

Evidence of Absence

Estrogenicity Assessment of a New Food-Contact Coating and its Monomer

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Food Packaging Forum

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Disclosure

- Paid consultant to advise Valspar Corporation on matters of science and testing strategy for new polymeric can-coating material.

Evidence of Absence: Estrogenicity Assessment of a New Food-Contact Coating and the Bisphenol Used in Its Synthesis

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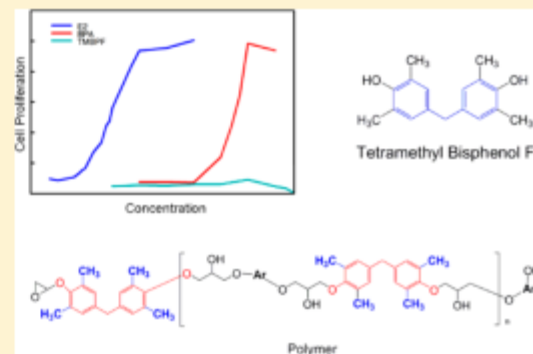
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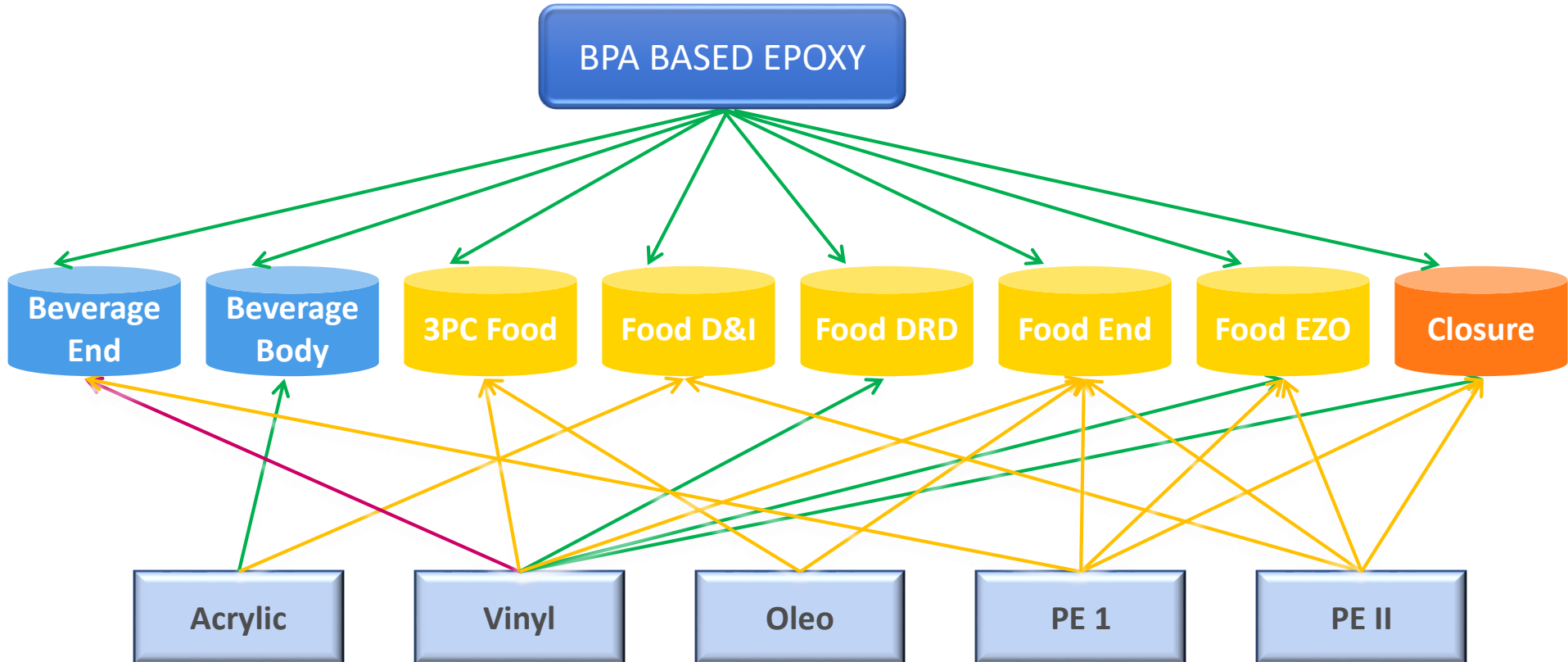
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Supporting Information

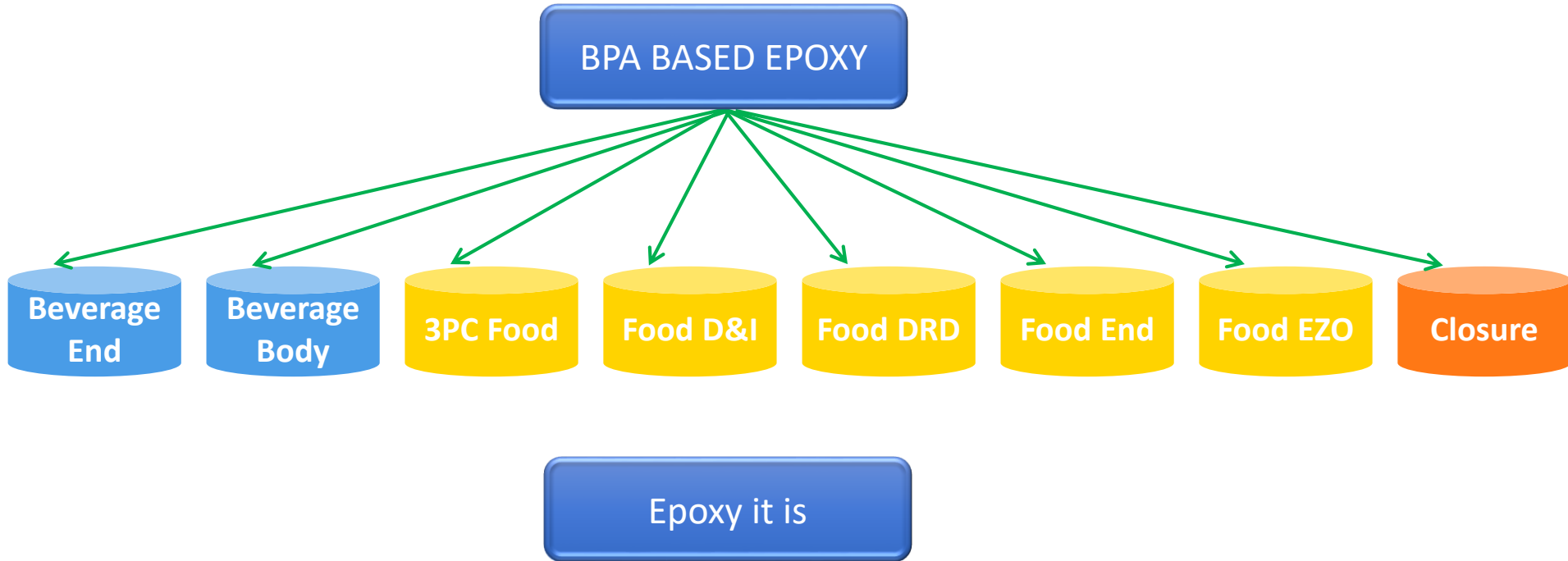
ABSTRACT: Consumer concerns about exposure to substances found in food contact materials with estrogenic activity (EA) have created substantial demand for alternatives. We assessed the potential EA of both a new bisphenol monomer used to synthesize polymeric coatings for metal food-contact applications and the nonintentionally added substances (NIAS) that may migrate into food. We evaluated tetramethyl bisphenol F (TMBPF) using *in vitro* and *in vivo* assays. We extracted the polymeric coating using food simulants ethanol (50% v/v) and acetic acid (3% w/v) and measured migration using tandem liquid chromatography (LC)/mass spectrometry (MS) and LC time-of-flight MS for TMBPF and NIAS, respectively. We also tested migrants for EA using the E-SCREEN assay. TMBPF did not show estrogenic activity in the uterotrophic assay and did not alter puberty in male and female rats or mammary gland development in female rats. Neither TMBPF nor the migrants from the final polymeric coating increased proliferation of estrogen-sensitive MCF7 cells. TMBPF did not show estrogen-agonist or antagonist activity in the estrogen receptor-transactivation assay. TMBPF migration was below the 0.2 parts per billion detection limit. Our findings provide compelling evidence for the absence of EA by TMBPF and the polymeric coating derived from it and that human exposure to TMBPF would be negligible.



If we replace BPA what choices do we have?



If we replace BPA what choices do we have?



Phenol

Acetone

Bisphenol A

Epichlorohydrin

Bisphenol A Diglycidyl Ether

1

2

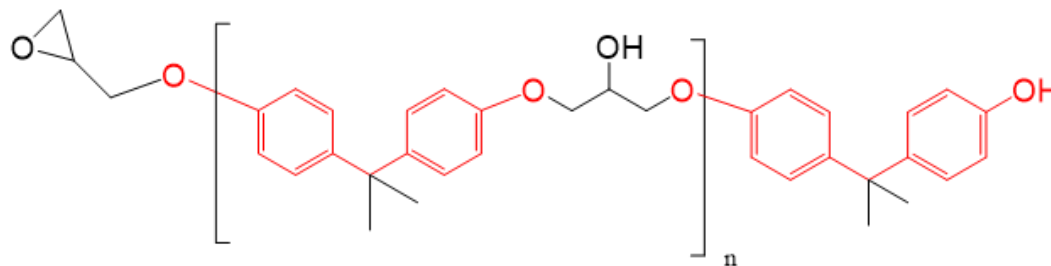
n

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

There are 2 sources of BPA in the polymer synthesis

4,4'-Methylenediphenol is needed for best performance

- Its polymer chain functionality imparts unique physical properties not available from other materials (e.g., olefins, acrylics, polyesters)
- Polymers are flexible, with high integrity barriers able to protect food for long periods, under extreme conditions and across an entire spectrum of metal food-contact applications



But, bisphenols are the only commercially viable source for 4,4'-methylendiphenol

So?

Bisphenols have either estrogenic activity or scant health-effects data

Challenge

To find a novel bisphenol that:

1- lacks estrogenic activity, and

2- imparts technical performance and integrity into polymers needed to ensure food safety

AND, if possible, no residual bisphenol in final product

What structural attributes best predict estrogen receptor binding?

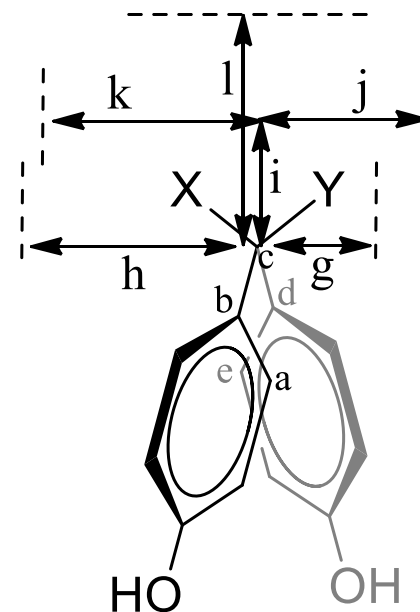
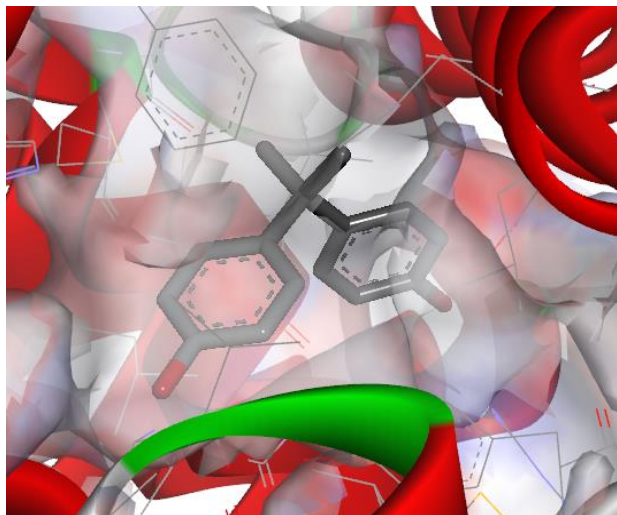
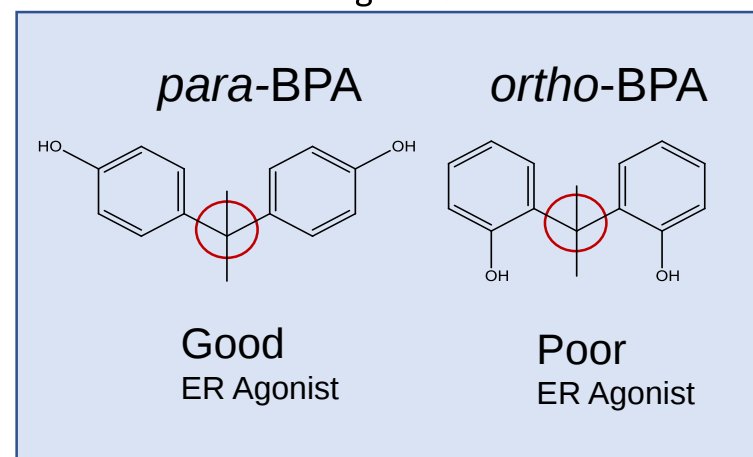
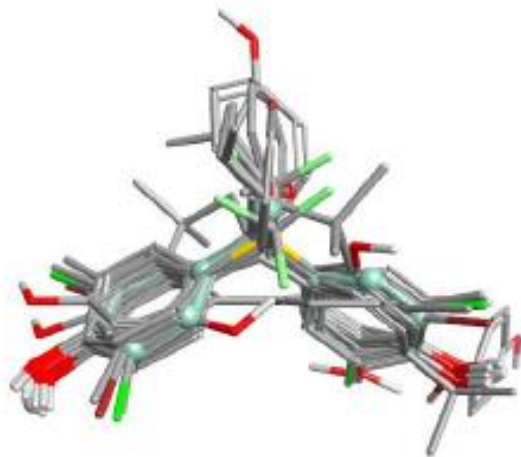
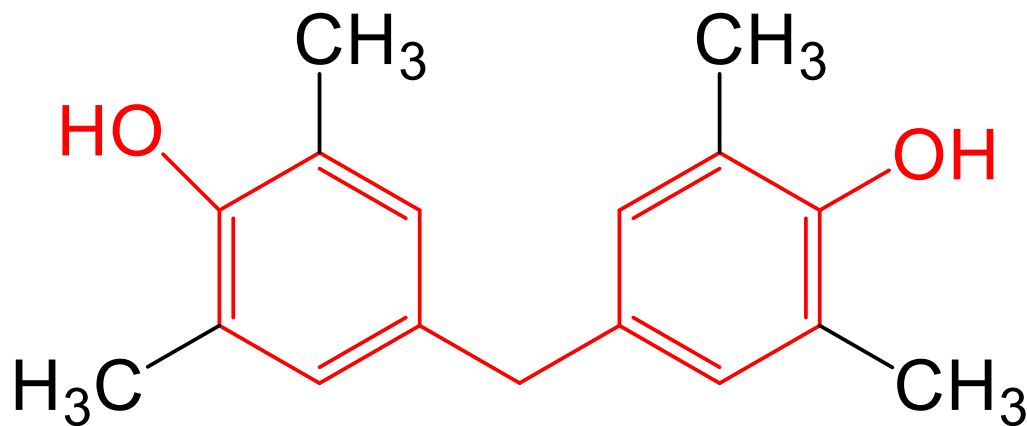


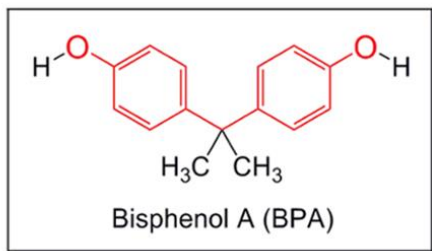
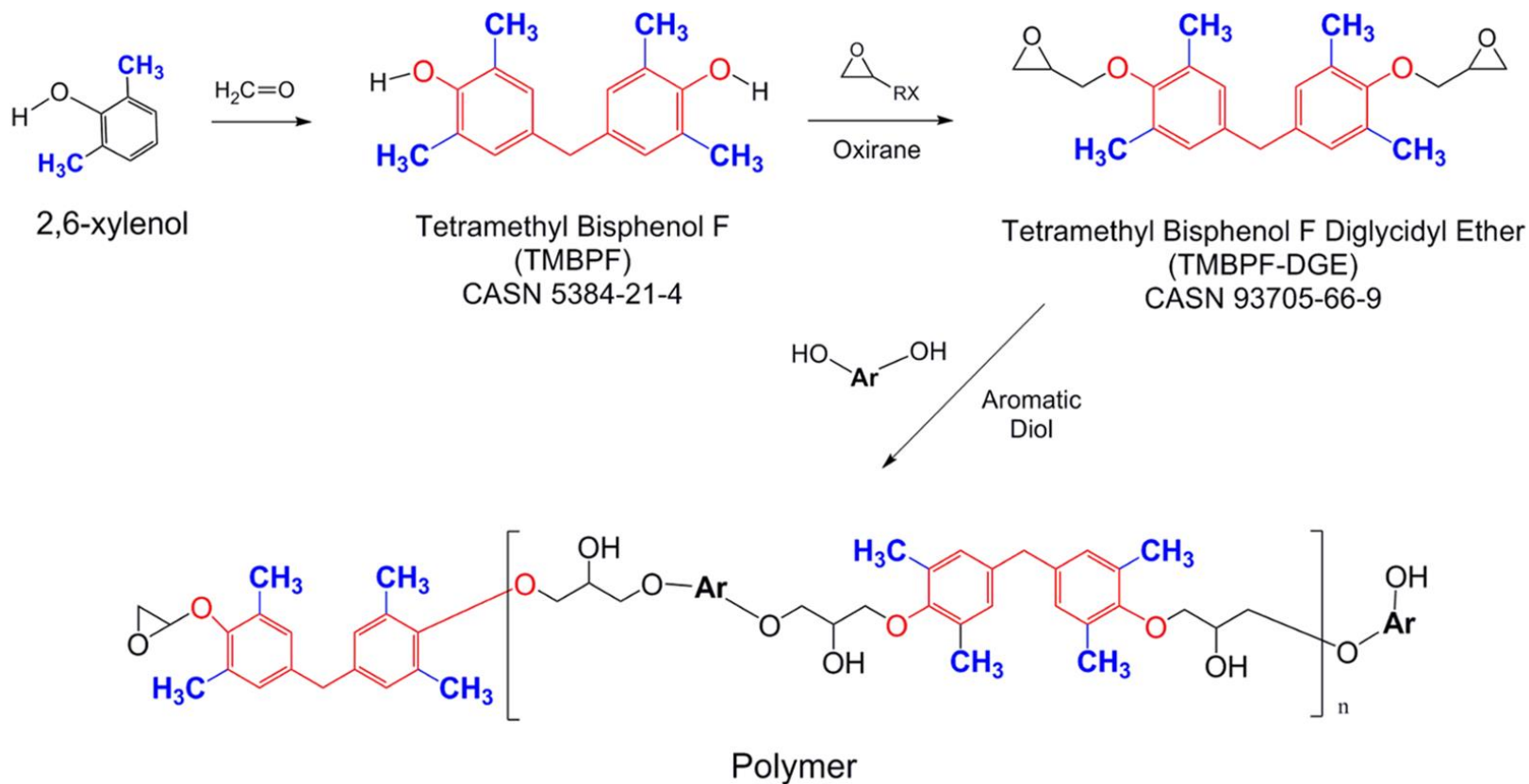
Figure 6.





Tetramethyl bisphenol F
(TMBPF) was selected

TMBPF-based Polymer Synthesis



Estrogenic activity testing approach

HYPOTHESES

TMBPF DOESN'T HAVE
ESTROGENIC ACTIVITY



IN VITRO AND IN VIVO
TESTING

TMBPF DOESN'T PERSIST
IN FINAL POLYMER



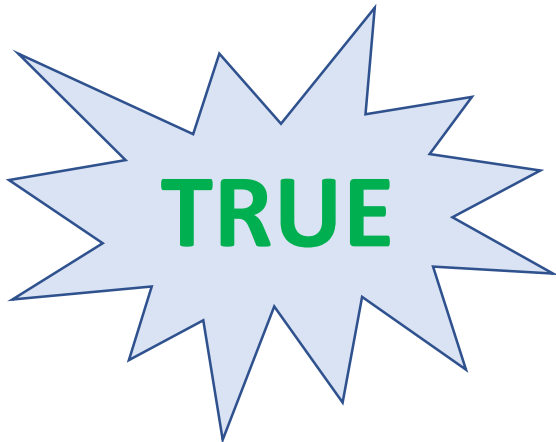
MIGRATION TESTING

HYPOTHESES

TMBPF DOESN'T HAVE
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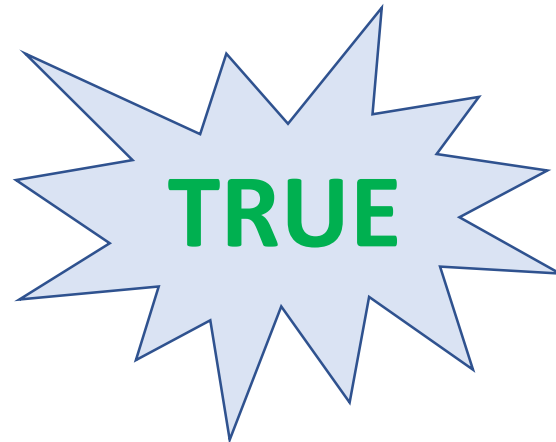
IN VITRO AND IN VIVO
TESTING



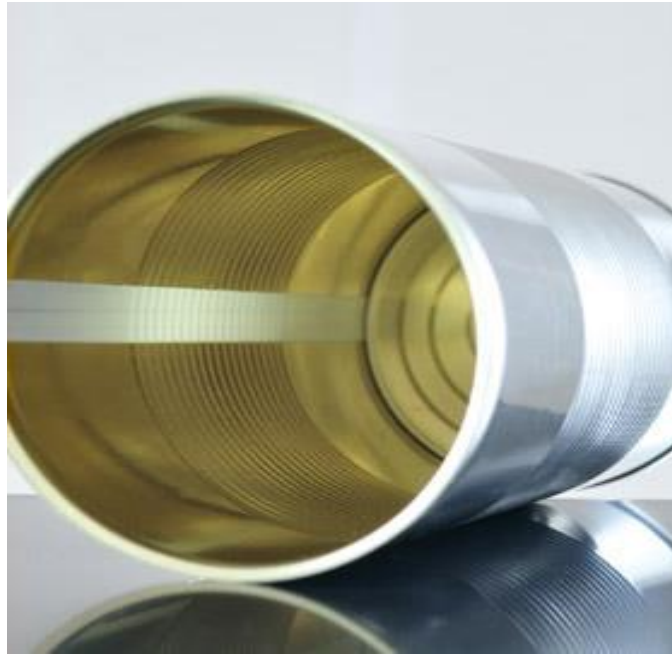
TMBPF DOESN'T PERSIST
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MIGRATION TESTING



HYPOTHESES



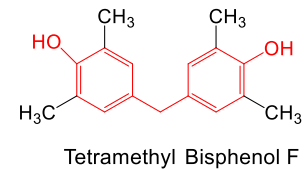
WHAT ABOUT THE POLYMERIC COATING?

IN VITRO TESTING  ANALYSIS OF MIGRANTS

Approaches to EA and migration assessment

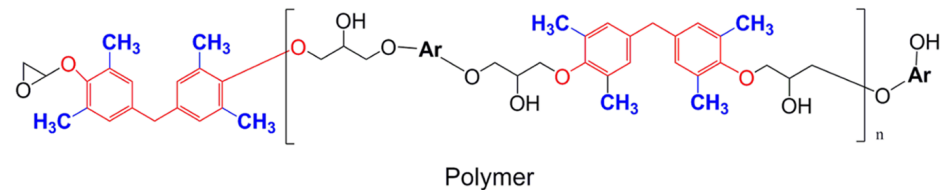
- Monomer TMBPF

- Estrogen activity
 - Guidance testing → Mammary gland biology
 - Collaborative testing → Mammary gland biology
- Migration quantification from final polymer

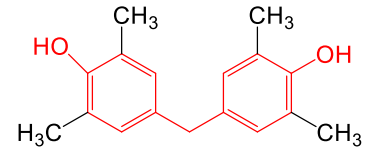


- Polymeric coating

- Estrogen activity
 - Collaborative testing
- Migrates profile



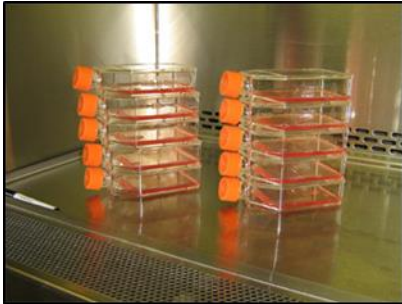
TMBPF testing



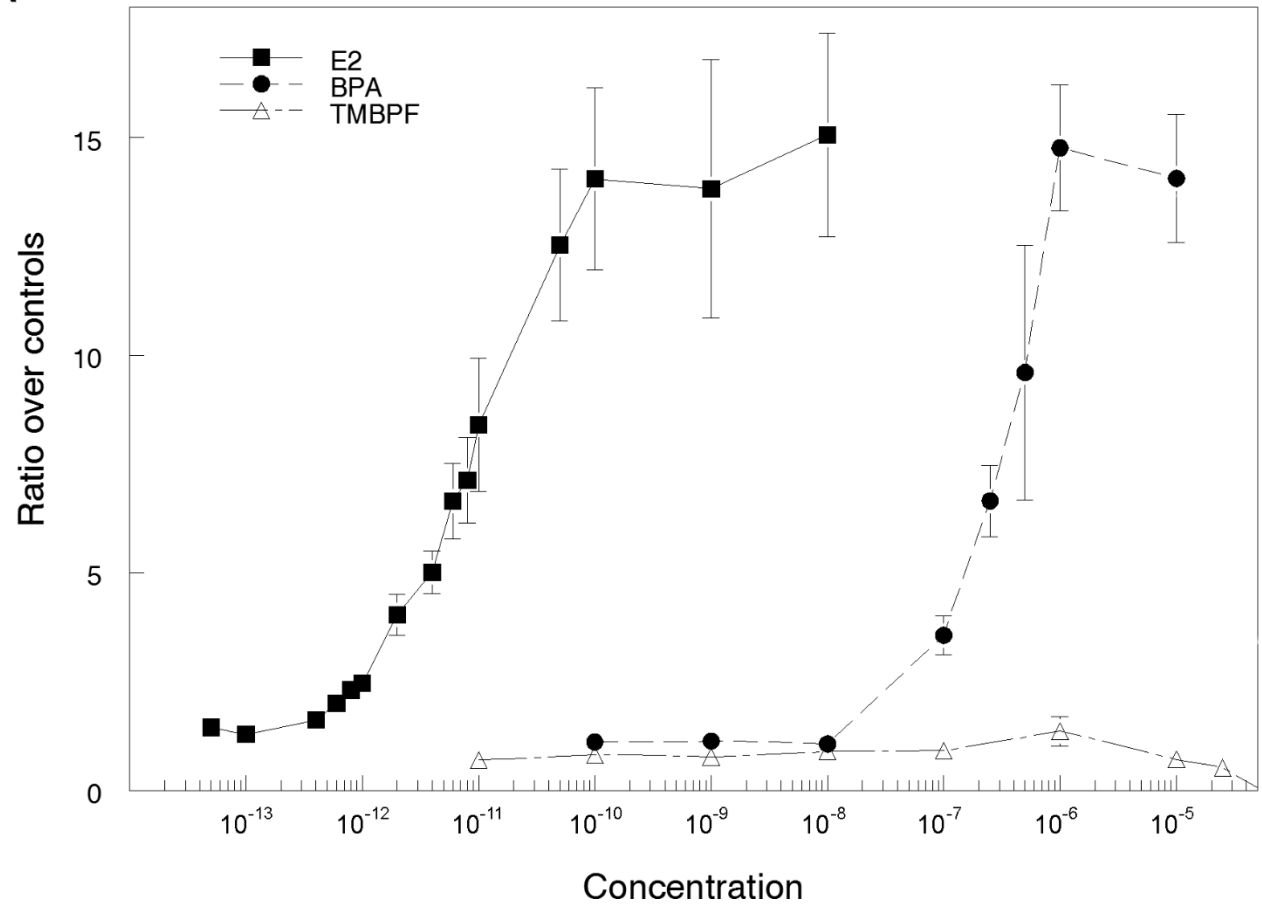
Tetramethyl Bisphenol F

- Cell proliferation E-SCREEN assay (Tufts)
- Immature rat uterotrophic assay (EPA+ mammary gland)
- Juvenile male and female pubertal assay (EPA+ mammary gland)

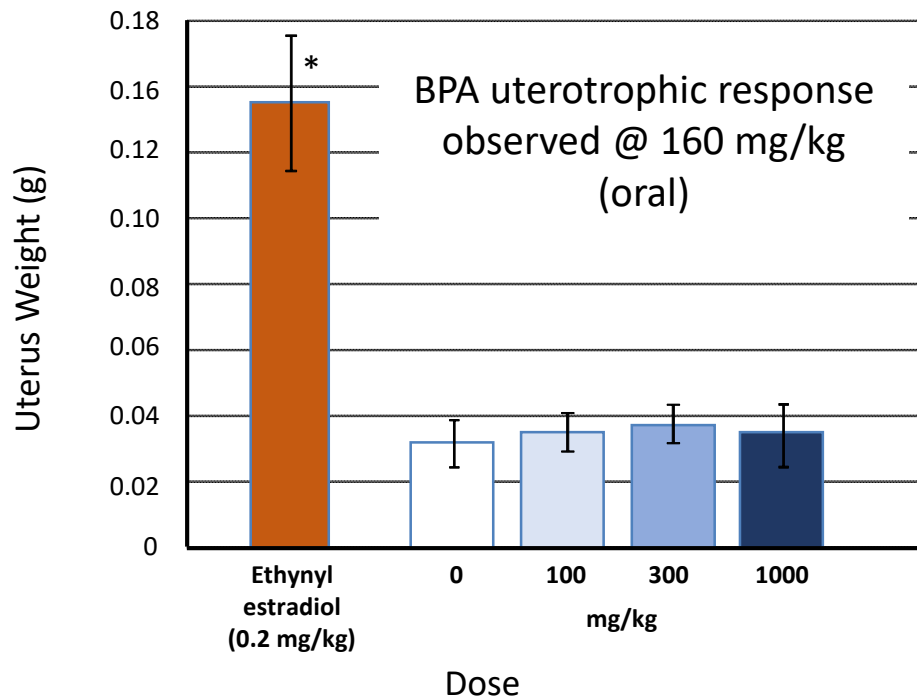
E-SCREEN



A



Uterotrophic assay (+)

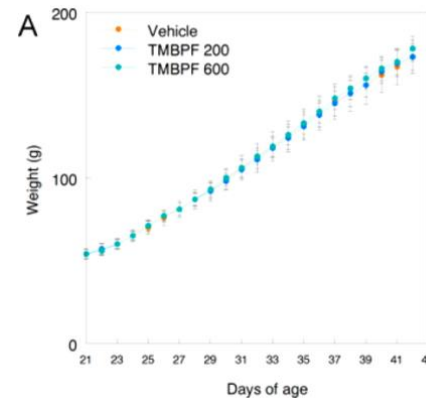
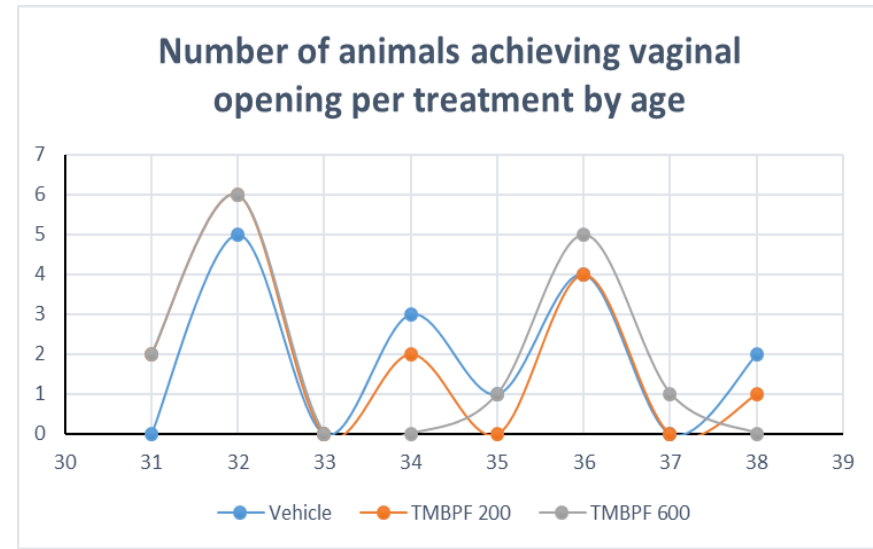


NORMAL MAMMARY
GLAND HISTOPATHOLOGY

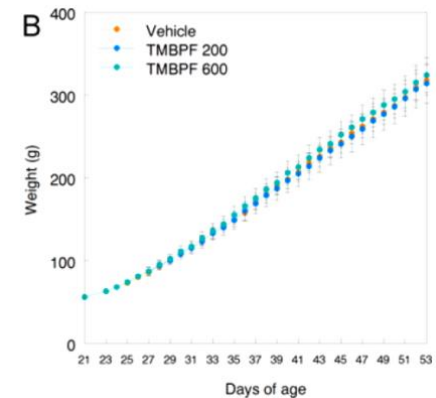
PUBERTAL ASSAY (+)



- 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
- No increase in body weight



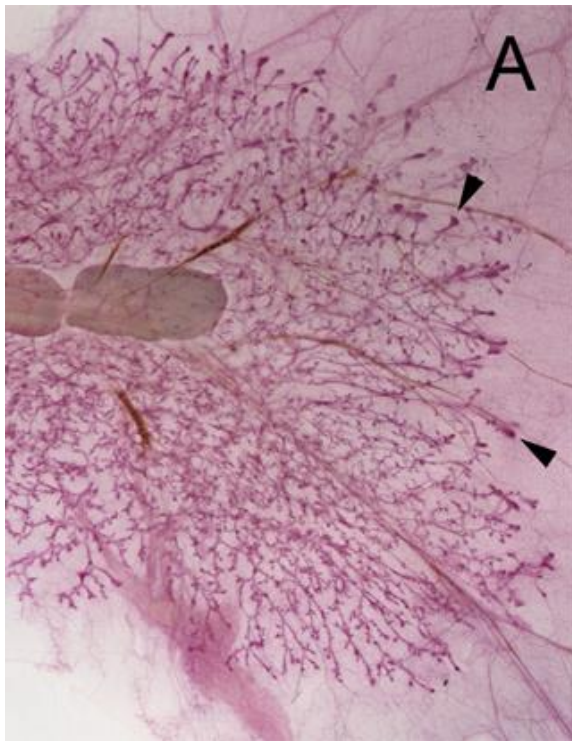
Females



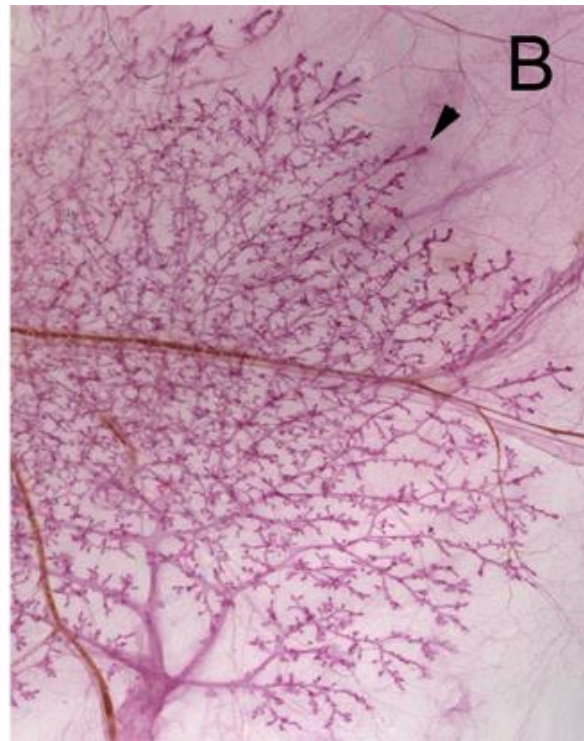
Males

PUBERTAL ASSAY (+)

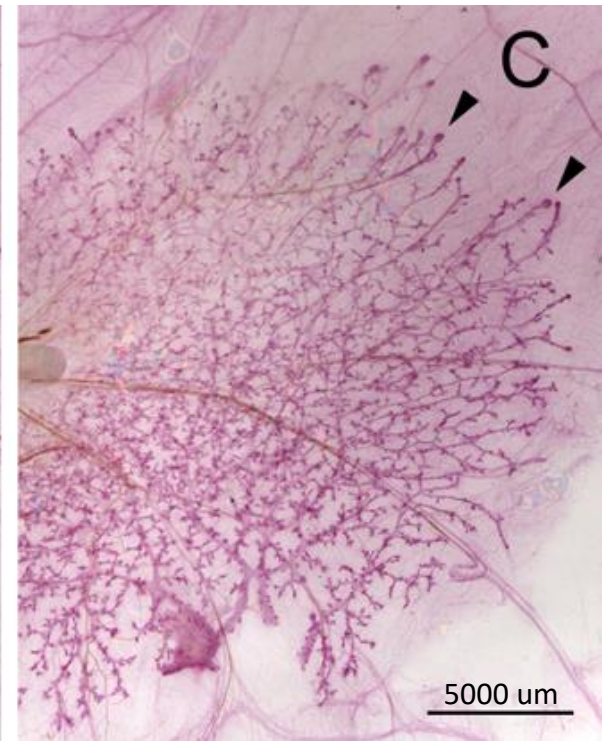
- No changes in mammary gland developmental pattern or histology



Untreated

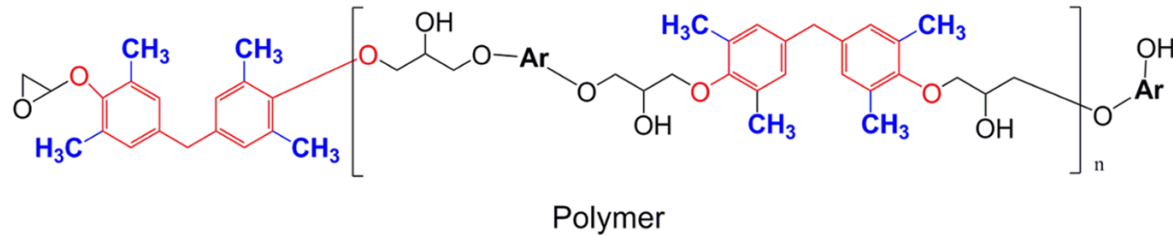


TMBPF 200

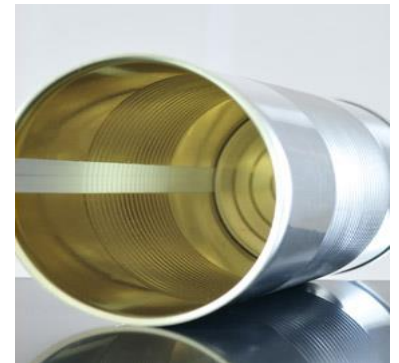


TMBPF 600

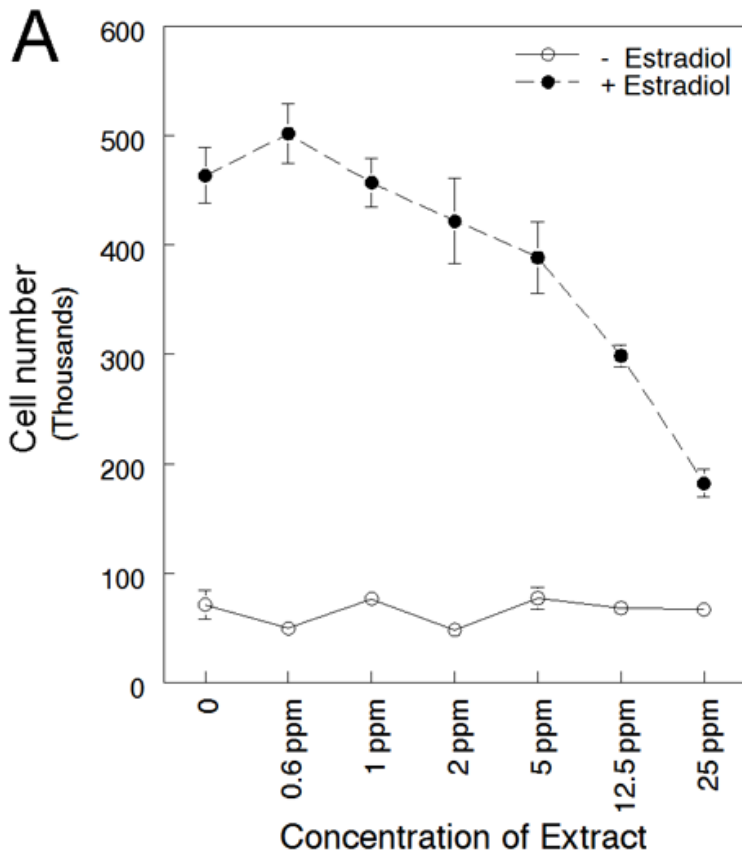
Polymer testing approach



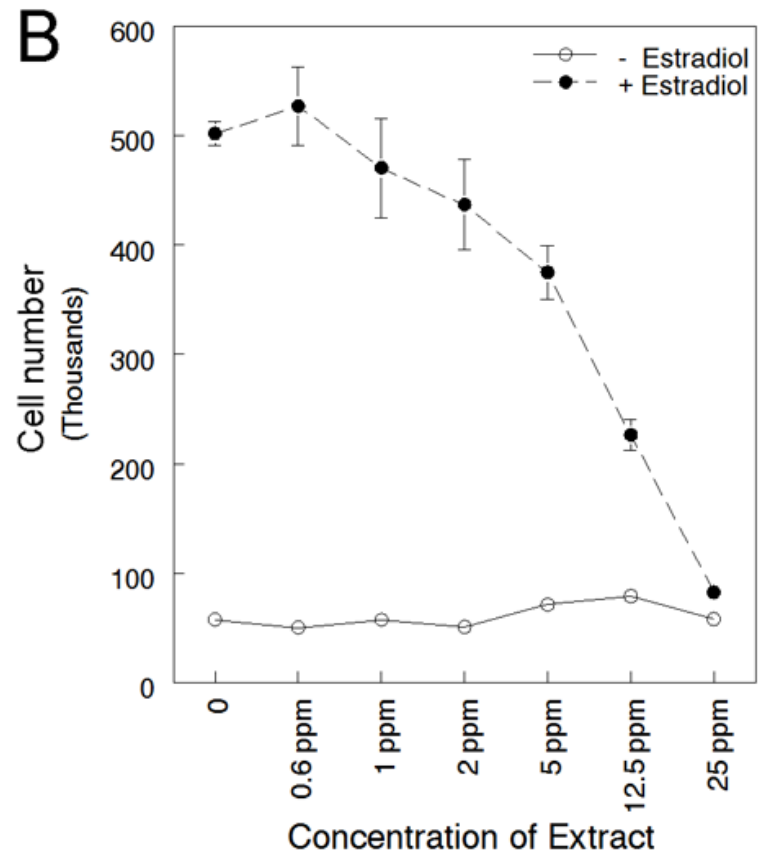
- Migrates from coating extracted using food simulants
 - 3% acetic acid: acidic food (tomatoes, fruits)
 - 50% ethanol: aqueous, fatty and alcoholic food
- Cell proliferation E-SCREEN assay (Tufts)



Extracted migrates E-SCREEN



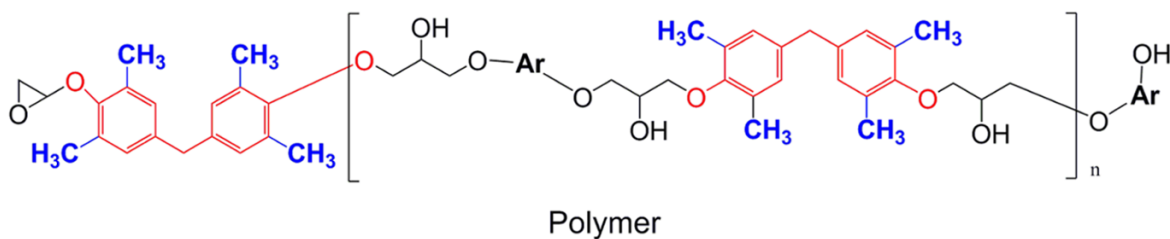
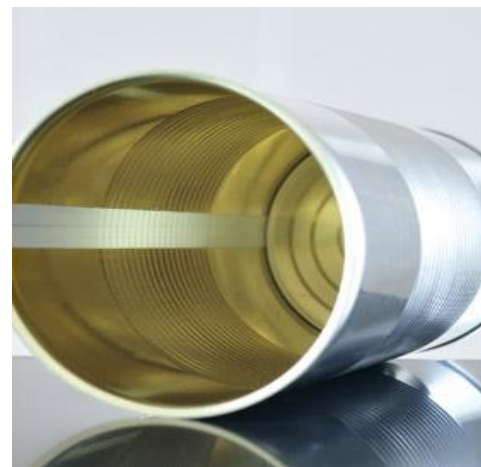
3% Acetic acid



50% Ethanol

Analysis of extracts

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



TMBPF migration quantification

Fera No.	Sample description	TMBPF concentration
S15-092945	3% acetic acid	< LOD (LOD = $0.2 \mu\text{g}/6 \text{ dm}^2 = 0.2 \text{ ppb}$)
S15-092946	50% ethanol	< LOD (LOD = $0.2 \mu\text{g}/6 \text{ dm}^2 = 0.2 \text{ ppb}$)
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 = $0.06 \mu\text{g}/6 \text{ dm}^2 = 0.06 \text{ ppb}$)
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 = $0.01 \mu\text{g}/6 \text{ dm}^2 = 0.01 \text{ ppb}$)

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

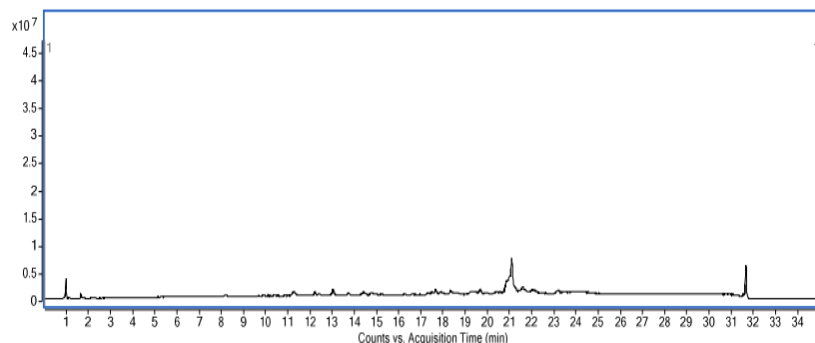
RL: reporting limit or limit of quantification, calculated as three times the response in the procedural blank.

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

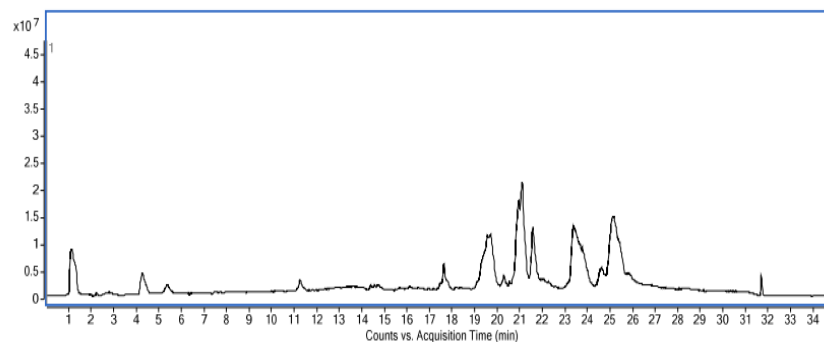
Liquid chromatography and tandem mass spectrometry (LC-MS/MS)

No-Intentionally Added Substances (NIAS) in food simulants

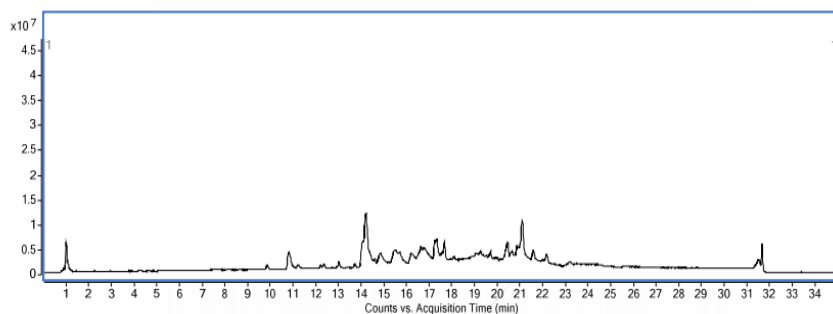
Procedural blank (50% ethanol)



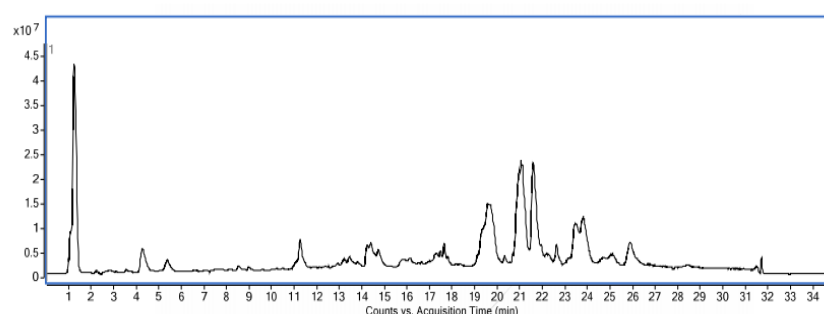
Procedural blank (3% acetic acid)



50% ethanol exposed to coated metal



3% acetic acid exposed to coated metal



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

Summary

- Hypothesis predicted by computational modeling was confirmed
- *In vitro* and *in vivo* data indicate there is strong evidence for absence of estrogenic activity by monomer TMBPF and polymer derived from TMBPF
- Migration of TMBPF below limit of detection
- Human exposure to TMBPF appears to be negligible

Thank you!

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