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Look Inside *ToxSci*

look inside ToxSci

From the Editor's desk

This issue of *Toxicological Sciences* features several exciting changes: a redesigned cover, revised category subheadings, and this "Look Inside *ToxSci*" feature. Together with my esteemed colleagues in the field of green chemistry, we have outlined some exciting opportunities for the field of toxicology in the editorial on green chemistry and toxicology. There are insightful articles on the regulatory challenges regarding mixtures that are being addressed by the European Union and on risk assessment of carbon nanotubes. In the past, we have highlighted a single article from each issue. It has become increasingly difficult to select just one article from the volume of high quality work, so beginning this month we will highlight multiple articles in each issue here in "Look Inside *ToxSci*." From solvents, pesticides, and nanoparticles to the microbiome, the highlighted articles span the breadth of our field. Of course, this issue contains something that will never change; our raison d'etre . . . the best original research in the field of toxicology. —Gary W. Miller

Editor's Highlights

Adverse outcome pathway of 2,4-dinitrotoluene: Nitrotoluenes are used in the production of explosives and are thus a concern for military personnel and those residing near sites of artillery production and testing. Wilbanks and colleagues (pp. 44–58) used an adverse outcome pathway-based approach to evaluate the toxicity of 2,4-dinitrotoluene. The team found that alterations in PPAR-alpha signaling led to alterations in energy metabolism and physical endurance with 2,4-dinitrotoluene causing weight loss and reduced exercise tolerance. The effects were mitigated in mice lacking PPAR-alpha. Increases in PPAR-gamma appeared to be a compensatory response in the 2,4-dinitrotoluene treated animals.

Insecticides and calcium channels: Pest control is an important public health intervention. Insecticides are designed to kill insects and many are designed to target the insect nervous system. Most are familiar with the ability of organophosphates to inhibit acetylcholinesterase or pyrethroids to inhibit sodium channels. In this issue Meijer *et al.* (pp. 103–111) report that many of these common insecticides also have a convergent point of toxicity, namely, voltage-gated calcium channels. The authors demonstrate that a variety of structurally diverse insecticides target these calcium channels. The disruption of depolarization-evoked calcium could wreak havoc on the process of neurotransmission, which relies on tight regulation of calcium homeostasis. The authors also demonstrated additive effects in binary mixtures of various insecticides. These findings suggest that evaluation of voltage-gated calcium channels may be an important aspect of neurotoxicity risk assessment.

Nanoparticles in paints: Once nanoparticles are incorporated into their intended product does their toxicity change? This is the question addressed by Smulders and colleagues (pp. 132–140). The authors compared the toxicity of nanoparticles in their pristine form and after they had been incorporated into paint. The authors examined titanium dioxide, silver, silicon dioxide engineered nanoparticles. The authors were able to show that the engineered nanoparticles induced a variety of inflammatory and immune responses (increase cytokines, increased neutrophil count). However, once the engineered nanoparticles were embedded into the paint these toxicological outcomes were not observed. This suggests that products that integrate nanoparticles into their structures may not pose specific nanotoxicity. However, the authors did not address the long-term effects that could occur as these products age. This will be an important area of research as the toxicological consequences of lead paint were observed only after deterioration of the lead-based paints.

Ochratoxin and the microbiome: Ochratoxins are produced by a variety of *Aspergillus* and *Penicillium* species. These mycotoxins can be found in grains, coffee, and dried fruit and some have been classified as human carcinogens. Thus, humans are exposed to these mycotoxins through the diet. Guo and coworkers (pp. 314–323) conducted metagenomic analysis on the microbial species found in the gut of exposed animals. Metagenomics consists of sequencing of all of the microbial species in given sample. The authors found that ochratoxin A caused a significant decrease in the diversity of the gut microbiota with a sharp increase in *Lactobacillus*. The Lactobacillus was then cultured and the identified strain was found to be able to readily absorb ochratoxin A, but not metabolize it. Using the combination of metagenomics and microbial culture, the authors demonstrate the importance of the gut microbiota in how food-based toxins impact human health.

Letters to the Editor

Invalid Controls Undermine Conclusions of FDA Studies

To the Editor,

We are writing in response to the recent paper on bisphenol A (BPA) by Delclos *et al.* (2014) and the related paper by Churchwell *et al.* (2014). These manuscripts represent some of the first data from an important multi-investigator initiative sponsored jointly by the U.S. Food and Drug Administration (FDA) and two divisions of the National Institute of Environmental Health Sciences, the National Toxicology Program (NTP), and the division of extramural research and training. Through this joint initiative, investigators from the FDA and academic researchers are working together for the first time to address important questions surrounding BPA and the risks to human health. This is a costly but critically important investment that will underpin future decisions to protect the public's health. Although we applaud both federal agencies for their investment, the Delclos *et al.* and Churchwell *et al.* publications raise serious concerns about whether meaningful data will result from this effort.

Delclos *et al.* report the results of an extensive preliminary study conducted by the FDA and designed to characterize the dose response for adverse effects induced over a wide range of BPA doses. A subsequent large-scale, multi-investigator study will involve many of our highly respected colleagues and assess a variety of endpoints. However, because this large initiative will be conducted in the same facility, the problems evident in the data published by Delclos *et al.* and Churchwell *et al.* raise serious concerns about the wisdom of investing research resources and expertise in this multi-investigator initiative. In the preliminary studies reported in these papers, a concerted effort was made to control for BPA contamination in both animal contact materials and those used in sample collection and analysis. Nevertheless, serum analyses revealed that both sets of negative control animals (naïve and vehicle only controls) had experienced significant BPA exposure, with serum BPA levels equivalent to those in the lowest BPA exposure groups. Positive and negative controls are essential for this study: Positive controls demonstrate that the animals are estrogen sensitive, and negative controls provide a point of reference for assessing adverse effects. The stated objective of the study was to examine low-dose effects of BPA (i.e., below the published NOAEL(No observed adverse effect level)), not just effects at very high, acutely toxic doses. Contamination in negative controls renders this control group useless for assessing low-dose effects.

Delclos *et al.* assessed a wide variety of endpoints and conclude that adverse effects only occur at extremely high BPA doses. This is a remarkable conclusion because in the absence of uncontaminated negative controls, it is impossible to determine if lower doses induced effects, especially because BPA levels in controls were similar to those in the four lowest dose groups (i.e., up to 80 μg/kg/day). Churchwell *et al.* state that the source of the contaminating BPA could not be identified, "but interpretation of the toxicological effects, observed only at the highest BPA doses, was not compromised." Essentially, the authors are arguing that they can make meaningful interpretations in the absence of controls. This not only violates basic scientific principles, it is untenable in view of the large body of published data demonstrating adverse effects at low doses and nonmonotonic responses for a variety of BPA-induced effects. In the absence of uncontaminated negative control animals, meaningful conclusions about the effects of low doses of BPA simply cannot be made.

Given the concerns in this field and the controversy already surrounding BPA, it is essential that researchers, reviewers, and editors maintain stringent standards. This is, however, particularly important for large-scale studies conducted using good laboratory practice (GLP) guidelines for toxicology studies, because these studies are generally accorded more weight in the regulatory arena. The studies by Delclos *et al.* and Churchwell *et al.* are particularly disappointing because they were conducted under the auspices of the FDA and will therefore—despite their significant limitations—be cited extensively. More importantly, the results reported in these manuscripts raise a very real concern: If the contamination problem has not or cannot be resolved, the subsequent large consortium effort seems destined to be a flawed study on an unprecedentedly grand scale.

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Response to Hunt *et al.*, Invalid Controls Undermine Conclusions of FDA Studies

To the Editor,

The comments of Hunt *et al.* on our recent manuscripts (Delclos *et al.* (2014). *Toxicol. Sci.* **139**, 174–197 and Churchwell *et al.* (2014). *Toxicol. Sci.* **139**, 4–20, hereafter Delclos *et al.* and Churchwell *et al.*) raise issues related to both completed and ongoing studies on bisphenol A (BPA) at the National Center for Toxicological Research (NCTR). We address these concerns separately.

Hunt *et al.* suggest that the results of the 90-day BPA subchronic study described in Delclos *et al.* are not interpretable with regard to effects <2700 μg/kg body weight (bw)/day, the study-defined "low dose" region, because of the data reported in the companion manuscript, Churchwell *et al.*, indicating that there was unintended exposure to BPA in the negative controls. As pointed out in Delclos *et al.*, exposure of the negative controls to low levels of the target compounds frequently occurs in the studies of ubiquitous environmental contaminants, with the critical factor being the differential exposure between negative controls and treatments. We concluded that the level of exposure in the negative control animals in our study, as evidenced by the presence of BPA-glucuronide, which can only be produced by inlife exposure, would compromise our ability to interpret any BPA-related treatment effects <8 μg/kg bw/day. However, this was not an issue in the Delclos *et al.*'s study because: (1) there were no effects observed for the endpoints measured in doses up to 2700 μg BPA/kg bw/day versus the negative controls; (2) the reference estrogen had clear effects, even though these animals were equally exposed to the unintended source of environmental BPA; and (3) clinical and histopathological observations in the negative controls were comparable to observations in multigenerational studies conducted at this institution using the same strain of rat fed the same base diet (NTP, 2008, 2010).

Hunt *et al.* are concerned that the detection of BPA-glucuronide in the blood of control animals in the 90-day study indicates that the ongoing chronic toxicity study, which is also providing animals and tissues to multiple academic investigators (Consortium Linking Academic and Regulatory Insights on BPA Toxicity; CLARITY-BPA, Schug *et al.*, 2013), will be of no use. The considerations discussed above would be applied to the interpretation of data from the chronic/CLARITY-BPA study. However, it should be made clear that the 90-day BPA subchronic study described in our two papers was conceived and implemented prior to the conception or formation of the chronic/CLARITY-BPA study. Although conduct at NCTR is common to both, and the issue of exposure of negative controls became evident only after the start of the chronic/CLARITY-BPA study, it is also the case that there are differences between the studies that significantly limit the potential for background exposure of controls in the ongoing study. The source of the reported unintended exposure in the 90-day study was proposed in Churchwell *et al.* to be related to the use of the broad BPA dose range, including very high doses (100,000 and 300,000 μg BPA/kg bw/day), in the same animal rooms as the control and low BPA doses (0 and 2.5 μg/kg bw/day). Consistent with our hypothesis, unpublished results of analyses of serum from animals in the chronic/CLARITY-BPA study rooms with a high dose of 25,000 μg BPA/kg bw/day indicate that the BPA-glucuronide levels in control rats, the critical measurement in distinguishing inlife exposure from postexposure sample contamination with aglycone BPA, are clearly distinguishable from levels in the lowest BPA dose group, 2.5 μg/kg bw/day. These data will be reported with the first results from the CLARITY-BPA studies.

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