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Environmental Toxicology

A Multiple Life-History Trait–Based and Time-Resolved Assessment of Imidacloprid Effects and Recovery in the Widely Distributed Collembolan *Folsomia quadrioculata*

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Abstract: Life-history traits determine individual fitness and the fate of populations. Imidacloprid, a widely used neonicotinoid insecticide, which persists in soil for more than 100 d at biologically relevant levels, may affect nontarget and ecologically important species, such as collembolans. In the present study, we determined the sublethal effects of short-term imidacloprid exposure and postexposure recovery in the collembolan *Folsomia quadrioculata*, which occurs abundantly across the northern hemisphere. We assessed survival, egg production, and hatching success in adult springtails exposed for 14 d through the diet to imidacloprid, followed by a 28-d postexposure phase. Survival and hatching success were high throughout the experiment in all the treatments, with no clear concentration dependence. However, egg production declined during the exposure phase and nearly stopped between 8 and 14 d in all the treatments (except the control) but resumed during the postexposure phase. Moreover, the resumption of egg production showed a concentration-dependent delay. Our findings suggest that low imidacloprid exposures can restrict reproduction, with potentially severe consequences for the population, notwithstanding the partial recovery in egg production. *Environ Toxicol Chem* 2021;40:139–147. © 2020 SETAC

Keywords: Insecticide; Neonicotinoids; Population-level effects; Recruitment; Soil invertebrates; Sublethal effects

INTRODUCTION

Recruitment and survival of organisms are important life-history traits linked to the success of their populations under natural conditions. Accordingly, these 2 life-history traits are often focused on as endpoints in the environmental risk assessment of anthropogenic chemicals, including insecticides (Organisation for Economic Co-operation and Development 2009). Recruitment is physiologically costly, and under stressful conditions, such as exposure to toxicants, the reduction of reproduction can improve adult survival (see Moriarty 1969; Haynes 1988; Sibly and Calow 1989; Lee 2000; and references therein). Therefore, reproduction can be particularly sensitive to toxicant exposure, and even sublethal doses of a toxicant can have adverse population-level effects.

In arthropods, egg production, egg development, and egg hatching success are important life-history traits determining recruitment. Because life-history traits are physiologically and genetically interlinked, they coadapt as life-history strategies, and their responses to stressors are dependent on each other (Sibly and Calow 1989; Stearns 1992). Studies on the responses of multiple traits often manifest as correlations and trade-offs (Forbes and Calow 1996; Fox and Czesak 2000; Knops et al. 2001). Moreover, these linked suites of traits can be affected by insecticides to different extents depending on the insecticide concentration, the duration of exposure, and the mode of action of the insecticide (Lee 2000). Accordingly, toxicity tests based on multiple endpoints might be a powerful way of elucidating the effects of sublethal toxicant doses on individual fitness and population dynamics.

Repeated or chronic exposure of nontarget organisms to sublethal doses of pesticides can threaten their populations (Desneux et al. 2007; de França et al. 2017; Braak et al. 2018). However, understanding the implications of sublethal effects for populations is laborious and challenging. Firstly, such studies require assessments of multiple endpoints, such as life

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history, behavior, and physiology, which are difficult to perform in a single study (de França et al. 2017; Braak et al. 2018; Anderson and Harmon-Threatt 2019). Secondly, not all endpoints may be equally affected by sublethal doses (Alves et al. 2014), and further studies may be essential. For example, altered behavior can lead to inefficient foraging, lack of energy acquisition, and consequently reduced reproductive investment (Dittbrenner et al. 2011). In addition, if allowed to recover after short-term exposure or pulsed dosing, organisms can show signs of recovery (Azevedo-Pereira et al. 2011; Dittbrenner et al. 2011), which adds to the challenge of providing ecologically realistic interpretations. Moreover, the occurrence of hormetic responses caused by sublethal exposure is not uncommon (Anderson and Harmon-Threatt 2019). Despite all the challenges involved, such studies can provide key insights into the mechanisms underlying the effects of toxicant exposure and our understanding of the effects of anthropogenic chemicals on natural populations (Desneux et al. 2007; Dittbrenner et al. 2011; Anderson and Harmon-Threatt 2019).

Neonicotinoid insecticides targeting the nervous systems of insects have been used widely in agriculture and forestry as sprays (Jeschke et al. 2010) or seed dressings (Douglas and Tooker 2015). These insecticides are known to contaminate both terrestrial (Wood and Goulson 2017; Silva et al. 2019) and aquatic (Hladik and Kolpin 2016) ecosystems. Several studies have reported adverse effects of these neurotoxic compounds on nontarget arthropods (Wood and Goulson 2017; Anderson and Harmon-Threatt 2019). Imidacloprid is a highly toxic insecticide that binds, presumably irreversibly, to the nicotinic acetylcholine receptor of insects (Abbink 1991; Ware and Whitacre 2004). Imidacloprid is photosensitive in the aqueous phase, but it can persist in soil for more than 100 d (van Gestel et al. 2017; Wood and Goulson 2017; Silva et al. 2019). Although banned in Europe, imidacloprid is still used in North America (Wood and Goulson 2017) and in other continents. Most of the imidacloprid applied for plant and crop protection can accumulate in soil as residues, resulting in high concentrations in soil (Wood and Goulson 2017; Anderson and Harmon-Threatt 2019; Silva et al. 2019). Toxic imidacloprid levels in soil can pose a serious challenge to nontarget soil organisms with important ecological functions, such as collembolans.

Collembolans (springtails) play an important role in soil nutrient recycling processes, and they are, therefore, regarded as a keystone group of soil communities (Rusek 1998). Collembolans are exposed to imidacloprid by direct diffusion of water through the cuticle (particularly in the ventral tube) and potentially through the diet by feeding on contaminated soil organic matter. The imidacloprid concentration in soil porewater can serve as a proxy of the severity of exposure for collembolans in soil (Ogungbemi and van Gestel 2018). The collembolan *Folsomia candida*, which is widely used in standard toxicity tests (Organisation for Economic Co-operation and Development 2009), is highly sensitive to imidacloprid in terms of reproduction and survival when exposed through soil or porewater (Idinger 2002; Ogungbemi and van Gestel 2018; de Lima e Silva et al. 2020), implying that

long-term chronic exposure can have strong adverse effects on natural collembolan populations. However, to the best of our knowledge, the importance of the dietary exposure route has not been addressed in detail. In addition, although *F. candida* is very useful for studying toxicological effects and mechanisms at the individual and cellular levels, it rarely occurs in natural soils, and its typical parthenogenetic reproductive strategy is adapted for very high reproductive efforts. Therefore, the ecological implications of imidacloprid exposure require validation across collembolan species more typical of natural environments.

Folsomia quadrioculata (Tullberg 1871) is a widely distributed collembolan, which is abundant in diverse habitat types across the northern hemisphere (Fjellberg 1994; Deharveng and Lek 1995; Ponge 2000). It reproduces sexually, and populations from different geographical regions and habitat types are known to differ in terms of thermal adaptation in several life-history traits (Sengupta et al. 2016, 2017). The temperature responses of *F. quadrioculata* populations suggest that an assessment of responses in multiple traits is essential for providing ecologically accurate explanations. Sexual reproduction and ease of handling in the laboratory make it a good model organism for toxicological studies. However, information on sublethal effects of imidacloprid on *F. quadrioculata*, as well as postexposure recovery (if any), is scarce.

In the present study, we aimed to understand the effects of dietary imidacloprid exposure on *F. quadrioculata* adults. We assessed 3 life-history traits linked to recruitment and population growth rate: adult survival, egg production, and hatching success. Based on previous knowledge of this species and studies on imidacloprid exposure of *F. candida*, we expected that 1) the focal traits will be affected by the selected imidacloprid concentrations and 2) egg production and hatching success will be more strongly affected than survival. In addition, we aimed to register signs of potential recovery after imidacloprid exposure. We discuss the implications of the observed sublethal effects on the fate of *F. quadrioculata* populations.

MATERIALS AND METHODS

Test organisms

Folsomia quadrioculata was extracted without heating in 2007 from randomly collected soil samples from the small northernmost island of Svalbard, Norway (Little Slate Island; 80.321°N, 20.360°E). This population belongs to a presumably highly pristine environment, and its life-history traits have been studied in detail previously (Sengupta et al. 2016, 2017). The animals were reared in small plastic boxes (diameter 3.4 cm, height 3 cm) with a moist base made of plaster of Paris and charcoal (Sengupta et al. 2016). The stock cultures were maintained at 15 °C in climate cabinets (Sanyo MIR 553; accurate to ± 0.5 °C). To stabilize the conditions further, the culture boxes were kept together inside a larger plastic box (11 × 17 × 3.5 cm). Temperature conditions were monitored constantly using HOBO data loggers (Onset Computer). The animals were fed ad libitum with small pieces of bark covered

with dark crusts of cyanobacteria and green algae, which were found to be highly favorable to the collembolans (Sengupta et al. 2016). We used a 24-h photoperiod (55 cm Osram 14 W, intensity $8 \mu\text{mol m}^{-2} \text{s}^{-1}$) to mimic the light regime of the growing season in the high arctic. Mass cultures were established with 100 to 200 animals. The same set of conditions was used for maintaining our experimental replicates (see section *Exposure method*).

Exposure method

Each experimental replicate (i.e., culture box described in the section *Test organisms*) started with 20 randomly selected adult *F. quadrioculata*. Randomized allocation of collembolans to the replicates was done to avoid systematic differences in body size which might have affected the results. For assessing the effect of dietary imidacloprid exposure across 5 imidacloprid concentrations and a control, 5 mg of food was spread out on the plaster substratum of each replicate (see section *Spiking of food*, for the method of spiking). All the replicates were kept at 15 °C. Food was renewed twice a week, and drops of distilled water were added to keep the plaster substratum moist.

Spiking of food

Branches of trees covered with dark crusts of cyanobacteria and algae were collected from the University of Oslo campus and defaunated by freezing at -80°C overnight. After 24 h at room temperature, the crusts were scraped off from the branches. The scraped powdery material was sieved with a 250- μm stainless steel mesh and subsequently soaked in aqueous solutions of imidacloprid (Chemical Abstracts Service no. 138261-41-3; Sigma-Aldrich; 0, 42.9, 128.7, 429, 128.7, and 4290 $\mu\text{g L}^{-1}$) overnight at 4 °C in darkness. The soaked food was filtered over polypropylene membranes (Merck; pore size 45 μm); the residue was removed from the filter and left to dry in darkness for 3 d. The dried residue was offered to the collembolans. We had no previous knowledge on dietary exposure effects of imidacloprid to *F. quadrioculata*. Therefore, our spiking solutions were prepared based on the imidacloprid concentrations achieved in standard LUFA 2.2 soil (0.01–1 mg kg^{-1} dry wt). This concentration range included median lethal concentration values for *F. candida* (van Gestel et al. 2017) and *Hypogastrura viatica* (S. M. Kristiansen et al., University of Oslo, Oslo, Norway, unpublished manuscript) exposed to imidacloprid in soil.

Chemical analysis of food

Subsamples of the spiked food were preserved in darkness at -20°C prior to high-performance liquid chromatographic–mass spectroscopic analysis of imidacloprid content. The homogenized food (10–30 mg) sample was weighed and added to a 15-mL tube. Prior to extraction, 50 μL of an internal standard (1 $\text{ng } \mu\text{L}^{-1}$ of d4-imidacloprid) was spiked into the sample. Two milliliters each of water and

acetonitrile (MeCN) were added to the tube. The sample present in the tube was mixed by vortexing for 1 min, followed by sonification for 30 min at room temperature. Approximately 1 g of NaCl (salt) was added to the tube, which was then shaken. The salt dissolved in the water, which then separated from the MeCN. The MeCN fraction was removed and evaporated until dryness by heating (60°C) under nitrogen. The remaining content was then dissolved in 0.5 mL 10% MeCN in water. All the samples were filtered with 20- μm Spin-X centrifuge nylon filter (Corning) and finally transferred into a liquid chromatography vial ready for ultraperformance liquid chromatographic (UPLC)–mass spectroscopic analysis.

Liquid chromatography was performed on an Acquity BEH C18 column (1.8 μm , $100 \times 2.1 \text{ mm}$; Waters), using an Acquity UPLC module (Waters). Separation was achieved using linear gradient elution at 0.5 mL min^{-1} starting with MeCN:water (10:90 v/v, water containing 0.1% formic acid), rising to 100% MeCN over 9 min. An isocratic elution with 100% MeCN was maintained for 2 min before the eluent was switched back to 10% MeCN. The UPLC system was coupled to a Xevo TQ-S tandem mass spectrometer operating with an ESI interface (Waters Micromass). Screening of imidacloprid was performed with multiple reaction monitoring in positive ionization mode; imidacloprid $256 > 175$, $256 > 209$ and deuterated $260 > 179$, $260 > 213$. The limit of detection of 0.1 ng g^{-1} imidacloprid was estimated to be 3 times the signal-to-noise ratio using spiked control samples.

Experimental design

Collembolans were exposed through the diet for 14 d (exposure phase) with 5 replicates per treatment. Thereafter, they were moved to new, clean culture boxes and fed ad libitum with pieces of bark covered with natural dark crusts. The postexposure phase lasted for 28 d; thus, the total duration of the experiment was 42 d. The treatments during the exposure phase consisted of food spiked with 5 imidacloprid concentrations (42.9–4290 $\mu\text{g L}^{-1}$ aqueous solutions) and a control (0 $\mu\text{g L}^{-1}$). Imidacloprid concentrations measured in the food were used as predictor variables in our analysis. Data were collected twice a week while renewing the feed (every 3–4 d).

Endpoints

We focused on the effects of imidacloprid on multiple life-history traits, and the main endpoints included survival and reproduction. Reproduction was studied by recording egg production and egg hatching success. Throughout the experiment, the number of eggs produced in each replicate was recorded twice a week, when food was renewed. Dead animals, if any, were counted and removed. Multiple egg batches (usually 3–5) were transferred to fresh culture boxes and incubated at 15 °C until hatching. We recorded the dates of egg laying and

counted the total number of eggs and the number of eggs hatched in each egg batch.

Data treatment

For each trait, we analyzed the response to treatment (i.e., mean imidacloprid concentrations measured in the food) over time using generalized linear models. To understand the temporal trends better, we assessed the responses in 4 periods: 0 to 7, 8 to 14, 15 to 27, and 28 to 42 d. Model selection and validation were done according to Zuur et al. (2009). In addition, dose–response models were fitted to the data using the *drc* package (Ritz et al. 2015) for estimating median effect concentration (EC50) values at appropriate time points (see section *EC50 estimation*). All the statistical analyses were carried out using R (R Development Core Team 2019).

Responses over time. Survival was analyzed using the Cox proportional hazards model with imidacloprid concentration as the fixed effect. This analysis was implemented using the *survival* package (Therneau 2020) in R. In addition, after considering the survival patterns observed in the first 14 d, we fitted similar models to survival data for the periods of 0 to 14, 15 to 27, and 28 to 42 d for analyzing the temporal trends.

The relationship of the cumulative number of eggs laid per animal with imidacloprid concentration and time was described using log-linear models fitted by generalized least squares (GLS; *gls* function, restricted maximum likelihood [REML] estimation) using package *nlme* (Pinheiro et al. 2020). The models included the imidacloprid concentration and its interaction with time as fixed effects. The models assumed independent variance per treatment using the *varIdent* function. Log-linear models were chosen a priori for this analysis because they do not predict values below zero, which is biologically meaningful for ratio-scaled response variables. Median values with 95% confidence intervals (CIs) were plotted. In addition, similar models were fitted to the cumulative number of eggs laid per animal during the following periods: 0 to 7, 8 to 14, 15 to 27, and 28 to 42 d.

Generalized linear mixed effects models (GLMMs) with binomial distribution and the logit link function were fitted to the data on hatching success to estimate concentration-dependent variation in this trait. The models included the interaction of time (from the start of the experiment to egg laying date) and imidacloprid concentration as fixed effects and replicate as a random effect. The models were fitted using the *glmer* function (REML fit) in package *lme4* (Bates et al. 2015). The fixed effects were standardized, as described in Zuur et al. (2013). The descriptor “time” included in these models served as a proxy for the physiological state of the parental generation at the time of laying the egg batches. However, because few eggs were laid for substantial periods during the experiment (see section *Results*), we performed a 2-phase analysis of temporal trends by fitting the binomial GLMMs to the data subsets comprising eggs laid during either the exposure phase (0–14 d) or the postexposure phase (15–42 d). To elucidate the effects of imidacloprid concentration and time, we plotted fitted descriptions from generalized linear models with binomial distribution,

which included concentration, time (exposure time or recovery time), and their interaction as fixed effects.

EC50 estimation. We aimed to estimate EC50s for effects on cumulative egg production per animal for the test periods of 0 to 7, 8 to 14, 15 to 27, and 28 to 42 d. The effects of exposure did not permit fitting of dose–response models to the 0- to 7- and 8- to 14-d data sets (see below, *Results*). However, dose–response models with imidacloprid concentration as the fixed effect were fitted to the combined exposure-phase data (0–14 d) and the data subsets for 15 to 27 and 28 to 42 d, which allowed us to estimate the EC50s until the end of the exposure phase and the ends of the 2 halves of the recovery phase, respectively. To further analyze possible recovery signs during the postexposure phase, we fitted dose–response models to the recovery time until resumption of reproduction (i.e., from day 15 until observation of the first egg batch in the clean environment). A 3-parameter log-logistic dose–response model was the best fit for these data. One replicate treated with the highest imidacloprid concentration, in which no eggs were produced during the recovery phase, was excluded from this analysis.

Because of the small effect of imidacloprid on survival and hatching success, dose–response models did not fit the data and EC50 values could not be estimated. All the dose–response model functions used were selected based on Akaike information criterion values with the *mselect* function in the *drc* package (Ritz et al. 2015). Assumptions of normality and homoscedasticity for the dose–response models were assessed using the Shapiro-Wilk test and Bartlett's test, respectively.

Trade-off between reproduction and survival. We aimed to assess whether recovery in egg production imposed physiological costs in terms of survival. Therefore, we first computed concentration-specific hazard ratios from a Cox proportional hazards model with imidacloprid concentration as the fixed effect and survival during the postexposure phase (15–42 d) as the response variable. Finally, we estimated the Pearson product–moment correlation of the estimated hazard ratios with median cumulative sum of eggs produced during the postexposure phase (log-linear GLS models).

RESULTS

Measured imidacloprid concentrations

Mean imidacloprid concentrations detected in the food used were 0.2, 6.6, 14.8, 48.8, 148, and 290 mg kg⁻¹ dry food after treatment of the food with 0, 42.9, 128.7, 429, 128.7, and 4290 µg L⁻¹ imidacloprid solutions, respectively (Supplemental Data, Table S1). Imidacloprid traces detected in the control (0.2 mg kg⁻¹) were substantially lower than the imidacloprid concentrations measured in the treatments (33–1450 times lower).

Survival

The imidacloprid concentrations used in the present study were within the sublethal concentration range for

FIGURE 1: Effects of dietary imidacloprid exposure on the number of eggs produced per surviving *Folsomia quadrioculata*, exposed for 14 d, followed by 28 d on clean food; $n = 5$ per treatment. Imidacloprid concentrations measured in the food are shown. Points and 95% confidence intervals are fitted predictions from a generalized least squares model (see *Data treatment*). The vertical stippled line indicates the end of the exposure phase (14 d). Gray horizontal lines correspond to 2 and 10 eggs per animal. A logarithmic scale was used in the vertical axes.

F. quadrioculata. No mortality was observed before 9 d of exposure, and survival until the end of the exposure phase (14 d) was >96% (Supplemental Data, Figure S1; Cox proportional hazards model, hazard ratio = 1.001, 95% CI 0.997–1.004, $df = 1$, $p = 0.69$). Imidacloprid had no significant effect on survival during the postexposure phase (Cox proportional hazards model, 15–27 d: hazard ratio = 1.000, 95% CI 0.994–1.006, $df = 1$, $p = 0.91$; 28–42 d: hazard ratio = 1.001, 95% CI 0.996–1.006, $df = 1$, $p = 0.71$). Survival was high throughout the experiment, and no concentration-dependent mortality was found (Supplemental Data, Figure S1; 93.5–96.8% survival).

Egg production

Egg production was significantly affected by imidacloprid concentration and time (Figure 1; log-linear GLS model, concentration: $F_{(1,416)} = 66.74$, $p < 0.001$; time: $F_{(1,416)} = 343.27$, $p < 0.001$; concentration $\times$ time: $F_{(1,416)} = 0.98$, $p > 0.05$). The controls produced eggs throughout the experiment (Figure 1). In all the other treatments, egg production was reduced during the second half of the exposure phase (i.e., 8–14 d; Supplemental Data, Figure S2) and halted. Egg production resumed in the postexposure phase, except for one replicate of

the highest imidacloprid concentration. Time had significant effects on egg production during the 0- to 7-d period; by contrast, concentration (but not time) had a significant effect in the 8- to 14-d period, thus indicating the onset of toxicity on egg production in the 8- to 14-d period (Supplemental Data, Table S2). Resumption of egg production during the postexposure phase was concentration-dependent (Figure 2), consistent with the detailed patterns in Figure 1 (EC50; Table 1) and the significant effects of concentration and time during 15- to 27- and 18- to 42-d periods (Supplemental Data, Table S2). Egg production in the imidacloprid treatments during the first week of the postexposure phase was low (Figure 1). Accordingly, the estimate of EC50 for egg production at 15 to 27 d was very low (Table 1). Imidacloprid concentration, time, and time $\times$ concentration effects were significant during this period (Supplemental Data, Table S2). Egg production improved during the second half of the postexposure phase (28–42 d; Figure 1), and the estimated EC50 value increased slightly (Table 1), indicating recovery. During this period, the effects of concentration and time were significant, whereas concentration $\times$ time interactions were not (Supplemental Data, Table S2). However, the hazard ratios and egg production were not significantly correlated during the postexposure phase (15–42 d; $r = -0.51$, 95% CI -0.93

FIGURE 2: Effect of 14-d dietary imidacloprid exposure on the recovery time required by the collembolan *Folsomia quadrioculata* until the resumption of egg laying (after transfer to clean food), as described by a 3-parameter log-logistic model. Points indicate data collected from each replicate ($n=5$ per treatment), the line indicates prediction, and the shaded region shows the 95% confidence interval of the prediction.

to 0.51, $t=-1.19$, $df=4$, $p=0.30$). In addition, the relative ranks of the treatments hardly changed throughout the experiment (Supplemental Data, Figure S2).

Hatching success

Hatching success did not differ among the treatments (Figure 3) at any stage of the experiment (Supplemental Data, Tables S3 and S4) and revealed no clear temporal trends (Supplemental Data, Figure S3).

DISCUSSION

The imidacloprid concentrations used in the present study were sublethal and affected the endpoints studied to different

TABLE 1: Median effect concentrations (EC50) with 95% confidence intervals for the effect of imidacloprid on egg production of *Folsomia quadrioculata* during 3 experimental phases and for recovery time required until resumption of egg production in the postexposure phase (refer to Figure 2 for concentration response of recovery pattern)^a

Egg production phases	Median EC50 (mg kg ⁻¹)	Model function
0–14 d	8.61 (4.3–12.9)	4-parameter log-logistic
15–27 d	3.1 (–3.0–9.3)	3-parameter log-logistic
28–42 d	33.6 (–30.2–97.5)	3-parameter Weibull type I
Time until resumption of egg production	638.6 ^b	3-parameter log-logistic

^aSpringtails were exposed for 14 d to imidacloprid through diet, followed by 28 d on clean food, and the model functions used for obtaining the EC50 values are indicated.

^bThis model did not meet the assumption of homogeneity. Therefore, the wide 95% confidence interval is not shown.

extents. Egg production was strongly affected, whereas egg hatchling success was hardly affected. Moreover, egg production partly recovered following the 14-d exposure to sublethal imidacloprid concentrations. Variations in sensitivity among the endpoints to a toxicant can be expected in risk-assessment studies (Alves et al. 2014), and a lack of observed mortality might not indicate absence of adverse effects at the population level (Desneux et al. 2007; Dittbrenner et al. 2011). Therefore, the assessment of sublethal effects on multiple endpoints with high temporal resolution can explain the consequence of sublethal effects better.

Survival

The highest dietary concentration used in the present study did not result in a decrease in survival. The high survival observed in the present study can be due to a short exposure time, consistent with short-term soil exposure studies on *F. candida* (Idinger 2002; Alves et al. 2014). In addition, it can

FIGURE 3: Effect of dietary imidacloprid exposure on the hatching success ($\pm 95\%$ confidence interval) of eggs produced by *Folsomia quadrioculata* during the exposure phase (0–14 d) and during the recovery phase (15–42 d). Concentrations measured in the food are shown. Point estimates and confidence intervals are described from generalized linear models, with binomial distribution and concentration as fixed effect.

be partially explained by the dietary exposure method used in our study, which minimizes the possibility of exposure by direct contact and porewater. This was, for instance, also shown for copper uptake effects on springtails by Bruus Pedersen et al. (2000). Soil porewater absorption through the cuticle and ventral tube is a major route of imidacloprid exposure, and Ogungbemi and van Gestel (2018) have shown that exposure through porewater can closely resemble the exposure scenarios in soil.

Egg production

Toxicant exposure strongly affects reproduction in collembolans (Schnug et al. 2014; van Gestel et al. 2017), and low EC50 values of imidacloprid for reproduction were reported in several studies on *F. candida* exposed through soil (Idinger 2002; Alves et al. 2014; van Gestel et al. 2017). Although our dietary exposure-based EC50 estimates in *F. quadrioculata* are low, these estimates are higher than the EC50 values reported in *F. candida*.

Life-history theory presumes that reproduction is physiologically costly; therefore, adult survival often trades off with reproductive investment, when exposed to toxicants (Moriarty 1969; Lee 2000). Consequently, reducing reproductive efforts might improve adult survival, which in turn can improve the population-level resilience for short periods. However, we found no such negative correlations supporting a trade-off in the present study. Consistent with the arguments of van Gestel et al. (2017) and the general reasons mentioned in Sibly and Calow (1989), we expect that a longer exposure duration or exposure through soil might elucidate trade-offs between survival and reproduction.

Temporary cessation of egg laying

In the present study, we found that imidacloprid exposure resulted in a transient cessation of egg production. Several mechanisms can drive this phenomenon. Firstly, the collembolans suffer from disoriented movements and spasms owing to the neurotoxic effects of imidacloprid (Ogungbemi and van Gestel 2018). This might lead to uncontrolled loss of energy, which reduces the resources available for reproduction (Anderson and Harmon-Threatt 2019) or altered sexual behavior (Haynes 1988). Changes in reproductive behavior following imidacloprid exposure have been studied in other arthropods (Desneux et al. 2007), but the effects on soil microarthropods have not been studied in detail. Moreover, imidacloprid might act as an environmental signal prompting *F. quadrioculata* to reduce egg production quite quickly after the initiation of exposure in favor of channelizing resources for maximizing adult survival. Nevertheless, it is known that honey bees cannot detect the presence of imidacloprid in the environment (Anderson and Harmon-Threatt 2019) and that the collembolans have a simpler brain structure (Kollmann et al. 2011). Therefore, further investigations into the mechanisms would require a separate study with a different experimental design.

Recovery in egg production and its demographic effects

We found that egg production resumed in the postexposure phase with a clear concentration-dependent delay. Recovery was further reflected as a slight increase in the estimated EC50 values at 28 to 42 d compared with that at 0 to 14 or 15 to 27 d (Table 1). The observed concentration dependence of the resumption of egg production suggests that the adult collembolans recovered to attain a physiological state suitable for reproductive investment. Previously, recovery after imidacloprid exposure was found in Chironomidae by Azevedo-Pereira et al. (2011).

The delay in resumption of egg production in the clean postexposure environment suggests that imidacloprid might delay maturity and the onset of reproduction if juvenile collembolans are exposed to it. Timing of reproduction is closely linked to fitness and population growth rate (Stearns 1992). Therefore, the delay in recovery to a state that allows reproduction, coupled with prolongation of the juvenile phase, might have important demographic consequences (Kammenga et al. 1996). The present recovery trend also highlights the importance and power of combining exploratory and rather descriptive analyses, such as the analysis of temporal trends, together with focused dose–response analyses of relevant time points.

Egg hatching success

Successful hatching of eggs is dependent on ambient abiotic conditions and reserves present within the eggs. The present study did not investigate the effects of contact exposure to imidacloprid on the eggs, nor did the eggs seem to be affected by maternal exposure conditions. The method used in the present study minimizes contact exposure, and imidacloprid, being highly water-soluble, might not be absorbed into the lipid-rich eggs, resulting in the observed lack of concentration-dependent effects. Consistent with this plausible explanation, lipophilic contaminants are easily transferred and accumulated in eggs through contact exposure and maternal transfer, and toxicants like pyrene show markedly different effects on life-history traits of collembolans than those observed in the present study (Sjursen and Holmstrup 2004).

Population-level effects of sublethal imidacloprid exposure

Impairment of movement caused by imidacloprid exposure can reduce foraging ability and increase susceptibility to predation (Desneux et al. 2007; Dittbrenner et al. 2011). Therefore, behavioral changes in *F. quadrioculata* caused by sublethal imidacloprid exposure should be assessed in future studies. Moreover, our ongoing microcosm and population modeling studies indicate that field populations, if exposed chronically to low imidacloprid concentrations, would face extinction owing to reduced egg production (J.J. Sanderson et al., University of Oslo, Oslo, Norway, unpublished manuscript, Y. Vindenes

et al., University of Oslo, Oslo, Norway, unpublished manuscript). Recovery, evident in the present study as the resumption of egg production during the postexposure phase (Figure 2), is likely to cause only small improvements in performance postexposure at the population level (J.J. Sanderson et al., University of Oslo, Oslo, Norway, unpublished manuscript; Y. Vindenes et al., University of Oslo, Oslo, Norway, unpublished manuscript). Consistent with the suggestion of Azevedo-Pereira et al. (2011), who reported recovery in an aquatic chironomid species after a short exposure period, it appears that long-term exposures to low imidacloprid concentrations or repeated exposure to low concentrations can have strong negative effects on populations. Despite the recovery trends observed, our findings are of ecological concern, as in the case of a study showing recovery in burrowing patterns of earthworms (Dittbrenner et al. 2011).

CONCLUSIONS

The sublethal effects of imidacloprid on *F. quadrioculata* highlight the importance of combining time-resolved analyses of exposure and recovery with concentration–response snapshots at selected time points. In addition, the present study clearly suggests that high survival at sublethal concentrations of a toxicant should not be treated as stand-alone evidence of its environmental safety. In addition, the partial recovery in egg production during the postexposure phase likely has little effect on population-level recovery. The present findings suggest that detailed information on life-history traits of soil organisms should be considered important criteria for evaluating the status and stages of soil contamination and remediation.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at <https://doi.org/10.1002/etc.4897>.

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Data Availability Statement—All data are available on request from the corresponding author (sagnik.sengupta@ibv.uio.no).

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