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Bridging boundaries: On the contributions of Dr. Michael Plewa to the disinfection byproduct field

William A. Mitch

Department of Civil and Environmental Engineering, Stanford University, California 94305, USA

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Why is a Professor in the Department of Crop Sciences such a dominant presence in the field of disinfection byproducts (DBPs)? I believe the answer to this question exemplifies the characteristics of impactful researchers. Most research fields rely on a set of core skills and tend to develop tunnel-vision with respect to research topics. These factors can be stultifying for the field, but present opportunities to those in other fields with the vision and fortitude to span research fields and fill unoccupied niches where their approach and skillset is needed and lacking. Dr. Michael Plewa has been one of these rare researchers.

At the start of his career in the mid-1970s, Dr. Plewa was squarely within the scope of the Department of Crop Sciences at the University of Illinois, with research that used plants as models to evaluate mutagenicity of pesticides and other contaminants (e.g., Gentile et al., 1977). This work even resulted in a publication in *Science* covering the metabolism of 2-aminofluorene into a mutagen within tobacco plants (Plewa et al., 1983).

The field of DBP research was founded in 1974 (Rook, 1974; Bellar et al., 1974) with the discovery of trihalomethanes (THMs) as byproducts of chlorine disinfection, just as Dr. Plewa's career was beginning. Subsequent epidemiological research indicated that consumption of chlorinated tap waters with high concentrations of THMs was associated with an elevated risk of bladder cancer (Costet et al., 2011). DBP research tended to focus on the small subset of DBPs currently regulated, particularly THMs and haloacetic acids (HAAs), with researchers divided into three pools based on their skillsets (Li and Mitch, 2018). Analytical chemists focused on characterizing the humic substance precursors believed to serve as the precursors for THMs and HAAs in pristine waters, with a view towards developing tools to predict the concentrations likely to form for different chlorine exposures (Liang and Singer, 2003). Toxicologists pursued studies using rats and mice to understand the mechanisms of toxicity associated with THMs and HAAs and differentiate the toxicity of their different chlorinated and brominated analogues (Boorman et al., 1999). Epidemiologists conducted additional studies to associate consumption of disinfected tap waters with adverse health outcomes using THMs as the metric for exposure (Costet et al., 2011).

In about 2000, several factors spurred a shift in the field. First, the highly potent carcinogen, *N*-nitrosodimethylamine (NDMA), was discovered in the effluent of Water Factory 21, the nation's first potable reuse plant, resulting in its temporary closure. This closure and subsequent research indicating that NDMA formed by fundamentally different pathways (i.e., from the reaction of chloramines with wastewater-derived precursors (Mitch et al., 2002; 2004)) illustrated that there was a broader universe of DBPs of critical importance to drinking water. Concurrently, Woo et al. (2002) developed structure-activity relationships to predict which of the unregulated DBPs (e.g., haloacetonitriles) might be most toxic,

E-mail: wamitch@stanford.edu

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and subsequent research demonstrated the widespread occurrence of these DBPs in disinfected waters (Krasner et al., 2006). Second, research documenting the occurrence of pharmaceuticals and personal care products in surface waters (Kolpin et al., 2002) demonstrated the widespread impact of wastewater discharges on surface waters used as drinking water supplies (i.e., *de facto* reuse). With respect to DBPs, these results indicated that the organic matter serving as precursors to DBPs could be fundamentally different from the naturally-occurring humic substances that had been the focus of research until that time. DBP chemists began to speak of effluent organic matter (EfOM) and algal organic matter (AOM) to distinguish this material from the conventional natural organic matter (NOM). Chemists began to demonstrate that EfOM and AOM promote the formation of different classes of DBPs (Huang et al., 2012), particularly nitrogen-containing DBPs such as haloacetonitriles (HANs). Lastly, utilities were increasingly switching from chlorination to novel combinations of disinfectants (e.g., ozone/chloramines) to minimize the formation of regulated THMs and HAAs. DBP chemists began to demonstrate that these novel combinations of disinfectants alter the array of DBPs formed (Shah et al., 2012).

These factors fostered a shift among DBP chemists towards using their analytical chemistry skills to identify novel DBPs and to understand how they formed. Over 600 DBPs were identified (Richardson, 2011), although usually each formed at lower concentrations relative to the regulated THMs and HAAs. This raised a fundamental challenge. How do we decide which DBPs are important, and thus which are worthy of further research and regulatory attention? With > 600 DBPs discovered, not all can be thoroughly evaluated; prioritization is critically important. *In vivo* toxicology studies and epidemiology studies are both expensive and time-consuming. Since cancer is associated with lifetime exposures, epidemiology studies also require knowledge of the concentrations of DBPs over several decades, and these concentrations typically are unavailable, particularly for the newly discovered DBPs.

Perhaps even more importantly, it should be noted that while chemical analyses can be highly precise (<10% error), *in vivo* toxicology and epidemiology studies are not; this higher precision provides chemical analyses higher power to resolve small differences in concentrations relative to the lower power to resolve differences in toxic potency provided by *in vivo* toxicology studies. The importance of a DBP ultimately reflects both its concentration and its toxic potency. A fundamental roadblock was how to combine high-resolution chemical analyses with low-resolution toxicological results to prioritize DBPs based on their potential for significant contributions to the toxicity of disinfected waters.

At about this same time, Dr. Plewa initiated a research collaboration with Dr. Roger Minear in the Department of Civil and Environmental Engineering at the University of Illinois. This research led to Dr. Plewa's keen insight that his fundamental skillset focusing on the development of high-resolution bioassays could be applied to fill this critical unoccupied niche in the DBP field. Dr. Plewa's hallmark has been a willingness to dive into research collaborations headfirst. He has not been afraid of working closely with DBP chemists and engineers despite coming from a completely different field. The Chinese hamster ovary (CHO) cell cytotoxicity and genotoxicity bioassays he developed as part of the collaboration with Dr. Minear (Plewa et al., 2002) were not "off-the-shelf" assays, but ones he developed and tailored to the needs of the DBP research field. Both endpoints are plausibly related to bladder cancer. However, a key principle was the focus on ensuring high-resolution. Dr. Plewa realized that the resolution of the bioassays needs to be comparable to the resolution of the chemical analyses (~10% error) to render the bioassays useful for prioritizing DBPs. Just as there is "analytical chemistry", so should there be "analytical biology". Biology is inherently more variable than chemistry. While many environmental toxicologists use log-transformed data due to this variability, Dr. Plewa has increased the number of replicates and conditions tested to enable resolution comparable to analytical chemistry.

Application of these assays over two decades has resulted in a library of cytotoxicity and genotoxicity metrics for > 100 DBPs (Wagner et al., 2017). The impact of this library on the DBP field has been profound. Researchers have weighted measured concentrations of DBPs with these toxicity metrics to estimate the relative importance of DBPs for the cytotoxicity and genotoxicity of disinfected waters (Plewa et al., 2017; Chuang et al., 2019). Additional research has demonstrated that the CHO cell cytotoxicity of DBP mixtures is additive, such that the toxic potency-weighted concentrations of measured DBPs can be used to predict the cytotoxicity of their mixture within ~12% (Lau et al., 2020). The results of these analyses have routinely indicated that some of the known, novel, but currently unregulated DBP classes (e.g., haloacetonitriles) are far more significant contributors to the cytotoxicity and genotoxicity of disinfected waters than the regulated THMs and HAAs.

These results have raised serious questions about the current regulatory focus on THMs and HAAs and suggest the need to broaden the scope of DBP research, including *in vivo* toxicology and epidemiology studies, to encompass novel DBP classes. There has been some pushback against these suggestions. *In vivo* toxicologists highlight that the *in vitro* assays employed by Dr. Plewa are not definitive, in that they do not cover all endpoints and do not incorporate pharmacokinetics (e.g., the potential for transformation of DBPs prior to reaching target organs). While true, should perfection be the enemy of the good? A similar criticism could be lodged against analytical chemistry. The > 600 novel DBPs characterized to date still account for ≤ 50% of total organic halogen (TOX), so how do we know that haloacetonitriles or other unregulated classes are more important than the uncharacterized TOX fraction? Why aren't we measuring everything? We may not score a touchdown, but we can still move the ball further down the field.

Epidemiologists sometimes indicate that THM concentrations correlate with those of other DBPs since greater chlorine contact time should form more DBPs across the board. Since historical records are routinely available only for regulated compounds such as THMs, they are the only convenient compounds with which to measure DBP exposure. Given that changes in precursor materials (e.g., EfOM) and disinfection practice may have altered the array of DBPs formed, we have recently demonstrated that THMs serve as a poor predictor for haloacetonitrile concentrations, and that this poor correlation could contribute to the rather low and variable odds ra-

tios (sometimes significant and sometimes not) observed in DBP epidemiology studies, even for bladder cancer (Furst et al., 2021). Shouldn't we start laying the groundwork for the epidemiology studies of the future by initiating consideration of alternative DBPs that may serve as toxicity drivers?

Whether or not CHO cytotoxicity and genotoxicity remain as the dominant bioassays in the DBP field, Dr. Plewa's lasting contribution will be the establishment of analytical biology using high-resolution bioassays as a cornerstone to help prioritize DBPs for the more expensive, and time-consuming *in vivo* toxicology and epidemiology studies. The relatively recent move throughout environmental science towards expecting research involving novel contaminants to incorporate toxicological bioassays concurs with his impact yet raises some troubling concerns. Since many bioassays are now incorporated within "off-the-shelf" kits, they can be relatively easy for those in other sub-disciplines (e.g., chemistry) to incorporate within their studies. However, we need to accord analytical biology comparable respect to analytical chemistry by ensuring that these bioassay studies retain the high-resolution needed to complement analytical chemistry. Bioassays that evaluate log-transformed data provide essentially qualitative results that are not very helpful for moving the field forward. Dr. Plewa has routinely demonstrated a willingness to train graduate students in other research labs, not just with respect to the CHO cell bioassays, but more importantly with regard to the experimental planning and statistical analysis needed to generate high-resolution results. Experts with this training will be sorely needed to continue progress in this field.

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