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Learned Discourses: Timely Scientific Opinions

Timely Scientific Opinions

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ARE ALL CURRENT ECOTOXICITY TEST RESULTS CONFOUNDED BY DESIGN AND IMPLEMENTATION ISSUES?

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Mammalian and ecological toxicologists share the primary goal of addressing Paracelcus' hypothesis: "All things are a poison. It is only the dose that makes a thing not a poison." (Deichmann et al. 1986). Thus, they strive to establish exposure scenarios whereby adverse effects occur and to distinguish those from exposures at which adverse effects do not occur.

For many centuries, toxicology has focused primarily on human medicine and pharmacology. Thus, the concept of dose is a deeply rooted paradigm where dose metrics are largely based on single or repeated bolus exposures (e.g., pills or injections). In contrast, dose metrics in environmental toxicology, particularly aquatic toxicity, are largely based on administration of continuous, constant concentrations (e.g., static or flow-through). Both employ a dose surrogate chain model where

the external exposure dose metric is a surrogate for the concentration of the substance in the organism as a whole. In turn, this is a surrogate for the concentration of the substance at lower levels of biological organization (organs, tissues, cells) and ultimately the concentration of the substance at the site or sites of toxic action in the organism. If the exposure produces a sufficient concentration of the substance at the site or sites of toxic action during the observation time frame, an observable adverse effect is produced. The concentration at the site of toxic action, however, is effectively unknown due to uncertainties in the kinetics of absorption, distribution, metabolism, and excretion (ADME) processes.

Virtually all standard toxicity tests used for research and regulatory purposes are based on this conceptual model. As is the case for all models, satisfying the underlying assumptions is critical for its valid application. First, the exposure dose metric is assumed to be a valid, consistent dose surrogate both for the whole-body concentration of test substance in the simplest case and, ultimately, for all organism compartments including the site or sites of toxic action. This applies for a single substance and test organism and, where relative toxicity comparisons are desired, for all substances and organisms being considered. Second, the design of the toxicity testing protocol is assumed to have controlled all toxicity modifying sources so as to virtually eliminate all differences in test results due to toxicokinetics. Thus, any differences in the results between test organisms and/or substances can be attributed to differences in toxicodynamics; therefore, reliable quantitative comparisons of relative toxicity data can be carried out.

Unfortunately, neither of these 2 assumptions is routinely validated in standard environmental and aquatic toxicity testing methods. Yet, despite this, they are generally assumed to be true. It is ironic that the statistical component of a toxicity test model is subject to more rigorous scientific scrutiny than the toxicological component. A consequence of this neglect is that it is difficult to retrospectively validate these assumptions on a case-by-case basis as appropriate data are not available, for example, a time series of whole body residues and toxicokinetic information, including time to exposure–organism steady-state (McCarty 2012).

To address this problem, a series of plausible, but hypothetical, organic chemicals were examined with a simple bioconcentration model for small fish. The possible influence of the interaction of toxicity test designs and common toxicity modifying factors was evaluated (Mackay et al. 2014). As definite influences were detected, a follow-up article quantified the nature and extent of these modifying factors (McCarty 2015). Hydrophobicity, exposure duration, body size, lipid content, mode of toxic action, and metabolic degradation can affect LC50s by approximately 10 to 1000 times.

It was judged that the varied and extensive range of influences found, and the considerable resulting variability in dose metrics, indicated that there is a significant amount of undocumented uncertainty in real testing data. Although the study focused on the LC50 test methodology, it should be expected in any other testing scheme employing an unvalidated dose surrogate design. Consequently, it was concluded "...results obtained with standard aquatic toxicity test protocols do not yield consistent, comparable measures of relative toxicity and are inappropriate for quantitative toxicology and risk applications" (McCarty 2015).

This strongly worded conclusion is based on the generally accepted 3 tiers of scientific validity framework (Borgert et al. 2011; McCarty et al. 2012). The primary tier consists of 3 decision criteria:

1) The identity and authenticity of scientific measurements must be verifiable within a defined range of precision.
2) Measurements and observations must not be confounded by extraneous factors and influences known to corrupt their accuracy and precision.
3) Measurements and observations must be replicable in independent hands.

It is difficult to suggest that an impartial evaluation of the nature and extent of the confounding influences reported would conclude anything other than a clear failure of the second criterion in the primary validity tier. Thus, the answer to the question posed in the title is an unequivocal yes; all current ecotoxicity test results are seriously confounded by design and implementation issues and are therefore inappropriate for many toxicology and regulatory risk applications.

Although there have been various discussions about other aspects of ecotoxicity testing—data quality, acute versus chronic response endpoints, lowest observed effect concentration and no observed effect concentration versus ECx, and investigator bias—the validity issue is a fundamental problem that affects all uses of ecotoxicity testing data. What is missing is a generic weight-of-evidence scheme for regulatory ecorisk decision making that considers quality, validity, and relevance. A 6-step outline, incorporating the tiered data quality framework of Borgert et al. (2011) and US Environmental Protection Agency (USEPA) guidance on relevance, is presented in McCarty et al. (2012). It is clearly time to begin this discussion in earnest.

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STATISTICAL HYPOTHESIS TESTING—TO TRANSFORM OR NOT TO TRANSFORM?

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Ecotoxicologists generate and analyze different types of data, for example, counts (nonnegative, integer-valued data) in

mesocosm studies or proportions (data bounded between 0 and 1, discrete) in acute toxicity laboratory tests. Such data are typically not normally distributed (Wang and Riffel 2011) and show a strong mean–variance relationship. To meet the assumptions of normality and variance homogeneity of statistical tests, the data are usually either transformed (for example log $[Ay+C]$ for count data [van den Brink et al. 2000] or arcsine $x^{0.25}$ for binomial data [Warton and Hui 2011]) or, if this fails to meet the test assumptions, nonparametric tests are used (Wang and Riffel 2011). However, such transformations can lead to biased estimates (O'Hara and Kotze 2010). Moreover, due to practical constraints, low sample sizes are common and lead to low power in hypothesis testing on which many ecotoxicological approaches rely (e.g., risk assessment for pesticides).

Generalized linear models (GLMs) extend the well-known linear model by allowing the use of other distributions than the normal distribution. For example, for count data, the Poisson or negative-binomial distribution represent appropriate data models (depending on the mean–variance relationship). For binomial data, the binomial distribution often represents a suitable model.

We compared the normal regression model with transformation, GLMs, and nonparametric methods in terms of Type I errors (claiming an effect when there is none) and statistical power (ability to detect an effect when it is present) using simulations (Szöcs and Schäfer 2015). All analyses were performed in a reproducible and open framework and are freely available at http://goo.gl/ygjK5Z. The provided simulation code can also easily be used to perform a priori power analyses.

We found (Szöcs and Schäfer 2015) that common experimental designs with small sample size and effect sizes of 50% reduction compared to the control showed undesirably low power (<<80%) for all valid methods (Figure 1, lower row). The quasi-Poisson GLM and the negative-binomial GLM with bootstrap both showed higher power than the normal regression model. The nonparametric Kruskal–Wallis test showed the least power (Figure 1, lower row). Surprisingly, the negative-binomial GLM had inflated Type 1 errors (Figure 1, top row). However, this could be fixed by using a parametric bootstrap test. The increased Type I error rate for the Poisson GLM (Figure 1, top row) was expected, because of overdispersed data and highlights the importance of model checking.

The general low power underpins the criticism toward endpoints like the NOEC that rely on hypothesis testing. Our results show that GLMs can increase the power to detect

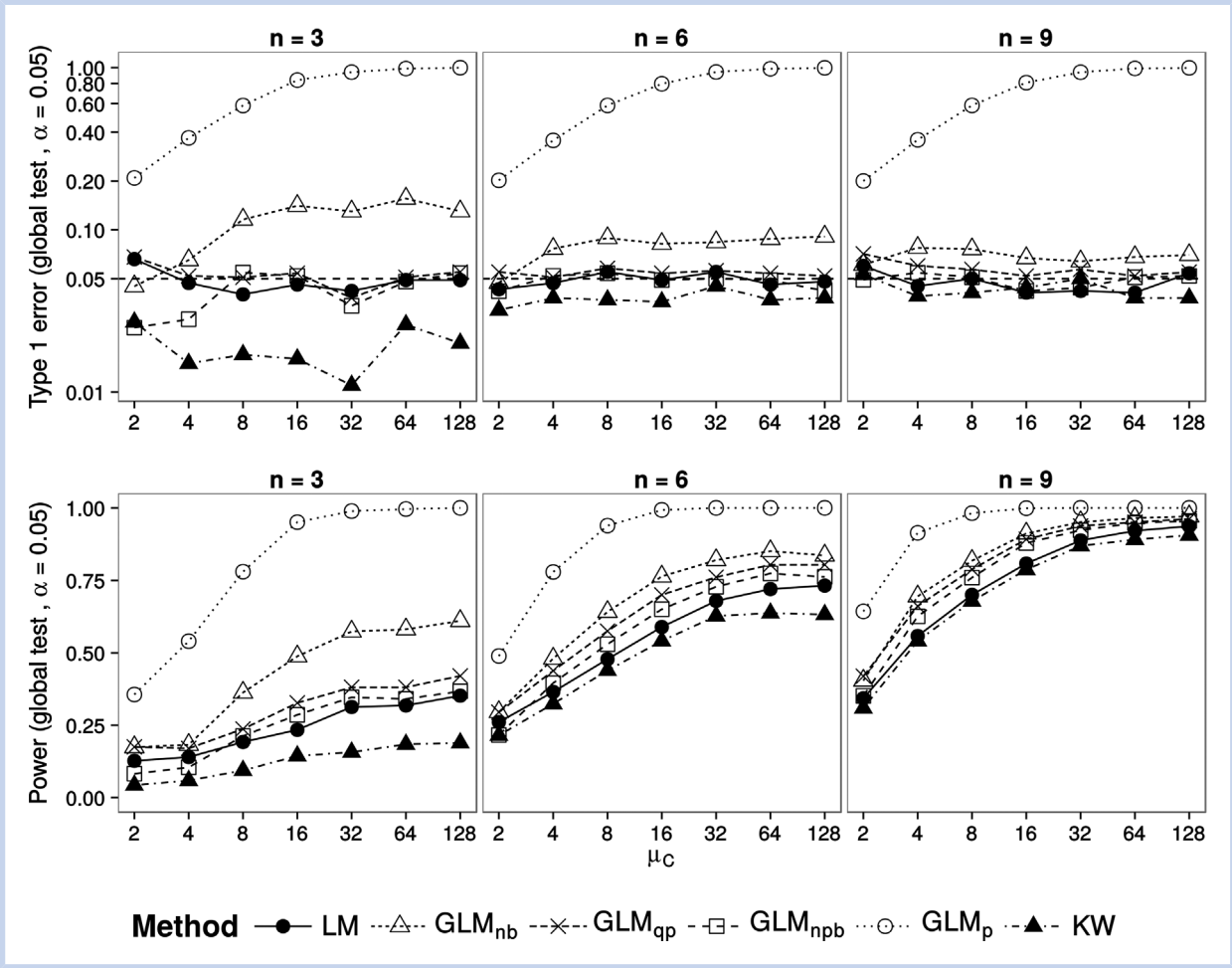

Figure 1. Results of count data simulations. Data have been simulated from a negative binomial distribution with moderate overdispersion, with an effect size of 50% and different sample sizes. Type I error (top) and power (bottom) for the test of a treatment effect for different models are displayed. Dashed horizontal line denotes the nominal Type I error rate at $\alpha = 0.05$. GLM_{nb}, negative binomial generalized linear model (GLM); GLM_p, Poisson GLM; GLM_{npb}, negative binomial GLM with parametric bootstrap; GLM_{qp}, quasi-Poisson GLM; KW, Kruskal–Wallis test; LM, linear model of log-transformed data. With kind permission from Springer Science+Business Media: Ecotoxicology is not normal, vol 22, 2015, 13990–13999, Szöcs E, Schäfer RB, Figure 2.

treatment effects. However, caution is advised with overdispersed data. The often used negative-binomial model is unreliable in these cases, and we recommend using bootstrapping or the quasi-Poisson GLM, which performed best in our study. Nevertheless, thorough model checking is indispensable in every data analysis. The normal regression model on transformed data had in all simulations appropriate Type I error levels but lower power (see also Ives 2015), suggesting that this method might be robust in different situations. However, there are a wide range of possible transformations available with possibly different properties. Moreover, if the aim of the study is modeling and prediction, rather than hypothesis testing, then GLMs are the preferred method because of lower bias.

We recommend that ecotoxicologists should: perform and report a priori power analyses, check the properties of their data and models, and change their models instead of their data.

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CITIZEN-BASED SCIENTIFIC DATA COLLECTION: FACT OR FICTION?

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Citizen-based scientific data collection is not well accepted by the general scientific community, perhaps most notably because of its overall perception as being unreliable. For citizen scientists engaged in environmental assessment, there are 2 key reliability components: the reliability of the analytical tool used and the reliability of the observer. We contend that tool reliability is no longer an issue, and with adequate training, the reliability of the citizen scientist can approach that of a well-trained scientist.

Over the last century, advances in analytical chemistry have produced rapid assessment tools of use to the citizen scientist. The earliest developed tools were targeted for the personal health market, including the home blood glucose test kit (Yamada 2011). Since their development, millions of kits have been used to the benefit of the public's health and well-being. Relative to water quality testing, the development of kits and assays by which the nonscientist can assess water quality, has undergone a similar, although slower, developmental trajectory. Commercial water quality testing kits are currently available, which allow the domestic user to measure water quality parameters ranging from pH, hardness, alkalinity, and nitrogenous compounds to trace contaminants, including metals and a variety of pesticides.

As the US Environmental Protection Agency (USEPA) has vetted many of these rapid assessment water quality tools, the analytical capability of the tool was never seriously in question. Rather, there appears to be an institutional bias within the scientific community against citizen scientists themselves as data collectors. If this bias can be overcome, we contend that the citizen science "work force" represents a massive data collection capacity that not only accumulates large amounts of data over small time periods but may also overcome some of the issues of data reliability through sheer sample size.

To produce accurate and reliable data from citizen scientists, training needs to be sufficient without creating a considerable burden to both the trainer and trainee. This can be achieved by an appropriate program design that allows a small number of professional scientists to train and orchestrate a large number of citizen scientists. For example, Rech et al. (2015) mobilized citizen scientists to quantify and qualify litter along a series of Chilean river systems. This study assessed the quality of categorical data (i.e., litter type) collected by citizen scientists and found that citizen scientists could provide reliable data comparable to professional scientists with appropriate training.

In our own experience, a brief training regime was sufficient to allow citizen scientists to collect and report reliable data generated by a semiquantitative triazine herbicide test strip (USEPA 2004). Reporting errors for 136 citizen scientists were assessed under laboratory conditions with 1.5% and 2.2% reporting a false positive or false negative result, respectively. In 2015, citizen scientists provided the opportunity to assess their reporting errors in the field by submitting 70 photographed results via Twitter or Facebook. Reporting errors from field-collected data were similar to laboratory conditions where 2.9% and 1.0% reported either a false positive or false negative, respectively (Cochran–Mantel–Haenszel test, $\chi^2 = 156.08$; $p < 0.001$). This low error rate demonstrates how appropriate selection of data acquisition tools by the experimental designer mitigates the potential for error by citizen scientists.

The strength of citizen science is not the individual participant but rather the overwhelming number of citizen scientists who can contribute to data collection, more or less simultaneously (Kolok et al. 2011). By increasing participation in data collection, the potential error is reduced, thereby improving overall reliability in the generated data set. A powerful example of this is the 115th Audubon Christmas Bird Count where 72 653 observers tallied 68 753 007 birds comprising 2106 species across the globe (National Audubon Society 2015). Given these final numbers, it is implausible to think that no mistakes occurred in the identification of bird species by citizen scientists. However, the annual records combined with replicated observation in many regions allow for identification of outliers and curation of the data set by experienced scientists. It is worth noting that data collected by this highly motivated group of citizen scientists have been used to support multiple peer-reviewed articles on migration patterns of birds (Link and Sauer 2007).

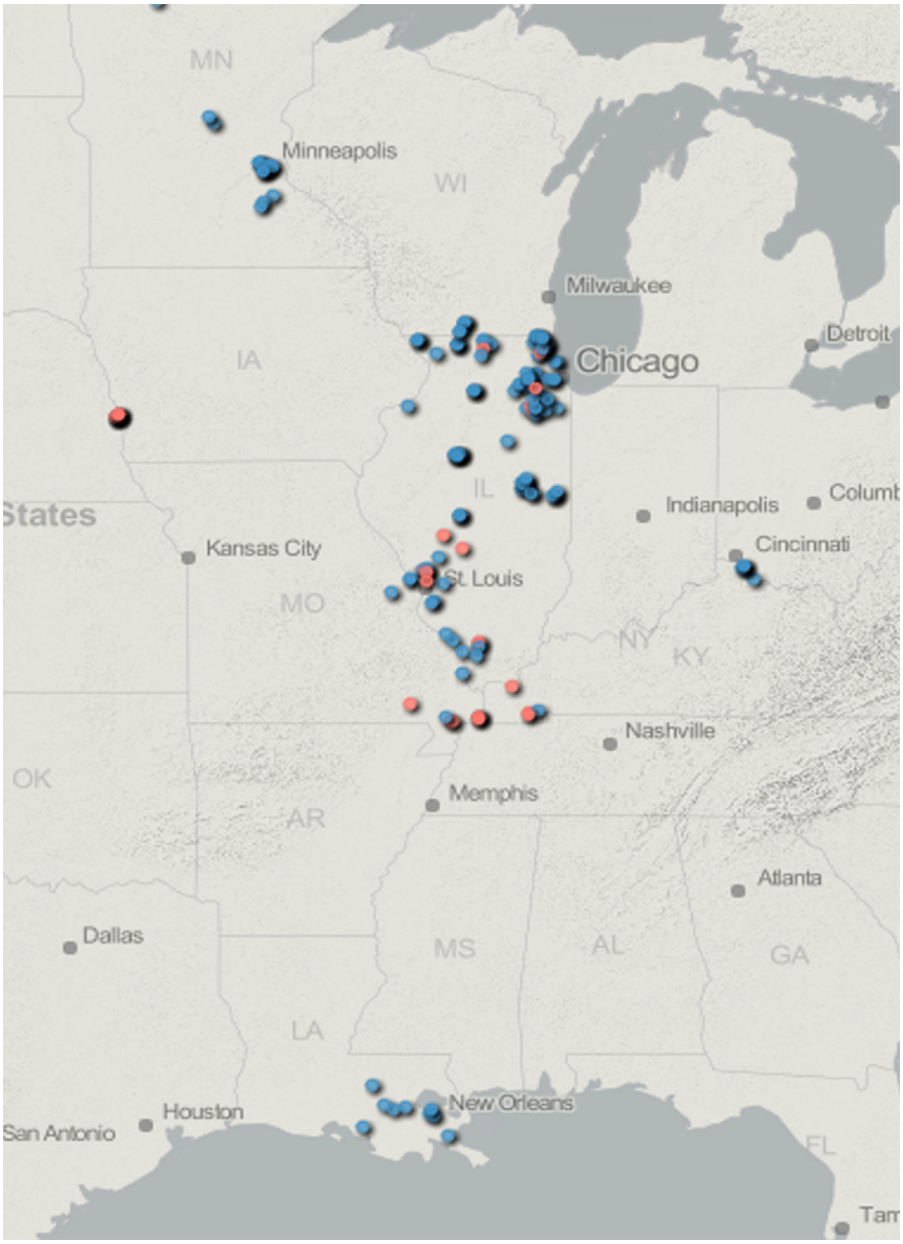

Figure 1. The distribution of positive (>3 μg/L, red circles) and negative (<3 μg/L, blue circles) atrazine hits across the Mississippi River ($n = 211$) on June 7, 2014.

To date, however, there have been few attempts to synchronize citizen-based data collection in water quality monitoring on a comparable scale to the Christmas Bird Count. We used citizen scientists from across the Mississippi River watershed to monitor for the occurrence of a commonly used herbicide, atrazine. During the spring of 2014, this survey simultaneously (all measurements collected on the same day) characterized the presence of atrazine within select regions of the watershed. University undergraduate students contacted individuals within these regions who we believed would be interested in water sampling, such as: teachers and professors from colleges and high schools; employees of zoos, museums, aquaria, and field stations; and, individuals associated with previously existing lake and stream monitoring programs. Once contacted, the participants were sent an information packet consisting of the number of strips that they requested, as well as practice strips and solutions, directions regarding strip determination (positive or negative atrazine measurement), and information regarding data recovery. The participants were given a number of options by which they could return their data to us, including email, text message, Twitter, and Instagram. On the date of the assessment, June 7, 2014, 211 atrazine samples were collected within the watershed from Lake Itasca, Minnesota to New Orleans, Louisiana (Figure 1). Although over 80% were negative for atrazine, geographic hot spots of atrazine were also located.

Given that the results from 2014 were all gathered on a single day, it is not possible to extrapolate from these data to a seasonal profile within the watershed over time. Nevertheless, they do allow for the elucidation of data-driven hypotheses that can be further tested. We contend that

rapid assessment tools, when used by citizen scientists, produce reliable data. These data in turn can be used as a form of first-tier testing that can generate testable hypotheses, which in turn can be tested using more conventional methods.

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ARE ALL CHEMICALS ENDOCRINE DISRUPTORS?

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Endocrine disrupting properties require specific evaluation under the European regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; 1907/2006) and the regulations on plant protection (Regulation [EC] 1107/2009) and biocidal (Regulation [EC] 528/2012) products. The development of specific criteria to "identify endocrine disrupting properties" is underway to enable hazard-based regulation in the European Union (EU). In the United States and Japan, scientific, risk-based approaches are being developed.

Regardless of the regulatory process, most geographies use the World Health Organisation International Programme on Chemical Safety (WHO IPCS 2002) definition of an endocrine disrupter or variants thereof. These definitions require that a substance is demonstrated to cause a change in endocrine function that consequently leads to an adverse effect in an intact organism to identify it as an endocrine disrupter. Such a definition is very broad, and at its most cautious, might capture many mechanisms that in general would not specifically be considered endocrine disruption. For instance, stress is a nonspecific, neuroendocrine response that can lead to adverse outcomes. In addition, other toxic mechanisms (e.g., liver toxicity) may also secondarily impact the endocrine system and tissues. Such factors should therefore be considered when screening and testing substances for potential endocrine activity or disruption, respectively. In fact, following the large scale screening of pesticides and pesticide inerts under the US Environmental Protection Agency's (USEPA) Endocrine Disruptor Screening Program (EDSP), practical experience with screening assays has highlighted some of these factors as important to data interpretation and future study design (Coady et al. 2014).

The misidentification of indirect effects as truly endocrine disrupting can have serious consequences in terms of triggering unnecessary higher tier testing, resulting in additional vertebrate animal use, and can be generally resource intensive. Additionally, misidentification of indirect effects as endocrine disruption could also result in product deselection by consumers and/or severe regulatory consequences in the EU, such as removal from the market. Thus, the ability to distinguish nonendocrine from endocrine modes of action is extremely important when operating in a purely hazard-based regulatory environment.

All organisms can experience systemic toxicity or stress at some level of exposure to any substance. These stressors are ultimately reflected in organismal responses—from reallocation of energy from nonessential processes such as growth, development, and reproduction to detoxification mechanisms. Ultimately, if the stressor is severe enough, the response will lead to death. Stress responses are a neuroendocrine cascade that has been well described in both mammalian and fish models. Stress leads to catecholamine release, corticotropin releasing factor (from the hypothalamus) causing pituitary synthesis and secretion of corticotropic hormone, which stimulates the synthesis and secretion of glucocorticoid hormones (cortisol in teleost fish or corticosterone in rats). Together, catecholamines and glucocorticoids initiate secondary and tertiary stress response factors (Figure 1).

The stress response in fish includes a number of endpoints that are also measured in screening studies that are designed to assess sexual endocrine activity and disruption. For instance, 11-ketotestosterone, estradiol and vitellogenin, female gonad histopathology, and Gonadal Somatic Index are key endpoints in the fish endocrine screening studies (guidelines OECD 229, 230 and OPPTS 890.1350) that are also known to be responsive to a generalized stress response (Aluru and Vijayan 2009; Milla et al. 2009). Adverse effects documented to be derived from stress, such as time to sexual maturity, fecundity, gamete quality, and sex reversal are also measured in higher tier fish studies, such as the fish full lifecycle and fish sexual development test (guidelines OECD 240 or OSCPP 890.2200 and OECD 234, respectively). Therefore, in screens and tests designed specifically to detect sexual endocrine activity and/or disruption, "endocrine responses" can be detected from broader, more generalized stress responses that are not specific to a particular endocrine mode-of-action.

This example with fish highlights that the stress response as a neuroendocrine cascade meets the requirements of the WHO/IPCs definition of an endocrine disrupter because both an altered endocrine function and adverse effect can be causally related. Because "the dose makes the poison," at a certain dose or concentration any chemical could meet the endocrine disruption definition. Clearly, screening and testing chemicals for endocrine activity or disruption needs careful consideration in regards to study design, interpretation, and regulatory decision-making.

It is important to separate the "generalized stress endocrine response" from those of direct endocrine interaction for which there may be a higher regulatory concern (e.g., due to particular hazards during sensitive windows of exposure with subsequent organizational effects on organism development). When assessing chemistries at the screening level for their potential to interact with specific aspects of the endocrine

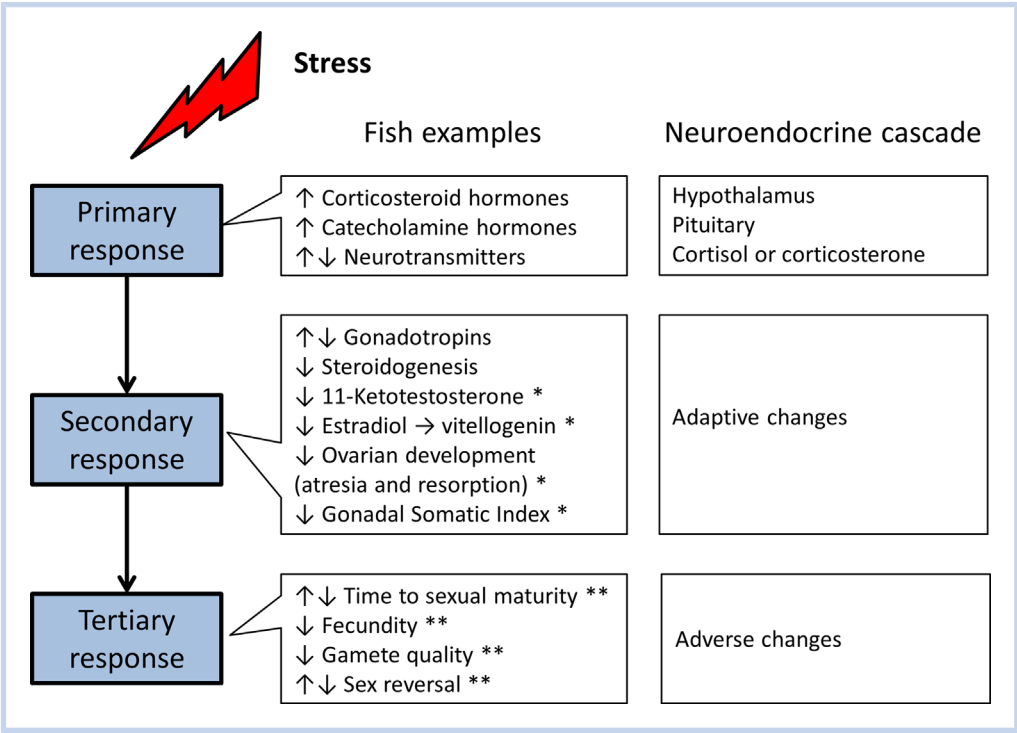

Figure 1. Generalized stress response highlighting the neuroendocrine cascade leading to both adaptive and adverse effects. Effects from the stress literature on fish indicate that responses are also endpoints in endocrine screening (*) and higher tier (**) studies.

system (i.e., estrogen, androgen, and thyroid hormone pathways), it is important to test at concentrations or doses that are as high as possible to maximize the chances of finding a true endocrine effect if it occurs. However, it is also necessary to avoid testing at concentrations that are confounded with systemic toxicity. Therefore, it is imperative to have an operationalized approach to determine the maximal tolerable dose or concentration and sufficient data and interpretation tools to separate general toxicity responses from specific endocrine interactions (Wheeler et al. 2013).

Other specific toxicities can also have indirect effects on the endocrine system that could potentially be mistaken for endocrine activity or disruption. Liver toxicity is one clear example common to both mammalian toxicological and ecotoxicological models. Liver toxicity modes of action have been described (Moslen 1996), and 2 of these mechanisms may be particularly influential in affecting endocrine endpoints: direct liver damage or degenerative changes leading to reduced functional capacity, and induction of biotransformation enzymes leading to increased hormone clearance. Because the liver plays a primary role in the metabolism of hormones, "interference" can lead to secondary effects on circulating hormone levels. This can lead to indirect effects on thyroid and sex steroid hormones, leading to impacts on endpoints related to such things as development, metamorphosis, vitellogenesis, and/or fecundity. Several of these endpoints are clearly relevant adverse effects that should be (and are) included in risk assessment. However, it would be unfortunate and potentially detrimental socio-economically if they were misidentified as primary endocrine responses that would be regulated on hazard alone in the EU.

Broad definitions of endocrine disruption are being used in different global regulatory programs. There are a number of stress-related and/or specific, but nonendocrine-mediated, toxicities that can lead to responses in endocrine screening and higher tier testing and that could be mistaken for primary endocrine effects. Misinterpretation could lead to unnecessary higher tier testing and have severe regulatory implications under the hazard-based regulations being finalized in the EU. By using hazard-based regulation alone, there is an implicit shift toward authorizations that are based solely on mode-of-action (in this case endocrine) that do not take into account the dose–concentration at which a particular effect occurs. Consequently, to avoid misidentification of a large host of chemicals as endocrine disrupters, it is extremely important that decisions are made on known primary endocrine effects that are not consequent to generalized stress responses or indirect toxicities.

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EXPLORING THE EFFECTS OF MICROPLASTICS IN FRESHWATER ENVIRONMENTS

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Littered plastic is one of the most conspicuous environmental pollution problems and is present in one form or another globally. In addition to the obvious aesthetic impacts of littering, the effects to organisms through entanglement and ingestion have been studied extensively (Gregory 2009). However, as plastic waste abrades, it disintegrates into smaller plastic fragments commonly termed "microplastics" (MPs) that are potentially more readily bioavailable (Lambert et al. 2014) (Figure 1).

MPs, particularly polyethylene, are also known to effectively sorb organic contaminants from surrounding waters, and internalized MPs might 1) lead to the direct physical injury of an organism, and 2) provide a vector for the transfer of sorbed co-occurring chemical compounds through the ingestion of contaminant-loaded MPs (Lambert et al. 2014; Wagner et al. 2014). Current investigations into the impacts of MPs have largely focused on the marine environment; scientific knowledge on the effects of MPs in freshwater ecosystems is lacking (Wagner et al. 2014).

We are currently working on a project that aims to assess the environmental risk of MPs in freshwater habitats. An investigation of MP environmental persistence will be carried out to provide environmental fate summaries for different polymer classes. This will be combined with laboratory studies to assess relevant sublethal endpoints such as reproduction and fitness for selected freshwater organisms. As certain polymer types are known to accumulate co-occurring organic contaminants, the toxicity of "virgin" MPs will be compared to MPs conditioned with relevant freshwater contaminants. Eventually, an outdoor mesocosm study will evaluate both MP fate and impacts in model ecosystems. Below, we highlight some of the key questions that this project will aim to address:

1. Are microscopic particles, specifically nano-sized plastic particles, formed during plastic degradation processes? To address this question we have initiated a long-term study that aims to quantify the formation of nanoplastics during the degradation of larger plastic samples in aqueous media. This study aims to test the hypotheses that 1) particle formation will be influenced by polymer type and polymer density, 2) particle formation will differ between the neat polymer and the final product, and 3) bio-based polymers will degrade at a quicker rate and produce more particles than petrochemical polymers of a similar density. First results using Nanoparticle Tracking Analysis indicate that nano-sized particles are formed during the degradation of polystyrene (Lambert and Wagner 2016).
2. How can effects from long-term exposure to environmentally relevant concentrations of MPs be assessed? Laboratory studies will be used to investigate the in vivo effects of MPs with representative freshwater organisms. The exposure scenarios will aim to address the hypothesis that MPs conditioned with co-occurring contaminants will have a greater effect on organism growth and functioning than virgin MPs. As current standard ecotoxicity testing approaches are probably inappropriate for assessing the effects of MPs, these studies will focus on population-level effects.
3. How can the environmental fate and effects of MPs be assessed under realistic conditions? This question will be addressed through a mesocosm study that will begin in

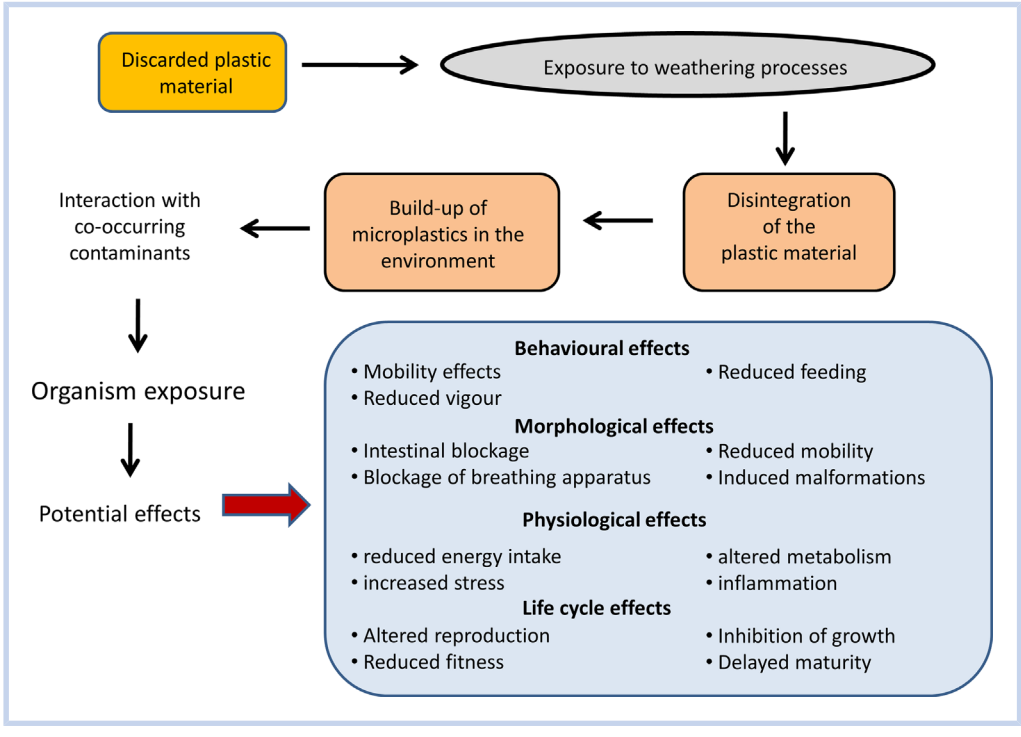

Figure 1. Microplastic formation and potential effects.

spring 2016. The study design will compare effects in mesocosm ponds exposed to virgin MPs and mesocosms exposed to the same concentration of MPs conditioned with a mixture of organic freshwater contaminants. The overall aims of the mesocosm study will be to: 1) build an environmental fate profile of the MPs used in the study, 2) quantify mesocosm-level impacts on macro-invertebrate species abundance and distribution, and 3) characterize community level impacts to case study sediment dwelling and water column organisms. The knowledge derived from the mesocosm study will be an important link bridging toxicity data from laboratory-scale experiments to the situation in the ecosystem.

4. How can the environmental risk of MPs be assessed? To address this question we will need to integrate both the project and literature data. On one level, plastic products can be assessed based on their chemical composition, including both the monomer and additives compounds. However, this type of approach does not take into consideration the effects that microscopic particles may exert. Therefore, a framework that takes into account the following may be appropriate: 1) the predicted uptake of MPs within a specified size range appropriate to the organism in question, 2) prediction of daily oral exposure to MPs by organisms from the consumption of contaminated food and/or prey, and 3) characterization of the ecotoxicological effects arising from exposure. This type of approach itself generates many more questions such as: 1) What are the most important exposure pathways for organisms of interest?, 2) How bioavailable are MPs of different size classifications once ingested?, 3) What are the risks of co-occurring contaminants and MP mixtures?, and 4) Will exposure impact ecosystem functioning?

It is hoped that this project will generate valuable information on the effects of MPs on species distribution and on bioaccumulation under conditions very close to the real world situation. The relevance of this proposed project is further highlighted by several stakeholders, including the United Nations (UNEP), the Oslo/Paris Convention (for the Protection of the Marine Environment of the North-East Atlantic) (OSPAR), and the International Maritime Organization (IMO) that have raised concerns about MPs. UNEP (2011) states with respect to MPs that "we lack adequate knowledge of their potential physical and chemical effects on marine organisms." OSPAR (2012) argues that "Setting a pressure target may be appropriate…". However, setting sensible pressure targets (i.e., ecological quality criteria) for freshwater ecosystems requires sound knowledge on the environmental hazards of MPs in those ecosystems, a gap in our current knowledge that the suggested project will help to address.

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CAN POLYCHLORINATED BIPHENYLS BE REMOVED FROM CHESAPEAKE BAY BY A COMMERCIAL FISHERY?

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The Chesapeake Bay is the largest estuary in the United States, receiving high nutrient inputs from various anthropogenic sources, in turn fueling eutrophication within its waters. Concurrently, the estuary receives significant inputs of PCBs, largely from point sources from its industrialized and urbanized tributaries (Velinsky and Baker 1999a). Phytoplankton provide organic C-rich sites for PCB sorption (both absorption and adsorption) and represent an important vector for PCB bioaccumulation within higher trophic level organisms such as zooplankton and planktivorous fishes.

Atlantic menhaden (*Brevoortia tyrannus*) are pelagic schooling fish found in abundance in the estuary and near coastal regions. As omnivorous filter feeders, juveniles and adults primarily feed on phytoplankton and zooplankton. By filtering vast quantities of planktonic organisms, these fish accumulate PCBs from this pelagic dietary route.

From 2004 to 2013, the sole commercial fishery operation for Atlantic menhaden in the US midAtlantic, Omega Protein, reported landing an annual average of 144 000 tons of fish, of which an average of approximately 50% came from fishing efforts within the Chesapeake Bay, with the other half from coastal regions in the midAtlantic. This reduction (whole body) fishery processes menhaden into fish oil and meal, products high in sought-after omega-3 fatty acids.

In a report detailing mass balance calculations of PCB inventories and input and loss vectors, Velinsky and Baker (1999a,b) concluded that PCBs could be removed from the mainstem Chesapeake Bay through volatilization, burial in sediment, and export to the ocean. Recognizing the large biomass of menhaden removed from the estuary through the commercial fishery, and the PCBs accumulated in these fish,

we pondered whether a fourth removal vector should be considered? Although menhaden are a financially and ecologically important species within the Chesapeake Bay and Eastern US coastal waters, reported PCB concentrations within this species are surprisingly scarce within published literature and elsewhere (e.g., monitoring data sets).

To address the paucity in contaminant data, an undergraduate research project at Philadelphia University quantified the individual PCB concentrations of 12 adult menhaden (~3 y; average length 28 cm) caught by purse seine from the southern Chesapeake Bay in October 2014 by Omega Protein. Using previously published analytical and instrumental methods (Ashley et al. 2000), the PCB concentrations (heads and tails removed, remaining body homogenized) of the sum of 90 individual or co-eluting PCBs (t-PCBs) congeners were found to range from 40 to 93 ng t-PCBs/g fish with a mean and standard deviation of 70 ± 17 ng/g. With t-PCB concentrations determined from this study, catch data reporting ktonnes of menhaden caught from the Chesapeake Bay from 2004 to 2013 (Figure 1, left-hand axis), supplied by Omega Protein, were used to estimate the mass of kg of PCBs removed by year from the Chesapeake Bay by this fishery (Figure 1, right-hand axis).

Based on our calculations, an average of 5 kg of PCBs have been removed annually from the Chesapeake Bay from 2003 to 2014. How impactful is this removal mechanism compared to other loss terms acting within the mainstem Chesapeake Bay? Using a mass balance model and available data at the time, Velinsky and Baker (1999b) estimated the mass of PCBs lost by 3 primary vectors to be: 340 kg/y by volatilization, 560 kg/y by export to the ocean, and 280 kg/y burial by sedimentation. Based on our estimates from this study, the menhaden fishery removed an average of 0.5% of the PCBs per year compared to the other removal mechanisms.

This pilot study is not the first to pose the question of contaminant reduction by commercial fisheries. Gustavson et al. (2010) discussed the feasibility of using fisheries as a method to decrease dioxin and dioxin-like contaminants in the Baltic. Mackenzie et al. (2004) found multiple fisheries within the Baltic Sea (e.g., cod, sprat, salmon) may account for 3.5% of identified PCB exports within that ecosystem. However, our study and the "back of the envelope" calculations outlined here represent the first attempt to estimate PCB removal from the Chesapeake Bay through the menhaden fishery.

This pilot investigation reveals the utility of mass balance estimates, coupled with contaminant data, as a cursory and useful first step in estimating the role of fisheries in contaminant reduction within aquatic systems. In the future, other contaminants, such as Hg and emerging chemicals of concern, and different species that are both commercially and recreationally important, such as blue crab and striped bass, should be included to fully capture the potential of multiple fisheries in removing PCBs from the Chesapeake Bay.

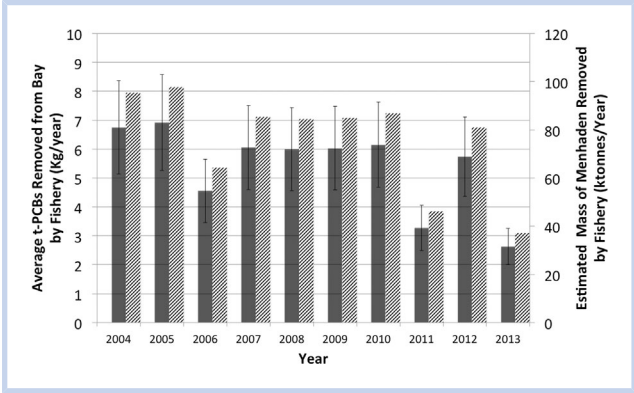

Figure 1. Solid bars (left axis) represent the calculated average t-PCBs removed from the Chesapeake Bay using the reported catch data from Chesapeake Bay caught menhaden (hatched bars, right axis). Error bars represent the range in estimated values for PCB removal based on the 25% relative standard deviation of PCB concentrations in menhaden caught in 2014.

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