NTP
National Toxicology Program

Evaluation of Bisphenol Analogues

Vicki Sutherland, PhD
Toxicology Branch
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors
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Presentation Outline

- Goals
- Portfolio – analogues/derivatives and evaluations
- Highlights from our data
- Future plans

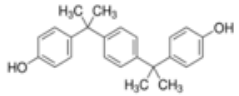
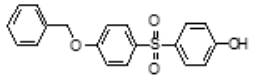
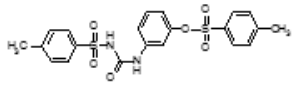
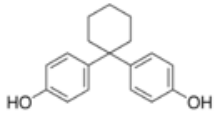
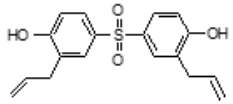
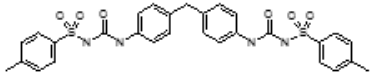
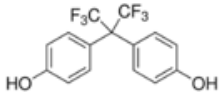
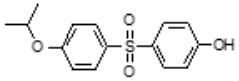
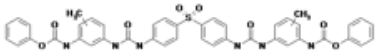
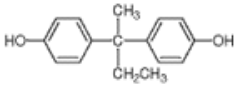
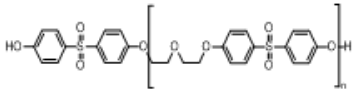
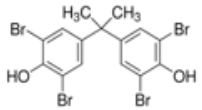
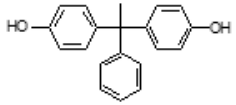
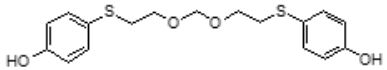
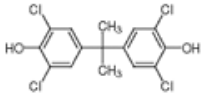
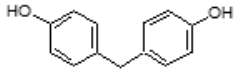
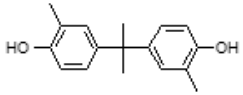
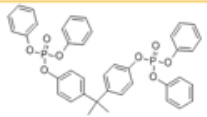
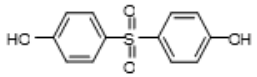
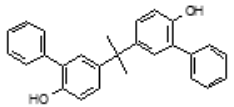
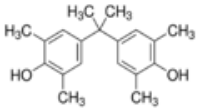
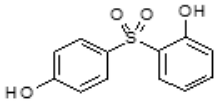
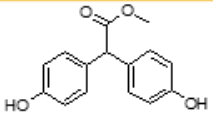
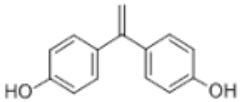
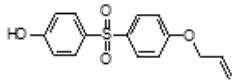
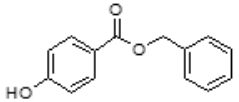
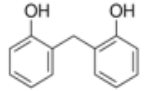
Bisphenol Analogue & Derivatives Program

1. What are the toxicological concerns related to the bisphenol analogues & derivatives?

2. How to develop an approach for analyzing classes of chemicals, avoiding full characterization of each chemical in that class?

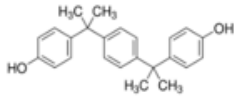
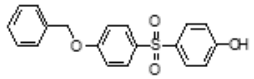
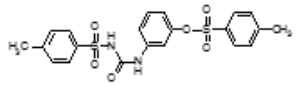
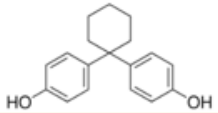
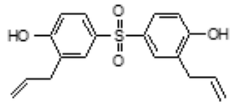
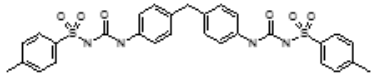
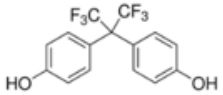
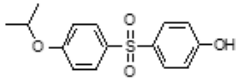
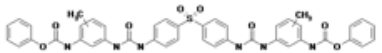
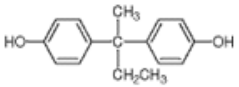
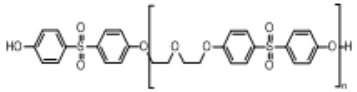
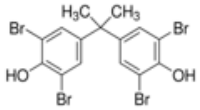
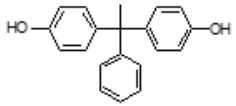
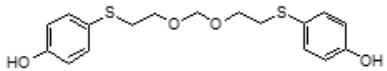
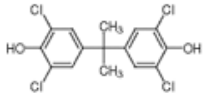
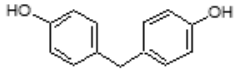
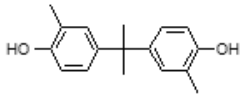
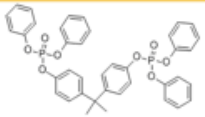
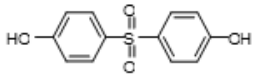
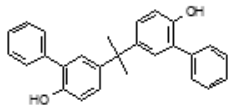
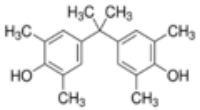
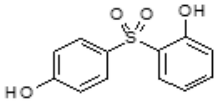
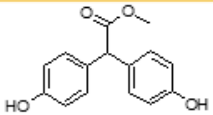
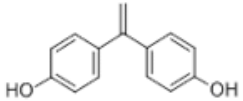
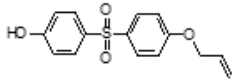
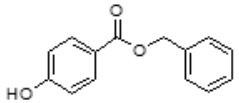
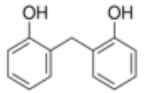
3. How does this feed into public health?

Testing Program

Structure	Chemical	Structure	Chemical	Structure	Chemical
	Bisphenol A				
	Bisphenol P		BPS-MPE		Pergafast 201
	Bisphenol Z		TGSA		BTUM
	Bisphenol AF		D-8		UU
	Bisphenol B		D-90		TBBPA
	Bisphenol AP		DD-70		TCBPA
	Bisphenol F (4,4)		Bisphenol C		BPA bis(diphenyl phosphate)
	Bisphenol S		BisOPP-A		TMBPA
	2,4-BPS		MBHA		Bisphenol E
	BPS-MAE		PHBB		Bisphenol F (2,2)

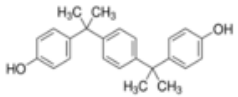
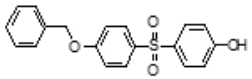
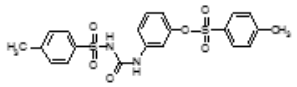
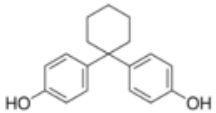
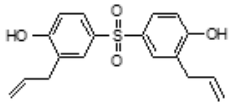
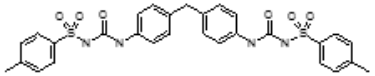
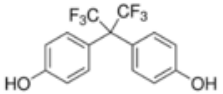
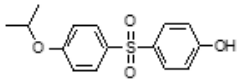
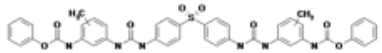
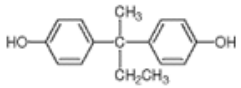
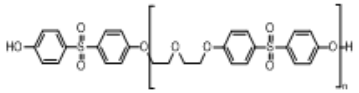
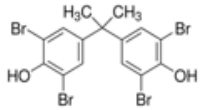
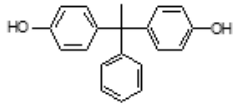
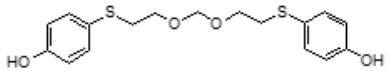
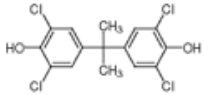
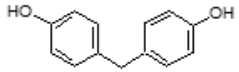
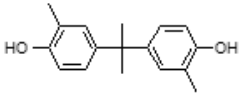
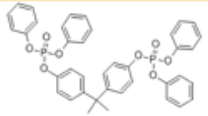
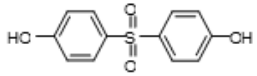
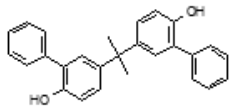
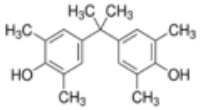
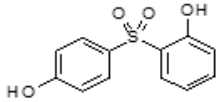
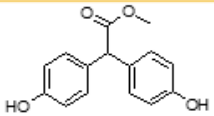
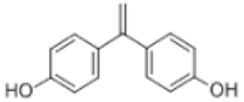
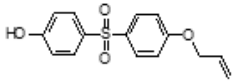
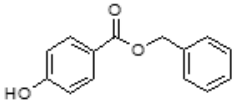
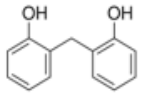
Testing Program

Red = potential use in thermal paper

Structure	Chemical	Structure	Chemical	Structure	Chemical
	Bisphenol P		***BPS-MPE***		***Pergafast 201***
	Bisphenol Z		***TGSA***		***BTUM***
	Bisphenol AF		***D-8***		***UU***
	Bisphenol B		***D-90***		TBBPA
	***Bisphenol AP***		***DD-70***		TCBPA
	***Bisphenol F (4,4)***		***Bisphenol C***		BPA bis(diphenyl phosphate)
	***Bisphenol S***		***BisOPP-A***		TMBPA
	***2,4-BPS***		***MBHA***		Bisphenol E
	***BPS-MAE***		***PHBB***		Bisphenol F (2,2)

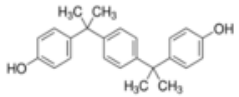
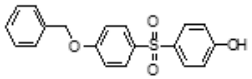
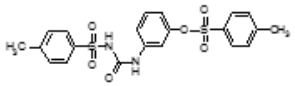
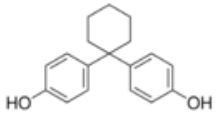
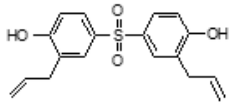
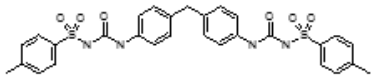
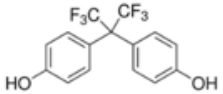
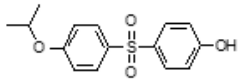
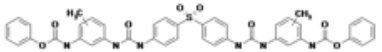
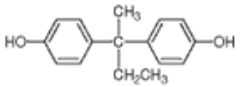
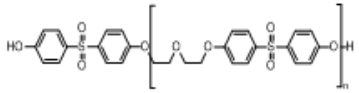
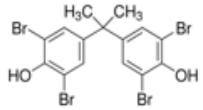
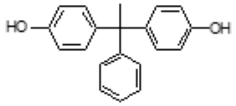
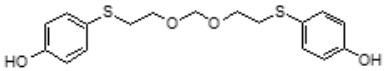
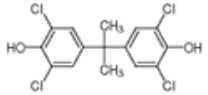
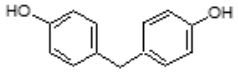
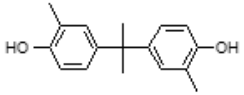
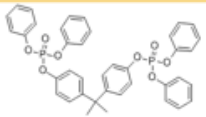
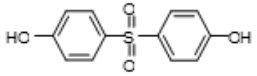
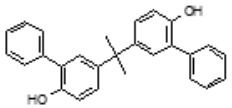
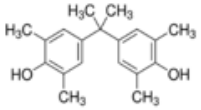
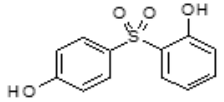
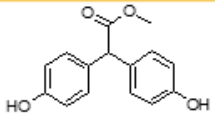
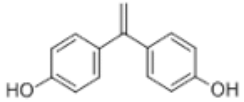
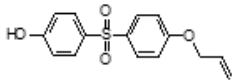
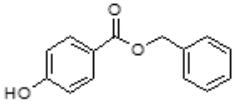
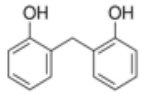
Green = flame retardants
Orange = plastic/resins/dental polymers

Testing Program

Structure	Chemical	Structure	Chemical	Structure	Chemical
	Bisphenol P		BPS-MPE		Pergafast 201
	***Bisphenol Z***		TGSA		BTUM
	***Bisphenol AF***		D-8		UU
	Bisphenol B		D-90		***TBBPA***
	Bisphenol AP		DD-70		***TCBPA***
	Bisphenol F (4,4)		***Bisphenol C***		***BPA bis(diphenyl phosphate)***
	***Bisphenol S***		BisOPP-A		***TMBPA***
	2,4-BPS		MBHA		***Bisphenol E***
	BPS-MAE		PHBB		Bisphenol F (2,2)

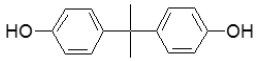
Testing Program

Blue = detected in environment or human samples

Structure	Chemical	Structure	Chemical	Structure	Chemical
	***Bisphenol P***		BPS-MPE		Pergafast 201
	***Bisphenol Z***		TGSA		BTUM
	***Bisphenol AF***		***D-8***		UU
	***Bisphenol B***		D-90		***TBBPA***
	***Bisphenol AP***		DD-70		***TCBPA***
	***Bisphenol F (4,4)***		Bisphenol C		BPA bis(diphenyl phosphate)
	***Bisphenol S***		BisOPP-A		***TMBPA***
	2,4-BPS		MBHA		***Bisphenol E***
	BPS-MAE		***PHBB***		Bisphenol F (2,2)

Testing Program

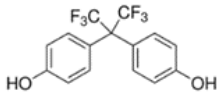
- **Bisphenol A** – Traditional carcinogenic evaluation (1982)



NTP-CERHR report (2008) on developmental and reproductive toxicity

Emerging issue …are there concerns with others in this class?

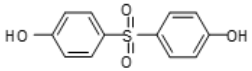
- **Bisphenol AF** – Survey of literature showing strong activity at ER and AR



ADME/TK likely different

When compared to other analogues, could this be the worst?

- **Bisphenol S** – Measurements reported in literature



Limited *in vitro* activity

Concern about use of analogues

Proposed Plan: use these 3 as the initial chemicals from this class for evaluation

Three primary workstreams for an integrated assessment of bisphenols:

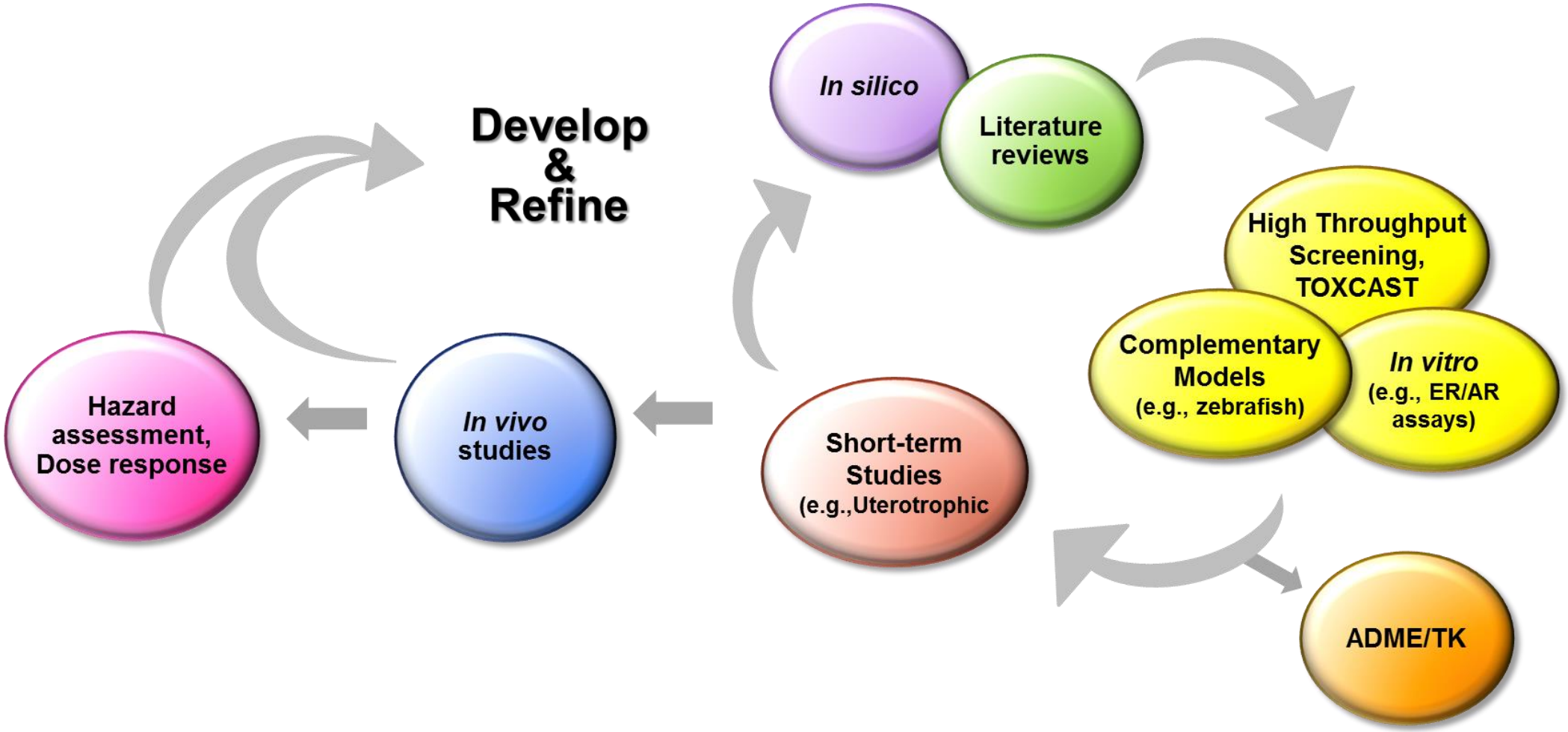
1. **Literature & *in vitro* evaluations – all analogues**
   a) Literature
   b) *In silico*, highthroughput screening (HTS), TOXCAST, *in vitro*, alternative models, etc.

2. ***In vivo* studies – initial chemicals first**
   a) BPA – CLARITY project with NCTR, ADME/TK
   b) BPAF – Dose range-finding (DRF) and definitive modified one-generation (MOG) study, ADME/TK
   c) BPS – DRF and definitive repro study, metabolomics (rat & human), ADME/TK

3. **Integrative Assessment – using all data**
   a) Develop a database for read-across/model (e.g., database showing which chemicals are structurally and biologically similar)
   b) Iterative process – what we learn will feed back into improving the process and model

Initial Testing Paradigm

Compare & contrast assays and data from BPA, BPAF, & BPS with information from other analogues to build a database of bisphenols



Literature & *In Silico*

Literature reviews

In silico

- Leadscope QSAR profiling

- **Literature Review of Bisphenol Analogues (2017):**
  NTP Research Report on Biological Activity of Bisphenols…
  https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr04_508.pdf

NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Research Report on
Biological Activity
of Bisphenol A (BPA)
Structural Analogues and
Functional Alternatives

NTP RR 4

OCTOBER 2017

Literature & *In Silico*

Literature reviews

In silico

Journal of Occupational and Environmental Hygiene

ISSN: 1545-9624 (Print) 1545-9632 (Online) Journal homepage: http://www.tandfonline.com/loi/uoeh20

Air, hand wipe, and surface wipe sampling for Bisphenol A (BPA) among workers in industries that manufacture and use BPA in the United States

Cynthia J. Hines, Matthew V. Jackson, Annette L. Christianson, John C. Clark, James E. Arnold, Jack R. Pretty & James A. Deddens

Annals of Work Exposures and Health, 2017, Vol. 61, No. 2, 164–182
doi: 10.1093/annweh/wxw021
Advance Access publication 1 January 2017
Original Article

Original Article

Urinary Bisphenol A (BPA) Concentrations among Workers in Industries that Manufacture and Use BPA in the USA

Cynthia J. Hines[1]*, Matthew V. Jackson[2], James A. Deddens[1], John C. Clark[1], Xiaoyun Ye[3], Annette L. Christianson[1], Juliana W. Meadows[1] and Antonia M. Calafat[3]

- **NIOSH Activities:**

  Air, hand wipe, and surface wipe sampling for BPA among workers… https://www.tandfonline.com/doi/full/10.1080/15459624.2017.1339164

  Urinary BPA concentrations among industry workers… https://academic.oup.com/annweh/article/61/2/164/2769471

Annals of Work Exposures and Health, 2017, 1–12
doi: 10.1093/annweh/wxy042
Original Article

Original Article

An Evaluation of the Relationship among Urine, Air, and Hand Measures of Exposure to Bisphenol A (BPA) in US Manufacturing Workers

Cynthia J. Hines[1]*, Annette L. Christianson[1], Matthew V. Jackson[2], Xiaoyun Ye[3], Jack R. Pretty[1], James E. Arnold[1] and Antonia M. Calafat[3]

In Vitro Assessments

In vitro
(e.g., ER/AR assays)

High Throughput Screening, TOXCAST

Alternative Models
(e.g., zebrafish)

- **Laboratory experiments on analogues**
  In vitro (genetic toxicology, ER/AR assays, Hepatic clearance, etc.)
- **HTS Similarity profiling of analogues**
  Tox21 & ToxCast structural and biological similarity profiling
- **Metabolomics**
- **Alternative assay evaluations**
  C. elegans and zebrafish

In Vivo Assessments

Short-term Studies (e.g.,Uterotrophic)

In vivo studies

ADME/TK

Hazard assessment, Dose response

- **Short-term evaluations**

  Uterotrophic & Hershberger assays

  Dose Range-Finding studies

  2-week evaluations

  Assessment of endocrine responsive tissues

  ADME/TK

- **Long-term evaluations**

  Subchronic studies

  Modified One-Generation assays

  Immunotoxicity assessments

  Neurobehavioral evaluations

  Human LifeCodes birth cohort biomonitoring (NIEHS, DIR)

In Vivo Assessments

Toxicology in Vitro 44 (2017) 287–302

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Binding of bisphenol A, bisphenol AF, and bisphenol S on the androgen receptor: Coregulator recruitment and stimulation of potential interaction sites

Lalith Perera[a,1], Yin Li[b,1], Laurel A. Coons[b], Rene Houtman[c], Rinie van B
Bonnie Goodwin[d], Scott S. Auerbach[e], Christina T. Teng[e,*]

Toxicology and Applied Pharmacology 351 (2018) 32–45

Contents lists available at ScienceD

Toxicology and Applied Pha

journal homepage: www.elsevier.com/

Disposition and metabolism of the bisphenol analogu
Harlan Sprague Dawley rats and B6C3F1/N mice and
from rats, mice, and humans

TOXICOLOGICAL SCIENCES 139(1), 4–20 2014
doi: 10.1093/toxsci/kfu021
Advance Access publication February 4, 2014

Comparison of Life-Stage-Dependent Internal Dosimetry for Bisphenol A, Ethinyl Estradiol, a Reference Estrogen, and Endogenous Estradiol to Test an Estrogenic Mode of Action in Sprague Dawley Rats

Mona I. Churchwell, Luísa Camacho, Michelle M. Vanlandingham, Nathan C. Twaddle, Estatira Sepehr, K. Barry Delclos, Jeffrey W. Fisher, and Daniel R. Doerge[1]

TOXICOLOGICAL SCIENCES 139(1), 174–197 2014
doi: 10.1093/toxsci/kfu022
Advance Access publication February 4, 2014

Toxicity Evaluation of Bisphenol A Administered by Gavage to Sprague Dawley Rats From Gestation Day 6 Through Postnatal Day 90

K. Barry Delclos,*,1 Luísa Camacho,* Sherry M. Lewis,† Michelle M. Vanlandingham,* John R. Latendresse,‡ Greg R. Olson,‡ Kelly J. Davis,‡ Ralph E. Patton,‡ Gonçalo Gamboa da Costa,* Kellie A. Woodling,* Matthew S. Bryant,* Mani Chidambaram,* Raul Trbojevich,* Beth E. Juliar,§ Robert P. Felton,§ and Brett T. Thorn§

*Division of Biochemical Toxicology, 3900 NCTR Road, Jefferson, Arkansas 72079; †Office of Scientific Coordination, 3900 NCTR Road, Jefferson, Arkansas 72079; ‡Toxicologic Pathology Associates, Jefferson, Arkansas 72079; and §Division of Bioinformatics and Biostatistics, National Center for Toxicological Research, 3900 NCTR Road, Jefferson, Arkansas 72079

Contents lists av

Toxicology and A

journal homepage: ww

Pharmacokinetics of bisphenol A in neo

Daniel R. Doerge [a,*], Nathan C. Twaddle [a], Michelle

Reproductive Toxicology 40 (2013) 35–40

Reproductive Toxicology 54 (2015) 120–128

Contents lists available at ScienceDirect

Reproductive Toxicology

omepage: www.elsevier.com/locate/reprotox

ental phenols and parabens in milk, urine
n Carolina women

Erin P. Hines[a,*], Pauline Mendola[b], Ondine S. von Ehrenstein[c], Xiaoyun Ye[d]

Contents lists available at SciVerse ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Review

A new approach to synergize academic and guideline-compliar research: The CLARITY-BPA research program

Thaddeus T. Schug[a,*], Jerrold J. Heindel[a], Luísa Camacho[b], K. Barry Delclos[b], Paul Howard[c], Anne F. Johnson[d], Jason Aungst[e], Dennis Keefe[e], Retha Newbold[f] Nigel J. Walker[f], R. Thomas Zoeller[g], John R. Bucher[f]

Reproductive Toxicology 58 (2015) 33–44

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Review

NIEHS/FDA CLARITY-BPA research program update

Jerrold J. Heindel[a,*], Retha R. Newbold[b], John R. Bucher[b], Luísa Camacho[c], K. Barry Delclos[c], Sherry M. Lewis[f], Michelle Vanlandingham[c], Mona I. Churchwell[c], Nathan C. Twaddle[c], Michelle McLellen[c], Mani Chidambaram[c], Matthew Bryant[c], Kellie Woodling[c], Gonçalo Gamboa da Costa[c], Sherry A. Ferguson[d], Jodi Flaws[e], Paul C. Howard[f], Nigel J. Walker[b], R. Thomas Zoeller[g], Jennifer Fostel[b], Carolyn Favaro[h], Thaddeus T. Schug[a]

Xenobiotica
the fate of foreign compounds in biological systems

ISSN: 0049-8254 (Print) 1366-5928 (Online) Journal homepage: http://www.tandfonline.com/loi/ixen20

Disposition of bisphenol AF, a bisphenol A analogue, in hepatocytes in vitro and in male and female Harlan Sprague-Dawley rats and B6C3F1/N mice following oral and intravenous administration

Suramya Waidyanatha, James M. Mathews, Purvi R. Patel, Sherry R. Black, Rodney W. Snyder & Timothy R. Fennell

Evaluation Efforts

Compounds

Bisphenol A (BPA)

- **Literature & QSAR**
- **HTS & *in vitro* assessments**
- **Alternative models**
- **ADME/TK**
- **Subchronic assessments**
- *Chronic CLARITY-BPA program*

Bisphenol AF (BPAF)

- **Literature & QSAR**
- **HTS & *in vitro* assessments**
- **Alternative models**
- **ADME/TK**
- **Modified OneGen assessment**
- *Immunotoxicity evaluation*
- *Neurobehavioral tests*

Bisphenol S (BPS)

- **Literature & QSAR**
- **HTS & *in vitro* assessments**
- **Alternative models**
- *ADME/TK*
- *Reproductive assessment*
- *Additional tests as needed*

Italicized – not completed

Sufficient Similarity Framework

Bisphenol analogues are sufficiently similar to the reference bisphenols (BPA, BPAF, and/or BPS)

Reference Bisphenols & analogues:

Compare within & across datastreams:
- Physiochemical/Structural
- Biological activity

Integrate data and evaluate similarity of analogue(s) to reference chemicals

Analogue(s) are different

De novo data needed: Data from reference chemicals does not apply, further testing of analogue(s) needed

Analogue(s) are similar

Comprehensive characterization not needed: Use data from reference bisphenols

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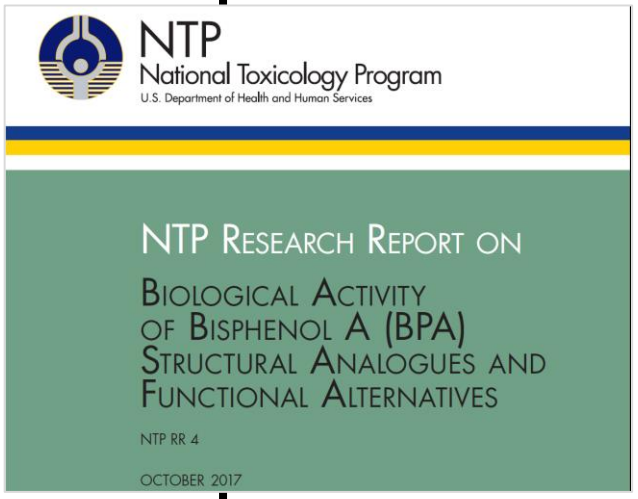
Comparisons Started

Bisphenol analogues are sufficiently similar to reference bisphenols (BPA, BPAF, and/or BPS)

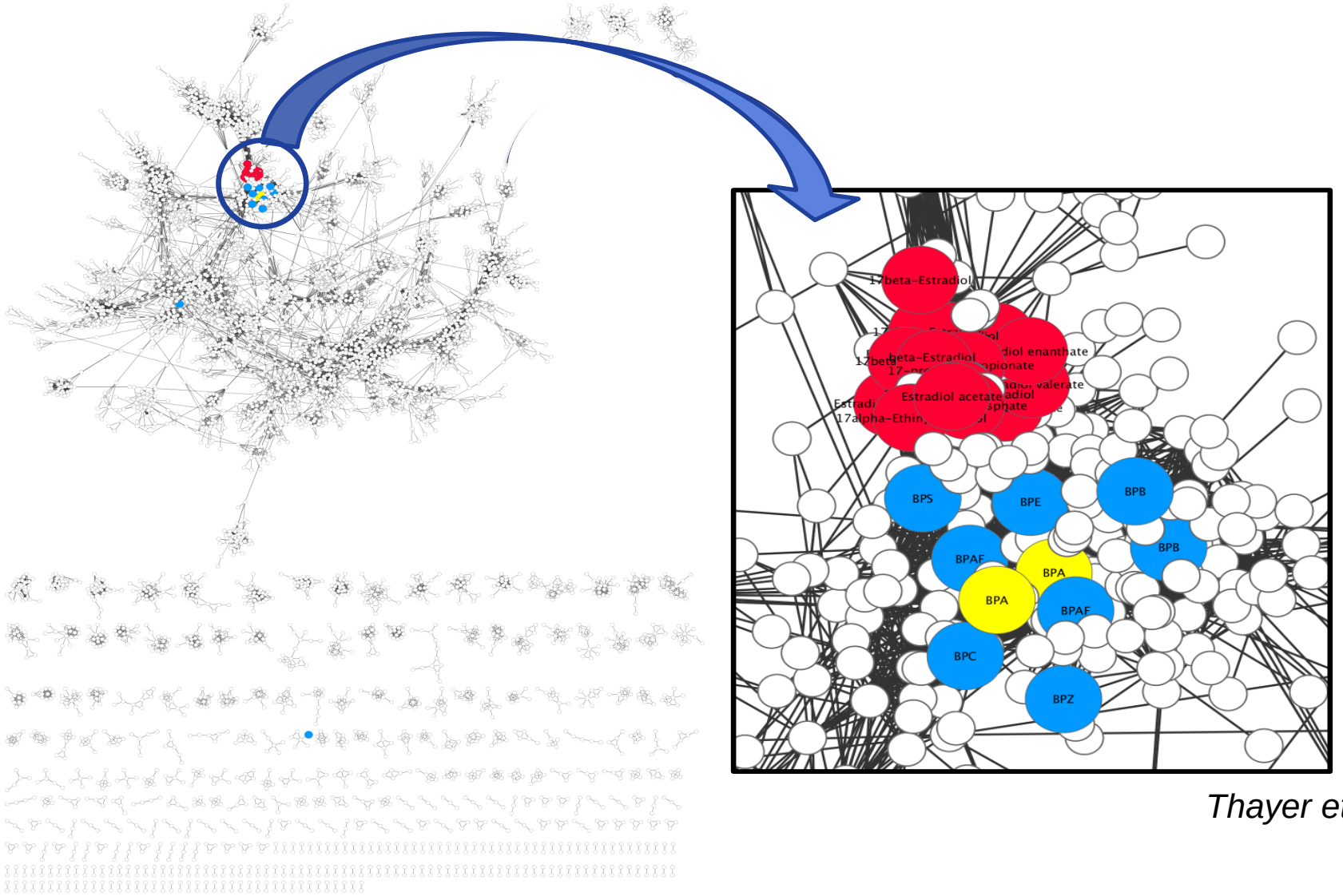
<u>Reference Bisphenols & analogues:</u>

Compare within & across datastreams:
- Physiochemical/Structural
- Biological activity

Integrate data and evaluate similarity of analogue(s) to reference chemicals
(e.g., structural and biological information plotted relative to BPA)



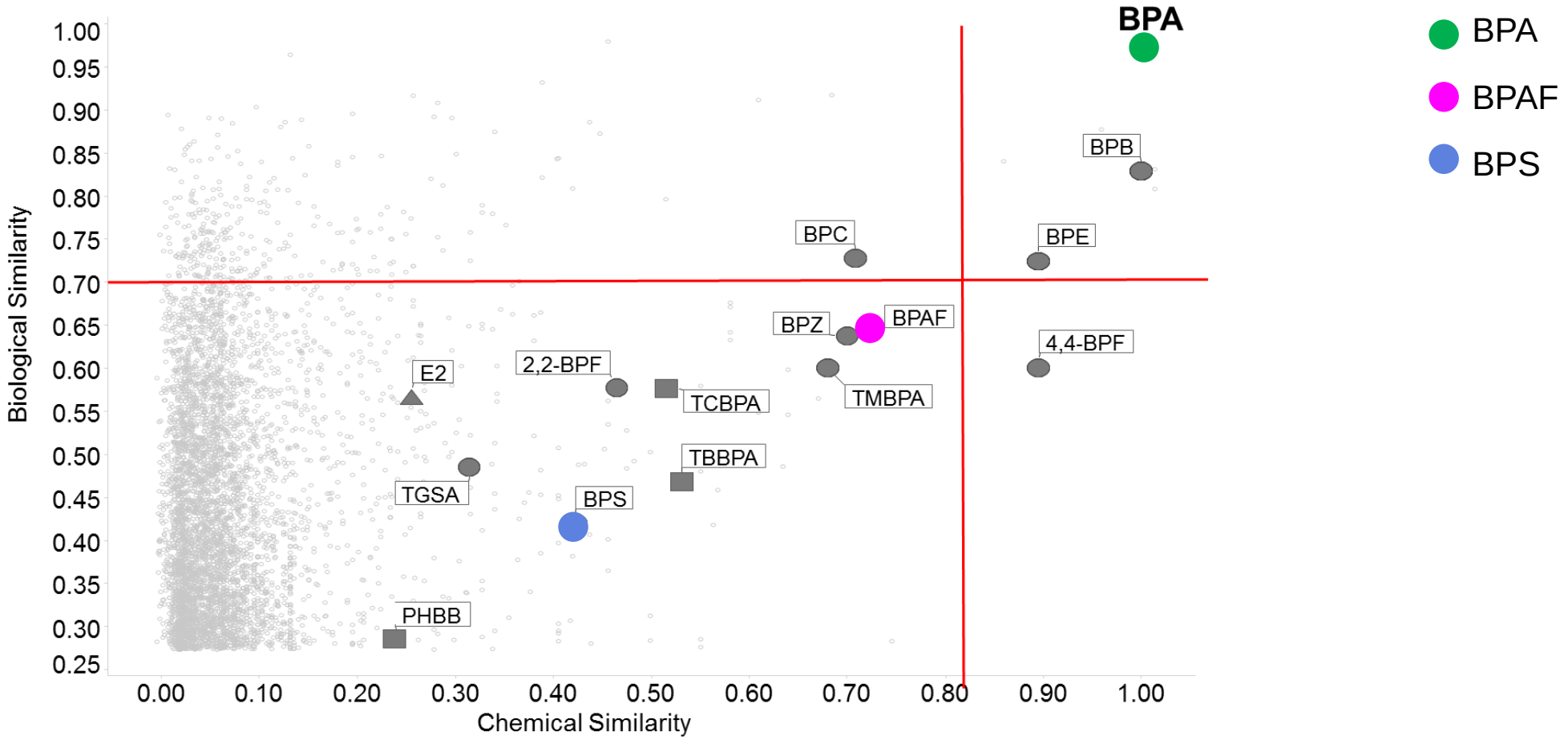
HTS Network Analysis



Thayer et al. 2016

HTS Similarity Analysis

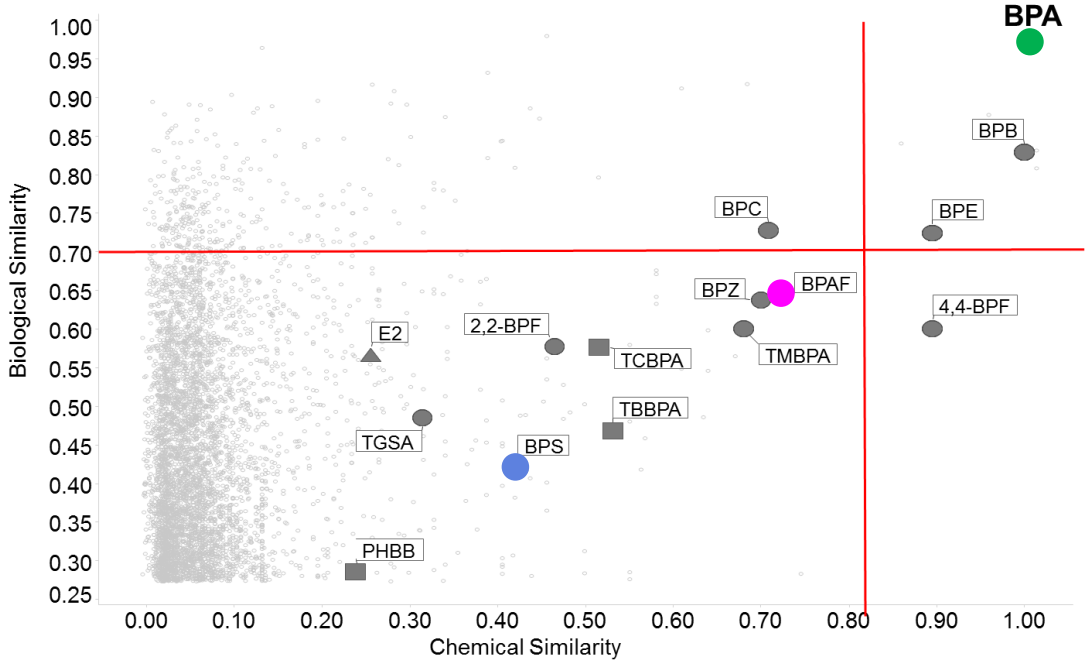
Respective relationship of analogues relative to BPA - chemical and biological similarity



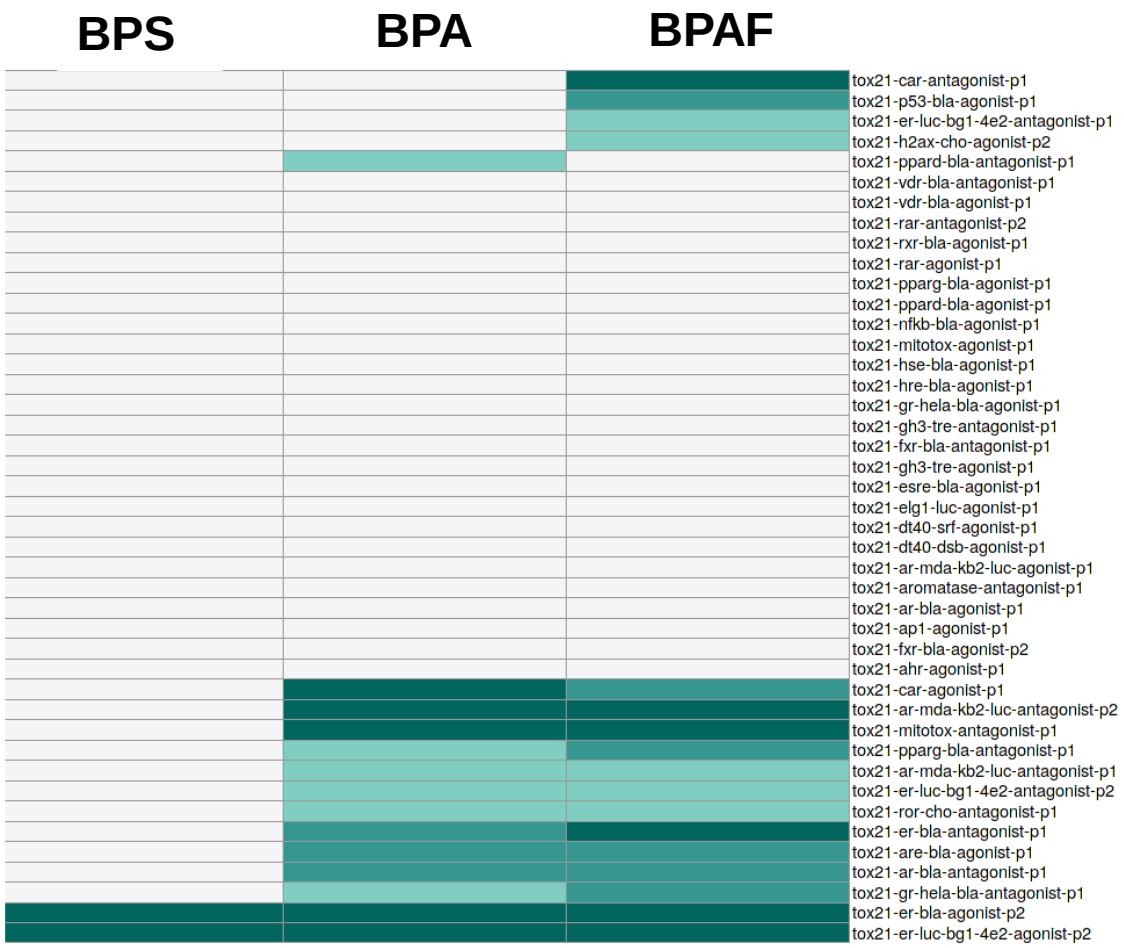
HTS Similarity Analysis

Respective relationship of analogues relative to BPA - chemical and biological similarity

- Where should the lines cross to best determine which analogues are most similar to BPA?
- What does the plot look like for each of the analogues?
- How do the analogues compare to other Tox21 chemicals?
- What does this look like when other data (e.g., in vivo) is added?



Tox21 Data – Comparison of Reference Chemicals



Bisphenol comparisons of Tox21 activities (including nuclear receptor and stress response pathways):

- Strong overlap for BPA and BPAF
- BPS activity only at ER

In Vivo Comparisons, Rats

	BPA (90-day) 0.0025, 0.008, 0.025, 0.08, 0.26, 0.84, 2.7, 100, or 300 mg/kg	**BPS (DRF)** ~300, 560, 850, or 1100 mg/kg, based on Food Consumption	**BPAF (MOG)** ~25, 80, or 280 mg/kg, based on Food Consumption
Dosing Equivalents	1.31 mmol/kg	1.20 mmol/kg*	0.83 mmol/kg*

*Mean chemical consumption during gestation was used to convert ppm to mg/kg

- Dosing equivalence is based on ~300 mg/kg
- BPAF is lower than BPS and BPA

In Vivo Comparisons, Rats

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*Mean chemical consumption during gestation was used to convert ppm to mg/kg

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Endocrine Endpoints:

Testicular Descent	**Delayed**	*Undescended testes*	**Delayed**
Balanopreputial Separation	No effect at doses tested	na	**Delayed**
Vaginal Opening	No effect at doses tested	Na	**Accelerated**

ADME/TK Comparison, Rats

BPA, BPS, and BPAF:

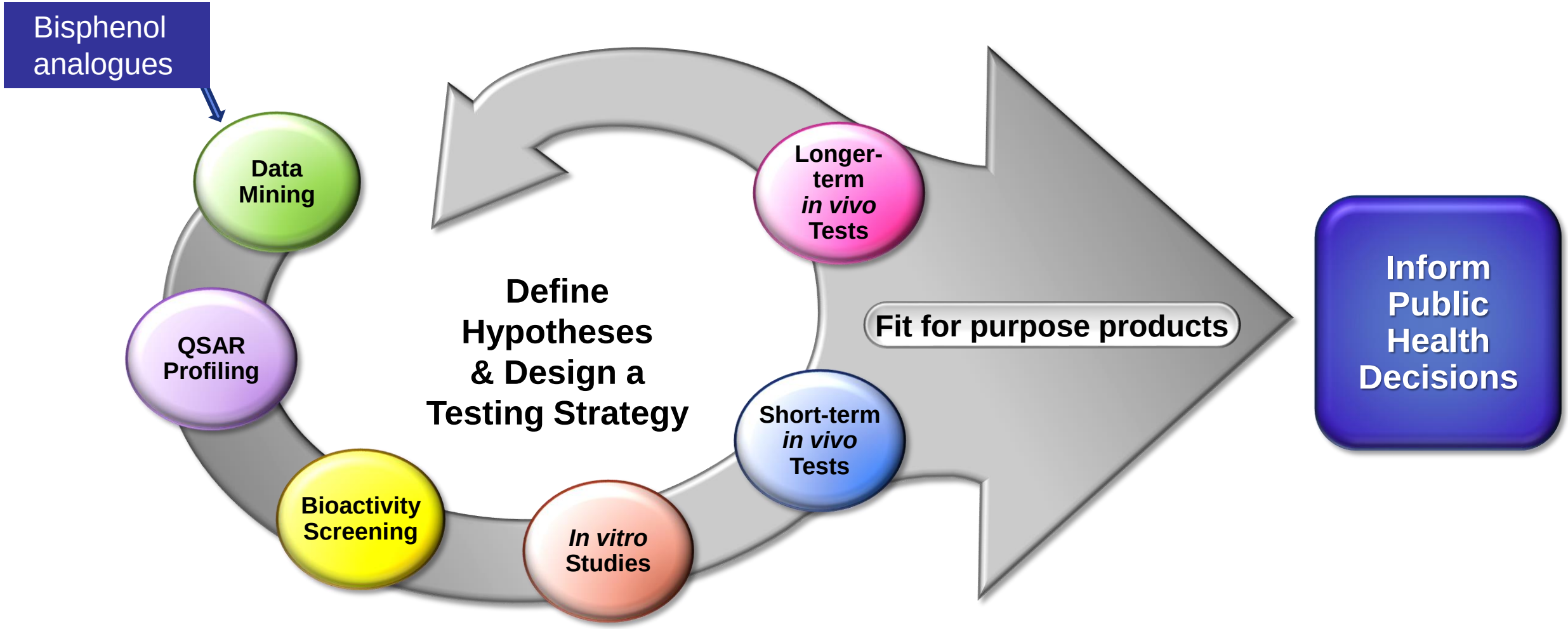
- Well absorbed following a single gavage administration (≥ 90%)
- Low oral bioavailability:
  - BPA ≤ 5%
  - BPS ≤ 16%
  - BPAF ≤ 3%
- Dose normalized C_{max} and AUC follow the rank order BPS >> BPAF > BPA
- Comparable plasma elimination half-lives:
  - BPA 3-5hrs
  - BPS 4-12hrs
  - BPAF 2-3hrs
- Metabolism is similar with glucuronide and sulfate conjugates being the major metabolites

Work is ongoing….

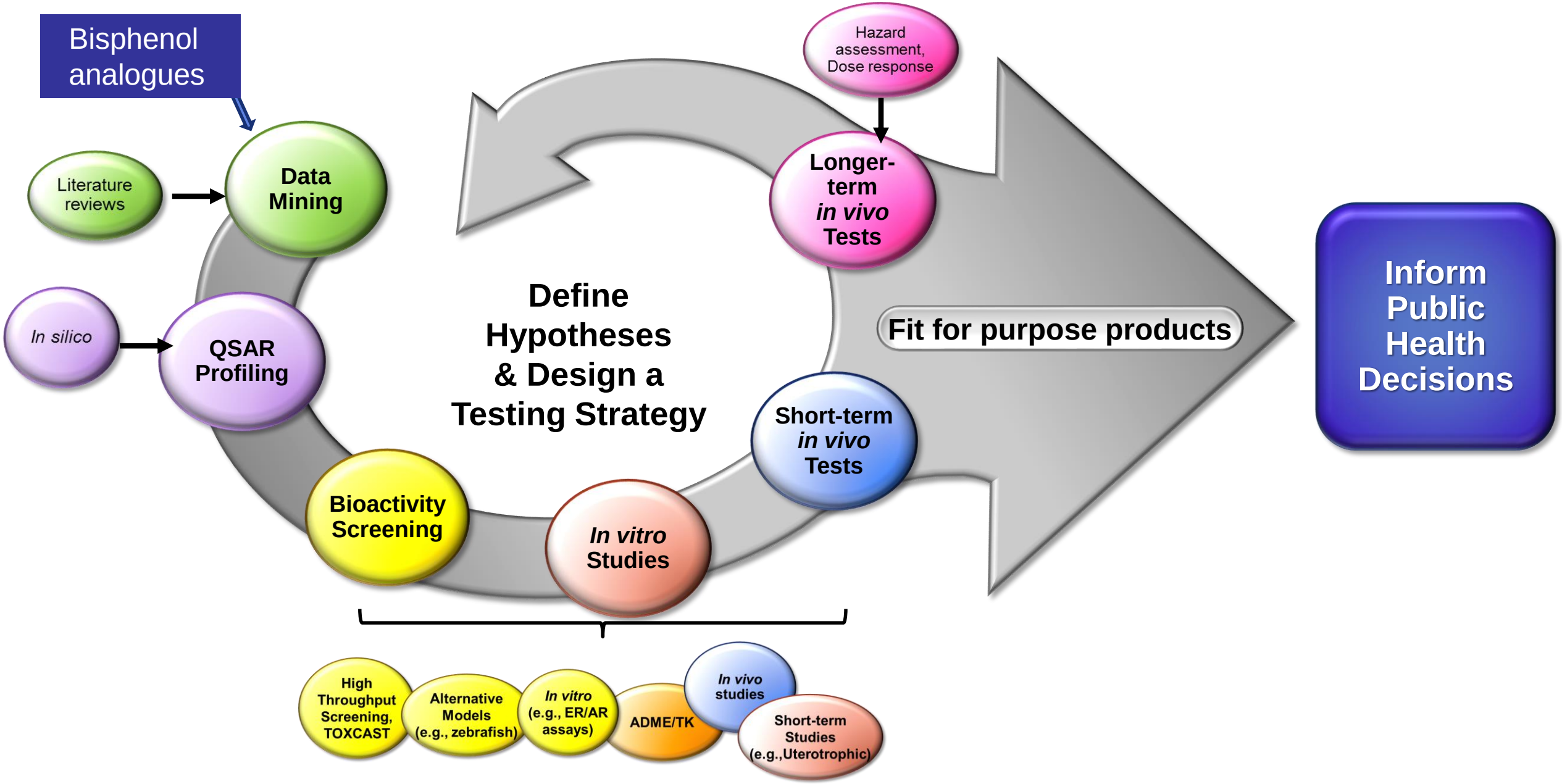
- Review of the literature, Tox21/TOXCAST structural and biological comparisons, and *in vitro* evaluations
  - NTP research report
  - Publications

- In vivo assessments of the initial compounds (reference chemicals):
  - BPA studies almost complete
  - BPAF and BPS studies ongoing
  - ADME/TK is ongoing

Assessment Plan

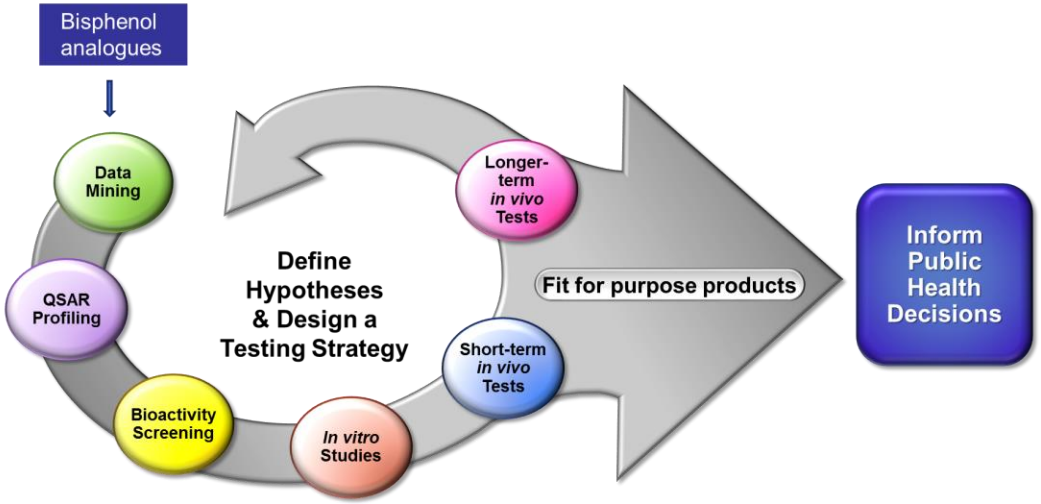


Combining Old and New



Plans

- Finish collecting *in vivo* data for the initial reference chemicals
- In depth comparison of all data streams for the analogues
- Work to develop an integrative assessment of the bisphenol class
- Iterative process – what we learn will feed back into improving class evaluations



It takes a village

Questions?