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Availability and toxicity of pendimethalin to aquatic microorganisms

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The characterization of bioavailability and toxicity of pesticides is necessary for the assessment of environmental risk caused by these chemicals. Pendimethalin is a dinitroaniline herbicide used for selective control of most annual grasses and many annual broad-leaved weeds in several crops. The technical formulation of this herbicide, Stomp 330, has been approved in Lithuania and is applied to winter rye, barley, maize, winter wheat, as well as to some vegetable crops.

The objective of this study was 1) to evaluate the bioavailability and toxicity of pendimethalin to three species of aquatic microorganisms: green microalgae *Selenastrum capricornutum*, ciliate protozoa *Tetrahymena thermophila* and luminescent bacteria *Vibrio fischeri*; 2) to compare the toxicity of pendimethalin-standard (pure compound) and technical formulation of herbicide Stomp 330 to the microorganisms tested. The growth of algae *S. capricornutum* was most sensitive to pendimethalin (the value of EC50 for pendimethalin-standard – 52 μg/l) In the tests with *T. thermophila* and *V. fischeri*, the values of EC50 for pendimethalin were above the water solubility level of this compound (0.3 mg/l). The effect of Stomp 330 on bacterial bioluminescence appeared in a shorter time as compared to the effect of pure pendimethalin. The difficulty in evaluating the bioavailability and toxicity of poorly water-soluble substances to microbial cells is discussed.

Key words: pendimethalin, Stomp, bioavailability, toxicity, bacterial bioluminescence

INTRODUCTION

The characterization of bioavailability and toxicity of pesticides continues to be of interest because this information is necessary for the assessment of environmental risk caused by these chemicals. The current environmental legislation regulating the issues of pollution is based on the total levels of pollutants. However, the presence of certain pollutants in an environmental sample does not mean that this chemical is available to biota. This is especially important for the assessment of soil pollution with hydrophobic organic toxicants, since these compounds tend to sorb into a solid matrix. The sorption–desorption process is affected by the physical and chemical properties of the pesticide and the soil. The availability of pesticides for transport, plant uptake and degradation is characterized by the incorporation of the sorption coefficient (Kd), which is the ratio of the amount of the chemical sorbed to its content in solution, as determined using batch slurry techniques [1].

Pendimethalin, the common name of N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine, is a dinitroaniline herbicide used for the selective control of most annual grasses and many annual broad-leaved weeds in several crops [2]. The technical formulation of this herbicide, Stomp 330 EC, has been approved in Lithuania and is applied to winter rye, barley, maize, winter wheat, as well as to some vegetable crops, mainly to cabbage, carrots, and parsley. Usual doses are from 2 to 6 kg/ha (or 0.6–2.0 kg of the active ingredient/ha). The sorption of pendimethalin was determined in seven regions of Lithuania where the soils are of different texture types. The values of Kd were high and ranged from 85.35 to 169.44 ml/g (unpublished observations). Leaching experiments carried out under natural climatic conditions during 1996–2001 at the Vokė Branch of Lithuanian Institute of Agriculture have shown that within fifteen months from the application of Stomp 330 in autumn at 1.0 kg (active ingredient)/ha in winter rye, only 0,01–0,03% of the herbicide was leached (unpublished observations).

The data obtained from the leaching experiments support the suggestion that pendimethalin presents minimal risk of groundwater contamination [3]. Nevertheless, some risk of surface water pollution remains. Due to the complexity of ecosystems and the multifunctional character of toxicity, the ecotoxicological hazard assessment

i s more informative when several biotests with organisms of different trophic levels are used together as a test battery [4]. Aquatic toxicity testing is usually performed with fish, crustaceans, algae and bacteria [5].

The aim of the current study was 1) to evaluate the availability and toxicity of pendimethalin to three species of aquatic microorganisms: algae, protozoa and bacteria; 2) to compare the toxicity of pure pendimethalin (pendimethalin-standard) and technical formulation of herbicide (Stomp 330 EC) to the microorganisms tested.

MATERIALS AND METHODS

Chemicals. Pendimethalin-standard-analytical grade, purity 97.1% from Cyanamid British Corporation, and Stomp 330 EC – a formulated product (emulsifiable concentrate) containing 330 g/l of the active ingredient (pendimethalin) were used in this study.

Toxicity testing. The bioassay with microalgae *Selenastrum capricornutum* was performed as described [6, 7]. The algae were incubated with toxicants in 20-ml glass vials (test volume was 5 ml) at 23 ± 2 °C at a constant illumination of 8000 lux for 72 h. The algal growth was followed by optical density at 670 nm.

The bioassay with ciliate *Tetrahymena thermophila* was performed according to the standard operational procedure of Protoxkit F™ [8]. The test was based on the turnover of the substrate into ciliate biomass. Test cuvettes with ciliate protozoa *T. thermophila* were incubated at 30 °C for 24 h in the dark, and the growth inhibition reflected by the reduction of food consumption was used as a toxicity endpoint. Turbidity was measured at 440 nm.

Bacterial bioassay. A luminescent bacterial strain *Vibrio fischeri* NRRL B-11177 was used. The bacteria were cultivated, harvested, and frozen as described in our previous paper [9]. After thawing, the bacterial suspension was used for toxicity test. Three to seven replicate experiments were performed with each concentration of the herbicide.

RESULTS AND DISCUSSION

Experiments with *S. capricornutum* revealed a significant effect of the herbicide on this green microalga: the population growth markedly decreased with an increasing pendimethalin concentration as presented in Fig.1. Effective concentration, which resulted in 50% growth inhibition (EC50) for pendimethalin-standard was 52 μg/l. When Stomp 330 vas used, the value of EC50 was 38 μg/l (if calculated to active ingredient).

High sensitivity of *S. capricornutum* was found by other investigators. For instance, with Stomp 400 SC the EC50 value for *S. capricornutum* was found to be markedly lower than EC50 for green alga *Stichococcus bacillaris* [10]. It can be suggested that the high sensitivity of some algal species is associated with a good

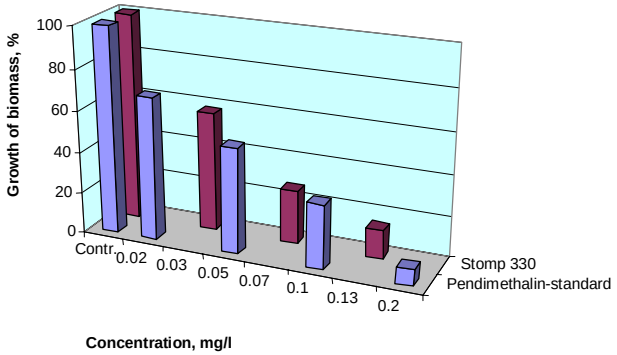

Fig. 1. Effects of pendimethalin-standard and technical formulation Stomp 330 on the growth of *Selenastrum capricornutum* microalgae

adsorbtion of chemical compounds to algal cells. A high adsorbtion capacity was observed in another algal species (of a similar sensitivity [5]) – *Scenedesmus subpicatus*: pendimethalin adsorbed to this alga was to 77% associated with the particulate (algae) fraction after 48 h and only 12% was recovered from the water phase [11].

The growth of ciliate *T. thermophila* was more tolerant to pendimethalin as compared to the algae: the EC50 value was 1.17 mg/l (Fig. 2, data from a pilot study). The test with *T. thermophila* is based on the turnover of food substrate into ciliate biomass. Thus, together with *T. thermophila* cells, there was a food suspension in the test vials where pendimethalin was also added. It is likely that an appropriate amount of pendimethalin was

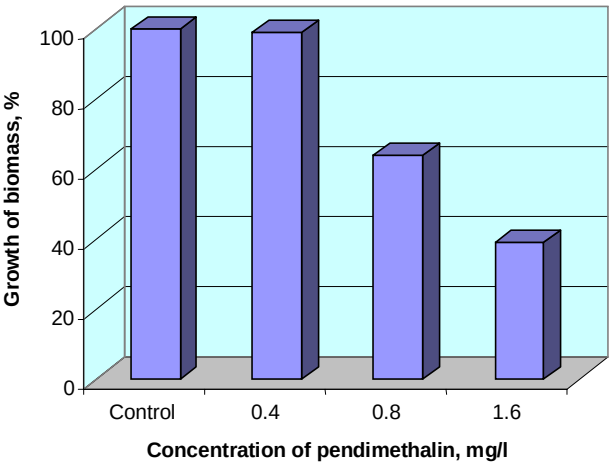

Fig. 2. Effects of pendimethalin-standard on the growth of *Tetrahymena thermophila* (data from a pilot study, without statistical treatment)

bound to the food particles and after that was incorporated into ciliate biomass (swallowed by ciliate).

The effects of pendimethalin on bioluminescence of *V. fischeri* were evaluated after 15, 30, 45, and 60 minutes of exposure. At the concentrations used in the experiments with *S. capricornutum* (0.02–0.2 mg/l) the effect of pendimethalin on bioluminescence of *V. fischeri* was rather negligible (data not shown). The further increase of pendimethalin doses added to the suspension of *V. fis-*

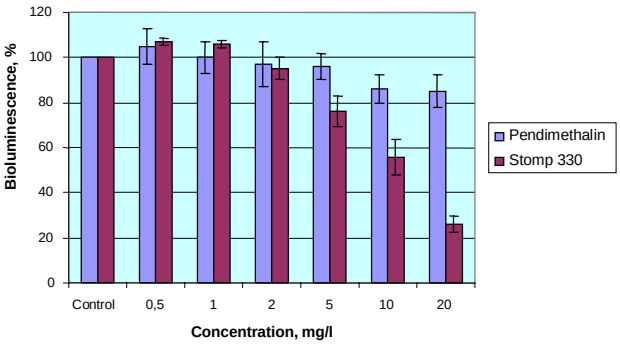

Fig. 3. Effects of pendimethalin-standard and Stomp 330 on the bioluminescence of *Vibrio fischeri* after 15 min of exposure

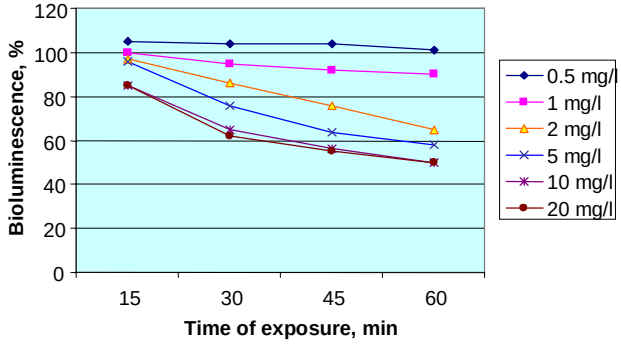

Fig. 4. Time-dependence of the effects of pendimethalin-standard on *Vibrio fischeri* bioluminescence

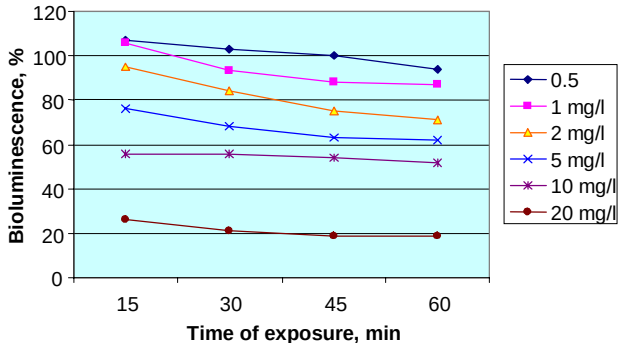

Fig. 5. Time dependence of the effects of Stomp 330 on *Vibrio fischeri* bioluminescence

heri led to an inhibition of bacterial bioluminescence. After 15 min of exposure (Fig. 3) the inhibitory effect of pendimethalin-standard differed from the effect of Stomp 330 significantly: for pendimethalin-standard no significant bioluminescence inhibition (effect ≤20%) up to a concentration of 20 mg/l was determined, while the inhibition of bacterial bioluminescence by Stomp 330 reached 74%. With prolonged exposure, an increase in inhibition of the bioluminescence of *V. fischeri* by pendimethalin-standard was observed (Fig.4). The effects of Stomp 330 on the bioluminescence of bacterial cells only slightly increased with the time of exposure (Fig.5). Thus, after 60 min of exposure, the values of bioluminescence inhibition for 2, 5 and 10 mg/l of pendimethalin were very close to each other. At a concentration of 20 mg/l the effect of Stomp 330 was significantly prominent as compared to pendimethalin-standard (81% and 50%, respectively).

In tests with *T. thermophila* and *V. fischeri,* the values of EC50 for pendimethalin were above the water solubility level of this compound (0.3 mg/l). As Fliedner [11] has noted, testing is quite difficult above the water solubility level: an adequate pretreatment of the test vessels or solubilizing agents must be identified which allow testing above the water solubility level without unrealistically altering the bioavailability and toxicity of the test substance. Inherent toxicity of the solvent itself or its interaction with the test substance may result in some alterations. Methanol and ethanol were used as solubilizing agents in this study, and the concentration of the solvent did not exceed 0.5% in the test sample. At such concentrations, the solvents are not likely to alter the toxicity of pendimethalin.

The luminescent bacterium *V. fischeri* is widely used in toxicity testing of various chemicals, herbicides included [12–14]. When handling unknown samples Microbics Corporation recommends to take the data of five and fifteen minutes [15]. According to data presented in this study, a 15-min exposure is insufficient to evaluate pendimethalin toxicity, especially in the case of pure compound. Acording to our findings, 15, 30, 45 and 60 minutes should be taken. Time intervals up to 90 min were used, but the data were similar to those at 60-min.

It has been shown that an increase in the dose of pendimethalin-standard above 10 mg/l did not increase the effect on bioluminescence. Such observation could be explained as follows: after addition of a small amount of pendimethalin applied as ethanol stock solution to an aqueous medium, an appropriate portion of pendimethalin gets into the water phase, while another portion makes colloid particles or even precipitates out. The further increase in the dose of pendimethalin leads to an increase in the amount of the precipitate but not of pendimethalin concentration in the water phase (as the amount of the chemical compound is very small, the precipitate is unlikely to be observable). Bacterial cells present in the medium are exposed not only to aqueous solute of pendimethalin; colloid particles of the chemical can be sorbed on the surface of the bacterial cells. During an appropriate time, pendimethalin can get into the cells. This assumption is supported by the fact that a prolonged incubation increases the inhibitory effect of pendimethalin on bacterial bioluminescence.

Due to poor water solubility, pure pendimethalin as well as many other herbicides is unsuitable as a commercial product. It must be refined by the manufacturer prior to sale and use. The final product, or its formulation, contains the active ingredient of the herbicide and some "inert" ingredients such as solvents, emulsifiers, and diluents [16]. Stomp 330 EC is an emulsifiable concentrate. It means that the herbicide-active ingredient (pendime-thalin) is dissolved first in an appropriate organic solvent and then an emulsifier is added to the solution. These "inert" ingredients enhance the availability of the

herbicide for transport and plant uptake [16]. It may be expected that the availability for microbial uptake also increases. The results of our research are in accordance with such assumption, i.e. the effect of Stomp 330 on the bacterial bioluminescence appeared in a shorter time as compared to the effect of pendimethalin-standard. For a better solubility of the emulsifiable concentrate, higher concentrations of the active ingredient could be tested.

The results of our research show that herbicide exposure above the water solubility level may be important when dealing with poorly water-soluble substances and should be considered in ecotoxicity testing. However, care must be taken not to alter the toxicity of the test substance when solubilizing agents are used.

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PENDIMETALINO PRIEINAMUMAS IR TOKSIŠKUMAS VANDENS MIKROORGANIZMAMS

Santrauka

Informacija apie herbicidų prieinamumą ir toksiškumą yra būtina norint įvertinti šių chemikalų pavojingumą aplinkai. Pendimetalinas yra dinitroanilinų grupės herbicidas, naudojamas daugeliui vienmečių piktžolių naikinti. Šis herbicidas komercinio produkto „Stomp 330 EC“ pavidalu yra registruotas ir Lietuvoje ir naudojamas daugeliui javų, taip pat ir kai kurioms daržovėms apsaugoti nuo piktžolių. Šio darbo tikslas buvo ištirti pendimetalino prieinamumą ir toksiškumą trijų rūšių vandens mikroorganizmams – žaliadumbliams *Selenastrum capricornutum,* infuzorijoms *Tetrahymena thermophila* ir liuminescuojančioms bakterijoms *Vibrio fischeri* – bei palyginti gryno pendimetalino ir techninio preparato „Stomp 330 EC“ toksiškumą tirtiems mikroorganizmams.

Tyrimo rezultatai rodo, kad dumbliai *S. capricornutum* buvo jautriausi šio herbicido poveikiui: pendimetalino EC50 – 52 µg/l. Poveikis infuzorijoms ir bakterijoms pasireiškė tik esant daug didesnėms (nei dumblių atveju) pendimetalino koncentracijoms, kurios viršijo pendimetalino tirpumo vandenyje ribą (0,3 mg/l). Pagal poveikio bakterijų bioliuminescencijai kinetiką techninis preparatas „Stomp 330 EC“ gerokai skyrėsi nuo gryno pendimetalino (pasireiškė per trumpesnį laiką). Straipsnyje aptariama mažai vandenyje tirpių herbicidų ekotoksiškumo vertinimo problema.