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Comparison of toxicity values across zebrafish early life stages and mammalian studies: Implications for chemical testing

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ABSTRACT

With the high cost and slow pace of toxicity testing in mammals, the vertebrate zebrafish has become a tractable model organism for high throughput toxicity testing. We present here a meta-analysis of 600 chemicals tested for toxicity in zebrafish embryos and larvae. Nineteen aggregated and 57 individual toxicity endpoints were recorded from published studies yielding 2695 unique data points. These data points were compared to lethality and reproductive toxicology endpoints analyzed in rodents and rabbits and to exposure values for humans. We show that although many zebrafish endpoints did not correlate to rodent or rabbit acute toxicity data, zebrafish could be used to accurately predict relative acute toxicity through the rat inhalation, rabbit dermal, and rat oral exposure routes. Ranking of the chemicals based on toxicity and teratogenicity in zebrafish, as well as human exposure levels, revealed that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), benzo(a)pyrene, and chlorpyrifos ranked in the top nine of all chemicals for these three categories, and as such should be considered high priority chemicals for testing in higher vertebrates.

1. Introduction

Rodent and rabbit toxicity testing has been the standard for assessing acute toxicity by the United States government since the 1950s. However, the process is costly and time consuming which has led to a backlog in chemical testing [1]. In addition, up to a thousand new chemicals are introduced to the market yearly [2]. Thus, new screening methods are needed to keep pace with the development of new chemicals and protect both human and environmental health. Zebrafish, *Danio rerio*, have emerged as a viable vertebrate organism for chemical risk testing with over 1490 papers published since the initial paper exploring the effect of zinc sulphate during different stages of zebrafish development in 1965 [3]. Zebrafish are a cost-effective model for chemical toxicity screening due to their high fecundity, rapid embryonic development, and high homology to mammalian species [4]. In addition, they provide a whole animal model advantage over cell lines allowing for metabolism and systemic interactions to mimic the processes in the human body. Because of its small size, the zebrafish embryo or larvae has been used successfully in high throughput toxicity screening. The embryo is also a preferred testing model to adult fish because it is anticipated that early life stages feel less pain and distress than adult fish. Therefore, zebrafish early life stages have been proposed as an effective model for toxicity studies (reviewed in [5]) and test guidelines have been developed and validated using zebrafish embryos as model organism (Fish Embryo Acute Toxicity (FET), OECD test guideline 236) [6].

Thus far, a few studies have assessed the reproducibility of rodent and rabbit toxicity with zebrafish embryo toxicity testing (reviewed in [5]). Some of these studies focus on a few chemicals in one class of compound. For example, one study showed that zebrafish embryo results from four organotin compounds accurately reflected the general developmental and neuro-developmental toxicity effects found in rodents [7]. Another study showed that six 1,2,4-triazole compounds tested in zebrafish embryos mimicked the results of rodent testing [8]. A single laboratory study by Ali and colleagues showed that there was a strong correlation between zebrafish embryo LC50 values and rodent LD50s for a set of 60 different compounds [9]. Another single laboratory study investigated both toxicity and teratogenicity of 18 compounds and concluded that toxic responses in zebrafish are comparable to those in mice [10]. However, whether zebrafish

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toxicity data can be collated from several different studies and correlated to rodent data has not been reported. Here, we set out to analyze how metadata from zebrafish toxicity during early life stages relates to rodent and rabbit toxicity. We first ranked chemicals tested in zebrafish embryo and larvae based on toxicity and teratogenicity, and we developed a potential hazard index based on human exposure data. Next, we performed a correlation analysis of rodent and rabbit acute toxicity and developmental toxicity data with zebrafish developmental toxicity data. These correlation and ranking findings have implications for future standards and testing guidelines. The results can be used for prioritizing chemical testing in mammalian species with the ultimate goal to protect human health.

2. Methods

2.1. Zebrafish toxicity and lethality data

We utilized the dataset of 120 publications from Ducharme et al. (2013) [11] and added 77 additional published studies from the literature available via PubMed through June 1, 2013 (listed in Supplemental Table 1). Inclusion criteria were: Publications describing exposure to environmental or industrial chemicals in zebrafish; Treatment and scoring period from 0 to 7 days post fertilization (dpf); Presentation of a statistically significant effect after exposure to the test chemical that was different from unexposed or vehicle exposed fish. The exclusion criteria were: Toxicity data only reported for pharmaceuticals (to keep within the scope of this study); Publications with non-significant data. All of these data were categorized according to our previously established system [11] with the addition of more subcategories for statistical analysis. For the different endpoints we listed the Lowest Observed Adverse Developmental Effect Dose (LOADED). This value represents the lowest reported dose that had an adverse outcome for a certain endpoint if a range of concentrations was tested or the treatment dose used if a range was not reported, and it should not be equalized to Lowest Adverse Effect Level (LOAEL), which is a calculated number often used in toxicology, but which requires a full dose response experiment. For the chemicals from Padilla and colleagues [12] we used the AC10 value instead of the LOADED. We also listed the Lethal Concentration for 50% of the embryos (LC50) values. In publications in which it was not possible to calculate an LC50, we listed the lower dose closest to 50% lethality. If multiple studies examined the same chemical and broad endpoint, we used the lowest reported dose that had an adverse effect for our analysis. In total, the data include 600 chemicals analyzed at 19 aggregated developmental endpoints, three physical parameters, and the no observed adverse effect dose yielding 2695 unique data points. The aggregated endpoints were also subdivided into individual endpoints (e.g. Behavioral Responses were subdivided into spontaneous movement, touch response, swimming, and other) such that 78 zebrafish endpoint categories were evaluated. A list of the endpoints that were chosen and how they were clustered into broader categories is shown in Supplemental Table 2. The data can be downloaded from http://cbl.uh.edu/Zebrafish

2.2. Mammalian lethality data

Mouse, rat, and rabbit LC50 (inhalation) and LD50 (all other exposure routes) values were collected for this study from both ChemIDplus and the Hazardous Substances Data Bank (HSDB), available through TOXNET (http://toxnet.nlm.nih.gov/index.html). If there were discrepancies between the databases, the lowest value was used. All mammalian acute toxicity data was converted to both mg/kg-day and M/day, assuming that 1 kg body weight was equivalent to 1 L.

2.3. Human exposure data

The theoretical daily dose (TDD) for humans from the 2011 Substance Priority List was downloaded from the ATSDR website (http://www.atsdr.cdc.gov/SPL/resources/index.html). It lists the substances that are most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure at these NPL sites. The reference doses were downloaded from the US EPA's IRIS database (http://www.epa.gov/iris/standal.html). All data was converted to M/day and mg/kg-day, assuming that 1 kg equals 1 L. Wetmore and colleagues gathered human exposure data from federal documents [13]. These exposure values were found in Supplementary Table 7 of Wetmore et al. [13], and converted to M/day and mg/kg-day, assuming that 1 kg equals 1 L.

2.4. Statistical analysis

Statistical analyses were performed as previously described [11]. Briefly, non-parametric Spearman rank correlation analysis was performed to identify the association between pairs of endpoints, where compounds formed the sample size. For outcome (affected endpoint) correlations, we required that each pair of outcomes have an overlap of at least 6 different chemicals in order to allow the p-value for the Spearman's rho to be equal or less than 0.05 [14]. All Spearman analyses were performed on consistent units of measure (molar values compared to molar, mg/kg-day compared to mg/kg-day).

3. Results

3.1. Ranking of chemicals based on zebrafish toxicity

We collected zebrafish developmental toxicity and lethality values for 600 chemicals from studies published in PubMed (www.ncbi.nlm.nih.gov/pubmed). First, we recorded the Lowest Observed Adverse Developmental Effect Dose (LOADED) of a chemical for any endpoint (listed in Supplemental Table 2) and the Lethal Concentration at which 50% of the embryos died (LC50) scored at different time points. We next set out to rank the chemicals based on zebrafish toxicity. The ranking based on LOADED is shown in Table 1 for the top 15 most toxic chemicals, and for 443 chemicals in Supplementary Table 3. From this ranking we conclude 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin, retinoic acid, and chlorpyrifos were the most toxic chemicals investigated in zebrafish so far, while sodium thiosulfate, saccharin, paraoxon-methyl, and acetone were the least toxic.

We next ranked the chemicals based on teratogenicity. We here define the teratogenicity as the capability of an agent to produce fetal malformation relative to lethality. A teratogen ratio was calculated by dividing the LC50 value with the LOADED value (excluding lethality) (LC50/LOADED), as described previously [11]. This ranking included 148 chemicals that had both a LOADED and LC50 value. The top 15 most teratogenic chemicals are shown in Table 2, and the full list is shown in Supplementary Table 4. This list clusters the chemicals into three distinct groups: highly teratogenic (1000 and above), mid-level (100–1000), and low-level (below 100) teratogens. We conclude that potassium perchlorate, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), diethyl dithiocarbamate disulfide (dithiocarbamate), carbon disulfide, and benzo(a)pyrene ranked as the most potent teratogens, while cadmium chloride, 2,6-dihydroxyphthalene, fenvalerate, ethanol, and