

The correspondence section is a public forum and, as such, is not peer-reviewed. EHP is not responsible for the accuracy, currency, or reliability of personal opinion expressed herein; it is the sole responsibility of the authors. EHP neither endorses nor disputes their published commentary.

### In Vitro Detection of Estrogen Activity in Plastic Products Using a Sensitive Bioassay: Failure to Acknowledge Limitations

doi:10.1289/ehp.1103894

Yang et al. (2011) used the *in vitro* E-SCREEN assay to infer that health risks from “estrogenic” plastics can be eliminated by using their proprietary materials, processes, and products to manufacture plastics. An *in vitro* cell proliferation assay such as the E-SCREEN is a sensitive indicator of *in vitro* estrogen agonist activity and potential estrogenic activity *in vivo* (e.g., in the rat uterotrophic assay). However, *in vitro* properties may not manifest in *in vivo* activity, and neither demonstrates a health risk. Without definitive evidence that *in vivo* activity leads to adverse health effects, the results of Yang et al. are unconvincing and fail to support changing current manufacturing processes for plastics.

The value of *in vitro* and *in vivo* estrogenic assays for predicting adverse health effects is largely untested but would need to account for actual exposure levels, metabolism, distribution, excretion, and the affinity of parent compounds and metabolites for estrogen receptor binding and transcriptional activation relative to and in competition with physiological levels of potent endogenous hormones. The combined effects of these exposures would also need to be assessed in the context of dietary (e.g., milk, cheeses, vegetables, meats, and other foodstuffs) and environmental estrogens. An excellent *in vitro/in vivo* study of combined effects (Charles et al. 2007) showed that while relatively high levels of a putative synthetic estrogen mixture increased the estrogenic action of common dietary phytoestrogens, low levels were without effect. Thus, sensitive *in vitro* detection may not portend estrogenic effects amid the endogenous and dietary hormonal milieu.

Yang et al. (2011) made inferences about the safety of plastic food packages, but it is unfortunate that they did not use an extraction method that was approved by the U.S. Food and Drug Administration (FDA 2007). This would have improved the reliability and applicability of their results. Although food typically contacts only the inside surface of containers, Yang et al. extracted materials from 4-mm squares of cut plastic, exposing the inside, outside, and cut surfaces to the extraction medium. Substances may leach into food from the exposed surface of a plastic container but do not typically migrate

through the plastic layer (Franz and Welle 2009); thus Yang et al.’s extraction method differs from FDA-approved methods and the way foods normally contact containers. Experimental error was not reported, making comparison of these results with standard methods impossible.

In the study by Yang et al. (2011), irradiation methods for simulating “stress” were not well characterized, but they appear to have involved all surfaces of the plastic squares. However, even clear plastics can filter ultraviolet (UV) rays, reducing the potential irradiation of inside container surfaces. Similarly, colorants were added to the extraction mixture; however, during the production of plastics, colorants are embedded and tightly linked. The extent to which these procedures may have confounded the data cannot be known, but the resulting tested extracts may be substantially different from residues that could enter food from plastic containers.

Yang et al. (2011) indicated that without increasing production costs, they can identify and/or have developed monomers, additives, and processing agents that lack estrogenic activity. This conclusion appears to derive from data for resins P1, P2, P3, P4, P19, and maybe P18 in their Table 3. In the text the authors noted six MCF-7 assays, but it is unclear whether a single assay was conducted for each of the six stressor and extraction combinations (microwave, UV, autoclave, saline, and ethanol) or whether the whole series was completed six times. Regardless, the authors provided no estimate of assay variance, making it difficult to differentiate real differences from experimental error. In addition, the relative safety of these new agents, particularly antiandrogenic potential, has yet to be resolved.

In conclusion, Yang et al. (2011) provided interesting observations but failed to acknowledge the significant limitations of their observations to human health risk assessment. They relied on a very limited *in vitro* screen to model a very complex system, and those reviewing the study should be aware of the limitations of the approach and the interpretation of such data.

*Funding for the writing of this letter was provided by the American Chemistry Council. The views expressed here are solely those of the authors.*

**William R. Kelce**  
Exponent  
Cary, North Carolina  
E-mail: wkelce@exponent.com

**Christopher J. Borgert**  
Applied Pharmacology & Toxicology Inc.  
Gainesville, Florida

#### REFERENCES

- Charles GD, Gennings C, Tornesi B, Kan HL, Zacharewski TR, Gallapudi BB, et al. 2007. Analysis of the interaction of phytoestrogens and synthetic chemicals: an *in vitro/in vivo* comparison. *Toxicol Appl Pharmacol* 218:280–288.
- FDA (Food and Drug Administration). 2007. Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations. Available: <http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm081818.htm> [accessed 10 August 2011].
- Franz R, Welle F. 2009. Can migration of endocrine disruptors from plastic bottles be the cause of estrogenic burden recently determined in bottled mineral water. *Deutsche Lebensmittel-Rundschau* 105(5):315–318.
- Yang CZ, Yaniger SI, Jordan VC, Klein DJ, Bittner GD. 2011. Most plastic products release estrogenic chemicals: a potential health problem that can be solved. *Environ Health Perspect* 119:989–996; doi:10.1289/ehp.11038220 [Online 2 March 2011].

### Estrogen Activity in Plastic Products: Yang et al. Respond

doi:10.1289/ehp.1103894R

In their letter, Kelce and Borgert raise points related to our methods, as well as the objective of our paper (Yang et al. 2011) and its significance.

Regarding our methods, our solvent extraction procedures were less stringent than U.S. Food and Drug Administration (FDA)-recommended methods for determining migration from plastic food packaging [37°C for 72 hr in our study (Yang et al. 2011) compared with 40°C for 240 hr for comparable FDA procedures (FDA 2002, 2007)]. Consequently, if we had used FDA-recommended procedures, we would expect to detect a higher frequency of chemicals with estrogenic activity (EA) leaching from plastic containers. At present, the FDA has no established standards regarding extraction of chemicals having endocrine-disrupting effects, including estrogenic activity (EA). In addition, Wagner and Oehlmann (2010) confirmed our data for polyethylene terephthalate (PET) plastics, moot other points made by Kelce and Borgert regarding our extraction procedures, and discussed the significance of such data in terms very similar to ours.

Kelce and Borgert question our method of using ultraviolet (UV) light as a stressor. In our study (Yang et al. 2011), UV exposures were only to one side of the plastic. The FDA has no established standards regarding exposure of food packaging to UV light. Because food packaging and containers are often exposed to various sources of UV light (e.g., sunlight, sterilization, high intensity UV curing of package decoration), we believe that a realistic evaluation of packaging hazards

should include UV exposure, even absent specific FDA requirements.

Our resin data (resins P1, P2, P3, P4, P19, and P18) cited by Kelce and Borgert came from at least three replications of stressing, extraction, and EA assays. As described in our “Methods” and “Supplemental Material,” the assay variance was very small: SEs were typically smaller than the diameter of the data points of the graphed means. The whole series of 49 assays was repeated only once, but no extract exhibited EA; more recent extracts of the same plastics confirm our original results.

Kelce and Borgert noted that colorants are “embedded” in plastics. However, “bound” colorants in plastic compounds can and do readily leach from plastics. They are additives, which—like most additives—are only rarely chemically bound to polymers. Hence, concerns about all additives are warranted because any can leach from a plastic product.

Regarding broader issues, the objective of our paper was to quantify the prevalence of xenoestrogen release from commonly used plastic products. These data are significant in part to help assess the risk of such products to human health and environmental contamination. Kelce and Borgert cite Charles et al. (2007), who examined some interactions between a small set of phytoestrogens and xenoestrogens. The limited negative results of that study have been contradicted by dozens of other studies (e.g., Patisaul and Jefferson 2010). However, our objective was not to establish definitive links between public health issues, environmental pollution, and exposure to xenoestrogens. This relationship is an active research area, and it will take many years to obtain definitive answers.

Kelce and Borgert’s concerns about the paucity of epidemiological data correlating EA exposure via use of plastics with adverse human health effects is analogous to the long-standing controversy for tobacco, which is now highly regulated, largely because increasing numbers of epidemiological studies correlated smoking with heart disease and lung cancer. For decades, it was common to hear tobacco industry spokespersons argue that “[epidemiological] correlation does not mean causation” and demand that molecular, cellular, and/or systemic mechanisms be extensively demonstrated before any action, regulatory or otherwise, be taken. One rarely hears spokespersons for the chemical and plastics industry make this argument for release of chemicals having EA from plastics, because the mechanisms by which tobacco has its effects are still much less well known compared to mechanisms by which chemicals having EA produce adverse health and environmental effects. Instead, we hear, “Where are the epidemiological correlations?”

Those correlations are fewer (but not non-existent) than for tobacco at this relatively young stage of the field, but the number of such publications is rapidly increasing. In the meantime, our study and hundreds to thousands of other *in vitro* studies demonstrate that chemicals having EA have easily measurable effects on all sorts of human cells (including MCF-7 cells). Most scientists in this field believe that such results suggest adverse health effects in humans and that, as such data continue to be gathered, these correlations will become as compelling as did those for the impact of tobacco smoking on public health.

Legislators, consumers, manufacturers, and scientists must judge current industry practices in this area based on available data. Reasonable people can differ. The American Chemistry Council takes the position that until definitive studies consistently show health and environmental hazards from chemicals with EA leaching from plastic products, no industry action need be taken. We disagree. Plastic items are essential consumer products, but we argue that they need to be made safer. Our most recent data show that there is very little extra expense to produce safer plastics that do not leach chemicals having EA; that is, it costs very little at this time to avoid a potential health risk.

*C.Z.Y. is employed by, and owns stock in, CertiChem (CCi) and PlastiPure (PPi). S.I.Y. and D.J.K. are employed by PPi. V.C.J. has no financial interests in CCi or PPi, but he was principal investigator for a subcontract at Northwestern Medical School to help develop the MCF-7 assay on NIH grant P30 CA051008 awarded to CCi. G.D.B. owns stock in and is the founder and chief executive officer of CCi and the founder and chief scientific officer of PPi. All authors had freedom to design, conduct, interpret, and publish research uncompromised by any controlling sponsor.*

**Chun Z. Yang**  
**George D. Bittner**  
CertiChem Inc.  
Austin, Texas

**Stuart I. Yaniger**  
**Daniel J. Klein**  
PlastiPure Inc.  
Austin, Texas

**V. Craig Jordan**  
Lombardi Comprehensive Cancer Center  
Georgetown University Medical Center  
Washington, DC

## REFERENCES

- Charles GD, Gennings C, Tornesi B, Kan HL, Zacharewski TR, Gallapudi BB, et al. 2007. Analysis of the interaction of phytoestrogens and synthetic chemicals: an *in vitro*/in vivo comparison. *Toxicol Appl Pharmacol* 218:280–288.  
FDA (Food and Drug Administration). 2002, 2007. Guidance for Industry: Preparation of Premarket Submissions

for Food Contact Substances: Chemistry Recommendations. Available: <http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm081818.htm> [accessed 10 August 2011].

Patisaul HB, Jefferson W. 2010. The pros and cons of phytoestrogens. *Front Neuroendocrinol* 31(4):400–419; doi:10.1016/j.yfme.2010.03.003 [Online 27 March 2010].

Wagner M, Oehlmann J. 2010. Endocrine disruptors in bottled mineral water: estrogenic activity in the E-screen. *J Steroid Biochem Mol Biol*; doi:10.1016/j.jsrb.2010.10.007 [Online 2 November 2010].

Yang CZ, Yaniger SI, Jordan VC, Klein DJ, Bittner GD. 2011. Most plastic products release estrogenic chemicals: a potential health problem that can be solved. *Environ Health Perspect* 119:989–996; doi:10.1289/ehp.1003220 [Online 2 March 2011].

## Environmental Factors Develop Different Patterns of Immune Disease

doi:10.1289/ehp.1104043

I read with interest the article by Schmidt (2011) on the sprawling explosion of autoimmune diseases and its link to environmental exposure. Schmidt (2011) summarized the problematic state of the field: Systemic autoimmune diseases are common but thought rare; their clinical identification is far from the medical school description; and they continue to be identified as an autoantibody–target–manifestation scheme. Experience shows that a patient develops different autoantibodies through the lifespan, with different clinical patterns within each phase; deeper investigation shows that organ autoimmune disease is in fact systemic. Likewise, allergy, food intolerance, cancer, and immunodeficiency (all broad diseases that are immune in nature) cross and share autoimmunity. This suggests that immature immune systems are promoted and prevented from natural selection in the era of antibiotics, but they pay the cost of fostered health dysfunctions or diseases exposed to the current complex hostile environment.

I noticed this complex scenario in a survey of 22 patients reporting sick building syndrome (Blasco 2011). Although reported data was limited to autoimmune cases and the involved substances were not yet identified, I found that the same environment triggered and worsened other immune disorders. The health of two patients with asthma inexplicably worsened when they started to work in the building. One patient developed gynecological cancer; another patient, who had a past history of Hodgkin’s lymphoma, developed chronic fever and fatigue again that lasted 3 years, until she was relocated. Some of the patients reported new adult onset of clinical intolerance of milk or other foods, and one patient was positive in a breath test for lactose intolerance. A review of family histories revealed that in 20% of the patients, more than one direct relative was affected by cancer. Personnel records showed that allergy