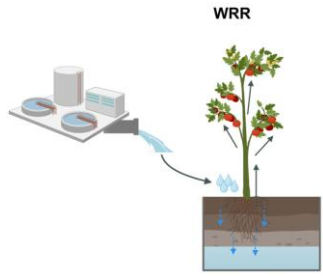
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- Uptake, translocation and accumulation of wide range of CEC from TWW in crop tissue: *de minimis* risk to human
- The use of reclaimed water is acceptable for several EU countries!
- TWW: a source for a reliable water supply WW reuse needs?



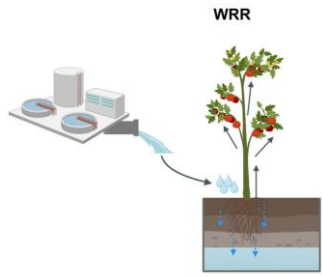
# Scientific literature review: Conclusion on RISks (CECs)

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- The use of reclaimed water is acceptable for several EU countries!
- TWW: a source for a reliable water supply WW reuse needs?
- Selected CEC (parent compounds)
- Other CEC including microbial contaminants, AMRG/B, M/NP....metabolites and TPs...
- Vicious circle of not being measured... additional TA, NTA, EDA...
- Risk: Additivity = mixtures of PPCP (CEC) present hazard
- Prioritisation of CEC (ML)!

# Scientific literature review: Conclusion on RISks (CECs)



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- TWW: a source for a reliable water supply WW reuse needs!

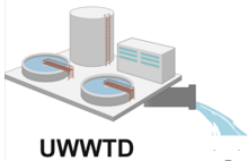
- Selected CEC (parent compounds)
- Other CEC including microbial contaminants, AMRG/B, M/NP....metabolites and TPs...
- Vicious circle of not being measured... additional TA, NTA, EDA...
- Risk: Additivity = mixtures of PPCP (CEC) present hazard
- Prioritisation of CEC (ML)

## MAIN BARRIER:

Lack of confidence in the health and env. safety of water reuse practices

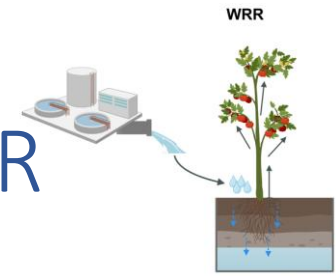


THE DEVELOPMENT OF THOROUGH MINIMUM QUALITY REQUIREMENTS FOR WATER REUSE FOR AGRICULTURAL IRRIGATION HAS THE AIM TO OVERCOME THIS BARRIER!



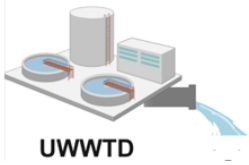
UWWTD

WRR



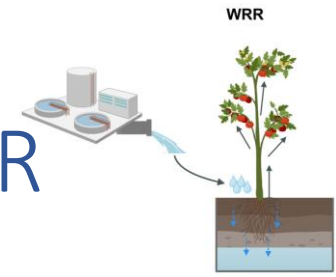
<https://www.linkedin.com/pulse/problems-drip-irrigation-system-senthil-kumar-domtf>





# UWWTD

# WRR



Aerobic  
biological  
treatment step

URBAN Wastewater Treatment Plant

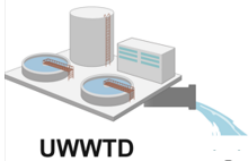
<https://www.ccn-domzale.si/>

## UWWTD: Urban Wastewater Treatment Directive



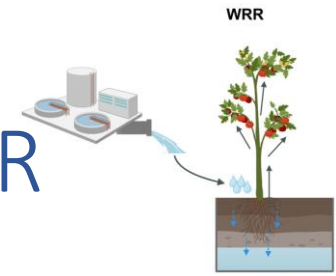
AGRICULTURAL IRRIGATION

<https://www.linkedin.com/pulse/problems-drip-irrigation-system-senthil-kumar-domtf>



# UWWTD

# WRR



RECLAMATION FACILITY

Aerobic  
biological  
treatment step

URBAN Wastewater Treatment Plant

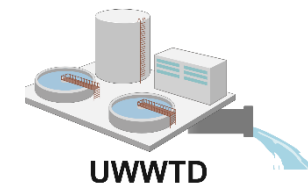
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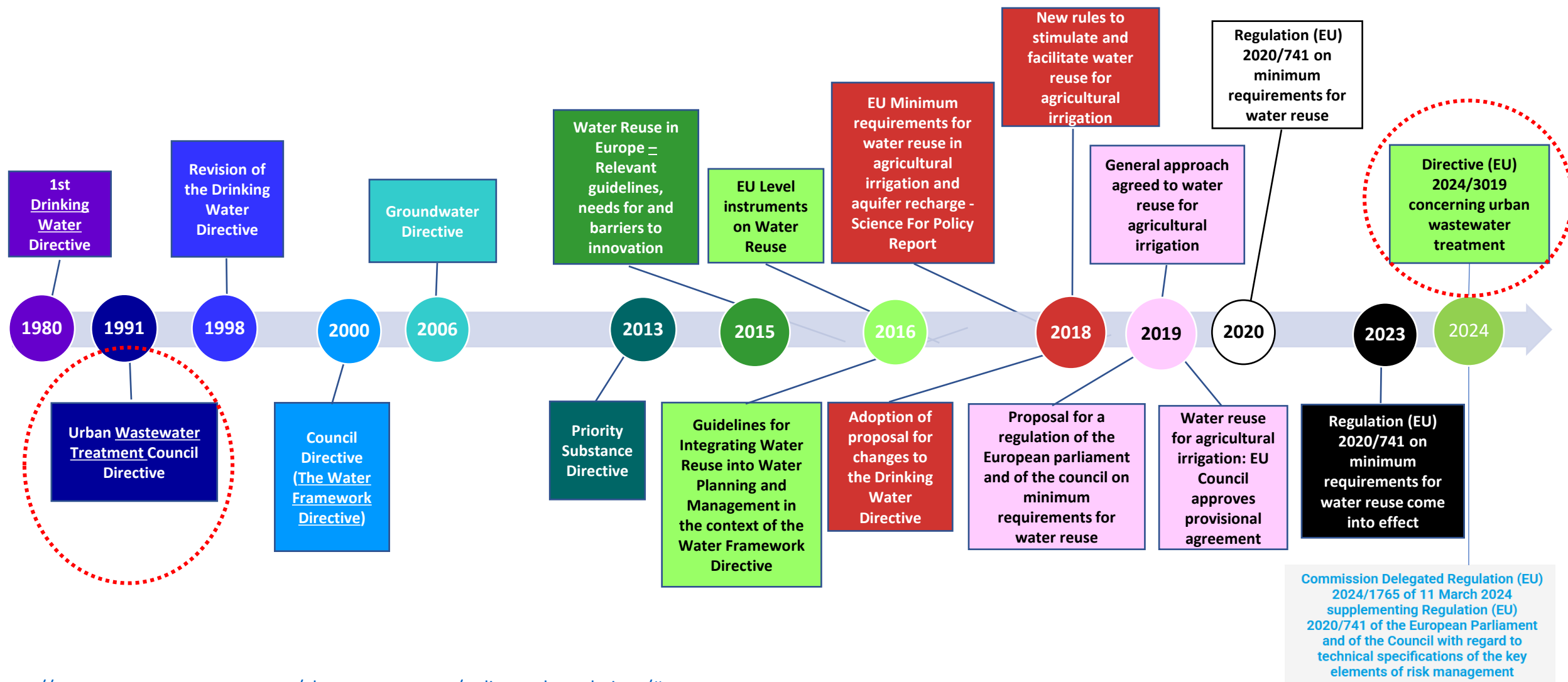


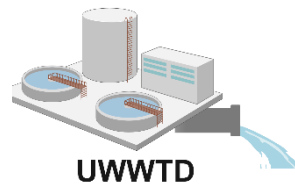


UWWTD

# Legislation: UWWTD

## Timeline of Regulatory Initiatives Relating to Water Reuse at EU Level





2024/3019

12.12.2024

DIRECTIVE (EU) 2024/3019 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 27 November 2024

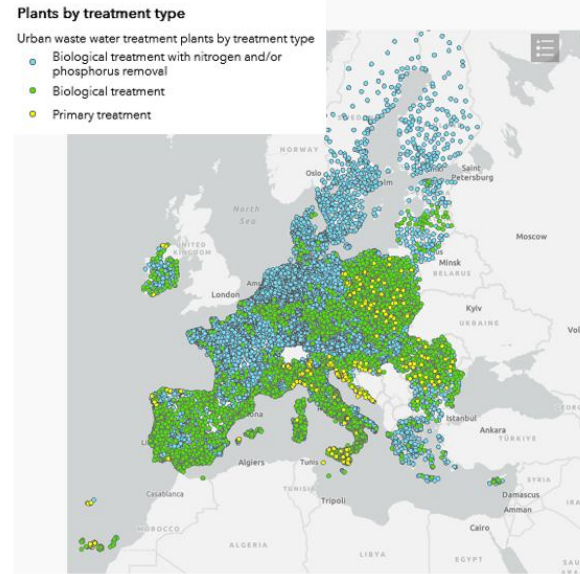
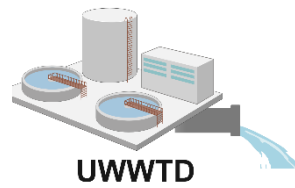
concerning urban wastewater treatment

(recast)

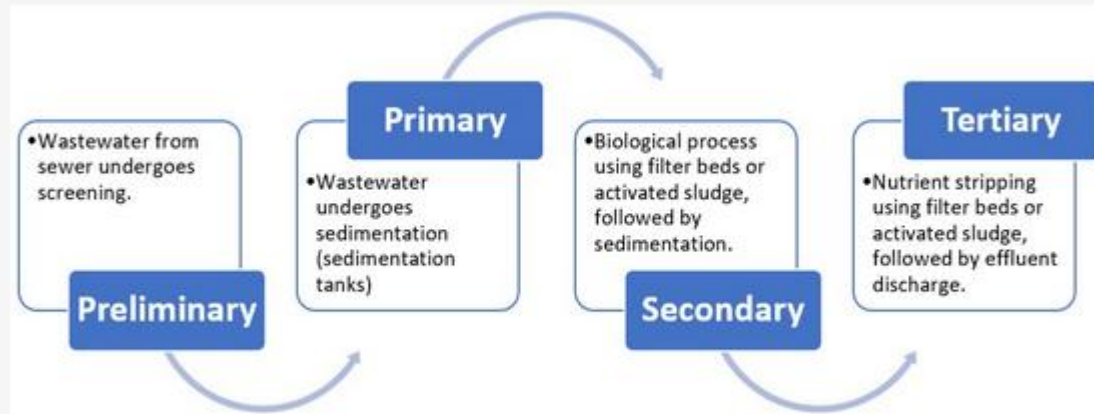


## - Revised Directive /2024

- **Protect** human health and environment (ensure cleaner rivers, lakes, groundwater, coast)
- **Strengthens treatment rules**, ensuring a higher level of protection for the public and the environment
- Applies to **broader number of areas** (1,000 PE)
- More nutrients removed, new std for micropollutants (QT!)
- **Systematic monitoring of MP (including PFAS, microplastic and public health parameters)**
- **Implementation of QT progressively by 2045 for PE>150.000 / PE>10.000** (sensitive areas)
- „**Polluter pays**“ (extended producer responsibility)
- Extreme weather (solution to reduce stormwater deriving pollution, PE> 100.000)
- **Stronger reuse of treated water** (no waste of resources, protect water supply, relieve pressure on supply chain)
- „**Zero pollution**“ ambition, **increased circularity** (e.g. P, further use in agriculture)
- Driving towards **energy and climate neutrality**, improving stormwater in cities (systematically...)



## WWT stages

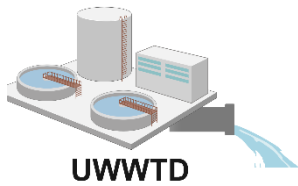


Additional: **Quaternary treatment (QT)** stage  
(CEC/MP removal)

## UWWTD

- Micropollutants (personal care products ingredients, pesticides, pharmaceuticals, MP, PFAS)
- > 80% removal 6/13 CEC (different deadlines according to WWTP PE, WWTPs  $\geq$  150,000 PE)
- Quaternary treatment stage (<80%)
- PFAS: Need for the harmonized methodologies for measuring 'PFAS Total' and 'Sum of PFAS' MP: removal



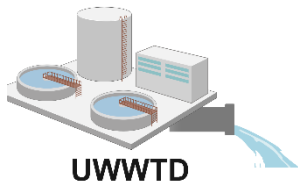


## UWWTD: 13 Micropollutants (MP)

1	Amisulprid	AMS	antipsychotic
2	Carbamazepine	CBZ	for epilepsy
3	Citalopram	CTL	antidepressant
4	Clarithromycin	CLR	antibacterial
5	Diclofenac	DCF	anti-inflammatory
6	Hydrochloro-thiazide	HDC	for hypertension
7	Metoprolol tartrate	MTP	for hypertension
8	Venlafaxine	VEN	antidepressant
9	Candesartan	CND	for hypertension
10	Irbesartan	IRB	for hypertension
11	Benzotriazole	BTR	corrosion inhibitor
12	4-Methyl-1H-benzotriazole	4MBT	corrosion inhibitor
13	5-Methyl-1H-benzotriazole	5MBT	corrosion inhibitor

### UWWTD

- Micropollutants (personal care products ingredients, pesticides, pharmaceuticals, MP, PFAS)
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## UWWTD: 13 MP

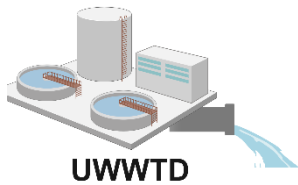
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CAT.1  
Pha:  
„very easy to treat“

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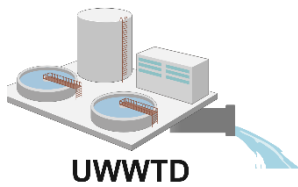


CAT.1  
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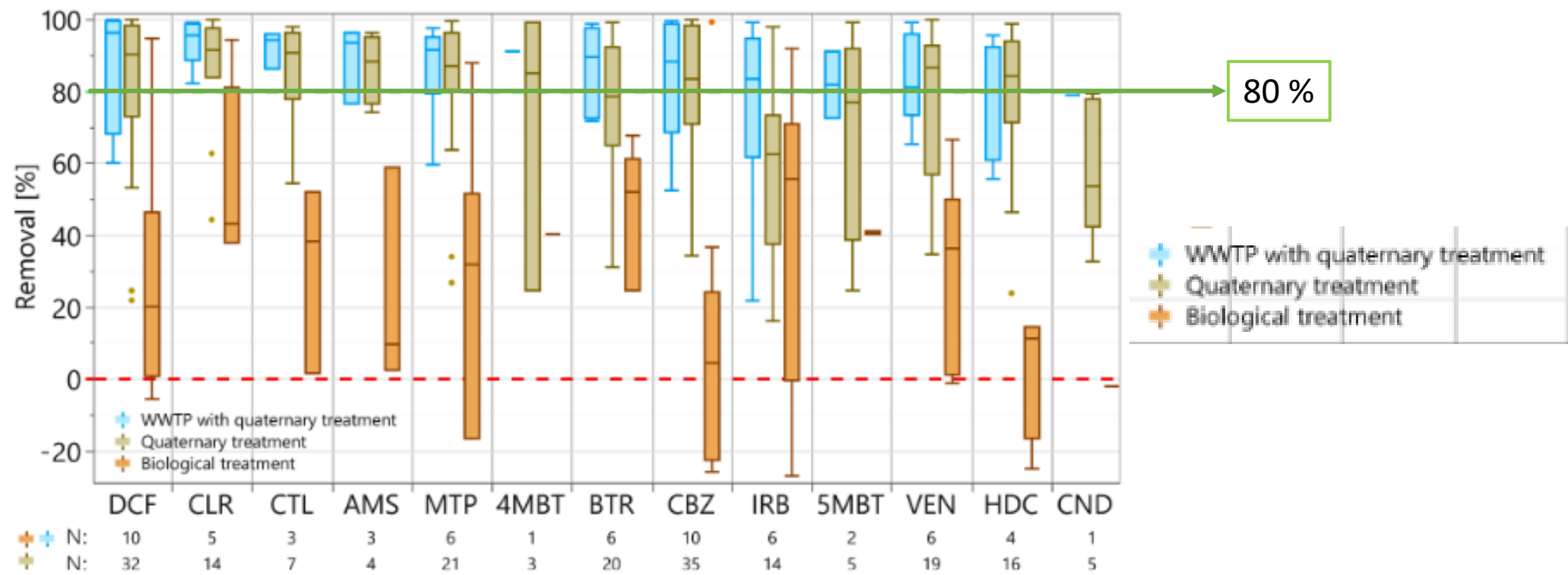
CAT.2  
2 Pha  
3 Ind. chem.  
„easily disposable“

### UWWTD

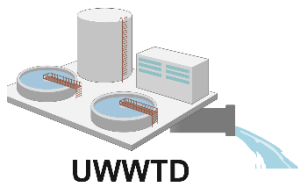
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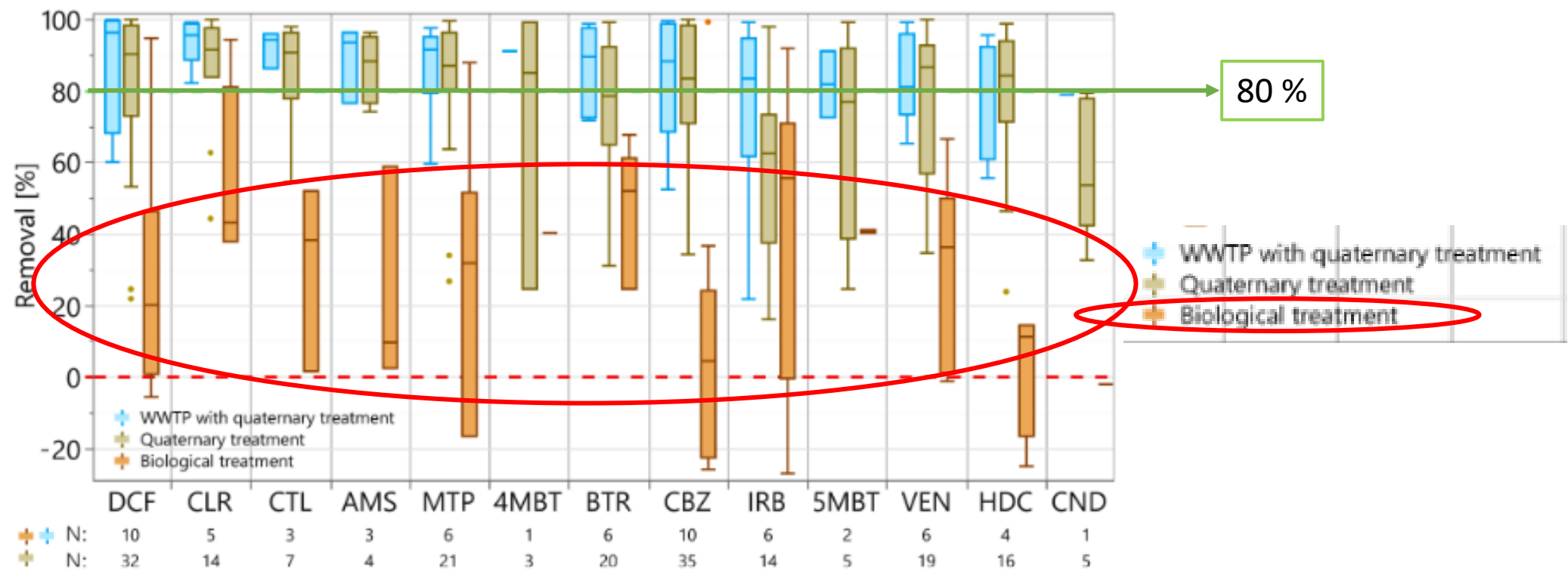
## Removal (%) at different stages of the WWTP: BT+QT



lanes et al., 2025 <https://doi.org/10.1016/j.wroa.2025.100334>



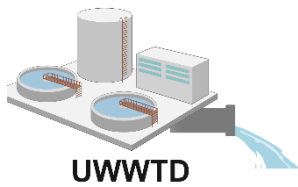
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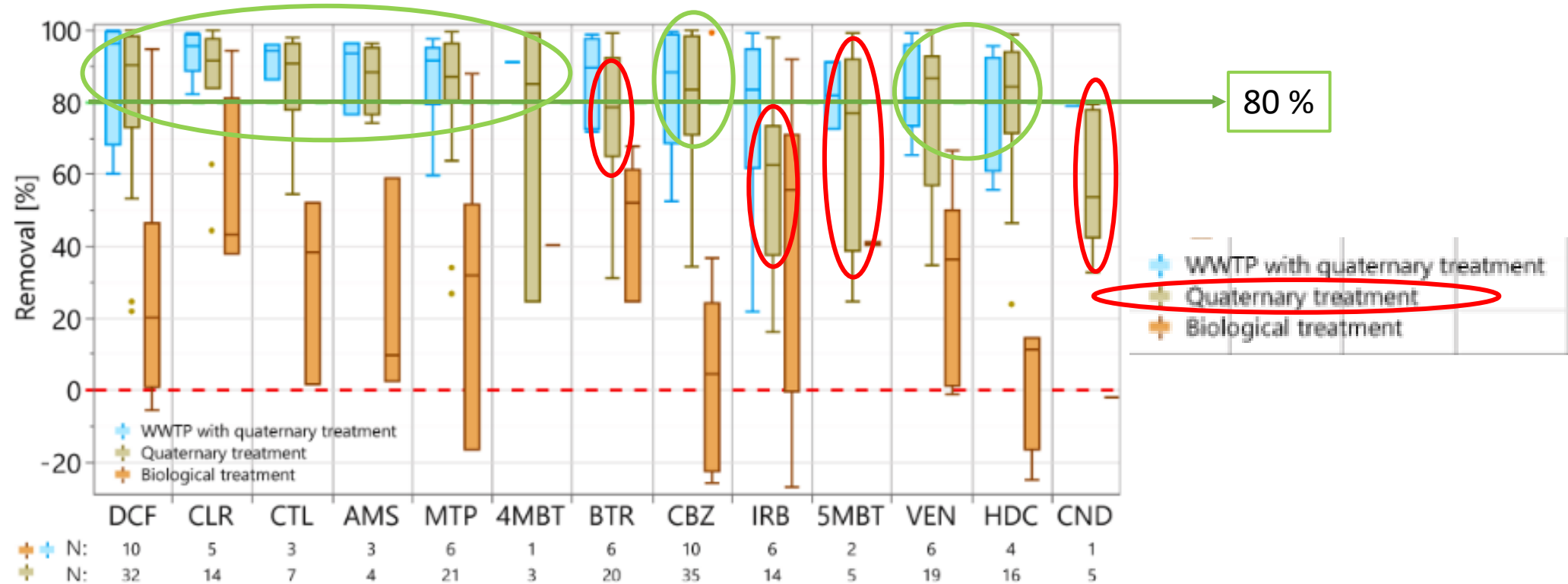
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BT: none of the target micropollutants lowers to the target outlet concentrations →→→ QT!!!



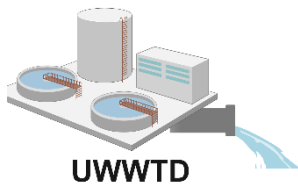


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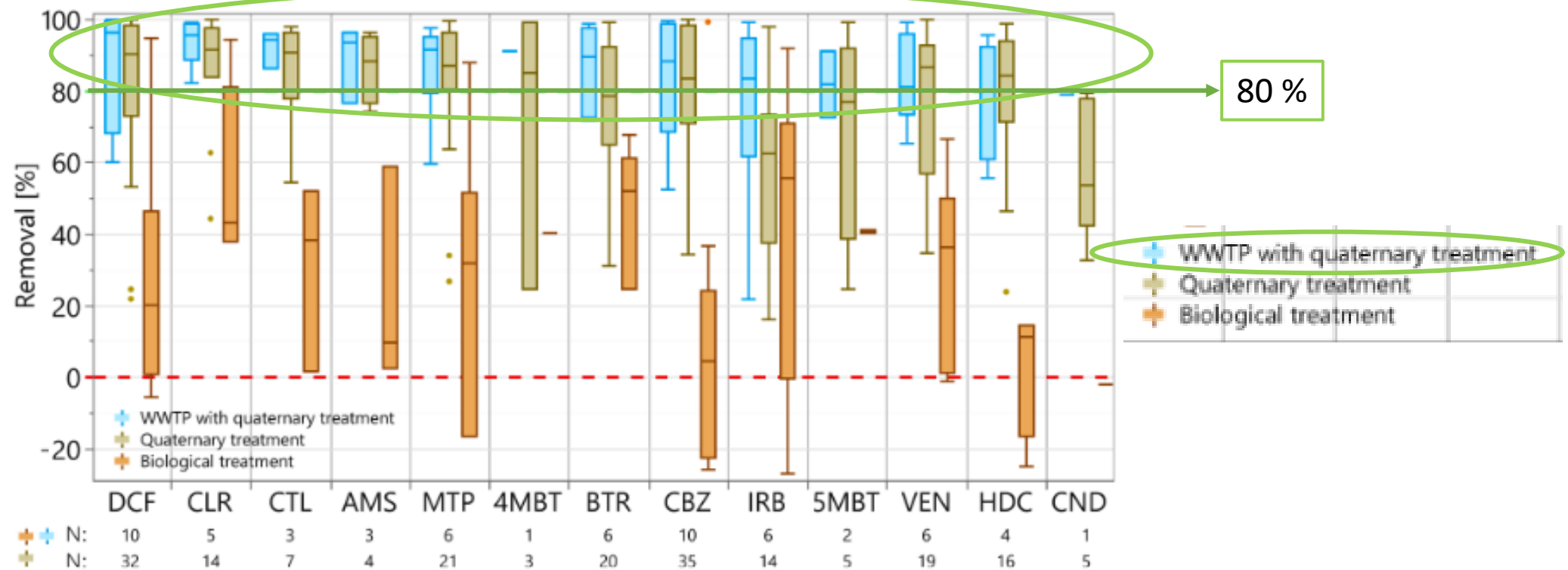


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QT: most yes (except: BTR and 5MBT, IRB, CND)



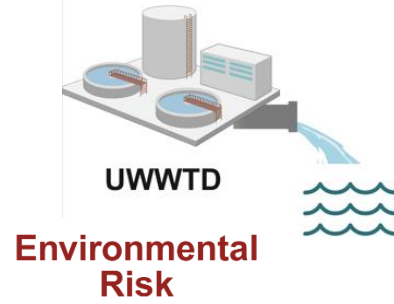
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QT: most yes (except: BTR, IRB, 5MBT and CND)  
BT+QT: yes!

# Literature review: ERA



## UWWTD:

- Limits on removal efficiency
- Safeguarding the environment → meeting removal eff insufficient
- ERA identifies env. threats: RQ

$$RQ = \frac{C_{out\_WWTP}}{Toxic\ Level}$$

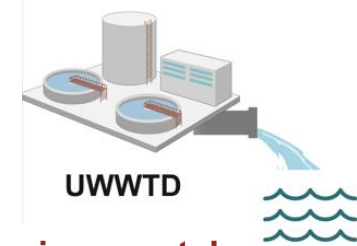
$$Required\ removal = \frac{C_{inWWTP} - Toxic\ Level}{C_{inWWTP}}$$

Toxic level....Norman network (2024) for each indicator substance

### QUESTION:

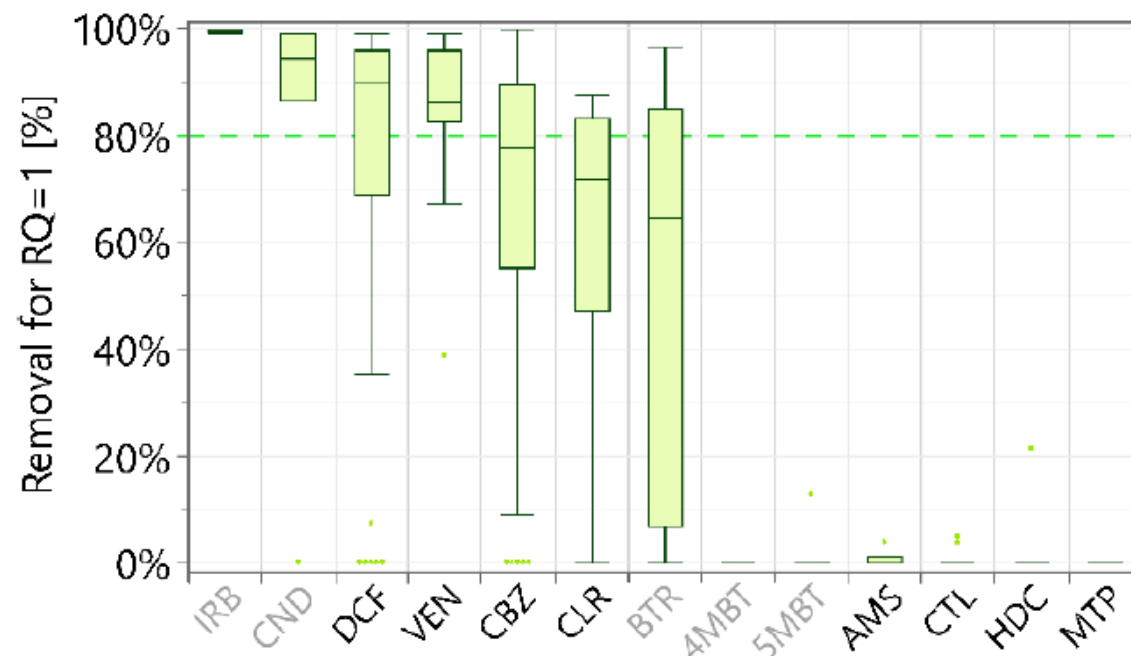
- 80% removal &  $RQ \leq 1$
- ? Removal &  $RQ \leq 1$

# Literature review: ERA



UWWTD  
Environmental Risk

- Required WWTP removal to obtain RQ=1 in the effluent (river dilution neglected)



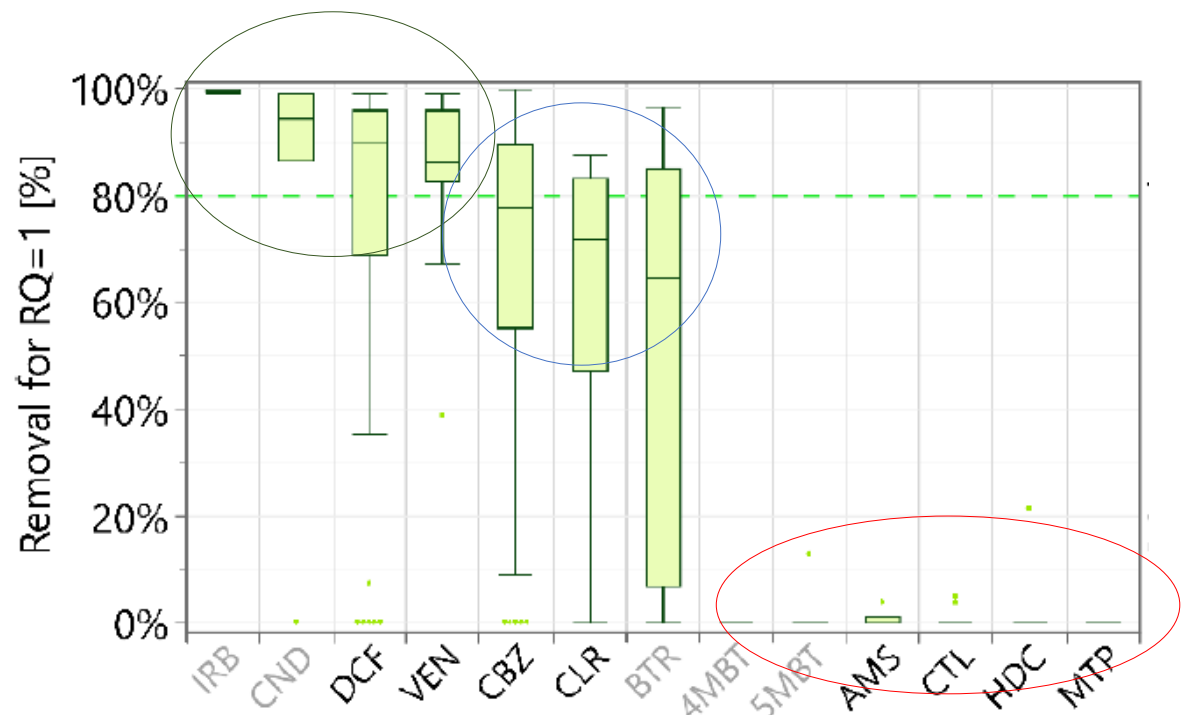
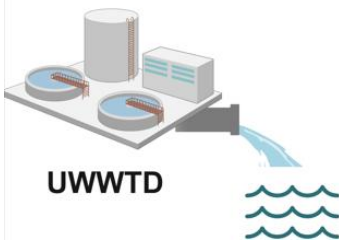
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$$RQ = \frac{C_{out\_WWTP}}{Toxic\ Level}$$

$$Required\ removal = \frac{C_{inWWTP} - Toxic\ Level}{C_{inWWTP}}$$

# Literature review: ERA

Environmental  
Risk



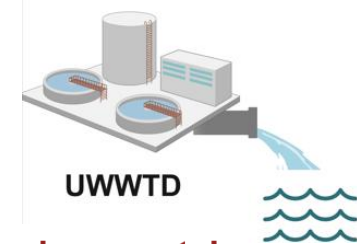
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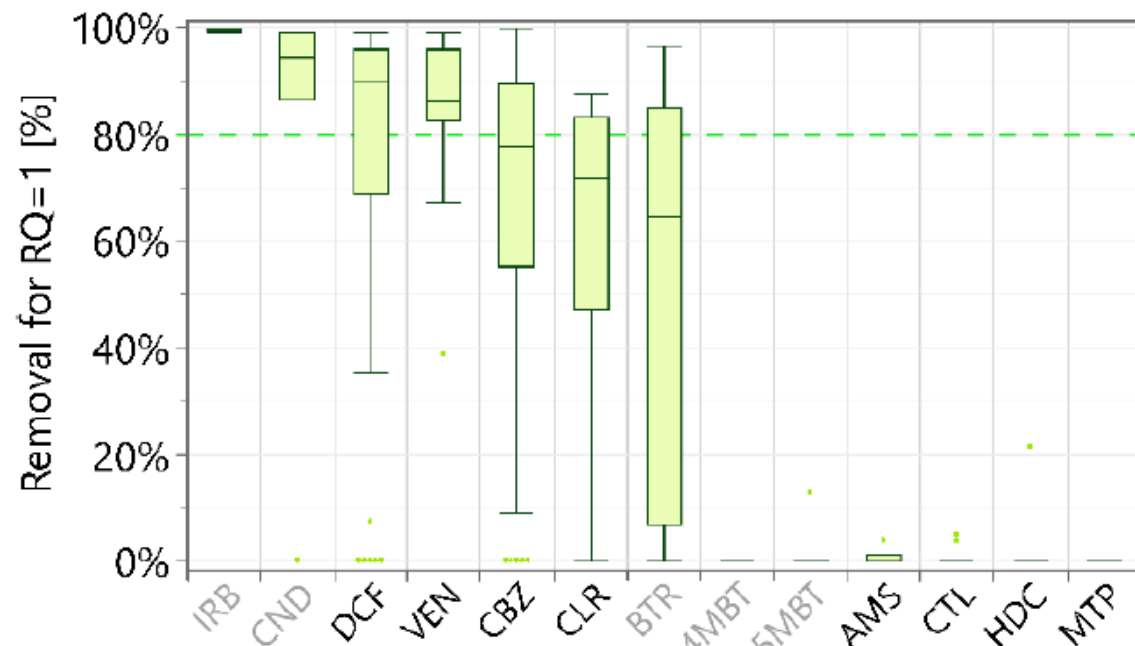
- Required WWTP removal to obtain RQ=1 in the effluent:
- 4,5-MBT, AMS, CTL, HDC, and MTP: low WWTP inlet concentrations with respect to their toxic level, P do not require specific treatments to RQ=1 (removal < 5%)
- CBZ, CLaR & BTR: < 80% removal OK for RQ=1
- IRB, CND, DCF & VEN: high WWTPs inlet conc. with respect to their toxic level: >80% removal needed for RQ<1



# Literature review: ERA



UWWTD  
Environmental Risk



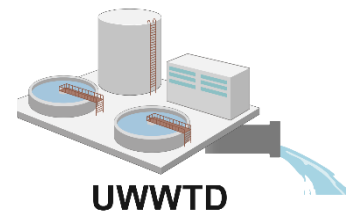
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Target pollutant = process indicators

ERA: identify CEC with 80% removal still environmental threats!

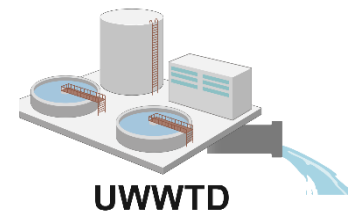
Typical dilution factor in ERA: 10

- Required WWTP removal to obtain RQ=1 in the effluent:
- 4,5-MBT, AMS, CTL, HDC, and MT: low WWTP inlet concentrations with respect to their toxic level, P do not require specific treatments to RQ=1 (removal < 5%)
- CBZ, CLR & BTR: < 80% removal OK for RQ=1
- IRB, CND, DCF & VEN: high WWTPs inlet conc. with respect to their toxic level: >80% removal needed



## UWWT

- Biological treatments (CAS, MBR) and combinations with membrane filtration methods (nanofiltration and reverse osmosis), ozonation, AOP and adsorption processes:  
**sufficient to very high removals of MP!**
- These combinations + widely used disinfection technologies (including oxidizing and physical agents) + emerging disinfection processes (peracetic and performic acid):  
**limitations!**
  - some „treatment trains“ are successful in removing parent compounds
  - generate transformation products (potentially more harmful than their parent compounds), toxicity, mutagenicity and endocrine disruption effects
  - selecting potentially pathogenic bacteria (repair and/or regrowth)
  - altering the microbial community structures of wastewater influent and of TWW
  - cost/feasibility



## UWWT

- Biological treatments (CAS, MBR) and combinations with membrane filtration methods (nanofiltration and reverse osmosis), ozonation, AOP and adsorption processes:  
**sufficient to very high removals of MP!**
- These combinations + widely used disinfection technologies (including oxidizing and physical agents) + emerging disinfection processes (peracetic and performic acid):  
**limitations!**

- some 1) FOCUSING ON 80% REMOVAL (UWWTD) → not effective env. protection
- gene 2) RQ>1: residual risk → case specific evaluation (WWTP characteristics, env. cond.)
- com 3) Mixture toxicity, case-specific risks, cumulative effects (parent comp + TP, bioassays?)
- sele
- alter
- cost
- „treatment trains“

# Legislation: UWWTD vs. WRR

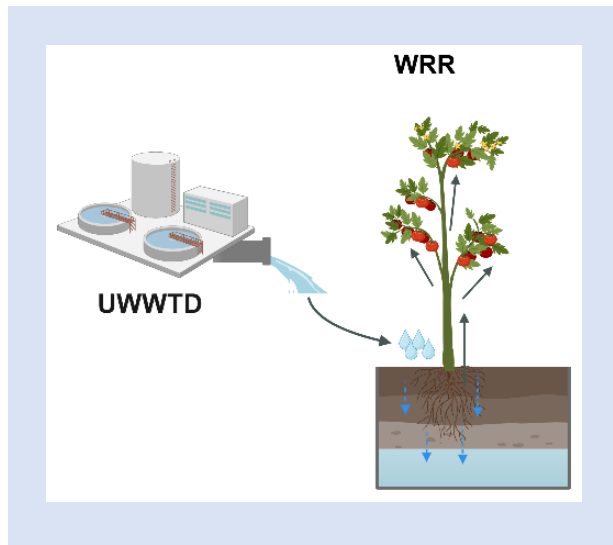
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## UWWTD:

- Defined MP (process indicator)
- QT: Effluent quality ↑
- Surface water quality ↑
- ERA ↓

## WRR:

- Min. requirements + Site-specific MP/CEC risk assessment (Member State)
- QT ↑ safety of use of TWW in agriculture
- HRA ↓



# Legislation: UWWTD vs. WRR

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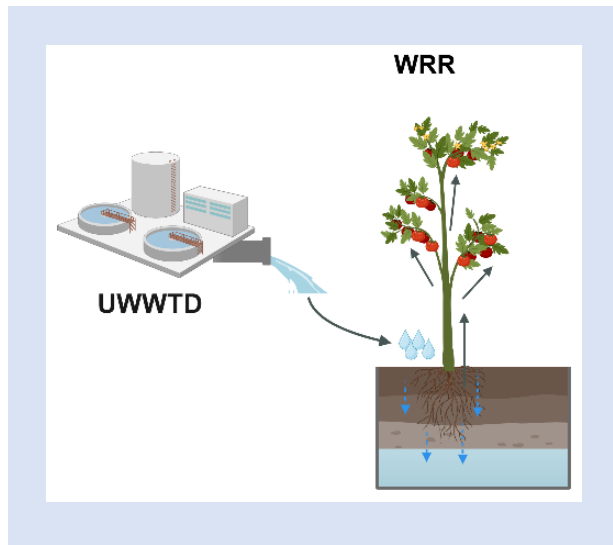
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- Sufficient action?
- Upgrading with time! (WFD principle)



# Legislation: UWWTD vs. WRR

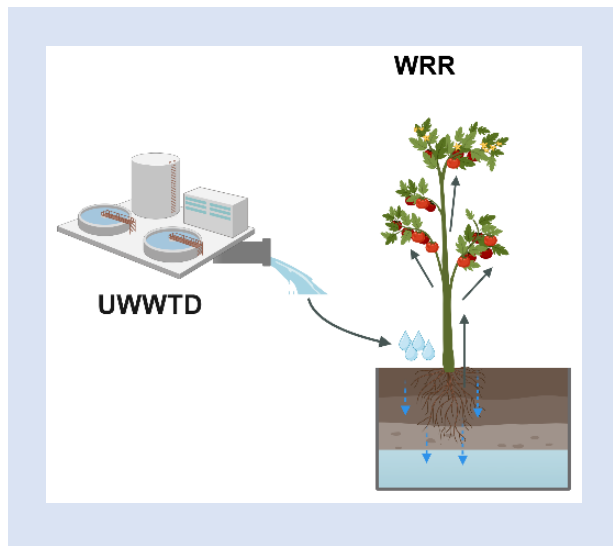
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## WRR:

- Site-specific MP/CEC risk assessment (Member State)
- QT ↑ safety of use of TWW in agriculture
- HRA ↓

- Sufficient action?
- Upgrading with time! (WFD principle)



## BARRIERS for UWWTD&WRR implementation:

1. Critical EU based data overview

UWWT: “real scale”

WRR: „living lab”/“real” scale

2. Fragmented data evaluation (Data QC, addressing missing data)

4. Prioritisation of CEC

5. Cost and time of implementation including analysis

(chemical and RA – national capacities and financial constrains)



# Our contribution...



Growth Exp. 2022

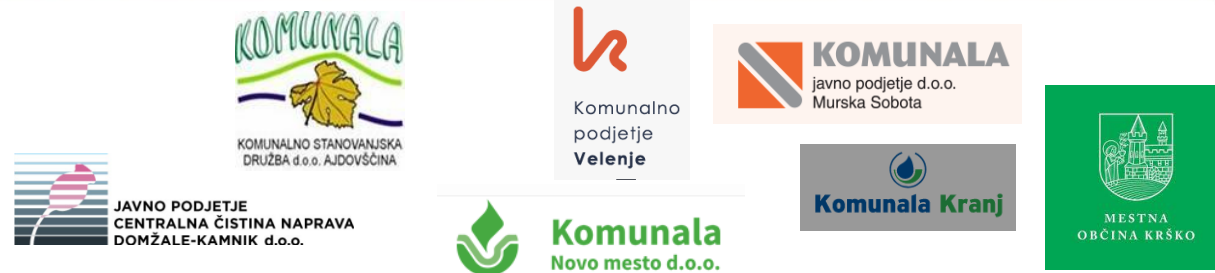
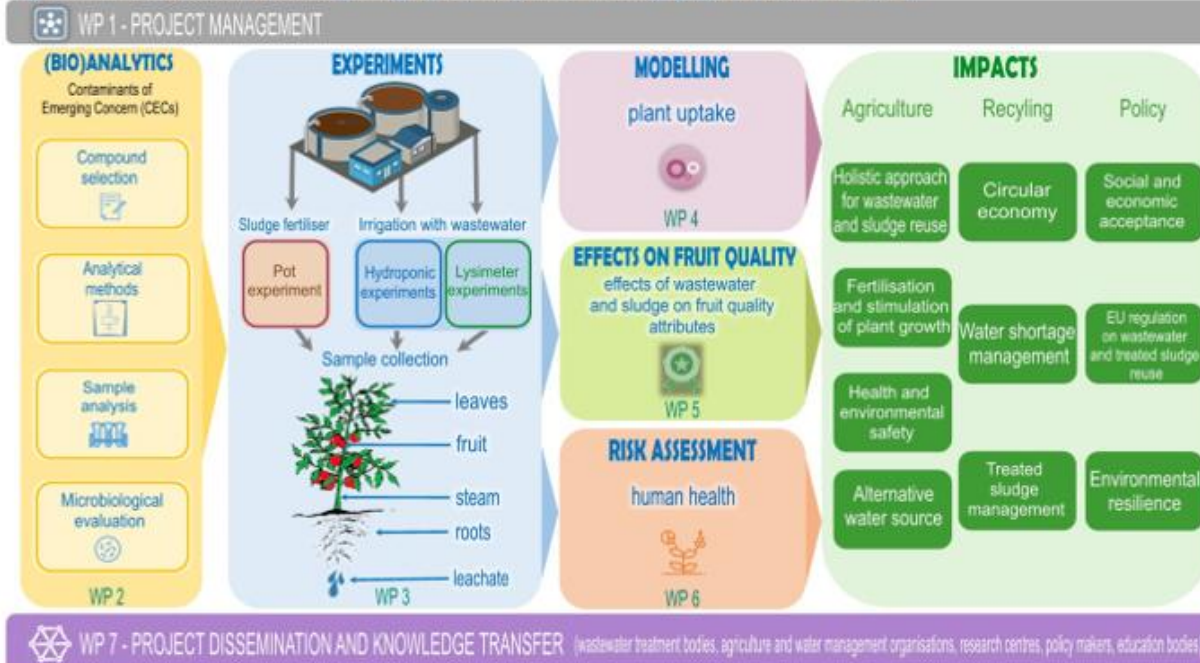
27 CECs were  
selected for studying  
TWW and TS reuse  
for tomato cultivation

Aim: Investigate  
uptake and  
translocation of CECs



# UPTAKE

Agricultural reuse of wastewater and sewage sludge: uptake and distribution of emerging organic contaminants in plants

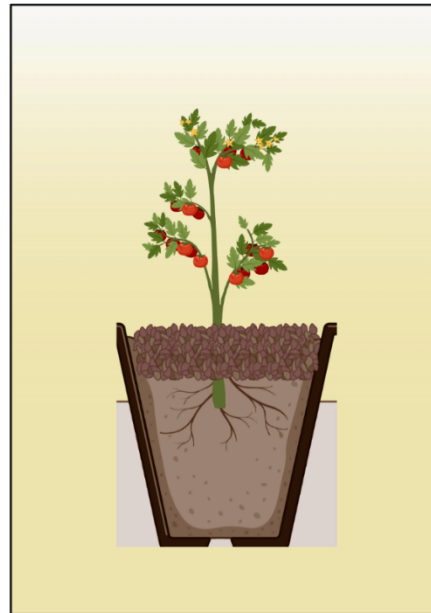


# Growth Experiment



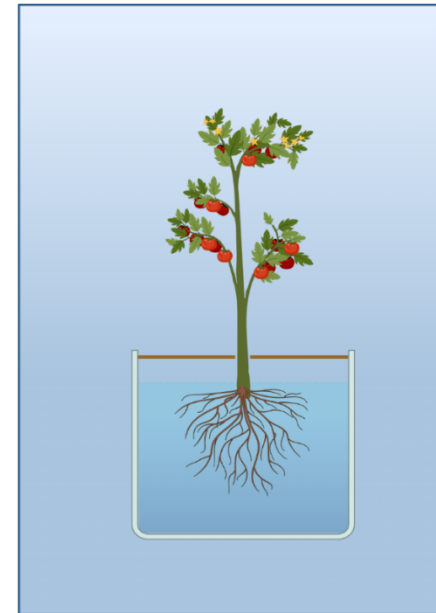
## ① Pot experiments

Tomatoes grown in treated sludge-amended substrate



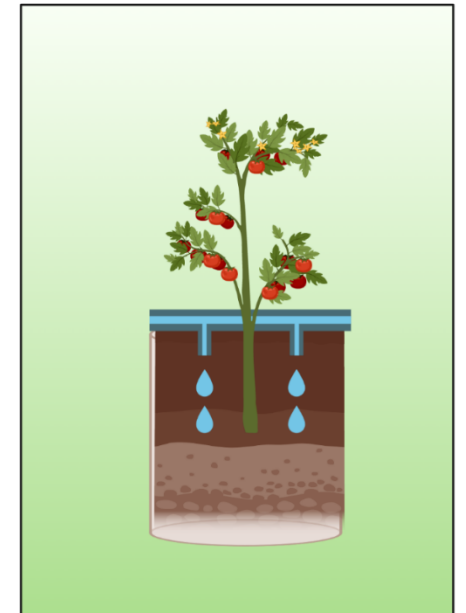
## ② Hydroponics

Tomatoes grown in potable water and wastewater without soil presence



## ③ Lysimeters

Tomatoes grown in soil, irrigated with potable water and wastewater

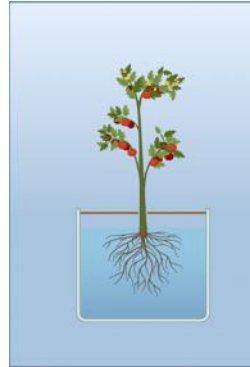


Unique experiment: similar conditions, different growing media



# Hydroponics Experiment

② Hydroponics  
Tomatoes grown in potable water  
and wastewater without soil  
presence



## HYDROPONICS

1. Potable Water (PW)
2. Potable Water with addition of CECs - 0.1 mg/L (PW+CEC Low)
3. Potable Water with addition of CECs - 1 mg/L (PW+CEC)
4. Treated Wastewater (TWW)
5. TWW with addition of CECs - 1 mg/L (TWW+CEC)



Greenhouse, UL BF

# Field Experiments



③ Lysimeters  
Tomatoes grown in soil, irrigated with  
potable water and wastewater



## LYSIMETERS

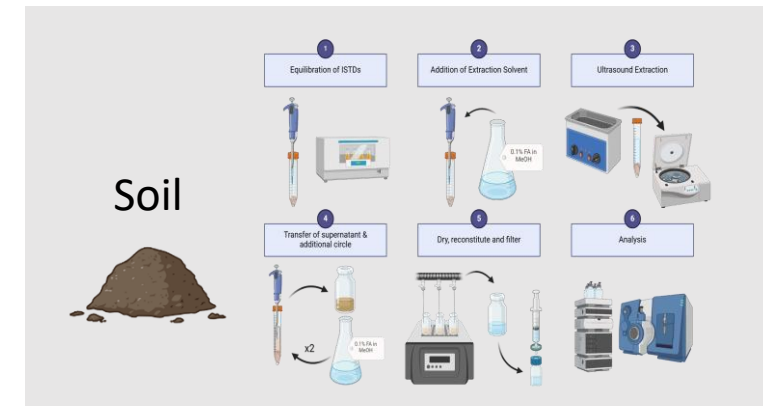
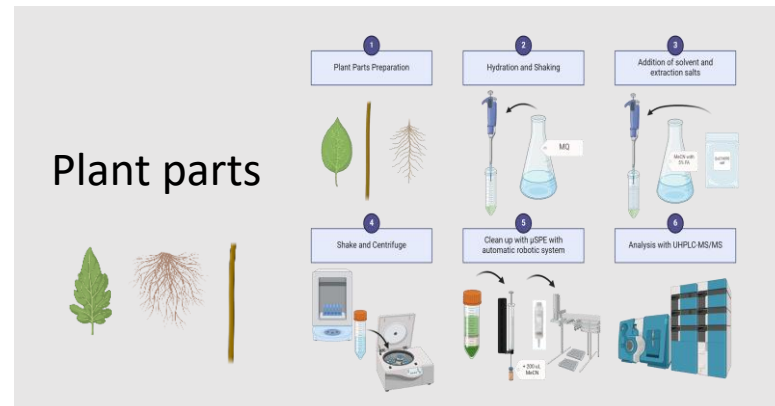
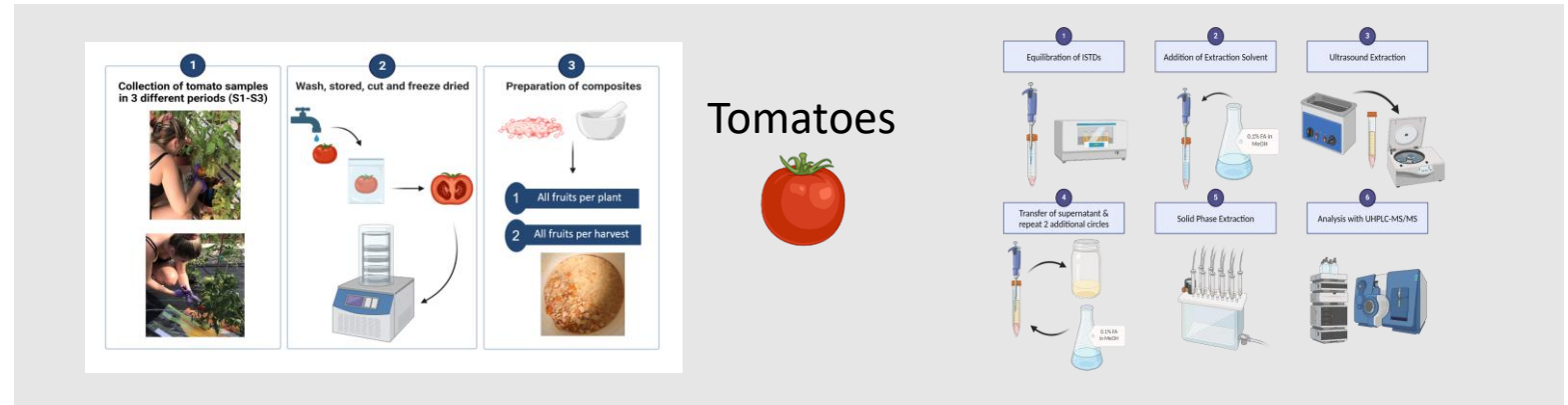
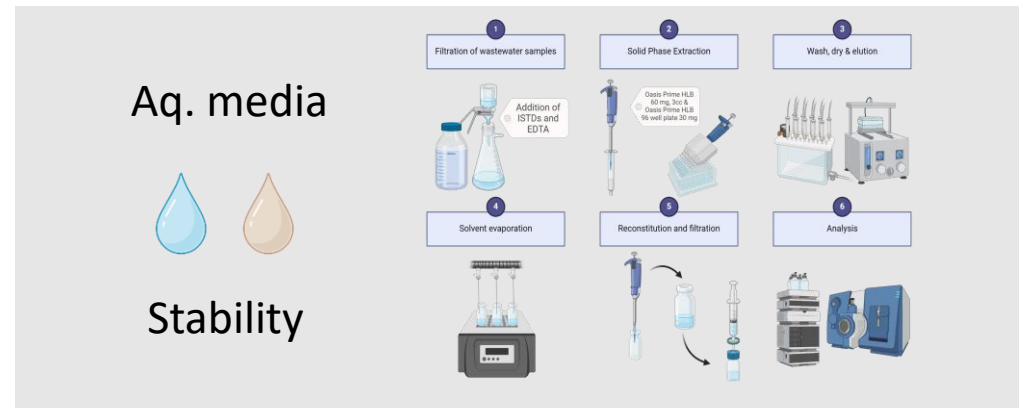
1. Potable Water (PW)
2. Potable Water spiked with CEC (1 mg/L, PW+CEC)
3. Treated Wastewater (TWW)
4. Treated Wastewater spiked with CEC (1 mg/L, TWW+CEC)



Demo site Ajdovščina (UL ZF & BF)



# Sample Preparation & Analysis



**FoodTraNet**

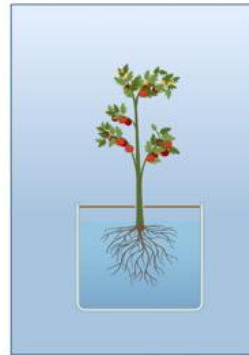
**Marie-Curie European Training Network**  
Food Quality, Safety, and Security

# Conclusions: UPTAKE and TRANSLOCATION

## CEC UPTAKE in fruit

	Hydroponics	Lysimeters
PW	3	-
PW+CEC	10	3
TWW	3	1
TWW+CEC	12	6

## TRANSLOCATION (plant parts)



**TWW: 5 CEC**  
BPA, Diclofenac,  
Ibuprofen, Triclocarban,  
Triclosan (37-266 ng/g)



**TWW:**  
CEC < LOQ

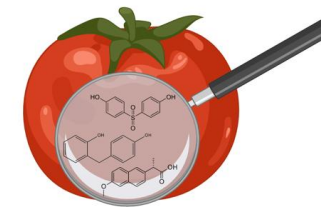
(soil) > roots > leaves > stems > fruits (spiked samples)

**TA: 27 CECs**

**SS/NTA under evaluation**

# HRA: Health Risk Assessment

## Human Health Risk



Compounds	µg/kg bw/day
17α-ethynylestradiol	0.001
Acetamiprid	5
Azithromycin	1.7
BPA	0.0002
BPF	0.0002
BPS	0.0002
Caffeine	5700
Carbamazepine	0.16
Ciprofloxacin	0.15
Clarithromycin	1.4
Dimethomorph	50
Erythromycin	5
Ibuprofen	0.29
Naproxen	1.43
Progesterone	30
Propylparaben	10000
Testosterone	2
Triclosan	8000
Triclocarban	25000

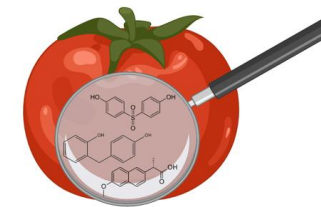
Population Group (L2)	Body weight (kg)	Exposure hierarchy (L5)	Number of subjects	Number of consumers	Mean (kg/day)	Standard Deviation	97.5th percentile (kg/day)
Adults	70	Tomatoes	385	189	0.0297	51.73	0.17742
Adolescents	53	Tomatoes	484	198	0.0158	34.28	0.11646
Toddlers	12	Tomatoes	343	156	0.0079	16.45	0.05429

$$\text{Dietary exposure (mg/kg bw/day)} = \frac{\sum \text{Concentration of chemical in food} \left( \frac{\text{mg}}{\text{kg}} \right) \times \text{Food consumption (kg/day)}}{\text{Body weight (kg)}}$$

- Dietary exposure: Combining data on CECs concentration in composite samples with the corresponding consumption levels from the EFSA Database
- Dietary exposure in fresh tomatoes was assessed deterministically
- EDI compared to **ADI**, **TDI** or **NOAEL**

# HRA: Hydroponics vs. Lysimeters

Human Health  
Risk



## HYDROPONICS

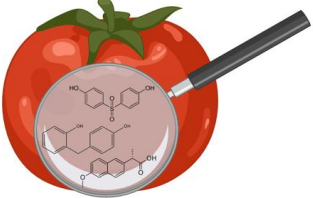
Treatment	Compounds Quantified	Concentration (ng/g dw)	Human Health Risk Assessment
PW	Azithromycin, BPS, Clarithromycin	27.7-274	BPS
PW+CEC Low	Acetamiprid, Azithromycin, BPS, Carbamazepine, Clarithromycin, Dimethomorph	29.5-220	BPS
PW+CEC	Acetamiprid, Azithromycin, BPF, BPS, Caffeine, Carbamazepine, Ciprofloxacin, Clarithromycin, Dimethomorph, Erythromycin	42.4-778	BPF
TWW	3 CEC: Azithromycin, BPS, Clarithromycin	16.7-50.5	BPS
TWW+CEC	Acetamiprid, Azithromycin, BPF, BPS, Caffeine, Carbamazepine, Ciprofloxacin, Clarithromycin, Dimethomorph, Erythromycin, Naproxen, Propylparaben	25.3-1117	BPF, BPS

## LYSIMETERS

Treatment	Compounds Quantified	Concentration (ng/g dw)	Human Health Risk Assessment
PW	-	-	-
PW+CEC	Acetamiprid, Carbamazepine, Dimethomorph	30.3-1034	-
TWW	1 CEC: Carbamazepine	14.4-22.0	-
TWW+CEC	Acetamiprid, Azithromycin, BPS, Carbamazepine, Clarithromycin, Dimethomorph	35.4-734.1	BPS

# HRA: Hydroponics vs. Lysimeters

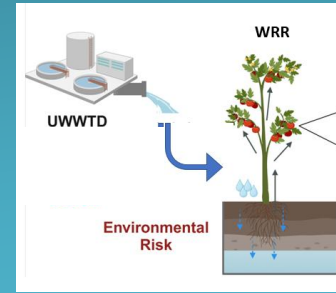
Human Health  
Risk



HYDROPONICS	Treatment	Compounds Quantified		Concentration (ng/g dw)	Human Health Risk Assessment
	PW	Azithromycin, BPS, Clarithromycin		27.7-274	BPS
	PW+CEC Low	Acetamiprid, Azithromycin, BPS, Carbamazepine, Clarithromycin, Dimethomorph		29.5-220	BPS
	PW+CEC	Acetamiprid, Azithromycin, BPF, BPS, Caffeine,			BPF
	TWW	<div> Realistic conditions (TWW): Low risk  Presence of non-targeted compounds (to be included...)  Synergistic/Additive effects of mixtures </div>			BPS
	TWW+CEC				BPF, BPS
LYSIMETERS	Treatment	<div> Realistic conditions (TWW): Low risk  Presence of non-targeted compounds (to be included...)  Synergistic/Additive effects of mixtures </div>		Risk Assessment	
	PW			<div> Introduction of QT! </div>	
	PW+CEC				
	TWW				
	TWW+CEC	Carbamazepine	14.4-22.0	-	
		Acetamiprid, Azithromycin, BPS, Carbamazepine, Clarithromycin, Dimethomorph	35.4-734.1	BPS	



# ERA: Environmental Risk Assessment - Soil



- PNEC values for soil: derived from aquatic toxicity data using EPM
- PNEC values for freshwater organisms ( $\mu\text{g/L}$ ) from Norman Ecotoxicology Database (lowest)
- PNECsoil ( $\mu\text{g/kg}$ ):

$$\begin{aligned} PNEC_{soil} \\ &= (0.1176 + 0.01764 \times K_{oc}) \\ &\times PNEC_{water} \end{aligned}$$

*CEC log K<sub>ow</sub>:3-6*

*Acceptable as conservative approximation*

$$RQ = \frac{MEC_{soil} \left( \frac{\mu\text{g}}{\text{kg}} dw \right)}{PNEC_{soil} \left( \frac{\mu\text{g}}{\text{kg}} dw \right)}$$

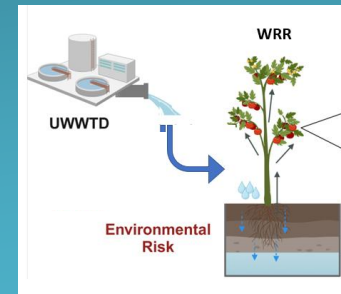
RQ:

0.01 - 0.1: low risk

0.1 - 1 medium risk

>1 high environmental risk

# ERA: Soil grown tomatoes (RQ)



Treatment	PW		TWW		PW+CEC		TWW+CEC	
Month	3	30	3	30	3	30	3	30
17 $\alpha$ -ethynylestradiol	-	-	-	-	<u>2794379</u>	<u>2237436</u>	<u>1840044</u>	<u>1754577</u>
Azithromycin	-	-	<u>1.4</u>	<u>1.2</u>	<u>27.8</u>	<u>56.8</u>	<u>88.6</u>	<u>85.8</u>
BPA	-	-	-	-	<u>3.6</u>	<u>4.3</u>	<u>5.1</u>	<u>7.9</u>
BPAF	0.4	0.05	0.3	0.05	1.9	2.6	1.9	1.5
BPF	-	-	-	-	0.1	0.1	0.1	0.1
BPS	-	-	-	-	1.3	0.03	0.2	0.03
Carbamazepine	-	-	-	-	54.6	22.3	42.6	21.5
Clarithromycin	-	-	0.2	0.2	4.8	9.5	13.9	13.2
Dimethomorph	-	-	-	-	94.7	50.4	78.0	35.6
Erythromycin	-	-	-	-	0.03	0.1	0.1	0.3
Estrone	-	-	-	-	2.0	1.0	1.6	0.9
Naproxen	-	-	-	-	3.3	0.5	0.4	0.4
Tonalide	-	-	-	-	624	425	425	300
Triclocarban	<u>830</u>	<u>205</u>	<u>446</u>	<u>217</u>	<u>77217</u>	<u>41500</u>	<u>100615</u>	<u>44034</u>
Triclosan	-	-	-	-	n.a	n.a	n.a	n.a

RQ > 1: high ecological risk

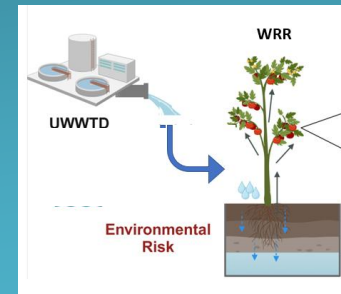
PW+other treatments:

Triclocarban  
(antibacterial agent)

TWW: Azitromycin  
(antibiotics can stimulate  
expression and persistence  
of resistance genes!!  
/affect soil microbiome!)

PW+CEC and TWW+CEC:  
17 $\alpha$ -ethynylestradiol (M3&30,  
potent EDC & low PNEC)  
Azithromycin  
BPA, BPAF

# ERA



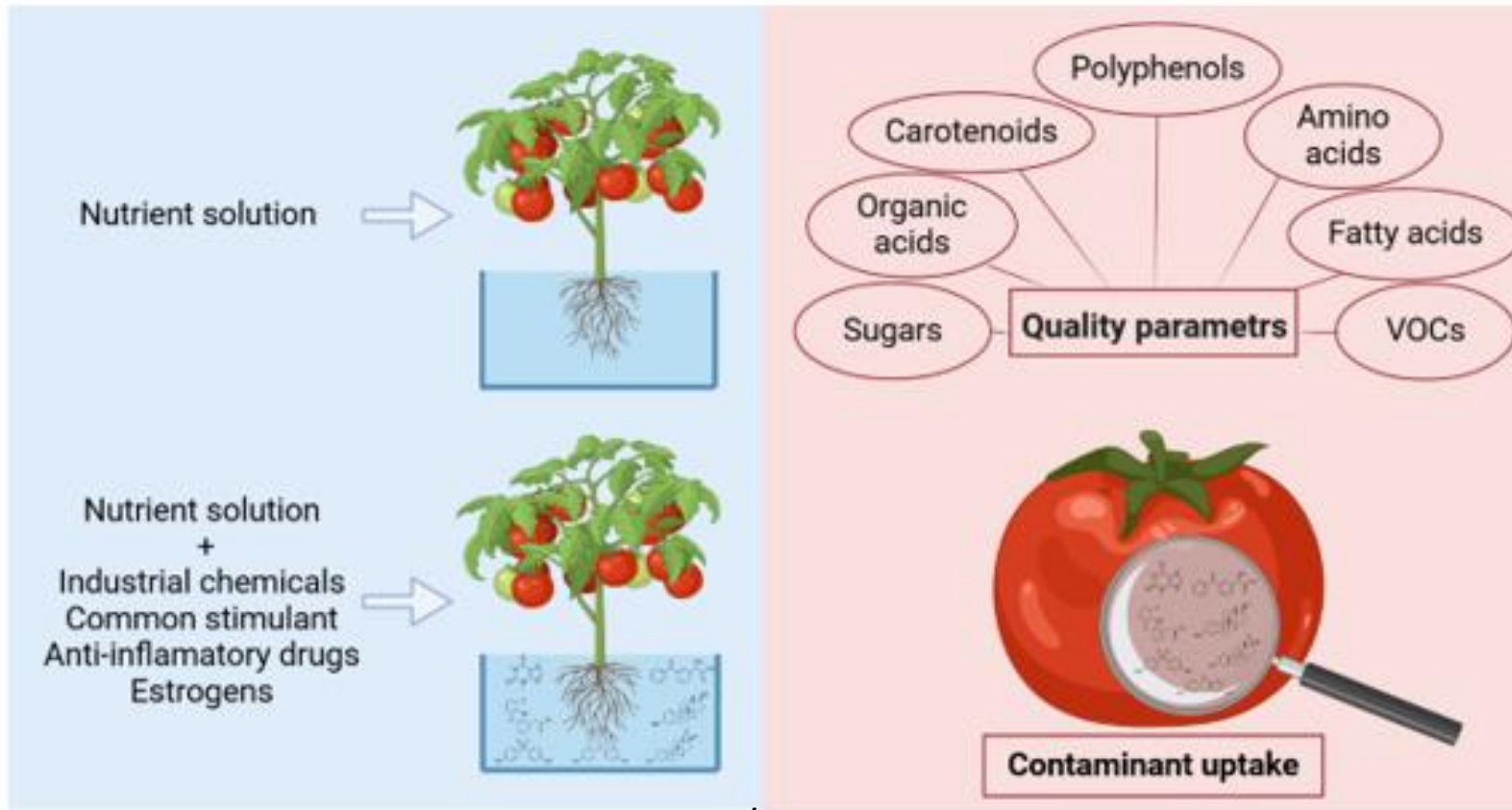
Treatment	PW		TWW		PW+CEC		TWW+CEC	
Month	3	30	3	30	3	30	3	30
17 $\alpha$ -ethynylestradiol	-	-	-	-	<u>2794379</u>	<u>2237436</u>	<u>1840044</u>	<u>1754577</u>
Azithromycin	-	-	<u>1.4</u>	<u>1.2</u>	<p><b>Realistic conditions: TWW reuse (min. risk?)</b>  <b>Diff. Month 3 and 30!</b>  <b>QT!</b>  <b>Accumulative effect with regular reuse practice</b>  <b>Non-targeted compounds to be included</b></p>			
BPA	-	-	-	-				
BPAF	0.4	0.05	0.3	0.05				
BPF	-	-	-	-				
BPS	-	-	-	-				
Carbamazepine	-	-	-	-				
Clarithromycin	-	-	0.2	0.2				
Dimethomorph	-	-	-	-				
Erythromycin	-	-	-	-				
Estrone	-	-	-	-	2.0	1.0	1.6	0.9
Naproxen	-	-	-	-	3.3	0.5	0.4	0.4
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Triclosan	-	-	-	-	n.a	n.a	n.a	n.a

RQ > 1: high ecological risk

PW: other treatments:

17 $\alpha$ -ethynylestradiol (MS&30, potent EDC & low PNEC)  
 Azithromycin  
 BPA, BPAF

# Quality Attributes

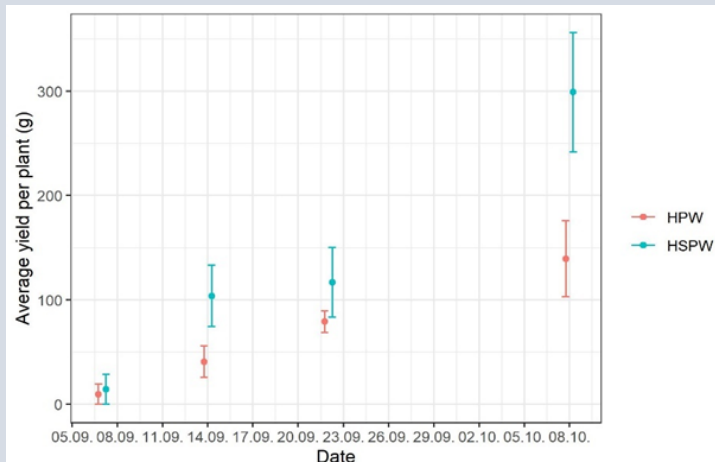
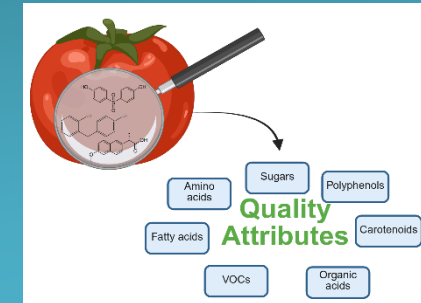


14 CEC at 0.1 mg/L

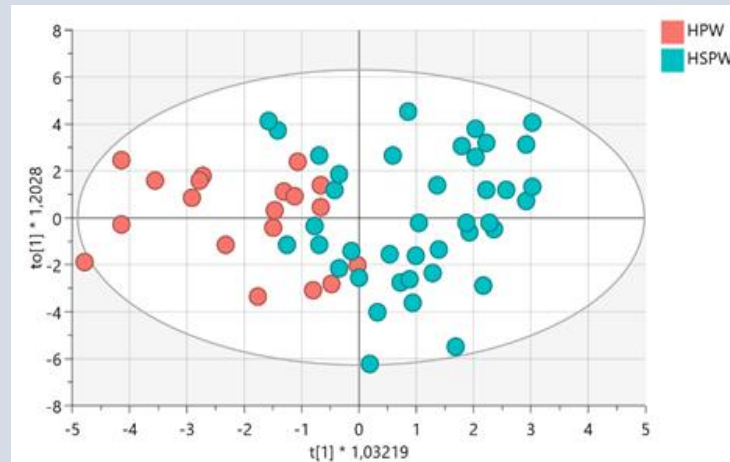
## Selection of parameters influences:

flavor, texture, structure, mouthfeel, the color of tomatoes, and the content of bioactive compounds and specific elements that promote good health

# Quality Attributes (2020)



Tomatoes grown in HSPW: significantly higher yield at 2. and 4. sampling period



28 VOC were significantly different in HPW (14 ↑) and HSPW (15 ↑)

- ↑ yield (115-154%)
- CECs alter the tomato fruit's volatile organic compound (VOC) profile
  - Some altered VOC related to plant stress (CEC?)
  - Some VOC altered not typically linked to stress
  - More complex interaction between CEC and tomato metabolism
- ↓ concentrations of specific amino acids (valine, leucine, aspartic acid and methionine, 7-14%)
- = sugars, organic acids, carotenoids and polyphenols
- = fatty acids



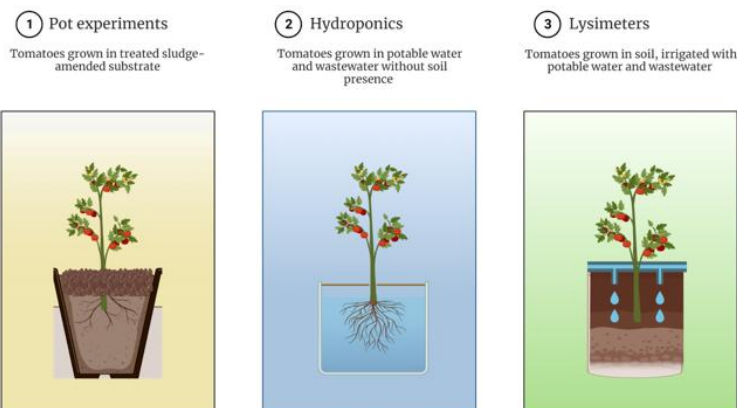
# Evaluating Tomato Quality and Machine Learning



## Does fruit quality depend on growing and irrigation media?

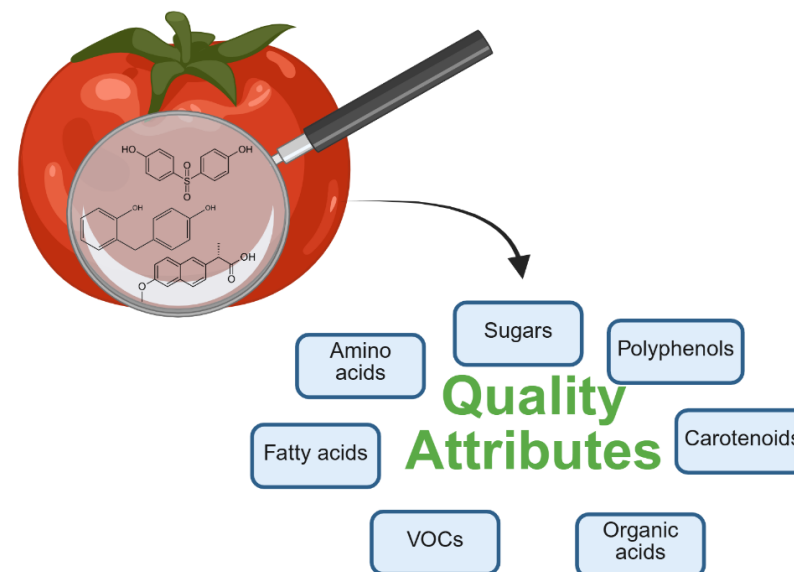
TOMATOES (2020)

LYSIMETERS (soil grown) vs  
HYDROPONICALLY grown tomatoes



TOMATOES (2022)

LYSIMETERS (soil grown) vs  
POTS (sludge) grown tomatoes



Explainable SHAP method: identifying the most important quality parameters related to each treatment

# CEC uptake: Modelling approach

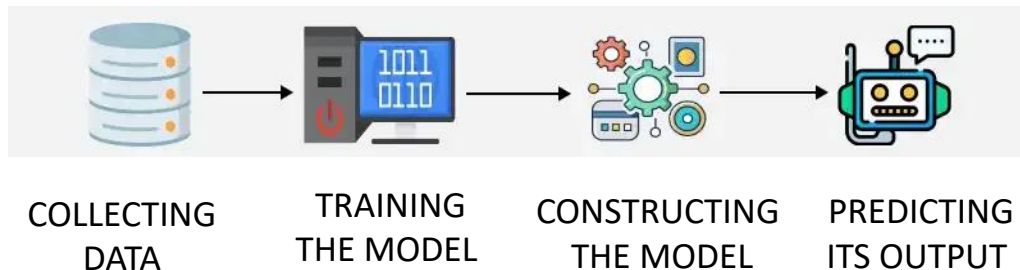


## Aim

- To build a model for CEC uptake in tomato plant depending on CEC phy-chem parameters
- Predict uptake (HRA) for CEC, for which we have no or little experimental data (CEC prioritisation)

## Modelling approaches

- Teoretical phy-chem model (multi-compartment model, extensive expert knowledge on involved processes, detailed measurements, demanding tuning of data to model)
- Experimental data-driven model (substantial exp. data, simple functional dependency, advanced statistics, ML)



# CEC uptake: Modelling approach



## Aim

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- Teoretical phy-chem model (multi-compartment model, extensive expert knowledge on involved processes, detailed measurements, demanding tuning of data to model)
- Experimental data-driven model (substantial exp. data, simple functional dependency, advanced statistics, ML)



COLLECTING  
DATA

TRAINING  
THE MODEL

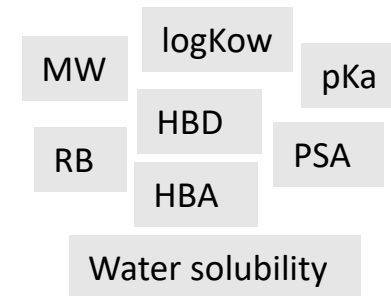
CONSTRUCTING  
THE MODEL

PREDICTING  
ITS OUTPUT

## CONDITIONS:

- VARIOUS GROWING (H, L, P)
- VARIOUS IRRIGATION (PW, TWW, +CEC) + SLUDGE

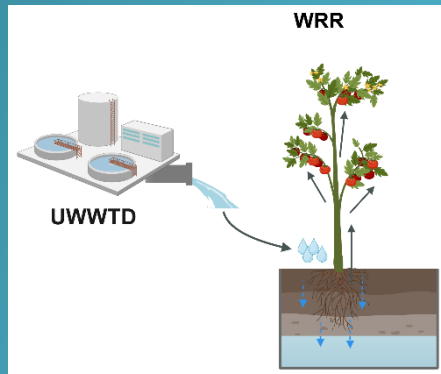
## CEC properties:



CEC  
uptake

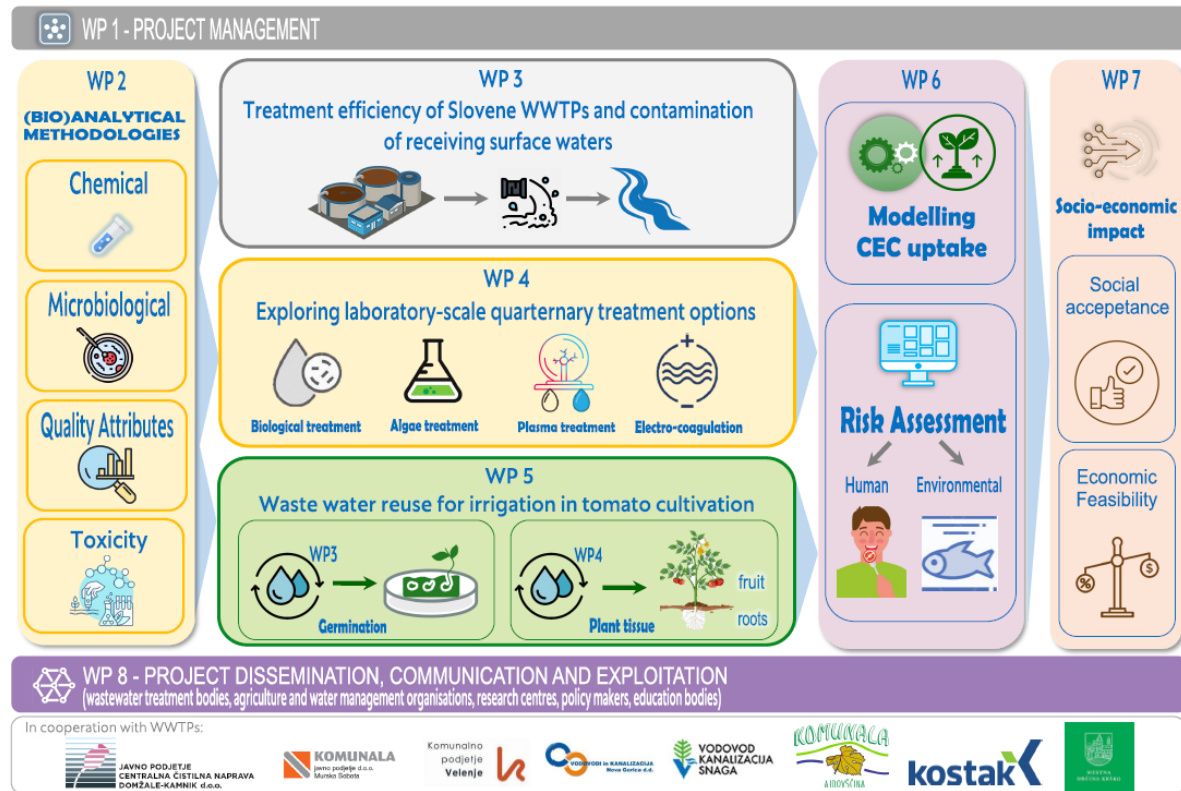


# Linking UWWTD and WRR....



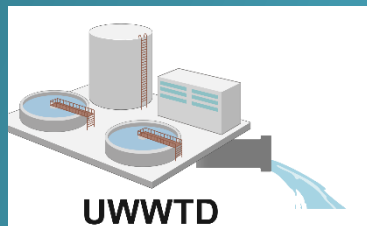
## WIDER UPTAKE

FROM DRAIN TO GRAIN: Quaternary wastewater treatment for safe water reuse in agriculture



Rezultati: oktober, 2025. Pričetek izvedbe 1.1.2026?

# UWWTD



## WP 3

Treatment efficiency of Slovene WWTPs and contamination of receiving surface waters



## WP 4

Exploring laboratory-scale quarternary treatment options



Biological treatment



Algae treatment



Plasma treatment



Electro-coagulation

## WP 5

Waste water reuse for irrigation in tomato cultivation



Germination



Plant tissue



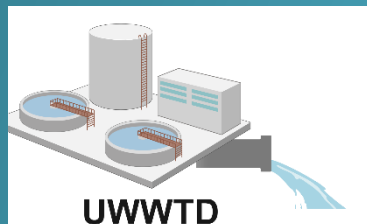
fruit  
roots

In cooperation with WWTPs:





# UWWTD



## WP 3 Treatment efficiency of Slovene WWTPs and contamination of receiving surface waters



## WP 4 Exploring laboratory-scale quarternary treatment options



Biological treatment



Algae treatment



Plasma treatment



Electro-coagulation

## WP 5 Waste water reuse for irrigation in tomato cultivation



Germination



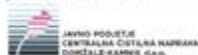
Plant tissue



fruit  
roots

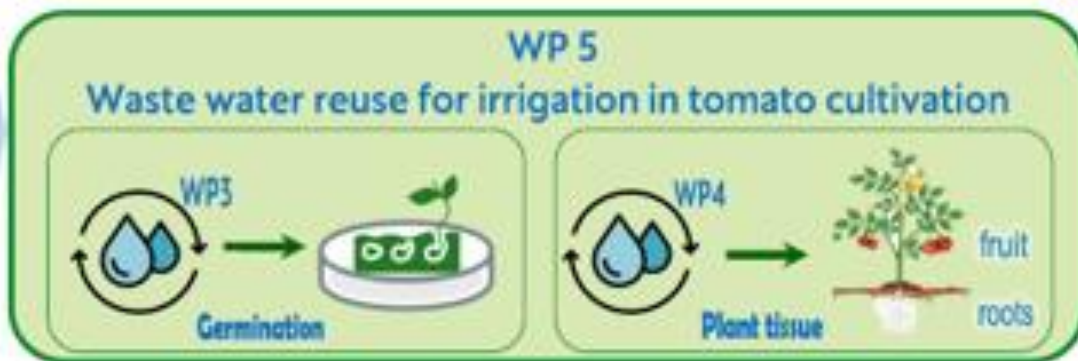
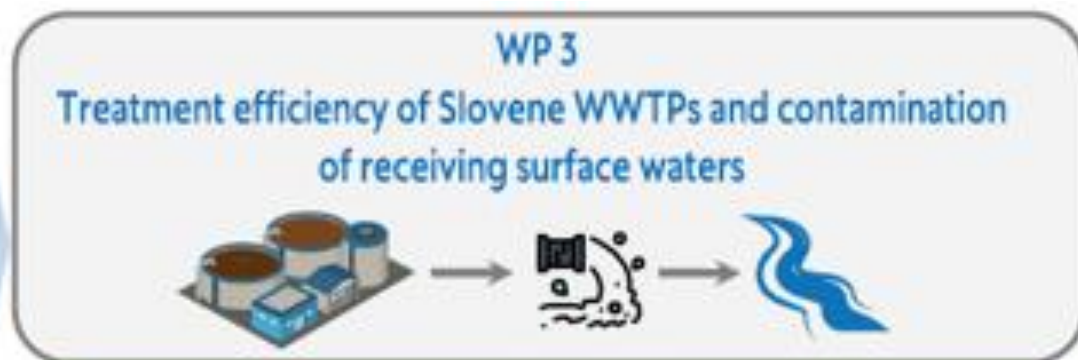
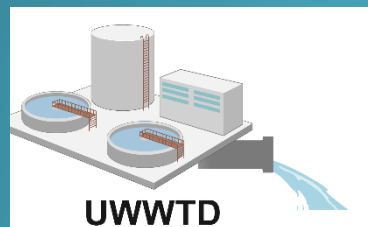


In cooperation with WWTPs:





# UWWTD



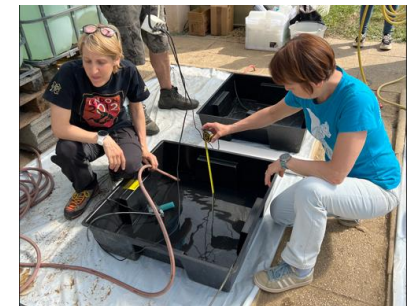
## Treatment efficiency of Slovene WWTPs and contamination of receiving surface waters



### Exploring laboratory-scale quarternary treatment options



### Waste water reuse for irrigation in tomato cultivation



**JAVNO PODJETJE  
CENTRALNA CISTILNA NAPRAVA  
DOMITILSKA KAMNICA, d.o.o.**



Epimorfo  
podjetje  
Velenje



# Acknowledgements





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Univerza v Ljubljani



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Agricultural reuse of wastewater and sewage sludge: uptake and distribution of emerging organic contaminants in plants



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FROM DRAIN TO GRAIN: Quaternary wastewater treatment for safe water reuse in agriculture





# Acknowledgements



