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The fate of isoproturon in a freshwater microcosm with *Lemna minor* as a model organism

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Abstract

Degradation, bioaccumulation and volatile loss of the ^{14}C-labeled phenylurea herbicide isoproturon (IPU) was examined in a freshwater microcosm with the free floating macrophyte species *Lemna minor* during a 21-day exposure time. Isoproturon volatilisation was very low with $0.13 \pm 0.01\%$ of the initially applied herbicide. Only a minor amount of the herbicide was completely metabolised, presumably by rhizosphere microorganisms and released as $^{14}CO_2$. In total, about 9% isoproturon was removed from the aquatic medium during 21 days. The major portion of the pesticide was removed by bioaccumulation of *Lemna minor* ($5.0 \pm 0.8\%$) and the bioconcentration factor (BCF) based on freshweight was 15.8 ± 0.2. However, this study indicated a high persistence of IPU in freshwater ecosystems and a potential hazard due to bioaccumulation in non-target species. The novel experimental system of this study, developed for easy use and multiple sampling abilities, enabled quantitatively studying the fate of isoproturon and showed high reproducibility with a mean average ^{14}C-recovery rate of $97.1 \pm 0.7\%$.

Keywords: Isoproturon; *Lemna minor*; Aquatic system; Mineralisation; Volatilisation; Bioconcentration factor

1. Introduction

Isoproturon (IPU) is a phenylurea derived systemic herbicide [3-(4-isopropylphenyl)-1,1-dimethylurea] for the control of annual grasses and broad-leaved weeds in agricultural fields. This herbicide is specific for monocots and mainly inhibits the electron transport in photosystem II (PS II) by binding to the D1 Protein in the thylakoid membrane (Arnaud et al., 1994). Spray-drift, drainage, erosion and runoff events may deport IPU primary to non-target sites, in first order to rills and ponds. Due to this effects, nearby freshwater ecosystems can be contaminated with high concentrations of herbicides (Mickelson et al., 2001). Laboratory simulations of worst case scenarios yielded runoff concentrations of IPU up to 60 mg l^{-1} (Lecomte et al., 2001). Isoproturon is one of the most common herbicide species, applied and to be found in water samples. High levels were observed in spring from April to May, the time herbicides are mainly applied and runoff events are very common (Nitschke and Schüssler, 1998).

Nitschke and Schüssler (1998) have shown that more than three quarters of the total herbicide residues load in the effluent of rural waste water treatment plants was isoproturon with a maximum burden of 42 μg l^{-1} in 14-days mixed samples. However, only a limited amount of studies have been reported regarding the fate of isoproturon in freshwater ecosystems (Feurtet-Mazel et al., 1996; Grollier et al., 1997). Degradation and bioaccumulation of IPU in the aquatic phase was scarcely determined. In the study presented here, the free floating macrophyte *Lemna minor* (duckweed) was chosen as non-target species which is common to occur in rural small-scale water bodies. Distribution of the family of duckweeds (*Lemnaceae*) is worldwide (Landolt, 1986) and they are well known to tolerate

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environmental stress and to accumulate xenobiotica. However, they also are sensitive in a way, that they show certain responses to various stress parameters (Mohan and Hosetti, 1999; Cleuvers and Ratte, 2002). This qualifies duckweeds for ecotoxicological studies especially *Lemna minor* (Hulsen et al., 2002), for which standardised test procedures are available (ISO, 2005). Further on, there is particular interest in duckweed for wastewater treatment systems due to their capacity in removing organic load and nutrients (Awuah et al., 2004). Duckweeds are monocot angiosperms, so that the PS II of *Lemna minor* should be targeted by IPU. However, IPU concentration was chosen to a tolerable degree of inhibition according to the seven day EC_{50} value for *Lemna minor* based on total frond number (Nitschke et al., 1999). It was the goal of this study, to quantitatively investigate accumulation, decomposition and volatilisation of IPU in a freshwater ecosystem with the non-target plant *Lemna minor*. Therefore, a novel microcosm test system was designed and qualified by this investigation. The basic setting was similar to a system already described by Huckins and Petty (1984) achieving ^{14}C-mass balance by using direct sampling from the aquatic medium, $^{14}CO_2$ and volatile ^{14}C-compound trapping. However, we additionally desired a separated gas phase sampling for light/dark regime and the use of laboratory standard equipment.

2. Material and methods

2.1. Maintenance of plants

A stock of *Lemna minor* ST (Jena stock collection) was obtained from the Bavarian Water Management Agency, Munich and kept in non-axenic laboratory culture under simulated outdoor conditions. Illumination was provided by four universal white fluorescence tubes with a 12 h photoperiod. Temperature for maintenance and experiments was 23 ± 1 °C. Precultures of *Lemna minor* clones were grown in 600 ml glass beakers filled with 250 ml Steinberg's medium mod. Altenburger (ISO, 2005). This corresponds to a surface area of approx. 60 cm^2 each. All beakers were covered with transparent foil to avoid microbial contaminations and evaporation.

2.2. Experimental design

Preliminary experiments have been conducted to test the system as a whole and to define an IPU concentration at which all occurring effects could be studied, without falling below detection limits.

Three equivalent systems were established and experiments run simultaneously. For each sampling from aqueous solutions, two replicates were analysed. Laboratory standard desiccators (10 l) were used simulating a small pond and were filled with 2 l Steinberg's medium mod. Altenburger (ISO, 2005), each. The light intensity measured on the water surface by closed lid was 43 ± 6 μmol m^{-2} s^{-1} (Light Meter, LI-COR LI-250). For modelling the environment of runoff events in spring, we considered a 12 h photoperiod. On each 2 l medium, 21 μl of a ^{14}C-labeled Arelone, [^{14}C-*u-phenyl*]Isoproturon (International Isotopes, Munich) standard with an IPU concentration of 5.07μg $μl^{-1}$, a specific radioactivity of 686.3 Bq $μg^{-1}$ and a radioactive purity of >98% were applied and homogenised intensively with water. However, due to systematic and statistic deviations, the volume was not used to rely on as basis for further calculations. Instead, the concentration of IPU was measured directly after application by ^{14}C liquid scintillation counting (Tri-Carb 1900 TR, Packard, Netherlands) using 1 ml sample plus 15 ml Ultima Gold (Packard, Netherlands) as scintillation cocktail.

Minimal invasive direct sampling from the medium during the experiments was enabled by a special sampling port at the side plug of the desiccator. The plastic plug was permanently connected to a tubing by a perforation as port for a syringe and sealed with a small stopper. The systems were continuously aerated (23 l h^{-1}) with air, which first was cleaned by passing through activated charcoal. Air inlet and outlet were provided via a gas bubbler plug at the top of the desiccator. For homogeneous mixing and avoiding perturbations of the water surface, gas was let in immediately at the top but withdrawn by a tube shortly above the water surface. The air from each plant system was trapped in three subsequent absorption tubes. In order to absorb organic ^{14}C-labeled compounds, the first tube was filled with 10 ml ethyleneglycolmonomethylether (Merck, Germany). The further two tubes were used for estimating biomineralisation by trapping $^{14}CO_2$ and were filled with 10 ml of a 1/1 (v/v) mixture of ethanolamine and diethyleneglycolmonobutylether, each. All traps were cooled by a cryostat at −10 °C to provide maximum trapping capacity.

The experimental set-up provided separated gas trapping for the 12 h light and dark regime: There were two arrays of absorption tubes per system, each with a pump and a gas flowmeter, so that they could separately be switched on and off over the day. They were automatically controlled by a timer, which was set according to the illumination timer with 55 min overlap, so that the gas exchange in each system was almost completed for each photoperiod before switching to the second array of absorption tubes. Assuming a model with immediate homogenous mixing of the incoming gas in the system and a gas phase of 8 l, a 55 min lasting aeration at a rate of 23 l h^{-1} should have resulted in 93% gas exchange as calculated by

$$V(t) = V_0 \cdot e^{-f \cdot \frac{t}{V_0}} \quad (1)$$

where $V(t)$ is the volume after a certain time of gas exchange, V_0 is the gas phase volume of the system, f is the flow rate and t is the time of gas exchange.

All tubing and connection pieces were either iso-versinic (viton) or glass with Teflon coatings to minimise sorption of IPU and its metabolites.