Environmental fate and behavior of transformation products of pesticides used in urban areas

Birte Hensen*, Oliver Olsson[a], Klaus Kümmerer[a]

[a] Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, Scharnhorststr. 1, 21335 Lüneburg, Germany

* Corresponding author: hensen@leuphana.de

Abstract

Pesticides like biocidal products are applied to urban outdoor materials. Due to rain events applied biocides are leached out and spread into the aqueous environment. Many processes like biodegradation and photolysis lead to the degradation and transformation of substances. Aqueous photolysis of the analyzed biocide octhilinone (OIT) leads to the degradation with a quantum yield of $\Phi = 0.01$ under simulated solar radiation. Biodegradation of the initial substance compared to the photolytic mixture shows an increased biological activity. Toxic effects on luminescent bacteria could be observed in low concentration ranges below 1 µg L^{-1}. This research demonstrates that new insights on the fate and behavior of TPs in the aquatic environment are necessary to provide the assessment of risk potential and the development of water pollution control measures.

XVI
World Water Congress
International Water Resources Association (IWRA)
Cancun. Quintana Roo. Mexico. 29 May- 3 June, 2017

Page 1 of 10

1.   Introduction

OIT (*2-octyl-1,2-thiazol-3-one*) is a biocidal active substance of the group of isothiazolinones. According to the EU Biocide Regulation No. 1062/2014 OIT is associated with the main group 2 ("preservatives") and the subordinated product types 6, 7, 9, 10, 11 and 13 which includes preserving agents, coating protection agents and masonry preservatives as well as leather, rubber and polymerized materials preservatives. OIT is the fifth most used biocidal product in Germany (Umweltbundesamt 2009; European Commission - Joint Research Centre - IEC Unit 2008).

Biocidal products are applied to outdoor materials and reaching the aquatic environment due to rain events. In the water phase various processes could influence the fate and behavior of the substances e.g. hydrolysis, biodegradation as well as direct and indirect photolysis. Photolysis is often the main degradation process of chemicals in the environment (Challis et al. 2014). Aqueous photolysis of environmental relevant chemicals leads to the development of transformation products (TPs) which characteristics are often unknown. In some cases there are huge differences between the TPs and initial substance regarding biodegradation processes and toxic effects (Barceló 1993; Escher und Fenner 2011; Fenner et al. 2002; Sinclair und Boxall 2003).

The leaching process of applied biocide to outdoor material like building facades by rain events have been analyzed in various studies (Burkhardt et al. 2012; Schoknecht et al. 2009; Wangler et al. 2012; Jungnickel et al. 2008; Breuer et al. 2012). The measured concentration of OIT in surface water of suburban areas in Denmark after heavy rain event was 5 - 50 ng L^{-1} (Bollmann et al. 2014). Other measured environmental concentrations of OIT have been analyzed in Chinese waste water treatment plants with mean concentration of 2.3 ng L^{-1} in influent and 0.5 ng L^{-1} in effluent (Chen et al. 2012). Burkhardt et al. considered a toxic effect of OIT on aquatic organisms at concentrations of 13 ng L^{-1} (Burkhardt et al. 2009).

To the best knowledge of the authors no photolysis studies of OIT were found in literature. This includes kinetically aspects of the photolytic degradation of the parent compound as well as qualitative aspects regarding the development of photo-TPs. Addressing these knowledge gaps this study focus the photolytic degradation of OIT under simulated solar radiation. Furthermore quantum yield and environmental lifetimes of photolysis will be calculated. Performed biodegradation test "Closed Bottle Test" (CBT) and toxicity test "Luminescent Bacteria Test" (LBT) of both initial substance and photolytic mixture will be compared. Finally initial substances and transformations products will be measured in environmental samples. Regarding this, the aim of the study is a first assay of the possible environmental behavior of OIT and its photo-transformation products.

Page 2 of 10

XVI
World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

2.   Material and methods

Analytical standard of OIT (CAS: 26530-20-1) and metamitron (CAS: 41394-05-2) purchased from Sigma Aldrich. Latter was used as an actinometer to receive the absolute spectral photon flux density of the used light sources to calculate the quantum yield of degradation.

Primary eliminations were analyzed using HPLC-UV-Vis (Prominence series Shimadzu, Duisburg, Germany) at wavelengths of 275 and 254 nm. A RP-column (Nucleodur 100-3, 125/2, c18 ec; Macherey Nagel, Düren, Germany) was used as stationary phase, while 0.01 % formic acid (A) and acetonitrile (B) were used as mobile phases with a flow of 0.25 ml/min and following gradient: 0-1 min (10 % B); 1-12 min (10-90 % B); 12-18 min (90 % B); 18-23 min (90-10 % B), 23-25 min (10 % B). Oven temperature was 30°C and injection volume was 10 µl. Measurements of environmental samples were conducted with a Triple Quadrupole (Agilent Technologies, 1200 Infinity LC-System and 6430 Triple Quad, Waldbronn, Germany).

2.1 Quantitative and qualitative photolysis

Photolysis experiments were implemented over a time period of eight hours at T = 293 K ± 2 with a xenon arc lamp (TXE 150, UV consulting Peschl, Mainz, Germany) and an optical bench (500 W Xenon lamp, 280 nm filter, AMKO-LTI, Tornesch, Germany). Solutions for the elucidation of the transformation products as well as biodegradation and toxicity tests were photolysed with $2 \cdot 10\text{-}5$ mol L^{-1}. To determine the rate constant lower concentrations of OIT ($4 \cdot 10\text{-}7$ mol L^{-1}) were prepared in ultra pure water (0.1 % acetonitrile) to get an optical thin solution (E < 0.02). Quantum yields ϕ were calculated following equation (1) by means of the experimental received rate constant k (s^{-1}) and the molar absorption coefficient ε (L mol^{-1} cm^{-1}) of the substance as well as the absolute light intensity I_{abs} (mol s^{-1} L^{-1} nm^{-1}) of the xenon lamps. Latter was determined by implementing actinometric measurements with metamitron as an actinometer. UV-Vis spectra (Lambda 45, Perkin-Elmer) were measured in quartz cuvettes (d = 1 cm) with a resolution of 2 nm in 1 nm steps, baseline correction was done with ultra pure water.

$$\phi = (k) / (\textstyle\sum(200nm - 400nm) \cdot (I_{(0,\lambda)} \cdot \varepsilon_\lambda) \cdot \ln(10)) \qquad (1)$$

The transfer of data acquired in laboratory to environmental rate constants was calculated as follows:

$$k_{env} = I_{solar} \cdot \ln(10) \cdot 1000 / 6.02 \cdot 10^{23} \cdot \phi \cdot \varepsilon \qquad (2)$$

with ϕ calculated in (1). I_{solar} is the global solar photon density (photons cm^{-2} s^{-1} nm^{-1}) and the molar absorption coefficient ε as described above. Values for the global photon flux density are received from the meteorological station Freiburg (48.0004 N, 7.5055 E).

Page 3 of 10

XVI
World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

Lifetimes are calculated by equation (3)

$$\tau = 1/k \qquad\qquad\qquad (3)$$

2.2 Biodegradation

CBT was performed according to the OECD-guidelines 301D. Inoculum was received from the municipal waste water treatment plant of Lüneburg (EWG = 70 000) (Gutowski et al. 2015). To obtain a theoretical oxygen demand of 5 mg L^{-1} a start concentration of OIT of 2.18 mg L^{-1} and of its photolytic mixture were prepared. Samples from the beginning (day 0) and the end of the test (day 28) were collected and analyzed by LC/MS. Oxygen decrease of all samples including blank and qualitative control was determined by measuring the fluorescent quenching of a luminophor by oxygen with oxygen sensor spots and an oxygen meter (Fibox 3, PreSens Precision Sensing, Regensburg, Germany).

2.3 Toxicity

Acute and chronic luminescent inhibition (testing after 30 minutes and 24 hours) as well as the inhibition of cell reproduction (after 14 hours) were tested in the LBT according to Menz et al. (2013). Dose-effect-relation was calculated for chronic luminescent inhibition after 24 hours.

2.4 Preparation of environmental samples

1 liter of each environmental sample was filtered with a folded filter (type 113 P Cellulose ø 240 mm). Supernatant was spiked with the external standard diuron-d6 (10 µl of 10 mg L^{-1}). Extraction procedure was a solid phase extraction (SPE). Cartridges (CHROMABOND® HR-X 6 mL/200 mg) were conditioned with 10 mL methanol and washed with 10 mL pure water.

Environmental samples were enriched on the cartridges via teflon capillary and a vacuum extraction unit. After enrichment of the samples cartridges were washed with 5 mL pure water and air dried about 5 - 10 minutes. Elution was done with a solvent mixture of methanol and chloroform (v:v; 1:1). The eluted phase was dried with nitrogen to dryness. 90 µL acetonitril as well as 10 µl of terbutryn-d5 as an internal standard were added. Samples were measured by Agilent Triple Quad.

Recovery was determined by spiking water samples with 100 µl of analytical standard of OIT (1 mg L^{-1}) and was found to be 95.5 %. The linearity between peak area and concentration of substances were obtained in the range of 0 - 5 µg L^{-1}. Hence limits of detection (LOD) and quantitation (LOQ) were calculated with DINTEST (2003) according to DIN 32645 and amounted to 0.2 and 0.7 ng L^{-1}, considering the enrichment factor of 5000.

XVI
World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

Page 4 of 10

3.   Results and discussion

OIT shows an absorption spectrum from < 200 to 310 nm with maximum absorption bands of ε = 11684 at 200 nm and ε = 10353 at 275 nm. Comparing the absorption spectrum with the emission spectrum of the global solar irradiation an overlap of theses spectra can be observed between 280 and 301 nm. Thus, the possibility to undergo environmental direct photolytic processes is given (see figure 1).

Figure 1: Molar absorption spectrum of OIT (left, black line) and absolute photon flux (right) of the TXe 150 xenon lamp (gray solid line) and the optical bench with filtered xenon light (gray dashed line) in a wavelength range of 200 - 400 nm.

3.1 Kinetic of photolysis

The calculation of the quantum yield by considering the absolute photon flux density of the lamp and the absorption spectrum of OIT follows equation (1). Thus a quantum yield of Φ = 0.01 was computed. By considering equation (2) and (3) this results in a maximum lifetime of τ = 15 days in winter and a minimum lifetime of τ = 0.3 days in summer (figure 1). Calculated values are just a conservative assumption for the top layer of water bodies. For closer inspection depth-depending turbidity due to humic substances needs to be considered.
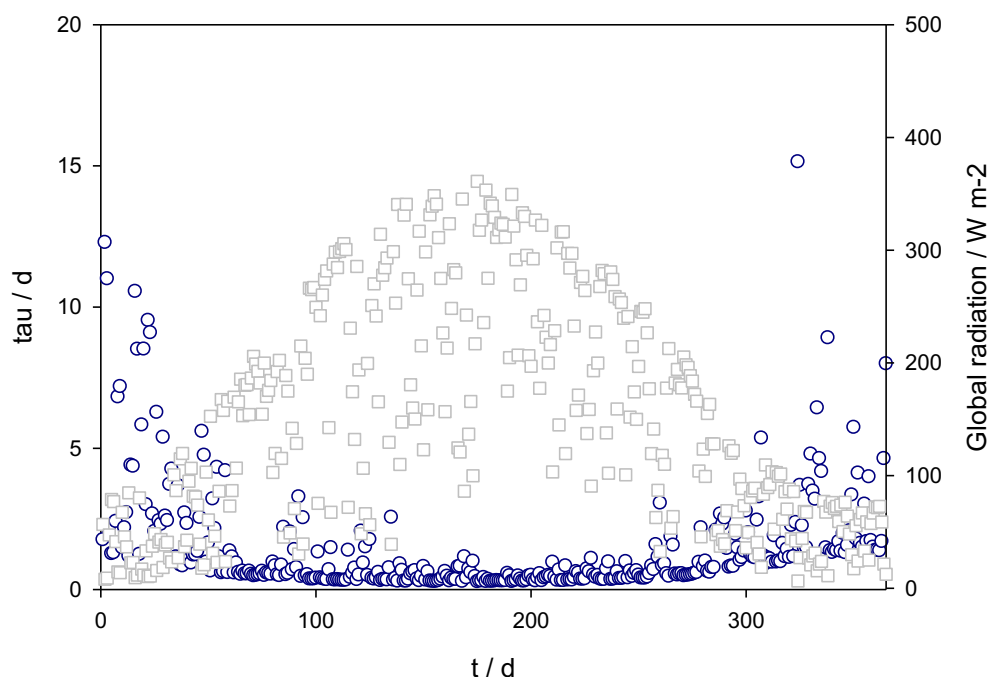
XVI
World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

Page 5 of 10

Figure 2: Mean day global radiation in W m^{-2} (grey squares) measured by the meterological station Freiburg) for 365 days in 2015 .Thereof calculated lifetimes in days (blue squares) of the photolytic degradation of the biocidal product OIT with φ = 0.01.

3.2 Transformation products

Photolysis of OIT results in the development of two TPs (table 2). Regarding the retention time of the analytical method (RP-column and a high aqueous gradient in the beginning) TP 1 has a higher polarity and TP 2 a lower than OIT. Further structure elucidation needs to be done.

3.3 Biodegradation

OIT is not readily biodegradable in the CBT. In contrast there is a low biological activity of the photolytic mixture of OIT after the test duration of 28 days by approx. 30 % (see figure 3). Just by comparison, it could be stated that the photo-TPs could be more biodegradable than the initial compound.

XVI
World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

Page 6 of 10

Figure 3: Biological degradation of OIT (left) and the photolytic mixture of OIT (right) in closed bottle test over a test duration of 28 days. Shown are quality control (orange line), toxicity control (green line) and OIT or photolytic mixture of OIT (red line).

A measurement of the biodegradation samples (LC-MS) of day 1 and day 28 shows, that the peak area of TP 1 could not be detected after 28 days. This basis of results leads to the conclusion that TP 1 seems to be biodegradable due to the conditions of the conducted CBT, e.g. bacteria abundance and composition.

3.4 Toxicity

First results of the luminescent bacteria test show approximately the same toxicity of both initial substance and photolytic mixture. A concentration of 0.85 µg L^{-1} shows an effect of 67 % (acute), 71 % (chronic) and 58 % (cell reproduction) inhibition of the luminescent. For the endpoint after 24 hours a dose-effect relation of EC_{50} = 0.45 mg L^{-1} could be calculated. Assuming an assessment factor (AF) of 1000 a PNEC (predicted no effect concentration) value of 450 ng L^{-1} can be roughly estimated through dividing EC_{50} by AF (Ferrari et al. 2004).

These results make clear, that OIT has a high toxic effect in low and environmental relevant concentrations. The toxicity of the photolytic mixture shows a slightly diminishing effect of about 9 % (acute toxicity) and 27 % (chronic toxicity). Taking into account that there is primary elimination of OIT about 25 % it could be stated, that TPs potentially have an equal acute toxicity and an increasing chronic effect.

3.5 Occurrence of TPs in environmental samples

By measuring the environmental samples with an Agilent Triple Quad, concentrations of OIT in a range of 60-100 ng L^{-1} were measured. Moreover TP1 could be observed in one environmental samples of the urban area of Freiburg in Germany. Verification remains to be done. Due to the fact that no analytical standard of TPs are available, no concentrations could be calculated.

4. Conclusion

OIT degrades by direct photolysis with a quantum yield of Φ = 0.01 and calculated annual mean lifetimes of τ = 1.5 ± 2.0 days (max. 15 day; min. 0.3 days). Considering the persistence criterion of the Stockholm Convention 2001 (Stockholm

Convention 2009) which amounted a half-life value of $t_{1/2} > 2$ month for water bodies, OIT seems to be non persistent. This conclusion is not taken into account, that environmental conditions like water turbidity and indirect photolytic processes could affect the kinetic behavior.

Abandon the primary elimination, degradation of OIT leads to the formation of two TPs within the applied analytical procedure. Estimation of their behavior was analyzed as well. Regarding this, TP 1 has a higher and TP 2 has a lower polarity than the initial substance. This fact leads to a changed mobility and therefore different spatial patterns compared to OIT. Moreover a low bacterial activity in the photolytic mixture could be observed and apparently TP 1 was degraded completely. Following structural transformation of the TP due to biodegradation processes could not be determined. Toxicity tests show unchanged or slightly lower toxicity effect of the photolytic mixture compared to the initial substance. Latter already shows toxic effects in a low concentration level with an $EC_{50} = 0.45$ mg L^{-1} and a roughly estimated PNEC of 450 ng L^{-1}, which is located near to the environmental measured concentration.

Even though transfer to environmental conditions needs to be investigated more closely, it could be stated, that the TPs of OIT have changed physicochemical properties such as sorptivity and solubility and therefore a deviating behavior regarding its temporal and spatial distribution in urban catchments. Moreover TPs show a slightly different behavior referring to biodegradation and toxicity. Hence, risk assessment on basis of the initial substance is insufficient and needs to be specified particularly with regard to environmental relevant TPs.

This research demonstrates that new insights on the fate and behavior of TPs in aquatic environments are necessary for the development of water pollution control measures to reduce the release of hazardous chemicals addressing the aims of Sustainable Development Goal 6.3.

5. References

Barceló, D. (1993): Environmental Protection Agency and other methods for the determination of priority pesticides and their transformation products in water. In: Journal of Chromatography 643, S. 117–143.

Bollmann, Ulla E.; Tang, Camilla; Eriksson, Eva; Jonsson, Karin; Vollertsen, Jes; Bester, Kai (2014): Biocides in urban wastewater treatment plant influent at dry and wet weather: concentrations, mass flows and possible sources. In: Water Research 60, S. 64–74. DOI: 10.1016/j.watres.2014.04.014.

Breuer, Klaus; Mayer, Florian; Scherer, Christian; Schwerd, Regina; Sedlbauer, Klaus (2012): Wirkstoffauswaschung aus hydrophoben Fassadenbeschichtungen. Verkapselte versus unverkapselte Biozidsysteme. In: Bauphysik 34 (1), S. 19–23. DOI: 10.1002/bapi.201200002.

XVI
World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

Page 8 of 10

Burkhardt, M.; Junghans, M.; Zuleeg, S.; Boller, M.; Schoknecht, U.; Lamani, X. et al. (2009): Biozide in Gebäudefassaden – ökotoxikologische Effekte, Auswaschung und Belastungsabschätzung für Gewässer. In: Umweltwiss Schadst Forsch 21 (1), S. 36–47. DOI: 10.1007/s12302-008-0033-1.

Burkhardt, M.; Zuleeg, S.; Vonbank, R.; Bester, K.; Carmeliet, J.; Boller, M.; Wangler, T. (2012): Leaching of Biocides from Façades under Natural Weather Conditions. In: Environ. Sci. Technol. 46 (10), S. 5497–5503. DOI: 10.1021/es2040009.

Challis, Jonathan K.; Hanson, Mark L.; Friesen, Ken J.; Wong, Charles S. (2014): A critical assessment of the photodegradation of pharmaceuticals in aquatic environments: defining our current understanding and identifying knowledge gaps. In: Environmental science. Processes & impacts 16 (4), S. 672–696. DOI: 10.1039/c3em00615h.

Chen, Zhi-Feng; Ying, Guang-Guo; Lai, Hua-Jie; Chen, Feng; Su, Hao-Chang; Liu, You-Sheng et al. (2012): Determination of biocides in different environmental matrices by use of ultra-high-performance liquid chromatography–tandem mass spectrometry. In: Anal Bioanal Chem 404 (10), S. 3175–3188. DOI: 10.1007/s00216-012-6444-2.

Escher, Beate I.; Fenner, Kathrin (2011): Recent advances in environmental risk assessment of transformation products. In: Environmental science & technology 45 (9), S. 3835–3847. DOI: 10.1021/es1030799.

European Commission - Joint Research Centre - IEC Unit (2008): The European Chemicals Bureau: an overview of 15 years experience in EU chemicals legislation. Institute for Health and Consumer Protection European Chemicals Bureau. Luxembourg.

Fenner, Kathrin; Kooijman, Cornelis; Scheringer, Martin; Hungerbühler, Konrad (2002): Including Transformation Products into the Risk Assessment for Chemicals. The Case of Nonylphenol Ethoxylate Usage in Switzerland. In: Environ. Sci. Technol. 36 (6), S. 1147–1154. DOI: 10.1021/es015576i.

Ferrari B., Mons R., Vollat B., Fraysse B., Paxéus N., Lo Giudice R., Pollio A., Garric J. (2004): Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? In: Environ Toxicol Chem. 23 (5), S. 1344-1354.

Gutowski, Lukasz; Olsson, Oliver; Leder, Christoph; Kümmerer, Klaus (2015): A comparative assessment of the transformation products of S-metolachlor and its commercial product Mercantor Gold® and their fate in the aquatic environment by employing a combination of experimental and in silico methods. In: Science of The Total Environment 506-507, S. 369–379.

XVI World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

Page 9 of 10

Jungnickel, Christian; Stock, Frauke; Brandsch, Thomas; Ranke, Johannes (2008): Risk assessment of biocides in roof paint. In: Environ Sci Pollut Res 15 (3), S. 258–265. DOI: 10.1065/espr2007.12.465.

Menz, J.; Schneider, M.; Kümmerer, K. (2013): Toxicity testing with luminescent bacteria – Characterization of an automated method for the combined assessment of acute and chronic effects. In: Chemosphere 93 (6), S. 990–996. DOI: 10.1016/j.chemosphere.2013.05.067.

Schoknecht, Ute; Gruycheva, Jana; Mathies, Helena; Bergmann, Hannelore; Burkhardt, Michael (2009): Leaching of biocides used in facade coatings under laboratory test conditions. In: Environmental science & technology 43 (24), S. 9321–9328. DOI: 10.1021/es9019832.

Sinclair, Chris J.; Boxall, Alistair B. A. (2003): Assessing the Ecotoxicity of Pesticide Transformation Products. In: Environ. Sci. Technol. 37 (20), S. 4617–4625. DOI: 10.1021/es030038m.

Umweltbundesamt: Texte 09/2009: Biozide in Gewässern: Eintragspfade und Informationen zur Belastungssituation und deren Auswirkungen.

Wangler, T. P.; Zuleeg, S.; Vonbank, R.; Bester, K.; Boller, M.; Carmeliet, J.; Burkhardt, M. (2012): Laboratory scale studies of biocide leaching from façade coatings. In: Building and Environment 54, S. 168–173. DOI: 10.1016/j.buildenv.2012.02.021.

Wick, Arne; Fink, Guido; Ternes, Thomas A. (2010): Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography–tandem mass spectrometry. In: Journal of Chromatography A 1217 (14), S. 2088–2103. DOI: 10.1016/j.chroma.2010.01.079.

XVI World Water Congress
International Water Resources Association (IWRA)
Cancun, Quintana Roo, Mexico, 29 May- 3 June, 2017

Page 10 of 10