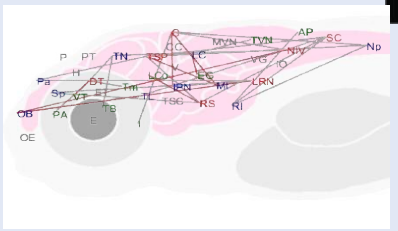
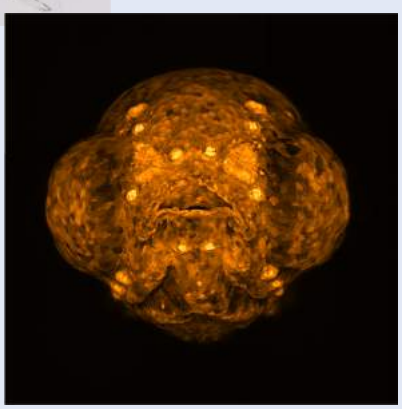

# *Waking up to Planetary Health 2020*

# *Chemicals and Environmental Health (and Zebrafish!).*

*Professor Charles R. Tyler*

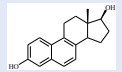


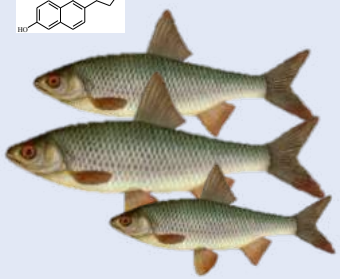



# Presentation Outline

*Waking up to Our Planetary Health*

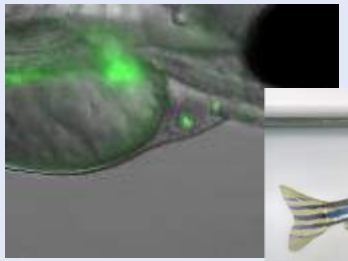
*Changing our Planetary Chemistry*





*Chemical Pollution and Environmental Health*

*Wildlife as Sentinels for Environmental Health- Case Examples for population level impacts of Endocrine Disrupting Chemicals*



*Zebrafish transgenics - health study models*

*Concluding thoughts*

*My Research Interests in the Environment*

*Planetary Health 2020*

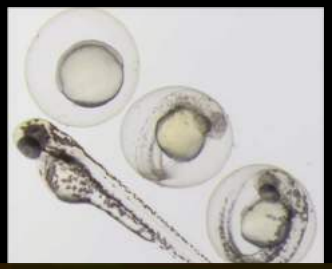
*Resources needs for declining bird species*

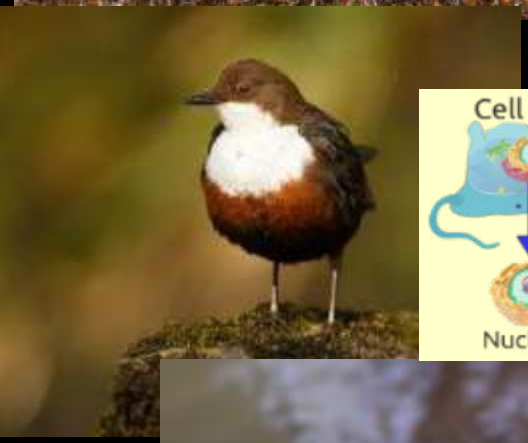
*Integrative research that seeks to better understand organism physiology, behaviour and ecology, how we affect them – and to use this information to better protect our natural environment.*

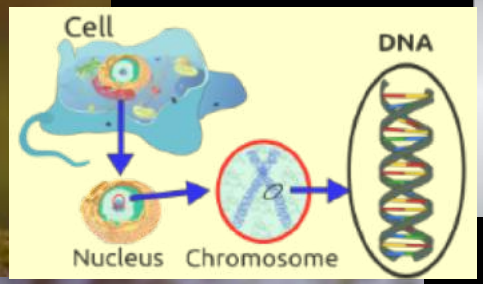
*Sustainable Aquaculture*

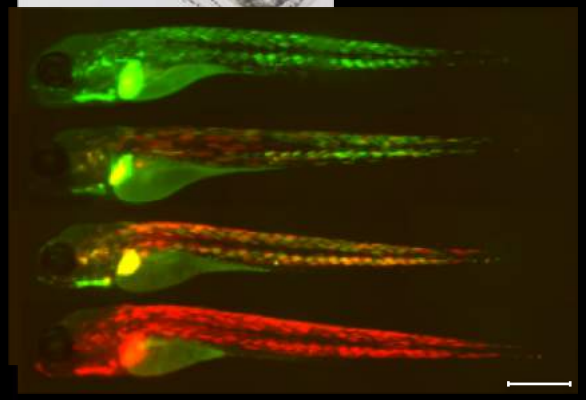
*Image – Charles R. Tyler*

# Chemical effect mechanisms and impacts: Ecotoxicology

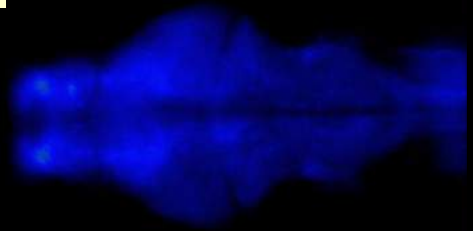


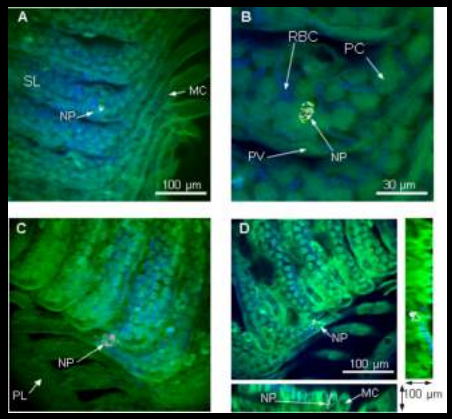




500 µm






*CARS: Identifying the presence of titanium dioxide nanoparticles in the gills of exposed rainbow trout*

*Images – Charles R. Tyler*

# *Reflecting on today's challenges for our environment*

# - ***Biodiversity***













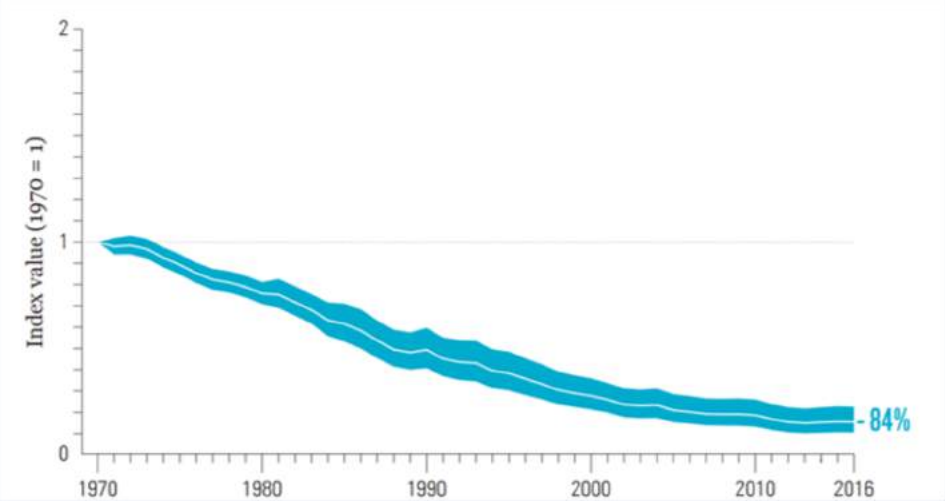


*Images – Charles R. Tyler*

# *Reflecting on today's challenges for our environment - **Biodiversity***

## ***Major (catastrophic) decline in Global Aquatic Biodiversity***

*Freshwater Biodiversity – an example*




*Freshwater LPI (Living Planet Index) measures biodiversity, calculating average change in abundance over time - based on trends in abundance data for* 3741 freshwater populations across 944 species of amphibians, fishes, reptiles, birds and mammals. *(WWF/ZSL, 2020)*

- *Freshwater species populations have dropped by over 80% since 1970 (compared with land -38%, and marine -36%).*
- *Extinction rates for well-studied freshwater animals are estimated to be as high as 4% per decade, five times greater than species losses in terrestrial systems*
- *Freshwater fish are among the most threatened vertebrates worldwide (35% classified as vulnerable or threatened).*
- *Factors underlying freshwater biodiversity declines include overexploitation, habitat destruction and degradation, and **pollution**, all of which are linked to human activities.*

*Reflecting on today's challenges for our environment -* ***Biodiversity***

## *Major (catastrophic) decline in Global Terestrial Biodiversity*

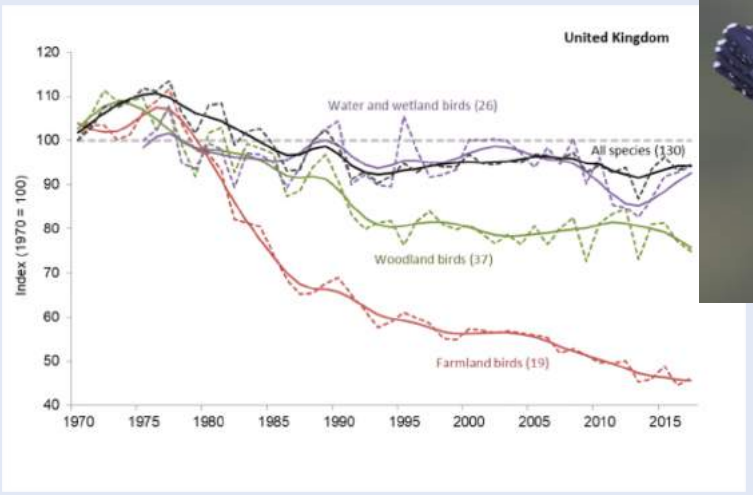
**Insects:** 75 studies covering a range of insect groups globally, -over 40% of all insects are declining and a third are endangered - rate of decline is at least 2.5% per year.

*Sanchez and Wyckhuys (2019) -. Biol Con 232 8-29*

More than 75 percent decline over 27 years in total flying insect biomass in protected areas. Hallmann et al., 2017. https://doi.org/10.1371/ journal.pone.0185809

Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances
**van Klink et al., 2020. *Science* 368, pp. 417-420**

## British Birds:




*Changes in the abundance of breeding birds of woodland, farmland, water and wetlands and all-species in the UK.*
*Source: BTO, Defra, JNCC, RSPB*

***Chemicals** have played a major role in many of these declines*

# *Chemical Pollution and Human Health Effects – The FACTS*

Pollution is the largest environmental cause of disease and premature deaths in humans; 9 million premature deaths in 2017 – 16% of all deaths worldwide ( > AIDS, TB and Malaria combined)

Nearly 92% of pollution –related deaths occur in low-income and middle income countries (and most prevalent in minorities everywhere)

Children are at the greatest overall risk

Pollution is costly:

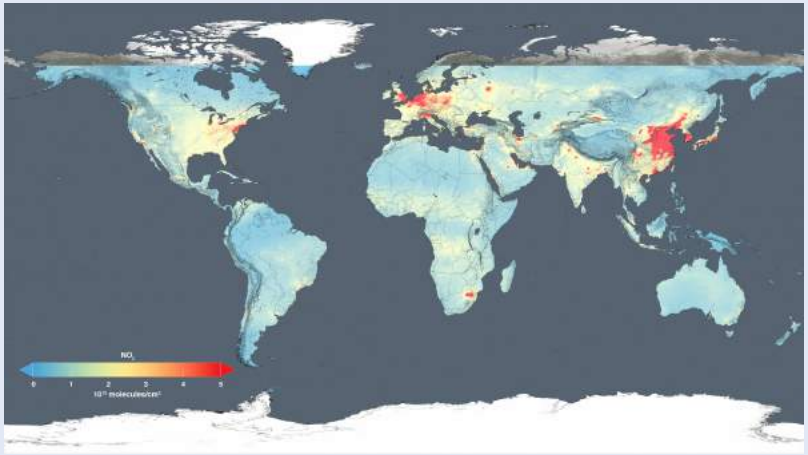
- Productivity loses due to pollution related diseases are at 2%GDP in LMIC
- Health spending: 1.7% in HIC and up to 7%in polluted, rapid developing MIC

Welfare losses US$4.6 trillion per year (6.2% economic output)

Health impact burdens are probably underestimated!

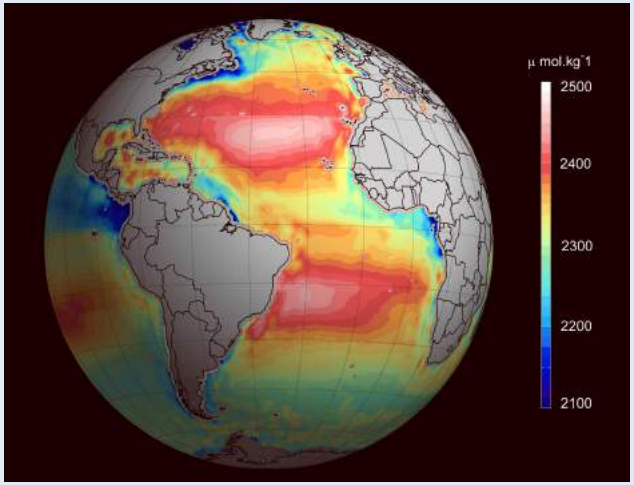
*Landrigan et al., : The Lancet Commission 2017*

# *We are fundamentally changing the chemical balance of our planet…..*

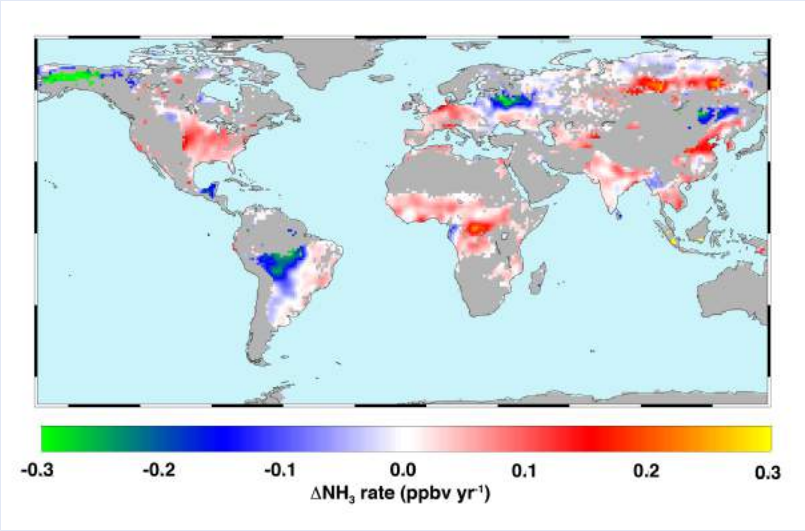


*Satellite images provide powerful insights into the extent of our chemical influence on the planet*

*Nitrogen dioxide*




*Ocean acidification*




*Ammonia*

## *Our Dependence on Chemicals*

We use tens of thousands of chemicals in products (a $3 trillion global enterprise)
..... plasticizers, fertilisers, pesticides, pharmaceuticals, etc.

- 140 000 new chemicals and pesticides synthesised since 1950
- 5000 are HPV – and only half of these have undergone any toxicity or safety testing

Chemicals enter human bodies via direct application (cosmetics), the food chain, drinking water or as aerosols.

Chemicals can also enter the bodies of wildlife and most notably animals living in, or closely associated with, aquatic environments.

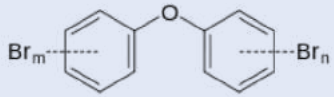
*Some chemicals build up in our bodies potentially increasing any biological effects they might have.*

# *Chemicals of Concern*

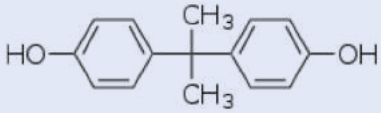
***Priority Substances*** have been identified for which we have concern because there is good evidence for adverse health effects.

***Other Chemicals for which there is increasing health concern include:***


*Perfluoro octanoic acid (PFOAs)* - associated with elevated cholesterol and chronic kidney disease




*Polybrominated diphenyl ethers* - Flame retardants associated with thyroid dysfunction

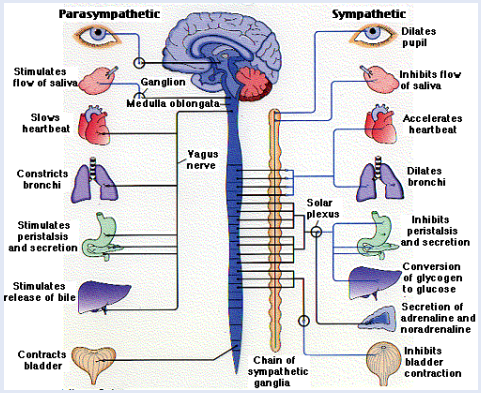


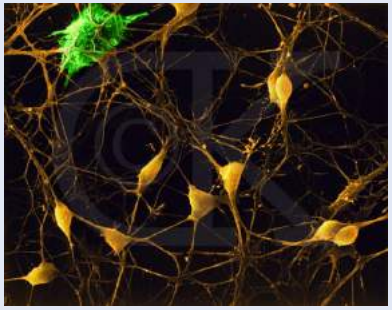

*Polycarbonate polymers* (e.g. Bisphenol A) and *epoxy resins*. Associated with various health disorders (e.g. obesity, reproduction)

*Pharmaceutical substances: 17 alpha-ethinyloestradiol (EE2), 17 beta-oestradiol (E2), Diclofenac, SSRIs (antidepressants)*
*Pharmaceutical substances - $800 billion industry.*

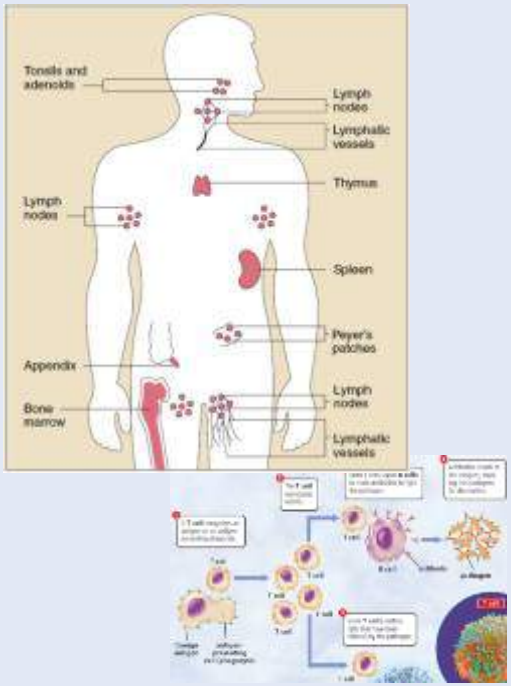
# *Identifying health effects for chronic exposure to low levels or toxicants (and their mixtures)*

***Endocrine Disrupters:*** *chemicals (man-made or natural) that mimics a hormone(s) and disrupts growth, development and/or reproduction in an individual or its progeny…*

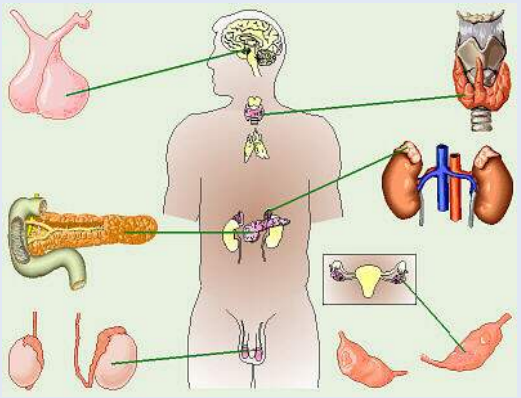


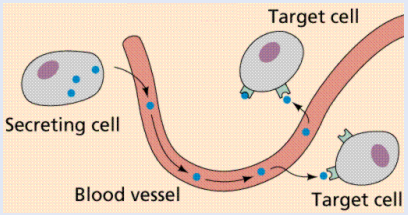



Nervous- *telephone network*




Immune system





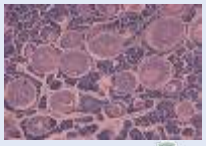

Endocrine- *postal system*

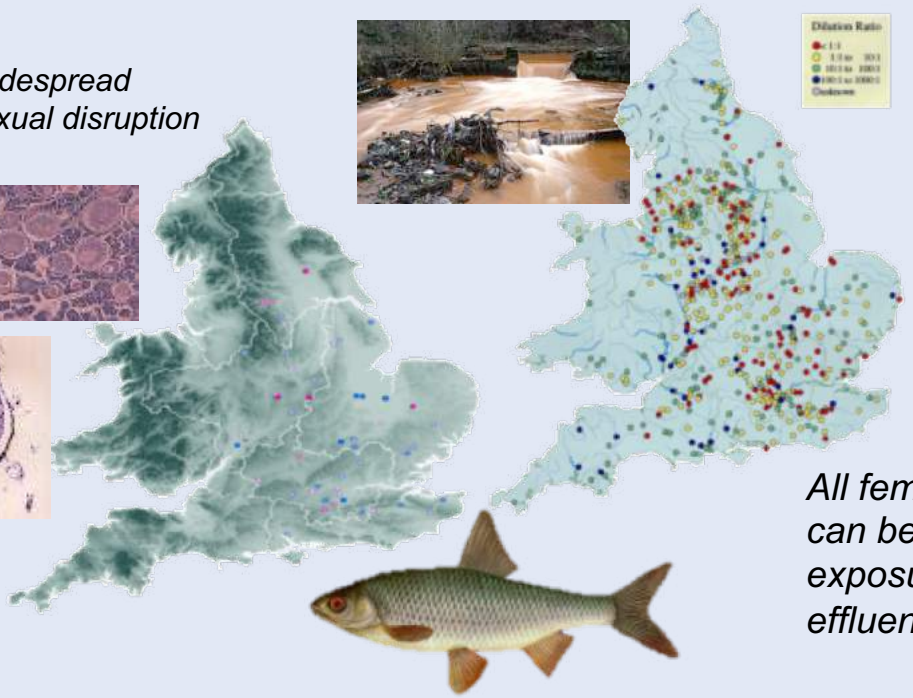
*A hormone is a chemical message that instructs a specific response*

# *Feminisation of Roach (Rutilus rutilus) in UK Rivers – A Brief Overview*

*Widespread sexual disruption*









*Impact of sewage treatment works effluents on fish health (endocrine active chemicals)*

- Intersex present at 44 (86%) of 51 sites
- Overall incidence of intersex in 'males' of 23%

*Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., and Sumpter, J.P. (1998).. Environmental Science and Technology 32: 2498-2506.*

*Jobling,S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nolan, M., Tyler, C.R,van Aerle, R., Santos, E.M.,and Brighty, G. (2006).Environmental Health Perspectives 114: 32-29*

*All feminised effects can be induced by exposure to WWTW effluents*

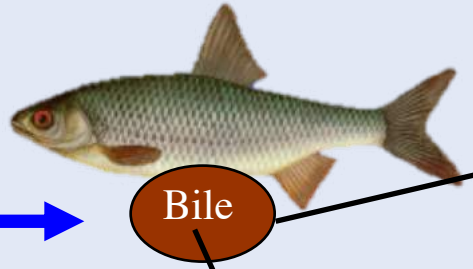
*Liney, K.E., Jobling, S. Shears, J., Simpson.P.,Tyler, C.R*. (2005)* .Environmental Health Perspectives 113 (10): 1299-1307.

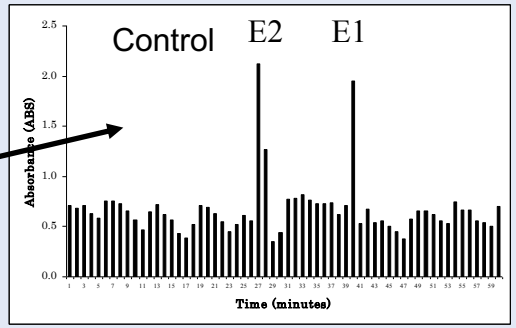
*Rodgers-Gray, T. P., Jobling, S., Kelly,C., Morris, S., Brighty, G., Waldock, M, Sumpter, J.P and Tyler C.R.* (2001).* Environmental Science and Technology 35:(3) 462- 470

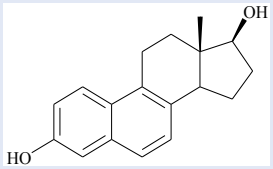
# *Environmental oestrogens entering fish from WwTW effluents*

*Mesocosms …*

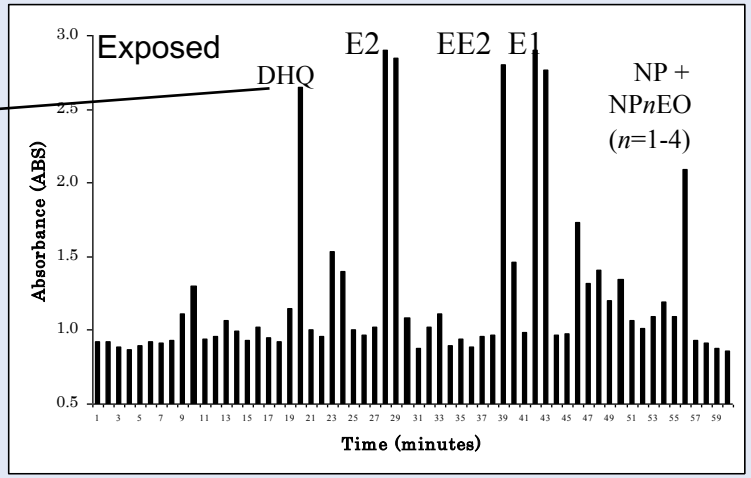
The oestrogenic activity of the test effluents measured

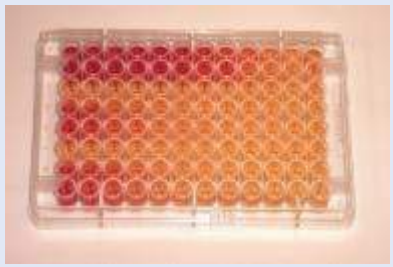



17β-dihydroequilenin
+ (Equilenin)



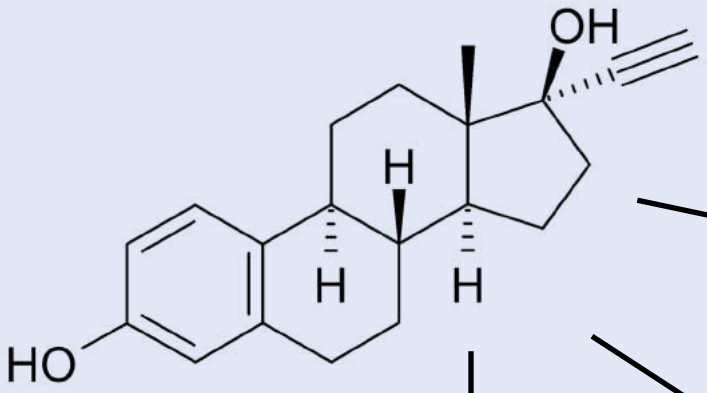



***Fish take up a very wide range of oestrogenic chemicals into their bodies from the WWTW effluent***

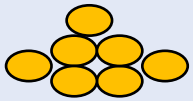
Bile analysed to identify (anti-)oestrogenic compounds and their metabolites ……Enzymic hydrolysis –RP-HPLC + yeast screens LC-NMR-MS/GC-MSMS

*Gibson, R., Smith, M.D., Spary, C., Tyler, C.R., Hill, E.M. (2005). ES&T 39(8) 2461-2471*
*Tyler C.R., Spary, C, Gibson, R., Shears, J., Santos, E., Sumpter, J.P., Hill, E.M. (2005). ES&T 39 (8) 2599-2607*
*Tyler, C.R, Filby, A.L., Hill, E.M., Liney, K.E., Katsu, Y., Bickley, L.K., Lange, A., Winter, M., Gibson, R., Shears, J.A., Iguchi, T.(2009). ES&T. 43:3897-3904*

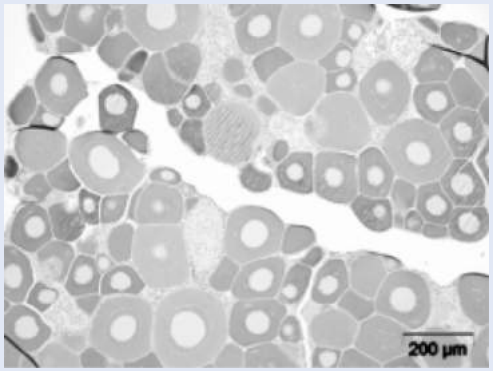
# *Environmental steroidal oestrogens can induce all the feminised phenotypes seen in wild roach.....*



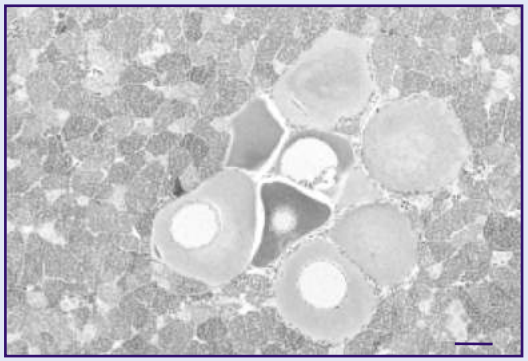

Ethinyloestradiol



Exposure to EE2 (0.3ng/L) can induce vitellogenin synthesis



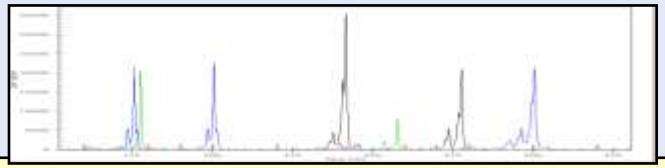

Life-long exposures to EE2 can complete sex reversal (4ng/L)



Exposure to EE2 (0.3ng/L) can induce ovarian cavity formation and intersex

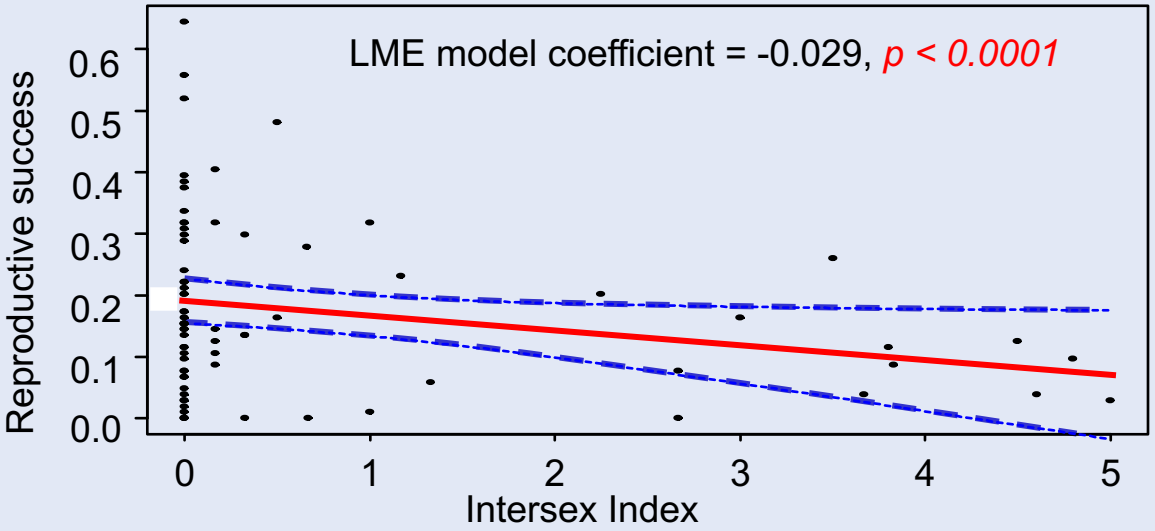
*Lange, A. Katsu ,Y. R. Ichikawa , Greg Paull, Laura L. Chidgey , Tobias S. Coe Iguchi, T, and Tyler, C.R. (2009).. Toxicological Sciences 106(1), 113–123*
*Lange, A., Katsu, Y., R. Ichikawa, Chidgey, L. Iguchi, T.and. Tyler, C.R. (2009). Environmental Science and Technology 43 (4), 1219-1225.*

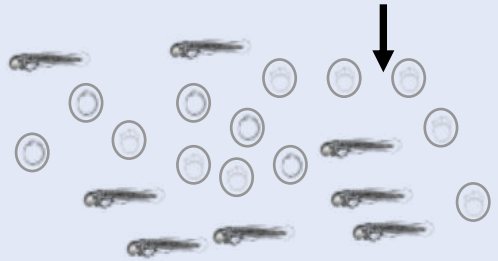
# *Individual Breeding Consequences of Feminisation in Roach*



DNA microsatellites - highly variable primers were developed and applied for parentage analysis (*100% success*)

*Breeding trials with wild roach*



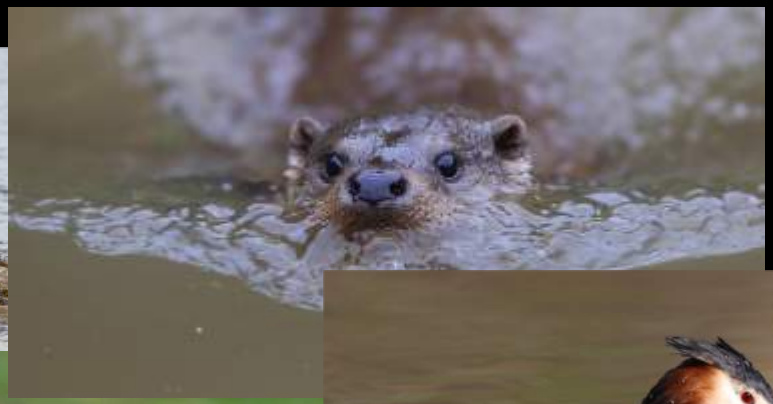
Genotyped all parents and offspring (50-104 fry from each tank). Histopathology analysis of adult gonads.

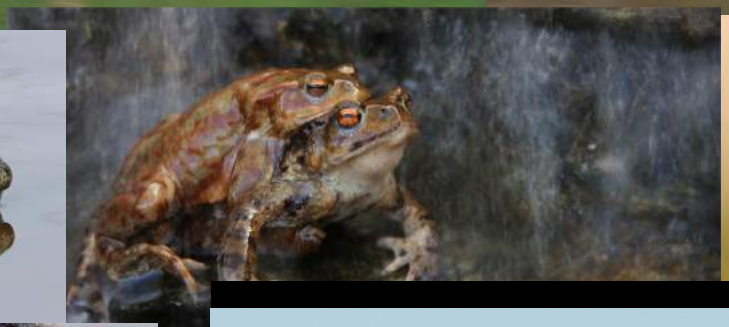
*For roach that are moderately to severely intersex (index >2) there was an estimated 15% reduction for each incremental increase in the intersex index (*75% reduction for the most feminised individuals included in this study).

*Harris, Hamilton, Runnalls, Jobling, Vinciotti, Henshaw, Hodgson. Coe, Tyler,. Sumpter (2010). EHP. 119:306–311*

*Endocrine Disruption in Wildlife*

# *The Evidence in Nature*

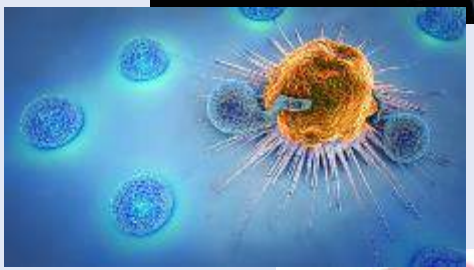


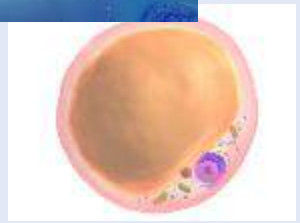


*Images – Charles R. Tyler*

# *Evidence for Endocrine Disruption in Humans*

*Exposure to EDCs during foetal life and/or puberty plays a role in:*





- *Proliferation of male and female reproductive problems,*
- *endocrine-related cancers*
- *Infections,*
- *Asthma*
- *Obesity,*
- *Diabetes,*
- *Behavioural and learning disorders,*

*WHO (World Health Organization)/UNEP (United Nations Environment Programme). 2013. The State-of-the-Science of Endocrine Disrupting Chemicals - 2012 (Bergman Å, et al) : http://www.who.int/ceh/publications/endocrine/en/index.html [accessed 12 March 2013].*

# *Roles of Oestrogens in the Body*

## ***Oestrogens mediate:***

- Female (and male) reproduction
- Growth
- Bone development
- Calcium Homeostasis
- Defence (Immune function)

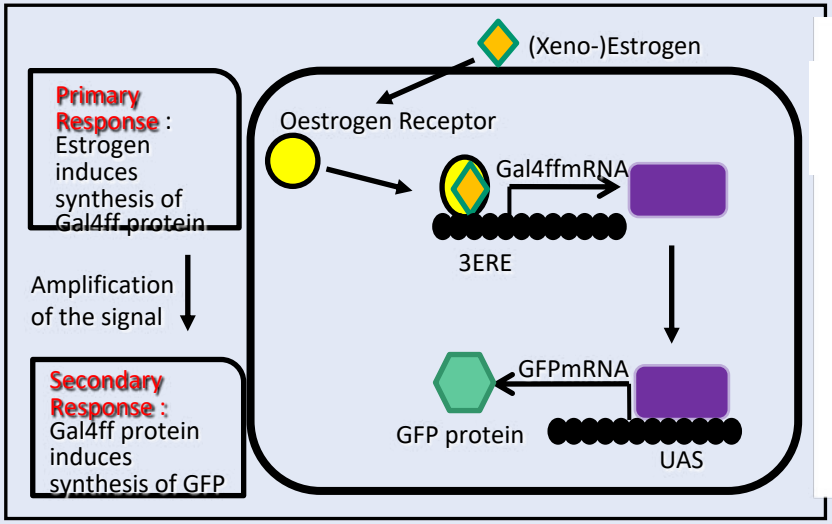
## ***Oestrogens signalling:***

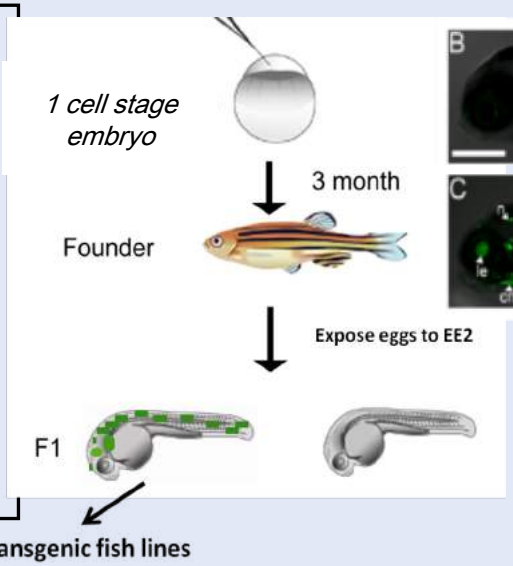
- Via specific receptors
  ( 3 subtypes in fish- ESR1,
  ESR2$\alpha$, ESR2$\beta$)
  (+ membrane receptors, oestrogen
  related receptors)
- Cross talk

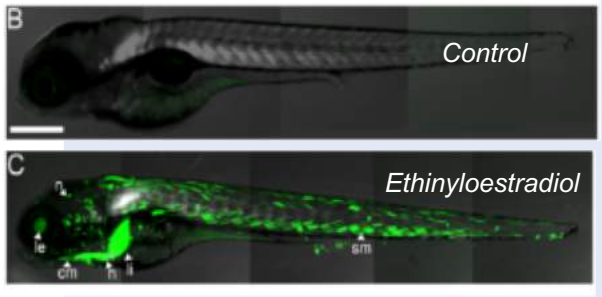
*Every cell in the body probably has an oestrogen receptor!*

*For exposures to chemical that mimic oestrogens there are much wider potential health effects that those on reproduction.*

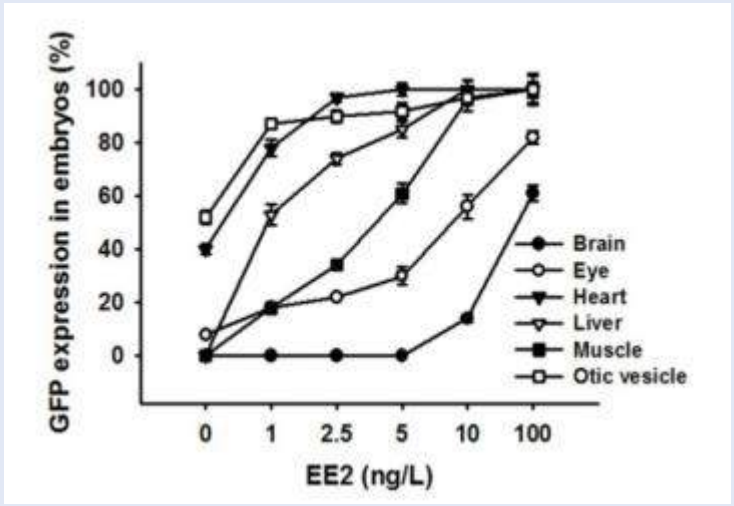
# *ERE-GFP Zebrafish - Assessing Environmental Oestrogens*



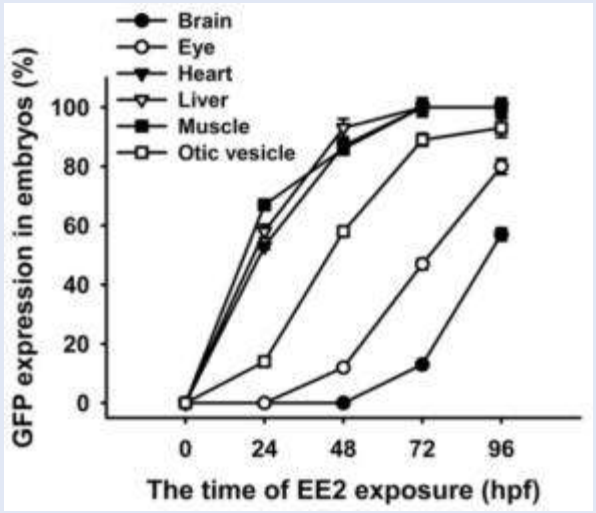




*Oestrogens have wide ranging functions – every cell in the body probably has an oestrogen receptor!*



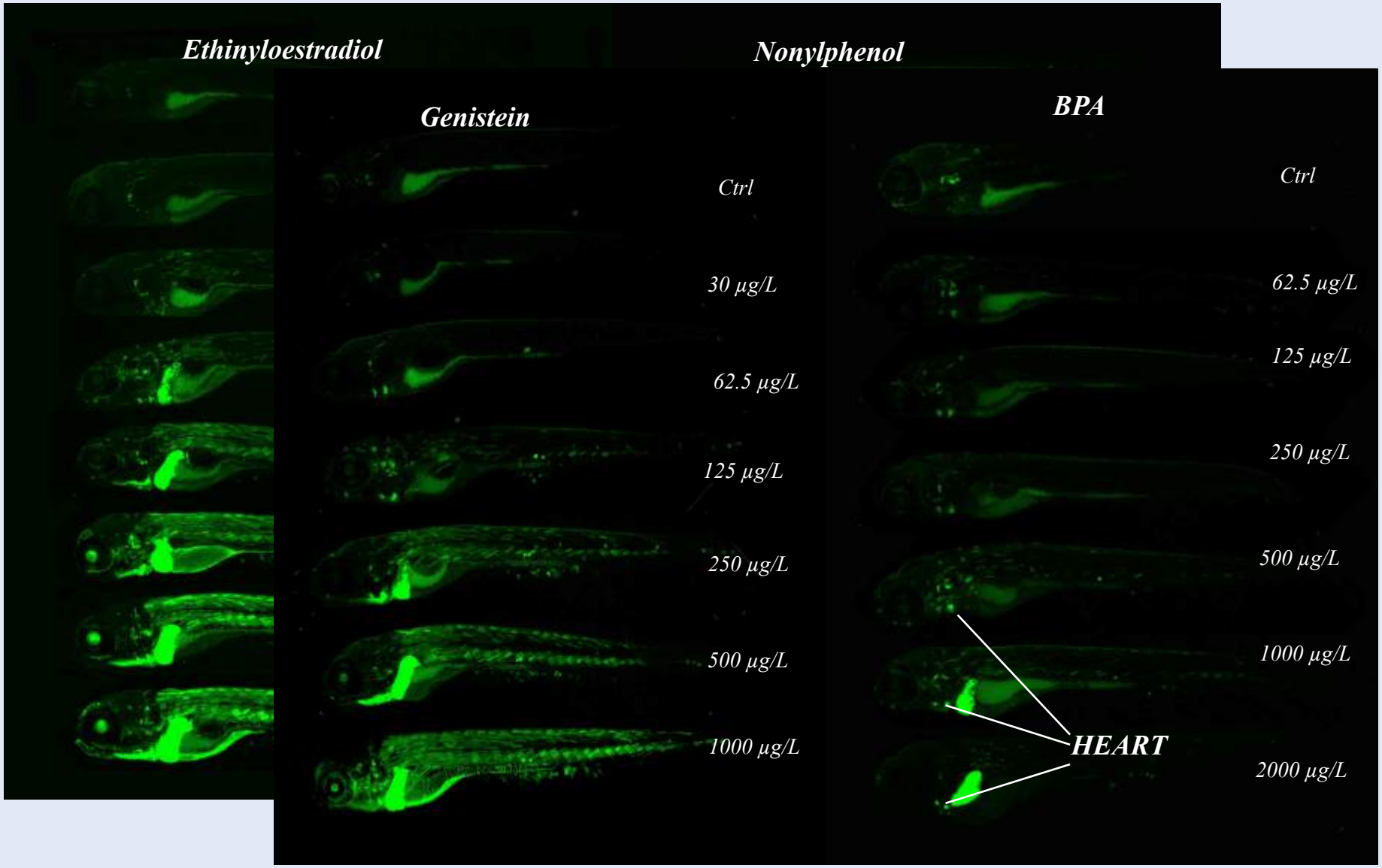

*Sensitivities in responding body tissues (96h)*




*Temporal dynamics in responding body tissues*

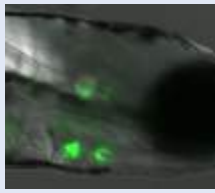
Okhyun Lee, Aya Takensano, Masa Tada, Charles R. Tyler and Tetsuhiro Kudoh (2012) *Environ Health Perspect.* ***120****, 990-996*

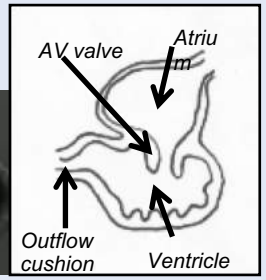
# Response patterns (different target tissues) in ERE-Transgenic Zebrafish



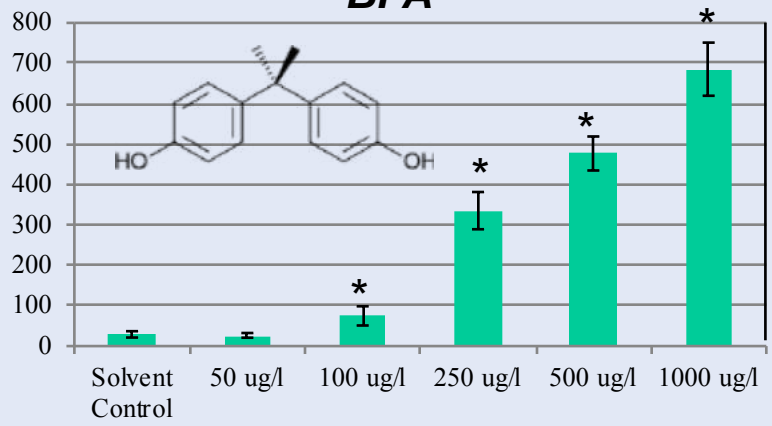

Green, Metz, Lee,Trznadel, Takesono,, Brown, Owen, Kudoh, Tyler (2016). Environ Sci & Technol 50 (12) 6536–6545

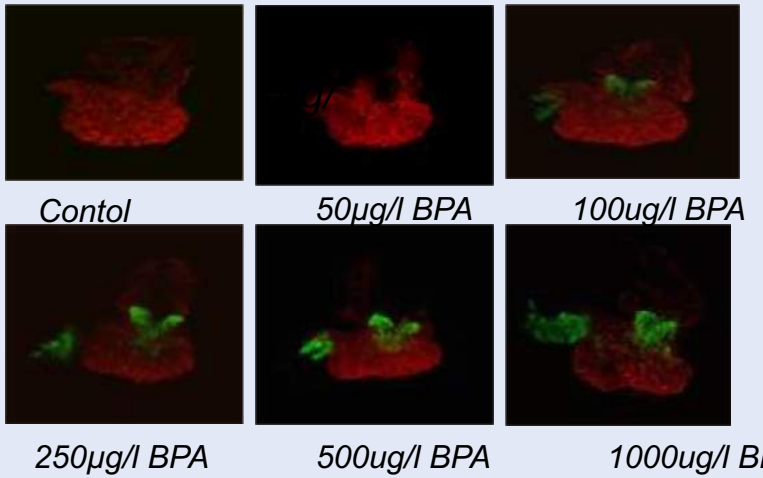
# GFP Expression in the Heart in ERE-Transgenic Zebrafish exposed to Bisphenol A and its metabolic product 4-methyl-2,4 -bis(4-hydroxyphenyl)pent-1-ene [MBP]



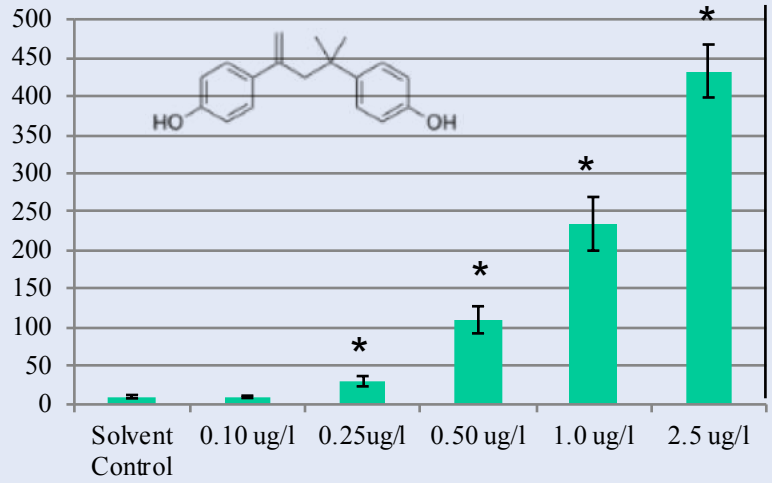


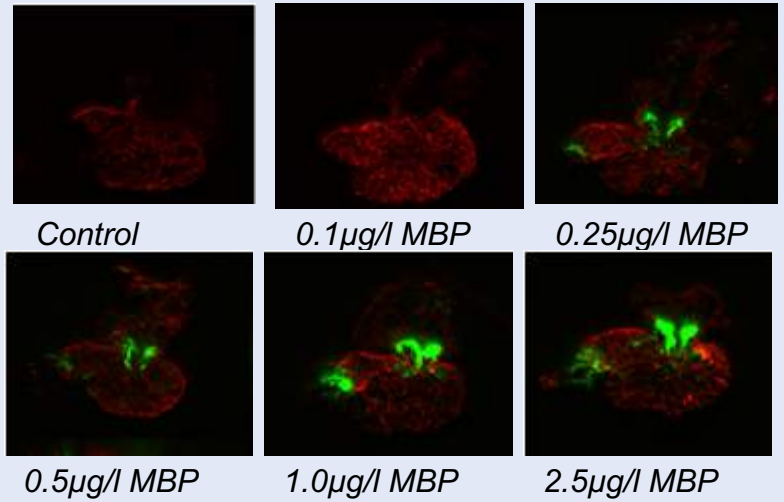

## BPA




## MBP




*Fluorescence intensity values measured from hearts of ERE-TG zebrafish (5dpf) $p<0.05$ compared to control*

Moreman, et al. (2018) Environ Sci Technol 52(11):6656-6665. doi: 10.1021/acs.est.8b01095

GFP, MF-20

# *Mixture Effects*

## *Humans and Wildlife are exposed to complex mixtures of chemicals*

***Babies are exposed to hundreds of industrial chemicals even before birth.*** Cord blood (North American general population);

Polybrominated biphenyl ethers (PBDEs);
Polychlorinated biphenyls (PCBs ); Organochlorine pesticides ;
Polyaromatic hydrocarbons (PAHs);
Polychlorinated dioxins and furans (PCDD/PCDFs).
Phthalates

***Fish bodies are blueprints of their chemical surroundings:***
*Testes content in fish exposure to WwTW effluents*

***NSAIDs*** *(diclofenac, ibuprofen, naproxen, mefenamic acid)*

***SSRIs*** *(fluoxetine, norfluoxetine, paroxetine, sertraline, norsertraline, citalopram, venlafaxine)*

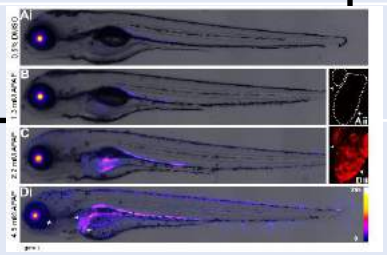
***EDCs*** *(bisphenol A, triclosan, chlorophene, propiconazole)*

***Other xenobiotics*** *(nordiazepam, clozapine, norclozapine, quetiapine, norquetiapine, warfarin, carbamazepine, propranolol, gemfibrozil, clopidogrel*

*David, A., Abdul-Sada , A; Al-Salhi ,R.,Lange, A., Tyler, C.R., Hill E.M. (2014).. J. Chromatography A 1365, 72-85.*
*David, Arthur, Lange, Anke, Abdul-Sada, Alaa, Tyler, C.R and Hill, Elizabeth M (2016) . Env Science & Technol. 51 (1). 616-624.*
*Arthur David, Anke Lange, Charles R. Tyler, Elizabeth M. Hill (2018). Sci. Tott Env, 621, 782-790*

# *Other models for (multiple) chemicals effects MoAs studies studies*

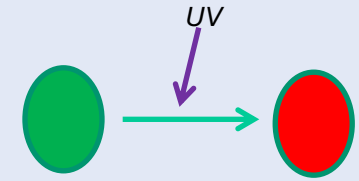
| | | |
|---|---|---|
| ***Oestrogen biosensors*** | Tg[ERE:GFP] | Live imaging of oestrogen responses |
| | Tg[ERE:GFP] Casper | |
| | Tg[ERE:mCherry] | |
| | Tg[ERE:mCherry];Tg[Cyp19:GFP] | ERE and Cyp19 double TG |
| | Tg[Cyp19:GFP] | Live imaging of aromatase gene induction |
| | Tg[ERE:Kaede] | Timing dependent oestrogen responses |
| | Tg[ERE:GCamp6m] | Ca++ responses of oestrogen responsive brain cells |
| | Tg[ERE:Zebrabow] | Cell lineage tracing of oestrogen responding cells |
| ***Oxidative stress/immune biosensors*** | Tg[EpRE:mCherry] | Live imaging of oxidative stress responses |
| | Tg[EpRE:mCherry];Tg[ERE:GFP] | EpRE and ERE double TG |
| | Tg[EpRE:mCherry];Tg[mpx:GFP] | EpRE and neutrophil double TG |



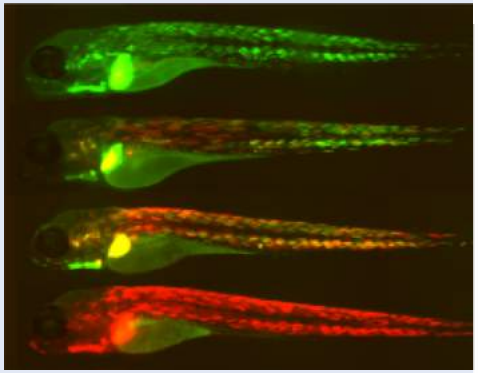
# *Mixtures Effects - repeat exposures*

## *Sensitivity to oestrogen is enhanced for repeated exposures.*

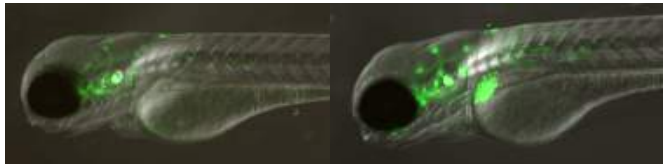
*ERE-Kaede Casper Model*



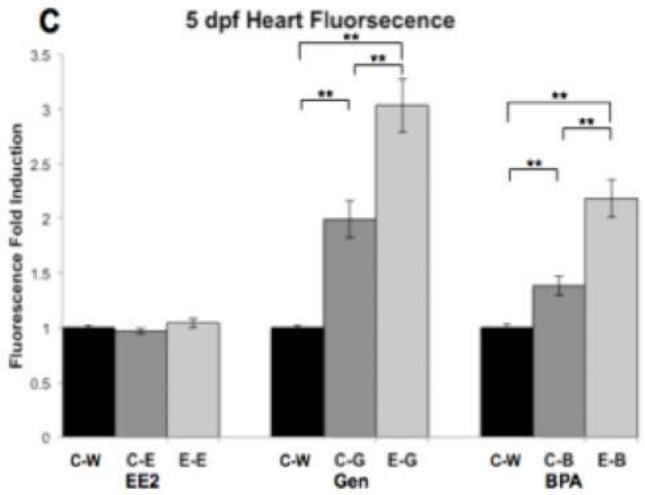

*Kaede: Japanese maple*

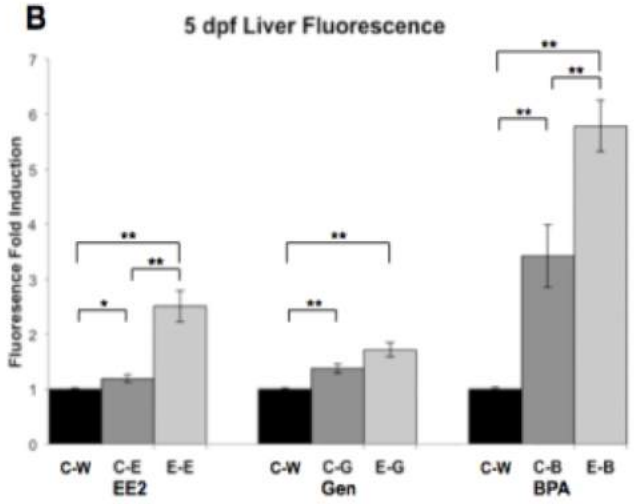


*Control (non exposed) larvae and larvae exposed initially to 10 ng EE2/L over the period of 48h (0-2 dpf) were imaged at 3 dpf (A) and the Kaede response was then converted fully from green to red fluorescence via UV exposure*



***C- EE2*** ***EE2 –EE2***




***Quantification of target tissue responses in ERE-Kaede-Casper transgenic zebrafish exposed to estrogen treatment, as determined by fluorescence induction.*** *Fluorescence intensity was quantified in liver and heart at 5 dpf. Responses were quantified after EE2, genistein and BPA exposures for 3-5 dpf. Data are reported as mean fold induction ± SEM (n=18). EE2-10ng/L, Genistein- 500μg/L, BPA- 2000μg/L)*

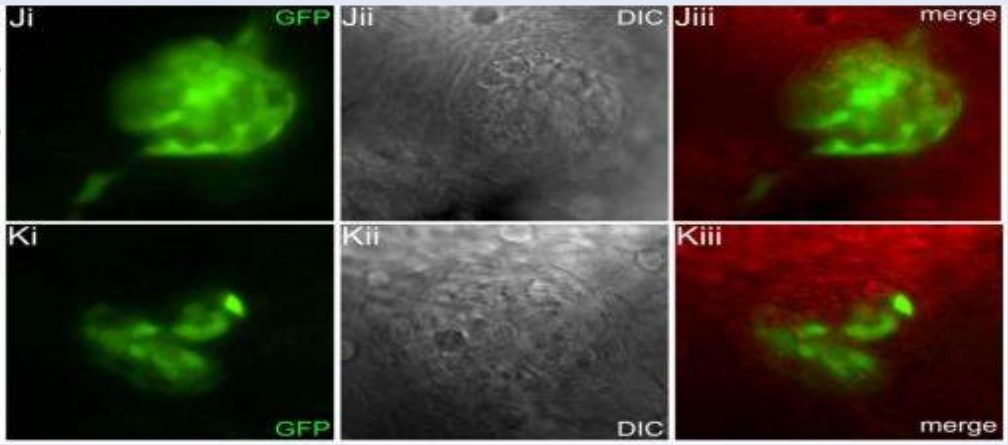
*Green, Lange, Scott, Trznadel, Wai, Takesono, Brown, Owen, Kudoh, Tyler (2018). Scientific Reports 8 Article number 2699*

# ***Neural /Endocrine Interactions ERE-TG zebrafish***

## *(responding neural tissues)*

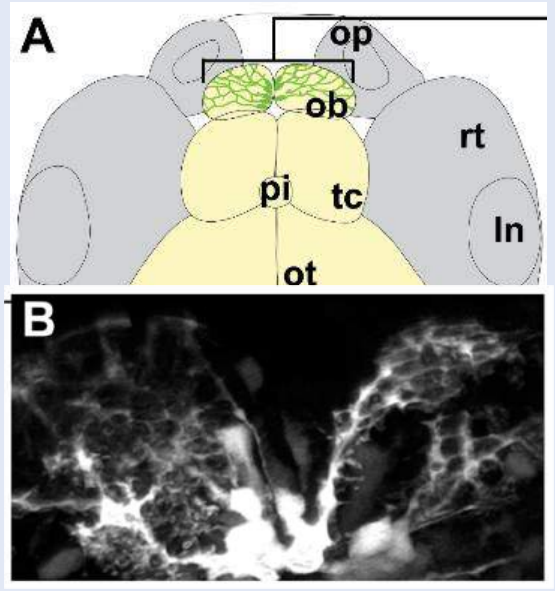
*ear ganglion*

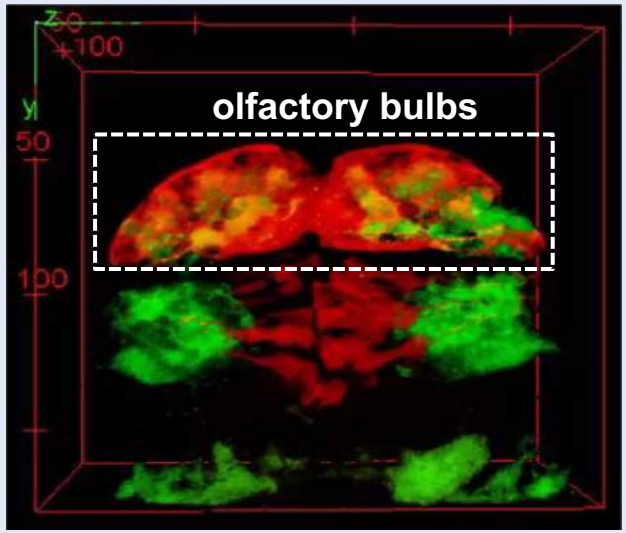
*neuromast*




Peripheral neural tissue

Effects relating to olfaction/sensing…….and behaviours



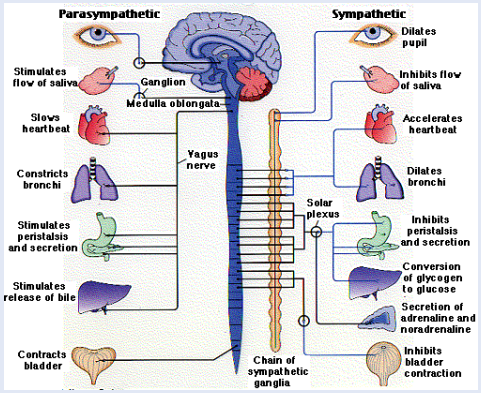



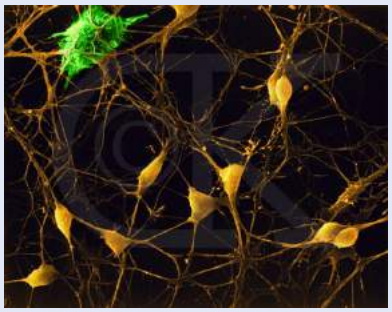

**Oestrogen responding cells in olfactory bulb (ER-OB cells) in zebrafish embryos**

***Aya Takesono**, Paula Schirrmacher, Aaron Scott, Jon M. Green**,** Okhyun Lee, Matt Winter, Olivier Kah, Tetsuhiro Kudoh and Tyler, C.R. (2020). Estrogen regulates early embryonic development of the olfactory sensory system via estrogen-responsive glia **Submitted***

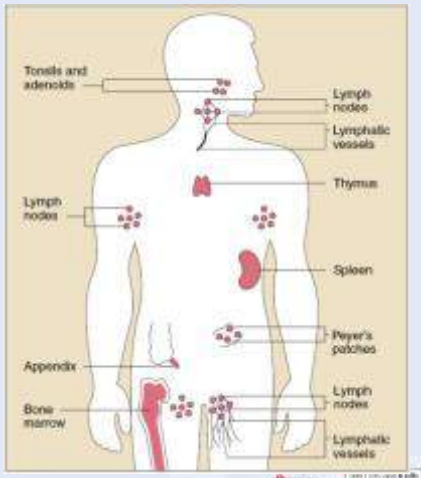
# *Chemical disruption of communication pathways in the body*

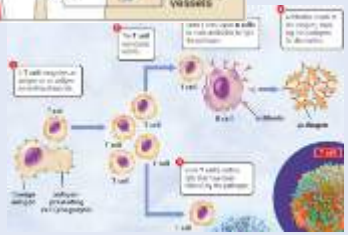
*The nervous, endocrine and immune systems are highly interconnected*



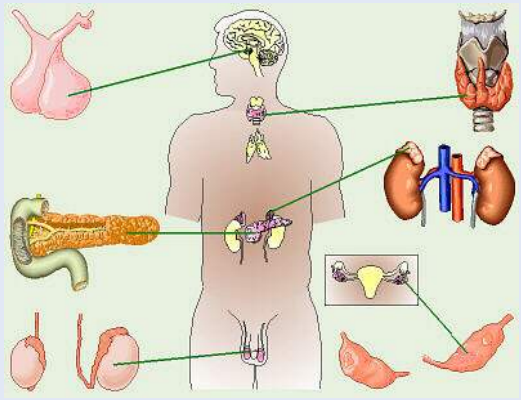



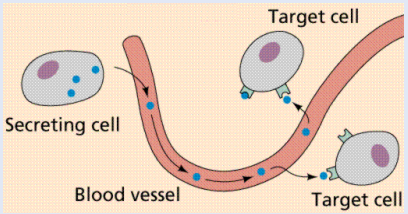
Nervous- *telephone network*



Immune system

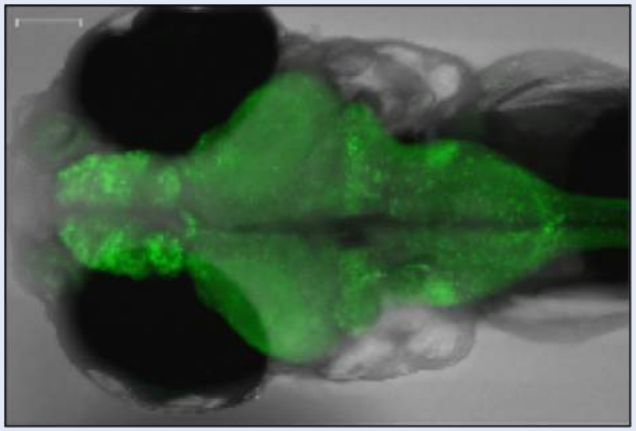





Endocrine- *postal system*

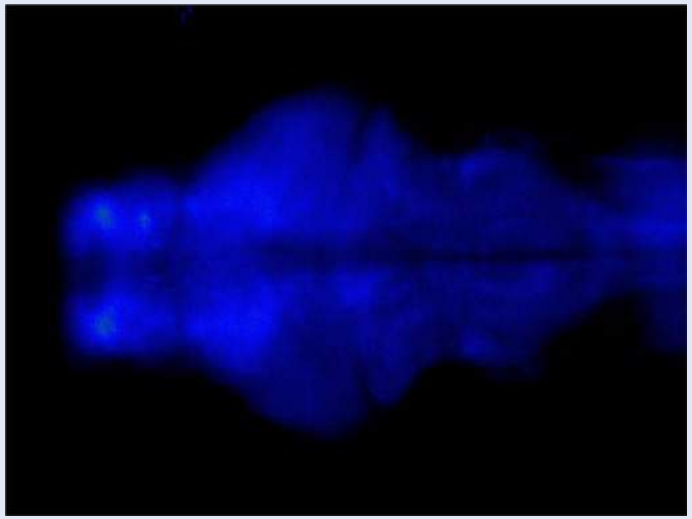
*A hormone is a chemical message that instructs a specific response*

# *Monitoring for chemical effects on the brain- linking with behaviour phenotypes*

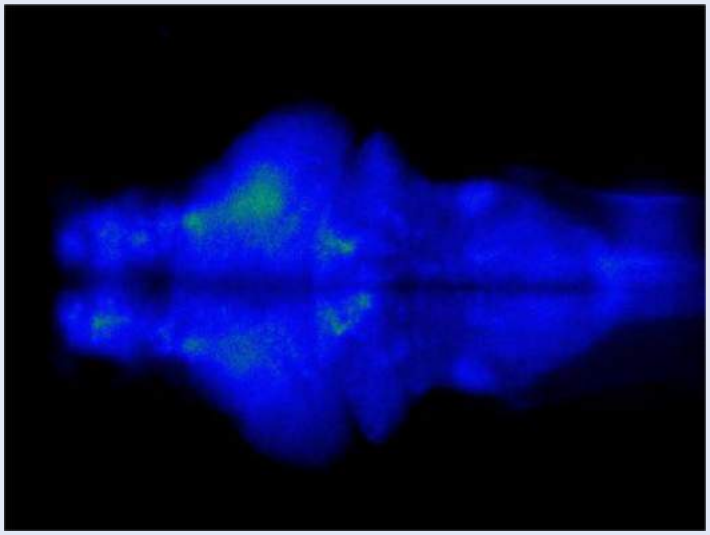


*elavl3:GCaMP6s larvae*

*(originally obtained from Misha B. Ahrens, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, Virginia, USA)*



***Untreated control***



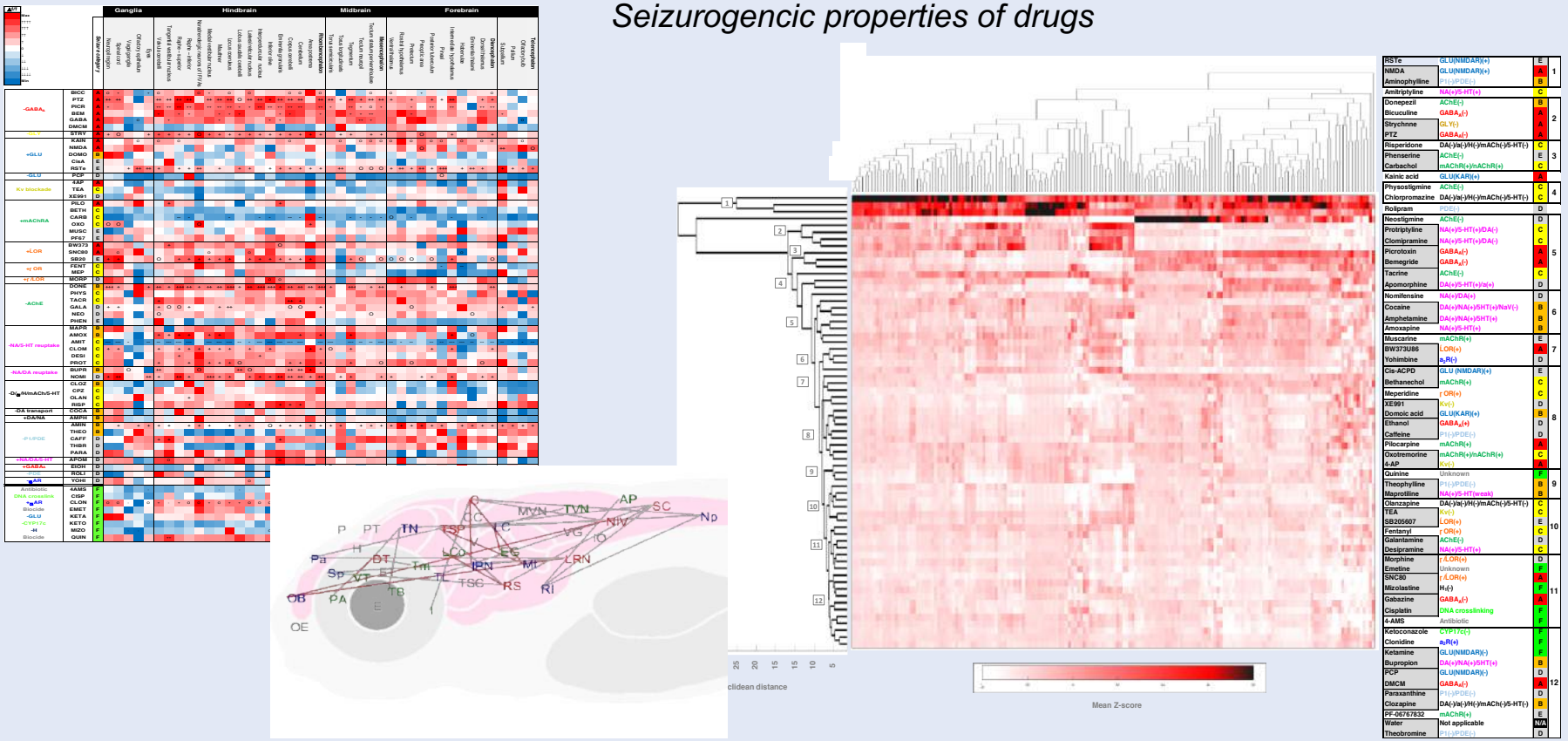
***4-AP treated***

*Winter; MJ, Windell, D., Metz, J., Matthews, P., Pinion, J., Brown, J., Hetheridge, M., Ball, J., Owen, S, Redfern, W., Moger, J., Randall, A., Tyler, C.R (2017).. **Scientific Reports**. DOI:10.1038/s41598-017-06646-6*

# *Monitoring for chemical effects on the brain- linking with behaviour phenotypes*

*Functional brain imaging in larval zebrafish reveals seizure propensity and pharmacological mechanisms of action of neuroactive chemicals.*

*Seizurogencic properties of drugs*

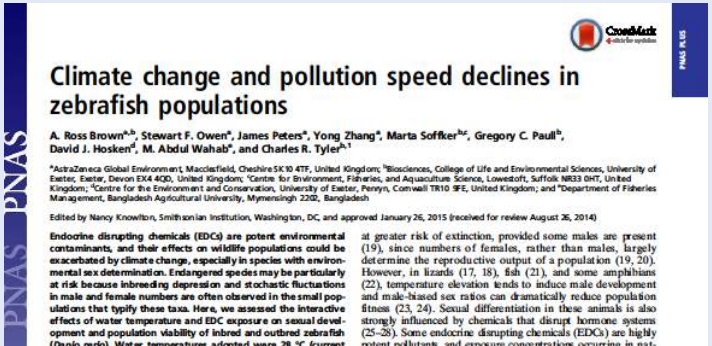



*Winter, MJ., Pinion, J., Tochwin, A., Takesono, A.,. Ball, J., Grabowski, P., Metz M., Trznadel, M., Tse, K., Redfern, WS, Hetheridge, MJ, Goodfellow, M, . Randall, , AD, Tyler , CR (2020). In review* ***British Journal of Pharmacology***

# *A few concluding thoughts*

- *Pollution is a major environmental threat to human and wildlife health.*
- *Chronic exposure effects and over generations are poorly established*
- *Effect analyses need to better consider effects for environmental relevant exposures (incl. timing, mixtures, interactions with other stressors etc.)*
- *Transgenic fish combined with imaging can provide advanced models for understanding where and how toxicants work in the body for informing more integrative approaches in health effects assessments.*
- *We (humans) are not that much different from fish – that provide us with sentinels for potential impact of chemicals on our own health!!*

*Brown, A.R., et al, 2015.* ***PNAS***
*DOI:10.1073/pnas.1416269112*



PNAS PNAS PLUS

Climate change and pollution speed declines in zebrafish populations

A. Ross Brown[a,b], Stewart F. Owen[a], James Peters[a], Yong Zhang[a], Marta Soffker[b,c], Gregory C. Paull[b], David J. Hosken[d], M. Abdul Wahab[e], and Charles R. Tyler[b,1]

[a]AstraZeneca Global Environment, Macclesfield, Cheshire SK10 4TF, United Kingdom; [b]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, Devon EX4 4QD, United Kingdom; [c]Centre for Environment, Fisheries, and Aquaculture Science, Lowestoft, Suffolk NR33 0HT, United Kingdom; [d]Centre for the Environment and Conservation, University of Exeter, Penryn, Cornwall TR10 9FE, United Kingdom; and [e]Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Edited by Nancy Knowlton, Smithsonian Institution, Washington, DC, and approved January 26, 2015 (received for review August 26, 2014)

**Endocrine disrupting chemicals (EDCs) are potent environmental contaminants, and their effects on wildlife populations could be exacerbated by climate change, especially in species with environmental sex determination. Endangered species may be particularly at risk because inbreeding depression and stochastic fluctuations in male and female numbers are often observed in the small populations that typify these taxa. Here, we assessed the interactive effects of water temperature and EDC exposure on sexual development and population viability of inbred and outbred zebrafish**

at greater risk of extinction, provided some males are present (19), since numbers of females, rather than males, largely determine the reproductive output of a population (19, 20). However, in lizards (17, 18), fish (21), and some amphibians (22), temperature elevation tends to induce male development and male-biased sex ratios can dramatically reduce population fitness (23, 24). Sexual differentiation in these animals is also strongly influenced by chemicals that disrupt hormone systems (25–28). Some endocrine disrupting chemicals (EDCs) are highly

# *Acknowledgements, Sponsors and Collaborators of the work presented:*


*The Contributing Team:*

*Aya Takesono,*
*Okhyun Lee,*
*Matthew Winter,*
*John Moreman,*
*Jon Green,*
*Ruth Cooper,*
*Sulay Mourabit,*
*Dylan Windell,*
*Rebekah Boreham*
*Charles Hamilton*
*Sophie Gould*
*Ross Brown,*
*Malcolm Hetheridge,*
*Jon Ball*
*Maciek Trznadel*
*Tetsuhiro Kudoh,*
*Patrick Hamilton,*
*Anke Lange*

*Max-Planck-Institut für Neurobiologie*
*Herwig Baier*

*University of Rennes*
*Olivier Kah*

*INERIS*
*Francois Brion*

*University College London*
*Masa Tada*
*Steve Wilson*

*Technische Universität Braunschweig*
*Reinhard Köster*

# Thank you for listening

Image: Charles R. Tyler

Image: Charles R. Tyler

# Questions?