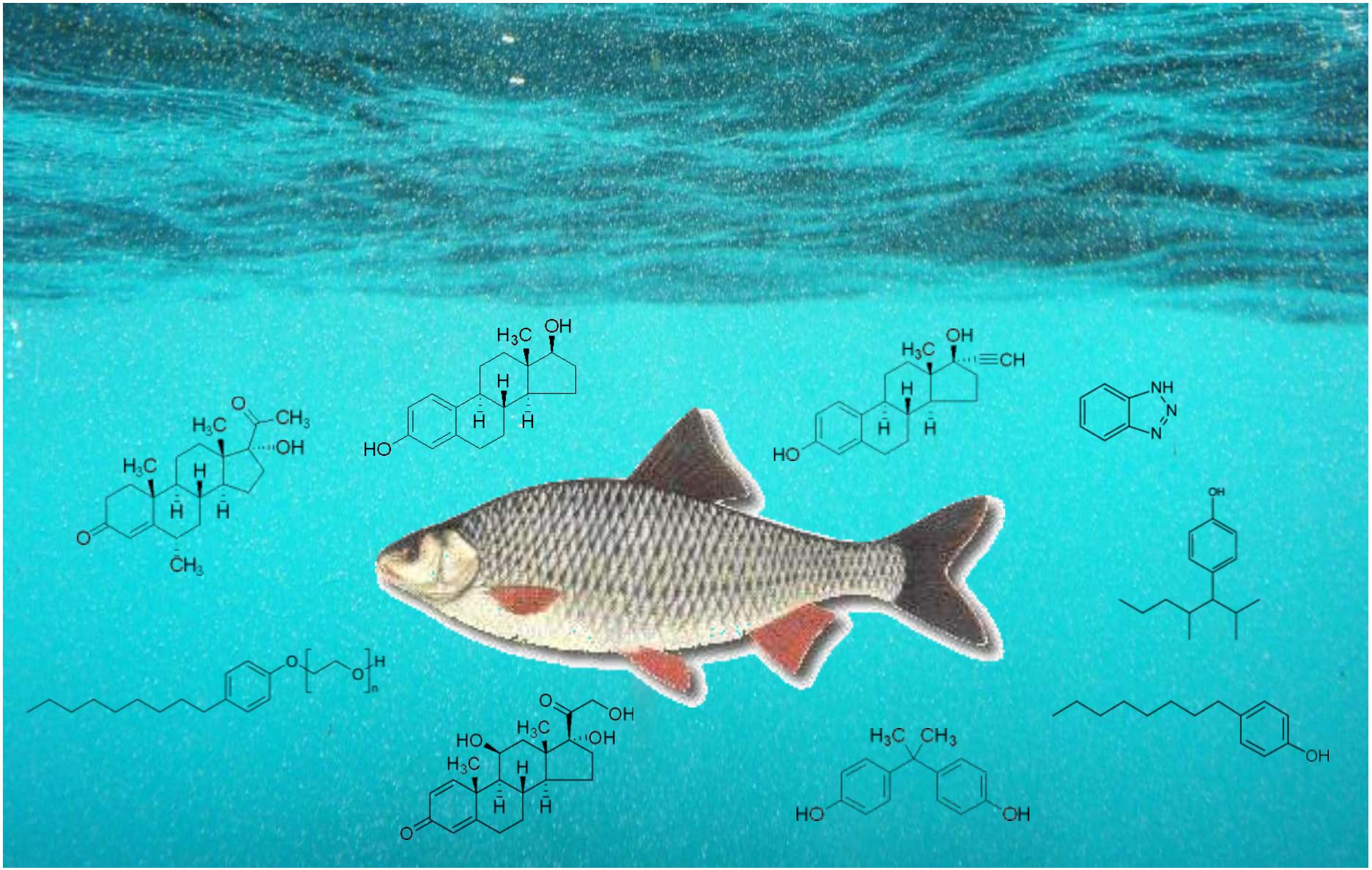
Case Study 2:
Complex Mixtures of Chemicals in the Aquatic Environment and their Effects on Fish.

John Sumpter,
Institute for the Environment,
Brunel University


Brunel
UNIVERSITY
WEST LONDON
Institute for the Environment
Brunel University, London

The River Thames as a Typical Modern River

- Catchment covers an area of 12,000 $Km^{2.}$
- The river flows through some densely populated areas.
- Population density is 447 people/Km^2.
- 352 sewage treatment plants discharge into the river, including 136 significant ones.
- They discharge the effluent of over 5.4 million people.
- In many locations the effluent is diluted less then 1 in 10, especially in periods of low flow.

- Conclusion: fish in the River Thames live permanently in diluted effluent.

Data from Williams et al. 2009. Environ. Toxicol. Chem. 28, 220-230.

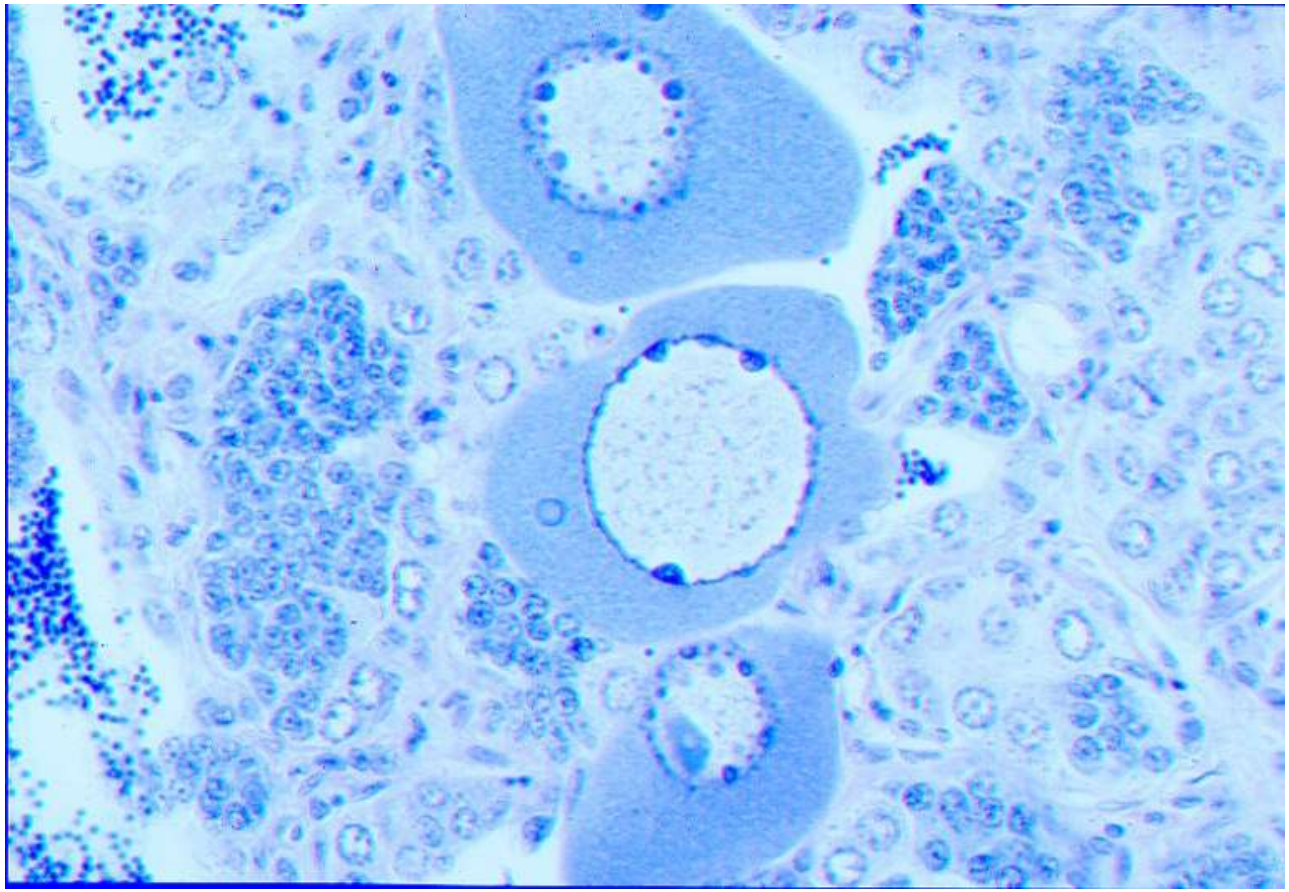
What does this mean for Aquatic Organisms?

- They are exposed continuously to highly complex mixtures of chemicals.
- These chemicals will be both natural and anthropogenic.
- The total number of chemicals is unknown, but it is probably very high.
- It is likely that there are thousands, if not tens of thousands, of anthropogenic chemicals, and their degradation products, in rivers.
- The concentrations of most of these chemicals will be extremely low.

And what Questions does this Raise?

- Are any of the chemicals present at a high enough concentration to have effects on wild fish?
- If they are, which ones? In other words, which ones are of concern?
- Do the chemicals of concern act independently, or do some, or all, of them interact, leading to greater than expected toxicity?

Evidence that Chemicals in Rivers can Affect Fish



Section through the “testis” of a feminised male roach.

The Cause(s) of Intersexuality in Wild Fish in English Rivers

- Exposure to “oestrogens”.
- But there are many chemicals with oestrogenic activity in STW effluents and rivers, including:

  (a) “Real” oestrogens

  e.g. Natural oestrogens, such as 17β-oestradiol.

  (b) Synthetic oestrogens

  e.g. Ethinyl oestradiol.

  (c) “False” oestrogens (xenoestrogens)

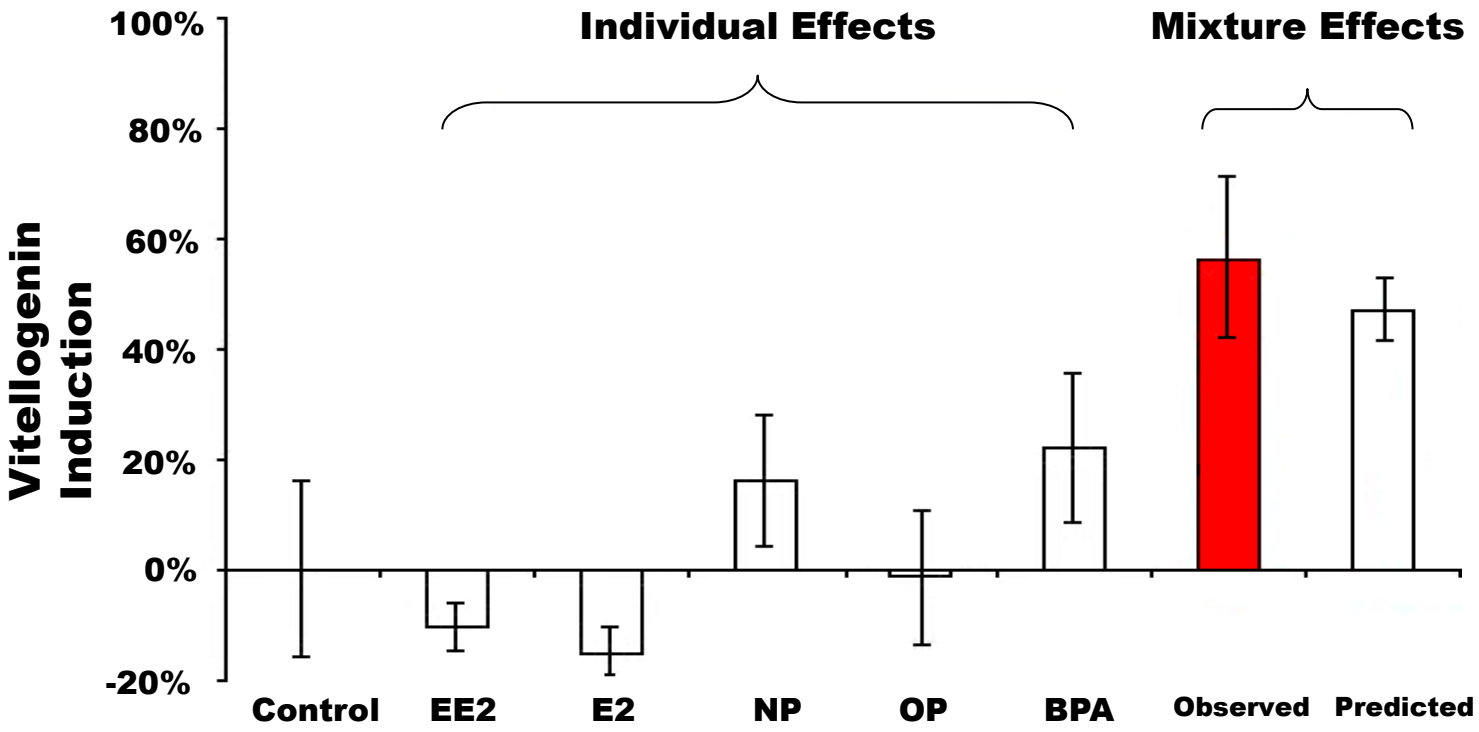
  e.g. Nonylphenol.

A Typical Cocktail of "Oestrogens" in an English River.

Name	Possible Concentrations
Oestrone	1 ng/L
Oestradiol	0.1 ng/L
Ethinyl Oestradiol	0.1 ng/L
Equine Oestrogens (e.g. equilenin)	0.2 ng/L
Nonylphenol	100 ng/L
Octylphenol	10 ng/L
Bisphenol-A	5 ng/L

etc (the list is almost endless)

Testing Mixtures of Oestrogenic Chemicals on Fish



Effects were additive, and predictable.

Data from Brian, J.V. et al. 2005. Environ. Health Perspect. 113, 712-728

Our Conclusions to Date

- Intersexuality is caused by a mixture of oestrogenic chemicals.
- That mixture contains natural (e.g. E2) and synthetic oestrogens (e.g. EE2), and also xenoestrogens (e.g. Bisphenol-A, NP).
- It probably also contains other oestrogenic chemicals, such as equine oestrogens used in post-menopausal therapy.
- The exact composition of the mixture causing the effects is almost certainly site-specific.

Are Oestrogens the Only Chemicals in Effluents and Rivers that can Adversely affect Fish Reproduction?

- No.
- There are probably many chemicals that can, if present at high enough concentrations, affect fish reproduction.
- Synthetic progestogens (widely used in hormonal contraceptives) are one group that might be expected to affect fish reproduction.

Concentrations of Synthetic Progestogens in the Aquatic Environment

- There are few data.
- But, a number of different synthetic progestogens have been detected in STW effluents (not, yet, in rivers).
- Reported effluent concentrations are in the low ng/L range.
- Hence, effluent concentrations are apparently not much lower than concentrations that stop fish reproducing.

Are Synthetic Progestogens of Concern?

- We do now know; too few data are available to judge.
- But the available data are worrying.
- Some synthetic progestogens can inhibit reproduction when present in the low ng/L range.
- As a number of synthetic progestogens are present simultaneously in effluents (and rivers?), additive effects should be anticipated.

What about a Mixture of a Synthetic Oestrogen and Synthetic Progestogen?

- This would, obviously be a very sensible mixture to test.
- It is very unlikely that one of these chemicals could be present in the environment without the other also being present.
- Both chemicals are reproductive toxicants, but they (presumably) have different modes-of-action.
- Would they interact additively, synergistically or antagonistically?
- I do not know. We need to find out!

Are Other Reproductive Toxicants Present in Rivers?

- Recently, it has been suggested that tributyltin (TBT) is a reproductive toxicant in fish.
- Environmentally-relevant concentrations (low ng/L) can both masculinise fish, and inhibit reproduction.
- It seems likely that many other reproductive toxicants will be present, but we know little or nothing about them.

Shimasaki, Y. et al. 2003. Environ. Toxicol. Chem. 22, 141-144.

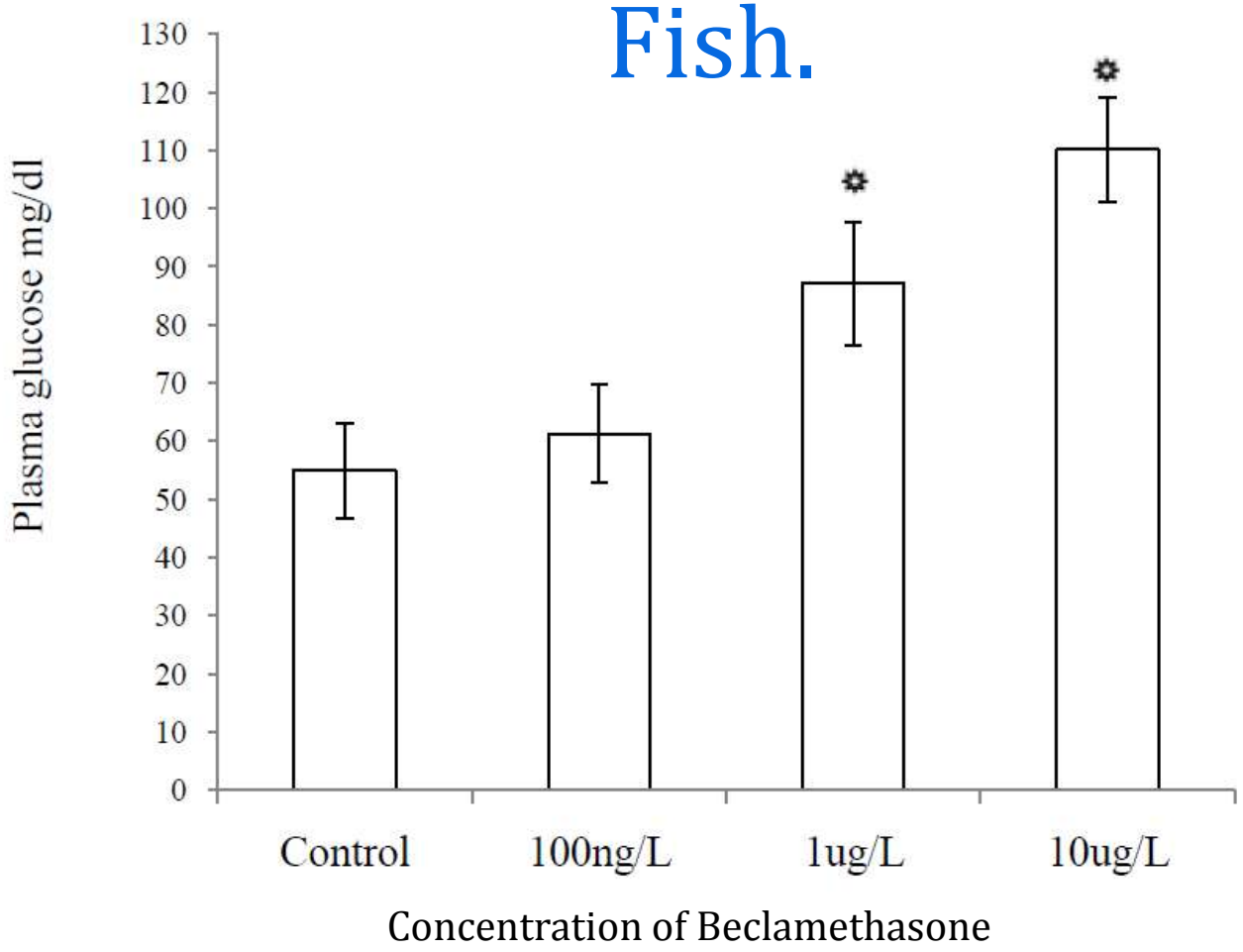
Zhang, J. et al. 2007. Aq. Toxicol. 83, 174-179.

Zhang, Z. et al. 2008. Environ. Sci. Technol. 42, 8133-8139.

Are Other Potent Steroid Pharmaceuticals Present in the Aquatic Environment?

- Glucocorticosteroids are used clinically in much larger amounts than any other group of steroids (e.g. progestogens, oestrogens).
- They are present in effluents, and so probably also in rivers.
- Many different ones (over 40) are in regular use to treat a variety of illnesses.
- Low concentrations can cause effects in fish.

Effects of a Synthetic Glucocorticoid on the Plasma Glucose Concentration of Fish.



Where are we now with all these Steroids?

- Many different steroids are present in the aquatic environment.
- Some, perhaps all, are very potent: they affect fish in the ng/L range.
- Some (e.g. oestrogens, progestogens) affect reproduction, others (e.g. glucocorticoids) may not.
- Essentially nothing is known about how these steroids do, or do not, interact when present as mixtures.

Yet more Complexity!

- Steroid antagonists, such as anti-oestrogens, may be present in effluents, and hence the aquatic environment.
- Laboratory studies have been conducted in which fish were exposed to an oestrogen and an anti-oestrogen simultaneously.
- The results are difficult to interpret, but under some circumstances, an anti-oestrogen may negate the effect of any oestrogen.
- The ratio of the two chemicals is probably very important in determining the overall effect.

Where are we now in our Understanding of Mixtures Toxicity?

- Progress has been made in both testing, and predicting, the effects of mixtures containing similarly-acting chemicals.
- Much less progress has been made in studying and predicting the effects of mixtures of dissimilarly acting chemicals (e.g. an oestrogen and a progestogen, or a metal, or a surfactant).
- All experimental research has, to date, been conducted with very simple mixtures (usually less than 10 chemicals and often only 2).

An Intractable Problem?

- Wild animals are exposed continuously to highly complex, ill-defined mixtures of chemicals.
- This exposure may, or may not, cause adverse effects.
- How can we find out if it does?
- How can we determine which chemical, or group of chemicals, in the mixture is responsible for any adverse effects?

How the Aquatic Mixtures "Problem" is Addressed Currently

- We study wildlife to assess their degree of health (c.f. intersexuality in fish). This strategy is sometimes called ecoepidemiology
- We test the mixture directly (e.g. whole effluent toxicity testing).
- We incorporate "safety factors" in environmental legislation.

Is this Adequate?

- In many, perhaps most, cases it probably is.
- But, if we are to significantly improve our understanding of how chemicals in the aquatic environment might affect fish, we must improve our knowledge of how mixtures of chemicals affect fish.
- Without that knowledge, protection of the aquatic environment from chemicals is based on ignorance and hope.

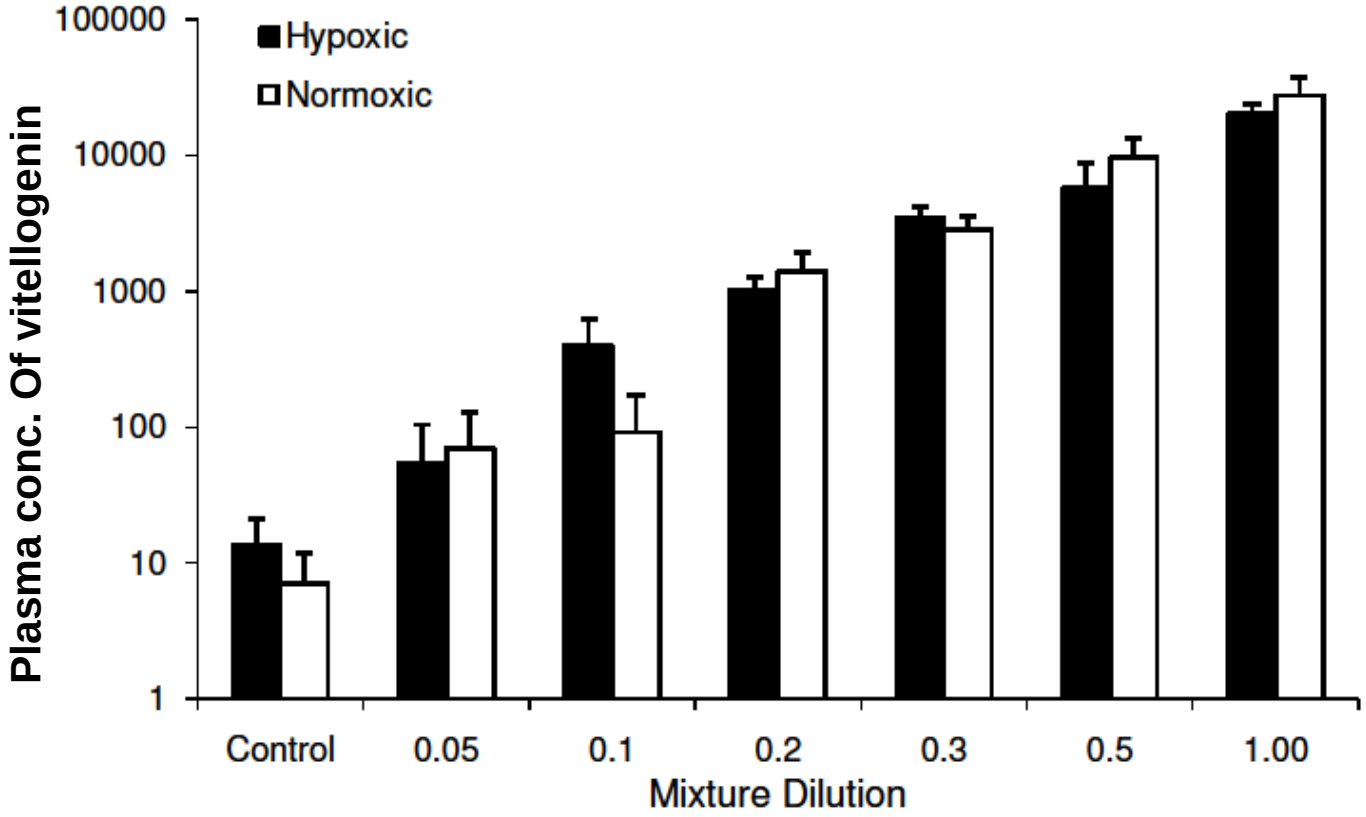
Some Suggested Next Steps

- Stop testing mixtures of similarly acting chemicals:
  - effects are additive.
- Start testing mixtures of dissimilarly acting chemicals (even simple mixtures of them).
  - E.g.
    - EE2 + Progestogen (use common endpoint)
    - Oestrogen + Androgen (use reproductive endpoints)
    - Oestrogen + Anti-oestrogen (common endpoints)
    - Oestrogen + Metal (or surfactant)
- Try to develop mathematics based on the results – move forward from C.A. and I.A. as the only two models.

Further Complexity

- There is more to mixtures toxicity than just chemicals.
- Wildlife face other threats (stressors) while simultaneously exposed to mixtures of chemicals.
- A fish may, for example, experience low oxygen levels, or elevated water temperature, together with a mixture of chemicals.

Effect of Low Oxygen Concentration on the response of fish to a mixture of estrogenic chemicals



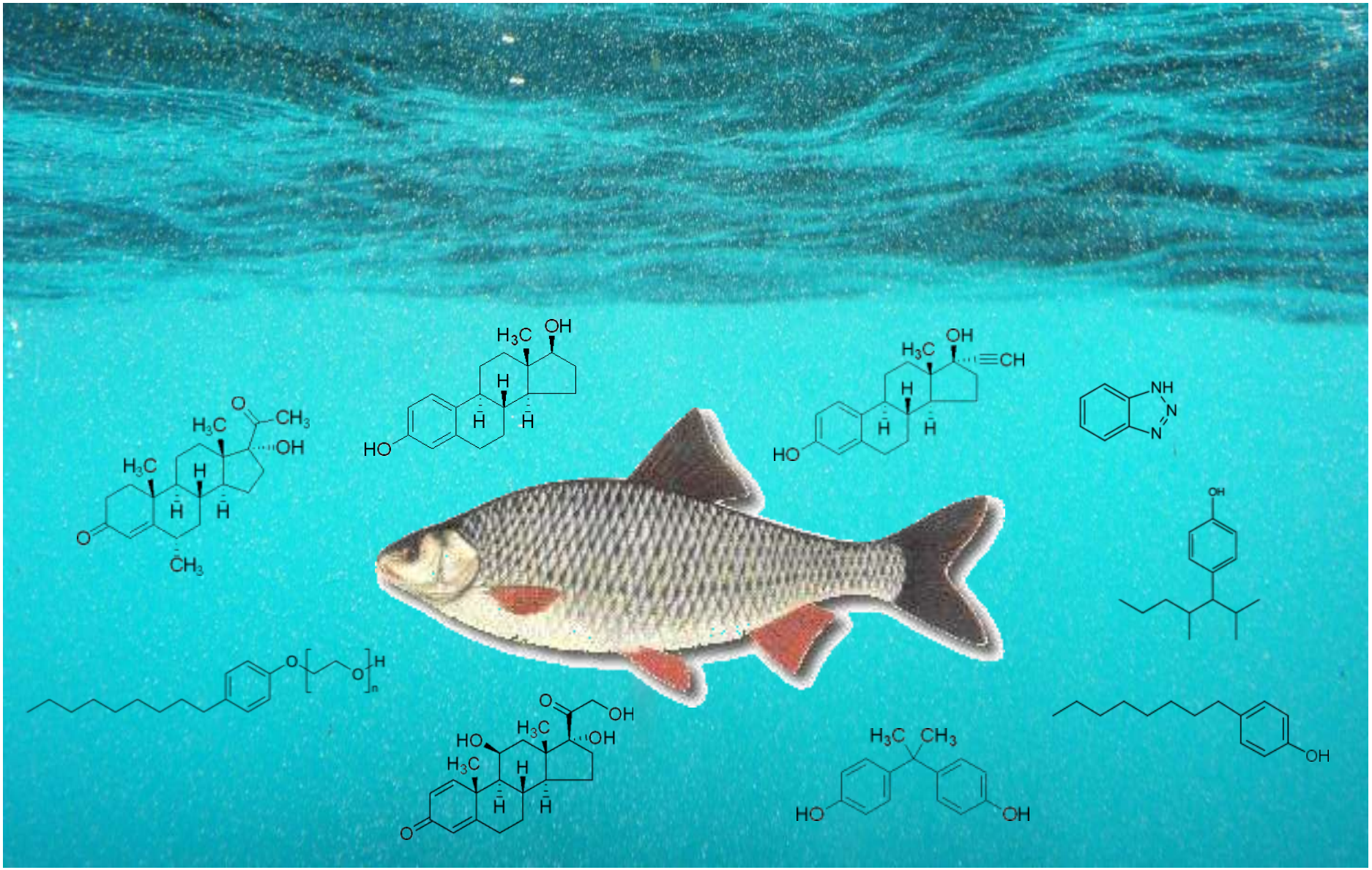
Data from Brian, J.V. et al. 2009. Environ. Sci. Technol. 43, 214-218.

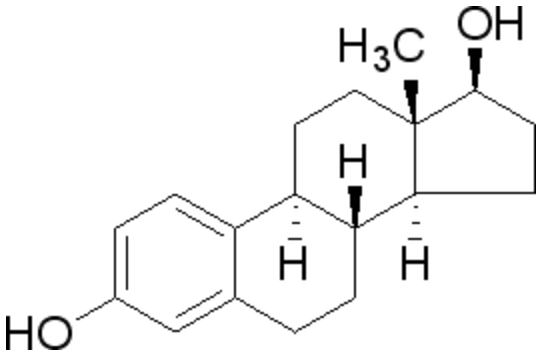
Summary of the Current Situation

- All wildlife, but especially aquatic species, are exposed to complex, ill-defined mixtures of chemicals.
- We very rarely know if this exposure is, or is not, of concern.
- Research is currently only scratching the surface of the "mixtures problem".
- Mixtures are the "real world" as far as all wildlife is concerned.

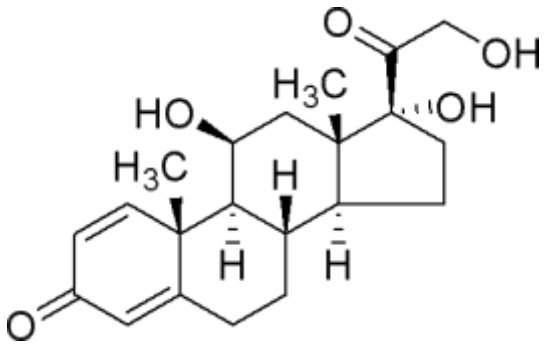
The Future

- The “mixtures issue” is a very difficult one!
- But we should not avoid it because it is difficult – we should rise to the challenge.
- It is an extremely important issue.
- We need to map out a way forward, and progress in a logical, step-by-step manner.
- To do so requires interdisciplinary research, involving (eco)toxicologists, chemists, mathematicians/statisticians and regulators.


Brunel
UNIVERSITY
WEST LONDON
Institute for the Environment
Brunel University, London

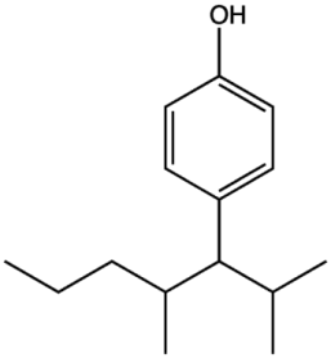


Oestradiol



Prednisolone

(a glucocorticoid)



Nonylphenol

(one isomer)

Thank you for Listening