

LETTER

Misuse of blood serum to assess exposure to bisphenol A and phthalates

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See related research by Sprague et al., <http://breast-cancer-research.com/content/15/3/R45>

We noted serious methodologic issues in the measurement of bisphenol A (BPA), phthalate diesters, and their metabolites in blood serum and other tissues, as reported in the recent *Breast Cancer Research* article by Sprague and colleagues [1]. Such measurements are analytically possible. However, for the reasons that follow, it is seldom possible to verify that serum concentrations of these compounds are valid measures of exposure.

BPA and phthalate diesters are non-persistent in the body; they metabolize quickly and, as a result, the levels of their polar, hydrophilic metabolites in blood can be several orders of magnitude lower than in urine (controlled human studies suggest 30- to 100-fold higher levels in urine than serum) [2-4]. Such low levels increase the possibility that contamination can obscure true exposures.

Extraneous sources of phthalate diesters include plastics, personal care and consumer products, and building furnishings [2,5]. Phthalate diesters derived externally can easily contaminate blood serum and other human matrices and overwhelm the very low levels in blood from daily exposures [2,4]. Hydrolytic enzymes are ubiquitous in blood and most other matrices (but not urine). These enzymes rapidly hydrolyze extraneous phthalate diesters to

their corresponding monoesters, beginning immediately after sample collection, and this can artificially elevate the concentrations of these hydrolytic monoesters, including monoethyl phthalate and mono-(2-ethylhexyl) phthalate. To the best of our knowledge, phthalate oxidative metabolites (such as mono-(2-ethyl-5-oxohexyl) phthalate) do not form as a result of recent external contamination. The oxidative metabolites were not measured in the study by Sprague and colleagues [1]. Therefore, in blood, saliva, or tissues other than urine, only the phthalate oxidative metabolites (not the phthalate diesters or the hydrolytic monoesters) are valid exposure biomarkers.

BPA also has extraneous sources such as plastics [3,5]. There are currently no comparable oxidative metabolites that can exclude recent contamination, but conjugated BPA (not 'free' BPA) is the most valid exposure biomarker and is not present to any significant degree in biological samples other than urine. For these reasons, urine is the best matrix for epidemiological assessment of exposure to BPA, phthalates, and other polar, non-persistent chemicals to whose exposures can be episodic in nature.

Moreover, because both BPA and the phthalate diesters have very short half-lives (regardless of the biomarker used),

Authors' response

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We thank Calafat and colleagues for their discussion of BPA and phthalate measurement. Their comments on the challenges of exposure assessment for rapidly metabolized chemicals echo the statements in our discussion. They

further emphasize the susceptibility of blood serum measures to contamination. We took several rigorous steps to avoid plasticizer contamination, including the use of glass labware, preparation steps to remove potential contaminants from labware, handling of labware and specimens in biosafety cabinets, and the assessment of method blanks as recommended in the literature [6]. Assessment of method blanks showed that iatrogenic contamination was lower than the limits of detection for BPA and phthalates.

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great care must be taken to ensure that measurements represent the daily habits of research subjects versus brief exposures from iatrogenic sources, such as collection devices, clinical apparatus, and tubing from medical procedures [2,5].

Non-differential measurement error due to contamination would tend to attenuate the observed associations between the measured chemicals and mammographic breast density. However, for contamination to explain the observed positive associations of BPA and monoethyl phthalate with breast density, the samples from patients with high breast density would need to be more greatly contaminated. The conditions for introducing a positive bias by sample contamination are not readily apparent.

Nevertheless, we agree that accurate biomarker assessment is essential for elucidating the role of environmental chemicals in the etiology of breast cancer. Future studies that measure metabolites less susceptible to contamination, use a variety of specimen types (including urine), and assess exposure levels at multiple points in time are needed.

Abbreviation

BPA: Bisphenol A.

Competing interests

The authors declare that they have no competing interests. The findings and conclusions in this letter are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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