How oestrogenic is the contraceptive pill to fish?

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10th ISRPF, Olhao Portugal, May 2014

UNIVERSITY OF LIVERPOOL

UNIVERSITY OF BIRMINGHAM

Cefas

Systems biology in toxicology

"This is a very dysfunctional company. No one will talk about the elephant in the boardroom!"

- Toxicological evaluation in general is confined to the assessment of chemical safety on an individual basis
- In reality neither humans nor wildlife are exposed to a single chemical at any time
- Testing of various chemical combinations at various concentrations is impossible
- Can systems toxicology predict mixture toxicity to aid chemical risk assessment in the aquatic environment?

Cefas

Data Management

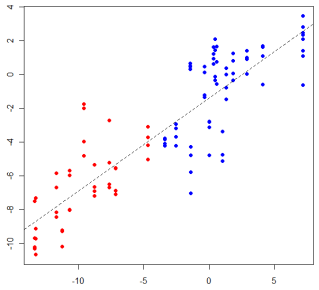
Chemicals

PC Features

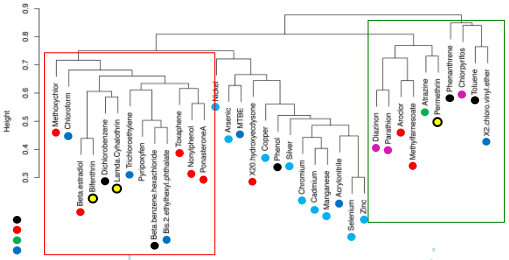
Omics Data

Physiology Toxicology

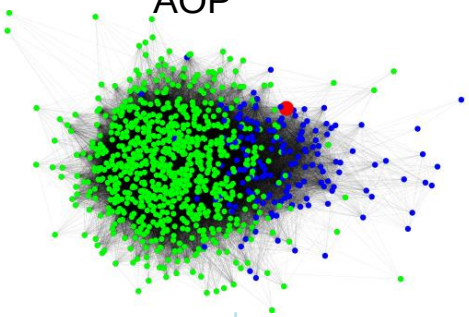
Multi-Level QSAR



Molecular Classification



AOP



NPs PCFs

Key representative NPs

AOP Modules

Identifying links between Molecular-Physiological Response and NPs PCFs

Time course Concentration response

Dynamical Models of NP Response

$$\dot{x}_i = \sum_{j=1}^{N} w_{ij} x_j + b_i x_i$$

$$E^{SQE} = \sum_{i=1}^{N} \sum_{t} \left(x_i^{measured} - x_i \right)^2$$

$$E^{Object} = \sum_{j} \sum_{t} \left(o_{ij} - w_{ij} \right)^2$$

efas

defra Department for Environment Food and Rural Affairs

The use of systems toxicology to reconstruct molecular pathways of adverse outcome

•Identify a priority list of 10 chemicals that will undergo extensive testing and construct/archive a toxicogenomic data set relevant to these chemicals by mining existing datasets

•To determine molecular signatures induced by these chemicals in the 3-spined stickleback

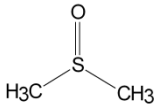
•Identify non-additive molecular responses in chemical mixtures exposures

•To determine the physiological effects of chronic exposure to single and mixed chemicals

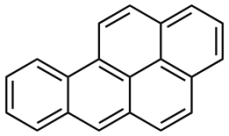
•Develop a computational model linking molecular targets of individual chemicals to non-additive molecular and physiological responses in mixture exposures

Cefas

The ten model chemicals acute-chronic (µg/L)



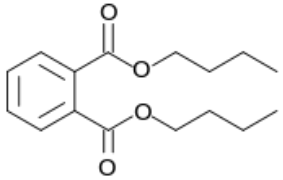
DMSO: 88-8.8 mg/l (0.008%-0.0008%)
Solvent



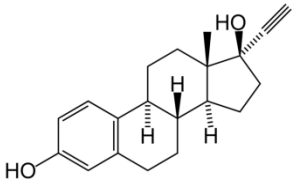
Benzo(a)pyrene: 10-1µg/l
PAH
LC50: 1200, HEC: 96 µg/l

Cd^{2+}

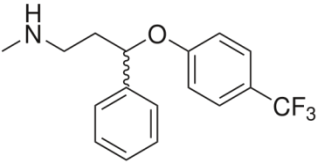
Cadmium: 65-6.5µg/l
Heavy metal
LC50: 6500, HEC: 4000 µg/l



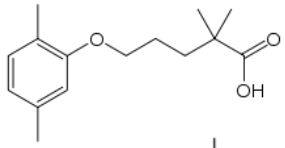
Dibutyl phthalate: 35-3.5µg/l
Plasticizer
LC50: 350, HEC : 170 µg/l



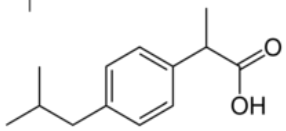
Ethinyloestradiol: 0.04 -0.004µg/l
Endocrine disrupter
LC50: 1600, HEC 0.04 µg/l



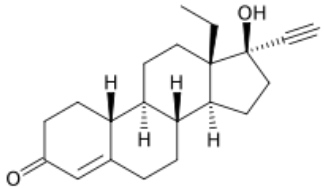
Fluoxetine: 10µg/l-1µg/l
SSRI antidepressant
LC50: 700, HEC 1 µg/l



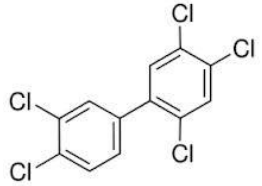
Gemfibrozil: 50-5µg/l
Fibrate
LC50: 22000, HEC : 5 µg/l



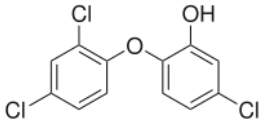
Ibuprofen: 50-5µg/l
Painkiller
LC50: 7100, HEC : 28 µg/l



Levonorgestrel: 0.05-0.005µg/l
Progestin
LC50: 6500, HEC : 0.015 µg/l



PCB-118: 1-0.1µg/l
PCB
LC50: 15 µg/l, HEC : 123 µg/kg (sed)



Triclosan: 20-2µg/l
Antibacterial/fungal
LC50: 260, HEC : 5 µg/l

LC50: Lowest found for stickleback or most sensitive fish species.
HEC: Highest environmental concentration found

Cefas

Experimental design

female

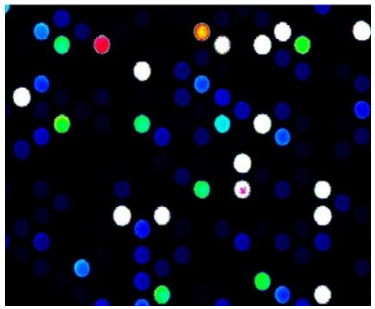
Stickleback Chemical Exposures

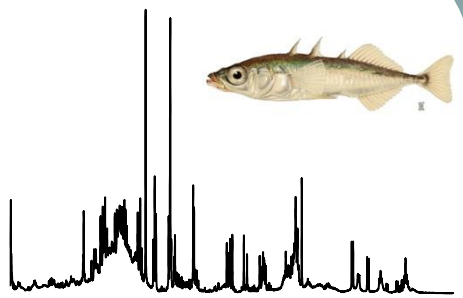
male

Individual Chemicals
and
Chemical Mixtures

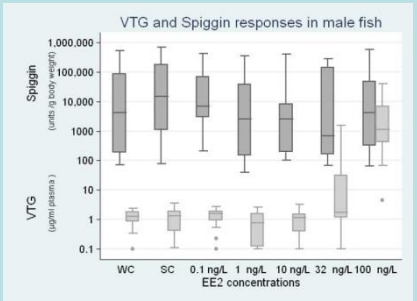
Acute-96h
January 2013

Chronic-12 w
January-April 2014





Transcriptomics:
Hepatic 8x15k Agilent stickleback microarray
Metabolomics:
Hepatic polar and non-polar FT-ICR Mass Spectrometry



Growth, morphology
Steroid levels (sex steroids, cortisol)
Vitellogenin and kidney hypertrophy
Gonadal histopathology
Reproductive behaviour (nest building)

Monster experiments

40 treatments
10 individual chemicals,
2x controls (water/solvent)
25 mixtures of 5 components plus solvent,
1 mixture with all 10 chemicals
All in duplicate tanks = 80 tanks (800 fish)
Fish (laboratory-bred over 12 generations)
10 sticklebacks per tank (mixed sex, pre-breeding)
Exposures
Flow-through (7 changes per day)
Chemical analysis:
4/7 chemicals weekly

Cefas

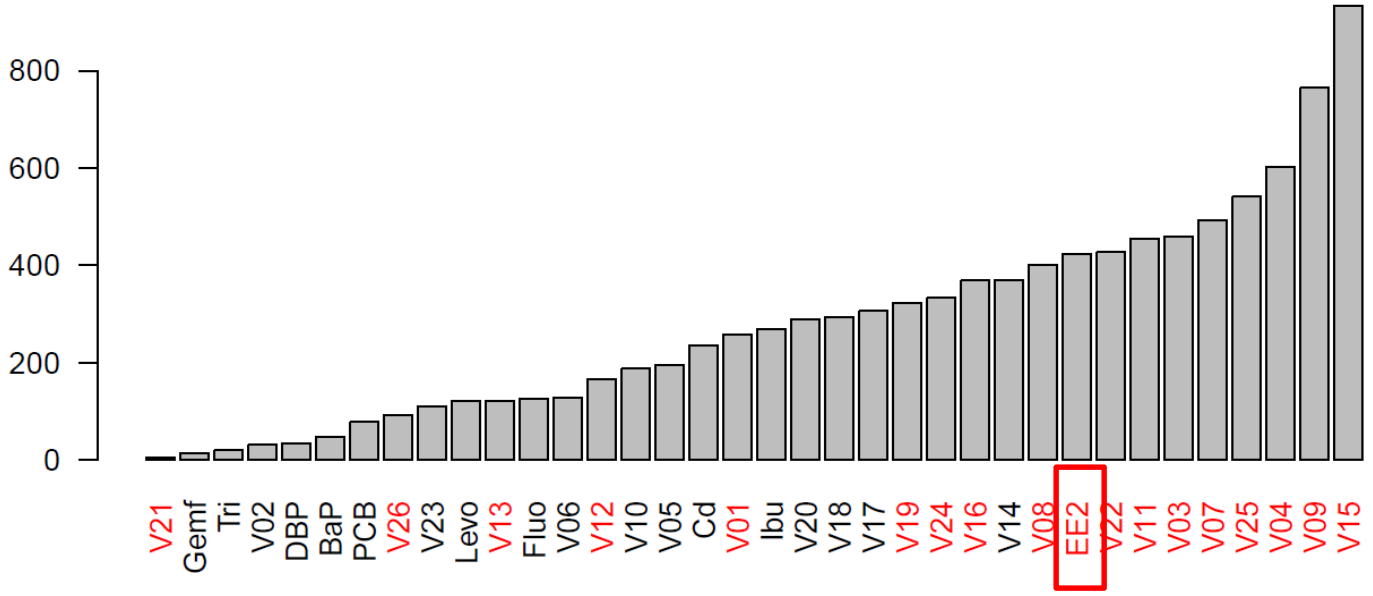
Mixture selection

1) Preferential selection of sets containing the pharmaceuticals Ibuprofen, Ethinyl-oestradiol and Levonorgestrel
2) A balanced combination of these three chemicals (maximising chemical variability),
3) A minimum representation of 3 sets for any compound,
4) Computer-generated combinations, which were checked for re-occuring combinations.

Exposure	WC	SC	BaP	Cd	DBP	EE2	Fluo	Gem	Ibu	Levo	PCB	Tri	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26
Solvent	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Benzo[a]pyrene	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0	1	1	1	0	0	1	0	0	0	1	1
Cadmium	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1	0	0	1	0	1	0	1
Dibutyl phthalate	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	0	1	1	1	1	1	1	1
Ethinyl-oestradiol	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1	1	1	0	1	1	0	0	1	0	1	1	0	1	1	1
Fluoxetine	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	1	1
Gemfibrozil	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1
Ibuprofen	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	0	1	0	0	1	0	1	1	0	0	0	1	1	1	1	1	0	0	1
Levonorgestrel	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1
PCB-118	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1
Triclosan	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	1	0	1	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	1

Cefas

All differentially expressed genes



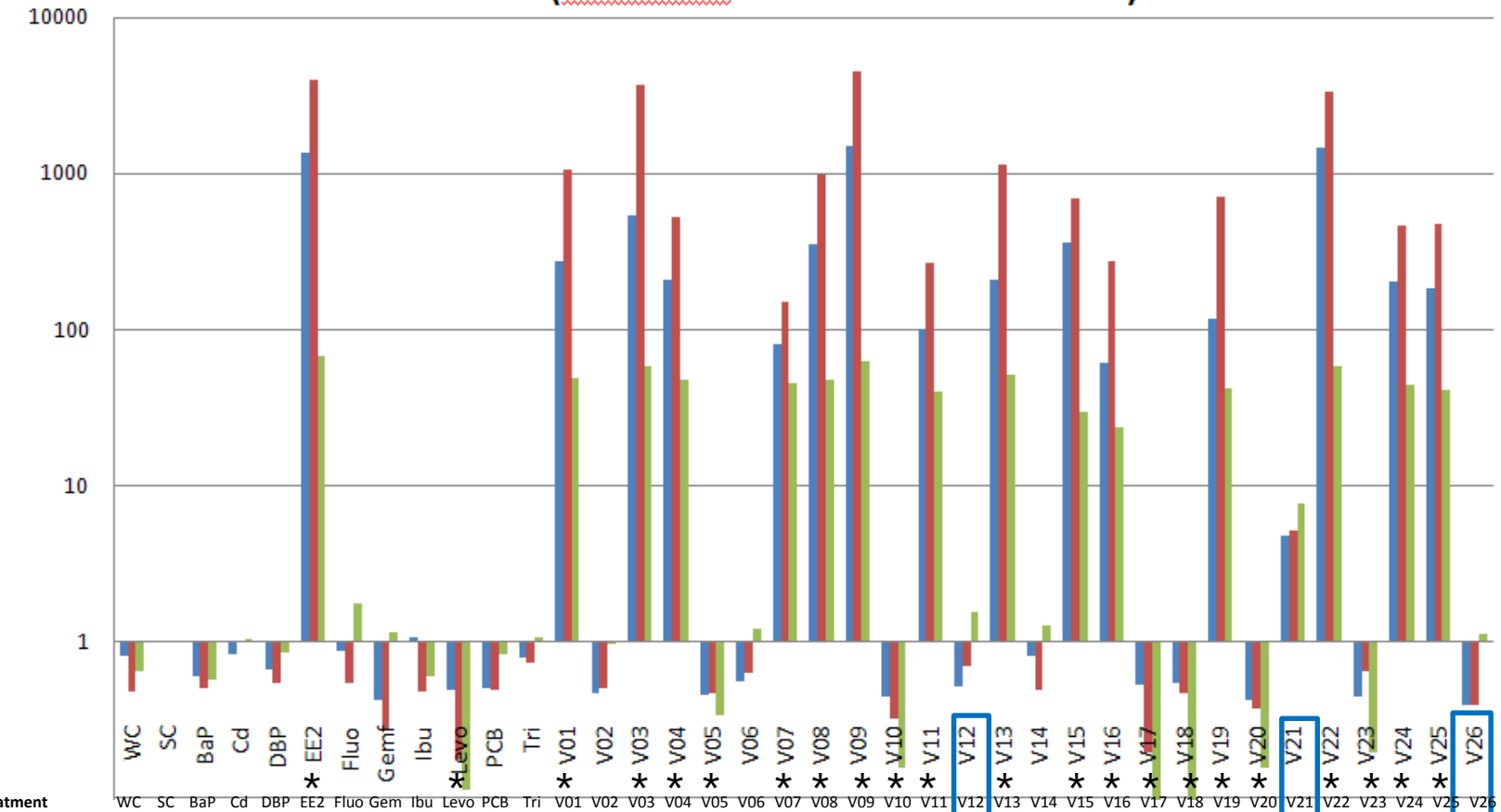
- Number of differentially expressed genes as compared to solvent control for all single and mixture exposures.

- **Exposures marked in red contain EE2, but some mixtures elicit fewer responses than EE2 alone: antagonistic effects?**

Cefas

Fold change in expression of Vitellogenin genes

(normalised to mean of solvent controls)



Treatment	WC	SC	BaP	Cd	DBP	EE2	Fluo	Gem	Ibu	Levo	PCB	Tri	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26
Solvent	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Benzo[a]pyrene	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0	1	1	1	0	0	1	0	0	0	1	1
Cadmium	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1	0	0	1	0	1	0	1
Dibutyl phthalate	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	0	1	1	1	1	1	1	1
Ethinyl-oestradiol	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1	1	1	0	1	1	0	0	1	0	1	1	0	1	1	1
Fluoxetine	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	1
Gemfibrozil	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1
Ibuprofen	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	0	1	0	0	1	0	1	1	0	0	0	1	1	1	1	1	0	0	1
Levonorgestrel	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1
PCB-118	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1
Triclosan	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	1	0	1	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	1

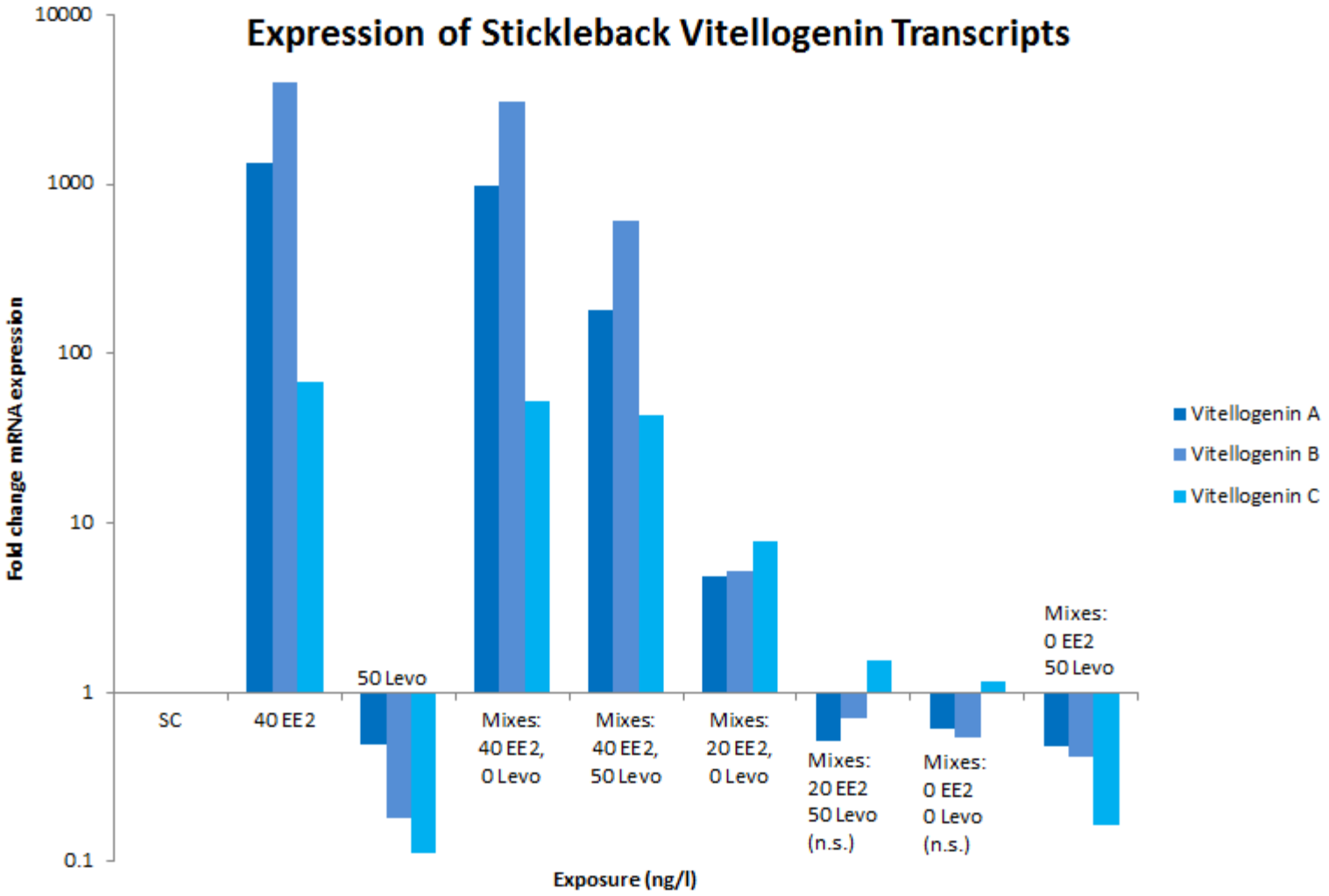
*= FDR<0.05
VTG A, B or C

EE2 20ng/l, B(a)P, Ibuprofen Levonorgestrel, Triclosan

EE2 20ng/l, B(a)P, Ibuprofen, Phthallate, PCB-118

All

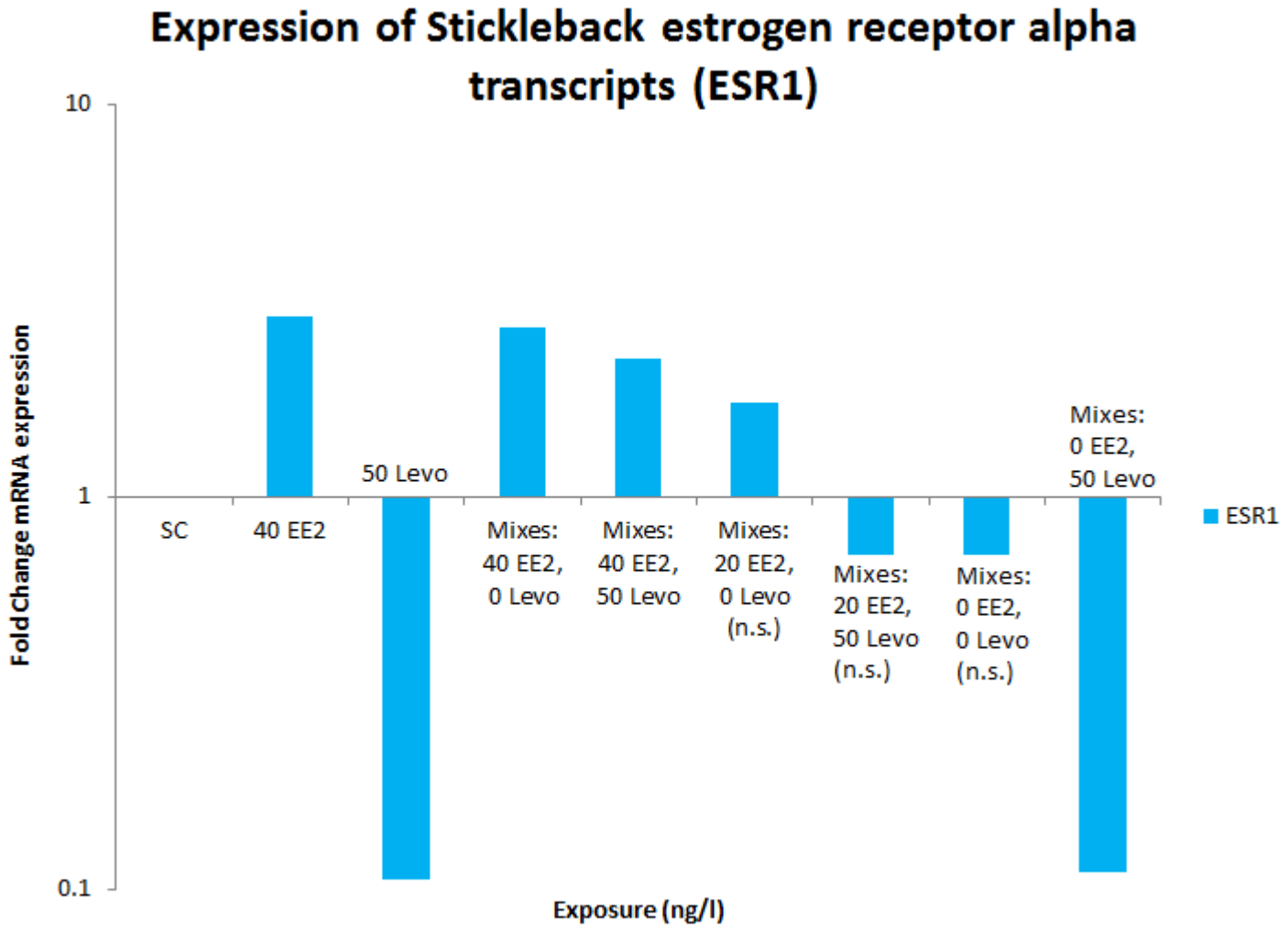
Cefas



Normalised to mean of solvent controls. All changes statistically significant except where noted (n.s.)

EE2 and Levonorgestrel give opposite responses. Each chemical can mask out the effect of the other at specific concentrations

Cefas



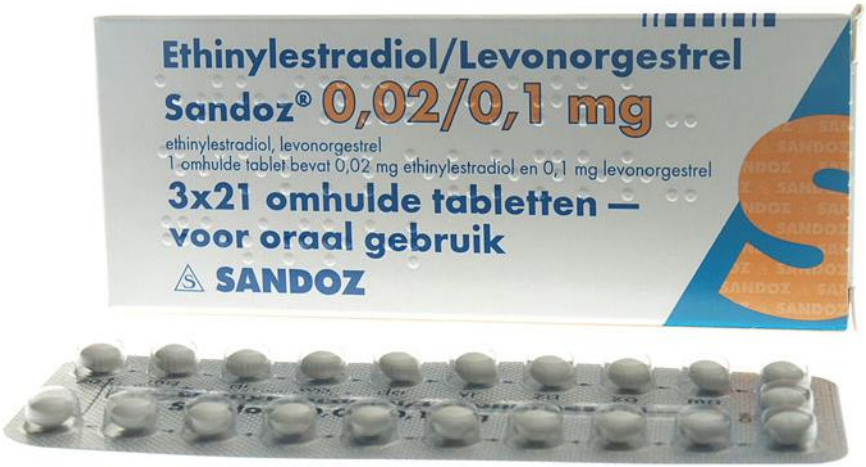
Normalised to mean of solvent controls. All changes statistically significant except where noted (n.s.)

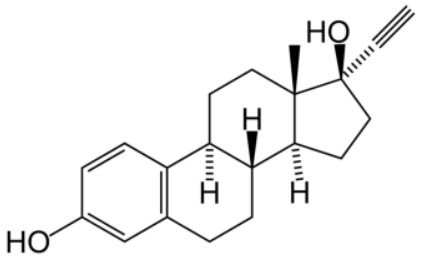
- Levonorgestrel (50ng/l) decreased expression of ESR1, but did not affect expression of androgen receptors so appears to act as an anti-estrogen.
- **EE2 and Levo show opposite effects on ESR1 expression, at a certain concentration of each the response is cancelled out.**

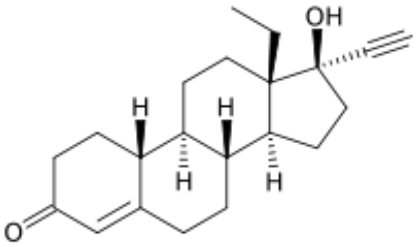
Cefas

First example of mixture effects

- **Active components of many oral contraceptives**





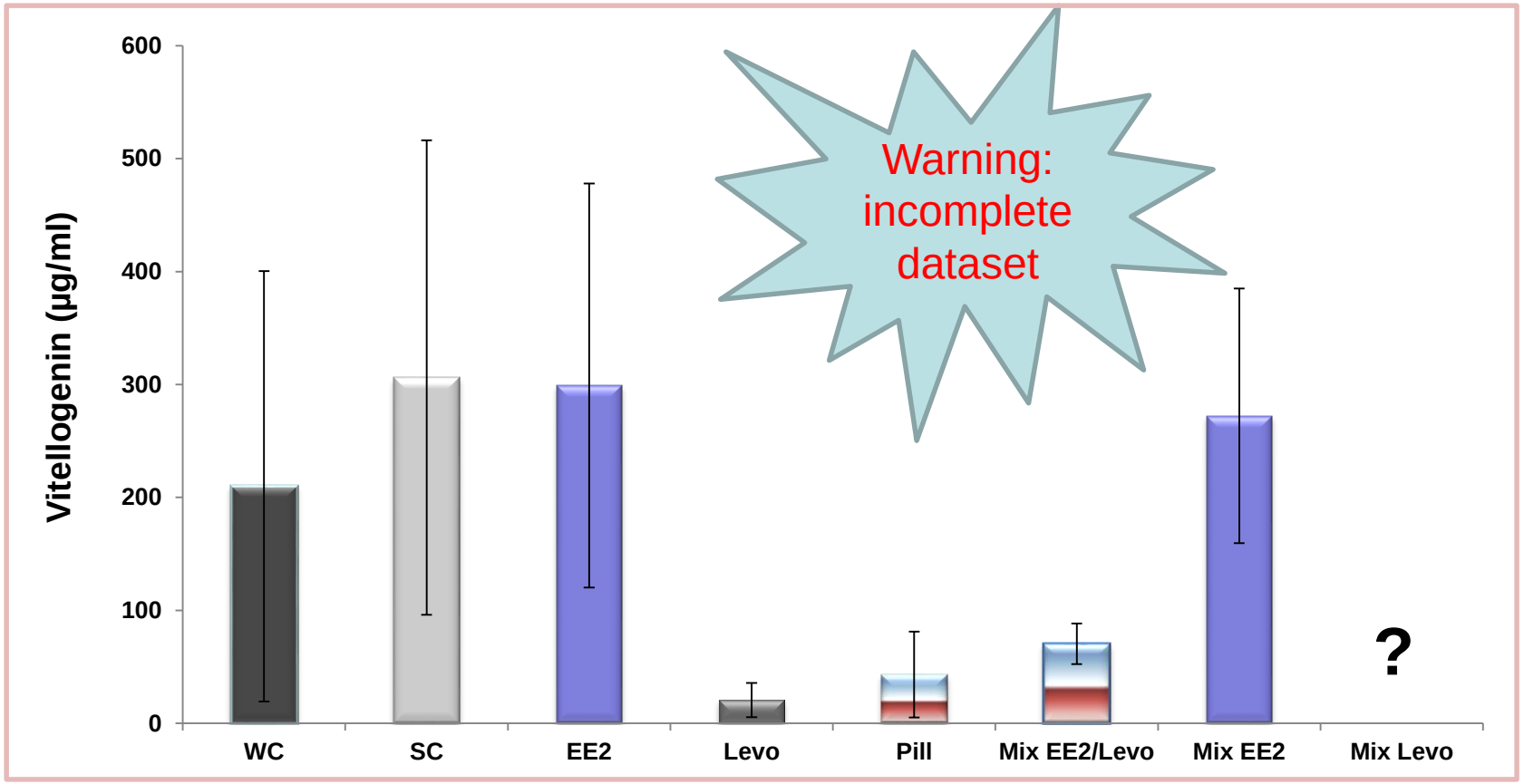


- **The ratio at which they used in contraceptives suggests overall effect is androgenic/anti-oestrogenic!**

- **Both are detected in aquatic environments at ng/l levels, particularly downstream of WWTPs**

Cefas

Chronic exposures-female plasma VTG



Cefas

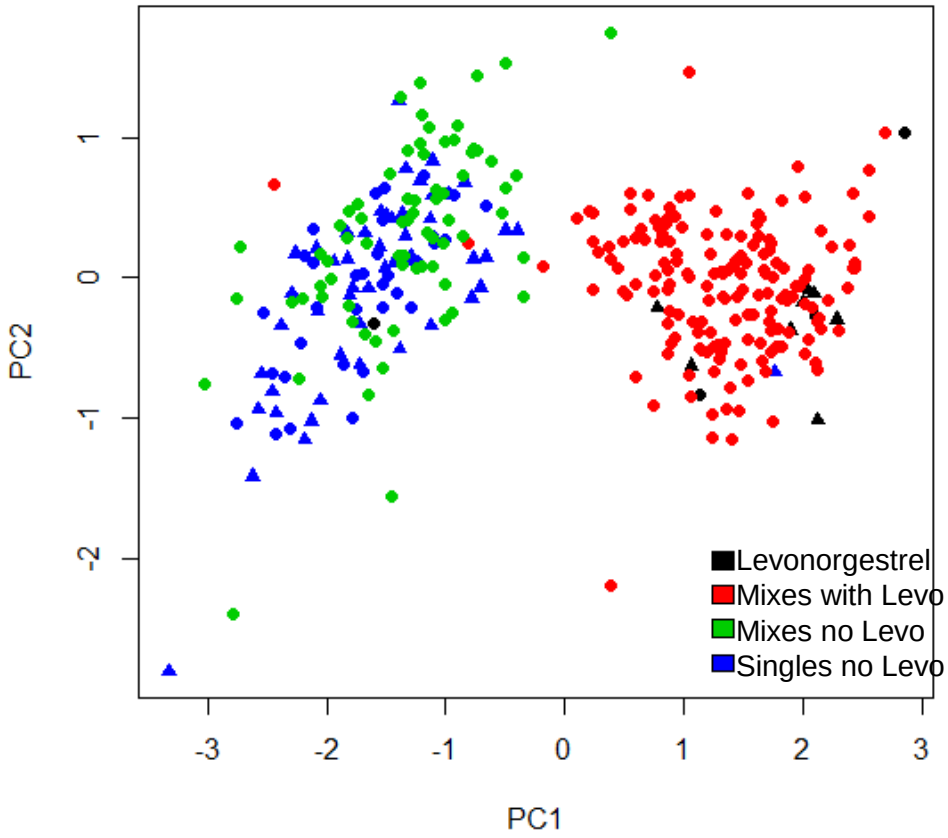
Chemical recovery of selected chemicals during the two exposures (in µg/L)

	Acute exposure (Jan 2013)					Chronic exposure (Jan 2014)					
Chemical	EE2	Tri	Cd	Flu	EE2	EE2	Tri	Cd	Flu	PCB	BaP
Nominal	0.04	20	65	10	0.02	0.004	2	6.5	1	0.1	1
WC	<0.00036	0.0185	0.15	0.2	<0.00036	<0.00036	0.00650	0.1	0.2	<0.001	<0.01
SC	<0.00036	0.022	0.1	0.2	<0.00036	<0.00036	0.00560	<0.1	0.2	<0.001	<0.01
Measured	0.033	3.14	65.90	6.94	0.018	0,0034	1.02	6.38	0.715	0.031	0.087
n (sample)	17	13	13	11	2	21	18	18	14	16	20
% recovery	82.27	15.71	101.38	69.38	90.2	85	51.3	98.13	71.50	31.04	8.73

Cefas

Predictive Modelling Approach

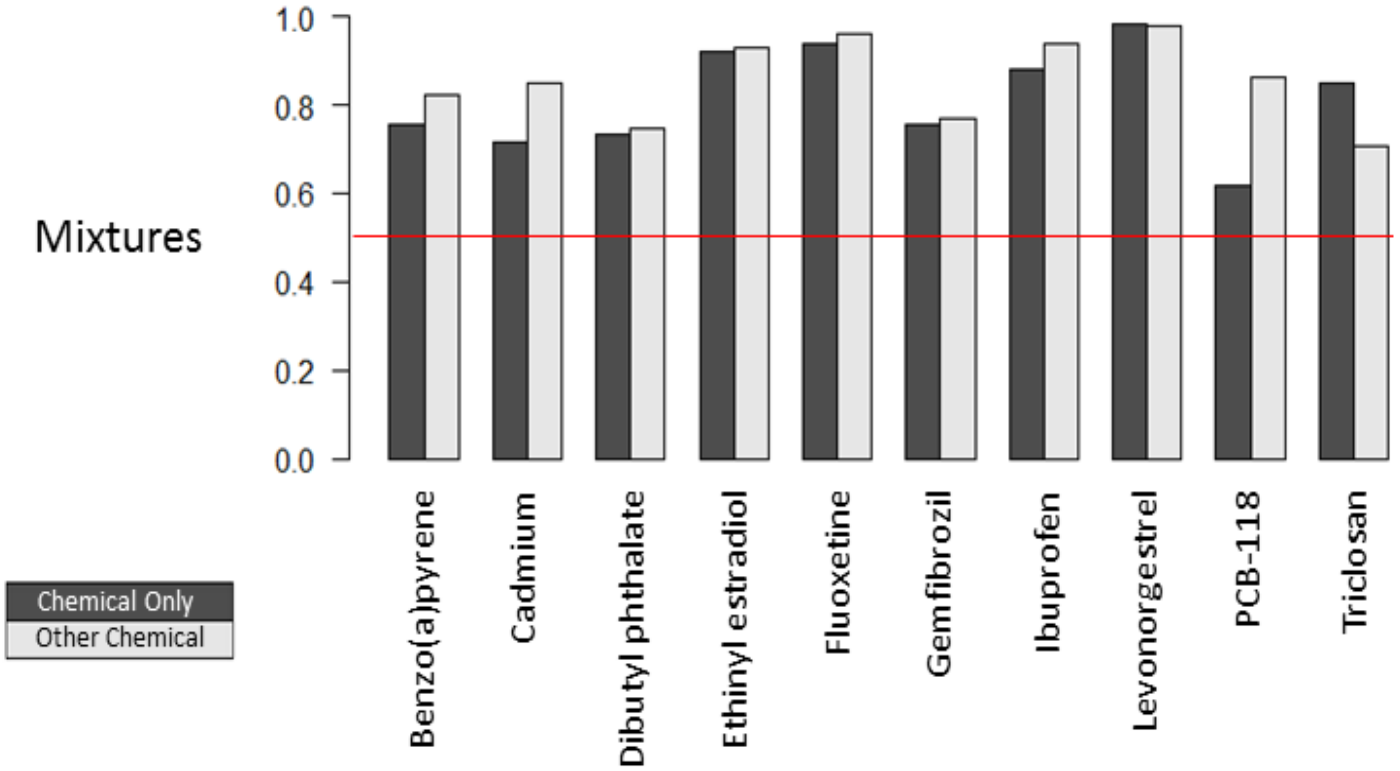
Single + Mixture Data → Genetic Algorithm GALGO → Models optimised for simultaneous prediction of either single or mixture exposure



The genetic algorithm (GALGO) develops models, based on a subset of genes, that discriminate between exposures with or without levonorgestrel.
This model is still effective for mixtures.

Cefas

Model developed to predict presence of individual chemicals within mixtures



Dark grey bars represent the specific accuracy for a specific compound while light grey represents the accuracy of prediction of all other compounds.

ENVIRONMENTAL Science & Technology
Article
pubs.acs.org/est

Molecular Toxicity Identification Evaluation (mTIE) Approach Predicts Chemical Exposure in *Daphnia magna*

Philipp Antczak,[†] Hun Je Jo,[‡] Seonock Woo,[¶] Leona Scanlan,[¶] Helen Poynton,[∥] Alex Loguinov,[¶] Sarah Chan,[⊥] Francesco Falciani,[*,†,#] and Chris Vulpe[*,¶,#]

Towards a System Level Understanding of Non-Model Organisms Sampled from the Environment: A Network Biology Approach

Tim D. Williams, Nil Turan, Amer M. Diab, Huifeng Wu, Carolynn Mackenzie, Katie L. Bartie, Olga Hrydziuszko, Brett P. Lyons, Grant D. Stentiford, John M. Herbert, Joseph K. Abraham, Ioanna Katsiadaki, Michael J. Leaver, [...], Francesco Falciani ✉, [view all]

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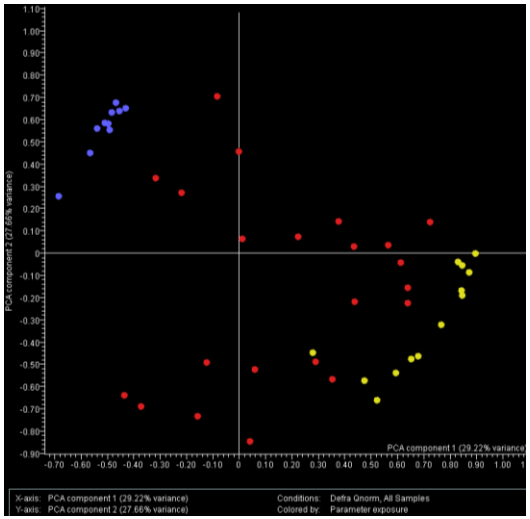
PLOS | COMPUTATIONAL BIOLOGY

Conclusions

- The overall oestrogenising potential of the contraceptive ‘pill’ has been long overestimated as almost all laboratory exposures to date focus on EE2 alone
- Progestins are extremely powerful steroids, exerting profound effects at low concentrations similar to those reported in males by EE2
- EE2 and Levo together, as used in contraceptives, are driving responses in opposite directions for key genes involved in reproduction (VTGs, Chgs, ERα,..)



•Chemical mixtures can not be assessed on the basis of concentration addition only, antagonism and synergism are possible and do happen!
•Combining chemical physicochemical properties, high-throughput omic data, pathway analysis and computational modeling is a powerful approach that could provide the only means of evaluating and predicting mixture toxicity, saving thousands and thousands of fish lives as well as money

Cefas

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Cefas

…..questions?

Cefas