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Q2 Method to assess component contribution to toxicity of complex mixtures: Assessment of puberty acquisition in rats exposed to disinfection byproducts

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ABSTRACT

A method based on regression-modeling was developed to discern the contribution of component chemicals to the toxicity of highly complex, environmentally realistic mixtures of disinfection byproducts (DBPs). Chemical disinfection of drinking water forms DBP mixtures. Because of concerns about possible reproductive and developmental toxicity, a whole mixture (WM) of DBPs produced by chlorination of a water concentrate was administered as drinking water to Sprague–Dawley (S–D) rats in a multigenerational study. Age of puberty acquisition, *i.e.*, preputial separation (PPS) and vaginal opening (VO), was examined in male and female offspring, respectively. When compared to controls, a slight, but statistically significant delay in puberty acquisition was observed in females but not in males. WM-induced differences in the age at puberty acquisition were compared to those reported in S–D rats administered either a defined mixture (DM) of nine regulated DBPs or individual DBPs. Regression models were developed using individual animal data on age at PPS or VO from the DM study. Puberty acquisition data reported in the WM and individual DBP studies were then compared with the DM models. The delay in puberty acquisition observed in the WM-treated female rats could not be distinguished from delays predicted by the DM regression model, suggesting that the nine regulated DBPs in the DM might account for much of the delay observed in the WM. This method is applicable to mixtures of other types of chemicals and other endpoints.

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Introduction

Although chemical disinfection effectively produces potable drinking waters, such processes generally form low concentrations (≤mg/L) of numerous disinfection byproducts (DBPs) (Richardson et al., 2007, 2008). These DBP mixtures include hundreds of known compounds, as well as compounds that have not yet been identified chemically and comprise the unidentified fraction. Two chemical classes of DBPs, the trihalomethanes (THMs) and haloacetic acids (HAAs), together typically account for approximately 40% of the total mass of organic halogen (TOX) formed in disinfected drinking waters (Richardson et al., 2008; Krasner et al., 2006). The unidentified fraction, which can comprise more than 50% of the TOX measured in some treated drinking waters (Pressman et al., 2010; Weinberg et al., 2002; Krasner et al., 2006), likely includes highly polar and high molecular weight compounds (Richardson et al., 2002).

A number of epidemiologic studies have evaluated associations between exposure to chemically disinfected drinking water and adverse reproductive and developmental effects. Some report significant associations between DBP exposure and still birth (King et al., 2000; Dodds et al., 2004), low birth weight (Danileviciute et al., 2012; Gallagher et al., 1998; Wright et al., 2003), premature birth (Bove, 1996; Hinckley et al., 2005), and pregnancy loss (Waller et al., 1998, 2001; Savitz et al., 1995, 2006). Other studies report negative results (*e.g.*, Nieuwenhuijsen et al., 2009; Hrudey, 2009). Using animal and *in vitro* bioassays, toxicologists have studied the reproductive and developmental effects of a small number of individual DBPs (Colman et al., 2011; U.S. EPA, 2000a; Hunter and Tugman, 1995; Hunter et al., 1996); there are even fewer *in vivo* developmental toxicity studies of complex DBP mixtures (Kavlock et al., 1979; Simmons et al., 2002; Teuschler and Simmons, 2003; Narotsky et al., 2008, 2013).

To address potential health concerns that cannot be answered by toxicological research on individual DBPs or defined mixtures, the U.S. EPA's Four Lab Study investigated the toxicity of environmentally realistic complex DBP mixtures. An important objective of this study was a thorough assessment of rodent reproductive and developmental endpoints, integrating these bioassay results with extensive quantitative and qualitative analyses of the chemicals present in the complex DBP mixture(s) (Simmons et al., 2002, 2004, 2008). To meet this objective, a multi-generational reproductive and developmental bioassay was conducted with a chlorinated concentrate in Sprague–Dawley (S–D) rats. The water concentrate was water produced using a procedure that concentrated a natural source water by reverse osmosis procedures; aliquots of this water concentrate were chlorinated as needed for the bioassay (Pressman et al., 2010). In the bioassay, this whole, complex DBP mixture (whole mixture, WM) was administered to the treatment group as the sole source of drinking water. When compared with controls, the WM-treated female offspring (F1 generation) experienced a slight, but significant delay in puberty acquisition, which was measured as their age at vaginal opening (VO) ($p < 0.05$); the WM-treated F1 males did not exhibit a significant delay in puberty acquisition, which was measured as their age at preputial separation (PPS) (Narotsky et al., 2013).

In a companion multi-generational bioassay, a defined mixture (DM) that contained the four regulated THMs and the five regulated HAAs was administered to S-D rats as the sole source of drinking water. Significant, dose-dependent delays in puberty acquisition were observed in both F1 males and females (Table 1) (Narotsky et al., 2015). This DM contained chloroform ($CHCl_3$), bromodichloromethane (BDCM), dibromochloromethane (DBCM), bromoform ($CHBr_3$), chloroacetic acid (CAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromoacetic acid (BAA), and dibromoacetic acid (DBAA). U.S. EPA regulations limit the sum of the four THMs to 80 μg/L and the sum of the five HAAs to 60 μg/L in U.S. drinking waters (U.S. EPA, 2006). Some bioassays of individual DBPs (*i.e.*, DBAA, bromochloroacetic acid [BCAA], and BDCM), administered as single chemicals in drinking water of S-D rats, also report significant delays in puberty acquisition (Table 1) (Klinefelter et al., 2004; Sloan et al., 2005; Christian et al., 2002).

Here, we present a method developed to assess the contribution of constituent chemicals and subset mixtures to the toxicity of highly complex environmental mixtures. We use assessment of puberty acquisition in rats exposed to DBP mixtures to illustrate methodology that allows comparison of the health effects from constituent chemicals and subset mixtures contained within more complex mixtures to the health effects associated with highly complex environmental mixtures. We specifically compare differences in the age of puberty acquisition in rats and evaluate whether any of the tested individual DBPs or the DM can account for the observed difference in the age at puberty acquisition associated with the WM. The method relies on component-based and whole-mixture approaches for determining if some of the delay in puberty acquisition is potentially due to the unknown fraction in the WM (Rice et al., 2008) and assumes that the relative proportions of the chemicals common to both the whole mixture and the defined mixture are similar.

1. Materials and methods

Fig. 1 depicts our method for comparing differences (relative to concurrent controls) in the age at puberty acquisition associated with the DM to those differences associated the WM and with individual DBPs. After completing the WM and DM bioassays, we undertook a literature search targeting studies that administered DBPs *via* the drinking water and reported age at puberty acquisition. Because differences among species and strains/stocks could influence the age of puberty acquisition, we targeted studies reporting the same endpoints (*i.e.*, age at VO or PPS) in the same test species/stock (S-D rats).

1.1. DBP bioassays reporting the age at puberty acquisition in S-D rats

Table 1 details the five studies, three studies of individual DBPs and two studies of DBP mixtures, meeting the search criteria. Four of the five studies (the exception being Klinefelter et al., 2004) met or exceeded the 20 litters/treatment recommended by toxicity testing guidelines (US EPA, US Environmental Protection Agency, 1998; OECD, Organization for Economic Co-operation and Development, 2012) and all met or exceeded the recommended number of offspring/sex/litter for VO or PPS evaluation.

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