metals to bats, so we assumed that bats had the same sensitivity as a rodent where toxicological data were available. Data could be generated from in vivo studies, but a more ethical approach in the case of heavily protected species would be to improve the extrapolations from rat and mouse data using toxicokinetic and toxicodynamic modeling methods.

7) How do we evaluate the modeling framework? It is critical that models are evaluated against empirical data. A range of approaches can be used to evaluate the exposure component of the modeling framework, including analysis of carcasses collected from the field, use of tissue banks or noninvasive sampling (e.g., of feathers, fur, nails, blood samples) of free-living animals. Measuring biomarkers in wild animals could enable an evaluation of the toxicological predictions from the modeling framework.
8) What are the ecological effects of exposure to metals? This cannot be answered with our current approach, which is focused on exposure, but we plan to explore consequences in an individual-based population model which will be developed according to the guidelines for Good Modeling Practice that are currently developed in the CREAM project (http://cream-itn.eu; Grimm et al. 2009; Schmolke et al. 2010).

Conclusion

The modeling framework discussed above is a valuable tool for identifying chemicals and scenarios that might pose a risk to wildlife health. To run the framework, an understanding is needed of the fate and behavior of a substance in the environment, uptake into prey items, toxicological effects in the target organism and the ecology of the system. In many cases appropriate data are currently not available, so major assumptions have to be made. By performing targeted research to address some of the questions listed above, it should in the future be possible to develop systems and modeling frameworks for better assessing threats to wildlife. This work needs to be highly co-ordinated and involve environmental chemists, toxicologists, soil scientists, ecologists, and modelers.

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INDIVIDUAL VERSUS POPULATION EFFECTS IN CONCENTRATION-RESPONSE MODELING

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Given the increasing mathematical and statistical complexity of models used to both describe and estimate toxicity effects, it is timely to momentarily pause and reflect on the distinction between sample *statistics* and population *parameters*.

The term ''parameter'' is used widely in several disciplines to denote quite specific, but oftentimes different things. For example, a water quality parameter for a chemist is a *random variable* for a statistician. Statisticians use probability models to describe the attributes of random variables and these probability models are in turn defined by one or more *parameters*. We suspect that this important (statistical) distinction is not always readily appreciated nor made clear in ecotoxicological modeling and may, in part, be responsible for some confusion about components of variation in concentration-response modeling.

We have both individually, and more recently, jointly been working on Bayesian methods for estimating the No Effect Concentration (NEC) as an alternative to the widely discredited No Observed Effect Concentration (NOEC). While the Bayesian paradigm has (in our view) many attractive features, it is not necessary for the estimation of a NEC. Indeed, conventional techniques such as the method of moments or maximum likelihood are equally suitable for this purpose. The critical distinction between a NEC and a NOEC is that the NEC is a *model parameter*, while the NOEC is a data point. Being a model parameter, the NEC describes some facet of an entire (statistical) population.

For the moment, let us assume that the population is a species. In keeping with conventional statistical practice, the NEC is assigned a Greek symbol in a concentration-response model. Let γ denote the *true* but unknown NEC for the defined population in our concentration-response models. To be clear, γ is a measure of toxicity for the *entire population*, but importantly it does not mean that every member of that population has an individual toxicological threshold of γ. By way of analogy, let X denote the random variable ''Intelligence Quotient'' (IQ). We know that IQs follow a normal distribution with some mean and some variance. To denote this we write $X \sim N(\mu, \sigma^2)$. In this statistical notation, the *parameters* are μ and σ where μ is the population mean and σ is the population standard deviation. While we all inhabit the ''IQ space'' described by this statistical model, we know all too well that we do not have a common IQ of μ. The variation in individual IQs is described by the probability model. In the same way, the variation in individual toxicological thresholds is defined by the (assumed) probability model. We thus find ourselves in disagreement with recent claims that ''estimates of the NEC assume that all

individuals have the same NEC'' and that ''all the different approaches implicitly assume that all individual organisms in a cohort have the same toxicological threshold'' (Baas et al. 2009). We acknowledge that these statements may have been intended to describe common practice in toxicokinetic and toxicodynamic modeling which, if true, only serves to reinforce our message that whatever the modeling framework, the *stochastic* component cannot be overlooked or assumed to be nonexistent. *Random* variation in concentration-response experiments is accounted for by an appropriate error term plus, in the case of the Bayesian framework, prior distributions on model parameters. In either case, the ''thing'' describing the scatter in concentration-response plot is a probability distribution which in turn is defined by its parameters.

Neglecting error models renders subsequent estimation and inference impossible. It is entirely possible to use ordinary least squares (OLS) to estimate model parameters in the absence of an error model (because OLS is a geometrical concept). However, an error model is necessary to compute confidence or prediction intervals or to test hypotheses about the true parameter values. That this type of inference can be performed even when there has been no explicit specification of any stochastic terms in the model (for example, Ashauer et al. 2010) is because the methods of OLS and maximum likelihood estimation (MLE) are equivalent under the assumption of normally distributed errors. Although this may not be a problem in some cases, there are situations where the assumption of a normally distributed error term is inappropriate—for example, modeling the number of surviving organisms in a concentration-response experiment when the sample sizes are very small (typically fewer than 10).

In our opinion, the stochastic part of modeling should be accorded as much attention as the deterministic part. Thinking about and identifying an appropriate error model underlies good statistical inference and may lead to additional insights and clarity that would otherwise remain undiscovered.

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IMPORTANCE OF CHARACTERIZING NANOPARTICLES BEFORE CONDUCTING TOXICITY TESTS

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Rapidly expanding growth in the field of nanotechnology has led to the development of numerous applications of nanomaterials in industrial (e.g., paints, electronics) and consumer (e.g., cosmetics, clothing treatments) products. These engineered nanoparticle (NP)-containing products have, however, the potential to release particles (single or aggregates) or ions by means of wastewater discharge into the aquatic environment. SCENIHR (2006) emphasized that the behavior of NPs is critically dependent on several particle characteristics, including size, surface area and surface reactivity, and that risk assessments for both human health and the environment have to be based on these characteristics. However, in practice, risks of NPs are in most cases assessed on the basis of their chemical composition alone and, to date, no widely accepted or well-defined risk assessment methods or test strategies exist explicitly designed for NPs.

There is a growing consensus on the necessity of proper and accurate characterization of NPs in environmental media and biological systems to ensure reliable and reproducible toxicity tests are performed. Without such characterization, nanotoxicity experiments will have limited value due to unknown variability in experimental conditions of the NPs (Warheit 2008). Some of the current divergent or conflicting results from nanotoxicological tests could also be better explained if there had been adequate characterization in all studies. However, exhaustive characterization of NPs is undoubtedly costly and time-consuming, and therefore, a sufficient but practical approach is needed. Some principal characteristics of NPs which have been considered to deserve quantification before conducting toxicity tests are size, shape, state of dispersion, physical and chemical properties (e.g., electronic and optical properties, chemical composition and reactivity), surface area, and surface chemistry (Powers et al. 2006). Whereas a significant number of papers list some of these characteristics for the powder or the initial dispersion media (usually in distilled water) few, if any, studies of aquatic nanotoxicity have provided a full characterization of the size distribution (especially hydrodynamic size), dispersion state (especially in biological media) or surface chemistry (like surface charge) of NPs in the actual test media. However, many NPs are likely to undergo significant size distribution or surface chemistry changes when they are transferred between media during experiments, such as from dispersion media (deionized water) to test media (e.g., sediment, freshwater, seawater, and cell culture media). Such changes may alter bioavailability or toxicity in ways that are not entirely understood.

We have characterized commercially available Ag NPs before conducting toxicity tests (Cong et al. unpublished data) and found a clear difference between the manufacturer's information (< 100 nm and 2 to 3.5 μm, respectively) and what we measured (20 to 200 nm and 8 nm to 3 μm in deionized water, respectively) for 2 Ag forms. This difference in size between that reported by the manufacturer and that measured in the laboratory was also observed by Scown et al. (2010). The reasons are most likely due to batch-to-batch variation during production, changes in material properties between synthesis and initial characterization, and particular experimental conditions when used (e.g., pH, ionic strength, and temperature). This observed variability highlights the importance of fully characterizing commercially obtained NPs before performing toxicity experiments, at the very least in the stock solutions used to prepare exposure treatments.