Integrating pharmacokinetics and pharmaco-dynamics in AOPs for next generation risk assessments

An Application to Ovarian Cycle Disruption by Mixtures of Aromatase Inhibitors

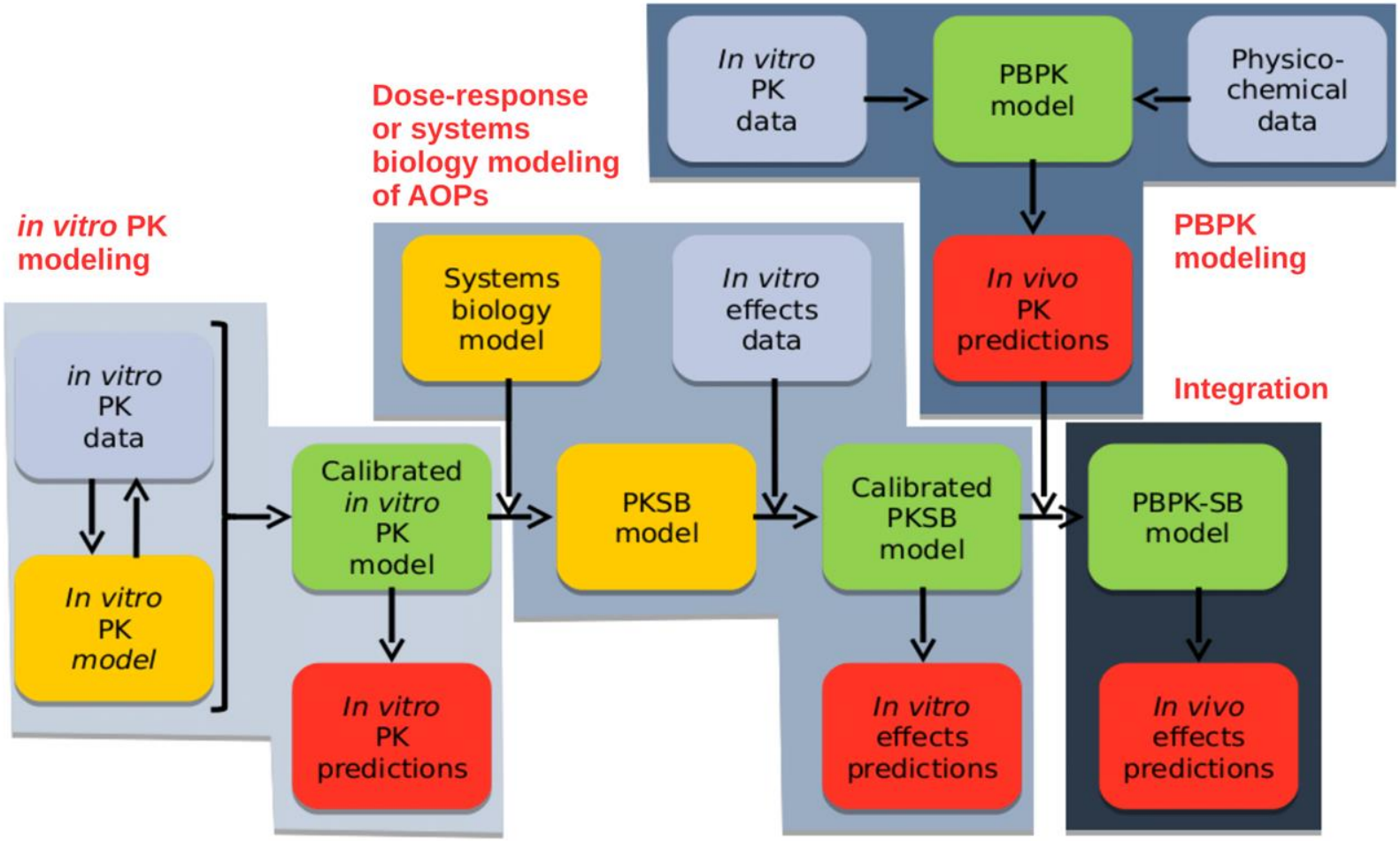
Frederic Y. Bois

frederic.bois@ineris.fr

INERIS COSMOS [:::] EUTOXRISK EuroMix

Context

Risk assessment is increasingly based on high throughput, high content *in vitro* data. That implies high-throughput quantitative *in vitro* to *in vivo* extrapolations.

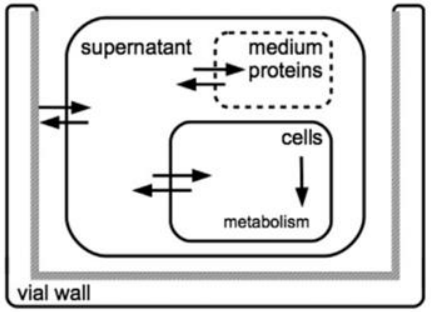


In Vitro pharmacokinetic modeling

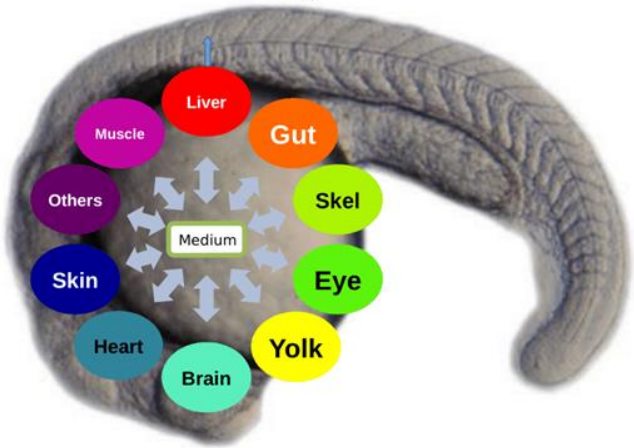
Proper analysis and extrapolation of *in vitro* toxicity requires data or estimates of intra-cellular concentrations. That applies to all cellular *in vitro* systems. *In vitro* PK models are indispensable tools.

Examples:

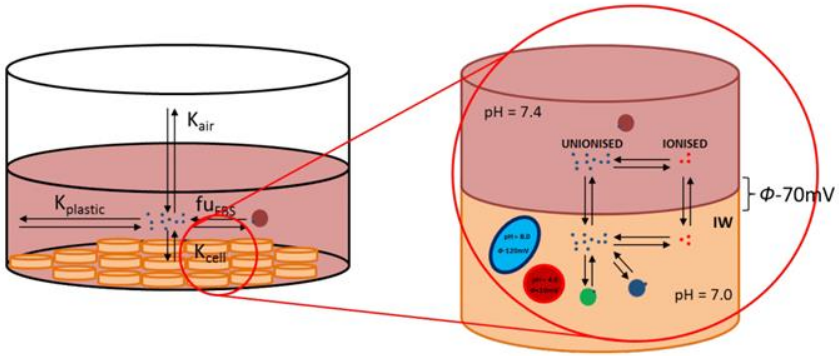
- Predict-IV *in vitro* PK model (INERIS)
- Armitage model (EPA), Comenges (JRC)
- Fisher model (CERTARA)
- Zebra fish model (INERIS)



(dynamic)



(dynamic, physiological, *ab initio*)



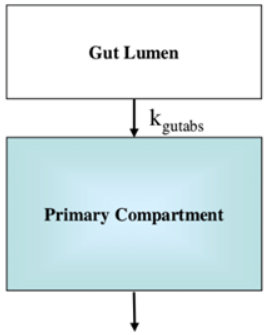
(static, *ab initio*)

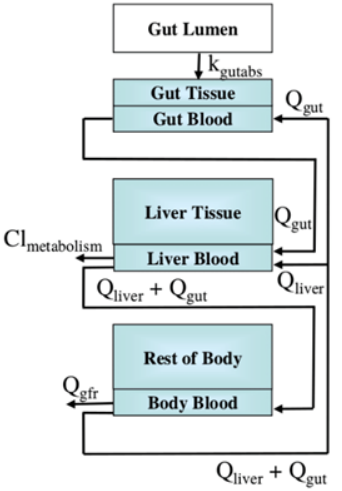
PBPK (in vivo) modeling

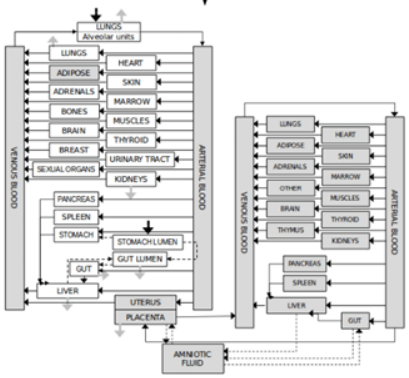
Physiologically based pharmacokinetic models, for extrapolation to animals or humans *in vivo*. They are increasingly generic for *ab initio* predictions

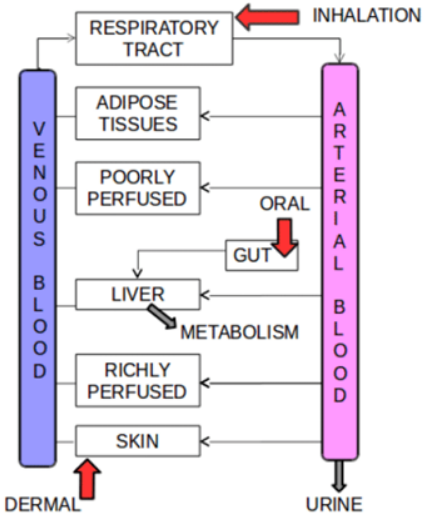
Examples:

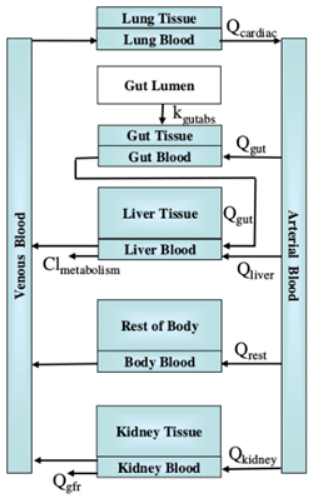
- HTTK model (EPA)
- Simcyp model (CERTARA)
- COSMOS model (INERIS, JRC)
- Pregnancy model (INERIS)





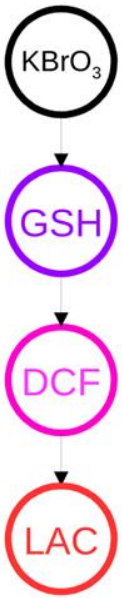




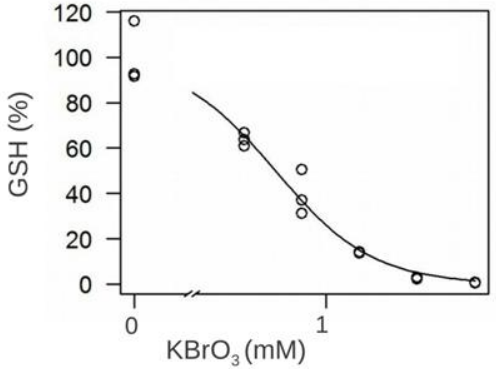


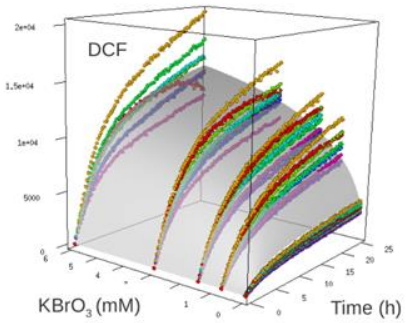
Quantitative AOP (pharmacodynamic) modeling

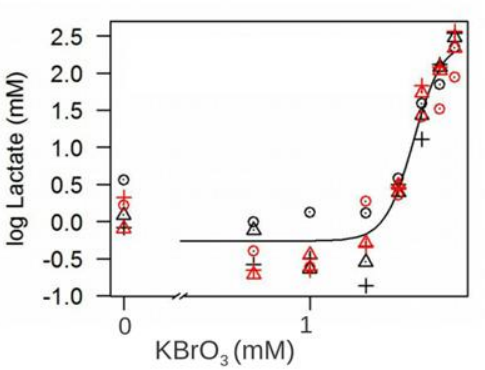
Here, the methods and the data are still under-developed (except in pharmacology). Several approaches:

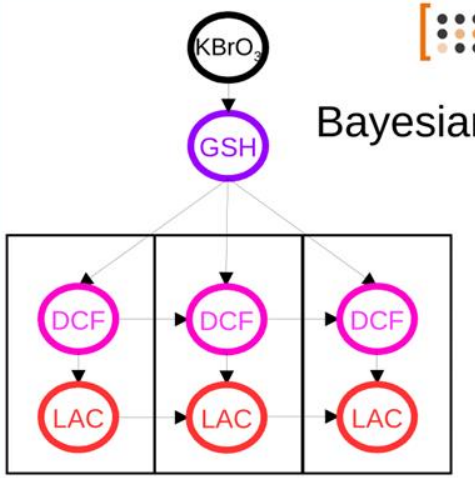


Chaining dose-response relationships









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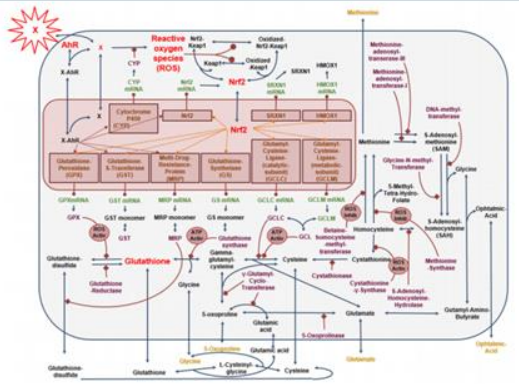
Bayesian networks



Mechanistic models

Example of application: endocrine disruption

Human exposure to aromatase inhibitors is susceptible to block estradiol synthesis, and therefore to perturb the ovarian cycle in females, potentially affecting fertility *via* endocrine disruption.

Exposure estimates are available from the US EPA ExpoCast project for about 300 molecules tested for aromatase inhibition in ToxCast.

We linked exposure, effects *in vitro*, and ovarian cycle perturbation in vivo through toxicokinetic and toxicodynamic modeling.

We used the overall model to assess the effect of exposures to random mixtures of aromatase inhibitors at realistic ExpoCast derived exposure levels.

EHP Environmental Health Perspectives

Research

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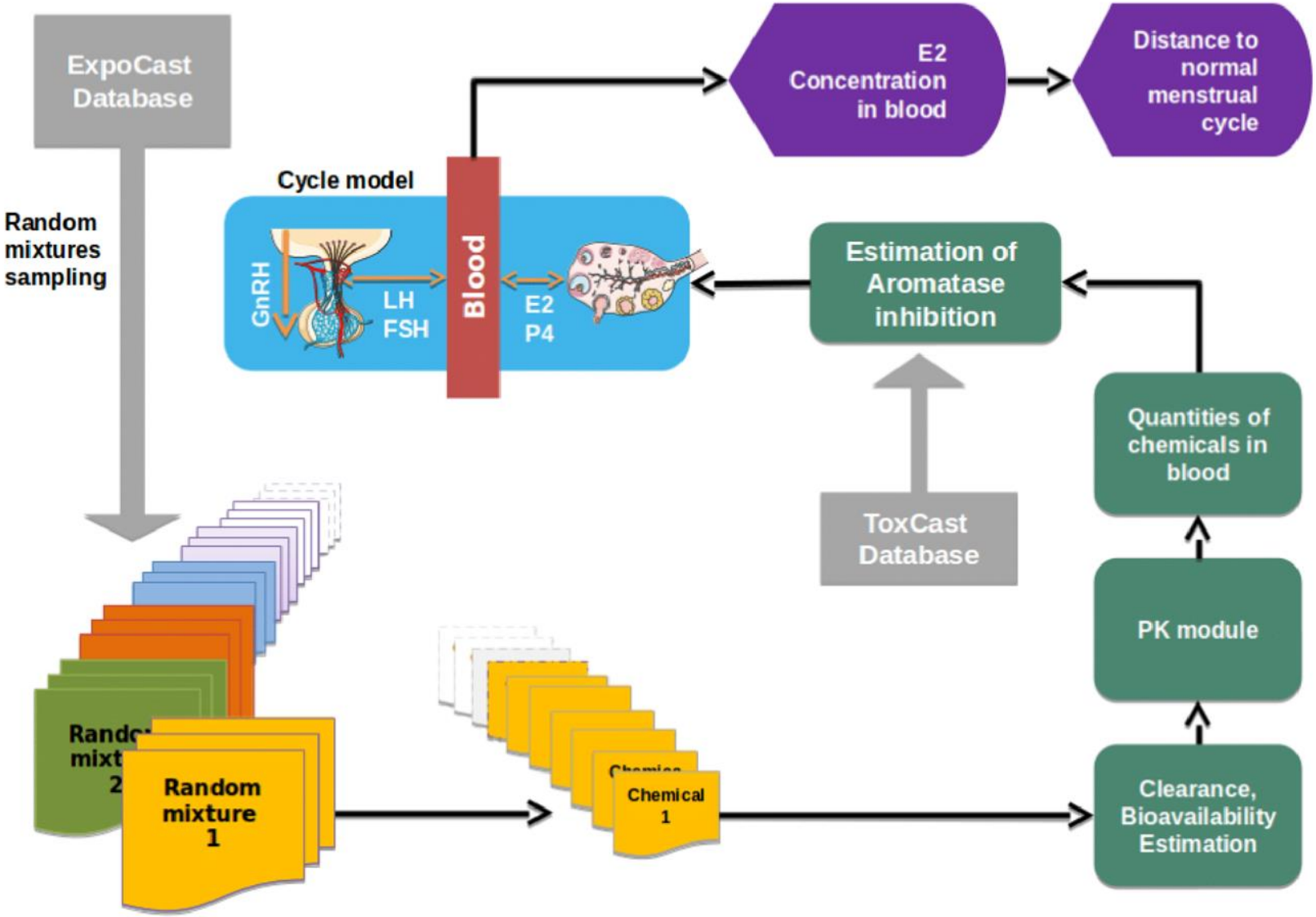
High-Throughput Analysis of Ovarian Cycle Disruption by Mixtures of Aromatase Inhibitors

Frederic Y. Bois,[1] Nazanin Golbamaki-Bakhtyari,[1] Simona Kovarich,[2] Cleo Tebby,[1] Henry A. Gabb,[3] and Emmanuel Lemazurier[1]

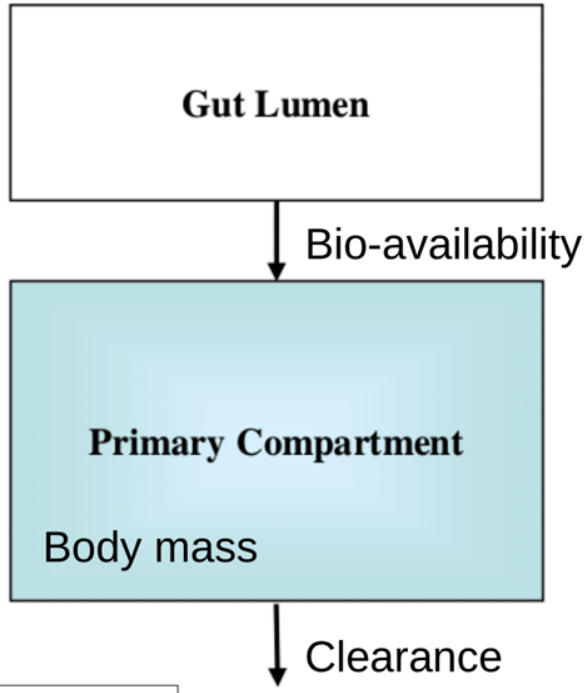
Workflow



86 aromatase inhibitors at the ExpoCast/ToxCast intersection

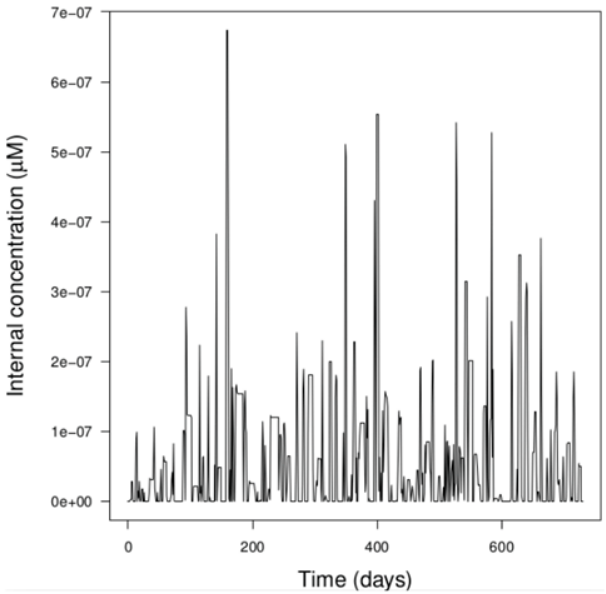
PK modeling

Simple one-compartment dynamic model to describe substance accumulation and elimination from the woman's body.
Distributions of parameter values were obtained by QSAR methods and Monte-Carlo sampled to account for uncertainty.



We simulate time-varying exposure profiles over 2 years for each chemical in each mixture (matching their time-weighted average to ExpoCast concentration distributions)

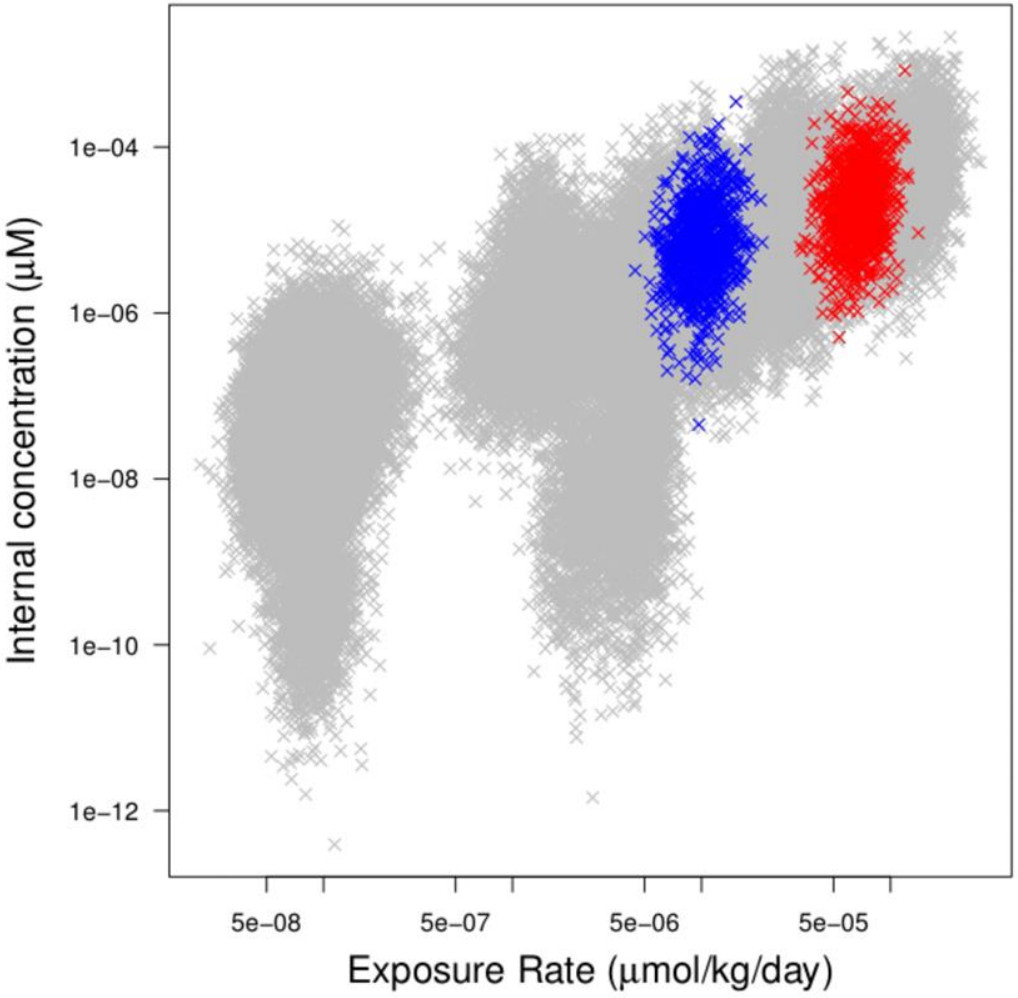
Example: lindane:



Internal dose *vs.* exposure

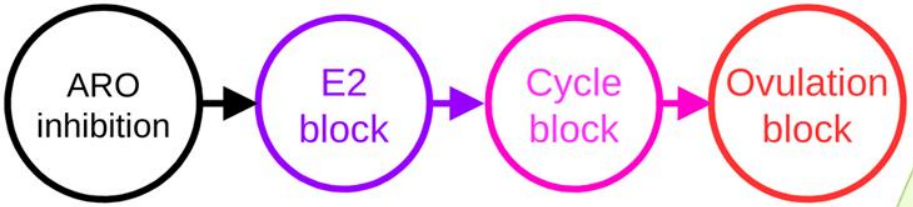
The Monte-Carlo combination of ExpoCast uncertainty about exposure levels and PK parameters uncertainty results in a large dispersion of the internal dose estimates.

- crystal violet in blue
- clotrimazole in red

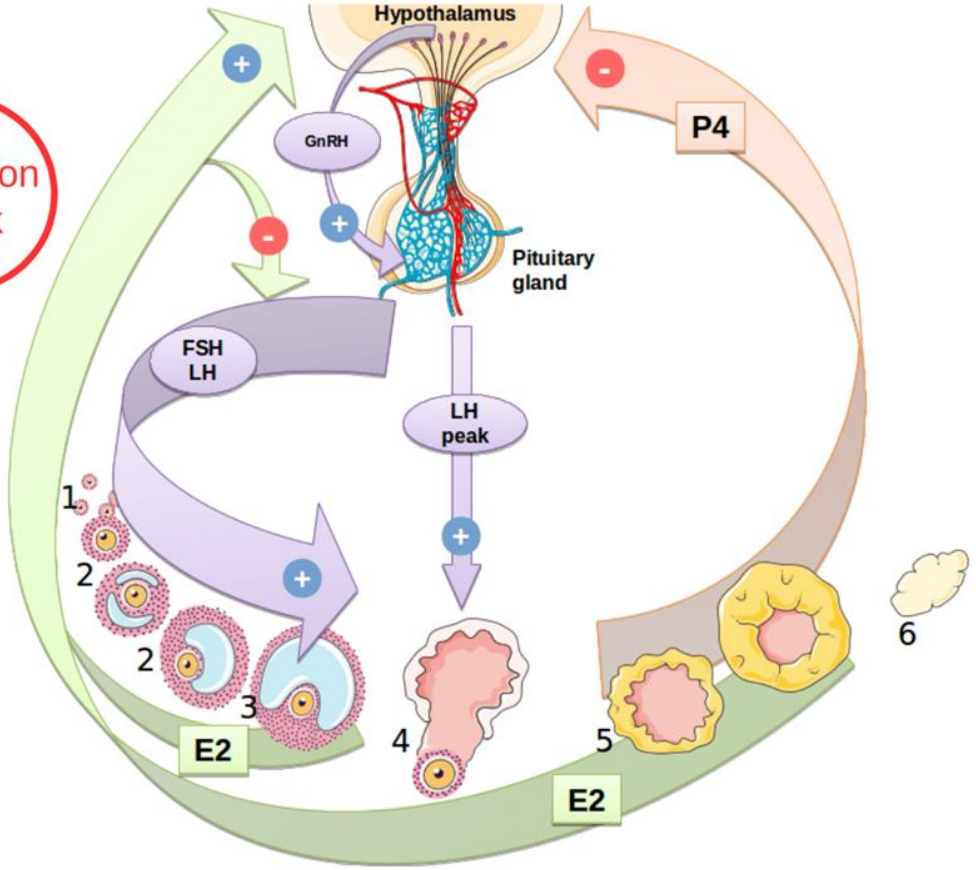


Mechanistic AOP model of the hormonal cycle

A dynamic model of cycle control adapted from Chen and Ward (2013).



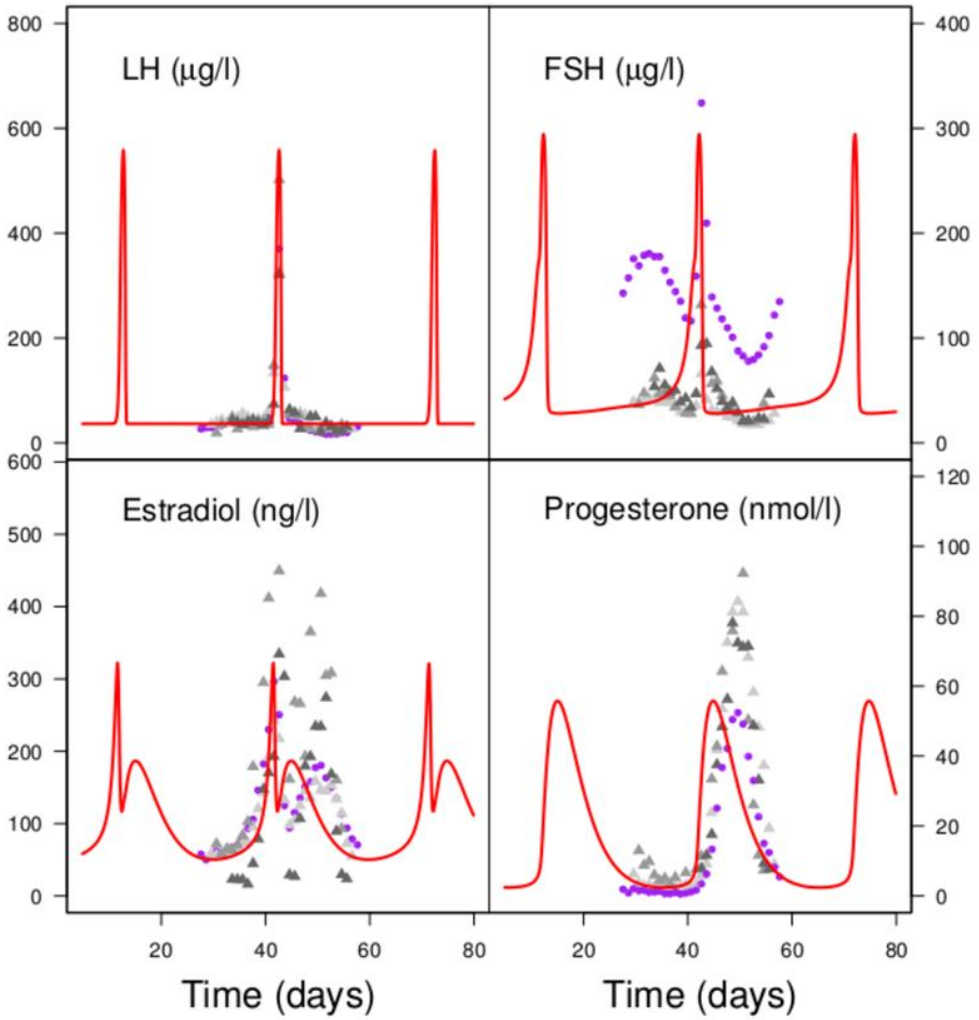
The levels of circulating hormones and <u>follicular mass</u> (which drops at ovulation) are computed.



LH: luteinizing hormone
FSH: follicle stimulating hormone
GnRH: gonadotropin-releasing hormone

Model predictions of "normal" cycles

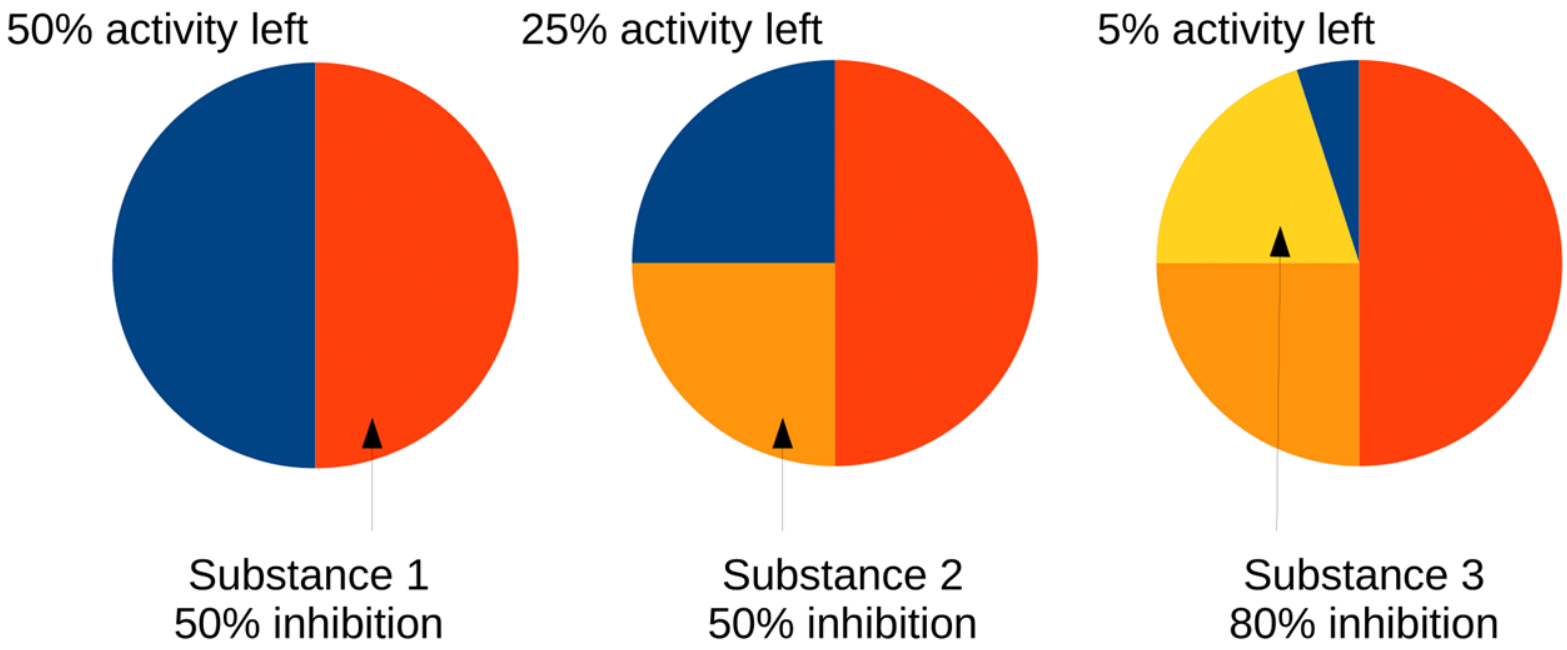
The model replicates historical data (but there are huge differences in reported hormonal profiles in the literature)



LH: luteinizing hormone
FSH: follicle stimulating hormone

Effects of a mixture on aromatase activity

Conceptual model:



Aromatase inhibition following exposure to single chemicals at constant concentration

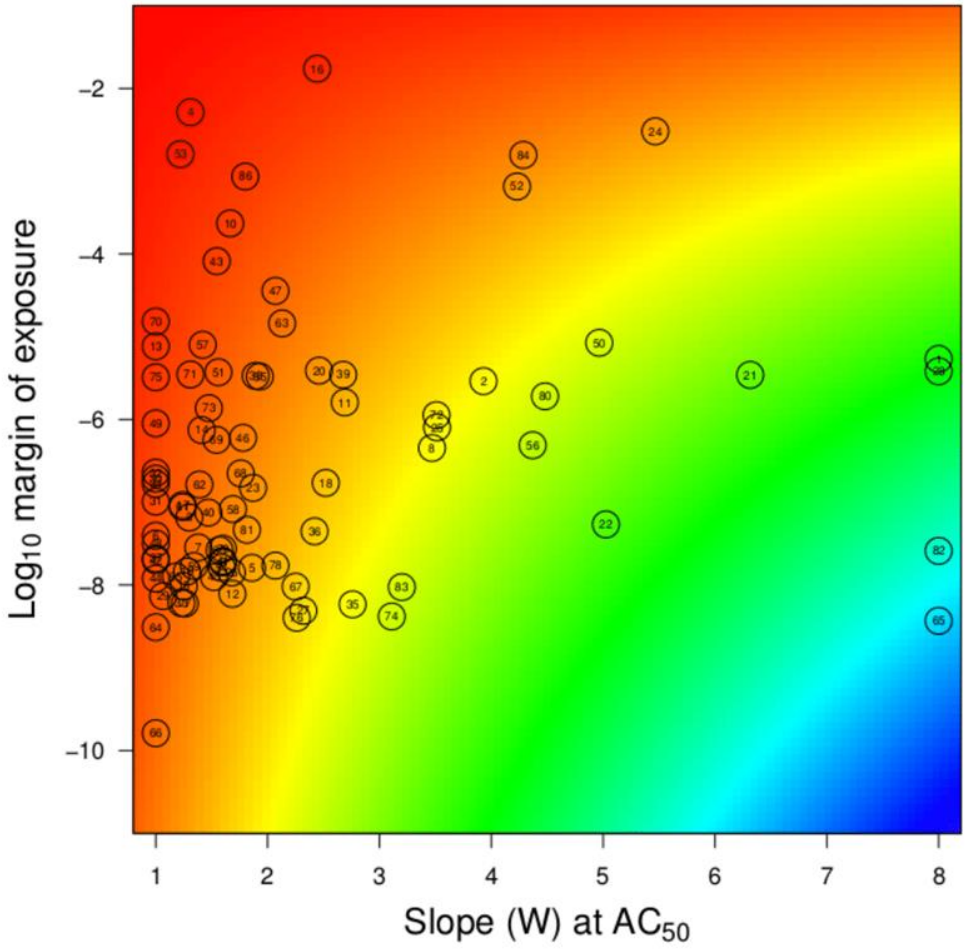
ToxCast dose-effect model:

$$Inhibition = \frac{C^W}{\left(AC_{50}^W + C^W\right)}$$

If C << AC_{50}:

$$\log_{10}\left(Inhibition\right) = W \times \log_{10}\left(\frac{C}{AC_{50}}\right)$$

We took the 97.5th percentile of internal *C*. *C/AC50* is a conservative "margin of exposure".
On the plot the color indicates the level of inhibition (from 1% in red to almost zero in blue). The dots mark the position of each chemical on that risk map.



Aromatase inhibition following exposure to single chemicals at constant concentration

We can investigate particular chemicals in the database:

No problem with any substance alone. The 10 "worst" lead to at most 0.1% aromatase inhibition at the high end of their exposure levels.

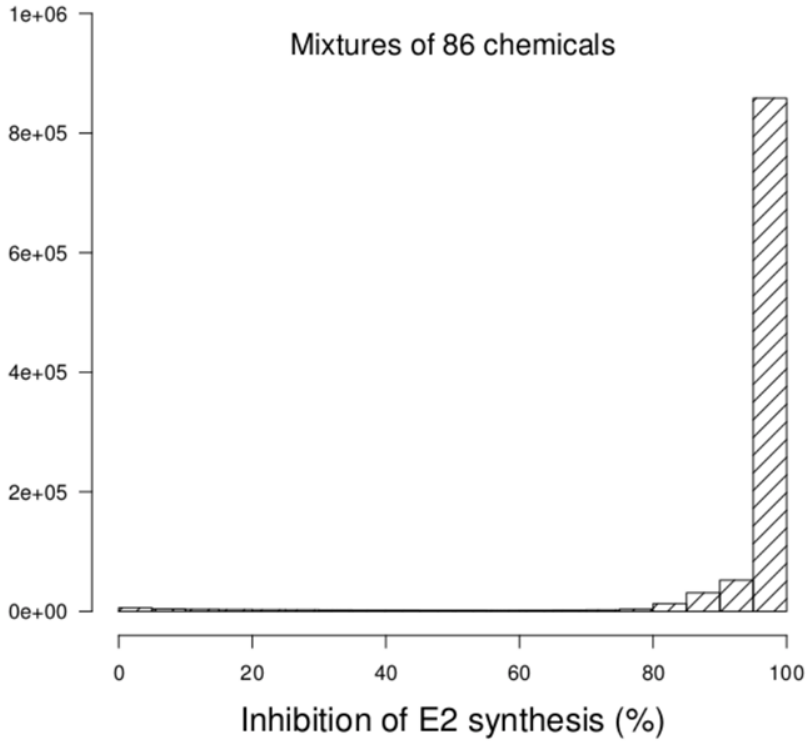
Name	Log10 inhibition
Letrozole	-2.99
Estrone	-3.41
Fulvestrant	-4.30
Triflumizole	-4.81
Tetramethyl-5-decyne-diol	-5.11
N-Methyl-2-pyrrolidone	-5.49
Rhodamine 6G	-5.51
Anastrozole	-6.04
Fenvalerate	-6.05
Imazalil	-6.31

Aromatase inhibition following exposure to mixtures of molecules at constant concentrations

1 million random mixtures of the 86 inhibitors:

Exposure levels are randomly sampled using ExpoCast distributions. ToxCast effect parameters uncertainty accounted for by Monte Carlo.

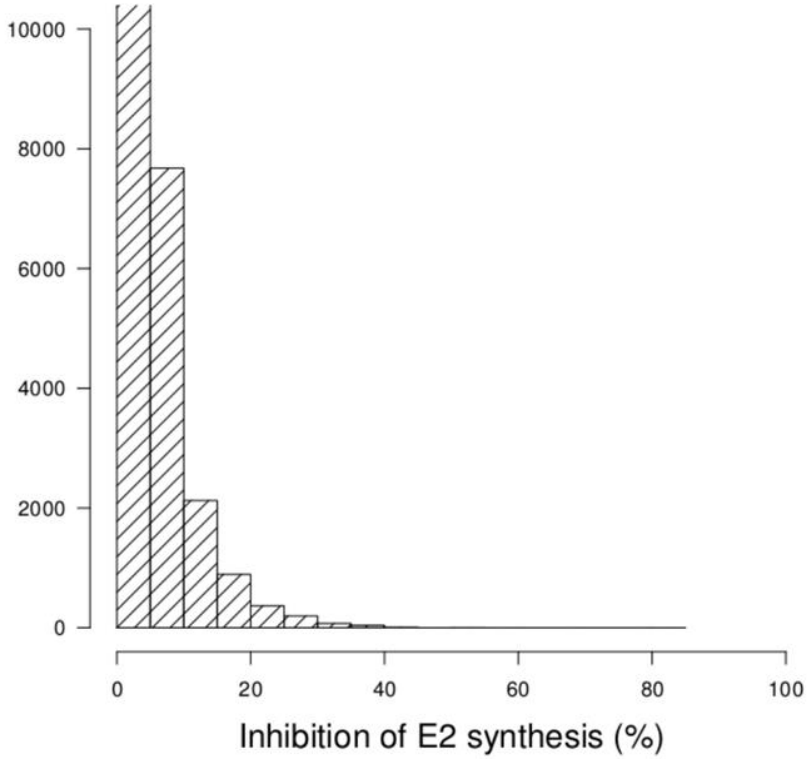
Complex mixtures can have a very large effect on aromatase inhibition. Effect on cycles are dramatic: there is no cycling above 50% continuous inhibition.



Aromatase inhibition following exposure to mixtures of molecules at time-varying concentrations but no co-exposures.

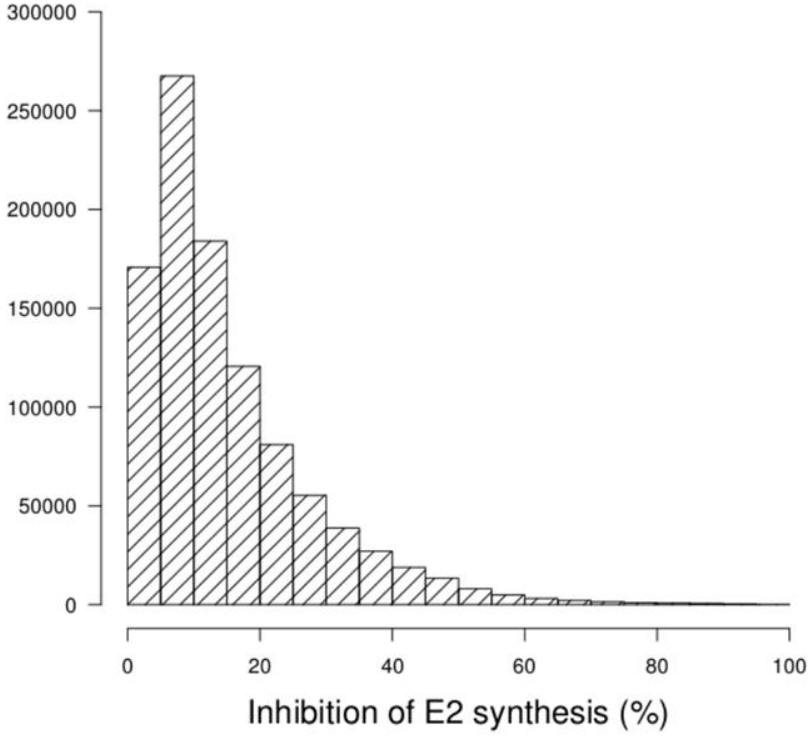
Unlikely optimistic case: no co-occurence of exposures (but a possible internal co-exposure by persistance in the body).

In 1 million simulations, only 0.3% of estradiol sysnthesis inhibitions are above 10%.



Aromatase inhibition following exposure to mixtures of molecules at time-varying concentrations with co-exposures

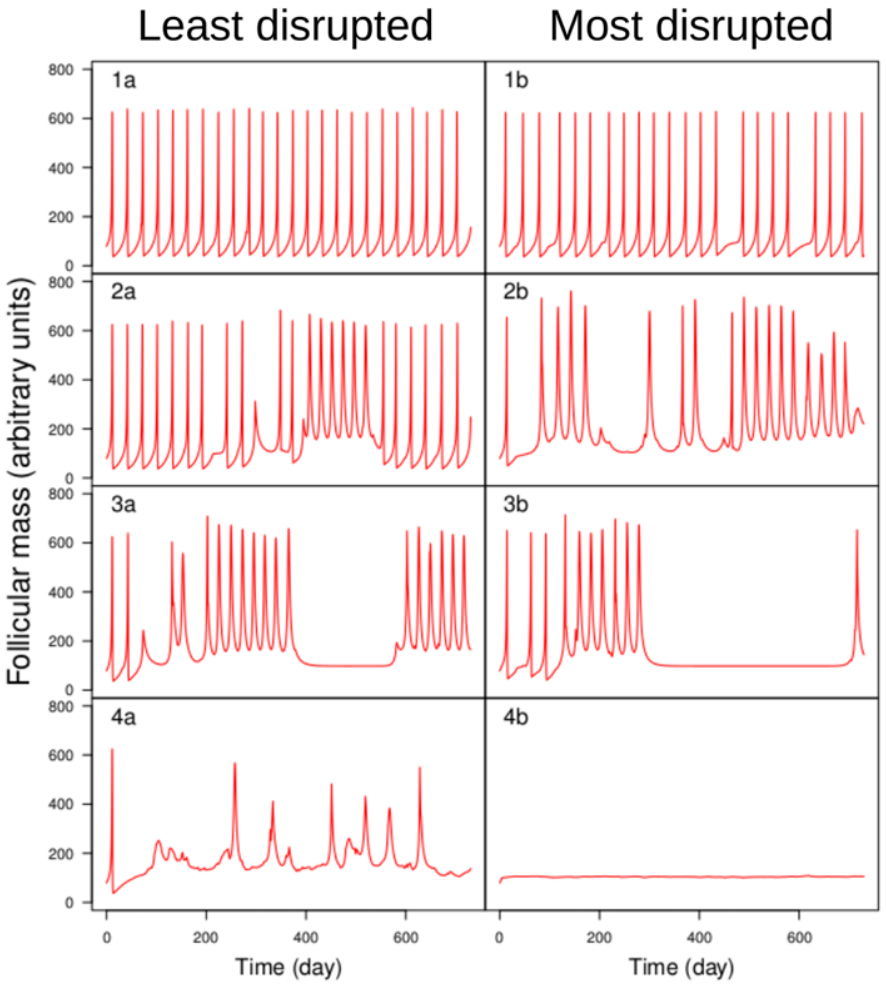
Recovery is possible in the case of time-varying exposures. Full inhibition was never predicted, but time-weighted average inhibition above 20% are still frequent. What does that mean exactly? It's just inhibition of an enzyme...



Cycle disruption following exposure to mixtures of molecules at time-varying concentrations

Effects on cycles have been computed for each mixtures. They are complex. We formed 4 groups of effects:

- Group 1 (35% of cases), cycles are practically normal.
- Group 2 (55% of cases) baseline shifts, no major irregularities.
- Group 3 (6% of cases) systematic baseline shifts and frequent or prolonged anovulations. Such cycling would clearly impair fertility.
- Group 4 (3 % of cases) total disruption.



Conclusions

- Tools development and data collection are rapidly progressing for QIVIVE and AOP quantification. High-throughput predictions can give us a glimse of the big picture.
- Results out exploratory full-chain exposure-effect assessment indicate a potential for pathological effects of mixtures of aromatase inhibitors in women. This, even though no single chemical seems to present a significant risk under the current use scenarios.
- There are still many limitations and opportunities for improvement:
- Limited exposure database for mixtures (but on-going efforts)
- Mix of uncertainty and variability in exposure distributions
- Lack of knowledge on exposure routes (ingestion assumed)
- Lack of *in vitro* pharmacokinetic data, simplistic model
- Lack of accounting for cycle variability in women

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