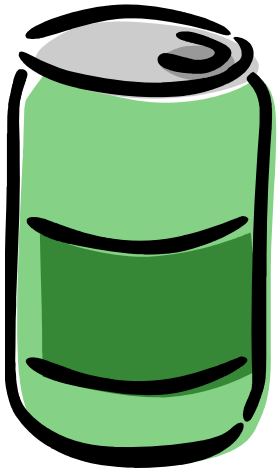


BISPHENOL A in OUR DIET: CONCENTRATIONS & CONTROVERSIES

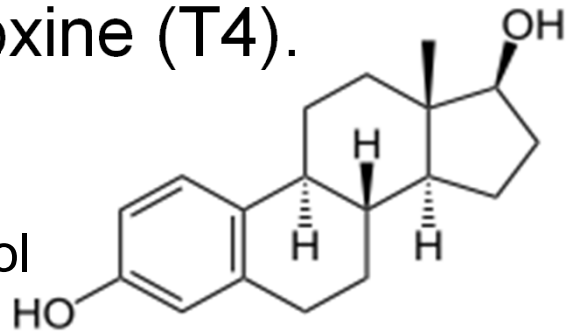
Martin Snider, MSc
Environmental Chemistry Laboratory
California DTSC
October 24, 2012

Today's Outline

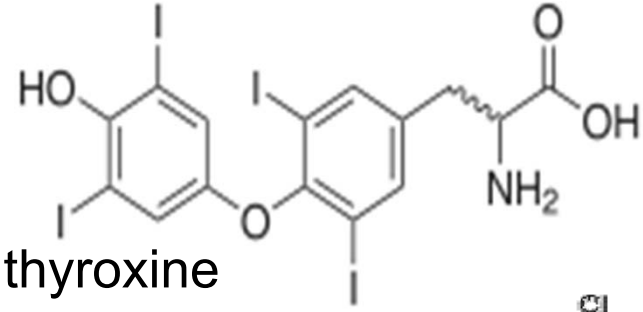
- Xenobiotic endocrine disrupting compounds
- Bisphenol A production and uses
- Exposure from foods and other sources
- Bisphenol A toxicology and epidemiology
- Regulation in foods, beverages & packaging
- Chemical analysis in foods, ECL 2012

Endocrine Disrupting Compounds (EDCs)

- Natural or synthetic compounds that act as agonists or antagonists at cellular hormone receptors, or interfere with hormone synthesis, transport or reactions.
- Structure and activity relationship (SAR) with endogenous hormones, eg. estradiol (E2) & thyroxine (T4).

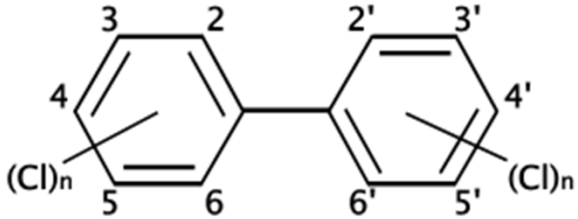


estradiol

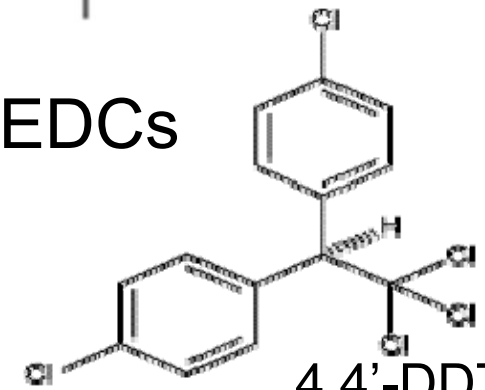


thyroxine

- PCBs and DDT are two “legacy” EDCs



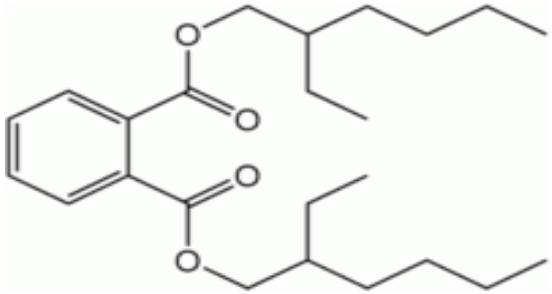
PCBs



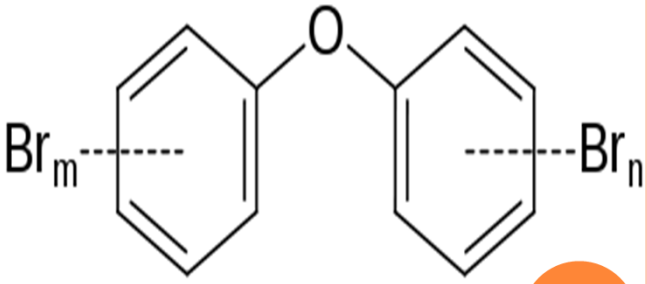
4,4’-DDT

Emerging EDCs
(many now banned or restricted in the EU & elsewhere)

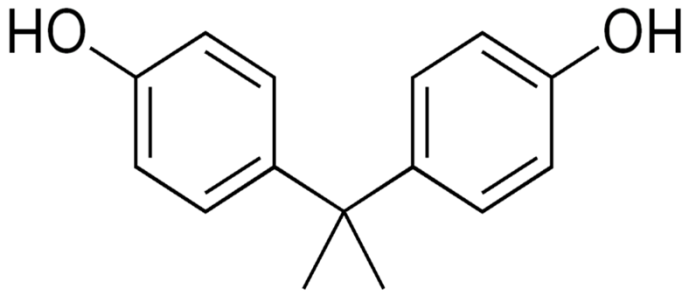
- Phthalates (eg. Bis-ethylhexyl phthalate aka DEHP) in plastics, toys, cosmetics, etc.



- Polybrominated diphenyl ethers (PBDEs) flame retardants in furniture, electronics, etc.

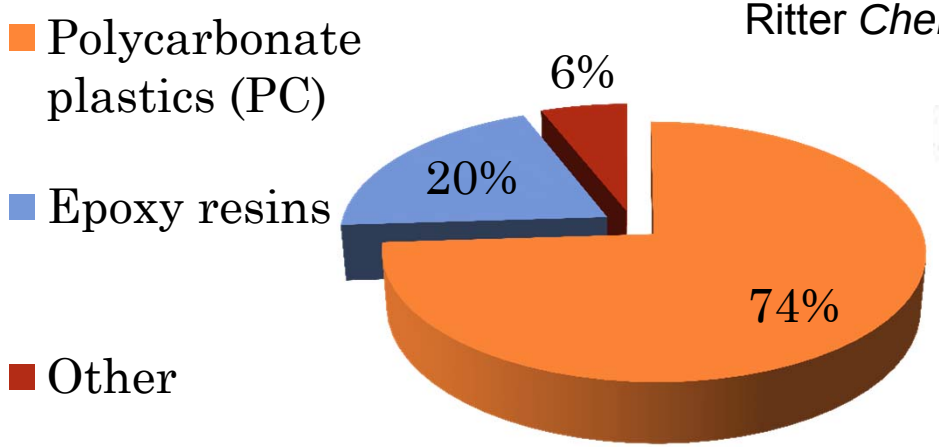


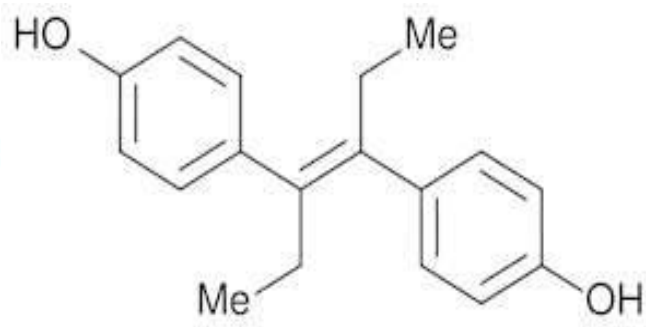
Bisphenol A (BPA)



- Has two aromatic rings with electron-rich, para-para' (4-4') substituents common to other EDCs
- Investigated as a pharmaceutical estrogen in 1930s; diethylstilbestrol (DES) used instead, until 1960s when linked to cancers in "DES daughters."
- 12 billions lbs/yr global use

Ritter *Chem. Eng. News* 89(23) 2011



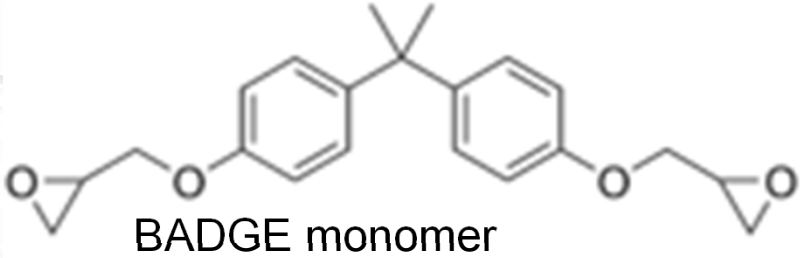


DES

BPA-based Polymers in Food & Beverage Containers

- PC was widely used for infant & sport beverage containers.

- BPA-diglycidyl ether (BADGE) resin is commonly used as a protective lining on metal food & beverage cans.
- Resin is sprayed on the sheet metal or finished can, and then “stoved” to further polymerize-cure before filling.
- But…BPA & BADGE have been found in the contents.



BADGE monomer

So how much BPA are we eating & drinking? I

Country of Study	Range BPA µg/kg	Sample with highest BPA	Tech-nique	Reference
US	0.1-13	all infant formula concentrates	HPLC-FLD	Biles, et al. 1998
UK	4-130	spaghetti bolognese	GC/MS (deriv.)	Goodson, et al. 2002
New Zealand	<10-192	coconut cream	"	Thompson, et al. 2005
Mexico	<7-103	tuna in oil	HPLC-FLD	Munguia-Lopez, et al. 2005
Austria	0.1-38	Tom Kha soup-Thai	"	Braunrath, et al. 2005
Singapore	33-165	sliced mango	"	Sun, et al. 2006
Czech R.	<3-389	cod liver	"	Poustka, et al. 2007
Italy	<LOQ-115	all peeled tomatoes	"	Grumetto, et al. 2008
Japan	nd-842	brown sauce	HPLC-	Sajiki, et al. 2007
Japan	nd-235	gratin sauce	MS/MS	Yonekubo, et al. 2008

So how much BPA are we eating & drinking? II

Country of Study	Range BPA µg/kg	Sample with highest BPA	Tech-nique	Reference
Canada	2.3-10	all infant formula (soy & milk)	GC/MS (deriv.)	Cao, et al. 2008
Canada	0.03-4.5	energy drink (all soft drinks)	"	Cao, et al. 2009
Canada	0.2-7.2	sweet potatoes & chicken, org. baby foods, jars-metal lid	"	Cao, et al. 2009
Belgium	<0.02-169	tuna in oil	GC/MS (deriv.)	Geens, et al. 2010
US	0.48-11	all infant formula (soy & milk)	HPLC-MS/MS	Ackerman, et al. 2010
US	<0.2-65	Del Monte brand green beans	GC/MS (deriv.)	Schecter, et al. 2010
Canada	0.2-106	fish, canned (composite)	"	Cao, et al. 2011
Spain	<0.005-0.6	orange soda (all soft drinks)	HPLC-MS/MS	Gallart-Ayala, et al. 2011
US	<2-730	green beans (national brand)	"	Noonan, et al. 2011

“I assure you there is no BPA on our menu, and if there is it probably won’t harm you.”

(graphic: Google images, caption added)

BPA Human Metabolism, Exposure & Biomonitoring

- Rapid first-pass metabolism in human liver, excreted in urine as non-bioactive glucoronide (> 90%) and sulfate conjugates.
- Found in >90% spot urines in US, Canada & Asia.
- Diet thought to be primary exposure source—urine levels positively correlated to canned food intake.
- Dermal contact (e.g. thermal paper receipts) & dust inhalation exposure thought to be much lower, but may be significant.

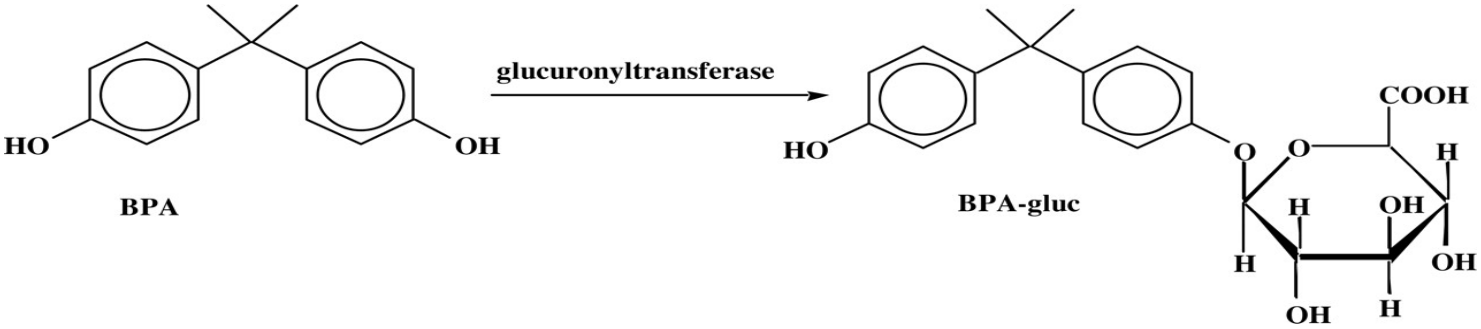


figure: Volkel, et al. *Drug Metabolism and Disposition* 2005, *33(11),1748-1757.*

BPA Epidemiology

- **Heart disease** associated with 10-year urine BPA levels Meltzer, D. et al. *Circulation* 2012, 125(12), 1482-1490.
- **Cardiovascular disease and diabetes** associated with higher urinary BPA, NHANES 2003-04 data Lang, I.A. et al. *JAMA* 2008, 300(11), 1303-1310.
- **Diabetes** positive assoc. with urinary BPA in NHANES 2003-08 data Shankar and Teppala, *J. Clin. Endocrin. Metab.* 2011, 96(12), 3822-3826.
- **Obesity and insulin resistance** associated with urinary BPA Wang, T. et al. *J. Clin. Endo. Metab. 2012,* 97(2), E223-E227.
- **Estrogenic gene expression in adults** assoc. with BPA exposure Melzer, D. et al. *Environ. Health Perspect.* 2011, 119, 1788-1793.
- **Worse behavior and executive function in 3-yr-olds,** especially girls, associated with *in utero* BPA exposure Braun, J.M. et al. *Pediatrics* 2011*, 128(5), 873-882.*

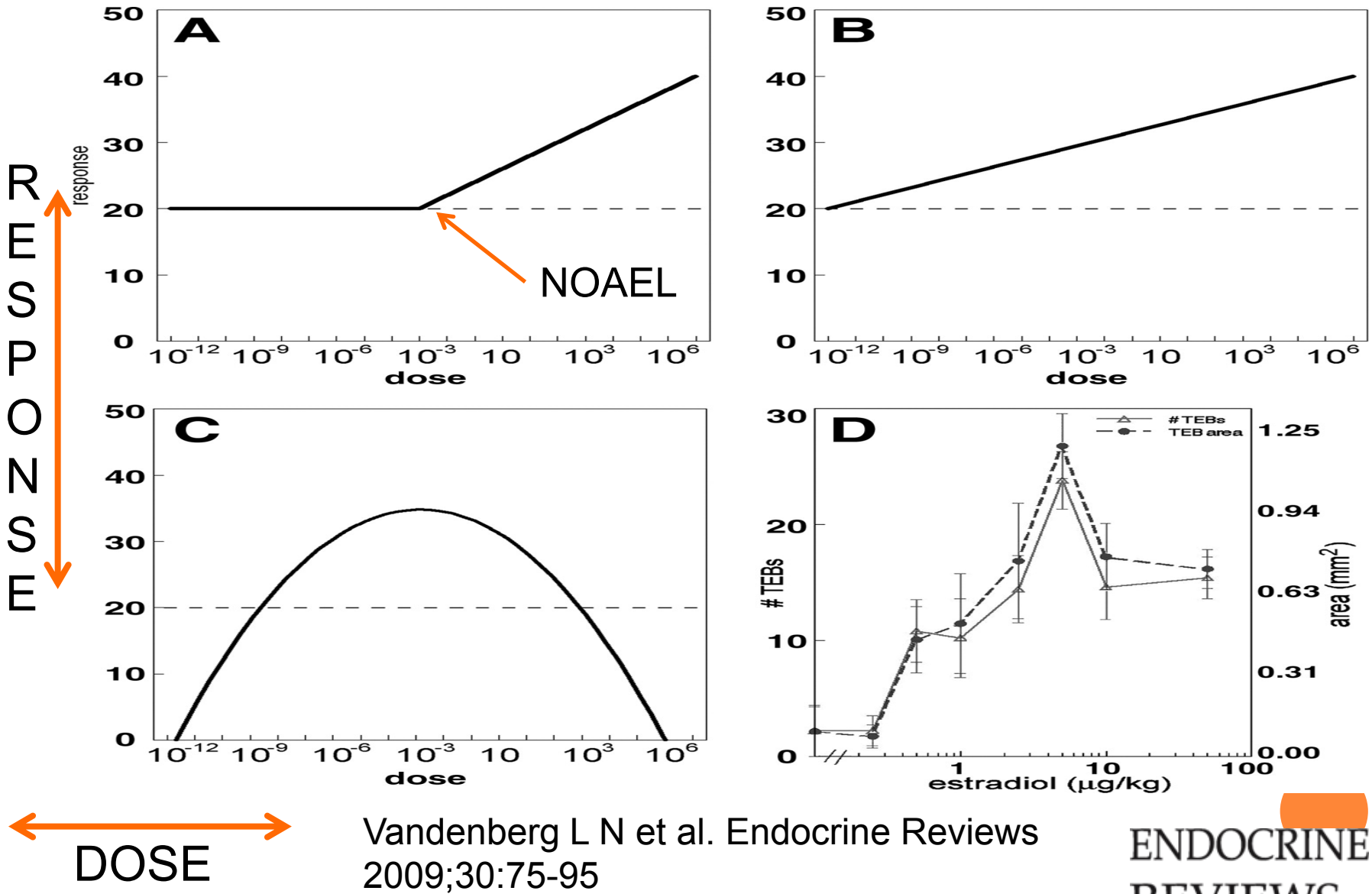
BPA *in vivo* Toxicology

- **Heart arrhythmia** in female adult rats from low-dose (10^{-9} M) exp. to BPA + estradiol Yan, S. et al. *PLoS ONE* 2011, 6(9), doi: 10.1371/journal.pone.0025455.
- Perinatal exposure to EU/US regulatory reference dose (50 µg/kg-bw/day) predisposed adult rats to **metabolic disorder** Wei, J. et al. *Endocrinol.* 2011, 152(8), 3049-3061.
- **Decreased fertility** in mice exposed perinatally to .025-25 µg/kg-bw/day Cabaton, N.J. et al. *Environ. Health Perspect.* 2011, 119 (4), 547-552.
- Perinatal exp. 0.6-1200 µg/kg-bw/day increased adult mice mammary gland cell no. & progesterone response (i.e. **may predispose to breast cancer**) Ayyanan, A. et al. *Mol. Endoc.* 2011, 25(11), 1915-1923.
- **Disruption of adult expression of sexually selected traits** by developmental exposure in male deer mice Jasarevic, E. et al. *Proc. Nat. Acad. Sci.* 2011, doi: 10.1073/pnas.1107958108
- **Behavioral & cognitive disturbance** in adult mice after single neo-natal dose Viberg, H. et al. *Toxicology* 2011, 290(2-3), 187-194.

The Low-Dose Hypotheses

- Hormones act at very low concentrations (pmol, ppt); EDCs (BPA, etc.) may also.
- EDCs may have non-monotonic dose-response curves.
- EDCs may affect hormone (estrogen, etc.) receptors on cell membrane as well as nucleus.
- Hormone mimics may work synergistically with each other and with endogenous hormones.
- In spite of rapid metabolism, constant low-dose exposure is of concern; toxicokinetics not fully known.
- EDCs may have epigenetic effects.
- ***Therefore***…
  Standard toxicological tests, concentrations, and endpoints are inappropriate for endocrine disruptors; new risk-assessment paradigms are needed.

Hypothetical model dose response curves: threshold (A), linear nonthreshold (B), and nonmonotonic (C).



Vandenberg L N et al. Endocrine Reviews 2009;30:75-95

ENDOCRINE REVIEWS

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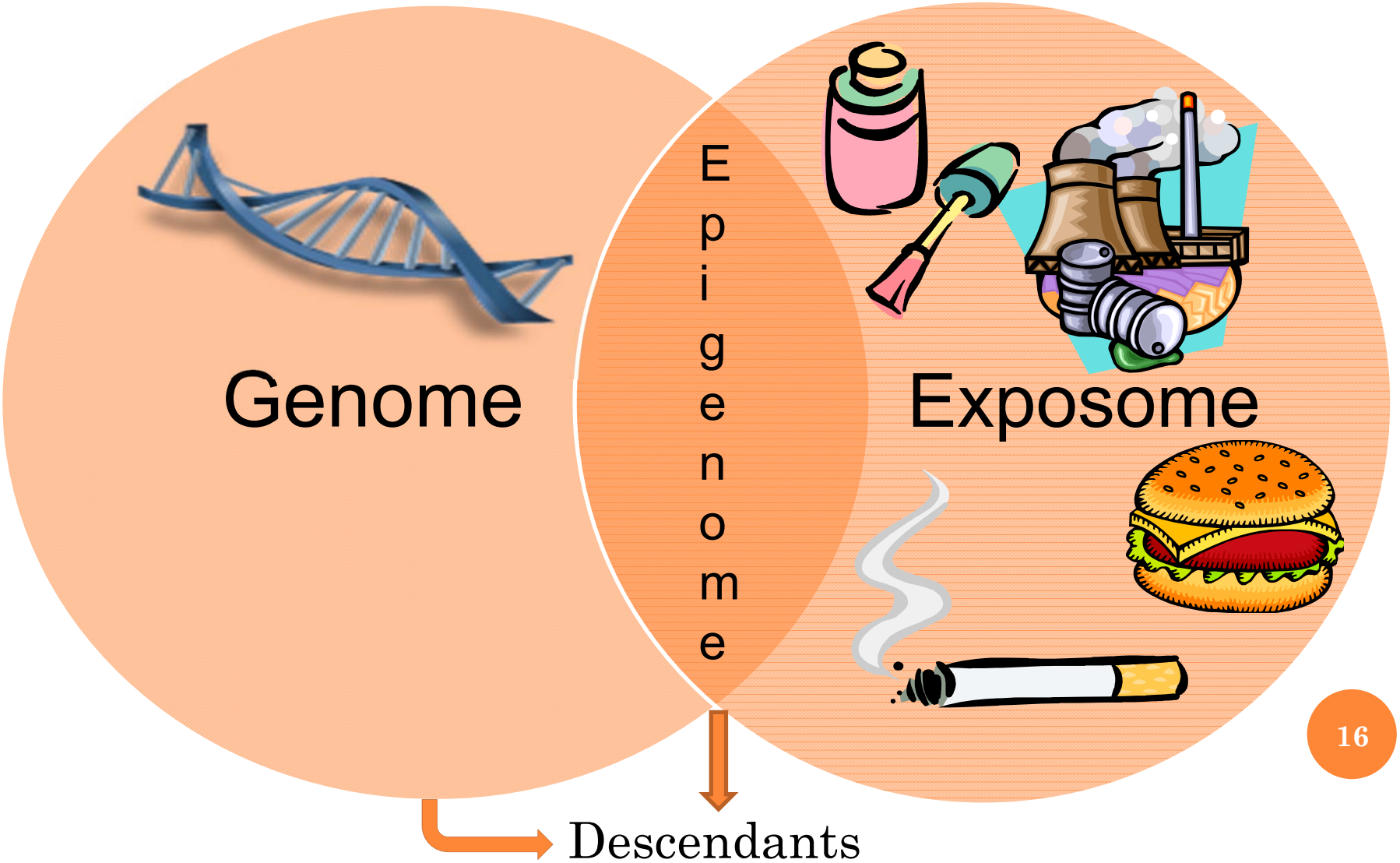
Epigenetic Effects from Environmental Toxins

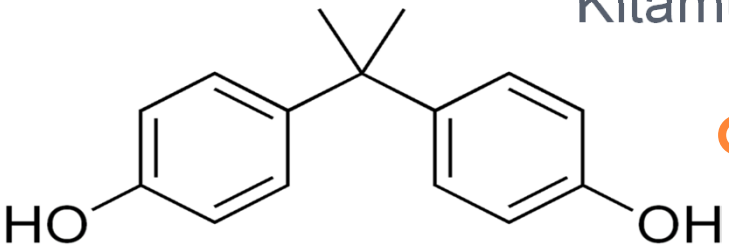
- Non-mutagenic: DNA base sequence unaltered
- DNA methylation and histone modification
- Developmental stage at exposure critical
- Changes in gene expression may occur long after exposure (e.g. in puberty or adulthood)
- Epigenetic changes to DNA may be heritable
  "Gestational Exposure to BPA Produces Transgenerational Changes in Behaviors and Gene Expression (in mice)"
  Wolstenholme, J.T. et al. *Endocrinology*, June 15, 2012

Epigenetics & Molecular Toxicology:
Intra- and Intergenerational Nature + Nurture

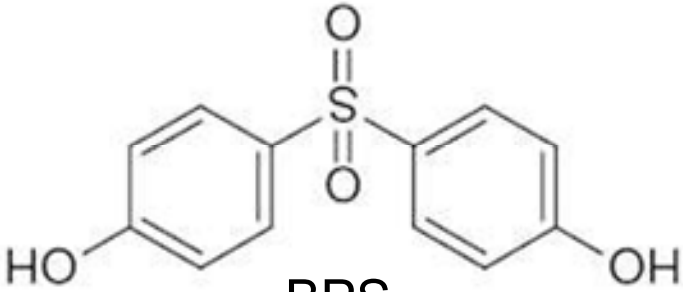


SAR: Estrogenicity Seems Related to 4-4' –OH

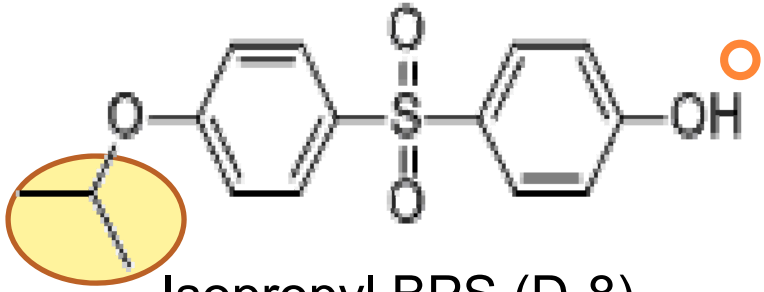
Kitamura, et al. *Toxicol. Sci. 2005, 84, 249-259*



BPA
CAS 80-05-7



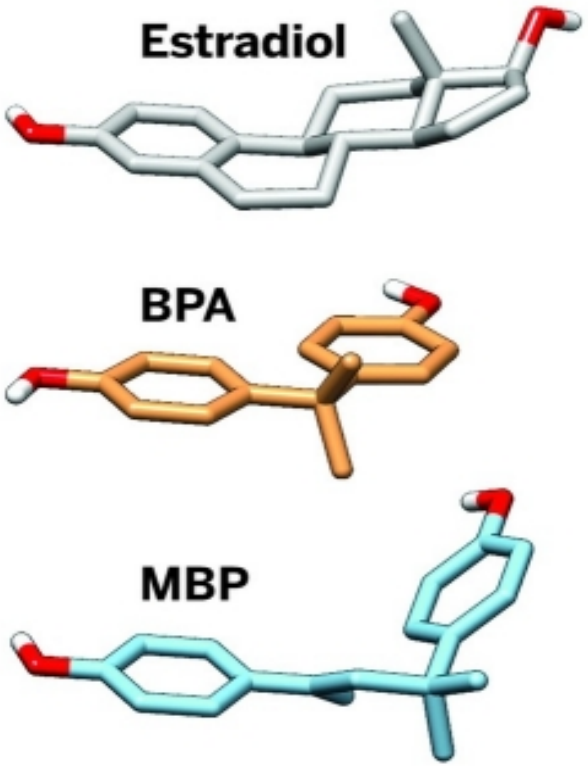
BPS
CAS 80-09-1

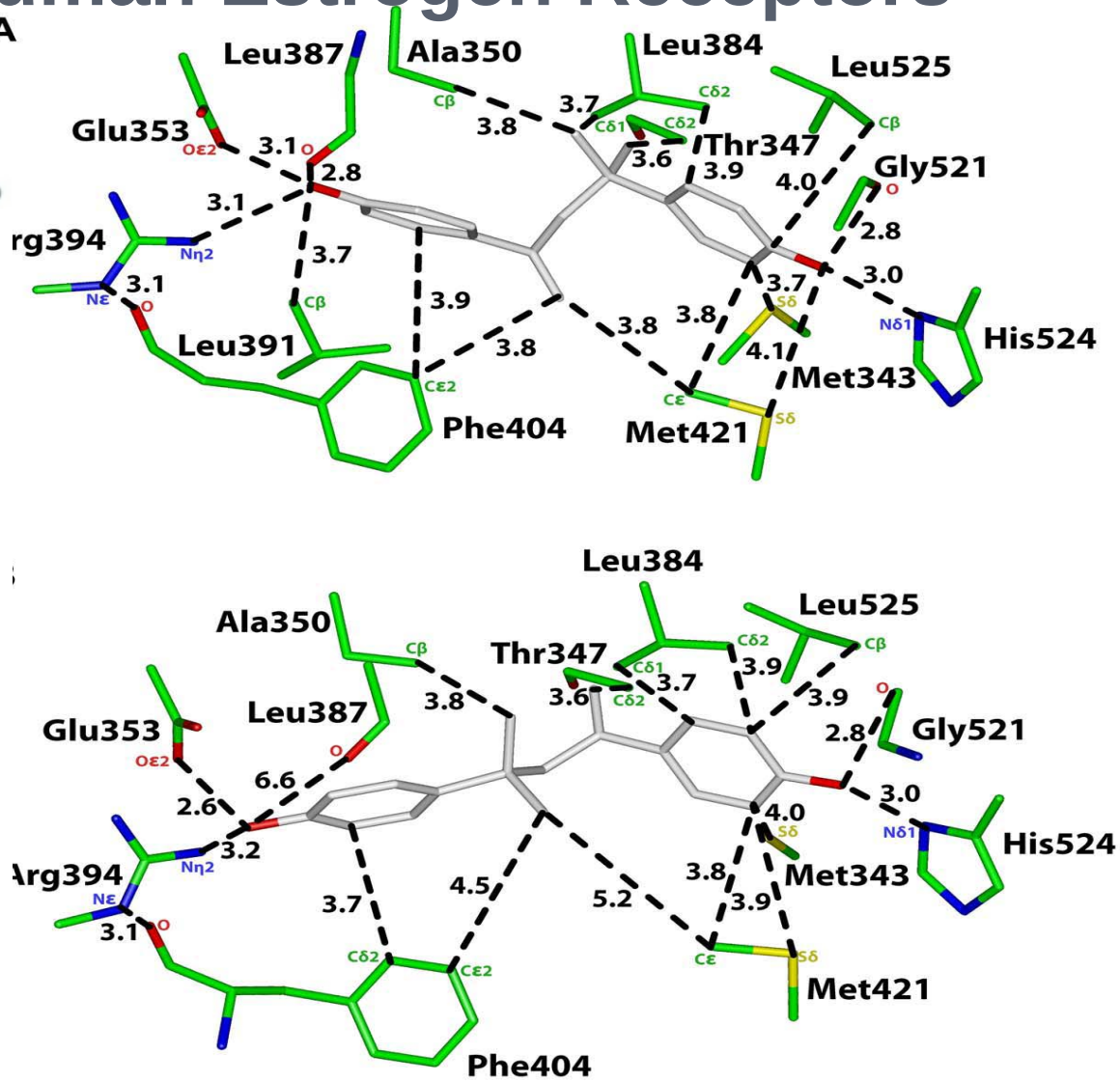


Isopropyl BPS (D-8)
CAS 95235-30-6

- BPS & D-8 are substitutes for BPA in thermal paper
- BPS estrogenicity similar to BPA (*in vitro)*
  Kuruto-Niwa, R. et al. *Environ.Toxicol. and Pharmacol.* 2005, 19,121–130.
- Isopropyl BPS (D-8) not estrogenic (*in vitro)*
  Terasaki. M et al. *Environ. Toxicol. and Chem.* 2007, 26 (11), 2356-66.

MBP (BPA Metabolite) May Have 1000X BPA's Activity in Human Estrogen Receptors





Chem. Eng. News 90(1) 2012

Baker, ME and Chandsawangbhuwana, C. *PloS One* 7(1) 2012: e46078.

Industry's Perspective

- Correlation is not causation; little or no proof that current BPA exposure is harming people.
- Animal toxicity studies flawed; physiology or dosing not relevant to humans.
- Metabolic clearance is fast and efficient.
- Human blood levels of unconjugated BPA vanishingly small, even after high dietary intake, in US gov't funded studies. (Teeguarden, et al. *Toxicol. Sci.* 2011,123(1), 48–57.)
- Public concern driven by questionable science & reporting.
- Regulations are based on sound science and are protective.
- Over-regulation will disrupt commerce, food supply.
- BPA-based resins have protected consumers for >50 years; few alternatives suitable for a wide range of foods.

European Food Contact Materials (FCM) Regs.

- BPA tolerable daily intake (TDI) of 50 µg/kg-body weight/day (NOAEL /100) (EFSA 2006)
- Specific Migration Level (SML) 600 µg/kg (ppb)
- PC infant bottles & cups banned (EC 2011/8/EU).
- EC 2011/10/EU consolidates & updates FCM regs. & tests.
- BPA ban in all FCM for kids< 4 (Denmark 2010,Sweden 2012, Belgium (?) 2013)
- France legislature passed BPA ban in all FCM by 2014.
- EFSA reviews risk assessment (2008, 2010); stands by its actions, but will review again by 2013.

http://www.efsa.europa.eu/en/topics/topic/bisphenol.htm

BPA Food Contact Regs. in U.S. & Canada

- Canada banned PC baby bottles & cups 2010, but reaffirmed BPA use in food packaging Sep. 2012.
- 11 states, incl. Calif., ban PC baby bottles & cups.
- CA AB 1319, < 0.1ppb BPA for kids< 4 (7/1/13); formula cans dropped. Requires “least toxic alternative.”
- BPA approved as a food additive for packaging in the US, 1960s. RfD is 50 ug/kg-bw/day, same as EC TDI. No SML analog.
- NTP (2008) & FDA (2010) express “some concern” for effects on brain, behavior & prostate gland.
- FDA bans PC bottle & cups, at industry request, July 2012.
- In response to NRDC suit, Mar. 2012, FDA said science did not support total BPA ban; awaiting ongoing studies.

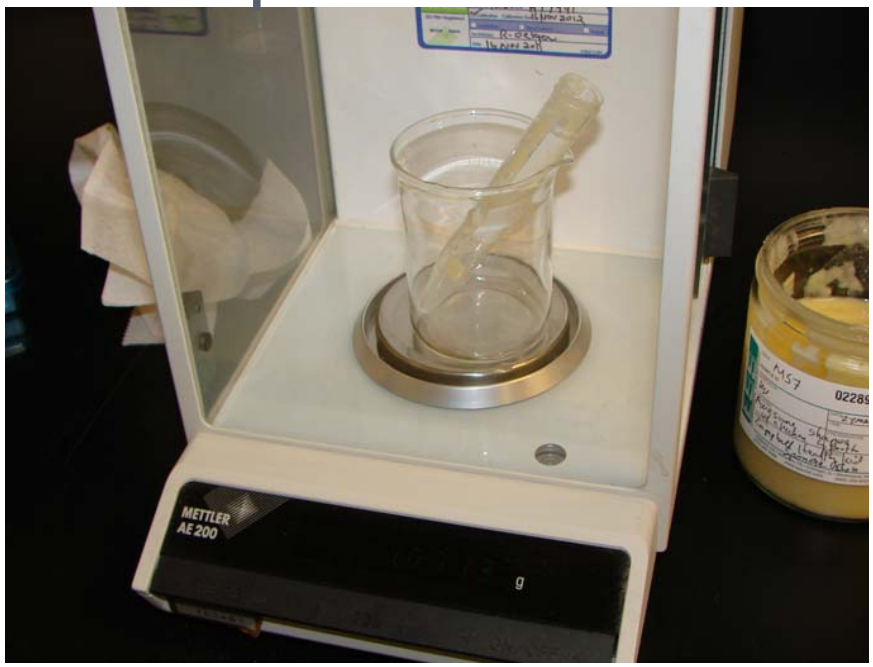
BPA in Foods Pilot Market Survey Sample

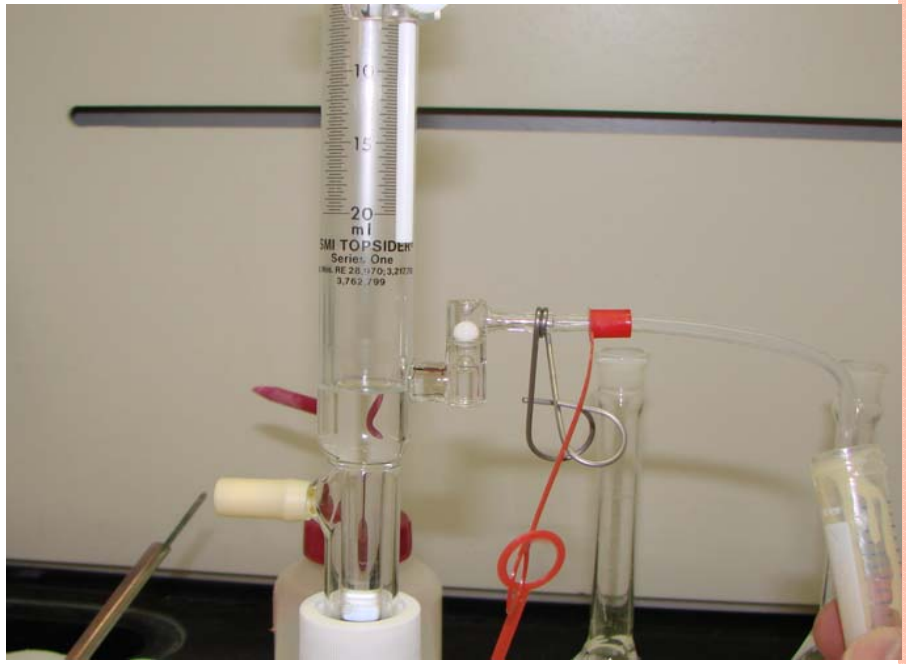
- Marketed to parents of young children
- Various US processors represented
- None claimed “BPA free”
- Similar to products tested by others (NGO, journal)
- Purchased 1 each of 12 products, Safeway 3/2012

Food Sample Preparation

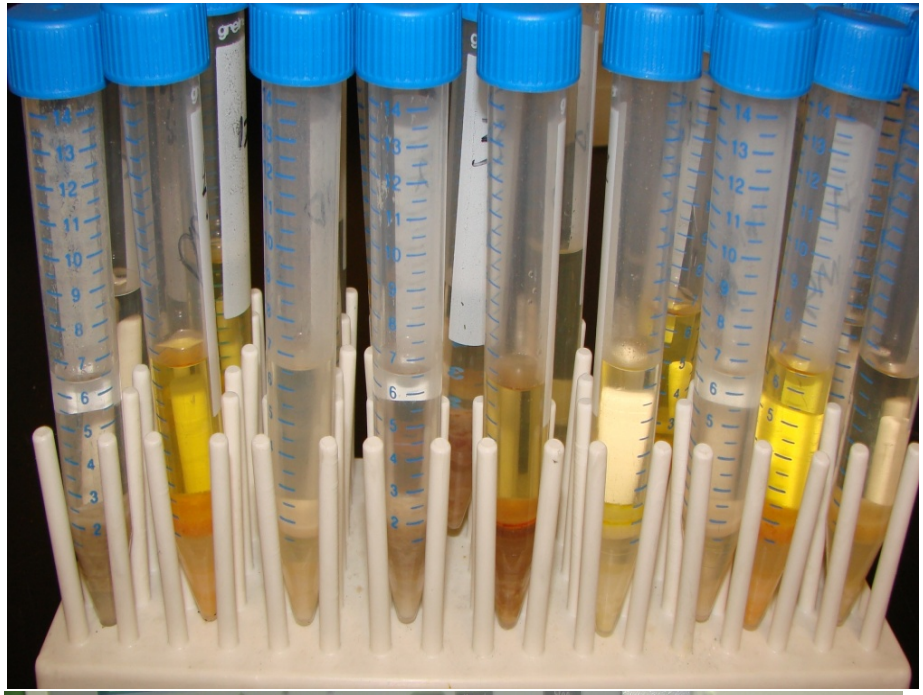
- Transfer food to clean jar
- Blend to smooth paste
- Clean & solvent rinse blender between samples
- Immerse blender in DI water for blank

Sample Extraction

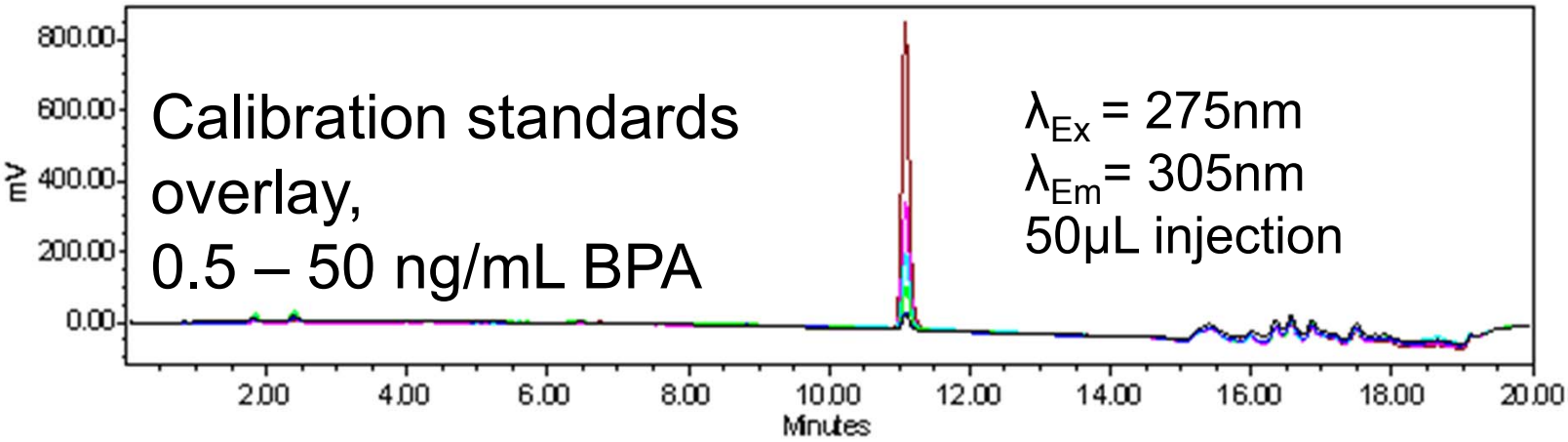


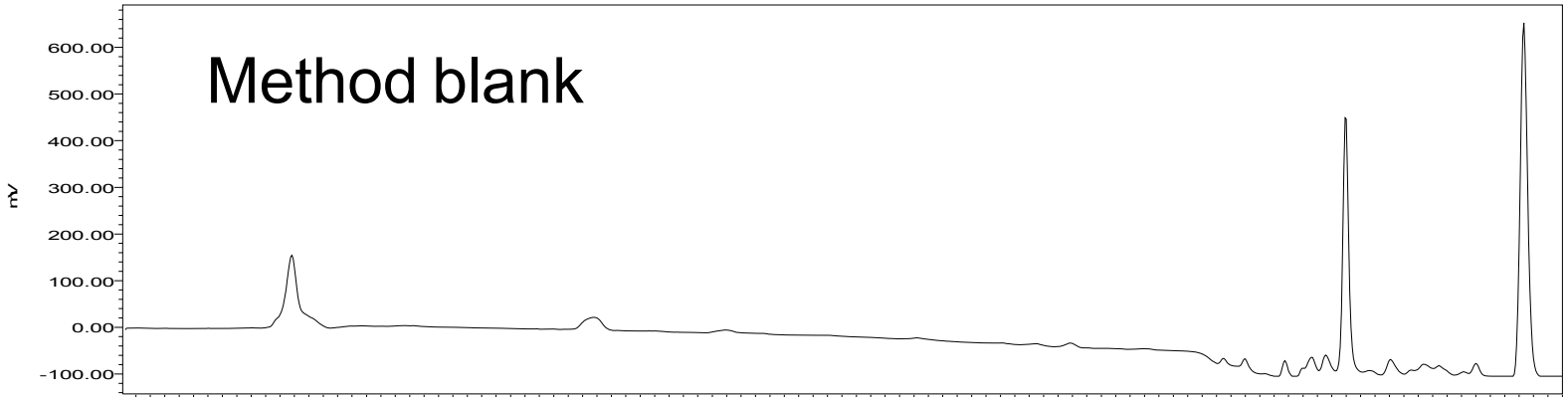


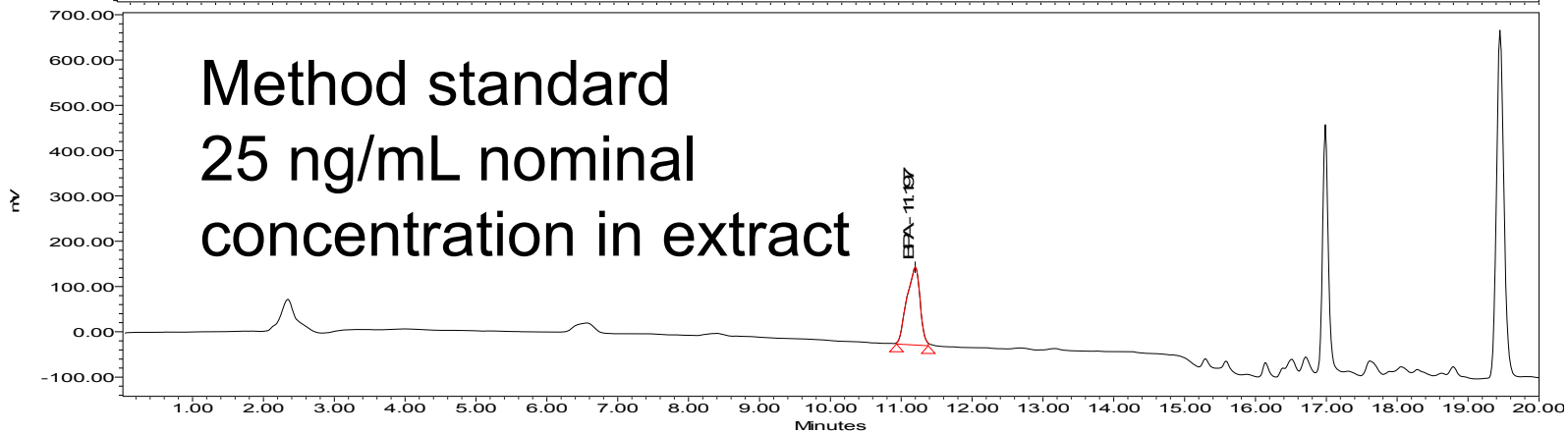
Sample Extract Filtration & HPLC-FLD Analysis



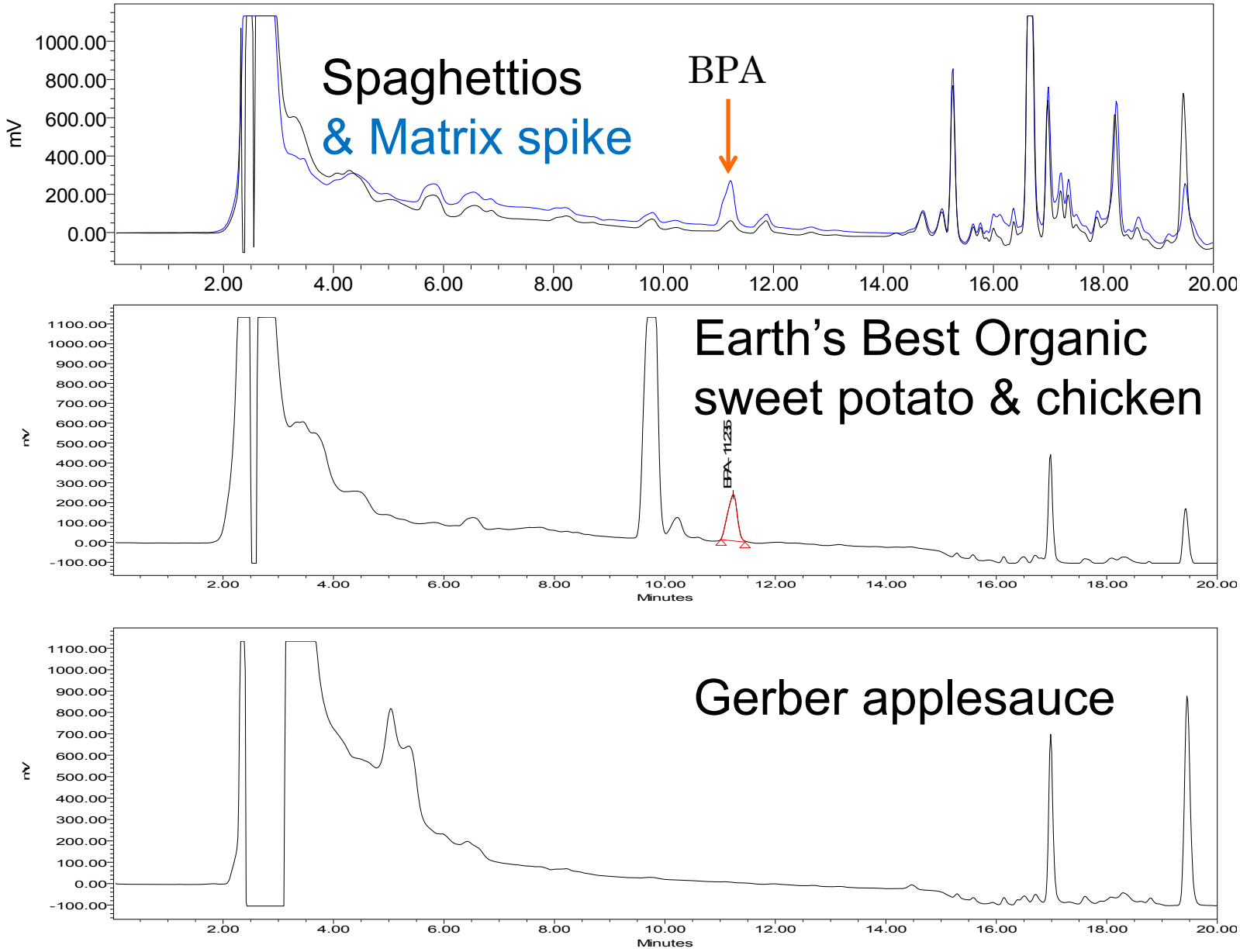
BPA QC HPLC-FLD Chromatograms



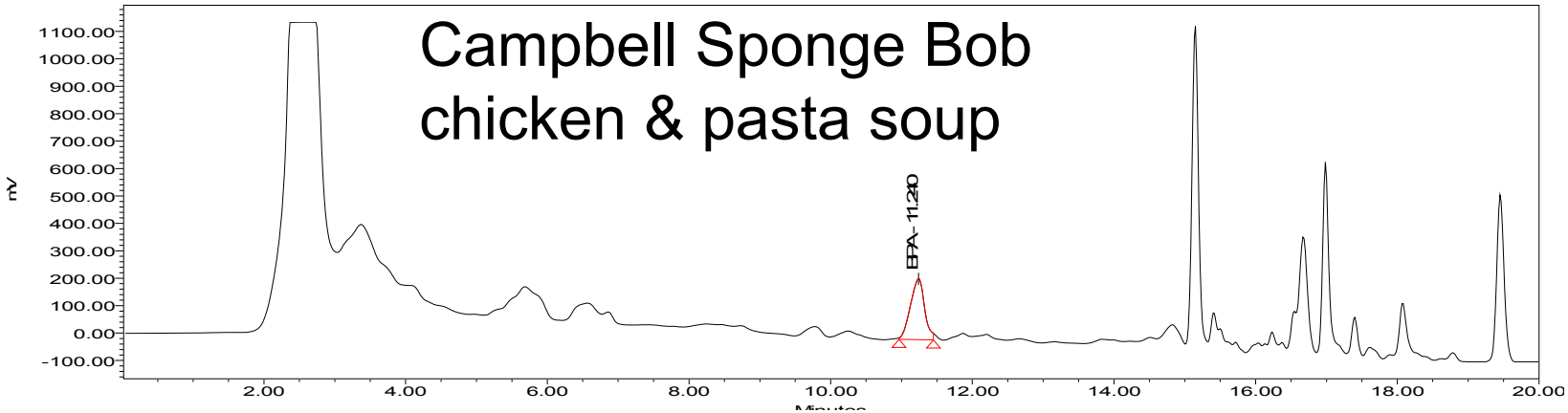


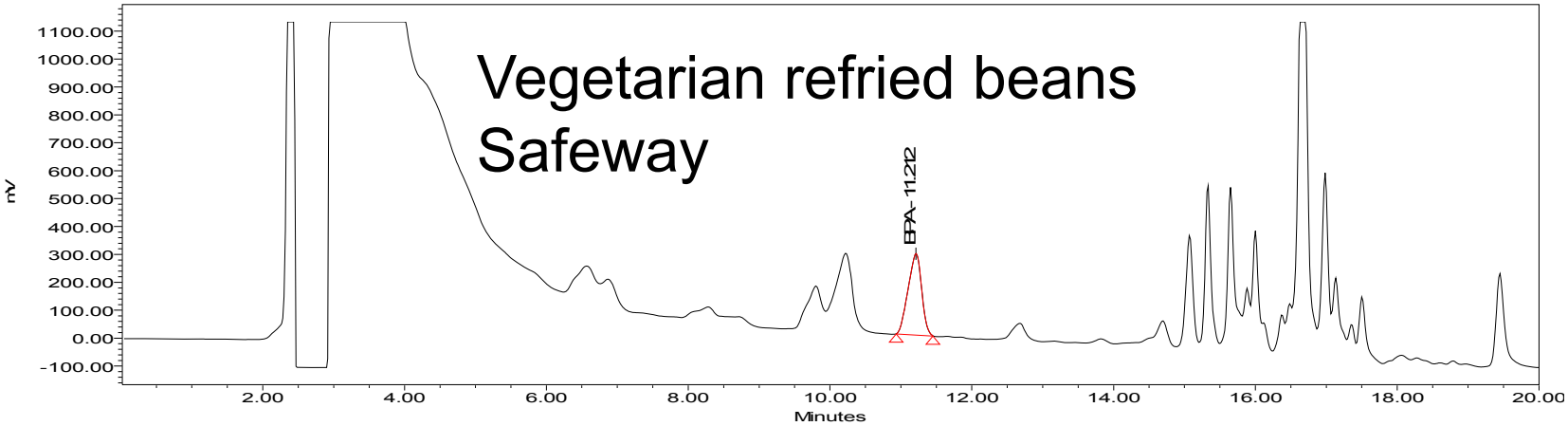


BPA Sample HPLC-FLD Chromatograms I



BPA Sample HPLC-FLD Chromatograms II





BPA in Food Preliminary Results-ECL 2012

Product	Brand	BPA µg/kg	serving size (g)	BPA µg serving
chili con carne with beans, original	Dennison's	108	256	27.7
refried beans, vegetarian	Safeway	115	130	15.0

EU SML = 600 µg/kg
EU TDI = US RfD = 50 µg/kg-body weight/day

Product	Brand	BPA µg /kg (sd)	serving size (g)	BPA µg serving
pasta, tomato & cheese sauce	Spaghettios (Campbell)	20.2 (2.7)	252	5.1
Mini ABCs &123s pasta & meatballs with tomato sauce	Chef Boyardee (ConAgra)	40.6	247	10.0
Awesome Shapes pasta w/ chicken in broth, Sponge Bob	Campbell Healthy Kids	85.6	120 mL con-densed	9.8
chicken & noodle soup	Progresso (Gen. Mills)	72.9	246	17.9

Product	Brand	BPA µg/ kg (sd)	serving size (g)	BPA µg/ serving
infant formula conc. milk based, (0-12 months)	Enfamil (Nestle)	< 2	74 mL concen-trate	nd
applesauce (infant)	Gerber 2nd Foods	< 2	113 (4 oz jar)	nd
peach, rice & banana (6 months & up)	O for Baby Organics Safeway	< 2	113 (4 oz jar)	nd
sweet potato & chicken dinner inf.	Earth's Best Organic	71.8 (13.9)	113 (4 oz jar)	8.1

Product	Brand	BPA µg /kg (sd)	serving size (g)	BPA µg serving
tuna, chunk light in water	StarKist	462 (37)	56	25.9
tuna, chunk light in water, liquid		< 2	n/a	n/a
tuna, chunk light in vegetable oil	StarKist	< 2	56	nd
tuna, chunk light in veg. oil, liquid		< 2	n/a	n/a

Similar Foods, ECL & Breast Cancer Fund Tests I

Study (Technique)	Product	Company	BPA µg/ kg
ECL 2012 (LC-FLD)	pasta with chicken in broth, Awesome Shapes Sponge Bob (condensed soup)	Campbell	86
BCF 2011 (GC-MS)	pasta with chicken in broth, (a) Fun Shapes Toy Story (b) Cool Shapes Disney Princess	Campbell	80, 140 (WI, CA) 71, 190 (WI,CA)

Source: BPA in Kids' Canned Foods http:/www.breastcancerfund.org /assets/pdf/publication/bpa-in-kids-food.pdf (accessed 11-02-11)

Similar Foods, ECL & Breast Cancer Fund Tests II

Study	Product	Company	BPA µg/ kg
ECL 2012 (LC-FLD)	Spaghettios Original	Campbell	20
BCF 2011 (GC-MS)	Spaghettios with Meatballs	Campbell	10, 16 (WI,CA)
ECL 2012 (LC-FLD)	Mini ABCs & 123s pasta & meatballs with tomato sauce	Chef Boyardee (ConAgra)	41
BCF 2011 (GC-MS)	Mini ABCs & 123s whole wheat pasta & meatballs with sauce	Chef Boyardee (ConAgra)	19,21 (WI,CA)

Conclusions

- Developed rapid, sensitive method for extraction and screening of BPA in foods.
- HPLC-FLD results agree with reported ppb levels.
- Infant formula & 2 of 3 jars food had BPA < 2ppb.
- Tuna in water was higher than expected.
- BPA was < EU 600µg/kg Specific Migration Level.
- Daily BPA from foods < 50 µg/kg-bw/day TDI, RfD.
- BPA risk assessment, regulation still unsettled.

Next Steps

- Develop LC-MS/MS confirmatory methods for BPA (& other compounds?) in food, packaging, etc. (MS confirmation was a recommendation of the FAO/WHO Expert Meeting on BPA, Ottawa 2010)
- Measure BPA & substitutes in thermal receipts.
- Ongoing ECL biomonitoring & Toxics in Product (TiP) studies
- Will BPA or other EDCs be among CA Safer Consumer Products regulations initial CoC?