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Published

2016

Book Title

Assessing Transformation Products of Chemicals by Non-Target and Suspect Screening - Strategies and Workflows

Version

Accepted Manuscript (AM)

DOI

10.1021/bk-2016-1242.ch004

Bioanalytical approaches in assessing transformation products

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Transformation products (TPs), including disinfection by-products (DBPs) produced from halogenenation of natural organic substances found in water, have been identified in disinfected waters at varying concentrations, depending on the source of the water. Normally for drinking water, the concentrations are very low, in the parts per trillion, but concentrations can be much higher in sewage treated waters. Methods for detecting these chemicals have improved over the past decade, but analytical chemistry methods generally lack the ability to detect new TPs and would work best if partnered with bioanalytical methods to evaluate genotoxicity, cytotoxicity and specific modes of action. The process of disinfection also destroys bioactive chemicals which can also be followed through bioanalytical assays. Bioanalytical tools are beginning to be used to

monitor and assess production of bioactive products in water quality.

Introduction

While natural and engineered systems for treating surface, ground or wastewater are effective in reducing pollutant concentrations and loads, they fall short in completely removing all chemicals that can pose health risks to humans and wildlife,[1] including so-called contaminants of emerging concern (CECs). Water and wastewater treatment processes, like those processes that occur in ambient water (*e.g.*, photolysis, hydrolysis), result in the transformation of parent CECs that can leave behind a suite of transformation products (TPs). Moreover, the resulting mixture of parent chemicals and TPs may or may not have reduced toxicity compared to the source water.[2] The disinfection of drinking water spawned a flurry of research activity during the last half of the 20th century to identify and assess the risk associated with disinfection by-products (DBPs). These DBPs arise from interactions of the oxidizing agent with naturally occurring substances (*e.g.*, residual organic C/N, halogen ions), while residuals of synthetic chemicals present in wastewater (*e.g.*, pharmaceutical and personal care products (PPCPs), consumer good additives, pesticides, flame retardants), or precursor molecules that leach from materials used

in the treatment process are refered to as TPs.[3] Moreover, some classes of transformation products have been shown to be exceedingly potent in eliciting unwanted biological responses.[4]

The occurrence of PPCPs in treated effluents in particular has raised new concerns about transformation of these chemicals by disinfection into more active forms. Most of these chemicals have acidic or basic functional groups that can easily be halogenated by chlorine, bromine or iodine during the disinfection process.[5-6] Bromination and iodination ensue because of natural high levels of bromide or iodide occurrence in different source waters.[6] Through reaction with hypochlorous acid, these halogens can be converted to reactive intermediates such as hypobromous acid.[6] Bromide concentrations will vary from one source to another, but levels as high as 0.3 mg/L have been measured in some rivers.[7-8] Bromine is much more effective than chlorine in the formation of TPs,[6,9] which is of great concern since the brominated TPs are more toxic than the corresponding chlorinated ones.[10] Iodinated analogs can also be found and these are even more toxic.[11] Both chlorine and chloramine are very effective at making TPs, but in general more are produced from chlorine treatment.[8]

Chlorination and chloramination during water treatment lead to the production of many TPs, some of which are known and regulated because of their carcinogenic potentials such as N-nitrosodimethylamine (NDMA),[12] but others have yet to be

discovered. These chemicals are made in minute quantities from precursors found in wastewater and some of them are potent carcinogens[13-14] or cause oxidative stress, driving adverse effects on aquatic biota. Depending on inputs into wastewater treatment plants, the TPs are diverse, with treatment plants generally producing a unique set of chemicals. NDMA is usually found in the 5-10 ng/L range in drinking water plants but can exceed 105 ug/L in some wastewater effluents, usually due to industrial inputs.[13-14]

Advanced oxidation processes (AOPs) that utilize ozone or UV radiation have been incorporated into treatment trains for indirect and direct potable reuse of treated municipal wastewater to destroy known problematic residual chemicals such as 1,4-dioxane and NDMA.[15-16] Whereas employing AOPs as a standalone treatment option can result in complex mixtures of TPs,[17] UV/ozone treatment positioned downstream of reverse osmosis (RO) can effectively minimize exposure to such known chemicals of health concern. However, knowledge gaps remain around the identity and health risks associated with CECs and their TPs.[18] This includes those formed early in the treatment train as a result of operational practices; TPs formed in RO concentrate (or "brine") that may be subject to disinfection and/or that maybe co-mingled with other waste streams prior to discharge to receiving waters; and TPs of parent CECs formed in ambient waters receiving

discharge from wastewater treatment and/or water recycling facilities.

Although evidence to date suggests little cause for concern is associated with recycled water subjected to RO/AOP, questions remain concerning the identity and biological activity of all possible TPs formed during recycled water treatment. In one study, *in vitro* bioassay results illustrated the gradient in water quality for samples taken across recycled water treatment trains; moreover, the measured bioactivity mirrored trends observed by monitoring known chemicals, including many CECs.[19] The amount of bioactivity measured in highly treated water that could be directly attributed to identified chemicals in the water varied widely depending on the *in vitro* endpoint, from >90% in specific responses (*e.g.*, estrogenicity, photosynthesis inhibition) to less than 3% for non-specific responses (*e.g.*, cytotoxicity, oxidative stress).[20] In a another study, bioanalytical tools revealed a gradation in water quality for samples from 2 water recycling facilities with different treatment trains, but the concentrations of known chemical agonists quantified in this study did not explain more than 10% of the measured bioactivity.[21] Clearly, additional work is needed to understand the formation and bioactivity of TPs for candidate unit processes and for the proposed treatment trains utilizing these processes.

As the source water for most recycling operations, treated wastewater effluent is known to contain measurable levels of CECs, and the evidence for transformation of CECs in effluent is growing. Endocrine active compounds such as estrogens, anti-androgens and glucocorticoids have been measured in treated wastewater effluent using both chemical and bioanalytical methods.[22-24] Most of the work on TPs and DBPs has been in advancing analytical chemistry methods to identify and then monitor chemicals of concern. In addition to targeted GC- and LC-MS/MS approaches to monitor known chemical products, a good bit of effort has also been put towards non-targeted approaches, using mass spectrometers that have very good duty cycles and that can identify chemicals based on precision measurements on their mass.[25-26]

Halogenation of gemfibrozil, a cholesterol lowering drug detectable in most treated wastewater effluent, was observed as a product of chlorine disinfection as well as by exposure to bromide; the resulting TPs were found to be anti-androgenic to fish.[27] The direct discharge of CECs contained in treated wastewater effluent into receiving waters and or engineered systems that take advantage of *in situ* processing (aka "natural attenuation") creates a third scenario whereby CECs and their TPs may impact water quality. Hijosa-Valsero et al.[28] observed *de novo* formation of TPs of selected PPCPs, including the analgesics ibuprofen and

diclofenac, in constructed wetland mesocosms. Whereas in many cases bioactivity and any subsequent toxic response may be mitigated with advanced treatment processes, the production of TPs may in some cases result in a different set of toxicity issues, even acute mortality.[29]

Bioanalytical tools such as receptor based *in vitro* assays possess the sensitivity to detect relatively low levels of endocrine activity in recycled water, wastewater effluent and surface waters that receive discharge of effluent and/or RO brine.[30] Because they screen for chemicals based on their common mode of biological activity (MOA), bioanalytical tools offer the promise of an efficient and comprehensive approach to detecting a wide universe of chemicals - legacy, CECs, and TPs alike – in potable reuse applications.[31] Applied in conjunction with advanced diagnostic tools (*e.g.*, non-targeted chemical analysis), a battery of *in vitro* bioassays targeting diverse MOAs is necessary to address complex mixtures and would complement existing chemical monitoring,[32] in providing a much needed additional line of evidence assuring good quality of recycled water for potable reuse.

Bioanalytical tools

In vitro bioassays have been developed and adapted to assess the presence of chemicals based on their biological effects.

Bioanalytical tools are very sensitive for their specific modes of action and add a new dimension to this approach. The assays provide an "effects-based" approach to identifying DBPs and TPs of biological concern and can add a measure of additive, synergistic and antagonistic effects for complex mixtures. In general for these assays to work well, waters containing the DBPs and TPs need to be extracted using a variety of methods including solid phase sorbents of various types for different analyses.

The USEPA and the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP) have developed a myriad of high throughput (HTP) assays for chemical screening purposes.[33] Additional assays have been developed through academic laboratories in recent years for HTP and high specificity and sensitivity and used for mode of action assessments of individual chemicals. With the concerted effort put forth by regulatory agencies in the US, Europe and Japan to develop pathways of toxicity[34-35] that relate to higher order endpoints in wildlife and humans, new attention has been devoted to developing sensitive and pathway-specific assays. Some of the assays include the potential for metabolism to take place, by adding a liver S9 fraction.[22,36] The liver S9 fraction refers to a liver homogenate that contains cytoplasm and microsomes and it is made by centrifuging the liver homogenate for a short time at 9,000 x g. This fraction contains all of the enzymes involved in phase 1 and phase 2

metabolism and thus can be used to test for activation of chemicals by metabolism. These new assays have been applied to a variety of environmental water projects.[37-39]

Among the biological activities that are of most concern for TPs are genotoxicity and oxidative stress and general cytotoxicity. Genotoxicity refers to chemicals which can damage DNA through several different mechanisms, including DNA breaks and mutations and which thereby interfere with the correct translation of the genetic code into functional proteins in the cell. Many chemicals that are genotoxic are highly reactive and form adducts with nucleotides leading to mutations, which can be detrimental to cells, depending on their location. DNA damage may be repaired through cellular repair mechanisms or the lesions can lead to cell death via apoptosis. Some mutations accumulate over time and lead to cancer. Thus to assess genotoxicity, there are a number of different assays that have been developed that focus on one or more of the specific endpoints described above.

Genotoxicity

For assessment of genotoxicity, one of the most commonly used assays is the bacterial umuC assay originally developed in 1985[40] and updated in 2000.[41] This assay is based on a recombinant Salmonella strain that has the lacZ reporter gene incorporated into the umuC operon, which responds to oxidative

stress and measures DNA repair. This assay, now known as the SOS/umuC assay (ISO 13829:2000) is used in many environmental applications.

Several commercial companies have developed assays using the tumor suppressor protein p53 in HTP format, of which several are used by EPA as part of the ToxCAST effort.[42-43] The p53 protein is an essential central protein in vertebrate cells that senses DNA damage in response to genotoxic stress.[44-45] Among its many functions, p53 can activate DNA repair or initiate apoptosis if repair cannot proceed and is thought to be a good screen for tumor formation. The cell-based assays that have been developed use reporter genes, such as the beta-lactamase gene, fused to the promoter for p53 in mammalian cells.[46]

Another very popular assay for wastewater applications is the micronucleus test, which uses the mammalian cell line V79 and which has gone through harmonization experiments to obtain a robust protocol that has been standardized[47] (ISO 21427-2:2006). The design of the test is relatively simple and can be easily picked up by new investigators. In the round robin test, ten different laboratories from academic institutions, government and industry were involved. For the test, cells are incubated for 24 h in test solutions and then examined for micronuclei formation by microscope.

Several groups use a variant of the COMET assay which looks directly at DNA fragmentation.[48] The basic principle is that DNA from a tissue is isolated and embedded into agarose and electrophoresed at high pH. Broken strands of DNA will migrate more slowly forming a comet tail which is reflective of DNA strand breaks. This assay has several versions but may not be as specific as other genotoxicity assays.

Oxidative stress

Oxidative stress results from chemicals that produce free radicals in cells at concentrations above the ability of the cell to counter the effects through natural antioxidants. These too can lead to DNA damage, but by free radicals. Several novel cell based assays have been developed to measure these activities in medium to HTP manners.[42-43] For direct measurement of oxidative stress, a popular assay is the AREc32, which is based on the Nrf2-Keap-ARE32 pathway.[22] This assay has worked well in wastewater applications and is relatively easy to perform.

In addition to free radical formation, there are a number of xenobiotics that deplete cells of internal reservoirs of glutathione (GSH). Intracellular concentrations of GSH have been measured between 0.1 mM and 15 mM[49] and this protects cellular proteins from chemicals that might target Cys residues in critical enzymes. However, it is known that intracellular concentrations can vary

with the lowest intracellular ratio of GSH to its oxidized version formed into glutathione disulfide (GSSG) measured in the rough endoplasmic reticulum,[49] making this cellular compartment more vulnerable to toxic insult. There are a number of assays to evaluate GSH-GSSG status of cells and these should be included in the overall bioanalytical assessment package. A modified bacterial assay (E. coli GSH±) that relies on growth differences between two strains of bacteria, one that expresses GSH and one that does not, has been used to measure highly treated waters with some success.[22,31]

Endocrine related activities

While the main focus for CECs has been on three endocrine related activities: estrogen, androgen and thryroid hormone, the endocrine system is much more complex and other endpoints can also be affected. All of the human receptor proteins have now been cloned into reporter-based transactivation assays, with many of these assays available commercially. Transactivation assays are powerful in that they depend on a multistep process, which is similar to what happens *in vivo*. First the chemicals must bind to the ligand-binding domain of the receptors, then they must alter the conformation of the receptor to allow it to homo- or hetero-dimerize and interact with other accessory factors in the cell. This is required before the receptors can bind to their recognition sites

in promoters of susceptible genes. The reporter assays have substituted a reporter gene for the normal gene that would be regulated in the cells. The reporter gene product can be detected easily by fluorescence or luminescence and the assays are amenable to HTP. The most effective assays are dependent on human estrogen receptors (ER), androgen receptors (AR), progesterone receptor (PR), peroxisome proliferator-activated receptors (PPARs), glucocorticoid receptor (GR), aryl hydrocarbon receptor (AhR), among others.[21-22] The efficacy and reproducibility of such assays have recently been studied and many have been applied to wastewater effluents with great success. [22,38,43,50]

An interesting variant of this approach are the CIS and TRANS factorial assays provided by ATTAGENE, Inc. This system depends on a multiplexed assay consisting of 48 human transcription factors for the CIS assay and 25 members of the human nuclear receptor superfamily for the TRANS assay working in human HepG2 liver hepatoma cells. (For a description of how the assay was constructed, please see Martin et al.[51]). Multiple endpoints can be determined with a single assay, as has been used by several investigators to monitor surface waters. [22,52-53]

Cytotoxicity

Cytotoxicity relates to overall general toxicity that leads to cell death. Chemicals that perforate membranes or that alter the ability

of cells to function are thought to be cytotoxic. Usually this effect is concentration dependent and may interfere with read outs of other cell based assays. Thus, cytotoxicity should be run in parallel with other assays to be able to exclude this as the primary mechanism of action. There are a variety of bacterial based assays that work well in this regard, but the most widely used is the Microtox assay (ISO 9001:2008), which is based on the inhibition of bioilluminescence by chemicals of *Vibrio fischeri.*[54] Other bacterial-based assays such as ToxScreen and BLT-Screen and a variety of mammalian cell-based assays are also sensitive and have been used in a wide variety of projects.[4,22]

As mentioned above, many chemicals are more toxic after undergoing metabolism by the cytochrome P450 enzymes, particularly present in the liver but also present in other tissues. In addition to assays that test the interaction of chemicals with the AhR, there are other nuclear receptors that should be considered, including the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), both of which dimerize with the retinoid X receptor (RXR) and have been identified as xenobiotic sensors[55]. In the presence of certain xenobiotics, these receptors increase transcription and activity of CYP2 and CYP3, both involved in metabolism of xenobiotics.[55] Thus, evaluating these activities is also important.

An issue for bioanalytical assays has been the ability to assay volatile TPs. A new method developed by Escher's laboratory that allows the use of closed systems for cell based assays was shown to be a workable solution.[56] This method was successfully applied to a cytotoxicity test with *Vibrio fisheri*, the UmuC assay for genotoxicity and the AREc32 assay for induction of oxidative stress. In each case, the captured volatile TPs improved the toxicity assessment. These assays were included into a risk-based framework for evaluating TPs and prioritizing further toxicological analyses.[57]

Case studies

As is clear by now, a reduction in the parent compound does not necessarily translate into a reduction of toxicity, as TPs can retain some of the toxicity of the parent compound and in some instances can be more toxic than the parent compound. Escher and Fenner[57] summarized the two main approaches to assess the toxicological significance of TPs as exposure-driven and effects-driven assessment. In exposure-driven assessment, TPs are identified and quantified by chemical analysis first, and this is followed by effects assessment only if an individual TP constitutes a significant portion (>10%) of the final mixture. In effect-driven assessment, the toxicity of the reaction mixture is tested first, and

only if the removal of the parent compound is not paralleled by the decrease of the reaction mixture (*i.e.*, the TP mixture) should an attempt be made to identify TPs. Unfortunately, it can be very difficult to identify the TPs in complex matrices, an issue that has plagued DBP research for many decades.

Several CECs have been shown to react with disinfectants such as chlorine to produce more bioactive TPs. For example, chlorine reacts quickly with bisphenol A and nonylphenol to produce chlorinated TPs, which as a mixture are more responsive in ER binding and reporter gene assays than the parent compounds [58-59] and have been detected in urban wastewater.[60] Chlorination of the pharmaceutical acetaminophen produces two highly toxic TPs, 1,4-benzoquinone and N-acetyl-*p*-benzoquinone imine.[61] Several PPCPs are quickly degraded by chlorination, but the resulting TP mixture is often (more) genotoxic and/or mutagenic than the parent compound (*e.g.*, cefazolin[62]; sildenafil and tadalafil).[63]

A recent study applied a battery of 11 *in vitro* bioassays covering a wide range of effects (specific endocrine responses, genotoxicity, cytotoxicity, xenobiotic metabolism and oxidative stress) to test the bioactivity of 8 CECs at environmentally relevant concentrations before and after chlorination.[64] In agreement with predictions from a combined chemistry and toxicology model (also applied to 12 other CECs), specific responses such as receptor mediated effects were significantly reduced after chlorination,

while non-specific effects (and in particular toxicity to bacteria) increased. Overall, the results suggested that disinfection of potent CECs would generate a large number of TPs, which were unlikely to produce compounds of increased specific toxicity (*e.g.*, endocrine activity) but may result in increased reactive (*e.g.*, mutagenicity and genotoxicity) and non-specific toxicity. An exception was noted for gemfibrozil, with the TP mixture producing a 39% increase in estrogenicity after chlorination. The latter is in agreement with the findings that chlorination of gemfibrozil produced chlorogemfibrozil, which has been shown *in vivo* to affect hormone levels in fish.[27]

While most of the work to date has focused on chlorine, other disinfectants also produce toxic TPs from CECs, which stands to reason as disinfectants generally do not completely mineralize the compounds. For example, both ozonation and advanced oxidation are effective techniques to degrade musk fragrances such as galaxolide and tonalide in water, but produce an increase in toxicity to bacteria.[65]

Natural attenuation pathways (*e.g.*, photodegradation, microbial degradation) can also produce more toxic TPs than the parent compound. In a series of studies, Isidori and co-workers showed that some of the photoproducts of the pharmaceuticals furosemide[66] and ranitidine[67] were more genotoxic and mutagenic than the parent compound using the SOS Chromotest and Ames

assay. Trovo et al.[68] showed a small increase in toxicity to bacteria using the Microtox assay after complete degradation of sulfamethoxazole by photolysis, indicating that the TPs are at least as toxic, or more, to bacteria as the parent compound. Roustan et al.[69] reported a 100-fold increase in genotoxicity in a micronucleus assay of a mixture of two herbicides (glyphosate and atrazine) and their main environmental degradation product (aminomethyl phosphoric acid and desethylatrazine, respectively) after exposure to light.

In addition, the antimicrobial agent triclosan provides a fascinating new perspective on the possible interplay between degradation in engineered systems followed by photolysis. Triclosan is found in a large number of products used in hospitals and for personal hygiene including soaps, deodorants and even in toothpaste. This ubiquitous additive in personal care products reacts with residual chlorine during wastewater treatment to form chlorinated triclosan derivatives, such as chloroform, 2,4-dichlorophenol and 2,4,6-trichlorophenol,[70] all of which could produce significant health effects. But more troubling is that it can also be transformed to highly toxic polychlorodibenzo-*p*-dioxins (PCDDs) by photolysis in surface waters.[71] We are only now starting to scratch the surface of this complex issue. For additional examples, see reviews by Radjenovic et al.[72] and Devier et al.[73]

Conclusions

We can no longer rely simply on targeted chemical analysis to determine the efficacy of water and wastewater treatment to "remove" toxic contaminants. Bioanalytical tools should be applied in parallel with targeted and non-targeted chemical analyses for a more comprehensive understanding of the removal of both the parent compound and its associated toxicity, while at the same time ensuring that the process has not created new and potentially more toxic chemicals. The most critical bioanalytical assays that should be employed cover the potential of TPs to produce genotoxicity, cytotoxicity and oxidative stress. However, since many effluents also seem to disrupt endocrine axes of aquatic organisms, assays that test for endocrine active TPs should also be employed.

It should be noted that we have highlighted examples of CECs that produce TPs with increased toxicity, but that there are likely many more examples of CECs that are degraded without producing more toxic TPs, or whose parent toxicity is reduced via disinfection. Bioanalytical assays can be used to monitor for these beneficial outcomes as well.

References

1. Prasse, C.; Stalter, D.; Schulte-Oehlmann, U.; Oehlmann, J.; Ternes, T. A. Spoilt for choice: A critical review on the chemical and biological assessment of current wastewater treatment technologies. *Water Res* 2015, *87*, 237-270.

2. Cwiertny, D. M.; Snyder, S. A.; Schlenk, D.; Kolodziej, E. P. Environmental designer drugs: when transformation may not eliminate risk. *Environ Sci Technol* 2014, *48*, 11737-11745.

3. Richardson, S. D.; Kimura, S. Y. Water Analysis: Emerging Contaminants and Current Issues. *Anal Chem* 2016, *88*, 546-582.

4. Plewa, M. J.; Wagner, E. D. Charting a new path to resolve the adverse health effects of DBPs. . In *Recent Advances in Disinfection By-Products*, Karanfil, T.; Mitch, B.; Westerhoff, P.; Xie, Y., Eds. American Chemical Society: Washington, DC, 2015; Vol. ACS Symposium Series vol. 1190, pp 3-23.

5. Snyder, S. A.; Westerhoff, P.; Yoon, Y.; Sedlak, D. L. Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry. *Environ Engr Sci* 2003, *20*, 449-469.

6. Richardson, S. D.; Thruston, A. D.; Caughran, T. V.; Chen, P. H.; Collette, T. W.; Floyd, T. L. Identification of new drinking water disinfection byproducts formed in the presence of bromide. *Environ Sci Technol* 1999, *33*, 3378-3383.

7. Neale, P. A.; Antony, A.; Bartkow, M. E.; Farre, M. J.; Heitz, A.; Kristiana, I.; Tang, J. Y. M.; Escher, B. I. Bioanalytical Assessment of the Formation of Disinfection Byproducts in a Drinking Water Treatment Plant. *Environ Sci Technol* 2012, *46*, 10317-10325.

8. Farre, M. J.; Day, S.; Neale, P. A.; Stalter, D.; Tang, J. Y. M.; Escher, B. I. Bioanalytical and chemical assessment of the disinfection by-product formation potential: Role of organic matter. *Water Res* 2013, *47*, 5409-5421.

9. Westerhoff, P.; Chao, P.; Mash, H. Reactivity of natural organic matter with aqueous chlorine and bromine. *Water Res* 2004, *38*, 1502-1513.

10. Plewa, M. J.; Kargalioglu, Y.; Vankerk, D.; Minear, R. A.; Wagner, E. D. Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products. *Environ Mol Mutagen* 2002, *40*, 134-142.

11. Richardson, S. D. Disinfection by-products and other emerging contaminants in drinking water. *Trac-Trends Anal Chem* 2003, *22*, 666-684.

12. Bartsch, H.; Montesano, R. Relevance of nitrosamines to human cancer. *Carcinogenesis* 1984, *5*, 1381-1393.

13. Mitch, W. A.; Sedlak, D. L. Characterization and fate of N-nitrosodimethylamine (NDMA) precursors during municipal wastewater treatment. *Abstracts of Papers of the American Chemical Society* 2003, *226*, U482-U482.

14. Mitch, W. A.; Sharp, J. O.; Trussell, R. R.; Valentine, R. L.; Alvarez-Cohen, L.; Sedlak, D. L. N-nitrosodimethylamine (NDMA) as a drinking water contaminant: A review. *Environ Engr Sci* 2003, *20*, 389-404.

15. Farre, M. J.; Doderer, K.; Keller, J.; Poussade, Y.; Mueller, J.; Gernjak, W. *N-nitrosodimethylamine (NDMA) in purified recycled water*; Urban Water Security Research Alliance, Technical Report No. 34: 2010; p 76.

16. Water-Research-Foundation-(WRF) *1,4-dioxane white paper accessed on July 1, 2016 at* http://www.waterrf.org/resources/StateOfTheScienceReports/1,4-dioxane.pdf.; Water Research Foundation: Denver, CO, 2014; p 6.

17. Reungoat, J.; Escher, B. I.; Macova, M.; Argaud, F. X.; Gernjak, W.; Keller, J. Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. *Water Res* 2012, *46*, 863-872.

18. National-Research-Council-(NRC) *Water reuse: potential for expanding the nation's water supply through reuse of municipal wastewater*; National Academies Press: Washington DC, 2012; p 363.

19. Leusch, F. D. L.; Khan, S. J.; Laingam, S.; Prochazka, E.; Froscio, S.; Trinh, T.; Chapman, H. F.; Humpage, A. Assessment of the application of bioanalytical tools as surrogate measure of chemical contaminants in recycled water. *Water Res* 2014, *49*, 300-315.

20. Tang, J. Y. M.; Busetti, F.; Charrois, J. W. A.; Escher, B. I. Which chemicals drive biological effects in wastewater and recycled water? *Water Res* 2014, *60*, 289-299.

21. Mehinto, A. C.; Jia, A.; Snyder, S. A.; Jayasinghe, B. S.; Denslow, N. D.; Crago, J.; Schlenk, D.; Menzie, C.; Westerheide, S. D.; Leusch, F. D.; Maruya, K. A. Interlaboratory comparison of in vitro bioassays for screening of endocrine active chemicals in recycled water. *Water Res* 2015, *83*, 303-309.

22. Escher, B. I.; Allinson, M.; Altenburger, R.; Bain, P. A.; Balaguer, P.; Busch, W.; Crago, J.; Denslow, N. D.; Dopp, E.; Hilscherova, K.; Humpage, A. R.; Kumar, A.; Grimaldi, M.; Jayasinghe, B. S.; Jarosova, B.; Jia, A.; Makarov, S.; Maruya, K. A.; Medvedev, A.; Mehinto, A. C.; Mendez, J. E.; Poulsen, A.; Prochazka, E.; Richard, J.; Schifferli, A.; Schlenk, D.; Scholz, S.; Shiraishi, F.; Snyder, S.; Su, G.; Tang, J. Y.; van der Burg, B.; van der Linden, S. C.; Werner, I.; Westerheide, S. D.; Wong, C. K.; Yang, M.; Yeung, B. H.; Zhang, X.; Leusch, F. D. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environ Sci Technol* 2014, *48*, 1940-1956.

23. Jarosova, B.; Ersekova, A.; Hilscherova, K.; Loos, R.; Gawlik, B. M.; Giesy, J. P.; Blaha, L. Europe-wide survey of estrogenicity in wastewater treatment plant effluents: the need for the effect-based monitoring. *Environ Sci Pollut Res* 2014, *21*, 10970-10982.

24. Suzuki, G.; Sato, K.; Isobe, T.; Takigami, H.; Brouwer, A.; Nakayama, K. Detection of glucocorticoid receptor agonists in effluents from sewage treatment plants in Japan. *Sci Tot Environ* 2015, *527*, 328-334.

25. Negreira, N.; Regueiro, J.; de Alda, M. L.; Barcelo, D. Degradation of the anticancer drug erlotinib during water chlorination: Non-targeted approach for the identification of transformation products. *Water Res* 2015, *85*, 103-113.

26. Pico, Y.; Barcelo, D. Transformation products of emerging contaminants in the environment and high-resolution mass spectrometry: a new horizon. *Anal Bioanal Chem* 2015, *407*, 6257-6273.

27. Bulloch, D. N.; Lavado, R.; Forsgren, K. L.; Beni, S.; Schlenk, D.; Larive, C. K. Analytical and biological characterization of halogenated gemfibrozil produced through chlorination of wastewater. *Environ Sci Technol* 2012, *46*, 5583-5589.

28. Hijosa-Valsero, M.; Reyes-Contreras, C.; Dominguez, C.; Becares, E.; Bayona, J. M. Behaviour of pharmaceuticals and personal care products in constructed wetland compartments: Influent, effluent, pore water, substrate and plant roots. *Chemosphere* 2016, *145*, 508-517.

29. Chen, P. J.; Kullman, S. W.; Hinton, D. E.; Linden, K. G. Comparisons of polychromatic and monochromatic UV-based treatments of bisphenol-A in water via toxicity assessments. *Chemosphere* 2007, *68*, 1041-1049.

30. Van der Linden, S. C.; Heringa, M. B.; Man, H. Y.; Sonneveld, E.; Puijker, L. M.; Brouwer, A.; Van der Burg, B. Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. *Environ Sci Technol* 2008, *42*, 5814-5820.

31. Leusch, F. D. L.; Snyder, S. A. Bioanalytical tools: half a century of application for potable reuse. *Environ Sci Water Res Technol* 2015, *1*, 606-621.

32. Jia, A.; Escher, B. I.; Leusch, F. D. L.; Tang, J. Y. M.; Prochazka, E.; Dong, B. F.; Snyder, E. M.; Snyder, S. A. In vitro bioassays to evaluate complex chemical mixtures in recycled water. *Water Res* 2015, *80*, 1-11.

33. EPA-ToxCast, US Environmental Protection Agency's National Center for Computational Toxicology ToxCast Project. Available from: http://www.epa.gov/ncct/toxcast. 2009.

34. Ankley, G. T.; Bennett, R. S.; Erickson, R. J.; Hoff, D. J.; Hornung, M. W.; Johnson, R. D.; Mount, D. R.; Nichols, J. W.; Russom, C. L.; Schmieder, P. K.; Serrrano, J. A.; Tietge, J. E.; Villeneuve, D. L. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 2010, *29*, 730-741.

35. Vinken, M. The adverse outcome pathway concept: A pragmatic tool in toxicology. *Toxicology* 2013, *312*, 158-165.

36. Sumida, K.; Ooe, N.; Nagahori, H.; Saito, K.; Isobe, N.; Kaneko, H.; Nakatsuka, I. An in vitro reporter gene assay method incorporating metabolic activation with human and rat S9 or liver microsomes. *Biochem Biophys Res Commun* 2001, *280*, 85-91.

37. Anderson, P.; Denslow, N.; Drewes, J. E.; Olivieri, A.; Schlenk, D.; Snyder, S. *Final report monitoring strategies for chemicals of emerging concern (CECs) in recycled water. Recommendations of a science advisory panel.* ; California State Water Resources Control Board: 2010 Jun 25.

38. Escher, B.; Leusch, F. *Bioanalytical tools in water quality assessment.* IWA publishing, London UK 2012, 272 pp.

39. Zegura, B.; Heath, E.; Cernosa, A.; Filipic, M. Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. *Chemosphere* 2009, *75*, 1453-1460.

40. Oda, Y.; Nakamura, S.; Oki, I.; Kato, T.; Shinagawa, H. Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens. *Mutat Res* 1985, *147*, 219-229.

41. Hamer, B.; Bihari, N.; Reifferscheid, G.; Zahn, R. K.; Muller, W. E.; Batel, R. Evaluation of the SOS/umu-test post-treatment assay for the detection of genotoxic activities of pure compounds and complex environmental mixtures. *Mutat Res* 2000, *466*, 161-171.

42. Knight, A. W.; Little, S.; Houck, K.; Dix, D.; Judson, R.; Richard, A.; McCarroll, N.; Akerman, G.; Yang, C.; Birrell, L.; Walmsley, R. M. Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCast chemicals. *Regul Toxicol Pharmacol* 2009, *55*, 188-199.

43. Kligerman, A. D.; Young, R. R.; Stankowski, L. F., Jr.; Pant, K.; Lawlor, T.; Aardema, M. J.; Houck, K. A. An evaluation of 25 selected ToxCast chemicals in medium-throughput assays to detect genotoxicity. *Environ Mol Mutagen* 2015, *56*, 468-476.

44. Lane, D. P. Cancer. p53, guardian of the genome. *Nature* 1992, *358*, 15-16.

45. Liu, Y.; Kulesz-Martin, M. p53 protein at the hub of cellular DNA damage response pathways through sequence-specific and non-sequence-specific DNA binding. *Carcinogenesis* 2001, *22*, 851-860.

46. Zlokarnik, G.; Negulescu, P. A.; Knapp, T. E.; Mere, L.; Burres, N.; Feng, L.; Whitney, M.; Roemer, K.; Tsien, R. Y. Quantitation of transcription and clonal selection of single living cells with beta-lactamase as reporter. *Science* 1998, *279*, 84-88.

47. Reifferscheid, G.; Ziemann, C.; Fieblinger, D.; Dill, F.; Gminski, R.; Grummt, H. J.; Hafner, C.; Hollert, H.; Kunz, S.; Rodrigo, G.; Stopper, H.; Selke, D. Measurement of genotoxicity in wastewater samples with the in vitro micronucleus test: results of a round-robin study in the context of standardisation according to ISO. *Mutat Res* 2008, *649* , 15-27.

48. Collins, A. R. The comet assay for DNA damage and repair: principles, applications, and limitations. *Mol Biotechnol* 2004, *26*, 249-261.

49. Deponte, M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochim Biophys Acta* 2013, *1830*, 3217-3266.

50. Leusch, F. D.; de Jager, C.; Levi, Y.; Lim, R.; Puijker, L.; Sacher, F.; Tremblay, L. A.; Wilson, V. S.; Chapman, H. F. Comparison of five in vitro bioassays to measure estrogenic activity in environmental waters. *Environ Sci Technol* 2010, *44*, 3853-3860.

51. Martin, M. T.; Dix, D. J.; Judson, R. S.; Kavlock, R. J.; Reif, D. M.; Richard, A. M.; Rotroff, D. M.; Romanov, S.; Medvedev, A.; Poltoratskaya, N.; Gambarian, M.; Moeser, M.; Makarov, S. S.; Houck, K. A. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. *Chem Res Toxicol* 2010, *23*, 578-590.

52. Buxton, H. T.; Reilly, T. J.; Kuivila, K. M.; Kolpin, D. W.; Villeneuve, D. L.; Mills, M. A. *Chemical mixtures and environmental effects: a pilot study to assess ecological exposure and effects in streams*; 2015, Open-file Report 2015, 1113.

53. Schroeder, A. L.; Ankley, G. T.; Houck, K. A.; Villeneuve, D. L. Environmental surveillance and monitoringThe next frontiers for high-throughput toxicology. *Environ Toxicol Chem* 2016, *35*, 513-525.

54. Parvez, S.; Venkataraman, C.; Mukherji, S. A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals. *Environ Int* 2006, *32*, 265-268.

55. Stanley, L. A.; Horsburgh, B. C.; Ross, J.; Scheer, N.; Wolf, C. R. PXR and CAR: nuclear receptors which play a pivotal role in drug disposition and chemical toxicity. *Drug Metab Rev* 2006, *38*, 515-597.

56. Stalter, D.; Dutt, M.; Escher, B. I. Headspace-free setup of in vitro bioassays for the evaluation of volatile disinfection by-products. *Chem Res Toxicol* 2013, *26*, 1605-1614.

57. Escher, B. I.; Fenner, K. Recent Advances in Environmental Risk Assessment of Transformation Products. *Environ Sci Technol* 2011, *45*, 3835-3847.

58. Hu, J. Y.; Aizawa, T.; Ookubo, S. Products of aqueous chlorination of bisphenol A and their estrogenic activity. *Environ Sci Technol* 2002, *36*, 1980-1987.

59. Hu, J. Y.; Xie, G. H.; Aizawa, T. Products of aqueous chlorination of 4-nonylphenol and their estrogenic activity. *Environ Toxicol Chem* 2002, *21*, 2034-2039.

60. Zafra, A.; del Olmo, M.; Suarez, B.; Hontoria, E.; Navalon, A.; Vilchez, J. L. Gas chromatographic-mass spectrometric method for the determination of bisphenol A and its chlorinated derivatives in urban wastewater. *Water Res* 2003, *37*, 735-742.

61. Bedner, M.; MacCrehan, W. A. Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and N-acetyl-p-benzoquinone imine. *Environ Sci Technol* 2006, *40*, 516-522.

62. Li, L. P.; Wei, D. B.; Wei, G. H.; Du, Y. G. Transformation of cefazolin during chlorination process: Products, mechanism and genotoxicity assessment. *J Hazard Matl* 2013, *262*, 48-54.

63. Temussi, F.; DellaGreca, M.; Pistillo, P.; Previtera, L.; Zarrelli, A.; Criscuolo, E.; Lavorgna, M.; Russo, C.; Isidori, M. Sildenafil and tadalafil in simulated chlorination conditions: Ecotoxicity of drugs and their derivatives. *Sci Tot Environ* 2013, *463*, 366-373.

64. Leusch, F. D. L.; van de Merwe, J.; Neale, P.; Escher, B.; Tang, J.; Card, M.; Ridgway, H.; Orbell, J.; Stewart, M.; Busetti, F.; Humpage, A.; Bull, R. *Transformation of EDCs/PPCPs during drinking water disinfection*; Water Research Foundation: Denver, CO, 2016; p 170.

65. Santiago-Morales, J.; Gomez, M. J.; Herrera, S.; Fernandez-Alba, A. R.; Garcia-Calvo, E.; Rosal, R. Oxidative and photochemical processes for the removal of galaxolide and tonalide from wastewater. *Water Res* 2012, *46*, 4435-4447.

66. Isidori, M.; Nardelli, A.; Parrella, A.; Pascarella, L.; Previtera, L. A multispecies study to assess the toxic and genotoxic effect of pharmaceuticals: Furosemide and its photoproduct. *Chemosphere* 2006, *63*, 785-793.

67. Isidori, M.; Parrella, A.; Pistillo, P.; Temussi, F. Effects of ranitidine and its photoderivatives in the aquatic environment. *Environ Int* 2009, *35*, 821-825.

68. Trovo, A. G.; Nogueira, R. F. P.; Aguera, A.; Sirtori, C.; Fernandez-Alba, A. R. Photodegradation of sulfamethoxazole in various aqueous media: Persistence, toxicity and photoproducts assessment. *Chemosphere* 2009, *77*, 1292-1298.

69. Roustan, A.; Aye, M.; De Meo, M.; Di Giorgio, C. Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. *Chemosphere* 2014, *108*, 93-100.

70. Rule, K. L.; Ebbett, V. R.; Vikesland, P. J. Formation of chloroform and chlorinated organics by free-chlorine-mediated oxication of triclosan. *Environ. Sci. Technol.* 2005, *39*, 3176-3185.

71. Buth, J. M.; Grandbois, M.; Vikesland, P. J.; McNeill, K.; Arnold, W. A. Aquatic photochemistry of chlorinated triclosan derivatives: potential source of polychlorodibenzo-p-dioxins. *Environ Toxicol Chem* 2009, *28*, 2555-2563.

72. Radjenovic, J.; Petrovic, M.; Barcelo, D. Complementary mass spectrometry and bioassays for evaluating pharmaceutical-transformation products in treatment of drinking water and wastewater. *Trac-Trends Anal Chem* 2009, *28*, 562-580.

73. Devier, M. H.; Mazellier, P.; Ait-Aissa, S.; Budzinski, H. New challenges in environmental analytical chemistry: Identification of toxic compounds in complex mixtures. *Comptes Rendus Chimie* 2011, *14*, 766-779.

Bioanalytical approaches in assessing transformation products

Journal:	*ACS Books*
Manuscript ID	bk-2016-00264b.R1
Manuscript Type:	Symposium Series Chapter
Date Submitted by the Author:	07-Aug-2016
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