The fish early-life stage sublethal toxicity syndrome – A high-dose baseline toxicity response

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Abstract

A large number of toxicity studies report abnormalities in early life-stage (ELS) fish that are described here as a sublethal toxicity syndrome ($TxSn_{FELS}$) and generally include a reduced heart rate, edemas (yolk sac and cardiac), and a variety of morphological abnormalities. The $TxSn_{FELS}$ is very common and not diagnostic for any chemical or class of chemicals. This sublethal toxicity syndrome is mostly observed at high exposure concentrations and appears to be a baseline, non-specific toxicity response; however, it can also occur at low doses by specific action. Toxicity metrics for this syndrome generally occur at concentrations just below those causing mortality and have been reported for a large number of diverse chemicals. Predictions based on tissue concentrations or quantitative-structure activity relationship (QSAR) models support the designation of baseline toxicity for many of the tested chemicals, which is confirmed by observed values. Given the sheer number of disparate chemicals causing the $TxSn_{FELS}$ and correlation with QSAR derived partitioning; the only logical conclusion for these high-dose responses is baseline toxicity by nonspecific action and not a lock and key type receptor response. It is important to recognize that many chemicals can act both as baseline toxicants and specific acting toxicants likely via receptor interaction and it is not possible to predict those threshold doses from baseline toxicity. We should search out these specific low-dose responses for ecological risk assessment and not rely on high-concentration toxicity responses to guide environmental protection. The goal for toxicity assessment should not be to characterize toxic responses at baseline toxicity concentrations, but to evaluate chemicals for their most toxic potential. Additional aspects of this review evaluated the fish ELS teratogenic responses in relation to mammalian oral LD50s and explored potential key events responsible for baseline toxicity.

Keywords

Fish early-life stage, sublethal toxicity syndrome, baseline toxicity, mechanism of action, teratogens

1. Introduction and Background

Methods and guidelines abound for characterizing fish toxicity for risk assessment and environmental protection. Full and partial life-cycle toxicity tests are important for assessing growth and reproduction over long-term exposure, but are labor intensive and costly. Tests with early-life stage (ELS) fish have become popular for their ease of use, low cost, and ability to generate a large amount of data, especially for an ever increasing number of compounds without basic toxicity information. Many ELS fish toxicity tests are short term (acute) and quantify lethality or indicators of lethality at 96 h (OECD 2013). Recently an increasing number of guidelines and research studies now include sublethal effects from acute tests, including many that are considered as teratogenic responses because of deformities observed during developmental stages. A large number of adverse effects in fish embryos are often assessed and reported as effective concentrations (e.g., EC50 or EC10) or lowest observed effect concentrations (LOECs) that can be used for comparison among species and chemicals. Overall, the fish embryo toxicity test is highly useful for acquiring basic toxicity data for myriad untested chemicals.

This review examines a subset of those sublethal embryo responses, which has been termed the fish early-life stage toxicity syndrome ($TxSn_{FELS}$). The data indicate that this toxicity syndrome is a common sublethal manifestation of baseline toxicity for a large number of chemicals occurring mostly at very high concentrations that are rarely environmentally relevant. Even though a high percentage of compounds can act by baseline toxicity, they can also elicit toxic responses at concentrations that are often orders of magnitude lower. These low-dose toxic response are frequently discovered with longer term life-cycle tests, but can also be detected with the same short-term fish embryo experiments.

Many authors group chemicals causing toxicity for fish and other taxa into modes or mechanisms of action (MoA) including baseline toxicants (non-specific acting) and those that are considered specific acting or reactive, which generally includes interaction with cellular receptors, other proteins, or genetic material (Escher and Hermens 2002; Meador et al. 2011). Data from the fish embryo test (FET) is often used to determine if a chemical acts as a baseline toxicant or specific-acting toxicant based on a comparison of the observed lethal dose and the predicted baseline lethal dose (Klüver et al. 2016, Scholz et al. 2018). As noted by some authors (Knöbel et al. 2012), the zebrafish embryo acute test is also a suitable surrogate for acute adult fish lethality, which occurs at similar exposure concentrations.

Some authors have found that the acute to chronic ratio (ACR) for toxicity metrics may be indicative of specific acting toxicants such as neurotoxic compounds and endocrine disruptors (Scholz et al. 2018). Acute values are usually lethality based occurring within 96 h of exposure and chronic values are generally lethal or sublethal values occurring at some later time. ACR values elicited by chemicals in the range of 1 – 10 are considered to be acting by baseline toxicity and those greater than 10 are classified as specific acting; however, specific acting toxicants do not always exhibit elevated ACR values (Ahlers et al. 2006). Most ACR data analyses for fish early-life stage (ELS) testing compare acute LC50 mortality values and chronic LOEC values such as survival or growth to separate baseline toxicity from a specific mechanism of action. The present analysis examines time independent ACR-type ratios to quantify the relationship between lethal and sublethal responses for ELS fish and the ELS sublethal responses in relation to additional low-dose sublethal non $TxSn_{FELS}$ responses that may be acting by a specific mechanism of action.

Another important component of ELS fish toxicity studies concerns the extrapolation of teratogenic responses in fish embryos to potential mammalian responses (McGrath 2012, Ducharme et al. 2013). In many cases, toxicity

metrics for fish ELS toxicity responses and that for mammals shows these effects occurring at near lethal values and therefore may not provide useful data for human risk assessment. An important goal of this review is to encourage future research to determine if the $TxSn_{FELS}$ for fish embryos is generally a non-specific, near lethal response for a large group of chemicals when applied at high doses. Such research delineating the role of ELS responses would be highly relevant for ecological risk assessment and setting threshold guideline values to protect fish or mammals.

1.1 The fish early-life stage toxicity syndrome

The $TxSn_{FELS}$ includes a suite of adverse sublethal effects observed in fish embryos and larvae exposed to a wide variety of chemicals at high, near lethal concentrations. The core responses generally include a reduced heart rate, yolk sac and cardiac edemas, and a variety of morphological abnormalities including spinal curvature, lack of swim bladder inflation, and jaw and eye abnormalities. These are the most commonly associated responses; however the full suite of responses remains to be defined. There are dozens of studies focusing on this suite of toxic effects in fish embryos exposed to myriad chemicals, which are sublethal effects frequently quantified in fish embryo-larval testing (Fraysse et al. 2006). Alterations to the developing heart and cardiac rhythm can lead to morphological abnormalities and edema of the heart and yolk sac. Incardona et al. (2004) described this as a characteristic suite of abnormalities that include cardiac dysfunction, edema, spinal curvature, and other morphological abnormalities in ELS fish exposed to PAHs. As noted by Incardona and Scholz (2018) these embryonic defects are linked by a reduction in peripheral circulation that can affect morphological development (e.g., spinal curve, enlarged eye, and reduced jaw) and also lead to kidney failure. Embryos can survive this severe effect because oxygen uptake and waste removal can occur by diffusion for the first several days of development (Burggren 2005). As discussed by Ducharme et al. (2013) in

their meta-analysis of 133 chemicals, most of the cardiovascular alterations including cardiac edema were positively correlated ($p<0.05$) with yolk sac edema and many of the morphological aberrations that constitute the $TxSn_{FELS}$. Other reviews have noted the high degree of positive correlation between these responses (McCollum et al. 2011; Jarque et al. 2020). While correlation among these responses was strong, they would have likely been even more pronounced if the dataset was limited to only those chemicals suspected of causing baseline toxicity. This list of responses associated with the $TxSn_{FELS}$ is likely not mutually exclusive because other responses may also appear correlated depending on the chemical and other potential mechanisms of action. As explained in this review, baseline toxicity is not an exclusive mechanism of action but one defined by high doses.

Even though there is overlap, these responses are only a subset of a large number of fish ELS responses that have been employed for assessing teratogenesis in vertebrates, especially zebrafish (Ducharme et al. 2013; McCollum et al. 2011). For this analysis, responses such as apoptosis, pigmentation changes, notochord abnormalities, and changes in signaling pathways may be useful for characterizing vertebrate teratogenesis, but are not considered to be part of the core suite of $TxSn_{FELS}$ responses. For example, dithiocarbamate pesticides such as thiram and disulfiram are known to affect notochord development in embryonic fish at concentrations far below baseline levels (Tilton et al. 2006), which likely occurs via a specific toxic mechanism.

1.2 Baseline toxicity

Baseline toxicity has been described and confirmed by a number of researchers (McCarty 1993; van Wezel and Opperhuizen 1995; Escher et al. 2002; van der Heijden et al. 2015; Antczak et al. 2015; Klüver et al. 2016; Escher et al. 2020) who have published extensively on this mechanism of action. It is essentially the default response that is observed in the absence of any receptor

interaction or activation. It can occur before receptor interaction can be expressed and it has been observed in diverse taxa ranging from bacteria to mammals. Baseline toxicity is also known as "narcosis"; however this is a poor term for this mechanism of action because it has been described as a behavioral response in organisms (loss of equilibrium and lethargy), which is not often observed. Baseline toxicity has been described as minimal toxicity, meaning the observed responses occur at some maximum concentration resulting in mortality. In general, it is an acute response that appears to occur in the absence of receptor interaction, which may be a function of exposure time or a complete lack of such interaction. In other cases it appears to occur before receptor interaction resulting in large differences for toxicity metrics. Toxic responses at lower concentrations for many of these compounds are usually a result of specific effects on receptors. Based on empirical data and quantitative-structure activity relationships (QSARs), mortality for the baseline response generally occurs at a relatively consistent mean value of 2 – 8 mmol/kg whole-body (McCarty and Mackay 1993; Di Toro et al 2000; McCarty et al. 2013; van Wezel and Opperhuizen 1995; van der Heijden et al. 2015) and approximately 40 – 343 mmol/kg lipid for membranes (Escher et al. 2002; van der Heijden et al. 2016; Endo 2016). These concentrations are characterized as an acute lethal response; however, chronic and sublethal whole-body concentrations occur in the range of 0.2 – 0.8 mmol/kg (McCarty and Mackay 1993). Baseline toxicity is also additive, meaning multiple compounds can contribute to achieve the critical whole-body or membrane concentration causing toxicity.

The most common predictor for QSAR analysis is the octanol-water partition coefficient (K_{ow}) that can be used to predict bioaccumulation of organic compounds in organisms. The K_{ow} is highly predictive of the bioconcentration factor that is used to determine a tissue concentration for a given water concentration. These QSARs can be used to predict both water and tissue

concentrations that produce toxicity because of the correlation between a toxicity metric such as the LC50 and the K_{ow}. This relationship can be used to define baseline toxicity values and determine when a compound is causing more specific toxicant responses at concentrations lower than that predicted or observed for baseline toxicity. Even though water concentrations are employed for such assessments, whole-body tissue concentrations are a better surrogate for characterizing toxicity (Escher and Hermens 2004; Meador et al. 2008; 2011, McCarty et al. 2011; Escher et al 2011). As noted by many authors (McCarty 1986; Escher and Hermens 2004) baseline toxicity is associated with bioaccumulation that is proportional to a compound's hydrophobic properties as determined by the K_{ow}. The result is a whole-body concentration that is proportional to aqueous exposure concentrations, which is also proportional to an amount within the organism and at the receptor causing toxicity. This assumes that internal tissue concentrations are not compromised in some way, such as high rates of metabolism, reduced internal kinetics, or inhibition of chemicals crossing membranes. Toxicity metrics for compounds that act by a specific mechanism of action are generally receptor-based and do not correlate with K_{ow}. Many authors use this correlation between toxicity and K_{ow} or related QSARs to judge if a compound is acting by non-specific baseline toxicity or is acting on a specific receptor (Scholz et al 2018, Klüver et al. 2016). As presented in this review, it appears that chemical toxicity can be both specific acting (likely receptor-based) and non-specific as a function of dose, time, and endpoint specificity. Baseline toxicity may be an exclusive mechanism of action for some chemicals; however, many chemicals may cause toxicity via multiple mechanisms.

Baseline toxicity is considered reversible by many authors (van Wezel and Opperhuizen 1995; Escher et al. 2011); however, baseline toxicity during fish ELS development is known to result in permanent damage to the heart leading to reduction in swimming performance in juveniles and adults (Hicken et al.

2011), assuming embryos survive the chemical insult. It is also unlikely that many of the morphological abnormalities observed for the $TxSn_{FELS}$, such as a reduced eye, jaw deformations, uninflated swim bladder, scoliosis, and others are reversible.

2. Analysis

2.1 Evaluating the available data

A number of studies were gleaned from the literature that reported on early-life stage toxicity for fish exposed to a variety of chemicals and related studies reporting on a diverse array of organismal responses for all life stages of fish for the same chemicals. This is a scoping review and was not intended to be comprehensive as required for a systematic review or meta-analysis. The intent was to explore the nature and extent of the available data and to identify key concepts, assumptions, and data gaps. A search of the literature was conducted via PubMed (PubMed 2020). There appears to be no database for all the relevant $TxSn_{FELS}$ responses, hence a search of the literature was necessary. A broad selection of various types of compounds was included to ensure that several classes of compounds were represented. These results were compared and evaluated as potential baseline toxicants and for their ability to cause toxic responses far below expected baseline levels. Fish ELS studies were selected if they reported most of the known $TxSn_{FELS}$ responses and an LC50. In many cases only some of the main ELS fish responses were quantified. For example, heart rate may not have been evaluated in a study that quantified edemas or spinal curvature. Almost all studies exposed ELS fish between 2 and 6 days and most started exposure within 24 h of fertilization. Another criterion for chemical selection was based on the availability of data that examined other toxic responses in fish that were not characterized as part of the $TxSn_{FELS}$. Additionally, a few low K_{ow} compounds were added to demonstrate the $TxSn_{FELS}$ for compounds that exhibit limited

bioaccumulation. Overall, there were very few additional compounds not included here that satisfied all the above criteria. Additional analyses came from four relevant review articles that presented $TxSn_{FELS}$ and mortality data for ELS fish (Ali et al. 2014; Knöbel et al. 2012, Yamashita et al. 2014; Jarque et al. 2020).

Many of these studies should be viewed cautiously because concentrations were not measured, small volumes were used, and exposure solutions were not replenished over the exposure period, which are all contrary to OECD guidelines (OECD 2013). Based on the protocols outlined in OECD TG236 (OECD 2013), exposure volumes are recommended to be 2 mL per well to avoid evaporation of the test solution and to ensure adequate oxygen for embryo respiration. They also recommend replacing test solutions every day to mitigate and maintain consistent exposure concentrations. Additionally, analytical confirmation of exposure concentrations is necessary to assure accuracy. This step is commonly omitted in studies resulting in high uncertainty regarding the reported toxicity metrics. As noted by Knöbel et al. (2012), very large differences can be observed between nominal and measured exposure concentrations.

In addition to the ELS toxicity data, corresponding toxicity results that were not part of the $TxSn_{FELS}$ for each compound were compiled for comparison to the ELS data and expressed as LOEC values. Toxicity results were generally limited to apical endpoints, such as growth and reproductive impairment that are likely to have population relevant consequences. These results were considered as specific-acting low-dose responses ($LOEC_{specific}$) in that they likely caused effects via a receptor at concentrations far below baseline toxicity levels. Altered hormone levels, a few physiological metabolites, and immune system parameters were included, especially if they supported the apical responses. Gene transcript alterations were not considered.

2.2 Approaches for characterizing baseline toxicity

There are several ways to determine if a compound is acting by baseline toxicity or a specific mechanism of action and two different approaches were used here. One approach is to compare observed responses to predicted values for baseline toxicity that are generated by simple QSARs. This can be accomplished by utilizing the relationship between the bioconcentration factor (BCF), LC50, and LR50, which is the whole-body lethal residue predicted to occur at the LC50 (equation 1) (McCarty 1986; Meador 2006, Klüver et al 2016). The baseline lethal critical body residue (CBR = LR50) (Meador 1997) value for fish (2.8 mM, wet weight, whole body, 5% lipid) was used for the calculation, which was based on measured whole-body concentrations determined for 78 observations for fish from all life stages (McCarty et al. 2013). This value (mean and range) agrees closely with previously published LR50 values (McCarty and Mackay1993; Di Toro et al. 2000, Kipka and Di Toro 2009; van der Heijden et al. 2015) reported for dozens of species and hundreds of organic compounds.

$$LR_{50} = BCF * LC_{50} \quad (1)$$

The BCF can be measured or predicted. QSAR modeling correlating the BCF to K_{ow} has been developed and refined over many years producing equations with high coefficients of determination (r^2) (Arnot and Gobas 2006; Fu et al. 2009). One commonly employed equation (Veith et al. 1979) (r^2=0.9) was used to model the BCF (equation 2). This predicted BCF assumes little or no metabolism and steady-state conditions and therefore predicts maximum whole-body concentrations. In some cases the amount of compound that is bioaccumulated will be overestimated and result in higher than expected toxicity metrics. As noted by Fu et al. (2009), the BCF is set to 1.41 for those $\log_{10}K_{ow}$ values less than 1, which is a more accurate representation of tissue concentrations for these compounds. The analysis was limited to compounds

with $\log_{10}K_{ow}$ values <6.5 due to uncertainty regarding bioaccumulation (Arnot and Gobas 2006). Hence, the $\log_{10}K_{ow}$ is a surrogate for bioaccumulation, which is used to predict tissue concentrations. For those ionizable compounds with a physiological charge other than 0, measured BCFs or those predicted with an ionization-corrected K_{ow} (D_{ow}) were used (Table S1). Most studies were conducted between pH 7 and 8, which is close to the physiological pH of 7.4.

$$BCF = 10^{(0.85*\log_{10}Kow - 0.7)} \quad (2)$$

The LR50 and BCF were used to predict an equivalent aqueous toxicity metric (LC50). Rearranging equation 1 we get equation 3, which is termed the Constant Critical Toxicity Concentration (CCTC) and is used to generate an aqueous baseline toxicity reference line that is based on tissue concentrations for lethality (LR50). In other words, it represents the water concentration that is expected to produce the mortality baseline tissue concentration (2.8 mM = LR50) for any compound as a function of its BCF.

$$LC50_{baseline} = LR50_{baseline} / BCF \quad (3)$$

Observed data can then be examined in terms of the toxicity ratio (TR), which is the ratio between the predicted baseline toxicity value (equation 3) and the observed fish ELS LC50 (equation 4).

$$TR_{LC50} = \frac{\text{LC50 baseline}}{\text{LC50 observed}} \quad (4)$$

Chemicals exhibiting a TR_{LC50} <10 are considered to be acting as baseline toxicants and those with a TR_{LC50} >10 are designated as specific acting (Klüver et al. 2016). Chemicals with TR_{LC50} values >10 are expected to be more potent and cause specific toxicity by receptor interaction at much lower concentrations than predicted by baseline toxicity models. Values <1 indicate a predicted toxic response that is higher than the benchmark response and is likely

exhibiting baseline toxicity. The predicted $LC50_{baseline}$ value may be higher than the benchmark value if the compound is metabolized; meaning higher water exposure concentrations are needed to achieve the baseline lethal whole-body concentrations (LR50). The predicted QSAR BCF is assumed to occur at equilibrium or steady-state conditions without significant metabolism. For many of these compounds the rates of metabolism are not defined and for high K_{ow} compounds, the time to steady state can occur beyond the experimental time frame resulting in over-estimated BCF values. Additionally, the yolk for fish embryos is known to sequester a very high percentage of the total whole-body concentration (Halbach et al. 2020). Values ranging from 50 – 95% were obtained depending on the compound and embryo age, which means higher than predicted whole-body concentrations are needed to elicit an effect because chemicals in the yolk are not toxicologically active. For both of the above cases, the TR_{LC50} will be less than 1. Ideally, the test for baseline toxicity should be made on measured tissue concentrations (whole body or membrane) in order to avoid inaccuracies in prediction, especially for highly metabolized or high K_{ow} compounds.

A second independent prediction for baseline toxicity was also selected. This approach predicts baseline toxicity in terms of water exposure concentration and the constant toxic membrane concentration (CTMC) reference line for baseline toxicity (Goss and Endo 2016; Scholz et al. 2018). These values are calculated with a membrane/water partition coefficient (equation 5); however a compound's K_{ow} is considered as a suitable surrogate for many compounds (Goss and Endo 2016), which was necessary due to a lack of data for many of the compounds examined in the present study.

$$C_{water} = \frac{100\ \mathrm{mmol/L}}{\mathrm{Kow}} \qquad (5)$$

The constant membrane concentration associated with baseline toxicity is 100 mmol/L in cell membranes (Scholz et al. 2018). C_{water} is the water

362 concentration that is predicted to result from the CTMC, which has been shown
363 to cause the baseline toxic response. Partitioning data (K_{ow}) was obtained
364 from PubChem (2020) and DrugBank (Wishart et al. 2017). It is important to
365 note that K_{ow} is not a fixed value and can vary several fold among databases
366 resulting in highly variable estimates for predicted tissue concentrations.

367 In the present analysis, a comparison was made between early-life stage lethal
368 responses ($LC50_{FELS}$) and the LOEC (mostly) or EC50 (noted when used) for
369 toxicity syndrome sublethal responses ($LOEC_{TxSn}$), which is a lethal to sublethal
370 ratio for early-life stage fish (LSR_{FELS}). It is similar to the classic acute to
371 chronic ratio (ACR), but qualitatively different. The LSR_{FELS} is a useful metric
372 that is used to quantify the relative closeness between the lethal and sublethal
373 responses for the same duration of exposure, which in most cases range from
374 2 – 5 days. In general terms, LOEC values are usually lower than EC50 values
375 and mainly fall somewhere between the EC20 and EC50, but can exceed the
376 EC50 in some cases (Moore and Caux 1997).

377 $$LSR_{FELS} = \frac{\text{LC50 observed}}{\text{EC50TxSn or LOECTxSn}} \quad (6)$$

378 The $TxSn_{FELS}$ response is also considered to result from baseline toxicity for any
379 chemical acting as a baseline toxicant (TR_{LC50} <10) and exhibits an LSR_{FELS}
380 value <10. LSR_{FELS} values <10 for specific-acting toxicants (defined as
381 exhibiting a TR_{LC50} >10) also highlight the closeness of the lethal and sublethal
382 $TxSn_{FELS}$ responses for this group of chemicals.

383 A more important comparison for this analysis is the ratio between the
384 $LOEC_{TxSn}$ (or $EC50_{TxSn}$) and what is termed the LOEC for specific or low-dose
385 toxicity ($LOEC_{specific}$). This ratio is termed the toxic ratio for sublethal responses
386 ($TR_{subleth}$). This is similar to the TR_{LC50} described above and is also used to
387 quantify the difference between short-term sublethal $TxSn_{FELS}$ effects for early

life-stage fish and additional sublethal apical responses for fish without regard to life-stage or exposure duration.

$$TR_{subleth} = \frac{\text{LOECTxSn or EC50TxSn}}{\text{LOECspecific}} \quad (7)$$

$TR_{subleth}$ values <10 indicate close agreement between other sublethal responses and the ELS toxicity syndrome responses indicating baseline toxicity. Values >10 indicate greater toxicant potency and responses that were likely elicited via a receptor (specific toxicity). The intent of the $TR_{subleth}$ values is to compare the concentrations known to cause the $TxSn_{FELS}$ responses to other sublethal LOEC values for fish for a variety of chemicals. Originally, the TR concept was developed to highlight differences between baseline and specific acting toxicants for a lethal response. In the present application, sublethal values are used to highlight the range in non-lethal responses for a given compound and to highlight the most sensitive responses.

Additional data are provided on the $TxSn_{FELS}$ in terms of measured whole-body tissue concentrations. Observed values for various early-life stage responses were compared to predicted values as outlined above. Tissue concentrations provided by each study were converted to mmol/kg values and compared to the predicted values determined above with equation 1 (substituting with sublethal EC or LOEC values to determine effective residue (ERp; p=proportion responding) values or lowest observed effect residue (LOER) values.

2.3 Comparing fish ELS toxicity metrics and mammalian LD50 values.

An additional analysis included a comparison between the predicted LR50 for early-life stage fish toxicity tests and mammalian oral LD50 values. The predicted LR50 values for ELS fish were determined with equation 1, using a QSAR modeled BCF (equation 2). Mammal oral LD50 data were obtained from PubChem (2020). The LR50 (=CBR) is determined with the acquired dose and is distinguished from the LD50, which is determined with the administered

dose (Meador et al. 2011). The LR50 accounts for variation attributable to uptake and elimination, whereas the LD50 does not. The oral LD50 is an acute response that is expected to be higher than the LR50 due to reduced uptake from the alimentary canal into the body and elimination loss of the toxicant before mortality is recorded. As a result of elimination (metabolism, passive diffusion, and excretion) or reduced uptake, the LD50 is usually lower than the acquired dose (LR50) associated with toxicity. In many cases values for the LR50 and LD50 can be very close if rates of elimination are relatively slow, most of the toxicant is assimilated, and the time course for toxicity to develop is short such as with an acute response.

3. Results

A large number of studies report effects for early-life stages of fish when exposed to high concentrations. The ELS fish syndrome of decreased heart rate, yolk sac and cardiac edemas, and a variety of morphological abnormalities are extremely common over a very wide range of compounds and species. The data presented in Figure 1 and Tables 1 and 2 include a variety of compounds, from ethanol to ethinylestradiol (EE2) demonstrated to cause the $TxSn_{FELS}$. Many of these compounds cause mortality and the morphological effects in embryos at high concentrations, but also cause effects at lower concentrations, possibly due to specific action on one or more receptors. A good example of this is EE2, exhibiting mortality and the $TxSn_{FELS}$ at baseline concentrations, but specific sublethal effects in fish at concentrations that are several orders of magnitude lower. Many of these compounds appear to be only baseline toxicants resulting in mortality and the $TxSn_{FELS}$ at high concentration with no known effects at lower concentrations. In some cases, this may be due to a lack of reported data by researchers and in other cases the conclusion supports only a baseline response (minimal toxicity) because no specific receptor has been identified.

443 The TR_{LC50} values for all compounds, except cyanide, diclofenac, retinoic acid,
444 and tralopyril (an antifouling biocide) in Table 1 were <10 indicating baseline
445 toxicity in terms of fish early-life stage mortality. Genistein exhibited mixed
446 results for the TR_{LC50}, therefore its action as a baseline toxicant is not certain.
447 The geometric mean value for all TR_{LC50} values in Table 1 was 1.37. Even for
448 those chemicals with a TR_{LC50} >10, all exhibited a LSR_{FELS} <10, except retinoic
449 acid (TR_{LC50} =0.1 and LSR_{FELS} = 19), but only for one of two studies. Overall, the
450 lethal to sublethal ratio (LSR_{FELS}) shows that the $TxSn_{FELS}$ values were very
451 close to the lethality values for almost all compounds (geometric mean = 1.35)
452 and most were consistent with baseline toxicity.

453 Most of the compounds examined exhibited both baseline toxicity in terms of
454 the $TxSn_{FELS}$ and a presumed specific response at exposure concentrations that
455 were often orders of magnitude lower (Table 2). In many cases the specific
456 response was characterized in a wider variety of species compared to the ELS
457 tests; however zebrafish accounted for more than half of the results and a high
458 percentage of all values were from ELS fish. Additionally, many of the specific
459 toxicity results were from fish embryos and many of the exposures were less
460 than 5 days. While species are known to exhibit variable responses to toxicants
461 as a result of variability in bioaccumulation or potency (McElroy et al. 2011;
462 Wassenaar et al. 2020; McCarty et al. 2013), in all cases specific toxicity
463 endpoints were always lower for a given compound and not correlated to K_{ow}.
464 Figure 2 shows all compounds considered in this analysis with ELS effect
465 concentrations plotted against $\log_{10}K_{ow}$ along with the two baseline toxicity
466 reference lines (CTMC and CCTC). Interestingly, the CBR-BCF based CCTC
467 provided good predictions for all chemicals except DMSO and ethanol, which
468 exhibited CBR toxicity values far above predicted values. The membrane-based
469 CTMC assumes linearity over all K_{ow} values and was better at predicting toxicity
470 for those two compounds. The regression between $\log_{10}K_{ow}$ and log LC50
471 without the four suspected non-baseline toxicants with TR_{LC50} values > 10

(tralopyril, cyanide, diclofenac, and retinoic acid) indicated a high correlation with an r^2=0.84 (slope coefficient p<0.0001). The regression between $\log_{10}K_{ow}$ and the $LOEC_{specific}$ values for specific toxicity resulted in an r^2=0.04 (slope coefficient p=0.26).

Table 2 also shows the ratio between the mean $TxSn_{FELS}$ value ($LOEC_{TxSn}$ or $EC50_{TxSn}$) and the specific toxicity value ($LOEC_{specific}$) for each compound ($TR_{subleth}$). Most values for this ratio indicated specific toxic response metrics in fish that were 2 – 4 orders of magnitude lower than those observed for the early-life stage fish responses and many that were more extreme. Interestingly, the $TR_{subleth}$ values for DMSO and ethanol were slightly higher than the >10 cutoff value. It is not clear if these two compounds can act via a receptor or if they are only capable of baseline toxicity; however one study observed large reductions in acetylcholinesterase for adult zebrafish exposed to both of these compounds (Audira et al. 2020). It is important to note that a far higher percentage of studies in Table 2 quantified exposure concentrations, which is the opposite of that for the acute studies in Table 1.

Interestingly DMSO, which is used as a carrier compound in many of the $TxSn_{FELS}$ toxicity studies to enhance compound solubility causes the same abnormal effects in fish embryos at 0.5% - 1.5% v/v (0.07 mM) (Selderslaghs et al. 2009), and may contribute to the baseline toxicity response. Safe levels are assumed to be less than 0.1% v/v (Selderslaghs et al. 2009); however, behavioral effects have been observed at that concentration (Audira et al. 2020).

As seen in Table 3, most of the predicted tissue concentrations for both $TxSn_{FELS}$ and the LR50 fall within the range of values determined for baseline toxicity (mean = 2.8; range 0.29 – 27 mmol/kg), with notable exceptions for DMSO and ethanol. Predicted values higher than expected for baseline toxicity can result from higher levels of elimination or metabolism (faster half-life),

500 inaccuracies in estimating bioaccumulation and K_{ow}, and higher concentrations
501 needed for a whole-body effect due to a high proportion of the toxicant being
502 associated with yolk (Halbach et al. 2020). Only the compounds identified as
503 non-baseline toxicants (cyanide, diclofenac, and retinoic acid) in this table
504 exhibited lower than the expected range, which likely indicates a specific
505 mechanism of action (e.g., uncoupling of oxidative phosphorylation) and not
506 baseline toxicity.

507 Several observed $TxSn_{FELS}$ and LR50 whole-body concentrations are shown in
508 Table 4, which are very close to expected baseline values. For those studies
509 with available aqueous concentrations, predicted tissue residue values were
510 generated for comparison. Even though most observed values were within the
511 range expected for baseline lethal toxicity, predicted values were
512 overestimated for many compounds, likely as a consequence of inaccurate
513 estimation of the BCF. Figure 4 shows all lethal, specific toxicity, and mean
514 $TxSn_{FELS}$ responses in terms of predicted whole-body tissue concentrations for
515 fish in mmol/kg listed in Table 3. Regressions for all values against $\log_{10}K_{ow}$
516 indicate no correlation for the LR50 (r^2 = 0.028) or $TxSn_{FELS}$ (r^2 = 0.032),
517 which is contrary to that for aqueous exposure concentrations (Figure 2) and
518 indicates the consistency of response across a variety of compounds and K_{ow}
519 when based on whole-body concentrations.

520 3.1 Fish ELS and mammalian lethal values

521 The data shown in Table 3 also highlight the fish early-life stage lethal toxicity
522 values in relation to those values for mammals. The mammalian oral LD50 for
523 most compounds occurs within the range described for baseline lethal toxicity
524 for fish (mean = 2.8 mM, range = 0.29 – 27 mM whole body) (McCarty et al.
525 2013) (Figure 3). Interestingly, the ratio of fish and mammalian LR50 and
526 LD50 values shown in Table 3 indicate a high degree of similarity for the lethal
527 response with most ratios falling within a factor of 5 (geometric mean = 1.6).

In light of the inaccuracies expected for predicting fish LR50s and the variability due to uptake and elimination kinetics for the mammalian oral LD50s, these values are considered very close. Notable exceptions include ethanol, diclofenac, and parathion with high ratios, indicating mammals are far more sensitive to these compounds physiologically or that elimination is much slower than that for fish embryos perhaps as a function of the surface to volume ratio. In the case of parathion, it is likely that zebrafish embryos at 24 hours post fertilization (hpf) accumulated baseline lethal concentrations before acetylcholinesterase could be inhibited resulting in the large disparity noted for mammals (Yen et al. 2011). Noteworthy is the large difference between the mammalian LD50 and fish LR50 for retinoic acid, indicating that ELS fish are far more sensitive to this compound compared to mammals. As noted above, LD50 or LR50 values above the benchmark of 2.8 mM likely indicate the need for higher exposure doses to achieve a toxic response consistent with the expected baseline concentration. The compounds with the greatest variation from the benchmark baseline toxicity value are DMSO and ethanol, which occurred for both fish and mammals. Tralopyril and genistein were not included because no verifiable mammalian toxicity data were found.

4. Discussion

While there are a large number of compounds causing lethality and the $TxSn_{FELS}$ at high concentrations, many of these same compounds elicit apical sublethal effects in fish at exposure concentrations that are orders of magnitude lower, indicating a potential specific MoA. Of course a number of compounds reported to cause only lethal and $TxSn_{FELS}$ effects in fish may also cause effects via specific MoAs at far lower exposure concentrations but have not been adequately tested.

4.1 Additional review data

555 Four large reviews of embryo toxicity support the conclusions of the present
556 analysis. Knöbel et al. (2012) examined lethality and several endpoints
557 comprising the $TxSn_{FELS}$ including cardiac and yolk sac edema, deformation of
558 axis, tail, and head, reduced heart rate and delayed development for zebrafish
559 exposed from 1.5 – 50 hpf. Of the 59 compounds examined, 24 had
560 quantifiable LC50 and EC50 values for these endpoints resulting in a geometric
561 mean lethal to sublethal ratio (LSR_{FELS}) of 1.5, indicating similar values for
562 lethality and the $TxSn_{FELS}$. All LSR_{FELS} values were <10. Only two compounds
563 exhibited toxicity that was 10 fold higher than the indicator for baseline toxicity
564 (TR_{LC50}), rotenone and menadione (TR_{LC50}=273 and 24, respectively). Excess
565 menadione can result in anemia by depolarizing mitochondria via redox cycling
566 or inhibition of complex I, which likely results in developmental effects.
567 Rotenone is an inhibitor of complex I resulting in a decline of ATP (Pinho et al.
568 2013).

569 Another study (Yamashita et al. 2014) exposed zebrafish embryos from 5 –
570 144 hpf and reported on lethal values (100% lethality) and a combined
571 morphological score (MS50) consisting of edemas, blood flow abnormalities,
572 and morphological anomalies. At these test concentrations, almost all
573 chemicals exhibited baseline toxicity in terms of lethality and the $TxSn_{FELS}$.
574 Only adriamycin exhibited a TR_{LC50} value > 10 (TR_{LC50} =11.8) indicating the
575 possibility of non-baseline toxicity. High concentrations of adriamycin are
576 cytotoxic affecting membrane integrity via lipid peroxidation. For the 27
577 compounds with quantifiable LSR_{FELS} values, all were less than 10 (geometric
578 mean = 3.0) indicating closeness in lethality and the $TxSn_{FELS}$.

579 The third review is from Jarque et al. (2020) who tested 31 compounds and
580 measured 16 ELS endpoints for zebrafish exposed from 0 – 96 hpf yielding 24
581 compounds with both an LC50 and EC50 for sublethal effects. Many of these
582 endpoints are part of the $TxSn_{FELS}$ described here. In that study the most
583 sensitive endpoint was used to determine teratogenicity; however the mean of

584 all endpoints is a more representative metric and consistent with other
585 reviews. Based on the mean of all ELS endpoints for each compound to
586 determine the EC50, the LSR_{FELS} comparing the lethal to sublethal metrics was
587 less than 5 for all compounds (geometric mean = 1.9), which supports the
588 conclusion of a severe effect for these responses. Two of the compounds
589 (Ochratoxin and diethylaminobenzaldehyde [DEAB]) exhibited TR_{LC50} values
590 >10 (20 and 29.4, respectively) and were therefore were considered to be
591 non-baseline toxicants. Ochratoxin is known to affect calcium homeostasis at
592 nanomolar concentrations (Kőszegi and Poór 2016).

593 The Ali et al. (2014) study reported results for 53 compounds with calculable
594 $LOEC_{TxSn}$ and LC50 values. All of the TR_{LC50} values were less than 10, except
595 for phenelzine (= 23.9), indicating that almost all were responding as baseline
596 toxicants. It is worth noting that 68% of the chemicals in that study exhibited
597 a $\log_{10}K_{ow}$ <2 (≈ 50% with a $\log_{10}K_{ow}$ < 1), supporting the observation of
598 baseline toxicity with limited bioaccumulation. All LSR_{FELS} values in Ali et al.
599 (2014) were less than 10 (geometric mean = 2.75), supporting the conclusion
600 that $TxSn_{FELS}$ is a severe, near lethal response. Another review that assessed
601 several $TxSn_{FELS}$ responses for zebrafish at 96 hpf (von Hellfeld et al. 2020)
602 was not used because specific data were not presented and details on toxicity
603 metric calculations were not provided. A cursory examination of the 42
604 chemicals with LC50 and EC50 values reported by von Hellfeld et al. (2020)
605 exhibited a very low LSR_{FELS} (geometric mean =1.97). Only ethanol exhibited
606 an LSR_{FELS} >10 (=14.1), which is contrary to the value seen in Table 1. Even
607 though some of these studies did not adhere to OECD (2013) guidelines, the
608 ratio between lethal and sublethal values was used because it was expected to
609 reflect the differences in those metrics even if exposure concentrations were
610 not accurate.

611 As proposed by Meador and Nahrgang (2019), the fish ELS toxicity syndrome
612 is the hallmark of sublethal effects for baseline toxicants and specific acting

613 compounds affecting calcium cycling. For most compounds, $TxSn_{FELS}$ effects
614 occur at water and tissue concentrations that are close to lethal or within a
615 factor of 10 (Meador and Nahrgang 2019). Of all the compounds examined in
616 this analysis, including the specific acting chemicals listed above, only one
617 exhibited a ratio between mortality and the suite of sublethal abnormalities
618 comprising the ELS fish toxicity syndrome (LSR_{FELS}) that was greater than 10
619 (retinoic acid) and most were between 1 and 2. This is compelling evidence
620 that the $TxSn_{FELS}$ is generally a severe effect occurring at concentrations just
621 below those causing mortality even for specific-acting chemicals.

622 The general conclusion here is that many organic compounds exhibit baseline
623 toxicity (minimal toxicity) at high concentrations that is consistent with
624 predicted values as a function of partitioning and possible membrane and ionic
625 disruption. Noteworthy is that many of these chemicals can also cause specific
626 effects at far lower concentrations that are likely receptor mediated. The
627 concept of categorizing chemicals as exhibiting one mechanism of action is not
628 supported for those exhibiting baseline and specific toxicity that is dose, time,
629 life-stage, and possibly species dependent. For example, time is an important
630 factor as seen for EE2 exposure in zebrafish. The LC50 was reported to be
631 1,700 ng/mL in larval zebrafish for a 96 hour exposure and 0.1 ng/mL for adult
632 zebrafish exposed for 28 days (Schäfers et al. 2007). When compared among
633 other fish ELS toxicity results, the data for EE2 are consistent with baseline
634 toxicity; however long-term exposure is more consistent with a specific
635 response because EE2 is known to interact with the estrogen receptor (Shved
636 et al. 2008).

637 One of the important issues here is the extrapolation of toxic responses at high
638 concentrations to those expected with low-level exposure concentrations. As
639 seen in Figure 2, it is not possible to predict specific toxic effect doses based on
640 those causing baseline toxicity. Many authors demonstrate toxic responses at
641 elevated concentrations that are severe and usually not environmentally

642 relevant and assume similar responses will occur in these same or related
643 species at lower concentrations for these chemicals or in mixtures of related
644 chemicals (Meador and Nahrgang 2019; Kankaya et al. 2015; Audira et al.
645 2020). This is an assumption that is not supported by toxicological mechanism
646 or empirical evidence.

647 4.2 The evidence for baseline toxicity

648 The $TxSn_{FELS}$ appears to be a generalized response to a variety of organic
649 chemicals acting by baseline toxicity. While the concept of baseline toxicity is
650 not universally accepted, it is difficult to imagine how so many different
651 compounds can elicit mortality and the $TxSn_{FELS}$ at narrowly defined whole-
652 body and membrane concentrations and be considered a receptor based, lock
653 and key type of toxicant response. These compounds vary so widely in
654 stereochemistry that such receptor interactions are highly unlikely at these
655 exposure levels. Similar toxic potency among such a large number of organic
656 compounds for a wide range of species provides strong support for a non-
657 specific mechanism of action that is a function of compound hydrophobicity. It
658 is clear that toxic potency for receptor based toxicity is not a function of
659 physical partitioning (r^2=0.04), which is evident in Figure 2.

660 The observation that this syndrome is correlated with a physicochemical
661 parameter (octanol-water partition coefficient, K_{ow}) leads many to conclude
662 that membranes are physically altered affecting ionophore function or
663 ionophores are directly altered as a function of hydrophobic partitioning
664 resulting in ionic imbalance. This feature of a high correlation between a toxic
665 response and the K_{ow} implies that the toxic response is related to water-tissue
666 partitioning. As K_{ow} increases so does compound hydrophobicity and
667 consequently bioaccumulation. Hydrophobic compounds mostly associate with
668 storage lipids or membrane lipids and that amount will increase with increasing
669 K_{ow}. Because compounds sequestered by storage lipids are not toxicologically

active, the membrane or some other hydrophobic entity must be the target for these compounds. Various theories abound regarding the mechanism of action including changes to membrane fluidity (Sikkema et al. 1995), disturbance of membrane bound proteins, specifically ligand-gated ion channels (Escher et al. 2002), and interaction with lipid bound proteins or hydrophobic pockets on proteins (Franks and Lieb 1987). Studies such as van Wezel and Opperhuizen (1995) and Escher et al. (2002) demonstrate that membrane burdens within a narrow range of 40 – 343 mmol/kg lipid are inducing toxicity (mortality) for a large number of compounds. These measured values are consistent with a whole-body tissue concentration of 2.8 mM (0.29 – 27 mM) as determined by McCarty et al. (2013), which is based on an approximate whole-body lipid content of 5%. These values have been determined for bacteria, invertebrates, and fish and likely apply to all species ranging from single cell organisms to higher vertebrates.

It is important to note, that baseline toxicity is more than a correlation between aqueous effect concentrations and K_{ow}, it is also a function of internal concentrations that have been empirically determined for mortality at approximately 2 – 8 mmol/kg wet weight (McCarty and Mackay 1993; Escher and Hermens 2004; McCarty et al. 2013; van der Heijden et al. 2015). Sublethal effects for baseline toxicants generally occur at internal concentrations that are 10 fold lower or less compared to the lethal values (McCarty and Mackay 1993; Vergauwen et al. 2015). As explained in many publications, the internal concentration causing toxic effects occurs in a very narrow range compared to aqueous effect concentrations because the highly variable aspect of uptake and elimination kinetics among species is accounted for (Escher and Hermens 2004; Meador et al. 2008, 2011). As seen in Figure 2, the $TxSn_{FELS}$ responses follow the predicted values for baseline toxicity as a function of K_{ow} and generally fall within a factor of 10 for lethality. Because this prediction line represents minimal toxicity, compounds are not generally

699 above this line. Those plotted above this line are likely a result from errors in
700 dosing, analytical errors, estimation of partition coefficients, reduced uptake,
701 higher than normal lipid levels, or metabolic conversion of the toxicant
702 requiring higher exposure concentrations to achieve the baseline levels.
703 Additionally, some compounds will not exhibit baseline toxicity because of
704 solubility limits that preclude bioaccumulation to baseline toxicity levels.
705 Chrysene is one example of this phenomenon (Lin et al. 2015). Most
706 compounds exhibiting adverse responses acting by receptor-mediated
707 processes always fall below these baseline prediction lines and do not correlate
708 with K_{ow} or other physicochemical parameters.

709 It is also important to note that myriad physiological and genomic changes
710 have been reported for the $TxSn_{FELS}$ and these are not an indication of
711 response sensitivity. There are dozens of studies that show changes in gene
712 expression or altered physiological pathways in embryos exposed at high
713 concentrations such that they elicit the baseline $TxSn_{FELS}$ (Schiller et al. 2013;
714 Maharajan et al. 2018; Jin et al. 2015; Schüttler et al. 2017). Even at lethal
715 concentrations, myriad genomic and physiological changes are evident.

716 Interestingly, fish embryo exposure to plastic and metal nanoparticles is also
717 known to result in the $TxSn_{FELS}$ and it is a relatively common response
718 (Rojanasakul et al. 1993; Warren et al. 2015; Chakraborty et al. 2016; Wu et
719 al 2010; Zhang et al. 2020; Bai and Tang 2020). Toxic responses to
720 nanoparticles are also assumed to be a result of membrane damage and
721 alteration of ionic homeostasis; however other mechanisms may be responsible
722 for developmental abnormalities. In support of this, Meindl et al. (2015)
723 proposes assessing intracellular calcium levels to detect nanoparticle toxicity.
724 It is certainly possible that multiple mechanisms for baseline toxicity occur
725 such that membranes may be physically altered (e.g., nanoparticles) or
726 membrane proteins altered, each resulting in changes to ion homeostasis and
727 the $TxSn_{FELS}$ responses.

4.3 Potential molecular initiating event for baseline toxicity

Based on early studies such as the Meyer and Overton correlation of anesthetic potency and lipid/water partitioning (Franks 2006) it is tempting to equate baseline toxicity (also called narcosis by some researchers) with anesthetic action; however these are likely very different. As noted by Franks (2006) anesthetics generally act on neural tissue and targets such as GABA and glycine receptors, and in some cases potassium channels, which is not consistent with recent studies on baseline toxicity and calcium homeostasis.

The prevailing theory explaining baseline toxicity is disruption of intracellular calcium homeostasis, which may be caused by ATPase pump dysfunction, specifically sarco/endoplasmic reticulum (SR/ER) Ca^{2+}-ATPase (SERCA) (Antczak et al. 2015). In that study, transcriptional alterations resulting from abnormal intracellular calcium levels due to baseline toxicant exposure was largely recreated with thapsigargin, a highly specific SERCA inhibitor (Antczak et al. 2015). Antczak et al. (2015) concluded that calcium mobilization was a key event for baseline toxicity. Additional work by Schüttler et al. (2017) also identified important alterations to genes associated with calcium homeostasis in their meta-analysis of 33 studies and 60 compounds from zebrafish embryo tests, including ryanodine receptor genes that control release of calcium from the sarcoplasmic reticulum. As noted by Schüttler et al. (2017), calcium is important for cell cycle progression and downregulation of calcium binding or transport genes and disruption of calcium homeostasis in fish embryos before 24 hpf is common and can affect the heart, muscles, brain, and eye during development. These are key anatomical components of the fish early-life stage toxicity syndrome. Another study (Tsuruwaka et al. 2015) reported many of the $TxSn_{FELS}$ responses (pericardial edema and morphological abnormalities such as reduced eye) in embryonic zebrafish that was associated with disruption of calcium dynamics as a consequence of a loss of function by the gene WWOX (via knockdown), which is important for development.

Additionally, the detailed work by researchers examining crude oil toxicity has shown that cellular calcium imbalance is associated with the responses seen for the $TxSn_{FELS}$ when fish embryos are exposed to the water soluble fraction of crude oil (Sørhus et al. 2016). As hypothesized in Meador and Nahrgang (2019), alteration of calcium homeostasis appears to explain the observed data for ELS fish and crude oil toxicity, which is consistent with baseline toxicity.

There are several ways to elicit the $TxSn_{FELS}$ phenotype and most data appear to implicate ion channel dysfunction, via specific or non-specific action as discussed above. Several lines of evidence point to a link between alteration in SERCA2 expression/function, cardiac dysfunction and eventual development of characteristics of the $TxSn_{FELS}$ phenotype. SERCA2 is a Ca^{+2} ATPase found in the sarcoplasmic reticulum of myocytes. For example, a recent study by Kuo et al. (2021) provides strong support for involvement of the Yulink gene in intracellular cycling of calcium in cardiomyocytes and demonstrates that knockdown of Yulink can lead to decreased SERCA2 expression and cardiac dysfunction. The phenotype of the zebrafish Yulink knockdown exhibited many of the $TxSn_{FELS}$ characteristics (bradycardia, edemas, and scoliosis in addition to heart arrhythmia). The same abnormalities were also observed in zebrafish embryos by Ebert et al. (2005) with a SERCA2 morpholino or SERCA inhibitor (cyclopiazonic acid). It should be noted that disruption of other ion channels, such as Na^{+}/K^{+} ATPase, which is structurally very similar to SERCA can also elicit many of the $TxSn_{FELS}$ responses (Keßler et al. 2012; Shu et al. 2003).

As noted by Wray and Burdyga (2010), SERCA has evolved to function optimally in the SR/ER membrane that is thin because of low cholesterol and has high fluidity both of which are required for large conformational changes and optimal performance. The mechanism for baseline toxicity focuses on membrane disruption at a critical chemical concentration (100 mmol/kg membrane) (Escher et al. 2002). In light of the high membrane fluidity requirement for SERCA, its function may be susceptible to alterations as

toxicant concentrations increase to critical levels. Important here is the observation of Endo et al. (2011) who noted that the membrane-water partition coefficient (K_{lipw}) was higher (10 fold) for the accumulation of organic chemicals in membranes with low cholesterol content. Because the SR/ER membrane contains a very low percentage of cholesterol (Wray and Burdyga 2010) to maintain fluidity it is likely to be impacted by high internal concentrations before other membranes achieve critical levels and thus exhibit heightened sensitivity to disruption, which may result in SERCA dysfunction. Based on the high fluidity requirement for SERCA, the recapitulation with gene knockouts and inhibitors, and the potential propensity of the SR/ER to accumulate higher organic xenobiotic concentrations compared to other membranes, disruption of calcium homeostasis due to SERCA inhibition is a compelling hypothesis for the mechanism describing baseline toxicity and the $TxSn_{FELS}$.

4.4 Specific acting toxicants and the $TxSn_{FELS}$

Based on this random selection of chemicals exhibiting $TxSn_{FELS}$ effects with quantifiable LOEC and LC50 values, only a few were found to cause responses at exposure concentrations below those associated with baseline levels. Specific acting compounds noted here include cyanide, tralopyril, diclofenac, and retinoic acid from Table 1, in addition to rotenone, phenelzine, menadione, adriamycin, ochratoxin, and DEAB from the four reviews, with TR_{LC50} values >10, but LSR_{FELS} <10. Cyanide was reported to cause edema, morphological abnormalities, and impaired swim bladder inflation at 1.75 ppm exposure concentration in 24 hpf zebrafish (Carbaugh et al. 2020). These concentrations were just below the LC50 of 2.3 ppm and toxicity values are approximately one order of magnitude lower than predicted baseline toxicity based on the $\log_{10}K_{ow}$ of 0.1. Cyanide is an uncoupler of oxidative phosphorylation and also appears to cause disruption in calcium homeostasis, which was noted by Johnson et al. (1987) who reported large increases in

cytosolic calcium in cells (PC12 cells) after exposure to cyanide. Another uncoupler, tralopyril, also causes mortality and the $TxSn_{FELS}$ in zebrafish embryos at concentrations below those for baseline toxicity (Chen et al. 2020); however the ratio between mortality and the $TxSn_{FELS}$ (LSR_{FELS}) is only 1.42. Diclofenac also exhibited non-baseline toxicity and is a suspected cardiovascular toxicant (Chen et al. 2014). All-trans retinoic acid has been shown to reduce intracellular calcium and affect Ca^{+2} channels (de Hoog et al. 2018), which is consistent with the proposed mechanism of action for baseline toxicity, likely a result of specific action on the retinoic acid receptor (RXR). RXR is also the target for tributyltin (TBT) causing adverse effects in fish and invertebrates at concentrations that are about 5 orders of magnitude below expected baseline toxicity values (Lagadic et al. 2017). As noted by Zhang et al (2011; 2012), TBT can cause spinal abnormalities and edema at very low water concentrations (0.1 ng/L) in rockfish embryos, which was attributed to significant decreases in Ca^{+2} ATPase. Pereira et al. (2019) also noted that TBT is cardiotoxic as a result of reduced sarcoplasmic reticulum calcium pump (SERCA) activity.

Compounds acting only at baseline water or tissue concentrations (TR_{LC50} <10) are considered non-specific disruptors of membranes, membrane proteins such as ionophores, or calcium flux, unless lower effect concentrations can be demonstrated. As noted above, only a few compounds were observed with $TxSn_{FELS}$ results at lower than baseline toxic concentrations (TR_{LC50} >10) and it most cases these occurred close to levels resulting in mortality. These responses may occur when the specific mechanism of action is alteration of calcium homeostasis such as direct inhibition of SERCA, Ca^{+2} ATPase, other pathways affecting calcium homeostasis, or specific effects on the cardiovascular system during development. Other MoAs and receptor interaction eliciting $TxSn_{FELS}$ responses at less than baseline concentrations are

likely; however in most cases the elicitation of these responses is generally a high-dose phenomenon resulting from baseline toxicity.

As reported by Scholz et al. (2018), a number of compounds caused mortality in fish embryos at concentrations far below those expected at baseline levels and were considered to act by a specific or reactive mechanism of action; however these were not evaluated for $TxSn_{FELS}$ responses. Many of the compounds with the highest TR_{LC50} values in Scholz et al. (2018) resulted in uncoupling of oxidative phosphorylation, methemoglobinemia, or were pesticides (dithiocarbamates and others), which are generally neurotoxic. The data presented by Klüver et al. (2016) also identifies a number of chemicals exhibiting a TR_{LC50} >10 for zebrafish embryos less than 120 hpf, indicating a specific or reactive mechanism of action. Many of these were pesticides, uncouplers, or reactive compounds. Future testing and analysis is needed to assess if compounds exhibiting a TR_{LC50} value >10 can also elicit the $TxSn_{FELS}$ at such low concentrations without specific action on calcium homeostasis and if an LSR_{FELS} value >10 is more than a rare occurrence.

4.5 Is the fish early-life stage toxicity syndrome relevant for mammalian species?

Comparison of tissue based toxicity metrics for fish embryos and mammals indicates that $TxSn_{FELS}$ assessment with fish embryos may not be informative given the generally high doses at which these effects occur. While zebrafish ELS are useful for studying a variety of human diseases and for drug discovery (Sarmah et al. 2016; Cassar et al. 2020), the toxicity syndrome occurring at baseline concentrations can be misinterpreted as a teratogenic response that is relevant for humans. Studies such as those by Brannen et al. (2010) and Selderslaghs et al. (2012) evaluating chemicals for teratogenic potential in zebrafish as surrogates for humans report the same suite of toxic responses as that for the $TxSn_{FELS}$. The list of endpoints shown in Brannen et al. (2010) for

zebrafish are those associated with the non-specific baseline response observed for a wide variety of compounds (Figure 1).

An examination of other research on teratogens indicates inconsistencies between the concentrations causing developmental effects in fish embryos and relevant concentrations for other species, such as humans. As seen in Sarmah and Marrs (2016), the zebrafish embryo studies are considered important for assessing cardiac effects for humans and other vertebrates after exposure to such chemicals as ethanol. The $TxSn_{FELS}$ tissue effect concentration for zebrafish embryos exposed to ethanol based is approximately 422 mM (Table 3), which is 3.2 times higher than the LD50 or lethal concentration for small mammals. Even though heart development is considered to be a very sensitive process (Sarmah and Marrs 2016), many of the chemicals studied using fish embryos cause effects only at concentrations that are close to lethal levels. For many chemicals, fish embryo toxicity tests highlighting developmental abnormalities do not appear to be especially sensitive or useful in risk assessment for higher vertebrates.

As seen in Table 1 of the present study, the fish embryo LC50 and $TxSn_{FELS}$ are very similar exhibiting a geometric mean for LSR_{FELS} values among all chemicals of 1.35 and their corresponding toxicity metrics in terms of whole-body tissue concentrations would also exhibit similar values. Also noteworthy is the similarity in the mean ratio between fish LR50 and mammal LD50 values for many compounds (Table 3), which fall within the range described for baseline toxicity (2.8 mM whole body). The point of this exercise is to examine the utility of studies claiming that the fish early-life stage test is useful for highlighting teratogenic effects that may be indicative of effects in humans and other mammals when based on a limited suite of endpoints (e.g., $TxSn_{FELS}$). The observation that these teratogenic effects in fish embryos occur at concentrations very close to those causing mortality in mammals renders them inefficacious for characterizing suspected effects in humans. Of course there

900 are known teratogens causing effects at concentrations far below baseline
901 levels in humans (e.g., Tilton et al. 2006; Ducharme et al. 2013); however,
902 using the zebrafish embryo test to discover such toxicants may not be
903 productive unless non-baseline toxicity effects can be demonstrated and
904 relevant teratogenic endpoints are assessed.

905 4.6 Confounding factors

906 It is important to note that the toxicity metrics (e.g., LCp or ECp) will vary with
907 time of exposure and developmental stage of the embryo. This is shown by
908 Selderslaghs et al. (2012) for several chemicals highlighting decreasing LC50
909 (increasing toxicity) and decreasing EC50 (teratogenicity) for zebrafish with
910 increasing embryo age (24 to 144 hpf). Additionally variability in test results
911 can occur by using inappropriate test volumes, insufficient solution renewal,
912 and excess biomass loading ratios.

913 Variability among studies for a given compound tested on the same species at
914 the same or similar life stage is common. One such example is for
915 phenanthrene and early-life stage fish responses. As noted in Zheng et al.
916 (2020) the $TxSn_{FELS}$ can vary by orders of magnitude as seen for zebrafish.
917 Unfortunately, many of these studies did not measure exposure
918 concentrations, which results in uncertainty regarding toxicity metrics. Even
919 for those that did measure water exposure concentrations the lack of tissue
920 concentrations in relation to toxic effects is another level of uncertainty
921 precluding definitive evaluation of toxic effects in relation to baseline toxicity
922 and for comparison among studies. Even measured tissue concentrations to
923 determine toxicity metrics (LR50, CBR) can exhibit variability as a function of
924 organism lipid content, types of lipids and proteins, partition coefficients (K_{ow}
925 or D_{ow}), toxic metabolites, and analytical extraction techniques (Endo 2016;
926 van der Heijden et al. 2014; McElroy et al. 2011). Improved experimental
927 design and consideration of confounding factors during experiments can reduce

928 variability considerably (van der Heijden et al. 2015). Estimated
929 bioaccumulation factors can also exhibit high variability. It is well known that
930 compounds with $\log_{10}K_{ow}$ values exceeding 6.5 (Arnot and Gobas 2006) may
931 take a long time to reach steady state or not bioaccumulate as much as
932 predicted due to steric hindrance.

933 An important physicochemical factor here is the partition coefficient used for
934 the QSARs. In most cases K_{ow} is sufficient to predict bioaccumulation, but not
935 always for ionizable organic compounds. The ten compounds with predicted
936 BCFs using the ionization corrected K_{ow} (D_{ow}) were generally similar to
937 observed BCFs; however some were not (Table S1). For example, propranolol
938 and pentachlorophenol exhibited far higher BCFs than expected based on their
939 predicted D_{ow}. Additional data and research on pH dependent toxicity, internal
940 effects concentrations, measured BCFs, and improved models such as the ion-
941 trapping model (Escher et al. 2020) are needed to correctly evaluate the
942 toxicity of ionized organic compounds.

943 **5. Conclusion**

944 The $TxSn_{FELS}$ consists of a variety of related responses including a reduced
945 heart rate, cardiac and yolk sac edema, and related morphological deformities
946 that are commonly induced by a generalized nonspecific toxicity response for
947 most chemicals occurring at high exposure concentrations. This does not
948 discount the fact that this syndrome of effects can also be induced at low
949 concentrations by specific receptor or ion channel mediated mechanisms;
950 however examples are not common. The key event for this baseline toxicity
951 response resulting in the ELS syndrome of toxic effects is hypothesized to
952 result from membrane disruption and alteration of Ca^{2+} ATPase (specifically
953 SERCA) leading to intracellular calcium imbalance. The $TxSn_{FELS}$ of effects may
954 also be caused by specific effects (receptor based) such as that from

uncouplers on Ca^{2+} ATPase or ionic gradients, in addition to receptor mediated action on other calcium dependent pathways or other cardiovascular effects.

The available data indicate that many of the responses noted for the $TxSn_{FELS}$ involve interrelated processes involving cardiac and circulatory dysfunction resulting in morphological abnormalities as a consequence of intracellular calcium imbalance. It is important to note that a variety of other responses for fish embryos are useful for determining teratogenicity and other impairments in vertebrates. The degree of commonality for this response syndrome from exposure to hundreds of chemicals is an important observation. It is unlikely that such a diverse suite of compounds can act via a lock and key style receptor interaction to result in this suite of correlated responses.

Of the more than two hundred chemicals considered in the present review, only one (retinoic acid) exhibited a lethal to sublethal ratio (LSR_{FELS}) >10, indicating that the $TxSn_{FELS}$ is generally a severe, near lethal response for almost all chemicals and is not indicative of toxicant potency. In general, the $TxSn_{FELS}$ is not a sensitive metric for the majority of compounds; however other apical responses observed in larvae, juveniles, or adults are usually more indicative of toxicodynamics or chemical potency. Even for those chemicals considered to be acting by non-baseline toxicity for ELS fish (e.g., several uncouplers including cyanide, rotenone, tralopyril, and dinitrophenol), essentially all exhibited $TxSn_{FELS}$ concentrations that were very close to exposure concentrations causing lethality. Most of those specific acting chemicals eliciting $TxSn_{FELS}$ type responses are suspected of altering calcium homeostasis, which is consistent with the hypothesized key initiating event for baseline toxicity. Since it is likely that other mechanisms of action are able to elicit the $TxSn_{FELS}$, it is therefore critical to separate baseline toxicity responses from those resulting from specific action in order to define the most relevant toxicity metrics.

Because the fish ELS toxicity syndrome generally occurs at concentrations just below those that are lethal, the results may not be useful for protecting species. Most of these compounds eliciting adverse effects at near lethal levels are also capable of causing population relevant responses at far lower environmentally relevant concentrations. Our goal should be to find the lowest toxic effect concentrations for use in risk assessment in support of environmental protection.

Acknowledgements

I appreciate the insightful reviews by Jasmine Nahrgang, UiT The Arctic University of Norway, Tromsø and 2 anonymous reviewers. This research was not funded by any specific grant or contract from agencies in the public, commercial, or not-for-profit sectors and was supported solely by NOAA NWFSC base funds.

Citations

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. doi: 10.1897/05-701r.1.

Ali S, van Mil HGJ, Richardson MK. 2011. Large-scale assessment of the zebrafish embryo as a possible predictive model in toxicity testing. PLoS ONE 6: e21076. https://doi.org/10.1371/journal.pone.0021076

Ali S., Aalders J., Richardson M. K. 2014. Teratological effects of a panel of sixty water-soluble toxicants on zebrafish development. *Zebrafish* 11:129–141. Doi:10.1089/zeb.2013.0901

Antczak, P., White, T.A., Giri, A., Michelangeli, F., Viant, M.R., Cronin, M.T.D., Vulpe, C., Falciani, F. 2015. Systems biology approach reveals a calcium-dependent mechanism for basal toxicity in *Daphnia magna*. Environ. Sci. Technol. 49:11132–11140. doi:10.1021/acs.est.5b02707

Arnot JA and Gobas APC. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14: 257–297. doi: 10.1139/A06-005

1015 Audira, G., Siregar, P., Chen, J.-R., Lai, Y.-H., Huang, J.-C., Hsiao, C.-D. 2020.
1016 Systematical exploration of the common solvent toxicity at whole organism
1017 level by behavioral phenomics in adult zebrafish. Environmental Pollution.
1018 doi: https://doi.org/10.1016/j.envpol.2020.115239.

1019 Bai, C., Tang, M. (2020). Toxicological study of metal and metal oxide
1020 nanoparticles in zebrafish. Journal of Applied Toxicology, 40:37-63. doi:
1021 10.1002/jat.3910

1022 Beker van Woudenberg, A., Snel, C., Rijkmans, E., de Groot, D., Bouma, M.,
1023 Hermsen, S., Piersma, A., Menke, A., & Wolterbeek, A. 2014. Zebrafish
1024 embryotoxicity test for developmental (neuro) toxicity: Demo case of an
1025 integrated screening approach system using anti-epileptic drugs. Repro Tox
1026 49:101–116. https://doi.org/10.1016/j.reprotox.2014.07.082

1027 Bittner L, Klüver N, Henneberger L, Mühlenbrink M, Zarfl C, Escher BI. 2019a.
1028 Combined ion-trapping and mass balance models to describe the pH-
1029 dependent uptake and toxicity of acidic and basic pharmaceuticals in
1030 zebrafish embryos (*Danio rerio*). Environ Sci Technol. 53:7877-7886. doi:
1031 10.1021/acs.est.9b02563.

1032 Bittner L, Teixidó E, Keddi I, Escher BI, Klüver N. 2019b. pH-dependent
1033 uptake and sublethal effects of antihistamines in zebrafish (*Danio rerio*)
1034 embryos. Environ Toxicol Chem 38:1012-1022. doi: 10.1002/etc.4395.

1035 Brannen KC, Panzica-Kelly JM, Danberry TL, Augustine-Rauch KA. 2010.
1036 Development of a zebrafish embryo teratogenicity assay and quantitative
1037 prediction model. Birth Defects Res B Dev Repro Toxicol. 89:66-77.
1038 doi:10.1002/bdrb.20223

1039 Bull CJ, McInenrey JE. 1974. Behavior of juvenile coho salmon (*Oncorhynchus*
1040 *kisutch*) exposed to Sumithion (fenitrothion), an organophosphate insecticide.
1041 J. Fish. Res. Board Can. 31: 1867 7872.

1042 Burggren WW. Developing animals flout prominent assumptions of ecological
1043 physiology. 2005. Comp Biochem Physiol A Mol Integr Physiol. 141:430-439.
1044 doi:10.1016/j.cbpb.2005.03.010

1045 Carbaugh CM, Widder MW, Phillips CS, Jackson DA, Valerie VT, DiVito T, van
1046 der Schalie WH, Glover KP. 2020. Assessment of zebrafish embryo
1047 photomotor response sensitivity and phase-specific patterns following acute-
1048 and long-duration exposure to neurotoxic chemicals and chemical weapon
1049 precursors. J Appl Toxicol. 40:1272-1283. doi:10.1002/jat.3984

1050 Cassar, S., Adatto, I., Freeman, J. L., Gamse, J. T., Iturria, I., Lawrence, C.,
1051 Muriana, A., Peterson, R. T., Van Cruchten, S., & Zon, L. I. 2020. Use of

1052 zebrafish in drug discovery toxicology. Chemical Res Tox 33:95–118.
1053 https://doi.org/10.1021/acs.chemrestox.9b00335

1054 Chakraborty, C., Sharma, A.R., Sharma, G., Lee, S.S., 2016. Zebrafish: A
1055 complete animal model to enumerate the nanoparticle toxicity. J.
1056 Nanobiotechnology 14:1–13. doi:10.1186/s12951-016-0217-6

1057 Chen, Y., Zhang, Y., Yu, Z., Guan, Y., Chen, R., Wang, C. 2021. Early life
1058 phenanthrene exposure inhibits reproductive ability in adult zebrafish and the
1059 mechanism of action, Chemosphere 272:129635.
1060 https://doi.org/10.1016/j.chemosphere.2021.129635

1061 Chen, J., Lei, L., Tian, L., Hou, F., Roper, C., Ge, X., Zhao, Y., Chen, Y., Dong,
1062 Q., Tanguay, R. L., & Huang, C. 2018. Developmental and behavioral
1063 alterations in zebrafish embryonically exposed to valproic acid (VPA): An
1064 aquatic model for autism. Neurotox terat 66:8–16.
1065 https://doi.org/10.1016/j.ntt.2018.01.002

1066 Chen, J. B., Gao, H. W., Zhang, Y. L., Zhang, Y., Zhou, X. F., Li, C. Q., & Gao,
1067 H. P. 2014. Developmental toxicity of diclofenac and elucidation of gene
1068 regulation in zebrafish (*Danio rerio*). Sci Repts 4:4841.
1069 https://doi.org/10.1038/srep04841

1070 Chen X, Teng M, Zhang J, Qian L, Duan M, Cheng Y, Zhao F, Zheng J, Wanget
1071 C. 2020. Tralopyril induces developmental toxicity in zebrafish embryo (*Danio*
1072 *rerio*) by disrupting the thyroid system and metabolism. Sci Tot Environ.
1073 141860. https://doi.org/10.1016/j.scitotenv.2020.141860

1074 de Hoog E, Lukewich MK, Spencer GE. 2018. Retinoic acid inhibits neuronal
1075 voltage-gated calcium channels.. Cell Calcium 72:51-61.
1076 doi:10.1016/j.ceca.2018.02.001

1077 Di Toro, D.; McGrath, J.; Hansen, D. 2000. Technical basis for narcotic
1078 chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and Tissue.
1079 Environ. Toxicol. Chem. 19:1951−1970. doi.org/10.1002/etc.5620190804

1080 Dixon DG, Leduc G. 1981. Chronic cyanide poisoning of rainbow trout and its
1081 effects on growth, respiration, and liver histopathology. Arch Environ Contam
1082 Toxicol. 10:117-31. doi: 10.1007/BF01057580.

1083 Duan Z, Zhu L, Zhu L, Kun Y, Zhu X. 2008. Individual and joint toxic effects of
1084 pentachlorophenol and bisphenol A on the development of zebrafish (*Danio*
1085 *rerio*) embryo. Ecotox Environ Saf. 71:774-780.
1086 doi:10.1016/j.ecoenv.2008.01.021

1087 Duarte, I. A., Reis-Santos, P., Novais, S. C., Rato, L. D., Lemos, M., Freitas, A.,
1088 Pouca, A., Barbosa, J., Cabral, H. N., & Fonseca, V. F. 2020. Depressed,

1089 hypertense and sore: Long-term effects of fluoxetine, propranolol and
1090 diclofenac exposure in a top predator fish. Sci Tot Environ 712:136564.
1091 https://doi.org/10.1016/j.scitotenv.2020.136564

1092 Ducharme NA, Peterson LE, Benfenati E, Reif D, McCollum CW, Gustafsson J-A,
1093 Bondesson M. 2013. Meta-analysis of toxicity and teratogenicity of 133
1094 chemicals from zebrafish developmental toxicity studies. Repro Toxicol.
1095 41:98-108. doi:10.1016/j.reprotox.2013.06.070

1096 Ebert AM, Hume GL, Warren KS, Cook NP, Burns CG, Mohideen MA, Siegal G,
1097 Yelon D, Fishman MC, Garrity DM. 2005. Calcium extrusion is critical for
1098 cardiac morphogenesis and rhythm in embryonic zebrafish hearts. Proc Natl
1099 Acad Sci 102(49):17705-10. doi: 10.1073/pnas.0502683102.

1100 Endo S. 2016. Re-analysis of narcotic critical body residue data using the
1101 equilibrium distribution concept and refined partition coefficients. Environ Sci
1102 Process Impacts. 18:1024-1029. doi: 10.1039/c6em00180g.

1103 Endo S, Escher BI, Goss KU. 2011. Capacities of membrane lipids to
1104 accumulate neutral organic chemicals. Environ Sci Technol. 45:5912-21. doi:
1105 10.1021/es200855w.

1106 Escher BI, Abagyan R, Embry M, Klüver N, Redman AD, Zarfl C, Parkerton TF.
1107 2020. Recommendations for improving methods and models for aquatic
1108 hazard assessment of ionizable organic chemicals. Environ Toxicol Chem.
1109 39:269-286. doi: 10.1002/etc.4602.

1110 Escher, B.I., Ashauer, R., Dyer, S., Hermens, J.L.M., Lee, J.H., Leslie, H.A.,
1111 Mayer, P., Meador, J.P., Warnekk, M.S.J., 2011. Crucial role of mechanisms
1112 and modes of toxic action for understanding tissue residue toxicity and
1113 internal effect concentrations of organic chemicals. Integr. Environ. Assess.
1114 Manag. 7:28–49. doi:10.1002/ieam.100

1115 Escher, B.I., Eggen, R.I.L., Schreiber, U., Schreiber, Z., Vye, E., Wisner, B.,
1116 Schwarzenbach, R.P. 2002. Baseline toxicity (narcosis) of organic chemicals
1117 determined by in vitro membrane potential measurements in energy-
1118 transducing membranes. Environ. Sci. Technol. 36:1971–1979.
1119 doi:10.1021/es015844c

1120 Escher, B.I., Hermens, J.L.M. 2002. Modes of action in ecotoxicology: Their
1121 role in body burdens, species sensitivity, QSARs, and mixture effects.
1122 Environ. Sci. Technol. 36:4201–4217. doi:10.1021/es015848h

1123 Escher BI, Hermens JL. 2004. Internal exposure: linking bioavailability to
1124 effects. Environ Sci Technol. 38:455A-462A. doi: 10.1021/es0406740.

1125 Faria M, Ziv T, Gómez-Canela C, Ben-Lulu S, Prats E, Novoa-Luna KA, Admon
1126 A, Piña B, Tauler R, Gómez-Oliván LM, Raldúa D. 2018. Acrylamide acute
1127 neurotoxicity in adult zebrafish. Sci Rep. 8:7918. doi: 10.1038/s41598-018-
1128 26343-2.

1129 Forner-Piquer, I., Beato, S., Piscitelli, F., Santangeli, S., di Marzo, V., Habibi,
1130 H.R., Maradonna, F., Carnevali, O., 2020. Effects of BPA on zebrafish gonads:
1131 Focus on the endocannabinoid system, Environmental Pollution 264:114710.
1132 doi: https://doi.org/10.1016/j.envpol.2020.114710

1133 Franks NP. Molecular targets underlying general anaesthesia. 2006. Br J
1134 Pharmacol. 147(Suppl 1):S72-81. doi: 10.1038/sj.bjp.0706441.

1135 Franks NP and Lieb WR. 1987. What is the molecular nature of general
1136 anaesthetic target sites? Trends in Pharmacological Sciences 8:169-174.
1137 doi.org/10.1016/0165-6147(87)90160-X.

1138 Fraysse B, Mons R, Garric J. 2006. Development of a zebrafish 4-day embryo-
1139 larval bioassay to assess toxicity of chemicals. Ecotoxicol Environ Saf.
1140 63:253-67. doi: 10.1016/j.ecoenv.2004.10.015.

1141 Fu W., Franco A., Trapp S. 2009. Methods for estimating the bioconcentration
1142 factor of ionizable organic chemicals. Env. Toxicol. Chem. 28:1372-1379.
1143 DOI: 10.1897/08-233.1

1144 Giltrow, E., Eccles, P. D., Winter, M. J., McCormack, P. J., Rand-Weaver, M.,
1145 Hutchinson, T. H., & Sumpter, J. P. 2009. Chronic effects assessment and
1146 plasma concentrations of the beta-blocker propranolol in fathead minnows
1147 (*Pimephales promelas*). Aquat Tox 95:195–202.
1148 https://doi.org/10.1016/j.aquatox.2009.09.002

1149 Goss K-U., Endo S. 2016. Comment on "Application of the Activity Framework
1150 for Assessing Aquatic Ecotoxicology Data for Organic Chemicals." Environ Sci
1151 Technol 50:4139–4140. DOI: 10.1021/acs.est.5b05534

1152 Guo, J., Wu, P., Cao, J., Luo, Y., Chen, J., Wang, G., Guo, W., Wang, T., & He,
1153 X. 2019. The PFOS disturbed immunomodulatory functions via nuclear
1154 Factor-κB signaling in liver of zebrafish (*Danio rerio*). Fish & shellfish
1155 Immunology 91:87–98. https://doi.org/10.1016/j.fsi.2019.05.018

1156 Halbach K, Ulrich N, Goss KU, et al. 2020. Yolk sac of zebrafish embryos as
1157 backpack for chemicals? Environ Sci Technol. 54:10159-10169.
1158 doi:10.1021/acs.est.0c02068

1159 Hicken, C.E., Linbo, T.L., Baldwin, D.H., Willis, M.L., Myers, M.S., Holland, L.,
1160 Larsen, M., Stekoll, M.S., Rice, S.D., Collier, T.K., Scholz, N.L., Incardona,
1161 J.P. 2011. Sublethal exposure to crude oil during embryonic development

1162 alters cardiac morphology and reduces aerobic capacity in adult fish. Proc.
1163 Natl. Acad. Sci. 108, 7086–7090. doi:10.1073/pnas.1019031108

1164 Hidaka H, Tatsukawa R. 1989. Avoidance by olfaction in a fish, medaka
1165 (*Oryzias latipes*), to aquatic contaminants. Environ Pollut. 56:299-309.
1166 doi:10.1016/0269-7491(89)90075-4

1167 Horie Y, Yamagishi T, Takahashi H, Shintaku Y, Iguchi T, Tatarazako N. 2017.
1168 Assessment of the lethal and sublethal effects of 20 environmental chemicals
1169 in zebrafish embryos and larvae by using OECD TG 212. J. Appl. Toxicol.
1170 37:1245–1253. doi:10.1002/jat.3487

1171 Huang, Q., Liu, Y., Chen, Y., Fang, C., Chi, Y., Zhu, H., Lin, Y., Ye, G., & Dong,
1172 S. 2018. New insights into the metabolism and toxicity of bisphenol A on
1173 marine fish under long-term exposure. Environ Poll 242:914–921.
1174 https://doi.org/10.1016/j.envpol.2018.07.048

1175 Incardona, J. Scholz, N.L. 2018. Case Study: The 2010 Deepwater Horizon Oil
1176 Spill and its environmental developmental impacts. In: Development and
1177 Environment, Burggren W. and Dubansky B. (eds). Chapter 10, pp. 235-283.
1178 doi:10.1007/978-3-319-75935-7

1179 Incardona JP, Collier TK, Scholz NL. 2004. Defects in cardiac function precede
1180 morphological abnormalities in fish embryos exposed to polycyclic aromatic
1181 hydrocarbons. Toxicol Appl Pharmacol. 196:191-205. doi:
1182 10.1016/j.taap.2003.11.026.

1183 Jantzen CE, Annunziato KA, Bugel SM, Cooper KR. 2016. PFOS, PFNA, and
1184 PFOA sub-lethal exposure to embryonic zebrafish have different toxicity
1185 profiles in terms of morphometrics, behavior and gene expression. Aquat
1186 Toxicol. 175:160-170. doi:10.1016/j.aquatox.2016.03.026

1187 Jarque S, Rubio-Brotons M, Ibarra J, Ordoñez V, Dyballa S, Miñana R,
1188 Terriente J. 2020. Morphometric analysis of developing zebrafish embryos
1189 allows predicting teratogenicity modes of action in higher vertebrates. Reprod
1190 Toxicol. 96:337-348. doi.org/10.1016/j.reprotox.2020.08.004

1191 Jin Y, Liu Z, Peng T, Fu Z. 2015. The toxicity of chlorpyrifos on the early life
1192 stage of zebrafish: a survey on the endpoints at development, locomotor
1193 behavior, oxidative stress and immunotoxicity. Fish Shellfish Immunol.
1194 43:405-414. doi:10.1016/j.fsi.2015.01.010

1195 Johnson JD, Conroy WG, Isom GE. 1987. Alteration of cytosolic calcium levels
1196 in PC12 cells by potassium cyanide. Toxicol Appl Pharmacol. 88:217-24. doi:
1197 10.1016/0041-008x(87)90007-x.

1198 Kankaya, E., Kaptaner, B., Doğan, A., Çelik, I. 2015. Toxicity of bisphenol a
1199 during the early life stages of *Chalcalburnus tarichi* (Pallas, 1811). Fresenius
1200 Environ. Bull. 24:977–985.

1201 Keßler M, Just S, Rottbauer W. 2012. Ion flux dependent and independent
1202 functions of ion channels in the vertebrate heart: lessons learned from
1203 zebrafish. *Stem Cells Int*. 2012:462161. doi:10.1155/2012/462161

1204 Kiparissis Y, Balch GC, Metcalfe TL, Metcalfe CD. 2003. Effects of the
1205 isoflavones genistein and equol on the gonadal development of Japanese
1206 medaka *Oryzias latipes*. Environ Health Perspect. 111:1158-1163.
1207 doi:10.1289/ehp.5928

1208 Kipka U and Di Toro DM. 2009. Technical basis for polar and nonpolar narcotic
1209 chemicals and polycyclic aromatic hydrocarbon criteria. III. A polyparameter
1210 model for target lipid partitioning. Environmental Toxicology and
1211 Chemistry28:1429–1438. DOI: 10.1897/08-364.1

1212 Keshari, V., Adeeb, B., Simmons, A.E., Simmons, T.W., Diep, C.Q. 2016.
1213 Zebrafish as a model to assess the teratogenic potential of nitrite. J. Vis. Exp.
1214 108, e53615, doi:10.3791/53615.

1215 Klüver N, Vogs C, Altenburger R, Escher BI, Scholz S. 2016. Development of a
1216 general baseline toxicity QSAR model for the fish embryo acute toxicity test.
1217 Chemosphere164:164-173. doi.org/10.1016/j.chemosphere.2016.08.079.

1218 Knöbel, M., Busser, F.J.M., Rico-Rico, Á., Kramer, N.I., Hermens, J.L.M.,
1219 Hafner, C., Tanneberger, K., Schirmer, K., Scholz, S., 2012. Predicting adult
1220 fish acute lethality with the zebrafish embryo: Relevance of test duration,
1221 endpoints, compound properties, and exposure concentration analysis.
1222 Environ. Sci. Technol. 46:9690–9700. doi:10.1021/es301729q

1223 Kőszegi T, Poór M. 2016. Ochratoxin A: Molecular interactions, mechanisms of
1224 toxicity and prevention at the molecular level. Toxins (Basel) 8:111.
1225 doi:10.3390/toxins8040111

1226 Kuo MW, Tsai HH, Wang SH, Chen YY, Yu AL, Yu J. 2021. *Yulink*, predicted
1227 from evolutionary analysis, is involved in cardiac function. *J Biomed Sci*
1228 28:7. doi.org/10.1186/s12929-020-00701-7

1229 Lagadic L, Katsiadaki I, Biever R, Guiney PD, Karouna-Renier N, Schwarz T,
1230 and Meador JP. 2017. Tributyltin: Advancing the science on assessing
1231 endocrine disruption with an unconventional endocrine-disrupting
1232 compound. Reviews Environmental Contamination Toxicology 245:65-127.
1233 DOI: 10.1007/398_2017_8

1234 Länge, R., Hutchinson, T. H., Croudace, C. P., Siegmund, F., Schweinfurth, H.,
1235 Hampe, P., Panter, G. H., & Sumpter, J. P. 2001. Effects of the synthetic
1236 estrogen 17 alpha-ethinylestradiol on the life-cycle of the fathead minnow
1237 (*Pimephales promelas*). Environ Tox Chem 20:1216–1227.
1238 https://doi.org/10.1897/1551-5028(2001)020<1216:eotsee>2.0.co;2

1239 Li S, Sun Q, Wu Q, Gui W, Zhu G, Schlenk D. 2019. Endocrine disrupting
1240 effects of tebuconazole on different life stages of zebrafish (*Danio rerio*).
1241 Environ Pollut. 249:1049-1059. doi:10.1016/j.envpol.2019.03.067

1242 Liu N, Jin X, Zhou J, Wang Y, Yang Q, Wu F, Giesy JP, Johnson AC. 2018.
1243 Predicted no-effect concentration (PNEC) and assessment of risk for the
1244 fungicide, triadimefon based on reproductive fitness of aquatic organisms.
1245 Chemosphere 207:682-689. doi: 10.1016/j.chemosphere.2018.05.093.

1246 Liu HC, Chu TY, Chen LL, Gui WJ, Zhu GN. 2017a. The cardiovascular toxicity
1247 of triadimefon in early life stage of zebrafish and potential implications to
1248 human health. Environ Pollut 231:1093-1103.
1249 doi:10.1016/j.envpol.2017.05.072

1250 Liu H, Chu T, Chen L, Gui W, Zhu G. 2017b. In vivo cardiovascular toxicity
1251 induced by acetochlor in zebrafish larvae. Chemosphere 181:600-608.
1252 doi:10.1016/j.chemosphere.2017.04.090

1253 Maharajan K, Muthulakshmi S, Nataraj B, Ramesh M, Kadirvelu K. 2018.
1254 Toxicity assessment of pyriproxyfen in vertebrate model zebrafish embryos
1255 (*Danio rerio*): A multi biomarker study. Aquat Toxicol. 196:132-145. doi:
1256 10.1016/j.aquatox.2018.01.010.

1257 Maharajan K, Muthulakshmi S, Karthik C, Nataraj B, Nambirajan K, Hemalatha
1258 D, Jiji S, Kadirvelu K, Liu K-C, Ramesh M. 2020. Pyriproxyfen induced
1259 impairment of reproductive endocrine homeostasis and gonadal
1260 histopathology in zebrafish (*Danio rerio*) by altered expression of
1261 hypothalamus-pituitary-gonadal (HPG) axis genes. Sci Tot Environ 139496.
1262 https://doi.org/10.1016/j.scitotenv.2020.139496

1263 McCarty, L.S., Arnot, J.A., Mackay, D. 2013. Evaluation of critical body residue
1264 data for acute narcosis in aquatic organisms. Environ. Toxicol. Chem.
1265 32:2301–2314. doi:10.1002/etc.2289

1266 McCarty, L.S., Landrum, P.F., Luoma, S.N., Meador, J.P., Merten, A.A.,
1267 Shephard, B.K., van Wezel, A.P. 2011. Advancing environmental toxicology
1268 through chemical dosimetry: external exposures versus tissue residues.
1269 Integr. Environ. Assess. Manag. 7:7-27. http://dx.doi.org/10.1002/ieam.98.

McCarty, L.S., Mackay, D. 1993. Enhancing ecotoxicological modeling and assessment: body residues and modes of toxic action. Environ. Sci. Technol. 27:1719-1728. doi.org/10.1021/es00046a001

McCarty, LS. 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. Environ Tox Chem 5:1071-1080.

McCollum CW, Ducharme NA, Bondesson M, Gustafsson JA. 2011. Developmental toxicity screening in zebrafish. Birth Defects Res C Embryo Today 93:67-114. doi:10.1002/bdrc.20210

McElroy, A.E., Barron, M.G., Beckvar, N., Kane Driscoll, S.B., Meador, J.P., Parkerton, T.F., Preuss, T.G., Steevens, J.A. 2011. A review of the tissue residue approach for organic and organometallic compounds in aquatic organisms. Integr. Environ. Assess. Manage. 7:50−74. Doi: 10.1002/ieam.132

McGrath P. 2012. Use of emerging models for developmental toxicity testing. In: McGrath P, editor. Zebrafish: Methods for assessing drug safety and toxicity. John Wiley & Sons, Inc. Chapter 3. pp. 27–44.

Meador JP. 1997. Comparative toxicokinetics of tributyltin in five marine species and its utility in predicting bioaccumulation and acute toxicity. Aquatic Tox 37:307-326. doi.org/10.1016/S0166-445X(96)00827-2

Meador, JP. 2006. Rationale and procedures for using the tissue-residue approach for toxicity assessment and determination of tissue, water, and sediment quality guidelines for aquatic organisms. Hum. Ecol. Risk Assess. 12:1018-1073. doi:10.1080/10807030600801535

Meador, J.P., Adams, W.J., Escher, B.I., McCarty, L.S., McElroy, A.E., Sappington, K.G. 2011. The tissue residue approach for toxicity assessment: Findings and critical reviews from a Society of Environmental Toxicology and Chemistry Pellston workshop. Integr. Environ. Assess. Manag. 7:2–6. doi:10.1002/ieam.133

Meador, J.P., McCarty, L.S., Escher, B.I., Adams, W.J., 2008. The tissue-residue approach for toxicity assessment: concepts, issues, application, and recommendations. J. Environ. Monit. 10:1486-1498. http://dx.doi.org/10.1039/b814041n

Meador JP and Nahrgang J. 2019. Characterizing crude oil toxicity to early-life stage fish based on a complex mixture: Are we making unsupported assumptions? Environmental Science and Technology 53:11080-11092. doi: 10.1021/acs.est.9b02889

1307 Meindl, C., Kueznik, T., Bösch, M., Roblegg, E., Fröhlich, E. 2015. Intracellular
1308 calcium levels as screening tool for nanoparticle toxicity. J. Appl. Toxicol.
1309 35:1150–1159. doi:10.1002/jat.3160

1310 Moore DRJ and Caux P-Y. 1997. Estimating low toxic effects. Environ. Toxicol.
1311 Chem. 16:794-801. DOI:10.1002/ETC.5620160425

1312 Moreman J, Lee O, Trznadel M, David A, Kudoh T, Tyler CR. 2017. Acute
1313 toxicity, teratogenic, and estrogenic effects of bisphenol A and its alternative
1314 replacements bisphenol S, bisphenol F, and bisphenol AF in zebrafish
1315 embryo-larvae. Environ Sci Technol. 51:12796-12805.
1316 doi:10.1021/acs.est.7b03283

1317 Mylroie JE, Wilbanks MS, Kimble AN, To KT, Cox CS, McLeod SJ, Gust KA,
1318 Moore DW, Perkins EJ, Garcia-Reyero N. 2021. Perfluorooctanesulfonic acid-
1319 induced toxicity on zebrafish embryos in the presence or absence of the
1320 chorion. Environ Toxicol Chem. 40:780-791. doi: 10.1002/etc.4899.

1321 Ni J, Wang H, Wei X, Shen K, Sha Y, Dong Y, Shu Y, Wan X, Cheng J, Wang F,
1322 and Liu Y. 2020. Isoniazid causes heart looping disorder in zebrafish embryos
1323 by the induction of oxidative stress. BMC Pharm Toxicol 21, 22.
1324 https://doi.org/10.1186/s40360-020-0399-2

1325 OECD (2013), *Test No. 236: Fish Embryo Acute Toxicity (FET) Test*, OECD
1326 Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris,
1327 https://www.oecd-ilibrary.org/environment/test-no-236-fish-embryo-acute-
1328 toxicity-fet-test_9789264203709-en

1329 Parrott JL, Balakrishnan VK. 2017. Life-cycle exposure of fathead minnows to
1330 environmentally relevant concentrations of the β-blocker drug propranolol.
1331 Environ Toxicol Chem. 36:1644-1651. doi: 10.1002/etc.3703.

1332 Pecquet AM, Maier A, Kasper S, Sumanas S, Yadav J. 2020. Exposure to
1333 perfluorooctanoic acid (PFOA) decreases neutrophil migration response to
1334 injury in zebrafish embryos. BMC Res Notes. 13:408. doi: 10.1186/s13104-
1335 020-05255-3.

1336 Peng X, Sun X, Yu M, Fu W, Chen H, Chen J. 2019. Chronic exposure to
1337 environmental concentrations of phenanthrene impairs zebrafish
1338 reproduction. Ecotoxicol Environ Saf. 182:109376.
1339 doi:10.1016/j.ecoenv.2019.109376

1340 Pereira CLV, Ximenes CF, Merlo E, A S Sciortino, AS, Monteiro JS, Moreira
1341 A, Jacobsen BB, Graceli JB, Ginsburg KS, Ribeiro Junior RF, Bers
1342 DM, Stefanon I. 2019. Cardiotoxicity of environmental contaminant
1343 tributyltin involves myocyte oxidative stress and abnormal Ca^{2+} handling.
1344 Environ Pollut. 247:371-382. doi:10.1016/j.envpol.2019.01.053

1345 Pinho BR, Santos MM, Fonseca-Silva A, Valentão P, Andrade PB, Oliveira JM.
1346 2013. How mitochondrial dysfunction affects zebrafish development and
1347 cardiovascular function: an in vivo model for testing mitochondria-targeted
1348 drugs. Br J Pharmacol. 169:1072-90. doi: 10.1111/bph.12186

1349 Pohl, J., Ahrens, L., Carlsson, G., Golovko, O., Norrgren, L., Weiss, J., & Örn,
1350 S. 2019. Embryotoxicity of ozonated diclofenac, carbamazepine, and
1351 oxazepam in zebrafish (*Danio rerio*). Chemosphere 225:191–199.
1352 https://doi.org/10.1016/j.chemosphere.2019.03.034

1353 PubChem (2020). National Center for Biotechnology Information. PubChem
1354 Database. https://pubchem.ncbi.nlm.nih.gov (accessed on July 24, 2021)

1355 PubMed (2020). National Center for Biotechnology Information.
1356 http://www.ncbi.nlm.nih.gov/pubmed. (accessed on July 24, 2021)

1357 Qiang L, Cheng J, Yi J, Rotchell JM, Zhu X, Zhou J. 2016. Environmental
1358 concentration of carbamazepine accelerates fish embryonic development and
1359 disturbs larvae behavior. Ecotoxicology 25:1426-1437. doi:10.1007/s10646-
1360 016-1694-y

1361 Qiu, W., Zhan, H., Tian, Y., Zhang, T., He, X., Luo, S., Xu, H., & Zheng, C.
1362 2018. The in vivo action of chronic bisphenol F showing potential immune
1363 disturbance in juvenile common carp (*Cyprinus carpio*). Chemosphere 205:
1364 506–513. doi.org/10.1016/j.chemosphere.2018.04.105

1365 Rojanasakul, Y., Wang, L., Malanga, C.J., Ma, J.Y.C., Banks, D.E., Ma, J.K.H.,
1366 1993. Altered calcium homeostasis and cell injury in silica-exposed alveolar
1367 macrophages. J. Cell. Physiol. 154, 310–316. doi:10.1002/jcp.1041540214

1368 Sancho E, Ferrando MD, Andreu E. 1997. Sublethal effects of an
1369 organophosphate insecticide on the European eel, *Anguilla anguilla*.
1370 Ecotoxicol Environ Saf. 36:57-65. doi:10.1006/eesa.1996.1488

1371 Sandahl JF, Baldwin DH, Jenkins JJ, Scholz NL. 2005. Comparative thresholds
1372 for acetylcholinesterase inhibition and behavioral impairment in coho salmon
1373 exposed to chlorpyrifos. Environ Toxicol Chem. 24:136-145. doi:10.1897/04-
1374 195r.1

1375 Sarasquete C, Úbeda-Manzanaro M, Ortiz-Delgado JB. 2018. Toxicity and non-
1376 harmful effects of the soya isoflavones, genistein and daidzein, in embryos of
1377 the zebrafish, *Danio rerio*. Comp Biochem Physiol C Toxicol Pharmacol.
1378 211:57-67. doi: 10.1016/j.cbpc.2018.05.012.

1379 Sarmah S, Marrs JA. 2016. Zebrafish as a vertebrate model system to evaluate
1380 effects of environmental toxicants on cardiac development and function. Int J
1381 Mol Sci. 17:2123. doi: 10.3390/ijms17122123.

1382 Schäfers C, Teigeler M, Wenzel A, Maack G, Fenske M, Segner H. 2007.
1383 Concentration- and time-dependent effects of the synthetic estrogen,
1384 17alpha-ethinylestradiol, on reproductive capabilities of the zebrafish, *Danio*
1385 *rerio*. J Toxicol Environ Health A. 70:768-779.
1386 doi:10.1080/15287390701236470

1387 Schiller V, Wichmann A, Kriehuber R, Schäfers C, Fischer R, Fenske M. 2013.
1388 Transcriptome alterations in zebrafish embryos after exposure to
1389 environmental estrogens and anti-androgens can reveal endocrine disruption.
1390 Reprod Toxicol. 42:210-223. doi:10.1016/j.reprotox.2013.09.003

1391 Schiwy S, Herber AK, Hollert H, Brinkmann M. 2020. New insights into the
1392 toxicokinetics of 3,4-dichloroaniline in early life stages of zebrafish (*Danio*
1393 *rerio*). Toxics 8:16. doi:10.3390/toxics8010016

1394 Scholz S, Schreiber R, Armitage J, Mayer P, Escher BI, Lidzba A, Léonard M,
1395 Altenburger R. 2018. Meta-analysis of fish early life stage tests - association
1396 of toxic ratios and acute-to-chronic ratios with modes of action. Environ
1397 Toxicol Chem. 37:955-969. doi: 10.1002/etc.4090.

1398 Schüttler, A., Reiche, K., Altenburger, R., Busch, W., 2017. The transcriptome
1399 of the zebrafish embryo after chemical exposure: A meta-analysis. Toxicol.
1400 Sci. 157, 291–304. doi:10.1093/toxsci/kfx045

1401 Schwaiger, J., Spieser, O. H., Bauer, C., Ferling, H., Mallow, U., Kalbfus, W., &
1402 Negele, R. D. 2000. Chronic toxicity of nonylphenol and ethinylestradiol:
1403 haematological and histopathological effects in juvenile common carp
1404 (*Cyprinus carpio*). Aquat Tox 51:69–78. https://doi.org/10.1016/s0166-
1405 445x(00)00098-9

1406 Schwaiger J, Ferling H, Mallow U, Wintermayr H, Negele RD. 2004. Toxic
1407 effects of the non-steroidal anti-inflammatory drug diclofenac. Part I:
1408 histopathological alterations and bioaccumulation in rainbow trout. Aquat
1409 Toxicol. 68:141-150. doi:10.1016/j.aquatox.2004.03.014

1410 Selderslaghs IW, Van Rompay AR, De Coen W, Witters HE. 2009. Development
1411 of a screening assay to identify teratogenic and embryotoxic chemicals using
1412 the zebrafish embryo. Reprod Toxicol. 28:308-320.
1413 doi:10.1016/j.reprotox.2009.05.004

1414 Selderslaghs IW, Blust R, Witters HE. 2012. Feasibility study of the zebrafish
1415 assay as an alternative method to screen for developmental toxicity and
1416 embryotoxicity using a training set of 27 compounds. Reprod Toxicol.
1417 33:142-154. doi:10.1016/j.reprotox.2011.08.003

1418 Shu X, Cheng K, Patel N, Chen F, Joseph E, Tsai HJ, Chen JN. 2003. Na, K-
1419 ATPase is essential for embryonic heart development in the zebrafish.
1420 Development. 130:6165-73. doi: 10.1242/dev.00844.

1421 Shved N, Berishvili G, Baroiller JF, Segner H, Reinecke M. 2008.
1422 Environmentally relevant concentrations of 17alpha-ethinylestradiol (EE2)
1423 interfere with the growth hormone (GH)/insulin-like growth factor (IGF)-I
1424 system in developing bony fish. Toxicol Sci. 106:93-102.
1425 doi:10.1093/toxsci/kfn150

1426 Sikkema J, de Bont JA, Poolman B. 1995. Mechanisms of membrane toxicity of
1427 hydrocarbons. Microbiol Rev. 59:201-222

1428 Sørhus, E., Incardona, J.P., Karlsen, Ø., Linbo, T., Sørensen, L., Nordtug, T.,
1429 Van Der Meeren, T., Thorsen, A., Thorbjørnsen, M., Jentoft, S., Edvardsen,
1430 R.B., Meier, S. 2016. Crude oil exposures reveal roles for intracellular calcium
1431 cycling in haddock craniofacial and cardiac development. Sci. Rep. 6:1–21.
1432 doi:10.1038/srep31058

1433 Tillitt DE, Papoulias DM, Whyte JJ, Richter CA. 2010. Atrazine reduces
1434 reproduction in fathead minnow (*Pimephales promelas*). Aquat Toxicol.
1435 99:149-159. doi:10.1016/j.aquatox.2010.04.011

1436 Tilton F, La Du JK, Vue M, Alzarban N, Tanguay RL. 2006. Dithiocarbamates
1437 have a common toxic effect on zebrafish body axis formation. Toxicol Appl
1438 Pharmacol. 216:55-68. doi:10.1016/j.taap.2006.04.014

1439 Truong, L., Bugel, S. M., Chlebowski, A., Usenko, C. Y., Simonich, M. T.,
1440 Simonich, S. L., & Tanguay, R. L. 2016. Optimizing multi-dimensional high
1441 throughput screening using zebrafish. Repro Tox 65:139–147.
1442 https://doi.org/10.1016/j.reprotox.2016.05.015

1443 Tsuruwaka Y, Konishi M, Shimada E. 2015. Loss of *WWOX* expression in
1444 zebrafish embryos causes edema and alters Ca^{2+} dynamics. PeerJ 3:*e727*
1445 https://doi.org/10.7717/peerj.727

1446 van der Heijden SA, Hermens JL, Sinnige TL, Mayer P, Gilbert D, Jonker MT.
1447 2015. Determining high-quality critical body residues for multiple species and
1448 chemicals by applying improved experimental design and data interpretation
1449 concepts. Environ Sci Technol. 49:1879-1887. doi: 10.1021/es505078r.

1450 van Wezel AP & Opperhuizen A. 1995 Narcosis due to environmental pollutants
1451 in aquatic organisms: residue-based toxicity, mechanisms, and membrane
1452 burdens. Crit Rev Tox 25:255-279. DOI:10.3109/10408449509089890.

1453 von Hellfeld R, Brotzmann K, Baumann L, Strecker R, and Braunbeck T. 2020.
1454 Adverse effects in the fish embryo acute toxicity (FET) test: a catalogue of

1455 unspecific morphological changes versus more specific effects in zebrafish
1456 (*Danio rerio*) embryos. Environ Sci Eur 32:122. doi.org/10.1186/s12302-020-
1457 00398-3

1458 Veith, G.D., DeFoe, D.L., Bergstedt, B.V., 1979. Measuring and estimating the
1459 bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. 36:1040-
1460 1048. doi.org/10.1139/f79-146.

1461 Vergauwen, L., Schmidt, S.N., Stinckens, E., Maho, W., Blust, R., Mayer, P.,
1462 Covaci, A., Knapen, D. 2015. A high throughput passive dosing format for the
1463 fish embryo acute toxicity test. Chemosphere 139:9–17.
1464 doi.org/10.1016/j.chemosphere.2015.05.041

1465 Vogs C, Johanson G, Näslund M, Wulff S, Sjödin M, Hellstrandh M, Lindberg J,
1466 Wincent E. 2019. Toxicokinetics of perfluorinated alkyl acids influences their
1467 toxic potency in the zebrafish embryo (*Danio rerio*). Environ Sci Technol.
1468 53:3898-3907. doi:10.1021/acs.est.8b07188.

1469 Wang, W., Ru, S., Wang, L., Wei, S., Zhang, J., Qin, J., Liu, R., & Zhang, X.
1470 2020. Bisphenol S exposure alters behavioral parameters in adult zebrafish
1471 and offspring. Sci Tot Environ 741:140448.
1472 https://doi.org/10.1016/j.scitotenv.2020.140448

1473 Wang Y, Chen J, Du C, Li C, Huang C, Dong Q. 2014. Characterization of
1474 retinoic acid-induced neurobehavioral effects in developing zebrafish. Environ
1475 Toxicol Chem. 33:431-7. doi: 10.1002/etc.2453.

1476 Warren, E.A.K., Payne, C.K., 2015. Cellular binding of nanoparticles disrupts
1477 the membrane potential. RSC Adv. 5:13660-13666 doi:10.1039/c4ra15727c

1478 Wassenaar PNH, Verbruggen, EMJ. Cieraad E, Peijnenburg WJGM, Vijver, MG.
1479 2020. Variability in fish bioconcentration factors: Influences of study design
1480 and consequences for regulation. Chemosphere 124731.
1481 DOI:10.1016/j.chemosphere.2019.124731

1482 Wiegand, C., Krause, E., Steinberg, C., & Pflugmacher, S. 2001. Toxicokinetics
1483 of atrazine in embryos of the zebrafish (*Danio rerio*). Ecotox Environ Safe
1484 49:199–205. https://doi.org/10.1006/eesa.2001.2073

1485 Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson
1486 D, Li C, Sayeeda Z, Assempour N, Iynkkaran I, Liu Y, Maciejewski A, Gale N,
1487 Wilson A, Chin L, Cummings R, Le D, Pon A, Knox C, Wilson M. 2017.
1488 DrugBank 5.0: A major update to the DrugBank database for 2018. Nucleic
1489 Acids Res. doi:10.1093/nar/gkx1037.

1490 Wray S, Burdyga T. 2010. Sarcoplasmic reticulum function in smooth muscle.
1491 Physiol Rev. 90:113-78. doi:10.1152/physrev.00018.2008.

1492 Wu, Y.; Zhou, Q.; Li, H.; Liu, W.; Wang, T.; Jiang, G. 2010. Effects of silver
1493 nanoparticles on the development and histopathology biomarkers of Japanese
1494 medaka (*Oryzias latipes*) using the partial life test. Aquat. Toxicol.
1495 100:160−167. DOI: 10.1016/j.aquatox.2009.11.014

1496 Xiong X, Luo S, Wu B, Wang J. 2017. Comparative developmental toxicity and
1497 stress protein responses of dimethyl sulfoxide to rare minnow and zebrafish
1498 embryos/larvae. Zebrafish 14:60-68. doi:10.1089/zeb.2016.1287

1499 Yamashita A, Inada H, Chihara K, Yamada T, Deguchi J, Funabashi H. 2014.
1500 Improvement of the evaluation method for teratogenicity using zebrafish
1501 embryos. J Toxicol Sci. 39:453-464. doi:10.2131/jts.39.453

1502 Yen J, Donerly S, Levin ED, Linney EA. 2011. Differential acetylcholinesterase
1503 inhibition of chlorpyrifos, diazinon and parathion in larval zebrafish.
1504 Neurotoxicol Teratol. 33:735-741. doi:10.1016/j.ntt.2011.10.004

1505 Zhang R, Silic MR, Schaber A, Wasel O, Freeman JL, Sepúlveda MS. 2020.
1506 Exposure route affects the distribution and toxicity of polystyrene
1507 nanoplastics in zebrafish. Sci Total Environ. 724:138065.
1508 doi:10.1016/j.scitotenv.2020.138065

1509 Zhang Y, Xue W, Long R, Yang H, Wei W. 2020. Acetochlor affects zebrafish
1510 ovarian development by producing estrogen effects and inducing oxidative
1511 stress. Environ Sci Pollut Res Int 27:27688-27696. doi:10.1007/s11356-
1512 020-09050-2

1513 Zhang J, Zuo Z, Wang Y, Yu A, Chen Y, Wang C. 2011. Tributyltin chloride
1514 results in dorsal curvature in embryo development of *Sebastiscus*
1515 *marmoratus* via apoptosis pathway. Chemosphere. 82:437-42. doi:
1516 10.1016/j.chemosphere.2010.09.057.

1517 Zhang J, Zuo Z, Sun P, Wang H, Yu A, Wang C. 2012. Tributyltin exposure
1518 results in craniofacial cartilage defects in rockfish (*Sebastiscus marmoratus*)
1519 embryos. Mar Environ Res 77:6-11. doi: 10.1016/j.marenvres.2011.12.008.

1520 Zheng, Y., Li, Y., Yue, Z., Samreen, Li, Z., Li, X., Wang, J. 2020. Teratogenic
1521 effects of environmentally relevant concentrations of phenanthrene on the
1522 early development of marine medaka (*Oryzia melastigma*), Chemosphere
1523 254: 126900. doi.org/10.1016/j.chemosphere.2020.126900.

1524 Zoupa M, Machera K. 2017. Zebrafish as an alternative vertebrate model for
1525 investigating developmental toxicity-the triadimefon example. Int J Mol Sci.
1526 18:817. doi:10.3390/ijms18040817

Figure legends

Figure 1. Plot showing effect concentrations (μM aqueous) for lethality (LC50 or lethal LOEC) (red X) and the fish early-life stage toxicity syndrome ($TxSn_{FELS}$) (all other symbols except circle). $LOEC_{specific}$ (circle) shows specific toxicity (non-syndrome) responses. Compounds ordered by increasing $\log_{10}K_{ow}$. Details provided in Tables 1 and 2.

Figure 2. Plot of effect concentrations against $\log_{10}K_{ow}$ for all compounds in Figure 1. Paired fish early-life stage toxicity effect concentrations for lethality (LC50 or lethal LOEC) (red X) and the $TxSn_{FELS}$ (all other symbols except circle). $LOEC_{specfic}$ values (circle) show other apical responses (non $TxSn_{FELS}$). Broken line represents the Constant CBR Toxicity Concentration (CCTC) determined with equation 3. The solid line shows the Constant Toxic Membrane Concentration (CTMC) determined with equation 5. All effect concentrations as $\log_{10}$ μM aqueous. The linear regression between $\log_{10}K_{ow}$ and log LC50 (without TR_{LC50} >10 compounds) was highly correlated (r^2=0.84, slope coefficient $p<0.0001$). The regression between $\log_{10}K_{ow}$ and the $LOEC_{specific}$ resulted in an r^2=0.04 (slope coefficient p=0.26). See text, Figure 1, and Tables 1 and 2 for details.

Figure 3. Comparison of fish early-life stage mortality response and mammal oral LD50 values. Blue circles are predicted lethal whole-body residue values for early life-stage fish (LR50) and red diamonds show oral LD50 values for mammals as mM whole-body. Compounds are ordered from left to right by increasing $\log_{10}K_{ow}$. Solid line represents LR50 baseline toxicity value (2.8 mM) (final non-PAH value) and broken lines show the range for those data (McCarty et al. 2013). Far right symbols show geometric mean and standard error for all plotted fish and mammal data. The full range of expected toxicity values shown on y-axis (0.1 nM to 1 M). See text for details on LR50 estimation and distinction between LR50 and LD50. All data from Table 3.

Figure 4. Lethal and sublethal toxicity values based on whole-body concentrations for fish. LR50, mean $TxSn_{FELS}$, and specific toxicity values as predicted whole-body tissue concentrations (mmol/kg) plotted for each chemical and ordered by $\log_{10}K_{ow}$ or D_{ow}. Dashed lines show upper and lower limits for expected lethal and sublethal baseline toxicity values. Upper solid line shows the regression for LR50 values versus $\log_{10}K_{ow}$ (r^2 = 0.028). Lower solid line shows regression for $TxSn_{FELS}$ endpoints versus $\log_{10}K_{ow}$ (r^2=0.032). Mean $TxSn_{FELS}$ values show the standard error (most bars not visible).

Table 1. Fish early life stage toxicity. Lethal and toxicity syndrome ($TxSn_{FELS}$) responses.

Chemical	K_{ow}/ D_{ow}	MW	Spp	Stage	Time (d)	$TxSn_{FELS}$ responses –$LOEC_{TxSn}$ or $EC50_{TxSn}$ (mg/L)					Lethal				
						Heart rate reduced	Edema	Morph	Swim bladder inflation	Other	LC50 or LOEC (mg/L)	LSR_{FELS}	TR_{LC50}	Conc meas	Ref
DMSO	-1.35	78.1	1	2 – 168 hpf	4	15,000	20,000	20,000	15,000		15,000	0.87	0.01	N	11
DMSO	-1.35	78.1	3	2 – 168 hpf	4	15,000	15,000	20,000	20,000		20,000	1.14	0.01	N	11
Isoniazid	-0.7	137	1	4 – 72 hpf	3	21.0	21.0	21.0			87.0	4.14	3.2	N	20
Acrylamide	-0.67	71.1	1	5 - 120 hpf	5	140	140			140 (bf)	140	1.0	1.0	N	19
Ethanol	-0.3	46.1	1	2 – 120 hpf	4		13,800	13,800	13,800		36,212	2.6	0.003	N	16, 17
Caffeine	-0.07	194.2	1	24 – 72 hpf	3	640		74		305	691	2.0	0.6	N	8
Cyanide	0.1	26.0	1	8 – 120 hpf	0.7		1.75	1.75	1.75	1.75 (eye & jaw)	2.3	1.31	22.6	N	15
Diclofenac	1.1	296	1	2 – 192 hpf	4-8	7.0	7.8	9.5	7.9		6.9	0.86	74	N	1
Diclofenac	1.1	296	1	2 – 96 hpf	4		1.0	1.0			3.0	3.0	170	Y +	2
Diclofenac	1.1	296	1	2 – 144 hpf	2 -6	15.0	7.5			7.5 (bf)	3.8	0.38	134	Y	6
Valproic acid	1.37	144.2	1	24 – 96 hpf	4	24.0					31	1.33	4.4	Y +	7
Valproic acid	1.37	144.2	1	24 – 72 hpf	3	113		113		113 (bf)	195	1.73	0.72	N	8
Valproic acid	1.37	144.2	1	8 – 120 hpf	5		216	72	72					N	9

Chemical	K_{ow}/ D_{ow}	MW	Spp	Stage	Time (d)	$TxSn_{FELS}$ responses –$LOEC_{TxSn}$ or $EC50_{TxSn}$ (mg/L)					Lethal				
						Heart rate reduced	Edema	Morph	Swim bladder inflation	Other	LC50 or LOEC (mg/L)	LSR_{FELS}	TR_{LC50}	Conc meas	Ref
PFOA	1.58	414.1	1	2 – 120 hpf	5		210	210						Y+	22
PFOA	1.58	414.1	1	1 – 48 hpf	4						300	1.4	0.78	Y	28
Bisphenol S	1.6	250.3	1	0 – 96 hpf	4		200	200			199.0	1.0	0.8	Y +	21
Genistein	2.1	270.2	1	2 – 48 hpf	2		2.4	2.4		2.4 (bf)				N	4
Genistein	2.1	270.2	1	2 – 96 hpf	4						2.4	0.99	16	Y	30
Genistein	2.1	270.2		2 – 96 hpf	4		5	5			4.4	0.88	8.7	N	31
Retinoic acid (all trans)	2.37	300.4	1	24 – 144 hpf	3	0.008		0.008		0.008 (bf)	0.17	19.1	239	N	8
Retinoic acid (all trans)	2.37	300.4	1	24 – 72 hpf			0.002	0.0007	0.001		0.005	3.5	7,889	N	29
Carbamazapine	2.5	236.3	1	2 – 144 hpf	2 -6	100.0	100.0	50.0			75.0	0.90	0.3	Y	6
Carbamazapine	2.5	236.3	1	24 – 96 hpf	4		43.7				45.5	1.04	0.6	Y +	7
Atrazine	2.6	215.7	1	2 – 48 hpf	3-4	30.0	5 - 10	10 - 20		10 (bf)	10	0.57	1.9	Y	10
Triadimefon	2.8	293.8	1	48 – 72 hpf	1	37.4	37.4			18.7 (bf)	47.2	1.51	0.4	N	13
Triadimefon	2.8	293.8	1	6 – 120 hpf	5	4.0	4.0	4.0	2.00	4.0 length				N	14
Bisphenol F	2.9	200.2	1	0 – 96 hpf	4		10.0	27.5			32.0	1.71	0.3	Y +	21
Bisphenol A	3.3	228.3	2	2 –120 hpf	5	0.75	0.75	0.75	0.75		3.96	5.28	1.3	N	3

Chemical	K_{ow}/ D_{ow}	MW	Spp	Stage	Time (d)	$TxSn_{FELS}$ responses –$LOEC_{TxSn}$ or $EC50_{TxSn}$ (mg/L)					Lethal					
						Heart rate reduced	Edema	Morph	Swim bladder inflation	Other	LC50 or LOEC (mg/L)	LSR_{FELS}	TR_{LC50}	Conc meas	Ref
Bisphenol A	3.3	228.3	1	2 – 48 hpf	2		8.5	8.5		8.5 (bf)				N	4
Bisphenol A	3.3	228.3	1	0 – 96 hpf	4		5.0		10.0		12.0	1.6	0.4	Y +	21
Fenitrothion	3.3	144	1	2 – 192 hpf	4-8	2.5	3.5	3.4	2.25		3.2	1.1	1.0	N	1
Propranolol	3.3	259.3	1	2 – 192 hpf	4-8	5.0	10.9	24.6	5.3		8.8	0.77	0.6	N	1
17α-ethinylestradiol	3.7	294.4	1	2 – 48 hpf	2		0.8			0.8 (bf)	0.8	1.0	3.8	N	4
17α-ethinylestradiol	3.7	294.4	1	6 – 120 hpf	5		19.0	19.0			19.0	1.0	0.2	Y	5
Tebuconazole	3.7	236	1	2 – 192 hpf	4-8	7.0	10.1	27.6	11.0		10.8	0.78	0.2	N	1
Parathion	3.8	291.3	1	24 – 120 hpf	0.7		29.1	29.1		29.1 (eye)	22.4	0.76	0.1	N	15
PFOS	3.85	500.1	1	2 – 120 hpf	5		1.9	1.9						Y+	22
PFOS	3.85	500.1	1	2 – 120 hpf	5			1.5	2.55		2.25	1.1	1.8	Y	27
Acetochlor	4.0	269.8	1	48 – 120 hpf	3	2.4	9.6			4.8 (bf)	11.5	2.05	0.1	N	23
Penta-chlorophenol	4.3	266.3	1	8 – 72 hpf	5		0.08			0.20 (bf)	0.76	5.43	1.0	Y	12
Tralopyril	4.3	349.5	1	2 – 120 hpf	5	0.004	0.004	0.005	0.004		0.006	1.42	182	N	26
Phenanthrene	4.5	179.2	1	2 – 120 hpf	5		0.36	0.052	0.059	0.36 (bf)	0.31	1.49	1.2	Y +	25
Pyriproxyfen	4.9	312.4	1	3 – 96 hpf	4	1.6	0.33	1.6			0.22	0.18	1.4	Y	1, 18

Chemical	K_{ow}/ D_{ow}	MW	Spp	Stage	Time (d)	$TxSn_{FELS}$ responses –$LOEC_{TxSn}$ or $EC50_{TxSn}$ (mg/L)					Lethal				
						Heart rate reduced	Edema	Morph	Swim bladder inflation	Other	LC50 or LOEC (mg/L)	LSR_{FELS}	TR_{LC50}	Conc meas	Ref
Chlorpyrifos	5.3	350.6	1	1 – 96 hpf	4	0.010	0.30	0.30			0.10*	0.61	1.5	N	24
4-nonylphenol	5.9	220.3	1	2 – 192 dpf	4-8	0.3	1.0	0.28	0.23		0.29	0.64	0.1	N	1
Geo mean												1.35	1.37		

LOEC is the lowest effect concentration and EC50 is the effective concentration at 50%. LC50 is the observed lethal concentration for 50% of the individuals in a test. K_{ow} is the log_{10} octanol-water partition coefficient or $Log_{10}D_{ow}$ at pH 7.5 listed for compounds with a physiological charge other than 0 (Table S1). MW is the molecular weight in daltons. Stage is the life stage period and Time is the duration of the test in days. Hpf is hours post fertilization. Fish early life stage toxicity syndrome ($TxSn_{FELS}$) includes; edema (yolk sac and cardiac edema), morph is generally spinal curvature and other morphological abnormalities, swim bladder inflation denotes a failure to inflate, others such as reduced blood circulation noted below. Bf is reduced blood flow, br is blood regurgitation, loop is unlooped heart. Conc meas, indicates if aqueous exposure concentrations were measured (+= whole-body concentrations). LSR_{FELS} is the lethal to sublethal ratio between the LC50 (or lethal LOEC) and the mean value for all $TxSn_{FELS}$ sublethal values ($LOEC_{TxSn}$ or $EC50_{TxSn}$) (equation 6). All values are LOEC except for references 1, 4, 7, 8, 12, 15, and 25 (=$EC50_{TxSn}$). TR_{LC50} is the ratio between the predicted water concentration for lethal baseline toxicity (equation 3) and the observed LC50 or LOEC for mortality. * hatchability as a surrogate for lethality.

References (Ref). 1. Horie et al. (2017); 2. Chen et al. (2014); 3. Kankaya et al. (2015); 4. Schiller et al. (2013); 5. Truong et al. 2016; 6. Pohl et al. (2019); 7. Beker van Woudenberg et al. (2014); 8. Selderslaghs et al. (2009); 9. Chen et al. (2018); 10. Wiegand et al. (2001); 11. Xiong et al. (2017); 12. Duan et al. (2008); 13. Liu et al. (2017a); 14. Zoupa et al. (2017); 15. Carbaugh et al. (2020); 16. Keshari et al. (2016); 17. Ali et al. (2011); 18. Maharajan et al. (2018); 19. Huang et al. (2018); 20. Ni et al. (2020); 21. Moreman et al. (2017); 22. Vogs et al. (2019); 23. Liu et al. (2017b); 24. Jin et al. (2015); 25. Vergauwen et al. (2015); 26. Chen et al. (2020); 27. Mylroie et al. (2021); 28. Pecquet et al. (2020); 29. Wang et al. (2014); 30. Bittner et al. (2019a); 31 Sarasquete et al. (2018). Species (spp). 1. Zebrafish (*Danio rerio*), 2. Cyprinidae (*Chalcalburnus tarichi)*, 3. Rare minnow (*Gobiocypris rarus*).

Table 2. Non-toxicity syndrome responses for fish ($LOEC_{specific}$).

						Toxic responses				
Chemical	**K_{ow}/ D_{ow}**	**MW**	**Spp**	**Stage**	**T (d)**	**Effect**	**$LOEC_{specific}$ (µg/L) ppb**	**$TR_{subleth}$**	**Conc meas**	**Ref**
DMSO	-1.35	78.1	4	Adult	10	Altered behavior (one conc.)	1,000,000	17.5	N	30
Acrylamide	-0.67	71.1	4	Adult	3	Neurotoxicity and behave (several parameters) (tested one conc.)	53,000	2.6	Y	21
Ethanol	-0.3	46.07	4	Adult	10	Altered behavior (one conc.)	1,000,000	13.8	N	30
Cyanide	0.1	26.0	2	Juv	18	Growth impairment, histological, respiration	10	175	Y	28
Diclofenac	1.1	296	1	Juv	30	ETS and CEA dec.	0.13	7,696	Y	1
Diclofenac	1.1	296	2	Adult	28	Abnormal histology gill and kidney	5	1,432	Y	2
Valproic acid	1.37	144.2	4	8 – 120 hpf	5	Altered behavior	720	143	N	12
PFOA	1.58	414.1	4	3 hpf – 14 dpf	5	Dec. growth	828	255	N	24
PFOA	1.58	414.1	4	3 hpf – 14 dpf	5	Altered behavior	83	2,539	N	24
Bisphenol S	1.6	250	4	Adult	14	Altered behavior (several parameters)	1	2.0E+05	Y	22
Genistein	2.1	270.2	7	Adult	100	Gonadal effects	10	240	N	10
Carbamazepine	2.5	236.3	4	4 – 82 hpf	3.3	Accelerated development, body length inc., yolk sac area dec., swim bladder appearance rate inc.	1	43,700	N	11
Carbamazepine	2.5	236.3	4	4 – 82 hpf	3.3	Altered behavior	1	43,700	N	11
Atrazine	2.6	215.7	5	Adult	14 – 30	Egg production rate and spawning events dec.	0.36	35,000	Y	13
Triadimefon	2.8	293.8	7	Adult	28	Fecundity dec.	10	3,117	Y	18
Bisphenol F	2.9	200.2	6	Juv	60	HSI inc.	1000	18.8	N	26

						Toxic responses				
Chemical	**K_{ow}/ D_{ow}**	**MW**	**Spp**	**Stage**	**T (d)**	**Effect**	**$LOEC_{specific}$ (µg/L) ppb**	**$TR_{subleth}$**	**Conc meas**	**Ref**
Bisphenol F	2.9	200.2	6	Juv	60	Alteration of immune parameters	100	188	N	26
Bisphenol A	3.3	228.3	3	Embryo 6 dpf	4	Heart rate inc.	50	15.0	Y	3
Bisphenol A	3.3	228.3	3	Adult	120	E2/T, GSI, egg number dec.	50	850	Y	3
Bisphenol A	3.3	228.3	4	Adult	21	Male GSI and vitellogenic oocytes inc., spermatogonia dec.	10	100	N	4
Fenitrothion	3.3	144	8	Juv	0.08	Altered behavior	100	29.1	Y	15
Fenitrothion	3.3	144	9	Sub-adult	0.08 – 0.5	Physiological changes, HSI	20	146	N	16
Fenitrothion	3.3	144	7	Adult	0.007	Avoidance	90	32.4	N	17
Propranolol	3.3	259.3	5	0.5 – 160 dph	160	GSI dec., eggs/female inc	7.8	11.5	Y	5
Propranolol	3.3	259.3	5	Adult	21	Male weight	1000	117	Y	6
Propranolol	3.3	259.3	5	Adult	21	GSI female & hatchability	98	117	Y	6
Propranolol	3.3	259.3	5	0.5 – 160 dph	160	Condition factor	0.76	1,468	Y	5
17α-ethinylestradiol	3.7	294.4	5	1 – 56 dph Fo	56	Weight and length red.	0.004	2.0E+05	Y	8
17α-ethinylestradiol	3.7	294.4	5	1 – 28 dph F1	28	Weight and length red.	0.0002	4.0E+06	Y	8
17α-ethinylestradiol	3.7	294.4	5	1 – 28 dph Fo	56	Sex ratio and intersex	0.004	2.0E+05	Y	8
17α-ethinylestradiol	3.7	294.4	4	0 – 75 dpf F1	75	Juv growth, eggs/day, fert success dec., Time to first spawn inc.	0.001	8.0E+05	Y	9
Tebuconazole	3.7	236	4	60 – 120 dpf	60	female length and HSI	50	279	Y	14
Tebuconazole	3.7	236	4	60 – 120 dpf	60	M&F bw and length, GSI male, egg production	200	69.6	Y	14

						Toxic responses				
Chemical	**K_{ow}/ D_{ow}**	**MW**	**Spp**	**Stage**	**T (d)**	**Effect**	**$LOEC_{specific}$ (µg/L) ppb**	**$TR_{subleth}$**	**Conc meas**	**Ref**
Parathion	3.8	291.3	4	25 hpf	0.04	Hyper activity (background phase) photomotor response	291	100	N	19
PFOS	3.85	500.1	4	Adult	7 – 14	Length and weight, immune-related enzymes, histological abnormalities	40	49	Y (tiss)	23
PFOS	3.85	500.1	4	3 hpf – 14 dpf	5	Dec. growth	100	20	N	24
PFOS	3.85	500.1	4	3 hpf – 14 dpf	5	Altered behavior	10	198	N	24
Acetochlor	4.0	269.8	4	Adult	7	Inc. ovarian weight	1	5,600	N	25
Acetochlor	4.0	269.8	4	Adult	21	Dec. GSI	100	56.0	N	25
Phenanthrene	4.5	179.2	1	Adult	120	GSI male, egg production	5	131	Y	29
Phenanthrene	4.5	179.2	1	Adult	120	HSI male, sex hormones	1	26	Y	29
Phenanthrene	4.5	179.2	4	1 hpf – 120 d	4	Reduce fertilization success, egg count, altered histology in adults	0.086	1,474	Y (tiss)	31
Pyriproxyfen	4.9	321.4	4	Adult	21	Dec. testosterone (male), dec. E2 (female), altered gonad histology	10	121	Y	20
Chlorpyrifos	5.6	350.6	8	Juv	4	Spontaneous swimming behavior	0.6	275	Y	27
Chlorpyrifos	5.6	350.6	8	Juv	4	Altered feeding behavior	1.2	138	Y	27
4-nonylphenol	5.9	220.3	6	Adult	70	Erythrocytes, leucocytes red.	4.7	90.5	Y	7

$LOEC_{specific}$ (all studies) is the lowest effect concentration found for sublethal effects other than $TxSn_{FELS}$ responses. K_{ow} is the log_{10} octanol-water partition coefficient or $Log_{10}D_{ow}$ at pH 7.5 listed for compounds with a physiological charge other than 0 (Table S1). Stage is the life stage exposed and hpf, dpf, and dph are hours or days post fertilization or hatch. T is the duration of the exposure in days starting at the first time value for stage. Conc meas, indicates if exposure concentrations were measured. E2/T = Estradiol:testosterone ratio, HSI = hepatosomatic index, GSI = gondosomatic index, ETS=Electron transport system activity and cellular energy allocation. $TR_{subleth}$ is the ratio between values for $TxSn_{FELS}$ ($LOEC_{TxSn}$ or $EC50_{TxSn}$) and specific toxicity $LOEC_{specific}$ for each compound (equation 7).

References (Ref). 1. Duarte et al. (2020); 2. Schwaiger et al. (2004); 3. Huang et al. (2018); 4. Forner-Piquer (2020); 5. Parrot and Balakrishnan (2017); 6. Giltrow et al. (2009); 7. Schwaiger et al. (2000); 8. Länge et al. (2001); 9. Schäfers et al. (2007); 10. Kiparissi et al. (2003); 11. Qiang et al.

(2016); 12. Chen et al. (2018); 13. Tillitt et al. (2010); 14. Li et al. (2019); 15. Bull and McInerney (1974); 16. Sancho et al. (1997); 17. Hidaka and Tatsukawa (1989); 18. Liu et al. (2018); 19. Carbaugh et al. (2020); 20. Maharajan et al. (2020); 21. Faria et al. (2018); 22. Wang et al. (2020); 23. Guo et al. (2019); 24. Jantzen et al. (2016); 25. Zhang et al. (2020); 26. Qiu et al. (2018); 27. Sandahl et al. (2005); 28. Dixon and Leduc (1981); 29. Peng et al. (2019); 30. Audira et al. (2020); 31. Chen et al. (2021).

Species (spp). 1. Croaker *Argyrosomus regius*, 2. Rainbow trout (*Oncorhynchus mykiss*), 3. Marine medaka (*Oryzias melastigma*), 4. Zebrafish (*Danio rerio*), 5. Fathead minnow (*Pimephales promelus)*, 6. Carp (*Cyprinus carpio*), 7. Medaka (*Oryzias latipes*), 8. Coho (*Oncorhynchus kisutch*), 9. Eel (*Anguilla anguilla*).

Table 3. Comparison of fish early life-stage toxicity and mammalian toxicity.

Chemical	K_{ow}/ D_{ow}	Fish Early life stage (mM)				Mammal oral LD50 (mM)							$LR50_{fish}$/ $LD50_{mammal}$
		LC50	Pred BCF	Pred tissue TxSn	Pred LR50	Rat	Mouse	Dog	Rabbit	Guinea pig	Human	Geom mean	
DMSO	-1.3	192	1.40	316	269	222.8		140.9		128.0		163.9	1.69
Acrylamide	-0.7	1.97	1.40	2.8	2.76	1.74	1.51					1.62	1.70
Isoniazid	-0.7	0.63	1.40	0.22	0.89	9.11	0.97	0.36	1.82	1.86	0.73	2.48	0.63
Ethanol	-0.3	786	1.40	422	1,108	151.9		119.4	136.8	119.4		131.9	8.39
Caffeine	-0.07	3.56	1.40	2.5	4.98	0.99	0.65		1.15	1.18	0.99	0.99	5.12
Cyanide	0.10	0.09	1.40	0.095	0.12		0.14	0.15	0.15			0.11	0.83
Diclofenac	1.1	0.02	1.6*	0.024	0.024	0.21	0.57					0.39	0.062
Valproic acid	1.37	0.22	2.9*	1.38	1.58	4.65	7.61					5.9	0.27
PFOA	1.58	0.73	5*	2.5	2.5	0.46						0.46	5.4
Bisphenol S	1.6	0.80	4.5	3.6	3.6	18.2	6.39		18.78			14.5	0.31
Retinoic acid	2.37	9.8E-5	21	2.5E-4	1.2E-5	13.3	11.3		6.52			10.4	1.2E-6
Carbamazepine	2.5	0.32	27	4.9	6.5	8.28	2.24	23.79	11.34	3.89		9.91	0.91
Atrazine	2.6	0.05	32	2.6	1.50	3.12	3.94		3.48			3.51	0.43
Triadimefon	2.8	0.16	48	5.1	7.69	1.24	3.40	1.70	1.70			2.01	4.12
Bisphenol F	2.9	0.16	58	5.4	9.30	24.7						24.7	0.38
Bisphenol A	3.3	0.02	127	4.2	3.85	10.9	10.5		9.77	17.5		12.2	0.32
Fenitrothion	3.3	0.02	127	2.6	2.83	1.74	1.59			3.47		2.27	1.3
Propranolol	3.3	0.03	133*	8.3	4.49	2.55	1.11	0.46				1.37	3.3
17α-ethinylestradiol	3.7	0.06	279	0.75	0.75	3.24	3.21					3.22	0.23
Tebuconazole	3.7	0.05	279	16.4	12.8	14.2	6.84		4.24			8.43	1.7
Parathion	3.8	0.08	339	33.8	25.7	0.03	0.02	0.01	0.03	0.03	0.01	0.02	1326
PFOS	3.85	0.004	373*	1.45	1.5	0.50						0.50	3.0
Acetochlor	4.0	0.04	501	10.4	21.4	2.83	5.75		2.22			3.60	6.46
Pentachlorophenol	4.3	0.0029	977*	0.51	2.6	0.10	0.44		0.75	0.63	1.51	0.69	4.1
Phenanthrene	4.5	0.002	1,622	0.4	2.3		3.93					3.93	0.72

Chemical	K_{ow}/ D_{ow}	Fish Early life stage (mM)					Mammal oral LD50 (mM)							$LR50_{fish}$/ $LD50_{mammal}$
		LC50	Pred BCF	Pred tissue TxSn	Pred LR50		Rat	Mouse	Dog	Rabbit	Guinea pig	Human	Geom mean	
Pyriproxyfen	4.9	0.0007	7,320	11.0	2.0		6.22						6.22	0.3
Chlorpyrifos	5.3	0.0003	6,383	3.0	1.82		0.20	0.21		2.85	1.44	0.86	1.11	2.7
4-nonylphenol	5.9	0.0013	20,654	42.4	27.2		7.35						7.35	3.7
Geometric mean (SE) all data					4.1 (3.9)								2.25 (2.2)	1.6 (3.2)

LC50 in mM observed for early life stage fish. Predicted whole-body tissue concentration for the LR50 (wet wt. whole body) in mmol/kg determined with equation 1, using modeled BCF (equation 2). * is measured BCF (Table S1). Predicted lowest effect tissue residue for the $TxSn_{FELS}$ in terms of tissue concentration determined with the observed or predicted BCF and the geometric mean value for all aqueous $LOEC_{TxSn}$ and $ER50_{txsn}$ values. Mammal oral LD50 data from PubChem (2020). Geometric mean (Geo mean) for each chemical based on data for all mammal species. The ratio of fish LR50s to mammal LD50s shown in last column. The LR50 is the fish whole-body tissue concentration associated with 50% mortality. The LD50 is based on the expected whole-body concentration at the time of dose (oral) administration. Last row is the geometric mean and standard error for all data (excluding retinoic acid).

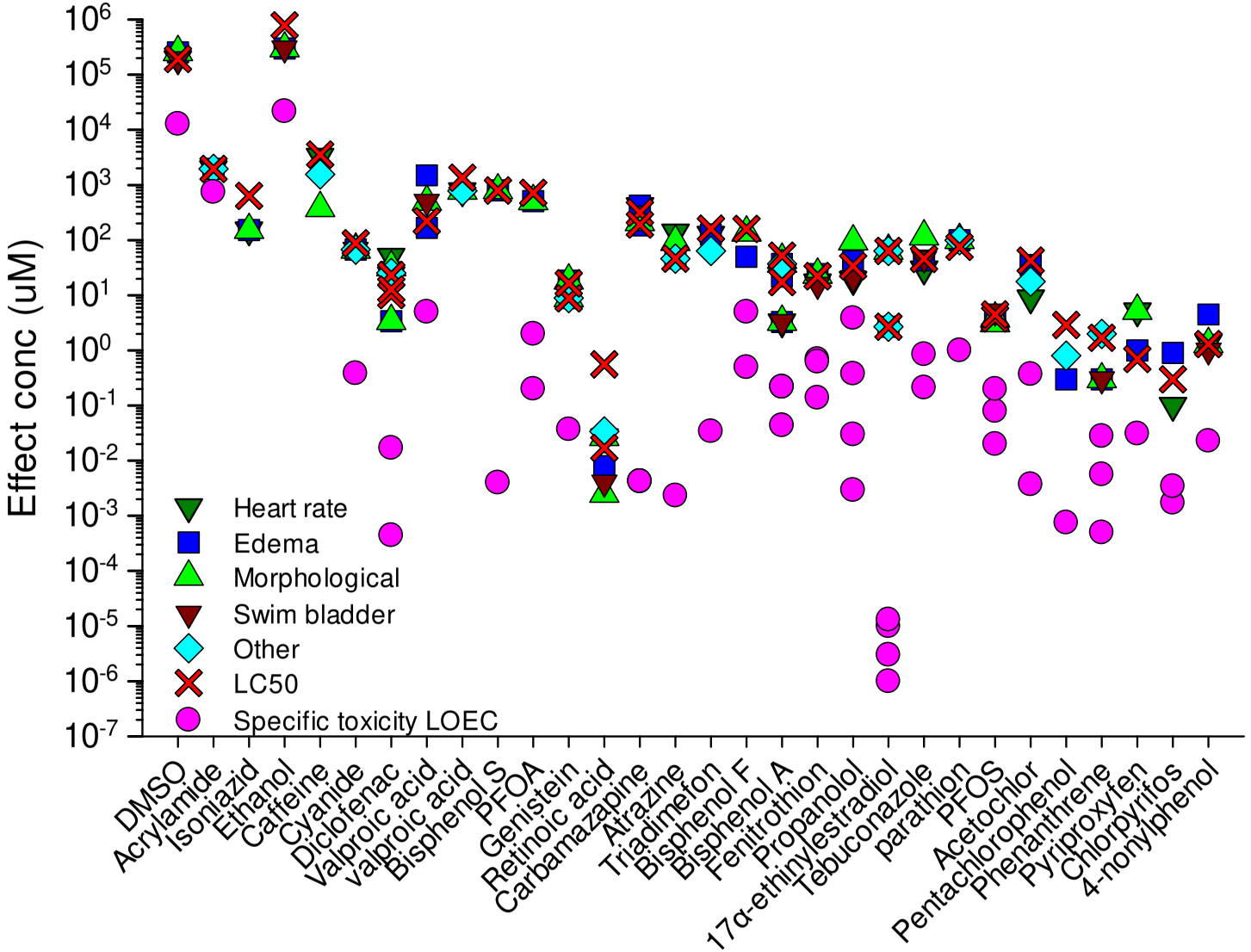
Table 4. Observed and predicted toxicity responses for early-life stage fish based on whole-body tissue concentrations (mmol/kg).

Chemical	Log_{10} Kow	Heart rate reduced		Edema		Morph		Swim bladder inflation		Other		LR50		Spp hpf	Ref
		obs	pred	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred		
Ethosuximide	0.4	11.2	25.2	5.7	8.0	-	-	-	-	-	-	-	-	1 96 hpf	2
Diclofenac~	1.1	-	-	-	-	-	-	-	-	-	-	0.05	0.024	1 96 hpf	3
Valproic acid~	1.37	-	-	0.29	0.5	-	-	-	-	-	-	0.39	0.64	1 96 hpf	2
Bisphenol S	1.6	-	-	0.18	3.6	0.18	3.6	-	-	-	-	0.18	3.6	1 96 hpf	1
PFOA~	1.65	-	-	2.6*	-	-	-	-	-	-	-	-	-	1 120 hpf	5
Cetirizine^	1.7	1.3	-	-	-	-	-	-	-	3.65+	-	2.7	-	1 96 hpf	7
PFHxS~	2.34	-	-	1.6*	-	-	-	-	-	-	-	-	-	1 120 hpf	5
Carbamazepine	2.5	-	-	0.45	4.9	-	-	-	-	-	-	0.4	6.5	1 96 hpf	2
Doxylamine^	2.5	0.2	-	2.3	-	2.2	-	-	-	2.2^{+}	-	1.98	-	1 96 hpf	7
3,4-dichloroaniline	2.7	-	-	-	-	-	-	-	-	-	-	0.32	0.55	1 120 hpf	6
Dimethindene^	2.7	0.15	-	0.56	-	0.54	-	-	-	0.47^{+}	-	1.1	-	1 96 hpf	7
Bisphenol F	2.9	-	-	2.9	2.9	8.1	8.0	-	-	-	-	9.4	9.3	1 96 hpf	1
Ketotifen^	3.2	0.05	-	-	-	2.4	-	-	-	1.2^{+}	-	2.3	-	1 96 hpf	7
Bisphenol A	3.3	-	-	0.22	2.8	0.45	5.6	-	-	-	-	0.54	3.85	1 96 hpf	1

Chemical	Log_{10} Kow	Heart rate reduced		Edema		Morph		Swim bladder inflation		Other		LR50		Spp hpf	Ref
		obs	pred	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred		
PFOS~	3.85	-	-	1.4*	-	-	-	-	-	-	-	-	-	1 120 hpf	5
Phenanthrene	4.5	-	-	0.21	0.39	0.21	0.39	0.27	0.41	-	-	2.7	2.3	1 120 hpf	4

All concentrations as mmol/kg whole-body wet weight. Observed values (obs) are tissue residue toxicity metrics determined by each study. Blank cells indicate endpoints not assessed. Predicted values (Pred) were determined with equations 1 and 2 based on water exposure concentrations provided by each study and paired only with obs values. Equation 1 was modified for EC50 and LOEC values to determine comparable ER50 and LOER residue values. Edema is yolk sac and cardiac edema, morph is generally spinal curvature and other morphological abnormalities, swim bladder inflation denotes a failure to inflate, circul is reduced blood circulation, and LR50 is the lethal residue (whole-body tissue concentration) associated with 50% mortality. Reduced hatch rate was used as a surrogate for mortality for reference 2. * Responses include pericardial and yolk sac edemas, scoliosis, and non-inflated swimbladder. ~ indicates predicted log_{10}Dow value determined with measured BCF (Table S1). ^All values are means of observations determined with measured BCF ($BCF_{apparent}$) at pH 7 and 8 and all are ER50 values except reduced heart rate (LOER). + is body length. References 1. Moreman et al. (2017); 2. Beker von Woudenberg et al. (2014); 3. Bittner et al. (2019a); 4. Vergauwen et al. (2015); 5. Vogs et al. (2019); 6. Schiwy et al. (2020); 7. Bittner et al. (2019b).

Figure 1


Effect conc (uM)
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
Heart rate
Edema
Morphological
Swim bladder
Other
LC50
Specific toxicity LOEC
DMSO
Acrylamide
Isoniazid
Ethanol
Caffeine
Cyanide
Diclofenac
Valproic acid
Valproic acid
Bisphenol S
PFOA
Genistein
Retinoic acid
Carbamazapine
Atrazine
Triadimefon
Bisphenol F
Bisphenol A
Fenitrothion
17α-ethinylestradiol
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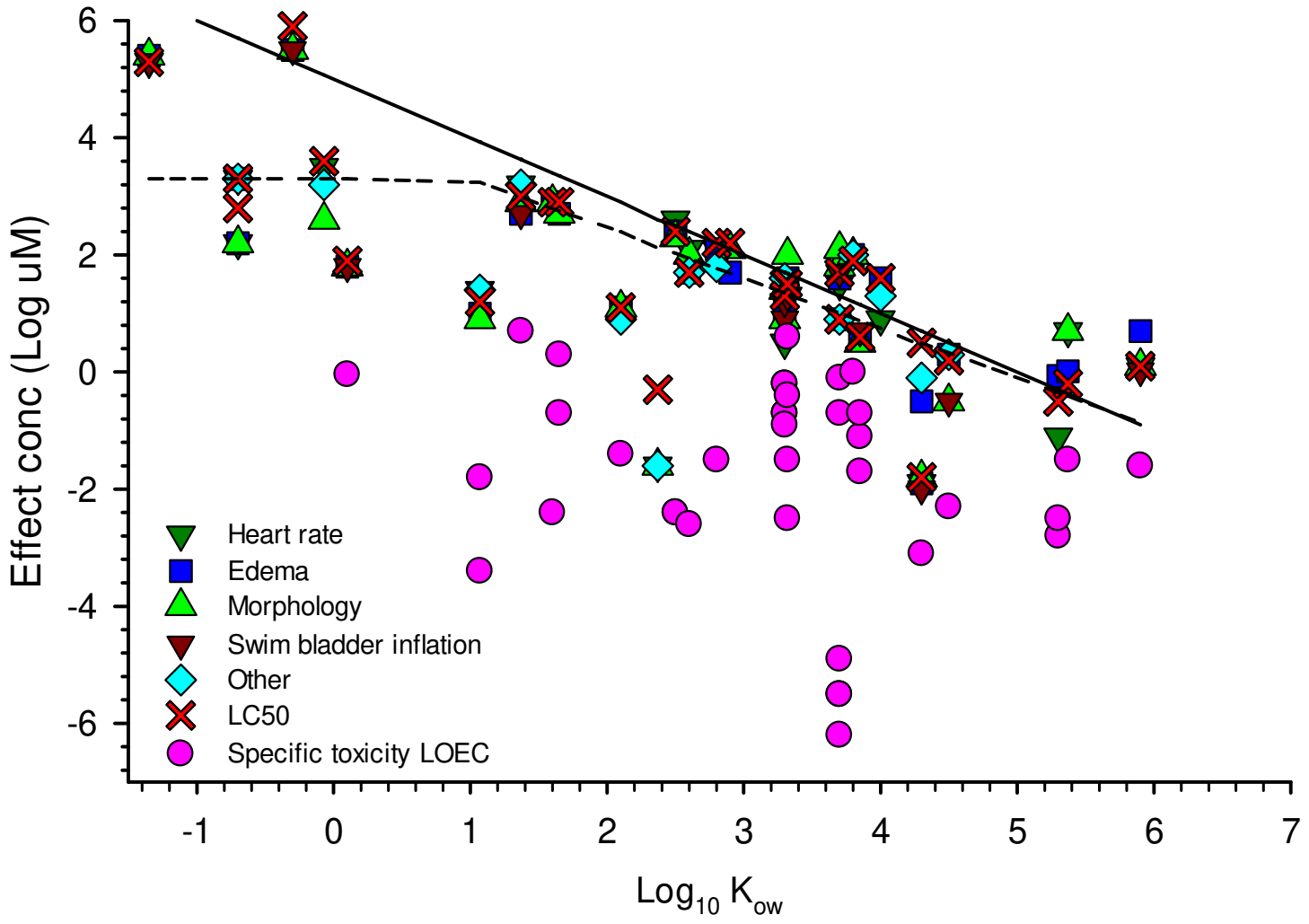
Phenanthrene
Pyriproxyfen
Chlorpyrifos
4-nonylphenol


Effect conc (Log uM)
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4
2
0
-2
-4
-6
-1
0
1
2
3
4
5
6
7
Log10 Kow
Heart rate
Edema
Morphology
Swim bladder inflation
Other
LC50
Specific toxicity LOEC

Fig. 2

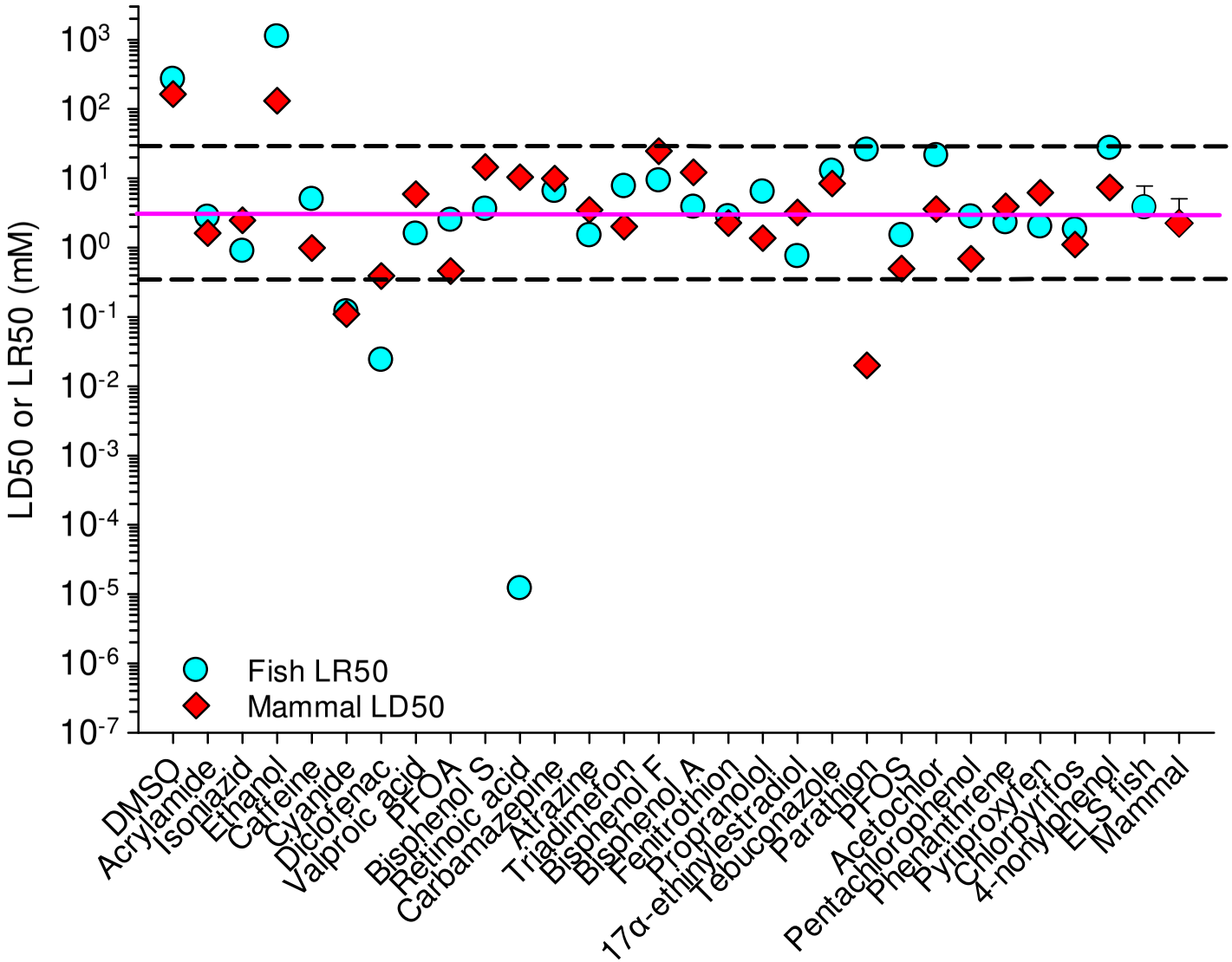
LD50 or LR50 (mM)
10^3
10^2
10^1
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
10^-6
10^-7
Fish LR50
Mammal LD50
DMSO
Acrylamide
Isoniazid
Ethanol
Caffeine
Cyanide
Diclofenac
Valproic acid
PFOA
Bisphenol S
Retinoic acid
Carbamazepine
Atrazine
Triadimefon
Bisphenol F
Bisphenol A
Fenitrothion
Propranolol
17α-ethinylestradiol
Tebuconazole
Parathion
PFOS
Acetochlor
Pentachlorophenol
Phenanthrene
Pyriproxyfen
Chlorpyrifos
4-nonylphenol
ELS fish
Mammal

Fig. 3

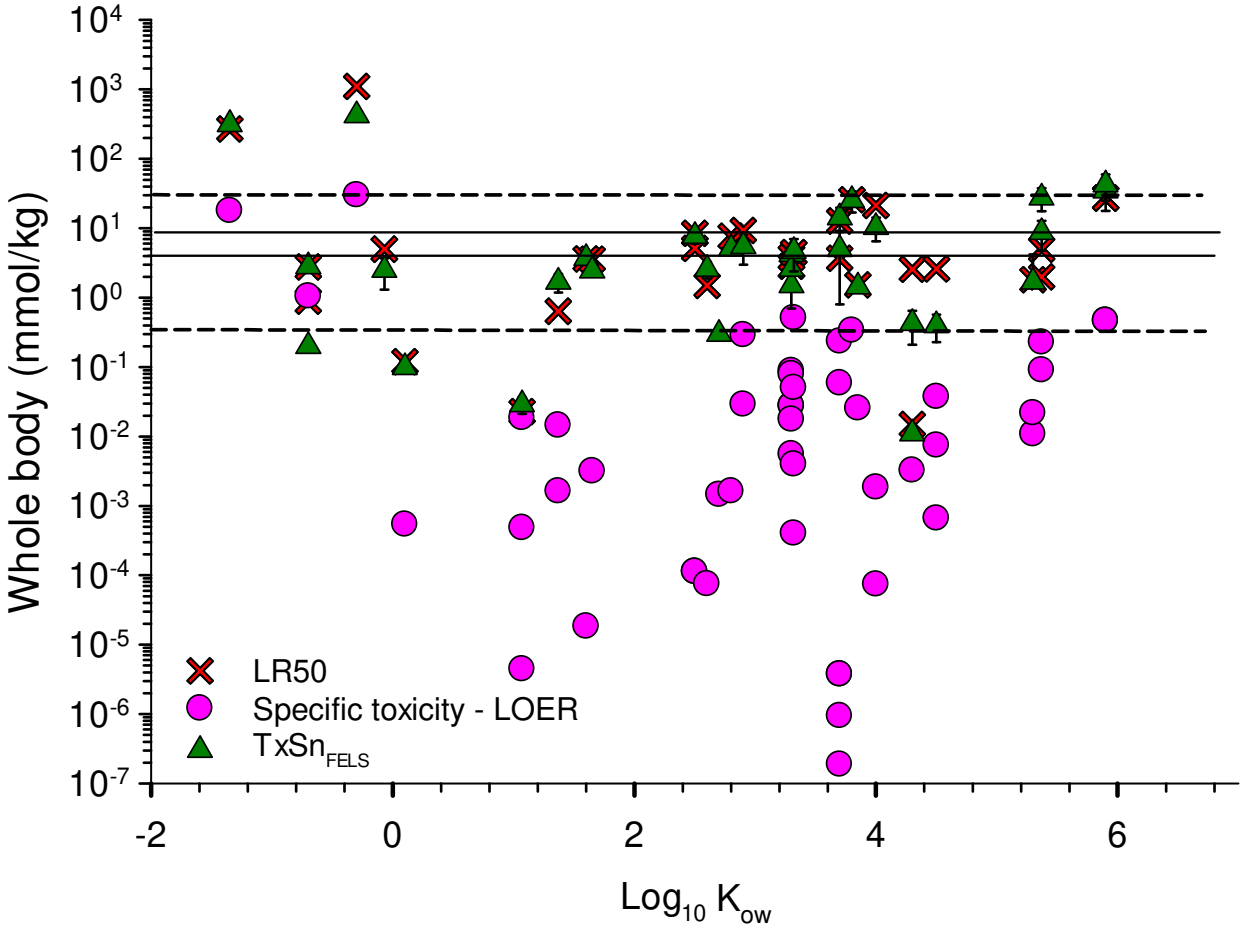
Whole body (mmol/kg)
10^4
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
LR50
Specific toxicity - LOER
$TxSn_{FELS}$
-2
0
2
4
6
$Log_{10}\ K_{ow}$

Fig 4.