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Male autism spectrum disorder is linked to brain aromatase disruption by prenatal BPA

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EDC Strategies Partnership Webinar 17th October 2024

The growing problem of chemical toxicants (e.g. plastics)

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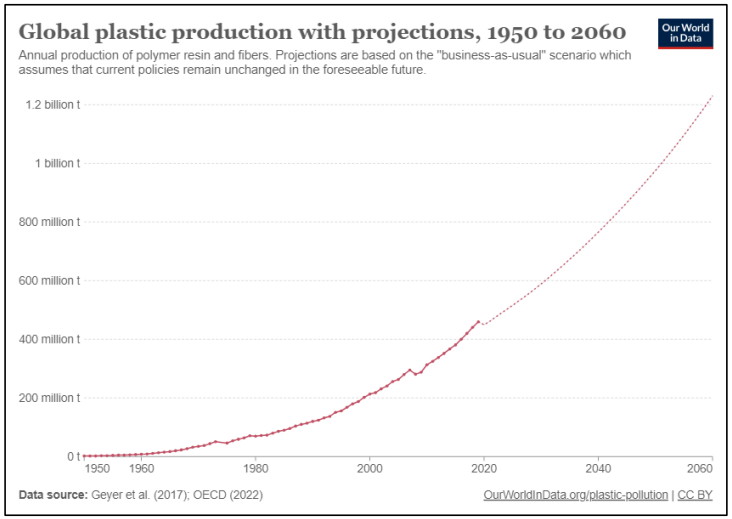
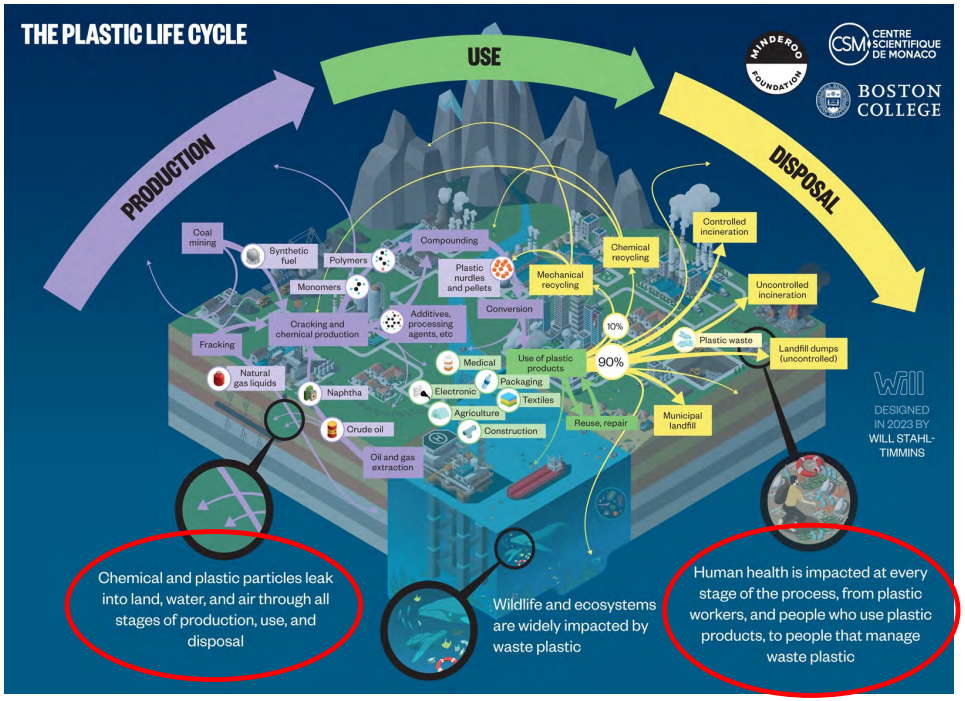
- Plastic – most utilised material in everyday life
- Annual global use (2019) of 460 million metric tons
- Expected to triple by 2060
- Regulation of chemicals generally assumes safety until proven otherwise





Ref: (Text) Seewoo et al 2023, Environ Int, 181:108255; (Figure) ourworldindata.org; (Image) Landrigan, Symeonides, et al 2023, Minderoo-Monaco Commission on Plastics and Human Health, Annals of Global Health

Increasing neurodevelopmental disorders

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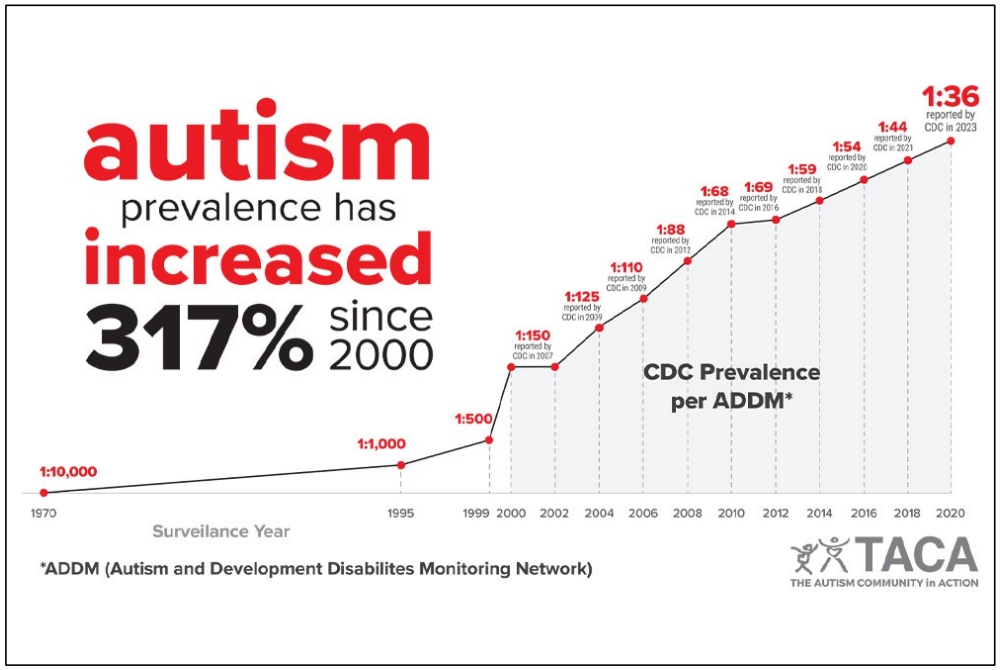

What has changed in our modern environment to drive the increase in neurodevelopmental disorders?

Plastic chemicals are one candidate factor set but not the only one.....

Example: In 1978 Coke and Pepsi introduced plastic bottles

Refs: coca-cola.com; (Figure) tacanow.org

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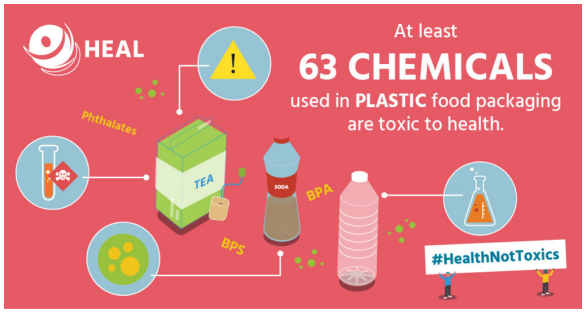
Manufactured chemicals: a widespread problem

- Plastics
  - Plastic-associated chemicals (e.g. bisphenols, phthalates)
  - Micro- and nano-plastics: fragments of any plastic <5 mm or <1 µm in size[1]
- 'Forever' chemical: PFAS (per- and poly-fluoroalkyl substances)[2]
  - Persistent organic pollutants; >7 million chemicals
  - Direct health effects due to their highly toxic nature[3]
    - Cancer (kidney, breast)
    - Endocrine disruption (thyroid disease, decreased fertility)
    - Children (reduced growth, developmental issues)
    - Metabolic (obesity, type 2 diabetes)
- Other toxicants:
  - Pesticides (e.g. organophosphates)
  - Heavy metals (mercury, lead, copper, arsenic)
  - Polychlorinated biphenyls (PCBs)
  - Tobacco smoke, etc



Health and Environment Alliance

Refs: 1) Bai et al 2024, Chemosphere, 142424; 2) Schymanski et al 2023, Environ Sci Technol, 57:16918; 3) Gaillard et al 2024, Trends Endocrinol Metab, S1043-2760(24)00202-9

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How do plastics, including microplastics and plastic-associated chemicals, affect human health?

- Plastics enter the body[1]
  - Micro- and nano-plastics (MNPs) found in human organs (brain, lung, placenta, breastmilk[2], cardiac tissue[3], testes[4])
- Plastic-associated chemicals detected in human biosamples[5]
  - Exposure occurs throughout lifespan, starting before conception
- Plastics are associated with adverse health impacts[5]
  - Related to disease: neurological, metabolic, reproductive, cardiovascular
  - e.g. neurodevelopmental changes in newborns, type 2 diabetes,
- Need to develop and implement global measures to protect public and planetary health[1]
  - e.g. elimination of plastic-associated chemicals, banning MNPs in personal care products, increased use of sustainable materials


Correspondence

https://doi.org/10.1038/s41591-024-03287-x

How do plastics, including microplastics and plastic-associated chemicals, affect human health?

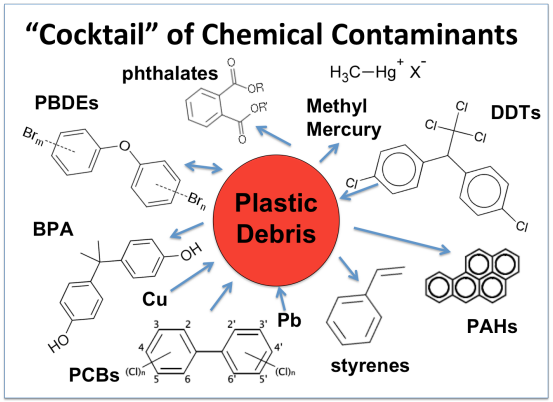
nature medicine

Bhedita J. Seewoo[1,2], Louise M. Goodes[1,2], Kevin V. Thomas[3,4], Cassandra Rauert[3,4], Ahmed Elagali[1,2], Anne-Louise Ponsonby[5,6], Christos Symeonides[1,6] & Sarah A. Dunlop[1,2]

Refs: 1) Seewoo et al 2024, Nat Med, doi: 10.1038/s41591-024-03287-x; 2) Bai et al 2024, Chemosphere, 142424; 3) Marfella et al 2024, New Eng J Med, 390:900-10; 4) Hu et al 2024, Toxicol Sci; 5) Symeonides et al 2024, Ann Global Health 90:52

How do plastics, including microplastics and plastic-associated chemicals, affect human health?

- Plastics composed of[1]:
  - polymer backbone
  - compounded with chemical additives (>16,000 exist)
    - e.g. plasticizers (bisphenols, phthalates), flame retardants (PCBs), stabilizers, colorants
  - array of non-intentionally added substances



- Most chemicals are not covalently bound to polymer and hence can leach out[2]
  - 1,500 chemicals known to leach out

- Safety data – absence of data is not an indication of safety[1,2]
  - 66% of chemicals do not have available hazard data
  - Of those with data, 75% (~4,200 chemicals) are known to be hazardous to human and/or environmental health
  - Limited data on complex real-world mixtures
  - Difficulty in accurately measuring chemicals, especially MNPs, due to contamination issues and lipid matrix interference
  - More research needed!

Refs: 1) Seewoo et al 2024, Nat Med, doi: 10.1038/s41591-024-03287-x; 2) Wagner et al 2024, Zenodo Report

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Bisphenol A

- Bisphenols – a class of chemicals used as a monomer in plastics
  - Includes hard plastics, can lining, thermal receipts and more
- Cross placenta and blood brain barrier
- Not well regulated
  - BPA is banned in some products, but analogues (BPS, BPAF, BPF) are used
- Over 60% of studies (which are cross sectional) show a relationship between bisphenols and neurodevelopment
  - Need to show mechanisms for causal evidence

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Minatoya M, Kishi R. *Int J Environ Res Public Health* 2021;18:3585.

A Review of Recent Studies on Bisphenol A and Phthalate Exposures and Child Neurodevelopment

Machiko Minatoya and Reiko Kishi *

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* Correspondence: rkishi@med.hokudai.ac.jp

"Overall, this review suggests that prenatal exposure to bisphenol A and phthalates **may** contribute to neurobehavioral outcomes in children"

Correlation is not causation but signals the need for for further work…

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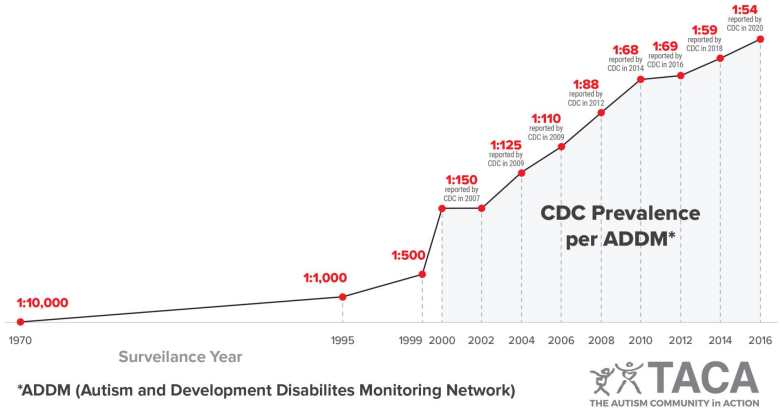

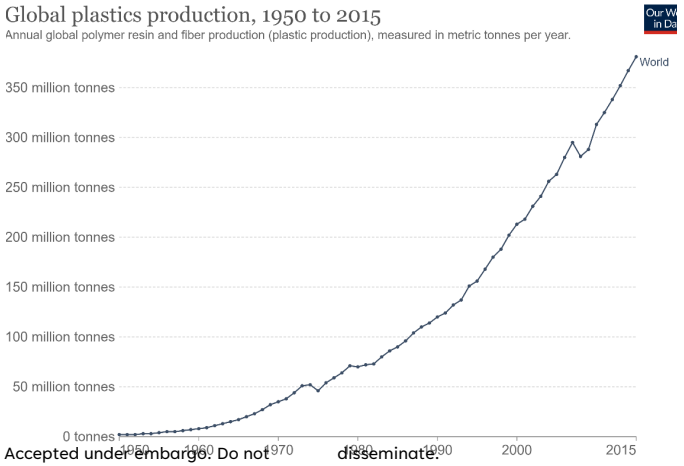

Prevalence of autism spectrum disorder (ASD) rising

ASD aetiology:

Gene x prenatal environment

Male excess in diagnosis

Aromatase/CYP19 is a genetic candidate.

30% lower in ASD prefrontal cortex post mortem

Epigenetics is sensitive to prenatal environment

Testosterone

17β-Estradiol

How do we build causal evidence on early life pollutant exposure (e.g. plastics) and neurodevelopmental harm?

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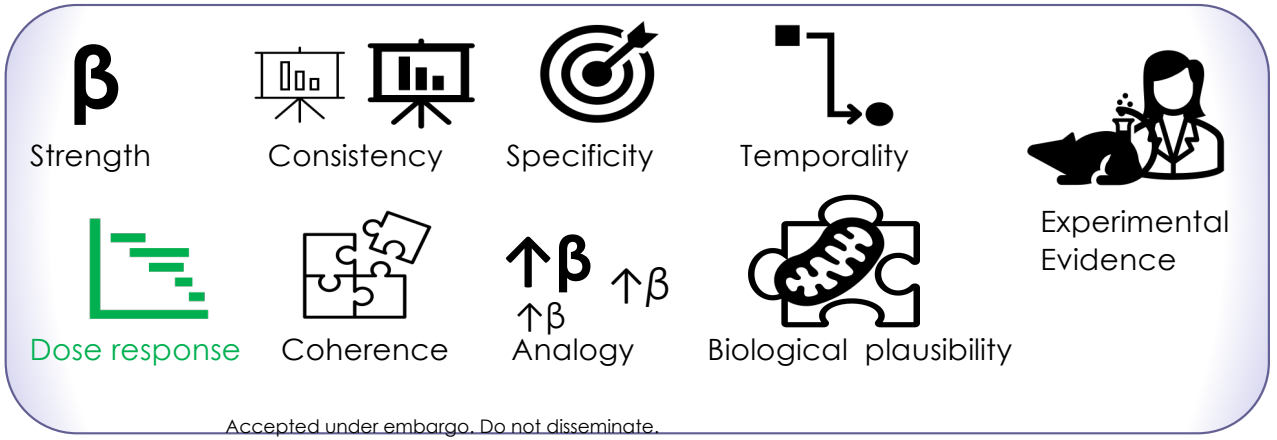
Experimental evidence

- Harmful early life exposures (.e.g. pollutants) are often not suitable for RCTs
- Plastic avoidance trials (at the individual level) have been unsuccessful
- At The Florey, we have moved to lab experimental findings to further assess observational cohort findings

Observational evidence

- Comprehensive, highly dimensioned, prospective cohorts **(these take decades to build)**
- Should examine:
  - The multifactorial causes of neurodevelopmental problems
  - Exclude confounding and other non causal explanations (e.g. poor nutrition)
- Need to be informative enough to provide data on Bradford Hill's criteria for causation



Key technique used – molecular mediation

IEA International Epidemiological Association

International Journal of Epidemiology, 2021, 1016–1029
doi: 10.1093/ije/dyaa174
Advance Access Publication Date: 17 February 2021
Education Corner

Education Corner

Reflection on modern methods: building causal evidence within high-dimensional molecular epidemiological studies of moderate size

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Editorial decision 21 July 2020; Accepted 17 August 2020

"A systematic approach is required to work through a question set and obtain insights on not only the exposure-disease association but also the multifactorial causal structure of the underlying data where possible. The appropriate inclusion of molecular findings will enhance the quest to better understand multifactorial disease causation in modern observational epidemiological studies"

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How do we build causal evidence on early life pollutant exposure (e.g. plastics) and neurodevelopmental harm?

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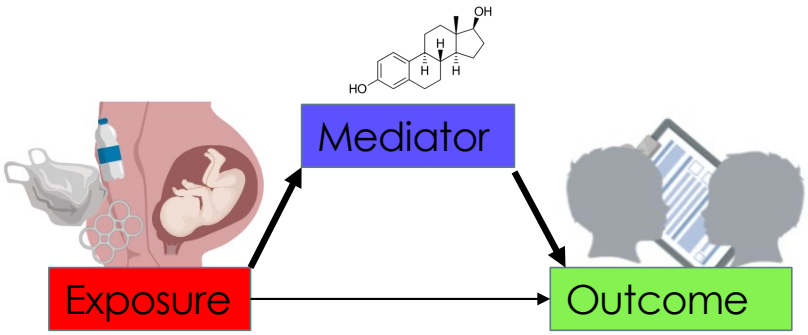
- Comprehensive, highly dimensioned, prospective cohorts **(these take decades to build)**
- Should examine:
  - The **multifactorial** causes of neurodevelopmental problems
  - Exclude confounding and other non causal explanations (e.g. poor nutrition)
- Need to be informative enough to provide data on Hill's criteria for causation
- **Need to incorporate modern causal inference approaches (e.g. molecular mediation)**
  - **Explain how it is operating at a molecular level**



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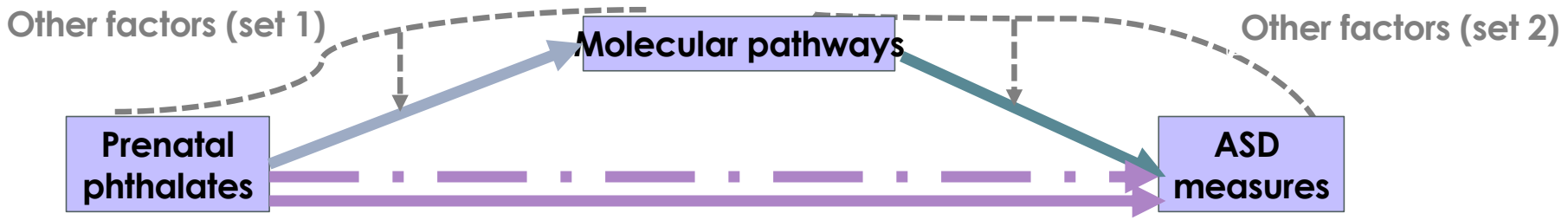
Key technique used – molecular mediation

1. Model where prenatal plastic effects on molecular markers is identified
2. Model where molecular markers are examined as predictors of neurodevelopment such as autism spectrum disorder
3. We then check the proportion of the exposure acting through a specific molecular pathway or signature (the indirect effect)



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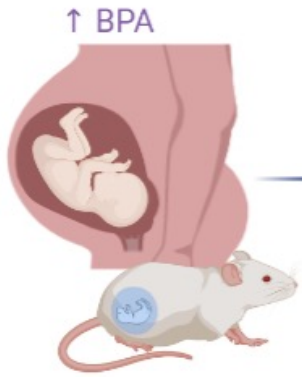
Hypothesis

We hypothesized that BPA exposure *in utero* reduces the expression of aromatase, affecting brain development, resulting in an ASD like phenotype.

Aromatase is particularly important in male brain development, we expect stronger impact on males.



- Once a molecular pathway is identified, it will be easier to find targets for ASD treatments.

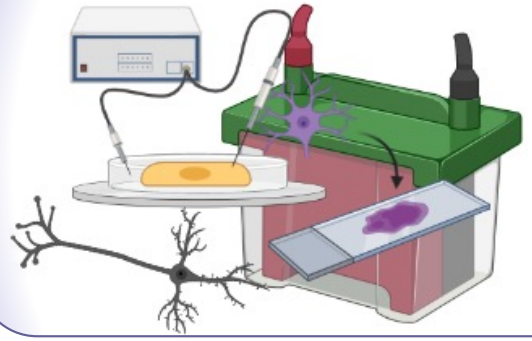
Aim

We tested across a multimodal program of laboratory and human prospective cohort studies.

This included:

- 28 lab studies
  - 18 mechanistic
- 3 human outcomes

Human and mouse participants



In vivo and *in vitro* studies

Human diagnosis, symptoms and mouse behaviour

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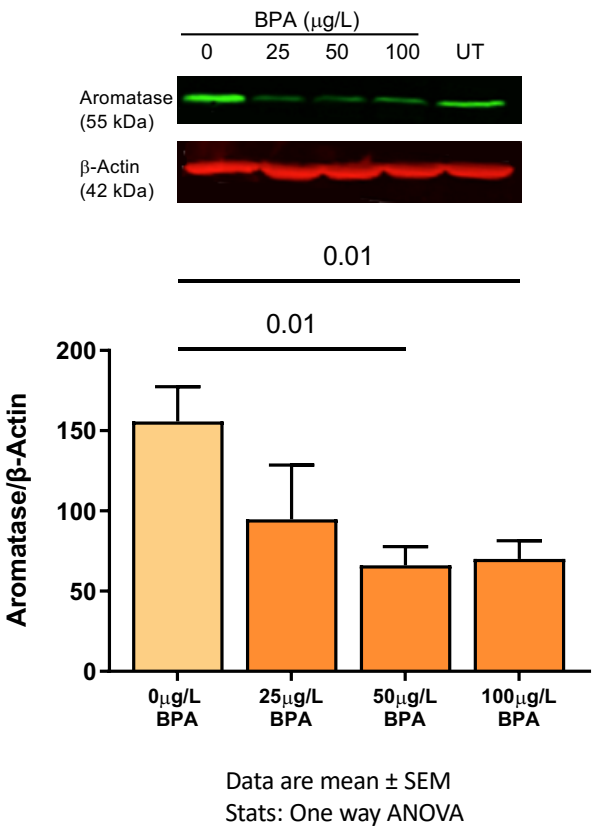
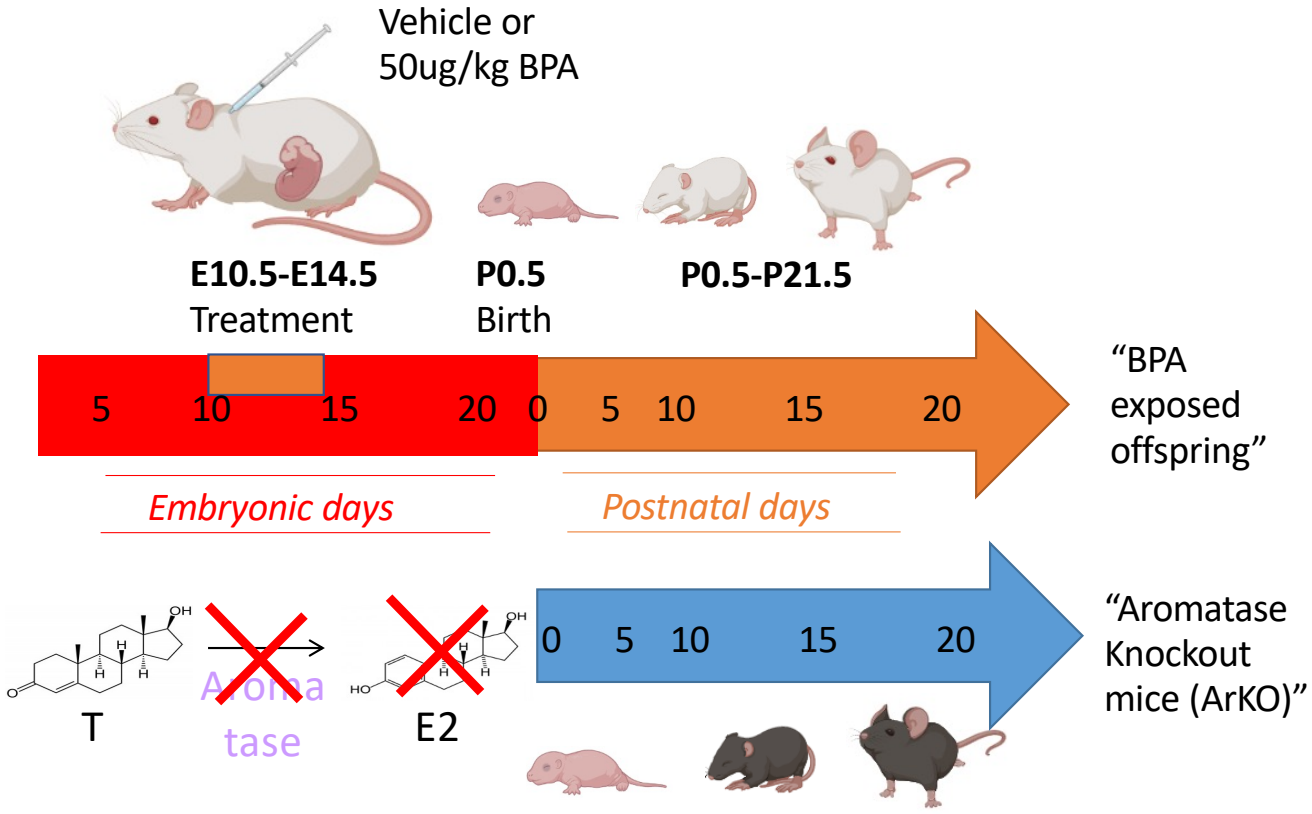
Preclinical Laboratory Studies

The animal models

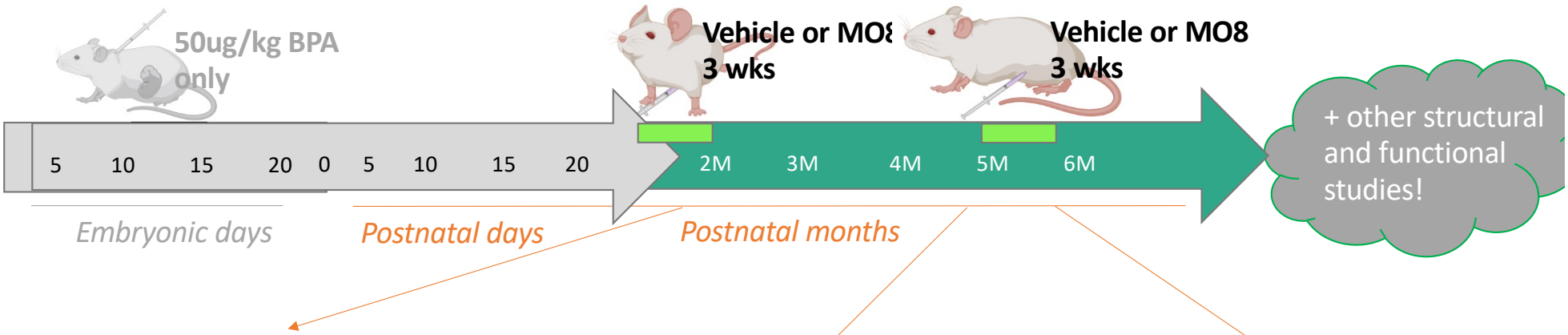


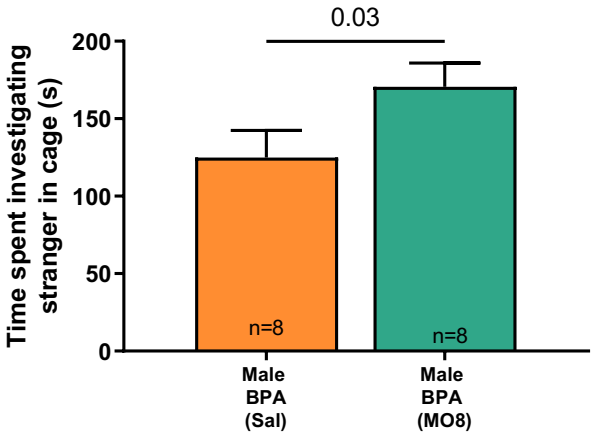
Data are mean ± SEM
Stats: One way ANOVA

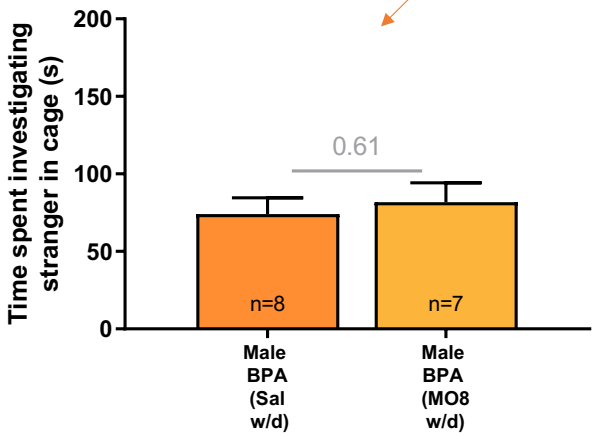


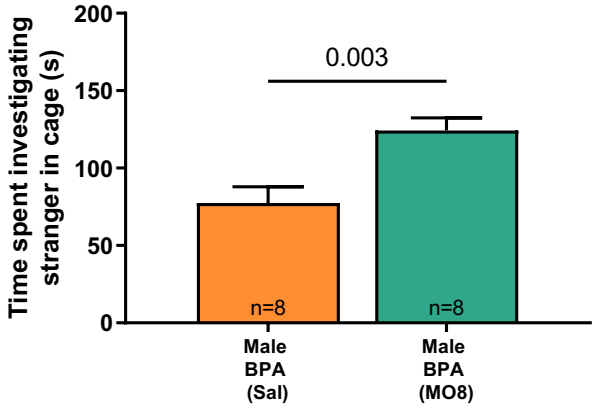
	BPA-exposed or ArKO Mouse	Autism Spectrum Disorder Human
Brain aromatase expression	↓ EGFP, marker for aromatase expression in CYP19-EGFP mice (Fig. 3C)	↓ Brain expression in post mortem ASD brain[18] ↑ Methylation of brain PI.f promoter in aromatase (associated with ↓ transcription) (Fig. 2)
Amygdala structure	↓ Dendritic length (Fig. 5A) ↓ Amygdala neuron number (BPA exposed, Fig S7)	↓ Amygdala cell number[19] ↓ Amygdala cell number in adults[20]
Amygdala hypo-responsiveness to social stimuli	↓ c-Fos (Fig. 5C) ↓ Multiple electrode analysis (Fig. 5D)	↓ fMRI[21]
Cortical layers	Excessive neurons in layer 4/5 in somatosensory cortex[8]	Excessive neurons in layer 4/5 in dorsolateral prefrontal cortex[22]
Electrophysiological activity	↑ ECoG power At 3-7Hz (Fig. 6D)	↑ EEG power at 3-9Hz[2336]
Social behavior	↓ Social approach: 3 chamber study (Fig. 2A and 2B)	↑ ASD problems at 2yrs in males with low aromatase GFS (Fig. 1; Table S3) ↑ ASD diagnosis at 9yrs in males with low aromatase GFS (Fig. 1; Table S3)

MO8 restored BPA induced phenotype



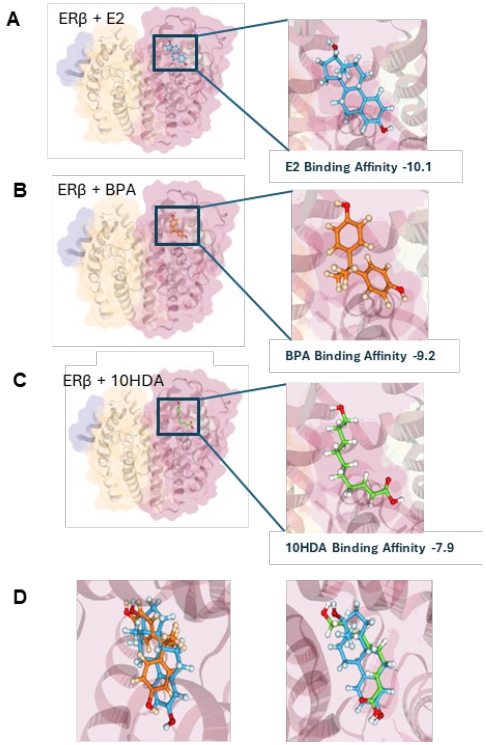






Data are mean ± SEM
Stats: Mann Whitney

Molecular docking of E2, BPA and 10HDA with estrogen receptor β.



In silico molecular docking analysis of estrogen receptor β (ERβ, Protein Data Bank (PDB) ID: 1YYE; encoded by the ES gene) using the DockThor platform, showing showing binding predictions for (A) the native ligand 17β-estradiol (E2), (B) bisphenol A (BPA), (C) Trans-10-hydroxy-2-decenoic acid (10HDA), and (D) E2 and BPA (left) and E2 and 10HDA (right) superimposed for spatial alignment comparison. While the molecular affinities of BPA and 10HDA for Erβ were comparable (-9.2 vs. -7.9, respectively), 10HDA aligns better with the binding conformation of the endogenous ligand E2, which activates the receptor. BPA is previously reported as sub-optimally estrogenic – more than 1000-fold less compared to natural estradiol - whereas 10HDA has an estrogenic role in nature. Thus, 10HDA may compensate for E2 deficiency caused by a reduction in aromatase enzyme, and in competition with binding by BPA.

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Human cohort studies

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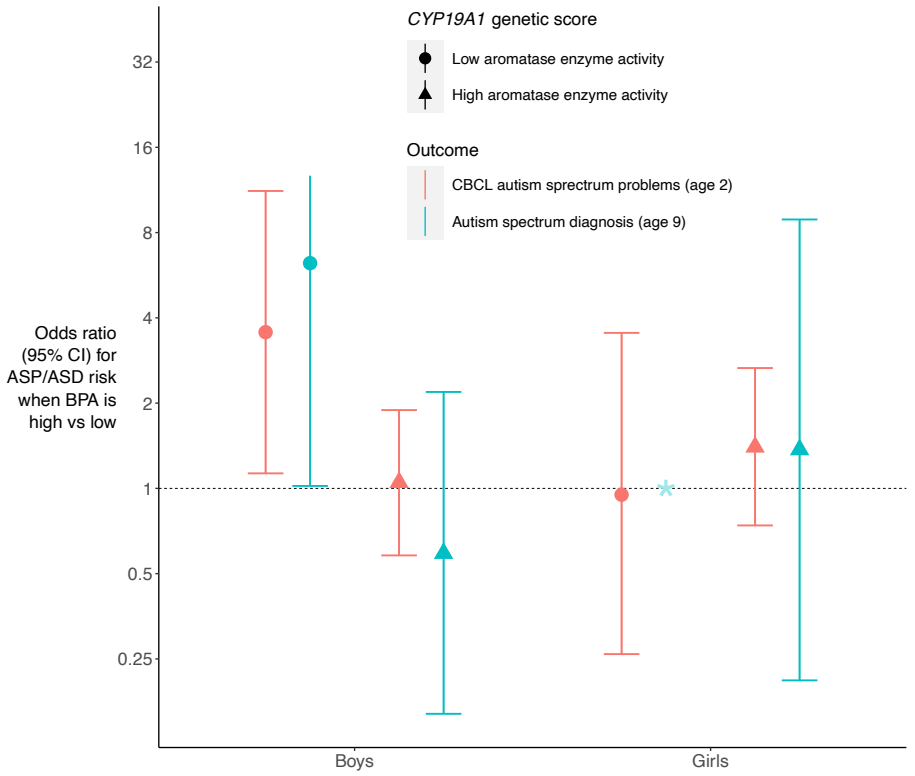
Odds ratio for ASP/ASD in high vs low BPA increases in boys with low aromatase enzyme activity



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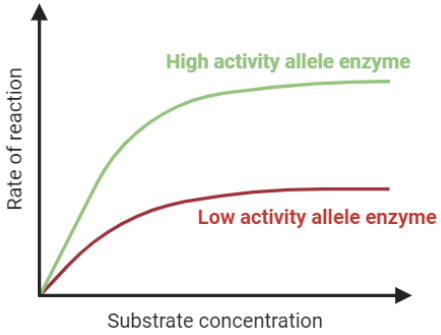
BPA silences brain promotor of aromatase

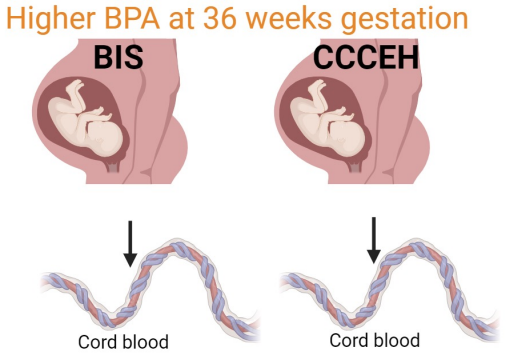
BPA blocks transcription(less enzyme protein made)



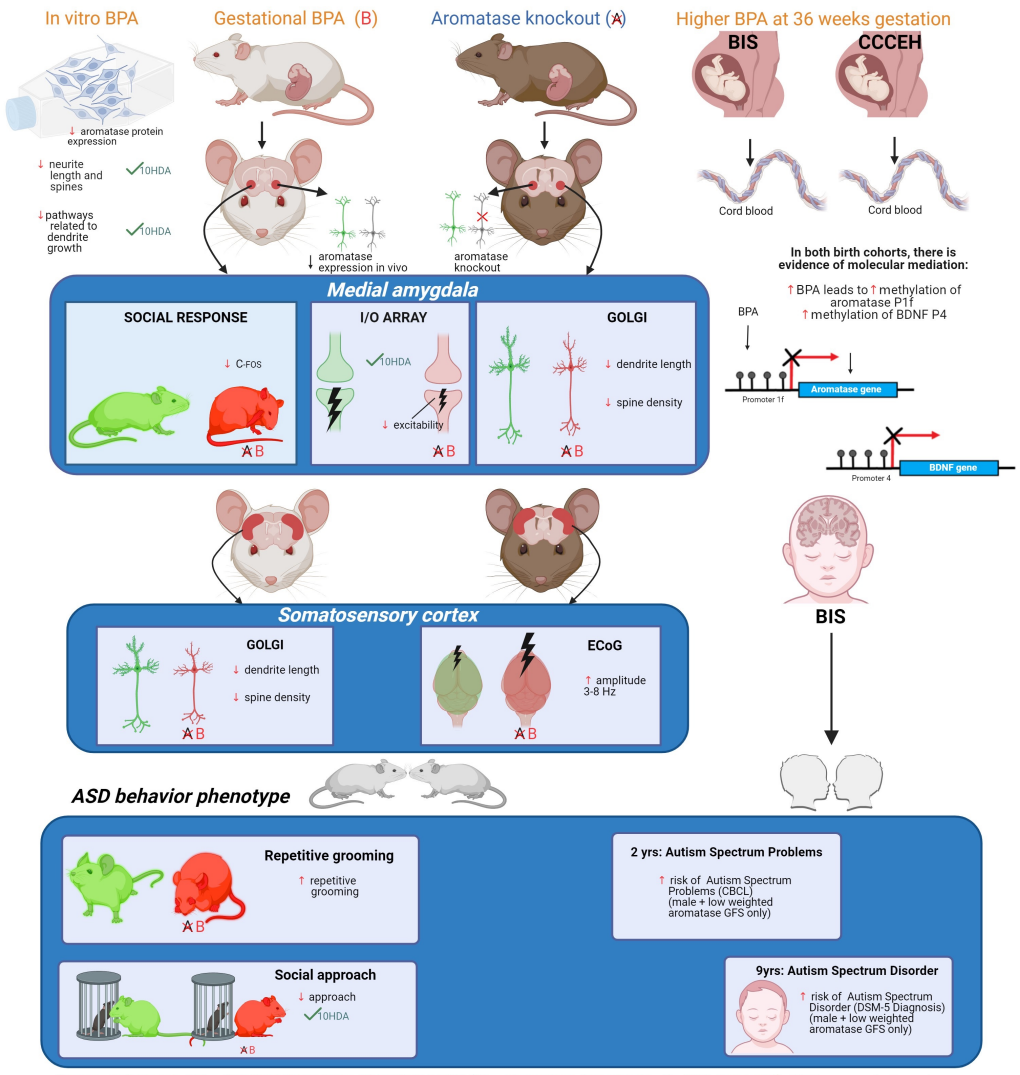
Low activity aromatase converts less T to E2
(The enzyme type is slower)

BIS= Barwon Infant Study (AUS)

CCCEH = Columbia Center for Children's Environmental Health



In both cohorts, there is evidence of molecular mediation:



Green = reference, red = Austism Spectrum Disorder model (A = aromatase knockout, B= BPA-exposed), GFS = genetic function score

✓10HDA = restored by 10HDA treatment

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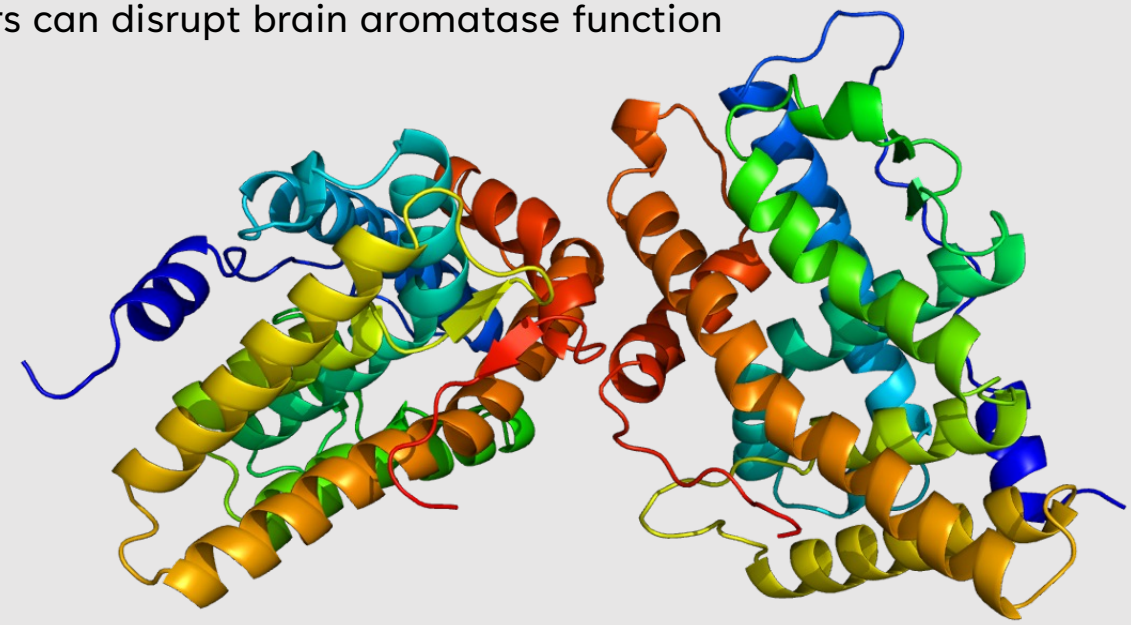
Aromatase as a common indicator

- Exposure to environmental factors can disrupt brain aromatase function
  - Bisphenols (BPA)[1]
  - Phthalates (DEHP)[2]
  - Vitamin D deficiency[3]

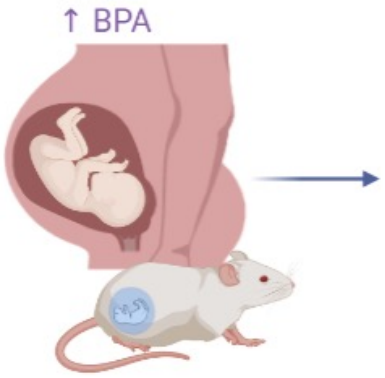


- References
- 1 Santangeli et al 2017 (Effects of BPA on female reproductive function: the involvement of epigenetic mechanism. *General and comparative endocrinology* **245**, 122-126);
  2 Andrade et al 2006 (A dose-response study following in utero and lactational exposure to DEHP: non-monotonic dose-response and low dose effects on rat brain aromatase activity. *Toxicology* **227**, 185-192);
  3 Ali et al 2020 (Developmental vitamin D deficiency increases foetal exposure to testosterone. *Mol Autism* **11**, 96)

Conclusion

We found that:

- BPA reduced aromatase expression in the medial amygdala
- Male BPA exposed and ArKO mice demonstrated ASD-like behavioural and brain changes at structural and functional levels
- We also observed in a prospective cohort study, males with low aromatase activity have greater odds of ASD diagnosis at age 9 and ASD symptoms at age 2 when exposed to high levels of BPA



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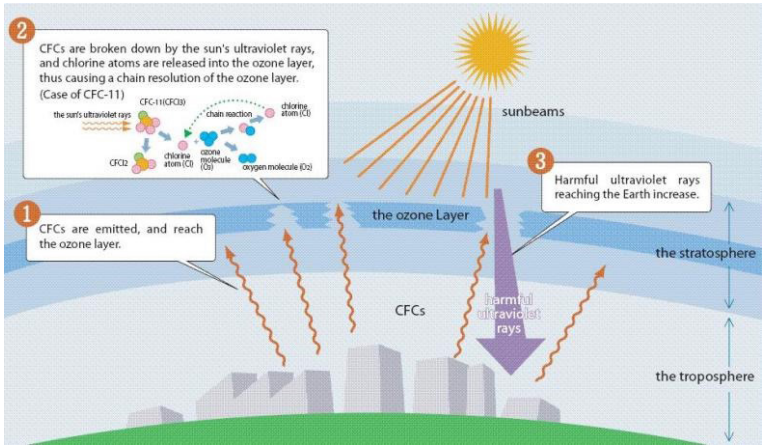
Example of how changing policy can result in real-world positive change: the hole in the ozone layer

- Ozone layer protects Earth's stratosphere by absorbing the sun's harmful ultraviolet-B radiation[1]
- Human-made chemicals began depleting the ozone layer (discovered in 1974)[2]
  - Chlorofluorocarbons (CFCs), halons, others (release chlorine, bromine)
  - CFCs - aerosol propellants (hairspray), refrigerants, foam blowing agents (insulation, packaging), industrial solvents, manufacturing
  - Halons – fire suppression systems (extinguishers, aircraft, military vehicles, industrial uses)
- In 1985, the "ozone hole" over Antarctica formally reported[2]
- Consequences of damage to ozone layer[1]:
  - increased UV radiation reaching Earth
  - higher risks of skin cancer (Australia/NZ world's highest rates), cataracts, harm to ecosystems



Refs: 1) www.epa.gov/ozone-layer-protection; 2) https://ozone.unep.org/ozone-timeline

Banning adverse chemicals

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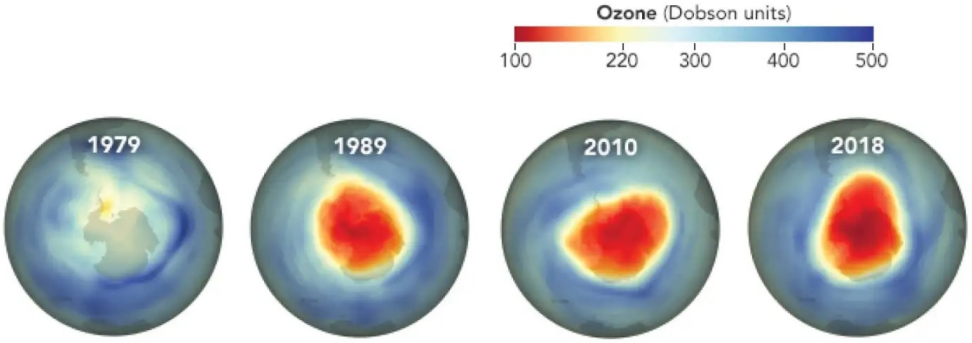
- Montreal Protocol (1987)[1]
  - International treaty aimed at phasing out the production of ozone-depleting substances (~100 substances)
  - Set legally binding, time-targeted and measurable commitments for the reduction and elimination of harmful substances in a step-wise manner
  - In 2016, amended to include HFCs – CFCs "safer" cousin – due to high global warming potential (2000x more harmful than carbon dioxide)
  - Impact: **significant global cooperation** (198 parties) led to **98% reduction in the use of substances** responsible for ozone depletion[1]
- Currently, ozone layer is on track to recover to its pre-1980 levels by 2040 (2066 for Antarctica)[1]
  - **20% decrease in ozone depletion** during winter months from 2005 - 2016[2]



Ozone[3]
Levels <220 DU indicates depletion.
Levels >300 DU indicates healthy.

1980's – rapidly growing hole (depth and area)
1994 – deepest hole, 73 DU
2000 – largest hole (area)
2000's – stabilising hole

Refs: 1) www.unep.org; 2) Mersmann 2018, nasa.gov; 3) nasa.gov, Ozone hole watch, and Earth observatory; (image) McGhee 2020, Australian Geographic

How do plastics, including microplastics and plastic-associated chemicals, affect human health?

- Global Plastics Treaty
  - Aims to address plastic pollution and mitigate human health impacts
  - International and legally binding
- Intergovernmental Negotiating Committee (INC)
  - Session 4 completed; Session 5 (Dec 2024)
  - Increased alignment
  - Major disagreements on whether Treaty should (i) include upstream measures to reduce fossil fuel-based plastics, (ii) tackle single-use plastic products, (iii) regulate plastics via criterion or non-criterion-based approaches, (iv) implement regulations globally or nationally
  - Countries agreed to conduct formal intersessional work prior to Session 5, to then finalise the Treaty
- Scientists Coalition for an Effective Plastics Treaty - Revised Zero Draft
  - Evidence-based document listing chemicals of concern and practical recommendations
- International Organisation, Health Care Without Harm
  - Open letter signed by >800 health professionals recommends:
    - limiting virgin plastic production, eliminating unnecessary and hazardous plastic products, ensuring transparency of chemicals in plastics

Ref: Seewoo et al 2024, Nat Med, doi: 10.1038/s41591-024-03287-x

The Florey

Nature Communications

Male autism spectrum disorder is linked to brain aromatase disruption by prenatal BPA in multimodal investigations and 10HDA ameliorates the related mouse phenotype

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