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QSAR-analysis and mixture toxicity as diagnostic tools: Influence of degradation on the toxicity and mode of action of diuron in algae and daphnids

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ABSTRACT

Even though the environmental occurrence of pesticide transformation products is well established, ecotoxicological data for transformation products are often lacking. Therefore, it remains an open question for regulators how to handle transformation products in the process of authorization and risk assessment. Transformation products may (1) possess a similar mode of toxic action as the parent compound, (2) exhibit unexpected effects towards non-target organisms or (3) contribute to overall mixture toxicity through baseline toxicity even if the specific activity of the parent compound is lost. In the present study, a systematic and integrated approach is presented to differentiate between these three options with the goal of identifying transformation products that significantly add to the risk posed by the parent compound. Quantitative structure–activity relationships (QSAR) and a toxic ratio (TR) analysis were used to evaluate the toxicity and mode of toxic action of the transformation products relative to the parent compound. In addition, mixture toxicity experiments were used as diagnostic tools to underpin the mode of action analysis and to elucidate whether the transformation products possess a similar risk potential as the parent compound. As an illustrative example, the phenylurea herbicide diuron was chosen since a sound basis of ecotoxicological data was available not only for diuron itself but also for most of its transformation products. Effects were investigated using the most sensitive species, algae, and the non-target organism *Daphnia magna*, for which a previous QSAR-analysis of literature data suggested a specific hazard. In the present study the primary transformation products 1-(3,4-dichlorophenyl)-3-methlyurea (DCPMU), 3-(3-chlorophenyl)-1,1-dimethylurea (MCPDMU), and 1-(3,4-dichlorophenyl)urea (DCPU) were identified as specific toxicants in algae, but as baseline toxicants in daphnids. The subsequent loss of the methylurea group during degradation, which formed 3,4-dichloroaniline, led to a clear detoxification in algae but to an increase in toxicity in daphnids. It could be shown that 3,4-dichloroaniline acted as baseline toxicant in algae, but showed a specific mode of toxic action in daphnids. Mixture toxicity experiments confirmed this mode of action analysis.

1. Introduction

When synthetic organic chemicals, such as pesticides, biocides, pharmaceuticals, and industrial chemicals, are released into the environment, they are subject to various transformation processes (Weyandt and Pressel, 2003; Boxall et al., 2004). This leads to the environment being exposed not only to mixtures of parent compounds but also to mixtures of parent compounds and their corresponding transformation products. If transformation products are more persistent and mobile than their parent compounds, they may be detected in even higher concentrations than their parent compound in the aquatic environment (Boxall et al., 2004; Gilliom et al., 2006). These mixtures of parent compounds and transformation products also present a challenge to regulators, who are responsible for the inclusion of transformation products in the process of authorization and risk assessment of chemicals (Boxall, 2009). For the risk assessment of mixtures an in-depth knowledge of the modes of toxic action of the mixture components is fundamental (Broderius et al., 1995; Altenburger et al., 2000; Backhaus et al., 2000). Transformation products may still possess a similar mode of toxic action as the parent compound, may exhibit unexpected effects towards non-target organisms, or, even if they lose their specific toxicity, can still contribute as baseline toxicants to mixture toxicity (Sinclair and Boxall, 2003; Escher et al.,

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2009a). To properly assess their contribution to risk in mixtures with the parent compound, the observed effects for each transformation product have to be clearly attributed to one of these three cases.

Therefore, this study presents an approach that facilitates and systematizes the ecotoxicological risk assessment of transformation products. To gain comprehensive knowledge about the mode of action of transformation products the strategy of the proposed approach is (1) to perform a mode of action analysis of experimental data using quantitative structure–activity relationships (QSAR) and a toxic ratio (TR) analysis to evaluate the toxicity and mode of action relative to the parent compound, and (2) to use mixture toxicity experiments as a diagnostic tool to underpin the mode of action analysis. The ultimate goal is to elucidate whether the transformation products possess a similar risk potential as the parent compound.

Since the authorization of pesticides is quite advanced, also with regard to the assessment of transformation products (European Commission, 2002; European Food Safety Authority, 2005), there exists a broad database on the ecotoxicological effects of pesticides, even including quite some information on the toxicity of their transformation products (Sinclair and Boxall, 2003). Nevertheless, discussions are still ongoing on how to handle changes in mode of action and mixtures of transformation products. Based on an analysis of existing ecotoxicity data, we chose the well-known phenylurea herbicide diuron, which is an inhibitor of photosystem II, as a case study compound to illustrate our proposed approach. Once released into the environment diuron is mainly degraded via aerobic (Ellis and Camper, 1982) and anaerobic (Attaway et al., 1982) microbial degradation (Fig. 1). N-demethylation leads to the formation of 1-(3,4-dichlorophenyl)-3-methlyurea (DCPMU) and 1-(3,4-dichlorophenyl)urea (DCPU), whereas reductive dechlorination leads to 3-(3-chlorophenyl)-1,1-dimethylurea (MCPDMU). The final known transformation product of the aerobic pathway is 3,4-dichloroaniline (3,4-DCA).

The analysis of the published ecotoxicological data for diuron and its transformation products using the approach developed in our earlier work (Lienert et al., 2007; Escher et al., 2009a) indicated that diuron (US EPA, 2003) and DCPMU (US EPA, 2006) act specifically towards algae, whereas 3,4-DCA (US EPA, 2006) acts as baseline toxicant in algae. In the non-target organism *Daphnia magna* DCPMU had somewhat surprisingly been found to be even more specifically toxic than in algae (Fernandez-Alba et al., 2002). Also, 3,4-DCA was identified as specifically acting in *D. magna* (Fernandez-Alba et al., 2002), presumably due to its *o*-unsubstituted aniline structure, which is a known toxicophore structure in water fleas (von der Ohe et al., 2005). In contrast, the parent compound diuron was shown to be a baseline toxicant in *D. magna*. For DCPU no data on its toxicity towards algae and daphnids is available. Analyzing the available data for MCPDMU (European Food Safety Authority, 2005), it is classified as specifically acting in algae, but as a baseline toxicant in *D. magna*. The analysis led to the conclusion that all transformation products would contribute equally to the mixture toxicity for algae, but for water flea DCPMU was identified as having greatest impact on the mixture toxicity due to a potentially specific mode of action.

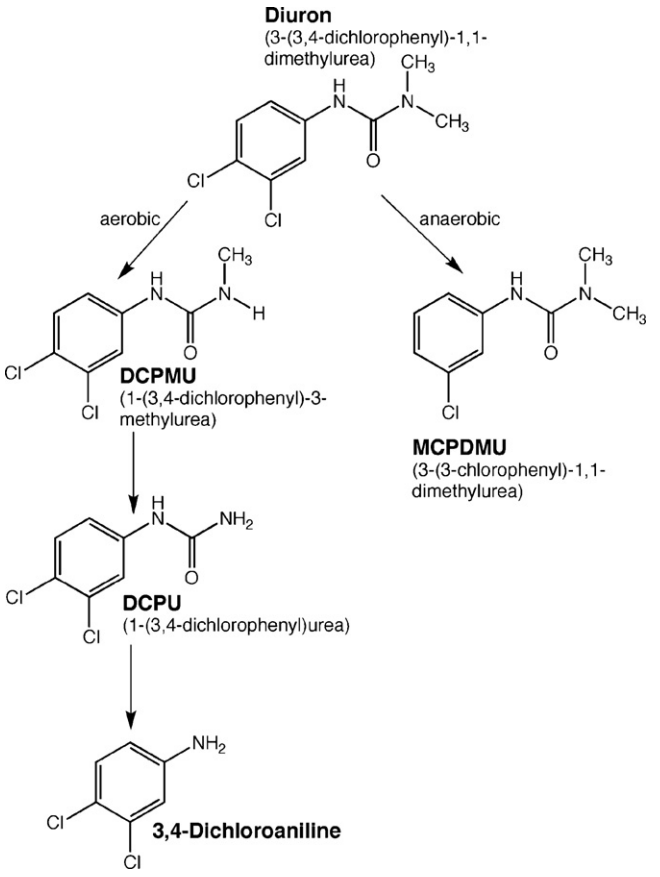

Fig. 1. Aerobic and anaerobic microbial degradation pathways of diuron (Attaway et al., 1982; Ellis and Camper, 1982).

Based on this preliminary data analysis, diuron was chosen as a suitable case study to demonstrate the applicability of our proposed approach of combining a QSAR-/TR-analysis with experimental and mixture toxicity studies to gain consistent information on the mode of action of the transformation products relative to the parent compound. Specifically, we show (1) whether the transformation products of diuron still exhibit the same mode of action as the parent compound itself, or (2) whether transformation leads to a loss of the specific toxic effect, or (3) to another specific mode of action. Algae served as illustrative case for a target organism for herbicides, where degradation is expected to lead to a decrease in toxicity, while *D. magna* was chosen to represent a non-target organism, for which the described literature analysis suggested a specific hazard of some transformation products and hence a change in mode of action upon transformation of the parent herbicide.

Table 1
Physicochemical properties of diuron and its transformation products.

Compound	Molecular weight[a]	Water solubility [mg/L, 25 °C][a]	pK_a[a]	log K_{ow}[a]	log D_{lipw} (pH 7) (predicted)
Diuron	233.09	42[b]	–	2.68[b]	2.94
DCPMU	219.07	107[c]	–	2.94[b]	3.17
MCPDMU	198.65	871[c]	–	2.00[b]	2.32
DCPU	205.04	225[c]	–	2.65[b]	2.91
3,4-DCA	162.02	92[b]	2.97	2.69[b]	2.95

[a] Data were derived from the Physprop Database (http://www.syrres.com/what-we-do/databaseforms.aspx?id=386).
[b] Experimental value.
[c] Calculated value.