Ecotoxicology and Environmental Safety 156 (2018) 463–475

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Estimation of population-level effect of the endocrine disruptor pyriproxyfen in *Daphnia magna* by using changes in sex ratio and reproductive output

Yoshinari Tanaka[a,b,*], Kensei Nakamura[a], Shigeto Oda[a], Haruna Watanabe[a], Norihisa Tatarazako[a,c]

[a] Center for Health and Environmental Risk Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki, Japan
[b] Sophia University, Graduate School of Global Environmental Studies, 7-1 Kioicho, Chiyoda-ku, Tokyo, Japan
[c] Ehime University, Graduate School of Agriculture, 10-13 Dogo-Himata, Matsuyama, Ehime, Japan

ARTICLE INFO

Keywords:
Endocrine disrupting chemicals
Daphnia magna
Sex change
Reproductive inhibition
Population-level effect
Ecological modeling

ABSTRACT

Here we developed an analytical means of estimating population-level effects of endocrine disruptors on *Daphnia magna*. Our approach was based on the fact that the endocrine-disrupting juvenile hormone analogs induce the production of male neonates if they are exposed to the analogs during a particular period in their prenatal development; the method also assumed that the abnormal production of male neonates in the sake of production of female neonates reduces population growth. We constructed a linear toxicodynamics model to elucidate the period in which *D. magna* neonates are sensitive to exposure to the analog and also the probability of an individual neonate changing sex under specific exposure concentrations. The proposed model was applied to *D. magna* reproduction test data obtained under time-varying exposure to pyriproxyfen to derive the maximum-likelihood estimates and the posterior distributions of the model parameters. To quantitatively assess the ecological risk at the population level, we conducted a population dynamics simulation under two time-varying exposure scenarios (i.e., constant or pulsed exposure) by using an age-structured population model. When the change in sex ratio was based on the time-weighted average concentration during the period of sensitivity, change in sex ratio caused approximately equivalent population-level effects as did reproductive inhibition (i.e., reduction in the total number of neonates per female parent) regardless of the exposure scenario. In contrast, when change in sex ratio was based on maximum concentration during the sensitive period, change in sex ratio caused only half the population-level effects as did reproductive inhibition under constant exposure, whereas it caused a much larger population-level effect than did reproductive inhibition under pulsed exposure.

1. Introduction

To assess the ecological risks posed by endocrine disruptors (EDs), amendment of the *Daphnia magna* reproduction test to include the additional endpoint of sex ratio has been proposed (OECD, 2012). This approach is based on the assumption that the abnormal production of male neonates, which is induced by endocrine disruption especially by juvenile hormone analogs (Baldwin et al., 2001; Olmstead and LeBlanc, 2003; Oda et al., 2005a, 2005b; Tatarazako and Oda, 2007; Matsumoto et al., 2008; Dang et al., 2012; Ginjupalli and Baldwin, 2013), brings about ecological risk in natural populations. However, there are no analytical procedures available to estimate ecological risk from observed changes in sex ratio. Furthermore, there is currently no means of comparing the ecological risks of endocrine disruption as measured by changes in sex ratio with those measured by using other endpoints in terms of ecologically relevant criteria. Therefore, a means of converting individual-level responses induced by EDs to population-level effects is urgently needed.

Several ecotoxicological and endocrinological studies have shown that the determination of neonate sex in cladoceran species is limited to within the early developmental stage, and *D. magna* neonates respond to EDs during this period of sensitivity by changing sex, which produces an increased number of male neonates (Oda et al., 2005b; Wang et al., 2005; Tatarazako and Oda, 2007; Matsumoto et al., 2008; Kato et al., 2011; Ginjupalli and Baldwin, 2013). Therefore, given that the period of sensitivity to EDs is limited, the population-level effects of EDs are likely to reflect whether the concentration of an ED changed over time or whether exposure occurred in a pulse-like manner (Ashauer et al., 2007).

Here we propose an analytical method of evaluating the ecological risk posed by EDs by examining the effects of an ED on sex ratio (i.e., the abnormal production of male neonates). Although we applied our

* Corresponding author at: Sophia University, Graduate School of Global Environmental Studies, 7-1 Kioicho, Chiyoda-ku, Tokyo, Japan.
E-mail address: y-tanaka-fo5@sophia.ac.jp (Y. Tanaka).

https://doi.org/10.1016/j.ecoenv.2018.03.044
Received 16 November 2017; Received in revised form 27 February 2018; Accepted 19 March 2018

approach to the standard *D. magna* reproduction test (OECD Test Guideline No. 211, Annex 7) with the insecticide pyriproxyfen as the test substance, our approach is applicable to the substances that induce endocrine disruption especially for parthenogenetically reproducing organisms. The aim of this study was to develop a means of converting changes in sex ratio (as a consequence of an individual-level effect on sex change) into a population-level effect to allow comparisons between the ecological risks estimated by using different endpoints or chemicals (Forbes and Calow, 1999; Tanaka and Nakanishi, 2000).

Previous studies have reported that juvenile hormones and their agonists (analogs), which have been developed as insect growth regulators, induce the production of males in some cladoceran species including *D. magna* (Olmstead and LeBlanc, 2002, 2003; Tatarazako et al., 2003; Oda et al., 2005a, 2005b). More current studies have also described the time- and age-dependent effects of pyriproxyfen (Ginjupalli and Baldwin, 2013; Abe et al., 2014), one of the major juvenile hormone analogs. This compound has two distinct effects to change sex (the production of males) and to reduce the reproductive capacity (the total number of neonates) in *D. magna* (Watanabe et al., 2018). We have chosen pyriproxyfen as the test substance for the present study, because this compound is repeatedly demonstrated to have the multiple effect on the change of sex and reproduction. Elucidation of ecological effects and development of risk assessment method for pyriproxyfen have practical importance as well, since this compound is widely used in household, and for agricultural and horticultural applications to control insect pests (Ishaaya and Horowitz, 1995). Nonetheless, our main purpose is to present a general framework of an ecologically sound procedure of risk estimation for EDs that have the adverse effects on daphnids similar to those pyriproxyfen induces.

The framework in the present study was composed of three parts including the final part in which the risk estimates were attempted to be compared between the sex ratio change and the reproductive inhibition induced by the same compound. The first part was a toxicodynamic model that predicted both the probability of a change of sex (from female to male) to occur for a particular neonate and the second part was to estimate the reduction of age-specific fecundity (the number of offspring with either sex) of that neonate when it matures due to time-dependent exposure to a chemical. All model parameters critical for predicting these responses were estimated by using Markov chain Monte Carlo (MCMC) simulations within a Bayesian framework (McCarthy, 2007; Billoir et al., 2008).

The third part of the framework was designed to convert the predicted change in sex ratio and age-specific fecundity to a population-level effect by using a matrix population model (Liess et al., 2006; Hanson and Stark, 2011). Using the estimated posterior distributions of the model parameters, we estimated the population-level effects and the probability distribution of the chemical with regard to specific exposure scenarios causing a reduction in the intrinsic population growth rate.

Finally, the population-level effects that were reflected by either the change in sex or the inhibition of reproduction or both were compared in terms of population growth rate. *D. magna* responds exposure to pyriproxyfen by reducing the total number of neonates produced by a female as well as by changing neonates' sex into male (Oda et al., 2005b; Watanabe et al., 2018), and the exposure concentrations which induce these responses noticeably overlap (Watanabe et al., 2018). Therefore, the insecticide pyriproxyfen provided a good system in which we could demonstrate the availability of a population-level risk analysis in measuring ecological risks induced by different modes of action by chemicals.

2. Materials and methods

2.1. Daphnia magna extended reproduction data

We used chronic reproduction data using *D. magna* that were obtained in our research project and has been published elsewhere (Watanabe et al., 2018). Here we outline the experimental procedure and the major results.

All experiments were conducted in accordance with OECD Test guideline 211. We used the NIES strain of *D. magna* (Oda et al., 2005b) as the test organism. In brief, less than 24-h-old offspring obtained from 2-week-old daphnids were exposed to each concentration of the test chemical with the semi-static procedure for 21 days. The rearing media including the food and the test chemical was renewed three times a week. Ten replicate glass vessels each containing a single neonate in 50 mL of the test solution were kept at 21 ± 1 °C under a 16-h light and 8-h dark photoperiod. The daphnias were fed daily with approximately 0.1 mg carbon of freshwater alga *Chlorella vulgaris*. All offspring produced were removed to be counted every day, and were morphologically sexed after the removal under a stereomicroscope on the basis of the length of the first antennae, as described in Annex 7 of OECD Test Guideline 211 (OECD, 2012).

As the test substance, we used pyriproxyfen (CAS 95737-68-1, 99.0% purity) and prepared a 10,000-fold stock solution for each test concentration in dimethylformamide and added it to the M4 medium at a concentration of 0.01% (v/v).

Experiments in the present study consists of two major parts that have alternative schemes of exposure, the constant exposure and the pulsed exposure. The pulsed-exposure experiment consists further of several subschemes of pulsed exposure: four kinds of single-pulse regime (one of the exposure regimes, which was the single-pulse exposure during the first two days at the start of experiment, "P1-1" see below, was not used for the analysis of sex change since it induced no male production) and one multiple-pulse regime (see Graphical abstract of Watanabe et al. (2018) for graphical representation of the exposure schemes used in this study).

For the constant exposure experiment, we conducted a standard 21-day toxicity assay to obtain a concentration–response relationship. This experiment was performed at nominal pyriproxyfen concentrations of 25, 74, 222, 677 and 2000 ng/L. Measured concentrations in these treatments, which were estimated using GC/MS (GCMS-QP2010, Shimadzu Co., Kyoto, Japan), are available in Watanabe et al. (2018; Supplementary Material). Three measurements were conducted at the start and at the end of the renewal period (2 days) for each nominal concentration, resulting in average values at the start of renewal as 28.7, 83.7, 230, 713 and 2290 ng/L. The measured concentrations faded rapidly during the renewal period, as indicated by the time-weighted average concentration was 59% of the nominal concentrations on average across concentrations. We used the nominal concentrations for modeling the response data including those obtained with the pulsed-exposure regime, because the measured concentrations did not show impermissible discrepancies with the nominal concentrations.

For the single-pulse exposure treatment, the daphnias were exposed to 525 ng/L pyriproxyfen for 2 days at 4 different age-classes; Day 0–1 (neonate), Day 5–6 (juvenile), Day 10–11 (adult), and Day 15–16 (adult), the scheme of which was respectively denoted as P1-1, P1–2, P1–3 and P1–4. For the multiple-pulse exposure treatment, which was denoted as P4, the daphnids were exposed to 131 ng/L pyriproxyfen for 2 days in each four age-class (Day 0–1, Day 5–6, Day 10–11, and Day 15–16), and then the total duration of exposure was 8 days.

All of the pulsed-exposure regimes were standardized to 50 ng/L in terms of time-weighted average concentration during the 21-day duration of the experiment, in order to exclude the effect of total amount of exposure that animals received from effects resulting from differences in exposure regimes.

From the constant exposure experiment EC_{50} was estimated as 137 ng/L [95% confidence interval (95% CI): 126–148 ng/L] for fecundity and 238 ng/L (95% CI: 173–302 ng/L) for proportion of male offspring. The constant exposure of 50 ng/L pyriproxyfen did not affect either the fecundity or the proportion of male offspring, while a single-