*Manuscript
Click here to view linked References

# Metabolism of oxybenzone in a hairy root culture: perspectives for phytoremediation of a widely used sunscreen agent

Feiran Chen[1], Christian Huber[1], Robert May[2], Peter Schröder[1]*

[1] Helmholtz Zentrum München, GmbH, German Research Center for Environmental Health, Research Unit Environmental Genomics, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany

[2] Labor Dr. Spranger & Partner, Lindberghstraße 9-13, 85051 Ingolstadt, Germany

* corresponding author: peter.schroeder@helmholtz-muenchen.de

## Abstract

Oxybenzone (OBZ), known as Benzophenone-3, is a commonly detected UV filter in sun tans and skin protectants, which enters aquatic systems mostly directly due to recreational activities or indirectly through wastewater treatment plants discharges. To study the potential degradation capacity of plants for OBZ in phytotreatment, a well-established model system of a hairy root culture (*Armoracia rusticana*) was treated with OBZ. More than 20% of the initial OBZ (100 μM) was eliminated from the culture medium by hairy roots after just 3 h of exposure. Two metabolites were identified as oxybenzone-glucoside (OBZ-Glu) and oxybenzone-(6-*O*-malonyl)-glucoside (OBZ-Mal-Glu) by LC-MS/MS and TOF-MS. The formation of these metabolites was further confirmed by enzymatic synthesis, as well as enzymatic and alkaline hydrolysis. Incubation with *O*-glucosyltransferase (*O*-GT) extracted from roots formed OBZ-Glu; whereas β-D-Glucosidase hydrolyzed OBZ-Glu. On the other hand, alkaline hydrolysis led to a cleavage of OBZ-Mal-Glu and yielded OBZ-Glu. In the hairy root culture system, an excretion of OBZ-Glu into the growth medium was observed while the corresponding OBZ-Mal-Glu remained stored in the root cells over the incubation time. In conclusion we propose that the pathway of oxybenzone in plants involves an initial conjugation with glucose to form OBZ-Glu which is followed by a malonylation to form OBZ-Mal-Glu. To our best knowledge this first finding presenting the potential of plants to degrade benzophenone type UV filters in phytoremediation based wastewater facilities.

Keywords

oxybenzone, glucosylation, malonylation, phytoremediation, time of flight mass spectrometry

## 1 Introduction

Personal care products including stimulants, fragrances, sunscreens, antimicrobials, and insect repellents are emerging contaminants, and have attracted much attention in recent years due to their presence in surface water and potential effects on ecosystems [1,2]. Only recently, UV sunscreens have become a topic of environmental research because they appear in cosmetic products up to 6% and may also be used as indirect additives in food contact substances [3,4]. Contamination of the aquatic environment by UV filters occurs either indirectly from wastewater treatment plant discharges, or directly via recreational activities due to the release of chemical compounds from skin to water.

OBZ is one of the most frequently found UV filters measured in wastewater, swimming pool water but also in surface water samples, occurring at concentrations ranging from 68-3300 ng•$L^{-1}$ [5–9]. These results indicate that OBZ is not fully eliminated during wastewater treatment and may be disseminated further into the environment. Hence, OBZ has been marked as an emerging contaminant since 2005 in Richardson's water analysis [10]. Studies demonstrate that OBZ and its metabolites accumulate in aquatic organisms (perch, roach and juvenile rainbow trout) [11,12], and may induce endocrine disrupting activities including antiestrogenic and antiandrogenic activities [13–16]. A recent paper even indicated that bleaching of coral reefs might be related to the release of sunscreens containing OBZ in coral reef areas [13].

Amongst attempts to reduce the amount of unwanted compounds in the environment, phytoremediation as an emerging technology which involves uptake, degradation and storage of contaminants by plants has been proposed. Roots are the main organ for absorption and transformation reactions [17]. In this context, hairy root cultures have been applied as a reliable experimental model to illustrate the metabolic processes and fate of contaminants of whole plants during phytoremediation. Products identified in hairy roots are considered to be formed in the roots of intact plants as well [18]. Hairy roots cell cultures have advantages of fast growth, free of photo- and microbial degradation, easy implementation and high

production of secondary metabolites [19].

Plant cells cope with foreign compounds mainly in successive phases which have been described by Sandermann's "Green Liver" concept [20]. Phase I activates compounds by oxidation, reduction or hydroxylation for the subsequent conjugation to reactive groups such as amino acids, sugars and peptides by phase II enzymes. Phase III (compartmentation) mostly refers to further conjugation or metabolism of phase II products for storage in vacuoles or cell walls [20,21]. In phase II, glucosylation is one of the most commonly observed detoxification mechanisms, which is employed by many organisms to maintain metabolic homeostasis; it is catalyzed by glucosyltransferases (E.C. 2.4.1.x) to attach an activated glucose on an acceptor molecule. By addition of sugars, the interactive aglycons are converted into stable, more water soluble and less toxic forms [22]. Plants are known to glucosylate a diverse range of endogenous and exogenous organic molecules, such as flavonoids, herbicides, pesticides and other xenobiotics [23–27]. *O*-glucosides are often further metabolized to form a malonate hemi-ester conjugate with the involvement of malonyl-CoA transferase (E.C. 2.3.1.x) to add an *O*-malonyl substituent to a hydroxyl group of a sugar residue of xenobiotics [20]. Malonylation is characterized by enhanced chemical stability, improved solubility and deposition of target compounds into vacuoles [28]. Malonylation of glucosides has been reported in several plant species including thale cress, tobacco, common duckweed, butterfly pea, and soybean [23,29–31].

So far, studies on the transformation of OBZ are mainly limited in mammals, hence, in this study we investigate the metabolism of OBZ in plant tissues by using hairy roots cells of *Armoracia rusticana* (horseradish) as a model. Structures of key metabolites are proposed and the formations of metabolites are presented as a function of time, and a recommendation on the phytoremediation potential is given.

# 2 Materials and methods

## 2.1 Chemicals

Oxybenzone (2-Hydroxy-4-methoxybenzophenone, pharmaceutical secondary standard) was

purchased from Fluka (Germany), acetonitrile (HPLC grade), water with 0.1% formic acid (LC-MS grade), acetonitrile with 0.1% formic acid (LC-MS grade) were obtained from Carl Roth (Germany). All water used for sample preparation was ultrapure (MilliQ, Millipore Corporation). Physiochemical properties of OBZ are shown in table 1.

## 2.2 Plant material

Horseradish (*A. rusticana* L.) hairy root culture transformed by *Agrobacterium rhizogenes* strain A4 [29] was grown in 100 ml full-strength Murashige and Skoog medium containing thiamine and inositol for 10 days [32]. OBZ was dissolved in ethanol and added to the growth medium to give a final concentration of 100 µM. After 3 h of treatment with OBZ, roots were washed twice with sterilized deionized water and transferred to fresh growth medium without OBZ. Samples were harvested at 2, 4, 6 and 24 after transferring the cells, dried with lint tissue paper, frozen in liquid nitrogen and stored at - 20°C.

## 2.3 Oxybenzone and metabolites extraction and sample preparation

0.5 g root material were ground under liquid nitrogen, and extracted with 1.5 mL $H_2O$ /Acetonitrile (30/70, *v/v*). Samples were vortexed for 1 min, treated in the ultrasonicator for 5 min and centrifuged at 13,000 ×*g* at 4°C for 30 min. Supernatants were collected and filtrated through 0.45 µm pore size PVDF syringe filters (Carl Roth, Germany) prior to loading on them on solid phase extraction (SPE) columns (3 $cm^3$ 60 mg Oasis HLB SPE cartridges, Waters, Germany) for further purification. Cartridges had been conditioned with 3 mL of methanol and equilibrated with 3 mL of water prior to use. 0.5 mL of samples were passed through the cartridges and 3 mL water were used to flush impurities. The cartridges were then dried under vacuum for 10 min, and analytes were subsequently eluted with two 0.7 mL and one 0.6 mL aliquots of acetonitrile. For the detection of OBZ and metabolites in medium, 200 µL of growth medium was filtered through PVDF syringe filters as mentioned above.

## 2.4 LC-MS analysis

LC-MS analysis was performed with a HPLC system (Varian ProStar 210) coupled to an ion

trap mass spectrometer (Varian 500-MS) as described elsewhere [33]. A Phenomenex HYDRO-RP column (C18, polar endcapped; particle size 4 µm; 50 mm × 2.0 mm) was applied for rapid separation of analytes using the following mobile phases for elution: $H_2O$, 0.1% formic acid as mobile phase A and acetonitrile, 0.1% formic acid as mobile phase B with following gradient: 0–2 min 97% Buffer A (isocratic); 2–10 min 95% Buffer B (linearly increasing); 10–12 min 95% Buffer B (isocratic); 12–12.5 min 97% Buffer A (linear decreasing); 12.5–17 min 95% A (isocratic). The flow rate was 0.3 mL/min. Concentration of OBZ was determined by an external standard calibration curve. The HPLC eluent was introduced to the mass spectrometer using a pneumatically assisted electrospray source. The mass spectrometer was operated in positive ESI mode. The interface was adjusted to the following conditions: capillary voltage, 63 V; needle voltage, 4500 V; drying gas temperature, 350 °C. MS/MS spectra were obtained by collision-induced dissociation using nitrogen as the collision gas. Mass transitions of OBZ and metabolites were as follows: $[M+H]^+$ *m/z* 229-151 (oxybenzone), $[M+Na]^+$ *m/z* 413-251 (oxybenzone-glucoside), $[M+Na]^+$ *m/z* 499-455 (oxybenzone-malonyl-glucoside).

LC-TOF-MS experiments were conducted on an Ultimate 3000 LC system (ThermoFisher) coupled to an ultra high resolution Maxis 4g plus TOF mass spectrometer (Bruker) equipped with an electrospray source. The LC conditions were identical as above. The TOF-MS was operated in the positive polarity mode with active focus under the following conditions: Capillary voltage, 5500 V; nitrogen dry gas temperature, 225 °C; dry gas flow, 10 L/min; nebulizer pressure, 2 bar. The TOF-MS was calibrated daily with ESI-L tuning mix (Agilent) using the enhanced quadratic algorithm. MS scans were recalibrated using Hexakis (1H, 1H, 4H-hexafluorobutyloxy) phosphazine (Agilent) as a lock mass.

### 2.5 Enzyme extraction

Extraction of an *O*-glucosyltransferase containing enzyme fraction was conducted with some modification as previously described [26]. In short, 3 g of hairy roots were homogenized in liquid nitrogen with mortar and pestle to yield a fine powder and extracted with 10 mL 100 mM sodium phosphate-buffer pH 6.5 containing 10 mM DTE, 2 mM $MgCl_2$, 1 mM EDTA, 1

mM PMSF and 1% PVP K90 at 4°C for 30 min. After centrifugation at 15,000 ×*g* for 30 min at 4 °C, proteins in the supernatant were precipitated progressively by addition of ammonium sulphate to 40% and 75% saturation in two subsequent steps. Samples were centrifuged at 18,500 ×g for 30min at 4°C after each step. Consequently, the pellets were resuspended in 2.5 mL 200 mM Tris/HCl buffer with 2 mM $MgCl_2$ and 1 mM DTE, pH 7.3. Proteins were desalted by chromatography through PD 10 columns (GE Healthcare, UK) and stored at –80°C for further use.

## 2.6 Enzyme assays

Enzymatic formation of the glucosides was performed by incubating 100 µM OBZ with 2 mM uridine diphosphate glucose (UDPG), 1.25 mM 4-NPG, 1,25 mM salicin in 200 mM Tris/HCl buffer, pH 7.5. The reaction was started by adding 100 µL glucosyltransferase containing enzyme extract to yield a final volume of 200 µL. The incubation lasted for 60 min at 30°C in a water bath [32]. Experiments without enzyme served as control. The reaction was stopped by precipitating the protein with 10 µL concentrated phosphoric acid. The samples were centrifuged at 13,000 ×*g* for 2 min and aliquots of the supernatants were applied to LC-MS for identification of products.

## 2.7 Enzymatic hydrolysis

Metabolite extracts prepared after SPE were freeze-dried (Speedvac, Savant Instr.) and re-dissolved in 400 µL of 50 mM sodium phosphate buffer (pH 5.0). Hydrolysis was started by incubating samples with 300 U of β-D-glucosidase (Fluka, Germany) at 36 °C for 2 h. Control experiments were free of glucosidase. Reaction was stopped by applying samples to the SPE columns. Samples were analyzed by LC-MS [33].

## 2.8 Alkaline hydrolysis

Plant extracts after SPE were incubated with 0.1 N sodium hydroxide for 6 h at room temperature, and control experiments were carried out with the absence of NaOH [25].

# 3 Results

## 3.1 Formation of glucose conjugate

### 3.1.1 Mass spectral analysis

In extracts of root material treated with OBZ, high resolution mass spectroscopy revealed a polar metabolite eluting well before the parent compound. It was identified as oxybenzone-glucoside (OBZ-Glu) with its pseudo-molecular ion at *m/z* 413.1218 $[M+Na]^+$ on TOF-MS, which corresponded to the molecular formula $C_{20}H_{22}O_8Na$ (413.1207) (table 2). Further MS/MS experiment with the ion trap systems of the parent compound resulted in two major fragments of *m/z* 185 and *m/z* 251 (figure 1A), corresponding to the dehydroglucose and OBZ with sodium adduct during fragmentation.

### 3.1.2 Enzymatic hydrolysis

To confirm this glucose conjugated metabolite, samples containing the product with a *m/z* of 413 were incubated with 300 U commercially available β-D-glucosidase for 2 h. This experiment resulted in the disappearance of the signal corresponding to OBZ-Glu in LC-MS/MS (figure 2), while samples incubated with 50 mM sodium phosphate buffer at same conditions still showed the peak representing OBZ-Glu.

### 3.1.3 Enzymatic synthesis

When glucosyltransferase extracts from horseradish roots were incubated with 100 μM OBZ in the presence of UDP-Glucose for 60 min, LC-MS/MS analysis revealed the same retention time and fragmentation pattern for the product formed that were previously observed in 3.1.1, which corresponds to OBZ-Glu.

## 3.2 Identification of malonylated glucoconjugate

### 3.2.1 Mass spectral analysis

Another molecular ion that was detected at *m/z* 499.1223 $[M+Na]^+$, had a molecular formula

confirmed by exact mass measurement on TOF-MS as $C_{23}H_{24}O_{11}Na$ (499.1221) with an error of just 2.1 ppm (table 2). On ESI-MS, this molecular ion was 86 units larger than that of OBZ-Glu (*m/z* 413 $[M+Na]^+$). The mass difference of 86 suggests an additional malonyl group, which was also proposed by MS/MS. During ESI-MS/MS of *m/z* 499, loss of 44 Da resulted in a major fragment of *m/z* 455, and fragments of *m/z* 413 $[M\text{-malonyl}+Na]^+$ and *m/z* 251 $[M\text{-malonyl-dehydroglucose}+Na]^+$ were also generated (figure 1B). The loss of $CO_2$ (*m/z* 44) due to the decarboxylation of malonic acid during fragmentation of a malonylglucopyranoside has been described in previous studies [23,34]. On this basis, our second metabolite was identified as oxybenzone-(6-*O*-malonyl)-glucoside, which was formed in a secondary conjugation step with conjugation of malonic acid on the first metabolite.

### 3.2.2 Alkaline hydrolysis

To confirm the structure of OBZ-Mal-Glu, root extracts containing OBZ-Mal-Glu were incubated with 0.1 N NaOH at room temperature for 6 h. The NaOH completely hydrolyzed the ester bond of OBZ-Mal-Glu (figure 3A, B), and released free OBZ-Glu. However, the OBZ-Glu is resistant to alkaline hydrolysis, therefore, an increase of the glucoconjguate in figure 3C, D was observed.

## 3.3 Time dependent formation of conjugated metabolites in roots

OBZ-Glu and OBZ-Mal-Glu were formed within the first 3 hours of treatment with OBZ, the OBZ-Glu was particularly dominant. The lack of reference materials of metabolites prevents the direct quantification of conjugates in plants; therefore, the concentrations are given as arbitrary units (Peak area, fig. 4). In the following hours the hairy roots were incubated in fresh medium without OBZ, but still concentrations of both metabolites increased with the incubation time (figure 4). After 24 h, the amount of OBZ-Glu and OBZ-Mal-Glu was 3.6 times and 17.3 times higher than 0 h, respectively. Moreover, the malonylated glucoconjugate became more abundant and accounted for a higher ratio of all metabolites after 24 h, whilst at 0 h only 15% had been identified as malonyl conjugate (figure 4).

## 3.4 Exudation of metabolite into medium

OBZ glucopyranoside was not only found in the root tissues, but also in the culture medium. After the transfer of cells into the fresh medium, OBZ-Glu released in the medium continued to increase up to 16.8 fold toward the end of the experiment compared to 2 h (figure 5). However, the distribution of metabolites differed from that in the cells, as no malonynated metabolite was detected in the medium.

## 4 Discussion

The degradation pathway of OBZ in mammals mainly depends on hydroxylation mediated by the cytochrome P450 enzyme system [35–38] and phase II enzymes to form glucuronide or sulfate conjugates [36,39]. Accordingly, in plants, transformation of OBZ quickly occurs via a direct glucosylation during phase II reactions (figure 6), which could be attributed to the already existing hydroxyl group of OBZ. However, no free phase I metabolites of OBZ have been detected in plants. Since plants do not possess microsomal UDP-glucuronosyltransferase [20], they use glucose as transfer partner, and hence, a glucose conjugate of OBZ was formed rapidly in the horseradish culture within just three hours of exposure. Although the stereoisomeric structure of the metabolites cannot be confirmed by mass spectrometry, the hydrolysis of OBZ-Glu by β-D-glucosidase supports that the hexose represents a β-D-glucopyranose group, which forms an *O*-glucoside. Enzymatic synthesis of OBZ-Glu with glucosyltransferases (GT) extracted from hairy roots proved the involvement of GT for glucose conjugation.

Glucosylation provides precursor molecules for further esterification with malonic acid, of which β-D-(6-*O*-malonyl)-glucoside is one of the most common products in plants [20]. Several studies have detected (6-*O*-malonyl)-glucoside of PCP, chlorophenols and triclosan in soybean, wheat, common duckweed and carrots, respectively [30,31,40]. Malonylation has been hypothesized to protect the saccharide conjugates against enzyme cleavage and to render the products ready for storage in vacuole or cell walls [31]. The release of free OBZ-Glu during alkaline hydrolysis supports our idea about the further malonyl conjugation of the phase II product.

The increasing formation of two metabolites within 24 h showed that plants have a high potential to transform and detoxify OBZ. Conjugation with glucose is important in the metabolism of xenobiotics, since glucosides are characterized for their potential of being stable and indigestibly incorporated in cell walls. Secondary conjugation of glucosides with malonic acid is of special interest, as malonylation may affect the release of xenobiotic glucosides from plant cells and on the contrary enhance vacuole sequestration [41]. Consistent with this theory, after 24 h incubation, only OBZ-Glu was excreted to the growth medium while leaving the malonylated metabolite inside the plants. Similar phenomena were observed in thale cress and tabacco treated with naphthols. In that case the knockout of the malonyltransferase gene (AtPMaT1) had increased the release of naphthol glucoside into media, while a force expression of AtPMaT1 decreased export and increased deposition of naphthols as malonates [29].

## 5 Conclusion

Our results indicate that plants may be able to take up and degrade the most common UV filter-oxybenzone. More than 20% of the initial OBZ (100 μM) was eliminated from the culture medium by hairy roots after just 3 h of exposure. Two novel metabolites were detected in the treated hairy roots as OBZ-Glu and OBZ-Mal-Glu. Metabolite formation increased with incubation time. The fragmentation pattern of metabolites in MS/MS was convincing for identification. Enzymatic synthesis and hydrolysis as well as alkaline hydrolysis contributed to the characterization of metabolites.

The horseradish hairy root system provides us with a simple way to explore the metabolism of oxybenzone in plants. It is an easy-to-handle culture, and allows equal distribution of compound throughout the tissue. This study contributes to a better understanding of detoxification pathways of oxybenzone in plants. The results suggest the use of plants for phytoremediation of UV filter compounds, and provide an appropriate alternative for treating emerging contaminants in wastewater. Research about oxybenzone metabolism in aquatic macrophytes and effects on detoxification defense enzyme systems are underway.

## Acknowledgement

Feiran Chen received funding from China Scholarship Council. We thank Mr. Rudolf Harpaintner for his expert technical assistance with the hairy roots culture and GT analysis.

## References

[1] L. You, V.T. Nguyen, A. Pal, H. Chen, Y. He, M. Reinhard, et al., Investigation of pharmaceuticals, personal care products and endocrine disrupting chemicals in a tropical urban catchment and the influence of environmental factors, Sci. Total Environ. (2015) 1–9. doi:10.1016/j.scitotenv.2015.06.041.

[2] J.A. Dougherty, P.W. Swarzenski, R.S. Dinicola, M. Reinhard, Occurrence of herbicides and pharmaceutical and personal care products in surface water and groundwater around Liberty Bay, Puget Sound, Washington, J. Environ. Qual. 39 (2006) 1173–1180. doi:10.2134/jeq2009.0189.

[3] List of indirect additives used in food contact substances, (2015) http://www.accessdata.fda.gov/scripts/FCN/fcnDetailNavigation.cfm?rpt=ialisting&id=1516 (accessed July 20, 2015).

[4] SSCP, Opinion on Benzophenone-3, COLIPA nr S38, Scientific committees, Brussels (2008). http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_159.pdf.

[5] C. Zwiener, S.D. Richardson, D.M. De Marini, T. Grummt, T. Glauner, F.H. Frimmel, Drowning in disinfection byproducts? Assessing swimming pool water, Environ. Sci. Technol. 41 (2007) 363–372. doi:10.1021/es062367v.

[6] W. Li, Y. Ma, C. Guo, W. Hu, K. Liu, Y. Wang, et al., Occurrence and behavior of four of the most used sunscreen UV filters in a wastewater reclamation plant, Water Res. 41 (2007) 3506–3512. doi:10.1016/j.watres.2007.05.039.

[7] M.E. Balmer, H.R. Buser, M.D. Müller, T. Poiger, Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes, Environ. Sci. Technol. 39 (2005) 953–962. doi:10.1021/es040055r.

[8] I. Tarazona, A. Chisvert, Z. León, A. Salvador, Determination of hydroxylated benzophenone UV filters in sea water samples by dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry, J. Chromatogr. A. 1217 (2010) 4771–4778. doi:10.1016/j.chroma.2010.05.047.

[9] A. Goksøyr, K.E. Tollefsen, M. Grung, K. Løken, E. Lie, A. Zenker, et al., Balsa raft crossing the pacific finds low contaminant levels, Environ. Sci. Technol. 43 (2009) 4783–4790. doi:10.1021/es900154h.

[10] S.D. Richardson, T.A. Ternes, Water Analysis: Emerging contaminants and current issues., Anal. Chem. 77 (2014) 3807–3838. doi:10.1021/ac500508t.

[11] M. Nagtegaal, T.A. Ternes, W. Baumann, R. Nagel, UV-Filtersubstanzen in Wasser und Fischen, UWSF-Z Umweltchem Ökotox. 9 (1997) 79–86. http://link.springer.com/content/pdf/10.1007/BF02937843.

[12] R. Al-Salhi, A. Abdul-Sada, A. Lange, C.R. Tyler, E.M. Hill, The xenometabolome and novel contaminant markers in fish exposed to a wastewater treatment works effluent, Environ. Sci. Technol. 46 (2012) 9080–9088. doi:10.1021/es3014453.

[13] R. Danovaro, L. Bongiorni, C. Corinaldesi, D. Giovannelli, E. Damiani, P. Astolfi, et al., Sunscreens cause coral bleaching by promoting viral infections, Environ. Health Perspect. 116 (2008) 441–447. doi:10.1289/ehp.10966.

[14] N. Blüthgen, S. Zucchi, K. Fent, Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (*Danio rerio*), Toxicol. Appl. Pharmacol. 263 (2012) 184–194. doi:10.1016/j.taap.2012.06.008.

[15] M. Coronado, H. De Haro, X. Deng, M.A. Rempel, R. Lavado, D. Schlenk, Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish, Aquat. Toxicol. 90 (2008) 182–187. doi:10.1016/j.aquatox.2008.08.018.

[16] K. Fent, P.Y. Kunz, E. Gomez, UV Filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish, Chim. Int. J. Chem. 62 (2008) 368–375. doi:10.2533/chimia.2008.368.

[17] M.L. Zhou, Y.X. Tang, Y.M. Wu, Plant hairy roots for remediation of aqueous pollutants, Plant Mol. Biol. Report. 31 (2013) 1–8. doi:10.1007/s11105-012-0465-z.

[18] J.V. Shanks, J. Morgan, Plant “ hairy root ” culture, Curr. Opin. Biotechnol. 10 (1999) 151–155. doi:10.1016/S0958-1669(99)80026-3.

[19] F. Bourgaud, A. Gravot, S. Milesi, E. Gontier, Production of plant secondary metabolites: a historical perspective, Plant Sci. 161 (2001) 839–851. doi:10.1016/S0168-9452(01)00490-3.

[20] H. Sandermann, Plant metabolism of xenobiotics., Trends Biochem. Sci. 17 (1992) 82–84. doi:10.1016/0968-0004(92)90507-6.

[21] G. Kvesitadze, G. Khatisashvili, T. Sadunishvili, J.J. Ramsden, Biochemical Mechanisms of Detoxification in Higher Plants, Springer, Germany, 2006.

[22] L.A. Kleczkowski, Glucose activation and metabolism through UDP-glucose pyrophosphorylase in plants, Phytochemistry. 37 (1994) 1507–1515. doi:10.1016/S0031-9422(00)89568-0.

[23] K. Kazuma, N. Noda, M. Suzuki, Malonylated flavonol glycosides from the petals of *Clitoria ternatea*, Phytochemistry. 62 (2003) 229–237. doi:10.1016/S0031-9422(02)00486-7.

[24] R. Winkler, H. Sandermann, N-Glucosyl conjugates of chlorinated anilines: spontaneous formation and cleavage, J. Agric. Food Chem. 40 (1992) 2008–2012. doi:10.1021/jf00022a054.

[25] D. Petroutsos, J. Wang, P. Katapodis, D. Kekos, M. Sommerfeld, Q. Hu, Toxicity and metabolism of p-chlorophenol in the marine microalga *Tetraselmis marina*, Aquat. Toxicol. 85 (2007) 192–201. doi:10.1016/j.aquatox.2007.09.007.

[26] S. Pflugmacher, H. Sandermann, Taxonomic distribution of plant glucosyltransferases acting on xenobiotics, Phytochemistry. 49 (1998) 507–511. doi:10.1016/S0031-9422(98)00139-3.

[27] P. Jones, T. Vogt, Glycosyltransferases in secondary plant metabolism: tranquilizers and stimulant controllers, Planta. 213 (2001) 164–174. doi:10.1007/s004250000492.

[28] U. Matern, W. Heller, K. Himmelspach, Conformational changes of apigenin 7-O-(6-O-malonylglucoside), a vacuolar pigment from parsley, with solvent composition and proton concentration, Eur. J. Biochem. 133 (1983) 439–448. doi:10.1111/j.1432-1033.1983.tb07483.x.

[29] G. Taguchi, T. Ubukata, H. Nozue, Y. Kobayashi, M. Takahi, H. Yamamoto, et al., Malonylation is a key reaction in the metabolism of xenobiotic phenolic glucosides in Arabidopsis and tobacco, Plant J. 63 (2010) 1031–1041. doi:10.1111/j.1365-313X.2010.04298.x.

[30] J.A. Day, E.M. Saunders, Glycosidation of chlorophenols by *Lemna minor*, Environ. Toxicol. Chem. 23 (2004) 613–620. doi:10.1897/02-649.

[31] R. Schmitt, J. Kaul, T. V.D. Trenck, E. Schaller, H. Sandermann, β-d-Glucosyl and O-malonyl-β-d-glucosyl conjugates of pentachlorophenol in soybean and wheat: identification and enzymatic synthesis, Pestic. Biochem. Physiol. 24 (1985) 77–85. doi:10.1016/0048-3575(85)90116-6.

[32] C. Götz, A. Fekete, I. Gebefuegi, S.T. Forczek, K. Fuksová, X. Li, et al., Uptake, degradation and chiral discrimination of N-acyl-D/L-homoserine lactones by barley (*Hordeum vulgare*) and yam bean (*Pachyrhizus erosus*) plants, Anal. Bioanal. Chem. 389 (2007) 1447–1457. doi:10.1007/s00216-007-1579-2.

[33] C. Huber, B. Bartha, P. Schröder, Metabolism of diclofenac in plants - hydroxylation is followed by glucose conjugation, J. Hazard. Mater. 243 (2012) 250–256. doi:10.1016/j.jhazmat.2012.10.023.

[34] D. Petroutsos, P. Katapodis, M. Samiotaki, G. Panayotou, D. Kekos, Detoxification of 2,4-dichlorophenol by the marine microalga *Tetraselmis marina*, Phytochemistry. 69 (2008) 707–714. doi:10.1016/j.phytochem.2007.09.002.

[35] D.J. Fediuk, T. Wang, Y. Chen, F.E. Parkinson, M.P. Namaka, K.J. Simons, et al., Metabolic disposition of the insect repellent DEET and the sunscreen oxybenzone following intravenous and skin administration in rats, Int. J. Toxicol. 31 (2012) 467–476. doi:10.1177/1091581812459893.

[36] L. Wang, K. Kannan, Characteristic profiles of benzonphenone-3 and its derivatives in urine of children and adults from the United States and China, Environ. Sci. Technol. 47 (2013) 12532–12538. doi:10.1021/es4032908.

[37] C.S. Okereke, M.S. Abdel-Rhaman, M.A. Friedman, Disposition of benzophenone-3 after dermal administration in male rats, Toxicol. Lett. 73 (1994) 113–122. doi:10.1016/0378-4274(94)90101-5.

[38] Y. Nakagawa, T. Suzuki, Metabolism of 2-hydroxy-4-methoxybenzophenone in isolated rat hepatocytes and xenoestrogenic effects of its metabolites on MCF-7 human breast cancer cells, Chem. Biol. Interact. 139 (2002) 115–128. doi:10.1016/S0009-2797(01)00293-9.

[39] C.J. Wegerski, S.S. Auerbach, J.M. Sanders, J.D. Mcdonald, Metabolism of 2-Hydroxy-4-Methoxybenzophenone depends on species and sex, (2011) http://dspace.lrri.org:8080/xmlui/bitstream/handle/123456789/1035/Wergerski-Metabolism%20of%202-Hydroxy-4-Methoxybenzophenone%20Depends%20on%20Species%20and%20Sex.pdf?sequence=1 (accessed Aug.03.2015)

[40] A. Macherius, T. Eggen, W. Lorenz, M. Moeder, J. Ondruschka, T. Reemtsma, Metabolization of the bacteriostatic agent triclosan in edible plants and its consequences for plant uptake assessment, Environ. Sci. Technol. 46 (2012) 10797–10804. doi:10.1021/es3028378.

[41] U. Matern, C. Feser, D. Hammer, Further characterization and regulation of malonyl-coenzyme A: flavonoid glucoside malonyltransferases from parsley cell

suspension cultures, Arch. Biochem. Biophys. 226 (1983) 206–217. doi:10.1016/0003-9861(83)90286-2.

**Figure**

Table 1 Chemical structure and physicochemical properties of oxybenzone. (single column fitting)

Table 2 Retention times (RT) of OBZ and its metabolites and suggested identities of OBZ metabolites based on mass spectrometric data. (single column fitting)

Figure 1 MS/MS spectra of $[M+Na]^+$ of A OBZ-Glu (*m/z* 413) and B OBZ-Mal-Glu (*m/z* 499) from hairy roots extracts. The product ion *m/z* 251 is formed via a cleavage of dehydroglucose; a loss of $CO_2$ resulted in the formation of *m/z* 455, and the cleavage of ester bond released free OBZ-Glu (*m/z* 413). The analysis was done in positive ionization mode. (2-column fitting)

Figure 2 LC-MS/MS chromatograms of samples containing OBZ-Glu (*SIM* mode for *m/z* 413). A is untreated sample, B represents sample treated with β-D-glucosidase. (single column fitting)

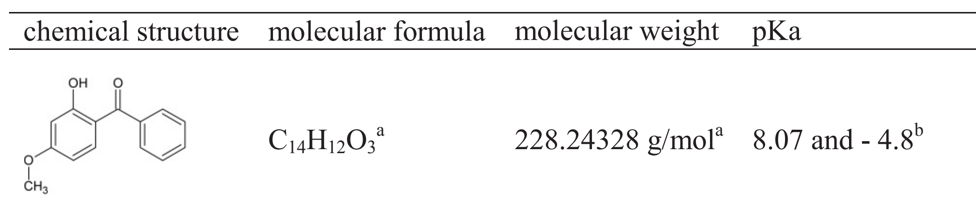
Figure 3 LC-MS/MS chromatograms (*MRM* mode for *m/z* 499 and *m/z* 413) of samples containing OBZ-Mal-Glu and OBZ-Glu after incubation without (A, C) and with (B, D) 0.1 N NaOH for 6 h. (2-column fitting)

Figure 4 Increase of OBZ-Glu and OBZ-Mal-Glu in hairy roots after 2, 4, 6 and 24 h transfer in medium without oxybenzone. (single column fitting)

Figure 5 Increase of OBZ-Glu in growth medium after 2, 4, 6 and 24 h transfer in medium without oxybenzone. (single column fitting)

Figure 6 Proposed metabolic pathway of Oxybenzone in *A. rusticana*. (2-column fitting)

Table 1

| chemical structure | molecular formula | molecular weight | pKa |
|---|---|---|---|
|  | $C_{14}H_{12}O_3$ [a] | 228.24328 g/mol [a] | 8.07 and - 4.8 [b] |

[a] Pubchem, [b] DrugBank

Table 2

| | RT (min) | molecular formula | accurate m/z (deviation [ppm]) |
|---|---|---|---|
| OBZ | 10.8 | $C_{14}H_{12}O_3$ | $[M+H]^+$ 229.0859 |
| OBZ-Glu | 6.9 | $C_{20}H_{22}O_8$ | $[M+Na]^+$ 413.1218 (2.6) |
| OBZ-Mal-Glu | 7.3 | $C_{23}H_{24}O_{11}$ | $[M+Na]^+$ 499.1223 (2.1) |

Figure 1

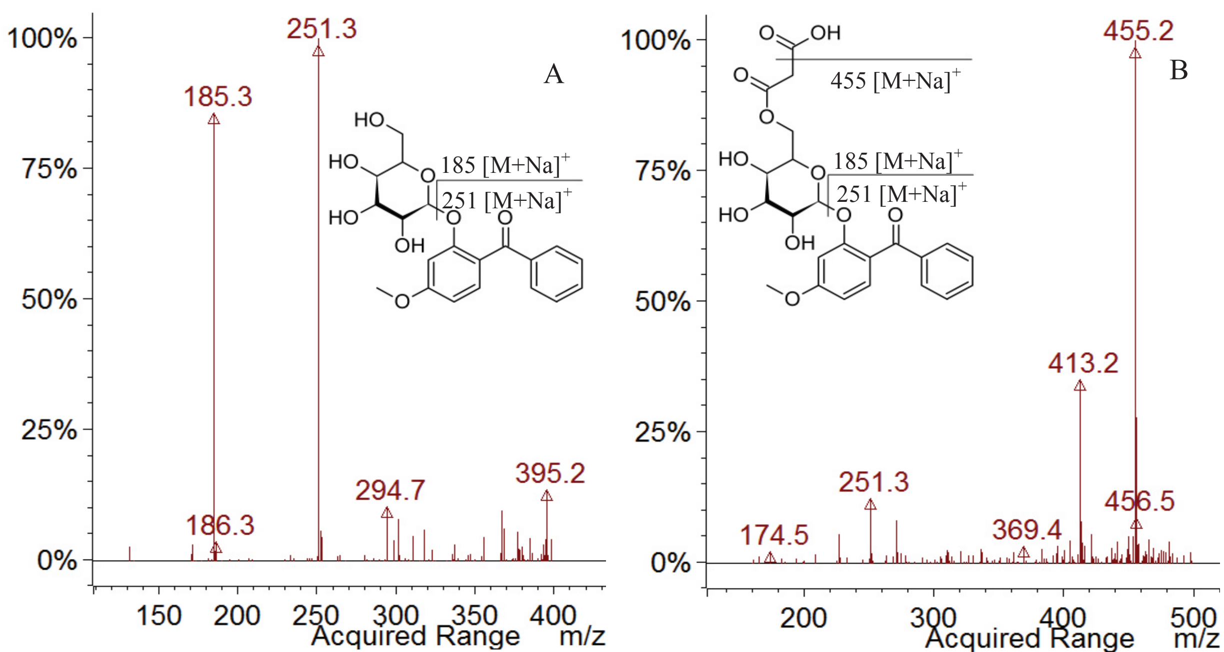


Figure 2

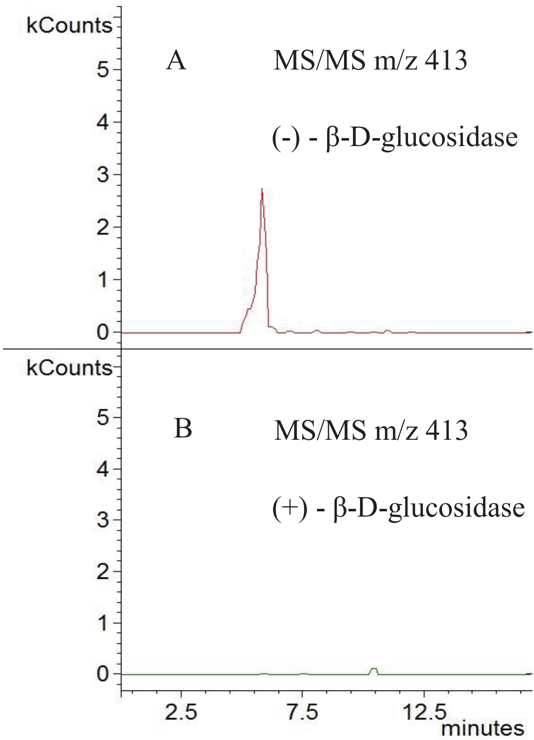


Figure 3

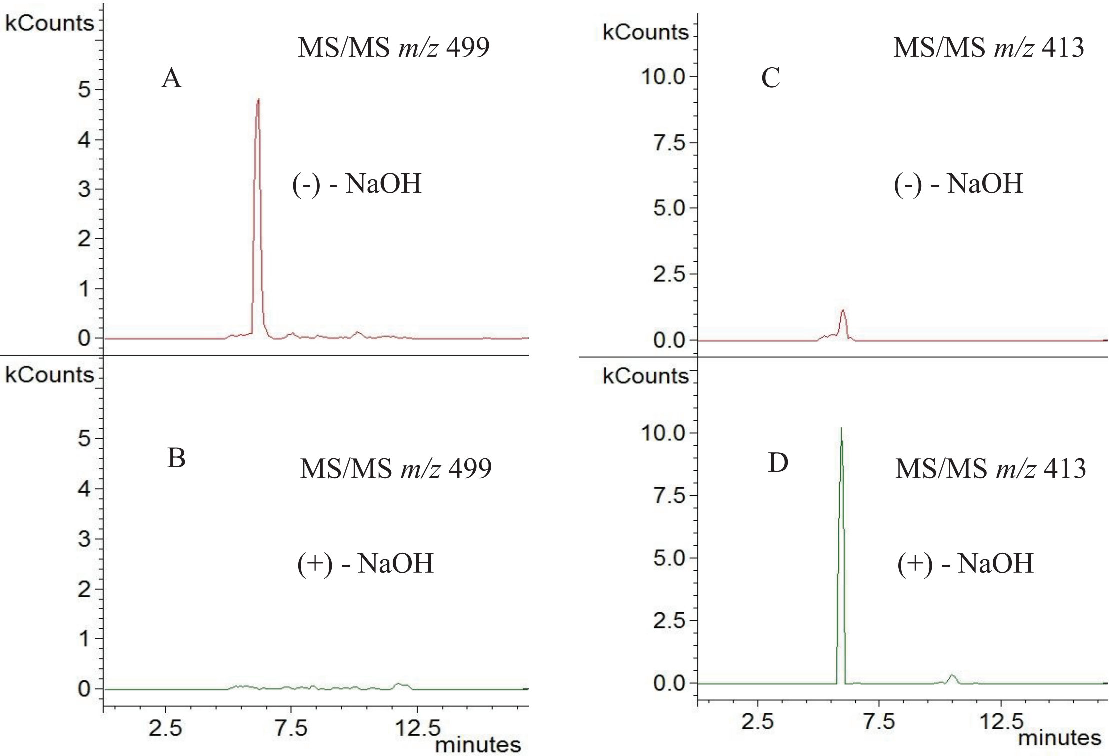


Figure 4

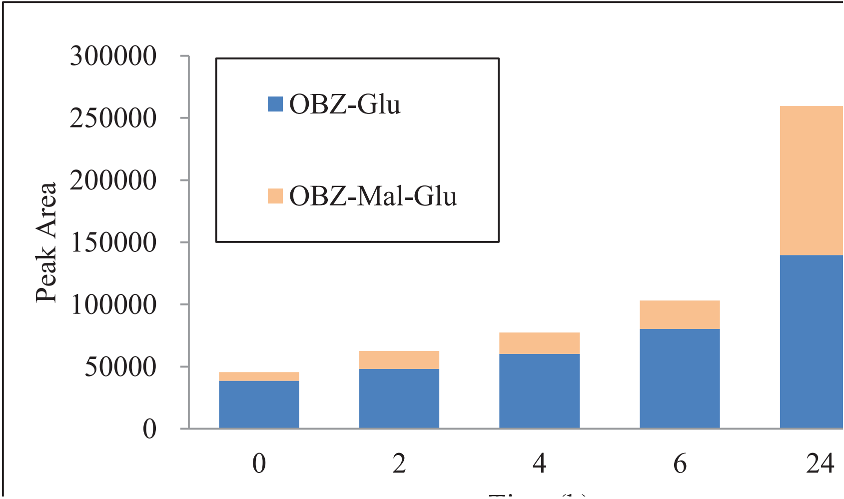


Figure 5

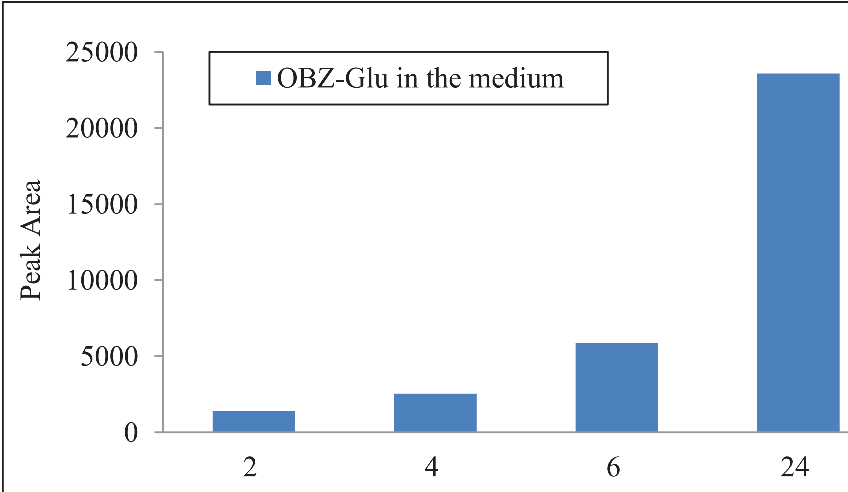
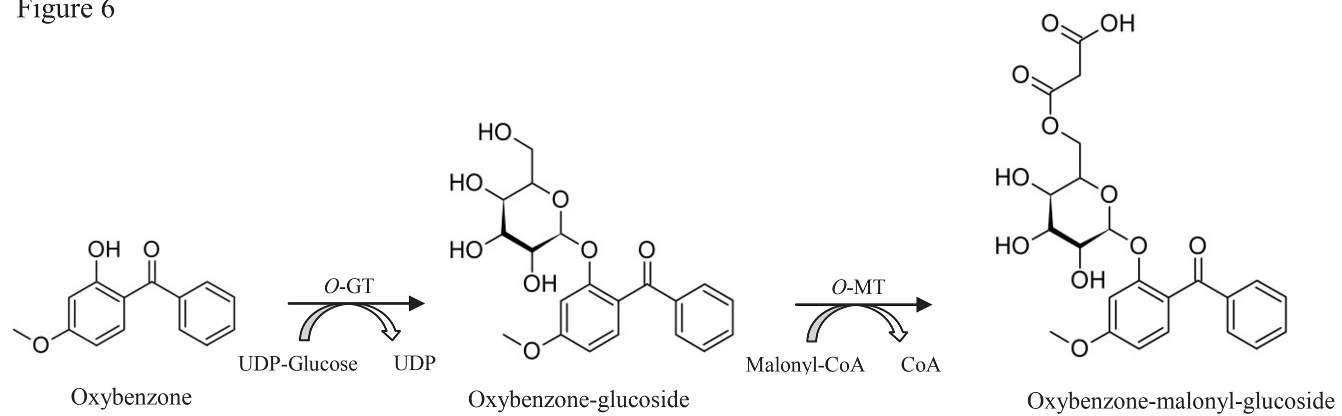


Figure 6



Highlights

- First finding on degradation of oxybenzone in plant tissues
- Two novel metabolites were identified
- Glucosylation and malonylation are main Phase II mechanisms
- Phytoremediation exhibits good potential for treating sunscreen compounds

*Novelty Statement

**Statement of novelty**

This manuscript covers the metabolism of a widely used sunscreen in cell cultures. Recently, sunscreens have attracted attention due to their persistence in water and their estrogenic effects. Oxybenzone is a typical UV filter detectable water bodies. Few studies show its degradation in mammals and partial metabolism by microbes. Evidence for alternative methods like phytoremediation is urgently required. We demonstrate for the first time that plants degrade oxybenzone in two successive steps. No comparable literature has been published, to our best knowledge. The scientific impact will be extremely high and meet the interest of a broad readership.