



Organic Microcontaminants in Tomato Crops Irrigated with Reclaimed Water Grown under Field Conditions

Martínez-Piernas, A. B., Plaza-Bolaños, P., Fernández-Ibáñez, P., & Agüera, A. (2019). Organic Microcontaminants in Tomato Crops Irrigated with Reclaimed Water Grown under Field Conditions: Occurrence, Uptake, and Health Risk Assessment. *Journal of Agricultural and Food Chemistry*, 67(25), 6930-6939. <https://doi.org/10.1021/acs.jafc.9b01656>

[Link to publication record in Ulster University Research Portal](#)

Published in:
Journal of Agricultural and Food Chemistry

Publication Status:
Published (in print/issue): 26/06/2019

DOI:
[10.1021/acs.jafc.9b01656](https://doi.org/10.1021/acs.jafc.9b01656)

General rights

Copyright for the publications made accessible via Ulster University's Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Ulster University's institutional repository that provides access to Ulster's research outputs. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact pure-support@ulster.ac.uk.

1 **Organic microcontaminants in tomato crops irrigated with reclaimed**
2 **water grown under field conditions: occurrence, uptake and health**
3 **risk assessment**

4
5 A.B. Martínez-Piernas,[†] P. Plaza-Bolaños,[†] P. Fernández-Ibáñez,[‡] A. Agüera^{*,†}

6 [†]*CIESOL (Solar Energy Research Center), Joint Centre University of Almeria-CIEMAT,*
7 *Carretera de Sacramento s/n, E-04120, Almeria, Spain*

8 [‡]*Nanotechnology and Integrated BioEngineering Centre, School of Engineering,*
9 *University of Ulster, Newtownabbey, BT37 0QB, Northern Ireland, United Kingdom*

10
11
12 *Corresponding author

13 Email: aaguera@ual.es, Tel: +34 950 015 531, Fax: +34 950 015 008

14 ORCID: 0000-0003-2649-6772

15

16 **ABSTRACT**

17 In many regions reuse of reclaimed water (RW) is a necessity for irrigation. Presence of
18 organic microcontaminants (OMCs) in RW and their translocation to plants may
19 represent a risk of human exposure. Nevertheless, information available about real field
20 crops is scarce and focused on a limited number of compounds. The novelty of this work
21 relies on the application of a wider-scope analytical approach based on a multi-analyte
22 target analysis (60 compounds) and a suspect screening (1300 compounds). This
23 methodology was applied to real field-grown tomato crops irrigated with RW. The study
24 revealed the presence of 17 OMCs in leaves (0.04 - 32 ng g⁻¹), and 8 in fruits (0.01 - 1.1
25 ng g⁻¹); 5 of them not reported before in real field samples. A health-risk assessment,
26 based on the toxicological threshold concern (TTC) concept, showed that RW irrigation
27 applied under the conditions given do not pose any threat to humans.

28

29

30 **KEY WORDS**

31 Organic microcontaminants

32 Plant uptake

33 Reclaimed water reuse

34 Health risk assessment

35 LC-MS target/suspect analysis

36

37 INTRODUCTION

38 The lack of fresh water resources for agriculture in arid and semiarid regions is a
39 worldwide problem that needs to be addressed in the 21st century. Factors such as climate
40 change and increasing population have led to severe droughts in areas where intensive
41 agriculture is the main economic activity. The reuse of reclaimed water (RW) for
42 agriculture irrigation seems to be an excellent approach to deal with water scarcity,¹⁻⁵
43 since it not only promotes efficient water usage, but also has other advantages such as
44 reducing the application of fertilizers and avoiding the discharge of waste into natural
45 water bodies, thus contributing towards the preservation of the environment.⁶

46 In Europe, the Mediterranean area is heavily influenced by low and irregular rainfall, a
47 fact that has worsened water shortages leading to a lower water supply for agricultural
48 purposes mainly during peak water demand periods. Nowadays, countries such as Cyprus,
49 France, Greece, Italy, Portugal and Spain, have adopted regulations regarding the reuse
50 of RW for crop irrigation due to the increasing application of this practice.⁷ So much so,
51 in Spain, the 10.8% of the RW is reused, being the 71% of it destined to agriculture.⁷ In
52 most cases, the national regulations include specific threshold values for either
53 microbiological (e.g. *E. coli*, intestinal nematodes) and physical-chemical parameters
54 (e.g. total suspended solids, turbidity) for any restricted use,⁸ being more strict for
55 agricultural uses. The European Commission has recently launched a proposal for a
56 regulation on minimum requirements for water reuse, which includes recommendations
57 based on a health and environmental risk management framework for future water reuse
58 legislation.⁹ Again, only microbiological and physical-chemical parameters have been
59 considered. However, in the last decade, the presence of organic microcontaminants
60 (OMCs) in RW, which are not completely removed during the treatments,¹⁰ have been
61 pointed out as a potential risk. It has been demonstrated that intensive use of RW in

62 agriculture leads to their accumulation in agricultural soils^{11,12} and their subsequent
63 uptake by plant roots, in some cases being able to translocate to aerial parts of plants such
64 as leaves and fruits through the vascular plant system.^{2,13-15} However, some knowledge
65 gaps and the lack of reliable data still prevent to make definite conclusions about their
66 risk posed to humans and the environment.

67 Numerous studies have shown translocation of OMCs to edible parts of crops in
68 simulated or controlled conditions.¹³⁻²¹ Nevertheless, little is known about their
69 occurrence and accumulation in real field crops exposed to RW irrigation for long time
70 periods. Recently, Picó et al.¹⁴ have evaluated the accumulation of OMCs in agricultural
71 soils and crops irrigated with treated wastewater, finding up to 6 pharmaceuticals in
72 different crops as cabbage, green beans or eggplants. Also Riemenschneider et al.⁵
73 reported the translocation in real field samples of 12 micropollutants and metabolites to
74 different plant organs such as roots, stems, leaves and fruits of 10 different vegetables
75 irrigated with river water mixed with effluent from a wastewater treatment plant
76 (WWTP). In another study, Wu et al.² monitored the accumulation of 19 OMCs in 8
77 vegetables irrigated with RW showing a detection frequency of 64% at concentrations in
78 the range of 0.01-3.87 ng g⁻¹, dry weight, (d.w.).

79 However, most of the reported studies analyze a low number of compounds or are
80 focused on certain pharmaceutical classes. In order to obtain a comprehensive evaluation
81 of the impact of OMCs in the food chain, it is necessary to apply multi-analyte/class
82 methodologies able to provide qualitative information for a wide range of compounds,
83 given the large number of OMCs reported in RW. Therefore, in addition to wider target
84 methods, non-target screening methodologies based on high resolution mass spectrometry
85 (HRMS) should be applied, leading to the identification of substances outside the limited
86 scope of the target analysis.^{12,14} This approach should contribute towards improving data

87 available regarding the occurrence/accumulation of OMCs in final products intended for
88 human consumption to ensure safe use of RWW in terms of health risk assessment.

89 Finally, the reported accumulation of OMCs in crops is in general low and no risk for
90 public health is expected to be associated to the until now, few known individual
91 compounds in crops grown under real field conditions.^{22,23} However, further work needs
92 to be carried out to assess the risk of not previously evaluated compounds that are present
93 in the edible tissues of plants grown under long-term and continuous exposition to these
94 microcontaminants.²⁴ This data will be valuable to study the risk associated with mixtures
95 of OMCs in end-products in future works.

96 The goal of this work was to increase the current information about the translocation of
97 OMCs derived from reuse by providing reliable data on their occurrence and fate in real
98 tomato crops (leaves and fruits) after long-term exposure to RW irrigation practices under
99 field conditions. Field-grown tomato plants were cultivated in agricultural soils
100 previously analyzed¹² and irrigated with RW for more than 10 years without soil
101 substitution. With this aim in mind, a combined strategy based on a multi-analyte target
102 analysis (including 60 compounds considered as contaminants of emerging concern)
103 together with a suspect screening methodology (covering a list of 1300 potential
104 contaminants) was applied. A simple and quick QuEChERS-based method was used for
105 sample preparation and liquid chromatography coupled to low and high resolution mass
106 spectrometry, were selected. A health-risk assessment approach was also applied to
107 evaluate human exposure of the RW-derived OMCs in tomato fruits.

108

109 **MATERIALS AND METHODS**

110 **Chemicals and Reagents.** A total of 60 OMCs (mainly pharmaceuticals from a variety
111 of therapeutic classes) (Table S1) were analyzed due to their frequent identification in

112 WWTP effluents.¹⁰ All reference standards (purity > 98%) were acquired from Sigma-
113 Aldrich (Steinheim, Germany). Methanol (MeOH), acetonitrile (ACN), water, formic
114 acid and acetic acid (LC-MS grade) were obtained from Sigma-Aldrich. Ultrapure water
115 for LC-MS/MS analysis was produced using a Milli-Q water purification system from
116 Millipore (Darmstadt, Germany). For the QuEChERS extraction method, anhydrous
117 magnesium sulfate (MgSO₄) and sodium acetate (NaOAc) were purchased from Sigma
118 Aldrich (all purity > 98%). Octadecyl-silyl-modified silica gel (C18) and primary-
119 secondary amine (PSA) were acquired from Supelco (Bellefonte, PA, USA).

120 Stock standard solutions of each compound were prepared at 1000-2000 mg L⁻¹ in
121 MeOH. Multi-compound working solutions were prepared at a concentration of 10 mg L⁻¹
122 in MeOH by diluting the individual stock solutions. All standard solutions were stored
123 in amber glass vials at -20°C. Matrix matched calibration solutions were daily prepared
124 and used for quantification purposes. Two surrogate standards, carbamazepine-d₁₀ and
125 ¹³C-caffeine, were used to check the extraction efficiency.

126 **Sample Collection.** To study the occurrence and distribution of OMCs in the plant
127 system, three greenhouses were selected (GH1, GH2 and GH3; intensive production;
128 13000–25000 m²), in which two different tomato varieties, ramyle (GH1, GH2) and
129 retinto (GH3) were grown. A fourth greenhouse dedicated to the experimental soilless
130 culture (SP1) of the cherry tomato variety, which was grown in pots filled with perlite
131 substrate, was also included in the study. All greenhouses were located in Almeria
132 province (Spain) and had been irrigated with RW for no less than ten years without soil
133 replacement. The RW was provided by a regeneration plant facility which treats
134 municipal wastewater secondary effluents by filtration (sand and anthracite filters) and
135 chlorination (NaClO). Treated water fulfilled the requirements of water quality according
136 to the Spanish regulation for water reuse.⁸ Drip irrigation was employed in all

137 greenhouses. Four sampling events during the commercial tomato campaign took place
138 from January (full plant growth) to May 2016 (removal of tomato plants). In each
139 sampling event, tomatoes at a mature stage of growth and leaves of tomato plant samples
140 (500 g in each case) of similar size were taken from different parts of the greenhouse
141 following a W sampling route. The subsamples were chopped and mixed to form a
142 homogeneous composite sample and were kept in the dark at -20°C until their analysis.
143 Three replicates of each sample were extracted for quantification purposes. RW was
144 analyzed coinciding with the first sampling of tomato fruits and leaves.

145 **Sample Extraction.** The extraction of OMCs in tomato fruit and leaves was carried out
146 by a modification of the QuEChERS acetate extraction method previously published by
147 our group.¹³ Briefly, a portion of 10 g of plant material were placed into a 50-mL
148 polypropylene centrifuge tube. After that, 10 mL of 1% acetic acid in ACN and 20 µL of
149 the extraction quality control solution were added to the sample and the tube was shaken
150 for 5 min and centrifuged at 3500 rpm (2054xg) for 5 min. Following the extraction
151 procedure, a clean-up step was carried out. An aliquot of 5 mL of the upper organic layer
152 was transferred to a 15-mL centrifuge tube containing 750 mg of anhydrous MgSO₄, 125
153 mg of primary-secondary amine (PSA) and 125 mg of C18. Then the tube was shaken for
154 30 s in a vortex and centrifuged at 3500 rpm for 5 min. Following this, the extract (4 mL)
155 was transferred to screw-cap vials adding 10 µL of ACN at 1% of formic acid per
156 milliliter of extract. Prior to injection into the LC-MS/MS system, 100 µL of the extract
157 was evaporated and reconstituted in 100 µL of ACN:H₂O (10:90, v/v).

158 **Liquid Chromatography-Mass Spectrometry.** *LC-MS/MS Target Analysis.* The
159 HPLC system (Agilent Series 1200, Agilent Technologies, Palo Alto, CA, USA)
160 consisted of a binary pump, a degasser and an autosampler. Chromatographic separation
161 was accomplished using a XDB C18 (50 x 4.6 mm, 1.8 µm particle size) column (Agilent

162 Technologies). Mobile phases were 0.1% formic acid in MilliQ water (solvent A) and
163 ACN (solvent B). The gradient used ranged from 10% to 100% of solvent B: initially it
164 was kept at 10% for 1 min, increased from 10% to 50% over 3 min and from 50% to
165 100% over 10 min; kept at 100% for 4 min and finally returned to its initial conditions.
166 The total analysis run time was 18 min. The injection volume was 10 μL and the flow rate
167 was set to 0.4 mL min^{-1} . The column outlet system was connected to a hybrid triple
168 quadrupole-linear ion trap-mass spectrometer 5500 QTRAP® (Sciex Instruments, Foster
169 City, CA, USA) equipped with an ESI source (TurboIon Spray) operating with positive
170 and negative polarities. The ionization settings used were: ionspray voltage, 5000 V;
171 curtain gas, 25 (arbitrary units); GS1, 50 psi, GS2, 40 psi; and a temperature, 500 °C.
172 Nitrogen was used as a nebulizer, curtain and collision gas. The multiple reaction
173 monitoring (MRM) mode was chosen for the analysis of the target compounds. To
174 increase the sensitivity for the acquisition performance, the schedule MRM™ algorithm
175 was applied with a retention time window of 40 s per transition. The optimal mass
176 spectrometric parameters for each compound are summarized in Table S2. Sciex Analyst
177 version 1.6.2 software was applied for data acquisition and processing, and MultiQuant
178 3.0.1 software for data quantification.

179 *LC-QTOF-MS/MS Suspect screening analysis.* Chromatographic separation was
180 performed using a HPLC (Agilent 1260 Infinity system) equipped with a Poroshell 120
181 EC-C18 (50 x 4.6 mm, 2.7 μm particle size) analytical column (Agilent Technologies).
182 0.1% formic acid in ultrapure water (solvent A) and ACN (solvent B) were used as mobile
183 phases. The injection volume was 20 μL and the flow rate was 0.5 mL min^{-1} . The gradient
184 used ranged from 10% to 100% of solvent B: initially it was kept constant at 10% for 2
185 min, then increased linearly from 10% to 100% for 9 min and finally it remained constant
186 for 4 min before being returning to initial conditions. The total analysis run time was 22

187 min. The LC system was coupled to a QTOF mass analyzer Triple TOF 5600+ (Sciex
188 Instruments), with a DuoSprayTM ion source consisting of an electrospray (ESI) interface
189 for sample injection and an atmospheric-pressure chemical ionization interface (APCI)
190 for calibrant delivery. Samples were analyzed in ESI+ and ESI- modes. The ESI source
191 parameters were: ionspray voltage, 4500 V; curtain gas, 25 (arbitrary units); GS1, 60 psi;
192 GS2, 60 psi; and temperature, 575°C. Nitrogen served as a nebulizer, curtain and collision
193 gas. The equipment worked via TOF MS survey scan (resolving power of 30000) with an
194 accumulation time of 250 ms followed by four IDA (Information Dependent Acquisition)
195 TOF MS/MS scans with an accumulation time of 100 ms. The IDA feature allows the
196 performance of MS/MS acquisitions simultaneously with the MS acquisition. The *m/z*
197 range was from 100 to 2000. IDA criteria considered dynamic background subtraction.
198 Collision energy of 30 eV with a ± 15 eV spread was applied for MS/MS fragmentation.
199 Diverse Sciex software (Analyst TF 1.5, PeakViewTM 2.2 and MasterView 1.1) was used
200 to record and process LC-QTOF-MS/MS data. A suspect list containing 1300 OMCs
201 commonly found in WWTP effluents was made before sample processing. The settings
202 considered for a final confirmation of the compounds were: a) a mass accuracy error for
203 the precursor ion < 5 ppm; b) an isotope ratio difference < 10%; c) a MS/MS spectra fit
204 $\geq 80\%$ when the acquired spectra was compared with the MS/MS spectra of the standard;
205 and d) a difference of ± 0.1 min in the retention time (RT) when it was compared with
206 the standard in matrix.

207 **Method Validation.** Concerning the quantitative method for tomato fruits and leaves,
208 the present methodology was validated assessing trueness (in terms of recoveries),
209 precision (expressed as relative standard deviation, RSD), linearity and limits of
210 quantification (LOQs). For method validation, tomato leaves and fruits not irrigated with
211 RW were used as blank matrices. Triplicate analyses of samples spiked at 0.5 ng g^{-1} were

212 used to calculate the recoveries. Satisfactory mean recovery values were considered in
213 the range 70-120% with an associated precision RSDs $\leq 20\%$. The linearity was studied
214 by matrix-matched standard calibration curves at six concentration levels ranging from
215 0.01 to 10 ng g⁻¹. Linearity was considered as acceptable when the determination
216 coefficients (R^2) were ≥ 0.990 . The LOQs were set as the lowest acceptable concentration
217 in the matrix-matched calibration curve which yielded the signal-to-noise (S/N) ratio
218 closer to 10 for the quantification transition (SRM 1). The quantification of the analytes
219 present in the samples was carried out by matrix-matched calibration curves of all
220 validated compounds. OMCs quantified in real samples fulfilled the requirements for
221 recoveries, precision and linearity (Table S3).

222 Regarding RW, the sample collected was analyzed per triplicate by direct injection
223 following the methodology reported elsewhere,¹⁰ which was previously validated for the
224 analysis of 115 OMCs in WWTP effluents.

225 **Health-risk Assessment.** The health risk associated with presence of OMCs in tomato
226 fruits was estimated using the threshold of toxicological concern (TTC) approach. This is
227 useful for assessing the risk involved with substances present in food at low
228 concentrations and for which toxicity data is still scarce.²⁵ TTC has previously been
229 applied to the risk assessment of OMCs in crops.^{3,20} In this study, an average body weight
230 of 70 kg for adults and 12 kg for toddlers was considered for the estimation of daily
231 consumption. The TTC values and compounds classification were estimated based on the
232 well-known Cramer classification tree. The Cramer method mainly utilizes chemical
233 structures and evaluates the total human intake to establish priorities for testing.²⁶ This
234 protocol considers a number of factors related to the presence of the chemical component
235 under study or the frequency of ingestion, including: a) different metabolic pathways for
236 either activation or deactivation of the chemicals under study; b) partial presence of a

237 target substance in a variety of standard foods and their metabolites; c) toxicity data for
238 each substance; and d) the level of exposure to humans via oral ingestion. This
239 information is then managed to obtain the TTC value of each compound in terms of $\mu\text{g-}$
240 ng kg^{-1} body weight (b.w.) day^{-1} .²⁷ For the OMCs translocation that were not reported
241 before, we considered as minimum tolerated exposure of each OMC as equals to the TCC
242 value given for the parent compound (Houeto et al, 2012; Munro et al., 1996; Stanard et
243 al., 2015).

244 TCC values and compound classification were estimated using ToxTree software
245 (ToxTree v.3.1.0, by JRC Computational Toxicology and Modeling and developed by
246 Ideaconult Ltd, Sofia, Bulgaria). The TTC values for all the compounds under study
247 were determined for the highest CEC concentrations of all greenhouses (SP1, GH1, GH2,
248 GH3) obtained in each sampling event (S1 - S4). Statistical analysis of all the samples
249 and repeated measurements in pairs ($p < 0.05$) were performed using ANOVA analysis.

250

251 **RESULTS AND DISCUSSION**

252 **Method validation results.** The proposed methodology was validated in tomato fruits
253 and leaves of tomato plant for a total of 60 OMCs. The validation results are presented in
254 Table S3. A total of 48 out of 60 compounds (80%) in fruit and 31 (51%) in leaves showed
255 acceptable recoveries in the 70-120% range with $\text{RSD} \leq 20\%$. Tomato results are in line
256 with the previous method validation of the same compounds in other vegetable matrices
257 such as lettuce, radish and strawberry.¹³ However, the number of successfully recovered
258 OMCs in leaves was lower than in fruits probably due to the complexity of this matrix.
259 The high content of chlorophylls and pigments may suppress OMCs extraction efficiency
260 in leaves case. In general, very low RSD values under 10% were found in the majority of
261 the cases regardless the recovery value. Solely for loratadine in leaves it was obtained a

262 RSD out of the acceptable values. This demonstrates the repeatability of the method. All
263 compounds presented good linearity with R^2 values higher than 0.991 and LOQs ranged
264 from 0.01 to 2 ng g⁻¹; showing more than the 50% of the analytes low LOQs below 0.1
265 ng g⁻¹ in both commodities. In spite of some OMCs such as clotrimazole, fenoprofen or
266 sulfapyridine do not fulfill the acceptable criteria for validation; they were maintained in
267 the method for qualitative purposes. Only those OMCs for which the method could be
268 fully validated adopting the criteria aforementioned were quantified in real samples.

269 **OMCs in Irrigation Water.** An analysis of the irrigation water was carried out at the
270 beginning of the study to obtain an overview of the potential exposure of the crops to the
271 tested OMCs. As can be observed in Table S4, up to 51 OMCs could be identified at
272 concentration values ranging from 15 to 14424 ng L⁻¹. The metabolites of dipyrrone, 4-
273 FAA and 4-AAA (14424 and 5396 ng L⁻¹, respectively), the diuretics hydrochlorothiazide
274 and furosemide (2758 and 1694 ng L⁻¹, respectively), and the beta-blocker atenolol (1279
275 ng L⁻¹), were detected at the highest concentrations. It was expected that OMC
276 concentrations in RW would vary throughout the study. However, overall these results
277 are in line with previous monitoring studies carried out on urban WWTP effluents from
278 Almeria^{10,13} and can be considered as representative of the type/concentration of
279 compounds usually present in the RW. The presence of 35 of these compounds has also
280 been previously reported by our group in soil and soilless perlite substrate samples taken
281 from the greenhouses monitored, which show average concentrations in the range 0.14 -
282 99 ng g⁻¹, d.w. (Table S4). Although the presence of OMCs in irrigation water and soils
283 cannot be directly related to their occurrence in plant tissues due to the diverse factors
284 involved in plant uptake, it can be assumed that their availability to be taken up by roots
285 and translocate to edible parts is feasible when RW is used in irrigation.

286 **Occurrence of OMCs in Tomato Plant Leaves.** Greater knowledge about the
287 occurrence of OMCs in vegetables irrigated with RW under field conditions is key to
288 evaluating the quality of crops and determining potential consequences of reusing RW in
289 agriculture irrigation. Moreover, the analysis of non-edible parts of the tomato crop, such
290 as leaves, which may be used as sustenance for livestock feeding, is also important since
291 it could represent another pathway for human exposure to OMCs. In this study, a total of
292 60 target compounds (Table S1) were monitored in real samples of tomato and tomato
293 plant leaves to evaluate their distribution throughout the plant-fruit system.

294 The average concentration levels of OMCs found in leaf samples during the four
295 sampling events are shown in Table 1. Up to 17 CECs were detected in leaves with
296 average concentrations ranging from 0.04 to 32 ng g⁻¹ wet weight (w.w.). The compounds
297 that eventually reached the higher concentrations were the metabolites of dypirone, 4-
298 AAA and 4-FAA (11 and 32 ng g⁻¹, respectively), the anticonvulsant drug carbamazepine
299 (8.9 ng g⁻¹), its metabolite carbamazepine epoxide (8.1 ng g⁻¹) and the antidepressant
300 venlafaxine (4.0 ng g⁻¹). Regarding the frequency of detection, only 7 OMCs were found
301 in all samples, namely caffeine, paraxanthine, carbamazepine, carbamazepine epoxide,
302 hydrochlorothiazide, mepivacaine and venlafaxine; evidencing their higher capability of
303 uptake and translocation within the plant. Nevertheless, their concentrations did not
304 increase during the sampling period; a fact that could demonstrate stable accumulation
305 despite constant irrigation with RW. Another group of OMCs were detected at very low
306 concentrations (<LOQ) and/or showed low frequency of detection. This was the case for
307 acetaminophen, antipyrin, diazepam, propranolol and the antibiotic trimethoprim.

308 In addition to the target analysis, samples were retrospectively analyzed by the acquired
309 LC-QTOF-MS/MS sample information. The strategy allowed the identification of 3 other
310 OMCs: flecainide, lidocaine and tramadol (Figures S1-S3). These compounds were also

311 found in the irrigation water and soil samples (Table S4).¹² Almost all of them were
312 identified in every sampling event, showing uptake from soil to leaf plant tissues. As the
313 methodology could not be validated for these analytes, estimated concentration values
314 had to be calculated (Table 1).

315 In general, no significant differences considering concentration levels were found
316 between the different tomatoes produced in the greenhouses. This suggests there is no
317 correlation between plant uptake and the tomato plant variety.

318

319 **Table 1. Average OMC concentrations (ng g⁻¹, w.w.) quantified in tomato plant leaves**

Compound	SP1 ^a				GH1 ^b				GH2				GH3			
	S1 ^c	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
4-AAA	<LOQ ^d	-	0.5	<LOQ	<LOQ	-	-	<LOQ	0.4	-	<LOQ	<LOQ	<LOQ	<LOQ	12	<LOQ
4-FAA	8	n.d. ^e	32	13	n.d.	n.d.	7	5	<LOQ	n.d.	4	3	<LOQ	3	4	2
Acetaminophen	n.d.	n.d.	<LOQ	2	n.d.	n.d.	n.d.	2	n.d.	n.d.	n.d.	3	n.d.	n.d.	n.d.	3
Antipyrine	<LOQ	<LOQ	1	<LOQ	<LOQ	<LOQ	0.7	n.d.	<LOQ	<LOQ	0.6	n.d.	<LOQ	<LOQ	<LOQ	n.d.
Caffeine	0.5	1	0.7	0.5	0.5	1	0.4	<LOQ	0.4	1	0.5	<LOQ	0.5	1	0.5	<LOQ
Carbamazepine	5	5	5	2	3	6	5	2	2	9	3	6	6	4	7	4
Carbamazepine epox	3	3	2	3	3	2	0.7	4	2	2	0.5	8	4	2	1	8
Flecainide^f	n.d.	2	n.d.	0.9	n.d.	2	4	4	2	4	4	4	3	2	3	4
Diazepam	<LOQ	<LOQ	0.06	0.04	<LOQ	<LOQ	<LOQ	0.01	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
Hydrochlorothiazide	1	1	1	0.6	1	1	2	1	0.9	1	0.9	0.6	1	0.6	1	0.6
Lidocaine^f	1	2	10	8	3	8	6	11	3	5	6	6	7	6	4	8
Mepivacaine	0.6	0.5	0.5	0.3	0.8	0.7	0.8	0.9	0.6	1	0.6	1	1	0.6	0.3	0.8
Paraxanthine	0.2	0.6	0.4	0.3	0.2	0.6	<LOQ	<LOQ	0.2	0.5	0.3	0.2	0.2	0.3	<LOQ	<LOQ
Propranolol	<LOQ	<LOQ	<LOQ	<LOQ	0.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.4	<LOQ	<LOQ	<LOQ
Tramadol^f	1	2	1	4	0.8	2	2	3	0.6	3	2	3	0.2	3	3	3
Trimethoprim	n.d.	n.d.	2	n.d.	n.d.	n.d.	0.7	n.d.	n.d.	n.d.	2	n.d.	n.d.	n.d.	n.d.	n.d.
Venlafaxine	2	1	2	0.7	2	2	2	3	2	4	3	4	4	2	2	4

320 ^aSP: soiless perlite culture; ^bGH: greenhouse; ^cS: sampling event; ^d<LOQ: concentration below the limit of quantification; ^en.d.: not detected; ^fEstimated OMC concentrations
 321 quantified by LC-QTOF-MS/MS

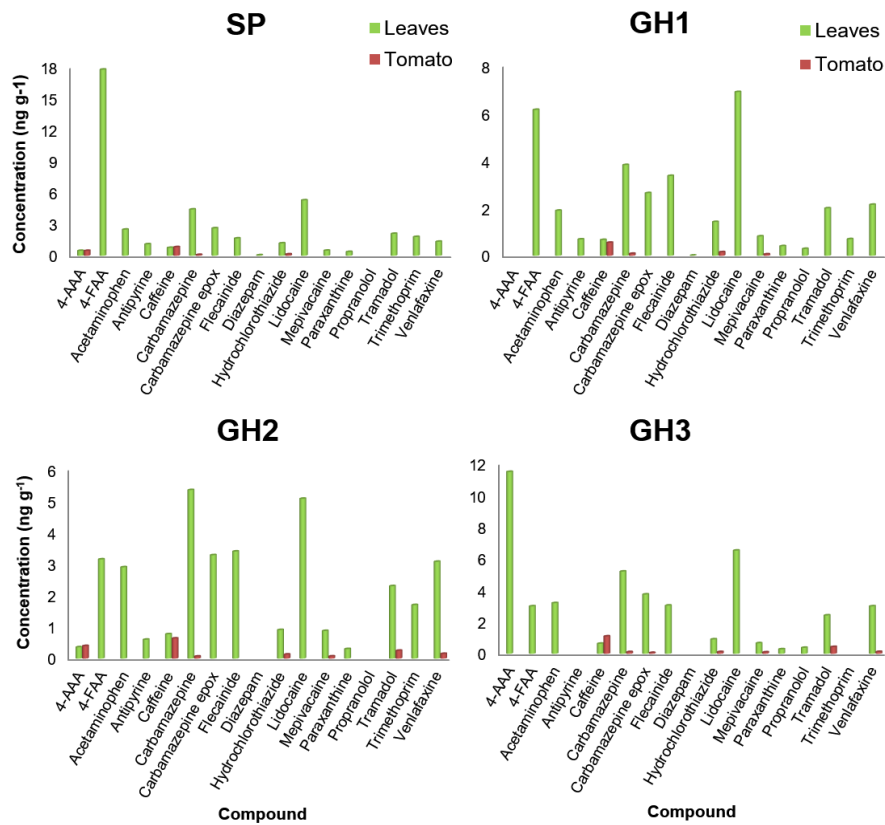
322

323 Results obtained in the field study concerning translocation of selected OMCs via plant
324 roots to other plant tissues, confirm previous results reported in studies under controlled
325 conditions. For instance, Martínez-Piernas et al.¹³ reported the accumulation of diverse
326 analytes such as 4-AAA, 4-FAA, caffeine, carbamazepine, carbamazepine epoxide,
327 hydrochlorothiazide, lincomycin, mepivacaine and venlafaxine, among others, in lettuce
328 and leaves of radish when RW was used as irrigation water. Wu et al.¹⁶ compared the
329 concentrations found for a group of OMCs such as acetaminophen, caffeine,
330 carbamazepine and diazepam in roots and leaves of lettuce, spinach, cucumber and pepper
331 irrigated with spiked water. The metabolism and plant uptake of diazepam has also been
332 evaluated by Carter et al.¹⁷ in radish and silverbeet cultivated with spiked soil. The
333 antibiotic trimethoprim has been reported by Dodgen et al.¹⁸ as being translocated to
334 lettuce, carrot and tomato leaves in an experiment carried out under controlled conditions
335 of temperature and humidity. Other studies have investigated the impact of soil
336 composition in OMCs' plant uptake in leafy crops when they were cultivated with spiked
337 water, observing correlations between soil characteristics and root uptake.^{19,20}

338 However, very few studies have analyzed real field samples exposed to OMCs. Wu et
339 al.² described the translocation of caffeine and carbamazepine within the different plant
340 organs in vegetables irrigated with RW and cultivated under field conditions. In addition,
341 Riemenschneider et al.⁵ observed the accumulation of caffeine, carbamazepine,
342 carbamazepine epoxide and hydrochlorothiazide in different vegetables and agricultural
343 plant tissues. In another study, levels of lincomycin were reported up to 20 $\mu\text{g kg}^{-1}$ (d.w.)
344 in leafy vegetables such as rape, celery and coriander grown in soil amended with
345 manure.²⁸

346 **Occurrence of OMCs in Tomato Fruit.** Concentrations of OMCs were found to a
347 lesser extent in tomato fruits, these generally being 10 times lower in fruit compared to

348 leaves (Figure 1). A total of 12 OMCs were detected in tomato samples. However, only
 349 8 compounds could be quantified in at least one sample (Table 2). In general, the
 350 compounds that showed higher frequencies of detection and concentrations in leaves were
 351 also present in tomatoes, showing mobility through the plant transpiration stream up to
 352 fruits. The highest concentration was observed for caffeine (1.1 ng g⁻¹), followed by the
 353 metabolite 4-AAA (0.4 ng g⁻¹), then carbamazepine (0.23 ng g⁻¹), hydrochlorothiazide
 354 (0.15 ng g⁻¹), venlafaxine (0.15 ng g⁻¹), mepivacaine (0.09 ng g⁻¹) and carbamazepine
 355 epoxide (0.07 ng g⁻¹). 4-FAA, acetaminophen, acetanilide and paraxanthine were
 356 identified at concentrations below the LOQ in at least one sample.



357
 358 **Figure 1. Average OMC concentrations found in leaves (green) and tomatoes (red)**
 359 **in each sampling site during the four sampling events.**

360

361 The retrospective analysis of tomato fruit samples revealed the presence of a previously
362 detected analyte in leaves by the same approach: tramadol (Figure S3). It is an opioid
363 analgesic generally used for moderate and severe pain. Tramadol was found in tomatoes
364 from two different greenhouses. Estimated concentrations of this compound are shown in
365 Table 2.

366 No remarkable differences were found between the concentrations observed for cherry
367 (SP1), ramyle (GH1, GH2) or retinto (GH3) tomato varieties. This fact evidences that
368 despite the higher size of the last two types and the different agricultural practices (soilless
369 culture for cherry and real soils for the rest), OMC accumulation was similar in all cases.

370

371 **Table 2. Average OMC concentrations (ng g⁻¹, w.w.) quantified in tomato fruit samples**

Compound	SP1 ^a				GH1 ^b				GH2				GH3			
	S1 ^c	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
4-AAA	-	<LOQ ^d	-	0.4	-	-	-	<LOQ	<LOQ	-	-	0.4	-	-	-	-
Caffeine	<LOQ	0.4	<LOQ	<LOQ	<LOQ	0.3	0.8	<LOQ	<LOQ	0.8	0.5	<LOQ	<LOQ	1	<LOQ	<LOQ
Carbamazepine	0.2	0.01	0.01	<LOQ	0.2	0.01	0.03	0.1	0.05	<LOQ	0.06	0.1	0.05	0.07	0.1	0.2
Carbamazepine epox	<LOQ	<LOQ	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.05	<LOQ	<LOQ	0.07
Hydrochlorothiazide	-	-	0.1	0.1	-	-	<LOQ	0.2	-	<LOQ	<LOQ	0.1	-	0.1	<LOQ	0.1
Mepivacaine	<LOQ	-	-	-	<LOQ	-	<LOQ	0.06	<LOQ	-	<LOQ	0.07	-	<LOQ	<LOQ	0.1
Tramadol ^a	-	-	-	-	-	-	-	-	-	0.2	-	-	-	0.1	-	0.7
Venlafaxine	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	0.1	<LOQ	<LOQ	<LOQ	<LOQ	0.1

372 ^aSP: soiless perlite culture; ^bGH: greenhouse; ^cS: sampling event; ^d<LOQ: concentration below the limit of quantification; ^eestimated OMC concentrations quantified by LC-
373 QTOF-MS/MS

374

375 As was observed in leaves, caffeine and carbamazepine were detected in all
376 greenhouses in every sampling event. Their plant uptake and translocation to the edible
377 parts of vegetables is well described in literature.^{2,13,29} Some studies have already reported
378 them in tomato crops cultivated under field and controlled conditions.^{5,21} Also metabolites
379 such as carbamazepine epoxide have been identified in tomatoes when plants were
380 irrigated with water spiked with carbamazepine under experimental conditions.³⁰
381 Hydrochlorothiazide has been reported in other vegetable tissues such as roots and leaves
382 of parsley cultivated under field conditions⁵ evidencing its high capability of translocation
383 through the plant system. OMCs such as 4-AAA, 4-FAA, mepivacaine and venlafaxine,
384 which were quantified in leaves, were also translocated to fruits and identified in certain
385 sampling events. This group of OMCs has been found in the edible parts of lettuce and
386 radish cultivated under controlled conditions submitted to RW irrigation.¹³ Considering
387 that 4-AAA and 4-FAA have exhibited toxicity,³¹ it is important to monitor their
388 occurrence and to evaluate their repercussions on human exposure.

389 To our knowledge, 4-AAA, mepivacaine, paraxanthine, tramadol and venlafaxine have
390 not previously been identified either in plant tissues or edible parts of real field samples,
391 which highlights the importance of applying wide-scope analytical methods for the
392 evaluation of reuse of RWW in agriculture under different conditions and crops, and the
393 potential of HRMS for the identification of non reported substances in environmental
394 analysis. These results contribute to cover the gap of knowledge regarding the possible
395 OMCs that can be present in edible parts of crops. This will help future studies dealing
396 with the evaluation of the environmental and human risks associated with mixtures of
397 analytes.

398 **Accumulation in Plant Tissues and Properties of Compounds.** It is well-known that
399 OMCs' uptake by roots is accessible for those compounds that are dissolved in the

400 solution of the soil pore water. In general, neutral and cationic species in the soil solution
401 are susceptible to uptake by roots and subsequently translocate to the aboveground parts
402 of plants by the transpiration stream.^{16,20,32} On the other hand, anions are considered less
403 transported to aerial parts due to their accumulation in cell roots by mechanisms such as
404 ion-trapping.³² The translocation of OMCs from roots to other plant organs is possible
405 due to their capability of moving through the transpiration streams. This mobility depends
406 on diverse analyte physical-chemical properties such as lipophilicity (K_{ow}), pK_a or the
407 type of crop, among others.^{3,33}

408 The results found in this study revealed that the OMC concentration values detected in
409 tomato leaves were significantly higher (up to ten times in some cases, Table 1) than those
410 found in tomatoes (Table 2). This behavior has been already reported in several
411 studies.^{2,13,20,33} This issue is explained by the greater water flow to leaves, leading to
412 higher accumulation of OMCs in leafy parts than in fruits.

413 In Table S5, the diverse lipophilic coefficients ($\log K_{ow}$ for neutral compounds and \log
414 D_{ow} for ions), pK_a and molecular charge (soil pore solution $pH = 7.5$) of the OMCs
415 identified in this work are shown. In general, moderate to strong bases ($pK_a \geq 7$), in its
416 cationic species or partially ionized (flecainide, hydrochlorothiazide, lidocaine,
417 mepivacaine, propranolol, trimethoprim and venlafaxine); weak bases ($pK_a < 6$) in neutral
418 form (4-AAA, 4-FAA, antipyrine, caffeine, carbamazepine, carbamazepine epoxide,
419 diazepam and paraxanthine) and a very weak acid ($pK_a > 7.5$) in its neutral form
420 (acetaminophen) were detected. The fact that these analytes are neutral or cations for a
421 wide range of pH values explains their good distribution through the transpiration streams
422 ($\sim 5.5 < pH < \sim 7.5$), being able to cross membranes, reaching leaves and fruits.²⁰ Although
423 some compounds were partially ionized, they were translocated via the transpiration-
424 derived mass flow subsequently being found in leaves, and in case of mepivacaine and

425 hydrochlorothiazide, in both leaves and fruits. No OMC in anionic form was detected in
426 either leaves or tomato. This is in accordance with the aforementioned reasons about the
427 expected low translocation of anions through the vascular system, making its distribution
428 less possible through plant streams.

429

430

431 As shown in Table S5, $\log K_{ow}$ and $\log D_{ow}$ of the OMCs identified, ranged from low to
432 medium lipophilic values ($-0.63 < \log K_{ow}, D_{ow} < 3.08$), demonstrating that the OMCs
433 observed have different affinities to lipid tissues. According to Miller et al.³³ non-ionized
434 compounds with $-1 < \log K_{ow} < 5$ are expected to translocate from roots to other plant
435 tissues, which is consistent with the results obtained for all the neutral compounds
436 identified in this study (Table S5).

437 **Human Exposure and Health-risk Assessment Analysis.** Tomato is one of the most
438 important crops around the world, with global production currently around 130 million
439 tons, of which 88 million is destined for the fresh market and 42 million processed.
440 Considering the intensive consumption of tomato worldwide, the evaluation of human
441 OMC exposure when RW is used as irrigation is of particular interest, even more when
442 this assessment focuses on real samples submitted to long-time RW irrigation.

443 In this study, an assessment of human exposure for each analyte quantified in samples
444 was carried out by the estimation of the daily tomato consumption required to reach TTC
445 levels in adults (average 70 kg) and toddlers (12 kg). All daily intakes were calculated
446 taking into account the worst-case scenario possible. To this aim, a single sampling event
447 with the highest value for TTC estimations was taking into account to provide the most
448 conservative considerations out of this study.

449 Regarding toxicological effects, substances were classified as follows. ‘Class I’ for
450 chemicals with simple structure and known metabolic pathways leading to innocuous end
451 products showing a low order of oral toxicity. Class II contains substances that are
452 intermediate. Very few compounds are included in this category, which is not very well
453 characterized and even questionable.³⁴ They have less innocuous structures than those in
454 Class I but they do not contain potentially toxic structural features. Class III contains
455 substances with complex chemical structures that provide no strong initial presumption
456 of safety and indeed may produce a significant toxicity effect, some of them being
457 genotoxic compounds. Examples of Class III are a number of pharmaceuticals and other
458 common used stimulants including, carbamazepine, caffeine, bezafibrate, clofibric acid,
459 ketoprofen, naproxen, and metoprolol.²⁰ TTC levels of these pharmaceuticals typically
460 reach values of around 1500 ng kg⁻¹ b.w. day⁻¹, while the TTC for genotoxic chemicals is
461 only 2.5 ng kg⁻¹ b.w. day⁻¹ or 0.15 µg person⁻¹ day⁻¹.^{27,34} Nevertheless, it is important to
462 remark that the consumption of a substance above the estimated TTC level would not
463 imply that there is a toxicological risk. It may even point out a demand for specific toxicity
464 analysis of the compound.

465 Some analytes quantified in tomato samples in this study are classified in Cramer Class
466 III (4-AAA, caffeine, carbamazepine, hydrochlorothiazide and mepivacaine). Regular
467 TTC values for these substances range from 1500 to 1800 ng kg⁻¹ b.w. day⁻¹.³⁵
468 Venlafaxine and tramadol are categorized as chronic toxic, being their TTC value
469 commonly set in 240 ng kg⁻¹ b.w. day⁻¹.³⁶ Carbamazepine epoxide has potential genotoxic
470 carcinogenicity. Therefore, TTC reported values are between 1.5 and 2.5 ng kg⁻¹ b.w. day⁻¹.³⁷

472 As can be observed in Table 3, the OMC concentrations found require an adult and
473 toddler consumption of tens to hundreds of kg to reach the TTC values in most cases.

474 Considering a reasonable tomato daily consumption (according to FAP the average is
 475 0.13 kg of tomatoes per adult per day,³⁸ depending on the dietary habits and country),
 476 these results do not bring along a health risk for the consumers.

477 As carbamazepine epoxide exhibit genotoxic carcinogenicity, it presented the lowest
 478 daily consumption of tomatoes per toddler and adult (400 g and 2.5 kg, respectively) to
 479 reach the TTC, despite its low concentration in the samples. These results are in
 480 agreement with the low amount intake of carrots to reach the estimated TTC reported by
 481 Malchi et al.²⁰

482 The results of this presented study clearly indicate that the estimated TTC values do not
 483 pose a health risk for any of the substances at the concentrations found. This contributes
 484 towards the safe usage of RW for tomato irrigation under the conditions presented even
 485 when the worst conditions were taking into account.

486

487 **Table 3. Health-risk assessment based on TTC levels of the OMCs quantified in**
 488 **tomato samples.**

Sampling	S1 ^a	S2	S3	S4
Maximum OMC concentration (ng g⁻¹, w.w.) detected in tomato samples				
4-AAA	<LOQ ^b	<LOQ	<LOQ	0.4
Caffeine	<LOQ	1	0.8	<LOQ
Carbamazepine	0.2	0.07	0.1	0.2
Carbamazepine epoxide	0.05	<LOQ	<LOQ	0.07
Hydrochlorothiazide	<LOQ	<LOQ	0.1	0.2
Mepivacaine	<LOQ	<LOQ	<LOQ	0.1
Tramadol	<LOQ	0.2	<LOQ	0.7
Venlafaxine	<LOQ	<LOQ	0.1	0.1
Daily consumption of tomatoes (kg) per adult (70 kg) to reach the TTC values				
4-AAA ^c	-	-	-	315
Caffeine ^c	-	114	150	-
Carbamazepine ^c	548	1800	1260	600
Carbamazepine epoxide ^d	3.5	-	-	2.5
Hydrochlorothiazide ^c	-	-	840	840
Mepivacaine ^c	-	-	-	1400
Tramadol ^d	-	67	-	22
Venlafaxine ^d	-	-	112	140
Daily consumption of tomatoes (kg) per toddler (12 kg) to reach the TTC values				
4-AAA ^c	-	-	-	54

Caffeine ^c	-	20	26	-
Carbamazepine ^c	94	309	216	103
Carbamazepine epoxide ^d	0.6	-	-	0.4
Hydrochlorothiazide ^c	-	-	144	144
Mepivacaine ^c	-	-	-	240
Tramadol ^d	-	67	-	4
Venlafaxine ^d	-	-	19	24

489 ^aS: sampling event; ^b<LOQ: concentration below the limit of quantification; ^ccompound classified
490 according to Munro *et al.* 1996;³⁵ ^dcompound classified according to Houeto *et al.* 2012.³⁷

491

492 Nevertheless, more studies are needed including the evaluation of exposure to other
493 hazards such as synergistic effects due to the addition of concentrations, mixtures of
494 compounds and the formation of metabolites and transformation products that may be
495 more toxic than the original compound. More information about OMCs identified in real
496 crop samples, agricultural procedures and the consideration of sensitive population
497 groups, should also be evaluated to conclude that reuse of RW in agriculture is a safe
498 approach.

499

500 **ABBREVIATIONS USED**

501 4-AAA: 4-acetyl-aminoantipyrine

502 4-FAA: 4-formyl-aminoantipyrine

503 APCI: Atmospheric pressure chemical ionization

504 ESI: Electrospray

505 GH: Greenhouse

506 IDA: Information dependent acquisition

507 LOQ: Limit of quantification

508 OMCs: Organic microcontaminants

509 PSA: Primary-secondary amine

510 RSD: Relative standard deviation

511 RW: Reclaimed water

512 SP: Soilless perlite culture
513 TTC: Toxicological threshold concern

514

515 **ASSOCIATED CONTENT**

516 **Supporting Information**

517 Information about experimental details: list of target analytes, LC-MS/MS details;
518 analytical method validation in tomato fruit and leaves information, OMCs found in RW
519 and agricultural soils, physico-chemical properties of compounds detected in samples
520 and extracted ion chromatograms and MS/MS spectra of the identified compounds by
521 suspect screening strategy (PDF).

522

523 **ACKNOWLEDGMENTS**

524 The authors would like to acknowledge the ALICE project (GA: 734560) “AcceLerate
525 Innovation in urban wastewater management for Cli-mate changE” supported by the
526 H2020-MSCA-2016. A.B. Martínez-Piernas gratefully acknowledges the Cooperation
527 agreement between the University of Almería and PSA-CIEMAT regarding financial
528 support for her PhD scholarship. P. Plaza-Bolaños acknowledges the University of
529 Almeria for her PhD research contract (Hipatia Program).

530

531 **REFERENCES**

- 532 (1) Lavrnić, S.; Zapater-Pereyra, M.; Mancini, M. L. Water Scarcity and Wastewater
533 Reuse Standards in Southern Europe: Focus on Agriculture. *Water. Air. Soil Pollut.*
534 **2017**, 228–251.
- 535 (2) Wu, X.; Conkle, J. L.; Ernst, F.; Gan, J. Treated Wastewater Irrigation: Uptake of
536 Pharmaceutical and Personal Care Products by Common Vegetables under Field

- 537 Conditions. *Environ. Sci. Technol.* **2014**, *48* (19), 11286–11293.
- 538 (3) Christou, A.; Karaolia, P.; Hapeshi, E.; Michael, C.; Fatta-Kassinos, D. Long-Term
539 Wastewater Irrigation of Vegetables in Real Agricultural Systems: Concentration
540 of Pharmaceuticals in Soil, Uptake and Bioaccumulation in Tomato Fruits and
541 Human Health Risk Assessment. *Water Res.* **2017**, *109*, 24–34.
- 542 (4) Pan, M.; Wong, C. K. C.; Chu, L. M. Distribution of Antibiotics in Wastewater-
543 Irrigated Soils and Their Accumulation in Vegetable Crops in the Pearl River
544 Delta, Southern China. *J. Agric. Food Chem.* **2014**, *62*, 11062–11069.
- 545 (5) Riemenschneider, C.; Al-Raggad, M.; Moeder, M.; Seiwert, B.; Salameh, E.;
546 Reemtsma, T. Pharmaceuticals, Their Metabolites, and Other Polar Pollutants in
547 Field-Grown Vegetables Irrigated with Treated Municipal Wastewater. *J. Agric.*
548 *Food Chem.* **2016**, *64* (29), 5784–5792.
- 549 (6) Vergine, P.; Salerno, C.; Libutti, A.; Beneduce, L.; Gatta, G.; Berardi, G.; Pollice,
550 A. Closing the Water Cycle in the Agro-Industrial Sector by Reusing Treated
551 Wastewater for Irrigation. *J. Clean. Prod.* **2017**, *164*, 587–596.
- 552 (7) Jörg E. Drewes, Uwe Hübner, Veronika Zhiteneva, S. K. *Characterization of*
553 *Unplanned Water Reuse in the EU*; 2017.
- 554 (8) *Spanish Royal Decree 1620/2007*; 2007; Vol. 294.
- 555 (9) *Proposal 2018/0169/COD*; 2018.
- 556 (10) Campos-Mañas, M. C.; Plaza-Bolaños, P.; Sánchez-Pérez, J. A.; Malato, S.;
557 Agüera, A. Fast Determination of Pesticides and Other Contaminants of Emerging
558 Concern in Treated Wastewater Using Direct Injection Coupled to Highly
559 Sensitive Ultra-High Performance Liquid Chromatography-Tandem Mass
560 Spectrometry. *J. Chromatogr. A* **2017**, *1507*, 84–94.
- 561 (11) Pan, M.; Chu, L. M. Fate of Antibiotics in Soil and Their Uptake by Edible Crops.

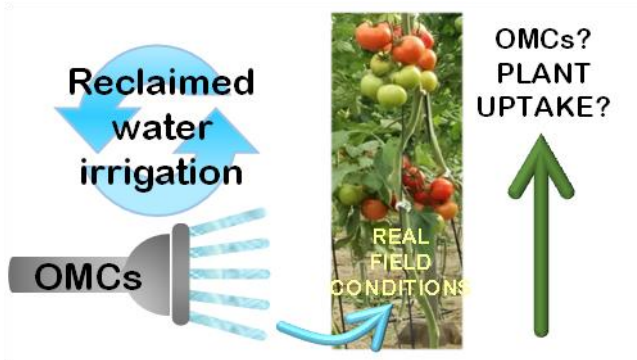
- 562 *Sci. Total Environ.* **2017**, 599–600, 500–512.
- 563 (12) Martínez-Piernas, A. B.; Plaza-Bolaños, P.; García-Gómez, E.; Fernández-Ibáñez,
564 P.; Agüera, A. Determination of Organic Microcontaminants in Agricultural Soils
565 Irrigated with Reclaimed Wastewater: Target and Suspect Approaches. *Anal.*
566 *Chim. Acta* **2018**, 1030, 115–124.
- 567 (13) Martínez-Piernas, A. B.; Polo-López, M. I.; Fernández-Ibáñez, P.; Agüera, A.
568 Validation and Application of a Multiresidue Method Based on Liquid
569 Chromatography-Tandem Mass Spectrometry for Evaluating the Plant Uptake of
570 74 Microcontaminants in Crops Irrigated with Treated Municipal Wastewater. *J.*
571 *Chromatogr. A* **2018**, 1534, 10–21.
- 572 (14) Picó, Y.; Alvarez-Ruiz, R.; Alfarhan, A. H.; El-Sheikh, M. A.; Alobaid, S. M.;
573 Barceló, D. Uptake and Accumulation of Emerging Contaminants in Soil and Plant
574 Treated with Wastewater under Real-World Environmental Conditions in the Al
575 Hayer Area (Saudi Arabia). *Sci. Total Environ.* **2019**, 652, 562–572.
- 576 (15) Calderón-Preciado, D.; Matamoros, V.; Savé, R.; Muñoz, P.; Biel, C.; Bayona, J.
577 M. Uptake of Microcontaminants by Crops Irrigated with Reclaimed Water and
578 Groundwater under Real Field Greenhouse Conditions. *Environ. Sci. Pollut. Res.*
579 **2013**, 20 (6), 3629–3638.
- 580 (16) Wu, X.; Ernst, F.; Conkle, J. L.; Gan, J. Comparative Uptake and Translocation of
581 Pharmaceutical and Personal Care Products (PPCPs) by Common Vegetables.
582 *Environ. Int.* **2013**, 60, 15–22.
- 583 (17) Carter, L. J.; Williams, M.; Martin, S.; Kamaludeen, S. P. B.; Kookana, R. S.
584 Sorption, Plant Uptake and Metabolism of Benzodiazepines. *Sci. Total Environ.*
585 **2018**, 628–629, 18–25.
- 586 (18) Dodgen, L. K.; Ueda, A.; Wu, X.; Parker, D. R.; Gan, J. Effect of Transpiration on

- 587 Plant Accumulation and Translocation of PPCP/EDCs. *Environ. Pollut.* **2015**, *198*,
588 144–153.
- 589 (19) Santiago, S.; Roll, D. M.; Ray, C.; Williams, C.; Moravcik, P.; Knopf, A. Effects
590 of Soil Moisture Depletion on Vegetable Crop Uptake of Pharmaceuticals and
591 Personal Care Products (PPCPs). *Environ. Sci. Pollut. Res.* **2016**, *23* (20), 20257–
592 20268.
- 593 (20) Malchi, T.; Maor, Y.; Tadmor, G.; Shenker, M.; Chefetz, B. Irrigation of Root
594 Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and
595 the Associated Human Health Risks. *Environ. Sci. Technol.* **2014**, *48* (16), 9325–
596 9333.
- 597 (21) Goldstein, M.; Shenker, M.; Chefetz, B. Insights into the Uptake Processes of
598 Wastewater-Borne Pharmaceuticals by Vegetables. *Environ. Sci. Technol.* **2014**,
599 *48* (10), 5593–5600.
- 600 (22) Prosser, R. S.; Sibley, P. K. Human Health Risk Assessment of Pharmaceuticals
601 and Personal Care Products in Plant Tissue Due to Biosolids and Manure
602 Amendments, and Wastewater Irrigation. *Environ. Int.* **2015**, *75*, 223–233.
- 603 (23) Paranychianakis, N. V.; Salgot, M.; Snyder, S. A.; Angelakis, A. N. Water Reuse
604 in EU States: Necessity for Uniform Criteria to Mitigate Human and
605 Environmental Risks. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45* (13), 1409–1468.
- 606 (24) Alcalde-Sanz, L.; Gawlik, B. M. *Minimum Quality Requirements for Water Reuse
607 in Agricultural Irrigation and Aquifer Recharge - Towards a Water Reuse
608 Regulatory Instrument at EU Level*; 2017.
- 609 (25) EFSA; WHO. *Review of the Threshold of Toxicological Concern (TTC) Approach
610 and Development of New TTC Decision Tree; Review of the Threshold of
611 Toxicological Concern (TTC) Approach and Development of New TTC Decision*

- 612 *Tree*; 2016.
- 613 (26) Cramer, G. M.; Ford, R. A.; Hall, R. L. Estimation of Toxic Hazard-A Decision
614 Tree Approach. *Food Cosmet. Toxicol.* **1976**, *16* (3), 255–276.
- 615 (27) Kroes, R.; Renwick, A. G.; Cheeseman, M.; Kleiner, J.; Mangelsdorf, I.; Piersma,
616 A.; Schilter, B.; Schlatter, J.; Van Schothorst, F.; Vos, J. G.; et al. Structure-Based
617 Thresholds of Toxicological Concern (TTC): Guidance for Application to
618 Substances Present at Low Levels in the Diet. *Food Chem. Toxicol.* **2004**, *42* (1),
619 65–83.
- 620 (28) Hu, X.; Zhou, Q.; Luo, Y. Occurrence and Source Analysis of Typical Veterinary
621 Antibiotics in Manure, Soil, Vegetables and Groundwater from Organic Vegetable
622 Bases, Northern China. *Environ. Pollut.* **2010**, *158*, 2992–2998.
- 623 (29) Riemenschneider, C.; Seiwert, B.; Goldstein, M.; Al-Raggad, M.; Salameh, E.;
624 Chefetz, B.; Reemtsma, T. An LC-MS/MS Method for the Determination of 28
625 Polar Environmental Contaminants and Metabolites in Vegetables Irrigated with
626 Treated Municipal Wastewater. *Anal. Methods* **2017**, *9*, 1273–1281.
- 627 (30) Riemenschneider, C.; Seiwert, B.; Moeder, M.; Schwarz, D.; Reemtsma, T.
628 Extensive Transformation of the Pharmaceutical Carbamazepine Following
629 Uptake into Intact Tomato Plants. *Environ. Sci. Technol.* **2017**, *51* (11), 6100–
630 6109.
- 631 (31) Gómez, M. J.; Sirtori, C.; Mezcua, M.; Fernández-Alba, A. R.; Agüera, A.
632 Photodegradation Study of Three Dipyrene Metabolites in Various Water Systems:
633 Identification and Toxicity of Their Photodegradation Products. *Water Res.* **2008**,
634 *42*, 2698–2706.
- 635 (32) Inoue, J.; Chamberlain, K.; Bromilow, R. H. Physicochemical Factors Affecting
636 the Uptake by Roots and Translocation to Shoots of Amine Bases in Barley. *Pestic.*

- 637 *Sci.* **1998**, *54*, 8–21.
- 638 (33) Miller, E. L.; Nason, S. L.; Karthikeyan, K. G.; Pedersen, J. A. Root Uptake of
639 Pharmaceuticals and Personal Care Product Ingredients. *Environ. Sci. Technol.*
640 **2016**, *50*, 525–541.
- 641 (34) Dewhurst, I.; Renwick, A. G. Evaluation of the Threshold of Toxicological
642 Concern (TTC) - Challenges and Approaches. *Regul. Toxicol. Pharmacol.* **2013**,
643 *65*, 168–177.
- 644 (35) Munro, I. C.; Ford, R. A.; Kennepohl, E.; Sprenger, J. G. Thresholds of
645 Toxicological Concern Based on Structure-Activity Relationships. *Drug Metab.*
646 *Rev.* **1996**, *28* (1–2), 209–217.
- 647 (36) Stanard, B.; Dolan, D. G.; Hanneman, W.; Legare, M.; Bercu, J. P. Threshold of
648 Toxicological Concern (TTC) for Developmental and Reproductive Toxicity of
649 Anticancer Compounds. *Regul. Toxicol. Pharmacol.* **2015**, *72* (3), 602–609.
- 650 (37) Houeto, P.; Carton, A.; Guerbet, M.; Mauclaire, A. C.; Gatignol, C.; Lechat, P.;
651 Masset, D. Assessment of the Health Risks Related to the Presence of Drug
652 Residues in Water for Human Consumption: Application to Carbamazepine.
653 *Regul. Toxicol. Pharmacol.* **2012**, *62* (1), 41–48.
- 654 (38) Tomato Worldwide Consumption <http://www.fao.org/faostat/en/#data/CC>.
655

656 **Table of Contents**



657