



## Comparing ecotoxicological effect concentrations of chemicals established in multi-species vs. single-species toxicity test systems

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### ABSTRACT

Most ecological effect assessment methodologies use effect concentrations derived from single-species testing ( $EC_{x,\text{single-species-test}}$ ) as the basis to estimate 'safe' environmental concentrations (such as environmental quality criteria). Here, we examined to what extent such  $EC_{x,\text{single-species-test}}$  are representative for population-level effect concentrations in a community setting ( $EC_{x,\text{multi-species-test}}$ ). Data from USEPA's ECOTOX database revealed the existence of considerable scatter around the relationship between  $EC_{x,\text{single-species-test}}$  (endpoint: mortality) and  $EC_{x,\text{multi-species-test}}$  (endpoint: population abundance). However, we demonstrate that this scatter is reduced when  $EC_{x,\text{single-species-test}}$  and  $EC_{x,\text{multi-species-test}}$  are determined simultaneously and by the same research group. Indeed, if these conditions are fulfilled, the quotient of both  $EC_x$  values for invertebrates approaches 1 for chemicals that directly target invertebrates. Unfortunately, comparable data for other classes of chemicals and/or taxonomic groups were not found. However, theoretical ecosystem model simulations, which confirmed the results based on the above-mentioned analysis of the ECOTOX database, indicated that for phytoplankton,  $EC_{10,\text{single-species-test}} > EC_{10,\text{multi-species-test}}$ , for chemicals that directly target invertebrates. For chemicals that directly target phytoplankton, the ecosystem model simulations suggest that  $EC_{x,\text{single-species-test}} > EC_{x,\text{multi-species-test}}$  for both phytoplankton and invertebrates. Hence, our observation based on the analysis of existing experimental data that the  $EC_{x,\text{single-species-test}}$  is similar to the  $EC_{x,\text{multi-species-test}}$  may be biased by the fact that only data were available for invertebrates and for chemicals targeting invertebrates. Experimental research is required to test the predictions made by the model simulations for phytoplankton as well as for chemicals directly targeting phytoplankton.

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### 1. Introduction

Environmental programs such as Registration, Evaluation and Authorization of Chemicals, REACH (European Commission, 2004), the Canadian Environmental Protection Act, CEPA and the Domestic Substances List, DSL (Environment Canada, 2003), and the USEPA high production chemicals assessments (Walker et al., 2004) encompass the risk assessment of large numbers of chemicals (more than 100,000). Assessing the risk that a chemical poses includes assessing the ecological effects of this chemical on aquatic ecosystems. One way to investigate ecological effects of a chemical is to expose an artificial ecosystem to different concentrations of the chemical while monitoring one or more biological parameters. Such multi-species toxicity tests, often

termed microcosm or mesocosm experiments, have features that promote their use as a realistic way of assessing chemical-caused stress (Boxall et al., 2002). The presence of multiple species as well as time-variable exposure scenario's and physicochemical water characteristics, all representative of field conditions, are examples of such features. Unfortunately, the amount of time and resources required to conduct such experiments impedes their routine use (Newman and Unger, 2003). Therefore, ecological effect assessments of chemicals often have to rely on ecotoxicity tests with single species, despite their lower degree of realism (Sanderson et al., 2004; Schindler, 1998). Usually, in most ecological effect assessment techniques, a set of single-species toxicity test results ( $EC_{x,\text{single species}}$ ) is then extrapolated to the community level to estimate a 'safe' environmental concentration of the chemical for an ecosystem or the potentially affected fraction of species at a given concentration of the chemical (Aldenberg and Slob, 1993; Vanstralen and Denneman, 1989; Wagner and Lokke, 1991). Obviously, the accuracy of the assessment with respect to effects occurring in the field will

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increase if the  $EC_{x,\text{single-species-test}}$  values used as the basis, of such extrapolations are similar to effect concentrations observed in a multi-species field situation ( $EC_{x,\text{multi-species}}$ ), i.e. if  $EC_{x,\text{single-species-test}} = EC_{x,\text{multi-species}}$ . There are, however, a number of biological and physicochemical reasons as to why this may not be true.

In a single-species toxicity test, the direct effect of a chemical on individuals of a single species is usually evaluated under controlled laboratory conditions. The resulting effect concentration, i.e. an  $EC_{x,\text{single-species-test}}$ , reflects the susceptibility of the monitored individuals to direct chemical effects. In multi-species toxicity tests such as microcosm and mesocosm experiments, the net result of direct and indirect effects is evaluated on a population level. The term ‘indirect effect’ is used here as in Fleeger et al. (2003). Examples of indirect effects include, but are not limited to, (i) an increased abundance of prey resulting from a decreased abundance of a predator due to a direct effect of a chemical on this predator, and (ii) a decreased abundance of a predator resulting from a decreased abundance of prey caused by a direct toxic effect on this prey. These examples clearly show that indirect effects may be both (numerically) positive (increased abundance) as well as negative (decreased abundance). Apart from the realm of predator–prey relationships in multi-species experiments, also differences in physicochemical conditions of the exposure media may cause  $EC_{x,\text{single-species-test}}$  to be different from  $EC_{x,\text{multi-species-test}}$ . For example, physicochemical properties like the dissolved organic carbon concentration (De Schampelaere and Janssen, 2004) and temperature (Heugens et al., 2001) have been shown to considerably alter the magnitude of direct effects of chemicals.

With the present study, we wished to investigate, using a database-analysis approach, the implicit assumption of many ecological effects assessments that  $EC_{x,\text{multi-species-test}} = EC_{x,\text{single-species-test}}$ . We did this by searching the USEPA database ECOTOX for population-level  $EC_{x,\text{multi-species-test}}$  values and corresponding  $EC_{x,\text{single-species-test}}$  values for the same chemical and species.

## 2. Material and methods

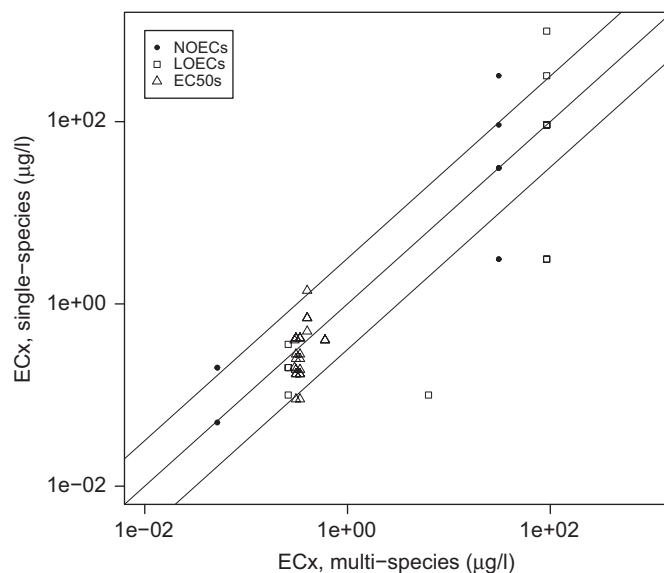
The search for  $EC_{x,\text{multi-species-test}}$  values in the aquatic part of the USEPA database ECOTOX (<http://cfpub.epa.gov/ecotox/>) was constrained by selecting concentration-based endpoints describing effects on a population level in freshwater (semi-)field tests. All data between 1915 and 2007 were considered. Both animal and plant data were collected. Population abundance and biomass constituted up to 88% of all reported endpoints within the population group.

Corresponding  $EC_{x,\text{single-species-test}}$  values were sought by constraining our ECOTOX search to concentration-based endpoints describing effects in freshwater single-species tests. CAS numbers of the chemicals for which  $EC_{x,\text{multi-species-test}}$  values were found were given as chemical entry to ensure correspondence. Again, all data between 1915 and 2007 were considered. Both animal and plant data were collected. Studies on benthic communities were omitted. The majority of the single-species toxicity data (85%) described the effects on survival of invertebrates.

Results of both described search operations were merged and a filtering operation was performed to ensure that species scientific names and the effect-magnitude ( $x$  in  $EC_x$ ) were identical for  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$ . In cases where a NOEC or a LOEC instead of an  $EC_x$  was reported, the effect-magnitude can be considered, literally, “no observable effect” or “lowest observable effect”, respectively, thus ensuring that NOEC and LOEC values were also useful for our analysis. In this way, we obtained a dataset where every record consisted of (1) a species’ scientific name, (2) its  $EC_{x,\text{single-species-test}}$  and (3) its  $EC_{x,\text{multi-species-test}}$ . All operations were performed using the free software R (<http://www.r-project.org/>).

## 3. Results

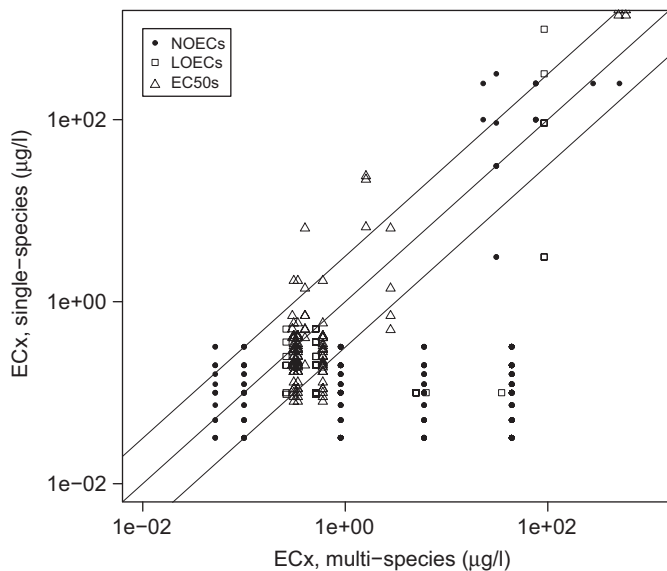
The dataset we obtained consisted of 49 records of which seven were insects and spiders and 42 were crustaceans (25 represented *Daphnia pulex*). Most  $EC_{x,\text{multi-species-test}}$  were within a factor two of the corresponding  $EC_{x,\text{single-species-test}}$  (Fig. 1). One



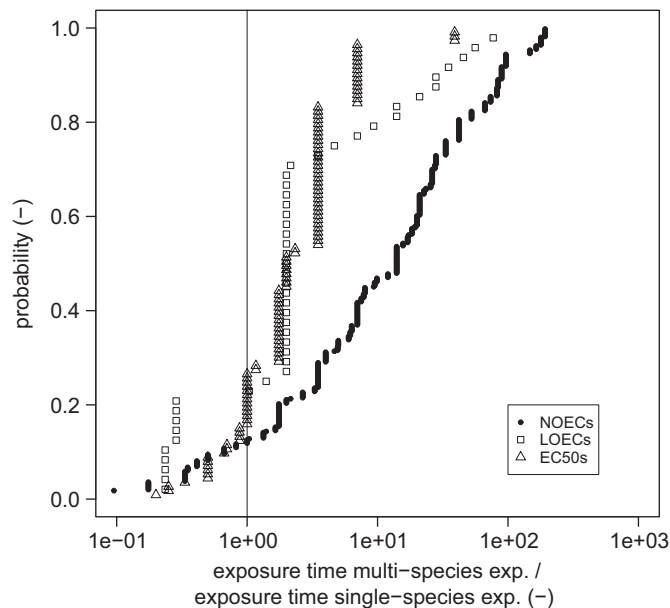
**Fig. 1.** A comparison between  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  using data from the aquatic part of the USEPA database ECOTOX. Only data were included for which species scientific name and effect magnitude were identical for both  $EC_x$  values. Different symbols denote different types of effect concentrations, as given by the legend.

NOEC and two LOECs were clearly located below the line of twofold error (up to two orders of magnitude difference), indicating that  $N/LOEC_{\text{multi-species-test}} > N/LOEC_{\text{single-species-test}}$ . Similarly, one NOEC and one LOEC are clearly located above the factor two-line and hence indicate  $N/LOEC_{\text{multi-species-test}} < N/LOEC_{\text{single-species-test}}$ . However, note that for the dataset presented in Fig. 1, confidence intervals were not available which makes it difficult to be decisive on whether or not  $EC_{x,\text{multi-species-test}}$  significantly differed from  $EC_{x,\text{single-species-test}}$ .

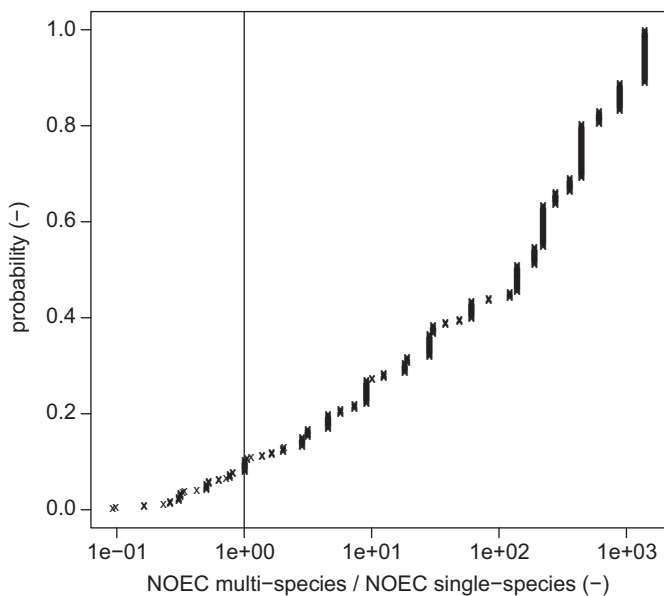
The dataset of 49 records only contained  $EC_x$  data for 2 chemicals (“1H-benzimidazol-2-yl carbamic acid, methyl ester”, and “phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester”), which is too limited to meaningfully address the questions posed here. The most limiting constraint posed on the ECOTOX search results appeared to be the requirement that reported species scientific name for  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  should be identical. Following closer inspection of the search results, we found that scientific names in ECOTOX were not always specified at the species level. This resulted in, for example, *Daphnia* sp. to be judged as taxonomically different from *Daphnia magna* by the filtering operations. Hence, the limited output of the filtering operations was primarily caused by different levels of the reported taxonomical resolution. To circumvent this problem, we redefined the filtering constraint on species equality for  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$ . Instead of using the attribute ‘species scientific name’, we used the attribute ‘species common name’ to decide whether the same species were used for  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  derivation. This yielded 610 extra records and 3 more chemicals for which  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  could be compared: “(1 alpha, 2 alpha, 3 beta, 4 alpha, 5 alpha, 6 beta)-1, 2, 3, 4, 5, 6-hexachlorocyclohexane”, “2, 3, 4, 6-tetrachlorophenol”, and “4-nonylphenol”. The majority of the examined species were crustaceans (83%), other species were insects and amphipods. Differences between  $EC_{50,\text{multi-species-test}}$  and  $EC_{50,\text{single-species-test}}$  were in most cases limited to a factor 2 (Fig. 2), while differences between  $N/LOEC_{\text{multi-species-test}}$  and  $N/LOEC_{\text{single-species-test}}$  show more deviation from the 1:1 line. We found that  $N/LOEC_{\text{multi-species}}$  was higher than  $N/LOEC_{\text{single-species}}$  in 90% of the cases (Fig. 3).



**Fig. 2.** A comparison between  $EC_{x, \text{multi-species-test}}$  and  $EC_{x, \text{single-species-test}}$  using data from the aquatic part of the USEPA database ECOTOX. Only data were included for which species common name and effect magnitude were identical for both  $EC_x$  values. Different symbols denote different types of effect concentrations, as given by the legend.



**Fig. 4.** Cumulative probability distribution of the quotient of duration of multi-species tests/duration of single-species tests. Different symbols denote different types of effect concentrations, as given by the legend. Data originate from the aquatic part of the USEPA database ECOTOX and have been filtered using the criteria given in the caption of Fig. 2 and in the material and method section. The vertical line is at the quotient equal to 1.



**Fig. 3.** Cumulative probability distribution of the quotient  $EC_{x, \text{multi-species-test}} / EC_{x, \text{single-species-test}}$  with 'NOEC' as effect magnitude. Data originate from the aquatic part of the USEPA database ECOTOX and have been filtered using the criteria given in the caption of Fig. 2 and in the material and method section. The vertical line is at quotient equal to 1.

Apparently, the (threshold) concentration resulting in (no) effects in a multi-species experiment is usually higher than in a single-species experiment. This finding is somehow counter-intuitive as exposure duration in the considered multi-species experiments is higher than in the single species, most notably for NOECs (Fig. 4).

#### 4. Discussion

A comparison between  $EC_{x, \text{multi-species-test}}$  and  $EC_{x, \text{single-species-test}}$  using publicly available datasets may increase our insight into

the influence that a species' biological and physicochemical environment has on the (adverse) effects it experiences when exposed to a chemical. Unfortunately, the relationship between  $EC_{x, \text{multi-species-test}}$  and  $EC_{x, \text{single-species-test}}$  exhibited a considerable amount of scatter (Fig. 2). Here, below, we will address the factors that may have lead to this scatter.

The magnitude of the direct effect of a chemical on a species has been shown not to be a constant, but variable depending on, among other things, the variability of a test population's fitness over time (Degraeve et al., 1991) or between genotype variation of sensitivity (Baird et al., 1991; Barata et al., 2002). As publicly available datasets include results from tests conducted by different research groups, which have possibly worked with populations or clones with different sensitivity or by the same research group, but where single-species and multi-species tests have not been conducted simultaneously, the direct effect reported to be experienced by a given species may differ between the single-species and multi-species study result. Hence, it is unsure if  $EC_{x, \text{multi-species-test}}$  and  $EC_{x, \text{single-species-test}}$  in our previous analysis differ, because direct effects are different between single-species and multi-species tests, or because of the presence of indirect effects in the multi-species test. As we wanted to investigate reliably the indirect effects of a chemical on a species by comparing  $EC_{x, \text{multi-species-test}}$  with  $EC_{x, \text{single-species-test}}$ , the data should come from experiments where the species experience the same magnitude of direct effects in both experimental settings.

Therefore, in an additional analysis, we used the ISI Web of Science [TS = (mesocosm\*OR microcosm\*OR enclosure\*OR semi-field OR semi field) AND (ecosystem\*OR communit\*) AND tox\*NOT soil AND effect\*] to analyse primary studies in which single-species toxicity tests were performed simultaneously with multi-species experiments by a single research group. We could only identify 5 such studies, i.e. Fairchild et al. (1992), Hose et al. (2003), Schroer et al. (2004), van der Hoeven and Gerritsen (1997), and van Wijngaarden et al. (1996), yet comprising 32 data points. In only one study a fish species was investigated, while all other studies investigated invertebrates. The four chemicals involved in

these studies, i.e. esfenvalerate, chlorpyrifos (2 studies), lambda-cyhalothrin, and endosulfan primarily target invertebrates.

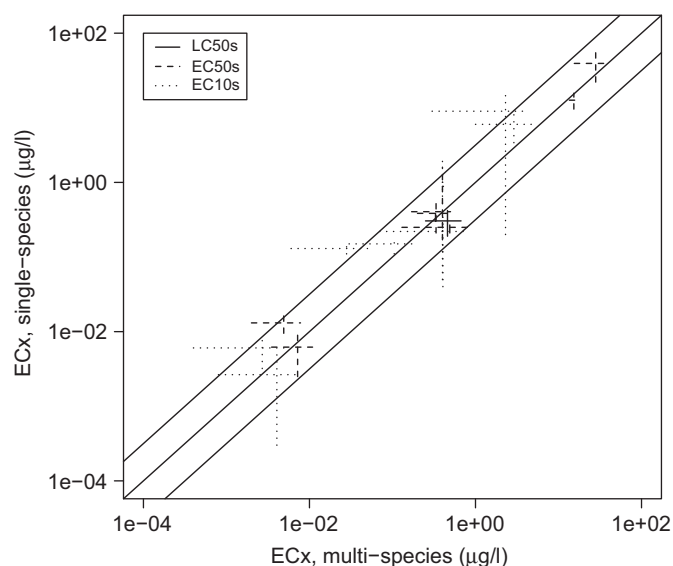
The data were clearly less scattered than what was found from the full ECOTOX database analysis (Figs. 1 and 2). This is illustrated by the high correlation coefficient between median  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  (0.94), compared to the correlation coefficient when using the ECOTOX data (0.44). This suggests that temporal and genotypic (or between population) variability of sensitivity can have an influence on how the relationship between  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  is interpreted.

Confidence intervals of all data indicate less than a factor two deviation from the 1:1 line, suggesting that  $EC_{x,\text{multi-species-test}} \approx EC_{x,\text{single-species-test}}$  (Fig. 5). In 3 out of the 5 studies found in the open literature data, single-species testing was performed in cages placed within the multi-species enclosure (Hose et al., 2003; Schroer et al., 2004; van Wijngaarden et al., 1996). The use of cages prevents contact with the other species in the experimental enclosures while the same physicochemical environment in multi- and single-species tests is guaranteed. Hence, the absence of marked differences between  $EC_{x,\text{single-species-test}}$  and  $EC_{x,\text{multi-species-test}}$  in the 5 reviewed studies suggests a limited influence of the biological environment, i.e. predator–prey interactions, on the response of the organisms to a chemical in a multi-species setting.

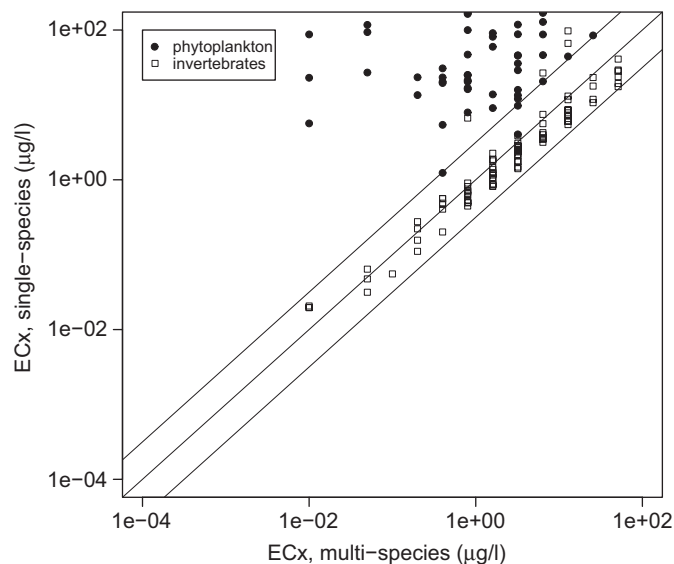
One should, however, bear in mind the limited number of chemicals for which this exercise was made. We found data for only 5 and 4 chemicals with the ECOTOX and the posterior ISI web of science approach, respectively. To examine if the results obtained can be generalised across chemicals, we performed a theoretical exercise with an ecosystem model methodologically identical to a previous study (De Laender et al., 2008). In that study, we randomly assigned mortality- $EC_{x,\text{single-species-test}}$  values to species from a virtual multi-species experiment consisting of fish, zooplankton, and phytoplankton. With a validated ecosystem model these  $EC_{x,\text{single-species-test}}$  values were transformed into abundance- $EC_{x,\text{multi-species-test}}$  values. Details on the methodology are described in De Laender et al. (2008). In the present paper, we choose  $x = 10$  which allowed us to examine if

$EC_{10,\text{single-species-test}} = EC_{10,\text{multi-species-test}}$ . The advantage of such an approach is that the  $EC_{10,\text{single-species-test}}$  values of the species in the virtual multi-species experiment are *a priori* known as they are parameters of the ecosystem model. Hence, we were sure that species in the (virtual) multi-species test experienced the same direct effects as in the (virtual) single-species test. Hence, a deviation of  $EC_{10,\text{multi-species-test}}$  from  $EC_{10,\text{single-species-test}}$  indicated the occurrence of indirect effects. Additionally, the use of a modelling approach allows to check if  $EC_{10,\text{single-species-test}} = EC_{10,\text{multi-species-test}}$  for many different chemicals. In line with the finding from our literature search (Fig. 5), the ecosystem model simulations suggested that  $EC_{10,\text{multi-species-test}}$  and  $EC_{10,\text{single-species-test}}$  for invertebrates did not differ more than a factor 2 (Fig. 6) and did not exhibit a lot of scatter. Note that in Fig. 6, only simulations for chemicals that primarily target invertebrates have been compiled, i.e. those chemicals for which  $\log(EC_{10,\text{single-species-test, invertebrates}}) - \log(EC_{10,\text{single-species-test, phytoplankton}}) \leq -1$ . These chemicals are representative for those used in our database-based analysis described in ‘Results’.

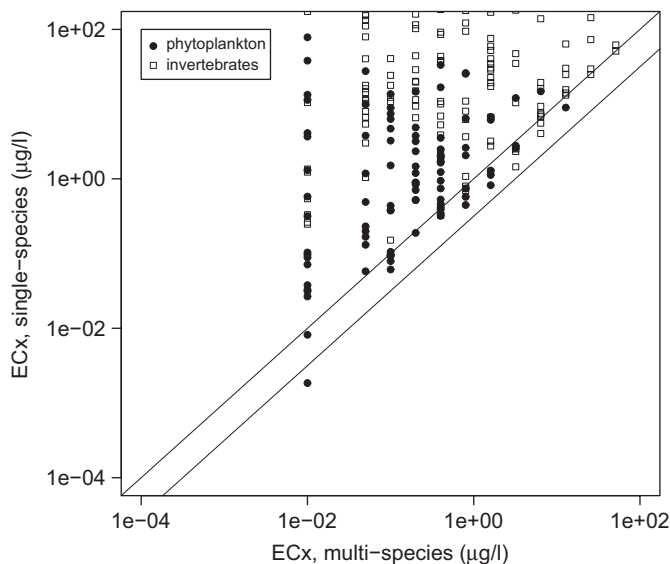
The majority of toxicity test results, whether they are derived in a single- or multi-species test, use invertebrates as a study object and mortality as an endpoint (Breitholtz et al., 2006). Hence, it is not surprising that our ECOTOX database approach as well as our literature search only reflects data on animals, the majority of which were invertebrates. However, phytoplankton blooms as an indirect effect from exposure to insecticides are often observed in experimental ecosystems (Hanazato, 2001). When  $EC_{10,\text{multi-species-test}}$  and  $EC_{10,\text{single-species-test}}$  from our model simulations are plotted for chemicals targeting invertebrates (i.e. as in this study), a difference can be seen between predictions for phytoplankton vs. invertebrates (Fig. 6). The trend shown in Fig. 6 for invertebrates agrees well with what was found in our presented database-based approach. However, predictions for phytoplankton suggest that  $EC_{x,\text{multi-species-test}} < EC_{x,\text{single-species-test}}$ . Hence, our database-based finding that  $EC_{x,\text{multi-species-test}} \approx EC_{x,\text{single-species-test}}$  (Fig. 5) may be skewed, because data availability itself is skewed towards invertebrates. More research is required, however, to investigate this statement.



**Fig. 5.** A comparison between  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  using data from an open literature search. Again, only data were included for which species common name and effect magnitude were identical for both  $EC_x$  values. Care was taken that single-species and multi-species tests were performed by the same authors at the same time. Different symbols denote different types of effect concentrations, as given by the legend.



**Fig. 6.** A comparison between  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  for chemicals that directly target invertebrates, predicted by an ecosystem model, as in a previous exercise (De Laender et al., 2008). Different symbols denote different taxonomic groups, as given by the legend.



**Fig. 7.** A comparison between  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  for chemicals that directly target phytoplankton, predicted by an ecosystem model as in a previous exercise (De Laender et al., 2008). Different symbols denote different taxonomic groups, as given by the legend.

All studies, which were re-analyzed, examined effects of substances which primarily target invertebrates. For the chemicals examined, no phytoplankton- $EC_{x,\text{single-species-test}}$  values were found that were in the range of the exposure concentrations. In the case of endosulfan, a 96h- $EC_{50}$  of  $427.8 \mu\text{g L}^{-1}$  for photosynthesis inhibition of the phytoplankton species *Pseudokirchneriella subcapitata* was reported (De Lorenzo et al., 2002). This  $EC_{50}$  is well above the range of the endosulfan concentrations tested in the ecosystem study conducted by Hose et al. (2003) ( $< 100 \mu\text{g L}^{-1}$ ). Hence, it is unlikely that phytoplankton species experienced direct effects in the latter study. Decreasing zooplankton concentrations, as a result from direct chemical effects on phytoplankton, have been observed, for example, for atrazine (Denoyelles et al., 1982). This suggests that for chemicals directly affecting phytoplankton,  $EC_{x,\text{multi-species-test}}$  of invertebrates is expected to be lower than the  $EC_{x,\text{single-species-test}}$ . This is confirmed by the theoretical ecosystem model simulations (Fig. 7). For such chemicals, a considerable fraction of the simulated cases exhibited a  $EC_{x,\text{multi-species-test}} < EC_{x,\text{single-species-test}}$ , both for invertebrates as for phytoplankton (Fig. 7). Again, the finding from our database-based approach, i.e. that  $EC_{x,\text{multi-species-test}}$  do not differ more than a factor two from  $EC_{x,\text{single-species-test}}$ , is thus partly due to a disproportionate attention in literature for effects of chemicals that directly target invertebrates.

## 5. Conclusions

From our exercise based on the ECOTOX database, it appears as if  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  for invertebrates do not differ more than a factor two for the chemicals examined, except for NOECs which seem to be lower in the single-species tests. The relationship between both  $EC_x$  values was less scattered when single-species and multi-species tests were conducted simultaneously and by the same research group. The relationship between  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  approached the 1:1 line, i.e. differences between most  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  values were limited. Because of the imposed constraints on our compiled data, data points were only available

for 4 and 5 chemicals, using the ECOTOX database and a literature search as primary data source, respectively. Theoretical ecosystem model simulations with different hypothetical chemicals confirmed our present findings for chemicals that primarily target invertebrates.

The majority of the studies involved in our databased approach presented here focused on invertebrates. Hence, the conclusions drawn should be interpreted as such, since predictions from theoretical simulations suggest  $EC_{10,\text{multi-species-test}} < EC_{10,\text{single-species-test}}$  for phytoplankton.

The chemicals that were involved in our database-based approach presented here exerted direct effects on invertebrates, while direct effects on phytoplankton most likely did not occur. Theoretical ecosystem model simulations suggested  $EC_{10,\text{multi-species-test}} < EC_{10,\text{single-species-test}}$  for all populations involved when exposed to chemicals that directly affect phytoplankton. These results illustrate that our interpretation of the relationship between  $EC_{x,\text{multi-species-test}}$  and  $EC_{x,\text{single-species-test}}$  may have been largely determined by data availability.

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