EA-Free™ Plastics: The only alternative for safer plastics

I: Biological Studies and Public Health Perspective

CertiChem, Inc. and PlastiPure, Inc
11212 Metric Blvd Suites 500, 600
Austin, TX
78758
www.plastipure.com

Executive Summary: Part I

The Problem

Plastics are a fundamental building block for the technologies that have improved life for billions of people and are an indispensable component of modern societies. However, most plastic products sold today release chemicals that have estrogenic activity (EA). While estrogens occur naturally in men and woman, many scientific studies have shown that significant health problems can occur when chemicals are ingested that mimic or block the actions of these sex hormones in mammals; the fetus, newborn, and young child are especially vulnerable. These health-related problems include early puberty in females, reduced sperm counts in males, altered functions of reproductive organs, obesity, altered behaviors, learning disorders, and increased rates of breast, ovarian, testicular, and prostate cancers.

The Focus on BPA and Phthalates

Estrogenic chemicals leach from most plastic products sold today. Bisphenol A (BPA) and phthalates are only two of the hundreds of chemicals that have EA and are commonly used in the large majority of plastics. Because of early publicity surrounding these estrogenic chemicals, the current commercial approach is to solve this health-related problem by producing BPA-free and/or phthalate-free plastic products. Unfortunately this incremental solution to replace an individual chemical does not provide a comprehensive health solution. Furthermore, substitutes for BPA or phthalate-containing products can leach other chemicals having more total EA than the EA released by the original products; the solution can be worse than the problem.

Legislation to Date

The call to ban BPA and phthalates is growing rapidly. California has passed legislation banning phthalates in certain products; similar bills are pending in Connecticut, New York, Pennsylvania, Maryland, Maine and Minnesota. The US Congress has passed an amendment to the Consumer Product Safety Commission Reform Act that would implement a similar ban. The European Union and Canada have already passed this legislation. However, all current regulatory attempts try to solve this EA problem by banning just one or two chemicals having EA at a time. More radical approaches that are based primarily on consumer sentiment, like banning the use of all plastics for certain products (e.g., plastic bag bans), ignore the performance and ecological benefits of plastics, and are counter-productive.

The Health-Related Solution

The most appropriate regulatory solution is to require that all plastics be made without estrogenic chemicals, rather than ban specific EA-causing ingredients one at a time. This is not a pie-in-the-sky solution, as the technology already exists to produce EA-Free™ plastics that also have the same performance and ecological advantages of existing EA-releasing conventional plastics. In fact, some of the advanced-technology EA-Free™ plastics are already in the marketplace.

Part II. The Design and Manufacture of EA-Free™ Plastics and Plastic Products

In the second part of this White Paper, the cause of EA from plastics is related to molecular structure, technological solutions for plastics producers and plastic product manufacturers are described, and some case studies are outlined.

Technical Summary: Part I

Using a highly sensitive method of *in vitro* biological assay, PlastiPure has compiled extensive data showing that most existing commercially available plastics release chemicals that exhibit EA the most significant form of endocrine disruption. Steroid hormones, like estrogen, can have significant adverse biological effects at very low concentrations (micromolar (~ppm) to nanomolar (~ppb) or even picomolar (~ppt)), especially on fetal and newborn mammals, including humans. (NIEHS, 2006; EDSTAC, 1998; NRC, 1999; NTP, 2000; Welshons et al, 2003; Kabuto et al., 2004; vom Saal and Hughes, 2005; Swan et al. 2005; Rubin et al. 2006; vom Saal, 2006). This raises significant concern for human exposure because most plastic products leach chemicals having EA at concentrations greater than this nanomolar to picomolar range (Takao et al., 1999; Howdeshell et al, 1999; Yang and Bittner, 2007).

In **Part I** of this White Paper, we outline the methods for comprehensive EA testing, demonstrate that these methods are reliable, repeatable, sensitive, and correlate with older less precise methods.

By far the large majority of plastics have tested positive for EA. Because of the lack of focus on endocrine disruption and use of much less sensitive EA testing modalities, reliable EA-Free™ plastics are only available from PlastiPure or its licensed partners. In fact, PlastiPure's data show that products advertised as BPA-free or phthalate-free can release chemicals that have more total EA than the total EA released by products containing BPA or phthalates. Products made from these highly estrogenic BPA-free materials are further contaminated by estrogenic colorants, additives, processing aids, and other materials. An example of this "marketing" solution to healthier plastics can be easily seen with the many reusable water and baby bottles currently being made from popular BPA-free materials, such as PES or PETG, which has replaced polycarbonate over the past two years but have consistently tested positive for significant EA. Millions of dollars have been spent to develop BPA-free plastics (and much more to market these materials) without addressing the underlying issue of EA.

However, it is possible to develop an extensive line of technologically advanced formulations and procedures for making safer plastics, food additives, and other products without sacrificing the desirable qualities of conventional plastics: flexibility, hardness, clarity, recyclability, small carbon footprint, etc. These materials can be produced from common chemicals, using existing tooling, and with only minor changes to operating procedures providing very price competitive solutions. The methods and materials for the design and manufacture of safer, EA-Free™ plastics are outlined in **Part II** of this White Paper.

Technical Presentation

Effects of endocrine disrupting chemicals (EDCs) on animals and humans

Many chemicals used in the manufacture of various products act as agonists or antagonists of androgenic or estrogenic hormones, while other chemicals interfere in multiple ways with the action of thyroid hormones (Brouwer, 1998; EDSTAC, 1998; NRC, 1999; ICCVAM, 2002a-c, 2003, 2006; Palanza et al., 2002; Singleton and Khan, 2003; Copenhagen, 2007; Janer and Porte, 2007; Tan and Zoeller, 2007; Adewale et al., 2009). Wildlife and laboratory animals exposed to such endocrine disrupting chemicals (EDCs) exhibit adverse effects on many physiological processes, such as brain activity (behavior), reproduction, immune response, growth, development, and metabolic rate (Tyler, 1998; McLachlan, 2001; Guillette and Gunderson, 2001; Hayes et al., 2002; Markey et al., 2003; Murray et al., 2006; Patisaul et al., 2006). Such ED effects can be either gross or subtle when tested in animal model systems. A significant probability exists that similar ED effects are produced in humans, since basic endocrine mechanisms have been highly conserved across all classes of vertebrates (Kavlock et al., 1996; NRC, 1999; Thornton, 2001; Calafat et al., 2005; vom Saal et al., 2005). EDCs can potentially produce abnormal physical and/or behavioral effects ranging from increased risk of hypospadias, cryptorchidism, and vaginal carcinoma to impaired mental development, particularly when exposure occurs during critical stages of development, from early fetal stages through puberty (Goldman, 2000; Baskin, 2001; Kawai et al., 2003; Markey et al., 2003; Goodman et al., 2006).

EA is the most common ED effect and can produce fetal pathophysiology, abnormal brain maturation, reduced sperm count, prostate enlargement, ovarian and uterine dysfunction, learning disabilities, disorders of attention, motivation, emotion, and cognitive development, including changes in sexual orientation (Hines, 1992; EDSTAC, 1998; NRC, 1999; Bonde and Storgaard, 2002; Calafat et al., 2005; Fujimoto et al., 2006; Copenhagen, 2007; Newbold et al., 2004, 2009; Patisaul et al., 2006, 2008, 2009; Garner et al., 2008; Monje et al., 2009; Spivey 2009). *In vivo* data from mice and rats have shown that exposure to estrogenic EDCs at various developmental stages is associated with alterations in the reproductive organs of infants and adults (Gray, 1998; Welshons et al., 1999; Baskin, 2001; Al-Hiyasat and Elbetieha, 2004; Newbold et al., 2004), the rate of growth and time to sexual maturation (Howdeshell et al., 1999, 2000), and aggressive behavior (Palanza et al, 1999, 2002; Kawai et al., 2003; Della Seta et al., 2006).

Federal Regulation of EDCs

Experimental data from *in vitro*, *in vivo*, ecological, and epidemiological studies showing that particular chemicals or chemical formulations possess varying degrees of ED activity have elicited concern from governmental bodies (EDSTAC, 1999; ICCVAM, 2002a, b, c, 2003, 2006), commercial entities, non-profit organizations, and scientific panels or meetings (NRC, 1999; Jordan et al., 2000; NTP, 2001; Copenhagen, 2007). In response to such concerns about ED activity on humans and wildlife, the US Congress passed amendments to the Food Quality Protection Act (1996) and the Safe Drinking Water Act (1996) that require that chemicals be tested for hormonal activity. To accomplish this goal, the EPA formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to examine whether current toxicological testing procedures are adequate to determine EDC activity.

EDSTAC recommended that many thousands of chemicals be tested in an Endocrine Disruptor Screening Program by a tiered set of *in vitro* and *in vivo* assays. As described in a report to the US Congress (EPA, 2000), the EDSTAC recommended a system consisting of two “Tiers” of EDC testing. Tier 1 *in vitro* and *in vivo* tests are designed to

identify EDCs. Tier 1 robotic *in vitro* screening tests are especially desired as a way to more quickly identify EDCs – and at lower costs. The robotic assays for EA used by PlastiPure's testing partner, CertiChem, Inc., meet these criteria.

ICCVAM was established in 1997 (Public Law P.L.103-43) to develop and validate new *in vitro* test methods and authorized in 2000 (P.L. 106-545), as a 15 agency permanent committee, to co-ordinate the development, validation and acceptance of toxicological tests throughout the Federal Government. As part of this mandate, ICCVAM and NICEATM [National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternate Toxicological Methods] formed an Endocrine Disruptor Working Group to assist ICCVAM in the evaluation of the validation status of assays for EDs. ICCVAM (2002a, b, 2006) recommended that ER-dependent Transcriptional Activation (TA) assays be developed because such "functional" assays are more sensitive than Relative Binding Affinity assays, can distinguish agonists from antagonists, and can be conducted with and without exogenous metabolic activation. The panel expressed a preference (ICCVAM, 2002b, 2003, 2006) for the use of human ER subtypes in any *in vitro* TA screening assay and developed minimum procedural standards for TA assays of EA.

Non-Federal Regulation of EDCs

Non-Federal legislation directed at EDCs has gained momentum recently. Last year's SB 484, also known as the California Safe Cosmetics Act, forces cosmetics makers to reveal harmful ingredients having endocrine disruptor activity. In New York, regulations in effect since September of 2006 now require all public and private schools to use chemicals that are free of reproductive hormonal activity. In late 2006, the European Union passed laws to protect people from thousands of toxic chemicals. REACH, or Registration Evaluation and Authorization of Chemicals will force industries to register chemicals, submit health and safety data, and replace the most hazardous ones with safer alternatives by 2009. A new European Chemicals Agency based in Helsinki, Finland will become the central regulatory authority. These regulations include the first steps to eliminate use of plastics containing polyvinylchloride (PVC), phthalates and many other chemicals; similar regulations have also been passed in Japan. The Economist (9/22/07) believes that these REACH regulations will quickly be accepted as world regulatory standards for chemicals having hormonal activities.

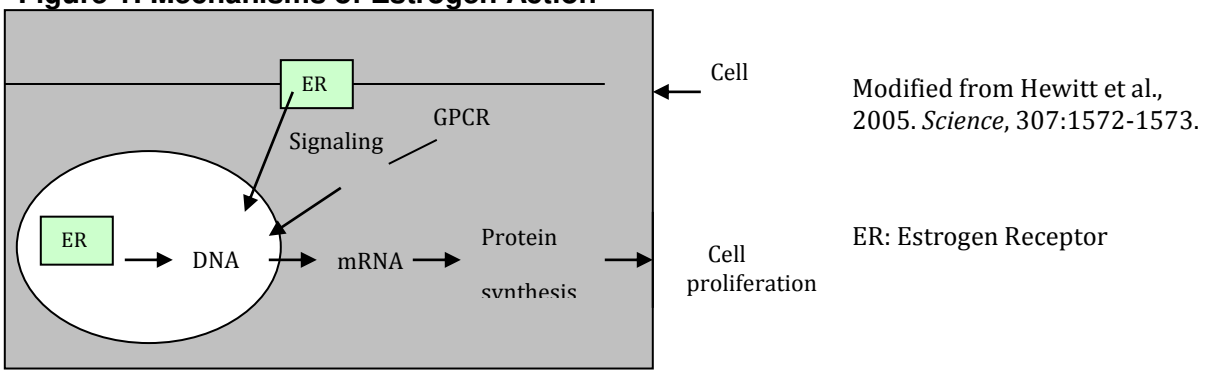
Cellular/Molecular mechanisms by which chemicals produce EA and Anti-EA

The cellular/molecular mechanisms of action of estrogenic and anti-estrogenic EDCs are shared with natural estrogens. Synthetic exogenous EDCs present in the environment, such as monomers or additives released from plastic products, mimic endogenous estrogenic hormones by affecting estrogen receptors (ERs) and other members of the nuclear receptor superfamily (Beato, 1989; Singleton and Khan, 2003; Hewitt et al., 2005). Estrogen Receptor-α (ER-α) and ER-β are promiscuous receptors, which bind a wide variety of natural and synthetic EDCs and activate transcription of estrogen-responsive genes, leading to cell proliferation (**Fig. 1**; Matthews et al., 2002; Revankar et al., 2005). Anti-EA effects may be produced by competitive inhibitors that bind to ERs but do not activate them (e.g., ICI 182,780 and ICI 164,384; Wakeling, 1993) or agonists that bind strongly to ERs, but do not activate as strong an ER response (Jordan and Murphy, 1990; Muller et al., 2002).

Furthermore, selective ER modulators (SERMs) bind to ERs, but subsequently activate cellular responses that differ from those activated by the endogenous estrogen, 17β-estradiol (E2) (Black et al., 1983; Yang et al., 1996; Shang and Brown, 2002; Lonard and Smith, 2002). It is also possible for a chemical to bind directly to an endogenous hormone, and thereby reduce its effect.

While binding affinities differ between estrogenic ligands (Kuiper, 1997), ER ligands typically bind to both receptors (ICCVAM, 2002a, 2003, 2006; Routledge et al., 2000). Both ERs bind to estrogen response elements, which are located upstream of the promoter regions of estrogen-activated genes (Paech et al., 1997; McDonnell and Norris, 2002). EDCs with EA or anti-EA can bind to nuclear or extra-nuclear receptors (**Fig. 1**; Hewitt et al., 2005; Evinger and Levin, 2005; Raz et al., 2008; Vasudevan and Pfaff, 2008).

Figure 1: Mechanisms of Estrogen Action



CertiChem's *In Vitro* EA and anti-EA Assays

The MCF-7 cell proliferation assay has been used in manual format as the gold standard for many years to measure EA: human breast-derived MCF-7 cells divide when stimulated by chemicals having EA. The ability of PlastiPure to develop EA-Free™ plastics has depended, in part, on the development by CertiChem of highly sensitive, reliable, and accurate EA and an *anti*-EA MCF-7 cell assays in robotic format that meet all duplicate wells, positive and negative controls, etc (See ICCVAM, 2002a, b, 2003, 2006, 2007 for details and references to CertiChem's EA assay).

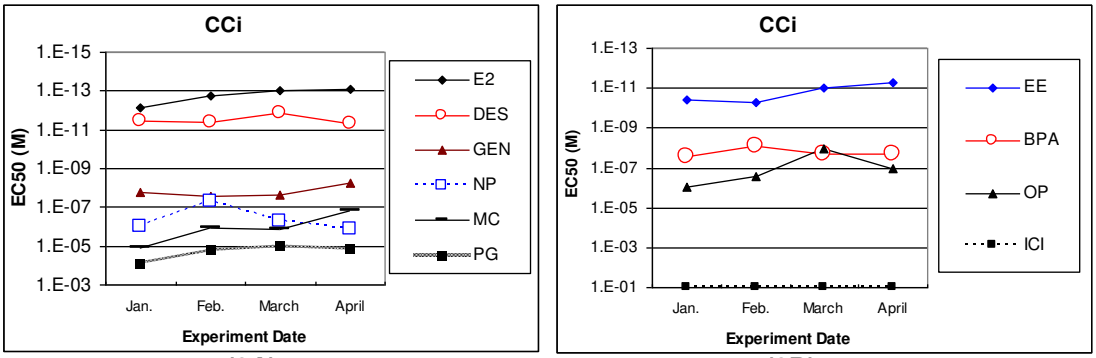
For example, CertiChem has extensively shown that its robotic MCF-7 assay for EA is repeatable (reproducible/reliable) both within its own laboratory (**Fig. 2A-B**) and among laboratories at CertiChem, U Missouri, and Northwestern Medical School (**Fig. 2C-D**). CertiChem has also shown that its cell proliferation assay is very versatile, i.e., is capable of assaying EA in many foodstuffs, feeds, plastics, etc (**Fig. 3**). In addition, CertiChem has completed extensive analyses of chemicals suggested by ICCVAM to show that this robotic assay is:

(1) extremely sensitive: concentration producing 50% of maximum response (EC50) for E2 =~10^{-13} M (see **Fig. 2, Table 1**);

And

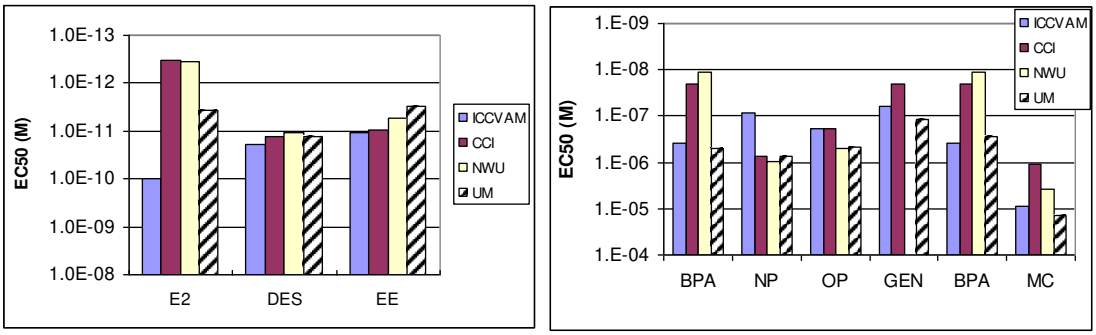
(2) very accurate (almost no false negatives or positives in over 75 chemicals analyzed to date; see **Table 2, Fig. 3**).

Figure 2: Reliability/Reproducibility of CertiChem's EC50 assays



(2A) (2B)

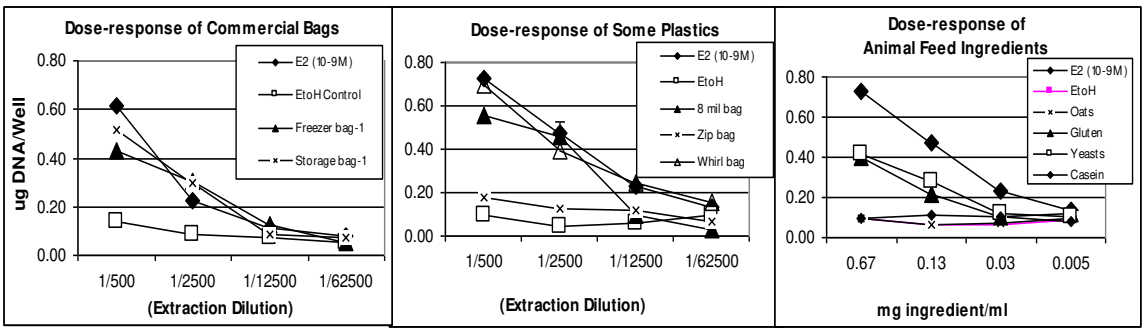
Figures 2A, B: Repetitive testing of 10 test chemicals by CertiChem laboratory once each month for four months using the current version of robotic EA assay.



(2C) (2D)

Figures 2C, D: Comparison of EC50's of 8 test chemicals as published by ICCVAM (2003 meta-analysis, median value of manual assays) vs. MCF-7 assay at CertiChem (average of robotic assays), Northwestern University Medical School (NWU) in the laboratory of Dr. V. Craig Jordan (average of manual assays), and UM in the laboratory of Dr. Wade Welshons (average of robotic assays). E2: 17β-Estradiol; DES: Diethylstilbestrol; EE: 17α-Ethyl estradiol; GEN: Genistein; BPA: Bisphenol A; MC: p, p'-methoxychlor; ICI: ICI 182,780; PG: Propyl gallate; NP: p-n-Nonylphenol; OP: 4-tert-Octylphenol. **Note the consistency (reliability/repeatability) of EC50 values among the three labs using this assay and its accuracy with respect to data from the ICCVAM (2003) meta-analysis.**

Figure 3: Versatility of CCi's Robotic EA assay



(3A) (3B) (3C)

Figures 3A-C: For plastics **(Figs. 3A-B)**, 1g finely cut plastic pieces are covered by 1ml 100% EtOH at 37°C for at least 24 hrs. For foodstuffs **(Fig. 3C)**, 1g of finely ground ingredient is dissolved in 1ml 100% EtOH for 30 minutes at 20°C; supernatant obtained by centrifuging at 3000 rpm for 10 min. The

supernatants from feed and plastics are then serially diluted in culture medium by robotics at 1:500 to 1/62,500 and applied to MCF-7 cells in 96 well plates as described for EC50 analyses.

Table 1: CertiChem or ICCVAM EC50 Values for ICCVAM Reference Chemicals

TEST CHEMICALS	CAS No.	CCi Mean EC50 (M)	CCi EA (+ or -)	ICCVAM Median EC50 (M)	ICCVAM EA (+ or -)	ICCVAM EC50 ranking	CCi EC50 ranking
17β-Estradiol	50-28-2	1.44E-13	+	1.00E-10	+	4	1
meso-Hexestrol	84-16-2	3.77E-12	+	2.00E-10	+	5	2
Estrone	53-16-7	8.27E-12	+	3.20E-09	+	8	3
17α-Ethinyl estradiol	57-63-6	1.95E-11	+	1.10E-11	+	1	4
Diethylstilbestrol	56-53-1	2.80E-11	+	1.90E-11	+	2	5
17α-Estradiol	57-91-0	2.90E-11	+	4.60E-11	+	3	6
Estriol	50-27-1	1.40E-10	+	7.10E-10	+	6	7
Zearalenone	17924-92-4	2.72E-10	+	2.00E-09	+	7	8
Coumestrol	479-13-0	4.86E-10	+	1.50E-08	+	9	9
Bisphenol B	77-40-7	1.21E-08	+	8.80E-08	+	12	10
Genistein	446-72-0	1.89E-08	+	6.20E-08	+	10	11
Bisphenol A	80-05-7	2.52E-08	+	4.00E-07	+	15	12
Flavone	525-82-6	3.51E-08	+	No EC50	+	No EC50	
4-Cumylphenol	599-64-4	4.70E-08	+	3.22E-07	+	14	13
Daidzein	486-66-8	5.11E-08	+	2.90E-07	+	13	14
Mifepristone	84371-65-3	6.84E-08	+	No EC50	-	No EC50	
o,p'–DDT	789-02-6	1.15E-07	+	6.60E-07	+	16	15
Kepone	143-50-0	1.91E-07	+	No EC50	+	No EC50	
Apigenin	520-36-5	2.14E-07	+	No EC50	+	No EC50	
4-tert-Octylphenol	140-66-9	3.31E-07	+	No EC50	+	No EC50	
p-n-Nonylphenol	104-40-5	5.01E-07	+	8.50E-08	+	11	16
Butylbenzyl phthalate	85-68-7	5.59E-07	+	No EC50	+	No EC50	
Kaempferol	520-18-3	7.70E-07	+	No EC50	+	No EC50	
p,p'-Methoxychlor	72-43-5	3.68E-06	+	8.85E-06	+	17	17
Fenarimol	60168-88-9	2.47E-06	+	2.70E-05	+	18	18
p,p'–DDE	72-55-9	4.27E-06	+	No EC50	+	No EC50	

No EC50: Reported as EA positive by ICCVAM meta-study, but no EC50s given and therefore no ranking comparison. The most active test compound (lowest EC50) in each set is assigned the lowest (1) rank number and the least active chemical is assigned the highest (18 or 26) rank number.

Table 2: Accuracy of CertiChem's MCF-7 EA Assay for All Chemicals Tested

24 EA Positive ICCVAM (2003, 2006) test chemicals assayed positive by CCi **0/24 false negatives**	17α-estradiol, 17β-estradiol, 4-Cumyphenol, 4-tert-Octylphenol, Apigenin, BPA, BPB, Butylbenzyl phthalate, Coumestrol, Daidzein, Diethylstilbestrol, o,p'-DDT, p,p'-DDE, Estriol, Estrone, Fenarimol, Flavone, Genistein, Kaempferol, Kepone, meso-Hexestrol, p,p'-Methoxychlor, p-n-Nonylphenol, Zearalenone.
15 EA negative ICCVAM (2003, 2006) test chemicals assayed negative by CCi **0/15 false positives**	Atrazine, Clomiphene citrate*, Corticosterone, Cycloheximide, Cyproterone acetate, Dexamethasone*, Flutamide, Haloperidol, Hydroxytamoxifen**, ICI 182, 780, Linuron, Procymidone, Progesterone, Trichloro-phenoxyacetic acid. *,**: Chemicals once considered EA positive by ICCVAM (2003), but now (ICCVAM, 2006) considered to be negative* or uncertain**.
34 other EA negative chemicals assayed by CCi. No EA has been reported for these chemicals by ICCVAM, nor do CCi's QSAR analyses predict any EA for these chemicals **0/34 false positives**	Acetaminophen, Acetonitrile, Acetylsalicylic acid, Amitriptyline HCl, Carbamazepine, Catechin, Cycloheximide, DL Propranolol, Eserine, Ethanol, Ethylene glycol, Glycerol, Glycyrrhizic acid, Haloperidol, Hexachlorophene, Lactic acid, Lithium carbonate, Methyl viologen, n-Phenylthiourea, Potassium chloride, Procainamide HCl, Sodium Chloride, Sodium fluoride, Sodium hypochlorite, Sodium oxalate, Sodium selenate, Strychnine, Tert-butylhydroquinone (TBHQ), Trichloroacetic acid, Trihydrobutyrophenone (TBHP), Triphenyltin hydroxide, Valprioc acid, Verapamil HCl, Vitamin E.

A least squares regression analysis (**Fig. 4a**) shows that the EC50 rankings produced by CertiChem's robotic EA assay and ICCVAM meta-analysis rankings for EC50 measures of EA of various chemicals do not differ significantly (null hypothesis, $p < 0.001$). CertiChem's robotic assay also includes several positive (E2, genistein) and negative (saline) controls, as well as an anti-EA control to insure that any cell growth is EA-dependent. As a check, this assay easily detects BPA (**Fig. 4b**).

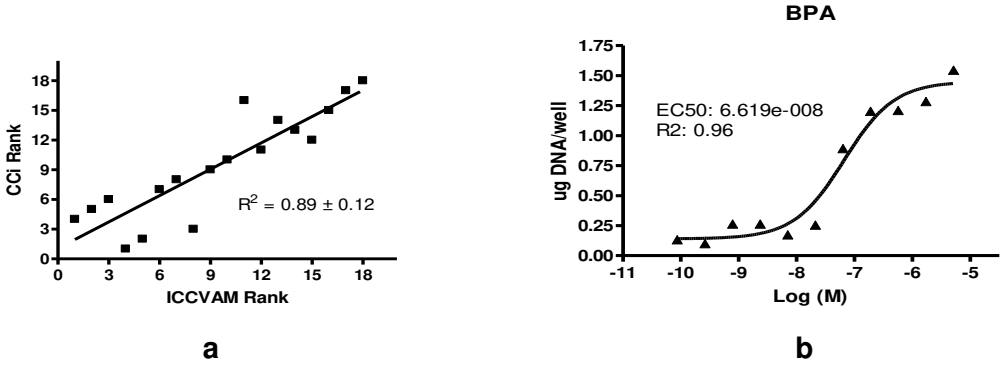


Figure 4: Accuracy of CCi's Robotic Assay a) CCi (mean) *versus* ICCVAM (median) EC50 rankings for 18 chemicals ranked by ICCVAM (2003) meta-study; b) Response of MCF-7 assay to BPA

Similar to its EA assay, CertiChem's anti-EA robotic *in vitro* assay is in compliance with ICCVAM recommended protocols. CCi's assay is highly sensitive, i.e. capable of detecting chemicals with high anti-EA (e.g., ICI, HDT) at picomolar concentrations and chemicals with low anti-EA (apigenin, flavone) at less than micromolar concentrations. This assay can measure the anti-EA of single chemicals, as well as complex mixtures of known and/or unknown chemicals in small amounts (data not shown).

CertiChem's EA assay is the most sensitive EA assay commercially available, having an EC50 of about 10^{-13} M for 17β–estradiol. For example, in contrast, the EC50 of 17β–estradiol is about 10^{-11} (100 times less sensitive) for the EA assay offered by some commercial laboratories; other ICCVAM reference test chemicals tested by CertiChem versus other commercial laboratory's assays usually show that CertiChem's assay is on average 20 to >100 fold more sensitive. In addition, CertiChem's most sensitive extraction protocols are also 5-20 times more robust than those published by other laboratories. Hence, CertiChem's sensitive EA assay can be100-10,000 times more sensitive in detecting EA in plastic extractives than other commercial laboratories.

Summary

- At least several thousand chemicals are now known to have EA, several hundred of which are commonly used in making plastics.
- Estrogens are the predominately female sex hormones, but are of critical importance in regulating multiple systems in both men and women. Chemicals with EA mimic or block the actions of these hormones.
- Many scientific studies have now shown that chemicals with EA produce a wide range of health problems in mammals, including early puberty in females, reduced sperm counts in males, altered functions of reproductive organs, obesity, altered behaviors, learning disorders, and increased rates of some breast, ovarian, testicular, and prostate cancers.
- PlastiPure, in conjunction with its testing partner, CertiChem, Inc, uses a cell proliferation *in vitro* assay protocol for determining EA in plastics, which is demonstrated to be highly sensitive, accurate, reliable, and repeatable. This method looks at EA directly through cell growth, rather than just individually assaying for one or two of the thousands of possible chemicals that can cause EA. These tests are generally much more sensitive than other *in vitro* (e.g. gene expression) or *in vivo* testing (e.g. Sprague-Dawley) modalities.

Cited References

Adewale HB, Jefferson WN, Newbold RR, Patisaul HB. 2009. Neonatal bisphenol-A exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin releasing hormone neurons. Environ. Health Perspectives. Epub ahead of print

Al-Hiyasat AS, Darmani H, Elbetieha AM. 2004. Leached components from dental composites and their effects on fertility of female mice. Eur J Oral Sci. 112:267-72.

Baskin LS, Himes K, Colborn T. 2001. Hypospadias and endocrine disruption: is there a connection? Environ Health Perspect. 109(11):1175-83.

Beato M. 1989. Gene regulation by steroid hormones. Cell. 56(3):335-44.

Black LJ, Jones CD, Falcone JF. 1983. Antagonism of estrogen action with a new benzothiophene-derived antiestrogen. Life Sci. 32(9):1031-6.

Bonde JP, Storgaard L. 2002. How work-place conditions, environmental toxicants and lifestyle affect male reproductive function. Int J Androl. 25(5):262-8.

Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. 1998. Interaction of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. Toxicol Ind Health. 14(1-2):59-84.

Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL. 2005. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perpect. 113(4):391-5.

Copenhagen. 2007. 4th Copenhagen workshop on the endocrine disruptors entitled endocrine disruptors and consumer products: Possible effect on human populations. 2007 May 28-May 31.

Della Seta D, Minder I, Belloni V, Aloisi AM, Dessi-Fulgheri F, Farabollini F. 2006. Pubertal exposure to estrogenic chemicals affects behavior in juvenile and adult male rats. Horm Behav. 50(2):301-7.

EDSTAC. 1998, 1999. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) final report. EPA. Available from: http://www.epa.gov/endo/pubs/edspoverview/finalrpt.htm

EPA. 2000. Report to Congress. August, 2000. Available from: http://www.epa.gov/oscpmont/oscpendo/index.htm.

Evinger AJ, Levin ER. 2005. Requirements for estrogen receptor alpha membrane localization and function. Steroids. 70(5-7):361-3.

Fujimoto T, Kubo K, Aou S. 2006. Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. Brain Res. 1068(1):49-55.

Garner M, Turner MC, Ghadirian P. Krewski D, Wade M. 2008. Testicular cancer and hormonally active agents. J. Toxicol. Environ. Health B Crit. Rev. 11(3-4): 260-275.

Goldman LR, Koduru S. 2000. Chemicals in the environment and developmental toxicity to children: a public health and policy perspective. Environ Health Perspect. 108 Suppl 3:443-8.

Goodman JE, McConnell EE, Sipes IG, Witorsch RJ, Slayton TM, Yu, CJ, Lewis AS, Rhomberg LR. 2006. An updated weight of the evidence evaluation of

reproductive and developmental effects of low doses of bisphenol A. Crit Rev Toxicol. 36(5):387-457.

Gray LE Jr, Ostby J, Wolf C, Lambright C, Kelce W. 1998. The value of mechanistic studies in laboratory animals for the prediction of reproductive effects in wildlife: Endocrine effects on mammalian sexual differentiation. Environ Toxicol Chem. 17:109-118.

Guillette LJ Jr, Gunderson MP 2001. Alterations in development of reproductive and endocrine systems of wildlife populations exposed to endocrine-disrupting contaminants. Reproduction. 122(6):857-64.

Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc Natl Acad Sci USA. 99(8):5476-80.

Hewitt SC, Deroo BJ, Korach KS. 2005. Signal Transduction. A new mediator for an old hormone? Science. 307(5715):1572-3.

Hines M. 1992. Surrounded by estrogens? Considerations For Neurobehavioral Development in Human Beings. In: Colborn T and Clement C, editors. Chemically induced alterations in sexual and functional development: the wildlife/human connection. Princeton, New Jersey: Princeton Scientific Publishing. p 261-81.

Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS. 1999. Exposure to bisphenol A advances puberty. Nature. 401(6755):763-4.

Howdeshell KL, vom Saal FS. 2000. Developmental exposure to bisphenol A alters postnatal phenotype: Interaction with endogenous estradiol in fetal mice. American Zoologist. 40(3):429-37.

ICCVAM. 2002a. Expert panel report on the current status of *in vitro* test methods for detecting endocrine disruptors. September, 2002. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/expertpanfinalrpt/panelrpt1102.pdf

ICCVAM. 2002b. Estrogen receptor binding. October 2002. NIH Pub. No 03-4504. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erbndbrd/ERBd034504.pdf

ICCVAM. 2002c. Estrogen receptor transcriptional activation. October 2002. NIH Pub. No 03-4505. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erta_brd/ERTA034505.pdf

ICCVAM. 2003. Evaluation of *in vitro* test methods for detecting endocrine disruptors. May, 2003. NIH Pub. 03-4503. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/edfinalrpt0503/edfinrpt.pdf

ICCVAM. 2006. Addendum March 2006 to evaluation of *in vitro* test methods for detecting endocrine disruptors. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/EDAddendFinal.pdf

ICCVAM. 2007. Validation of CertiChem Inc. MCF-7 Cell Proliferation Assay of Estrogenic Activity. Available from: http://iccvam.niehs.nih.gov/methods/endocrine/end_eval-CChem.htm

Janer G, Porte C. 2007. Sex steroids and potential mechanisms of non-genomic endocrine disruption in invertebrates. Ecotoxicology. 16(1):145-60.

Jordan VC, Murphy CS. 1990. Endocrine pharmacology of anti-estrogens as anti-tumor agents. Endocr Rev. 11(4):578-610.

Jordan VC. 2000. Tamoxifen: personal retrospective. Lancet Oncology. 1(1):43-9.

Kabuto H, Amakawa M, Shishibori T. 2004. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci. 74(24):2931-40.

Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo C. 2003. Aggressive Behavior and Serum Testosterone Concentration during the Maturation Process of Male Mice: The Effects of Fetal Exposure to Bisphenol A. Environ Health Perspect. 111(2):175-8.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, et al. 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report pf the U.S. EPA-sponsored workshop. Environ Health Perspect. 104 Suppl 4:715-40.

Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology. 138(3):863-70.

Lonard DM, Smith CL. 2002. Molecular perspectives on selective estrogen receptor modulators (SERMs): progress in understanding their tissue-specific agonist and antagonist actions. Steroids. 67(1):15-24.

Loder N. 2000. Royal Society warns on hormone disrupters. Nature. 406(6791):4.

Markey CM, Coombs MA, Sonnenschein C, Soto AM. 2003. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. Evol Dev. 5(1):67-75.

Matthews JB, Fertuck KC, Celius T, Huang YW, Fong CJ, Zacharewski TR. 2002. Ability of structurally diverse natural products and synthetic chemicals to induce gene expression mediated by estrogen receptors from various species. J Steroid Biochem Mol Biol. 82(2-3):181-94.

McDonnell DP, Norris JD. 2002. Connections and regulation of the human estrogen receptor. Science. 296(5573):1642-4.

McLachlan JA. 2001. Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. Endocr Rev. 22(3):319-41.

Monje L, Varayoud J, Munoz-de-Toro M, Ramos JG. 2009. Neonatal exposure to bisphenol-A alters estrogen-dependent mechanisms governing sexual behavior is the adult female rat. Reprod Toxicol 2009 Epub

Muller P, Kietz S, Gustafsson JA, Strom A. 2002. The anti-estrogenic effect of all-trans-retinoic acid on the breast cancer cell line MCF-7 is dependent on HES-1 expression. J Biol Chem. 277(32):28376-9.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. 2006. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod Toxicol. 23(3):383-90.

Newbold RR, Jefferson WN, Padilla-Banks E, Haseman J. 2004. Developmental exposure to diethylstilbestrol (DES) alters uterine response to estrogens in prepubescent mice: low versus high dose effects. Reprod Toxicol. 18(3):339-406.

Newbold RR, Jefferson, WN, Padilla-Banks E. 2009. Prenatal exposure to bisphenol A at environmentally relevant doses adversely affects the murine reproductive tract later in life. Env Health Persp 117:879-85

NIEHS. 2006. Endocrine disruptors. Available from: http://www.niehs.nih.gov/health/topics/agents/endocrine/docs/endocrine.pdf

NRC. 1999. Hormonally active agents in the environment. Washington, D.C.: Nat Acad Press. 430p.

NTP. 2000, 2001. National Toxicology Program (NTP) National Institute of Environmental Health Sciences (NIEHS) Endocrine Disruptors Low-Dose Peer Review, Research Triangle Park, NC 27709, October 10-12. Available from: http://ntp.niehs.nih.gov/index.cfm?objectid=06F5CE98-E82F-8182-7FA81C02D3690D47.

Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, Scanlan TS. 1997. Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. Science. 277(5331):1508-10.

Palanza P, Parmigiani S, Liu H, vom Saal FS. 1999. Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p'-DDT alters aggressive behavior of male and female house mice. Pharmocol Biochem Behav. 64(4):665-72.

Palanza PL, Howdeshell KL, Parmigiani S, vom Saal FS. 2002. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ Health Perspect. 110 Suppl 3:415-22.

Patisaul HB, Fortino AE, Polston EK. 2006. Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. Neurotoxicol Teratol. 28(1):111-8.

Patisaul HB, Todd KL, Mickens JA, Adewale HB. 2009. Impact of neonatal exposure to the ERalpha agonist PPT, bisphenol-A or phytoestrogens on hypothalamic kisspeptin fiber density in male and female rats. Neurotoxicology 30(3):350-7.

Raz L, Khan MM, Mahesh VB, Vadlamudi RK, Brann DW. 2008. Rapid estrogen signaling in the brain. Neurosignals. 16:140-53.

Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. 2005. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science. 307(5715):1625-1630.

Routledge EJ, White R, Parker MG, Sumpter JP. 2000. Differential effect of xenoestogens on coactivator recruitment by estrogen receptor (ER) α and ERβ. J Biol Chem. 275(46):35986 - 35993.

Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. 2006. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinology. 147(8):3681-91.

Shang Y, Brown M. 2002. Molecular determinants for the tissue specificity of SERMs. Science. 295(5564):2465-8.

Singleton DW, Khan SA. 2003. Xenoestrogen exposure and mechanisms of endocrine disruption. Front Biosci. 8:s110-8.

SPI. 2007. Plastics Fact Sheet. The Society of the Plastics Industry, Inc. Available from: http://www.plasticsdatasource.org.

Spivey A. 2009. Prenatal preview: early bisphenol a exposure may spawn late-life reproductive problems. Environ Health Perspect. 117(6):A256.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL. 2005. Decrease in anogenital distance among male Infants with prenatal phthalate exposure. Environmental Health Perspect. 113(8):1056-61.

Takao Y, Lee HC, Ishibashi Y, Kohra S, Tominaga N, Arizono K. 1999. Fast screening method for bisphenol-A in environmental water and in food by solid-phase microextraction. J Health Sci. 45(1):P-39.

Tan SW, Zoeller RT. 2007. Integrating basic research on thyroid hormone action into screening and testing programs for thyroid disruptors. Crit Rev Toxicol. 37(1-2):5-10.

The Freedonia group. 2007. U.S. food container demand to rise 3.3% annually through 2011. Packaging World Magazine. October; 13.

Thornton JW. 2001. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci USA 98(10):5671-6.

Tyler CR, Jobling S, Sumpter JP. 1998. Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol. 28(4):319-61.

USA Today. 2007. 'Everywhere chemicals' in plastics alarm parents. October 31, 2007.

Vasudevan N, Pfaff DW. 2008. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. Frontiers Neuroendocrinol. 29:238-57.

vom Saal FS, Hughes C. 2005. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect. 113(8): 926-33.

vom Saal FS, Nagel SC, Timms BG, Welshons WV. 2005. Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public. Toxicology. 212(2-3):244-52.

vom Saal FS. 2006. Bisphenol A eliminates brain and behavior sex dimorphisms in mice: how low can you go? Endocrinology. 147(8):3679-80.

Wakeling AE. 1993. The future of new pure antiestrogens in clinical breast cancer. Breast Cancer Res Treat. 25(1):1-9.

Welshons WV, Nagel SC, Thayer KA, Judy BM, vom Saal FS. 1999. Low-dose bioactivity of xenoestrogens in animals: Fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health. 15(1-2):12-25.

Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111(8):994-1006.

Yang CZ, Bittner GD. 2007. Production of plastic, cosmetic, paper and other consumer products with desired levels of estrogenic activity. 4th Copenhagen Workshop on Endocrine Disruptors. Abs. II-7, p72.

Yang NN, Venugopalan M, Hardikar S, Glasebrook A. 1996. Identification of an estrogen response element activated by metabolites of 17beta-estradiol and raloxifene. Science. 273(5279):1222-25.

Zweifel H. 2001. Plastics Additive Handbook, 5th Ed. Munich: Hanser. p1-136.

Scientific References

Adverse Health Effects of EDCs Used to Make Plastics

Acevedo R, Parnell PG, Villaneuva H, Chapman LM, Gimenez T, Gray SL, Baldwin WS. 2005. The contribution of hepatic steroid metabolism to serum estradiol and estriol concentrations in nonylphenol treated MMTVneu mice and its potential effects on breast cancer incidence and latency. J Appl Toxicol. 25(5):339-53.

Angerer J, Bird MG, Burke TA, Doerrer NG, Needham L, Robison SH, Sheldon L, Zenick H. 2006. Strategic biomonitoring initiatives: Moving the science forward. Toxicol Sci. 93(1):3-10.

Baskin LS, Himes K, Colborn T. 2001. Hypospadias and endocrine disruption: is there a connection? Environ Health Perspect. 109(11):1175-83.

Bonde JP, Storgaard L. 2002. How work-place conditions, environmental toxicants and lifestyle affect male reproductive function. Int J Androl. 25(5):262-8.

Borch J, Metzdorff SB, Vinggaard AM, Brokken L, Dalgaard M. 2006. Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. Toxicology. 223(1-2):144-55.

Brody JG, Rudel RA. 2003. Environmental pollutants and breast cancer. Environ Health Perspect. 111(8):1007-19.

Brody JG, Rudel RA, Melly SJ, Maxwell NI. 1998. Endocrine disruptors and breast cancer. Forum for Applied Research and Public Policy. 13(3):24-31.

Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. 1998. Interaction of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. Toxicol Ind Health. 14(1-2):59-84.

Cox DG, Blanche H, Pearce CL, Calle EE, Colditz GA, Pike MC, Albanes D, Allen NE, Amiano P, Berglund G, et al. 2006. A comprehensive analysis of the androgen receptor gene and risk of breast cancer: results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BCP3). Breast Cancer Res. 8(5):R54.

Della Seta D, Minder I, Belloni V, Aloisi AM, Dessi-Fulgheri F, Farabollini F. 2006. Pubertal exposure to estrogenic chemicals affects behavior in juvenile and adult male rats. Horm Behav. 50(2):301-7.

Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, Munoz-de-Toro M. 2007. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. Environ Health Perspect. 115(1): 80-6.

EDSTAC. 1998, 1999. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) final report. EPA. Available from: http://www.epa.gov/endo/pubs/edspoverview/finalrpt.htm

EPA. 2000. Report to Congress. August, 2000. Available from: http://www.epa.gov/oscpmont/oscpendo/index.htm.

Fang H, Tong W, Branham WS, Moland CL, Dial SL, Hong H, Xie Q, Perkins R, Owens W, Sheehan DM. 2003. Study of 202 natural, synthetic, and environmental chemicals for binding to the androgen receptor. Chem Res Toxicol. 16(10): 1338-58.

Fenton SE. 2006. Endocrine-disrupting compounds and mammary gland development: Early exposure and later life consequences. Endocrinology 147(6 Suppl):S18-S24.

Foster PM. 2006. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl. 29(1):140-7.

Fujimoto T, Kubo K, Aou S. 2006. Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. Brain Res. 1068(1):49-55.

Goodman JE, McConnell EE, Sipes IG, Witorsch RJ, Slayton TM, Yu, CJ, Lewis AS, Rhomberg LR. 2006. An updated weight of the evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. Crit Rev Toxicol. 36(5):387-457.

Gross L. 2007. The toxic origins of disease. PLoS Biol. 5(7):e193.

Guillette LJ Jr, Gunderson MP 2001. Alterations in development of reproductive and endocrine systems of wildlife populations exposed to endocrine-disrupting contaminants. Reproduction. 122(6):857-64.

Giusti RM, Iwamoto K, Hatch EE. 1995. Diethystilbestrol revisited: A review of the long-term health effects. Ann Intern Med. 122(10):778-88.

Hines M. 1992. Surrounded by estrogens? Considerations For Neurobehavioral Development in Human Beings. In: Colborn T and Clement C, editors. Chemically induced alterations in sexual and functional development: the wildlife/human connection. Princeton, New Jersey: Princeton Scientific Publishing. p 261-81.

Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS. 1999. Exposure to bisphenol A advances puberty. Nature. 401(6755):763-4.

Howdeshell KL, vom Saal FS. 2000. Developmental exposure to bisphenol A alters postnatal phenotype: Interaction with endogenous estradiol in fetal mice. American Zoologist. 40(3):429-37.

Ibarluzea Jm J, Fernandez MF, Santa-Marina L, Olea-Serrano MF, Rivas AM, Aurrekoetxea JJ, Exposito J, Lorenzo M, Torne P, Villalobos M, et al. 2004. Breast cancer risk and the combined effects of environmental estrogens. Cancer Causes Control. 15(6):591-601.

ICCVAM. 2002a. Expert panel report on the current status of *in vitro* test methods for detecting endocrine disruptors. September, 2002. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/expertpanfinalrpt/panelrpt1102.pdf

ICCVAM. 2002b. Estrogen receptor binding. October 2002. NIH Pub. No 03-4504. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erbndbrd/ERBd034504.pdf

ICCVAM. 2002c. Estrogen receptor transcriptional activation. October 2002. NIH Pub. No 03-4505. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erta_brd/ERTA034505.pdf

ICCVAM. 2003. Evaluation of *in vitro* test methods for detecting endocrine disruptors. May, 2003. NIH Pub. 03-4503. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/edfinalrpt0503/edfinrpt.pdf

ICCVAM. 2006. Addendum March 2006 to evaluation of *in vitro* test methods for detecting endocrine disruptors. Available from: http://iccvam.niehs.nih.gov/docs/endo_docs/EDAddendFinal.pdf

ICCVAM. 2007. Validation of CertiChem Inc. MCF-7 Cell Proliferation Assay of Estrogenic Activity. Available from: http://iccvam.niehs.nih.gov/methods/endocrine/end_eval-CChem.htm

Jiang JT, Ma L, Yuan L, Wang XR, Zhang W. 2007. Study on developmental abnormalities in hypospadiac male rats induced by maternal exposure to di-n-butyl phthalate (DBP). Toxicology. 232(3): 286-93.

Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. 1995. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect.103(6): 582-7.

Kabuto H, Amakawa M, Shishibori T. 2004. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci. 74(24):2931-40.

Kang SC, Lee BM. 2005. DNA methylation of estrogen receptor alpha gene by phthalates. J Toxicol Environ Health A. 68(23-24):1995-2003.

Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo C. 2003. Aggressive Behavior and Serum Testosterone Concentration during the Maturation Process of Male Mice: The Effects of Fetal Exposure to Bisphenol A. Environ Health Perspect. 111(2):175-8.

Kim IY, Han SY, Moon A. 2004. Phthalates inhibit tamoxifen-induced apoptosis in MCF-7 human breast cancer cells. J Toxicol Environ Health A. 67(23-24):2025-35.

Kim JB, O'Hare MJ, Stein R. 2004. Models of breast cancer: is merging human and animal models the future? Breast Cancer Res. 6(1):22-30.

Latini G, Del Vecchio A, Massaro M, Verrotti A, De Felice C. 2006. Phthalate exposure and male infertility. Toxicology 226(2-3):90-8.

Loder N. 2000. Royal Society warns on hormone disrupters. Nature. 406(6791):4.

Lovekamp-Swan T, Davis BJ. 2003. Mechanisms of phthalate ester toxicity in female reproductive system. Environ Health Perspect. 111(2):139-45.

Maffini MV, Rubin BS, Sonnenschein C, Soto AM. 2006. Endocrine disruptors and reproductive health: The case of bisphenol-A. Mol Cell Endocrinol. 254-255:179-86.

Markey CM, Coombs MA, Sonnenschein C, Soto AM. 2003. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. Evol Dev. 5(1):67-75.

Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM. 2001. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. Biol Reprod. 65(4):1215-23.

Matsumoto H, Adachi S, Suzuki Y. 2005. Bisphenol A in ambient air particulates responsible for the proliferation of MCF-7 human breast cells and its concentration changes over 6 months. Arch Environ Contam Toxicol. 48(4):459-66.

Matthews JB, Fertuck KC, Celius T, Huang YW, Fong CJ, Zacharewski TR. 2002. Ability of structurally diverse natural products and synthetic chemicals to

induce gene expression mediated by estrogen receptors from various species. J Steroid Biochem Mol Biol. 82(2-3):181-94.

McLachlan JA. 2001. Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. Endocr Rev 22(3): 319-41.

Moon HJ, Han SY, Shin JH, Kang IH, Kim TS, Hong JH, Kim SH, Fenton SE. 2007. Gestational exposure to nonylphenol causes precocious mammary gland development in female rat offspring. J Reprod Dev. 53(2):333-44.

Munoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM. 2005. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology 146(9):4138-47.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. 2006. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod Toxicol. 23(3):383-90.

Nagel SC, vom Saal FS, Thayer KA, Dahr MG, Boechler M, Welshons WV. 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ Health Perspect. 105(1):70-6.

Nagel SC, vom Saal FS, Welshons, WV. 1998. The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity. Proc Soc Exp Biol Med 217(3):300-9.

National Academy Press. 1999. Hormonally active agents in the environment. ISBN-0309-06419-8.

Newbold RR, Jefferson WN, Padilla-Banks E, Haseman J. 2004. Developmental exposure to diethylstilbestrol (DES) alters uterine response to estrogens in prepubescent mice: low versus high dose effects. Reprod Toxicol. 18(3):339-406.

Newbold RR, Jefferson WN, Padilla-Banks E. 2007. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reprod Toxicol. 24(2):253-8.

NIEHS. 2006. Endocrine disruptors. Available from: http://www.niehs.nih.gov/health/topics/agents/endocrine/docs/endocrine.pdf

NTP. 2000, 2001. National Toxicology Program (NTP) National Institute of Environmental Health Sciences (NIEHS) Endocrine Disruptors Low-Dose Peer Review, Research Triangle Park, NC 27709, October 10-12. Available from: http://ntp.niehs.nih.gov/index.cfm?objectid=06F5CE98-E82F-8182-7FA81C02D3690D47.

Palanza P, Parmigiani S, Liu H, vom Saal FS. 1999. Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p'-DDT alters aggressive behavior of male and female house mice. Pharmocol Biochem Behav. 64(4):665-72.

Palanza PL, Howdeshell KL, Parmigiani S, vom Saal FS. 2002. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ Health Perspect. 110 Suppl 3:415-22.

Patisaul HB, Fortino AE, Polston EK. 2006. Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. Neurotoxicol Teratol. 28(1):111-8.

Rivas A, Lacroix M, Olea-Serrano F, Laios I, Leclercq G, Olea N. 2002. Estrogenic effect of a series of bisphenol analogues on gene and protein expression in MCF-7 breast cancer cells. J Steroid Biochem Mol Biol 82(1): 45-53.

Routledge EJ, White R, Parker MG, Sumpter JP. 2000. Differential effect of xenoestogens on coactivator recruitment by estrogen receptor (ER) α and ERβ. J Biol Chem. 275(46):35986 - 35993.

Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. 2006. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinology. 147(8):3681-91.

Singleton DW, Khan SA. 2003. Xenoestrogen exposure and mechanisms of endocrine disruption. Front Biosci. 8:s110-8.

Soto AM, Chung KL, Sonnenschein C. 1994. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ Health Perspect. 102(4):380-3.

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. 2003. The E-SCREEN Assay as a Tool to Identify Estrogens: An Update on Estrogenic Environmental Pollutants. Environ Health Perspect. 103 Suppl 7:113-22.

Soule HD, Vazquez J, Long A, Albert S, Brennan M. 1973. A human cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst. 51(5):1409-16.

Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K. 2005. Exposure to bisphenol A is associated with recurrent miscarriage. Hum Reprod. 20(8):2325-9.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL. 2005. Decrease in anogenital distance among male Infants with prenatal phthalate exposure. Environmental Health Perspect. 113(8):1056-61.

Thomas P, Dong J. 2006. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: A potential novel mechanism of endocrine disruption. J Steroid Biochem Mol Biol. 102(1-5):175-9.

Vandenberg LN, Maffini MV, Wadia PH, Sonnenschein C, Rubin BS, Soto AM. 2007. Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. Endocrinology 148(1):116-27.

vom Saal FS. 2006. Bisphenol A eliminates brain and behavior sex dimorphisms in mice: how low can you go? Endocrinology. 147(8):3679-80.

vom Saal FS, Hughes C. 2005. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect. 113(8): 926-33.

vom Saal FS, Nagel SC, Timms BG, Welshons WV. 2005. Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public. Toxicology. 212(2-3):244-52.

vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette LJ Jr, Hauser R, Heindel JJ, et al. 2007. Chapel Hill Bisphenol A Expert Panel Consensus Statement: Integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol. 24(2):131-8.

Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM. 2007. Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse species. Environ Health Perspect. 115(4):592-8.

Welshons WV, Nagel SC, Thayer KA, Judy BM, vom Saal FS. 1999. Low-dose bioactivity of xenoestrogens in animals: Fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health. 15(1-2):12-25.

Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111(8):994-1006.

EDCs in Plastics in the Environment

McKelvey W, Brody JG, Aschengrau A, Swartz CH. 2004. Association between residence on Cape Cod, Massachusetts, and breast cancer. Ann Epidemiol. 14(2):89-94.

Norwegian RoHS, 18 substances banned. Reported July 23, 2007. Available from: http://www.evertiq.com/news/read.do?news=8237&cat=7

NRC. 1999. Hormonally active agents in the environment. Washington, D.C.: Nat Acad. Press. 430p.

Rodriguez-Mozaz S, Lopez de Alda ML, Barcelo D. 2005. Analysis of bisphenol A in natural waters by means of an optical immmunosensor. Water Res. 39(20):5071-9.

Rudel RA, Camann DE, Spengler JD, Korn LR, Brody JG. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environ Sci Technol. 37(20):4543-53.

Rudel RA, Melly SJ, Geno PW, Sun G, Brody JG. 1998. Identification of alkylphenols and other estrogenic phenolic compounds in wastewater, septage, and groundwater on Cape Cod, Massachusetts. Environ Sci Technol. 32(7):861-9.

Rudel RA, Brody JG, Spengler JD, Vallarino J, Geno PW, Sun G, Yau A. 2001. Identification of selected hormonally active agents and animal mammary carcinogens in commercial and residential air and dust samples. J Air Waste Manag Assoc. 51(4):499-513.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL. 2005. Decrease in anogenital distance among male Infants with prenatal phthalate exposure. Environmental Health Perspect. 113(8):1056-61.

Soto AM, Chung KL, Sonnenschein C. 1994. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ Health Perspect. 102(4):380-3.

Swartz CH, Reddy S, Benotti MJ, Yin H, Barber LB, Brownawell BJ, Rudel RA. 2006. Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. Environ Sci Technol. 40(16):4894-902.

EDC Chemicals in Plastics Found in Humans

Boyle CA, Decoufle P, Yeargin-Allsopp M. 1994. Prevalence and health impact of developmental disabilities in US children. Pediatrics 93(3):399-403.

Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL. 2005. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perpect. 113(4):391-5.

2005. Third National Report on Human Exposure to Environmental Chemicals. Atlanta: Centers for Disease Control and Prevention.

Commission of the European Communities (2004). Commission staff working document on implementation of the community strategy for endocrine disruptors – a range of substances suspected of interfering with the hormone systems of humans and wildlife. (Accessed online 7/5/07) Available from: http://ec.europa.eu/environment/endocrine/documents/sec_2004_1372_en.pdf

Copenhagen. 2007. 4th Copenhagen workshop on the endocrine disruptors entitled endocrine disruptors and consumer products: Possible effect on human populations. 2007 May 28-May 31.

Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. 1993. Medical hypothesis: Xenoestrogens as preventable causes of breast cancer. Environ Health Perspect. 101(5):371-7.

DeBruin LS, Pawliszyn JB, Josephy PD. 1999. Detection of monocyclic aromatic amines, possible mammary carcinogens, in human milk. Chem Res Toxicol. 12(1):78-82.

Fenton SE, Condon M, Ettinger AS, LaKind JS, Mason A, McDiarmid M, Qian Z, Selevan SG. 2005. Collection and use of exposure data from human milk biomonitoring in the United States. J Toxicol Environ Health A. 68(20):1691-712.

Fernandez MF, Molina-Molina JM, Lopez-Espinosa MJ, Freire C, Campoy C, Ibarluzea J, Torne P, Pedraza V, Olea N. 2007. Biomonitoring of environmental estrogens in human tissues. Int J Hyg Environ Health. 210(3-4):429-432.

Goldman LR, Koduru S. 2000. Chemicals in the environment and developmental toxicity to children: a public health and policy perspective. Environ Health Perspect. 108 Suppl 3:443-8.

Grandjean P, Bellinger D, Bergman A, Cordier S, Davey-Smith G, Eskenazi B, Gee D, Gray K, Hanson M, van den Hazel P, et al. 2007. The Faroes Statement: Human health effects of developmental exposure to chemicals in our environment. Basic Clin Pharmacol Toxicol. 102(2):73-5.

Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. 2002. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod. 17(11):2839-41.

Kato K, Silva MJ, Reidy JA, Hurtz D 3rd, Malek NA, Needham LL, Nakazawa H, Barr DB, Calafat AM. 2003. Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxhexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. Environ Health Perspect. 112(3): 327-30.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, et al. 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report pf the U.S. EPA-sponsored workshop. Environ Health Perspect. 104 Suppl 4:715-40.

Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. 2002. Parent Bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect. 110(11):A703-7.

Shen H, Main KM, Virtanen HE, Damggard IN, Haavisto AM, Kalevea M, Boisen KA, Schmidt IM, Chellakooty M, Skakkebaek NE, et al. 2006. From mother to child: Investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and placenta biomonitoring. Chemosphere 67(9):S256-62.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL. 2005. Decrease in anogenital distance among male Infants with prenatal phthalate exposure. Environmental Health Perspect. 113(8):1056-61.

Soto AM, Chung KL, Sonnenschein C. 1994. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ Health Perspect. 102(4):380-3.

Swartz CH, Reddy S, Benotti MJ, Yin H, Barber LB, Brownawell BJ, Rudel RA. 2006. Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. Environ Sci Technol. 40(16):4894-902.

Leaching of Chemicals with EA from plastic products

Al-Hiyasat AS, Darmani H, Elbetieha AM. 2004. Leached components from dental composites and their effects on fertility of female mice. Eur J Oral Sci. 112:267-72.

Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N. 1995. Xenoestrogens released from lacquer coating in food cans. Environ Health Perspect. 103(6):608-12.

Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, vom Saal FS, Welshons WV. 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ Health Perspect. 111(9):1180—7.

Institute for Agriculture and Trade Policy. (2005). Smart Plastics Guide. October 2005. Available from: http://www.healthobservatory.org/library.cfm?RefID=102202

Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D. 1993. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132(6):2279-86.

Slack RJ, Gronow JR, Voulvoulis N. 2005. Household hazardous waste in municipal landfills: contaminants in leachate. Sci Total Environ. 337(1-3):119-37.

SNO. 1999. Food for thought: What's coming out of baby's bottle? Science News Online. 156: 1-4.

Soto AM, Justicia H, Wray JW, Sonnenschein C. 1991. p-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. Environ. Health Perspect. 92:167-73.

Takao Y, Lee HC, Ishibashi Y, Kohra S, Tominaga N, Arizono K. 1999. Fast screening method for bisphenol-A in environmental water and in food by solid-phase microextraction. J Health Sci. 45(1):P-39.

Till DE, Ehntholt DJ, Reid RC, Schwartz PS, Sidman KR, Schwope AD, Whelan RH. 1982. Migration of BHT antioxidant from high density polyethylene to foods and food simulants, Ind. Eng., Chem. Prod. Res. Dev. 21:106-13.

Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS. 2005. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the mouse prostate and urethra. Proc Natl Acad Sci USA 102(19):7014-9.

Yang CZ, Bittner GD. 2007. Production of plastic, cosmetic, paper and other consumer products with desired levels of estrogenic activity. 4th Copenhagen Workshop on Endocrine Disruptors. Abs. II-7, p72.

Recent relevant papers by CertiChem and PlastiPure Scientists

C. Z. Yang, S. I. Yaniger, V. C. Jordan, D. Klein and G.D. Bittner. 2011. Most Plastic Products Release Estrogenic Chemicals: A Potential Health Problem That Can Be Solved. Environmental Health Perspectives 119: 989-996. doi: 10.1289/ehp.1003220. Epub 2011 Mar 2

C.Z. Yang, W. Casey, M. Stoner, G.J .Kollessery, A.W. Wong and G.D. Bittner, 2014. A robotic MCF-7:WS8 cell proliferation assay to detect agonist and antagonist estrogenic activity. Toxological Sci. 137:335-349. doi: 10.1093/toxsci/kft250. PubMed PMID: 24213142; PubMed Central PMCID: PMCPMC3908721.

S.L. Myers, C.Z.Yang, G.D. Bittner, K.L. Witt, R.R. Tice, D.D. Baird. 2014. Estrogenic and Anti-Estrogenic Activity of Off –The-Shelf Hair and Skin Products. Journal of Exposure Science and Environmental Epidemiology. 25:271-277. doi: 10.1038/jes.2014.32. PubMed PMID: 24849798.

M.A.Stoner, C.Z.Yang, and G.D.Bittner. 2014. A Robotic BG1Luc Reporter Assay to Detect Estrogen Receptor Agonists. Toxicology in Vitro. 28: 916–925. . doi:

10.1016/j.tiv.2014.03.013. PubMed PMID: 24747293; PubMed Central PMCID: PMCPMC4088324

G.D.Bittner, M. A. Stoner, C. Z. Yang. 2014. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. Environmental Health 13:41-54. doi: 10.1186/1476-069X-13-41. PubMed PMID: 24886603; PubMed Central PMCID: PMCPMC4063249.

G.D. Bittner, M.S. Denison, C. Z. Yang, M.A.Stoner, G. He. 2014. Chemicals having estrogenic activity can be released from some BPA-free, hard and clear, thermoplastic resins. Environmental Health. 13:103-121. doi: 10.1186/1476-069X-13-41. PubMed PMID: 24886603; PubMed Central PMCID: PMCPMC4063249.