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Changes of chemical chronic toxicity to *Daphnia magna* under different food regimes

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ABSTRACT

In aquatic ecosystems several stressors may act together and affect the life traits of organisms. Pesticide runoffs are usually associated with high inputs of organic matter and depletion of oxygen in aquatic systems. This study aimed at combining anthropogenic stress (chemicals) and natural stress (food availability) and evaluates their joint effect to the life traits of *Daphnia magna*. The neonicotinoid insecticide imidacloprid and the heavy metal nickel chloride were used and a 21 d chronic test was carried out to obtain reproduction and growth data. The conceptual model Independent action, usually used for assessing response patterns in chemical mixtures, was used for data interpretation.

Results showed an increase in the reproduction and growth pattern of *D. magna* as food levels increased. Both chemicals significantly impaired the reproduction as well as the somatic growth of the organism while the same happened with food concentrations lower than 3×10^5 cells/mL. It was also observed that food availability did not change the toxicity of imidacloprid and nickel chloride when food levels were higher than 3×10^5 cells/mL. When combined with low food levels, imidacloprid showed a slight increase in toxicity, showing that daphnids become more sensitive with reduced food availability, however in a non-significant way. However, toxicity of nickel appeared to be independent of the food level. Both chemicals induced mortality to the organisms exposed in the absence of food only at the end of the test.

1. Introduction

In aquatic ecosystems several stressors may act together and affect the life traits of organisms. Pesticides and metals can frequently be found in the aquatic environment, in combination with several natural stressors such as different food levels (Antunes et al., 2004), extreme temperatures, dissolved oxygen depletion (Ferreira et al., 2008) and UV radiation (Ribeiro et al., 2011).

Zooplankton populations can periodically be found in the environment under conditions of natural stress such as predation (Hanazato, 2001; Pestana et al., 2010) and variable food availability (Antunes et al., 2003; Pereira et al., 2007; Pieters and Liess, 2006). *Daphnia* avoid predation in lakes by migrating downward into dark waters (Bastos et al., 2013) during the day, where shortage of food and the presence of other stressors (e.g. oxygen depletion) occurs, and upwards during the afternoon in order to feed (Hanazato, 2001). According to Hanazato (2001), due to that diel vertical migration, daphnids are exposed to low food concentrations, among other stressors, resulting in increased sensitivity to pesticides that can also be part of the stressors already mentioned. According to some studies (Buikema et al., 1980; McCauley et al., 1990; Pieters and Liess 2006) low food concentrations occur in the environment altering the sensitivity of organisms, like *Daphnia*, to contaminants (Antunes et al., 2003; Chandini 1988, 1989; Pieters and Liess 2006). Interactions of different pesticides and low food levels have already been investigated by several studies demonstrating an interaction between food limitations and chemical presence resulting to sensitivity of the organism (Beketov and Liess, 2005; Knillmann et al., 2012; Postma et al., 1994). Beketov and Liess (2005) shows how survival of the mayfly *Cloeon dipterum* is affected by food limitations. Postma et al. (1994) in their study exposing the midge, *Chironomus riparius*, to different concentrations of cadmium and different food levels, showed how the organism adjusted its fitness to the negative effects of cadmium and food limitations. Algae concentrations up to 10^6 cells/mL can be found in eutrophic environments, and therefore studies using algal densities are needed in order to evaluate the dynamics of toxicants in polluted ecosystems, (Rodgher and Gaeta Espíndola, 2008) that can affect various individual traits (e.g. growth, reproduction, survival).

Regarding contamination of aquatic systems, several compounds from different sources can be found. From agricultural practices, imidacloprid (a neonicotinoid insecticide) is widely used

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in crops and slowly taking over the market and replacing organophosphorous pesticides (Jemec et al., 2007). In addition, domestic wastewater effluents and non-ferrous metal smelters are considered to be the major sources of trace metal pollution in aquatic ecosystems (Cempel and Nikel, 2006). Nickel is an abundant heavy metal throughout the planet and it is essential in many forms of life. Nickel metal and its alloys are used widely in the metallurgical, chemical and food processing industries. According to Barceloux and Barceloux (1999), the main sources of exposure of nickel for the general population are through the drinking water and food: for example, the average American diet contains about 300 μg Ni/d. Nevertheless, recent reports mention that environmental mean concentrations of nickel in relatively uncontaminated surface waters of six European regions ranged between 0.33 and 5.13 μg/L of dissolved Ni (Holmstrup et al., 2010; Spears, 1984). Values for nickel can vary from 69.3 to 110.7 μg/L during winter and from 41.0 to 60.7 μg/L during spring in the River Karoon, the biggest river in the South of Iran (Cempel and Nikel, 2006), while in seawater the dissolved nickel ranges from 0.1 to 0.5 μg/L (Bencko, 1983; Cempel and Nikel, 2006; Pavlaki et al., 2011; Takahashi and Hanazato, 2007).

This study aimed at evaluating the effects of different food levels on the toxicity of two chemicals, imidacloprid and nickel chloride, to the life traits of a non-target organism, *Daphnia magna*. In addition to the calculation of ECx (effective concentration) endpoints to compare toxicities, the independent action model was used to fit our data and predict the joint toxicity of combined stressors. This approach has already been used by other authors with accurate results (e.g. Ferreira et al., 2008; Long et al., 2009; Martin et al., 2009). Additionally, deviations from the conceptual model for synergism or antagonism patterns were also assessed. The single toxicity data used in this study were based on a previous study done by Pavlaki et al. (2011).

2. Materials and methods

2.1. Test organism

The cladoceran *Daphnia magna*, clone K6 (Antwerp, Belgium) was used to perform all bioassays in this study. Glass beakers (1 L capacity) were kept with ASTM (American Society for Testing and Materials) hard water (ASTM, 1998) and daphnids, 10–12 individuals/L, were fed daily with the algae *Raphidocelis subcapitata* at a rate of 3×10^5 cells/mL and an organic additive (Marinure seaweed extract, supplied by Glenside Organics Ltd.) (Baird et al., 1989). The cultures were maintained at a temperature of 20 °C in a 16:8 light: dark cycle. New cultures of daphnids as well as bioassays were initiated using the neonates from the third to the fifth brood.

2.2. Test chemicals

Chemical compounds used in this study were the neonicotinoid insecticide, imidacloprid, ≥95 percent purity, (CAS no. 138261-41-3, Bayer, Germany) and the heavy metal, nickel (II) in the form of nickel chloride hexahydrate, ≥98 percent purity, (CAS no. 7791-20-0, Merck, Germany).

Chemical analysis was carried out by high performance liquid chromatography with UV detector (HPLC–UV) for imidacloprid and by inductively coupled plasma-mass spectrometry (ICP-MS) for nickel. For the imidacloprid analysis, the analytes were extracted from the sample by Solid Phase Extraction (SPE). About 90 ml of the sample were applied to a SPE Cartridge (STRATA X, 1 mL/100 mg, Phnomenex). The apparatus used was HPLC-PDA-System (Shimadzu, Japan), including two HPLC pumps model LC-10ADvp, Autosampler SIL-10ADvp, Column oven CTO-10ASvp, Photodiodenarray-Detector (PDA) SPD-M10Avp. The column used was a LUNA C18, 250 mm × 3.0 mm, 5 μm (Phenomenex, Ascgaffenburg, Germany) with a temperature of 40 °C. The flow rate was 0.6 mL/min and the injection volume 10 μL of Acetonitril-extract. The limit of detection was 0.05 μg/L and the limit of quantification was 0.1 μg/L. For the nickel analysis, a calibration curve was made with seven standards. Calibration curve tested (verified) with a CRM (Certified Reference Material) – NIST 1643-e. The limit of detection was 0.333 μg/L and the limit of quantification was 1.0 μg/L. Chemical analysis was performed in two samples of the lowest and the highest concentrations and two samples of the stock solution at the beginning of the experiment, due to the complex experimental design used for combination experiments.

2.2.1. Chronic test with natural stressor

The OECD 211 guideline for chemical testing, for *Daphnia magna* (OECD 211 Guideline, 2008) was adapted in order to evaluate the effects of different food levels to reproduction and somatic growth.

Low and high food concentrations were tested ranging from absence (only seaweed extract and no green algae) to 2×10^5 cells of *R. subcapitata* per mL of medium and from 4×10^5 to 6×10^5 cells of *R. subcapitata* per mL of medium, respectively. The control was based on the concentration of food, the unicellular green algae *R. subcapitata,* used in *D. magna* cultures (3×10^5 cells/mL). For each treatment ten replicates with one organism each (<24 h old) was used in the experimental set up for all food level. The food level treatments included 0 cells/mL (only seaweed extract and no green algae), 1×10^5 cells/mL, 2×10^5 cells/mL, 3×10^5 cells/mL, 4×10^5 cells/mL, 5×10^5 cells/mL and 6×10^5 cells/mL. In all the treatments, even in the starvation conditions (0 cells/mL), seaweed extract was used in the same quantity as in the cultures, to ensure similar conditions and no to few mortality of the organisms in the starvation conditions.

Tests were maintained in a controlled chamber at 20 °C, in a 16:8 light: dark cycle, fed everyday with the corresponding amount of food for each food level and the medium renewed every other day for 21 days. Reproduction output, as the number of live neonates was assessed daily. Mortality was recorded and neonates were removed from the vessels daily. At the end of the test, the length of each adult daphnia was measured from the point above the eyespot to the base of the dorsal spine with the use of a stereo-microscope.

2.3. Values for pH, dissolved oxygen and temperature were obtained weekly to check for the validity criteria

2.3.1. Chronic toxicity tests with chemicals

Chronic toxicity was assessed for each chemical compound individually following the OECD 211 guideline, for the *Daphnia magna* reproduction test (OECD 211 Guideline, 2008). This approach was used simultaneously with the combined toxicity tests in order to derive what was the expected toxicity in the combined exposures (see below). The nominal concentrations for imidacloprid ranged from 2 mg/l to 10 mg/L, five concentrations in total and for nickel from 100 μg/L to 350 μg/L, six concentrations in total and the results were presented on a previous study (Pavlaki et al., 2011).

2.3.2. Binary combination toxicity tests

In the combined toxicity tests using chemicals and food levels, the number of replicates per each treatment was decreased from ten to one to allow the use of more treatments per test, in order to obtain a broader range of the chemical's response to the combination, as advised in several studies where data is modelled using conceptual models for chemical mixtures or combination of stressors (Cedergreen et al., 2006; Cedergreen and Streibig, 2005; Ferreira et al., 2008; Jonker et al., 2004, 2005; Long et al., 2009; Loureiro et al., 2010; Vandenbrouck et al., 2009). Both combination sets, imidacloprid – food levels, and nickel – food levels, followed an experimental full factorial design, which included also the individual exposure tests to chemicals and food levels. In this setup, imidacloprid concentrations ranged from 2 to 10 mg/L and nickel from 100 to 300 μg/L. Total number of treatments used were 35 for imidacloprid and 42 for nickel. Food levels ranged from 0 to 6×10^5 cells/mL, but they were divided in two sets, with low food levels ranging from 0 to 3×10^5 cells of algae per mL of medium and high levels ranging from 4 to 6×10^5 cells of algae per mL of medium from using 3×10^5 as control. For the low food level experiment, to obtain a concentration–response curve, where the toxicity increases as concentrations increase, a transformation of $[3-x]$ was made, where 3 corresponds to the control concentration (3×10^5 cells/mL of food) and x is the real concentrations tested (0, 0.5, 1, 1.5 and 2×10^5 cells/mL of food), while for the high food level no transformation was made.

2.4. Data analysis

For the natural stressor (low food concentration) exposure of *D. magna*, the EC_{50} values obtained, as well as the EC_{50} (effective concentration that causes 50 percent of the effect to the organism) values of nickel and imidacloprid to each food level after 21 days of exposure, were calculated by fitting the data to the sigmoidal logistic model, $Y_{(ci)}=Y_{max}/[1+(c_i/EC_{50})^{\beta}]$, with a $Y_{(ci)}$ response as a function of the maximum response Y_{max}, the exposure concentration c_i, the EC_{50} value and the slope β of the response curve (Systat, 2006). A One Way Analysis of Variance (ANOVA) and multiple comparisons Dunnett's Method were used to detect differences between data that follow a normal distribution, and No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values derived for each stressor (Systat, 2006). When the normality test failed, a Kruskal–Wallis One Way Analysis of Variance on Ranks was performed and multiple comparisons Dunn's Method used to isolate group or groups that differ from the control group.