Metabolism of Pharmaceutical and Personal Care Products by Carrot Cell Cultures

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Abstract

With the increasing use of treated wastewater and biosolids in agriculture, residues of pharmaceutical and personal care products (PPCPs) in these reused resources may contaminate food produce via plant uptake, constituting a route for human exposure. Although various PPCPs have been reported to be taken up by plants in laboratories or under field conditions, at present little information is available on their metabolism in plants. In this study, we applied carrot cell cultures to investigate the plant metabolism of PPCPs. Five phase I metabolites of carbamazepine were identified and the potential metabolism pathways of carbamazepine were proposed. We also used the carrot cell cultures as a rapid screening tool to initially assess the metabolism potentials of 18 PPCPs. Eleven PPCPs, including acetaminophen, caffeine, meprobamate, primidone, atenolol, trimethoprim, DEET, carbamazepine, dilantin, diazepam, and triclocarban, were found to be recalcitrant to metabolism. The other 7 PPCPs, including triclosan, naproxen, diclofenac, ibuprofen, gemfibrozil, sulfamethoxazole, and atorvastatin, displayed rapid metabolism, with 0.4–47.3% remaining in the culture at the end of the experiment. Further investigation using glycosidase hydrolysis showed that 1.3–20.6% of initially spiked naproxen, diclofenac, ibuprofen, and gemfibrozil were transformed into glycoside conjugates. Results from this study showed that plant cell cultures may be a useful tool for initially exploring the potential metabolites of PPCPs in plants as well as for rapidly screening the metabolism potentials of a variety of PPCPs or other emerging contaminants, and therefore may be used for prioritizing compounds for further comprehensive evaluations.

1. Introduction

Treated wastewater and biosolids are widely used for agricultural irrigation and soil fertilization, respectively, especially in regions next to large metropolitan areas. Studies show that many pharmaceutical and personal care products (PPCPs) are not completely removed during wastewater treatment and appear ubiquitously in treated wastewater or biosolids (Clarke and Smith, 2011; Kim et al., 2007; Vanderford and Snyder, 2006). Through irrigation or biosolid amendment, PPCPs may contaminate food produce via plant uptake, leading to potential human exposure (Wu et al., 2015). In greenhouse and field studies, PPCPs were found to be taken up and accumulated in plants (Goldstein et al., 2014; Malchi et al., 2014; Wu et al., 2013, 2014). However, an apparent bottleneck in understanding the health risk from these practices is the sheer number of PPCPs present in treated wastewater or biosolids. As such, an urgent task is to establish a short list of “priority” PPCPs that have the highest potential for plant accumulation, so that monitoring and risk assessment may become better targeted and focused.

The levels of PPCPs in plants depend not only on their uptake and translocation, but also on their metabolism in plants. Studies have shown that xenobiotics, typically herbicides such as atrazine and metribuzin, may undergo metabolism pathways in plants that can be divided into three phases (Coleman et al., 1997; Sandermann, 1992). During phase I metabolism, xenobiotics usually undergo hydroxylation, hydrolysis or other types of oxidation, producing intermediates with increased polarity or reactivity (Coleman et al., 1997). Phase II metabolites are the conjugates of parent compound or phase I metabolites with polar biomolecules such as amino acids, glutathione, or carbohydrates (Coleman et al., 1997). For PPCPs, similar

metabolism pathways may also be expected after they enter a plant. Phase I/II metabolites of acetaminophen, diclofenac, carbamazepine, and triclosan have been detected in plants (Goldstein et al., 2014; Huber et al., 2009, 2012; Macherius et al., 2012). To date, the PPCPs that have been examined for their metabolism in plants are limited, likely due to the fact that whole plant experiments are labor-intensive and identification of trace levels of PPCPs, especially their metabolites, in plant tissues often presents a great analytical challenge due to the presence of plant lipids, pigments, and other materials that may result in severe matrix interferences.

Plant cell cultures, which contain simpler matrices and are more easily manipulated than whole plants, have been used as model systems to investigate the metabolism of various xenobiotics, such as 4-nonylphenol (Bokern et al., 1996), bisphenol A (Schmidt and Schuphan, 2002), polycyclic aromatic hydrocarbons (Huckelhoven et al., 1997; Kolb and Harms, 2000), and fungicides (Myung et al., 2013). So far only 3 PPCPs have been studied for their plant metabolism using plant cell cultures. Macherius et al. (2012) studied the metabolism of triclosan and triclocarban in carrot cell cultures and identified 8 phase II metabolites of triclosan. Huber et al. (2012) exposed diclofenac to horseradish root cell cultures and identified one phase I metabolite (4-OH-diclofenac) and one phase II metabolite (4-OH-diclofenac glucopyranoside). Moreover, the metabolism of xenobiotics in plant cell cultures may resemble that in plants (Kolb and Harms, 2000; Macherius et al., 2012). It was reported that metabolites of fluoranthene that were identified in plant cell cultures were also formed in both roots and shoots of intact plants (Kolb and Harms, 2000). Based on these advantages as indicated by earlier researchers (Bokern et al., 1996; Kolb and Harms, 2000;

Schmidt and Schuphan, 2002), the plant cell cultures may serve as a useful tool to study the plant metabolism of various PPCPs.

Although metabolism rates of xenobiotics may differ in cell cultures from different plant species (Kolb and Harms, 2000), the major metabolites in different plant species were reported to be similar (Goldstein et al., 2014; Malchi et al., 2014). In this study, the carrot callus tissues were easily cultivated in the lab and were chosen as the representative plant cells. We applied carrot cell suspension cultures to explore the metabolites of an important PPCP, carbamazepine, which is one of the most frequently detected PPCPs in wastewater effluent and biosolids (Zhang et al., 2008). Moreover, carbamazepine has been proven to have high potential for plant uptake (Shenker et al., 2011; Wu et al., 2013) and was widely detected in crops receiving treated wastewater irrigation or biosolids application (Wu et al., 2010, 2014; Malchi et al., 2014). We also used the cultures to rapidly screen and initially assess the metabolism potentials of 18 PPCPs encompassing a wide range of physicochemical properties. Glycoside conjugates (phase II metabolites) of these PPCPs were further investigated following enzymatic hydrolysis. Results from this study not only provide novel information on metabolism of the selected PPCPs in plants, but also offer a rapid and inexpensive option for evaluating the plant metabolism of other emerging contaminants.

2. Materials and methods

2.1 Chemicals and enzymes

Acetaminophen, caffeine, meprobamate, atenolol, trimethoprim, carbamazepine, diazepam, gemfibrozil and *N,N*-diethyl-*meta*-toluamide (DEET) were purchased from

Sigma-Aldrich (St. Louis, MO). Primidone was obtained from Spectrum Chemical (Gardena, CA). Sulfamethoxazole was provided by MP Biomedicals (Solon, OH). Dilantin, diclofenac and triclocarban were from TCI America (Portland, OR). Naproxen, ibuprofen and triclosan were from Alfa Aesar (Ward Hill, MA). (3*S*, 5*S*)-Atorvastatin sodium salt was from Santa Cruz Biotechnology (Santa Cruz, CA). The corresponding deuterated standards of these 18 PPCPs, which were used as surrogates for analysis, were purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada), except for diazepam-*d*₅, which was purchased from Cerilliant (Round Rock, TX).

A total of 8 carbamazepine metabolites were purchased for identification of transformation intermediates. 10,11-Dihydro-10,11-epoxy carbamazepine (carbamazepine-epoxide) was purchased from Sigma-Aldrich (St. Louis, MO). The standards of rac *trans*-10,11-dihydro-10,11-dihydroxy carbamazepine (carbamazepine-diol), 10-hydroxy carbamazepine (10-OH-carbamazepine), and 3-hydroxy carbamazepine (3-OH-carbamazepine) were obtained from TRC Inc. (Toronto, Ontario, Canada). Acridine and 5H-dibenzo[b,f]azepine were from Santa Cruz Biotechnology (Santa Cruz, CA). 9(10H)-acridone was from TCI (Tokyo, Japan). 10,11-Dihydrocarbamazepine was obtained from Alfa Aesar (Ward Hill, MA).

Two glycosidases, α -glycosidase (from *Saccharomyces cerevisiae*) and β -glycosidase (from almonds), were purchased from Sigma-Aldrich and used for enzymatic hydrolysis. The solvents used in this study were from Fisher (Fair Lawn, NJ). Ultrapure water (at 18.2 M Ω • cm resistivity) was produced using a Barnstead E-Pure water purification system (Thermo Scientific, Dubuque, IA).

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126 *2.2 Metabolism experiments with carrot cell cultures*

127 *2.2.1 Cultivation of carrot callus tissue*

128 The carrot callus tissue, which was used for the preparation of carrot cell suspension
129 culture, was cultivated in the laboratory based on the method reported by Mustafa et al.
130 (2011). Seeds of carrot (Scarlet Nantes) were purchased from a local nursery. The seeds were
131 surface sterilized by immersion in 70% ethanol for 10 s, followed by three washes using
132 sterile distilled water and immersion in 50% bleach for 15 min. The seeds were subsequently
133 rinsed several times with sterile distilled water, and then cultured aseptically on Murashige
134 and Skoog solid media (Mustafa et al., 2011; Supplementary Material) at 25 °C in the dark
135 until emergence. The seedlings were cut into small segments, transferred to fresh Murashige
136 and Skoog solid media, and grown under the same conditions. The primary callus rose on
137 stem segments and was selected for further cultivation.

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139 *2.2.2 Incubation of carbamazepine in carrot cell cultures*

140 The carrot cell suspension culture was prepared by measuring 0.4 g of fresh carrot callus
141 tissue into an Erlenmeyer flask containing 20 ml of Murashige and Skoog liquid media
142 (Supplementary Material) at an initial cell density of 20 mg/ml. The stock solution of
143 carbamazepine was prepared in DMSO at 50 mg/L. An aliquot of 0.2 ml of stock solution was
144 spiked into the cell culture to yield an initial concentration of 0.5 mg/L. The final
145 concentration of DMSO in the culture was 1% (v/v). The carrot cell culture was grown on a
146 rotary shaker (180 rpm) in the dark at 25 °C.

An aliquot of the culture (250 µl) was withdrawn at 4, 10, 15, 22 day for analyzing carbamazepine and its metabolites in the aqueous phase. The withdrawn culture was diluted to 500 µl with methanol and injected directly into an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) system (Waters, Milford, MA) after filtration through a polytetrafluoroethylene (PTFE) membrane (13 mm, 0.2 µm, Millipore). In this study, all treatments were performed in 3 replicates with control blanks, which contained the same medium and chemicals as the treatments but without carrot cells.

2.2.3 Incubation of multiple PPCPs in carrot cell cultures

The carrot cell cultures and stock solution of mixed PPCPs (50 mg/L) were prepared the same as described above. The PPCP mixtures were added to the carrot cell cultures to reach a final concentration of 0.5 mg/L for each PPCP. The incubation conditions were also the same as above. An aliquot of the culture (250 µl) was withdrawn at 0, 1, 3, 4, 17, 24, and 90 h for the analysis of remaining PPCP levels in the aqueous phase. The withdrawn culture was diluted to 500 µl with methanol and injected directly into UPLC-MS/MS system after filtration through a PTFE membrane.

At the end of the incubation (90 h), cell tissues were separated by centrifugation at 3000 rpm for 30 min, and then freeze-dried. The dried cell tissues were spiked with mixed deuterated PPCPs (200 ng for triclosan-*d*₃, and 50 ng/each for other deuterated PPCPs), and then extracted with 10 ml methanol in a sonication bath for 20 min, followed by centrifugation at 3000 rpm for 20 min. The supernatant was decanted into another container and the residue was extracted two more times using fresh methanol. The combined

supernatant was dried under nitrogen gas at room temperature and recovered in 1.0 ml methanol. High-speed centrifugation or filtration was used to remove suspended cell tissues before UPLC-MS/MS analysis.

2.2.4 Enzymatic hydrolysis

The method for enzymatic hydrolysis was modified based on the method reported by Kolb and Harms (2000). After 90-h incubation, an aliquot (100 µl) of the remaining culture was withdrawn and its aqueous phase (obtained from centrifugation) was subjected to enzymatic hydrolysis to deconjugate the glycoside conjugates. An aliquot of 100 µl cell extracts (as described above) was evaporated to near dryness under nitrogen gas, and then re-dissolved in 100 µl of sodium acetate buffer (100 mM; pH=5.0). The culture aqueous phase or cell extracts were then added with 100 µl of the enzyme solution (20 U/ml, in sodium acetate buffer) and 100 µl of sodium acetate buffer. The mixtures were then incubated at 30 °C for 24 h. The reaction was stopped by adding 200 µl methanol, and the mixtures were injected directly into UPLC-MS/MS system for chemical analysis.

2.3 LC-MS/MS analysis

Samples were analyzed using a Waters ACQUITY UPLC system in combination with Waters Micromass tandem quadrupole detector equipped with electrospray ionization (ESI). Chromatographic separation of compounds was performed at 40 °C using an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm particle size, Waters).

For the analysis of carbamazepine and its metabolites, 0.1% formic acid in water and

methanol were used as mobile phase A and B, respectively. The following gradient program (with respect to mobile phase B) was used: 0–0.5 min, 10 to 40% B; 0.5–8 min, 40 to 100% B; 8–9 min, 100% B; and 9–11 min, re-equilibration with 10% B. The flow rate was 0.2 ml/min, and the sample injection volume was 5 μ l. Data acquisition was performed in the positive ESI mode. The optimized MS parameters were as follows: source temperature, 120 $^{\circ}$ C; desolvation temperature, 350 $^{\circ}$ C; capillary voltage, 3.2 kV; cone voltage, 30 V; desolvation gas flow, 600 L h⁻¹ and cone gas flow, 50 L h⁻¹. The collision gas (Argon, 99.999%) flow in the collision cell was kept at 0.2 ml min⁻¹. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode and the optimized parameters are listed in Table S1 (Supplementary Material). All data were acquired and processed using the MassLynx 4.1 software. For the analysis of 18 PPCPs, the LC conditions and MS parameters may be found in Wu et al. (2012).

3. Results and Discussion

3.1 Metabolism pathways of carbamazepine in carrot cell cultures

In this study, we applied carrot cell cultures to investigate potential phase I metabolites of a typical PPCP, carbamazepine, during a 22-d incubation. The results showed that only a small portion of carbamazepine was metabolized by carrot cells, with >95% carbamazepine still remaining in the aqueous phase of the culture at the end of the experiment. Five metabolic intermediates of carbamazepine were identified using authentic standards (Figure 1), and their amounts were found to increase during the incubation (Figure 2), indicating that carbamazepine was slowly metabolized. Among the identified metabolites,

3-OH-carbamazepine, 10-OH-carbamazepine, and 10,11-dihydrocarbamazepine have not been reported in plants. Based on these metabolites, a tentative phase I metabolism pathway map was given for carbamazepine (Figure 3). Similar to the pathways in human body (Breton et al., 2005), the main metabolism route of carbamazepine in carrot cell cultures was the formation of carbamazepine-epoxide, which was further converted into carbamazepine-diol (Figure 3). A second metabolism route was hydroxylation, leading to the formation of hydroxylated compounds such as 3-OH-carbamazepine and 10-OH-carbamazepine (Figure 3). Different from the pathways in human body, in which formation of 5H-dibenzo[b,f]azepine was considered as the third minor route of metabolism (Breton et al., 2005), 5H-dibenzo[b,f]azepine was not detected in this study (Figure 1). Instead, 10,11-dihydrocarbamazepine was observed at trace levels (Figures 1 and 2).

It was reported that the metabolism of xenobiotics, e.g., fluoranthene, in plant cell cultures resembled that in plant roots, while higher amounts of metabolites existed in the shoots than in the roots (Kolb and Harms, 2000). Similarly, the metabolism of carbamazepine in carrot cell cultures in this study was close to its metabolism in the roots of sweet potato and carrot, in which a small fraction of carbamazepine taken up by the plants was metabolized and carbamazepine-epoxide was the dominant metabolite (Malchi et al., 2014). PPCPs may be more extensively metabolized in plant leaves and fruits than in plant cell cultures. In the leaves of tomato, cucumber, sweet potato, and carrot, and fruits of cucumber and tomato, carbamazepine may be significantly metabolized and transformed into carbamazepine-epoxide and carbamazepine-diol (Goldstein et al., 2014; Malchi et al., 2014), both of which were also found to be the main metabolites of carbamazepine in carrot cell

cultures in this study, suggesting that the major metabolic pathway of carbamazepine in plant cell cultures were similar to that in plant leaves and fruits. This suggests that the plant cell cultures can serve as a simple, rapid tool to study the metabolism of PPCPs or other emerging contaminants in plants.

3.2 Dissipation of PPCPs in carrot cell cultures

The metabolic stabilities of 18 PPCPs were measured by observing their disappearance in the culture throughout the incubation. During the 90-h incubation, little or slow dissipation in the aqueous phase of cultures was observed for the following 10 PPCPs: trimethoprim, acetaminophen, atenolol, diazepam, carbamazepine, dilantin, DEET, primidone, caffeine, and meprobamate (Figure S1, Supplementary Material). After 90 h, a majority of these PPCPs (77.3–102.9% of the initially spiked amounts, Table 1) still remained in the aqueous phase, and the fractions that were associated with cell tissues were 4.1–21.4% (Table 1). Although triclocarban was found rapidly depleted in the aqueous phase of the culture, with only a small fraction (< 4%) remaining in the aqueous phase after incubation, it was mostly detected in the cell tissues (Table 1), indicating that the loss of triclocarban from the aqueous phase was mainly due to sorption and uptake by carrot cells. For these 11 PPCPs, most of the initially spiked amounts (88.3–125.2%, Table 1) existed in the unaltered parent form in the whole culture (i.e., both aqueous phase and cell tissues) at the end of incubation, probably due to their recalcitrance to metabolism and/or their poor association with the cells (except triclocarban). This observation was in agreement with Macherius et al. (2012), who also reported that no appreciable metabolism occurred with triclocarban in carrot cell cultures.

On the other hand, triclosan, naproxen, diclofenac, ibuprofen, gemfibrozil, sulfamethoxazole, and atorvastatin displayed rapid dissipation in both aqueous phase of the culture and cell tissues. In the aqueous phase, triclosan disappeared at the fastest rate, with an estimated 50% dissipation time (DT_{50}) of only 0.2 h (Figure 4). This rapid dissipation of triclosan was similar to the findings in Macherius et al. (2012), where more than 75% of triclosan was depleted in a carrot cell culture medium within 2 h. Naproxen was also eliminated at a rapid rate, with a DT_{50} of only 0.8 h. In addition, diclofenac, ibuprofen, and gemfibrozil also disappeared relatively quickly, with DT_{50} values of 1.4 h, 2.2 h, and 2.8 h, respectively. Sulfamethoxazole and atorvastatin dissipated at a relatively slower rate, with DT_{50} values estimated at 7.7 h and 17 h, respectively (Figure 4). These 7 PPCPs exhibited moderate to strong metabolism potentials in carrot cell cultures, with only 0.4–47.3% of the initially spiked amounts remaining in the whole culture at the end of the experiment (Table 1). The disappearance of PPCPs in the whole culture may be attributed to the following potential routes: adsorption to the inner wall of the container; abiotic degradation; and/or metabolism by carrot cells. The carrot cells were cultured under sterile conditions, and therefore microbial degradation, if any, should be negligible. As shown in Figure 4, six PPCPs, i.e., triclosan, naproxen, diclofenac, ibuprofen, gemfibrozil, and sulfamethoxazole showed little depletion in the aqueous phase of control groups (without carrot cells), indicating that they barely adsorbed to the inner wall of the container and were stable in the medium. Therefore, their depletion in the whole culture was mainly due to the metabolism by carrot cells. The metabolism potentials of triclosan, diclofenac, and naproxen have been observed in intact plants. For example, triclosan was found to be rapidly metabolized in carrots after being taken

up, and the amounts of triclosan conjugates were estimated to be five-times that of triclosan (Macherius et al., 2012). Similarly, diclofenac was reported to undergo rapid metabolism in plants (Huber et al., 2012), and the majorities of diclofenac and naproxen that were taken up by plants were converted to unknown non-extractable residues (Dodgen et al., 2013). On the other hand, about 30% of atorvastatin disappeared in the aqueous phase in control groups at the end of the experiment (Figure 4), indicating that atorvastatin may partly adsorb to the container surfaces and/or undergo abiotic degradation. In our previous greenhouse and field studies, these 7 PPCPs were generally found to display low accumulation in vegetables (Wu et al., 2013, 2014). Results from this study suggested that the limited plant accumulation of these PPCPs may be partly due to their rapid metabolism in plants.

It was noted that the PPCPs which contained functional groups like carboxyl group (naproxen, diclofenac, ibuprofen, gemfibrozil, and atorvastatin), phenolic hydroxyl group (triclosan), or *N*-aryl amine group (sulfamethoxazole) were generally prone to metabolism in carrot cell cultures (Figure 4). Studies on metabolism of herbicides in plants revealed that herbicides containing carboxyl (e.g., 2,4-D), phenolic hydroxyl (e.g., 6-OH-bentazon), or *N*-aryl amine groups (e.g., chloramben) might formed sugar conjugates (phase II metabolites) such as glucose esters or glucosides via these functional groups (Hall et al., 2000). Therefore, the PPCPs containing these functional groups may probably undergo similar metabolism pathways and also formed sugar conjugates in plant cell cultures and intact plants.

3.3 Glycosylation of PPCPs in carrot cell cultures

In this study, two glycosidases, β -glycosidase and α -glycosidase, were used to hydrolyze

the glycoside conjugates of PPCPs that may have been formed during incubation. After enzymatic hydrolysis, increased amounts of naproxen were found in cultures as compared to those prior to the enzymatic treatment. As shown in Figure 5A, naproxen couldn't be detected by LC-MS/MS in the aqueous phase of the culture after 90-h incubation. After hydrolysis with glycosidases, a significant peak of naproxen was observed (Figure 5B and 5C), indicating the formation of glycoside conjugates of naproxen in carrot cell cultures. Similar glycoside conjugates were also observed for diclofenac, ibuprofen, and gemfibrozil, but with lower amounts than naproxen conjugates (Table 2). Hydrolysis with β -glycosidase produced much higher amounts of parent compounds than hydrolyzing with α -glycosidase (Table 2), suggesting that the glycosides were mainly in the form of β -glycoside conjugates. Glycoside conjugates, however, only accounted for 20.6%, 3.5%, 3.32%, and 1.28% of the initially spiked amounts of naproxen, diclofenac, ibuprofen, and gemfibrozil, respectively (Table 2), and no parent compound after hydrolysis was detected for other PPCPs. This suggested that the amounts of these PPCPs generated from glycosidase hydrolysis were low under the experimental condition in this study, and/or other metabolites may have been formed in the carrot cell cultures. For example, in the cell cultures of horse radish, Huber et al. (2012) reported that diclofenac was first transformed into 4-OH-diclofenac (phase I metabolite) and conjugated subsequently forming 4-OH-diclofenac-glucopyranoside (phase II metabolite). Advanced techniques such as time-of-flight (TOF) MS, high resolution MS, and nuclear magnetic resonance spectroscopy (NMR) may be needed for further identification and verification of potential metabolites, as reported by Macherius et al. (2012) and Myung et al. (2013).

In this study, we applied carrot cell suspension cultures as a tool to identify potential phase I metabolites of carbamazepine in plants. We also used the carrot cell cultures to rapidly screen the metabolism potentials of 18 PPCPs. Seven PPCPs were found to be comparably easy to be metabolized in the culture, in which naproxen, diclofenac, ibuprofen, and gemfibrozil may form phase II metabolites such as glycosides. Compared to whole plants, the use of plant cells is less expensive, requires much less space and simpler sample preparation procedures, and has much shorter experimental cycles. These characteristics enable the use of plant cell cultures for high-content screening of PPCPs (and other emerging contaminants) for their metabolism potentials, and exploring their phase I and II metabolites. Compounds with recalcitrance to plant metabolism, or with a potential to form biologically active metabolites, may then be selected as candidates for more comprehensive evaluation through greenhouse or field-scale experiments, thus expediting risk assessment of PPCPs and other emerging contaminants in a cost-effective manner.

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Reference

- Bokern, M., Nimtz, M., Harms, H.H., 1996. Metabolites of 4-n-nonylphenol in wheat cell suspension cultures. *J. Agric. Food Chem.* 44, 1123-1127.
- Breton, H., Cociglio, M., Bressolle, F., Peyriere, H., Blayac, J.P., Hillaire-Buys, D., 2005. Liquid chromatography-electrospray mass spectrometry determination of carbamazepine, oxcarbazepine and eight of their metabolites in human plasma. *J.Chromatogr. B* 828, 80-90.
- Clarke, B.O., Smith, S.R., 2011. Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ. Int.* 37, 226-247.
- Coleman, J.O.D., BlakeKalff, M.M.A., Davies, T.G.E., 1997. Detoxification of xenobiotics by plants: Chemical modification and vacuolar compartmentation. *Trends in Plant Sci.* 2, 144-151.
- Dodgen, L.K., Li, J., Parker, D., Gan, J.J., 2013. Uptake and accumulation of four PPCP/EDCs in two leafy vegetables. *Environ. Pollut.* 182, 150-156.
- Goldstein, M., Shenker, M., Chefetz, B., 2014. Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. *Environ. Sci. Technol.* 48, 5593-5600.
- Hall, J.C., Wickenden, J.S., Yau, K.Y.F., 2000. Biochemical conjugation of pesticides in plants and microorganisms: An overview of similarities and divergences, in: Hall, J.C., Hoagland, R.E., Zablotowicz, R.M (Eds.), *Pesticide Biotransformation in Plants and Microorganisms*. ACS Symposium Series; American Chemical Society, Washington, DC, pp. 89-118.
- Huber, C., Bartha, B., Harpaintner, R., Schroeder, P., 2009. Metabolism of acetaminophen (paracetamol) in plants-two independent pathways result in the formation of a glutathione and a glucose conjugate. *Environ. Sci. Pollut. Res.* 16, 206-213.
- Huber, C., Bartha, B., Schroeder, P., 2012. Metabolism of diclofenac in plants - Hydroxylation is followed by glucose conjugation. *J. Hazard. Mater.* 243, 250-256.
- Huckelhoven, R., Schuphan, I., Thiede, B., Schmidt, B., 1997. Biotransformation of pyrene by cell cultures of soybean (*Glycine max L*), wheat (*Triticum aestivum L*), jimsonweed (*Datura stramonium L*), and purple foxglove (*Digitalis purpurea L*). *J. Agric. Food Chem.* 45, 263-269.
- Kim, S.D., Cho, J., Kim, I.S., Vanderford, B.J., Snyder, S.A., 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. *Water Res.* 41, 1013-1021.

- Kolb, M., Harms, H., 2000. Metabolism of fluoranthene in different plant cell cultures and intact plants. *Environ. Toxicol. Chem.* 19, 1304-1310.
- Macherius, A., Eggen, T., Lorenz, W., Moeder, M., Ondruschka, J., Reemtsma, T., 2012. Metabolization of the bacteriostatic agent triclosan in edible plants and its consequences for plant uptake assessment. *Environ. Sci. Technol.* 46, 10797-10804.
- Malchi, T., Maor, Y., Tadmor, G., Shenker, M., Chefetz, B., 2014. Irrigation of root vegetables with treated wastewater: Evaluating uptake of pharmaceuticals and the associated human health risks. *Environ. Sci. Technol.* 48, 9325-9333.
- Mustafa, N.R., de Winter, W., van Iren, F., Verpoorte, R., 2011. Initiation, growth and cryopreservation of plant cell suspension cultures. *Nat. Protoc.* 6, 715-742.
- Myung, K., Williams, D.A., Xiong, Q., Thornburgh, S., 2013. Metabolism of strobilurins by wheat cell suspension cultures. *J. Agric. Food Chem.* 61, 47-52.
- Sandermann, H., 1992. Plant metabolism of xenobiotics. *Trends Biochem. Sci.* 17, 82-84.
- Schmidt, B., Schuphan, I., 2002. Metabolism of the environmental estrogen bisphenol A by plant cell suspension cultures. *Chemosphere.* 49, 51-59.
- Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B., 2011. Uptake of carbamazepine by cucumber plants — a case study related to irrigation with reclaimed wastewater. *Chemosphere* 82, 905-910.
- Vanderford, B., Snyder, S.A., 2006. Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. *Environ. Sci. Technol.* 40, 7312-7320.
- Wu, C., Spongberg, A.L., Witter, J.D., Fang, M., Czajkowski, K.P., 2010. Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated Water. *Environ. Sci. Technol.* 44, 6157-6161.
- Wu, X., Conkle, J.L., Gan, J., 2012. Multi-residue determination of pharmaceutical and personal care products in vegetables. *J. Chromatogr. A* 1254, 78-86.
- Wu, X., Ernst, F., Conkle, J.L., Gan, J., 2013. Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables. *Environ. Int.* 60, 15-22.
- Wu, X., Conkle, J.L., Ernst, F., Gan, J., 2014. Treated wastewater irrigation: Uptake of pharmaceutical and personal care products by common vegetables under field conditions. *Environ. Sci. Technol.* 48, 11286-11293.

428 Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and
429 personal care products from recycled water and biosolids: a review. *Sci. Total Environ.* 536,
430 655-666.

431
432 Zhang, Y.J., Geissen, S.U., Gal, C., 2008. Carbamazepine and diclofenac: Removal in
433 wastewater treatment plants and occurrence in water bodies. *Chemosphere* 73, 1151-1161.

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Figure Legends

Figure 1. LC-MS/MS chromatograms of carbamazepine metabolites in methanol (left) and in the aqueous phase of carrot cell cultures after 22-day incubation (right).

Figure 2. Amounts of carbamazepine metabolites in the aqueous phase of carrot cell cultures during incubation.

Figure 3. Possible metabolism pathways of carbamazepine in carrot cell cultures.

Figure 4. Dissipation of PPCPs in the aqueous phase of cultures without carrot cells (Δ) and with 20 mg/ml of carrot cells ($\bullet$). C_i : chemical concentration at sampling time; C_0 : chemical concentration at initial time.

Figure 5. LC-MS/MS chromatogram of naproxen in the aqueous phase of carrot cell cultures after 90-h incubation: (A) without enzyme treatment; (B) after β -glycosidase treatment; (C) after α -glycosidase treatment.

454 Table 1. Distribution of PPCPs in carrot cell cultures after 90-h incubation.
 455

Compound	Remaining proportion (%)		
	Aqueous phase	Cell tissues	Total
Triclocarban	3.7±0.6	121.5±12.6	125.2±13.8
Trimethoprim	102.9±6.2	9.5±0.8	112.4±5.4
Acetaminophen	94.5±11.7	13.4±7.3	107.9±12.4
Atenolol	97.4±8.0	6.6±0.2	104.0±7.8
Diazepam	86.8±8.8	17.1±1.5	103.9±10.2
Carbamazepine	91.6±5.6	7.8±0.1	99.4±5.5
Dilantin	77.3±5.0	21.4±2.6	98.7±7.6
DEET	91.2±7.5	5.8±1.2	97.0±8.6
Primidone	77.7±13.3	13.4±0.7	91.1±14.1
Caffeine	84.2±11.7	4.1±3.1	88.3±8.6
Meprobamate	81.6±4.1	6.7±1.0	88.3±3.2
Atorvastatin	23.9±4.2	23.4±0.8	47.3±5.0
Triclosan	4.5±1.2	40.0±2.5	44.5±3.7
Sulfamethoxazole	1.0±0.02	7.3±6.1	8.3±6.1
Naproxen	n.d.	2.8±1.0	2.8±1.0
Diclofenac	0.9±0.03	0.8±0.2	1.7±0.2
Ibuprofen	0.1±0.04	0.5±0.2	0.6±0.1
Gemfibrozil	0.1±0.01	0.3±0.06	0.4±0.05

456 n.d.: not detected.
 457

Table 2. PPCPs in carrot cell cultures after enzymatic hydrolysis ^a.

Enzyme		Proportion of initial spiked amounts (%)			
		Naproxen	Diclofenac	Ibuprofen	Gemfibrozil
β-Glucosidase	Aqueous phase	18.7±0.1	2.3±0.02	3.3±0.1	1.1±0.02
	Cell tissues	0.8±0.4	0.4±0.1	0.02±0.1	0.07±0.002
α-Glucosidase	Aqueous phase	1.1±0.01	0.7±0.002	n.d.	0.09±0.01
	Cell tissues	n.d.	0.1±0.03	n.d.	0.02±0.01
Total		20.6±0.2	3.5±0.04	3.32±0.12	1.28±0.04

^a The amounts in the culture before enzyme treatment had already been subtracted.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.