BPA: have flawed analytical techniques compromised risk assessments?

Experimental and epidemiological studies provide compelling evidence of a causal link between increasing exposure to endocrine-disrupting chemicals (environmental contaminants with the potential to perturb the development and function of the endocrine system) and increases in non-communicable diseases, including most aspects of metabolic syndrome.[1] Indeed, increasing concern about the health effects posed by endocrine-disrupting chemicals has prompted two position statements from the Endocrine Society.[2]

By virtue of extensive research attention, bisphenol A (BPA) has become the so-called poster child for endocrine-disrupting chemicals. This high production (approximately 9 million tons per year) molecule is used in a wide range of consumer products (eg, plastics, epoxy resins, and thermal receipts), resulting in daily human exposure. Experimental data suggest BPA can affect a variety of endocrine signalling pathways, including those mediated by oestrogens, androgens, progestins, and thyroid hormone.[3] Exposure during gestation has been linked to changes in a wide array of developing tissues, with corresponding postnatal effects on growth, metabolism, behaviour, fertility, and cancer risk.[2,3] However, the health risks posed by BPA have remained controversial because adverse effects evident in experimental studies have not been corroborated by traditional regulatory guideline studies to determine toxicity. This lack of corroboration prompted a collaborative initiative, convened in 2012—the Consortium Linking Academic and Regulatory Insights on Toxicity of BPA (CLARITY-BPA). The study was designed to determine the basis of the discrepancy in findings by undertaking both guideline and experimental studies on the same set of animals. CLARITY data provide evidence of significant adverse effects at the lowest dose examined (2·5 µg/kg per day), far lower than the lowest observed adverse effect level (5000 µg/kg per day) used to establish the tolerable daily intake for BPA.[4] However, based on the assumption that human exposure to BPA is negligible, the US Food and Drug Administration (FDA) has not taken into account the adverse low dose effects in CLARITY data and many other studies.

Understanding the amount of BPA that enters the human body is essential for risk assessment. However, rapid metabolism of orally ingested BPA means accurate assessment in humans requires not only measurement of BPA but also of its major conjugated metabolites. The primary metabolite, BPA glucuronide, and secondary metabolite, BPA sulfate, are excreted in urine. Thus, biomonitoring of urine over time provides the best insight to human exposure to BPA. Although the parent compound, BPA, can be measured directly using authentic reference standards, standards for primary metabolites only became available in the early 2000s. Thus, previous biomonitoring studies have relied on indirect analytical methods to estimate metabolite levels. Specifically, a crude enzyme solution from the snail *Helix pomatia* is used to hydrolyse BPA glucuronide and BPA sulfate to unconjugated (free) BPA, and the free BPA is assayed by liquid chromatography-mass spectrometry (LC-MS) to obtain total BPA (free and conjugated) levels in urine. Development of direct methods with synthesised BPA glucuronide and BPA sulfate standards has simplified analysis, but early evidence suggested results obtained using direct and indirect techniques differ.[5,6] The present analysis was prompted by questions about the accuracy of historical data based on indirect analysis and the validity of the long-held assumption that human exposure to BPA is negligible.

Direct methods provide a tool to assess the accuracy of the widely used indirect methods. We first monitored the efficiency of deconjugation for indirect methods used by the FDA (appendix pp 1–2)[7] and the US Centers for Disease Control and Prevention (CDC; appendix).[8] We monitored the disappearance of BPA glucuronide when 50 ng/mL of the conjugate in synthetic human urine was subjected to enzymatic deconjugation by *H pomatia* glucuronidase type III (figure A). The FDA method, using a low concentration of enzyme, resulted in less than 10% deconjugation, while deconjugation was complete using the CDC method and a variation of the method we devised (UCSF method; appendix p 1).[9]

We next tested the assumption that deconjugation of BPA glucuronide by these different methods results in formation of similar amounts of free BPA. We measured free BPA concentrations obtained when 1, 10, 50, 500 ng/mL of BPA glucuronide was deconjugated using the two methods that yielded complete deconjugation (ie, the CDC method and our method variation). The lowest starting concentration (1 ng/mL BPA glucuronide) resulted in only two-thirds of the expected free BPA (figure B). Furthermore, the yield of free BPA diminished substantially with increasing starting concentrations of BPA glucuronide between 1 ng/mL and 10 ng/mL.

Because our data suggested BPA glucuronide can be efficiently deconjugated by the *H pomatia* enzyme but is converted to products other than free BPA, we created a database of 80 BPA metabolites predicted to result from hydroxylation, oxidation, sulfonation, or glucuronidation, and used it to query total ion chromatograms obtained

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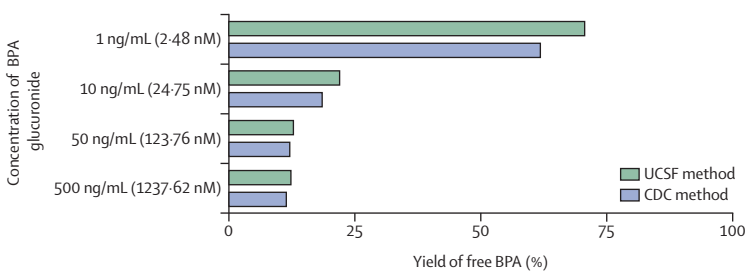
See Online for appendix

A

Method	*Helix pomatia* enzyme concentration	Incubation time (h)	BPA glucuronide remaining (ng/mL)	Percentage conversion
FDA	2 U	2	45·9	8·3%
CDC	46·3 U	24	<LOD	100%
UCSF	450 U	2	<LOD	100%

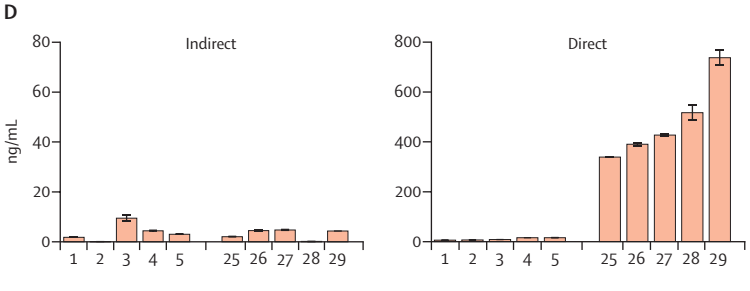
B



C

	Pregnant women (n=29)		Men (n=5)		Non-pregnant women (n=5)	
	Indirect	Direct	Indirect	Direct	Indirect	Direct
Mean (ng/mL)	4·21	129·45	4·38	41·12	1·94	260·86
Geometric mean (ng/mL)	2·77	51·99	0·82	38·96	0·85	170·12
Median (ng/mL)	3·58	33·58	0·87	45·20	0·66	235·95
SEM	0·67	34·58	2·95	6·30	0·98	97·08

D



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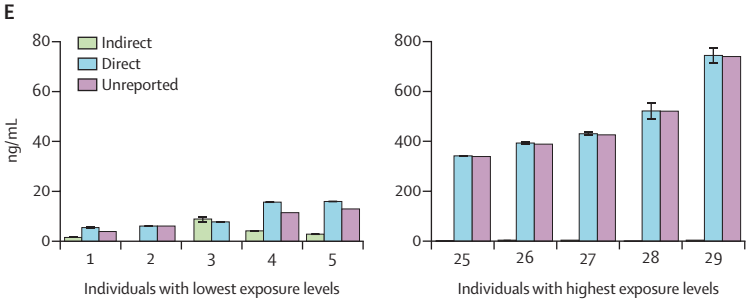

Figure: **Indirect analysis of BPA does not accurately measure concentrations in human urine**
(A) Deconjugation of 50 ng/mL of BPA glucuronide in synthetic urine using *Helix pomatia* glucuronidase type III method. (B) Yield of free BPA via different methods of deconjugation. (C) Concentration of BPA in urine samples from pregnant women, men, and non-pregnant women by direct and indirect methods. Direct analysis resulted in higher mean values, reflecting the inability of the indirect method to accurately measure BPA metabolites (appendix p 3). (D) Subset of urine samples from pregnant women with the lowest (1–5) and highest (22–29) results with increasing BPA exposure. Errors bars show SD (E) Amount of BPA reported by indirect and direct methods, and of BPA not reported by indirect method in the subset of urine samples from pregnant women. Error bars show SD. A similar trend is seem in the small sample of men and non-pregnant women (appendix p 3). BPA=bisphenol A. FDA=US Food and Drug Administration. CDC=US Centers for Disease Control and Prevention. UCSF=University of California San Francisco. LOD=limit of detection.

from running aliquots of reaction products from urine deconjugation in liquid chromatography-quadrupole time-of-flight mass spectrometry. This platform facilitates screening for additional products because it calculates the exact mass of candidate products using their chemical formula. However, we did not find a consistent match to any candidate compound (data not shown).

The conclusion from our analyses is that previous studies using indirect techniques requiring deconjugation underestimated actual human levels of BPA. Accordingly, we undertook a comparative analysis of 29 urine samples from pregnant women in their second trimester using both indirect and direct methods (figure C; appendix pp 1–2). Using the direct method, we obtained a geometric mean for these urine samples of 51·99 ng/mL total BPA, 44-times higher than the latest geometric mean for adults in the USA reported by the National Health and Nutrition Examination Survey (NHANES).[10] By contrast, the indirect method yielded a geometric mean for total BPA of 2·77 ng/mL, nearly 19-times lower than the direct method but in good agreement with NHANES data. Importantly, disparity between indirect and direct results increased substantially as exposure increased (figure D and E). Because pregnancy induces physiological changes that might affect metabolism of BPA, we also compared indirect and direct measurements on urine samples from five adult men and five non-pregnant women (figure C). The same trends were evident in this small sample (appendix p 3) and, as in the samples from the pregnant women, the difference in BPA levels was a reflection of the inability of the indirect method to accurately measure the levels of metabolites of BPA.

Indirect analytical methods have provided the bulk of data on human BPA levels. To our knowledge, our data provide the first evidence that this is a flawed analytical tool

for measurement of BPA levels. Surprisingly, despite its widespread use, the efficiency of conversion of BPA glucuronide to free BPA using enzyme deconjugation with *H pomatia* glucuronidase has never been assessed. Our findings of both a low conversion rate in spiked synthetic urine samples and significantly lower BPA levels obtained by indirect analysis of samples from both non-pregnant and pregnant women and men challenges the widely held assumption that human levels of BPA can be assessed using enzymatic deconjugation. Importantly, because estimates of human exposure have been based almost exclusively on data from indirect methods, these findings provide compelling evidence that human exposure to BPA is far higher than has been assumed previously. Because negligible exposure levels have been a cornerstone of regulatory decisions, including the FDA conclusion that BPA poses little health risk, the present data raise urgent concerns that risks to human health have also been dramatically underestimated. In addition to offering a new dimension to the dispute over the safety of BPA and structural analogues that have replaced it, our findings have broader implications. Currently, measurements of a wide range of chemicals, including replacement bisphenols, other environmental phenols (eg, parabens, benzophenone, triclosan), and phthalate metabolites rely on indirect methods.[8] Thus, the problem identified here for BPA might extend to other environmental contaminants. Accordingly, determining the extent to which errors in estimation of human exposure extend to other chemical contaminants that are measured with indirect methods is essential. This effort will require a coordinated approach to provide funding for the synthesis of standards for individual chemicals and their metabolites for accurate measurement of chemicals of concern to the health of humans and wildlife.

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Exendin-based glucagon-like peptide-1 receptor agonists and anaphylactic reactions: a pharmacovigilance analysis

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Glucagon-like peptide-1 (GLP-1) receptor agonists are commonly used as second-to-third line drugs in the treatment of type 2 diabetes.[1] They include exenatide and lixisenatide, which are structurally similar to exendin-4, a peptide found in the saliva of the Gila monster lizard (exendin-based GLP-1 receptor agonists). In contrast, liraglutide, dulaglutide, albiglutide, and semaglutide are structurally analogous to the human GLP-1 molecule (human-analogue GLP-1 receptor agonists).[2] Although potentially life-threatening allergies, such as anaphylactic reactions, have been documented with all GLP-1 receptor agonists,[2,3] whether differences exist between exendin-based and human-analogue GLP-1 receptor agonists is unclear.

We did a pharmacovigilance analysis using VigiBase, WHO's individual case safety report (ICSR) database.[4] The ICSRs are spontaneously generated accounts of adverse drug reactions, as reported by health professionals, consumers, or the drug manufacturers. The VigiBase database stores more than 21 million ICSRs from more than 130 countries, which are entered after rigorous quality checks and deduplication.[4]

For **Vigibase** see https://www.who-umc.org/vigibase/vigibase/

We considered ICSRs registered from Jan 1, 2008, to April 1, 2018, of all patients of at least 18 years of age. Cases were ICSRs containing all sub-terms retrieved using the MedDRA term "anaphylactic reaction", whereas non-cases were all other ICSRs retrieved during the same period. Using a case-non-case design, we did multivariate logistic regressions to estimate crude and adjusted reporting odds ratios of anaphylactic reactions, comparing exendin-based