PEER-REVIEWED RESEARCH AT CERTICHEM AND PLASTIPURE

COMPANY OVERVIEWS

Sister companies CertiChem and PlastiPure were founded by Dr. George Bittner in 2000 to begin addressing the significant concern that scientists, NGOs, and a growing number of consumers had (and have) regarding hazardous chemicals leaching from products that can enter our bodies and our environment. CertiChem's mission has been to develop the most sensitive and reliable robotized assays to detect hormonal activity, especially for chemicals having estrogenic activity (EA) or androgenic activity (AnA). PlastiPure's mission is to develop comprehensive processes and formulations to help companies make safer EA-free products. The research of both companies has received extensive scientific and financial support from many US Federal agencies including the NIH, NSF, NTP, and ICCVAM/NICEATM. This research is designed to improve the safety of products by detecting and avoiding the use of potentially harmful hormonal chemicals that are often found in cosmetics, plastic packaging, consumer products, medical supplies and devices, food additives, and other highly utilized products.

CertiChem – CertiChem scientists develop state-of-the-art *in vitro* assays to detect chemicals with estrogenic activity (EA) or androgenic activity (AnA). Many of these assays have been developed using about $4M in funds received from the National Institutes of Health (NIH) and the National Science Foundation (NSF) after peer-review evaluation by scientists and business representatives who serve on panels that typically fund only 10-15% of all such grant applications. CertiChem has received the following grants:

- NIH R43/R44 ES011469 "*In Vitro* Robotic Assay for Estrogenic Activity"
- NIH R43/R44 ES014806 "*In Vitro* Robotic Assay for Anti-Estrogenic Activity"
- NSF IIP-1127553/1026904 "Food Antioxidants (AOs) With or Without Estrogenic Activity"
- NIH R43ES025075 "Safer Personal Care Products"

PlastiPure – PlastiPure scientists develop processes and formulations that can be used to produce consumer products, packaging, and materials that do not release chemicals having EA or AnA. Much of this research has been in collaboration with CertiChem scientists. Similar to CertiChem, many of these EA-free formulations have been developed with the support of about $4.5MM in funds from peer-reviewed NIH/NIEHS and NSF grants:

- NIH R43/R44 ES016964 "Estrogen Free Polymer Formulations for Food Packaging and Baby Products"
- NIH R43/R44 ES018083 "A Hard and Clear, Estrogen-Free Replacement for Bisphenol-A Based Polycarbonates"
- NSF IIP-1013865/1127553 "Flexible Plastic Packaging Without Estrogenic Activity (EA)"
- R43/R44 ES019442 "Baby Bottles That Release No Chemicals Having Estrogenic Activity"

Some of this research has been recognized as scientifically important for inclusion in peer-reviewed papers in major journals. What follows are abstracts and highlights of six selected papers. Please see the original papers for detail including figures, tables, and references. These articles are available from the publishers (links provided) or from PlastiPure's website.

PUBLISHED PAPERS FOR BIOASSAYS

C. Z. Yang, W. Casey, M. Stoner, G.J .Kollessery, A.W. Wong and G.D. Bittner, A Robotic MCF-7:WS8 Cell Proliferation Assay to Detect Agonist and Antagonist Estrogenic Activity. Toxicological Sciences 137, 335-349 (2014).
http://toxsci.oxfordjournals.org/content/137/2/335.full.pdf

ABSTRACT: *"Endocrine-disrupting chemicals with estrogenic activity (EA) or anti-EA (AEA) have been extensively reported to possibly have many adverse health effects. We have developed robotized assays using MCF-7:WS8 cell proliferation (or suppression) to detect EA (or AEA) of 78 test substances supplied by the Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program's Interagency Center for the Evaluation of Alternative Toxicological Methods for validation studies. We also assayed ICI 182,780, a strong estrogen antagonist. Chemicals to be assayed were initially examined for solubility and volatility to determine optimal assay conditions. For both EA and AEA determinations, a Range-Finder assay was conducted to determine the concentration range for testing, followed by a Comprehensive assay. Test substances with potentially positive results from an EA Comprehensive assay were subjected to an EA Confirmation assay that evaluated the ability of ICI 182,780 to reverse chemically induced MCF-7 cell proliferation. The AEA assays examined the ability of chemicals to decrease MCF-7 cell proliferation induced by nonsaturating concentrations of 17β-estradiol (E2), relative to ICI or raloxifene, also a strong estrogen antagonist. To be classified as having AEA, a saturating concentration of E2 had to significantly reverse the decrease in cell proliferation produced by the test substance in nonsaturating E2. We conclude that our robotized MCF-7 EA and AEA assays have accuracy, sensitivity, and specificity values at least equivalent to validated test methods accepted by the U.S. Environmental Protection Agency and the Organisation for Economic Co-operation and Development."*

This paper describing our robotic version of a well-known and widely accepted assay for EA was published in collaboration with ICCVAM/NICEATM. This interagency group of 15 federal research and regulatory agencies is charged with developing and validating *in vitro* assays. The authors of the paper report the following: "*We conclude that our robotized MCF-7 EA and AEA Assays have accuracy, sensitivity, and specificity values at least equivalent to validated test methods accepted by the US EPA and the Organisation for Economic Co-operation and Development (OECD)."* In fact, this paper demonstrates that the MCF-7 assay is perhaps the most accurate and sensitive *in vitro* assay to detect EA and anti-EA.

M. A. Stoner, C. Z. Yang, G. D. Bittner, A Robotic BG1Luc Reporter Assay to Detect Estrogen Receptor Agonists. Toxicology In Vitro 28, 916-925 (2014).
http://www.sciencedirect.com/science/article/pii/S0887233314000629

ABSTRACT: *"Endocrine disrupting chemicals with estrogenic activity (EA) have been associated with various adverse health effects. US agencies (ICCVAM/NICEATM) tasked to assess in vitro*

transcription activation assays to detect estrogenic receptor (ER) agonists for EA have recently validated a BG1Luc assay in manual format, but prefer robotic formats. We have developed a robotic BG1Luc EA assay to detect EA that demonstrated 100% concordance with ICCVAM meta-analyses and ICCVAM BG1Luc results in manual format for 27 ICCVAM test substances, i.e. no false negatives or false positives. This robotic assay also consistently assessed other, more problematic ICCVAM test substances such as clomiphene citrate, L-thyroxin, and tamoxifen. Agonist responses using this robotic BG1Luc assay were consistently inhibited by the ER antagonist ICI 182,780, confirming that agonist responses were due to binding to ERs rather than to a non-specific agonist response. This robotic assay also detected EA in complex mixtures of substances such as extracts of personal care products, plastic resins or plastic consumer products. This robotic BG1Luc assay had at least as high accuracy and greater sensitivity and repeatability when compared to its manual version or to the other ICCVAM/OECD validated assays for EA (manual BG1Luc and CERI)."

This paper describes a robotic version of an assay recently validated by US (ICCVAM) and EU (OECD) agencies. This paper, along with the earlier MCF-7 publication, validates the reliability of CertiChem's robotized protocols, in addition to showing the very high sensitivity of the two cell lines as compared with other assays.

As discussed above, this robotic BG1Luc assay has very high accuracy (100% concordance with ICCVAM meta-analyses, BG1Luc manual assay, CERI test, and MCF-7 assay data). This is in contrast to the Yeast Estrogen Screening (YES) assay which has only 47% concordance with any of these other assays.

When sensitivities of these different assays are compared to detect the EA of the same test chemical as defined by its EC50 [half maximum response], this robotic BG1Luc assay is more sensitive than the BG1Luc manual, CERI, and YES assays while being slightly less sensitive than the robotic MCF-7 assay.

PUBLISHED PAPERS FOR PERSONAL CARE PRODUCTS

S. L. Myers, C.Z.Yang, G.D. Bittner, K.L. Witt, R.R. Tice, D.D. Baird., Estrogenic and Anti-Estrogenic Activity of Off-the-Shelf Hair and Skin Care Products. Journal of Exposure Science and Environmental Epidemiology (2014).
http://www.nature.com/jes/journal/vaop/ncurrent/full/jes201432a.html

ABSTRACT: *"Use of personal care products is widespread in the United States but tends to be greater among African Americans than whites. Of special concern is the possible hazard of absorption of chemicals with estrogenic activity (EA) or anti-EA (AEA) in these products. Such exposure may have adverse health effects, especially when it occurs during developmental windows (e.g., prepubertally) when estrogen levels are low. We assessed the ethanol extracts of eight commonly used hair and skin products popular among African Americans for EA and AEA using a cell proliferation assay with the estrogen sensitive MCF-7:WS8 cell line derived from a human breast cancer. Four of the eight personal care products tested (Oil Hair Lotion, Extra-dry Skin Lotion, Intensive Skin Lotion, Petroleum Jelly) demonstrated detectable EA, whereas three (Placenta Hair Conditioner, Tea-Tree Hair Conditioner, Cocoa Butter Skin Cream) exhibited AEA.*

Our data indicate that hair and skin care products can have EA or AEA, and suggest that laboratory studies are warranted to investigate the in vivo activity of such products under chronic exposure conditions as well as epidemiologic studies to investigate potential adverse health effects that might be associated with use of such products."

This paper was published in collaboration with scientists at the National Toxicology Program (NTP) and is particularly interesting from a public health standpoint. As stated by the authors:

"Personal care products are a $40 billion dollar industry in the United States, while world-wide, the industry is worth more than $200 billion (1). Environmental and human health concerns regarding these products are increasing (2, 3), but they do not fall under regulatory scrutiny in the United States unless a health claim is made for a product or damage to the environment warrants investigation.

Personal care products enter the environment in a number of ways including down-the-drain disposal, leaching from landfills, and direct dispersal (e.g., sunscreen wash-off from swimmers). Components of these products are now among the most commonly detected trace chemicals (in the nanogram to microgram per liter range) in surface water (4, 5) and groundwater (6). Since some ingredients of personal care products degrade slowly and some ingredients concentrate in lipids, these substances have the potential to bioaccumulate in the environment, thus indirectly exposing wildlife and humans (4, 7). However, substantially higher exposures to humans would be expected from personal use of these products than from environmental exposures, and reports linking direct exposures to adverse health effects have begun to appear. Use of products with estriol concentrations ranging from 9 to 24 mg/g (8-13) or containing components with estrogenic or antiandrogenic activity has been associated with premature sexual development and prepubertal gynecomastia in case reports. More extensive observational studies have indicated a relationship between self-reported product use and hormonally mediated outcomes.

In the United States, African Americans report heavier use of many personal care products compared to Caucasians (10, 14, 15), and may therefore be at higher risk for any possible adverse health effects. National Health and Nutrition Examination Survey (NHANES) data show that African Americans have higher levels of urinary parabens (16) and monoethyl phthalate, a metabolite of diethyl phthalate (17); these chemicals are common ingredients in personal care products (18). The ethnic disparity may be especially marked for children, with black children exhibiting far higher levels than Caucasian children for the two most commonly detected parabens in NHANES (16).

Of most concern are the potential endocrine disrupting effects of these products, given that some may contain endogenous estrogens (19) or ingredients found to have estrogenic effects (20). However, estrogenic (or anti-estrogenic) effects of the personal care products as commercial mixtures have not been evaluated. In this study, we used an in vitro roboticized version of the E-SCREEN assay that quantifies estrogen-receptor (ER)-mediated proliferation of breast cancer MCF-7 cells (21) to perform a hazard assessment of ethanol extracts of eight off-

the-shelf hair and skin care products that are popular among African American women for estrogen activity (EA) or anti-estrogenic activity (AEA). "

PUBLISHED PAPERS FOR CONSUMER PRODUCTS

C. Z. Yang, S. I. Yaniger, V. C. Jordan, D. J. Klein, G. D. Bittner, Most Plastic Products Release Estrogenic Chemicals: A Potential Health Problem That Can Be Solved. Environmental Health Perspectives 119, 989-996 (2011). http://ehp.niehs.nih.gov/1003220/

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

Objectives: 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 robotized 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."

This is the first paper describing the release of chemicals having EA from many different types of plastic products that do ***not*** contain Bisphenol-A (BPA), a chemical having EA that had received much attention because of its widespread use to make polycarbonate plastics. The authors concluded that BPA-free did not mean EA-free for most of the over 400 products tested.

G. D. Bittner, C. Z. Yang, M. A. Stoner, Estrogenic Chemicals Often Leach From BPA-Free Plastic Products That Are Replacements For BPA-Containing Polycarbonate Products. Environmental Health 13, 41 (2014). http://www.ehjournal.net/content/13/1/41

ABSTRACT: *"Background*
Xenobiotic chemicals with estrogenic activity (EA), such as bisphenol A (BPA), have been reported to have potential adverse health effects in mammals, including humans, especially in

fetal and infant stages. Concerns about safety have caused many manufacturers to use alternatives to polycarbonate (PC) resins to make hard and clear, reusable, plastic products that do not leach BPA. However, no study has focused on whether such BPA-free PC-replacement products, chosen for their perceived higher safety, especially for babies, also release other chemicals that have EA.

Methods
We used two, well-established, mammalian cell-based, assays (MCF-7 and BG1Luc) to assess the EA of chemicals that leached into over 1000 saline or ethanol extracts of 50 unstressed or stressed (autoclaving, microwaving, and UV radiation) BPA-free PC-replacement products. An EA antagonist, ICI 182,780, was used to confirm that agonist activity in leachates was due to chemicals that activated the mammalian estrogen receptor.

Results
Many unstressed and stressed, PC-replacement-products made from acrylic, polystyrene, polyethersulfone, and Tritan™ resins leached chemicals with EA, including products made for use by babies. Exposure to various forms of UV radiation often increased the leaching of chemicals with EA. In contrast, some BPA-free PC-replacement products made from glycol-modified polyethylene terephthalate or cyclic olefin polymer or co-polymer resins did not release chemicals with detectable EA under any conditions tested.

Conclusions
This hazard assessment survey showed that many BPA-free PC- replacement products still leached chemicals having significant levels of EA, as did BPA-containing PC counterparts they were meant to replace. That is, BPA-free did not mean EA-free. However, this study also showed that some PC-replacement products did not leach chemicals having significant levels of EA. That is, EA-free PC-replacement products could be made in commercial quantities at prices that compete with PC-replacement products that were not BPA-free. Since plastic products often have advantages (price, weight, shatter-resistance, etc.) compared to other materials such as steel or glass, it is not necessary to forgo those advantages to avoid release into foodstuffs or the environment of chemicals having EA that may have potential adverse effects on our health or the health of future generations."

Since our 2011 paper was published, various products have been manufactured and sold as substitutes for hard and clear polycarbonate plastics that are BPA-free, some even advertised as (or implied to be) EA-free. This paper reported that the large majority of these products continue to leach chemicals with EA regardless of their adverstising claims.

PUBLISHED PAPERS FOR BPA-FREE RESINS

G.D. Bittner, M.S. Denison, C. Z. Yang, M.A. Stoner, G. He, Chemicals Having Estrogenic Activity Can Be Released From Some Bisphenol A-Free, Hard and Clear, Thermoplastic Resins. Environmental Health 13, 103 (2014). http://www.ehjournal.net/content/pdf/1476-069X-13-103.pdf

ABSTRACT: *"Background: Chemicals that have estrogenic activity (EA) can potentially cause adverse health effects in mammals including humans, sometimes at low doses in fetal through juvenile stages with effects detected in adults. Polycarbonate (PC) thermoplastic resins made from bisphenol A (BPA), a chemical that has EA, are now often avoided in products used by babies.*

Other BPA-free thermoplastic resins, some hypothesized or advertised to be EA-free, are replacing PC resins used to make reusable hard and clear thermoplastic products such as baby bottles.

Methods. We used two very sensitive and accurate in vitro assays (MCF-7 and BG1Luc human cell lines) to quantify the EA of chemicals leached into ethanol or water/saline extracts of fourteen unstressed or stressed (autoclaving, microwaving, UV radiation) thermoplastic resins. Estrogen receptor (ER)-dependent agonist responses were confirmed by their inhibition with the ER antagonist ICI 182,780.

Results: Our data showed that some (4/14) unstressed and stressed BPA-free thermoplastic resins leached chemicals having significant levels of EA, including one polystyrene (PS), and three Tritan™ resins, the latter reportedly EA-free. Exposure to UV radiation in natural sunlight resulted in an increased release of EA from Tritan™ resins. Triphenyl-phosphate (TPP), an additive used to manufacture some thermoplastic resins such as Tritan™, exhibited EA in both MCF-7 and BG1Luc assays. Ten unstressed or stressed glycolmodifiedpolyethylene terephthalate (PETG), cyclic olefin polymer (COP) or copolymer (COC)thermoplastic resins did not release chemicals with detectable EA under any test condition.

Conclusions: This hazard survey study assessed the release of chemicals exhibiting EA as detected by two sensitive, widely used and accepted, human cell line in vitro assays. Four PC replacement resins (Tritan™ and PS) released chemicals having EA. However, ten other PC replacementresins did not leach chemicals having EA (EA-free-resins). These results indicate that PC-replacement plastic products could be made from EA-free resins (if appropriate EA-free additives are chosen) that maintain advantages of re-usable plastic items (price, weight, shatter resistance) without releasing chemicals having EA that potentially produce adverse health effects on current or future generations."

This paper reports that the EA in many polycarbonate replacement products comes from the resin, not just some of the intended "ingredients" used to manufacture plastic products. As stated by the authors:

"…each plastic part is a rather unique combination of (typically) 10 or more chemicals, and a plastic product with many parts (e.g., a baby bottle) often consists of 30 - 100 chemicals [20,21]. Any of these chemicals might have EA [8,16,17] and leach from the final product because polymerization is almost always incomplete, leaving residual unincorporated monomers and/or because most additives (e.g., antioxidants) are not chemically part of the polymeric structure [20,21]. Various stresses such as UV light, microwave radiation, and moist heat can also cause chemical changes in resins or plastic products [20,21], possibly converting EA-free monomers or additives into chemicals exhibiting EA [16,17]. Accounting for such factors may individually appear obvious or mundane, but are essential to producing EA-free resins or plastic parts, and

to our knowledge have not been explicitly considered individually, much less in aggregate, by any publication of which we are aware other than [16,17] for products. Furthermore, if a resin or product contains chemicals that have EA, then the final finished resin or product will almost-certainly leach chemicals having EA, since no additive or process known to us could prevent their leaching, sometimes especially if stressed [17]. All previous relevant publications [16,17] have examined plastic products, not the resins used to make such products."

The assessment and conclusion of this research are also very interesting in that they are consistent with those set forth in earlier published studies by the authors but are in conflict with the Eastman Chemical-funded study authored by consultant Thomas Osimitz. The authors have reported:

"Assessments of EA from other studies of plastic resins

Our data demonstrating that unstressed Tritan™ leaches chemicals with EA are consistent with the more-limited set of previous data showing that four water bottles and a baby bottle made from Tritan™ resins release chemicals having EA, especially when stressed with UVC radiation (identified as PETG resins in Fig. 3 of [16]). Our data are also consistent with data reported in a recent study [17] that products made from Titan™ resins almost always (23/25 products) released chemicals having EA, as did all (9/9) products made from PS resins exposed to UV. The two exceptions were two types of green bottles made from Tritan™ for which the particular green colorant(s) used during manufacture blocked the penetration of UV radiation [17]. In contrast, many products made from COC (2/2), COP (1/1), and PETG (2/3) resins did not release chemicals having significantly-detectable levels of EA (Table 1 in [17}. That is, if a resin releases chemicals having EA, then the product will almost-certainly release chemicals exhibiting EA.

Our data demonstrating that unstressed Tritan™ leaches chemicals with EA are not consistent with a conclusion reported in a recent study by Osimitz et al. [30] that Titan™ resins should not release chemicals having EA because the three monomers (dimethyl-terephthalate (DMT), 1,4-cyclohexanedimethanol (CHDM), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD)) used in various ratios by Eastman Chemical to manufacture various Tritan™ resins purportedly had no detectable EA. In this Eastman-funded study, a combination of in silico, in vitro and in vivo methods were reportedly used to examine the estrogenic and androgenic activity of DMT, CHDM and TMCD. Although the lack of positive EA results reported for the three monomers in their yeast cell ER-transactivation bioassay [30] were interpreted to mean that these monomers have no EA, yeast bioassays have low sensitivity, a high rate of false negative results, and often do not respond appropriately to some ER ligands/antagonists [40]. Accordingly, while positive EA results from yeast assays are generally acceptable, negative EA results do not provide meaningful evidence that a test substance lacks EA [1,2,40]. Additionally, while Osimitz et al. [30] reported that the three Tritan™ monomers were inactive in ER-transactivation assays in recombinant human T47D cells, they only reported ER-transactivation results for CHDM, and these results actually revealed a CHDM concentration-dependent enhancement of E2-dependent luciferase gene expression; the effect of CHDM alone on ER-dependent gene expression was not shown. Furthermore, chemicals other than the three tested monomers are used in the manufacture of Tritan™ resins [18,19,33], and at least one of them (TPP) has EA ([32]; Fig. 7).

No in vivo assay data is available for resin EA

*The results presented in this study and other described above examine the EA of extracts of plastic products utilizing a variety of in vitro cell-based assays. There are no studies of which we are aware examining the EA of extracts of any BPA-free PC-replacement resin (or product) using an in vivo assay (i.e. measurement of the ability of an extract to stimulate uterine growth or ER-dependent gene expression). Osimitz et al. [30] reported that a very dilute mixture of the three monomers that comprise part of the chemical composition of Tritan™ showed no EA in an uterotrophic assay and concluded that the manufactured resin should therefore leach no chemicals having EA. There were major problems with the experimental design of this in vivo study, including: (**1**) They evaluated the EA of the mixture of the three monomers at dosages below their no observable effect level (NOEL) instead of their maximum tolerated dose as recommended by OECD [41] and is standard practice in the field [42]. Using their protocol, E2 would be expected to have no EA. (**2**) They utilized an insensitive strain of rat for these studies [10], and (**3**) they tested only three ingredients of Tritan™ resins instead of all of the 8-10 chemicals that go into the preparation of the resins. (**4**) They never tested extracts of the resin which could release additional chemicals that could form during the high pressures and temperatures experienced polymer synthesis. No effort to identify such chemicals has been reported. Therefore, the negative in vivo test results obtained by Osimitz and coworkers [30] using only a mixture of three pure monomers provide little meaningful information as to whether extracts of unstressed or stressed resins would release chemicals having EA [42].*

CONCLUSIONS

In this survey of PC-replacement resins, we recognize that we quantify the maximum effects of total EA (%RME2) in extracts relative to the maximum effect of E2 using two sensitive assays, at least six extraction protocols, and at least six stress protocols. We define a resin as releasing chemicals having EA if an EA value > 15 %RME2 is observed in at least one assay condition. In fact for the resins reported in this paper, resins labeled as EA positive had at least three independent assays greater than 15% RME2 for a given assay condition and others at least three consistently-positive in at least three similar assay conditions (yellow-highlighted cells in Tables 1,2). Therefore, the current paper is an in vitro study that reports the existence of a possible hazard for consumption of chemicals with EA leaching from plastic products made from PS and Tritan™ resins that leach chemicals having detectable levels of EA.

This study does not assess the risk that such consumption might have for human health. In fact, we believe that this risk cannot be adequately assessed at this time because neither we nor any other scientists or entity to our knowledge have identified and characterized the EA and anti-EA of all chemicals and their metabolites in these extracts, including those formed during different stresses. How much of the total EA that leach from plastics made from these resins or other sources is consumed and absorbed by human subjects is another unknown as are short-term and long-term effects at different life stages (fetus, infant juvenile, adult, etc.). These are areas that need in depth analyses and evaluation by scientists or entities that do not have a financial or ideological stake in a particular set of results.

Nevertheless, the results of our potential-hazard study are important because other studies have reported that chemicals with EA in mammals can produce various adverse health effects such as early menarche, reduced sperm counts and other altered functions of reproductive organs, obesity, and increased rates of some cancers. Some of these effects occur at very low

doses in fetal, infant, and juveniles, but are often only detected in the adult stage [4,8,11]. These animal studies are relevant to humans [12], as dramatically and unfortunately demonstrated by the adverse health effects on the offspring of mothers given diethylstilbestrol, a chemical exhibiting high EA [3,4,8,10,11,36].

In summary, our MCF-7 and BG1Luc assays demonstrate that extracts of four unstressed and/or stressed BPA-free thermoplastic resins, one PS and three Tritan™ resins, release chemicals that can activate ER-dependent cell signaling. These data and our conclusion on Tritan™ resins reported herein, combined with data for those products assessed in [17], are in stark contrast to those of Osimitz et al. [30], whose data are not relevant to assess the EA in extracts of Tritan™ resin. Considering all the available data, we conclude that these four BPA-free thermoplastic resins are not EA-free. This conclusion is especially important because our data on products made from other BPA-free, BPA-replacement resins assayed in a related survey study [17], show that it is possible to synthesize thermoplastic resins in commercial quantities that are usable to manufacture hard and clear products that could be EA-free, assuming that chemicals added, used, or created in the manufacturing process are also EA-free [16,17]. Given that plastic products have advantages (weight, cost, impact strength, energy footprint, etc.) in various combinations compared to other materials such as steel or glass, our data suggest that these advantages of plastics can be maintained while avoiding potential adverse health effects of release of chemicals having EA into foodstuffs or the environment."

ADDITIONAL INFORMATION

Each of these papers is available from their respective journals cited within this document. Copies have been posted in full on http://plastipure.com/company/news-about-the-health-environmental-concerns-of-chemicals-with-ea. For additional information on these studies, please contact Dr. George Bittner at bittner@austin.utexas.edu.