Environmental Pollution 220 (2017) 1014–1023

Contents lists available at ScienceDirect

Environmental Pollution

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

Does long term low impact stress cause population extinction?☆

M.J.B. Amorim [a, *], C. Pereira [a], A.M.V.M. Soares [a], J.J. Scott-Fordsmand [b]

[a] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark

ARTICLE INFO

Article history:
Received 24 April 2016
Received in revised form
10 November 2016
Accepted 14 November 2016
Available online 19 November 2016

Keywords:
Multigenerational
Tolerance
Population size distribution
Skewness
Kurtosis

ABSTRACT

This study assessed and monitored 40 consecutive reproduction tests - multigenerational (MG) - of continuous exposure to Cd (at 2 reproduction Effect Concentrations (EC): EC10 and EC50) using the standard soil invertebrate *Folsomia candida*, in total 3.5 years of data were collected. Endpoints included survival, reproduction, size and metallothionein (MTc) gene expression. Further, to investigate adaptation to the toxicant, additional standard toxicity experiments were performed with the MG organisms of F_6, F_{10}, F_{26}, F_{34} and F_{40} generations of exposure. Exposure to Cd EC10 caused population extinction after one year, whereas populations survived exposure to Cd EC50. Cd induced the up-regulation of the MTc gene, this being higher for the higher Cd concentration, which may have promoted the increased tolerance at the EC50. Moreover, EC10 induced a shift towards organisms of smaller size (positive skew), whereas EC50 induced a shift towards larger size (negative skew). Size distribution shifts could be an effect predictor. Sensitivity increased up to F_{10}, but this was reverted to values similar to F_0 in the next generations. The maximum Cd tolerance limits of *F. candida* increased for Cd EC50 MG. The consequences for risk assessment are discussed.

1. Introduction

Long term exposure to chemicals is a common scenario, with consequent multigenerational effects on organisms. This is particularly the case in the context of persistent chemicals at sub-lethal concentrations and, especially, within a solid compartment (e.g. soil).

Current guidelines to assess environmental effects of chemicals are based on toxicity within one generation, even for POPs (Persistent Organic Pollutants). Moreover, even when effects are assessed at long term chronic levels, this constitutes an exposure during a small fraction of the organisms' life cycle, e.g. from mature adults till offspring reproduction (e.g (OECD, 2004).). Obviously, rather than using only a fragment of a life cycle, a full life cycle test would be preferable, but such tests are seldom available for terrestrial invertebrates (Bicho et al., 2015). When it comes to assessing effects over more life-cycles, i.e. for more than one generation, the tests documenting the possible effects are even scarcer. Hence, given that the hazard is predicted based on current standard duration tests, the Environmental Risk Assessment (ERA) framework will also, in principle, only predict shorter term risk and will not include potential effects of multigenerational (MG) exposure. This is a gap and a concern, as sustainability is the aim.

The ability of organisms to survive and reproduce in contaminated habitats has been widely reported and has caused, among others, increased resistance of pests and pathogens to pesticides and antibiotics (Bickham et al., 2000). For instance, studies by Ward and Robinson (2005) showed an increased resistance in *Daphnia magna* populations when exposed to Cd along eight generations. Studies from historically polluted sites have shown that the impact can persist years after original source input (Roelofs et al., 2009). Effects can vary, e.g. species selection, development of resistance to higher levels of the source pollutant through adaptation or tolerance, epigenetics, etc. For instance, changes in genetic variability and allele frequencies of populations, which result from induced mutations and population bottlenecks (Bickham et al., 2000), can induce increased survival in contaminated environments (Timmermans et al., 2005). However, the associated changes often carry a decrease in the overall fitness, i.e. less viability to secondary stresses: resistant animals may be less fit and eliminated when exposed to different stressors (Ward and Robinson, 2005).

Overall, few multigenerational studies have been conducted.

☆ This paper has been recommended for acceptance by Prof. von Hippel Frank A.

* Corresponding author.
E-mail addresses: mjamorim@ua.pt (M.J.B. Amorim), CeciliaManuela.Pereira@UGent.be (C. Pereira), asoares@ua.pt (A.M.V.M. Soares), jsf@bios.au.dk (J.J. Scott-Fordsmand).

http://dx.doi.org/10.1016/j.envpol.2016.11.044
0269-7491/

Some of the progress has been driven by endocrine disruptors research (e.g. (OECD, 2005; Segner et al., 2003)), where effects are not observed until a second generation. In terms of soil invertebrates, the examples include mainly few (1–3) generation studies, e.g. *Enchytraeus crypticus* (Menezes-Oliveira et al., 2013), *Enchytraeus albidus* (Lock and Janssen, 2002), *Folsomia candida* (Campiche et al., 2007; Ernst et al., 2016) with one example of 10 generations (Paumen et al., 2008), *Eisenia fetida* (Spurgeon and Hopkin, 2000), *Lumbricus rubellus* (Langdon et al., 2009, 2003). Menezes-Oliveira et al. (2013) studied Cu exposed *E. crypticus* along 2 generations, showing an increase in sensitivity in the 2nd generation. Studies using *F. candida* showed that pre-exposure to certain endocrine disrupters (not all) caused an increase in sensitivity in the 2nd generation (Campiche et al., 2007). In an other study with *F. candida* (Ernst et al., 2016), a 2 generation design is proposed to differentiate between substances of potential long-term or low risk. Also with *F. candida*, exposure to phenantrene EC50 showed complete reproductive failure and subsequent extinction of the population after four generations (Paumen et al., 2008). Spurgeon and Hopkin (Spurgeon and Hopkin, 2000) showed that *E. fetida* developed resistance to Zn after pre-exposure up to 2 generations (higher LC50, LC90, LC99). Also, studies over 3 generations with another earthworm species, *L. rubellus* (Langdon et al., 2009) showed resistance to As for pre-exposed animals. On the other hand, no resistance to Cu was demonstrated (Langdon et al., 2003) showing the metal specificity. Lock and Janssen (2001) showed that *E. albidus* populations pre-exposed to Cd (18 months) had increased metallothionein levels, but had no changes in acute and chronic toxicity.

Hence, as outlined above it is envisaged that even in a laboratory setting a multigenerational experiment will provide additional information on the long-term consequences of pollution of natural populations [such phenomena are notoriously difficult to study directly on natural populations, i.e. in natural environments].

The aim of this study was to assess and monitor the impact along multigenerational exposures and to do this along a substantial period of time fully monitored.

The standard soil invertebrate *Folsomia candida* was used. Cadmium (Cd) was selected as a test chemical because it is both relevant and well-studied in this species and is also non-essential and persistent. This was a very long term study, which lasted 41 consecutive reproduction tests (each test was 28 days) of continuous exposure to Cd, i.e. more than 3 years of data was collected. The recovery potential was also assessed in each generation by transfer to control soil tested in parallel. Moreover, to investigate adaptation to the toxicant, additional standard toxicity experiments were performed after 6, 10, 26, 34 and 40 generations of exposure.

2. Materials and methods

2.1. Test organism

The standard soil test organism *Folsomia candida* (Collembola) was used. Organisms were kept in laboratory in petri dishes in a substrate consisting of a mixture of plaster of Paris and activated charcoal (8:1). Cultures were kept at 19 °C, photoperiod 16:8 h (light-dark) and were fed twice a week with dried bakers' yeast. For testing, organisms were of synchronized age (10–12 days old).

2.2. Test soil

The natural standard soil LUFA 2.2 was used. The properties can be summarised as follows: pH = 5.5, organic matter = 3.9%, grain size distribution: 6% clay; 17% silt; 77% sand.

2.3. Test chemicals and spiking procedures

Cadmium chloride anhydrous ($CdCl_2$, Sigma-Aldrich, 99%) was used. Cadmium was added to the test soil as aqueous solution in deionised water. Test concentrations were 32 and 60 mg Cd/kg dry soil, i.e. the known approximate EC10 and EC50 for reproduction (van Gestel and Mol, 2003). The spiked soil was allowed to equilibrate for three days prior to test start (McLaughlin et al., 2002). Moisture was adjusted to 50% of the maximum water holding capacity (WHC).

2.4. Experimental procedures

2.4.1. Effect concentration response exposure

The standard guideline (OECD, 2009) was followed. In short, 10 juvenile organisms were selected randomly from cultures of synchronized age (10–12 days) and introduced in each test vessel containing the moist test soil and food supply. Four replicates per treatment were performed. One additional container was prepared for pH measurements at the beginning and at the end of the experiment. Test concentration range was 0-32-64-128-256 mg Cd/kg soil DW, following the same described spiking procedures. Exposure lasted 28 days, at 20 ± 2 °C, 16:8 h photoperiod. Water content and food were replenished weekly. At test end, the adults (F_0) and hatched juveniles (F_1) were recovered by flotation and counted. The process consisted of adding water into the test vessel, transferring all into a 500 ml glass beaker and gently stirring with a spatula to maximize flotation and spread the organisms on the water surface. The adults were counted and removed. A picture of the organisms on the water surface was recorded with a digital camera and the number (and size (length) in the MG test) of organisms was obtained using image analysis software (SPSS, 2003).

Concentration response experiments, to assess the Effect Concentration (ECx), were assessed at generations F_6, F_{10}, F_{26}, F_{34} and F_{40}, always performing additional F0 in parallel, i.e. using organisms from synchronized cultures.

2.4.2. Multigenerational (MG) exposure

The standard guideline (OECD, 2009) was followed with adaptations. The maturity of *F. candida* is usually reached around 15–16 days (6th instar (Snider, 1973),) in control animals, but this may take longer time under exposure conditions. In short, 10 juvenile organisms were selected randomly from cultures of synchronized age (10–12 days) and introduced in each test vessel containing the moist test soil and food supply. Samples were named as Ct_EC10 and Ct_EC50 (for the controls of the respective Cd treatment) and Cd_EC10 and Cd_EC50 (for the Cd treatments). In terms of replicates, four replicates were performed for control and ten replicates per Cd treatments (32 and 60 mg/kg) to ensure enough organisms to continue sequential generations, i.e. 4 replicates of Ct_EC10, 10 replicates of Cd_EC10, 4 replicates of Ct_EC50 and 10 replicates of Cd_EC50.

At each test end, after removing the adults (F_x), F_{x+1} juveniles were transferred from the water surface using a small spoon to a recipient with a layer of mixed plaster of Paris and activated charcoal, adsorbing extra existing water. After this, 10 juvenile organisms were selected within similar size (selecting the larger sized animals) and transferred to a new test vessel with soil spiked under the exact same conditions as the previous generation, including the water control. Extra juvenile organisms were snap frozen in liquid nitrogen and kept at −80 °C for further analysis.

This procedure was repeated over all generations for EC10 and EC50, respectively. A schematic representation of the experimental design is given in Fig. 1.

For an overview, the experimental set up included the following