

Identifying and Evaluating Alternative Materials:

Case Study

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4- Nov-2016

GROWTH

INNOVATION

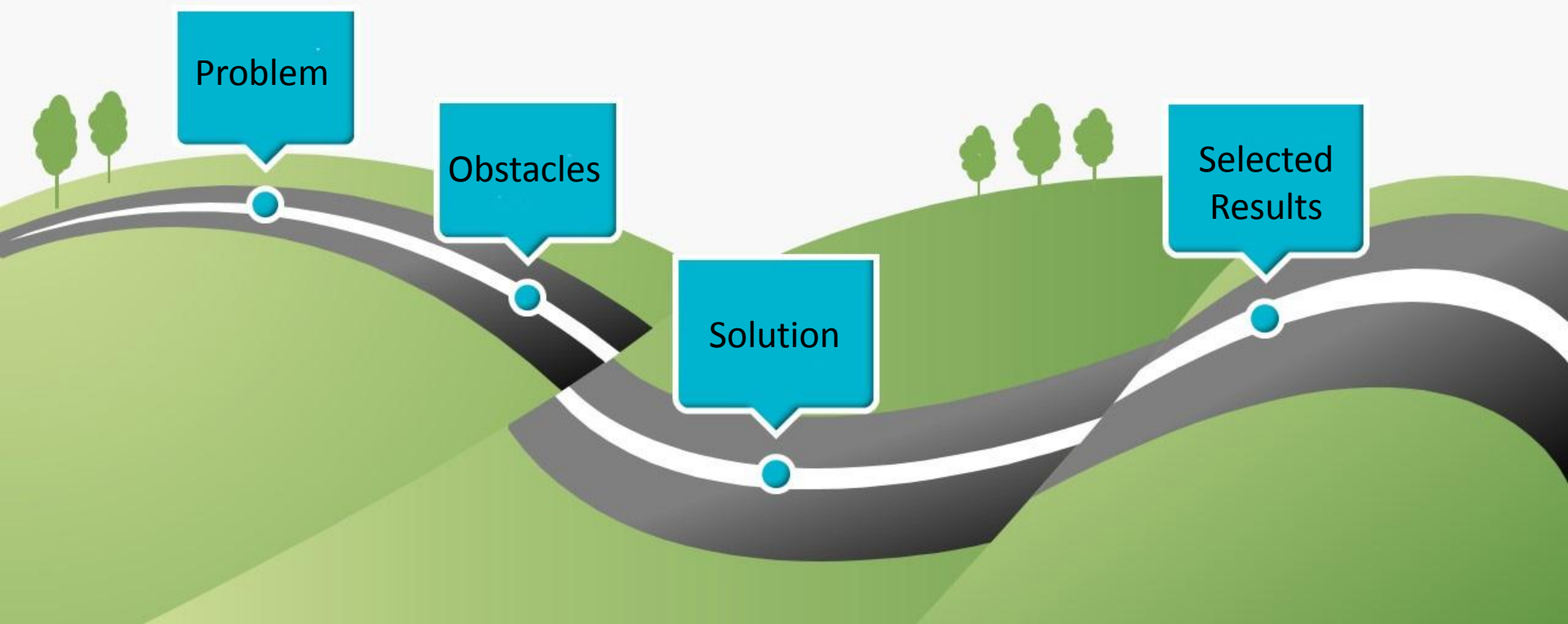
SOLUTIONS

SUCCESS



Journey of Reverse Drug Discovery...

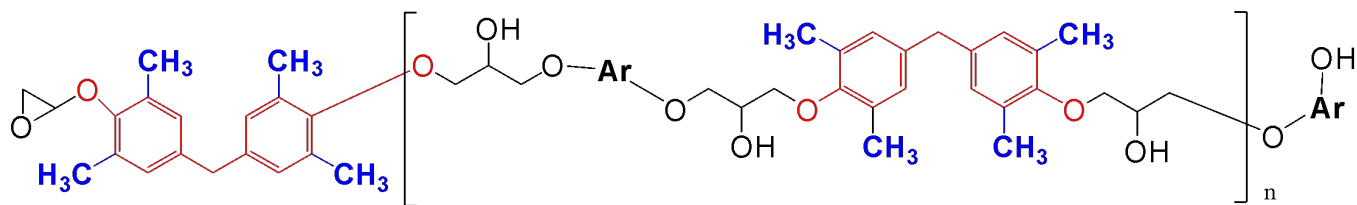
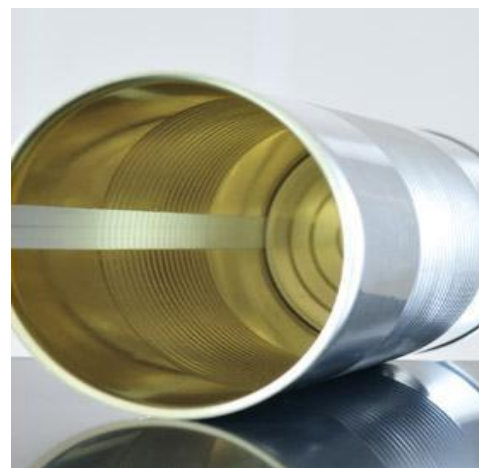
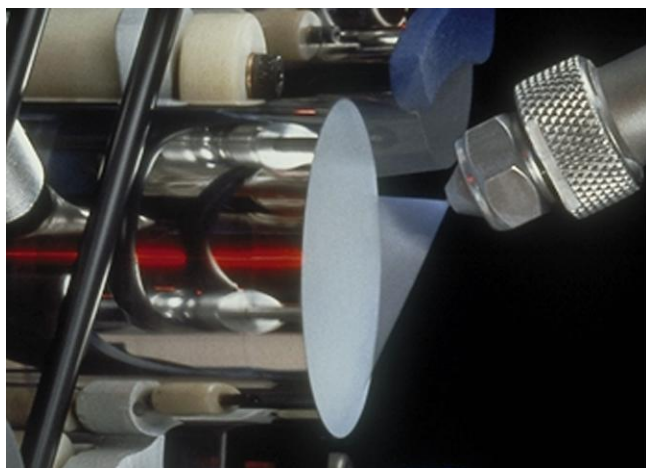
Valspar non-BPA Replacement





Finding New Materials

Non-BPA Food-Contact Epoxy



V70 Polymer

Historically

Both Valspar and its competitors made new coatings using pre-authorized monomers listed “in the box”



Monomer Lists

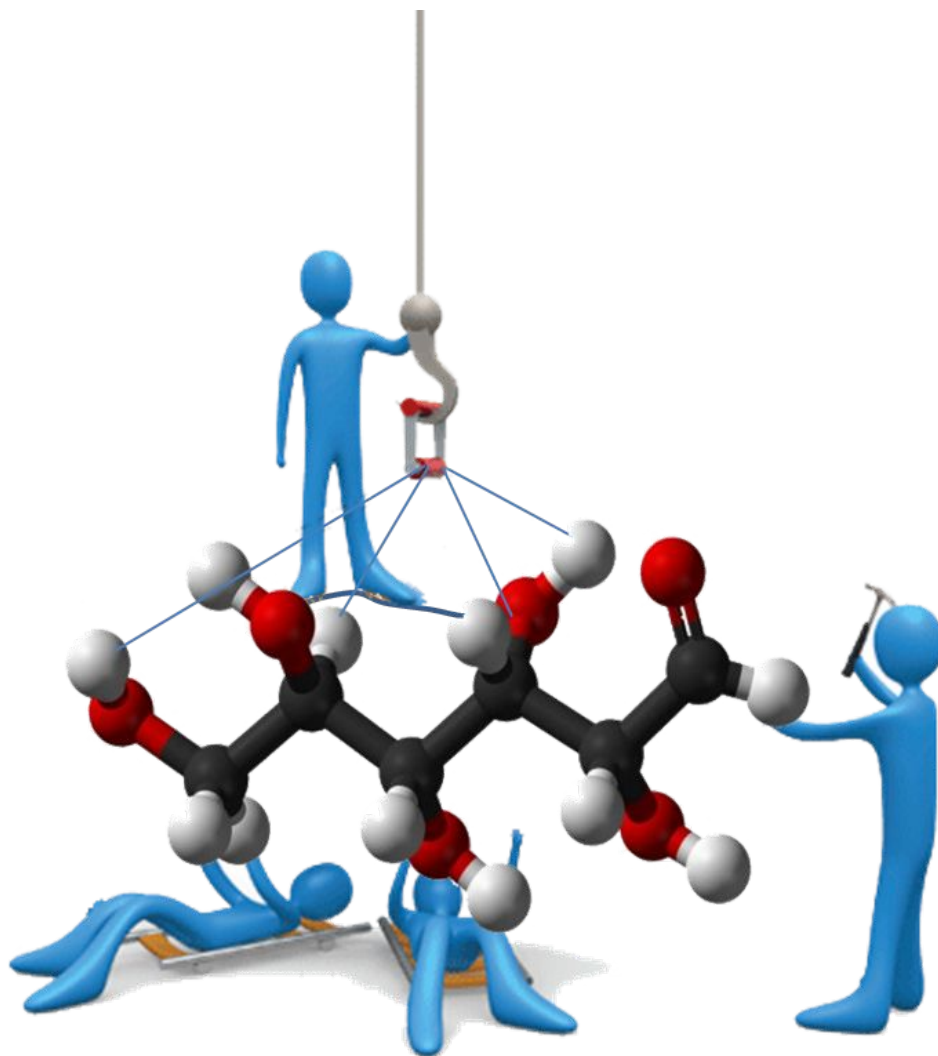
EU Commission Regulations

FDA 21CFR175.300

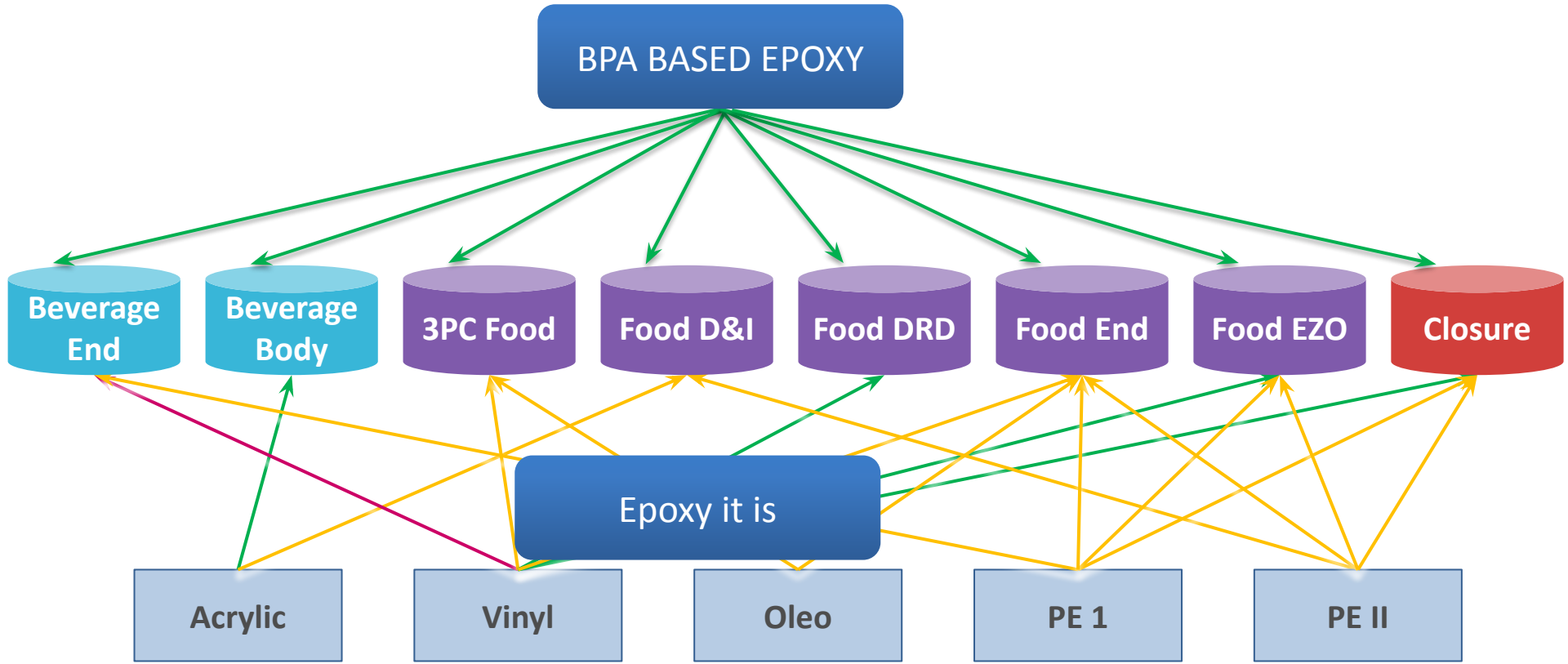
To accomplish performance goals, Valspar had
to get out of the box



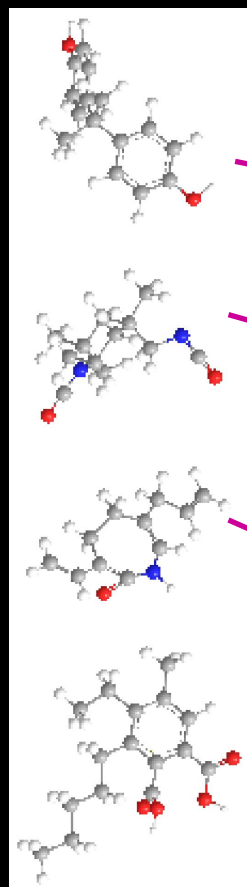
Which meant new molecules with a lot of requirements....



To replace BPA what choices are there?



*If we replace BPA
what has to
happen?*



**Technical
Performance**

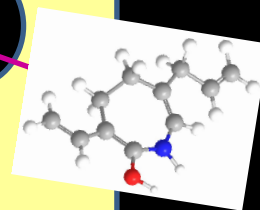
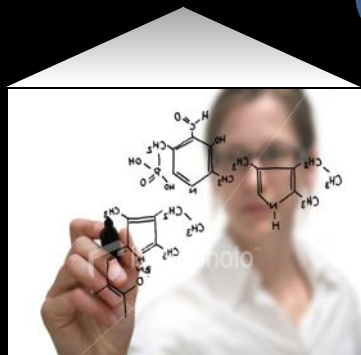
**Toxicology
Migration**

**Supply
Chain**

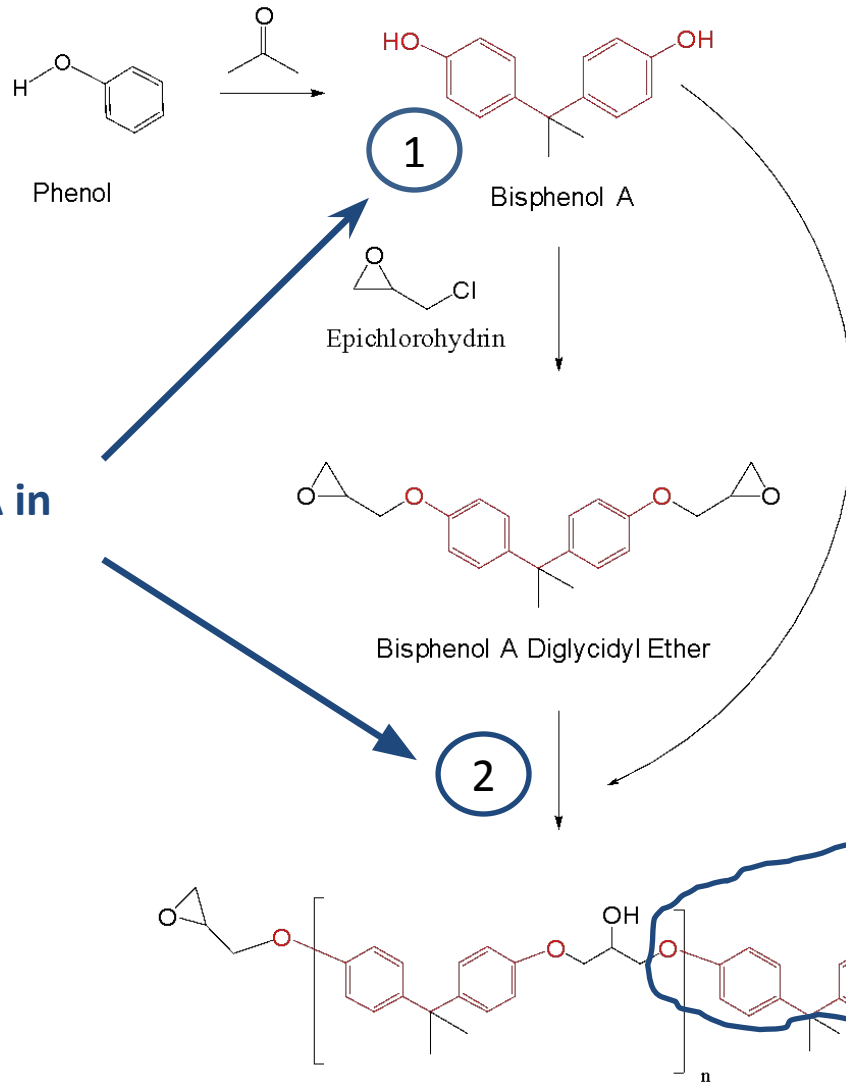
**Food
Regulatory**

**Chemical
Regulatory**

**Candidate
Molecules**



What do we know from BPA epoxy?

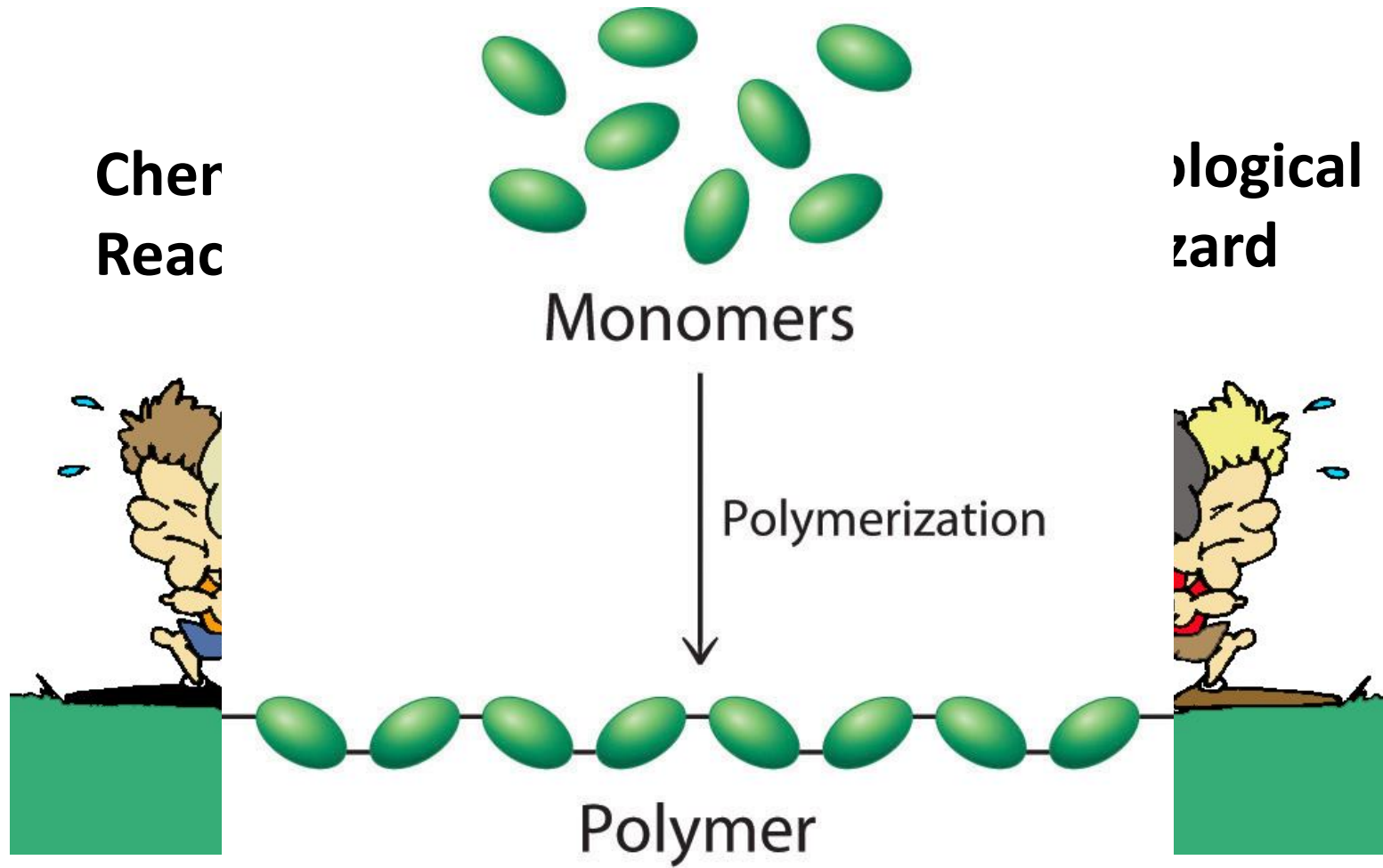


Bisphenol A is Estrogenic

4,4'-methylenediphenol functionality found in BPA is essential

There are 2 sources of BPA in the polymer synthesis

The Challenge with Monomers Used to Make Food Contact Polymers

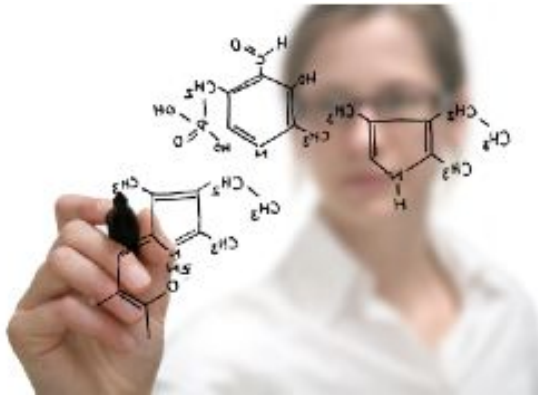


...SO we needed molecules that react with each other
...but like reverse drug discovery
physiologically, do...



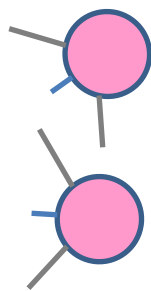
Now What?!!



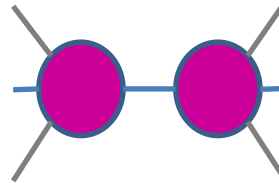


Find some molecules that aren't

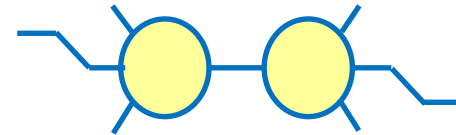
- Estrogenic
- Genotoxic



Xylenols



Bisphenols

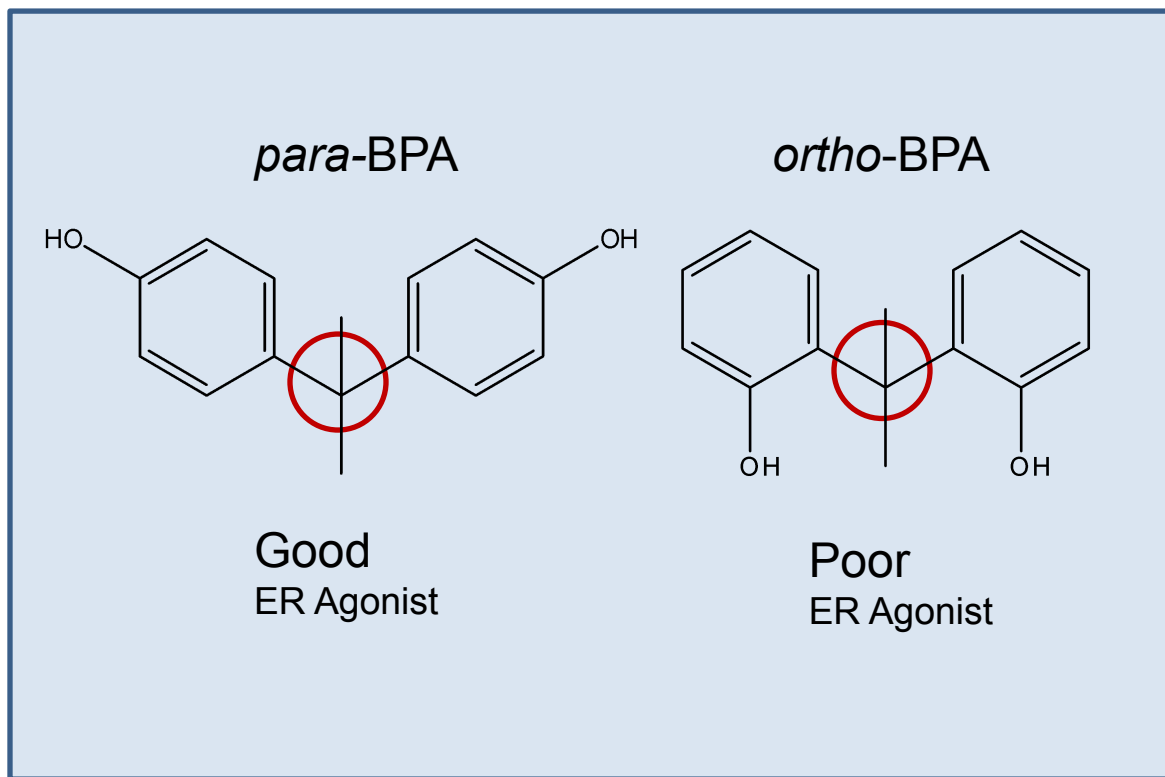


Diglycidyl ethers

Problem

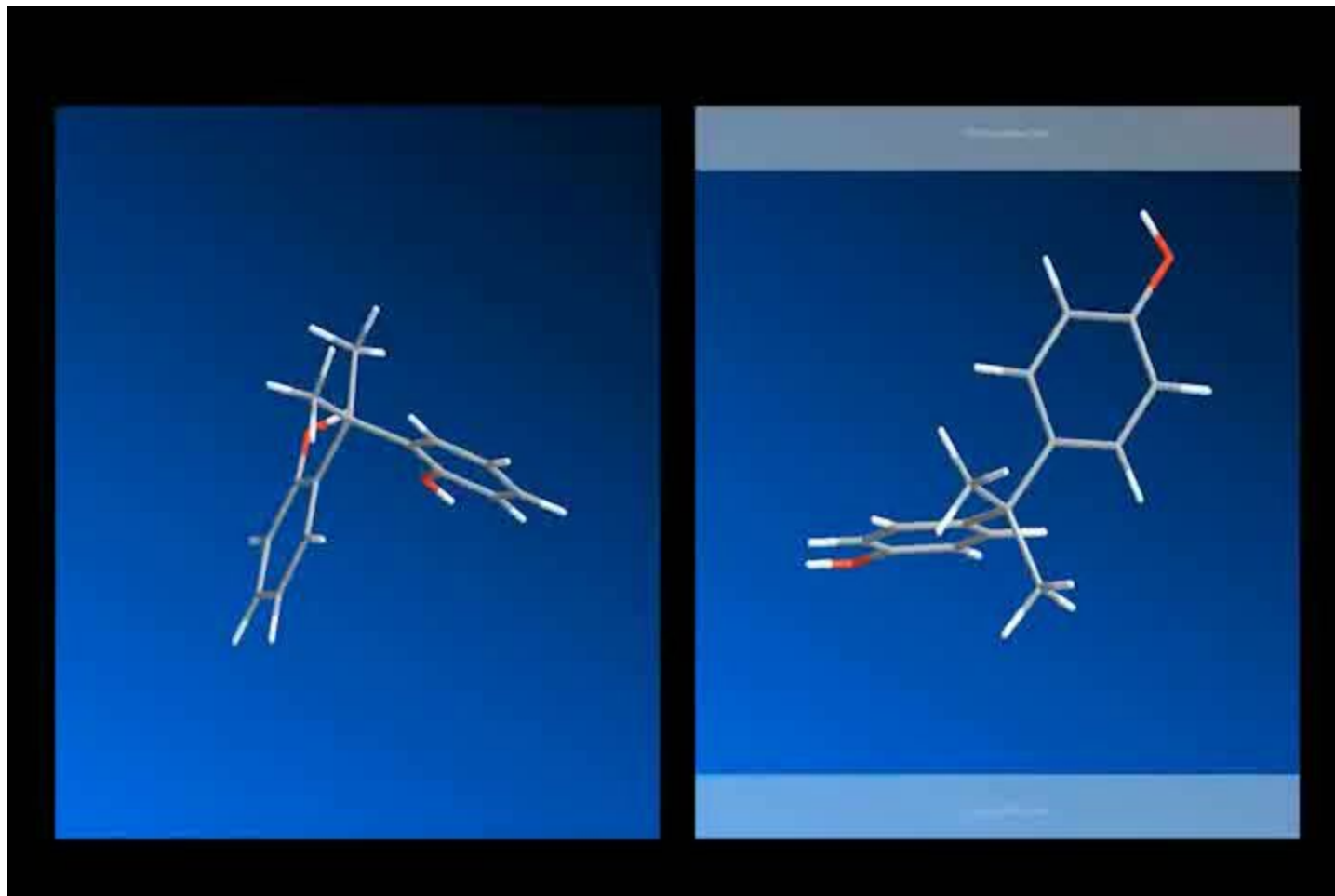
- ...
- Bisphenols are the only commercially viable source for **4,4'-methylenediphenol** functionality, many are estrogenic
- Diglycidyl ethers alert for mutagenicity

We started Screening Bisphenols for Activity by asking what's with BPA?



Inactive - Ortho BPA

Active - Para BPA



What structural attributes best predict ER inactivity?

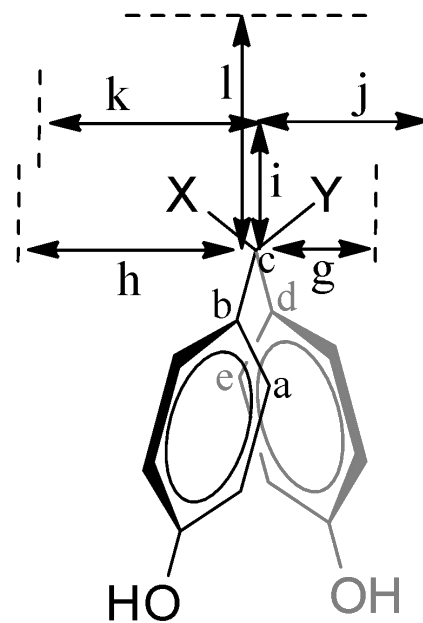
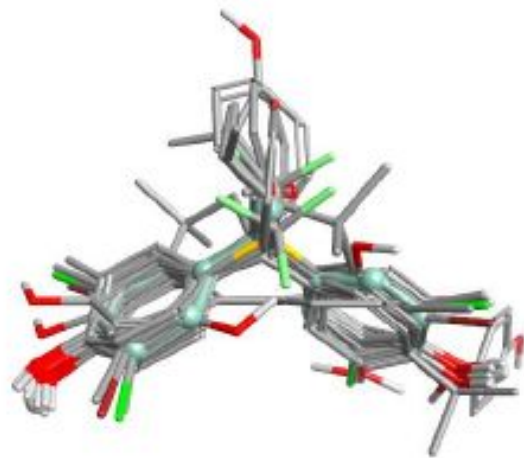
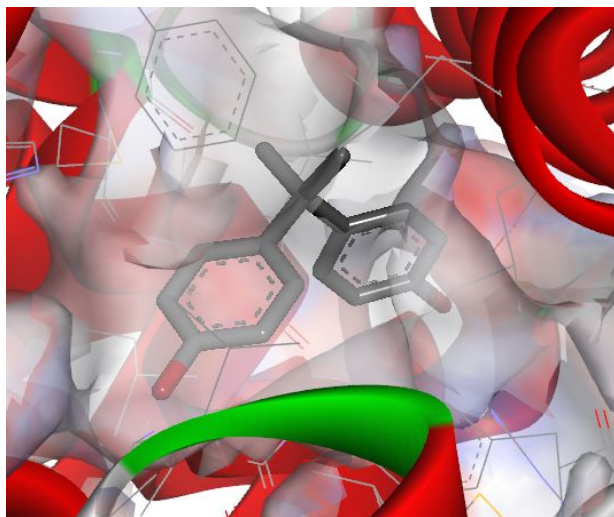
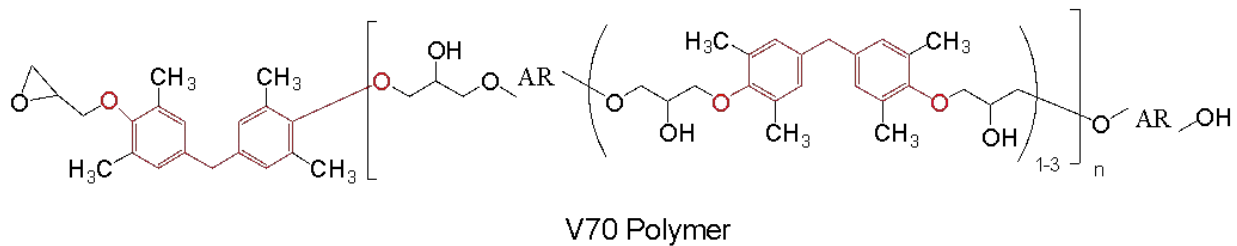
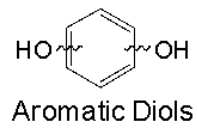
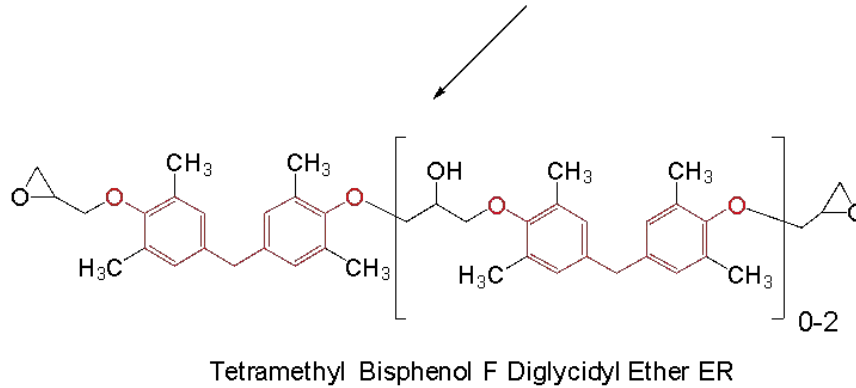
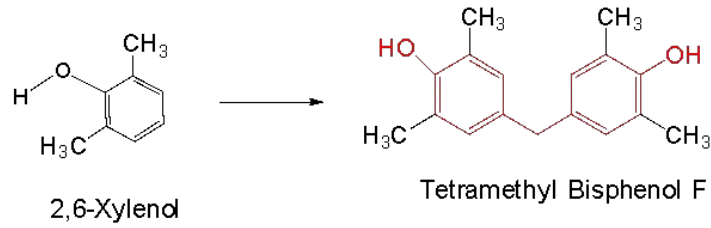


Figure 6.

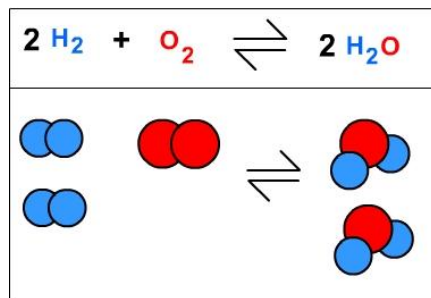
V70 Route of Synthesis



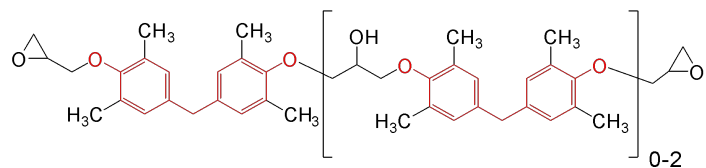
Why should people believe these molecules are safe?



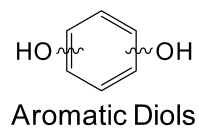
Keeping in mind reactants \neq products



\neq



Tetramethyl Bisphenol F Diglycidyl Ether ER



\neq





GreenScreen[®]



GreenScreen[®] Sodium

Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
C	M	R	D	E ¹	AT	ST	N	SnS	SnR	IrS	IrE	IM	AA	CA	P	B	Rx	F
L	L	L	L	L	H	M	L	H	H	H	H	L	H	H	L	L	H	H

GreenScreen[®] Chlorine

Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
C	M	R	D	E ¹	AT	ST	N	SnS	SnR	IrS	IrE	IM	AA	CA	P	B	Rx	F
M	M	L	L	L	H	H	H	H	H	H	H	L	H	H	L	L	H	L

Figure 1: GreenScreen® Hazard Ratings for TMBPF DGE Resin

Group I Human					Group II and II* Human								Ecotox		Fate		Physical		
C	M	R	D	E	AT	ST		N		SnS ⁺	SnR ⁺	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeated*	single	repeated*										
L	L	L	M	DG	L	L	<i>M</i>	DG	L	H	DG	L	L	H	H	<i>vH</i>	<i>vL</i>	L	L

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated (modeled) values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in **BOLD** font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L)

Tiered Protocol for Endocrine Disruption (TiPED)

*Designing endocrine disruption out of the next generation of chemicals.
Schug et al. Green Chem., 2013,15, 181-198*



“While *in silico* and *in vitro* assays offer less costly starting points, *in vivo* assays are necessary to conclude that a chemical is unlikely to have EDC activity.”

http://www.tipedinfo.com/tiped_tier/guiding-principles/

Organizations with whom Valspar openly shared data for comment, approval, or assistance
 (Does not necessarily imply endorsement, approval, or future activity)



Regulatory Authorizations Received



Toxicity Data Summary

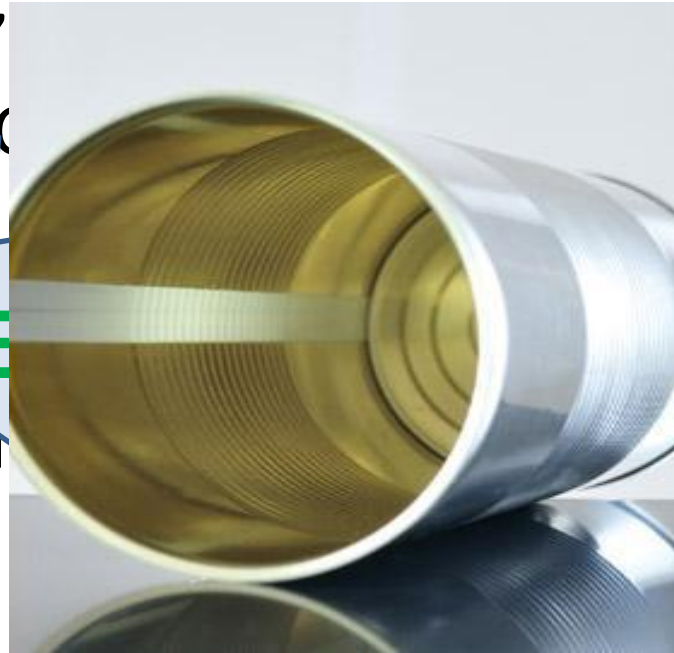
	Xylenols	Aromatic Diols	TMBPF	TMBPFEDGE monomer/res	V70 Migrants	
Migration (exposure)	Not found – volatile < LOQ = 0.05 ppb	Not found – hydrolytically unstable and volatile < LOQ = 0.05 ppb	Not found in food simulant migrants (< LOQ = 0.05 ppb)	8 ppb	< 50 ppm	
Estrogenicity	Literature SAR	Genomic Array (α,β)	SAR Transactivation (2) Redistribution Genomic Array (α,β) EScreen Uterotrophic Immature Pubertal Estrogen mimic	SAR Transactivation Redistribution Genomic Array (α,β) Read-across Genomic Array Estrogen mimic	EScreen	
Androgen		Genomic array	Genomic Array Redistribution Transactivation Hershberger	Genomic Array Redistribution Transactivation		
Thyroid		Literature SAR		Immature Pubertal		SAR Read across
Enzyme				Aromatase Steroidogenesis		SAR Read across
Toxicity				From other in vivo		Acute 28-Day
Genotoxicity				SAR		Ames/GS multi-formats Lymphoma In vivo-Comet/MN
EcoTox				Biodegradability Kow Solubility Hydrolysis/pH Dissociation Aquatic toxicity-fish, daphnia, algae		Biodegradability Kow Solubility Hydrolysis/pH Dissociation Aquatic toxicity-fish, daphnia, algae
Other				Microsomal metabolism AhR activation		Microsomal metabolism In vitro – skin Eye hazard LLNA AhR activation

HYPOTHESES

TMBPF DOESN'T
ESTROGENIC AC

IN VITRO
TESTING

TRUE



DOESN'T PERSIST
POLYMER

TESTING

TRUE

WHAT ABOUT THE POLYMERIC COATING?

IN VITRO TESTING



ANALYSIS OF MIGRATES

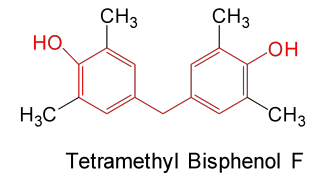
Approaches to EA and migration assessment

- Monomer TMBPF

- Estrogen activity

- Guidance testing Mammary gland biology
- Collaborative testing Mammary gland biology

- Migration quantification from final polymer

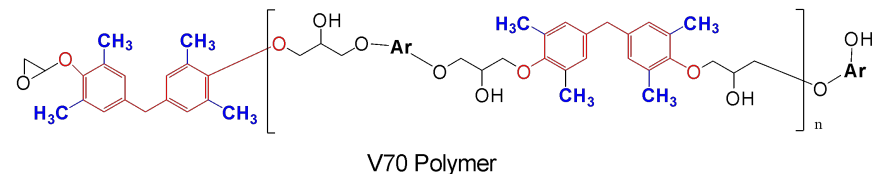


- Polymeric coating

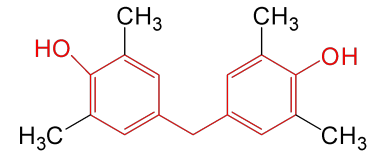
- Estrogen activity

- Collaborative testing

- Migrates profile



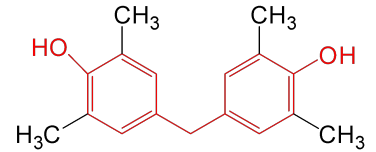
TMBPF testing



Tetramethyl Bisphenol F

- Estrogen receptor binding assays (OECD)
 - Agonist and antagonist
- High Content Microscopy Prolactin Array (Texas A&M and Baylor)
- Cell proliferation E-SCREEN assay (Tufts)
- Immature rat uterotrophic assay (EPA+ mammary gland)
- Juvenile male and female pubertal assay (EPA+ mammary gland)

TMBPF testing



Tetramethyl Bisphenol F

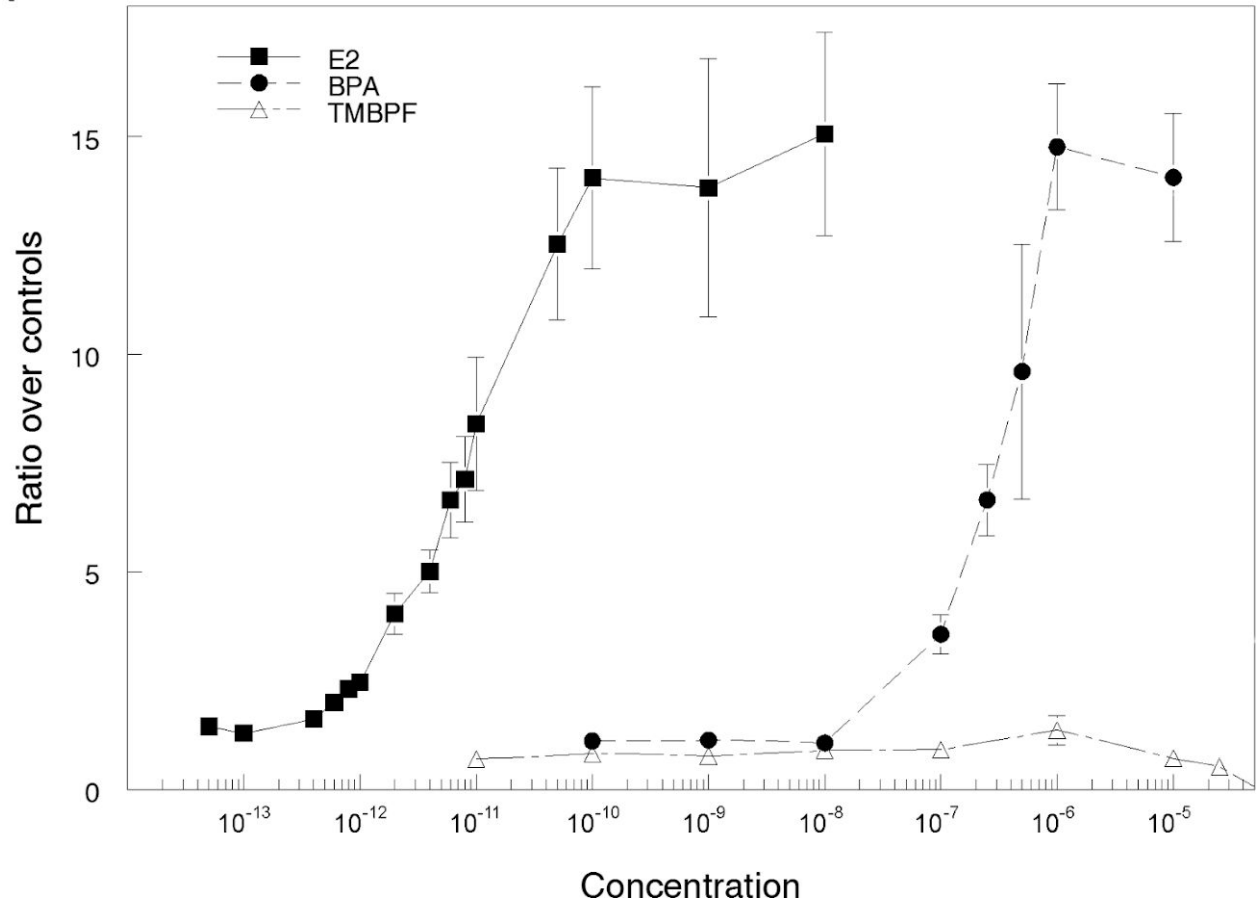
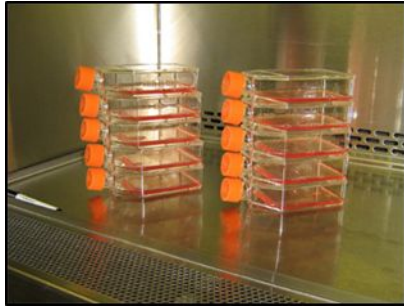
- Estrogen receptor binding assays (OECD)
 - Agonist and antagonist
- Chromatin transcription-factor binding arrays for prolactin (Texas A&M and Baylor)
- **Cell proliferation E-SCREEN assay (Tufts)**
- Immature rat uterotrophic assay (EPA+ **mammary gland**)
- Juvenile male and female pubertal assay (EPA+ **mammary gland**)

E-SCREEN

- Estrogen-sensitive MCF7 cells
 - Day 1: known number of cells
 - Day 5: count final number of cells
- Positive control: 17-beta E2 10^{-14} to 10^{-8} M
- Positive EA chemical: BPA 10^{-10} to 10^{-5} M
- TMPBF: 10^{-11} to 10^{-5} M
- *ENDPOINT: INCREASED CELL PROLIFERATION*

E-SCREEN

A



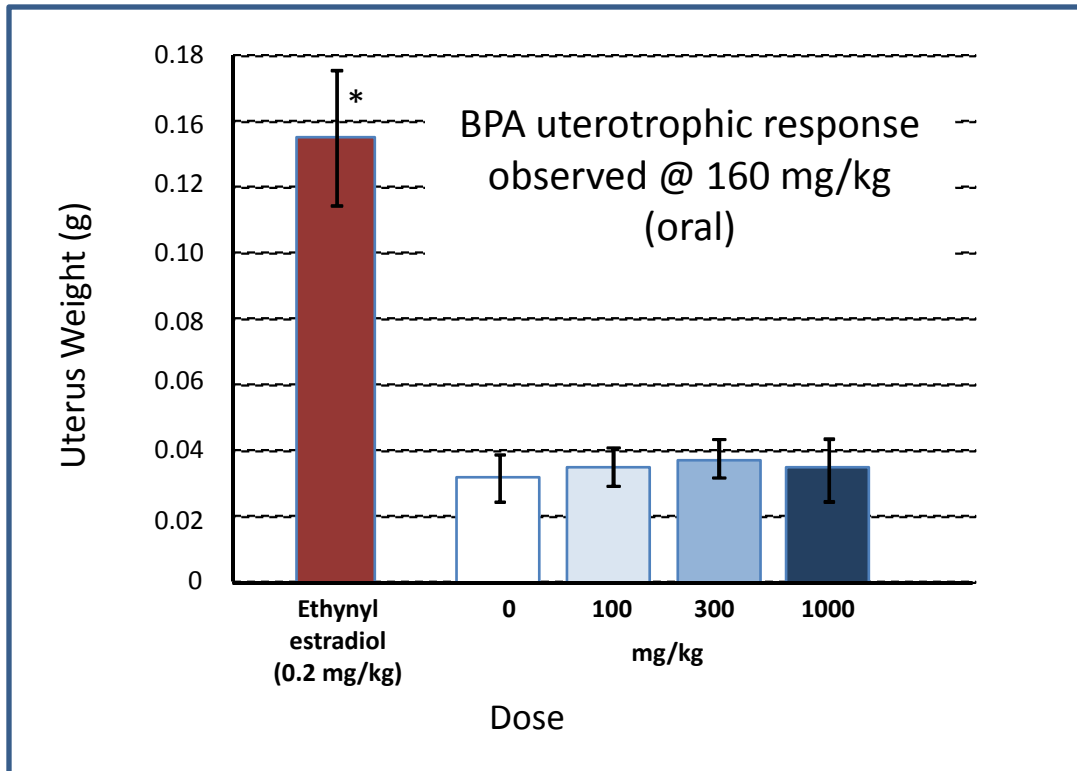
E-SCREEN

TMBPF DID NOT INCREASE MCF7 CELL
PROLIFERATION

Uterotrophic assay (+)

- Immature female rats
- 3-days daily oral (gavage) treatment
- Positive control: Estradiol (0.2 mg/kg bw)
- TMBPF: 0, 100, 300, 1000 mg/kg bw
- *ENDPOINT: INCREASED UTERINE WEIGHT*
- *NEW ENDPOINT: MAMMARY GLAND DEVELOPMENT*

Uterotrophic assay (+)



NORMAL MAMMARY
GLAND HISTOPATHOLOGY

Uterotrophic assay (+)

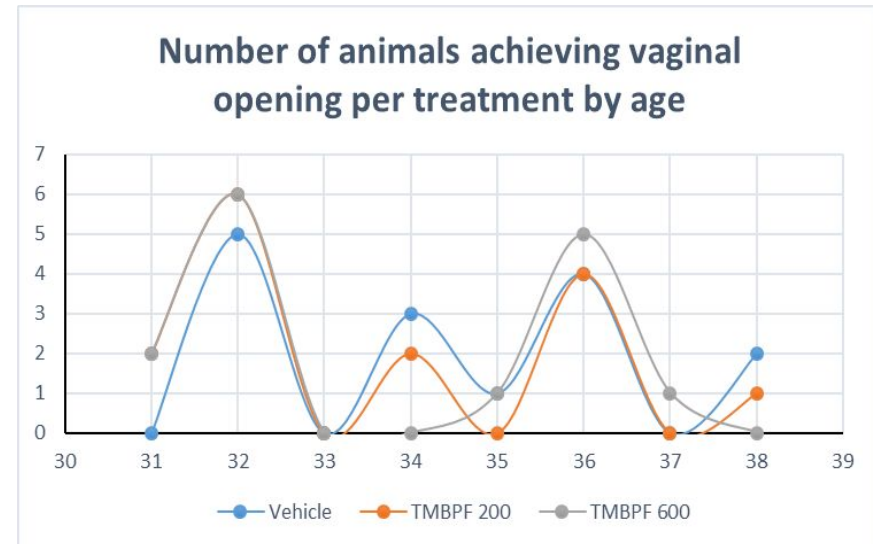
TMBPF DID NOT INCREASE UTERINE
WEIGHT AND DID NOT ALTER FEMALE
MAMMARY GLAND DEVELOPMENT

PUBERTAL ASSAY (+)

- Juvenile male and female rats
- Daily oral (gavage) treatment: 20 and 30 days females and males, respectively
- TMBPF: 0, 200, 600 mg/kg bw
- *ENDPOINTS: VAGINAL OPENING, PREPUTIAL SEPARATION, ESTROUS CYCLE, REPRODUCTIVE ORGANS WEIGHT AND HISTOPATHOLOGY*
- *NEW ENDPOINTING: MAMMARY GLAND DEVELOPMENT*

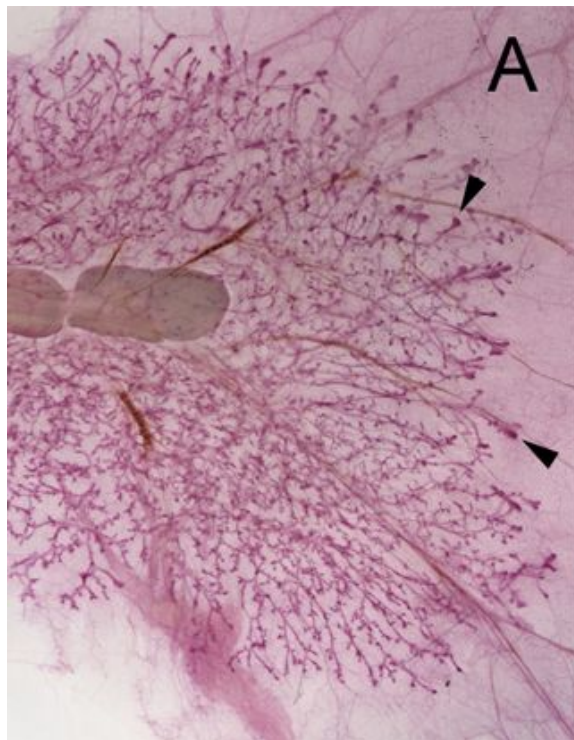
PUBERTAL ASSAY (+)

- No delay in preputial separation; mean ages (days)
 - Control: 47.27
 - 200 mg: 48.20
 - 600 mg: 48.67
- No differences in vaginal opening; mean ages (days)
 - Control: 34.47
 - 200 mg: 33.60
 - 600 mg: 33.73
- No differences in estrous cycle
- No histological findings in male or female reproductive organs

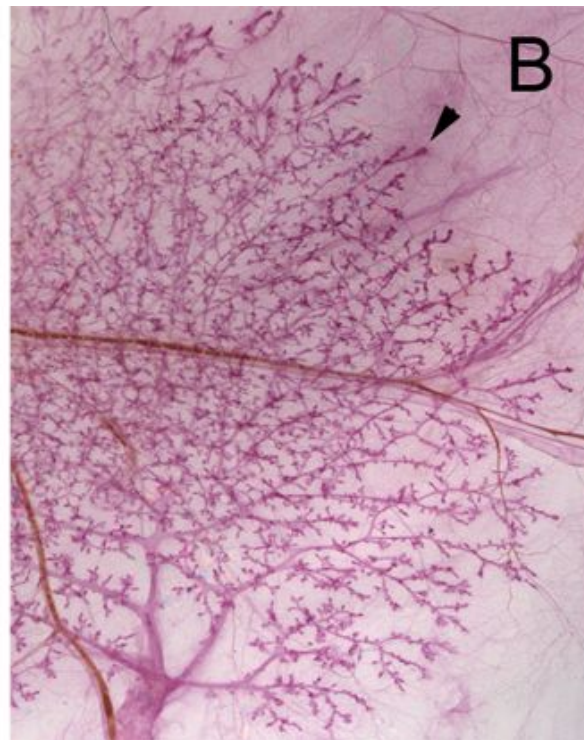


PUBERTAL ASSAY (+)

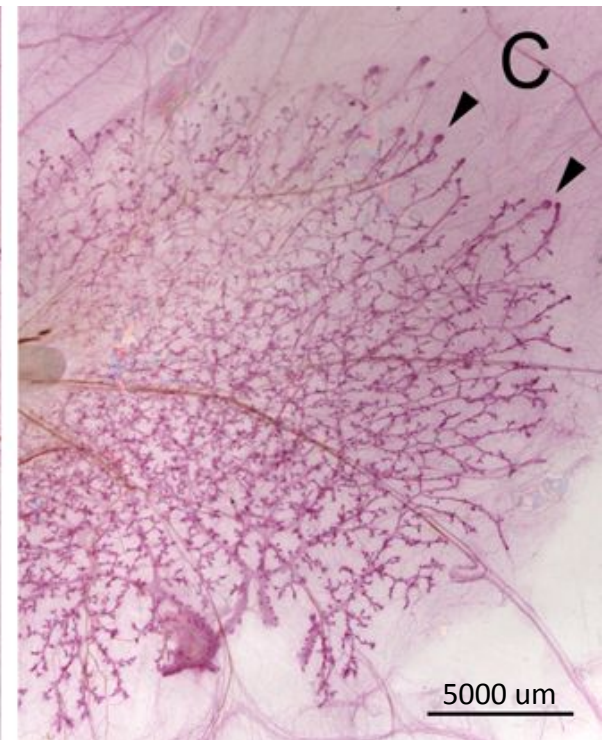
- No changes in mammary gland developmental pattern or histology



Untreated



TMBPF 200

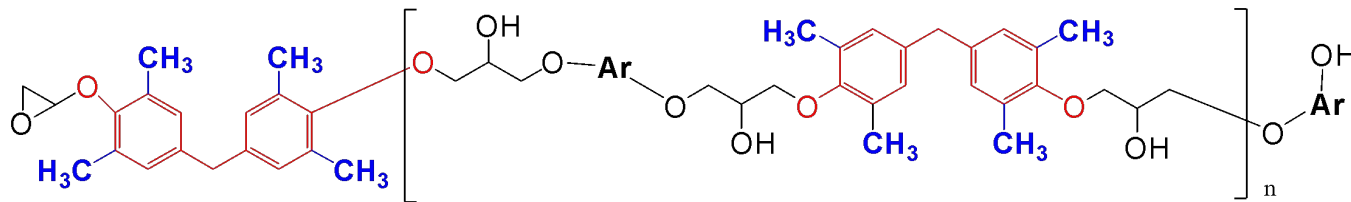


TMBPF 600

PUBERTAL ASSAY (+)

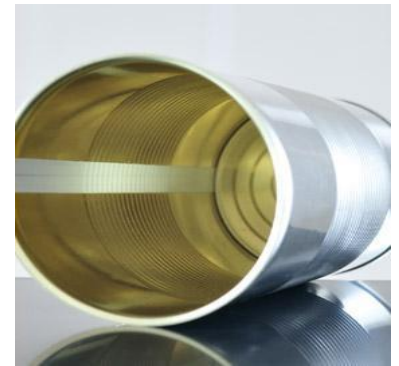
TMBPF DID NOT ALTER:
PUBERTY IN MALE AND FEMALE RATS
FEMALE MAMMARY GLAND DEVELOPMENT

Polymeric coating testing approach

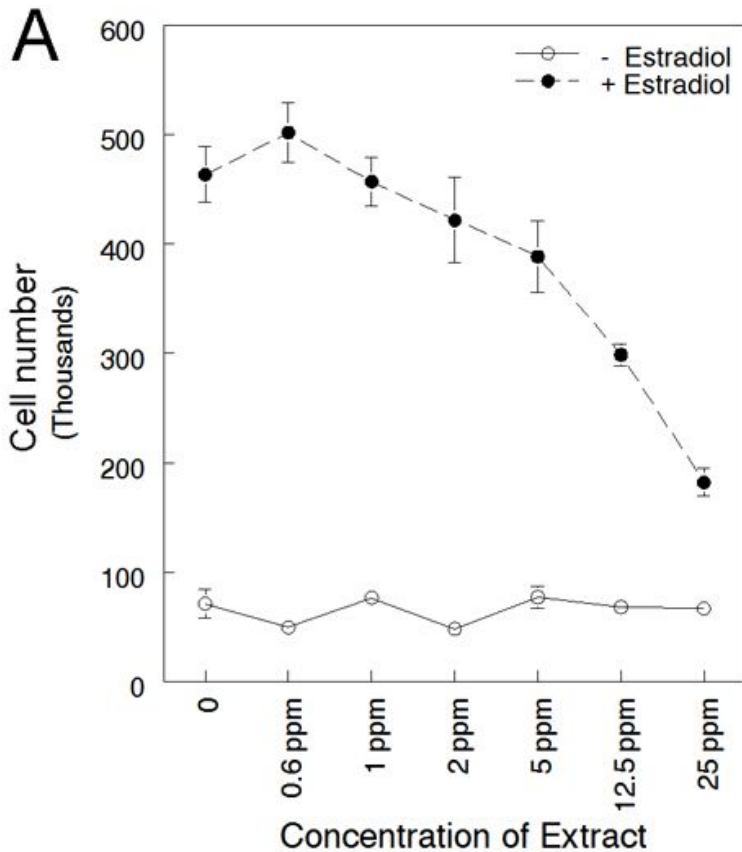


V70 Polymer

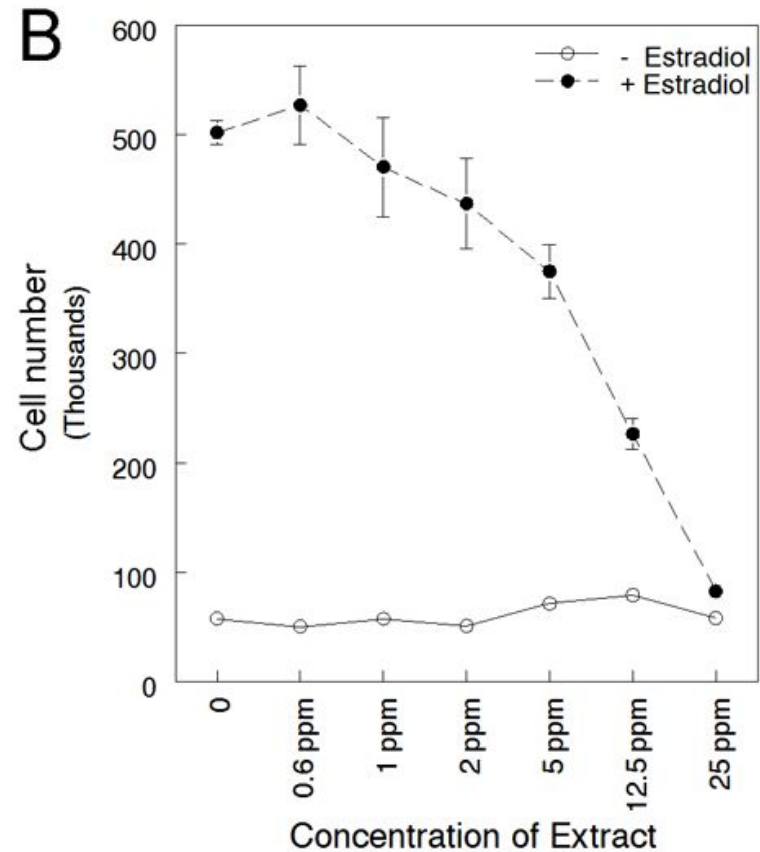
- Migrates from coating extracted using food simulants
 - Acidic foods: 3% acetic acid (FDA)
 - Fatty foods: 50% ethanol (FDA)
- Cell proliferation E-SCREEN assay (Tufts)



Extracted migrates E-SCREEN



3% Acetic acid



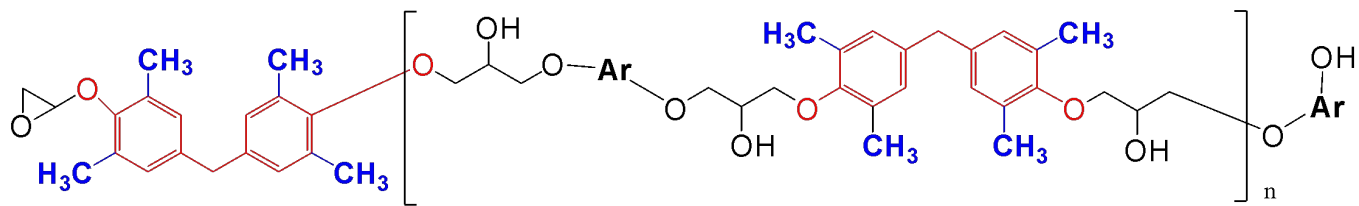
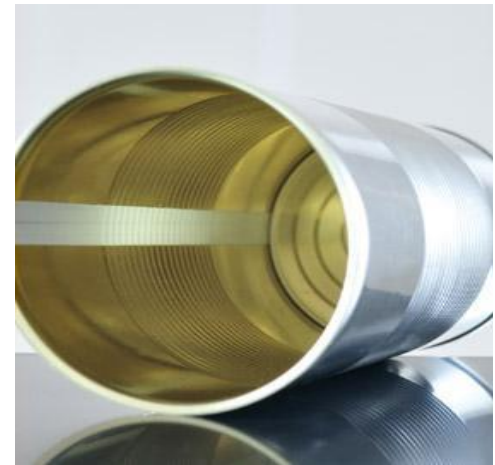
50% Ethanol

Extracted migrates E-SCREEN

POLYMERIC COATING EXTRACTS DID NOT
INCREASE PROLIFERATION OF
ESTROGEN-SENSITIVE MCF7 CELLS

Analysis of extracts

- TMBPF quantification (extracts spiked with TMBPF)
- Chromatographic analysis of migrates from both simulants (FERA)



V70 Polymer

TMBPF migration quantification

Fera No.	Sample description	TMBPF concentration
S15-092945	3% acetic acid	< LOD (LOD = 0.2 µg/6 dm ² = 0.2 ppb)
S15-092946	50% ethanol	< LOD (LOD = 0.2 µg/6 dm ² = 0.2 ppb)
S15-092947	1.78mL of 5 part per thousand oligomer migrant (NIAS) concentrate in DMSO derived from 3% acetic acid/water (8.9 mg/1.78 mL)	<RL (RL = 0.06 µg/6 dm ² = 0.06 ppb)
S15-092948	1.28 mL of 5 part per thousand oligomer migrant (NIAS) concentrate in DMSO derived from 50% ethanol/water (6.5 mg/1.28 mL)	< LOD (LOD = 0.01 µg/6 dm ² = 0.01 ppb)

LOD: limit of detection, calculated as three times the signal to noise of the TMBPF response in an over spiked aliquot of the simulant

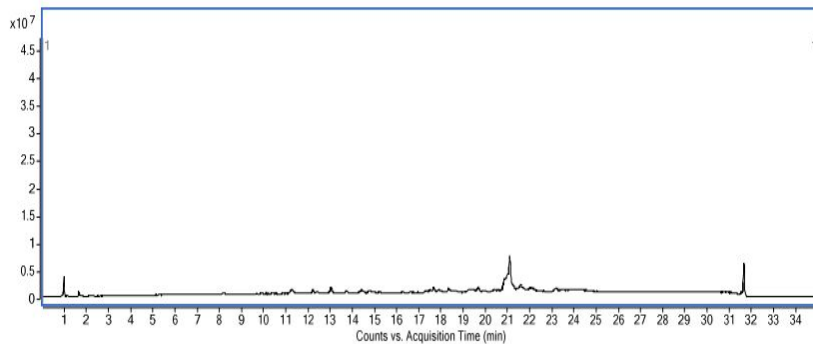
RL: reporting limit or limit of quantification, calculated as three times the response in the procedural blank.

NIAS: no intentionally added substances that migrated from polymeric-coated metal the presence of food simulants.

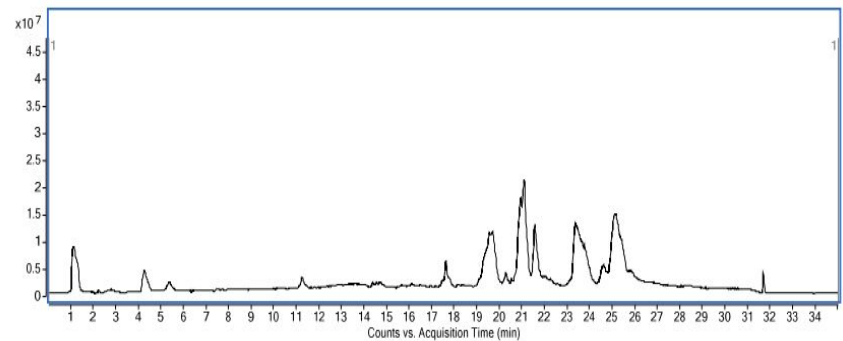
Liquid chromatography and tandem mass spectrometry (LC-MS/MS)

No-Intentionally Added Substances (NIAS) in food simulants

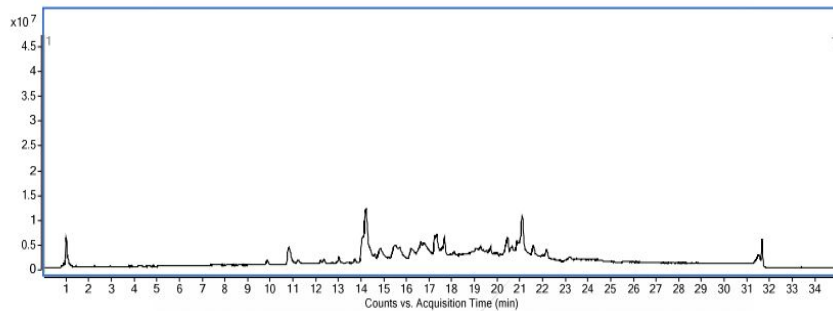
Procedural blank (50% ethanol)



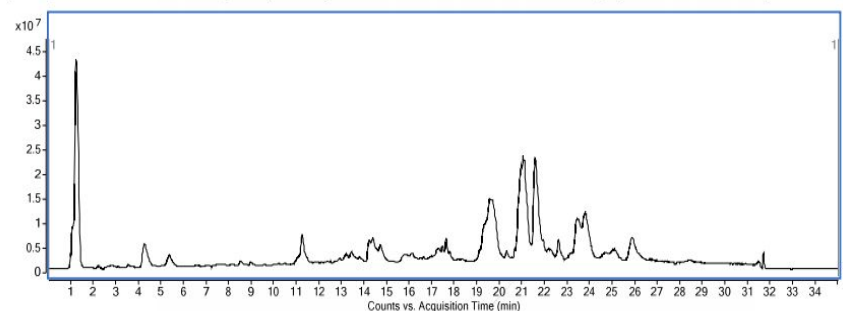
Procedural blank (3% acetic acid)



50% ethanol exposed to coated metal



3% acetic acid exposed to coated metal



Liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS)

What we learned

- Safety requires *evidence of absence*
- **Absence of evidence** has little value
- *Evidence of absence* requires thinking partners who are not like you
- Hire critics and ask them to prove you are wrong
- Get your IP protections in place and freely share your toxicity data
- Be patient and be ready for conflict
- Prepare to change your mind

GROWTH

INNOVATION

SOLUTIONS

SUCCESS

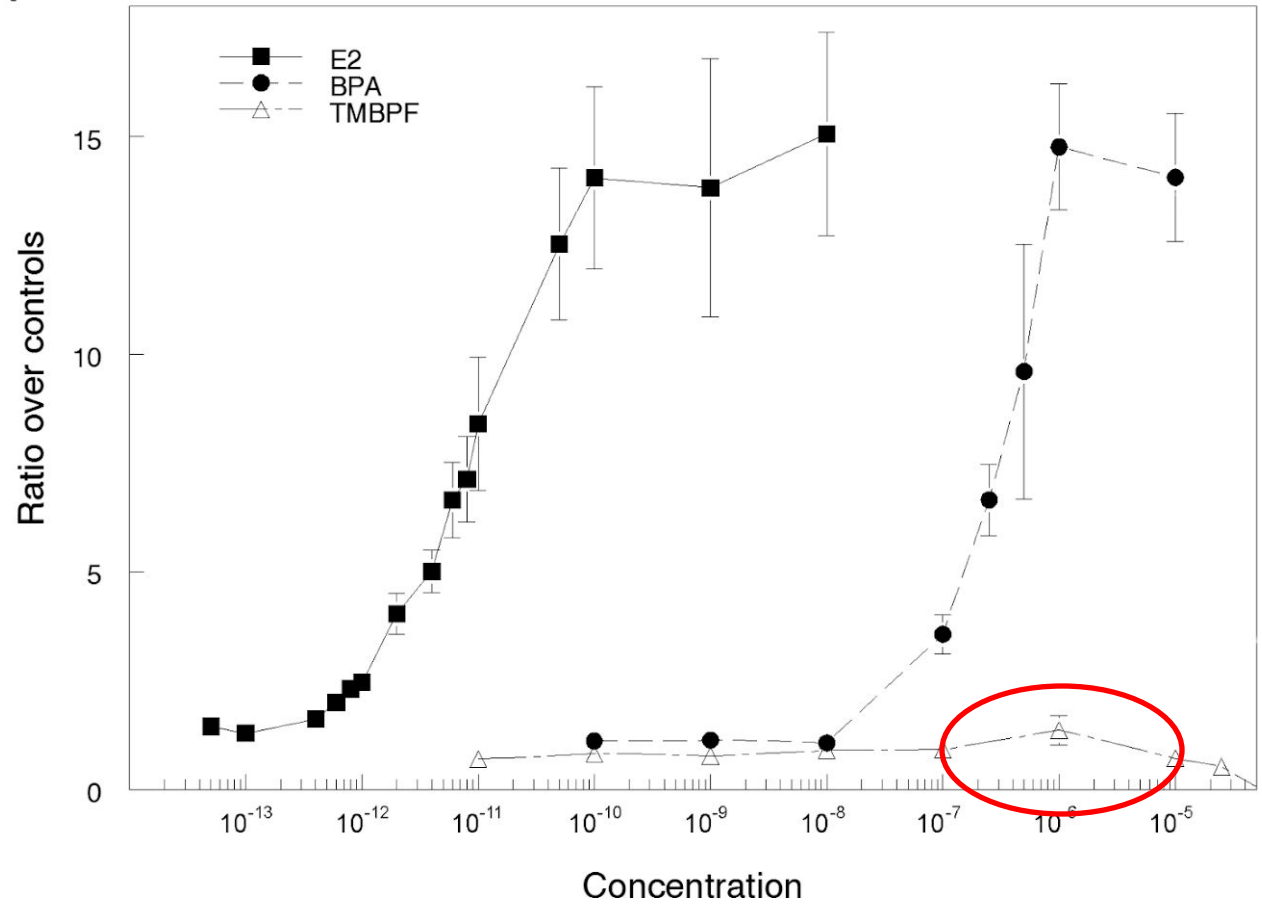
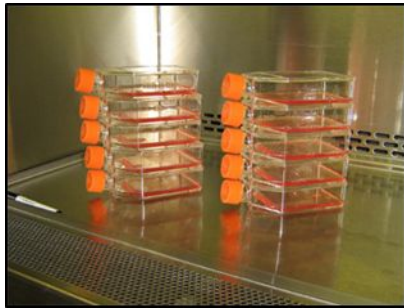


[Valsparpackaging.com > ValPure > Non-BPA Epoxy > Study Reports](http://www.valsparpackaging.com/valpure/item/our-materials/#om)

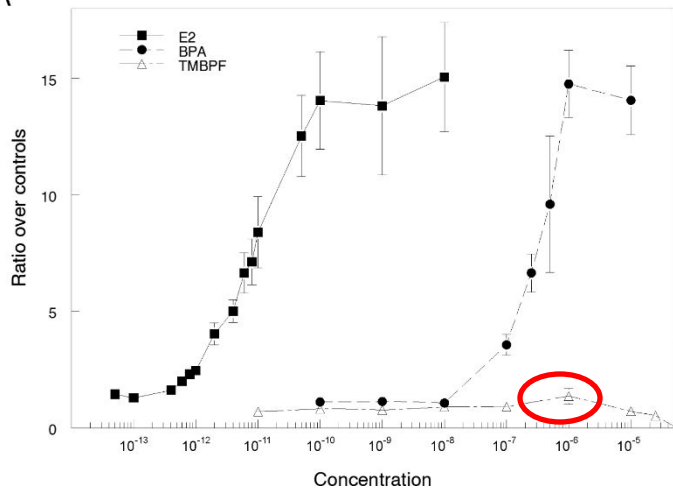
<http://www.valsparpackaging.com/valpure/item/our-materials/#om>

E-SCREEN

A

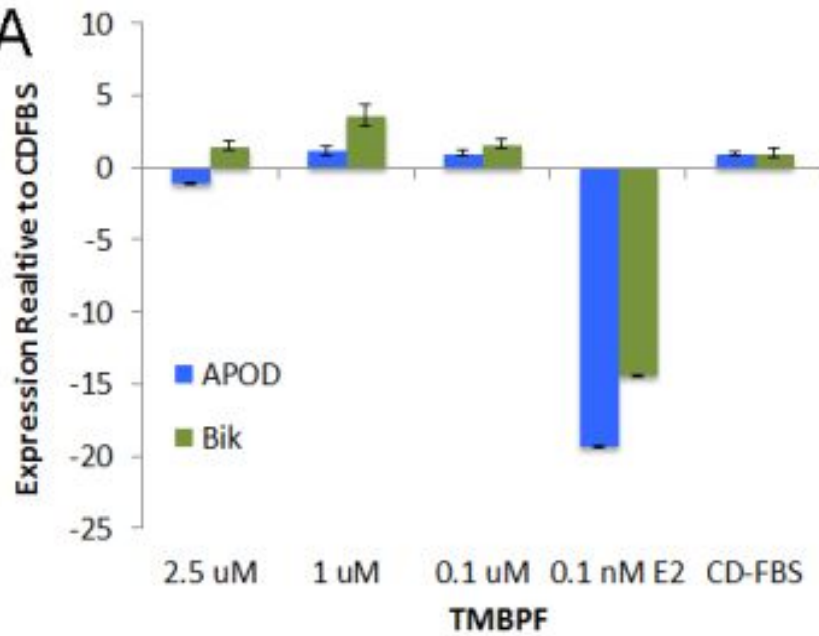


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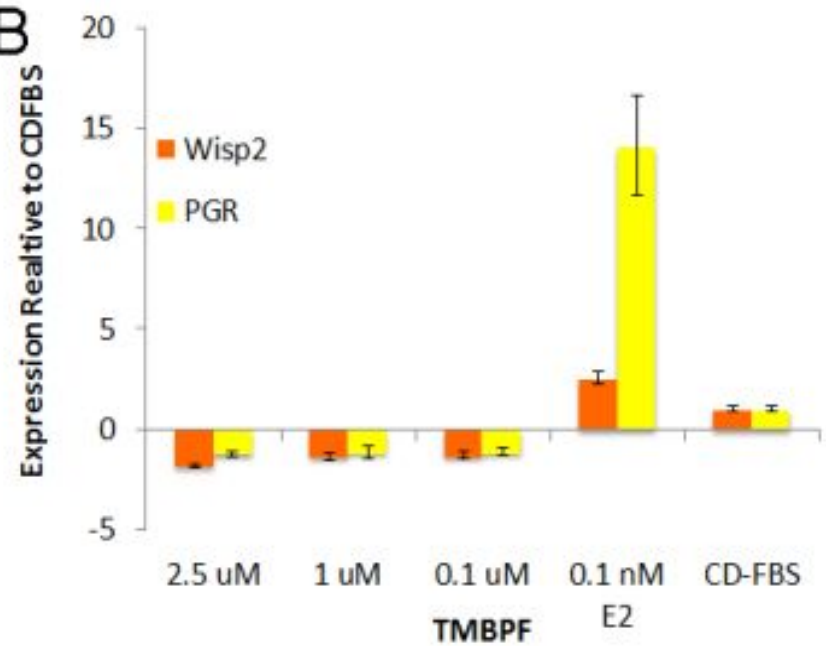


E-SCREEN

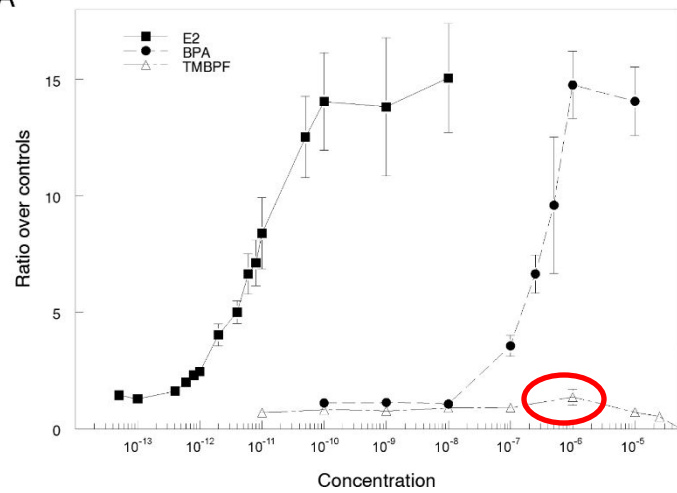
A



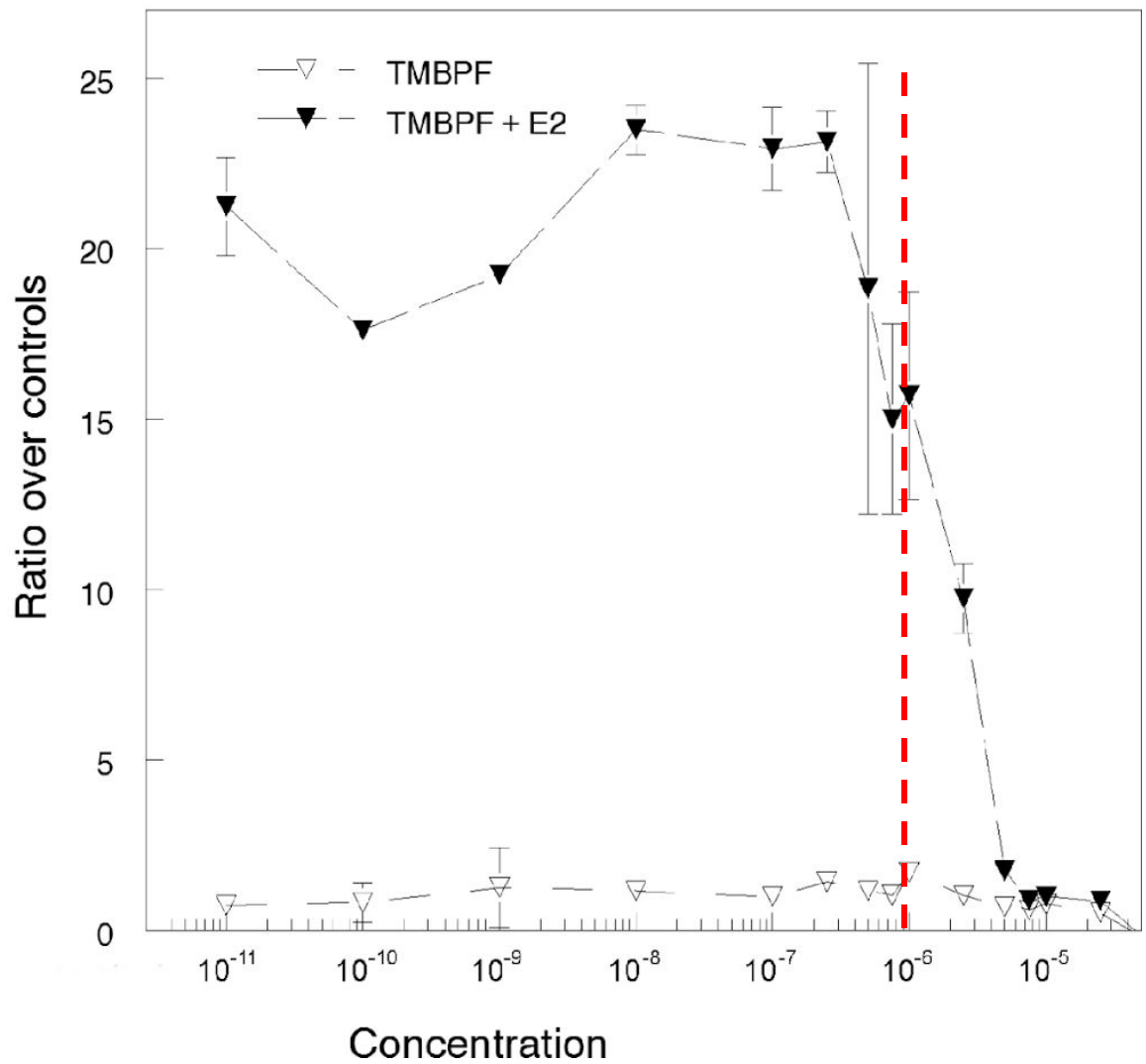
B



A

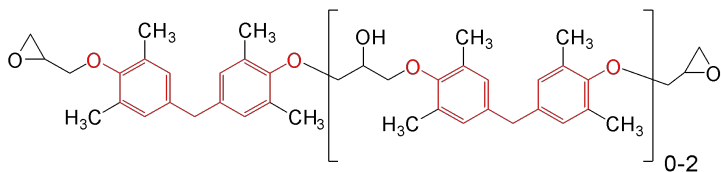


E-SCREEN



We need the bisphenol out of the last synthesis step

Read-Across Analysis for ER



Tetramethyl Bisphenol F Diglycidyl Ether ER

Table III. Summary of analysis and assay results for ER (read-across target) and read-across sources TMBPFDEG, BADGE, NOGE and oligo-BFDGE.

Green = Negative experimental result, Red = Positive experimental result
 RA = Read-across (only applies to “target” substance, ER)
 ND = no data available
 Numbers indicate relative potency in the standard Ames assay (Table IV)

In vitro			ER	TMBPFDEG	BADGE	NOGE	oligo-BFDGE
MLA	-S9	culture				ND	
MLA	+S9	culture				ND	
TA-98	-S9	plate					
TA-100	-S9	plate					
TA-1535	-S9	plate					
TA-1537	-S9	plate					
TA-98	+S9	plate					
TA-100	+S9	plate	3.9	4.7	25.0		
TA-1535	+S9	plate	33.6	35.5	117.8		
TA-1537	+S9	plate					
Mutagenicity	CAESAR	Model	Model	Model	Model	Model	Model
TA-98	-S9	mpf	RA		ND	ND	ND
TA-100	-S9	mpf	RA		ND	ND	ND
TA-1535	-S9	mpf	RA		ND	ND	ND
TA-1537	-S9	mpf	RA		ND	ND	ND
TA-98	+S9	mpf	RA		ND	ND	ND
TA-100	+S9	mpf	RA		ND	ND	ND
TA-1535	+S9	mpf	RA		ND	ND	ND
TA-1537	+S9	mpf	RA		ND	ND	ND
GS GADD45	-S9	culture	RA		ND	ND	ND
GS GADD45	+S9	culture	RA		ND	ND	ND
In vivo							
Micronucleus			Oral	Oral	Oral	ND	Oral
Comet Assay or other DNA damage			Oral	Oral	Oral	Oral	Oral
Chromosomal aberration			RA	ND	Oral	Oral	Oral
Transgenic Mouse			RA	ND	ND	Oral	
Carcinogenicity			RA	ND		ND	Dermal
Carcinogenicity			Model	Model	Model	Model	Model
Carcinogenicity			QSAR	QSAR	QSAR	QSAR	QSAR
Repeated Oral Dose (mg/kg-bw/d)			28-56Day NOAEL = 100 LOAEL = 300 Repro/Dev NOAEL = 300	ND	Chronic NOAEL = 15 Subchronic NOAEL = 50; LOAEL = 250	ND	90-Day NOAEL = 250 (limit dose) 14-day NOAEL = 300; LOAEL = 1000