Published in final edited form as:

Integr Environ Assess Manag. 2017 January ; 13(1): 208–210. doi:10.1002/ieam.1858.

AGE-RELATED DIFFERENCES IN SENSITIVITY TO METALS CAN MATTER FOR *DAPHNIA MAGNA* NEONATES

Elizabeth M Traudt^{†,*}, James F Ranville[†], and Joseph S Meyer^{†,‡}

[†]Department of Chemistry, Colorado School of Mines, Golden, Colorado, USA

[‡]Applied Limnology Professionals LLC, Golden, Colorado, USA

The invertebrate *Daphnia magna* is frequently used in the evaluation of the toxicity of chemicals to freshwater organisms. Because organisms of different developmental stages may differ in their sensitivity to a chemical, many regulatory agencies (USEPA 2002; OECD 2004) specify that acute toxicity tests should be started with *D. magna* younger than 1 day old. However, we have observed relatively high variability in the toxicity of Cd and Zn to *D. magna* neonates (Meyer et al. 2015; Traudt et al. 2015) and have demonstrated that this variability is at least partly caused by major changes in sensitivity to the metals even within their first day postbirth (Traudt et al. 2016). This methodologically induced variability can have important consequences, especially for predictions of metal-mixture toxicity when using results from individual-metal tests. This Learned Discourse is an extension of the work presented in Traudt et al. (2016); however, herein we elaborate on the increased uncertainty that may arise when predicting the toxicity of mixtures if the toxicity of at least 1 component in the mixture is relatively high.

We previously exposed *D. magna* to binary and ternary mixtures of Cd, Cu, Ni, and Zn in 48-h, static, nonrenewal lethality tests following USEPA-recommended procedures (Meyer et al. 2015; Traudt et al. 2015). We observed high variability in mortality in the binary and ternary mixtures that contained either Cd or Zn but relatively lower variability in mixtures containing Cu or Ni. It is difficult enough to interpret and/or predict mixture toxicity when the metals interact geochemically and/or physiologically, but generating accurate predictions becomes substantially more challenging when the toxicity of at least 1 of the metals in the mixture is highly variable.

To test our hypothesis that even very early age-related differences in sensitivity contribute to high variability in the toxicity of some metals, we conducted acute toxicity tests with Cd, Cu, Ni, and Zn using *D. magna* neonates in several different 4-h age windows after birth. By collecting neonates within these short time intervals, we were able to compare the individual-metal toxicity experienced by young neonates (first exposed 0–4 h postbirth) to the toxicity experienced by older neonates (first exposed 20–24 h postbirth) and to the toxicity in the traditional age range (0–24 h postbirth).

* etraudt@mymail.mines.edu.

In repeated toxicity tests, the variability of the median effect concentrations (EC50 values) increased in the order Cu < Zn < Ni < Cd (Figure 1). The range of Cd EC50 values with neonates 0 to 24 h old was especially broad, spanning nearly 2 orders of magnitude. In the age-controlled tests, the Cd EC50 values in total spanned a similar range; however, the results were stratified based on the organisms' age at the start of the metal exposure. The youngest neonates (exposed 0–4 h postbirth) were the most tolerant of Cd, whereas the oldest neonates (exposed 20–24 h postbirth) had a statistically significant 7-fold greater sensitivity to Cd. These results have important implications.

Around the world, environmental regulatory decisions are widely based on the results of individual-metal toxicity tests. Therefore, it is important to have accurate and precise EC50 (or other ECx) values for individual metals. Additionally, significant uncertainties in individual-metal toxicity tests may have rippling effects for predicting the toxicity of metal mixtures. Mixture effects usually are predicted using either the independent-action (IA) or concentration-addition (CA) models, both of which use information from individual-metal toxicity tests as inputs. Increased uncertainty in individual-metal toxicity thus leads to increased uncertainty in predictions of metal-mixture toxicity. Furthermore, non-additivity of mixture toxicity is usually evaluated by statistically testing for a significant difference between the toxicity experimentally observed in a mixture and the toxicity predicted from a null model such as IA or CA. Large uncertainties in the toxicity of each component of a mixture can thus make it difficult to differentiate between additive and nonadditive interactions. Unfortunately, in many past studies, the EC50 of each metal in a mixture has been treated as a deterministic (i.e., invariant) value, thus ignoring important components of uncertainty that should be incorporated in the statistical analysis to appropriately test for significant differences between observed and predicted mixture toxicity. Our results indicate that even individual-metal toxicity results may vary greatly when the age of *D. magna* neonates is not tightly controlled, potentially leading to a methodologically inflated Type II error probability (i.e., erroneously not identifying nonadditive metal interactions that actually exist).

In summary, age-dependent sensitivity differences to some metals (e.g., Cd, Zn) exist in *D. magna* even within the 1-d postbirth period. The variability in individual-metal sensitivities has an important effect on the uncertainty associated with results of predictive toxicity models. Although not all uses of EC50 values might require relatively low uncertainty in toxicity results, low uncertainty can be very important in the rapidly developing field of bioavailability-based interpretation and modeling of metal-mixture toxicity. Subtle but real nonadditive mixture toxicity effects may be missed when uncertainty is high. That kind of Type II error can offset advances in understanding the geochemical and/or physiological interactions of metals with each other and with other water chemistry parameters, because refined models become moot if they cannot be tested with precision.

We also note that, although not tested by us, such age-related differences in sensitivity might also occur with other species (e.g., *Ceriodaphnia dubia*) and other metals. Researchers, regulators, and the regulated community need to be aware of this possibility and evaluate the level of precision needed for their various uses of acute metal-toxicity data. Even very early age-related differences in sensitivity to metals can matter.

References

- Meyer JS, Ranville JF, Pontash M, Gorsuch JW, Adams WJ. Acute toxicity of binary and ternary mixtures of Cd, Cu, and Zn to *Daphnia magna*. Environ Toxicol Chem. 2015; 34:799–808. [PubMed: 25336231]
- [OECD] Organization for Economic Co-operation and Development. Test no. 202: *Daphnia* sp. acute immobilisation test. Paris (FR): OECD; 2004.
- Traudt EM, Ranville JF, Smith SA, Meyer JS. A test of the additivity of acute toxicity of binary-metal mixtures of Ni with Cd, Cu, and Zn to *Daphnia magna*, using the inflection point of the concentration-response curves. Environ Toxicol Chem. 2015; 35:1843–1851.
- Traudt EM, Ranville JF, Meyer JS. Effect of age on acute toxicity of Cd, Cu, Ni, and Zn in individual-metal exposures to *Daphnia magna* neonates. Environ Toxicol Chem. 2016; doi: 10.1002/etc.3507
- [USEPA] US Environmental Protection Agency. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5. Washington (DC): USEPA; 2002. EPA-821-R-02-012

Figure 1.

Median effect concentrations (EC50 values) for *Daphnia magna* neonates exposed to Cd, Cu, Ni, or Zn as a function of age at the start of 48-h static, nonrenewal lethality tests. “0–24 h” refers to individual-metal toxicity tests conducted from September 2012 through November 2013, in which the age range of the neonates at the start of the test was 0 to 24 h. In age-controlled trials with each of the 4 metals, two 4-h age windows of neonates (0–4 h and 20–24 h postbirth) were used to start the toxicity tests. A third 4-h age window (10–14 h postbirth) was used in one of the Cd toxicity tests. Error bars are the range of EC50 values across the number of tests (*n*) conducted with each age group. Within each metal, EC50 values that have the same letter did not differ significantly ($p < 0.05$).