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Laboratory tests on sorption and transformation of the insecticide flubendiamide in Japanese tea field soil

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HIGHLIGHTS

- Laboratory tests on sorption, leaching, microbial and photo-induced microbial transformation were performed.
- Strong sorption was revealed by batch equilibrium and column tests.
- High persistence was found in aerobic biotransformation tests.
- An enhanced biotransformation by photo-induced impacts could not be confirmed.
- Field studies are necessary to elucidate fate and behavior of flubendiamide in Japanese tea field soil.

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ABSTRACT

Flubendiamide belongs to the modern insecticides applied in Japanese tea cultivation to control smaller tea tortrix and tea leaf roller. Since fate and behavior in soil have been only monitored sparsely and fragmentarily until today, laboratory tests were performed on sorption, leaching, biotransformation and photo-induced biotransformation of flubendiamide in two different soils. In batch equilibrium tests, K_d and K_{OC} values were 15 and 298 L kg^{-1} for the Japanese tea field soil as well as 16 and 1610 L kg^{-1} for the German arable field soil classifying flubendiamide to be moderately mobile and slightly mobile, respectively. The affinity to the tea field soil was additionally confirmed by soil column tests where flubendiamide was predominantly retarded in the topsoil layers resulting in a percolate contamination of only 0.002 mg L^{-1}. In the aerobic biotransformation tests, flubendiamide did not substantially disappear within the 122-d incubation period. Due to $DT_{50} > 122$ d, flubendiamide was assessed very persistent. Supplementary, photo-induced impacts on biotransformation were studied in a special laboratory irradiation system. Despite a 14-d irradiation period, photo-induced biotransformation in the tea field soil was not identifiable, neither by HPLC/DAD nor by LC/MS/MS. 3-d irradiation tests in photosensibilizing acetone, however, showed that the primary photo-transformation product desiodo-flubendiamide was formed. How far this photochemical reaction may also occur in soil of perennial tea plant stands, however, has to be checked in field studies.

1. Introduction

Due to the requirements of regulatory procedures, fate and behavior of novel pesticides in different environmental compartments have to be thoroughly investigated by the industrial producers before pesticides can be placed on the market. Hence, their environmental fate is principally well-known. Data, however, very often are not published. This is the fact for flubendiamide, the first example of a modern insecticide belonging to the group of benzenedicarboxamides or phthalicacidiamides with an extremely high activity against a broad spectrum of lepidopterous insects (Tsubata et al., 2007). This compound exhibits a novel mode of action because flubendiamide disrupts proper muscle functions in larvae. Ryanodine receptors are intracellular Ca^{2+} channels, specialized for the rapid and massive release of Ca^{2+} from intracellular stores. This release is an essential step in the muscle contraction process leading to rapid feeding cessation, lethargy, partial paralysis, cardiac muscle failure and regurgitation (Masaki et al., 2006). Flubendiamide is the active substance of the plant protection products Phoenix® produced by Nihon Nohyaku (Tokyo, Japan) and Belt® produced by Bayer CropScience (Monheim, Germany) (Tohnishi et al., 2005). Due to its effects against, e.g., smaller tea tortrix and tea leaf roller, it is currently applied in Japanese tea cultivation.

Published studies on the environmental fate of flubendiamide are rare. Its disappearance from chili, cabbage, tomatoes and eggplants was reported (Sahoo et al., 2009; Mohapatra et al., 2010, 2011; Chawla et al., 2011). Fate and behavior in soil, however, have been only monitored sparsely and fragmentarily (Das and Mukherjee,

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2011, 2012a,b). On this existing database, the behavior of flubendiamide in Japanese tea field soil cannot be evaluated. Therefore, this modern insecticide was selected for fate monitoring in two different soils under laboratory conditions. Thus, sorption and leaching were studied in batch equilibrium and column tests. Incubation experiments were performed in the dark and under irradiation to check for microbial and photo-induced microbial transformation.

2. Materials and methods

2.1. Reference chemical and standard solutions

Flubendiamide (3-iodo-*N*′-(2-methyl-1-(methylsulfonyl)propan-2-yl)-*N*-(2-methyl-4-(per-fluoropropan-2-yl)phenyl)phthalamide; 97% purity) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The stock standard solution of 10 μg μL^{-1} was prepared in acetonitrile (HPLC grade; all solvents: Merck, Darmstadt, Germany) and was stored in the dark at 4 °C at maximum for 1 month. According to the application rate of flubendiamide in green tea cultivation in Japan (400 g active ingredient ha^{-1} in 2000–4000 L water ha^{-1}) (FAO, 2010), this stock standard solution was used for spiking the soil samples of the transformation tests. Since a rapid disappearance of flubendiamide in soil was reported (Sahoo et al., 2009; Mohapatra et al., 2010, 2011), the concentration of 1 mg 50 g^{-1} soil of each batch test was adjusted without any adverse effect on the soil microbial activity in order to follow the disappearance of the applied parent compound and the formation of its primary metabolite desiodo-flubendiamide (*N*′-(2-methyl-1-(methylsulfonyl)propan-2-yl)-*N*-(2-methyl-4-(per-fluoropropan-2-yl)phenyl)phthalamide). Due to its unavailability as a reference chemical, it was only semi-quantitatively screened using HPLC/DAD and LC/MS/MS. For recording external calibration curves by means of HPLC/DAD and LC/MS/MS, flubendiamide standard solutions were prepared at 10 to 200 ng μL^{-1} and 1 to 20 pg μL^{-1}, respectively.

2.2. Soil samples

Topsoil samples (0–15 cm) were taken from a tea field at Shizuoka Tea Research Center, Kikugawa, Japan. This Relic soil was characterized as a silty clay soil with 3.6% sand, 59.8% silt and 36.5% clay. Further parameters were organic carbon content: 5.4%, maximum water holding capacity (WHC_{max}): 44%, pH($CaCl_2$): 3.8, substrate induced respiration (glucose): 9 mg O_2 kg^{-1} dry soil h^{-1}. Additional samples were taken from an arable field at the Julius Kühn-Institut, Bundesforschungsanstalt für Kulturpflanzen, Braunschweig, Germany. This Luvisol was characterized as a silty sand soil with 47.0% sand, 46.7% silt and 6.3% clay. Further parameters were organic carbon content: 0.9%, WHC_{max}: 19%, pH($CaCl_2$): 5.6, substrate-induced respiration (glucose): 23 mg O_2 kg^{-1} dry soil h^{-1}.

The soil samples of both investigation sites were sieved <2 mm and homogenized. Thereafter, samples were stored at −20 °C up to 3 months at maximum. Before experimental processing, samples were re-conditioned at 4 °C for 5 to 7 d. For this purpose, samples were additionally moistened with demineralized water and temporarily homogenized by mixing. This procedure was continued at room temperature during the last 3 d prior to the transformation tests. Finally, the soil samples were checked for microbial activity by substrate-induced respiration.

2.3. Sorption tests

In order to assess the mobility of flubendiamide in both soils under study, sorption tests in 4 replicates were performed in accordance with the OECD guideline 106 (1981a, 2000). Thus, 50 μL of the stock standard solution (10 μg μL^{-1}) were homogenized with 5 mL 0.01 M calcium chloride solution in centrifuge tubes. After addition of 25 g soil each, additional 30 mL of calcium chloride solution was added to adjust a soil/water ratio of 1:1.4 (Rütters et al., 1999). The suspensions were shaken at 220 rpm for 24 h and subsequently centrifuged at 4000 rpm (Megafuge 1.0; Haereus, Hanau, Germany) for 30 min. The aqueous phases were taken, filtrated and liquid–liquid extracted using a cyclohexane/ethyl acetate (1:1) mixture. The organic phases were dried over anhydrous sodium sulfate, rotary evaporated to 2 mL, subsequently evaporated to near dryness using a gentle stream of nitrogen and redissolved in acetonitrile. Flubendiamide was finally analyzed by means of HPLC/DAD.

2.4. Column tests

In accordance with the OECD guideline 312 (2004), soil column tests were conducted in order to check for leaching tendencies of flubendiamide in Japanese tea field soil under worst-case conditions. Glass columns were filled with disturbed Relic soil samples (sieved <2 mm; soil column: 30 cm length, 5 cm ID), covered at top and bottom by sea sand layers. The test substance dissolved in acetonitrile was homogeneously spiked on topsoil layer at 0.23 mg, equivalent to 0.4 mg kg^{-1} soil. For irrigation, 800 mL 0.01 M calcium chloride solution was introduced under saturated water flow to simulate an extreme precipitation event of 400 mm within 48 h. The percolate was sampled as a bulk sample. Thereafter, the soil samples were differentiated into 5-cm layers and analyzed as described in Section 2.7.

2.5. Biotransformation tests

Aerobic biotransformation tests were performed using a laboratory batch system traced back to the OECD guideline 304 A (1981b). This system was equipped with an Erlenmeyer flask, an internal carbon dioxide trap with inlet and outlet valves. Its performance was specified by Kreuzig et al. (2007) in comparison to the flow-through system and the biometric flask with soda lime trap both described in the OECD guideline 307 (2002). For the batch experiments, 50-g aliquots of soil (ds) were adjusted to a water content of approximately 40% of WHC_{max} by adding demineralized water and spiked with 100 μL of the stock standard solution. The samples prepared in duplicate were stored in the dark in an incubator at 20 ± 1 °C at intervals of 0, 1, 3, 7, 14, 28, 48, 56, 100 and 122 d. The samples were aerated once the week to remain aerobic conditions indicated by redox potentials of E_h > 400 mV (Kreuzig et al., 2007). The subsequent sample preparation is described in Section 2.7.

2.6. Photo-induced biotransformation tests

The experiments on photo-induced transformation were performed in the laboratory irradiation system described by Kreuzig et al. (2003). Its performance has been already specified for studies on the photo-induced microbial and chemical transformation of the azole fungicide prochloraz and the sulfonamide antibiotic sulfadiazine (Höllrigl-Rosta et al., 1999; Kreuzig and Höltge, 2005). For the direct comparison between the biotransformation batch experiments and the photo-induced biotransformation tests, the same soil sample amount of 50 g (ds) and 100 μL of the stock standard solution were used. After the sample preparation described in Section 2.7, the concentration of flubendiamide was determined using HPLC/DAD. For the identification of the primary metabolite, LC/MS/MS experiments were additionally performed. The irradiation tests operated for 3, 7 and 14 d with 9-h light/15-h dark intervals.

In order to check for the potential photo-sensitivity of flubendiamide, additional photochemical tests in demineralized water and acetone were carried out. For this purpose, 20 mL water or 20 mL acetone spiked with 200 μL of the stock standard solution was used. After 1, 2, 4 and 6 h and 3 d, 1-mL samples were taken. The aliquots of the water samples were directly analyzed. The acetone samples were evaporated in a gentle stream of nitrogen. Each sample was dissolved in 1 mL acetonitrile for HPLC/DAD analysis. For LC/MS/MS analysis, the samples were