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Toxicity of imidacloprid to the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea)

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Abstract

Imidacloprid is a neonicotinoid insecticide with neurotoxic action that, as a possible alternative for commonly used organophosphorus pesticides, has gained registration in about 120 countries for use in over 140 agricultural crops. Only few data are available on its toxicity for soil invertebrates. We therefore assessed the effects of imidacloprid on survival, weight gain, feeding rate, total protein content, glutathione *S*-transferase activity (GST), and digestive gland epithelial thickness in juveniles and adults of the terrestrial isopod *Porcellio scaber*. After two weeks of feeding on imidacloprid-dosed food, weight gain (NOEC 5 μg/g dry food) and feeding rate (NOEC 10 μg/g) in juveniles, and feeding rate (NOEC < 10 μg/g) and digestive gland epithelial thickness (NOEC < 10 μg/g) in adults were most affected. In juveniles induction of GST activity and increase of total protein content per wet animal weight was detected at 5 μg/g dry food, whereas in adults a reduction of GST was observed at 25 μg/g (NOEC 10 μg/g). An estimate of actual intake rates suggests that imidacloprid affects isopods at similar exposure concentrations as insects. The toxicity of imidacloprid was similar to that of the organophosphorus pesticide diazinon, tested earlier using the same methods [Stanek, K., Drobne, D., Trebse, P., 2006. Linkage of biomarkers along levels of biological complexity in juvenile and adult diazinon fed terrestrial isopod (*Porcellio scaber*, Isopoda, Crustacea). Chemosphere 64, 1745–1752]. At actual environmental concentrations, diazinon poses a higher risk to *P. scaber*. Due to its increasing use in crop protection and higher persistence in soil, imidacloprid might however, be potentially more dangerous after long-term application. We conclude that toxicity testing with *P. scaber* provides relevant, repeatable, reproducible and comparable toxicity data that is useful for the risk assessment of pesticides in the terrestrial environment.

Keywords: Biomarkers; Toxicity testing; Glutathione *S*-transferase; Digestive gland epithelium; Risk quotient; Environmental risk characterization

1. Introduction

The market for pesticides is changing as a result of the US EPA phase out of most urban uses of two of the most commonly used organophosphorus (OP) pesticides, diazinon and chlorpyrifos (TDC Environmental, 2003). However, agricultural uses of diazinon continued after December 2004 in a wide range, what led the US EPA to conduct a cumulative risk assessment of all OPs. The US EPA has reached the decision that diazinon poses unacceptable risk to agricultural workers, birds and other wildlife species (Cobb et al., 2000). The situation is similar in the European Union and will probably lead to a progressive withdrawal of diazinon from use also in Europe. In the UK, diazinon is being progressively phased out because of a lack of data to support its continued use (APVMA, 2003).

The US EPA created a candidate list of ten alternatives to diazinon (TDC Environmental, 2003). Among them,

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imidacloprid succeeded to have the world's fastest growing sales (TDC Environmental, 2003).

Imidacloprid belongs to a major new class of insecticides, called neonicotinoids, which are accounting for 11–15% of the total insecticide market (TDC Environmental, 2003). Since its launch in 1991, products containing imidacloprid have gained registrations in about 120 countries and are marketed for use in agriculture (for over 140 agricultural crops), on turf, on pets, and for household pests (Liu et al., 2005). Imidacloprid is marketed under variety of names including Gaucho, Merit, Admire, Confidor and Winner. Although imidacloprid has been in use for a relatively short period of time compared to other common pesticides, it is considered to being used in the largest volume globally of all insecticides (Cox, 2001; Ware and Whitacre, 2004). Imidacloprid can be used for seed dressing, foliar application, trunk injection, soil injection and soil drench. Application rates of imidacloprid for different crops range from 0.05 to 0.4 kg of active ingredient/ha (Bayer Technical Information and Confidor®, 2000), while 0.03 kg a.i./ha is the recommended application rate for tea fields in India (Anatra-Cordone and Durkin, 2005).

Imidacloprid is a nicotinic acetylcholine receptor (nAChR) agonist (Buckingham et al., 1997). Its mechanism of action has been studied extensively, and is relatively well known. In essence, imidacloprid activates nAChR through binding at or near the sites where nicotine and acetylcholine bind, resulting in dysfunction of the nervous system, immobilization or death (Anatra-Cordone and Durkin, 2005).

Imidacloprid has different affinities to nAChRs of different organisms. The selectivity of vertebrate and invertebrate nAChR is attributed to structural heterogeneity of neuronal nAChRs and leads to differences in the sensitivity to imidacloprid (Lindstrom et al., 1995; Lind et al., 2001; Matsuda et al., 2001; Tomizawa and Casida, 2005). Available data indicate that imidacloprid can be highly toxic to some aquatic crustaceans, but generally less toxic to fish (TDC Environmental, 2003; Jemec et al., 2007a). Also, the LD_{50}s for mammals and birds are much higher than for invertebrates (Anatra-Cordone and Durkin, 2005). Due to the high insecticidal activity and low mammalian toxicity, imidacloprid is considered safer compared to traditional OP, carbamate and pyrethroid insecticides (Felsot and Ruppert, 2002). In addition imidacloprid can be applied at very low rates. However, very little data are available on the toxicity of imidacloprid to non-target organisms, especially those inhabiting terrestrial ecosystems. A few comparative toxicity studies indicate high species-specific response to imidacloprid, which suggests that imidacloprid toxicity data may not be generalized.

Terrestrial isopods may be suitable test organisms due to their well-known biology and physiology, relative ease of laboratory maintenance, and possibility to acquire individual toxicity data. Also, due to their important ecological role as decomposers of organic material, terrestrial isopods are widely accepted as test organisms in terrestrial ecotoxicology and ecophysiology (Drobne, 1997; Løkke and Van Gestel, 1998; Lapanje et al., 2007). Isopods also allow measuring biomarkers at different levels of biological complexity. Biomarkers may be divided into biomarkers of exposure and biomarkers of effect. By a definition posed by Kammenga et al. (2000), biomarkers of effect are those measured alterations in an organism that are associated with possible health impairment or disease. Biomarkers of exposure are those measured responses of an organism that indicate the interaction between a xenobiotic agent and some target molecule or cell (Roberts and Oris, 2004). Biomarkers of exposure in our study are *S*-transferase (GST) activity and total protein content per wet animal weight and biomarkers of effects are hepatopancreas epithelial thickness, feeding rate, weight change and mortality.

The aim of our work was:

(a) To assess toxicity of imidacloprid in two-week laboratory toxicity tests by analyzing biomarkers at different levels of biological complexity in juvenile and adult terrestrial isopod *Porcellio scaber*;
(b) To compare toxicity data on imidacloprid and toxicity data on diazinon tested in the same experimental set-up;
(c) To discuss relative toxicity of imidacloprid to diazinon on the basis of data obtained in our study and those reported in the literature;
(d) To evaluate the potential of the toxicity testing protocol with the terrestrial isopod *P. scaber* test system for providing toxicity data for risk characterization of pesticides in the terrestrial environment.

2. Materials and methods

2.1. Chemicals

Imidacloprid (99.8%) was provided by Bayer Crop Science Slovenia. Sodium hydrogen phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Fluka. Bovine serum albumin (albumin fraction V) was purchased from Merck, Bradford reagent, 1–chloro-2,4-dinitrobenzene (CDNB), and L-glutathione (reduced form) (GSH) were purchased from Sigma Aldrich and concentrated sulphuric acid from Carlo Erba. Methanol (HPLC grade), abs. ethanol and chloroform were obtained from J.T. Baker.

2.2. Test organisms

Terrestrial isopods (*P. scaber*, Isopoda, Crustacea) were collected from the litter layer of uncontaminated woodlands near Nova Gorica (Slovenia) and Ljubljana. In the laboratory, the animals were kept in a terrarium ($20 \times 35 \times 20$ cm) filled with a layer of moistened sand and soil (2–5 cm) and a thick layer of partly decomposed hazelnut tree leaves (*Corylus avellana*). The substratum in