Comparative Assessment of the Sensitivity of Fish Early-Life Stage, *Daphnia*, and Algae Tests to the Chronic Ecotoxicity of Xenobiotics: Perspectives for Alternatives to Animal Testing

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Abstract: No-observed-effect concentrations (NOECs) are used in environmental hazard classification and labeling of chemicals and their environmental risk assessment. They are typically obtained using standard tests such as the fish early-life stage (FELS) toxicity test, the chronic *Daphnia* reproduction test, and the algae growth inhibition test. Given the demand to replace and reduce animal tests, we explored the impact of the FELS toxicity test on the determination of effect concentrations by comparing the FELS toxicity test and the *Daphnia* and algae acute or chronic toxicity tests. Lowest-observed-effect concentrations (LOECs) were used instead of NOECs for better comparison with median lethal or effect concentration data. A database of FELS toxicity data for 223 compounds was established. Corresponding *Daphnia* and algae toxicity tests were identified using established databases (US Environmental Protection Agency ECOTOX, Organisation for Economic Co-operation and Development QSAR Toolbox, eChemPortal, EnviroTox, and OpenFoodTox). Approximately 9.5% of the investigated compounds showed a 10-fold higher sensitivity with the FELS toxicity test in comparison with the lowest effect concentrations obtained with any of the other tests. Some of these compounds have been known or considered as endocrine disrupting, or are other non-narcotic chemicals, indicating that the higher sensitivity in the FELS toxicity test is related to a specific mechanism of action. Targeting these mechanisms by alternative test systems or endpoints, using fish embryos for instance, may allow reduction or replacement of the FELS toxicity test or may allow us to prioritize compounds for conduction of the FELS toxicity test. *Environ Toxicol Chem* 2020;39:30–41. © 2019 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals, Inc. on behalf of SETAC.

Keywords: Fish early-life stage toxicity test; Adverse outcome pathways; Mode of action; Alternatives to animal testing; Fish embryo test

INTRODUCTION

Classification and labeling of industrial chemicals, biocides, and plant protection products are conducted for acute and long-term aquatic hazards to aquatic life, as requested by the Global Harmonization System for classification and labeling of chemicals (United Nations 2011) and its European application (Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures; European Commission 2008). Long-term effects are measured in standard tests covering 3 trophic levels: typically, the fish early-life stage (FELS) toxicity test, the chronic *Daphnia* reproduction test, and the algae growth inhibition test. The no-observed-effect concentration (NOEC) or median effect concentration (EC50) obtained with the most sensitive of the 3 organisms is used for the environmental hazard classification. This NOEC or EC50, when divided by safety factors, allows calculation of the predicted no-effect concentration needed for the environmental risk assessment (Ahlers et al. 2006).

For long-term aquatic hazard estimation in fish, the determination of chronic toxicity is conducted using a specific test setup ( Organisation for Economic Co-operation and Development [OECD] test guideline 210, Fish early-life stage [FELS] toxicity test; 2013a). With respect to fish, ethical concerns have been raised with regard to the use of vertebrate animal tests. Therefore, various approaches had been proposed to improve the statistical performance of the FELS toxicity test (Oris et al. 2012) and avoid or reduce the need to conduct these tests. For instance, a tiered...
testing strategy and the use of the adverse outcome pathway (AOP) concept as a foundation to replace and reduce FELS toxicity tests was proposed, and potential AOPs that lead to FELS toxicity have been discussed (Volz et al. 2011; Villeneuve et al. 2014). A systematic analysis regarding modes of action that may result in enhanced FELS toxicity revealed that baseline toxicity in the FELS toxicity test was similar to baseline toxicity in acute fish and fish embryo toxicity (Scholz et al. 2018). Enhanced toxicity (i.e., effect concentrations below baseline toxicity levels and a high acute-to-chronic ratio [ACR]) was particularly caused by compounds with a specific or reactive mode of action such as neuromuscular toxicity, methemoglobin formation, extracellular matrix inhibition, or endocrine disruption (Scholz et al. 2018).

Some of these modes of action may be captured by alternative endpoints such as behavior or phenotypic assessment in the fish embryo toxicity (FET) test (Organisation for Economic Cooperation and Development test guideline 236; 2013b) and could be used to predict the chronic fish toxicity appropriately. However, in the context of a comparative assessment with Daphnia and algae tests, the FELS toxicity test may not be the most sensitive, and even for compounds with a specific mode of action, the FELS test may exhibit a weak impact for subsequent environmental hazard classification and risk assessment. This minor impact of fish tests has recently been shown for some chemicals in a comparative assessment of acute fish (embryo and later life stages) and acute Daphnia and algal toxicity (Rawlings et al. 2019). Daphnia and algae were shown to be usually more sensitive than fish, and fish embryo and acute juvenile/adult fish toxicity exhibited similar sensitivity. Hence, it was concluded that replacement of acute toxicity tests by fish embryo tests would result in most cases in a very similar classification and labeling.

It is also important to understand the relation of the FELS toxicity test effect concentrations to other chronic aquatic toxicity endpoints, for 2 reasons: 1) in case the FELS toxicity test would not represent the test with the highest sensitivity, the impact on environmental hazard classification and risk assessment would be low, because the compounds with weak sensitivity in the FELS toxicity test may provoke the strongest effects in daphnids or algae (similar to those observed for acute toxicity in Rawlings et al. 2019); and 2) the development of alternative test systems such as the FET could be optimized and prioritized for modes of action that exhibit a significantly higher toxicity in the FELS toxicity test compared with other aquatic toxicity endpoints (i.e., algae and Daphnia toxicity).

Such an analysis may lead to the identification of compound characteristics that are the most influential and that would provide a basis for the potential development of new endpoints in alternative test systems and assessment strategies with sufficient protection to environmental hazards but without the requirement to conduct (vertebrate) animal tests.

Our study had 3 main objectives: First, we wished to extend an existing FELS toxicity test database (Scholz et al. 2018) and provide corresponding acute and chronic Daphnia and chronic algae toxicity data by searching European Chemicals Agency (ECHA) registration dossiers, and the European Food Safety Agency (EFSA) OpenFoodTox database, and using various other search tools to identify potential data on FELS, Daphnia, and algae toxicity (eChem Portal [the global portal to information on chemical substances], the EnviroTox database, and the AMBIT Cheminformatics Data Management System database).

Second, we compared the effect concentrations of the different taxonomic levels (fish, Daphnia, algae), aiming at the identification of cases in which the FELS toxicity test would have the highest influence on the environmental hazard classification and risk assessment. We used a factor of 10 to discriminate compounds with higher sensitivity in the FELS toxicity test versus those that exhibited similar or higher sensitivity in Daphnia or algae tests. According to the European Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) guidance document R7B, no further requirements for fish toxicity testing are indicated if there is compelling evidence to suggest that the fish value is likely to be at least a factor of approximately 10 less sensitive than that of invertebrates or algae (European Chemicals Agency 2017). It was anticipated that the use of this threshold would allow us to identify potential major modes of action leading to high sensitivity in the FELS toxicity test compared with other aquatic toxicity tests.

Third, we wished to provide a strategy to improve prediction of FELS toxicity by an alternative testing strategy or identification of the compounds for which a FELS toxicity test may have importance for environmental hazard classification and risk assessment.

MATERIALS AND METHODS

Compilation of toxicity data

Toxicity data were collected from 5 public databases, the US Environmental Protection Agency (USEPA) ecotoxicology knowledgebase (ECOTOX; 2018), databases included in the OECD QSAR Toolbox (Dimitrov et al. 2016; Organisation for Economic Co-operation and Development & European Chemicals Agency 2018), the database in eChem Portal (Organisation for Economic Co-operation and Development 2018), the EnviroTox database (Connors et al. 2019; Health and Environmental Sciences Institute 2018), and the OpenFoodTox database (European Food Safety Authority 2018) available via the AMBIT search tool (European Chemical Industry Council 2018). The search in the OECD QSAR Toolbox was limited to the chemical inventory lists of available databases. It was restricted to databases that include data on aquatic toxicology, such as the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Aquatic Hazard Assessment database (Solbé et al. 1998), the Aquatic Japan Existing Chemical Database, the Aquatic OASIS database (contributed by the Laboratory of Mathematical Chemistry, Bourgas, Bulgaria), and the Bioaccumulation Environment Canada database (Existing Substances Division, Environment Canada, Toronto, ON, Canada). To search for available toxicity data, each of these databases had to be analyzed separately. Therefore, the entire list of compounds of each database was used to search for aquatic ecotoxicity data in the whole set of the databases. The USEPA ECOTOX database was not included in the search of the OECD QSAR Toolbox but was analyzed separately. The data we obtained were subsequently filtered for availability of
the appropriate endpoints. Given that many results were represented in more than one database, duplicate entries were removed. Entries found in the USEPA ECOTOX, the OECD QSAR Toolbox, and EnviroTox databases were manually inspected in the original literature for their similarity to OECD test guidelines 210, 211, 202, and 201; Organisation for Economic Co-operation and Development 2004, 2011, 2012, 2013a.

The ECHA registration dossiers (European Chemicals Agency 2018a) were searched via the OECD eChemPortal. Datasets were retrieved using the query provided in the Supplemental Data (Table S1). Data entries in the ECHA database are generated for chemical registration under REACH by the registrant; thus data provided by the registrants are partially confidential, and the primary data source could not be evaluated. For quality control, the search was limited to high-quality data (reliability 1, studies well documented and conducted according to or similar to international guidelines representing 59 studies; reliability 2, studies deviating from international guidelines but well documented and scientifically acceptable representing 13 studies) and not assigned quality (reliability 4 represented by 1 study), as indicated by the Klimisch score provided (Klimisch et al. 1997). Studies with reliability level 3 (inappropriate method, insufficient documentation) were excluded from the database. For data obtained from ECHA registration dossiers, the underlying data are not directly available from the online supplementary information, due to property and confidentiality reasons; all those data are available on the ECHA dissemination website (European Chemicals Agency 2018b).

Data from the OpenFoodTox database (Bassan et al. 2018) were retrieved using a KNIME (Berthold et al. 2008) node that queries the AMBIT database (Jeliazkova and Jeliazkov 2011; European Chemical Industry Council 2018). This node was developed by IdeaConsult (2018) and is freely available through GitHub.

For the comparative analyses, FELS, Daphnia, and algae tests were not considered if the purity of the test chemical was reported to be below 90%. Furthermore, effect concentrations reported as “less than” were also excluded from comparative analysis. Only monoconstituent organic and organometallic compounds were included in the final FELS toxicity test database.

Compilation of FELS toxicity data

In compliance with the existing FELS database (Scholz et al. 2018), the search for additional FELS data was restricted to studies that were conducted like that described in OECD test guideline 210 (Organisation for Economic Co-operation and Development 2013a). Furthermore, the search was limited to the fish species Pimephales promelas, Oncorhynchus mykiss, Cyprinodon variegatus, Jordanella floridae, Danio rerio, Orzyias latipes, and Fundulus heteroclitus, which had been previously identified to represent the most abundant fish species used for FELS toxicity testing. The FELS toxicity tests were identified by an initial collection of all studies that reported the lowest-observed-effect concentration (LOEC), the NOEC, the lowest-observed-effect level, and the no-observed-effect level as endpoints. Only results based on laboratory tests (no field studies) were accepted. Studies exceeding the exposure recommendation of OECD test guideline 210 (Organisation for Economic Co-operation and Development 2013a) up to approximately 50% were accepted as well. Hence, datasets with a mean observation duration from 60 to 100 d (for rainbow trout) or 28 to 40 d for other species were included. No recommendations were available for mummichog and flagfish, and exposure durations up to 60 d were accepted. However, studies exceeding recommended exposures were also considered if growth and survival data were reported for time points falling into the recommended durations. If available, the original literature or report was reviewed to verify that the FELS toxicity tests were conducted in a fashion similar to OECD test guideline 210 (Organisation for Economic Co-operation and Development 2013a). Datasets obtained under conditions strongly deviating from OECD requirements were not included in the subsequent correlation analysis.

The lowest effect concentration that caused a statistically significant effect in comparison with control treatments was considered as the LOEC. Accordingly, the highest tested concentration that did not provoke an effect was considered as the NOEC. The LOEC of the most sensitive endpoint (survival or growth) was used for a comparative assessment with EC50 values obtained for Daphnia and algae. Although the NOEC is used for deriving classification, we preferred to use the LOECs for the comparative assessment because LOECs are closer to median lethal concentration (LC50) or EC50 values and hence allow a better comparison of the different types of effect concentration reported for fish, Daphnia, and algae (Scholz et al. 2018). Typically, LOECs were available for the FELS toxicity test, whereas EC50s and LOECs were available for daphnids and EC50s for algae. However, in 2 cases no LOEC or NOEC was provided for the FELS toxicity test, and the reported LC50 or EC50 values were used. If more than one study for the same chemical was available, a geometric mean was calculated. Given the limited availability of FELS toxicity test data and the minor differences in effect concentrations between freshwater and saltwater species (Wheeler et al. 2002), the effect concentrations of saltwater fish species (C. variegatus, F. heteroclitus) were included in the assessment. For compounds with a 10-fold higher sensitivity in the FELS toxicity test compared with Daphnia or algae effect concentration, the original data were inspected for a concentration-dependent effect. However, for 3 chemicals (thiram, estradiol, and peracetic acid), no information on concentration dependency was available because individual concentration data could not be obtained from the reported dossiers.

Only chemicals (n = 223) with available FELS studies were considered for subsequent identification of corresponding acute and chronic daphnid toxicity and algal toxicity. For this search the CAS registry numbers were used. Both ionic and neutral form CAS numbers were used to link effects of different taxa. (A list of CAS numbers is available as Supplemental Data, Table S2.)
The baseline FELS toxicity test LOEC was calculated based on a previously established regression for narcotic compounds (Scholz et al. 2018) using the following equation:

\[
\log \text{LOEC survival (mM)} = 1.04 \times \log D + 1.36
\]

The log distribution coefficient (D) was used instead of the log octanol/water partition coefficient (K\text{OW}) to account for dissociation.

**Compilation of acute and chronic Daphnia toxicity data**

For acute toxicity data, the datasets generated from tests with various *Daphnia* species and a duration of 48 h were considered. *Daphnia* species included were *D. pulex*, *D. magna*, *D. obtusa*, *D. carinata*, *D. Galeata*, and *D. longispina*. When an individual study reported EC50s separately for replicates, the geometric mean EC50 was calculated. Only results from laboratory tests were accepted. For details on endpoints and identifiers that were considered for retrieving EC50s in each database, see the Supplemental Data, Table S3.

For chronic toxicity, only data on reproductive effects obtained with *D. magna* and a test duration of 21 d were accepted. Studies identified via the OECD Toolbox were inspected for assignment of the reproductive effect endpoints used in the USEPA database (see Supplemental Data, Table S3). Furthermore, after manual inspection of the original literature, reproductive effects were subdivided based on 3 commonly measured endpoints: 1) the total number of living offspring at the end of the test, 2) the number of living offspring/day or brood, and 3) the time required to production of the first brood. From the data obtained via eChemPortal, the endpoint “reproduction” was used for analysis. The LOECs were used for analysis, but when no LOEC was reported, the EC50 value was used. The most sensitive endpoint among reproductive endpoints or mortality was used for the comparison. For studies that reported a separate LOEC or EC50 for each replicate, a geometric mean was calculated. Furthermore, when chemicals were tested in more than one study, the geometric mean of all studies was calculated. Effect measures were limited to direct measures of algal growth (according to OECD test guideline 201; Organisation for Economic Co-operation and Development 2011) for details on species and the effect measurement identifiers that were considered as indicators for growth in the different databases, see the Supplemental Data, Tables S3 and S4.

When a reference reported more than one effect concentration for a certain compound, the geometric mean was calculated and included in the final database. The data selection for sensitivity comparison was conducted as follows: 1) the most sensitive EC50 was selected from the algal endpoints (Supplemental Data, Table S4); and 2) if no EC50 but a pair of NOEC and LOEC values was available, the most sensitive LOEC was used. When more than one study was available for the same chemical, the geometric mean was calculated and used for comparative assessment.

**Fish juvenile/adult and embryo acute toxicity data**

Fish juvenile/adult and embryo toxicity data were collected for those chemicals for which the FELS toxicity test was 10-fold or more sensitive than *Daphnia* (acute or chronic) and algal chronic toxicity tests. Acute fish toxicity was identified from the aforementioned databases (ECOTOX, QSAR Toolbox, EChemPortal, EnviroTox, and OpenFoodTox), from Klüver et al. (2015), and from Sobanska et al. (2018). Tests following OECD test guideline 203 (Organisation for Economic Co-operation and Development 1992) or other International Standards Organization and USEPA guidelines were used. Fish embryo acute toxicity data (LC50 and EC50 values for sublethal and additional endpoints) were identified from Sobanska et al. (2018) and from the open literature on PubMed. The LC50 values recorded after 96 or 120 h of exposure were used preferentially. If no values for 96- or 120-h exposure duration were available, the LC50 values recorded after 72 or 48 h were used.

**Physicochemical properties**

The physicochemical properties of compounds tested in the FELS toxicity test were estimated using the software ACD/Percepta, Ver 14.0.0.2726 (ACD/Labs; molecular weight, logP, logSW, logD at pH 7, dissociation constant [pK\text{a}], first strongest acid and first strongest base) and EPIWEB, Ver 4.1 (log Henry’s low coefficient, bond method). The following prediction models of ACD/Percepta were used: Consensus LogP, LogS, LogD, ACD/pK\text{a}, Classic GALAS and classical ACD consensus model.

**Identification of modes of action**

Modes of action were assigned by searching databases (e.g., Drugbank, IRAC), a recently established database for predictive model development (Barron et al. 2015), and available literature for the primary mode of action of the chemical. If no data on the primary mode of action were available, the potential mode of action for acute fish toxicity was identified using a structural alert QSAR based on algorithms of Russom et al. (1997) and Verhaar et al. (1992). This analysis was conducted using the software ChemProp, Ver 6.8 (license available
Correlation analysis
The compiling, grouping, and statistical analysis of data in the different databases were conducted using the analytical workflow program KNIME, Ver 3.7 (Zurich, Switzerland).

Regression analysis of molar effect concentrations was conducted using a Deming (type II) regression to consider variability for both the independent and dependent variables. The regression analysis was performed using the software Sigma Plot, Ver 13.0 (Systat Software) or the R package mrc (R Core Development Team 2014). Statistically significant deviation of the regression slope from 1 or −1 was calculated with the F test in Sigma Plot, Ver 13.0 (p < 0.05).

RESULTS
Availability of data
The search for newly published FELS toxicity tests not included in an existing dataset (Scholz et al. 2018) revealed 79 additional studies representing 72 chemicals. Because 3 of these chemicals had already been tested in other studies included in the existing dataset, the update added a total number of 69 chemicals to the existing FELS test database. One chemical (polymeric ethylenethiuramdisulfide [CAS# 30394140]) from the existing dataset without a specified molecular weight was not included in the sensitivity comparison because the analysis was conducted based on molar concentrations (µmol/L). The result was a final number of 223 chemicals with FELS data that could be used for comparative analyses. (Detailed information on CAS reference numbers, chemical properties, effect concentrations, modes of action, and references for each compound is given in the Supplemental Data, Tables S5–S10.) These chemicals represent 301 entries because some compounds were studied in more than one species or study. We found FELS toxicity data for 163 (P. promelas), 46 (O. mykiss), 33 (D. rerio), 26 (C. variegatus), 22 (O. latipes), 8 (J. floridana), and 3 (F. heteroclitus) compounds. Only datasets with availability of both a NOEC and a LOEC, or an EC50, were considered for the final comparative assessment. For approximately 18% of the FELS data entries with available NOEC/LOEC pairs, nominal exposure concentrations were not verified by chemical analysis. Information on the purity of the test chemical was lacking for 33.4% of the data entries. For 184 of 223 compounds, toxicity data fulfilling the quality criteria were available for at least one of the investigated alternative test systems (Daphnia acute toxicity, Daphnia chronic toxicity, or algae chronic toxicity; Supplemental Data, Figure S1). The greatest compound overlap was observed between FELS and acute Daphnia toxicity test data (Table 1).

Comparison of Daphnia chronic and acute toxicity
Daphnia toxicity data used for long-term aquatic hazard classification are typically derived from chronic toxicity tests.

| | No. of study entries | No. of chemicals |
|---|---|---|
| Test | Database | Total entries | Entries after application of quality criteria/removal of duplicates | Total chemicals | Entries after application of quality criteria/removal of duplicates | FELS toxicity test compound overlap |
| FELS test | ECOTOX/QSAR Toolbox | 328 | 234 | 206 | 165 | n/a |
| | eChem Portal | 381 | 65 | 265 | 58 | |
| | EnviroTox | 717 | 3 | 317 | 3 | |
| | OpenFoodTox | 16 | 0 | 14 | 0 | |
| Daphnia acute toxicity | ECOTOX/QSAR Toolbox | 422 | 344 | 140 | 134 | 155 |
| | eChem Portal | 146 | 116 | 99 | 83 | |
| | EnviroTox | 272 | 27 | 85 | 19 | |
| | OpenFoodTox | 21 | 3 | 16 | 2 | |
| Daphnia chronic toxicity | ECOTOX/QSAR Toolbox | 179 | 136 | 97 | 82 | 123 |
| | eChem Portal | 92 | 76 | 79 | 68 | |
| | EnviroTox | 377 | 8 | 95 | 8 | |
| | OpenFoodTox | 16 | 0 | 13 | 0 | |
| Algae chronic toxicity | ECOTOX/QSAR Toolbox | 339 | 235 | 124 | 99 | 130 |
| | eChem Portal | 198 | 119 | 102 | 87 | |
| | EnviroTox | 669 | 6 | 86 | 6 | |
| | OpenFoodTox | 18 | 1 | 8 | 1 | |

*The search was limited to chemicals for which FELS data were available. Then subsequent searches were conducted, first via the ECOTOX QSAR Toolbox, followed by the eChemPortal and EnviroTox databases. This resulted in a decreasing number of newly identified chemicals in the subsequent searches. Quality criteria refer to purity of the test chemical (below 90%) or deviation from the test guidelines. The search for Daphnia and algae data was limited to compounds for which FELS data were available. n/a = not available.
In our study the most sensitive endpoint among reproductive endpoints and mortality in the Daphnia chronic toxicity test was used. For 16 studies of 220, only the LOEC for mortality was available—either because there were no effects on reproduction or the LOEC for reproduction was not reported. There were 43 studies with reported LOEC values for mortality and reproduction, and in only 4 cases was mortality the most sensitive endpoint, with a maximum of 2.5 times increased sensitivity (Supplemental Data, Figure S2). However, chronic Daphnia toxicity data were not available for many compounds, and restriction to compounds for which chronic toxicity was available would have reduced the number of compounds for comparative assessment to 97 (Supplemental Data, Table S11). In a previous systematic assessment, May et al. (2016) observed that acute and chronic Daphnia toxicities were highly correlated, with a median ACR of 8.8 and a range from 1 to 1500. This observation was confirmed by our dataset, for which chronic and acute Daphnia toxicity data were available for 103 compounds. The regression analysis of logarithmic values indicated a high correlation of acute and chronic Daphnia toxicity, with a data correlation coefficient (R) of 0.88 (Figure 1). The slope of the regression was not significantly different from 1, and the average difference in sensitivity was approximately 4-fold higher for reproduction in the Daphnia chronic toxicity test. Compounds with larger deviation (more than 100-fold difference) included chlorotetracycline, fenitrothion, afdopyr-open, dimethyl disulfide, and chloroacetic acid (5% of all compounds, total number of test compounds = 103). Despite the difference in sensitivity, acute Daphnia data were used as a surrogate to increase the database. Hence, in some cases in which the FELS toxicity test represents the test with the highest sensitivity, the lack of chronic Daphnia toxicity data should be considered as a potential bias. Therefore, we analyzed cases with higher FELS sensitivity for the lack of Daphnia chronic toxicity, to estimate the number of compounds for which this bias may apply (see the following section).

### Compounds with high sensitivity in the FELS toxicity test

To study the potential impact of the FELS toxicity test for environmental hazard classification and risk assessment, the effect concentrations of this test were compared with the most sensitive effect concentration between acute or chronic Daphnia toxicities and algae chronic toxicity. Acute toxicity data for Daphnia were used to increase the size of the dataset (see previous section for the comparative analysis between acute and chronic Daphnia toxicity tests). The FELS toxicity test data were compared with the most sensitive effect concentration of any of the other tests. The line of unity and a threshold value of 10 were used to compare the overall sensitivity of the FELS test with respect to the most sensitive endpoint of Daphnia or algae tests.

A total of 125 compounds were used for comparative analysis, that is, for these compounds, data for FELS toxicity, algae chronic toxicity, and Daphnia toxicity tests (chronic or acute) were available (Figure 2). A total of 12 chemicals

![FIGURE 1: Correlation of Daphnia chronic and acute toxicity. The indicated sample numbers (n) refer to the number of compounds used for regression analysis. For details on compounds and data sources, see the Supplemental Data (Tables S7 and S8). The table summarizes the parameters of the linear regression. EC50 = median effective concentration; LOEC = lowest-observed-effect concentration; IMBL = immobile endpoint.](image1)

![FIGURE 2: Comparison of effect concentrations in fish early-life stage toxicity (FELST) tests and the most sensitive test concentration between Daphnia sp. (chronic [DCT] or acute [DAT] toxicity), and algae chronic toxicity (ACT). Toxicity data are given in µmol/L. Comparison of all data for which both a chronic algae toxicity test and one Daphnia (acute or chronic) test—in addition to the FELST test—were available (n = 125). The type of test yielding the most sensitive effect concentration can be identified from the graph by the symbol preceding the abbreviation of the compound name. § = DAT; * = DCT; ~ = ACT. Compound name abbreviations can be found in Table 2. Dashed lines represent the line of unity ± 10-fold difference (1 log). LOEC = lowest-observed-effect concentration; EC50 = median effect concentration.](image2)
TABLE 2: Compounds with more than 10-fold higher sensitivity in fish early-life stage (FELS) toxicity test compared with Daphnia acute toxicity or Daphnia magna chronic toxicity and algae chronic toxicity tests (for comparison, the toxic ratio based on the FELS test baseline toxicity is shown)\(^a\)

| CAS no. | Common chemical name | 3-Letter abbrev. | Mode of action | FELS test species | Sensitivity of Daphnia acute/ FELS test | Sensitivity of Daphnia chronic/ FELS test | Sensitivity of algae chronic/ FELS test | TR\(^b\) |
|---------|----------------------|-----------------|----------------|------------------|--------------------------------------|------------------------------------------|----------------------------------------|-------|
| 57-63-6 | 17Alpha-ethynylestradiol | AEE | Endocrine disruption | PP | 6404 | 52 500 | 73166 |
| 613-62-7 | 2-(Phenylmethoxy) naphthalene | 2NP | Narcosis | PP | 28 | >31 | 26 |
| 1072957-71-1 | Benzoindiflupyr | BVF | Succinate dehydrogenase inhibitor | PP | 47 | 19 | >494 | 264 |
| 109-46-6 | Dibutyl thiourea | DBT | Out of structural alert domain | OM | 38 | 69 | 196 |
| 15307-79-6 | Diclofenac\(^b\) | DCF | Cyclooxygenase inhibition | DR | 1387 (13.87) | 828 (8.28) | 1000 (10) | 1684 |
| 105-53-3 | Diethyl malonate | DEM | Reactive electrophiles/ pro-electrophiles | PP | 205 | 28 | 544 | 1055 |
| 50-28-2 | Estradiol | ETD | Endocrine disruption | PP | 10630 | 117 | >3700 | 9180 | 5842 |
| 79-21-0 | Peracetic acid | PAA | Out of structural alert domain | DR | 117 | 106 | | 590293 |
| 1918-02-1 | Pilocram | PCL | Methemoglobin formation | OM | 78 | 55 | | 23465 |
| 835621-07-3 | Regorafenib\(^c\) | RGF | Other mode of action | PP | 988 | 127 | | 15882 |
| 137-26-8 | Thiram | THI | Extracellular matrix formation | OM/PP | 37 | 47 | 38 | 51235 |
| 76-87-9 | Triphenylstannanol | TPS | Endocrine disruption | PP | 53 | 61 | | 872109 |

\(^a\)The toxic ratio (TR = baseline toxicity\(_{FELS test}/\text{LOEC}_{FELS test}\)) was calculated for the FELS test. Color code: blue = most sensitive; red = lowest sensitive; gray = either acute or chronic toxicity data were available for daphnids. Sensitivity values represent the ratio of effect concentrations (Daphnia or algae toxicity vs FELS test). A “>” indicates that no toxicity was observed; in these cases the highest tested concentration was used to calculate the effect ratio.

\(^b\)The high ratios are based on a zebrafish study with weak concentrations dependency and similar partially nonsignificant growth reductions observed over a wide range. The ratios in parentheses are based on the LOEC for survival of zebrafish and trout.

\(^c\)A weak concentration dependency with mortality reaching a plateau of approximately 50% was observed.

LOEC = lowest-observed-effect concentration; PP = Pimephales promelas; DR = Danio rerio; OM = Oncorhynchus mykiss.

(9.5%) showed a higher sensitivity in the FELS test (effect concentration less than 10-fold lower) compared with daphnids or algae. This also included compounds that did not provoke any toxicity in daphnids or algae, if the highest tested concentration was at least 10-fold above the FELS LOEC. Five of the 12 compounds with higher FELS sensitivity (4%) showed an effect concentration that was 100-fold higher (Table 2). Three compounds did not provoke any toxicity to algae and are not represented in Figure 2 (total n = 122). For 23 compounds (18%), algae or Daphnia toxicity tests displayed a 10-fold or higher sensitivity. Ten of those compounds exhibited more than 100-fold higher sensitivity (Supplemental Data, Table S13). All compounds with higher sensitivity in the FELS test were associated with a high toxic ratio in the FELS test (toxic ratio = baseline toxicity\(_{FELS test}/\text{LOEC}_{FELS test}\)), a ratio that reached levels of 10\(^3\) to 10\(^6\) for some compounds.

**Relation of high sensitivity in the FELS test with modes of action**

Table 2 shows the modes of action that were associated with compounds of 10-fold higher sensitivity in the FELS test. To link FELS sensitivity to a specific mode of action, one can compare the range of sensitivities for each mode of action. Previous analyses have indicated that certain modes of action are associated with a high toxic ratio or ACR in the FELS test (e.g., neurotoxicity, methemoglobin formation, extracellular matrix formation inhibition, and endocrine disruption). When the FELS test sensitivity was compared with modes of action, particularly 2 modes of action, then endocrine disruption and inhibition of extracellular matrix formation appeared to be associated with higher FELS sensitivity ratios, with median values between 1 and 10 and peak values of 6400 and 37, respectively (Figure 3 and Supplemental Data, S12).

**Fish juvenile/adult and embryo toxicity of compounds with high sensitivity in the FELS test**

In many cases FELS test concentrations relate to acute effect concentrations (Scholz et al. 2018). Furthermore, the FELS toxicity test includes embryonic stages, and thus chronic effects may already be indicated by (sublethal) effects of compounds in fish embryos. Therefore, we investigated how reported effect concentrations for acute fish toxicity and sublethal and lethal effect concentrations of fish embryos relate to effect concentrations in the FELS test, with a focus on compounds that exhibit the highest sensitivity in the FELS test (see the previous section, *Compounds with high sensitivity in the FELS test; n = 12*).
induction of cyp19a1b was considered. 

ethinylestradiol) showed an effect concentration close to the

transgenic zebra 

measured with reporter gene 

related to estrogenic effects (i.e., induction of cyp19a1b 

FELS test were endocrine disruptors. Therefore, an endpoint 

sublethal effects was compared with the chronic 

fl 

sh embryo test and the chronic FELS test when the EC50 for 

parative assessment. Thus, for some individual datasets for 

limited dataset that would compromise a quantitative com-

mortalities and data quality. However, restricting the analysis to data for 

assessments that allows to reduce or replace FELS tests, either by predicting effect 

concentrations or by indicating the compounds for which the 

duction of a FELS test may finally be required (Scholz et al. 2018). However, given that FELS tests are used in the context of and for comparison with endpoints of other taxonomic levels (typically represented by algae and Daphnia toxicity), even for compounds with a specific mode of action the FELS toxicity may not be relevant for risk assessment, classification, and labe-

therefore, it is also critical to understand how FELS

 toxicity relates to algae and Daphnia toxicity and for which type 

of compound or mode of action the higher sensitivities for the 

FELS test are observed. Such a comparative assessment rep-

resents the rationale for the development of the threshold 

approaches for reduction of acute fish toxicity tests (Hoeger et al. 2003; Organisation for Economic Co-operation and De-

velopment 2010; Creton et al. 2014). The threshold approach 

acknowledges that algae and Daphnia represent in many cases the 

most sensitive models. Hence, it was proposed that 

chemicals first be tested in Daphnia and algae, with the lowest 

effect concentration of these models used for a limit test of 

acute fish toxicity. A full range of concentrations would only be 

tested for acute fish toxicity if mortality were to occur in the 

limit test (Rawlings et al. 2019).

To compare effect concentrations of the FELS test with 

algae and Daphnia, we made use of a previously established 

database with effect concentrations of the FELS toxicity test for 

183 compounds (Scholz et al. 2018). However, given that for 

these compounds only a limited number of algae and 

Daphnia 

effect concentrations were available and to increase the data 

basis for a comparative assessment, the database was ex-

 tended by searching additional databases. This search lead to 

the identification of 69 additional compounds and also an 

increased number of corresponding algae and Daphnia effect 

concentrations. Although our database content was increased, 

it may also contain a higher degree of uncertainty than the 

previously established dataset. The reason is that our assess-

ment was based on data retrieved from other databases 

without an accompanying publication and/or limited availability of the original studies or raw data (e.g., from dossiers sub-

mitted to ECHA), and it was difficult to assess the quality and 

reliability of the data in detail. Hence, for individual compounds 

there might be a bias with regard to experimental protocols 

and data quality. However, restricting the analysis to data for 

which original data sources were available would result in a very 

limited dataset that would compromise a quantitative com-

parative assessment. Thus, for some individual datasets for

The FELS test is the most demanding vertebrate animal test 

routinely conducted for environmental hazard and risk

Figure 3: Relation of FELST test sensitivity to the mode of action. The fish early-life stage toxicity (FELST) test sensitivity is described by the ratios of the lowest effect concentration found in the chronic algae and acute or chronic Daphnia test to the effect concentration of the FELST test. The dashed line represents a ratio of 10. The numbers inside the parentheses indicate the number of chemicals present in each class. For details on the compounds and data sources, see the Supplemental Data (Tables S5–S10 and S12). Inh. extracellular matrix = inhibition of extracellular matrix formation by lysyl oxidase inhibition; LOEC = lowest-observed-effect concentration; MoA = mode of action; Ox. = oxidative.

Figure 4 shows the EC50 or LOEC values, for fish, algae, Daphnia, and fish embryo toxicity tests. Fish embryo toxicity data were available for 6 of 12 compounds (see detailed effect concentrations in the Supplemental Table, Table S14). For 2 compounds (benzovindiflupyr and peracetic acid), the effect concentrations of acute fish toxicity were already close (i.e., in the range of 10-fold difference) to the FELS toxicity test (Figure 4). Thiram, a dithiocarbamate associated with inhibition of cellular matrix formation, showed a similar sensitivity for the fish embryo test and the chronic FELS test when the EC50 for sublethal effects was compared with the chronic fish LOEC.

Some of the compounds (3 of 12) with high sensitivity to the FELS test were endocrine disruptors. Therefore, an endpoint related to estrogenic effects (i.e., induction of cyp19a1b measured with reporter gene fluorescence in embryos of a transgenic zebrafish strain) was included (Brion et al. 2012). Figure 4 shows the sensitivity ratios using the most sensitive EC50 values for malformations or cyp19a1b induction in the fish embryo test. Two compounds (estradiol and 17alpha-ethinylestradiol) showed an effect concentration close to the FELS test (Figure 4 and Supplemental Data, Table S14) when the induction of cyp19a1b was considered.

DISCUSSION

The FELS test is the most demanding vertebrate animal test...
which we did not have access to the original data, a subsequent analysis of the original study data or replication of the study may be required to confirm the reliability. Detailed dossiers submitted to agencies may represent a potential source of data associated with sufficient details for quality assessment. However, for this kind of data, the identity of the compound must often be kept confidential (e.g., Ahlers et al. 2006). This would hamper the release of mode of action information on chemicals showing high sensitivity and would interfere with a mode of action analysis of the FELS test. Hence, the use of confidential datasets was not considered as an option for obtaining more data on FELS toxicity tests.

The present study demonstrated that in many cases Daphnia and algae chronic toxicity tests revealed similar effect concentrations as the FELS test. Hence, for these compounds the FELS test would have a weak or no impact on the determination of the lowest NOEC value. For approximately 9.5% of the test compounds, the FELS test revealed an at least 10-fold higher sensitivity. The compounds for which the higher FELS toxicity was observed appeared to exhibit a specific mode of action, indicated by their toxic ratios and the known mode of action, such as endocrine disruption or extracellular matrix inhibition. For 4 of the compounds with high FELS sensitivity (dibutyl thiourea, peracetic acid, picloram, and triphenylstannanol), however, only acute Daphnia studies were available. The availability of chronic effect data may reveal that FELS tests may no longer represent the test with highest sensitivity. For the 22 other compounds with availability of only acute Daphnia toxicity, the FELS test did not represent the most sensitive test. Hence, the use of acute Daphnia data appears to have a minor impact on the overall assessment.

A bias may have been introduced by including saltwater species for retrieving FELS effect concentrations (C. variegatus and F. heteroclitus) and comparing these data with freshwater Daphnia and algae data (29 studies representing 28 compounds). Principally, speciation under saltwater conditions could

FIGURE 4: Differential sensitivity of 16 chemicals to 6 toxicity tests (chronic fish early-life stage toxicity [FELST] test, Daphnia acute and chronic test, algae chronic test, and acute and embryo fish toxicity test). In the case of the fish embryo test, 2 types of endpoints are displayed, the median lethal concentration (LC50) and the median effect concentration (EC50) for sublethal effects (malformations, locomotor response, or cyp19a1b induction; see Supplemental Data Table S14 for details). The dashed line indicates 10-fold sensitivity difference from the FELST test. In case more than one study was available, the bars represent median values, and the range of values for the toxicity studies is represented by error bars. No bars indicate lack of data or that no toxicity was observed (denoted by a #). DAT = Daphnia acute test; DCT = Daphnia chronic test; ACT = algae chronic test; FET = fish embryo toxicity test; baseline chronic fish toxicity = FELS baseline toxicity.
result in a differential toxicity of ionic compounds compared with freshwater. However, species sensitivity distributions for organic compounds have been previously reported to be on average very similar between fresh- and saltwater species, only approaching a factor of 10 in a few cases (Wheeler et al. 2002). Furthermore, none of the FELS test data on compounds with a high sensitivity identified by our comparative analysis were generated by saltwater species. Hence, specification is unlikely to result in a higher sensitivity for the FELS toxicity test data presented here.

As indicated by the previous assessment of toxic ratios and ACRs of the FELS test, typically compounds with a specific mode of action provoked enhanced toxicity. Similarly, sensitivity (compared with algae and Daphnia) in the FELS test appeared to be associated with a non-narcotic and specific mode of action. Some of the mode of actions leading to high ACRs or high toxic ratios in the FELS test, such as extracellular matrix inhibition and endocrine disruption, were also characterised by a high sensitivity in the FELS test compared to Daphnia and algae toxicity (Figure 3). Some of the modes of action displayed a wide range of sensitivity ratios with the FELS test (Supplemental Data, Table S11), which could be due, for example, to experimental variability, species-specific sensitivities in the FELS test, and other (unknown) modes of action assigned in the present study.

The inhibition of extracellular matrix synthesis inhibition was linked to enhanced FELS toxicity via its impact on embryonic development and impaired swimming and feeding, but the relation of endocrine disruption to growth is not well understood (Scholz et al. 2018). However, there is some evidence that environmental estrogens can affect postembryonic growth in fish through an impact on the growth hormone/insulin-like growth factor system (Hanson et al. 2012; Reindl and Sheridan 2012). For compounds with other modes of action, such as benzovindiflupyr, a fungicide that inhibits succinate dehydrogenase, there were also concerns that such compounds may exhibit endocrine-disrupting properties (European Food Safety Authority 2015). We also identified one cyclooxygenase (COX) inhibitor (diclofenac) with high sensitivity (based on growth reduction; LOEC 0.11 µM; Memmert et al. 2013) in the FELS test. Prostaglandins have been hypothesized to play an important role in fish reproduction (Martinović-Weigelt et al. 2017), but the relation to the potential higher sensitivity in FELS test observed especially for diclofenac is not known. However, the data on high sensitivity for diclofenac are conflicting. First, it has only been observed for zebrafish and not in a parallel study conducted with rainbow trout (Memmert et al. 2013). Second, there was a weak concentration dependency, with similar partially nonsignificant growth reductions observed over a wide concentration range (0.11–11 µM). Third, there was no parallel increase in the mortality that often occurs at similar concentration ranges (Scholz et al. 2018). Based on survival, both rainbow trout and zebrafish indicated a LOEC of 11 µM, close to effect concentrations (8–10-fold) of chronic Daphnia and algae tests. Fourth, a juvenile growth test on zebrafish (Praskova et al. 2014) did not indicate any effects on growth up to a concentration of 17 µM. Finally, 2 other COX inhibitors included in the comparative assessment did not exhibit higher FELS sensitivity. A weak concentration dependency with a plateau reaching a survival rate of approximately 50% was also reported for one other compound (regorafenib) tested in fathead minnows. Hence, independent studies would be needed to confirm the high sensitivity for growth or mortality observed in these studies.

For some of the additional modes of action (e.g., reactive electrophiles) that were identified in the present study, appropriate knowledge about why they are related to higher sensitivity in the FELS test is lacking, and research may be required to provide data for an understanding of the mechanistic process. Two compounds for which no specific or reactive mode of action was identified were outside the structural alert domain for prediction of (acute) modes of action (dibutyl thiourea and peracetic acid). However, the high toxic ratios found for the FELS test indicate that these compounds are likely to exhibit an unknown specific or reactive mode of action for FELS toxicity. Interestingly, although neurotoxicity is known to be associated with high toxic ratios (Scholz et al. 2018), the comparative analysis did not reveal that this mode of action leads to high sensitivity in the FELS test. It is likely that many neuroactive compounds have an impact primarily on invertebrates. Hence, many neurotoxic compounds could be identified among those compounds that did not provoke highest sensitivity in the FELS test, particularly for compounds for which Daphnia represented the most sensitive species (with ~45.5% [5/11] of the neuroactive chemicals displaying a 10-fold higher sensitivity in acute or chronic Daphnia tests; Supplemental Data, Table S13 and Figure 3). This high sensitivity of neurotoxic compound in Daphnia has also been observed for the comparison of acute toxicity data (Rawlings et al. 2019).

Overall, the comparative assessment of FELS toxicity provides the perspective that measurement of endpoints related to key events and AOPs in alternative test systems could contribute to a reduction in animal tests. The assessment of sublethal endpoints in embryonic stages is particularly promising, as has already been indicated for the prediction of fish acute toxicity (Sobanska et al. 2018). With respect to high FELS sensitivity, analysis of malformations and markers for endocrine disruption in fish embryos could provide an endpoint with similar sensitivity related to or representing key events of AOPs leading to high FELS toxicity. This was indicated for 3 compounds, for which fish embryo EC50 concentrations were found in the range of the FELS test LOEC. Appropriate corresponding data in fish embryos are still lacking for most of the other compounds, and other targets or sublethal endpoints may be relevant as well. The examples provided in the present study primarily indicate that inclusion of sublethal endpoints could provide a perspective to improve prediction of FELS toxicity at least for the identification of compounds for which conduction of the FELS test would still be required. Similarly, it was found that an in vitro method of identifying endocrine-disrupting chemicals often revealed effects at the same concentration as reported for reproductive effects (Scholz et al. 2012). However, a systematic assessment of alternative endpoints would be needed to develop a strategy based on the prediction of FELS toxicity by fish embryo tests or other alternative approaches.
Even though assessment of alternative endpoints may not be used to predict effect concentrations, such an assessment in fish embryos or other alternative test systems may at least lead to the identification of compounds for which a FELS toxicity test should be conducted. Such an approach may be combined with the threshold approach proposed for the reduction of acute toxicity tests and help to reduce the need for conducting FELS tests to assess the long-term aquatic hazards of chemicals.

**Supplemental Data**—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4607.

**Acknowledgment**—The present study was supported by the L’Oréal Environmental Research Department (service agreements C160386, C151422, and C180899). We acknowledge L. Ma and the Wiley Online Library at DOI: 10.1002/etc.4607.

**REFERENCES**

Ahlers J, Riedhammer C, Vogliano M, Ebert RU, Kühne R, Schüürmann G. 2006. Acute to chronic ratios in aquatic toxicity—Variation across trophic levels and relationship with chemical structure. Environ Toxicol Chem 25:2937–2945.

Barron MG, Lilavos CR, Martin TM. 2015. MOAtox: A comprehensive mode of action and acute aquatic toxicity database for predictive model development. Aquat Toxicol 161:102–107.

Bassan A, Ceriani L, Richardson J, Livianou A, Ciacci A, Baldin R, Kovarich S, Fioravanzo E, Pavan M, Gibin D, Di Piazza G, Pisanato L, Cappé S, Verhagen H, Robinson T, Dorne J Lou. 2018. OpenFoodTox: EFSA’s chemical hazards database. [cited 2019 July 2]. Available from: https://zenodo.org/record/1252752#.XZyUth_O1s

Berthold MR, Cebron N, Dill F, Gabriel TR, Kötter T, Meini T, Ohi P, Sieb C, Thiel K, Wiswedel B. 2008. KNIME: The Konstanz Information Miner. In Klüver N, König M, Ortmann J, Massei R, Paschke A, Kühne R, Scholz S, Thiel K, Wiswedel B. 2008. KNIME: The Konstanz Information Miner. Springer, Berlin, Germany, pp 319–326.

Brion F, Le Page Y, Piccini B, Cardoso O, Tong S-K, Chung B, Kah O. 2012. Screening estrogenic activities of chemicals or mixtures in vivo using transgenic (cyp19a1b-GFP) zebrafish embryos. PLoS One 7:e36069.

Connors KA, Beasley A, Barron MG, Belanger SE, Bonnell M, Brill JL, de Zwart D, Kienzler A, Kräaller J, Otter R, Phillips JL, Embry MR. 2019. Creation of a curated aquatic toxicity database: EnviroTox. Environ Toxicol Chem 38:1062–1073.

Cretson S, Cloop M, Wheeler JR. 2014. Application of the threshold approach for acute fish toxicity testing to plant protection products: A proposed framework. Chemosphere 96:196–200.

Dmitrov SD, Diderich R, Sobanski T, Pavlov TS, Chankov GV, Chapkanov AS, Karakolov YH, Temelkov SG, Vaseva RA, Gerova KD, Kuseva CD, Todorova ND, Mehmeh AM, Rassenberg M, Mekenyan OG. 2016. QSAR Toolbox—Workflow and major functionalities. SAR QSAR Environ Res 27:203–219.

European Chemicals Agency. 2017. Chapter R.7b: Endpoint specific guidance. In Guidance on Information Requirements and Chemical Safety Assessment. Helsinki, Finland. [cited 2018 December 20]. Available from: https://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf/1a551efc-bd6a-4d1f-b719-16e0d3a01919

European Chemicals Agency. 2018a. Registered substances. Helsinki, Finland. [cited 2018 November 5]. Available from: https://echa.europa.eu/information-on-chemicals/registered-substances

European Chemicals Agency. 2018b. Search for chemicals. Helsinki, Finland. [cited 2018 November 5]. Available from: https://echa.europa.eu/home

European Chemical Industry Council. 2018. The LRI AMBIT Read-Across Tool. Brussels, Belgium. [cited 2019 March 4]. Available from: https://ambitinfra.ideaconsult.net/tool2

European Commission. 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official J Eur Union L353:1–1355.

European Food Safety Authority. 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA J 1313:107.

European Food Safety Authority. 2018. OpenFoodTox. Parma, Italy. [cited 2019 February 19]. Available from: https://www.efsa.europa.eu/en/microstrategy/openfoodtox

Hanson AM, Kittlison JD, Shyrman MA. 2012. Effects of 17β-estradiol, 4-nonylphenol, and 17α-ethinylestradiol on growth hormone-insulin-like growth factor system and seawater adaptation of rainbow trout (Oncorhynchus mykiss). Aquaculture 362–363:241–247.

Hoeger B, Jeram S, Holt M, Douben P, Halder M. 2003. Reduction of animal testing by the use of transgenic (cyp19a1b−) zebrafish embryos or other alternative test systems may at least lead to the identification of compounds for which a FELS toxicity test should be conducted. Such an approach may be combined with the threshold approach proposed for the reduction of acute toxicity tests and help to reduce the need for conducting FELS tests to assess the long-term aquatic hazards of chemicals.

**Data Availability Statement**—Data, associated metadata, and calculation tools are available from the corresponding author (elisabet.teixido@gmail.com).

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Sensitivity comparison of FELS with Daphnia and Algae tests—Environmental Toxicology and Chemistry, 2020;39:30–41

ENVI/JM/TOG(2010)/7. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2011. Test No. 201: Freshwater alga and cyanobacteria, growth inhibition test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2012. Test No. 211: Daphnia magna reproduction test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2013a. Test No. 210: Fish, early-life stage toxicity test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2013b. Test No. 236: Fish embryo acute toxicity (FET) test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2018. eChemPortal: The global portal to information on chemical substances. Paris, France. [cited 2018 December 20]. Available from: https://www.echemportal.org

Organisation for Economic Co-operation and Development & European Chemicals Agency. 2018. QSAR Toolbox. Paris, France. [cited 2018 November 26]. Available from: http://www.qsartoolbox.org/

Oris JT, Belanger SE, Bailer AJ. 2012. Baseline characteristics and statistical implications for the OECD 210 fish early-life stage chronic toxicity test. Environ Toxicol Chem 31:370–376.

Praskova E, Phalova L, Chromova L, Stepanova S, Bedanova I, Blahova J, Hostovsky M, Skoric M, Maršálek P, Voslarova E, Svobodova Z. 2014. Effects of subchronic exposure of diclofenac on growth, histopathological changes, and oxidative stress in zebrafish (Danio rerio). ScientificWorldJournal 645737.

R Core Development Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rawlings JM, Belanger SE, Connors KA, Carr GJ. 2019. Fish embryo tests and acute fish toxicity tests are interchangeable in the application of the threshold approach. Environ Toxicol Chem 38:671–681.

Reindl KM, Sheridan MA. 2012. Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comp Biochem Physiol A Mol Integr Physiol 163:231–245.

Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE, Drummond RA. 1997. Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow (Pimephales promelas). Environ Toxicol Chem 16:948–967.

Scholz S, Renner P, Belanger SE, Busquet F, Davi R, Demeneix BA, Denny JS, Léonard M, McMaster ME, Villeneuve DL, Embry MR. 2012. Alternatives to in vivo tests to detect endocrine disrupting chemicals (EDCs) in fish and amphibians—Screening for estrogen, androgen and thyroid hormone disruption. Crit Rev Toxicol 43:45–72.

Scholz S, Sela E, Blaha L, Braunbeck T, Galay-Burgos M, García-Franco M, Gueane J, Klüver N, Schirmer K, Tanneberger K, Tobor-Kaplon M, Witters H, Belanger S, Benfenati E, Creton S, Cronin MTD, Eggen RIL, Embry M, Ekman D, Gourmelon A, Halder M, Hardy B, Hartung T, Hubesch B, Jungmann D, Lampi MA, Lee L, Léonard M, Küster E, Lillcrap A, Luckenbach T, Murk AJ, Navas JM, Peijnenburg W, Repetto G, Salinas E, Schüermann G, Spielmann H, Tollefsen KE, Walter-Rohde S, Whale G, Wheeler JR, Winter MJ. 2013. A European perspective on alternatives to animal testing for environmental hazard identification and risk assessment. Regul Toxicol Pharmacol 67:506–530.

Scholz S, Schreiber R, Armitage J, Mayer P, Escher BI, Lidzba A, Léonard M, Altenburger R. 2018. Meta-analysis of fish early life stage tests—Association of toxic ratios and acute-to-chronic ratios with modes of action. Environ Toxicol Chem 37:955–969.

Sobanska M, Scholz S, Nyman AM, Cesnaitis R, Gutierrez Alonso S, Klüver N, Kühne R, Tyle H, de Knecht J, Dang Z, Lundbergh I, Carlson C, De Coen W. 2018. Applicability of the fish embryo acute toxicity (FET) test (OECD 236) in the regulatory context of Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH). Environ Toxicol Chem 37:657–670.

Solbè J, Mark U, Buyle B, Guhl W, Hutchinson T, Kloepper-Sams P, Länge R, Scholz N, Bontinck W, Niessen H. 1998. Analysis of the ECETOC Aquatic Toxicity (EAT) database I—General introduction. Chemosphere 36:99–113.

US Environmental Protection Agency. 2018. ECOTOX Knowledgebase. Washington, DC. [cited 2018 July 2]. Available from: http://cfpub.epa.gov/ecotox/

Verhaar HJM, Van Leeuwen CJ, Hermens JLM. 1992. Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. Chemosphere 25:471–491.

Villeneuve D, Volz DC, Embry MR, Ankley GT, Belanger SE, Léonard M, Schirmer K, Tanguay R, Truong L, Wehmas L. 2014. Investigating alternatives to the fish early-life stage test: A strategy for discovering and annotating adverse outcome pathways for early fish development. Environ Toxicol Chem 33:158–169.

Volz DC, Belanger S, Embry M, Padilla S, Sanderson H, Schirmer K, Scholz S, Villeneuve D. 2011. Adverse outcome pathways during early fish development: A conceptual framework for identification of chemical screening and prioritization strategies. Toxicol Sci 123:349–358.

Wheeler JR, Leung KMY, Morritt D, Sorokin N, Rogers H, Toy R, Holt M, Whitehouse P, Crane M. 2002. Freshwater to saltwater toxicity extrapolation using species sensitivity distributions. Environ Toxicol Chem 21:2459–2467.