Applied Soil Ecology 53 (2012) 56–64

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Landsnail eggs bioassays: A new tool to assess embryotoxicity of contaminants in the solid, liquid or gaseous phase of soil

Coline Druart[a], Renaud Scheifler[a], Maurice Millet[b], Annette de Vaufleury[a,*]

[a] Department of Chrono-Environment, University of Franche-Comté, UMR UFC/CNRS 6249 usc INRA, Place Leclerc, 25030 Besançon Cedex, France
[b] Department of Physical-Chemistry of the Atmosphere, LMSPC (UMR 7515 CNRS-University of Strasbourg), Blessig 1, 67084 Strasbourg Cedex, France

ARTICLE INFO

Article history:
Received 23 June 2011
Received in revised form
10 November 2011
Accepted 13 November 2011

Keywords:
Helix aspersa
Embryotoxicity
Terrestrial environment
Pesticides
Metals

ABSTRACT

Bioassays for ecotoxicity testing for the same non-target soil organism are not currently available for contaminants that can be present in the liquid, solid or gaseous phase of soil. Here, three bioassays with three modes (liquid, solid or gaseous phase, LPB, SPB and GPB, respectively) allowing the assessment of the embryotoxicity of chemicals or pluri-contaminated matrices on land snail eggs are presented. Eight pesticides commonly used in vineyards (based on 10 active ingredients: copper, sulfur, metiram-zinc + pyraclostrobin, cymoxanil + folpet + fosetyl-Aluminium, tebuconazole, glyphosate, glufosinate) were tested by LPB: all of them reduced egg hatching success at concentrations lower than those applied in the field. The SPB was developed with one artificial ISO substrate and seven natural soils: three non-contaminated agricultural soils (Agr1–3) and four metal-contaminated soils (Me1–4). The moisture content (from 40 to 60% of the water holding capacity) in the natural soils did not influence the hatching success. Hatching success did not vary in the three agricultural soils suggesting the relative insensitivity of eggs to some soil properties. Among the two pesticides tested in SPB, Corail® (tebuconazole based-fungicide) was more toxic than Bypass® (glyphosate based-herbicide) to snail embryos with EC50 values of 1 and 219 mg kg^{-1} respectively in a natural soil (Agr2). Both pesticides were less toxic when tested in the ISO substrate (EC50 of 7.8 and higher than 400 mg kg^{-1}, respectively for Corail® and Bypass®), highlighting the influence of the organic matter content (lower in soil Agr2) on the bioavailability and thus the toxicity of the chemicals. LPB showed that soluble compounds of the most toxic soil in SPB (Me4) did not affect embryos. Similarly, GPB did not reveal toxic volatile compounds from this soil. These bioassays are complementary and efficient tools for soil risk assessment.

1. Introduction

Recent European Community guidance on chemical risk assessment requests new bioassays that should be sensitive, easy to perform and an alternative to vertebrate animal testing (EC, 2006). Embryotoxicity tests with oviparous organisms meet with these criteria because they are sensitive (Hallare et al., 2005a; Sawasdee and Köhler, 2009) and considered as *in vitro* systems (Weisbrod et al., 2008). Most of the bioassays currently available to assess the effects of chemicals on embryogenesis and hatching success use aquatic organisms, like pond snail, oyster, zebrafish, ascidian and sea urchin (Gomot, 1998; Geffard et al., 2002; Hallare et al., 2005b; Pennati et al., 2006; Arslan et al., 2007). As stated in the Guidance on Information Requirements and Chemical Safety Assessment (ECHA, 2008), and despite the fact that soil is often the first receptor of numerous contaminants, embryotoxicity data on terrestrial organisms are scarce. Several authors have begun to develop embryotoxicity tests based on the incubation of eggs of vertebrate species in soil, e.g. the lizard *Sceloporus undulatus* (Brasfield et al., 2004) or the turtle *Trachemys scripta elegans* (Sparling et al., 2006), and assessing embryo mortality, size of hatchlings or hatching success. A bioassay with the bee *Apis mellifera* (Aupinel et al., 2007) is classically used for pesticide risk assessment, but does not directly concern soil contaminants. Some other methods have also been developed to determine effects of contaminants on reproduction (egg laying and hatching success) of soil invertebrates (ISO 10872:2010; ISO 11268-2:1998). However, no standardized terrestrial bioassay exists to really assess the embryotoxicity of pollutants for soil invertebrates, and only few experiments with metals or organic compounds have been performed on the slugs *Deroceras reticulatum* (Iglesias et al., 2000, 2002) or the snails *Helix aspersa* or *Monacha obstructa* (Druart et al., 2010; Shoaib et al., 2010). Although snails are not considered in the guidelines for the implementation of REACH (EC, 2006) as a major group of soil organisms to be considered in risk assessment (ECHA, 2008), they do belong to various terrestrial food webs (Eeva et al., 2010) and contribute

* Corresponding author. Tel.: +33 381 665 788; fax: +33 381 665 797.
E-mail address: annette.devaufleury@univ-fcomte.fr (A. de Vaufleury).

0929-1393/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2011.11.006

to nutrient cycling in soil (Dallinger et al., 2001). Snails colonize many habitats, e.g. vineyards (Snyman et al., 2005) or forest borders (Kerney et al., 2006), where they lay eggs at 2–3-cm depth in soil, one to three times a year during spring and summer. Land snails are also used in ecotoxicology as bioindicators of pollution and for soil quality assessment (de Vaufleury et al., 2006) but few data are available on the effects of soil contamination on their embryogenesis. However, snail eggs are suitable for this type of experimentation and can be obtained year round in the lab. Snail eggs are in contact with the contaminant fractions dissolved in pore water and leachates, sorbed to soil particles and in the gaseous phase of the soil.

The aim of this study is to develop relevant bioassays (i.e. liquid, solid and gaseous phase bioassays: LPB, SPB, GPB) to assess and compare the effects of contaminants on snail embryos exposed by these three sources of exposure. LPB and SPB have been presented in Druart et al. (2010) and Kramarz et al. (2007) respectively and till now, they were only used with a limited number of compounds (pesticides and Cry1 Ab protein). More data are then needed to validate their interest in ecotoxicity testing for various chemicals or complex matrices as soils and soil leachate. Thus, the LPB was performed with eight commercial formulations of pesticides (five fungicides and three herbicides) commonly used in vineyards and a leachate of a metal-contaminated soil. To document the influence of the soil matrix on the toxicity of metals and pesticides tested in LPB, two of the eight pesticide formulations (chosen according to their high toxicity in LPB) and a metal-contaminated soil were assessed in SPB using two different soil matrices. To determine the main parameters that can influence the results obtained with this bioassay, the effects of various soils (natural or artificial, contaminated by metals or not) with different characteristics (pH, organic matter, size of particles, moisture) were studied on the hatching success. Finally, a new configuration (GPB) was experimented to test the toxicity of volatile substances that could be emitted by the soil.

2. Materials and methods

2.1. Chemicals

The data concerning the commercial formulations of pesticides are listed in Table 1. Pesticide solutions were prepared with demineralized water.

2.2. Soil preparation

An artificial soil substrate was prepared according to ISO procedure (ISO 11268-1:1993). The seven other soils studied were natural: three agricultural soils (Agr1–Agr3), respectively collected in Foulum (Denmark), Bergbieten (France) and Chambornay (France) and four metal-contaminated soils (Me1–Me4), sampled in the surroundings of the former smelter of Metaleurop Nord (France). Soils showed contrasted characteristics (Table 2), e.g. for organic matter contents (OMC) and metal concentrations. More information can be found in Andersen et al. (2007) for Agr1, Druart et al. (2011a) for Agr2, de Vaufleury et al. (2006) for Agr3, Gimbert et al. (2008) for Me4 and in Douay et al. (2009) for Me soils at large. All soils were dried and sieved to 4 mm.

2.3. Snail eggs

Adult snails *Helix aspersa aspersa* Müller (*syn. Cantareus aspersus aspersus* Müller, 1774), aged between 4 months and 2 years came from our laboratory rearing (more details are available in ISO 15952) and their eggs were obtained as described by Druart et al. (2010). The maximum time lapse between the end of laying and the beginning of the experiments was 6–8 h. For all tests, the conditions of the incubation room were $20 \pm 2\,^{\circ}C$, 80% humidity and 18 h light per day. Embryonic development for LPB, SPB and GPB (Fig. 1) lasts 14 days and the measured endpoint is the hatching success (determined after about 20 days of incubation). In one clutch, corresponding to one replicate, 10 (LPB), 15 (SPB) or 20 (GPB) eggs were dealt for each treatment or concentration. From 3 to 6 clutches were used for each experiment, depending on the number of clutches available at the beginning of the experiments and on the number of validated replicate (hatching success of controls higher than 70%) at the end of the experiments.

2.4. Liquid phase bioassay (LPB)

Ten eggs from a single clutch were exposed in Petri dishes (Greiner Bio-one, 35 mm × 10 mm, crystal polystyrene, triple vent) containing four layers of filter papers (Whatman grade 1; 32 mm diameter) dampened with 0.8 ml of demineralized water (for control) or tested solution (Druart et al., 2010). Eight pesticides were assessed in LPB to determine NOEC (no observed effect concentration), LOEC (lowest observed effect concentration), EC10 and EC50 (effective concentrations causing 10% or 50% effect, e.g. hatching success inhibition, compared with controls). Two repetitions (test 1 and test 2) were performed for each formulation (Table 3), with 1 month of minimum interval between both. The tests were usually performed with five replicates except for Corail® test 1 and Basta® test 1 (three replicates), Bordeaux mixture, Bypass® test 1 and Valiant® Flash test 1 (four replicates) and Roundup® test 1 (six replicates).

In a first attempt to determine the nature of the toxic substances contained in soil Me4, water-soluble fractions were tested. In this aim, a leachate from soil Me4 was prepared according to European standard EN14735 to dissolve the compounds present. After agitation of 100 g of soil in 1000 ml of demineralized water during 24 h, the sample was decanted for 24 h. The supernatant was centrifuged during 30 min at 2500 × *g*. The retrieved supernatant was filtered at 0.45 μm. In order to obtain more information about the composition of the leachate, the concentrations of six metals (Al, Cd, Cr, Cu, Ni and Zn) were determined using a furnace atomic absorption spectrophotometer AAS (220Z, Varian, Les Ulis, France). The leachate was tested by the LPB in five replicates and demineralized water was used as control.

2.5. Solid phase bioassay (SPB)

Eggs were exposed in similar devices used by Kramarz et al. (2007) and modified to obtain a quick bioassay: 15 eggs were deposited on the surface of the soil, contained in small glass containers (about 70–80 g of wet soil with demineralized water at 40–60% WHC per 130-ml pot; Fig. 1b) covered by a wet lid. Eggs were not covered by an upper layer of soil, like in Kramarz et al. (2007), because it was observed that this mode reduced hatching success and increased the time of emergence.

2.5.1. Effect of various parameters on hatching

The influence of moisture was studied with soils Agr1, Agr3 and Me1–4. Two moisture levels (expressed as percentages of the respective WHC of the different soils, Table 2) were tested with four replicates for each moisture level: 40% and 50% for soils Me2–4 and 50% and 60% for the soils Agr1, Agr3 and Me1. Very high (≥70% of the WHC) or very low moistures (≤30% of the WHC) were not tested because the test primarily concerns the study of soils with a typical moisture level (and not desiccated or saturated soils). In desiccated or saturated soils, eggs swelled or shriveled, leading to abnormal hatching success independent of the test compounds. Hatching success was compared between three agricultural