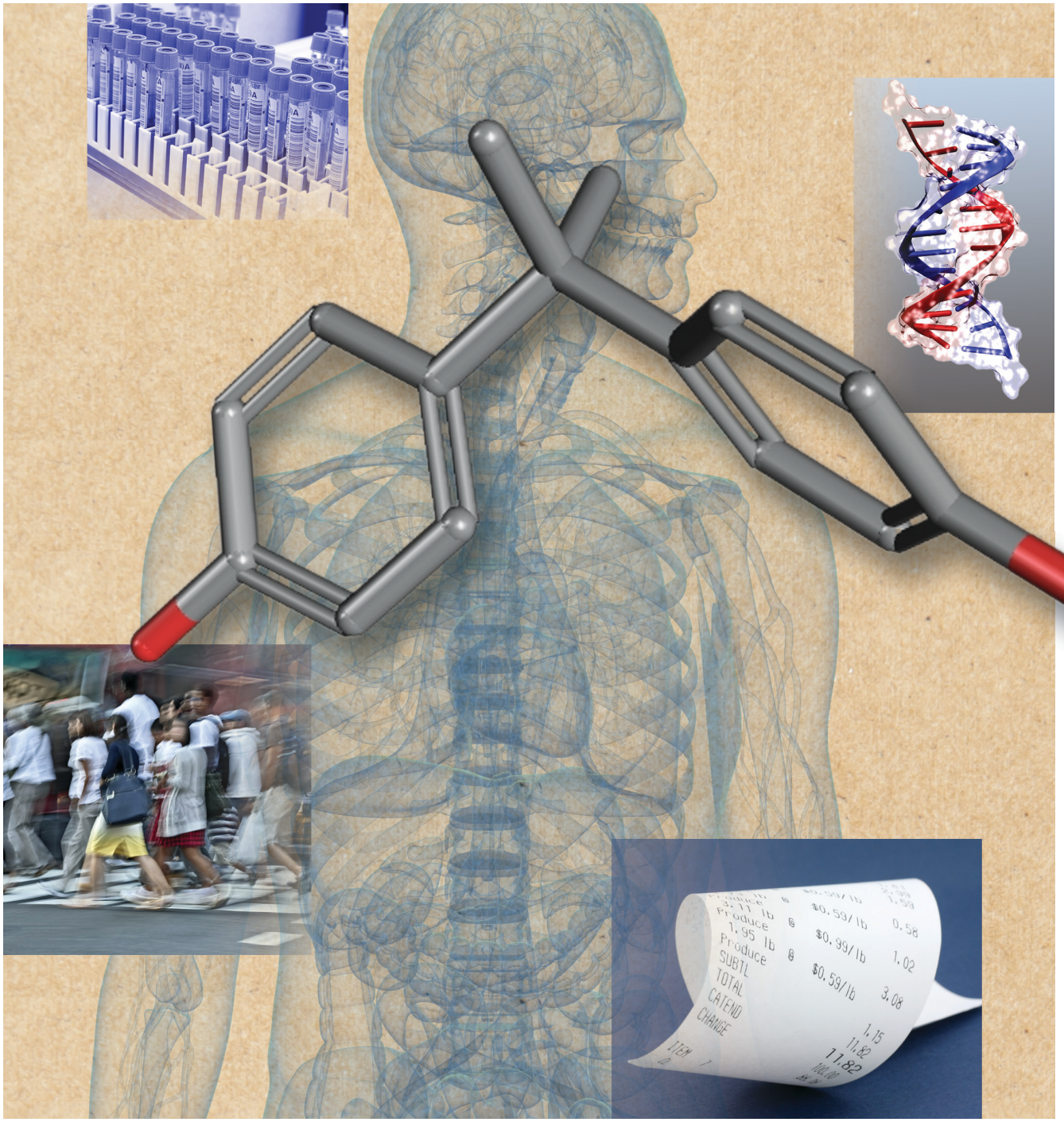
ENVIRONMENTAL HEALTH PERSPECTIVES
ehp

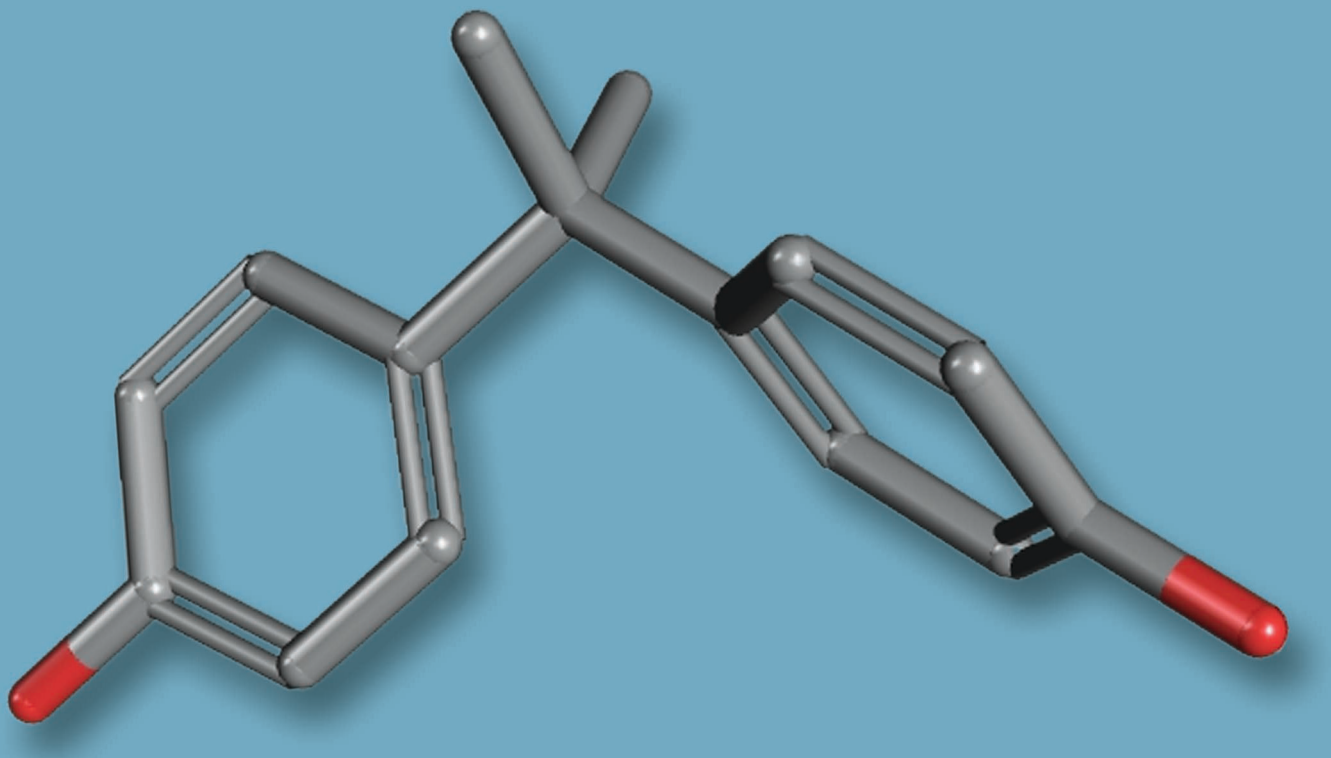
COLLECTION

Bisphenol A

www.ehponline.org

January 2007–December 2011





What is bisphenol A?

Bisphenol A (BPA) is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins.

Where is BPA found?

Polycarbonate plastics have many applications including use in some food and drink packaging, e.g., water and infant bottles, compact discs, impact-resistant safety equipment, and medical devices. Epoxy resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some dental sealants and composites may also contribute to BPA exposure.

How does BPA get into the body?

The primary source of exposure to BPA for most people is through the diet. While air, dust, and water are other possible sources of exposure, BPA in food and beverages accounts for the majority of daily human exposure.

Bisphenol A can leach into food from the protective internal epoxy resin coatings of canned foods and from consumer products such as polycarbonate tableware, food storage containers, water bottles, and baby bottles. The degree to which BPA leaches from polycarbonate bottles into liquid may depend more on the temperature of the liquid or bottle, than the age of the container. BPA can also be found in breast milk.

Why are people concerned about BPA?

One reason people may be concerned about BPA is because human exposure to BPA is widespread. The 2003–2004 National Health and Nutrition Examination Survey (NHANES III) conducted by the Centers for Disease Control and Prevention (CDC) found detectable levels of BPA in 93% of 2,517 urine samples from people six years and older. The CDC NHANES data are considered representative of exposures in the United States. Another reason for concern, especially for parents, may be because some animal studies report effects in fetuses and newborns exposed to BPA.

Reprinted from: National Toxicology Program. Since You Asked—Bisphenol A (BPA). Questions and Answers about Bisphenol A. Updated 20 Feb 2012.

ENVIRONMENTAL HEALTH PERSPECTIVES
ehp COLLECTION

Bisphenol A

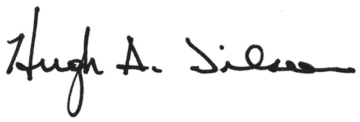
January 2007–December 2011

PREFACE

Bisphenol A (BPA) is a component of polycarbonate plastics and epoxy resins and is one of the highest-volume chemicals produced globally. Researchers have found this chemical in many places in the environment, including the drinking water, air, food, and house dust. Many studies also have shown that BPA has estrogenic activity in several *in vitro* and *in vivo* preparations. The possibility that exposure to BPA might be associated with adverse health effects in humans has prompted safety evaluations by regulatory agencies in the United States and the European Union. Both the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have declared that exposure to BPA is safe at current levels. However, these decisions have been challenged by some members of the environmental health science community on the basis that hundreds of potentially relevant studies were summarily excluded from the risk assessment.

It is clear that BPA continues to be a topic of interest to the toxicology and regulatory communities. For this reason, *EHP* has developed this collection of BPA-related commentaries, reviews, and research articles published in the journal between 2007 and 2011; we capped the collection at the past five years for the sake of timeliness and maneuverability. The collection is divided into four sections: Toxicology, Epidemiology, Exposure, and Regulatory. Abstracts are provided for each article as well as a hyperlink to the full article on *EHP*'s website (http://www.ehponline.org). Readers can also search the website for additional information on BPA, including editorials, correspondence, and news.

EHP is committed to providing the latest cutting-edge research on the effects of environmental factors on human health. We hope this BPA collection will be of use to scientists, regulators, and decision makers. As a service to our readers and to public health professionals, *EHP* intends to build a comprehensive, searchable collection of research and news articles about BPA that will be updated on an annual basis.



Hugh A. Tilson, PhD
Editor-in-Chief
Environmental Health Perspectives

BPA TABLE OF CONTENTS

TOXICOLOGY

EPIDEMIOLOGY

EXPOSURE

REGULATORY

Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats

Milena Durando, Laura Kass, Julio Piva, Carlos Sonnenschein, Ana M. Soto, Enrique H. Luque, Mónica Muñoz-de-Toro

2007 115(1):80–86 | http://dx.doi.org/10.1289/ehp.9282

Background: Humans are routinely exposed to bisphenol A (BPA), an estrogenic compound that leaches from dental materials, food and beverage containers, and other consumer products. Prenatal exposure to BPA has produced long-lasting and profound effects on rodent hormone-dependent tissues that are manifested 1–6 months after the end of exposure.

Objective: The aim of the present work was to examine whether *in utero* exposure to BPA alters mammary gland development and increases its susceptibility to the carcinogen *N*-nitroso-*N*-methylurea (NMU).

Methods: Pregnant Wistar rats were exposed to BPA (25 µg/kg body weight per day) or to vehicle. Female offspring were sacrificed on postnatal day (PND) 30, 50, 110, or 180. On PND50 a group of rats received a single subcarcinogenic dose of NMU (25 mg/kg) and they were sacrificed on either PND110 or PND180.

Results: At puberty, animals exposed prenatally to BPA showed an increased proliferation/apoptosis ratio in both the epithelial and stromal compartments. During adulthood (PND110 and PND180), BPA-exposed animals showed an increased number of hyperplastic ducts and augmented stromal nuclear density. Moreover, the stroma associated with hyperplastic ducts showed signs of desmoplasia and contained an increased number of mast cells, suggesting a heightened risk of neoplastic transformation. Administration of a subcarcinogenic dose of NMU to animals exposed prenatally to BPA increased the percentage of hyperplastic ducts and induced the development of neoplastic lesions.

Conclusions: Our results demonstrate that the prenatal exposure to low doses of BPA perturbs mammary gland histoarchitecture and increases the carcinogenic susceptibility to a chemical challenge administered 50 days after the end of BPA exposure.

Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains

Perinaaz R. Wadia, Laura N. Vandenberg, Cheryl M. Schaeberle, Beverly S. Rubin, Carlos Sonnenschein, Ana M. Soto

2007 115(4):592–598 | http://dx.doi.org/10.1289/ehp.9640

Background: Studies of low-dose effects of xenoestrogens have yielded conflicting results that may be attributed to differences in estrogen sensitivity between the rodent strains examined. Perinatal exposure of CD-1 mice to low doses of the xenoestrogen bisphenol A (BPA) alters peripubertal mammary gland development. Future studies to assess the role of estrogen receptors as mediators of BPA action require estrogen receptor knock-out mice that were generated on a C57Bl6 background. The sensitivity of the C57Bl6 strain to estradiol and BPA is unknown.

Objectives: In the present study we examined whether the mammary glands of CD-1 and C57Bl6 mice exhibited similar responses to 17β-estradiol (E_2) and whether perinatal exposure to BPA equally enhanced sensitivity of the mammary glands to E_2 at puberty.

Methods: Immature mice were ovariectomized and treated for 10 days with one of eight doses of E_2. Morphological mammary gland parameters were examined to identify doses producing half-maximal effects. Mice were exposed perinatally to 0 or 250 ng BPA/kg body weight (bw)/day from gestational day 8 until postnatal day (PND) 2. On PND25, female offspring were ovariectomized and given an estrogen challenge of 0, 0.5, or 1 µg E_2/kg bw/day for 10 days. Morphometric parameters of the mammary gland were compared between strains.

Results: Both strains exhibited similar responses to E_2. Perinatal BPA exposure altered responses to E_2 at puberty for several parameters in both strains, although the effect in CD-1 was slightly more pronounced.

Conclusion: Both mouse strains provide adequate models for the study of perinatal exposure to xenoestrogens.

Estradiol and bisphenol A stimulate androgen receptor and estrogen receptor gene expression in fetal mouse prostate mesenchyme cells

Catherine A. Richter, Julia A. Taylor, Rachel L. Ruhlen, Wade V. Welshons, Frederick S. vom Saal

2007 115(6):902–908 | http://dx.doi.org/10.1289/ehp.9804

Background: Hormonal alterations during development have lifelong effects on the prostate gland. Endogenous estrogens, including 17β-estradiol (E_2), and synthetic estrogenic endocrine disruptors, such as bisphenol A (BPA), have similar effects on prostate development. Increasing exposure to estrogens within the low-dose, physiologic range results in permanent increases in the size and androgen responsiveness of the prostate, whereas exposure within the high-dose, pharmacologic range has the opposite effects.

Objective: We tested the hypothesis that the low-dose effects of estrogens on the developing prostate are associated with increased expression of androgen receptor (*Ar*) and estrogen receptor 1 (α) (*Esr1*) genes in mesenchyme cells.

Methods: Ar and Esr1 mRNA levels were quantified in primary cultures of fetal mouse prostate mesenchyme cells treated with E_2 and BPA.

Discussion: Ar and Esr1 mRNA expression increased in response to E_2, with thresholds of 0.001 and 0.037 nM, respectively; and in response to BPA, with a threshold of 1 nM for both mRNAs. We did not observe the expected inhibition of Ar mRNA expression by pharmacologic levels of E_2 relative to unexposed cells.

Conclusions: The observed induction of gene expression occurred at concentrations within the range of free E_2 previously shown to permanently increase prostate size, thus supporting the involvement of direct effects of estrogens on gene expression in prostate mesenchyme. The effects of BPA occurred within the range of concentrations currently measured in human serum, demonstrating the vulnerability of developing tissues to xenoestrogens.

Unique bisphenol A transcriptome in prostate cancer: novel effects on ERβ expression that correspond to androgen receptor mutation status

Janet K. Hess-Wilson, Siobhan L. Webb, Hannah K. Daly, Yuet-Kin Leung, Joanne Boldison, Clay E.S. Comstock, Maureen A. Sartor, Shuk-Mei Ho, Karen E. Knudsen

2007 115(11):1646–1653 | http://dx.doi.org/10.1289/ehp.10283

Background: Prostatic adenocarcinomas are dependent on androgen receptor (AR) activity for growth and progression, and therapy for disseminated disease depends on ablation of AR activity. Recurrent tumors ultimately arise wherein AR has been re-activated. One mechanism of AR restoration is via somatic mutation, wherein cells containing mutant receptors become susceptible to activation by alternative ligands, including bisphenol A (BPA). In tumors with specific AR mutations, BPA promotes therapeutic bypass, suggesting significant negative impact to the clinical management of prostate cancer.

Objective: Our goal was to determine the mechanism of BPA action in cancer cells carrying BPA-responsive AR mutants.

Methods: The molecular signature of BPA activity in prostate cancer cells harboring mutant AR was delineated via genetic microarray analysis. Specificity of BPA action was assessed by comparison with the molecular signature elicited by dihydrotestosterone (DHT).

Results: BPA and DHT elicited distinct transcriptional signatures in prostate cancer cells expressing the BPA-responsive mutant AR-T877A. BPA dramatically attenuated estrogen receptor beta (ERβ) expression; this finding was specific to prostate tumor cells in which BPA induces cellular proliferation.

Conclusions: BPA induces a distinct gene expression signature in prostate cancer cells expressing somatic AR mutation, and a major molecular consequence of BPA action is down-regulation of ERβ. Since ERβ functions to antagonize AR function and AR-dependent proliferation, these findings reveal a novel mechanism by which BPA likely regulates cellular proliferation. Future investigation directed at dissecting the importance of ERβ in the proliferative response to BPA will establish the contribution of this event to adverse effects associated with human exposure.

Endocrine-disrupting potential of bisphenol A, bisphenol A dimethacrylate, 4-*n*-nonylphenol, and 4-*n*-octylphenol *in vitro*: new data and a brief review

Eva C. Bonefeld-Jørgensen, Manhai Long, Marlene V. Hofmeister, Anne Marie Vinggaard

2007 115 Suppl 1:69–76 | http://dx.doi.org/10.1289/ehp.9368

Background: An array of environmental compounds is known to possess endocrine disruption (ED) potentials. Bisphenol A (BPA) and bisphenol A dimethacrylate (BPA-DM) are monomers used to a high extent in the plastic industry and as dental sealants. Alkylphenols such as 4-*n*-nonylphenol (nNP) and 4-*n*-octylphenol (nOP) are widely used as surfactants.

Objectives: We investigated the effect *in vitro* of these four compounds on four key cell mechanisms including transactivation of *a*) the human estrogen receptor (ER), *b*) the human androgen receptor (AR), *c*) the aryl hydrocarbon receptor (AhR), and *d*) aromatase activity.

Results: All four compounds inhibited aromatase activity and were agonists and antagonists of ER and AR, respectively. nNP increased AhR activity concentration-dependently and further increased the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin AhR action. nOP caused dual responses with a weak increased and a decreased AhR activity at lower (10^{-8} M) and higher concentrations (10^{-5}–10^{-4} M), respectively. AhR activity was inhibited with BPA (10^{-5}–10^{-4} M) and weakly increased with BPA-DM (10^{-5} M), respectively. nNP showed the highest relative potency (REP) compared with the respective controls in the ER, AhR, and aromatase assays, whereas similar REP was observed for the four chemicals in the AR assay.

Conclusion: Our *in vitro* data clearly indicate that the four industrial compounds have ED potentials and that the effects can be mediated via several cellular pathways, including the two sex steroid hormone receptors (ER and AR), aromatase activity converting testosterone to estrogen, and AhR; AhR is involved in syntheses of steroids and metabolism of steroids and xenobiotic compounds.

Mixtures of estrogenic chemicals enhance vitellogenic response in sea bass

Ana D. Correia, Sandro Freitas, Martin Scholze, José F. Goncalves, Petra Booij, Marja H. Lamoree, Evaristo Mañanós, Maria A. Reis-Henriques

2007 115 Suppl 1:115–121 | http://dx.doi.org/10.1289/ehp.9359

Background: The potential impact of natural and synthetic estrogens on aquatic ecosystems has attracted considerable attention because it is currently accepted that their joint effects are more severe when they are present in mixtures. Although it is well-known that they occur as mixtures in the marine environment, there is little information about the combined effects of estrogenic chemicals on marine biota.

Objective: In 14-day tests with juvenile sea bass, we analyzed singly and in combination the estrogenic activity of estradiol (E_2), ethynylestradiol (EE_2), and bisphenol A (BPA) using vitellogenin induction as an end point.

Methods: Fish were exposed to each compound, and on the basis of these concentration–response data, we predicted mixture effects by applying the model of concentration addition. The mixtures were tested using a fixed-ratio design, and the resulting mixture effects were compared to the predictions.

Results: EE_2 was the most potent steroid, with an EC_{50} (median effective concentration) of 0.029 µg/L, 3.6 times more potent than E_2 (EC_{50} = 0.104 µg/L); BPA was the least potent chemical, with an EC_{50} of 77.94 µg/L. The comparative assessment yielded a good agreement between observed and predicted mixture effects.

Conclusions: This study demonstrates the potential hazard of these compounds to seawater life by their ability to act together in an additive manner. It provides evidence that concentration addition can be used as a predictive tool for assessing the combined effects of estrogenic chemicals in marine ecosystems.

Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-γ

Hiroyuki Okada, Takatoshi Tokunaga, Xiaohui Liu, Sayaka Takayanagi, Ayami Matsushima, Yasuyuki Shimohigashi

2008 116(1):32–38 | http://dx.doi.org/10.1289/ehp.10587

Background: Various lines of evidence have shown that bisphenol A [BPA; HO-C_6H_4-$C(CH_3)_2$-C_6H_4-OH] acts as an endocrine disruptor when present in very low doses. We have recently demonstrated that BPA binds strongly to human estrogen-related receptor-γ (ERR-γ) in a binding assay using [^{3}H]4-hydroxytamoxifen ([^{3}H]4-OHT). We also demonstrated that BPA inhibits the deactivation activity of 4-OHT.

Objectives: In the present study, we intended to obtain direct evidence that BPA interacts with ERR-γ as a strong binder, and also to clarify the structural requirements of BPA for its binding to ERR-γ.

Methods: We examined [^{3}H]BPA in the saturation binding assay using the ligand binding domain of ERR-γ and analyzed the result using Scatchard plot analysis. A number of BPA derivatives were tested in the competitive binding assay using [^{3}H]BPA as a tracer and in the luciferase reporter gene assay.

Results: [^{3}H]BPA showed a K_D of 5.50 nM at a B_{max} of 14.4 nmol/mg. When we examined BPA derivatives to evaluate the structural essentials required for the binding of BPA to ERR-γ, we found that only one of the two phenol-hydroxyl groups was essential for the full binding. The maximal activity was attained when one of the methyl groups was removed. All of the potent BPA derivatives retained a high constitutive basal activity of ERR-γ in the luciferase reporter gene assay and exhibited a distinct inhibitory activity against 4-OHT.

Conclusion: These results indicate that the phenol derivatives are potent candidates for the endocrine disruptor that binds to ERR-γ.

Exposure to bisphenol A prenatally or in adulthood promotes T_H2 cytokine production associated with reduction of $CD4^+CD25^+$ regulatory T cells

Huimin Yan, Masaya Takamoto, Kazuo Sugane

2008 116(4):514–519 | http://dx.doi.org/10.1289/ehp.10829

Background: Bisphenol A (BPA) is a widespread endocrine-disrupting chemical that can affect humans and animals.

Objectives: We investigated the effects of adult or prenatal exposure to BPA on T-helper (T_H)1/T_H2 immune responses and the mechanisms underlying these effects.

Methods: To evaluate the effects of exposure to BPA in adulthood, male *Leishmania major*–susceptible BALB/c and –resistant C57BL/6 mice were subcutaneously injected with 0.625, 1.25, 2.5, and 5 μmol BPA 1 week before being infected with *L. major*. To evaluate prenatal exposure, female mice were given BPA-containing drinking water at concentrations of 1, 10, and 100 nM for 2 weeks, then mated, and given BPA for another week. Male 10-week-old offspring were infected with *L. major*. Footpad swelling was assessed as a measure of the course of infection.

Results: Mice exposed to BPA prenatally or in adulthood showed a dose-dependent increase in footpad swelling after being infected with L. major. Exposure to BPA in adulthood significantly promoted antigen-stimulated production of interleukin (IL)-4, IL-10, and IL-13 but not interferon-γ (IFN-γ). However, mice prenatally exposed to BPA showed increased production of not only IL-4 but also IFN-γ. The percentages of $CD4^+CD25^+$ cells were decreased in mice exposed to BPA either prenatally or in adulthood. Effects of prenatal BPA exposure were far more pronounced than effects of exposure in adulthood.

Conclusion: BPA promotes the development of T_H2 cells in adulthood and both T_H1 and T_H2 cells in prenatal stages by reducing the number of regulatory T cells.

Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes

Eric R. Hugo, Terry D. Brandebourg, Jessica G. Woo, Jean Loftus, J. Wesley Alexander, Nira Ben-Jonathan

2008 116(12):1642–1647 | http://dx.doi.org/10.1289/ehp.11537

Background: The incidence of obesity has risen dramatically over the last few decades. This epidemic may be affected by exposure to xenobiotic chemicals. Bisphenol A (BPA), an endocrine disruptor, is detectable at nanomolar levels in human serum worldwide. Adiponectin is an adipocyte-specific hormone that increases insulin sensitivity and reduces tissue inflammation. Thus, any factor that suppresses adiponectin release could lead to insulin resistance and increased susceptibility to obesity-associated diseases.

Objectives: In this study we aimed to compare *a*) the effects of low doses of BPA and estradiol (E_2) on adiponectin secretion from human breast, subcutaneous, and visceral adipose explants and mature adipocytes, and *b*) expression of putative estrogen and estrogen-related receptors (ERRs) in these tissues.

Methods: We determined adiponectin levels in conditioned media from adipose explants or adipocytes by enzyme-linked immunosorbant assay. We determined expression of estrogen receptors (ERs) α and β, G-protein–coupled receptor 30 (GPR30), and ERRs α, β, and γ by quantitative real-time polymerase chain reaction.

Results: BPA at 0.1 and 1 nM doses suppressed adiponectin release from all adipose depots examined. Despite substantial variability among patients, BPA was as effective, and often more effective, than equimolar concentrations of E_2. Adipose tissue expresses similar mRNA levels of *ER*α, *ER*β, and *ERR*γ, and 20- to 30-fold lower levels of *GPR*30, *ERR*α, and *ERR*β.

Conclusions: BPA at environmentally relevant doses inhibits the release of a key adipokine that protects humans from metabolic syndrome. The mechanism by which BPA suppresses adiponectin and the receptors involved remains to be determined.

Bisphenol A at low nanomolar doses confers chemoresistance in estrogen receptor-α–positive and –negative breast cancer cells

Elizabeth W. LaPensee, Traci R. Tuttle, Sejal R. Fox, Nira Ben-Jonathan

2009 117(2):175–180 | http://dx.doi.org/10.1289/ehp.11788

Background: Resistance to chemotherapy is a major problem facing breast cancer patients, and identifying potential contributors to chemoresistance is a critical area of research. Bisphenol A (BPA) has long been suspected to promote carcinogenesis, but the high doses of BPA used in many studies generated conflicting results. In addition, the mechanism by which BPA exerts its biological actions is unclear. Although estrogen has been shown to antagonize anticancer drugs, the role of BPA in chemoresistance has not been examined.

Objective: The objective of our study was to determine whether BPA at low nanomolar concentrations opposes the action of doxorubicin, cisplatin, and vinblastine in the estrogen receptor-α (ERα)-positive T47D and the ERα-negative MDA-MB-468 breast cancer cells.

Methods: We determined the responsiveness of cells to anticancer drugs and BPA using the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) cytotoxicity assay. Specific ERα and ERβ inhibitors and real-time polymerase chain reaction were used to identify potential receptor(s) that mediate the actions of BPA. Expression of antiapoptotic proteins was assessed by Western blotting.

Results: BPA antagonizes the cytotoxicity of multiple chemotherapeutic agents in both ERα-positive and -negative breast cancer cells independent of the classical ERs. Both cell types express alternative ERs, including G-protein–coupled receptor 30 (GPR30) and members of the estrogen-related receptor family. Increased expression of antiapoptotic proteins is a potential mechanism by which BPA exerts its anticytotoxic effects.

Conclusions: BPA at environmentally relevant doses reduces the efficacy of chemotherapeutic agents. These data provide considerable support to the accumulating evidence that BPA is hazardous to human health.

Alkylphenol xenoestrogens with varying carbon chain lengths differentially and potently activate signaling and functional responses in GH_3/B_6/F10 somatomammotropes

Mikhail Y. Kochukov, Yow-Jiun Jeng, Cheryl S. Watson

2009 117(5):723–730 | http://dx.doi.org/10.1289/ehp.0800182

Background: Alkylphenols varying in their side-chain lengths [ethyl-, propyl-, octyl-, and nonylphenol (EP, PP, OP, and NP, respectively)] and bisphenol A (BPA) represent a large group of structurally related xenoestrogens that have endocrine-disruptive effects. Their rapid nongenomic effects that depend on structure for cell signaling and resulting functions are unknown.

Objective: We compared nongenomic estrogenic activities of alkylphenols with BPA and 17β-estradiol (E_2) in membrane estrogen receptor-α–enriched GH_3/B_6/F10 pituitary tumor cells. These actions included calcium (Ca) signaling, prolactin (PRL) release, extracellular-regulated kinase (ERK) phosphorylation, and cell proliferation.

Methods: We imaged Ca using fura-2, measured PRL release via radioimmunoassay, detected ERK phosphorylation by fixed cell immunoassay, and estimated cell number using the crystal violet assay.

Results: All compounds caused increases in Ca oscillation frequency and intracellular Ca volume at 100 fM to 1 nM concentrations, although long-chain alkylphenols were most effective. All estrogens caused rapid PRL release at concentrations as low as 1 fM to 10 pM; the potency of EP, PP, and NP exceeded that of E_2. All compounds at 1 nM produced similar increases in ERK phosphorylation, causing rapid peaks at 2.5–5 min, followed by inactivation and additional 60-min peaks (except for BPA). Dose–response patterns of ERK activation at 5 min were similar for E_2, BPA, and PP, whereas EP caused larger effects. Only E_2 and NP increased cell number. Some rapid estrogenic responses showed correlations with the hydrophobicity of estrogenic molecules; the more hydrophobic OP and NP were superior at Ca and cell proliferation responses, whereas the less hydrophobic EP and PP were better at ERK activations.

Conclusions: Alkylphenols are potent estrogens in evoking these nongenomic responses contributing to complex functions; their hydrophobicity can largely predict these behaviors.

Neonatal exposure to bisphenol A alters reproductive parameters and gonadotropin releasing hormone signaling in female rats

Marina Fernández, Maria Bianchi, Victoria Lux-Lantos, Carlos Libertun

2009 117(5):757–762 | http://dx.doi.org/10.1289/ehp.0800267

Background: Bisphenol A (BPA) is a component of polycarbonate plastics, epoxy resins, and polystyrene and is found in many products. Several reports have revealed potent *in vivo* effects because BPA acts as an estrogen agonist and/or antagonist and as an androgen and thyroid hormone antagonist.

Objective: We analyzed the effects of neonatal exposure to BPA on the reproductive axis of female Sprague-Dawley rats.

Methods: Female rats were injected subcutaneusly, daily, from postnatal day 1 (PND1) to PND10 with BPA [500 µg/50 µL (high) or 50 µg/50 µL (low)] in castor oil or with castor oil vehicle alone. We studied body weight and age at vaginal opening, estrous cycles, and pituitary hormone release *in vivo* and *in vitro*, as well as gonadotropin-releasing hormone (GnRH) pulsatility at PND13 and in adults. We also analyzed two GnRH-activated signaling pathways in the adults: inositol-triphosphate (IP_3), and extracellular signal-regulated $kinase_{1/2}$ ($ERK_{1/2}$).

Results: Exposure to BPA altered pituitary function in infantile rats, lowering basal and GnRH-induced luteinizing hormone (LH) and increasing GnRH pulsatility. BPA dose-dependently accelerated puberty onset and altered estrous cyclicity, with the high dose causing permanent estrus. In adults treated neonatally with BPA, GnRH-induced LH secretion *in vivo* was decreased and GnRH pulsatility remained disrupted. *In vitro*, pituitary cells from animals treated with BPA showed lower basal LH and dose-dependently affected GnRH-induced IP_3 formation; the high dose also impaired GnRH-induced LH secretion. Both doses altered $ERK_{1/2}$ activation.

Conclusions: Neonatal exposure to BPA altered reproductive parameters and hypothalamic–pituitary function in female rats. To our knowledge, these results demonstrate for the first time that neonatal *in vivo* BPA permanently affects GnRH pulsatility and pituitary GnRH signaling.

Differential regulation of dopamine transporter function and location by low concentrations of environmental estrogens and 17β-estradiol

Rebecca A. Alyea, Cheryl S. Watson

2009 117(5):778–783 | http://dx.doi.org/10.1289/ehp.0800026

Background: The effects of 17β-estradiol (E_2) and xenoestrogens (XEs) on dopamine transport may have important implications for the increased incidence of neurologic disorders, especially in women during life stages characterized by frequent hormonal fluctuations.

Objective: We examined low concentrations of XEs [dieldrin, endosulfan, *o´, p´*-dichlorodiphenyl-ethylene (DDE), nonylphenol (NP), and bisphenol A (BPA)] for nongenomic actions via action of membrane estrogen receptors (ERs).

Methods: We measured activity of the dopamine transporter (DAT) by the efflux of ^{3}H-dopamine in nontransfected nerve growth factor–differentiated PC12 rat pheochromocytoma cells expressing membrane DAT, ER-α, ER-β, and G-protein–coupled receptor 30. We used a plate immunoassay to monitor trafficking of these proteins.

Results: All compounds at 1 nM either caused efflux or inhibited efflux, or both; each compound evoked a distinct oscillatory pattern. At optimal times for each effect, we examined different concentrations of XEs. All XEs were active at some concentration < 10 nM, and dose responses were all nonmonotonic. For example, 10^{-14} to 10^{-11} M DDE caused significant efflux inhibition, whereas NP and BPA enhanced or inhibited efflux at several concentrations. We also measured the effects of E_2/XE combinations; DDE potentiated E_2-mediated dopamine efflux, whereas BPA inhibited it. In E_2-induced efflux, 15% more ER-α trafficked to the membrane, whereas ER-β waned; during BPA-induced efflux, 20% more DAT was trafficked to the plasma membrane.

Conclusions: Low levels of environmental estrogen contaminants acting as endocrine disruptors via membrane ERs can alter dopamine efflux temporal patterning and the trafficking of DAT and membrane ERs, providing a cellular mechanism that could explain the disruption of physiologic neurotransmitter function.

Increased expression of histone proteins during estrogen-mediated cell proliferation

Zheying Zhu, Robert J. Edwards, Alan R. Boobis

117(6):928-34 2009 | http://dx.doi.org/10.1289/ehp.0800109

Background: There is concern about the potential risk posed by compounds with estrogen-like activity present in the environment. As previous studies have shown that combined exposure to such compounds results in dose additivity, it should be possible to assess estrogen exposure with suitable biomarkers of effect.

Objective: Our goal was to identify candidate protein biomarkers of effect for estrogenic compounds.

Methods: In the search for biomarkers, we assessed the effect of several estrogenic compounds on the expression profile of proteins in breast-derived cell lines varying in their estrogen receptor (ER) phenotype using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. We identified responsive proteins, after separating them by SDS-polyacrylamide gel electrophoresis, and analyzing the trypsin-digested proteins by tandem mass spectrometry.

Results: The estrogenic compounds 17β-estradiol, genistein, bisphenol A, and endosulfan produced similar protein profile changes in MCF-7 cells (phenotype: $ER\alpha^+/ER\beta^+$), but had no effect on MDA-MB-231 ($ER\alpha^-/ER\beta^+$), MCF-10F ($ER\alpha^-/ER\beta^+$), or MCF-10A ($ER\alpha^-/ER\beta^-$) cells. The most responsive proteins in MCF-7 cells were identified as histones H2A, H2B, H3, and H4. Histone levels were not increased in cell lines that showed no proliferative response to estrogens despite their rapid intrinsic growth rate in culture.

Conclusion: Our results indicate that ER-mediated cell proliferation results in up-regulation of core histone proteins.

Oral exposure to bisphenol A increases dimethylbenzanthracene-induced mammary cancer in rats

Sarah Jenkins, Nandini Raghuraman, Isam Eltoum, Mark Carpenter, Jose Russo, Coral A. Lamartiniere

2009 117(6):910–915 | http://dx.doi.org/10.1289/ehp.11751

Background: Bisphenol A (BPA) is widely used in the manufacture of polycarbonate plastics, including infant formula bottles.

Objective: Based on the reported endocrine disruptor activity of this polyphenol, we hypothesized that exposure to BPA early in life would elicit developmental changes in the mammary tissue and cause a predisposition for mammary cancer.

Methods: We exposed neonatal/prepubertal rats to BPA via lactation from nursing dams treated orally with 0, 25, and 250 µg BPA/kg body weight/day. For tumorigenesis studies, female offspring were exposed to 30 mg dimethylbenzanthracene (DMBA)/kg body weight at 50 days of age.

Results: The combination of DMBA treatment with lactational exposure to BPA demonstrated a dose-dependent increase in mammary tumor multiplicity and reduced tumor latency compared with controls. In the absence of DMBA treatment, lactational BPA exposure resulted in increased cell proliferation and decreased apoptosis at 50 but not 21 days postpartum (shortly after last BPA treatment). Using Western blot analysis, we determined that steroid receptor coactivators (SRCs) 1–3, Akt, phosphorylated Akt, progesterone receptor A (PR-A), and erbB3 proteins were significantly up-regulated at 50 days of age.

Conclusions: The data presented here provide the first evidence that maternal exposure to BPA during lactation increases mammary carcinogenesis in a DMBA-induced model of rodent mammary cancer. Changes in PR-A, SRC 1–3, erbB3, and Akt activity are consistent with increased cell proliferation and decreased apoptosis playing a role in mammary cancer susceptibility. These alterations provide an explanation of enhanced mammary carcinogenesis after lactational BPA exposure.

Prenatal exposure to bisphenol A at environmentally relevant doses adversely affects the murine female reproductive tract later in life

Retha R. Newbold, Wendy N. Jefferson, Elizabeth Padilla-Banks

2009 117(6):879–885 | http://dx.doi.org/10.1289/ehp.0800045

Background: Exposure to endocrine-disrupting chemicals during critical developmental periods causes adverse consequences later in life; an example is prenatal exposure to the pharmaceutical diethylstilbestrol (DES). Bisphenol A (BPA), an environmental estrogen used in the synthesis of plastics, is of concern because its chemical structure resembles that of DES, and it is a "high-volume production" chemical with widespread human exposure.

Objective: In this study we investigated whether prenatal BPA causes long-term adverse effects in female reproductive tissues in an experimental animal model previously shown useful in studying effects of prenatal DES.

Methods: Timed pregnant CD-1 mice were treated on days 9–16 of gestation with BPA (0.1, 1, 10, 100, or 1,000 µg/kg/day). After delivery, pups were held for 18 months; reproductive tissues were then evaluated.

Results: Ovarian cysts were significantly increased in the 1-µg/kg BPA group; ovarian cyst-adenomas were seen in the other three BPA-treated groups but not in corn-oil controls. We observed increased progressive proliferative lesions of the oviduct after BPA treatment, similar to those described in response to DES. Further, although not statistically different from the controls, prominent mesonephric (Wolffian) remnants and squamous metaplasia of the uterus, as well as vaginal adenosis, were present in BPA-treated mice, similar to lesions reported following DES treatment. More severe pathologies observed in some BPA-treated animals included atypical hyperplasia and stromal polyps of the uterus; sarcoma of the uterine cervix; and mammary adenocarcinoma. We did not observe these lesions in controls.

Conclusions: These data suggest that BPA causes long-term adverse reproductive and carcinogenic effects if exposure occurs during critical periods of differentiation.

Low doses of bisphenol A promote human seminoma cell proliferation by activating PKA and PKG via a membrane G-protein–coupled estrogen receptor

Adil Bouskine, Marielle Nebout, Françoise Brücker-Davis, Mohamed Benahmed, Patrick Fenichel

2009 117(7):1053-1058 | http://dx.doi.org/10.1289/ehp.0800367

Background: Fetal exposure to environmental estrogens may contribute to hypofertility and/or to testicular germ cell cancer. However, many of these xenoestrogens have only a weak affinity for the classical estrogen receptors (ERs,) which is 1,000-fold less potent than the affinity of 17β-estradiol (E_2). Thus, several mechanisms have been suggested to explain how they could affect male germ cell proliferation at low environmental relevant concentrations.

Objective: In this study we aimed to explore the possible promoting effect of bisphenol A (BPA) on human testicular seminoma cells. BPA is a well-recognized estrogenic endocrine disruptor used as a monomer to manufacture polycarbonate plastic and released from resin-lined food or beverage cans or from dental sealants.

Methods and results: BPA at very low concentrations (10^{-9} to 10^{-12} M) similar to those found in human fluids stimulated JKT-1 cell proliferation *in vitro*. BPA activated both cAMP-dependent protein kinase and cGMP-dependent protein kinase pathways and triggered a rapid (15 min) phosphorylation of the transcription factor cAMP response-element–binding protein (CREB) and the cell cycle regulator retinoblastoma protein (Rb). This nongenomic activation did not involve classical ERs because it could not be reversed by ICI 182780 (an ER antagonist) or reproduced either by E_2 or by diethylstilbestrol (a potent synthetic estrogen), which instead triggered a suppressive effect. This activation was reproduced only by E_2 coupled to bovine serum albumin (BSA), which is unable to enter the cell. As with E_2-BSA, BPA promoted JKT-1 cell proliferation through a G-protein–coupled nonclassical membrane ER (GPCR) involving a $G\alpha_s$ and a $G\alpha_i/G\alpha_q$ subunit, as shown by the reversible effect observed by the corresponding inhibitors NF449 and pertussis toxin.

Conclusion: This GPCR-mediated nongenomic action represents—in addition to the classical ER-mediated effect—a new basis for evaluating xenoestrogens such as BPA that, at low doses and with a high affinity for this GPCR, could interfere with the developmental programming of fetal germ cell proliferation and/or differentiation when they cross the placenta.

Perinatal exposure to bisphenol A alters early adipogenesis in the rat

Emmanuel Somm, Valérie M. Schwitzgebel, Audrey Toulotte, Christopher R. Cederroth, Christophe Combescure, Serge Nef, Michel L. Aubert, Petra S. Hüppi

2009 117(10):1549–1555 | http://dx.doi.org/10.1289/ehp.11342

Background: The causes of the current obesity pandemic have not been fully elucidated. Implication of environmental endocrine disruptors such as bisphenol A (BPA) on adipose tissue development has been poorly investigated.

Objective: The aim of the present study was to evaluate the effects of perinatal exposure to BPA on early adipose storage at weaning.

Methods: Pregnant Sprague-Dawley rats had access to drinking water containing 1 mg/L BPA from day 6 of gestation through the end of lactation. Pups were weaned on postnatal day (PND) 21. At that time, we investigated perigonadal adipose tissue of pups (weight, histology, gene expression). For the remaining animals, we recorded body weight and food intake for animals on either standard chow or a high-fat diet.

Results: Gestational exposure to BPA did not alter the sex ratio or litter size at birth. On PND1, the weight of male and female BPA-exposed pups was increased. On PND21, body weight was increased only in females, in which parametrial white adipose tissue (pWAT) weight was increased about 3-fold. This excess of pWAT was associated with adipocyte hypertrophy and overexpression of lipogenic genes such as *C/EBP-α* (CAAT enhancer binding protein alpha), *PPAR-γ* (peroxisome proliferator-activated receptor gamma), *SREBP-1C* (sterol regulatory element binding protein-1C), *LPL* (lipoprotein lipase), FAS (fatty acid synthase), and *SCD-1* (stearoyl-CoA desaturase 1). In addition, gene expression of *SREBP-1C*, *FAS*, and *ACC* (acetyl-CoA carboxylase) was also increased in liver from BPA-exposed females at PND21, without a change in circulating lipids and glucose. After weaning, perinatal BPA exposure predisposed to overweight in a sex- and diet-dependent manner. We observed no change in food intake due to perinatal BPA exposure in rats on either standard chow or a high-fat diet.

Conclusions: Perinatal exposure to a low dose of BPA increased adipogenesis in females at weaning. Adult body weight may be programmed during early life, leading to changes dependent on the sex and the nutritional status. Although further studies are required to understand the mechanisms of BPA action in early life, these results are particularly important with regard to the increasing prevalence of childhood obesity and the context-dependent action of endocrine disruptors.

Maternal bisphenol A exposure promotes the development of experimental asthma in mouse pups

Terumi Midoro-Horiuti, Ruby Tiwari, Cheryl S. Watson, Randall M. Goldblum

2010 118(2):273–277 | http://dx.doi.org/10.1289/ehp.0901259

Background: We recently reported that various environmental estrogens induce mast cell degranulation and enhance IgE-mediated release of allergic mediators *in vitro*.

Objective: We hypothesized that environmental estrogens would enhance allergic sensitization as well as bronchial inflammation and responsiveness. To test this hypothesis, we exposed fetal and neonatal mice to the common environmental estrogen bisphenol A (BPA) via maternal loading and assessed the pups' response to allergic sensitization and bronchial challenge.

Methods: Female BALB/c mice received 10 µg/mL BPA in their drinking water from 1 week before impregnation to the end of the study. Neonatal mice were given a single 5 µg intraperitoneal dose of ovalbumin (OVA) with aluminum hydroxide on postnatal day 4 and 3% OVA by nebulization for 10 min on days 13, 14, and 15. Forty-eight hours after the last nebulization, we assessed serum IgE antibodies to OVA by enzyme-linked immunosorbent assay (ELISA) and airway inflammation and hyperresponsiveness by enumerating eosinophils in bronchoalveolar lavage fluid, whole-body barometric plethysmography, and a forced oscillation technique.

Results: Neonates from BPA-exposed mothers responded to this "suboptimal" sensitization with higher serum IgE anti-OVA concentrations compared with those from unexposed mothers ($p < 0.05$), and eosinophilic inflammation in their airways was significantly greater. Airway responsiveness of the OVA-sensitized neonates from BPA-treated mothers was enhanced compared with those from unexposed mothers ($p < 0.05$).

Conclusions: Perinatal exposure to BPA enhances allergic sensitization and bronchial inflammation and responsiveness in a susceptible animal model of asthma.

Peroxisome proliferator-activated receptor-γ mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells

Jakub Kwintkiewicz, Yoshihiro Nishi, Toshihiko Yanase, Linda C. Giudice

2010 118(3):400-406 | http://dx.doi.org/10.1289/ehp.0901161

Background: Bisphenol A (BPA), a chemical used as a plasticizer, is a potent endocrine disruptor that, even in low concentrations, disturbs normal development and functions of reproductive organs in different species.

Objective: We investigated whether BPA affects human ovarian granulosa cell function.

Methods: We treated KGN granulosa cells and granulosa cells from subjects undergoing *in vitro* fertilization (IVF) with follicle-stimulating hormone (FSH), BPA, or BPA plus FSH in a dose- and time-dependent manner. We then evaluated expression of insulin-like growth factor 1 (IGF-1), aromatase, and transcription factors known to mediate aromatase induction by FSH [including steroidogenic factor-1 (SF-1), GATA4, cAMP response element binding protein-1 (CREB-1), and peroxisome proliferator–activated receptor-γ (PPARγ)], as well as 17β-estradiol (E_2) secretion. KGN cells were transfected with a PPARγ-containing vector, followed by assessment of aromatase and IGF-I expression.

Results: BPA reduced FSH-induced IGF-1 and aromatase expression and E_2 secretion in a dose-dependent fashion. Similar effects on aromatase were observed in IVF granulosa cells. SF-1 and GATA4, but not CREB-1, were reduced after BPA treatment, although PPARγ, an inhibitor of aromatase, was significantly up-regulated by BPA in a dose-dependent manner, with simultaneous decrease of aromatase. Overexpression of PPARγ in KGN cells reduced FSH-stimulated aromatase and *IGF-1* mRNAs, with increasing concentrations of the transfected expression vector, mimicking BPA action. Also, BPA reduced granulosa cell DNA synthesis without changing DNA fragmentation, suggesting that BPA does not induce apoptosis.

Conclusions: Overall, the data demonstrate that BPA induces PPARγ, which mediates down-regulation of FSH-stimulated IGF-1, SF-1, GATA4, aromatase, and E_2 in human granulosa cells. These observations support a potential role of altered steroidogenesis and proliferation within the ovarian follicular compartment due to this endocrine disruptor.

Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus

Miyu Nishikawa, Hidetomo Iwano, Risa Yanagisawa, Nanako Koike, Hiroki Inoue, Hiroshi Yokota

2010 118(9):1196–1203 | http://dx.doi.org/10.1289/ehp.0901575

Background: Bisphenol A (BPA), a well-known endocrine disruptor, is highly glucuronidated in the liver, and the resultant BPA-glucuronide (BPA-GA) is excreted primarily into bile. However, in rodents, prenatal exposure to low doses of BPA can adversely affect the fetus, despite the efficient drug-metabolizing systems of the dams. The transport mechanisms of BPA from mother to fetus are unknown.

Objective: To test our hypothesis that BPA-GA—an inactive metabolite—is passed through the placenta to the fetus, where it affects the fetus after reactivation, we investigated the placental transfer of BPA-GA and reactivation to BPA in the fetus.

Methods: After performing uterine perfusion with BPA-GA in pregnant rats, we examined the expression and localization of the placental transporters for drug metabolites in the perfusate by reverse-transcriptase polymerase chain reaction and immunohistochemistry. We also investigated the deconjugation of BPA-GA in the fetus and examined uridine 5′-diphospho-glucuronosyltransferase (UGT) activity toward BPA and the expression of UGT isoforms in fetal liver.

Results: We detected BPA-GA and deconjugated BPA in the fetus and amniotic fluid after perfusion. In the trophoblast cells, organic anion-transporting polypeptide 4a1 (Oatp4a1) was localized on the apical membrane, and multidrug resistance-associated protein 1 (Mrp1) was localized to the basolateral membrane. We observed deconjugation of BPA-GA in the fetus; furthermore, we found the expression of UGT2B1, which metabolizes BPA, to be quite low in the fetus.

Conclusions: These results demonstrate that BPA-GA is transferred into the fetus and deconjugated in the fetus because of its vulnerable drug-metabolizing system.

Neonatal exposure to bisphenol A and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats

Marina Fernández, Nadia Bourguignon, Victoria Lux-Lantos, Carlos Libertun

2010 118(9):1217–1222 | http://dx.doi.org/10.1289/ehp.0901257

Background: Bisphenol A (BPA), an endocrine disruptor, is a component of polycarbonate plastics, epoxy resins, and polystyrene. Several studies have reported potent *in vivo* effects, because BPA behaves as an estrogen agonist and/or antagonist and as an androgen and thyroid hormone antagonist.

Objective: We investigated the effects of neonatal exposure to BPA on the reproductive axis in adult female Sprague-Dawley rats.

Methods: Female rats were injected subcutaneously, daily from postnatal day 1 (PND1) to PND10 with BPA in castor oil at 500 µg/50 µL [BPA500; ~ 10^{-4} M, a dose higher than the lowest observed adverse effect level (LOAEL) of 50 mg/kg], 50 µg/50 µL (BPA50), or 5 µg/50 µL (both BPA50 and BPA5 are doses lower than the LOAEL), or castor oil vehicle alone. In adults we studied *a*) the release of gonadotropin-releasing hormone (GnRH) from hypothalamic explants, *b*) serum sex hormone levels, and *c*) ovarian morphology, ovulation, and fertility.

Results: Neonatal exposure to BPA was associated with increased serum testosterone and estradiol levels, reduced progesterone in adulthood, and altered *in vitro* GnRH secretion. Animals exposed to BPA500 had altered ovarian morphology, showing a large number of cysts. Animals exposed to BPA50 had reduced fertility without changes in the number of oocytes on the morning of estrus, whereas animals exposed to BPA500 showed infertility.

Conclusions: Exposure to high doses of BPA during the period of brain sexual differentiation altered the hypothalamic–pituitary–gonadal axis in female Sprague-Dawley rats. These results have the potential to link neonatal exposure to high doses of BPA in rats with the development of polycystic ovarian syndrome. Studies of doses and routes of administration more consistent with human exposures are needed to determine the relevance of these findings to human health.

Bisphenol AF is a full agonist for the estrogen receptor ERα but a highly specific antagonist for ERβ

Ayami Matsushima, Xiaohui Liu, Hiroyuki Okada, Miki Shimohigashi, Yasuyuki Shimohigashi

2010 118(9):1267–1272 | http://dx.doi.org/10.1289/ehp.0901819

Background: Bisphenol AF has been acknowledged to be useful for the production of CF_3-containing polymers with improved chemical, thermal, and mechanical properties. Because of the lack of adequate toxicity data, bisphenol AF has been nominated for comprehensive toxicological characterization.

Objectives: We aimed to determine the relative preference of bisphenol AF for the human nuclear estrogenic receptors ERα and ERβ and the bisphenol A–specific estrogen-related receptor ERRγ, and to clarify structural characteristics of receptors that influence bisphenol AF binding.

Methods: We examined receptor-binding activities of bisphenol AF relative to [^{3}H]17β-estradiol (for ERα and ERβ) and [^{3}H]bisphenol A (for ERRγ). Functional luciferase reporter gene assays were performed to assess receptor activation in HeLa cells.

Results: We found that bisphenol AF strongly and selectively binds to ERs over ERRγ. Furthermore, bisphenol AF receptor-binding activity was three times stronger for ERβ [IC_{50} (median inhibitory concentration) = 18.9 nM] than for ERα. When examined using a reporter gene assay, bisphenol AF was a full agonist for ERα. In contrast, it was almost completely inactive in stimulating the basal constitutive activity of ERβ. Surprisingly, bisphenol AF acted as a distinct and strong antagonist against the activity of the endogenous ERβ agonist 17β-estradiol.

Conclusion: Our results suggest that bisphenol AF could function as an endocrine-disrupting chemical by acting as an agonist or antagonist to perturb physiological processes mediated through ERα and/or ERβ.

Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring

Paloma Alonso-Magdalena, Elaine Vieira, Sergi Soriano, Lorena Menes, Deborah Burks, Ivan Quesada, Angel Nadal

2010 118(9):1243–1250 | http://dx.doi.org/10.1289/ehp.1001993

Background: Bisphenol A (BPA) is a widespread endocrine-disrupting chemical used as the base compound in the manufacture of polycarbonate plastics. In humans, epidemiological evidence has associated BPA exposure in adults with higher risk of type 2 diabetes and heart disease.

Objective: We examined the action of environmentally relevant doses of BPA on glucose metabolism in mice during pregnancy and the impact of BPA exposure on these females later in life. We also investigated the consequences of *in utero* exposure to BPA on metabolic parameters and pancreatic function in offspring.

Methods: Pregnant mice were treated with either vehicle or BPA (10 or 100 μg/kg/day) during days 9–16 of gestation. Glucose metabolism experiments were performed on pregnant mice and their offspring.

Results: BPA exposure aggravated the insulin resistance produced during pregnancy and was associated with decreased glucose tolerance and increased plasma insulin, triglyceride, and leptin concentrations relative to controls. Insulin-stimulated Akt phosphorylation was reduced in skeletal muscle and liver of BPA-treated pregnant mice relative to controls. BPA exposure during gestation had long-term consequences for mothers: 4 months post-partum, treated females weighed more than untreated females and had higher plasma insulin, leptin, triglyceride, and glycerol levels and greater insulin resistance. At 6 months of age, male offspring exposed *in utero* had reduced glucose tolerance, increased insulin resistance, and altered blood parameters compared with offspring of untreated mothers. The islets of Langerhans from male offspring presented altered Ca^{2+} signaling and insulin secretion. BrdU (bromodeoxyuridine) incorporation into insulin-producing cells was reduced in the male progeny, yet β-cell mass was unchanged.

Conclusions: Our findings suggest that BPA may contribute to metabolic disorders relevant to glucose homeostasis and that BPA may be a risk factor for diabetes.

In utero exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat

Angela M. Betancourt, Isam A. Eltoum, Renee A. Desmond, Jose Russo, Coral A. Lamartiniere

2010 118(11):1614–1619 | http://dx.doi.org/10.1289/ehp.1002148

Background: Bisphenol A (BPA) is a ubiquitous environmental chemical with reported endocrine-disrupting properties.

Objective: Our goal in this study was to determine whether prenatal exposure to BPA predisposes the adult rat mammary gland to carcinogenesis.

Methods: Pregnant rats were treated orally with 0, 25, or 250 µg BPA/kg body weight (BW) from gestation day (GD) 10 to GD21. For tumorigenesis experiments, prenatally exposed female offspring received a single gavage of 7,12-dimethylbenz(*a*)anthracene (DMBA; 30 mg/kg BW) on postnatal day (PND) 50, or PND100.

Results: Prenatal exposure of the dam to 250 µg BPA/kg BW combined with a single exposure of female offspring to DMBA on PND100, but not on PND50, significantly increased tumor incidence while decreasing tumor latency compared with the control group. Prenatal exposure of the dam to 250 µg BPA/kg BW, in the absence of DMBA to the female offspring, increased cell proliferation and elicited differential effects at the protein level at PND100 compared with PND50. Differentially regulated proteins in the mammary gland included estrogen receptor-α, progesterone receptor-A, Bcl-2, steroid receptor coactivators, epidermal growth factor receptor, phospho-insulinlike growth factor 1 receptor,and phospho-Raf.

Conclusions: Our study demonstrates that oral prenatal exposure to BPA increases mammary cancer susceptibility in offspring and shifts the window of susceptibility for DMBA-induced tumorigenesis in the rat mammary gland from PND50 to PND100. These changes are accompanied by differential effects of prenatal BPA exposure on the expression of key proteins involved in cell proliferation.

Estrogenic activity of bisphenol A and 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) demonstrated in mouse uterine gene profiles

Sylvia C. Hewitt, Kenneth S. Korach

2011 119(1):63–70 | http://dx.doi.org/10.1289/ehp.1002347

Background: Interest and concern regarding potentially estrogenic substances have resulted in development of model systems to evaluate mechanisms of such chemicals. Microarray studies have indicated that estradiol (E_2)-stimulated uterine responses can be divided into early and late phases. Comparison of E_2 uterine transcript profiles and those of other estrogenic chemicals of interest *in vivo* indicates mechanisms and activities of test compounds.

Objective: We compared transcript responses and mechanisms of response using mouse reproductive tracts after treatment with E_2, estriol (E_3), bisphenol A (BPA), and 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE).

Methods: Uterine RNA from ovariectomized wild-type mice, estrogen receptor α (ERα) knockout (α ERKO) mice, and mice expressing a DNA-binding–deficient ERα (KIKO) treated with E_2, E_3, BPA, or HPTE for 2 or 24 hr was analyzed by microarray. Resulting regulated transcripts were compared by hierarchical clustering and correlation analysis, and response patterns were verified by reverse-transcription real-time polymerase chain reaction (RT-PCR).

Results: Both xenoestrogens, BPA and HPTE, showed profiles highly correlated to that of E_2 in the early response phase (2 hr), but the correlation diminished in the later response phase (24 hr), similar to the known weak estrogen E_3. Both xenoestrogens also mimicked E_2 in samples from KIKO mice, indicating that they are able to utilize the indirect tethering mode of ERα signaling. No response was detected in ERα-null uteri, indicating that ERα mediates the responses.

Conclusion: Our study forms a basis on which patterns of response and molecular mechanisms of potentially estrogenic chemicals can be assessed.

Combinations of physiologic estrogens with xenoestrogens alter ERK phosphorylation profiles in rat pituitary cells

Yow-Jiun Jeng, Cheryl S. Watson

2011 119(1):104–112 | http://dx.doi.org/10.1289/ehp.1002512

Background: Estrogens are potent nongenomic phosphoactivators of extracellular-signal–regulated kinases (ERKs). A major concern about the toxicity of xenoestrogens (XEs) is potential alteration of responses to physiologic estrogens when XEs are present simultaneously.

Objective: We examined estrogen-induced ERK activation, comparing the abilities of structurally related XEs (alkylphenols and bisphenol A) to alter ERK responses induced by physiologic concentrations (1 nM) of estradiol (E_2), estrone (E_1), and estriol (E_3).

Methods: We quantified hormone/mimetic-induced ERK phosphorylations in the GH_3/B6/F10 rat pituitary cell line using a plate immunoassay, comparing effects with those on cell proliferation and by estrogen receptor subtype-selective ligands.

Results: Alone, these structurally related XEs activate ERKs in an oscillating temporal pattern similar (but not identical) to that with physiologic estrogens. The potency of all estrogens was similar (active between femtomolar and nanomolar concentrations). XEs potently disrupted physiologic estrogen signaling at low, environmentally relevant concentrations. Generally, XEs potentiated (at the lowest, subpicomolar concentrations) and attenuated (at the highest, picomolar to 100 nM concentrations) the actions of the physiologic estrogens. Some XEs showed pronounced nonmonotonic responses/inhibitions. The phosphorylated ERK and proliferative responses to receptor-selective ligands were only partially correlated.

Conclusions: XEs are both imperfect potent estrogens and endocrine disruptors; the more efficacious an XE, the more it disrupts actions of physiologic estrogens. This ability to disrupt physiologic estrogen signaling suggests that XEs may disturb normal functioning at life stages where actions of particular estrogens are important (e.g., development, reproductive cycling, pregnancy, menopause).

Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice

Nicolas J. Cabaton, Perinaaz R. Wadia, Beverly S. Rubin, Daniel Zalko, Cheryl M. Schaeberle, Michael H. Askenase, Jennifer L. Gadbois, Andrew P. Tharp, Gregory S. Whitt, Carlos Sonnenschein, Ana M. Soto

2011 119(4):547–552 | http://dx.doi.org/10.1289/ehp.1002559

Background: Perinatal exposure to low doses of bisphenol A (BPA) results in alterations in the ovary, uterus, and mammary glands and in a sexually dimorphic region of the brain known to be important for estrous cyclicity.

Objective: We aimed to determine whether perinatal exposure to environmentally relevant doses of BPA alters reproductive capacity.

Methods: Female CD-1 mice that were exposed to BPA at 0, 25 ng, 250 ng, or 25 µg/kg body weight (BW)/day or diethylstilbestrol (DES) at 10 ng/kg BW/day (positive control) from gestational day 8 through day 16 of lactation were continuously housed with proven breeder males for 32 weeks starting at 2 months of age. At each delivery, pups born to these mating pairs were removed. The cumulative number of pups, number of deliveries, and litter size were recorded. The purity of the BPA used in this and our previous studies was assessed using HPLC, mass spectrometry, and nuclear magnetic resonance.

Results: The forced breeding experiment revealed a decrease in the cumulative number of pups, observed as a nonmonotonic dose–response effect, and a decline in fertility and fecundity over time in female mice exposed perinatally to BPA. The BPA was 97% pure, with no evidence of contamination by other phenolic compounds.

Conclusions: Perinatal exposure to BPA leads to a dose-dependent decline in the reproductive capacity of female mice. The effects on the cumulative number of pups are comparable to those previously reported in mice developmentally exposed to DES, a compound well known to impair reproduction in women. This association suggests the possibility that early BPA exposure may also affect reproductive capacity in women.

Comparison of serum bisphenol A concentrations in mice exposed to bisphenol A through the diet versus oral bolus exposure

Paizlee T. Sieli, Eldin Jašarević, Denise A. Warzak, Jiude Mao, Mark R. Ellersieck, Chunyang Liao, Kurunthachalam Kannan, Séverine H. Collet, Pierre-Louis Toutain, Frederick S. vom Saal, Cheryl S. Rosenfeld

119(9):1260–1265 2011 | http://dx.doi.org/10.1289/ehp.1003385

Background: Bisphenol A (BPA) is a widely produced endocrine-disrupting chemical. Diet is a primary route of exposure, but internal exposure (serum concentrations) in animals and humans has been measured only after single oral bolus administration.

Objective: We compared serum concentrations of BPA over a 24-hr period after oral bolus administration or *ad libitum* feeding in mice and assessed for buildup with dietary exposure.

Methods: Adult female mice were administered [dimethyl-d_6]-BPA (BPA-d_6) as a single oral bolus (20 mg/kg body weight) or fed a diet containing 100 mg BPA-d_6/kg feed weight *ad libitum* for 1 week. Serum concentrations were analyzed using isotope dilution liquid chromatography coupled with electrospray tandem mass spectrometry and compared between exposure groups over the first 23 hr and after 7 days of dietary exposure.

Results: Maximum concentration (C_{max}) for BPA-d_6 during the first 24 hr was reached at 1 hr and 6 hr for oral bolus and diet groups, respectively. Relative BPA-d_6 bioavailability (unconjugated BPA-d_6) was higher in diet-exposed mice than in the bolus group despite a relative lower absorption, a phenomenon consistent with an inhibitory effect of food on first-pass hepatic metabolism. In mice with ongoing dietary exposure, unconjugated BPA-d_6 was higher on day 7 than on day 1.

Conclusions: This is the first report of serum BPA concentrations in an animal model exposed to this chemical via the diet. Although bolus administration of BPA-d_6 led to peak concentrations within 1 hr, C_{max} for diet-exposed mice was delayed for several hours. However, bolus administration underestimates bioavailable serum BPA concentrations in animals—and presumably humans—than would result from dietary exposure. Exposure via diet is a more natural continuous exposure route than oral bolus exposure and is thus a better predictor of BPA concentrations in chronically exposed animals and humans.

Chronic oral exposure to bisphenol A results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice

Sarah Jenkins, Jun Wang, Isam Eltoum, Renee Desmond, Coral A. Lamartiniere

2001 119(11):1604–1609 | http://dx.doi.org/10.1289/ehp.1103850

Background: Bisphenol A (BPA) is a synthetic compound used to produce plastics and epoxy resins. BPA can leach from these products in appreciable amounts, resulting in nearly ubiquitous daily exposure to humans. Whether BPA is harmful to humans, especially when administered orally in concentrations relevant to humans, is a topic of debate.

Objective: In this study, we investigated the role of chronic oral exposure to BPA during adulthood on mammary carcinogenesis by using a transgenic mouse model that spontaneously develops tumors through overexpression of wild-type erbB2 [mouse mammary tumor virus (MMTV)-erbB2].

Methods: MMTV-erbB2 mice were exposed to 0, 2.5, 25, 250, or 2,500 µg BPA/L drinking water from 56 until 112 days of age (for mechanism of action) or 252 days of age (for tumorigenesis). Cellular and molecular mechanisms of BPA action in the mammary gland were investigated via immunohistochemistry and immunoblotting.

Results: Only low doses of BPA significantly decreased tumor latency and increased tumor multiplicity, tumor burden, and the incidence of metastasis. All BPA doses significantly increased the cell proliferation index, but only the higher doses also increased the apoptotic index in the mammary gland. At the molecular level, 25 µg BPA/L, but not 2,500 µg BPA/L, increased phosphorylation of erbB2, erbB3, insulin-like growth factor 1 receptor, and Akt in the mammary gland.

Discussion: Low, but not high, BPA doses significantly accelerated mammary tumorigenesis and metastasis in MMTV-erbB2 mice. The combined ratio of cell proliferation and apoptosis indices and alterations in protein expression best predicted the ability of each dose of BPA to alter tumorigenesis in this model.

Low concentrations of bisphenol A induce mouse spermatogonial cell proliferation by G-protein–coupled receptor 30 and estrogen receptor-α

Zhi-Guo Sheng, Ben-Zhan Zhu

2011 119(12):1775-1780 | http://dx.doi.org/10.1289/ehp.1103781

Background: Bisphenol A (BPA) is one of the most prevalent chemicals in daily-use materials; therefore, human exposure to BPA is ubiquitous. The estrogenicity of BPA is generally mediated by nuclear estrogen receptors (ERs). However, low concentrations of BPA stimulate seminoma cell proliferation by an uncertain mechanism that does not involve activation of ERs.

Objective: We investigated the possible promoting effects of low-concentration BPA and the possible mechanism(s) using the murine ER-β negative spermatogonial GC-1 cell line.

Methods and results: Using the specific signaling inhibitor, BPA at test concentrations ranging from 10^{-10} to 10^{-8} M markedly induced proliferation of GC-1 cells by activating both cGMP-dependent protein kinase (PKG) and epidermal growth factor receptor (EGFR) extracellular regulated kinase (ERK) pathways. BPA stimulated a rapid (15-min) phosphorylation of the transcription factor cAMP response element binding protein (CREB) and the cell cycle regulator retinoblastoma protein (Rb). Interestingly, ER-α phosphorylation is involved in the proliferation, whereas BPA does not directly transactivate ER-α in gene reporter assays. Using specific agonists and gene silencing, we further observed that BPA mediates the proliferation and *fos* gene expression of GC-1 cells by G protein–coupled receptor 30 (GPR30) and ER-α.

Conclusions: Our data suggest that low concentrations of BPA activate the PKG and EGFR/ERK/*c-fos* pathways through a cross-talk between GPR30 and ER-α, which in turn stimulates GC-1 cell proliferation. The present study provides a novel insight regarding the potential role of GPR30 and ER-α in mediating the proliferative effects of BPA in male germ cells.

REVIEW |

Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure

Julia A. Taylor, Frederick S. vom Saal, Wade V. Welshons, Bertram Drury, George Rottinghaus, Patricia A. Hunt, Pierre-Louis Toutain, Céline M. Laffont, Catherine A. VandeVoort

2011 119(4):422-430 | http://dx.doi.org/10.1289/ehp.1002514

Objective: Daily adult human exposure to bisphenol A (BPA) has been estimated at < 1 μg/kg, with virtually complete first-pass conjugation in the liver in primates but not in mice. We measured unconjugated and conjugated BPA levels in serum from adult female rhesus monkeys and adult female mice after oral administration of BPA and compared findings in mice and monkeys with prior published data in women.

Methods: Eleven adult female rhesus macaques were fed 400 μg/kg deuterated BPA (dBPA) daily for 7 days. Levels of serum dBPA were analyzed by isotope-dilution liquid chromatography–mass spectrometry (0.2 ng/mL limit of quantitation) over 24 hr on day 1 and on day 7. The same dose of BPA was fed to adult female CD-1 mice; other female mice were administered ^{3}H-BPA at doses ranging from 2 to 100,000 μg/kg.

Results: In monkeys, the maximum unconjugated serum dBPA concentration of 4 ng/mL was reached 1 hr after feeding and declined to low levels by 24 hr, with no significant bioaccumulation after seven daily doses. Mice and monkeys cleared unconjugated serum BPA at virtually identical rates. We observed a linear (proportional) relationship between administered dose and serum BPA in mice.

Conclusions: BPA pharmacokinetics in women, female monkeys, and mice is very similar. By comparison with approximately 2 ng/mL unconjugated serum BPA reported in multiple human studies, the average 24-hr unconjugated serum BPA concentration of 0.5 ng/mL in both monkeys and mice after a 400 μg/kg oral dose suggests that total daily human exposure is via multiple routes and is much higher than previously assumed.

Prenatal bisphenol A exposure and early childhood behavior

Joe M. Braun, Kimberly Yolton, Kim N. Dietrich, Richard Hornung, Xiaoyun Ye, Antonia M. Calafat, Bruce P. Lanphear

2009 117(12):1945–1952 | http://dx.doi.org/10.1289/ehp.0900979

Background: Prenatal exposure to bisphenol A (BPA) increases offspring aggression and diminishes differences in sexually dimorphic behaviors in rodents.

Objective: We examined the association between prenatal BPA exposure and behavior in 2-year-old children.

Methods: We used data from 249 mothers and their children in Cincinnati, Ohio (USA). Maternal urine was collected around 16 and 26 weeks of gestation and at birth. BPA concentrations were quantified using high-performance liquid chromatography–isotope-dilution tandem mass spectrometry. Child behavior was assessed at 2 years of age using the second edition of the Behavioral Assessment System for Children (BASC-2). The association between prenatal BPA concentrations and BASC-2 scores was analyzed using linear regression.

Results: Median BPA concentrations were 1.8 (16 weeks), 1.7 (26 weeks), and 1.3 (birth) ng/mL. Mean (± SD) BASC-2 externalizing and internalizing scores were 47.6 ± 7.8 and 44.8 ± 7.0, respectively. After adjustment for confounders, $\log_{10}$-transformed mean prenatal BPA concentrations were associated with externalizing scores, but only among females [$\beta = 6.0$; 95% confidence interval (CI), 0.1–12.0]. Compared with 26-week and birth concentrations, BPA concentrations collected around 16 weeks were more strongly associated with externalizing scores among all children ($\beta = 2.9$; 95% CI, 0.2–5.7), and this association was stronger in females than in males. Among all children, measurements collected at ≤ 16 weeks showed a stronger association ($\beta = 5.1$; 95% CI, 1.5–8.6) with externalizing scores than did measurements taken at 17–21 weeks ($\beta = 0.6$; 95% CI, –2.9 to 4.1).

Conclusions: These results suggest that prenatal BPA exposure may be associated with externalizing behaviors in 2-year-old children, especially among female children.

Are environmental levels of bisphenol A associated with reproductive function in fertile men?

Jaime Mendiola, Niels Jørgensen, Anna-Maria Andersson, Antonia M. Calafat, Xiaoyun Ye, J. Bruce Redmon, Erma Z. Drobnis, Christina Wang, Amy Sparks, Sally W. Thurston, Fan Liu, Shanna H. Swan

2010 118(9):1286–1291 | http://dx.doi.org/10.1289/ehp.1002037

Background: Rodent and *in vitro* studies have demonstrated the estrogenicity of bisphenol A (BPA). However, few studies have examined the relationship between human exposure to BPA and male reproductive function.

Objective: We investigated the relationships between environmental BPA exposure and reproductive parameters, including semen quality and male reproductive hormones, in prospectively recruited fertile men.

Methods: Participants ($n = 375$) were partners of pregnant women who participated in the Study for Future Families in four U.S. cities, and all of the men provided blood, semen, and urine samples. BPA was measured in urine. Serum samples were analyzed for reproductive hormones, including follicle-stimulating hormone, luteinizing hormone (LH), testosterone, inhibin B, estradiol, and sex hormone–binding globulin (SHBG), as well as the free androgen index (FAI). Semen analyses were performed according to World Health Organization criteria. Pearson correlations were used for unadjusted analyses, and multiple linear regression analyses were used to examine associations controlling for age, body mass index, smoking, ethnicity, urinary creatinine concentration, time of sample collection, and duration of abstinence.

Results: After multivariate adjustment, we observed no significant associations between any semen parameter and urinary BPA concentration. However, a significant inverse association was found between urinary BPA concentration and FAI levels and the FAI/LH ratio, as well as a significant positive association between BPA and SHBG.

Conclusions: Our results suggest that, in fertile men, exposure to low environmental levels of BPA may be associated with a modest reduction in markers of free testosterone, but any effects on reproductive function are likely to be small, and of uncertain clinical significance.

Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study

Tamara Galloway, Riccardo Cipelli, Jack Guralnik, Luigi Ferrucci, Stefania Bandinelli, Anna Maria Corsi, Cathryn Money, Paul McCormack, David Melzer

2010 118(11):1603–1608 | http://dx.doi.org/10.1289/ehp.1002367

Background: Bisphenol A (BPA) is a high production volume chemical widely used in packaging for food and beverages. Numerous studies have demonstrated that BPA can alter endocrine function in animals, yet human studies remain limited.

Objective: We estimated daily excretion of BPA among adults and examined hypothesized associations with serum estrogen and testosterone concentrations.

Methods: We conducted cross-sectional analyses using data from the InCHIANTI Study, a prospective population-based study of Italian adults. Our study included 715 adults between 20 and 74 years old. BPA concentrations were measured by liquid chromatography–mass spectrometry in 24-hr urine samples. The main outcome measures were serum concentrations of total testosterone and 17β-estradiol.

Results: Geometric mean urinary BPA concentration was 3.59 ng/mL [95% confidence interval (CI), 3.42-3.77 ng/mL], and mean excretion was 5.63 µg/day (5th population percentile, 2.1 µg/day; 95th percentile, 16.4 µg/day). We found higher excretion rates among men, younger respondents, and those with increasing waist circumference ($p = 0.013$) and weight ($p = 0.003$). Higher daily BPA excretion was associated with higher total testosterone concentrations in men, in models adjusted for age and study site ($p = 0.044$), and in models additionally adjusted for smoking, measures of obesity, and urinary creatinine concentrations (β = 0.046; 95% CI, 0.015-0.076; $p = 0.004$). We found no associations with the other serum measures. We also found no associations with the primary outcomes among women, but we did find an association between BPA and sex hormone–binding globulin concentrations in the 60 premenopausal women.

Conclusion: Higher BPA exposure may be associated with endocrine changes in men. The mechanisms involved in the observed cross-sectional association with total testosterone concentrations need to be clarified.

Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage

John D. Meeker, Tiffany Yang, Xiaoyun Ye, Antonia M. Calafat, Russ Hauser

2011 119(2):252–257 | http://dx.doi.org/10.1289/ehp.1002238

Background: Parabens are commonly used as antimicrobial preservatives in cosmetics, pharmaceuticals, and food and beverage processing. Widespread human exposure to parabens has been recently documented, and some parabens have demonstrated adverse effects on male reproduction in animal studies. However, human epidemiologic studies are lacking.

Objective: We investigated relationships between urinary concentrations of parabens and markers of male reproductive health in an ongoing reproductive epidemiology study.

Methods: Urine samples collected from male partners attending an infertility clinic were analyzed for methyl paraben (MP), propyl paraben (PP), butyl paraben (BP), and bisphenol A (BPA). Associations with serum hormone levels ($n = 167$), semen quality parameters ($n = 190$), and sperm DNA damage measures ($n = 132$) were assessed using multivariable linear regression.

Results: Detection rates in urine were 100% for MP, 92% for PP, and 32% for BP. We observed no statistically significant associations between MP or PP and the outcome measures. Categories of urinary BP concentration were not associated with hormone levels or conventional semen quality parameters, but they were positively associated with sperm DNA damage (p for trend = 0.03). When urinary BPA quartiles were added to the model, BP and BPA were both positively associated with sperm DNA damage (p for trend = 0.03). Assessment of paraben concentrations measured on repeated urine samples from a subset of the men ($n = 78$) revealed substantial temporal variability.

Conclusions: We found no evidence for a relationship between urinary parabens and hormone levels or semen quality, although intraindividual variability in exposure and a modest sample size could have limited our ability to detect subtle relationships. Our observation of a relationship between BP and sperm DNA damage warrants further investigation.

The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003–2006

Erin M. Rees Clayton, Megan Todd, Jennifer Beam Dowd, Allison E. Aiello

2011 119(3):390–396 | http://dx.doi.org/10.1289/ehp.1002883

Background: Exposure to environmental toxicants is associated with numerous disease outcomes, many of which involve underlying immune and inflammatory dysfunction.

Objective: To address the gap between environmental exposures and immune dysfunction, we investigated the association of two endocrine-disrupting compounds (EDCs) with markers of immune function.

Methods: Using data from the 2003–2006 National Health and Nutrition Examination Survey, we compared urinary bisphenol A (BPA) and triclosan levels with serum cytomegalovirus (CMV) antibody levels and diagnosis of allergies or hay fever in U.S. adults and children ≥ 6 years of age. We used multivariate ordinary least squares linear regression models to examine the association of BPA and triclosan with CMV antibody titers, and multivariate logistic regression models to investigate the association of these chemicals with allergy or hay fever diagnosis. Statistical models were stratified by age (< 18 years and ≥ 18 years).

Results: In analyses adjusted for age, sex, race, body mass index, creatinine levels, family income, and educational attainment, in the ≥ 18-year age group, higher urinary BPA levels were associated with higher CMV antibody titers ($p < 0.001$). In the < 18-year age group, lower levels of BPA were associated with higher CMV antibody titers ($p < 0.05$). However, triclosan, but not BPA, showed a positive association with allergy or hay fever diagnosis. In the < 18-year age group, higher levels of triclosan were associated with greater odds of having been diagnosed with allergies or hay fever ($p < 0.01$).

Conclusions: EDCs such as BPA and triclosan may negatively affect human immune function as measured by CMV antibody levels and allergy or hay fever diagnosis, respectively, with differential consequences based on age. Additional studies should be done to investigate these findings.

Renal function, bisphenol A, and alkylphenols: results from the National Health and Nutrition Examination Survey (NHANES 2003–2006)

Li You, Xiangzhu Zhu, Martha J. Shrubsole, Hong Fan, Jing Chen, Jie Dong, Chuan-Ming Hao, Qi Dai

2011 119(4):527–533 | http://dx.doi.org/10.1289/ehp.1002572

Background: Urinary excretion of bisphenol A (BPA) and alkylphenols (APs) was used as a biomarker in most previous studies, but no study has investigated whether urinary excretion of these environmental phenols differed by renal function.

Objective: We estimated the association between renal function and urinary excretion of BPA and APs.

Methods: Analyses were conducted using data from the National Health and Nutrition Examination Survey (NHANES) 2003-2006. Renal function was measured as estimated glomerular filtration rate (eGFR) calculated by the Modification of Diet in Renal Disease (MDRD) Study equation and by the newly developed Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Regression models were used to calculate geometric means of urinary BPA and APs excretion by eGFR category (≥ 90, 60–90, < 60 mL/min/m^2) after adjusting for potential confounding factors.

Results: When we used the MDRD Study equation, participants without known renal disease ($n = 2,573$), 58.2% ($n = 1,499$) had mildly decreased renal function or undiagnosed chronic kidney disease. The adjusted geometric means for urinary BPA excretion decreased with decreasing levels of eGFR (p for trend = 0.04). The associations appeared primarily in females (p for trend = 0.03). Urinary triclosan excretion decreased with decreasing levels of eGFR (p for trend < 0.01) for both males and females, and the association primarily appeared in participants < 65 years of age. The association between BPA and eGFR was nonsignificant when we used the CKD-EPI equation.

Conclusions: Urinary excretion of triclosan, and possibly BPA, decreased with decreasing renal function. The associations might differ by age or sex. Further studies are necessary to replicate our results and understand the mechanism.

CASE REPORT | High prenatal bisphenol A exposure and infant neonatal neurobehavior

Sheela Sathyanarayana, Joe M. Braun, Kimberly Yolton, Stacey Liddy, Bruce P. Lanphear

2011 119(8):1170–1175 | http://dx.doi.org/10.1289/ehp.1003064

Context: Most of the U.S. population is exposed to the high-production-volume chemical bisphenol A (BPA), but targetable sources of exposure remain to be determined. Animal studies and one human study suggest that BPA is a neurotoxicant.

Case presentation: A mother in the Health Outcomes and Measures of the Environment (HOME) Study, a prospective birth cohort examining prenatal and postnatal environmental toxicants and childhood health outcomes, had a urinary BPA concentration of 583 µg/g creatinine at 27 weeks of pregnancy, which was the highest concentration observed in this cohort (median, 2.0 µg/g creatinine) and the general population. We used prenatal questionnaire data and a follow-up interview to identify potential sources of exposure that included daily plastic use and consumption of canned beverages and foods. Her male infant had a normal newborn neurobehavioral assessment but presented with abnormalities at the 1-month examination that prompted physician referral. Subsequently, the child had normal neurobehavioral testing results at annual evaluations from 1 to 5 years of age.

Discussion: Investigations into sources of high gestational urinary BPA concentrations provide an opportunity to identify potential targets for reduction of BPA exposure. This case highlights a potential link between gestational BPA exposure and transient neurobehavioral changes that is hypothesis generating and can serve to alert researchers to potential areas for examination in future studies.

Relevance to clinical practice: It is important to educate health care practitioners regarding potential sources of BPA exposure and anticipatory guidance on minimization of exposures during vulnerable periods of development.

Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007–2008

John D. Meeker, Kelly K. Ferguson

2011 119(10):1396–1402 | http://dx.doi.org/10.1289/ehp.1103582

Background: Limited animal, *in vitro*, and human studies have reported that exposure to phthalates or bisphenol A (BPA) may affect thyroid signaling.

Objective: We explored the cross-sectional relationship between urinary concentrations of metabolites of di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and BPA with a panel of serum thyroid measures among a representative sample of U.S. adults and adolescents.

Methods: We analyzed data on urinary biomarkers of exposure to phthalates and BPA, serum thyroid measures, and important covariates from 1,346 adults (ages ≥ 20 years) and 329 adolescents (ages 12–19 years) from the National Health and Nutrition Examination Survey (NHANES) 2007–2008 using multivariable linear regression.

Results: Among adults, we observed significant inverse relationships between urinary DEHP metabolites and total thyroxine (T_4), free T_4, total triiodothyronine (T_3), and thyroglobulin, and positive relationships with thyroid-stimulating hormone (TSH). The strongest and most consistent relationships involved total T_4, where adjusted regression coefficients for quintiles of oxidative DEHP metabolites displayed monotonic dose-dependent decreases in total T_4 (p-value for trend < 0.0001). Suggestive inverse relationships between urinary BPA and total T_4 and TSH were also observed. Conversely, among adolescents, we observed significant positive relationships between DEHP metabolites and total T_3. Mono(3-carboxypropyl) phthalate, a secondary metabolite of both DBP and di-*n*-octyl phthalate, was associated with several thyroid measures in both age groups, whereas other DBP metabolites were not associated with thyroid measures.

Conclusions: These results support previous reports of associations between phthalates—and possibly BPA—and altered thyroid hormones. More detailed studies are needed to determine the temporal relationships and potential clinical and public health implications of these associations.

Bisphenol A exposure is associated with *in vivo* estrogenic gene expression in adults

David Melzer, Lorna Harries, Riccardo Cipelli, William Henley, Cathryn Money, Paul McCormack, Anita Young, Jack Guralnik, Luigi Ferrucci, Stefania Bandinelli, Anna Maria Corsi, Tamara Galloway

2011 119(12):1788-1793 | http://dx.doi.org/10.1289/ehp.1103809

Background: Bisphenol A (BPA) is a synthetic estrogen commonly used in polycarbonate plastic and resin-lined food and beverage containers. Exposure of animal and cell models to doses of BPA below the recommended tolerable daily intake (TDI) of 50 µg/kg/day has been shown to alter specific estrogen-responsive gene expression, but this has not previously been shown in humans.

Objective: We investigated associations between BPA exposure and *in vivo* estrogenic gene expression in humans.

Methods: We studied 96 adult men from the InCHIANTI population study and examined *in vivo* expression of six estrogen receptor, estrogen-related receptor, and androgen receptor genes in peripheral blood leukocytes.

Results: The geometric mean urinary BPA concentration was 3.65 ng/mL [95% confidence interval (CI): 3.13, 4.28], giving an estimated mean excretion of 5.84 µg/day (95% CI: 5.00, 6.85), significantly below the current TDI. In age-adjusted models, there were positive associations between higher BPA concentrations and higher *ESR2* [estrogen receptor 2 (ER beta)] expression (unstandardized linear regression coefficient = 0.1804; 95% CI: 0.0388, 0.3221; $p = 0.013$) and *ESRRA* (estrogen-related receptor alpha) expression (coefficient = 0.1718; 95% CI: 0.0213, 0.3223; $p = 0.026$): These associations were little changed after adjusting for potential confounders, including obesity, serum lipid concentrations, and white cell subtype percentages. Upper-tertile BPA excretors (urinary BPA > 4.6 ng/mL) had 65% higher mean *ESR2* expression than did lower-tertile BPA excretors (0–2.4 ng/mL).

Conclusions: Because activation of nuclear-receptor–mediated pathways by BPA is consistently found in laboratory studies, such activation in humans provides evidence that BPA is likely to function as a xenoestrogen in this sample of adults.

Pilot study of urinary biomarkers of phytoestrogens, phthalates, and phenols in girls

Mary S. Wolff, Susan L. Teitelbaum, Gayle Windham, Susan M. Pinney, Julie A. Britton, Carol Chelimo, James Godbold, Frank Biro, Lawrence H. Kushi, Christine M. Pfeiffer, Antonia M. Calafat

2007 115(1):116–121 | http://dx.doi.org/10.1289/ehp.9488

Background: Hormonally active environmental agents have been measured among U.S. children using exposure biomarkers in urine. However, little is known about their variation by race, age, sex, and geography, and no data exist for newly developed biomarkers.

Objective: Our goal was to characterize relevant, prevalent exposures for a study of female pubertal development.

Methods: In a pilot study among 90 girls from New York City, New York, Cincinnati, Ohio, and northern California, we measured 25 urinary analytes representing 22 separate agents from three chemical families: phytoestrogens, phthalates, and phenols. Exposures occur chiefly from the diet and from household or personal care products.

Results: Participants represented four racial/ethnic groups (Asian, black, Hispanic, white), with mean age of 7.77 years. Most analytes were detectable in > 94% of samples. The highest median concentrations for individual analytes in each family were for enterolactone (298 µg/L), monoethylphthalate (MEP; 83.2 µg/L), and benzophenone-3 (BP3; 14.7 µg/L). Few or no data have been reported previously for four metabolites: mono(2-ethyl-5-carboxypentyl) phthalate, triclosan, bisphenol A (BPA), and BP3; these were detected in 67–100% of samples with medians of 1.8–53.2 µg/L. After multivariate adjustment, two analytes, enterolactone and BPA, were higher among girls with body mass index < 85th reference percentile than those at or above the 85th percentile. Three phthalate metabolites differed by race/ethnicity [MEP, mono(2-ethylhexyl) phthalate, and mono-3-carboxypropylphthalate].

Conclusions: A wide spectrum of hormonally active exposure biomarkers were detectable and variable among young girls, with high maximal concentrations (> 1,000 µg/L) found for several analytes. They varied by characteristics that may be relevant to development.

Exposure of the U.S. population to bisphenol A and 4-*tertiary*-octylphenol: 2003–2004

Antonia M. Calafat, Xiaoyun Ye, Lee-Yang Wong, John A. Reidy, Larry L. Needham

2008 116(1):39–44 | http://dx.doi.org/10.1289/ehp.10753

Background: Bisphenol A (BPA) and 4-*tertiary*-octylphenol (tOP) are industrial chemicals used in the manufacture of polycarbonate plastics and epoxy resins (BPA) and nonionic surfactants (tOP). These products are in widespread use in the United States.

Objective: We aimed to assess exposure to BPA and tOP in the U.S. general population.

Methods: We measured the total (free plus conjugated) urinary concentrations of BPA and tOP in 2,517 participants ≥ 6 years of age in the 2003–2004 National Health and Nutrition Examination Survey using automated solid-phase extraction coupled to isotope dilution–high-performance liquid chromatography–tandem mass spectrometry.

Results: BPA and tOP were detected in 92.6% and 57.4% of the persons, respectively. Least square geometric mean (LSGM) concentrations of BPA were significantly lower in Mexican Americans than in non-Hispanic blacks ($p = 0.006$) and non-Hispanic whites ($p = 0.007$); LSGM concentrations for non-Hispanic blacks and non-Hispanic whites were not statistically different ($p = 0.21$). Females had statistically higher BPA LSGM concentrations than males ($p = 0.043$). Children had higher concentrations than adolescents ($p < 0.001$), who in turn had higher concentrations than adults ($p = 0.003$). LSGM concentrations were lowest for participants in the high household income category (> $45,000/year).

Conclusions: Urine concentrations of total BPA differed by race/ethnicity, age, sex, and household income. These first U.S. population representative concentration data for urinary BPA and tOP should help guide public health research priorities, including studies of exposure pathways, potential health effects, and risk assessment.

Temporal variability and predictors of urinary bisphenol A concentrations in men and women

Shruthi Mahalingaiah, John D. Meeker, Kimberly R. Pearson, Antonia M. Calafat, Xiaoyun Ye, John Petrozza, Russ Hauser

2008 116(2):173–178 | http://dx.doi.org/10.1289/ehp.10605

Background: Bisphenol A (BPA) is used to manufacture polymeric materials, such as polycarbonate plastics, and is found in a variety of consumer products. Recent data show widespread BPA exposure among the U.S. population.

Objective: Our goal in the present study was to determine the temporal variability and predictors of BPA exposure.

Methods: We measured urinary concentrations of BPA among male and female patients from the Massachusetts General Hospital Fertility Center.

Results: Between 2004 and 2006, 217 urine samples were collected from 82 subjects: 45 women (145 samples) and 37 men (72 samples). Of these, 24 women and men were partners and contributed 42 pairs of samples collected on the same day. Ten women became pregnant during the follow-up period. Among the 217 urine samples, the median BPA concentration was 1.20 µg/L, ranging from below the limit of detection (0.4 µg/L) to 42.6 µg/L. Age, body mass index, and sex were not significant predictors of urinary BPA concentrations. BPA urinary concentrations among pregnant women were 26% higher (–26%, +115%) than those among the same women when not pregnant ($p > 0.05$). The urinary BPA concentrations of the female and male partner on the same day were correlated ($r = 0.36$; $p = 0.02$). The sensitivity of classifying a subject in the highest tertile using a single urine sample was 0.64.

Conclusion: We found a nonsignificant increase in urinary BPA concentrations in women while pregnant compared with nonpregnant samples from the same women. Samples collected from partners on the same day were correlated, suggesting shared sources of exposure. Finally, a single urine sample showed moderate sensitivity for predicting a subject's tertile categorization.

Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants

Antonia M. Calafat, Jennifer Weuve, Xiaoyun Ye, Lily T. Jia, Howard Hu, Steven Ringer, Ken Huttner, Russ Hauser

2009 117(4):639–644 | http://dx.doi.org/10.1289/ehp.0800265

Objective: We previously demonstrated that exposure to polyvinyl chloride plastic medical devices containing di(2-ethylhexyl) phthalate (DEHP) was associated with higher urinary concentrations of several DEHP metabolites in 54 premature infants in two neonatal intensive care units than in the general population. For 42 of these infants, we evaluated urinary concentrations of several phenols, including bisphenol A (BPA), in association with the use of the same medical devices.

Measurements: We measured the urinary concentrations of free and total (free plus conjugated) species of BPA, triclosan, benzophenone-3, methyl paraben, and propyl paraben.

Results: The percentage of BPA present as its conjugated species was > 90% in more than three-quarters of the premature infants. Intensity of use of products containing DEHP was strongly associated with BPA total concentrations but not with any other phenol. Adjusting for institution and sex, BPA total concentrations among infants in the group of high use of DEHP-containing products were 8.75 times as high as among infants in the low use group ($p < 0.0001$). Similarly, after adjusting for sex and DEHP-containing product use category, BPA total concentrations among infants in Institution A were 16.6 times as high as those among infants in Institution B ($p < 0.0001$).

Conclusion: BPA geometric mean urinary concentration (30.3 µg/L) among premature infants undergoing intensive therapeutic medical interventions was one order of magnitude higher than that among the general population. Conjugated species were the primary urinary metabolites of BPA, suggesting that premature infants have some capacity to metabolize BPA. The differences in exposure to BPA by intensity of use of DEHP-containing medical products highlight the need for further studies to determine the specific source(s) of exposure to BPA.

Predicting plasma concentrations of bisphenol A in children younger than 2 years of age after typical feeding schedules, using a physiologically based toxicokinetic model

Andrea N. Edginton, Len Ritter

2009 117(4):645–652 | http://dx.doi.org/10.1289/ehp.0800073

Background: Concerns have recently been raised regarding the safety of potential human exposure to bisphenol A (BPA), an industrial chemical found in some polycarbonate plastics and epoxy resins. Of particular interest is the exposure of young children to BPA via food stored in BPA-containing packaging.

Objectives: In this study we assessed the age dependence of the toxicokinetics of BPA and its glucuronidated metabolite, BPA-Glu, using a coupled BPA–BPA-Glu physiologically based toxicokinetic (PBTK) model.

Methods: Using information gathered from toxicokinetic studies in adults, we built a PBTK model. We then scaled the model to children < 2 years of age based on the age dependence of physiologic parameters relevant for absorption, distribution, metabolism, and excretion.

Results: We estimated the average steady-state BPA plasma concentration in newborns to be 11 times greater than that in adults when given the same weight-normalized dose. Because of the rapid development of the glucuronidation process, this ratio dropped to 2 by 3 months of age. Simulation of typical feeding exposures, as estimated by regulatory authorities, showed a 5-fold greater steady-state BPA plasma concentration in 3- and 6-month-olds compared with adults, reflecting both a reduced capacity for BPA metabolism and a greater weight-normalized BPA exposure. Because of uncertainty in defining the hepatic BPA intrinsic clearance in adults, these values represent preliminary estimates.

Conclusions: Simulations of the differential BPA dosimetry between adults and young children point to the need for more sensitive analytical methods for BPA to define, with greater certainty, the adult hepatic BPA intrinsic clearance, as well as a need for external exposure data in young children.

COMMENTARY | What additional factors beyond state-of-the-art analytical methods are needed for optimal generation and interpretation of biomonitoring data?

Antonia M. Calafat, Larry L. Needham

2009 117(10):1481–1485 | http://dx.doi.org/10.1289/ehp.0901108

Background: The routine use of biomonitoring (i.e., measurement of environmental chemicals, their metabolites, or specific reaction products in human biological specimens) to assess internal exposure (i.e., body burden) has gained importance in exposure assessment.

Objectives: Selection and validation of biomarkers of exposure are critical factors in interpreting biomonitoring data. Moreover, the strong relation between quality of the analytical methods used for biomonitoring and quality of the resulting data is well understood. However, the relevance of collecting, storing, processing, and transporting the samples to the laboratory to the overall biomonitoring process has received limited attention, especially for organic chemicals.

Discussion: We present examples to illustrate potential sources of unintended contamination of the biological specimen during collection or processing procedures. The examples also highlight the importance of ensuring that the biological specimen analyzed both represents the sample collected for biomonitoring purposes and reflects the exposure of interest.

Conclusions: Besides using high-quality analytical methods and good laboratory practices for biomonitoring, evaluation of the collection and handling of biological samples should be emphasized, because these procedures can affect the samples integrity and representativeness. Biomonitoring programs would be strengthened with the inclusion of field blanks.

Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both

Richard W. Stahlhut, Wade V. Welshons, Shanna H. Swan

2009 117(5):784–789 | http://dx.doi.org/10.1289/ehp.0800376

Background: It is commonly stated in the literature on human exposure to bisphenol A (BPA) that food is the predominant BPA exposure source, and that BPA is rapidly and completely cleared from the body. If this is correct, BPA levels in fasting individuals should decrease with increased fasting time.

Objective: We set out to investigate the relationship between urine BPA concentration and fasting time in a population-based sample.

Methods: We modeled log BPA urine concentration as a function of fasting time, adjusted for urine creatinine and other confounders, in 1,469 adult participants in the 2003–2004 National Health and Nutrition Examination Survey. We estimated the BPA "population-based half-life" (pop½) for a fasting time of 0–24 hr, < 4.5 hr, 4.5–8.5 hr, and > 8.5 hr.

Results: The overall pop½ for the 0- to 24-hr interval was 43 hr [95% confidence interval (CI), 26–119 hr]. Among those reporting fasting times of 4.5–8.5 hr ($n = 441$), BPA declined significantly with fasting time, with a pop½ of 4.1 hr (95% CI, 2.6–10.6 hr). However, within the fasting time intervals of 0–4.5 hr ($n = 129$) and 8.5–24 hr ($n = 899$), we saw no appreciable decline. Fasting time did not significantly predict highest (> 12 ng/mL) or lowest (below limit of detection) BPA levels.

Conclusions: Overall, BPA levels did not decline rapidly with fasting time in this sample. This suggests substantial nonfood exposure, accumulation in body tissues such as fat, or both. Explaining these findings may require experimental pharmacokinetic studies of chronic BPA exposure, further examination of BPA levels and effects in fat, and a search for important nonfood sources.

Polycarbonate bottle use and urinary bisphenol A concentrations

Jenny L. Carwile, Henry T. Luu, Laura S. Bassett, Daniel A. Driscoll, Caterina Yuan, Jennifer Y. Chang, Xiaoyun Ye, Antonia M. Calafat, Karin B. Michels

2009 117(9):1368–1372 | http://dx.doi.org/10.1289/ehp.0900604

Background: Bisphenol A (BPA) is a high-production-volume chemical commonly used in the manufacture of polycarbonate plastic. Low-level concentrations of BPA in animals and possibly in humans may cause endocrine disruption. Whether ingestion of food or beverages from polycarbonate containers increases BPA concentrations in humans has not been studied.

Objective: We examined the association between use of polycarbonate beverage containers and urinary BPA concentrations in humans.

Methods: We conducted a nonrandomized intervention of 77 Harvard College students to compare urinary BPA concentrations collected after a washout phase of 1 week to those taken after an intervention week during which most cold beverages were consumed from polycarbonate drinking bottles. Paired *t*-tests were used to assess the difference in urinary BPA concentrations before and after polycarbonate bottle use.

Results: The geometric mean urinary BPA concentration at the end of the washout phase was 1.2 µg/g creatinine, increasing to 2.0 µg/g creatinine after 1 week of polycarbonate bottle use. Urinary BPA concentrations increased by 69% after use of polycarbonate bottles ($p < 0.0001$). The association was stronger among participants who reported ≥ 90% compliance (77% increase; $p < 0.0001$) than among those reporting < 90% compliance (55% increase; $p = 0.03$), but this difference was not statistically significant ($p = 0.54$).

Conclusions: One week of polycarbonate bottle use increased urinary BPA concentrations by two-thirds. Regular consumption of cold beverages from polycarbonate bottles is associated with a substantial increase in urinary BPA concentrations irrespective of exposure to BPA from other sources.

Variability and predictors of urinary bisphenol A concentrations during pregnancy

Joe M. Braun, Amy E. Kalkbrenner, Antonia M. Calafat, John T. Bernert, Xiaoyun Ye, Manori J. Silva, Dana Boyd Barr, Sheela Sathyanarayana, Bruce P. Lanphear

2011 119(1):131–137 | http://dx.doi.org/10.1289/ehp.1002366

Background: Prenatal bisphenol A (BPA) exposure may be associated with developmental toxicity, but few studies have examined the variability and predictors of urinary BPA concentrations during pregnancy.

Objective: Our goal was to estimate the variability and predictors of serial urinary BPA concentrations taken during pregnancy.

Methods: We measured BPA concentrations during pregnancy and at birth in three spot urine samples from 389 women. We calculated the intraclass correlation coefficient (ICC) to assess BPA variability and estimated associations between $\log_{10}$-transformed urinary BPA concentrations and demographic, occupational, dietary, and environmental factors, using mixed models.

Results: Geometric mean (GM) creatinine-standardized concentrations (micrograms per gram) were 1.7 (16 weeks), 2.0 (26 weeks), and 2.0 (birth). Creatinine-standardized BPA concentrations exhibited low reproducibility (ICC = 0.11). By occupation, cashiers had the highest BPA concentrations (GM: 2.8 µg/g). Consuming canned vegetables at least once a day was associated with higher BPA concentrations (GM = 2.3 µg/g) compared with those consuming no canned vegetables (GM = 1.6 µg/g). BPA concentrations did not vary by consumption of fresh fruits and vegetables, canned fruit, or store-bought fresh and frozen fish. Urinary high-molecular-weight phthalate and serum tobacco smoke metabolite concentrations were positively associated with BPA concentrations.

Conclusions: These results suggest numerous sources of BPA exposure during pregnancy. Etiological studies may need to measure urinary BPA concentrations more than once during pregnancy and adjust for phthalates and tobacco smoke exposures.

Environmental chemicals in pregnant women in the United States: NHANES 2003–2004

Tracey J. Woodruff, Ami R. Zota, Jackie M. Schwartz

2011 119(6):878–885 | http://dx.doi.org/10.1289/ehp.1002727

Background: Exposure to chemicals during fetal development can increase the risk of adverse health effects, and while biomonitoring studies suggest pregnant women are exposed to chemicals, little is known about the extent of multiple chemicals exposures among pregnant women in the United States.

Objective: We analyzed biomonitoring data from the National Health and Nutritional Examination Survey (NHANES) to characterize both individual and multiple chemical exposures in U.S. pregnant women.

Methods: We analyzed data for 163 chemical analytes in 12 chemical classes for subsamples of 268 pregnant women from NHANES 2003–2004, a nationally representative sample of the U.S. population. For each chemical analyte, we calculated descriptive statistics. We calculated the number of chemicals detected within the following chemical classes: polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), organochlorine pesticides, and phthalates and across multiple chemical classes. We compared chemical analyte concentrations for pregnant and nonpregnant women using least-squares geometric means, adjusting for demographic and physiological covariates.

Results: The percentage of pregnant women with detectable levels of an individual chemical ranged from 0 to 100%. Certain polychlorinated biphenyls, organochlorine pesticides, PFCs, phenols, PBDEs, phthalates, polycyclic aromatic hydrocarbons, and perchlorate were detected in 99–100% of pregnant women. The median number of detected chemicals by chemical class ranged from 4 of 12 PFCs to 9 of 13 phthalates. Across chemical classes, median number ranged from 8 of 17 chemical analytes to 50 of 71 chemical analytes. We found, generally, that levels in pregnant women were similar to or lower than levels in nonpregnant women; adjustment for covariates tended to increase levels in pregnant women compared with nonpregnant women.

Conclusions: Pregnant women in the U.S. are exposed to multiple chemicals. Further efforts are warranted to understand sources of exposure and implications for policy making.

Food packaging and bisphenol A and bis(2-ethyhexyl) phthalate exposure: findings from a dietary intervention

Ruthann A. Rudel, Janet M. Gray, Connie L. Engel, Teresa W. Rawsthorne, Robin E. Dodson, Janet M. Ackerman, Jeanne Rizzo, Janet L. Nudelman, Julia Green Brody

2011 119(7):914–920 | http://dx.doi.org/10.1289/ehp.1003170

Background: Bisphenol A (BPA) and bis(2-ethylhexyl) phthalate (DEHP) are high-production-volume chemicals used in plastics and resins for food packaging. They have been associated with endocrine disruption in animals and in some human studies. Human exposure sources have been estimated, but the relative contribution of dietary exposure to total intake has not been studied empirically.

Objective: To evaluate the contribution of food packaging to exposure, we measured urinary BPA and phthalate metabolites before, during, and after a "fresh foods" dietary intervention.

Methods: We selected 20 participants in five families based on self-reported use of canned and packaged foods. Participants ate their usual diet, followed by 3 days of "fresh foods" that were not canned or packaged in plastic, and then returned to their usual diet. We collected evening urine samples over 8 days in January 2010 and composited them into preintervention, during intervention, and postintervention samples. We used mixed-effects models for repeated measures and Wilcoxon signed-rank tests to assess change in urinary levels across time.

Results: Urine levels of BPA and DEHP metabolites decreased significantly during the fresh foods intervention [e.g., BPA geometric mean (GM), 3.7 ng/mL preintervention vs. 1.2 ng/mL during intervention; mono-(2-ethyl-5-hydroxy hexyl) phthalate GM, 57 ng/mL vs. 25 ng/mL]. The intervention reduced GM concentrations of BPA by 66% and DEHP metabolites by 53–56%. Maxima were reduced by 76% for BPA and 93–96% for DEHP metabolites.

Conclusions: BPA and DEHP exposures were substantially reduced when participants' diets were restricted to food with limited packaging.

Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections

Xiaoyun Ye, Lee-Yang Wong, Amber M. Bishop, Antonia M. Calafat

2011 119(7):983–988 | http://dx.doi.org/10.1289/ehp.1002701

Background: Human exposure to bisphenol A (BPA) is widespread. After exposure, BPA is rapidly metabolized and eliminated in urine. Therefore, there is considerable within-person and between-person variability of BPA concentrations in spot urine samples. However, no information exists on the within-day variability of urinary BPA concentrations.

Objective: We examined the between-person and within-person and between-day and within-day variability in the urinary BPA concentrations of eight adults who collected all voids for 1 week to investigate the impact of sampling strategy in the exposure assessment of BPA using spot, first morning, or 24-hr urine collections.

Methods: We determined the urinary concentrations of BPA using on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography/tandem mass spectrometry.

Results: The between-day and within-person variability was the primary contributor to the total variance both for first morning voids (77%) and 24-hr urine collections (88%). For the spot collections, we observed considerable within-day variance (70%), which outweighed the between-person (9%) and between-day and within-person (21%) variances.

Conclusions: Regardless of the type of void (spot, first morning, 24-hr collection), urinary BPA concentrations for a given adult changed considerably—both within a day and for the 7 days of the study period. Single 24-hr urine collections accurately reflect daily exposure but can misrepresent variability in daily exposures over time. Of interest, when the population investigated is sufficiently large and samples are randomly collected relative to meal ingestion times and bladder emptying times, the single spot–sampling approach may adequately reflect the average exposure of the population to BPA.

Most plastic products release estrogenic chemicals: a potential health problem that can be solved

Chun Z. Yang, Stuart I. Yaniger, V. Craig Jordan, Daniel J. Klein, George D. Bittner

2011 119(7):989-996 | http://dx.doi.org/10.1289/ehp.1003220

Background: Chemicals having estrogenic activity (EA) reportedly cause many adverse health effects, especially at low (picomolar to nanomolar) doses in fetal and juvenile mammals.

Objective: We sought to determine whether commercially available plastic resins and products, including baby bottles and other products advertised as bisphenol A (BPA) free, release chemicals having EA.

Methods: We used a roboticized MCF-7 cell proliferation assay, which is very sensitive, accurate, and repeatable, to quantify the EA of chemicals leached into saline or ethanol extracts of many types of commercially available plastic materials, some exposed to common-use stresses (microwaving, ultraviolet radiation, and/or autoclaving).

Results: Almost all commercially available plastic products we sampled—independent of the type of resin, product, or retail source—leached chemicals having reliably detectable EA, including those advertised as BPA free. In some cases, BPA-free products released chemicals having more EA than did BPA-containing products.

Conclusions: Many plastic products are mischaracterized as being EA free if extracted with only one solvent and not exposed to common-use stresses. However, we can identify existing compounds, or have developed, monomers, additives, or processing agents that have no detectable EA and have similar costs. Hence, our data suggest that EA-free plastic products exposed to common-use stresses and extracted by saline and ethanol solvents could be cost-effectively made on a commercial scale and thereby eliminate a potential health risk posed by most currently available plastic products that leach chemicals having EA into food products.

REVIEW | Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A

Laura N. Vandenberg, Ibrahim Chahoud, Jerrold J. Heindel, Vasantha Padmanabhan, Francisco J.R. Paumgartten, Gilbert Schoenfelder

2010 118(8):1055–1070 | http://dx.doi.org/10.1289/ehp.0901716

Background: Bisphenol A (BPA) is one of the highest-volume chemicals produced worldwide, and human exposure to BPA is thought to be ubiquitous. Thus, there are concerns that the amount of BPA to which humans are exposed may cause adverse health effects. Importantly, results from a large number of biomonitoring studies are at odds with the results from two toxicokinetic studies.

Objective: We examined several possibilities for why biomonitoring and toxicokinetic studies could come to seemingly conflicting conclusions.

Data sources: We examined > 80 published human biomonitoring studies that measured BPA concentrations in human tissues, urine, blood, and other fluids, along with two toxicokinetic studies of human BPA metabolism.

Data extraction and synthesis: The > 80 biomonitoring studies examined included measurements in thousands of individuals from several different countries, and these studies overwhelmingly detected BPA in individual adults, adolescents, and children. Unconjugated BPA was routinely detected in blood (in the nanograms per milliliter range), and conjugated BPA was routinely detected in the vast majority of urine samples (also in the nanograms per milliliter range). In stark contrast, toxicokinetic studies proposed that humans are not internally exposed to BPA. Some regulatory agencies have relied solely on these toxicokinetic models in their risk assessments.

Conclusions: Available data from biomonitoring studies clearly indicate that the general population is exposed to BPA and is at risk from internal exposure to unconjugated BPA. The two toxicokinetic studies that suggested human BPA exposure is negligible have significant deficiencies, are directly contradicted by hypothesis-driven studies, and are therefore not reliable for risk assessment purposes.

COMMENTARY | Why public health agencies cannot depend on good laboratory practices as a criterion for selecting data: the case of bisphenol A

John Peterson Myers, Frederick S. vom Saal, Benson T. Akingbemi, Koji Arizono, Scott Belcher, Theo Colborn, Ibrahim Chahoud, D. Andrew Crain, Francesca Farabollini, Louis J. Guillette, Jr., Terry Hassold, Shuk-mei Ho, Patricia A. Hunt, Taisen Iguchi, Susan Jobling, Jun Kanno, Hans Laufer, Michele Marcus, John A. McLachlan, Angel Nadal, Jörg Oehlmann, Nicolás Olea, Paola Palanza, Stefano Parmigiani, Beverly S. Rubin, Gilbert Schoenfelder, Carlos Sonnenschein, Ana M. Soto, Chris E. Talsness, Julia A. Taylor, Laura N. Vandenberg, John G. Vandenbergh, Sarah Vogel, Cheryl S. Watson, Wade V. Welshons, R. Thomas Zoeller

2009 117(3):309–315 | http://dx.doi.org/10.1289/ehp.0800173

Background: In their safety evaluations of bisphenol A (BPA), the U.S. Food and Drug Administration (FDA) and a counterpart in Europe, the European Food Safety Authority (EFSA), have given special prominence to two industry-funded studies that adhered to standards defined by Good Laboratory Practices (GLP). These same agencies have given much less weight in risk assessments to a large number of independently replicated non-GLP studies conducted with government funding by the leading experts in various fields of science from around the world.

Objectives: We reviewed differences between industry-funded GLP studies of BPA conducted by commercial laboratories for regulatory purposes and non-GLP studies conducted in academic and government laboratories to identify hazards and molecular mechanisms mediating adverse effects. We examined the methods and results in the GLP studies that were pivotal in the draft decision of the U.S. FDA declaring BPA safe in relation to findings from studies that were competitive for U.S. National Institutes of Health (NIH) funding, peer-reviewed for publication in leading journals, subject to independent replication, but rejected by the U.S. FDA for regulatory purposes.

Discussion: Although the U.S. FDA and EFSA have deemed two industry-funded GLP studies of BPA to be superior to hundreds of studies funded by the U.S. NIH and NIH counterparts in other countries, the GLP studies on which the agencies based their decisions have serious conceptual and methodologic flaws. In addition, the U.S. FDA and EFSA have mistakenly assumed that GLP yields valid and reliable scientific findings (i.e., "good science"). Their rationale for favoring GLP studies over hundreds of publically funded studies ignores the central factor in determining the reliability and validity of scientific findings, namely, independent replication, and use of the most appropriate and sensitive state-of-the-art assays, neither of which is an expectation of industry-funded GLP research.

Conclusions: Public health decisions should be based on studies using appropriate protocols with appropriate controls and the most sensitive assays, not GLP. Relevant NIH-funded research using state-of-the-art techniques should play a prominent role in safety evaluations of chemicals.

COMMENTARY | Basic exploratory research versus guideline-compliant studies used for hazard evaluation and risk assessment: bisphenol A as a case study

Rochelle W. Tyl

2009 117(11):1644–1651 | http://dx.doi.org/10.1289/ehp.0900893

Background: Myers et al. [Environ Health Perspect 117:309–315 (2009)] argued that Good Laboratory Practices (GLPs) cannot be used as a criterion for selecting data for risk assessment, using bisphenol A (BPA) as a case study. They did not discuss the role(s) of guideline-compliant studies versus basic/exploratory research studies, and they criticized both GLPs and guideline-compliant studies and their roles in formal hazard evaluation and risk assessment. They also specifically criticized our published guideline-compliant dietary studies on BPA in rats and mice and 17β-estradiol (E_2) in mice.

Objectives: As the study director/first author of the criticized E_2 and BPA studies, I discuss the uses of basic research versus guideline-compliant studies, how testing guidelines are developed and revised, how new end points are validated, and the role of GLPs. I also provide an overview of the BPA guideline-compliant and exploratory research animal studies and describe BPA pharmacokinetics in rats and humans. I present responses to specific criticisms by Myers et al.

Discussion and conclusions: Weight-of-evidence evaluations have consistently concluded that low-level BPA oral exposures do not adversely affect human developmental or reproductive health, and I encourage increased validation efforts for "new" end points for inclusion in guideline studies, as well as performance of robust long-term studies to follow early effects (observed in small exploratory studies) to any adverse consequences.

COMMENTARY | A clash of old and new scientific concepts in toxicity, with important implications for public health

John Peterson Myers, R. Thomas Zoeller, Frederick S. vom Saal

2009 117(11):1652–1655 | http://dx.doi.org/10.1289/ehp.0900887

Background: A core assumption of current toxicologic procedures used to establish health standards for chemical exposures is that testing the safety of chemicals at high doses can be used to predict the effects of low-dose exposures, such as those common in the general population. This assumption is based on the precept that "the dose makes the poison": higher doses will cause greater effects.

Objectives: We challenge the validity of assuming that high-dose testing can be used to predict low-dose effects for contaminants that behave like hormones. We review data from endocrinology and toxicology that falsify this assumption and summarize current mechanistic understanding of how low doses can lead to effects unpredictable from high-dose experiments.

Discussion: Falsification of this assumption raises profound issues for regulatory toxicology. Many exposure standards are based on this assumption. Rejecting the assumption will require that these standards be reevaluated and that procedures employed to set health standards be changed. The consequences of these changes may be significant for public health because of the range of health conditions now plausibly linked to exposure to endocrine-disrupting contaminants.

Conclusions: We recommend that procedures to establish acceptable exposure levels for endocrine-disrupting compounds incorporate the inability for high-dose tests to predict low-dose results. Setting acceptable levels of exposure must include testing for health consequences at prevalent levels of human exposure, not extrapolations from the effects observed in high-dose experiments. Scientists trained in endocrinology must be engaged systematically in standard setting for endocrine-disrupting compounds.

COMMENTARY | Does rapid metabolism ensure negligible risk from bisphenol A?

Gary Ginsberg, Deborah C. Rice

2009 117(11):1639–1643 | http://dx.doi.org/10.1289/ehp.0901010

Background: Bisphenol A (BPA) risks are being evaluated by many regulatory bodies because exposure is widespread and the potential exists for toxicity at low doses.

Objective: We evaluated evidence that BPA is cleared more rapidly in humans than in rats in relation to BPA risk assessment.

Discussion: The European Food Safety Authority (EFSA) relied on pharmacokinetic evidence to conclude that rodent toxicity data are not directly relevant to human risk assessment. Further, the EFSA argues that rapid metabolism will result in negligible exposure during the perinatal period because of BPA glucuronidation in pregnant women or sulfation in newborns. These arguments fail to consider the deconjugation of BPA glucuronide *in utero* by β-glucuronidase, an enzyme that is present in high concentrations in placenta and various other tissues. Further, arylsulfatase C, which reactivates endogenous sulfated estrogens, develops early in life and so may deconjugate BPA sulfate in newborns. Biomonitoring studies and laboratory experiments document free BPA in rat and human maternal, placental, and fetal tissues, indicating that human BPA exposure is not negligible. The pattern of these detections is consistent with deconjugation in the placenta, resulting in fetal exposure. The tolerable daily intake set by the EFSA (0.05 mg/kg/day) is well above effect levels reported in some animal studies.

Conclusion: This potential risk should not be dismissed on the basis of an uncertain pharmacokinetic argument. Rather, risk assessors need to decipher the BPA dose response and apply it to humans with comprehensive pharmacokinetic models that account for metabolite deconjugation.

COMMENTARY | Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A

Laura N. Vandenberg, Ibrahim Chahoud, Vasantha Padmanabhan, Francisco J.R. Paumgartten, Gilbert Schoenfelder

2010 118(8):1051–1054 | http://dx.doi.org/10.1289/ehp.0901717

Background: Within the past 3 years, four major evaluations of bisphenol A (BPA) safety have been undertaken. However, these assessments have arrived at quite different conclusions regarding the safety of BPA at current human exposure levels.

Objective: We compared the reasons provided by the European Food Safety Authority (EFSA) BPA risk assessment panel for their conclusion that human exposures are negligible with the conclusions reached by the other panels, with all panels having the same body of literature at their disposal.

Discussion: The EFSA panel dismissed ≥ 80 biomonitoring studies that documented significant levels of BPA exposure in humans, including internal exposures to unconjugated BPA, on the basis that they did not match a model of BPA metabolism. Instead, the EFSA panel relied on two toxicokinetic studies—conducted in 15 adults administered BPA—to draw conclusions about exposure levels in the population, including exposures of neonates.

Conclusions: As with all exposure assessments, models should be developed to explain actual data that are collected. In the case of BPA, samples from a large number of human subjects clearly indicate that humans are internally exposed to unconjugated BPA. The dismissal of these biomonitoring studies simply because their results do not conform to a model violates scientific principles. Expert panels should evaluate all data—including human biomonitoring studies—to make informed risk assessments.

Environmental Health Perspectives (ISSN 0091-6765) is a peer-reviewed publication of the Public Health Service, U.S. Department of Health and Human Services. *EHP* is an open-access monthly journal of peer-reviewed research and news on the impact of the environment on human health. *EHP* also publishes a quarterly *Chinese Edition* (ISSN 1542-6351) and occasional special issues. The Secretary of Health and Human Services has deemed *EHP* to be necessary in the transaction of the public business required by law of this department.

Correspondence: Send all correspondence to Hugh A. Tilson, PhD, Editor-in-Chief, *EHP*, NIEHS/NIH, Mail Drop K3-01, PO Box 12233, Research Triangle Park, NC 27709 USA; phone: 919-541-3280; e-mail: EHPEditor@niehs.nih.gov.

Submissions: See Instructions to Authors online at http://www.ehponline.org/instructions/.

Subscriptions: Call 1-866-541-3841, or go online to http://www.ehponline.org/subscribe.

Address Changes: Send your mailing label(s) for each periodical with corrected information or a description of your subscription problem to Brogan & Partners, Attn: Order Processing, 14600 Weston Parkway, Suite 100, Cary, NC 27513 USA; fax: 919-678-8696; phone (toll-free) 1-866-541-3841.