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Review

Cumulative effects of antiandrogenic chemical mixtures and their relevance to human health risk assessment

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ABSTRACT

Toxicological studies of defined chemical mixtures assist human health risk assessment by establishing how chemicals interact with one another to induce an effect. This paper reviews how antiandrogenic chemical mixtures can alter reproductive tract development in rats with a focus on the reproductive toxicant phthalates. The reviewed studies compare observed mixture data to mathematical mixture model predictions based on dose addition or response addition to determine how the individual chemicals in a mixture interact (e.g., additive, greater, or less than additive). Phthalate mixtures were observed to act in a dose additive manner based on the relative potency of the individual phthalates to suppress fetal testosterone production. Similar dose additive effects have been reported for mixtures of phthalates with antiandrogenic pesticides of differing mechanisms of action. Overall, data from these phthalate experiments in rats can be used in conjunction with human biomonitoring data to determine individual hazard indices, and recent cumulative risk assessments in humans indicate an excess risk to antiandrogenic chemical mixtures that include phthalates only or phthalates in combination with other antiandrogenic chemicals.

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Contents

1. Introduction

Risk assessment has traditionally been conducted on an individual chemical basis. However, human biomonitoring and environmental monitoring studies have detected the presence of

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multiple chemical exposures, including many endocrine disrupting chemicals. Of special interest, multiple chemical exposures have been documented for sensitive periods of human development, including in pregnant women (Enke et al., 2013; Mitro et al., 2015; Woodruff et al., 2011; Ye et al., 2009), amniotic fluid samples (Silva et al., 2004), infants (Enke et al., 2013), and children (Becker et al., 2009; Blount et al., 2000; Eskenazi et al., 1999; Koch et al., 2011; Teitelbaum et al., 2008). Environmental monitoring studies have documented exposure to multiple chemicals in wildlife, including fish and birds (Ankley et al., 2007; Baxter et al., 2015; Jaspers et al., 2006; Jobling and Tyler, 2006; Kendall et al., 2010; Tyler et al., 1998). Furthermore, environmental monitoring efforts have detected multiple chemicals in freshwater samples, including: pesticides (Hela et al., 2005; Maruya et al., 2016), hormones (Kolok et al., 2007; Kolpin et al., 2002), pharmaceuticals and personal care products (Kolpin et al., 2002; Wu et al., 2014), and industrial chemicals (Durhan et al., 2006; Maruya et al., 2016).

Regulatory agencies have begun to consider how to conduct cumulative risk assessment for toxic chemicals. In 1996, the United States Congress implemented the Food Quality Protection Act (FQPA), which mandated that the United States Environmental Protection Agency (US EPA) consider the cumulative risk of pesticides found in food that operate via a common mechanism of toxicity (US Congress, 1996). For example, the US EPA has risk assessment programs in the area of mixtures toxicology in the Office of Water, the Office of Air and Radiation, Superfund, and the Office of Research and Development's National Center for Environmental Assessment (Sexton, 2012; US EPA, 2015). In addition, many international health agencies have either recently established or are actively developing cumulative risk assessment guidelines (Boobis et al., 2008; Health Canada, 2016; RIVM, 2016; Solecki et al., 2014).

Many of the chemicals detected in human biomonitoring can disrupt normal hormone-signaling; for example, in utero exposure to several phthalates induces reproductive toxicity in male rats due to inhibition testosterone production during sexual differentiation. In 2006, the US EPA requested that the National Academy of Sciences (NAS) establish a panel to provide the US EPA with recommendations on whether to perform a cumulative risk assessment for the phthalates due to their ubiquitous presence in the environment and human urine samples. The NAS panel concluded that the US EPA should conduct cumulative risk assessments on phthalates that are known to inhibit fetal testosterone production (National Academies of Science, 2008). Furthermore, the NAS panel recommended that the US EPA also include other antiandrogenic environmental chemicals in their risk assessment of phthalates based on their common adverse outcome of impaired androgen-dependent male reproductive tract development, instead of cumulative risk assessment based on a narrowly-defined common mechanism of action (US EPA, 2002).

In this paper, we review published animal research from our laboratory on impaired male rat reproductive tract development following in utero exposure during the critical period of sexual differentiation. This research was intended to contribute to the development of a guidance framework for assessing the cumulative risk of mixtures of phthalates, or phthalates in combination with other antiandrogenic chemicals. Finally, we describe how our data have been applied to assess human hazard identification and assessment for phthalate and antiandrogenic chemical mixtures.

2. Adverse outcome pathways for antiandrogenic chemicals

There are multiple mechanisms (i.e., molecular initiating events) by which chemicals can interfere with the androgen signaling pathway and, thereby, disrupt male reproductive development. In risk assessment, this series of events with multiple molecular initiating events leading to an adverse outcome can be outlined as an adverse outcome pathway (AOP) network (Edwards et al., 2016)(Fig. 1). Among these mechanisms are androgen receptor (AR) antagonism and inhibition of androgen synthesis enzymes, as well as the unknown mechanism by which phthalates reduce testosterone production. Depending on the timing of the exposure, these chemicals can disrupt development of androgen-sensitive reproductive organs (e.g., reducing organ weights or inducing malformations (e.g., hypospadias, undescended testes)), lead to reduced fertility, and, possibly, testicular cancer. Exposure to antiandrogenic chemicals in utero can cause many different alterations in the developing male rodent. Developmental effects of antiandrogenic chemicals include a shortening of the anogenital distance (AGD) at birth relative to control males, and the retention of areolae and/or nipples in juvenile and adult males (absent in control male rats). While these developmental effects (reduced AGD or retention of areolae and/or nipples) are not considered adverse, they are predictive of changes in adult reproductive tissues following in utero exposure to antiandrogenic chemicals during the period of sexual differentiation (Hotchkiss et al., 2004; McIntyre et al., 2001).

The effects of antiandrogenic chemicals (e.g., phthalates) in the rat share a striking similarity to the human testicular dysgenesis syndrome (TDS) (Sharpe and Skakkebaek, 2008). Developmental exposure to phthalates has also been reported to negatively impact reproductive development in humans (Arbuckle et al., 2014)(reviewed in (Marie et al., 2015)) and in animal models other than the rat (e.g., rabbit, African clawed frog and mouse) (reviewed in (Howdeshell et al., 2008a)). Some studies have suggested possible species specificity in responsiveness to phthalates; however, the dose, route, and timing of exposure may have been significant contributing factors to the lack of inhibition of testosterone observed in these experiments (reviewed in (CHAP, 2014; Zarean et al., 2016)).

3. Mixture models

As interest in the study of chemical mixtures has grown over the past several decades, so too have efforts to accurately predict mixture responses based on individual chemical data. Predicted mixture responses can be compared to empirical mixture data to test hypotheses regarding the types of interactions occurring between multiple chemicals. These models are based on the concepts of dose addition, response addition (also called independent action or independent joint action), or integrated addition. Each of these models and their applications are discussed briefly below. In comparing empirical data to modeled predictions, the data can either fit one or more of the models, or diverge from modeled predictions to indicate potential greater than additive or less than additive interactions among mixture constituents.

Dose addition is commonly used for chemicals which share the same mechanism of action (Altenburger et al., 2003; Rider et al., 2008, 2010; Silva et al., 2002). The current US EPA guidance for cumulative risk assessment of chemical mixtures recommends that the chemicals share a common narrowly-defined mechanism of action (US EPA, 2002); for example, inhibition of acetylcholinesterase by phosphorylation and induction of cholinergic effects were identified as a common mechanism of action for organochlorine pesticides. There are many different approaches for calculating predicted mixture responses based on the concept of dose addition. However, all approaches involve adding together individual chemicals at the dose level. Often, this is accomplished by converting individual chemical doses to equivalent terms (Rider et al., 2008, 2010). Here, we accomplish this by dividing the dose of the individual chemical in the mixture by the effective dose 50%