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Hazard/Risk Assessment

Harnessing Modeling for Assessing the Population Relevance of Exposure to Endocrine-Active Chemicals

Charles R. E. Hazlerigg,[a,b,*] Katie S. Mintram,[c,d] Charles R. Tyler,[d] Lennart Weltje,[e] and Pernille Thorbek[e]

[a]Enviresearch, Newcastle-Upon-Tyne, UK
[b]School of Natural Environmental Sciences, Newcastle University, Newcastle-Upon-Tyne, UK
[c]Department of Computer Science, Brunel University, London, UK
[d]Biosciences, University of Exeter, Exeter, UK
[e]Agricultural Solutions—Ecotoxicology, BASF SE, Limburgerhof, Germany

Abstract: The presence of endocrine-active chemicals (EACs) in the environment continues to cause concern for wildlife given their potential for adverse effects on organisms. However, there is a significant lack of understanding about the potential effects of EACs on populations. This has real-world limitations for EAC management and regulation, where the aim in environmental risk assessment is to protect populations. We propose a methodological approach for the application of modeling in addressing the population relevance of EAC exposure in fish. We provide a case study with the fungicide prochloraz to illustrate how this approach could be applied. We used two population models, one for brown trout (*Salmo trutta*; inSTREAM) and the other for three-spined stickleback (*Gasterosteus aculeatus*) that met regulatory requirements for development and validation. Effects data extracted from the literature were combined with environmentally realistic exposure profiles generated with the FOCUS SW software. Population-level effects for prochloraz were observed in some modeling scenarios (hazard-threshold [HT]) but not others (dose–response), demonstrating the repercussions of making different decisions on implementation of exposure and effects. The population responses, defined through changes in abundance and biomass, of both trout and stickleback exposed to prochloraz were similar, indicating that the use of conservative effects/exposure decisions in model parameterization may be of greater significance in determining population-level adverse effects to EAC exposure than life-history characteristics. Our study supports the use of models as an effective approach to evaluate the adverse effects of EACs on fish populations. In particular, our HT parameterization is proposed for the use of population modeling in a regulatory context in accordance with Commission Regulation (EU) 2018/605. *Environ Toxicol Chem* 2023;42:1624–1640. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Endocrine-active chemicals (EACs) are exogenous substances that can interact or interfere with normal hormonal action. When this consequently causes adverse health effects in an intact organism, or its progeny, or a (sub)population, it is further identified as an endocrine disruptor (World Health Organization [WHO] 2002; hereafter described as an endocrine-disrupting chemical [EDC]). Endocrine-active chemicals continue to be a source of concern due to their observed presence in the environment at toxicologically relevant concentrations (see Kasonga et al., 2021; Matthiessen et al., 2018). Endocrine-active chemicals may cause sublethal effects in individual organisms at low concentrations (e.g., ng/L for steroidal estrogens) on a wide range of biological endpoints, most notably relating to reproduction (see Vos et al., 2008). Population-level effects in fish have also been observed in an experimental lake system following a targeted dosing with 17α-ethinylestradiol (EE2; Kidd et al., 2007) and also in natural fish populations exposed to concentrated chemical spills (wastewater effluent; Hamilton et al., 2016), although these data are limited to very few EDCs. Indeed, our understanding of wildlife population and community relevance of EAC exposure is still very limited (Windsor et al., 2017).

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* Address correspondence to Charles.hazlerigg@enviresearch.com
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There is now an urgent need for the development of approaches to support effective regulations to protect wildlife from potentially harmful effects of EACs and to better understand population responses to EAC exposure (Godfray et al., 2018). It has been highlighted that the effects on wildlife of many legacy chemicals with endocrine activity are generally greater than those caused by current-use chemicals, with the exception of EE2 and other estrogens found in sewage effluents, which are causing widespread effects on fish populations (Matthiessen et al., 2018). To emphasize the population relevance of the adverse effects, we use the term EAC to describe chemicals for which no effect on the population has been reported, whereas EDC is used when population-relevant effects of exposure have been confirmed. This is consistent with the WHO (2002) terminology and the regulatory definitions used in Europe (according to Commission Regulation (EU) 2018/605).

Authorizations of plant protection products (PPPs) under European Commission Regulation 1107/2009, and biocides under European Commission Regulation 528/2012, state that substances cannot be given authorization if they are regarded as having endocrine-disrupting properties that may be harmful to humans or nontarget organisms. Commission Regulation (EU) 2018/605 specifies the three criteria that are necessary to positively identify an EDC, and a joint guidance document by the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) provides the methods and approaches used to evaluate endocrine-disrupting properties under the aforementioned regulations (ECHA/EFSA, 2018). In brief, the substance must show an adverse effect on humans or nontarget organisms, and this adverse effect must be a consequence of an endocrine mode of action. In the nontarget organism assessment, the adverse effect is considered at the population level (European Commission, 2018). By default, it is assumed that effects observed on individual organisms (e.g., on reproduction or growth in a laboratory study) are relevant at the population level, unless there is evidence suggesting otherwise. In such cases, effectively a "4th criterion" applies, whereby the adverse effect is at the population level and results from population models may be considered in the assessment. This reflects the growing acceptance of the use of effects modeling in PPP risk assessment, and these models are already used to support higher tier risk assessments in non-EAC situations (Pastorok et al., 2016).

However, statements in the ECHA–EFSA guidance on the practicality of how to use population models in this context are absent. This omission will inevitably lead to inconsistencies in model implementation and application, also likely bringing doubt into the regulatory acceptability of the different approaches that may be undertaken. In part, this reflects the more fundamental problem that there is no agreed-on approach in the scientific community to dealing with some of the issues specific to EACs (e.g., hazard- or risk-based assessments) and compelling those on one side of the debate to meet on the other side, but this has appeared to further polarize the two camps of thought (McIlroy-Young et al., 2021).

Over the last decade, much effort has gone into developing scientifically sound good practice modeling guidelines for chemical risk assessment (Grimm et al., 2014; Schmolke, Thorbek, DeAngelis, et al., 2010; Schmolke, Thorbek, DeAngelis, Chapman, 2010). In parallel, many population models have been developed specifically for use within chemical assessments in fish (see David et al., 2019; Hazlerigg et al., 2014; Mintram et al., 2018) and other taxonomic groups (e.g., Johnston et al., 2014; Liu et al., 2014). There has been considerable progress in population models regarding our understanding of the mechanisms of toxicity and their effects on resource allocation decisions in organisms harmonized within the dynamic energy budget theory (Baas et al., 2018), as well as greater acknowledgement that properties of a population emerge from decisions and interactions at an individual level (Forbes et al., 2008; Uchmanski & Grimm, 1996). Crane et al. (2019) suggest a hazard-based framework for population modeling of EACs. Their method proposes the use of population models within an adverse outcome pathway (AOP) framework. Once an endocrine mechanism leads to an adverse outcome in individuals, can focal species assessments be undertaken using population models to assess whether thresholds for population-level impacts are exceeded? Applying population models within an EAC context, however, provides additional challenges. Forbes et al. (2019) used the individual-based Stream Trout Environmental Assessment Model (inSTREAM) model to predict the impacts of an EDC (EE2) on two trout species in the context of provision of ecosystem services (i.e., angling) and the population viability of a threatened species (greenback cutthroat trout). In the model, they imposed effects from EE2 exposure on egg fertility and male gonadal development based on laboratory data. This provides one example of how the population relevance of endocrine effects on individuals could be modeled and evaluated; however, the approach is not compatible with the chemical hazard assessment paradigm of European Commission Regulation (EU) 2018/605.

In terms of exposure, EE2 concentrations in the system were implemented as a constant loading, which is not expected from proposed uses of PPPs in agriculture. The effects imposed were also based on literature data that did not differentiate between endocrine modalities and systemic toxicity, the latter of which is not directly relevant to the hazard assessment of endocrine-disrupting properties of chemicals (European Commission, 2018). However, the regulation stipulates that "adverse effects that are nonspecific secondary consequences of other toxic effects shall not be considered for the identification of the substance as an endocrine disruptor." This highlights the need for a clear discussion of the issues regarding how population modeling could be used in the hazard-based assessment for EACs.

In the present study, we assessed how population modeling approaches can be used in the hazard-based assessment for EACs. We first give an overview of the specific issues relating to EACs to comply with the current regulations, and in particular, how to use modeling in a hazard assessment context. We then illustrate an approach for harmonizing modeling for assessing population-level impacts of EACs in fish, using a case study with the fungicide prochloraz. We also provide the same case study using the standard risk assessment methodology to allow

comparisons between the two approaches and to demonstrate the level of conservatism with a hazard-based approach. A key purpose was to bring closer attention to the unresolved issues relating to the application of population modeling in a hazard context so that a consensus can be reached for a standardized population modeling approach for application in EAC effects analysis and regulatory practice.

METHODS

The recent publication of the Population Modeling Guidance (Pop-GUIDE) tool (Raimondo et al., 2020) provides a comprehensive guideline designed to meet the needs of population model development for use in chemical risk assessment. Aspects of this framework were applied in our study, focusing on the use of previously developed models for assessment of EAC impacts on fish populations. The elements of this process are illustrated in Figure 1. The three areas of *Model choice*, *Data*, and *Application* are discussed in broad terms, before being specifically applied in a case study with prochloraz in fish.

Decision framework

Model choice. In vivo tests to determine adverse effects of EACs on individuals are predominantly based on fish (see Organisation for Economic Co-operation and Development [OECD], 2011, 2015b), amphibians (see OECD, 2015a), and rodents (see OECD, 2001). Because water is an important repository for EACs in the environment, fish were used for our study due to the availability of suitable models, the availability of appropriate effects data, and the relevance of the EAC assessment to wildlife. Matrix models have been used to link vitellogenin (an estrogen-dependent yolk protein widely used as a biomarker of exposure to estrogenic EACs) and reductions in fecundity to changes in population abundance in the fathead minnow (Miller & Ankley, 2004) as well as to assess their vulnerability to pesticide exposure in 23 different European fish species (Ibrahim et al., 2014). More recently, individual-based models (IBMs) have been developed for zebrafish (*Danio rerio*; Beaudouin et al., 2015; Hazlerigg et al., 2014), three-spined stickleback (*Gasterosteus aculeatus*, David et al., 2019; Mintram et al., 2018, 2020), and brown trout (*Salmo trutta*; Railsback et al., 2009) among others.

Ultimately, any models selected for use in assessing EAC effects at the population level must 1) capture the relevant processes and dynamics of the modeled system to provide reliable outputs (i.e., the model must be validated); and 2) be capable of using and implementing effects observed in ecotoxicity testing (e.g., effects on apical endpoints in individuals, such as fecundity) to extrapolate to effects on populations. Any model can be assessed against these criteria using the checklist from the EFSA Good Modelling Practice Scientific Opinion (EFSA, 2014). This strategy is commonly applied when one is proposing models for use in regulatory risk assessment (see Tarazona et al., 2021).

Data. There are a range of sources that could provide relevant effects data for use in population modeling of EACs. A literature search for any relevant material may be the first step. Suitable search methodologies include the use of search "strings" in appropriate databases and search platforms (EFSA, 2011a). Furthermore, the reliability of any relevant studies should be evaluated (see Klimisch et al., 1997; Moermond et al., 2016). A second source of data may include read-across effects data from similar substances, as can be derived from toxophore

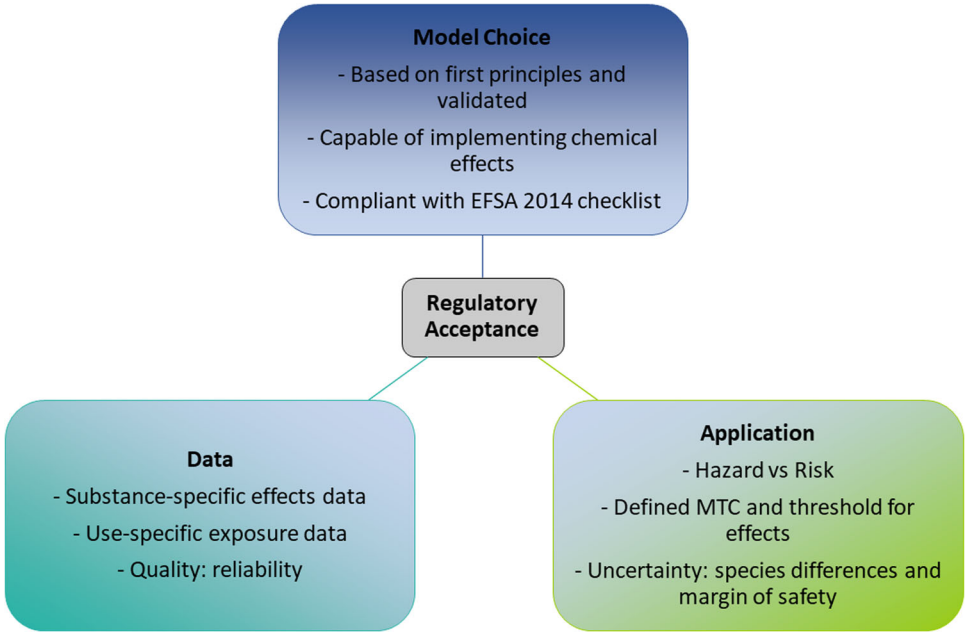

FIGURE 1: Application, in a condensed format, of the Pop-GUIDE conceptual framework for the use of population models in the assessment of endocrine active chemicals. EFSA = European Food Safety Authority; MTC = maximum tolerated concentration.

(Sinclair, 2009) and quantitative structure–activity relationship (QSAR) analyses (e.g., ECOSAR, DEMETRA). These approaches are commonly proposed as a way of reducing animal testing, although the accuracy and reliability of these approaches may vary depending on the underlying data set and applicability domain of the QSAR model (European Commission, 2010). Finally, regulatory studies performed under Good Laboratory Practice may be available. These can provide substance-specific effects data on endocrine-mediated endpoints. As such, they are considered the most useful when parameterizing effects data for use in population modeling, but they are not always available.

To assess for biological effects in a population model, a suitable exposure profile of the substance is required. The simplest option for selecting an appropriate temporal scale of exposure is to assume a constant exposure scenario. This may be considered relevant for hazard assessments because it is not explicitly considering temporal scales of exposure, although it creates a further challenge in defining the magnitude of effect associated with exposure. One alternative is to base exposure data for fish and other aquatic organisms on the presence of the substance from water monitoring schemes (see European Commission, 2000). These data sets provide environmentally relevant concentrations of a substance. If, however, the substance of interest is not monitored or this is a prospective risk assessment, then monitoring data may be unavailable. Alternatively, the FOrum for Co-ordination of pesticide fate models and their USe developed surface water models (FOCUS SW) that may be used to calculate the predicted environmental concentrations of pesticides in waterbodies over time following their application (FOCUS, 2001, 2015). Ten different scenarios (D1–D6, R1–R4) with three different waterbodies (ditches, streams, and ponds) using different combinations of soil properties, climate, and topography have been developed to define a "realistic worst-case" scenario for exposure in edge-of-field waterbodies in the European Union. These models and their outputs are currently the regulatory standard for predicting pesticide concentrations in edge-of-field surface waters in Europe. As such, the outputs from FOCUS SW Step 3 calculations provide a reasonable exposure profile to use in population modeling of EACs.

Application. Once appropriate models have been identified and suitable data for parameterizing effects of a chemical on fish are available, decisions need to be made about how these effects are integrated into the population model and the way in which the results are analyzed. This includes decisions regarding the type of assessment being performed (e.g., hazard or risk), what magnitude of effects need to be implemented into the model (e.g., maximum observed or magnitude associated with the maximum tolerated concentration [MTC]), at what exposure concentration (e.g., zero or some other threshold), and against what exposure profile (e.g., hypothetical, FOCUS prediction, or monitoring). Considerations that form part of the decision process for each of these factors are now briefly discussed.

Regarding the question of hazard or risk, ECHA/EFSA (2018) state that a hazard assessment should be performed while also acknowledging that many EAC effects are dose dependent (so a risk assessment could be justified scientifically). A hazard assessment focuses on the inherent toxicity of a substance, whereas a risk assessment focuses on the potential for that inherent toxicity to have an effect in a real-world setting taking account of predicted exposure (i.e., a dose–response [DR] relationship). In a regulatory context, performing population modeling with a hazard approach is most consistent with the regulation, whereas scientifically, performing population modeling with a risk approach is more consistent with the literature on EAC effects, which are dose dependent. In a risk assessment, a DR curve, fitted to the available data for an effect on individual fish, can be implemented in a population model to apply the appropriate magnitude of effect associated with a given concentration based on the concentration in the model at that time. Performing a hazard assessment in population modeling is more challenging. Selecting a magnitude of effect in a hazard assessment is more complex because a single effect value is imposed on the model at any time when an individual is considered "exposed" rather than as a direct consequence of the specific concentration at that time. The largest observed effect from laboratory studies (a worst-case assumption) could be selected, but this is somewhat arbitrary because it may depend on the dose setting used in the study (e.g., assuming a standard DR curve; if the concentrations tested reaches a high enough level, many studies will report 100% effect). Moreover, at the highest dosing levels—exposure concentration—some of the observed effects on endpoints result from systemic toxicity rather than from endocrine activity. Alternatively, the magnitude of effect associated with the MTC may be used, defined as the concentration at which effects from systemic toxicity are starting to be observed and above which one cannot reliably study endocrine effects (Wheeler et al., 2013). For a hazard assessment, implementing the effects associated with the MTC for a constant exposure over a certain duration of time may be considered most appropriate (ECHA/EFSA, 2018). However, this would not match expected exposure to PPPs where concentrations are variable over time, and furthermore, effects would be imposed in the population model at concentrations that had not resulted in observed effects in laboratory tests. However, use of an exposure profile then requires that the concentration at which effects associated with the MTC be imposed on the population model must be determined (the "effects threshold"). Although precisely what the effects threshold for an effect is set at (defined as the concentration above which effects associated with the MTC are imposed in the model), or what duration of exposure should be considered are unclear. However, given the DR observed for endocrine effects, a risk assessment based on an appropriate exposure profile may also be scientifically justified (e.g., FOCUS SW as a regulatory accepted approach in other areas).

Irrespective of the decisions made in the parameterization and implementation of endocrine effects in population models, some assessment of the conservativeness and uncertainty in the assessment is required. The margin-of-safety approach is a

widely accepted method in the regulatory sector. Based on the method first used by Ashauer et al. (2013) for assessing toxicokinetics/toxicodynamic models (later renamed lethal profile, [LPx] and effect profile, [EPx] ECHA/EFSA, 2018), this involves applying various multiplication factors (MFs) to any exposure profile used in population modeling and assessing how the population response changes. Whereas other model types use this method to define an LPx, because of the complexities of many IBMs, it is often not possible to work backward from the results obtained to arrive at an MF that leads to a specific deviation from the control (i.e., linearity of output cannot be assumed and simulation run-time can be long). Thus, rather than identifying the lowest MF that leads to more than a specific change in the population endpoint, performing simulations with a few select MF values can give a good indication of the potential margin of safety.

Case study

Substance chosen—Prochloraz. Azoles such as prochloraz are primarily used in PPPs as fungicides and act by inhibiting the enzyme 14-demethylase (CYP51) that has a role in the biosynthesis of ergosterol and thereby hindering cell wall formation in target fungi. Azoles have been identified as a class of chemicals with the potential to cause endocrine effects on nontarget organisms including fish (Matthiessen & Weltje, 2015). The dominant nontarget endocrine-mediated effect in organisms, including fish, is the inhibition of aromatase (CYP19) activity leading to increased levels of testosterone and reduced levels of estradiol (Ankley et al., 2005). However, whereas impairment of reproductive variables has been attributed to reduced aromatase activity in roach (*Rutilus rutilus*) and chub (*Leuciscus cephalus*) populations in polluted surface waters (Gerbron et al., 2014; Hinfray et al., 2010), it is not clear to what extent fish populations are impacted by the endocrine activity of azoles (Matthiessen & Weltje, 2015). Through a review of the effects of five azoles (prochloraz, propiconazole, fadrozole, fenarimol, and ketoconazole) on female fecundity in fish, we chose prochloraz as a potent representative for azole-type effects.

Prochloraz is an imidazole fungicide originally registered for use in the European Union (EFSA, 2011b), although it is no longer employed (European Commission, 2021). Due to its reported endocrine activity, it is one of the most well-studied chemicals for endocrine-mediated effects on fish, and data are available for the required model parameterization. Furthermore, there are comprehensive data sets on prochloraz concentrations detected in natural waterways, most notably for the Netherlands, Italy, Switzerland, and Sweden between 1991 and 2010. In the latter surveys, the majority of observations measured prochloraz at peak concentrations below 1 μg/L, but up to a maximum peak concentration of 2.1 μg/L (Weltje, 2013). These are concentrations associated with sublethal effects under sustained exposure, including through endocrine mechanisms. Thus, concerns regarding its endocrine potential in combination with its presence in natural waterways suggest it is an ideal chemical for the present case study to assess population relevance.

Model choice. Two models were chosen for use in our case study: a three-spined stickleback (*Gasterosteus aculeatus*) IBM (Mintram et al., 2018) and a brown trout (*Salmo trutta*) Energy Budget IBM—inSTREAM (Railsback et al., 2009). The original model description and code for the stickleback model was available in the Supporting Information from Mintram et al. (2018), and Ver 7 of the inSTREAM model (available in Netlogo) was provided by Steve Railsback following contact via the Ecomodel website (Cal Poly Humboldt, 2021). These two models were deemed acceptable for use in chemical risk assessments based on the EFSA's Good Modelling Practice guidelines (EFSA, 2014). The main reasons for this conclusion on both models were: 1) extensive data was used in the parameterization of both models; 2) both models were based on fundamental ecological principles and included relevant processes (e.g., density dependence, competition); 3) model validation against field census (and in the case of inSTREAM, further validation of internal processes using pattern-oriented modeling); 4) a mechanistic basis for population dynamics to emerge from apical endpoints (stickleback) and energy budgets (inSTREAM) in individuals; and 5) the inclusion of all relevant apical endpoints on which endocrine effects could be imposed.

The full EFSA (2014) checklist for each model is provided in the Supporting Information. Although the stickleback model does not include an energy budget component (and the flexibility of environmental scenarios and mechanistic implementation of chemical effects associated with this), it does contain the relevant apical endpoints (sex ratio, growth, and egg production) and essential density dependence and individual-level behaviors necessary to perform a population assessment of the effects of EACs. Although the inSTREAM model was not originally developed for assessing the population-level effects of exposure to EACs, it has been shown previously to be applicable to this type of investigation (Forbes et al., 2019). In addition to the changes made to the model to implement toxicant effects, discussed later in this section, we modified flow and physicochemical data features in the ecological model in inSTREAM. The flow and physicochemical data (i.e., water temperature, water flow, and water turbidity) used as input to this model was originally based on a time-series data set with natural, observed variability, over 70 years. The flow and physicochemical data associated with a single year was used for every year in our study to remove this natural variability and make it more likely to be able to discern any population-level effects of prochloraz (i.e., a single year of climate data was repeated every year for 23 years in the model simulations). The year selected for input of flow data into the model, 1960, reflected a generally average (neutral) flow regime that would most likely allow for population-level effects of prochloraz to be observed if they occurred.

Exposure. Standard regulatory FOCUS SW Step 3 modeling was used to generate exposure profiles for prochloraz following a hypothetical spray application of 125 g a.s./ha to winter cereals in the spring (BBCH 30), a use representative of Central European agriculture. Full FOCUS SW modeling

parameterizations are provided in the Supporting Information. FOCUS SW Step 3 modeling was performed for scenarios D1–D6, R1, R3, and R4. (R2 is not relevant for winter cereals.) From these scenarios, three exposure profiles were selected (D2 ditch, R1 pond, R4 stream) for use in population modeling. This combination of scenarios was illustrative of the different types of exposure profile (worst-case exposure) including one short, high peak concentration (D2 ditch), multiple short, high peak concentrations (R4 stream), and one long, low peak concentration (R1 pond), rather than the waterbodies being representative of the habitats of the fish species modeled. A few minor adjustments to the exposure profiles generated by FOCUS SW were required to ensure compatibility with the population models. First, two of the exposure profiles did not include leap years, so in population models, the concentration on 28 February was repeated on 29 February, where required. Second, the duration of the exposure profile generated for the D2 ditch scenario was 18 months, whereas the population models run on an annual cycle. The first 12 months of the exposure profile were used in the population models because this included the highest concentration of prochloraz (>0.8 μg/L). Third, the exposure profiles for each scenario produced in FOCUS SW differ in their start date (January in D2 ditch, March in R1 pond, October in R4 stream). In all population modeling, the start of the exposure period was timed to coincide with the spawning period of each species (May in stickleback, October in brown trout). Finally, as the stickleback model uses a 360-day year, an additional 5 days were removed from each exposure profile for use in this model. The days removed were associated with concentrations below the lowest effect threshold (<5 μg/L) even when using an MF of 1000 to ensure no potentially significant exposure was removed. To assess the margin-of-safety, simulations were performed for each exposure profile with four different MFs. These MFs were 1, 10, 100, and 1000. In each case, the concentration on a given day from the exposure profile was multiplied by the relevant MF, and the resultant higher concentration (and associated effects on fish) was used in the population model on that simulation day (Figure 2).

Effects. Publicly available data from the primary literature were used to determine the relationships of the effects of prochloraz on fish. When raw data were not available, a graph digitizer tool was used to extract data points from graphs as accurately as possible. An initial review of the available data showed prochloraz had effects on egg production, sex ratio, and potentially growth rate in fish (see the Supporting Information). The four-parameter log-logistic function was fitted to the data for each endpoint in R (Ver 3.6.2, R Foundation for Statistical Computing, DRC Package), with this relationship used in the DR simulations (see Figure 3). The magnitude of effects used in the hazard-threshold (HT) simulations (i.e., magnitude of effects associated with the MTC) was defined for each endpoint. The effects threshold (the concentration above

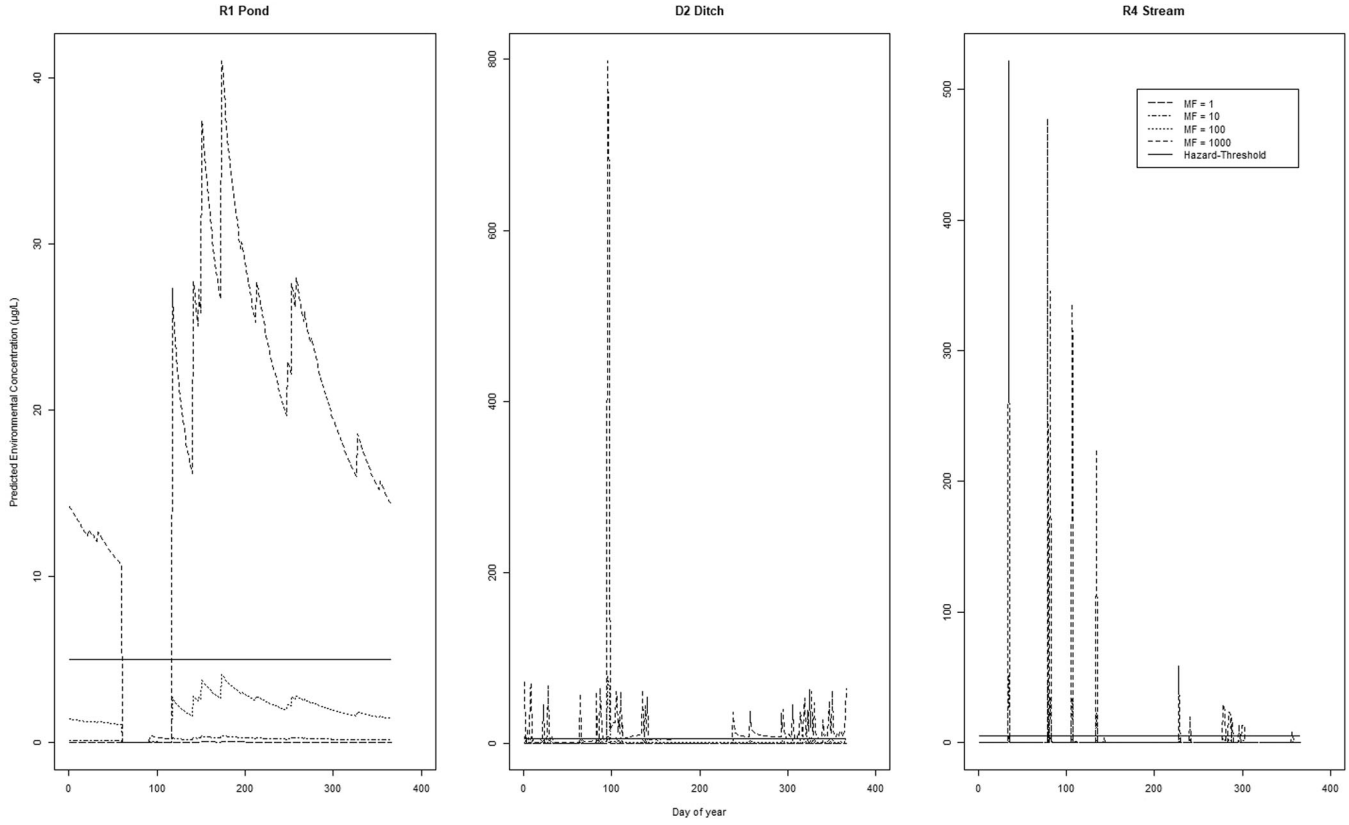

FIGURE 2: FOCUS exposure profiles for R1 pond, D2 ditch, and R4 stream, generated for a representative use of prochloraz. Exposure profiles for each scenario are displayed using different multiplication factors (MF). The threshold above which effects are imposed on individual fish (based on the lowest overall laboratory no-observed-effect concentration of 5 μg/L) in the hazard-threshold simulations is also shown.

which effects associated with the MTC are imposed in the HT simulations) was also defined based on evidence of apical and nonapical effects in the published studies.

Fecundity. All published studies on fish reported significant effects of prochloraz on egg production (Ankley et al., 2005; OECD, 2006; Zhang et al., 2008). Although fecundity is under endocrine control, a decrease in fecundity can be caused by several mechanisms and is not diagnostic for endocrine activity of a chemical (Ankley & Jensen, 2014). To aid in the identification of the MTC, oocyte atresia was identified as a biomarker for systemic toxicity for our case study. Atresia is commonly measured in 21-day reproduction studies via gonadal histopathology and is an indicator of apoptosis and consequently cellular stress, making it suitable for use in this case, even though theoretically it might be affected by endocrine activity (e.g., via disruption of growth hormones maintaining early stage oocytes). In two prochloraz studies, oocyte atresia was observed to commence at 311 and 279 µg/L and was associated with very high levels of reduction in cumulative egg production in fathead minnow (88%; Ankley et al., 2005) and medaka (89%; OECD, 2006). Following the MTC approach, the effect magnitude was defined as an 88.5% reduction in fecundity. Therefore, in the HT simulations whenever the chemical was present in the population model above the effects threshold, an 88.5% reduction in fecundity was imposed. Meanwhile, in the DR modeling the reduction in fecundity was dependent on the concentration at the time of spawning and the DR relationship (Figure 3).

Sex ratio. Prochloraz has been reported to cause a male-skewed sex ratio in multiple laboratory studies for a range of species, including fathead minnow, medaka, and zebrafish (Baumann et al., 2015; Holbech et al., 2012; Kinnberg et al., 2007; Thorpe et al., 2011). Phenotypic sex is under hormonal control, and it can therefore be assumed in the absence of mortality that any effect on sex ratio reported in these studies is likely caused by an endocrine mechanism of prochloraz (i.e., aromatase inhibition) rather than systemic toxicity. The greatest effect reported in any prochloraz study was an all-male (i.e., no females) population (at a very high concentration). Fish species vary greatly in the mechanisms of sex determination and subsequent differentiation, as well as the life stage/age at which sex is most susceptible to the effects of EACs. For example, in zebrafish sex is determined by environmental and genetic factors and can be influenced by EAC exposures up to the juvenile/subadult stage (Santos et al., 2017). In the population models, the effect on sex is implemented on embryos

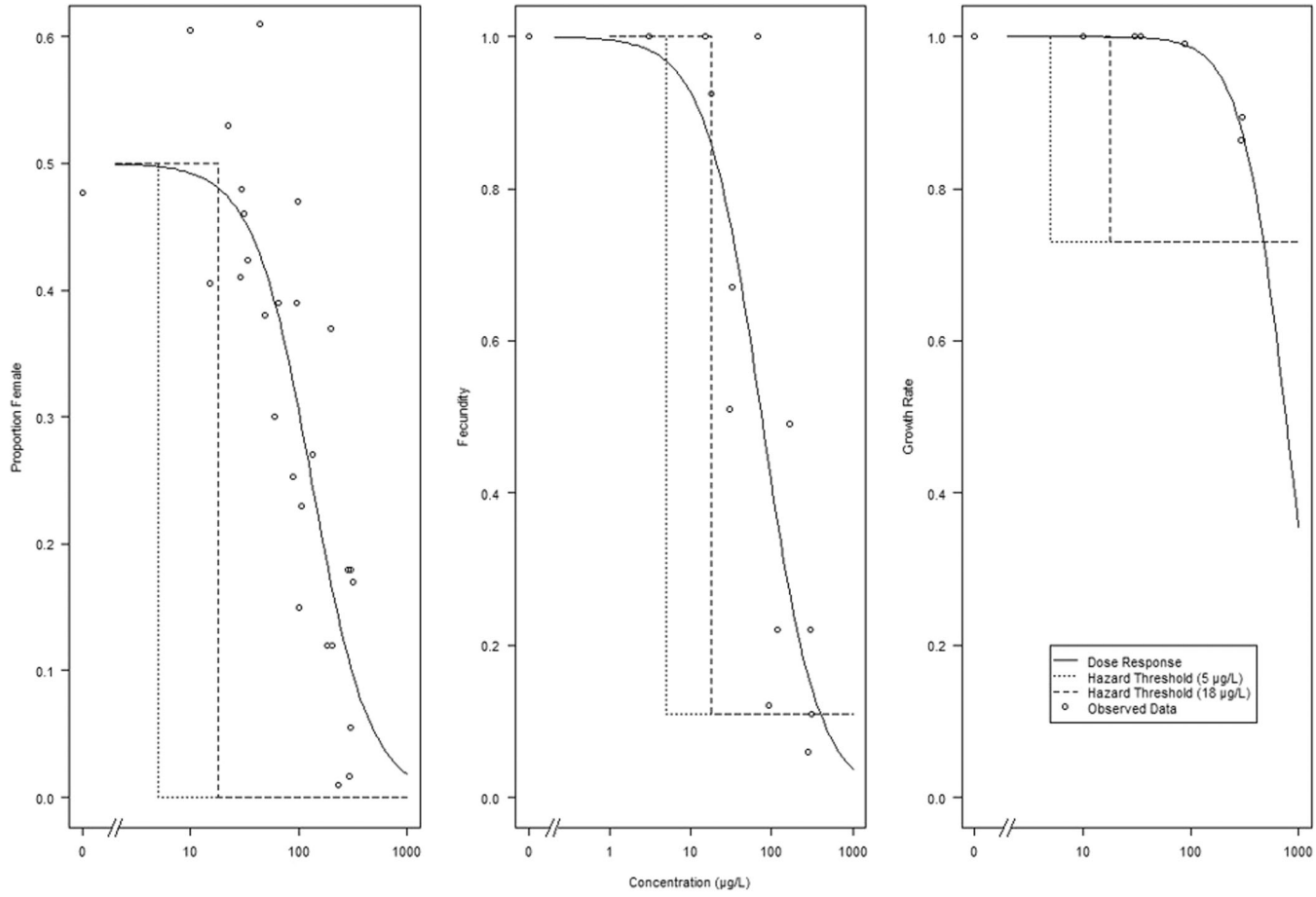

FIGURE 3: The four-parameter log-logistic function fitted to effects of prochloraz on sex ratio, fecundity, and growth in fish. The effects at each concentration associated with both effects threshold in the hazard-threshold simulations are also illustrated.

exposed to prochloraz because this is a critical life stage of sexual development (e.g., sexual differentiation has been observed in female salmonids from 3 weeks posthatch; Nakamura & Nagahama, 1993). In the HT simulations, whenever the chemical was present above the effects threshold, full masculinization of all fish undergoing sexual differentiation (i.e., embryos) was imposed. Meanwhile, in the DR modeling the proportion of males and females was dependent on the concentration at the time of sexual differentiation (i.e., embryos) and the DR relationship (Figure 3).

Growth. The effect of prochloraz on growth in the literature is highly variable, with some studies showing no effects on growth (see Hill & Caunter, 1991; Kinnberg et al., 2007), whereas others report significant growth effects (i.e., Thorpe et al., 2011). The extent to which the effects of prochloraz on growth are a result of endocrine mechanisms or systemic toxicity are unclear. Growth is primarily controlled by growth hormone, which is secreted by the pituitary gland, and via thyroid hormones; however, there have been very few studies that directly investigate this mode-of-action for prochloraz. One study on zebrafish (Liu et al., 2011) reported effects on the hypothalamic–pituitary–thyroid axes, whereas another (Le Gac et al., 2001) found that prochloraz disrupted cell membrane receptivity to peptide hormones like growth factors in trout. However, sex hormones such as testosterone and 17β-estradiol are also involved in somatic growth in fish (Matty, 1986), and prochloraz has been shown to suppress blood concentrations of these hormones (a Marca Pereira et al., 2011; Ankley et al., 2005; Thorpe et al., 2011). Because the mechanisms behind the reported effects on growth are largely unknown, it is difficult to apply the MTC approach for this endpoint. The only endpoint generally taken in fish early life-stage studies (from which growth effects were identified) that could be indicative of systemic toxicity is survival. Although this is not an endpoint generally affected by prochloraz, a 20% mortality was observed in one study with zebrafish exposed at 300 μg/L (OECD, 2006). This may indicate that the reduction in growth observed (by 27%) in fathead minnow exposed at a concentration of 294 μg/L by Thorpe et al. (2011) is potentially the result of systemic toxicity, rather than endocrine activity. In support of this conclusion, Wheeler et al. (2013) estimated that test concentrations for prochloraz in the Fish Short-Term Reproductive Assay of only between 48.5 and 220 μg/L were needed to match the MTC. Integrating all this available information, in conjunction with the calculated DR relationship (Figure 3), we concluded that effects on growth are most likely a result of systemic toxicity rather than endocrine activity. Nevertheless, effects of prochloraz on growth of all fish life stages were included in the population modeling as a conservative, worst-case situation. In the HT simulations, whenever the chemical was present in the population model above the effects threshold, the greatest effect reported within the empirical studies (−27% total length; Thorpe et al., 2011) was applied to all fish. Meanwhile, in the DR simulations the reduction in growth was dependent on the daily concentration present and the DR relationship (Figure 3).

The effects threshold concentration selected to accommodate the HT simulations was based on worst-case considerations. In short, the lowest no-observed-effect concentration (NOEC) from all available endpoints (apical and nonapical), associated with endocrine activity of prochloraz in fish, was selected for the simulations. A full set of HT simulations was performed using this conservative NOEC value of 5 μg/L (US Environmental Protection Agency, 2013) as the effects threshold concentration. Given this, modeling for each scenario with an MF of 1000 was also performed with a revised effect threshold concentration of 18 μg/L, which derives from effects based on apical endpoints (i.e., reduction in fecundity in medaka; OECD, 2006). The effects imposed in the model associated with different choices for the effects threshold are illustrated in Figure 3.

The model setup. The population model versions used in our study are available in the Supporting Information, in conjunction with a modeling log detailing all changes made to each model from their original form. In brief, these changes included preparation of a text file containing the relevant exposure profile for each scenario; preparing new selectors in the Graphical User Interface to allow the different exposure/effect options to be chosen (e.g., MF); parameterizing new global and fish parameters to implement the necessary endocrine effects (Table 1); and implementing these effects in the reproduction, development, and growth submodels. At the start of each timestep, the concentration of prochloraz and the magnitude of effect associated with that concentration are updated. These values and their effect on each endpoint are then used in the relevant submodels, which are called in the same order as the original model versions.

Simulation experiments. Initialization of each model was left unchanged from the original versions. To allow the populations to stabilize, a period of nonexposure was implemented (3 years in inSTREAM, 10 years in the stickleback model). Then the population was exposed to the chosen exposure profile for 10 years, followed by a 10-year recovery phase without exposure. Thus, in total each replicate run of inSTREAM consisted of 23 years of simulations and 30 years for the stickleback model. A negative control (without exposure) was performed, and the population abundance/biomass from these simulations was used as the "baseline" against which the results from the exposure simulations were compared. A scenario control (with toxicity included and an MF of 1) was performed with the R1 pond scenario only, to check first that when the exposure profile in the HT scenarios had no exceedance of the HT then there was no difference from the control, and second, to assess the variability in population outputs due to stochastic aspects within the model. A full outline of the simulation plan is provided in Table 2. Simulations were performed for either of the following: 1) HT, in which effects were imposed using the MTC approach and an effects threshold; or 2) DR, in which no threshold was set and effects were imposed using standard

TABLE 1: New model parameters and their values used in the prochloraz exposure simulations

Parameter	Description	Unit (µg/L)	Value at start of simulation (whole range)
Pond-concentration-list/reach-concentration	Daily prochloraz concentration		0 (0–unbounded)
Stickleback-SexRatio-Female/Trout-SexRatio-Female	Proportion of embryos developing as female	—	0.5 (0–1)
Stickleback-Fec-Effect/Trout-Fec-Effect	Variable to implement prochloraz effect on fecundity	—	1 (0–1)
Stickleback-Growth-Effect/Trout-Growth-Effect	Variable to implement prochloraz effect on growth	—	1 (0–1)
Toxicant-Hazard-Fec-Effect	Prochloraz effect on fecundity when exposure exceeds the effect threshold concentration in HT simulations	—	0.115
Toxicant-Hazard-SexRatio-Effect	Prochloraz effect on sex ratio when exposure exceeds the effect threshold concentration in HT simulations	—	0
Toxicant-Hazard-Growth-Effect	Prochloraz effect on growth when exposure exceeds the effect threshold concentration in HT simulations	—	0.73
Toxicant-Hazard-Threshold	Effect threshold concentration in HT simulations		5 or 18
Toxicant-DR-Fec-upper	Upper limit in 4-parameter log-logistic DR equation for effects on fecundity	—	1
Toxicant-DR-Fec-lower	Lower limit in 4-parameter log-logistic DR equation for effects on fecundity	—	0
Toxicant-DR-Fec-slope	Slope in 4-parameter log-logistic DR equation for effects on fecundity	—	1.25597
Toxicant-DR-Fec-ED50	Inflection point in 4-parameter log-logistic DR equation for effects on fecundity		75.03026
Toxicant-DR-SR-upper	Upper limit in 4-parameter log-logistic DR equation for effects on sex ratio	–	0.5
Toxicant-DR-SR-lower	Lower limit in 4-parameter log-logistic DR equation for effects on sex ratio	–	0
Toxicant-DR-SR-slope	Slope in 4-parameter log-logistic DR equation for effects on sex ratio	—	1.61599
Toxicant-DR-SR-ED50	Inflection point in 4-parameter log-logistic DR equation for effects on sex ratio		131.73372
Toxicant-DR-Growth-upper	Upper limit in 4-parameter log-logistic DR equation for effects on growth	–	1
Toxicant-DR-Growth-lower	Lower limit in 4-parameter log-logistic DR equation for effects on growth	–	0
Toxicant-DR-Growth-slope	Slope in 4-parameter log-logistic DR equation for effects on growth	—	2.1048
Toxicant-DR-Growth-ED50	Inflection point in 4-parameter log-logistic DR equation for effects on growth		753.6327

DR = dose–response; ED50 = median effective concentration; Fec = fecundity; HT = hazard threshold; SR = sex ratio.

DR relationships. Effects were implemented on growth and fecundity when the chemical was present and immediate recovery was assumed with no delayed effects. For sex ratio, if an embryo was exposed when hatching, the effect was implemented for the remainder of the organism's life.

Data analysis. Population-level effects were analyzed by comparing control with exposed model populations. Analyses were performed using continuous time series during exposure and recovery. Four population metrics were evaluated: 1) the mean and minimum abundance (number of individuals) during the exposure period; 2) the mean and minimum biomass (cumulative weight of the individuals) during the exposure period; 3) the time to recovery; and 4) the area under curve during exposure and recovery (biomass only). Model outputs represent mean values of 15 runs. Population-level effects were considered significant if they exceeded a 10% change from control, and the population was considered to have recovered once differences were less than 10%.

RESULTS

Control simulations

The negative control (no exposure) confirmed that a stable population was established after a 3-year simulation period for the trout model and a 10-year simulation period for the stickleback model. After these periods, there followed a 10-year oscillation in the population abundance and biomass around a consistent average, reflecting life-history characteristics and environmental variability. The total population abundance fluctuated between approximately 250 and 600 (average 360) and 40 and 2200 (average 503) for trout and stickleback, respectively. The total population biomass fluctuated between approximately 9–17 kg (average 11.5 kg) and 36–167 g (average 89 g) for trout and stickleback, respectively. The total population biomass was comprised predominantly of mature fish (e.g., >95% of the total population biomass in the trout population was made up of mature fish), whereas the total population abundance was composed predominantly of early

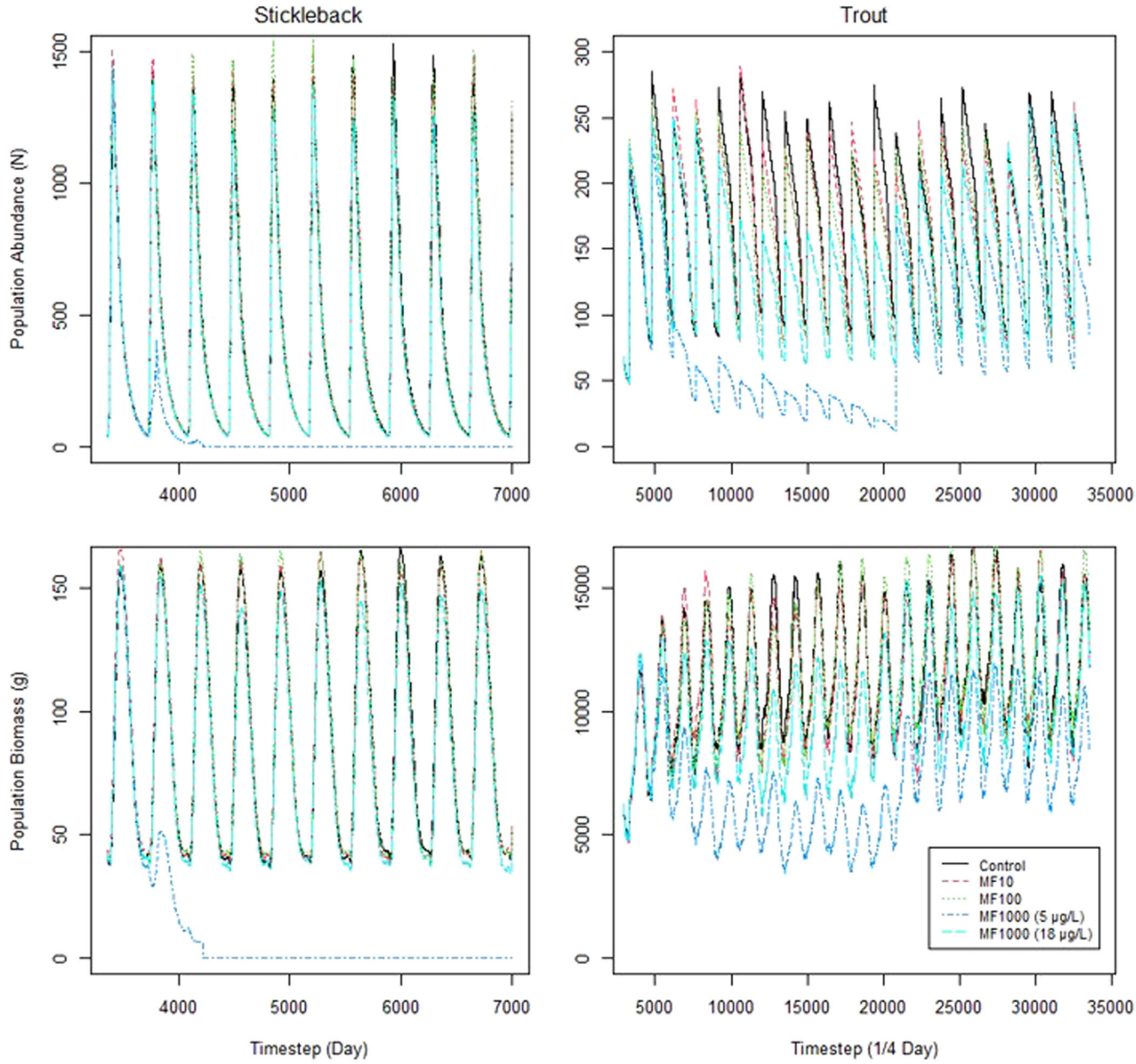

FIGURE 4: The abundance and biomass of the recruited population of stickleback and trout in the D2 ditch scenario with a range of multiplication factors (MF) applied to the exposure profile, (Note that exposure was commenced on timestep 3720 and 5472 in the stickleback and trout models, respectively).

life-stage fish (i.e., larval and juvenile fish). The R1 pond scenario control (exposure included with an MF of 1) resulted in small fluctuations in abundance and biomass compared with the negative control. In the HT simulations (in which no exposure exceeded the threshold, so no ED effects on individual fish were imposed), the greatest fluctuation of +4.6% (minimum total population biomass) and –3% (minimum total population abundance) for the stickleback and trout models, respectively, was observed compared with the negative control. This reflects the inherent variability in each model due to stochastic processes. Knowledge of the magnitude of this stochastic noise can further inform our conclusions derived from the results from all simulations, because irrespective of exposure, the results from one set of simulations to another may differ by up to 4.6% due to natural variability in model processes and not as a result of toxic effects. Meanwhile, similar fluctuations from the negative control were observed in the DR simulations, with +5% (minimum total population abundance) and –3.8% (minimum adult population abundance) in stickleback and trout, respectively. These figures reflect the inherent variability in the models, but also some small toxic effects (e.g., the daily concentrations of prochloraz were all <1 µg/L, which caused a change in sex ratio from 0.5 to 0.498 and in relative egg production from 1 to 0.99). As presented, the differences compared with the negative control were sometimes positive (greater abundance or biomass) or negative (lesser abundance or biomass).

TABLE 2: Summary of simulations performed with each model

Scenario	Effects scenario	Negative control–no exposure	Multiplication factor (applied to the FOCUS exposure profile)			
			1	10	100	1000
D2 ditch	No effects	X	—	—	—	—
	HT (NOEC = 5 µg/L)	—	—	X	X	X
	HT (NOEC = 18 µg/L)	—	—	—	—	X
	DR	—	—	X	X	X
R1 pond	HT (NOEC = 5 µg/L)	—	X[a]	—	—	X
	HT (NOEC = 18 µg/L)	—	—	—	—	X
	DR	—	X	—	—	X
R4 stream	HT (NOEC = 5 µg/L)	—	—	X	X	X
	HT (NOEC = 18 µg/L)	—	—	—	—	X
	DR	—	—	X	X	X

Each combination was performed with 15 runs.
HT = hazard threshold; NOEC = no-observed-effect concentration; DR = dose–response.
[a]The scenario control (R1 Pond, MF = 1) showed no significant difference (<10%) from the negative control, confirming no simulations were necessary when the selected exposure profile multiplied by the MF was below the HT (i.e., no simulations were performed for any other scenario with an MF of 1, nor were simulations performed with the R1 pond scenario with an MF of 10 or 100).

The maximum percentage changes in the fish population (considering abundance and biomass) in both models under different exposure regimes are presented in Table 3.

Population effects in HT versus DR scenarios

As expected, the greatest population effects were observed in the HT simulations, with population extinction (stickleback, R1 pond, MF = 1000) or no population recovery (trout, D2 ditch, MF = 1000, −86.9% minimum total population abundance) within 10 years after exposure at the highest simulated prochloraz concentrations. In total, 59% (13/22) of scenario–MF combinations simulated resulted in significant effects on at least one population-relevant endpoint in the HT simulations. Meanwhile, the greatest population effect in any of the DR simulations was only −24.9% (stickleback, R1 pond, MF = 1000, average total population abundance). Furthermore, only 12.5% (2/16) of scenario–MF combinations simulated resulted in significant effects on at least one population-relevant endpoint in the DR simulations.

Population effects in each exposure scenario

Applying an MF of 10 to the exposure profiles resulted in no population endpoint deviating from the negative control by more than 10% in any of the three scenarios for either the HT or the DR simulations.

Applying an MF of 100 to the exposure profiles resulted in population effects exceeding 10% in only one scenario: the HT D2 ditch simulations with trout. During exposure there was a reduction in the average total population abundance of −11.5% and a reduction in minimum total population abundance of −13.4%. No effects on the population were observed in the R1 pond and R4 stream scenarios when applying an MF of 100. The number of days on which prochloraz concentration exceeded the HT of 5 µg/L in each scenario (thus resulting in effects on individual fish) when using different MFs is presented in Table 4. The D2 ditch scenario only has one major peak (of 0.8 µg/L); however, it has many minor peaks of approximately 0.05–0.08 µg/L. These do not exceed the HT in the simulations with a low MF; however, once an MF of 100 is used, these minor peaks now exceed the threshold and the prochloraz

TABLE 3: Population response (% change from control) to different exposure scenarios in both trout and stickleback models

Scenario	Multiplication factor	Stickleback		Trout	
		Hazard threshold	Dose–response	Hazard threshold	Dose–response
R1 pond	1	4.6^{B}	5.0^{A}	−3.0^{A}	−3.8^{RA}
	1000 (NOEC = 5 µg/L)	**Extinction**	**−24.9^{A}**	**−80.3^{A}**	−5.9^{RA}
	1000 (NOEC = 18 µg/L)	**Extinction**	n/a	**−33.5^{RA}**	n/a
D2 ditch	10	4.4^{AB}	−2.2^{A}	−3.7^{A}	−6.1^{RB}
	100	5.2^{A,RA}	−1.7 ^{B,RB}	**−13.4^{A}**	5.6^{RB}
	1000 (NOEC = 5 µg/L)	**Extinction**	**−10.3^{A}**	**−86.9^{A}**	−9.2^{A}
	1000 (NOEC = 18 µg/L)	**−11.7^{A}**	n/a	**−30.3^{A}**	n/a
R4 stream	10	5.9^{B}	3.7^{A}	−3.4^{RA}	6.3^{RA}
	100	−3RA	9.8^{A}	−4.9^{A}	5.5^{RB}
	1000 (NOEC = 5 µg/L)	**−27.9^{B,RB}**	−4.0^{A,RA}	**−79.9^{A}**	5.8^{RB}
	1000 (NOEC = 18 µg/L)	**−22.4^{RB}**	n/a	**−35.5^{RA}**	n/a

Each combination was performed with 15 runs. Responses highlighted in bold indicate a deviation of more than 10% from the control simulation. Only the results of the most sensitive population endpoint in each case are presented, with full results in the Supporting Information.
n/a = simulations with a change in the hazard threshold to 18 µg/L are not relevant to dose–response because this parameter is only used in the hazard-threshold simulations; Symbol indicates population endpoint: A = population abundance; RA = recruited population abundance; B = population biomass; RB = Recruited population biomass; NOEC = no-observed-effect oncentration.

TABLE 4: Number of days that exceed the hazard threshold of 5 μg/L in each scenario when using different multiplication factors

	Multiplication factor			
Scenario	1	10	100	1000
D2 ditch	0	3	22	259
R1 pond	0	0	0	310
R4 stream	0	1	7	25

effects are imposed regularly throughout the year (22 days in total). This is far higher than in the other two scenarios, in which, although the R4 stream has more major peaks (4 over 0.5 μg/L), it has very few of these minor peaks so the total number of days in exceedance of the HT is fewer than the D2 ditch scenario when using an MF of 100 (7 days in total).

Applying an MF of 1000 results in large, widespread effects in both the trout and stickleback populations in all three FOCUS scenarios for the HT simulations. The fish populations were most sensitive to the exposure in the R1 pond and D2 ditch scenarios, with >80% effects on abundance and/or biomass in all cases. In both scenarios, the stickleback population went extinct, whereas in the D2 ditch scenario the trout population did not recover to pre-exposure levels after 10 years of recovery (no exposure) (Figure 4). Population effects were noticeably lower in the R4 scenario, especially in the stickleback model, in which a maximum effect of −27.9% on biomass was observed and recovery was within 2 years.

Finally, a revised set of modeling was performed for the HT assessment using a higher threshold before effects are imposed on the fish in the model (based on apical endpoints). Specifically, the threshold level before effects are imposed was increased to 18 μg/L (from the worst-case 5 μg/L in the original set of simulations). In all cases, significant effects on the modeled population were still observed, although the magnitudes of these effects were equal to or lower than those using the lower threshold. This was more apparent for the trout, for which maximum effects were approximately –30% compared with –80% (minimum total population abundance), whereas for the stickleback the change in threshold made little difference to the population effects (i.e., the population still went extinct in the R1 pond and D2 ditch scenarios). Although the outcome of this specific assessment is the same irrespective of the choice of threshold level, what is not clear is whether this threshold based on apical or nonapical endpoint NOECs could make a difference in assessments for other chemicals with different magnitudes of effect and exposure profiles. For example, a lower threshold associated with a nonapical endpoint NOEC or an exposure profile with higher concentrations will lead to greater exceedance of the threshold and potentially result in a greater population response and vice versa.

Comparison of effects in stickleback and trout

Generally, whether effects on the fish population were considered significant (>10% change compared with the negative control) or not for each scenario did not differ between species. Only 3/19 scenario–MF combinations modeled resulted in a different conclusion for the assessment for each species (i.e., DR, R1 pond, MF = 1000 significant effects in stickleback, but not trout; HT, D2 ditch, MF = 100 significant effects in trout, but not stickleback; DR, D2 ditch, MF = 1000 significant effects in stickleback, but not trout). Of these, two were marginal, with the significant effects only just exceeding the threshold of 10% (maximum of −13.4%). Thus the only scenario–MF combination that resulted in clear differences between the population responses of the two species was the DR, R1 pond, MF = 1000 scenario, in which effects on stickleback abundance were up to −24.9%.

However, with an MF = 1000, the maximum magnitude of population effects did differ, with the stickleback population going extinct in two scenarios, whereas the trout population remained extant, althought recovery to pre-exposure levels was not recorded over the 10 years following the end of the prochloraz exposure. The modifications to the NOEC for the HT assessment also resulted in small differences between the species, with the increase in the threshold ubiquitously resulting in a decrease in population effect in the trout, whereas in the stickleback the change in threshold resulted in variable changes in population response, ranging from no change (extinct in R1 pond) to a marginally significant population effect (11.7% in D2 ditch). Thus population response was a result of interacting mechanisms among exposure profile, HT and species life history rather than simply exposure alone.

DISCUSSION

The objective of our study was to explore methodologies to conduct population modeling for EACs and evaluate how different assumptions in model setup may influence the outcome of an assessment of EACs. The study was carried out with an applied aim for supporting the use of population modeling approaches in a regulatory context, but also to stimulate a wider discussion about the scientific basis for how to conduct population modeling for studies on EACs and their effects on fish populations. Our study further included considerations on how the ecology and life-history differences between species may affect their population responses to exposure, which affects our ability to read across between species when assessing for population-level effects of EACs.

Modeling challenges

The two population models we used were not originally designed for use in the assessment of EACs. The benefits to using previously developed models, include their community acceptance, established validation, and reduced costs of development. However, a critical element of model development is defining the purpose of the model, which then informs all aspects of model design (Grimm & Railsback, 2005; Raimondo et al., 2020). Without development of a specific model for the intended use, therefore, an understanding and acceptance of any limitations in model structure is required. For example, the

literature on prochloraz indicated that effects on growth were only observed on juvenile fish (this is not uncommon because although adult fish continue to grow throughout their life span, growth slows down considerably with increasing age; von Bertalanffy, 1957). However, due to the model structure (the inSTREAM trout model uses the same growth processes for juvenile and mature trout), growth effects were imposed on all life stages, including adults. Because we are presenting an illustrative case study in which the effect on growth is relatively minor and potentially not actually due to endocrine activity, this implementation was considered appropriate (i.e., worst case). However, in future modeling for regulatory submission, appropriate model choice and how the code of those models may allow the consistent implementation of effects should be evaluated.

Generally, it was necessary to ensure that any model meets the requirements of the specific scientific question, which was determined using the EFSA (2014) checklist. Although this approach allowed for the evaluation of the underlying ecological models, implementation of prochloraz effects was newly coded in our study. Thus it was necessary to select models capable of accommodating these changes. Many of the fish IBMs considered for use in our study implement changes in individuals through the acquisition and use of energy (i.e., energy budget), thereby using a mechanistic approach consistent with the AOP concept (Ankley et al., 2010) and providing further opportunity for model validation (i.e., pattern-oriented modeling; Grimm & Railsback, 2012; Grimm et al., 1996). This model structure also affects how chemical effects are implemented in these models. Energy-budget models would allow prochloraz effects to be implemented on energy-allocation decisions based on a known AOP (see Mintram et al., 2020).

Alternatively, a simplifying assumption can be made and effects are instead implemented directly on an apical endpoint, and the energy associated with the change is simply lost rather than allocated elsewhere (see Hazlerigg et al., 2014). Although the implementation of effects on energy resource decisions may provide more mechanistic realism to the population response to exposure and link more tightly to the AOP framework, there are significant challenges with this approach (Murphy et al., 2018) that are often exacerbated by a lack of knowledge regarding the AOPs relevant to a given molecule (Lagadic et al., 2020). These unresolved limitations at the present time lend support to a more generalized approach (i.e., effects on apical endpoints as used in our study), although developments in the fundamentals of imposing toxicity effects in energy-budget population models should be explored further.

In this study, we only considered fish models. Toxicity data on rodents are more commonly used in a human health EAC assessment in which the focus is on an individual rather than the population. However, suitable mammalian population models are available for assessing the population relevance of endocrine effects (see Wang, 2012), and an analysis similar to ours would make an interesting comparison against the outcomes observed for fish. Although laboratory testing of EACs on amphibians has been established, the development of amphibian population models is still in its infancy (e.g., the simple matrix models in Awkerman & Raimondo, 2018). This lack of validated amphibian population models precludes their use for the assessment of population responses to EACs at the present time. However, this should change in the future as appropriate models become available (see Awkerman et al., 2019 for guidance on developing amphibian population models for chemical risk assessment).

Regulatory application

Our study presents a number of different ways that population modeling could be performed and analyses conducted in a regulatory context. We have endeavored to avoid prescribing one parameterization over another, however, one possibility was not considered appropriate and was not explored further. An effect threshold of 0 µg/L for the HT simulations was not considered in the modeling presented. The EC Regulation 1107/2009 specifically states that if exposure to an EDC is negligible, then that would not prevent the substance from being approved for use. There is no reference to this point in the ECHA/EFSA (2018) guidance and thus no definition of "negligible exposure." Nevertheless, it is clearly more than 0 µg/L. Therefore, although the guidance states the process is a hazard-based assessment, we interpreted this to mean exposure could be more than 0 µg/L. Scientifically, this makes sense and is consistent with the conclusion that EACs do show a DR effect. The community is still divided on whether these DRs are monotonic or can show nonmonotonic tendencies (see Beausoleil et al., 2013; ECHA/EFSA, 2018), but either way, the concentration must be greater than 0 µg/L to elicit an effect. Therefore, we do not consider an effect threshold of 0 µg/L appropriate, but have provided alternative options for defining what it might be based on; empirical evidence of effects on nonapical or apical endpoints. However, it is widely accepted that population-relevant effects and hence the choice of endpoint, should impact survival, reproduction, growth, and development (see Hutchinson et al., 2006; Weltje, Wheeler, et al., 2013; Wheeler et al., 2020).

Population models are commonly seen as a higher tier option for addressing the risk to nontarget organisms (e.g., Tier 4 in EFSA [2013] guidance on edge-of-field surface waters). Higher tier methods commonly are more complex and require more investment, but they are also expected to provide more realism in the actual risk to a nontarget organism. This greater understanding of the risk is reflected in the arbitrary assessment factors (AFs) applied at all tiers, because lower AFs are applied at the higher tiers (e.g., an AF of 10 is used with a NOEC from a standard chronic *Daphnia* test, but this is reduced to 2–5 when higher tier mesocosm data are considered). Importantly, the conservativeness of the population modeling options we provide vary. This may provide an opportunity for applying the same paradigm of a tiered scheme for population modeling in the assessment of potential EACs. For example, a screening level assessment might require population modeling with the worst-case combination of HT assessment, highest magnitude of effect observed in an empirical study (effects

associated with the MTC), lowest threshold for effect observed in an empirical study (potentially even including nonapical endpoints), and worst-case FOCUS exposure profile (FOCUS Step 3 without refinements or mitigations) for only one fish species (sufficient because both models tested responded similarly in the worst-case scenarios). Options to refine any of these inputs may then be made as a higher tier refinement and may necessitate modeling more than one species.

We emphasize that there are a number of sources of uncertainty in the model parameterization provided in the prochloraz case study. Assessing these are critical for the use of population models in a regulatory application. When effects data are considered, a fundamental aim is to replace, reduce, or refine testing on vertebrates (European Commission, 2010; EFSA, 2011a). Although regulatory requirements will still result in generation of new data (e.g., a fish EAC study) read-across and nontesting methods may be necessary for data-poor substances. However, the use of effects data that are not substance-specific increases uncertainty in the analysis. The use of the MTC to define the magnitude of effects for use in a HT assessment is not without debate. In reality, there is no clear line where effects caused by endocrine activity stop and systemic toxicity starts, and it can also vary between studies, depending on the exposure duration, test species, and chemicals used (Matthiessen et al., 2017). This makes it challenging to determine the magnitude of effects to implement in an HT assessment. It can also be challenging to identify which FOCUS scenario may be the worst case for population-level effects without performing the modeling (e.g., the interaction of the MF and the exposure profile in relation to the HT resulted in different results between the D2 ditch and R4 stream scenarios with an MF of 100). However, it is impractical to perform population modeling with all FOCUS exposure profiles associated with the proposed use of a substance.

In conjunction with the uncertainty of the assessment, our case study also illustrates the conservativeness of the assessment depending on different decisions. The HT assessment results in far greater population level impacts (Table 3), although this is based on a magnitude of effects on individual fish far greater than any effects observed in empirical studies (Figure 3). The margin-of-safety approach provides a mechanism for the uncertainty and conservativeness of the assessment to be accounted for in any regulatory evaluation, although we do not make any recommendations on which MFs should be used to demonstrate an "acceptable use" and leave this to the appropriate risk managers.

Species differences

The differences in population responses to exposure in the stickleback and trout models were not as different as we perhaps imagined they might have been. In the worst-case exposures (HT with high MF), significant effects on the population were observed for both species. This suggests that very high effects of chemical exposure on individual fish may saturate any compensatory processes that regulate populations under stress irrespective of differences between species. Extreme stressors commonly overwhelm species differences, as reflected in the almost ubiquitous decline of fisheries following overfishing (Pauly, 2008). This provides an indication of the severity (and conservativeness) of the exposure implemented in these scenarios. However, a species difference observed was that whilst the stickleback population became extinct under extreme exposure scenarios, the trout remained existent. Analysis of the age-distribution in the trout population showed only a few mature fish were surviving during high levels of exposure, but those that did survive were growing into old age (8–9 years). The effects of prochloraz were effectively suppressing intraspecific competition enabling the remaining trout to optimize their fitness with access to good foraging areas with low mortality risk (i.e., reduced predation). These compensatory mechanisms are common in fish populations to a greater or lesser extent (e.g., Rose et al., 2002) making some populations more resilient to stressors. However, although a few individuals may maintain a population in terms of abundance/biomass, issues with reduced genetic diversity may still lead to extinction (Bickham et al., 2000) and/or be an issue of potential concern for adaptation to future climates (Hamilton et al., 2016, 2017). The stickleback life history involves a shorter life span, and this led to extinction during the simulation. The surviving old trout provided an opportunity for population recovery. How recovery to EDCs is implemented may vary, with effects on fish sex generally associated with a specific life stage and the sex commonly irreversible (see Morthorst et al., 2010), whereas effects on fecundity or growth may be reversible to a greater or lesser extent due to the removal of endocrine activity at the binding site (see Nash et al., 2004) and seasonal resorption and growth of reproductive tissue in fish from temperate regions. This has implications for future empirical studies, with the inclusion of a recovery period at the end of exposure allowing the opportunity to explore the potential for recovery of different endpoints and thereby use this information in population modeling, which in turn may alter the resilience of a population to the effects of EDCs. Recovery may not be acceptable in the current regulatory assessment, because effects must be negligible. However, should the regulatory procedure for EACs align with a more standard risk assessment in the future, then recovery within a certain time period may be accepted (e.g., recolonization/recovery of nontarget arthropods to a field within 1 year of pesticide application is acceptable under EC 1107/2009, ESCORT II).

CONCLUSIONS

We demonstrate the possible use of IBMs to assess the population relevance of EAC effects on individual fish. We have identified a number of critical study design decisions that have significant impacts on the results of any assessment. We anticipate that population models will become a standard regulatory method/toolbox for the assessment of EACs, although we acknowledge the need for a consensus to emerge as to the appropriate nature of this approach. We envisage that future work will further establish this consensus as well as extending

the scope of population models to include other groups of organisms and other EAC effect mechanisms.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5640.

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Disclaimer—Pernille Thorbek and Lennart Weltje are employed by BASF, and the substance used for our case study, that is, prochloraz, is a product of BASF.

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Data Availability Statement—The stickleback and trout population model code and a log of changes from the original versions are available in the Supporting Information. The FOCUS output files from Toxic Substances in Surface Waters (TOXSWA) are also available, with details of how these were used in the population models.

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