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Historical Perspective

HISTORICAL PERSPECTIVE

Bisphenol A and Phthalates: How Environmental Chemicals Are Reshaping Toxicology

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Bisphenol A (BPA) and phthalates are important chemical building blocks in the plastics industry. They are also ubiquitous contaminants in the human body, wildlife, and the environment (CDC and NCEH, 2018; Teuten *et al.*, 2009). Despite longstanding questions over their safety, BPA and phthalates are still used in consumer products around the globe because it has been difficult to develop economical and safe replacements (Lowell Center for Sustainable Production, 2011; Ritter, 2011). One reason for their continued use is that during initial toxicity testing over 50 years ago, BPA and phthalates were not identified as harmful using traditional toxicological methods. Historically, toxicity was evaluated using high-dose testing under the assumption of a linear dose response curve. However, this paradigm of "the dose makes the poison" does not hold for BPA, phthalates, and other endocrine disrupting chemicals. The unique properties of BPA and phthalates, including low-dose effects, nonmonotonic dose response curves (NMDRCs), and quick metabolism, disobey traditional principles of toxicology (Vandenberg *et al.*, 2012). Our journey to understand and incorporate these properties into toxicological methods has triggered a paradigm shift within the field of toxicology (Kuhn, 1970).

The first phthalate ester, di(2-ethylhexyl)phthalate (DEHP), was introduced as a plasticizer for the newly developed hard plastic polyvinyl chloride (PVC) in the 1930s (Graham, 1973). Phthalate production quickly diversified and grew in parallel with PVC throughout the 20th century. By the 1970s, phthalate production totaled over 1 billion pounds for use in the construction, home furnishings, transportation, apparel, food, and medical industries (Graham, 1973). BPA was first synthesized in late 1800s, but was not commercially used until the 1940s when it became a component of epoxy resins. The development of polycarbonate plastics made from polymerized BPA occurred in the following decade. By the 1970s, BPA production reached over half a billion pounds per year (Vogel, 2009).

Standard toxicology testing in the 20th century for high production volume industrial chemicals such as BPA and phthalates involved high-dose adult animal studies to evaluate general toxicity (Krewski *et al.*, 2010). Once the no observable adverse effect level (NOAEL) was determined, a safety threshold of 100–1000× was added at which the chemical was presumed safe for humans. Carcinogenicity and occupational exposure risk were also assessed using high doses. The estrogen mimicking properties of BPA were established in the 1930s, but were not considered problematic and were not further investigated until the 1990s after the discovery of nuclear hormone receptors (Dodds and Lawson, 1936). High-dose testing in the 1950s identified only mild dermatitis from occupational exposure to BPA as a toxic effect (Hine *et al.*, 1958). Due to rising production, carcinogenicity assays on BPA using rats were undertaken in the 1970s to investigate occupational exposure (NTP, 1982). The initial study was problematic due to maintenance issues, poor pathology practices, and chemical and animal handling practices that could lead to contamination (United States Government Accountability Office, 1979). In an effort to avoid adulteration of future studies on BPA, pesticides, and other chemicals, the government adopted "Good Laboratory Practice" (GLP) protocols in 1978. GLP includes guidelines for animal care, data collection, and many other organizational aspects of research studies to prevent misconduct and expedite regulation (Services, 2000).

The tendency of phthalates to migrate out of plastics into the environment was first observed in 1970, whereas for BPA, this discovery did not occur until the 1990s (Jaeger and Rubin, 1970; Krishnan and Stathis, 1993). After the realization that phthalates readily leach, studies were conducted to assess if leaching could result in human exposure; phthalates were detected in blood transfusion patients, healthy human volunteers, environmental waters, fish, and even in unmanned NASA

spacecraft (Gross and Colony, 1972; Marcel and Noel, 1970; Marx, 1972). Publications on phthalate toxicity surged following these observations. Previous high-dose toxicity analyses had indicated low chronic toxicity for phthalates, but new research indicated "subtle" developmental toxicity in animal models (Autian, 1973). However, these effects were considered to be innocuous because the mode of action was unknown and indicators that subtle early effects could lead to permanent biological changes were undiscovered (Tepper, 1973).

The discovery in 1993 of estrogenic BPA leachate and emerging concern over environmental estrogens brought BPA and phthalates to the attention of the new field of endocrine disruption (Colborn *et al.*, 1993). Endocrine disruptors mimic or disrupt the action of endogenous hormones, which are active at low doses in the body and frequently have multiphasic dose response curves (Gore *et al.*, 2015). Suddenly, the estrogenicity of BPA, which in the 1930s was considered favorable with potential for pharmaceutical application, became an important property to study. Thus began investigation into the low-dose effects of BPA and phthalates in a significant break from traditional toxicology, which focused on high doses and linear dose response curves.

Low-dose effects, defined as effects present at or below the levels of average human exposure, are often accompanied by NMDRCs, in which the slope of the dose-response curve changes sign (Vandenberg *et al.*, 2012). Although these characteristics are common for natural hormones and pharmaceuticals, they are a newer concept in toxicology (Vandenberg *et al.*, 2009, 2013). NMDRCs disobey the principle of the dose makes the poison; the fact that low-level exposure may be more hazardous than high exposure means that high doses can no longer be considered predictive, especially for any suspected reproductive toxicants. These counterintuitive dose response curves can occur when, for example, different mechanisms of action are at play at different doses. Hormone disrupting toxicity is typically observed within the physiologic concentration of hormones in the body (picomolar to nanomolar), which is also similar to environmental exposure levels (Vandenberg *et al.*, 2012).

More hormones in the body exist than just estrogen and these hormones can be disrupted by mimics. For example, BPA can bind to estrogen, androgen, thyroid, estrogen-related, and peroxisome proliferator-activated receptors (Vandenberg *et al.*, 2012). Phthalates, of which there are more than 10 congeners in commerce and countless metabolites, can also interact with multiple hormone systems. Endocrine disrupting action may or may not be receptor driven and may be agonistic, antagonistic, or a mixture of both.

In addition to the plethora of mechanisms, endocrine disrupting effects also vary widely depending on age of exposure and species. Arguably, timing of exposure is even more critical than dose. Pre- and perinatal exposure can contribute to lifelong disease outcomes that adult toxicity testing cannot predict (Bern, 1992). Critical windows of development, including sex determination and organ formation, are especially vulnerable to insult by low doses of xenobiotics (Heindel and Vandenberg, 2015). Historically, high-dose toxicity tests were performed on adult male animals only, but sex specific effects are also so important that the National Institutes of Health now requires reporting of both sexes in all proposals (Clayton and Collins, 2014). Effects also vary widely by species, as some model animals are well known to be less sensitive to estrogens than other models (Vandenberg *et al.*, 2013). All of these factors are important to consider when designing toxicity studies to understand chemicals like BPA and phthalates.

A unique characteristic of BPA and phthalates compared with persistent organic pollutants (POPs) is their quick metabolism and lack of persistence and bioaccumulation. The estimated half-life of both BPA and phthalate metabolites in the human body is on the order of days (Hauser and Calafat, 2005; Stahlhut *et al.*, 2009). The rapid metabolism of these chemicals in the human body made us quick to discount their potential toxicity (Shaffer *et al.*, 1945). However, we now know that exposure is constant and that the metabolites are the true toxic agents (Heudorf *et al.*, 2007; Stahlhut *et al.*, 2009; Vandenberg *et al.*, 2010). Additionally, rapid metabolism complicates biomonitoring because the timing of sample collection can result in huge variations in metabolite detection (Hormann *et al.*, 2014; Koch *et al.*, 2004). Phthalate esters with long side chains also have more metabolites, which can lead to underestimation of exposure if only a few are measured as biomarkers (Koch *et al.*, 2005).

Another factor that has further complicated the understanding BPA and phthalate toxicity is GLP (Myers *et al.*, 2009). GLP includes strict standards for data collection and provides uniformity to studies to facilitate regulatory interpretation, but is independent of study design. For example, perfect execution of GLP guarantees that comprehensive records were kept, but not that proper controls were included (Myers *et al.*, 2009). Policymakers generally place high weight on GLP studies. However, academic labs often do not follow GLP because of the high cost and excessive paperwork and instead rely on peer review to evaluate research quality. As a result, a divide in the endocrine disruption literature exists between academic and regulatory studies. The Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA) project, a consortium between government and academic researchers, was designed to surmount this problem by combining the rigor of GLP with the specialization of academic methods (Schug *et al.*, 2013). In one branch of the project, animals were dosed in a government facility following GLP and the tissues were sent to academic labs for analysis. Academic CLARITY-BPA results from this study published or under review report low-dose effects on gene expression and hormone signaling in the developing brain, ovarian follicle counts, and prostate stem cell differentiation, with many more endpoints yet to be published (Arambula *et al.*, 2016, 2018; Heindel *et al.*, 2015; Patel *et al.*, 2017). Data from another branch of the study performed solely at the FDA under GLP have not been peer reviewed yet, but preliminary data show some low-dose effects that the FDA has dismissed as "minimal" (National Toxicology Program, 2018; vom Saal *et al.*, 2018).

Regulatory and academic scientists must reconverge for the field to move forward and to protect human health and the environment. In the case of BPA and phthalates, the public has not been protected as we have grappled with this over the past 50 years, as exemplified by the accompanying review (Strakovsky and Schantz). It is vital that we do not repeat history as we continue to develop replacements, as current replacements such as bisphenols S and F and the long chain phthalate diisononyl phthalate (DiNP) have proven to have many of the same toxicities (Gray *et al.*, 2000; Rochester and Bolden, 2015). Effective collaboration between risk assessors, government researchers, and academic researchers will be key to properly assessing the low-dose toxicity of future chemical products.

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