Parma Summer School 2019

"Risk-benefit in food safety and nutrition"

Case study: risk-benefit of alternatives to bisphenol A

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Unit of Nutritionn and Health

OUTLINE

- ✓ **Introduction**
  - **Bisphenol A (BPA): benefits and risks in brief**
- ✓ **Searching for BPA replacement**
  - **A computational approach**

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Bisphenol A (BPA): benefits in brief (1a)

Bisphenol A (BPA) or 4,4'-isopropylidenediphenol

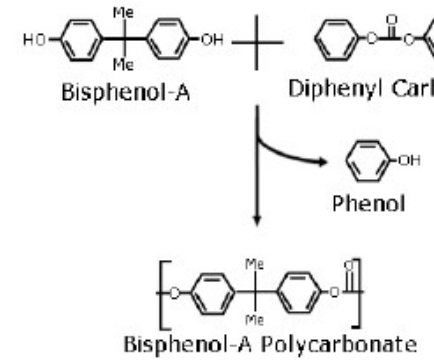
CAS no. 80-05-7

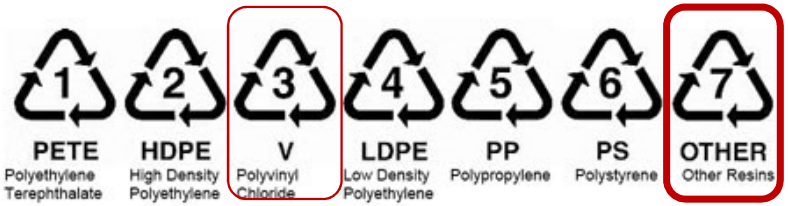
Man-made chemical used mainly to manufacture:

- ✓ **polycarbonate-based plastics** (BPA is the building block), to make food [including returnable beverage bottles, infant feeding (baby) bottles, tableware and mugs] and storage containers,

BUT ALSO

- ✓ DVDs, CDs, cell phones, eye glass lenses, automobile parts





FDA data 2011:
about 74% total usage

Bisphenol A (BPA): benefits in brief (1b)

Bisphenol A (BPA) or 4,4'-isopropylidenediphenol

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  and so on…

- ✓ **epoxy resins**, to make protective coatings and linings for food and beverage cans and vats

FDA data 2011:
about 20% total usage

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- ✓ **epoxy resins**, to make protective coatings and linings for food and beverage cans and vats
- ✓ **thermal paper**, used in thermal printers present in very common devices, such as adding machines, cash registers and credit card terminals

FDA data 2011:
about 6% total usage

Bisphenol A (BPA): benefits in brief (2)

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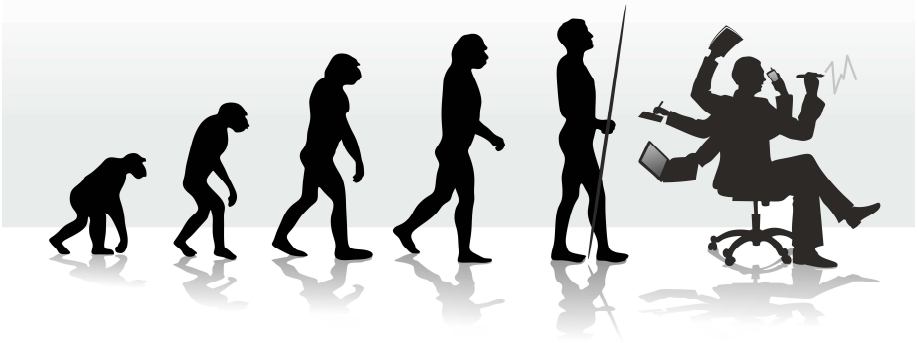
dietary exposure

- ✓ **thermal paper**, as a dye developer used in thermal printers present in very common devices, such as adding machines, cash registers and credit card terminals

dermal exposure

Bisphenol A (BPA): benefits-to-risks

Since 1891 / 1934



2023 estimates

- ✓ The "The Global Bisphenol A Market" report:
  The global bisphenol A market is projected to reach **approximately 7,348 Kilotons by the end of 2023**, increasing at a CAGR of around 3% per year in the period 2017-2023
- ✓ In particular, the largest share of bisphenol A consumption is for the **production of polycarbonates**, which **accounted for around 64.05% of the total in volume terms**.

https://www.researchandmarkets.com/research/hl86rz/global_bisphenol?w=5
https://www.prnewswire.com/news-releases/global-bisphenol-a-market-report-2018-analysis-2013-2017--forecasts-2018-2023-300757673.html

Bisphenol A (BPA): risks in brief (1)

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- ✓ **epoxy resins**, to make protective coatings and linings for food and beverage cans and vats
- ✓ **thermal paper**, as a dye developer used in thermal printers present in very common devices, such as adding machines, cash registers and credit card terminals

- ➢ BPA use in EU permitted in **FCMs with a Specific Migration Limit** (0.05 mg/kg)
- ➢ Since Jan 2011, it exists **an EU ban on BPA to manufacture of polycarbonate infant feeding bottles,** extended in Jan 2018 to **plastic bottles and packaging containing food for babies and children under 3 years old**
- ➢ Moreover, it exists **in toys a lower Specific Migration Limit** (0.04 mg/kg)

Regulation EU 10/2011 on plastic materials and food contact materials
Directive 2011/8/EU restricting the use of bisphenol A in plastic infant feeding bottles

Bisphenol A (BPA): risks in brief (2)

European Food Safety Authority
EFSA Journal 2015;13(1):3978

SCIENTIFIC OPINION

Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2,3]

- ✓ The **estimated BPA dietary intake in infants and toddlers** (up to 0.875 µg/kg bw per day), **in women of childbearing age** comparable to men of the same age (up to 0.388 µg/kg bw per day).
- ✓ The **highest aggregated exposure** of 1.449 µg/kg bw per day was **estimated for adolescents**.
- ✓ **Biomonitoring data were in line with estimated internal exposure to total BPA from all sources**.
- ✓ It established a **temporary Tolerable Daily Intake (t-TDI) of 4 µg/kg bw per day** (considering adverse effect on mammary gland, reproductive, neurobehavioural, immune and metabolic system).
- ✓ By comparing this t-TDI with the exposure estimates, the CEF Panel concluded that there is **no health concern for any age group from dietary exposure** and **low health concern from aggregated exposure**.
- ✓ The CEF Panel noted **considerable uncertainty in the exposure estimates for non-dietary sources**, whilst the uncertainty around dietary estimates was relatively low.

Bisphenol A (BPA): risks in brief (3)

Classified as toxic for human reproduction

Bisphenol A is classified in the EU as a substance that has toxic effects on our ability to reproduce. All manufacturers, importers, or suppliers of BPA must classify and label mixtures containing BPA as toxic for reproduction category 1B by 1 March 2018. This means that companies will be better informed about the potential hazardous effects and how workers can be protected.

Identified as an endocrine disruptor for human health and environment

Bisphenol A was listed in the Candidate List of substances of very high concern (SVHCs) due to its toxic for reproduction properties in January 2017. In June 2017, ECHA's Member State Committee supported the French proposal to additionally identify Bisphenol A as a substance of very high concern also because of its endocrine disrupting properties which cause probable serious effects to human health which give rise to an equivalent level of concern to carcinogenic, mutagenic, toxic to reproduction (CMRs category 1A or 1B) substances. In January 2018, the BPA entry was updated to reflect an additional reason for inclusion in the Candidate List due to its endocrine disrupting properties causing adverse effects to the environment, as proposed by Germany.

https://echa.europa.eu/hot-topics/bisphenol-a

Bisphenol A (BPA): risks in brief (5)

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An EU ban on BPA to manufacture thermal paper will apply from 2020

USING WHAT ?

https://echa.europa.eu/hot-topics/bisphenol-a

Bisphenol A (BPA): looking for BPA replacement (1)

From 2020, EU ban on BPA to manufacture thermal paper

- ✓ In December 2016, the European Commission decided **to restrict BPA in thermal paper in the EU**. This ban will take effect in 2020, giving manufacturers, importers and users of thermal paper **the time to phase it out and find an alternative**.
- ✓ As a result of the restriction, paper manufacturers will need **to replace BPA with other dye developers**.
- ✓ One potential replacement that is being considered by industry is the chemical **Bisphenol S (BPS)**. **However, concerns have been expressed that it may cause similar health problems to BPA. To make sure that one hazardous chemical is not being replaced by another, BPS is currently under substance evaluation and the European Commission has also asked ECHA to further investigate the use of BPS as a substitute for BPA in thermal paper.**

https://echa.europa.eu/hot-topics/bisphenol-a

REACHing REPLACEMENT: A RECOMMENDATION

➢ EU regulatory framework

The EU has introduced specific legislative obligations aimed at ***phasing out endocrine disruptors*** in **water** (*Water Framework Directive 2000/60/EC),* **industrial chemicals** (*REACH Regulation 2006/1907/EC, Food Contact Materials Regulation 2011/10/EU* and following amendments, …), **plant protection products** (*Plant Protection Products Regulation 2009/1107/EC*) and **biocides** (*Biocidal Products Regulation 2012/528/EU*).

➢ Importantly, EU regulations strongly recommended **the use of *in vitro* alternative** (to animal experimentation) **methods**, at least as a prioritizing screening approach to identify ***endocrine disrupting properties of Endocrine Active Substances*** (**EAS**).

➢ REACH Regulation

- In REACH, Endocrine Disrupting Chemicals (EDCs) are considered of **similar regulatory concern as *Substances of Very High Concern (SVHC)***.
- REACH also calls for the **progressive substitution *of*** the most dangerous chemicals (referred to as ***SVHC***) ***when suitable alternatives have been identified***.

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Case study: risk-benefit of alternatives to bisphenol A

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Francesca CAVALIERE
Pietro COZZINI

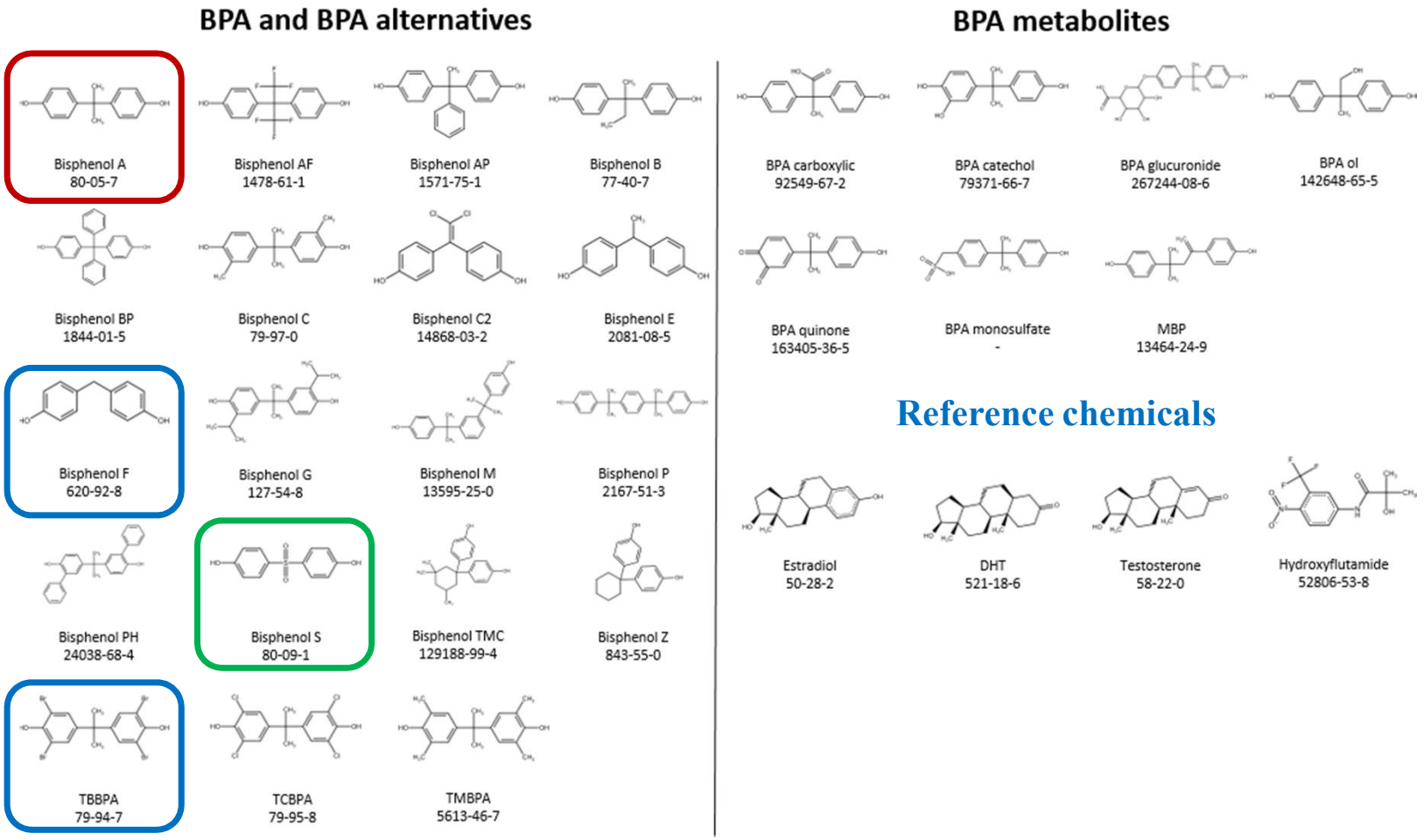
Cavaliere et al., 2019. "Non-statistical computational methods in food safety: bisphenols as case study".
Manuscripit in preparation

Bisphenol A (BPA): looking for BPA replacement (2)

Common name, acronym	IUPAC name	M.W. (g/mol)	CAS no.	EC no.	ECHA registration: total tonnage band (tonnes per annum)[1]
Bisphenol A, BPA	4,4'-isopropylidenediphenol	228.29	80-05-7	201-245-8	registered, 1 000 000 - 10 000 000
Bisphenol AF, BPAF	4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol	336.23	1478-61-1	216-036-7	registered, 100 - 1 000
Bisphenol AP, BPAP	1,1-bis(4-hydroxyphenyl)-1-phenylethane	290.36	1571-75-1	433-130-5	registered, confidential
Bisphenol B, BPB	4,4'-(1-methylpropylidene)diphenol	242.31	77-40-7	201-025-1	not registered
Bisphenol BP, BPBP	4,4′-(diphenylmethylene)diphenol	352.43	1844-01-5		
Bisphenol C, BPC	4,4'-isopropylidenedi-o-cresol	256.34	79-97-0	201-240-0	registered, 0 - 10
Bisphenol C 2, BPC2	Bis(4-hydroxyphenyl)-2,2-dichloroethylene	281.13	14868-03-2		
Bisphenol E, BPE	4,4′-ethylidenediphenol	214.26	2081-08-5		
Bisphenol F, BPF	4,4′-methylenediphenol	200.23	620-92-8	210-658-2	not registered
Bisphenol G, BPG	4,4′-isopropylidenedi(2-isopropylphenol)	312.45	127-54-8		
Bisphenol M, BPM	4,4'-(1,3-phenylene-bis(1-methylethylidene))diphenol	346.47	13595-25-0	428-970-4	registered, 0 – 10 confidential
Bisphenol P, BPP	4,4′-(1,4-phenylenediisopropylidene)diphenol	346.46	2167-51-3		
Bisphenol PH, BPPH	5,5′-isopropylidenedi-2-biphenylol	380.48	24038-68-4		
Bisphenol S, BPS	4,4'-sulphonyldiphenol	250,27	80-09-1	201-250-5	registered, 10 000 - 100 000 intermediate use only
Bisphenol TMC, BPTMC	4,4′-(3,3,5-trimethylcyclohexane-1,1-diyl)diphenol	310.43	129188-99-4	404-140-7 603-320-4	
Bisphenol Z, BPZ	4,4′-cyclohexylidenediphenol	268.35	843-55-0	212-677-1	not registered
Tetrabromo BPA, TBBPA	2,2',6,6'-tetrabromo-4,4' isopropylidenediphenol	543.87	79-94-7	201-236-9	registered, 1 000 - 10 000
Tetrachloro BPA, TCBPA	2,2-bis-(3,5-dichloro-4-hydroxyphenyl)propane, 2,2',6,6'-tetrachloro-4,4'-isopropylidenediphenol	366.07	79-95-8	201-237-4	not registered
Tetramethyl BPA, TMBPA	4,4'-isopropylidenedi-2,6-xylol, 2,2',6,6'-tetramethyl-4,4'-isopropylidenediphenol	284.39	5613-46-7	227-033-5	registered, 10 - 100

Bisphenol A (BPA): looking for BPA replacement (3)

BPA, 18 BPA-like chemicals, 7 BPA metabolites, 4 reference chemicals (Cavaliere et al, 2019, *manuscript in preparation*)



Bisphenol A (BPA): looking for BPA replacement (4)

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HOW TO TEST THEM in silico, computationally ? QSAR vs molecular docking

Quantitative structure-activity relationship (QSAR)

- a ligand-based approach
- needs a SAR database
- predict the properties of new chemical compounds without the need to synthesize and test them
- broadly utilized for the prediction of physicochemical properties in the chemical, industrial, pharmaceutical, biological, and environmental fileds
- QSAR strategies save resources and accelerate the process of developing new molecules for use as drugs, materials, and additives or for whatever purposes

Molecular docking

- a computational method used to determine the binding strength between the active site residues and specific molecule(s).
- expedient tool used in the drug discovery field to investigate the binding compatibility of molecules (ligands) to target (receptor)

https://www.hindawi.com/journals/jpath/2018/1018694/

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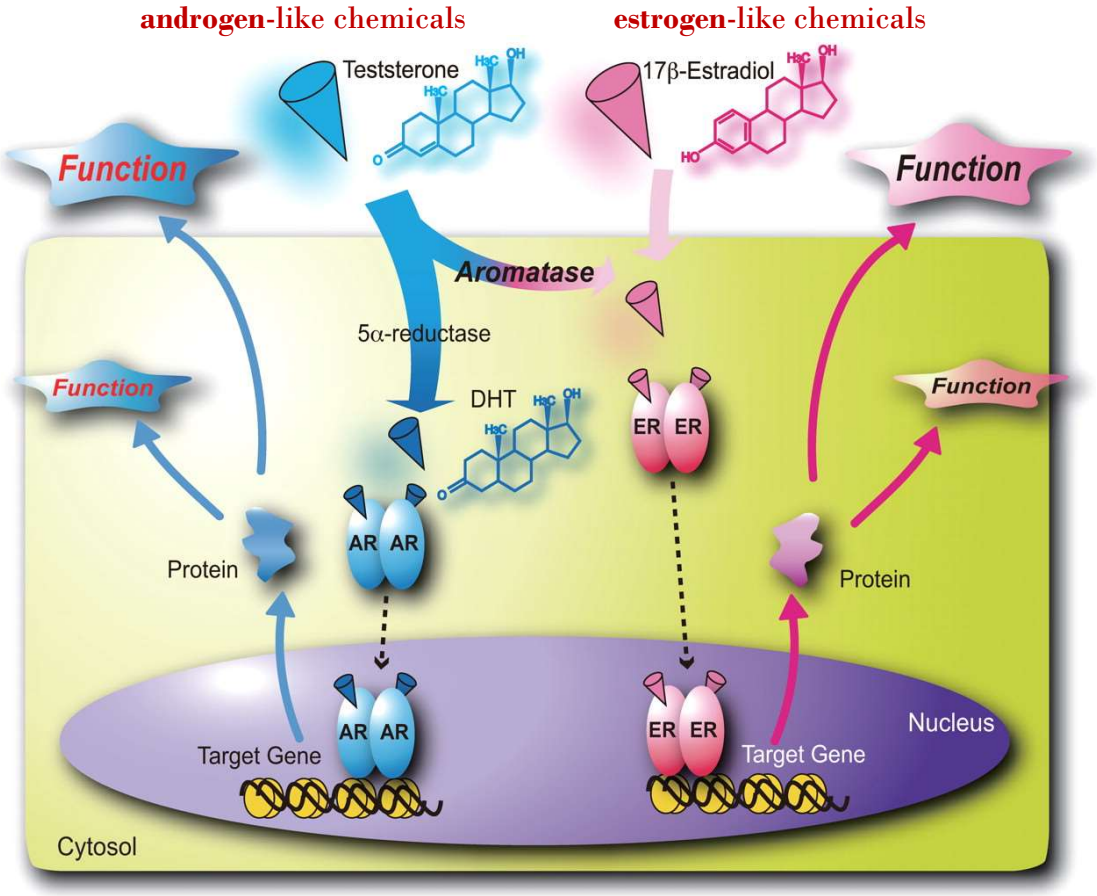

Imai Y et al. 2010

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✓ **molecules (ligands) = BPA and BPA-like chemicals**

✓ **target (receptor) = nuclear receptors (NRs), such as the estrogen (ERs) and the androgen (AR) receptors**

✓ **the active site residues = those ones in the LBD (Ligand Bindind Domains) of ERs and ARs**

Bisphenol A (BPA): looking for BPA replacement (6)

BPA, 18 BPA-like chemicals, 7 BPA metabolites, 4 reference chemicals (Cavaliere et al, 2019, *manuscript in preparation*)

DOCKED in the Ligand Binding Domain of 6 Nuclear Receptors

- **Processing of the crystallographic structures** of human ERα, ERβ, ERRγ, AR, AR^{T877A} and AR^{W741L} taken from the RCSB Protein Data Bank (PDB) https://www.rcsb.org/pdb/home/sitemap.do and *www.tripos.com (Sybyl software v8.1)*
- **Docking simulation by two different docking programs** (GOLD and AutoDock) **and four different scoring functions** (GoldScore, ChemScore, and HintScore plus AutoDock score)
- **BPA Relative Predicted Activity (RPA) calculation** for each chemical as follow:

$$BPA\ Relative\ Predicted\ Activity\ (RPA) = \frac{food\ contaminant\ score}{reference\ compound\ score} = \frac{BP\ score}{BPA\ score}$$

- **The chemicals with RPA greater and lower than 1 were considered respectively, as higher (H) and lower (L) EDC-like chemicals compared to BPA or, in other words, BPA-like chemicals.**

Bisphenol A (BPA): looking for BPA replacement (10)

SOME PRELIMINARY CONCLUSIONS

- ✓ **Computational ERα and ERβ binding affinities of BPs by molecular docking predicted as expected that:**
  E2 as higher interactor of BPA placing the two BPA metabolites (BPA sulfate and BPA glucuronide) as well as bisphenol S (BPS) among those ones in the lower ranking.
- ✓ Accordingly to published *in vitro* data (gene reporter assays) , the *in silico* prediction suggests to consider BPS as a safer chemical for human health.

- ✓ **Computational ERRγ binding affinities of BPs by molecular docking predicted as expected that:**
  E2 does not bind ERRγ,
- ✓ BPA instead resulted as a strong interactor, whereas *in silico* preditcion for BPA closely resembles E2, hence also in this case suggesting that BPS might be a safer chemical for human health.

- ✓ **Computational AR binding affinities of BPs by molecular docking predicted as NOT expected that:**
  BPA is a strong AR interactor, even better than androgens, a result in accordance with some published *in vitro* data BUT not all of them. **IT DESERVES FURTHER ATTENTION**
- ✓ **As expected**, in accordance with some previous published *in vitro* data, the mutated ARs are recognized by more BPs.
- ✓ Inn any case, BPS resulted a weaker binder than BPA and androgens, although the *in silico* prediction closely resembles the pharmacological anti-androgen 2OH-FTA, hence, it does not appear so safe for human health.

Bisphenol A (BPA): looking for BPA replacement (10)

SOME PRELIMINARY CONCLUSIONS AND A QUESTION MARK

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BPS ia safer chemical than BPA for estrogen-like activities but not for androgen-like ones ?

Case study: risk-benefit of alternatives to bisphenol A

SCREENING ENDOCRINE DISRUPTORS in vitro: EFFECT-based approaches

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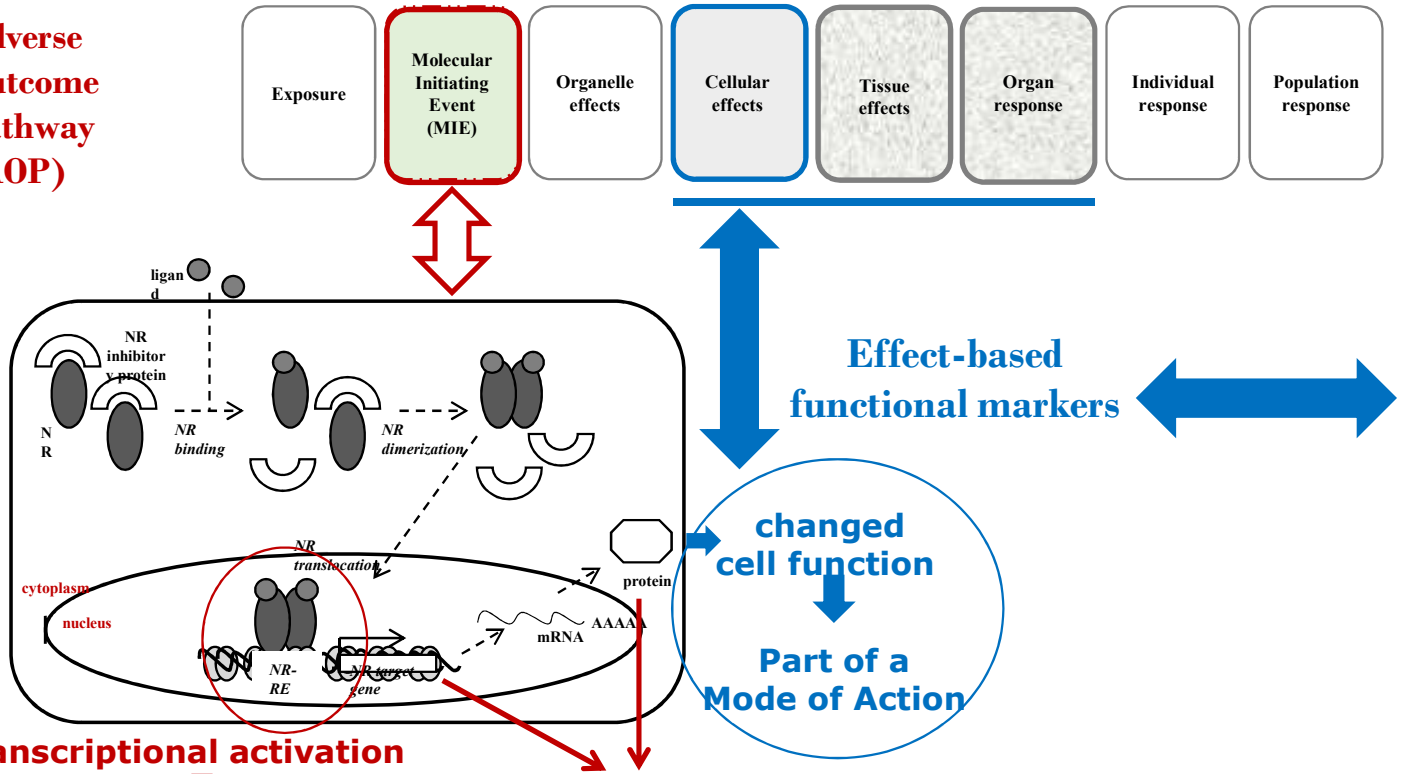
Adverse Outcome Pathway (AOP)



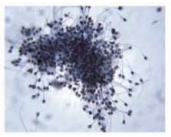
Effect-based functional markers

WHICH BIOMARKERS TO SCREEN FOR:

- **AN ENDOCRINE ACTIVITY ?**
- **AN ADVERSE EFFECT ?**

cell-specific, clinically relevant, hormone-dependent biomarkers of effects

- **PSA secretion – androgen disruption in prostate epithelium**
- **βhCG secretion – estrogen disruption in trophoblast-like cell (placenta)**
- **AFP secretion – metabolic disruption in liver**



ACKNOWLEDGEMENTS

Francesca CAVALIERE
Pietro COZZINI

UNIVERSITÀ DI PARMA

efsa
European Food Safety Authority

Endocrine Disruptors *in silico / in vitro* - Evaluation and Substitution for Industrial Applications
LIFE12 ENV/IT/000633
http://www.iss.it/life/

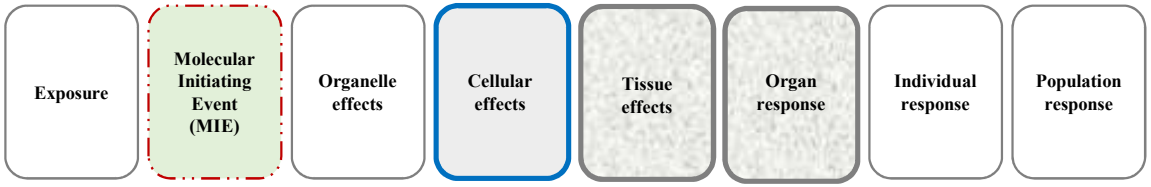
MAN-MADE CHEMICALS

ISTITUTO SUPERIORE DI SANITÀ

Chemical biodistribution in LNCaP cells. Data are expressed as mean % values upon 3 independent experiments. Cell and culture medium were harvested at 72 hrs upon treatment.

SCREENING ENDOCRINE DISRUPTORS: mechanism-based approaches - 3

Adverse Outcome Pathway (AOP)



… *in vitro* screening:
so far, mostly by GENE REPORTER ASSAYS

***In vitro* Nuclear Receptor binding & regulation of gene transcription (*gene reporter assays*)**
IS SUFFICIENT TO DEFINE…

- **AN ENDOCRINE ACTIVITY ?**

NO, if an endocrine activity is a Mode-of-Action

WHO/IPCS 2002 – Weybridge definition
STATE OF THE ART ASSESSMENT OF ENDOCRINE DISRUPTERS (EC)

- **AN ADVERSE EFFECT ?**

NO, because a binding to a Nuclear Receptor (or its transcriptional regulation) does not define any cellular output(s) in terms of ADVERSITY

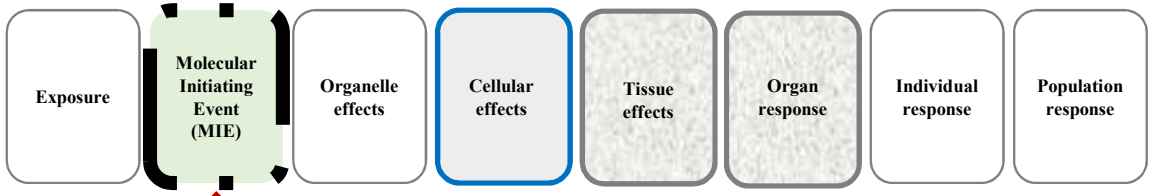
SCREENING ENDOCRINE DISRUPTORS: a mechanism-based misleading concept

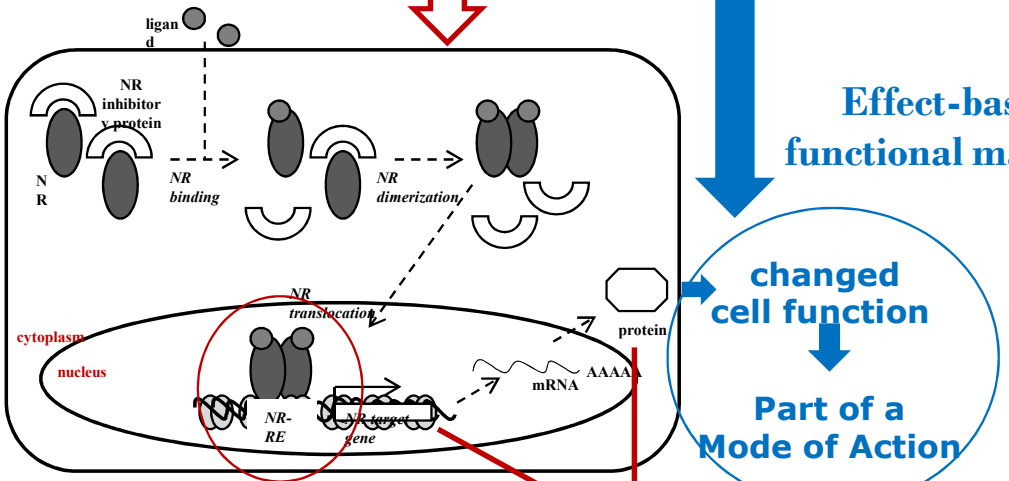
In vitro screening of EDs by Androgen Receptor (AR)-transcriptional activation assay (OECD TG 458)

... it will detect a lack of binding to AR (no activation of AR-mediated gene transcription)

BUT it will never detect its already known Mode-of Action: anti-androgenicity !

Adverse Outcome Pathway (AOP)





Effect-based functional markers

changed cell function

Part of a Mode of Action

transcriptional activation

Part of a Mechanism of Action (incl. toxicogenomics)

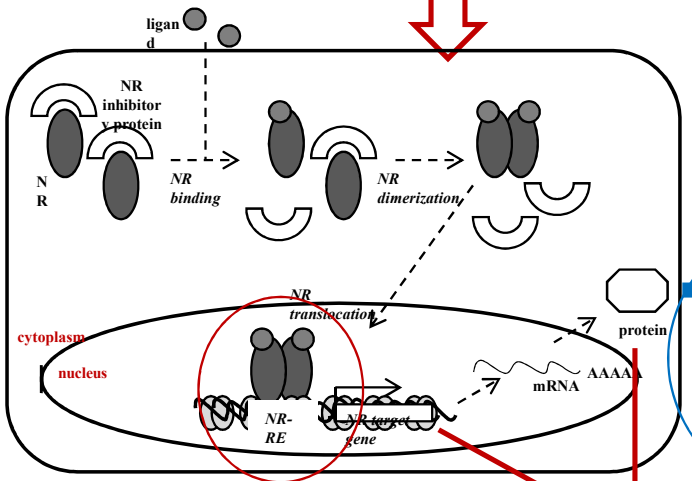
molecular markers:
mRNA, miRNA, proteins, metabolites

Adapted from Lorenzetti and Narciso, 2012
DOI: 10.1039/9781849735353

SCREENING ENDOCRINE DISRUPTORS: EFFECT-based approaches - 1

Adverse Outcome Pathway (AOP)

Exposure | Molecular Initiating Event (MIE) | Organelle effects | Cellular effects | Tissue effects | Organ response | Individual response | Population response



transcriptional activation

Part of a Mechanism of Action (incl. toxicogenomics)

molecular markers:
mRNA, miRNA, proteins, metabolites

Effect-based functional markers

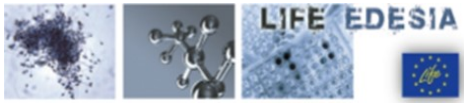
changed cell function

Part of a Mode of Action

WHICH BIOMARKERS TO SCREEN FOR:

- **AN ENDOCRINE ACTIVITY ?**
- **AN ADVERSE EFFECT ?**

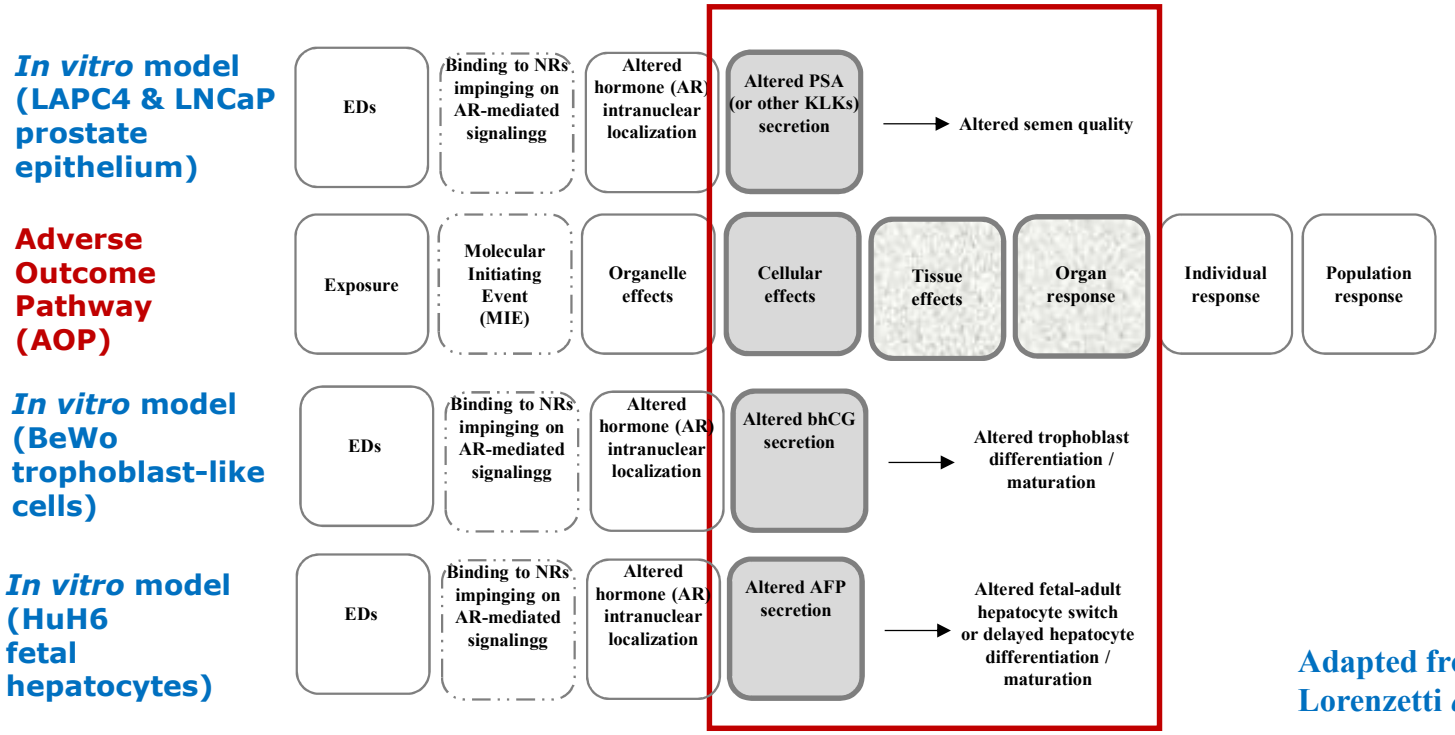
cell-specific, clinically relevant, hormone-dependent biomarkers of effects



- **PSA secretion – androgen disruption in prostate epithelium**
- **βhCG secretion – estrogen disruption in trophoblast-like cell (placenta)**
- **AFP secretion – metabolic disruption in liver**

SCREENING ENDOCRINE DISRUPTORS: EFFECT-based approaches - 2

Endocrine-dependent, cell-specific biomarkers to build an AOP for Endocrine Disruption



Adapted from Lorenzetti *et al.*, Annals 2015

Monolayers and 3D-cultured cells STOP here

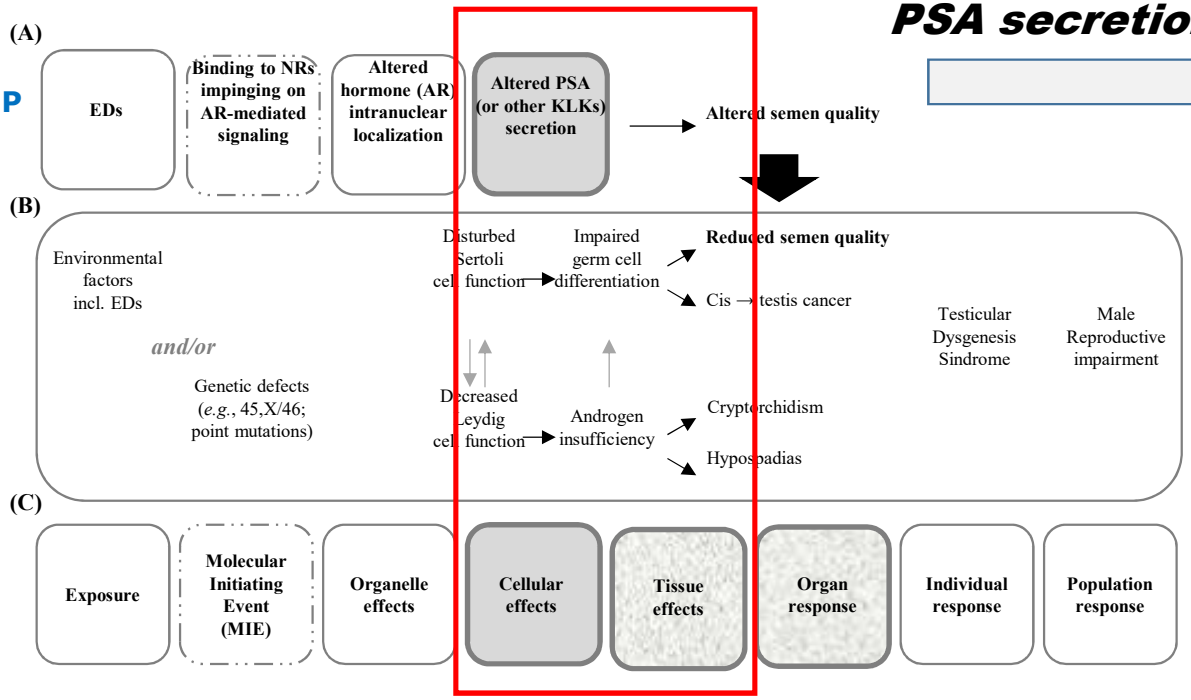
Co-cultured cells & organoids can eventually STOP here

SCREENING ENDOCRINE DISRUPTORS: EFFECT-based approaches - 3

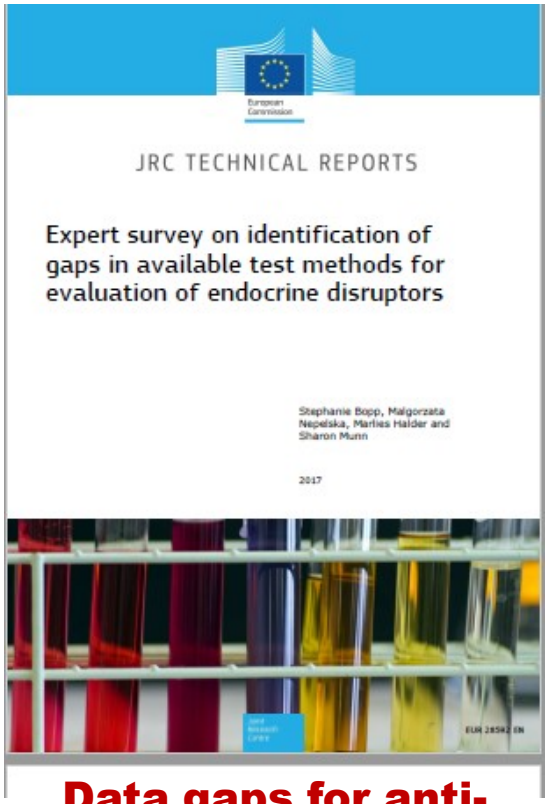
***In vitro* model (LAPC4 & LNCaP prostate epithelium)**

Testicular Dysgenesis Syndrome (TDS)

Adverse Outcome Pathway (AOP)



PSA secretion test



Data gaps for anti-androgenicity in male accessory glands

Adapted from Lorenzetti *et al.*, Annals 2015