Letter to the Editor

Comment on Plugge et al. 2021 "Toward a Universal Acute Fish Threshold of Toxicological Concern"

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To the Editor:

Precedent: an earlier event or action that is regarded as an example or guide to be considered in subsequent similar circumstances.

Recently, a study by Plugge et al. (2021) was published by *Environmental Toxicology and Chemistry*. The authors conflate several related terms with the intent to improve predictions of fish acute toxicity based on large historical data sets, to reduce the numbers of fish required for testing in the future. The authors term the outcome a "universal threshold of toxicologic concern (TTC)" for acute fish toxicity. We believe that their approach inaccurately frames the approach as an ecological (eco)-TTC and includes multiple scientific errors. Terminology built on precedent is important.

What is an eco-TTC?

Belanger et al. (2015) launched a new series of investigations to establish ecologically relevant TTCs for environmental species. As stated in that study, this builds on, but is somewhat different from, TTCs derived for human safety endpoints (Kroes et al. 2004). The eco-TTCs were thus defined to be predictions of thresholds based on the (necessary) inclusion of multiple test species used to derive predicted no-effect concentrations (PNECs) for ecosystems using statistical distributions and cutoff values of the 5th percentile lowest values (consistent with human safety TTCs; Belanger et al. 2015). Subsequent research under a multisector group of the Committee on Animal Alternatives in Environmental Risk Assessment, Health and Environmental Sciences Institute (HESI; 2019) resulted in a curated collection of data (the EnviroTox Database, which is referenced in Plugge et al. 2021), an assessment of the implications regarding mode of action (Kienzler et al. 2017, 2019), and a detailed study on the database itself (Connors et al. 2019). In addition, an invited review was recently published in a special issue of *Frontiers in Toxicology* (Barron et al., 2021). Reference materials related to the background of all concepts explored can be downloaded directly from the EnviroTox website (Health and Environmental Sciences Institute 2019).

An international workshop on the eco-TTC concept was cohosted by HESI and Environment and Climate Change Canada in September 2017 in Ottawa, Canada, and was attended by >40 ecotoxicology and risk assessment experts. Attendees debated the status of the science, the deployment of the database, and the means to improve the eco-TTC concept. One significant outcome of the workshop was to pause the release of the website and tools to develop a chemical toxicity distribution (CTD) tool. The CTD tool was specifically created to focus on the distribution of toxicity values from a defined group of compounds (e.g., chemical category or mode of action) toward a specific organism group (e.g., fish, *Daphnia*, or green algae). The term CTD was coined by Williams et al. (2011), who recognized the additional importance of mode of action and chemical category on the outcome of the statistical distributions for toxicity. The CTDs differ from the eco-TTCs in that they do not utilize application factors before being plotted in the distribution. Application factors take into account the function of the data quality, the breadth, and the study type and can vary by region (i.e., different regions have different application factors depending on their regulatory approach and precedent). The workshop participants recognized the differences and potential utilities of both the eco-TTC and CTD approach and delayed the release of the EnviroTox database until November 2019, when both approaches were fully developed and able to be documented properly.

We highlight below some of the major technical issues with the approach and conclusions in the Plugge et al. (2021) study.

Accuracy

The methods and results utilized do not accurately represent an eco-TTC, but rather more closely resemble a CTD with a (somewhat arbitrary) application factor applied to the data. As just explained, CTDs are taxon or trophic group specific and

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do not represent a PNEC extrapolation. This is an important clarification for 2 main reasons. 1) PNECs are intended to be protective of the ecosystem (e.g., the ability to integrate data from multiple taxa, and both acute and chronic exposures) with a systematic way of incorporating conservatism and uncertainty by the use of application factors. Hence, the definition of an eco-TTC is based on a distribution of the PNECs, with an eye toward identifying levels below which there would be de minimis risk to the ecosystem as a whole. 2) A CTD is not equivalent to an eco-TTC. Importantly, no application factors are included in the generation of a CTD; it is a distribution of individual effect values (or a geometric mean) for each CAS or chemical grouping. Plugge et al. (2021) quote EnviroTox as creating an eco-TTC of 9 μg/L for "chemicals with a direct mechanism of action." This is simply not correct. The cited value of 9 μg/L was derived via a $CTD_{0.05}$, which does not apply application factors to the toxicity data and is, by definition, *not* an eco-TTC.

It is not our purpose in the present Letter to the Editor to present a series of comparative extrapolations, but some limited examples are illuminating. For example, in Table 1 we show results from extrapolations for acute fish toxicity data sets for chemicals that act by narcosis (nonspecifically) versus specifically acting chemicals, expressed as a $CTD_{0.05}$. To provide better context for the extrapolation, numbers of data points and fish species are identified as well. If this was positioned as an eco-TTC, an application factor of 1000 would be applied because only one taxon is considered per chemical (in our view, this would also be very conservative because we ignore information on algae and invertebrates, as well as chronic toxicity data that are also available).

TABLE 1: Results from extrapolations for acute fish toxicity data sets for chemicals that act by narcosis (nonspecifically) versus specifically acting chemicals, expressed as a $CTD_{0.05}$

Consensus mode of action	No. of CAS #s	No. of entries	No. of fish species	$CTD_{0.05}$ (μg/L)
Narcosis	1150	10 523	195	119
Specifically acting	481	11 544	249	9.2

CTD = chemical toxicity distribution.

Data

There are some fundamental issues with the data used for the analysis. The authors assume that "All US Environmental Protection Agency and US Geological Survey data were considered curated." This assumption is not fully justified, and the arbitrary assignment of Klimisch 1 or 2 to these data, without additional detailed examination of the original studies, is not appropriate. For example, in the development of the EnviroTox database, numerous data sources were utilized in the original data pull. We began with a data set of 220 000 records and after extensive curation (see Connors et al. 2019), only 40% of the original records were included in the EnviroTox database.

After an examination of the data set used for the Plugge et al. (2021) calculations (provided as their Supplemental Data), the following findings were made. 1) The original data set was 47 694 records for 6133 unique substances. 2) Approximately 9000 of those records had records with qualifier values (< or >) on the effect measurements; this is problematic because those values cannot be reliably utilized for quantitative analysis. 3) Approximately 3900 substances either had no CAS number or were known mixtures. 4) Tests above the chemical solubility limit are populated throughout many databases (including the US Environmental Protection Agency [USEPA] ECOTOX); clear violations of this ecotoxicity rule of thumb (testing above the solubility limit) were deleted from EnviroTox. 5) When one is downloading data from ECOTOX, tests with multiple time points are frequent, usually as a consequence of studies citing progressive toxicity (e.g., for a 96-h fish acute assay, 24-, 48-, and 72-h data are also included); summaries of a study on a test chemical should rely on a single time point when used in these types of distribution analyses. 6) Many duplicate values were identified in the data tables (e.g., their Supplemental Data, Table S1). This would mean that the distributions were made with multiple entries for each chemical, and not the geometric mean. However, geometric means are mentioned in their *Materials and Methods* section. This has major implications for the distributions and makes the results very difficult to interpret.

The authors state that for metals, "…because these were "big data," no speciation separation was performed." It is well known that speciation is critically important for metal toxicity. The idea that the analysis is based on "big data" is not a sound justification to eliminate consideration of water quality on test outcomes for metals with a resulting impact on distribution analyses. If proper and appropriate handling of metals data was not possible (e.g., normalizing to a set hardness value for the purpose of aggregating metals data in distributions), the authors should have excluded metals from this analysis. Although we have not re-analyzed the Plugge et al. (2021) data set with this in mind, we expect that a grouping structure based on the dissociated metal ion driving toxicity would result in a significant portion of the data being collapsed together and thus alter the $CTD_{0.05}$ distribution.

Mode of action

Mode of action is not appropriately discussed/addressed. The authors do note that mode of action is an important consideration, yet state that "…mechanism of action is not a consideration because the aim is to derive a universal TTC applicable to all chemicals, even those without mechanism of action information." Various tools have been developed specifically based on fish acute toxicity information to assign mode of action (Verhaar, TEST, OASIS, ASTER; see Kienzler et al. 2017). As is well known, pesticides and other specifically acting chemicals dominate the lower end of the distribution. In Plugge et al. (2021), there is no analysis of the mode of action make-up of their database, nor do they specifically test whether mode of action makes a difference. In fact, their Supplemental Data, Table S1, contains a list of chemicals that fall below the hazardous concentration for 5% of the species, and, not surprisingly, these are specifically acting chemicals and metals (which were not normalized in the analysis). Ignoring mode of action is a step back, not a step forward, in developing either CTDs or eco-TTCs.

Sensitivity

Zebrafish/fathead minnow sensitivity comparisons are inconsistent with other published interspecies correlations based on best data. The consequences of the data handling procedures we have just described in Plugge et al. (2021) are evident in one of the many conclusions that we feel lack scientific justification. Zebrafish have not previously been postulated to be 10× more tolerant than fathead minnow. The examples in Belanger et al. (2013), and other sources, including data held within the USEPA Web-Interspecies Correlation Estimation program (US Environmental Protection Agency 2016) show that they are similar in sensitivity (less than a factor of 2). The importance of this observation contributes to confidence by Organisation for Economic Co-operation and Development member nations that both species are appropriate surrogates for acute and chronic fish toxicity.

Precedents are important in science. They mark the initiation of new theories and discoveries of significance, and they guide us in relating these to historical information. Science progresses methodically through the establishment of precedents and associated building blocks that are proximate to support the link between older and newer information. Linnean taxonomy relies heavily on precedent. Older names are given precedence when it is clear that the recent naming convention is already covered by existing nomenclature. Technical precision also plays an important role. Precision is defined as the quality, condition, or fact of being exact and accurate. Furthermore, in science we make a distinction between accuracy and precision, whereby in a set of measurements, accuracy is closeness of the measurements to a specific value, whereas precision is the closeness of the measurements to each other. These are all important concepts that keep us on the road to scientific improvement. For example, it makes a difference when we speak of mode of action, mechanism of action, and adverse outcome pathways.

Robust data curation and appropriate use of accepted scientific terms and approaches contribute to the legitimization and utility of either CTD or eco-TTC for use as an animal alternative strategy. These terms were developed for a specific purpose: to address a significant and technically challenging issue in the ecological risk assessment space. It is important that the progress we have made not be compromised by inappropriate use and application.

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