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EFFECTS OF LINURON TO AN AQUATIC PLANT IN SEDIMENT-DOSED TEST SYSTEMS

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Abstract: *The availability of standardised methodologies to assess the environmental risks of pesticides to non-target plants is currently limited. Aquatic plant test guidelines are only available for algae and Lemna. These studies may not be sufficient especially in the case when contamination via sediment is an important exposure pathway. The present study was initiated to investigate the effects of exposure via the sediment on a sediment-rooted aquatic plant, i. e. Myriophyllum spicatum. Plants of Myriophyllum spicatum were cultivated in linuron spiked sediment under similar conditions for 21 days. At days 7, 14 and 21 of the test, various morphological endpoints were measured and dose-response relationships, EC_{50} values and NOECs were estimated. During the test period, linuron concentrations in sediment, water and plants were monitored to quantify exposure in the aquatic plant test and concentrations in the compartments. The results from these experiments were used to quantify risks of a herbicide to sediment-rooted aquatic plants and may lead to recommendations for improvement of test designs and risk assessment methods.*

Keywords: herbicides, aquatic macrophytes, risk assessment, sediment, linuron

INTRODUCTION

Spreading of pesticides via spray drift, runoff and leaching from agricultural fields can affect non-target terrestrial and aquatic plants in adjacent land and water ecosystems. The availability of standardized methodologies to assess the environmental risks of pesticides to non-target plants is currently limited. Aquatic plant test guidelines are only available for algae and *Lemna* as representatives of primary producers.

These studies may not be sufficient especially in the case when contamination via sediment is an important exposure pathway. Information on toxicity to sediment-rooted aquatic macrophytes may be required because the endpoints reflecting root growth can be more sensitive than growth/biomass and shoot endpoints (Arts et al. 2008). Therefore a standardized test protocol for the

rooted aquatic macrophyte, *Myriophyllum* sp. has currently been proposed in Maltby et al. (2010). This test method enables the study of exposure to a substance either via the water phase or via the sediment.

A study was initiated to investigate the effects of exposure via the sediment on a sediment-rooted aquatic plant, i. e. *Myriophyllum spicatum*. A second objective of the experiment was to quantify exposure in the aquatic plant test. During the test period herbicide concentrations in sediment, water and plants were monitored

MATERIALS AND METHOD

The methods and conditions of the experiment follow test protocol for the rooted aquatic macrophyte, *Myriophyllum* sp., as proposed in Maltby et al. (2010). The protocol was slightly modified.

As a contaminant the herbicide linuron was chosen. Linuron is a photosynthetic electron transport inhibitor that has the photosystem II receptor site as target site. It is a selective systemic herbicide, absorbed principally by the roots but also by the foliage, with translocation primarily via the xylem.

The sediment was spiked with a concentration range of linuron (concentrations 10.7; 107.3; 1072.7; 10726.7; 21453.3 µg/kg) by addition of a solution of the test substance directly to the sediment. The standard sediment was prepared from 5 % peat powder, 74 % quartz sand and 20 % kaolin clay, 1 % $CaCO_3$ powder (on weight basis). Nutrients $Na_3PO_4 \cdot 12H_2O$ and NH_4Cl were added in a concentration of 300 mg/kg.

Planting pots (ca. 9 cm in diameter and 8 cm in height) were filled with sediment in the following sequence: first a filter paper was adjusted on the bottom of the pot. Afterwards sediment was added as an approximately 1 cm layer without any contaminants. On top of this a 4 cm layer of spiked sediment was introduced. This was again covered with 1 cm of the standard sediment without any contaminants and with a very thin layer of sand on top in order to reduce suspension of sediment particles into the water.

For the experiment plants of *Myriophyllum spicatum* were collected from outdoor stock cultures and acclimatized in the laboratory for three weeks. Three healthy shoot apices were clipped off at a length of ca 10 cm and directly added to the test pots (two nodes within the sediment) without a prior rooting phase. The pots with sediment and plants were carefully submersed into the beaker including two litres of the Smart and Barko (1985) medium. The accurate length, wet and dry weight of 12 % of the shoots was measured at the beginning of the test and was set as initial values.

The plants were cultivated in a climate room for 21 d at a temperature of 20 ºC ± 2 ºC, a photoperiod of 16/8 h light/dark and a light intensity of 190 (+/- 20) µE/m^2/s with a wavelength of 400 – 700 nm (see fig. 1). Temperature, pH and O_2 concentrations were measured regularly.

At the days 7, 14, 21 of the experiment four test systems from each linuron treatment and six controls were evaluated. The plants were harvested and the morphological endpoints were measured. The samples of the water medium, sediment and shoots were collected for linuron analyses.

Endpoints recorded were chlorosis, necrosis, morphological changes, wet and dry weight of the root and shoot, length and number of the roots, length of the main shoot, number and length of the side shoots. From these endpoints a dose-response relationship was established and ECx, NOEC and LOEC were estimated for individual endpoints.

In the samples of water medium the linuron concentrations were directly analysed using a high performance liquid chromatography technique (HPLC). Analysis of linuron concentrations in plant shoots and sediments were performed according to Crum et al. (1998). Linuron was extracted by shaking with ethyl acetate and water. The subsamples were then cleaned up on florisil columns. In final samples linuron was analysed by HPLC method.

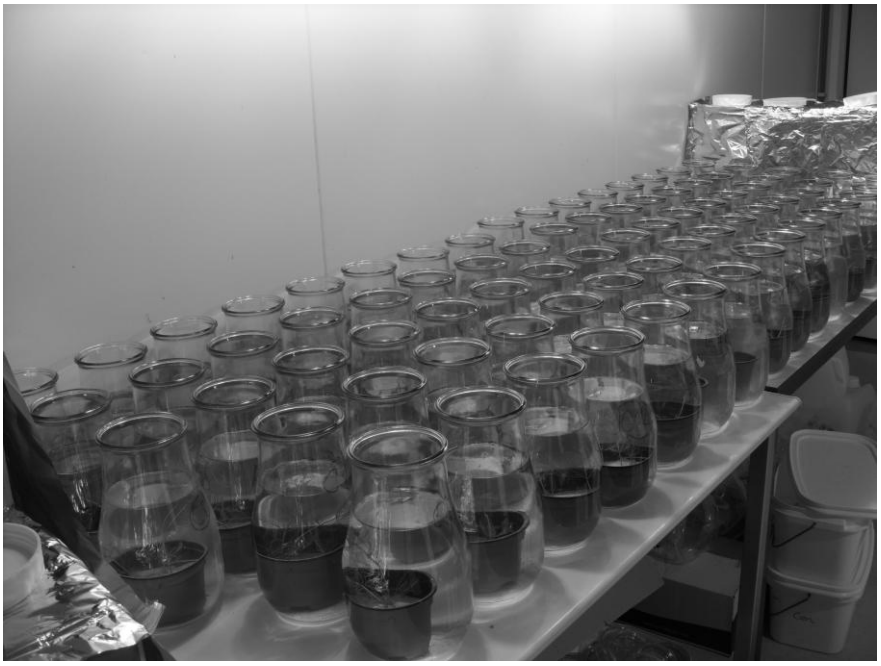

Fig. 1: Beakers with sediment pots and plants in water medium

RESULTS AND DISCUSSION

Although the artificial sediment was covered with a thin layer of sand and 1 cm layer of non-contaminated sediment in order to minimize the exchange between sediment and the overlying water, linuron was already measured in the

water layer in toxic concentrations at day 7. It was evident that linuron has not only effects on the plant through sediment exposure but also through exposure in the water medium.

The recovery rate of linuron by the method from macrophyte samples was 96 % ± 10 % (n = 4) and from sediments 105 % ± 2 % (n = 3).

Overall effects of linuron on *Myriophyllum spicatum* will be presented and discussed.

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REFERENCES

Arts G. H. P., Belgers J. D. M., Hoekzema C. H., Thissen J. T. N. M. 2008. Sensitivity of submersed freshwater macrophytes and endpoints in laboratory toxicity tests. Environ. Pollut. 153, pp. 199-206.

Crum S. J. H., Aalderink G. H., Brock T. C. M. 1998. Fate of the herbicide linuron in outdoor experimental ditches. Chemosphere 10, Vol. 36, pp. 2175-2190.

Maltby L., Arnold D., Arts G., Davies J., Heimbach F., Pickl C., Poulsen V. 2010. Aquatic macrophyte risk assessment for pesticides. Society of Environmental Toxicology and Chemistry. Penascola, USA.

Smart R. M., Barko J. W. 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquat. Bot. 21, pp. 251-263.

Tomlin C. (ed.) 2003. The pesticide manual: A world compendium. 13th ed. British Crop Protection Council, Farnham, UK, pp. 599-600.