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Review

Evidence that bisphenol A (BPA) can be accurately measured without contamination in human serum and urine and that BPA causes numerous hazards from multiple routes of exposure

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A B S T R A C T

There is extensive evidence that bisphenol A (BPA) is related to a wide range of adverse health effects based on both human and experimental animal studies. However, a number of regulatory agencies have ignored all hazard findings. Reports of high levels of unconjugated (bioactive) serum BPA in dozens of human biomonitoring studies have also been rejected based on the prediction that the findings are due to assay contamination and that virtually all ingested BPA is rapidly converted to inactive metabolites. NIH and industry-sponsored round robin studies have demonstrated that serum BPA can be accurately assayed without contamination, while the FDA lab has acknowledged uncontrolled assay contamination. In reviewing the published BPA biomonitoring data, we find that assay contamination is, in fact, well controlled in most labs, and cannot be used as the basis for discounting evidence that significant and virtually continuous exposure to BPA must be occurring from multiple sources.

Contents

Abbreviations: ACC, American Chemistry Council; AUC, area under the concentration time curve; BPA, bisphenol A; CERHR, Center for the Evaluation of Risks to Human Reproduction; EDSP, US-EPAs Endocrine Disruptor Screen and Testing Program; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; FDA, US Food and Drug Administration; GLP, Good Laboratory Practices; LC–MS/MS, HPLC followed by tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation.

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Highlights

- The basis for the FDA declaring BPA safe is shown to be false by findings reported in a large number of studies.
- The FDA claims that BPA is a ubiquitous environmental contaminant that is so pervasive that their BPA assay is contaminated.
- The FDA states that all other scientists who detect bioactive BPA in human blood must also have BPA assay contamination from unknown sources.
- Although BPA is proposed to be a ubiquitous contaminant, the FDA also claims that there is virtually no bioactive BPA found in human blood.
- Based on findings from numerous uncontaminated assays, human blood BPA levels are in fact high and associated with many common human diseases.
- The FDA's assay contamination hypothesis is a manufactured controversy in order to reject all human disease findings related to exposure to BPA.
- A significant problem is that conclusions in abstracts by authors of articles relating to BPA safety are often discordant with their own data.

1. Introduction

Bisphenol A (BPA) is a high volume production chemical, with 15-billion pounds reported being produced in 2013 (GrandViewResearch, 2014). BPA is used in a wide variety of consumer products, including polycarbonate and other forms of plastics, resins used to line food and beverage containers, thermal print papers, and composites used in dentistry. Based on data from the National Health and Nutrition Examination Survey (NHANES), virtually all people in the USA are exposed to measurable levels of BPA (Calafat et al., 2008). BPA contaminates our air, water, and soil (EnvironmentCanada, 2008), and thus the pervasiveness of human exposure is not disputed (Calafat et al., 2008; Vandenberg et al., 2010a). BPA exposure appears to be from multiple routes on near continuous basis, since only a portion the urine total BPA drops as a function of fasting time (Stahlhut et al., 2009).

Beginning in 1999, studies were published of the results of methods to measure BPA in human serum (Table 1). These initial studies reported determinations solely of the unconjugated (also referred to as aglycone or parent) BPA that is the biologically active endocrine disrupting molecule; BPA has estrogenic and anti-estrogenic activity and also disrupts other aspects of endocrine function (Reif et al., 2010). Endogenous hormones are evaluated clinically by the parent, hormonally active compound, not by less active or inactive metabolites, and BPA conjugates were reported to be devoid of estrogenic activity by a number of groups (Welshons et al., 2006). However, the possibility of *in vivo* deconjugation has not been examined, and recent work suggests other BPA conjugates have biological activities; specifically, BPA conjugates disrupt non-genomic, rapid estrogen-response systems associated with the cell membrane (Viñas et al., 2013).

The approaches required in method development for hormonally active chemicals such as BPA include controls, typically required for publication by endocrine journals, for specificity, accuracy, precision and sensitivity. Sensitivity is typically defined in assays of hormones as two or three standard deviations above background; therefore, to achieve high sensitivity, endocrine assays minimize variance and minimize or preferably eliminate background, i.e., contamination.

The reason that this issue is so important to endocrinologists is that the circulating levels of hormones, with their research and clinical implications, are very low, often below levels of detection using the most sensitive approaches in analytical chemistry. For example, free estradiol in fetal mouse and rat serum (measured by highly sensitive and specific radioimmunoassay and ultrafiltration dialysis) is below 1 pg/ml (Montano et al., 1995; vom Saal et al., 1997). Therefore, contamination, with what are by chemical analysis invisible levels, is a very substantial and serious problem in endocrine assays and taken very seriously by laboratories involved in endocrine assay development and use (vom Saal et al., 1990). For this reason, investigators studying hormones and endocrine active chemicals assume a high level of awareness and management of contamination, which may be missing or not assumed to be necessary in non-endocrine laboratory investigations. We will address these issues in this review.

2. Routes and sources of BPA exposure: Is assay contamination a significant problem?

2.1. Background

As will be discussed in more detail below, over the last 17 years there has been a huge amount of research conducted on the hazards of BPA in a wide variety of animal models, and there have also been a large number of studies conducted relating BPA to numerous diseases in humans. The published literature showing adverse effects of BPA is thus vast, and only a very small number of studies report being unable to detect any effects of BPA within the "low dose" range (Myers et al., 2009; Richter et al., 2007; vom Saal and Welshons, 2006; Vandenberg et al., 2013a); "low dose" refers to administered doses that are below the lowest levels typically examined in guideline studies for regulatory purposes (NTP, 2001).

In 2006 the National Institute of Environmental Health Sciences (NIEHS) sponsored a workshop that resulted in a consensus statement (The Chapel Hill Consensus Statement) signed by 38 experts from the USA, Europe and Japan that concluded the following about human exposure and blood levels of BPA: "Based on existing data we are confident of the following. 1. Human exposure to BPA is widespread. 2. Human exposure to BPA is variable, and exposure levels cover a broad range [central tendency for unconjugated BPA: 0.3–4.4 ng/ml (ppb)] in tissues and fluids in fetuses, children and adults." (vom Saal et al., 2007). Together with the extensive hazard data (Richter et al., 2007), which was supported by extensive evidence concerning the underlying mechanisms based on *in vitro* studies (Welshons et al., 2006; Wetherill et al., 2007), it seemed as if the argument about the safety of BPA and need to regulate it was over. However, the reasons that this did not happen will be discussed later.

2.2. Exposure models are used to reject BPA biomonitoring data

Estimates of sources and amounts of exposure to BPA differ markedly (Dekant and Volkel, 2008; Taylor et al., 2011; Vandenberg et al., 2010a). Of importance is that the different estimates of exposure to BPA are based on the exposure models that are used (Gies et al., 2009), with one set of pharmacokinetic models being based entirely on single intra-gastric gavage exposure (LaKind et al., 2008; Volkel et al., 2002). In contrast, other exposure models assume that gavage exposure alone is inadequate to explain human serum levels of bioactive BPA (Vandenberg et al., 2010a, 2010b, 2013b, 2014b).

Central to our review is an examination of data in studies (Table 1) reporting significant (ng/ml or parts-per-billion) concentrations of unconjugated, bioactive BPA in human serum (also see tables listing studies in Vandenberg et al., 2007, 2010a). These data have been rejected by industry-funded studies (Dekant and Volkel, 2008; LaKind et al., 2008) and subsequently in studies supported by the FDA (Patterson et al., 2013). The position that the FDA took in its 2008 draft risk assessment (FDA, 2008a) has not changed in spite of a dramatic increase in data over the last 6 years (Rochester, 2013; Vandenberg et al., 2013a). The FDA draft risk assessment did not adequately explain the basis for ignoring all of the published biomonitoring and hazard data by academic investigators, and the draft was rejected in the October 31, 2008 report by the FDA Science Board Subcommittee Report on Bisphenol A (FDA, 2008b). The Board stated in their review of the FDA's risk assessment that: "The draft FDA report does not articulate reasonable and appropriate scientific support for the criteria applied to select data for use in the assessment." The rejection of the published biomonitoring data because the data were not consistent with exposure models was also criticized by other scientists (Gies et al., 2009; Vandenberg et al., 2010b). Nevertheless, the current position of the FDA remains that rejection of published biomonitoring data reporting measurable unconjugated BPA in humans serum is justified based on the hypothesis that any study that reports finding unconjugated BPA in human blood must have experienced contamination (Churchwell et al., 2014).

The FDA's position regarding contamination may reflect the fact that they recently acknowledged that they have not been able to eliminate sources of contamination from their BPA LC–MS/MS assay: "Mean BPA aglycone levels in vehicle and naive control rat serum

Table 1
Controls for contamination in human biomonitoring papers 1999–2013 (Y = yes; N = no).

Authors	Year	Detection method	Sensitivity (ng/ml)	Sample volumes	Std curve range (ng/ml)	Human Endpoint(s)	Levels found [ng/ml (ppb), mean ± SD or SEM]	Other chemicals measured	Contam eval before data collection?
Sajiki et al., 1999	1999	HPLC/ Electrochemical detection	0.2	0.2–0.5 ml serum or plasma	0–500	Healthy human serum:	0–1.6		Y
		HPLC/MS/ESI	0.1			12 women 9 men	0.33 ± ***0.16*** (0–1.6) 0.59 ± ***0.07*** (0.38–1.0)		
		MS/ESI	0.1						
Inoue et al., 2000	2000	HPLC/electro-chemical detection, or with - Coulometric array	LOQ 0.01 in solvent, 0.05 in serum 19844	3 ml/1.5 ml eq	0.1–100	Healthy human serum $n = 5$	0.32 ± 5%		
Schonfelder et al., 2002	2002	Derivatization- GC/MS	0.01 in serum			Fetal (cord) serum	2.9 ± 0.411		Y
						Maternal serum Placenta	4.4 ± 0.641 11.2 ± 1.5 12 ng/g tissue		
Yoshimura et al., 2002	2002	Derivitization-GC/MS GC/ECD	0.005 "0.15 pg/ml"	1 ml	0.01–10 pg/ml in serum	Pooled 20 human serum samples of ≥5 individuals/ serum pool	0.54 ± 0.037; range 0.39–0.92		Y
Kuroda et al., 2003	2003	HPLC fluorescence derivitization, column switching clean-up	0.04 (at signal/ noise = 3)	0.1 ml	0.1–7.0	Maternal serum from 9 healthy women at delivery	0.46 ± 0.067 range 0.21–0.79		
						Fetal cord serum	0.62 ± 0.043 range 0.45–0.76		
						21 Sterility female serum	0.46 ± 0.044 range 0.22–0.87		
						Ascitic (peritoneal) fluid	0.56 ± 0.041 range 0.15–0.88		
Tan and Mohd, 2003	2003	GC-MS	0.05	1 ml	0 - 50	Fetal cord plasma	ND – 4.05 (88% with positive detection)	Nonylphenol pesticides, other alkylphenols; 7 chemicals total	
Padmanabhan et al., 2008	2008	HPLC-ESI-MS/MS	0.5	0.8–1 ml	0.2–100	Maternal blood from 40 women at delivery	5.9 ± 0.94; range, ND-22.3		Y
Sprague et al., 2013a, 2013b	2013	SPE, HPLC tandem MS	0.24	4.5 ml		Human serum 264 postmenopausal women	Median 0.55;(ND – 8.77); two "outlier values" of 10.7 and 14.5 excluded from analyses; 69% pos for BPA	Mono-alkyl phthalates, propyl and butyl parabens, octylphenol, nonylphenol; estradiol	Y
Gerona et al., 2013	2013	SPE, HPLC-tandem MS, with direct simultaneous measures of BPA, BPA glucuronide and BPA sulfate	0.05 (LOQ 0.1)	0.25 ml	0.1–80	Human fetal cord serum, mid-21013 pregnancies	2.18 ± 8.10 GM 0.16 GSD 7.22 range ND – 52.26 (47% positive) total BPA 0.08–62.77, GM 0.79	BPA GlucuronideBPA SulfateTotal BPA as sum of direct measures	Y, Extensive

(continued on next page)

Table 1 (*continued*)

Authors	Contamination and other evaluated endpoints	Contam detected… (ng/ml)	…that was removed before assay?	Samples collected into vessels pre-tested <LOD or <LOQ?	Contam Control Blanks in Each Assay?	Blanks < LOD or <LOQ?	Pre-tested sample assay equipment to < LOQ?	Lab Bkgd BPA (ng/ml)	Assay "contamination-free"?
Sajiki et al., 1999	Commercial fetal bovine serum Commercial sheep plasma Bovine serum albumin Fresh sheep plasma Polycarbonate tubes (incubated with fresh sheep plasma)	Y Y Y N Y	Y	Y	Y	Y	Y (glass syringes, glass tubes, SPE cartridges)	<LOD	Y "In the experiments, no contamination was observed during clean-up and analysis of BPA. . . ." (Sajiki et al., 1999), p. 257
Inoue et al., 2000	Tap water Milli-Q DI water Disc-purified water SPE catridges	Y (0.01) Y (0.02) N Y	YY; MeOH pre-rinse	Y				<LOD	Y
Schonfelder et al., 2002		Y	Y	Y	Y	Y	Y	<LOQ	Y
Yoshimura et al., 2002	BPA-free water prepared by SPE filtration; SPE columns MeOH-washed		Y					<LOD	Y
Kuroda et al., 2003	Background noise = sensitivity defined 0.04/3 = 0.013 ng/ml							<LOD	Noise background = sensitivity 0.04/3 = 0.013 ng/ml
Tan and Mohd, 2003	Blank sample extractions parallel to serum sample extractions				YBlank extractions < LOD	Y	Y	<LOD	Y for BPA, and Y for all six other chemicals measured
Padmanabhan et al., 2008	Procedural blank; sheep serum; glass vs. plastic pipetting	<0.1 ng/ml	subtracted	Y	Y	Y	Y	<0.1 ng/ml (<LOD)	Y
Sprague et al., 2013a, 2013b	Solvent and method blanks < LOD; use of glass labware, prep-arative steps and 450 °C to remove potential con-taminants from lab-ware, handling of lab-ware and specimens in biosafety cabinets, and assessment of method blanks; serum collected into glass	all < LOD	Y	Y	Y	Y	Y	<LOD	Y"Assessment of method blanks showed that iatrogenic contamination was lower than the limits of detection for BPA and phthalates.", published letter (Sprague et al., 2013b), p. 403
Gerona et al., 2013	Glass or polypropylene sample collection, hand-ling; all sample contact-ing or extracting materials and assay equip-ment; field control blanks with synthetic human serum	All < LOD	Y	Y	YProcedural quality controls and blanks in each assay	Y	Y	<LOD	YExtensive contamination evaluation and controls published with the report

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(0.02–0.5 ng/ml) indicated sample processing artifact, consistent with literature reports of a propensity for post-exposure blood contamination by BPA." (Churchwell et al., 2014). Other recent FDA-sponsored publications also report that their assays for BPA in the circulation of lab animals were contaminated by levels of BPA in a variable range of 2 ng/ml and even higher in adult and neonatal rats (Doerge et al., 2010a) and rhesus monkeys (Patterson et al., 2013). These authors insist that because they were unable to eliminate sources of contamination from their BPA assay, all other laboratories that measure BPA in blood must also be experiencing similarly uncontrolled contamination. The FDA scientists also indicated that contamination was present even after taking all precautions, despite the fact that other laboratories nationally and internationally achieve contamination-free BPA assays as a matter of course (Table 1).

In sharp contrast to the position of the FDA, a NIH-sponsored round robin study to assess whether laboratories could accurately measure BPA in human serum showed that, in fact, laboratories could accurately measure BPA without contamination (Vandenberg et al., 2014a). Similarly, the issue of assay performance was also examined using a round robin validation process in Europe for a number of chemicals, including BPA, which identified that some laboratories were able to accurately assay BPA and other chemicals without contamination, while other laboratories were unable to assay BPA or other chemicals accurately (Vanderford et al., 2014).

2.3. The NIH-sponsored round robin analysis of BPA in human serum

The recently published NIH round robin study addressed this issue of the ability to accurately assay BPA without contamination in human serum. Four participating laboratories screened materials to identify BPA contamination in collection and analysis materials. Serum was spiked with concentrations of unconjugated BPA and/or BPA glucuronide ranging from 0.09 to 19.5 ng/ml (unconjugated BPA) and 0.5 to 32 ng/ml (glucuronidated BPA). Samples were coded and provided by NIEHS blind to laboratories for LC–MSMS analysis. To determine whether inadvertent hydrolysis of BPA metabolites occurred, samples spiked with only BPA glucuronide were analyzed for the presence of unconjugated BPA.

The results of the round robin clearly contradicted the FDA universal contamination hypothesis (Vandenberg et al., 2014a). The round robin authors reported that: "BPA contamination can be controlled during sample collection and inadvertent hydrolysis of BPA conjugates can be avoided during sample handling". The study reported that (1) extensive sampling of collection materials indicated that contaminating BPA was not ubiquitous in collection materials or analytical methods, (2) precautions of screening and selection of materials and reagents, if taken before sampling, could manage BPA contamination to below the limit of detection (LOD), and (3) three labs of the four total were able to achieve contamination-free measures of unconjugated BPA in human serum. All laboratories were able to distinguish low, moderate and high concentrations of unconjugated BPA and glucuronidated BPA. Linear relationship between the amount spiked and the amount measured by the four participating labs ranged from 0.920 to 0.999 for unconjugated BPA and from 0.976 to 1.0 for BPA glucuronide (Vandenberg et al., 2014a).

In the NIH-sponsored round robin study, the limit of detection (LOD) was defined as three times (3 SD), and limit of quantitation (LOQ) was defined as 10 times (10 SD) of the standard deviations (SD) of three replicate analyses using the lowest calibration standard of BPA. The LOQ ranged from 0.01 to 0.13 among the laboratories participating in the round robin. The round robin authors stated that: "Each laboratory independently analyzed their sample extraction protocol, processing materials (including pipet tips, test tubes, cartridges, conical tubes, sample vials, water, methanol) and their liquid chromatography (injection needle, injection port, capillaries, column, mobile phase solvents, mobile phase reservoir) and mass spectrometry procedures (injection valve, ion source, collision cell, quadrupole detector) to ensure that their materials and reagents did not introduce BPA contaminations in the laboratory. In subsequent testing of the collection materials selected for the remainder of the Round Robin experiments, BPA was not observed in either water or stripped human serum, with the exception of a low concentration (0.17 ng/ml) measured in the water sample in one laboratory" (Vandenberg et al., 2014a).

The authors of the NIH-sponsored round robin made it clear that it was essential that equipment used in the sample collection process should be determined prior to sample collection to not contain potentially contaminating BPA. This should be examined for BPA using a solvent appropriate for extraction of a lipophilic compound. Thus, assays of stored samples from previously conducted experiments should only be assumed to be valid if potential sources of contamination had been examined and eliminated.

2.4. Claims that assay contamination is common are not supported by the published literature and data

Given the successful measurement of serum BPA in this round robin, an important question is why the FDA lab cannot do the same. The FDA chemists were initially participants in the NIH-sponsored BPA round robin analysis but withdrew prior to completion of the study. A question is why do experienced FDA scientists publish studies in which BPA contamination above 1 ng/ml is tolerated, accompanied by repeated claims that contamination is unavoidable? The contamination with BPA should be addressed and controlled to below the LOD before any further studies begin, and significant contamination with the chemical of study should not be tolerated in any further publications. The NIH-sponsored round robin and descriptions of assay control procedures in the prior biomonitoring literature demonstrate that this is feasible.

Importantly, it is clear that there are potential sources of BPA contamination that need to be determined and eliminated prior to assaying samples for BPA, which is also the case for other ubiquitous environmental pollutants. For example, in developing and validating our LC–MSMS BPA assay, we identified and found clean substitutes for sources of water that were found to contain detectable background levels of BPA. Sources of BPA contamination have been identified and eliminated by other investigators, which demonstrates the ever-present need to examine equipment and utilize field blanks in experiments that measure BPA or other ubiquitous contaminants.

There are publications devoted to accomplishing this set of required preliminary studies prior to conducting analyses of samples (Salgueiro-Gonzalez et al., 2012). As correctly noted in a chemical-industry study, adequate control of contamination is required in order to publish results if analysis of human serum and urine levels are to be believed (Markham et al., 2010). Supporting the contention that BPA contamination can be controlled in human biomonitoring studies is a report from the CDC in which sources of contamination were identified and systematically eliminated during the successful development of LC–MS/MS assays for BPA and three other chemicals (Ye et al., 2013).

The potential for assay contamination is thus not unique to BPA, and simply requires the use of standard assay procedures and appropriate controls that should be routinely employed, which has also been the conclusion reached by others (Calafat and Needham, 2009; Markham et al., 2010; Vandenberg et al., 2014a). It is important to note that the two groups of scientists that have promoted the belief that BPA cannot be assayed in human serum without

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contamination, the FDA laboratory (Doerge et al., 2010a) (Churchwell et al., 2014), and a plastic-industry funded laboratory (Dekant and Volkel, 2008; Volkel et al., 2002), are the two laboratories that in their publications have reported uncontrolled contamination and high background BPA in their assays.

FDA scientists (Patterson et al., 2013) stated in a recent publication that: "a significant body of evidence has shown that contamination from ubiquitous environmental sources of BPA during sample collection, storage, and analysis has a propensity to introduce artifactual aglycone [unconjugated] BPA in extracts from blood and tissues". Given this statement as a pillar for their argument, along with the fact that it is contradicted by data we have presented earlier and inconsistent with standard analytic laboratory procedures, it is crucial to identify what exactly was stated in the eight studies Patterson et al. (2013) cited as support for their statement (the articles cited were: Doerge et al., 2012; Koch et al., 2012; Markham et al., 2010; Salgueiro-Gonzalez et al., 2012; Teeguarden et al., 2011; Twaddle et al., 2010; Vandentorren et al., 2011; Volkel et al., 2002). In fact, examination of these publications reveals that the Patterson et al. (2013) statement is not consistent with the conclusions drawn by most of the authors that they cite.

First, Koch et al. (2012) stated for the assay of BPA in urine: "... we were able to keep the laboratory blank value of BPA (caused by contamination with omnipresent BPA) below the LOD of 0.05 µg/L", and they also identify that this was true for plasma. Second, the objective of the Markham et al. (2010) study was "to account for and/or eliminate background contamination from all sources." Markham et al. reported on a comparison of BPA serum and urine assay performance in two laboratories, and they reported very good accuracy and precision, although this was reduced at the very lowest dose examined compared to higher doses, consistent with typical assay performance. Third, Salgueiro-Gonzalez et al. (2012) conducted an analysis of sources of blank contamination in BPA, as well as nonylphenol and octylphenol, assays and concluded that: "the main contamination sources in DLLME–LC–MS/MS were considered and >90% of the contamination could be removed by following the guidelines described here". These were standard procedures for identifying and eliminating sources of contamination in assays.

Another study cited by Patterson et al. was by Teeguarden et al. (2011), which is particularly interesting. Their study reports results of assays conducted by the CDC laboratory that subsequently reported on development of the BPA assay and procedures used to successfully eliminate sources of contamination for BPA as well as three other chemicals (Ye et al., 2013). In Teeguarden et al. (2011) study some serum samples that had been assayed by the CDC lab were re-assayed at the FDA lab, and again problems with contamination were identified.

The Vandentorren et al. (2011) study that was cited by Patterson et al. regarding ubiquitous contamination of samples in biomonitoring studies concerned contamination from urinary catheters and is thus irrelevant to the general issue of analysis of urine by methods other than via indwelling catheters. The Doerge et al. (2012) and Twaddle et al. (2010) studies involved the administration of deuterated BPA to mice and rats and were thus also not relevant to the issue of contamination of assays by authentic BPA from environmental sources (although information about contamination in another study from this lab using a BPA isotope is to be discussed later). In summary, eliminating contamination is required if the objective is a sensitive assay, and if the contamination is variable and a low constant background value cannot be subtracted from all assayed values, then accuracy and precision will be impacted and the assay will not produce valid results (Salgueiro-Gonzalez et al., 2012).

As discussed earlier, for a number of the studies cited by Patterson et al. (2013), assay contamination was not identified as interfering with measurement of unconjugated BPA. However the two laboratories that experienced contamination and a high background level of BPA included an industry lab (Volkel et al., 2002) and the FDA lab (Doerge et al., 2010a). For example, assay sensitivity was compromised by assay contamination in an industry-funded study designed to measure BPA in human blood and urine after placing BPA contained in a capsule into the stomach of adult men and women (Volkel et al., 2002). Volkel et al. stated that: "When this method was used to quantify bisphenol A content from "unexposed" individuals, a small background of bisphenol A (up to 50 nM [11.4 ng/ml]) was detected in all urine and blood samples analyzed. Identical background concentrations as seen in the human blood and urine samples were also observed when water samples purified by a Millipore Water purification system were subjected to the workup procedures." In the Doerge et al. (2010a) study, they reported that: "All dosing was done using stable isotope labeled BPA to avoid contamination with unlabeled BPA from laboratory materials or other sources, which were found to be significant (buffer blanks contained approximately 2 ng/ml, data not shown)."

Even though a number of the authors or studies described earlier reported that contamination was identified and controlled, some of these authors then went on to state that contamination was, in fact, a problem. For example, in their abstract Markham et al. (2010) stated that: "Trace contamination of BPA from exogenous sources or hydrolysis of BPA-G to free BPA, either during or after biomonitoring specimen collection, may have contributed to the reported concentrations of free BPA." However, these authors actually reported that hydrolysis of conjugated BPA, which would lead to overestimation of unconjugated BPA, was not observed in their study, similar to findings reported in the NIH-sponsored round robin study (Vandenberg et al., 2014a). While Koch et al. (2012) agreed that: "circulating unconjugated BPA in blood (or serum) is of special interest for toxicological and mechanistic valuations, because only unconjugated BPA is regarded as hormonally active", they went on to accept that because gavage exposures lead to such a low percent of unconjugated BPA in serum (<1%), any findings of serum unconjugated BPA cannot be valid based on accepting that assays must be experiencing BPA contamination, even though they did not have a contaminated assay.

In the Ye et al. (2013) study from the CDC, despite the successful control of contamination, they strangely contradict themselves in the paper's title: "Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: an elusive laboratory challenge". The casual reader would not expect that the conclusion drawn from their data was that eliminating contamination by BPA or other chemicals was, in reality, not "an elusive laboratory challenge". Thus, even though Ye et al. systematically identified and eliminated potential sources of BPA contamination, they emphasized in their conclusion that: "Unfortunately, until all of the environmental sources of these chemicals are known, totally eliminating external contamination is practically impossible. However, judicious application of the measures below will allow the identification of contamination scenarios, thus facilitating the implementation of measures to isolate and track external contamination and minimize as much as possible its recurrence and impact." A similar argument by the senior (corresponding) author on the Ye et al. study that serum BPA assays must always be contaminated recently led to a criticism that is was "misuse of blood serum" to study the relationship between serum unconjugated BPA and mammographic breast density in postmenopausal women (Calafat et al., 2013). The authors of the original study had reported that there was a significant relationship between serum BPA and increased breast density (Sprague et al., 2013a), which is a marker of increased risk for breast cancer that has been shown to be a consequence of very low dose BPA exposure during development in a number of studies with laboratory mice, rats and monkeys (Soto et al., 2013; Tharp et al., 2012; Vandenberg et al.,

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2013a). In responding to this criticism the authors of the breast density study pointed out that numerous steps had been taken to ensure the absence of contamination (also identified in the initial published article), that method blanks were below the limit of detection, and that random contamination would tend to decrease, not increase, the likelihood of a statistically significant association (Sprague et al., 2013b). The conclusion by Ye et al. (2013) that eliminating contamination by environmental BPA "is practically impossible" directly contradicts that they did successfully eliminate BPA contamination, as did laboratories that participated in the Markham et al. (2010) study, the NIH round robin study, and many other laboratories for both biological (Gerona et al., 2013; Schonfelder et al., 2002) and environmental (Watabe et al., 2004) samples.

3. Unknown sources of contamination of control animals is unacceptable in experimental research

The FDA scientists promoting the idea that BPA contamination was to be expected and was the basis for finding unconjugated BPA in serum have acknowledged problems with BPA contamination not only in their assay procedures (Churchwell et al., 2014), but in addition, the animal research facility at the FDA's toxicology center (National Center for Toxicological Research, NCTR) also has an unidentified source of contamination (Delclos et al., 2014). Churchwell et al. acknowledged that their negative controls in an experiment with rats had been contaminated with BPA in the preliminary study conducted as part of an ongoing collaborative research program (paradoxically identified by the acronym CLARITY). The contamination was from a source that they were unable to identify, and that serum levels of unconjugated BPA in the negative controls were not different from serum BPA levels detected in all six low dose groups in animals at postnatal day 80, and total serum BPA in the negative controls overlapped with total serum BPA levels in the lowest two BPA dose groups (Churchwell et al., 2014). The FDA scientists drew the conclusion that there were no adverse effects at low doses and stated: "Clear adverse effects of BPA . . .were observed only at the two high doses of BPA" (Delclos et al., 2014). This conclusion was harshly criticized as violating the basic principle of experimental research that a valid negative (reference) control is required to draw any conclusion of safety from an experiment and that the results of their contaminated animal experiment cannot be interpreted (Hunt et al., 2014).

The use of data from fundamentally flawed studies that are intended to be used to assure the public that BPA does not pose a risk to the public health has been ongoing under the supervision of product protection firms funded by chemical industry lobbying organizations, such as the American Chemistry Council (ACC), since the publication of "low dose" BPA findings beginning in 1997 (Nagel et al., 1997; Steinmetz et al., 1997; vom Saal et al., 1998). The results of these Nagel et al. study were disputed by two industry-funded studies (Ashby et al., 1999; Cagen et al., 1999). However, both of these industry-funded studies were declared flawed and rejected for inclusion by the National Toxicology Program CERHR panel's review of published BPA findings. In its final 2007 draft report to the NTP, the CERHR panel stated with regard to both the Ashby et al. and Cagen et al. studies that with regard to: "Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to absence of response of the positive control group" (CERHR, 2007).

Importantly, the initial findings by Nagel et al. (1997) were replicated and extended by Gupta (2000a, 2000b). This replication was acknowledged by a senior FDA scientist as resolving the dispute over the validity and reliability of our original findings (Sheehan, 2000). The issue of the need for appropriate controls (both negative and positive controls) in experimental research on endocrine disrupting chemicals such as BPA has been previously reviewed (vom Saal and Welshons, 2006; Welshons et al., 2003).

Since the study with contaminated negative controls was published by FDA scientists (Delclos et al., 2014), the concern is that the conclusions drawn by the authors will be uncritically accepted as valid, even though they have been harshly criticized, as were the editorial policies of the journal for allowing a flawed study to be published (Hunt et al., 2014). Previously, a group of 24 scientists, including us, identified the need for the editors of this same journal to understand, and incorporate into journal editorial policy, the importance of appropriate controls in experimental research (vom Saal et al., 2010). The second issue relating to publication of the Delclos et al. study by the FDA is that it was conducted using Good Laboratory Practices (GLP). A large group of scientists pointed out that GLP does not guarantee good science, just good record keeping, since the use of GLP was instituted as a result of fraud at commercial laboratories (Myers et al., 2009; vom Saal and Myers, 2010). The logical question here is: can a study with contaminated negative controls even be considered GLP-compliant?

An issue that should be considered in the future is whether regulatory agencies that are charged with making decisions regarding the safety of chemicals in commerce should be tasked with conducting scientific research (such as the FDA's CLARITY collaboration) that could reveal that the agency's prior assessments of safety were incorrect. This can create a potential conflict of interest.

4. The impact of age on pharmacokinetics of BPA

The most concern with exposure to endocrine disrupting chemicals is during fetal development and during early postnatal life through adolescence. During these critical periods in organ development permanent adverse effects can occur, including effects that can be transmitted across generations due to changes in the germ line (see Skinner review in this theme issue). The maxim in pediatric medicine is that babies are not little adults, and it is well understood that fetuses and babies are more susceptible to toxic exposures than adults (Bearer, 1995a, 1995b). A major factor in a criticism of the inadequacy of the US-EPAs Endocrine Disruptor Screen and Testing Program (EDSP) in a position paper by the Endocrine Society (Zoeller et al., 2012) was the absence of developmental assays that took the issue of age-related changes in susceptibility into account.

The increased susceptibility of fetuses and infants to exposure to BPA and other chemicals is, in part, due to the age-related change that occurs in the ability to metabolize BPA in all species that have been examined. The primary phase 2 BPA metabolizing enzyme, UDP-glucuronosyltransferase, is not expressed in the human fetal liver until after birth (de Wildt et al., 1999), and infant (5-day-old) rhesus monkeys have 3.8-fold higher unconjugated serum BPA values relative to adult monkeys after oral administration of the same single oral dose, based on the area under the concentration time curve or AUC (Doerge et al., 2010b). We have published that there is a virtually identical age-related increase in phase II metabolism of BPA in mice, with infant (3-day-old) mice (Taylor et al., 2008) having a 4.0-fold higher serum unconjugated BPA (based on the AUC) relative to adults (Taylor et al., 2011) after oral administration of the same dose of BPA. Matsumoto et al. (2002) reported that the BPA-conjugating enzyme (UDP-glucuronosyltransferase) was not detected in the liver of fetal rats, but showed a linear 4.5-fold increase between postnatal days 3 and 21, at which age adult levels of BPA-glucuronide were reached.

In adult rats there is approximately 10-fold higher serum unconjugated BPA after IV administration relative to gavage administration (Pottenger et al., 2000); the lower unconjugated BPA in serum after gavage administration is a result of direct transport via the mesenteric vessels of BPA from the GI tract to the liver.

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However, due to the limited BPA conjugating activity of the liver in infant rats and mice, the effect of route of administration on the levels of serum unconjugated BPA that are achieved is greatly reduced (Prins et al., 2011; Taylor et al., 2008). Although, similar to other species, there was a steady increase in phase II metabolism of BPA between infancy and adulthood in rhesus monkeys (Doerge et al., 2010b), consistent with an age related change in BPA pharmacokinetics reported in other species, the authors of this study concluded in the abstract that: "No age-related changes were seen in internal exposure metrics for aglycone [unconjugated] BPA in monkeys." Thus, there was discordance between this statement in the abstract and actual findings concerning the fact that oral exposure to the same amount of BPA resulted in higher serum unconjugated BPA in infants relative to adults.

5. BPA pharmacokinetics in humans is similar to other species

The only previously published attempt at a human pharmacokinetic study involved placing BPA directly into the stomach by administering it in a capsule (gavage administration) (Volkel et al., 2002). This experiment was of limited value because the assay was about 10-fold less sensitive than current LC–MS/MS BPA serum assays (Vandenberg et al., 2014a). In addition, the study by Volkel et al. did not allow levels of serum unconjugated BPA to be determined because of the lack of control of background contamination (Volkel et al., 2002). While the study by Volkel et al. has been used to justify the conclusion that there should be no unconjugated BPA detected after exposure to BPA in humans, the insensitivity of the assay used precludes drawing this conclusion. We have thus had to rely on rodent, primate and other species, such as sheep (Corbel et al., 2013) to estimate human pharmacokinetics of BPA. The available evidence is that both rodent and primate data are relevant to human pharmacokinetics without allometric adjustment of pharmacokinetic parameters for body size, which is unexpected. The conclusion that there was a lack of need for allometric scaling across species was based on comparison of conjugated BPA levels in serum after oral administration in mice, monkeys and humans, where it was found that there was no significant difference in pharmacokinetic parameters over a 24-h period (Taylor et al., 2011).

Pregnancy involves complex physiological changes and there are species differences in the interaction between the mother, placenta and fetus (Corbel et al., 2013; Gerona et al., 2013; Inoue et al., 2005; vom Saal et al., 2014). However, unconjugated BPA, but not conjugated BPA, readily passes across the placenta from the maternal circulation into the fetus in all species examined (Gerona et al., 2013; Miyakoda et al., 1999; Padmanabhan et al., 2008; vom Saal et al., 2014; Zalko et al., 2003).

6. Important routes of exposure to BPA that are not modeled by intra-gastric gavage administration: Relevance of the serum conjugated/unconjugated BPA ratio

6.1. *Sublingual exposure to BPA bypasses first-pass metabolism in the liver*

Gayrard et al. (2013) reported that BPA is rapidly absorbed in the mouth, which is a known method for rapid (and virtually complete) uptake of drugs such as nitroglycerine. The Gayrard et al. study was conducted in dogs, which are an accepted model for human oral exposures. Gayrard et al. reported high sublingual absorption and bioavailability of BPA (about 70%), close to that from IV administration, but much higher than the <1% absorption and bioavailability values following gavage administration in side-by-side comparisons (Gayrard et al., 2013). This finding by Gayrard et al. thus contradicts the prediction by others that virtually all BPA exposure can be modeled by pharmacokinetics based on a single gavage administration, which bypasses sublingual absorption and leads to very low serum unconjugated BPA (Patterson et al., 2013).

The disagreement about the safety of BPA has been over the substantial difference between the estimated and measured human blood levels of unconjugated BPA. The estimate of very low, virtually undetectable, serum unconjugated BPA is based on exposure estimates from urine total BPA data coupled with the prediction that all human exposure to BPA can be modeled by an acute gavage administration that results in significant first-pass metabolism in the liver and less than 1% bioavailability of the administered dose. In contrast, there are numerous biomonitoring studies that report what would clearly be high enough unconjugated BPA to cause adverse effects (Vandenberg et al., 2007, 2010a). In fact, given the typical LOQ of 0.1 ng/ml for serum unconjugated BPA in LC–MS/MS assays (Vandenberg et al., 2014a), any detection of unconjugated BPA in human serum would be biologically active, based on a very large *in vitro* and experimental animal *in vivo* literature (reviewed in Vandenberg et al., 2013a; Welshons et al., 2003, 2006; Wetherill et al. 2007): as well as a large epidemiology literature [reviewed in Rochester, 2013). For example, Prins et al. (2011) reported that a 10 µg/kg oral dose of BPA (fivefold below the EPA's reference or safe daily dose 0f 50 µgkgday) resulted in a maximum concentration (Cmax) of 0.26 ng/ml unconjugated BPA in neonatal rat pups, with the consequence of an increased incidence of prostate intraepithelial neoplasia in adulthood. Angle et al. (2013) reported that a 20 µg/kg oral dose to pregnant mice resulted in a Cmax of 14 pg/ml unconjugated BPA in fetal blood and an average serum level over 24 h (based on the area under the concentration time curve or AUC) of 7 pg/ml (these data are based on administering tritiated BPA to the pregnant females as these levels would be undetectable by LC–MS/MS). Serum BPA within this range resulted in a wide range of hormonal and metabolic abnormalities, including glucose intolerance, in male offspring when examined during postnatal life (Angle et al., 2013). These data also emphasize that estimates of pharmacokinetics based on studies in adults are not valid for predicting pharmacokinetics in fetuses or infants, nor are they predictive of the types of adverse outcomes that can occur at very low serum concentrations of unconjugated BPA; clearly the results identified earlier would be labeled as adverse and were caused by concentrations of serum unconjugated BPA in the pg/ml (parts-per-trillion) range.

The importance of the data in Gayrard et al. is that they are the first experimental evidence that provides an answer regarding how relatively low exposures can lead to high blood concentrations of unconjugated BPA that have escaped first pass metabolism in the liver and that are clearly in the bioactive range. Regarding the model Gayrard et al. challenge, a paper funded by the trade organization Polycarbonate/BPA Global Group presents estimates concerning amounts and routes of human exposure to BPA (Lakind and Naiman, 2008). These estimates have become the basis for predicting the levels of unconjugated BPA in human blood from urine BPA data. This "leap of faith", as anyone knowledgeable about pharmacokinetics knows, is impossible unless one is confident that all routes and amounts of exposure are known and have been accounted for in the exposure model. Gayrard et al. reported that sublingual exposure can lead to very high absorption and bioavailability of BPA, resulting in high concentrations of unconjugated BPA in arterial blood, which is the blood circulating directly to tissues and cells, while bioavailable BPA in blood is less than 1% after an acute gavage administration in dogs. This should not be surprising, since sublingual administration is the route used to rapidly deliver nitroglycerine into the blood (Narang and Sharma, 2011).

The current FDA model that all BPA exposure can be modeled by a single bolus gavage administration (Patterson et al., 2013) not only has to now take into account the high absorption and bioavailability of BPA associated with sublingual exposure

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reported by Gayrard et al., but also underestimates BPA exposure via other routes that can bypass first-pass metabolism, such as dermal exposure from thermal receipt paper coated with milligram levels of free BPA per gram paper (Hormann et al., 2014; Mendum et al., 2011). If one accepts the NHANES data that fasting time does not show the predicted inverse relationship to urine BPA (Stahlhut et al., 2009), then there has to be concern that exposure estimates based on an acute gavage exposure are significantly underestimating human exposure to BPA.

6.2. Thermal receipt paper as a source of transdermal exposure to BPA that bypasses first-pass metabolism in the liver

Free, unpolymerized BPA is present in the print surface of thermal paper, which is used for airline ticket, gas, ATM, cash register and other types of receipts. The print surface of thermal paper is coated with milligrams of free BPA per gram paper as a heat-activated print developer (Hormann et al., 2014; Mendum et al., 2011), and free BPA appears to be readily transferred to anything that the thermal paper contacts (Liao and Kannan, 2011), although the characteristics of the material contacted by thermal paper impact the amount of BPA transferred (Hormann et al., 2014). We have recently completed a study of the consequence of adult men and women holding a thermal receipt coated with BPA after using a commonly used skin-care product containing dermal penetration enhancing chemicals (these are chemicals used to enhance transdermal drug delivery but are commonly found in skin-care products). The volunteers also ate French fries that were picked up with the BPA-contaminated hand that had held the receipt paper (Hormann et al., 2014). We found rapid transfer (due to holding a thermal receipt for 2 s) of hundreds of micrograms of free BPA from the surface of thermal receipt paper to the hand. Maximum levels of free BPA were swiped off of the surface of the hand after holding a receipt for only 45 s (over 500 µg of BPA was swiped from the surface of the hand). Importantly, many people touch thermal receipts multiple times per day and may hold the receipts for variable periods of time.

There are many factors that impact the ability of compounds to pass through skin, including differences due the location of skin on the body, gender, age, molecular weight and lipophilicity (Singh and Morris, 2011), in addition to the use of personal care products that contain chemicals that impact the integrity of the dermal barrier (Funke et al., 2002; Karande and Mitragotri, 2009). While lipophilic compounds such as BPA ($\log P = 3.4$) can pass through skin (Zalko et al., 2011), regulatory agencies have assumed that this route of human BPA exposure should be limited in spite of the lack of data and acknowledged "significant uncertainties" around the issue of human exposure to BPA from thermal paper (EFSA, 2013). However, a factor that has not been considered in estimating transdermal exposure to BPA from thermal paper is that many skin-care products, including hand sanitizers, lotions, soaps and sunscreens, contain mixtures of chemicals that are also used as dermal penetration enhancers to increase the transdermal delivery of drugs. The dermal penetration enhancing chemicals present in personal care products as well as hand sanitizers cause a breakdown of the dermal barrier that reduces transdermal absorption (Funke et al., 2002; Karande and Mitragotri, 2009).

Our data provide the first evidence that the use of very large amounts of free BPA as a developer on the print surface of thermal paper (~20 mg BPA/g paper) could be an important factor in accounting for the high levels of bioactive serum unconjugated BPA reported previously in human biomonitoring studies (Vandenberg et al., 2010a). We conducted this study to mimic aspects of the behavior of people in a fast-food restaurant where we observed people handling a thermal receipt prior to picking up and eating food with their hands after using hand sanitizer. In both men and women there was a dramatic increase in serum unconjugated BPA after holding thermal receipt paper and then eating French fries with the BPA-contaminated hand. While we only examined five men and five women, the data suggest that absorption through the skin is more rapid in females relative to males, consistent with men having a thicker stratum corneum (the outermost layer of the epidermis) relative to women (Fitzmaurice and Maibach, 2010; Polak et al., 2012). Thus, the skin of females may allow greater transdermal transport of BPA relative to males due to sex differences in skin permeability (Singh and Morris, 2011). One possible contributor to the sex differences we observed would be a greater use of skin cream in females than in males, which could impact both the transfer of BPA to the hand from the surface of thermal paper as well as transdermal penetration of BPA.

Our finding that thermal receipt paper is a potential source of high exposure to BPA are supported by data showing that environmental contamination caused by the use of unpolymerized (free) BPA in thermal paper is widespread (Liao and Kannan, 2011). While BPA was reported to be absorbed through pig and human skin *in vitro* (Zalko et al., 2011), our data show that use of hand sanitizer containing dermal penetration enhancing chemicals significantly enhanced by over 100-fold extraction of free BPA from the surface of thermal receipt paper, thus providing a much greater amount of BPA to be absorbed through the skin.

6.3. The significance of high vs. low ratio of conjugated BPA/unconjugated BPA in serum

When examining all of our data for serum unconjugated and conjugated BPA, a critical finding is that the ratio of conjugated BPA/unconjugated BPA was similar to the ratio observed by Gayrard et al. (2013) after sublingual administration in dogs; the ratio we observed and Gayrard et al. observed was close to 1:1 rather than >100:1 observed after gavage administration in dogs (Gayrard et al., 2013) and rhesus monkeys (Patterson et al., 2013). These new findings have to be considered in relation to prior findings that thermal paper also poses an occupational risk with regard to increased BPA exposure (Braun et al., 2011; Ehrlich et al., 2014). Together with the findings by Gayrard et al. regarding high serum levels of unconjugated BPA after sublingual absorption of BPA (Gayrard et al., 2013), these findings further challenge predictions that high levels of biologically active unconjugated BPA in blood are not possible.

Rhesus monkey data showed that continuous exposure to BPA via Silastic capsules produced a profile of conjugated BPA/unconjugated BPA in maternal serum that ranged from 0.99:1 to 3.87:1 during pregnancy (vom Saal et al., 2014). Because the ratios obtained from continuously exposed animals are more similar to the profiles observed in cross-sectional studies in people, where the ratio of conjugated BPA to unconjugated BPA is less than 10:1 (Gerona et al., 2013; Liao and Kannan, 2012a), our results suggest that continuous exposure (via subcutaneously implanted capsule) may better model human exposures than oral bolus exposure one time per day. This is important because in the prior NTP-CERHR panel analysis of the BPA literature up to 2007, all studies that used non-oral sources of BPA administration were eliminated from consideration in the assessment of potential hazards caused by BPA, which dramatically reduced their level of concern in assessing the hazards posed by BPA (CERHR, 2007).

6.4. Incompatibility between observations in human exposure data and predictions based on pharmacokinetic models based on gavage

There are four kinds of paradoxes between the observations in the current human exposure data and the predictions of the current pharmacokinetic model for BPA of sole-oral exposure and acute-rapid metabolism. First, the published biomonitoring data require at least some kind of non-oral exposures to be accepted as valid (as discussed in the prior sections) because circulating levels of unconjugated BPA in human serum are higher than are predicted from acute oral intake models; reviewed median serum unconjugated BPA values are reported to be 1 or 2 ng/ml, extending from high sub ng/ml to over 10 ng/ml (Vandenberg et al., 2007, 2010a). As shown in Table 1, the prediction of contamination for many of these studies is not credible given the method development and explicit contamination controls, including field blanks, in many of these studies.

Second, as reviewed in Vandenberg et al. (2010a), human urinary BPA and human serum BPA (nonpregnant and pregnant) concentrations are reported in similar overlapping ranges of low ng/ml, which vary substantially from the acute studies where ratios of up to 250-fold higher in urine than in serum are reported (Teeguarden et al., 2011). Rapidly cleared chemicals are found with a high ratio in urine relative to serum, but bioaccumulated chemicals, such as PCBs, are found at similar concentrations in both urine and serum. The discordant urinary-serum ratio from acute studies further suggests that acute pharmacokinetics do not model the reality of human BPA exposure.

Third, the decline in urinary BPA with time of fasting, which would be predicted to be rapid based on data from acute pharmacokinetic studies, was in sharp contrast, only partial with a decrease of approximately one-third between 4 and 8.5 h fasting time, and with no decrease in urinary BPA being observed after 8.5 h fasting time in an evaluation of the NHANES BPA data set (Stahlhut et al., 2009). This evaluation of the NHANES BPA data set could be explained by continuous BPA exposure, or by reduced clearance rates after prolonged exposures or both, but the data are not consistent with current models of rapid clearance within a few hours of ingestion and that all human exposure is from food and beverage packaging and modeled by gavage exposure.

Fourth, there are a small number of current reports and some unpublished data of the ratio of circulating BPA conjugates to unconjugated BPA, ratios which are much smaller than predicted by solely oral route of exposure. For example, Schonfelder et al. (2002) published the first data on unconjugated BPA in maternal serum with a median of 3.1 ng/ml (mean 4.4 ng/ml), while Lee et al. (2008) published the first total BPA (after enzymatic hydrolysis) in maternal serum with a median value of 2.73 ng/ml (mean 9.04 ng/ml). Additional unpublished human serum data sets have been reported at meetings with ratios of conjugated BPA/unconjugated BPA of 6 or 10, discordant with acute oral pharmacokinetic predictions of ratios over 100:1. These new data will have to be published for this issue to be resolved. While this issue is being resolved, an unbiased approach needs to be applied in analyzing serum BPA data. In the Teeguarden et al. (2011) study, some serum samples were eliminated from consideration because the ratio of conjugated BPA to unconjugated BPA did not match the profile that would be predicted after gavage administration. Discarding data that do not fit your model is clearly unacceptable (Gies et al., 2009).

7. Extensive BPA hazard data dispute the assumption that bioactive levels of BPA cannot occur in humans due to rapid first-pass metabolism

There is a complete lack of knowledge of exposure information and hazards for the great majority of even high volume chemicals; the lack of information was pointed out in the 1997 report "Toxic Ignorance" (EDF, 1997), and the situation has not changed since then. Thus, the huge amount of information based on hundreds of BPA publications reporting data on human and wildlife exposure, *in vitro* mechanisms, developmental and adult health effects in laboratory animal studies, as well as a large number of epidemiological studies, is highly unusual. One fascinating aspect of why this is the case is the controversy created by the aggressive response of the chemical industry to the initial findings that a dose 25,000-times lower than had been previously tested in animals disrupted development of the male reproductive system in mice, associated with an unexpectedly high free concentration of BPA in serum relative to estradiol due to limited binding to plasma estrogen binding proteins (Nagel et al., 1997; vom Saal et al., 1998). The elevated free serum BPA due to reduced binding to plasma estrogen-binding proteins is similar to another estrogenic drug with which BPA shares many characteristics, diethylstilbestrol or DES (Nagel et al., 1999; Sheehan and Young, 1979; Welshons et al., 2003, 2006).

The idea that the high dose testing paradigm used in chemical risk assessments, followed by linear extrapolation using safety factors, was an approach that could not be applied to any endocrine active compound challenged the core assumptions of chemical risk assessments (vom Saal and Sheehan, 1998; Vandenberg et al., 2012; Welshons et al., 2003; Zoeller et al., 2012). If these challenges to the assumptions used in chemical risk assessments were to be accepted as valid, it would mean that the FDA in the USA and European Food Safety Authority (EFSA) in Europe, as well as other regulatory agencies around the world, would have to acknowledge that their assessments of "safe" exposure levels for endocrine disrupting chemicals were no longer valid. The aggressive reaction to the possibility that very low doses of endocrine disrupting chemicals could have effects that were unpredicted by high dose studies (vom Saal and Hughes, 2005), which everyone knowledgeable about hormonally active compounds knows to be possible (Vandenberg et al., 2012), led scientists from a wide range of disciplines outside of toxicology to test for effects of BPA. BPA turned out to impact such a wide range of systems than just nuclear estrogen receptors based on both *in vitro* and *in vivo* experiments. For example, in the EPA's ToxCast program Reif et al. (2010) developed a weight-of-evidence Toxicological Priority Index (ToxPi) score based positive findings using a battery of 467 *in vitro*, high-throughput screening assays, and BPA had the third highest ToxPi score out of 309 environmental chemicals that were tested. This finding is consistent with the now enormous published literature reporting adverse effects of BPA at low exposure levels (Peretz et al., 2014; Richter et al., 2007; Rochester, 2013; vom Saal et al., 2007; Vandenberg et al., 2013a). When combined with massive amount of hazard information, biomonitoring data identifying high levels of unconjugated BPA in human serum are clearly of concern (Vandenberg et al., 2007, 2010a, 2010b).

The problem from a public health perspective of the blanket rejection of human biomonitoring data is that if there are sources of BPA exposure that are not modeled by gavage exposure, then there should be great concern with the health effects of BPA that have been identified. Our concern with this issue is based on published findings from hundreds of experimental animal studies for "low dose" effects of BPA; there was a NIH-sponsored review of published studies up to 2007 (Richter et al., 2007; vom Saal et al., 2007). Follow-up reviews of additional hundreds of studies published between 2007 and 2013 were recently published (Peretz et al., 2014; Vandenberg et al., 2013a). In addition, there have been over 90 epidemiological studies, both cross-sectional and prospective, reporting relationships between total BPA in urine and a wide array of adverse health outcomes, including a significant increase in the likelihood of developing cardiovascular disease and type 2 diabetes, obesity, impaired liver function, impaired immune and kidney function, inflammation, reproductive effects in women (polycystic ovary syndrome, altered ovarian response to hormones, reduced fertilization success, implantation failure, endometrial disorders, reduced embryo quality, miscarriage, premature delivery and breast cancer), reproductive effects in men (reduced libido, sperm quality, altered sex hormone concentrations and embryo quality), altered thyroid hormone concentrations, and neurobehavioral deficits such as aggressiveness, hyperactivity and impaired learning (Rochester, 2013;

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Vandenberg et al., 2013a). The estimate of the costs per year of additional cases of just cardiovascular disease in the USA attributable to BPA is $1.5 billion (Trasande, 2014).

For government public health agencies to reject such a massive amount of information about the hazards of BPA, that are remarkably consistent across hundreds of *in vitro* mechanistic studies and experimental animal studies as well as dozens of human studies, one would imagine that there would be a very high level of certainty regarding routes of BPA exposure rather than this decision being based on estimates derived from models (Lakind and Naiman, 2008) that are contradicted by other findings (Gayrard et al., 2013; Hormann et al., 2014; Stahlhut et al., 2009; Vandenberg et al., 2010b).

The majority of experimental studies of the hazards due to exposure to BPA have been conducted with rodents, which are often criticized as not being predictive of effects in primates. However, unconjugated BPA levels in pregnant rhesus monkeys (vom Saal et al., 2014) that were within the range reported in numerous human biomonitoring studies (Vandenberg et al., 2007, 2010a) were reported to have adverse effects in a series of studies examining fetal tissues (ovary, mammary gland, brain, lung, uterus and heart) from the female fetuses carried by pregnant rhesus monkeys (Calhoun et al., 2014; Chapalamadugu et al., 2014; Elsworth et al., 2013; Hunt et al., 2012; Tharp et al., 2012; Van Winkle et al., 2013). The monkey fetus findings for ovary, mammary gland and brain recapitulate previously reported effects from numerous studies in rodents (reviewed in Vandenberg et al., 2013a). This underscores the importance of pharmacokinetic data based on measuring unconjugated BPA in serum rather than estimating serum unconjugated BPA based on levels of total BPA in urine. Data from this cohort of monkeys demonstrate that low, human-relevant concentrations of unconjugated BPA in maternal and fetal serum disrupt normal fetal development in a primate model and produce effects similar to those observed in rodents.

8. Conclusions

The universal contamination hypothesis appears to be a "manufactured controversy" (Michaels and Monforton, 2005) without a basis in the literature (Table 1). The data concerning the validity of contamination-free assays demonstrating that unconjugated BPA can be accurately measured in human serum obtained with contamination-free sample collection procedures have now been confirmed (Vandenberg et al., 2014a). The prediction that any assay that detected unconjugated BPA in human serum must have had an unknown source of BPA contaminate the sample is thus not supported by data and ignores that numerous published biomonitoring studies were careful to examine potential sources of contamination.

Regulatory agencies in the USA and Europe are using models and estimates of human exposure based on a single gavage route of administration to reject published data (Gies et al., 2009) by holding on to the assumption that all detected unconjugated BPA in human serum is due to contamination (Churchwell et al., 2014). Here we reviewed evidence demonstrating that this assumption has been falsified by controlled studies from a number of laboratories (Markham et al., 2010; Vandenberg et al., 2014a). Since there remain some laboratories that report being unable to control assay contamination (Churchwell et al., 2014), there is legitimate concern about the usefulness of data from studies in which there is inadequate information provided regarding assay performance or high background contamination is evident.

A significant problem encountered in reviewing publications related to these issues is that conclusions stated in abstracts are often discordant with data in the results section of articles, and those interested in these issues thus must carefully review the actual data before drawing conclusions. Similar problems were faced as debate occurred over the safety of the use of lead in products such as paint (Markowitz and Rosner, 2000) and the safety of second-hand tobacco smoke (Ong and Glantz, 2001).

In summary, contamination of assays from environmental sources should not be tolerated in studies of endocrine disrupting chemicals such as BPA. Statements that elimination of contaminating BPA cannot be achieved in biomonitoring studies are disputed by laboratories in round robin blinded studies reporting the ability to eliminate contamination. Assay development before sample analysis to accepted standards for clinical laboratories in which environmental sources of contamination are identified and eliminated needs to be a requirement for publication of data.

Competing financial interest declaration

FVS and WVW have no conflicts to declare.

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