To regulate or not to regulate? What to do with more toxic disinfection by-products?

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Abstract
Since the first regulation of disinfection by-products (DBPs) in the 1970s, >700 DBPs have been identified, and many of these are much more toxic than those regulated. Moreover, drinking water today is not the same as it was in the past, with increasing use of alternative disinfectants like chloramine, ozone, chlorine dioxide, and UV (which can form other types of DBPs), as well as new impacts on our source waters from climate change, population increases, wastewater intrusion, and energy exploration. The question today is whether we are regulating the right DBPs to protect human health, and if not, what should be done. New approaches may involve (1) the use of *in vitro* data and a Precautionary Principle approach, (2) using surrogate metrics of finished waters, such as total organic bromine/iodine, total nitrosamines, or total organic nitrogen rather than creating longer lists of regulated DBPs, and (3) using toxicity assays for whole drinking water extracts to pinpoint potential problems, then using chemical analyses to identify the toxic agents, and finally, (4) invoking different treatment strategies to reduce the toxicity. While the early regulations likely significantly improved the safety of drinking water, DBP exposure is a constant in modern life, and there is more that we can do.

Background

Disinfection of drinking water is recognized as a principal public health triumph of the 20th Century, as it significantly reduced waterborne illness and increased life expectancy. However, an unintended consequence was the generation of DBPs that are formed by the reaction of disinfectants (chlorine, chloramine, ozone, chlorine dioxide, UV) with natural and anthropogenic organic matter, bromide, and iodide [1-3]. These are not chemicals made for any particular purpose, but are formed during drinking water treatment. More than 700 DBPs have been identified to date, and of those, approximately 100 have been rigorously studied for their occurrence, formation, and quantitative analytical biological toxicity [1, 3-7].

Back in the early 1970s, only a handful of DBPs were known—primarily trihalomethanes (THMs)—which were first discovered by Rook and Bellar et al. in 1974 [8, 9]. Soon afterwards, these THMs were found to be widespread in chlorinated drinking water and were also reported to be carcinogenic in laboratory animals. As a result, the first DBP regulations were born. The fledgling U.S. Environmental Protection Agency (EPA), which was inaugurated in 1970, began to regulate THMs (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) under the 1979 Safe Drinking Water Act. Later, additional toxicity data were obtained for five haloacetic acids, bromate, and chlorite, and these were added to the regulation under the Stage 1 and Stage 2 Disinfectants/DBP Rules in 1998 and 2005, respectively. Interestingly, while most DBPs are regulated based on animal cancer data, bromoacetic acid has no cancer data, and chlorite is regulated based on a different endpoint (developmental neurotoxicity)[10]. The World Health Organization, European Union, and several other countries also have guidelines or regulations for a small number of DBPs in drinking water.

Are we controlling the right DBPs?

Human epidemiology studies bolster the idea of regulating and controlling DBPs, as several studies reported a risk of bladder cancer, colorectal cancer, miscarriage, and birth defects [11-22].

In fact, DBPs belong to a small group of contaminants for which adverse human health effects were consistently demonstrated. Recently, however, there is significant discussion as to *whether the right DBPs are being controlled*. A multidisciplinary Gordon Research Conference on drinking water DBPs (now titled 'Water Disinfection, Byproducts, and Health') was formed around these very issues in 2006. The controversy stems from the fact that while most of the regulated DBPs are carcinogenic in test animals, they do not cause the same kind of cancer observed in the human studies. Test animals show primarily liver cancer, whereas humans show primarily bladder cancer. This begs the question whether we are controlling the right DBPs. Another line of thought is that the test animals may not be good models for drinking water exposure in humans. For example, because rats and mice (the most common test animals) do not hold their urine like humans do [23, 24], DBPs may not reside in their bladders long enough to produce bladder cancer.

Drinking water is changing

Another issue is that drinking water is not what it used to be…*it's changing*. While regulations and environmental protection measures considerably improved the safety of our water, drinking water is different from what it was in the 1970s. First, chloramine, ozone, chlorine dioxide, and UV are increasing in use, and much less is known regarding DBPs from these alternative disinfectants. Ironically, the driver for this switch to other disinfectants are the regulations, as many drinking water treatment plants struggle to meet the tightened regulations using chlorine as a disinfectant. A switch to chloramine is the easiest. Drinking water treatment plants only have to add ammonia to their existing chlorination treatment to form chloramines, which can lower the regulated DBPs by as much as 90%. Thus, a simple and cheap change can help plants meet regulatory limits with no problem.

If only all the other DBPs were also lowered with the regulated DBPs, all would be good. Unfortunately, the more toxic iodo-DBPs and nitrogen-containing DBPs (N-DBPs) [25] can be formed at much higher levels when plants switch to chloramine [26-31]. A switch to ozone can also result in increased formation of bromate, as well as unregulated halonitromethane, haloaldehyde, haloketone, and haloacid DBPs, with secondary chlorination. Even UV can react with natural organic matter to increase the formation of highly toxic halonitromethanes when secondary chlorine is applied [32]. Because ozone and UV do not leave a residual disinfectant, chlorine or chloramine is often applied as a secondary disinfectant to maintain disinfection in the distribution system.

Population increases, climate change, water and wastewater reuse, and new energy exploration are also impacting our water sources. With population increases come increased wastewater impacts on our fresh water sources, which can be exacerbated in times of drought, providing less dilution for toxic contaminants not well removed in wastewater treatment [33, 34]. In fact, some heavily impacted rivers with little rainfall and little dilution may consist of 90-100% treated wastewater during certain months of the year (e.g., Santa Ana River and Trinity River in the U.S.) [35]. Most cities are downstream of other cities, such that the upstream city's treated wastewater enters the drinking water source of the downstream city. Climate change (with increasing drought) is likely to further concentrate these wastewater contaminants in drinking water sources, as well as concentrate natural organic matter and bromide and iodide, creating conditions for enhancing the toxic impacts of drinking water. Increased use of desalinated drinking waters poses risks from DBPs [36]. Finally, new energy exploration, such as hydraulic fracturing, can introduce new geogenic and manmade chemicals, as well as very high levels of bromide and iodide, into drinking water sources. This can result in new DBPs not previously imagined [37].

Many unregulated DBPs are more toxic than the ones we regulate

Since the first DBP toxicity studies began in the 1970s, more than 100 additional DBPs were rigorously analyzed for cytotoxicity, genotoxicity, endocrine disruption and carcinogenicity. With >100 DBPs analyzed, the largest quantitative comparative analytical toxicity database was developed by Plewa and colleagues [5, 38]. These *in vitro* data include cytotoxicity and genotoxicity endpoints conducted using non-neoplastic Chinse hamster ovary (CHO) cells. A series of important conclusions were derived from these systematic studies. First, in general, the order of declining toxicity follows: I-DBPs > Br-DBPs >> Cl-DBPs, such that I- and Br-DBPs are much more of a concern than Cl-DBPs [1, 28, 38, 39]. In CHO cells iodoacetic acid (unregulated) is the most genotoxic DBP discovered [5, 28, 39], it is tumorigenic [40], impacts cell hormonal systems [41], and induces adverse reproductive effects [42-45]. Extensive biochemical and toxicogenomic studies were conducted on iodoacetic acid. It inhibits glyceraldehyde-3-phospate dehydrogenase (GAPDH) and causes oxidative stress in cells, leading to cytotoxicity and genomic DNA damage [46-50]. An animal carcinogenicity study on iodoacetic acid is currently in progress; those results should be available soon.

Second, it is clear that N-DBPs are significant drivers of much of the toxicity in drinking water [5, 25, 51, 52]. These include haloacetonitriles (HANs)[53, 54], haloacetamides (HAMs)[55], halonitromethanes (HNMs)[56], and nitrosamines [57]. For example, THMs are relatively low toxic agents (but are regulated), while halonitromethanes are high toxicity agents that are not regulated [56, 58]. *In fact, no I-DBPs or N-DBPs are regulated in the U.S*. Unregulated haloacetaldehydes have also been found to be important toxicity drivers [59, 60].

If the regulated DBPs were good surrogates for all these other more toxic DBPs, everything would be good. In fact, when EPA created its initial regulations for the THMs in the 1979 Safe Drinking Water Act, they knew there would be other DBPs generated, but assumed that the THMs could serve as good surrogates for all DBPs. Unfortunately, this did not prove true, as EPA had to add the haloacetic acids, bromate, and chlorite, which don't always track with THMs. It is now clear that *many other DBPs don't track* with the 11 regulated in the U.S. [27, 61].

A new approach called 'TIC-Tox' has been gaining in popularity for determining toxicity drivers in drinking water. This method involves multiplying the measured DBP with cytotoxicity or genotoxicity metrics of that DBP in CHO cells [62]. These analyses were applied to drinking waters in the U.S. and Europe and have revealed important groups of toxicity drivers for drinking water [63-67]. However, because TIC-Tox is limited to the ~100 DBPs for which quantitative, comparative cellular toxicity data exists (using an identical biological platform) and also for DBPs that can be quantified or semi-quantified, it is missing both unknown/unquantified DBPs and DBPs that have not yet been studied for toxicity. Thus, there is more to do.

What should we do?

Despite all the toxicity and occurrence data showing the widespread presence of these DBPs in drinking water, no new regulations were promulgated [7, 68]. The U.S. EPA appeared to be on the brink of potentially regulating nitrosamines about 10 years ago, based on national occurrence data and carcinogenicity data, but appears to have backed off for now. And, while EPA announced that it would regulate perchlorate several years ago, there is still no maximum contaminant level (MCL) established and no new regulation.

Regulations generally take many years to develop, with EPA wanting animal (*in vivo)* toxicology data (typically in two types of test animals) and significant national occurrence data to justify a meaningful

opportunity for improvement of human health. While that approach should be lauded, some are questioning whether we should take a more precautionary approach as done in Europe. Rather than waiting 20-30 years for all of the animal data and further testing, it may be better policy to be precautious and not wait to find out 30 years later to discover those highly toxic DBPs cause adverse human health effects [7]. *However, to our knowledge, EPA has never regulated any DBPs based only on in vitro toxicology data.*

In fact, animal toxicity data is increasingly hard to come by. In the early days of new environmental regulations, many scientists were generating loads of toxicology data. This enabled the five haloacetic acids, bromate, and chlorite to be regulated. In addition, significant cancer data was obtained for nitrosamines 40-50 years ago (including *N*-nitrosodimethylamine, NDMA), which is the main reason that nitrosamines could be considered for regulation, despite not knowing they were DBPs until the early 2000s. Now, that we are in the 2020s, there are fewer "classical" toxicologists in regulatory agencies and elsewhere to conduct these studies. Mostly due to the significant time and expense required for multigenerational animal cancer studies, there is a push toward high throughput screening approaches [69, 70]. While these screening approaches are rapid and sensitive, they have some drawbacks in that they use genetically unstable human tumor cell lines; precise, focused concentration-response curves are rarely conducted; and it may be difficult to integrate these approaches with cell-based metrics on cytotoxicity and genotoxicity. However, integrating these high-throughput approaches with quantitative comparative mammalian cell analyses may provide a positive impact on improving the safety of drinking water [6, 48, 52, 71-73]).

In light of this, how do we move forward? If we switch to using *in vitro* data in the U.S., some may argue that we may regulate a lot of DBPs unnecessarily, causing undue burden and expense for drinking water treatment plants and consumers. A cell is not a tissue, a cell is not an organ and a cell is not an organism. A chemical that is genotoxic or cytotoxic in cell culture does not necessarily mean it will induce adverse health effects in humans. Also, if we regulate on *in vitro* data, what toxicity endpoints should be used? Clearly a battery of analytical biological assays is needed; however, they must be quantitative and directly comparable to identify those DBP classes and individual DBPs that express toxicity. Furthermore, it is important that this battery of assays can ultimately define the molecular mechanisms for DBP toxicity [6, 7, 52, 70, 72, 74].

Another concept recently discussed is whether we should switch from chemical-by-chemical regulation to surrogates. This could help simplify future regulations, such that easy-to-measure surrogates are used rather than creating longer and longer lists of DBPs to regulate. Examples would include using total organic bromine (TOBr) and total organic iodine (TOI), measured using combustion-ion chromatography, which could account for the known, toxic Br- and I-DBPs, *as well as unknown Br- and I-DBPs not yet identified* [64, 67, 75-77]. The total nitrosamine (TONO) assay could be used as a surrogate measure for all nitrosamines [78], including ones we currently know and those still unknown. Finally, total organic nitrogen (TON) might also be a way of capturing the more toxic N-DBPs. However, these surrogate approaches would invariably incorporate many low-toxic or non-toxic compounds into the measurement, and will likely include DBPs that may not pose significant health risks.

Another approach could be to have a toxicity assay or multiple assays applied to the extracted organics from finished water. This has been useful in identifying toxic responses associated with water characteristics and disinfection methods [28, 29, 33, 34, 61, 79-83]. Those waters that express high levels of toxicity would be candidates for detailed chemical analyses (using high resolution-mass spectrometry) to identify the forcing agents leading to the toxicity. However, it is important to

understand that for any *in vitro* system, it is impossible to describe a human health risk factor based on cellular assays alone. Yet, these quantitative *in vitro* assays allow for precise comparisons of the relative cytotoxicity, genotoxicity, or toxicogenomic effects of a series of drinking waters. Identifying the forcing factors that lead to enhanced toxicity and then removing these agents or minimizing their formation would reduce the health risks of the finished water. In addition, the use of acellular thiol-reactivity as a surrogate for DBP toxicity could also be applied [80, 84, 85]. This new assay is a chemical reaction and does not require cells, making it accessible for many laboratories. It has shown good correlation with real *in vitro* measurements across a wide variety of waters with different impacts [80].

Ultimately, there would need to be treatment strategies to lower the DBP levels, whether regulated individually or through surrogates. Promising approaches that we favor include the use of granular activated carbon (GAC) or membranes to remove DBP precursors (then applying a lower dose of chlorine for disinfection), using UV with a lower dose of chlorine, or using chlorine dioxide, which tends to generate fewer DBPs overall. Finally, finding an inexpensive way to remove bromide and iodide would go a long way toward reducing toxic Br- and I-DBPs.

DBP exposure is a constant in modern life. Concerns of adverse health effects from these toxic agents is increasing with the increased level of compromised source waters used in the generation of drinking water. With the advent of climate change-mediated stress, there is increased reliance upon direct or indirect wastewater recycling or disinfected desalinated water, and new emerging DBP classes are entering the drinking water stream. Biologists, chemists, and engineers must form interdisciplinary teams to address problems posed by hazardous DBPs and other micropollutants in water. Systematic, comparative *in vitro* toxicology must be integrated as a feed-back information loop into innovative engineering processes to remove and degrade micropollutants and disinfect water. *In vitro* toxicity, the mode of toxic effects, and analytical chemistry must be integrated in future epidemiologic studies. Interdisciplinary systems must be implemented to prevent unintended adverse human health consequences as we move forward in the implementation of new methods to desalinate, decontaminate, reuse, and disinfect water.

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