Does ERA underestimate the risk of mixtures of APIs?

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## Bayesian Networks

* How to construct a Hierarchical model (Bayesian?)
* What other ERA BNs are there?

# Questions that need Answering

## Ecotoxicology

* Who invented TUs? (How) do they work?
* Likewise for EC50s. How intercomparable/applicable are they? Across species, chronic vs acute, etc?
* **What’s the existing literature on estrogen/progestogen ecotoxicity and mixture toxicity?**
  + **How do we deal with metabolism, e.g. of estradiol into estrone & estriol?**
* What do people think of ΣRQ?
* Fit log-normal distributions to all the relevant EC50s you can find
  + Get done, put in teams

Some key questions

* What exactly are we trying to show vis-à-vis mixture toxicity?
  + The current EMA risk assessment guidelines underestimate risks of important pharmaceutical groups (estrogens, statins, etc.)
  + They also over-estimate individual risk, so do we need to show doing so doesn’t sufficiently protect against mixture risk?
* Are we shoehorning Bayesian networks in there? How to make them an organic part of the paper?
  + Use of Value of Information analysis to quantify the added value of mixture toxicity approaches in this?
* There are two issues here: mixture toxicity and data scarcity – is the advantage of BNs that we can tackle both?
  + How (if at all) can we exploit quantification of uncertainty to predict toxicity/risk of unassessed substances
  + QSARs? Is that within scope?
* Furthermore, how do we fit PMBT concepts in? The simplest way is just to append them in another section, but is that novel/a value add?
* With a small subset of APIs it may actually be worth breaking down PNECs into component organism toxicity, which may give us more options?

# Measuring Toxicity

PNECs are obviously rubbish, but what do people think about EC50s? And how do I integrate them from multiple tests, across a variety of species and conditions?

[Statistical strategies for averaging EC50 from multiple dose–response experiments](https://link.springer.com/article/10.1007/s00204-014-1350-3)

Jiang & Kopp-Schneider (2014)

* Two strategies raised: mixed effect models and meta-analysis
* Under two sets of conditions: everybody has a clear DRC, and only some
* Basically you can stick all your points on a graph then plot a mixed-effect 4-parameter llme over all of them
* Or you can take your EC50s and their variance and calculate a weighted average with 95% Cis
* I don’t think I have enough variance data to do anything with this, although I could perhaps get it

### [Improving the regulatory assessment of combination effects of chemicals - Mixture Assessment Factor](https://www.youtube.com/watch?v=U7NGyk7V31g)

Backhaus (2021)

### [Mixture toxicity and the Threshold of Toxicological Concern (TTC)](https://www.youtube.com/watch?v=DjxvCdyL97o)

Backhaus (2018)

* The Threshold of Toxicological Concern is the lower 5% percentile of a pool of log-normally distributed NOELs, divided by an AF of 100
  + Chart

    Description automatically generatedThis implies a 5% risk that **one** compound is a false negative
  + The more compounds, the higher the overall risk becomes (Risk = 1 – 0.95n)
  + At 14 compounds you already have a 50% risk of false negative
  + Conversely, the more compounds in the mixture, the lower we’d need the TTC to be to have a 5% **overall** risk
* Mixtures are of specific concern because
  + Small, negligible effects multiply up (regardless of endpoint, species)
  + Compliance with individual thresholds doesn’t necessarily safeguard against mixture effects
* The Scientific Opinion on Mixture Toxicity assessment has a nice flow diagram I should check
  + But it assumes stuff without similar MoA doesn’t add to toxicity
  + Compounds with different MoAs don’t contribute to mixture toxicity if below their TTC
  + Under IA you can just get the TTC for the least risky compound, and keep everything below that
    - But this only works if the TTC truly describes a 0-effect concentrations. NOELs don’t.
    - Does the lower 5% percentile divided by an AF of 1000? TB doesn’t know, and doesn’t think anyone can know
* Basic pharmacology (apparently) tells us the only concentration we don’t have an effect is true – on a chemical level
* Also, for IA to be protective we need to be 100% sure the compounds have independent action
  + Knowing the complexity of the human body, can we really really assume independent action
  + Even if we’re really really sure, as we add more compounds to the mixture we will fill the finite space that is a living organism’s metabolism and physiology
* Concentration Addition makes life much simpler, because you can take their TTC and divide it by the number of compounds (?)
* If you can’t prove totally dissimilar MoA, you should use CA
  + MoA-driven mixture assessment is very data demanding
  + IA is of very limited use for a multi-component mixture
    - Can’t assume that mixture effects are absent at low doses

# Concentration Addition (CA)

[Toxicity and Assessment of Chemical Mixtures](https://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf) – SCHER/SCENIHR/SCCS (2011)

* CA requires an EC50 for each substance, and assumes a common mode of action
  + Fair assumption for statins, estrogens, etc., but we don’t have EC50s for much of these
  + Thomas Backhaus says non-additive effects are rare and shouldn’t be your first concern

## Hazard Index / Adjusted Hazard Index

* Sum of Hazard Quotients – presumably applicable to Risk Quotients also
* Bodged together out of a whole load of Assessment Factors, which are subsequently lost
* Possible to subtract HQs where evidence of antagonism (not relevant here, though)

## Toxic Units

* Ratio of PEC to acute (LC50) or chronic (long-term NOEC) endpoint
* TUm is simply the sum of TUs
* Turns out TUs are to HIs as RQs are to HQs

### Measurement of Pollutant Toxicity to Fish. II. Utilising and Applying Bioassay Results

Sprague (1969)

* A review of state of the art mixture toxicity (…in 1969)
* Cites Sprague & Ramsey (1965) on Toxic Units

### Lethal Levels of Mixed Copper-Zinc Solutions for Juvenile Salmon

Sprague & Ramsey (1965)

* Seems to be the first use of Toxic Units in the English-language literature, but cites
* Bergström & Vallin (1937) as the original source, which measures strength of waste in *giftenhet*
  + I couldn’t find a Swedish transcript for this, nor an English translation, but it perhaps exists somewhere
* Also, whoops! Toxic Units are used previously by Lloyd (and sometimes Herbert), (1961, 62)

## The toxicity of mixtures of zinc and copper sulphates to rainbow trout (*Salmo gaidnerii* Richardson)

Lloyd (1961)

* Fish were dosed with zinc and copper sulphate at a ratio of 6:1 (mg/L)
* The term “toxic units” isn’t used anywhere in the paper
* Instead, ratio of metal concentrations
* I don’t know why I bother

## Vattenförorening genom avloppsvattnet från sulfatcellulosafabriker

Bergström & Vallin (1937)

* [Helpfully scanned](https://gupea.ub.gu.se/bitstream/handle/2077/48919/gupea_2077_48919_1.pdf;jsessionid=F5C02B9D2972BB929928DF1995384F0D?sequence=1) and OCR’d by Göteborgs Universitet, but not indexed
* Describes *giftenheter* in a confusing way related to dilution – Google Translate has perhaps not been kind here
* Of questionable use to me
* Was translated at some point into English, but I can’t find that either
  + Scratch that, found it

### [Water Pollution due to Waste From Sulphate Pulp Factories](https://science-catalogue.canada.ca/record=3917337~S6)

Bergström & Vallin (1937), translated by Lars Ohman (1965)

* Charmingly misaligned typewriting
* Contains the word perspicuity – “lucidity, clearness of style”
* This initial definition of *giftenhet* – here translated as *poison unit* – is defined using an LD50 of a 2-3 year salmon fry over a 5 day exposure (they’re more sensitive than roach and perch)
* This was a fun little rabbit hole, but isn’t especially relevant

## Relative Potency Factor/Toxic Equivalence Factor/etc.

* If you know your toxicities, then you can normalise them against a toxicity prime
* Then simply add together
* I still don’t understand why we can’t look at the distribution of PNECs

## RQMix

* Based on CA and Toxicity Units

# Independent Action

## Cumulative Risk

* Just a bunch of nodes for p(RQ > 1), etc. across all the RQs
  + Thoroughly dissatisfying
* Does the assumption hold up for substances with similar/identical MoAs?

## [The neglected threat of toxic mixtures and how to fix it](https://chemtrust.org/wp-content/uploads/Chemical-cocktails_CHEMTrust-report_March-2022.pdf)

CHEM Trust, 2022

# Paper Thoughts

* How about if we only use well-tested APIs. E.g.:
  + Estrogens: Levonorgestrel, ethinylestradiol, estradiol
  + Progestogens: ulipristal, norelgestromin, nomegestrol, drospirenone, dienogest, desogestrel
* Salts are possibly an issue with at least some of these APIs, but with a subset we can afford to look more in-depth
  + (How) do we fit this into the BN, though?
* Apropos of nothing – do these substances all have regular dose-response relationships?
* There’s also plenty of potential to look at IA between progestogens and estrogens, because they’re so often taken as combinations?
* Some drugs that target the same receptors are antagonists (tamoxifen, estradiol, etc.); worth checking the literature for these so you can nuance the assumption of additivism
* If we have receptor affinity data (e.g. norethisterone has 150% the affinity for progesterone receptors as progesterone), can we use that to predict toxicity?
  + This is what Raoul did in Adam’s ED project

## Value of Information

* From what I’ve read, the Value of Information is a cash value a decision maker assigns to a piece of information (certain or otherwise)
* I don’t see how we can figure this out from a BN?
* Additionally, it seems difficult to employ within our existing models of BNs? Would we need to model decisions instead?
* An attempt to adapt the example from <https://www.youtube.com/watch?v=4N0FFzGYfTs> (Canessa et al, 2015) to a hypothetical toxicant for which tox data isn’t available
  + The example uses number of frogs as a measure of outcome (more frogs = better)
  + How do we measure the desirability of our outcome in a simple metric?

|  |  |  |  |
| --- | --- | --- | --- |
|  | **State of the System** | |  |
|  | Significant Toxicity | Negligible Toxicity |  |
| **Prior Belief** | 0.8 | 0.2 |  |
| Tox test Substance | Good  100 | Bad  50 | = 0.8 \* 100 + 0.2 \* 50 = **90** |
| Do nothing | Worst  25 | Good  100 | = 0.8 \* 25 + 0.2 \* 100 = **40** |
|  | Expected Value Under Certainty =  100 \* 0.8 + 100 \* 0.2 = **100** | | Expected value under uncertainty = **90** |
| Value of Perfect Information = 100 – 90 = **10** | | | |

## A brief summary of the paper might then be…

* **We mixture assessed progestogens and estrogens (separately and together) and determined current API-based RA under/over/correctly estimates risk in comparison**
* **We used value of information (somehow) to assess the cost-benefit analysis of not doing mixture toxicity, and of not having tox data available for relevant APIs**
* **In conclusion: more research needed…**

## Journal Options

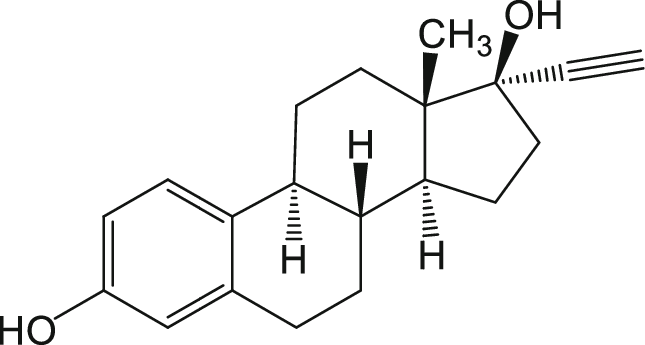
* ET&C – Original Article (8000 words)

# Diagram, venn diagram Description automatically generatedEcotoxicologically-relevant Drug Groups

### [Steroid hormones in the aquatic environment](https://www.sciencedirect.com/science/article/pii/S0048969721033775?via%3Dihub)

Ojoghoro, Scrimshaw & Sumpter (2021)

* Enviromnent has lots of chemical in
* No discussion of steroid hormone antagonists, as “we are unaware of any evidence to date that steroid hormone antagonists are present in the aquatic environment at concentrations that would cause concern”
* EE2 has an ethinyl group at carbon 17 that blocks oxidation of the C17-hydroxyl group, making it far more resistant to metabolism



### [Screening level mixture risk assessment of pharmaceuticals in STP effluents](https://doi-org.ezproxy.uio.no/10.1016/j.watres.2013.11.005)

Backhaus & Karlsson (2014)

* Pharmaceutical risk in the environment has repeatedly been assessed as negligible, outside of special cases
  + But, mixtures
* Various studies have shown various greater-than-additive mixture effects between multiple toxicants
* Backhaus & Faust developed two possible mixture ERA approaches
  + Sum RQ
  + Sum TU, then pick the most sensitive trophic level, and apply an appropriate AF to calculate a final RQSTU
* In this paper, Backhaus & Karlsson apply both techniques to a dataset of 7 European STP effluents from Andreozzi et al., 2003
* They note that EMA ERA guidelines say “use chronic toxicity data”, but there was sufficiently little available that they elected to use acute EC50s with AF = 1000 in line with REACH guidelines on ERA of single substances
* Toxicity EC50s were gather from reviews, EPA ECOTOX, MistraPharma, and Scopus
  + Where multiple EC50s were available, the lowest was used
  + Where no EC50s were available, NOECs were used
  + Where neither were available, ECOSAR was used to predict QSARs
* Algal TU exceeding the FDA threshold of 10-3 (tier 1) were found for antibiotics in several STPs, driven by blue-green algae (apparently the EMA suggests antibiotic ERA should be based on blue-green algae)
* Fish TUs never exceeded the 10-3 threshold – this is suggested to be down to fish-human similarity, as APIs are also designed not to be acutely toxic to people
* Gemfibrozil & ibuprofen exceeded 10-3 TUinvert in several cases
* Conclusions: acute tests aren’t very useful, but perhaps neither are chronic, if they exclude important endpoints like behaviour
* Following REACH/WFD guidelines (AF 1000), RQSTU­ values were found to be between 16-48. Final risk would thus depend on actual dilution of effluent in the recipient stream.
* TUs only allow you to identify the “most risky” substance towards a taxa
* The ratio of RQSTU to RQMEC/PNEC never exceeded 1.3 (with identical AFs), because the APIs driving risk had similar ecotoxicological profiles, and risk estimates were driven by risk to algae. With a more diverse range of substances, this ratio could be as high as 3 (since 3 taxa are assessed)
* Real IA can’t be carried out, as there are not DRCs available for most APIs
  + They did some maths I don’t understand by “estimating the maximum error that occurs by simply ignoring IA”
* One could calculate a Maximum Cumulative Ratio (MCR) by dividing STU by max (TU), and use it as a trigger for mixture toxicity assessment, but it’s not useful here as you have to include *all* the toxicants in a mixture to assess this
* In conclusion, we only looked at a small subset of APIs with poor data, and still RQ < 1 in several cases.

### [EurEau Position on Environmental Quality Standards for pharmaceuticals](https://www.eureau.org/resources/position-papers/6061-position-paper-on-environmental-quality-standrds-eqs-for-pharmaceuticals/file)

European federation of national associations of water services (2021)

* We support the setting of EQSs for pharmaceuticals, as long as we’re not left carrying the can
  + Specifically, don’t make WWTPs responsible for bridging the gap between the end of the pipe and the EQS
* Priority, harmful pharmaceuticals should be banned from OTC sales
  + Where medications are already prescription-only, the EMA should publish recommendations for less hazardous alternatives
* As with pesticides, exceedance of EQS should trigger a formal review & mitigation process
* Please publish ERA data properly
* Polluter Pays Principle
* Risk assess mixtures of chemicals and their transformation products

### [Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects](https://doi-org.ezproxy.uio.no/10.1016/S0378-4274(03)00068-7)

Cleuvers (2003)

* 10 prescription drugs - Clofibrinic acid (metabolite of several lipid lowering drugs), Carbamazepine (anti-epileptic), Propranolol and Metoprolol (β-blocker), Ibuprofen sodium, Diclofenac sodium and Naproxen sodium (analgesics/anti-inflammatory drugs), Captopril (anti-hypertensive), and Metformin (anti-diabetic)
* 3 species – *Daphnia magna*, *Lemna minor* & *Desmodesmus subspicatus*
* Generally 5 concentrations per substance, plus various binary mixtures of APIs with shared modes of action
* Ibuprofen/Diclofenac/Naproxen are all COX inhibitors in humans, but act by non-polar narcosis in other species
* Bit of a wonky paper, and I don’t really understand it

### [Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S016041200900186X?via%3Dihub)

Gros et al. (2010)

* 4 sampling periods over 3 years, various WWTPs across the Ebro River Basin (NE Spain), 73 APIs analysed
* At the time of writing, in Spain, WWT was relatively primitive compared to N Europe
  + Included primary (filtration of large solids) & secondary (biofiltration/aeration/oxidation)
  + Lacked, by contract, tertiary (further filtration, disinfection)
* Analysis of 12 analgesics, 6 statins, 5 psychoactives, 4 antihistamines, 4 tetracycline, 8 macrolide, 3 sulphonamide, 6 fluoroquinolone & 4 other antibiotics, etc
  + Notably no hormonal drugs
* WWTP removal of APIs was a mixed bag, even within categories, and a few APIs even increased in concentration
  + NSAIDs were well-removed, though
  + An interesting adjunct – apparently higher removal efficiency is observed in summer than winter
* Measured dilution factors from 30-40 (Ebro), to as low as 5 (river Arga, Pamploma)
* HQs based on MEC / PNEC calculated (but the PNECs were based on acute EC50 \* AF = 1000)
* “it could be concluded that dilution of wastewaters once pharmaceuticals are discharged in receiving river water efficiently mitigate possible environmental hazards”
  + Standard disclaimer about mixture toxicity

### [Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions?](https://doi.org/10.1289/ehp.1104477)

Boxall et al. (2012)

* The world’s worst website article layout
  + Not really, but it is pretty bad
* 1. Prioritisation – focusing of limited resources
* 2. Identification of pathways of exposure – especially those currently excluded from ERA
* 3. Uptake of ionisable APIs – we know even less than we do for unionisable APIs
* 4. Many PPCPs turn into Non-Extractable Residues – what’s the deal
* 5. How do we use clinical data for ERA?
* 6. How do we use receptor conservation for ERA, or at least flagging?
* 7. How do we translate molecular/histological endpoints into adversity endpoints?
* 8. How do we avoid missing important toxicity with OECD-based tests?
  + Standard Vulture OECD test?
* 9. What about long-term exposure to low-level mixtures?
* 10. How do we do all this and still cut down on animal testing?
* 11. How can we identify where PPCPs do and will pose the biggest risk?
* 12. How important are PPCPs compared to other stressors?
* 13. What about all the non-OECD standard wildlife?
* 14. What about metabolites and environmental transformation products?
* 15. How do we assess if current ERA works:
* 16. Does environmental exposure drive AMR, and significantly so?
* 17. How do we put this in ERA?
* 18. How can we mitigate/manage PPCP risk?
* 19. What WWTP techs reduce effects without accidentally increasing effects?
* 20. How do we assess the efficacy of risk management approaches?

### [Arrrrrghghg](https://www.researchgate.net/profile/Thomas-Ter-Laak/publication/233773122_Prediction_of_concentration_levels_of_metformin_and_other_high_consumption_pharmaceuticals_in_wastewater_and_regional_surface_water_based_on_sales_data/links/5d19be10299bf1547c8cec78/Prediction-of-concentration-levels-of-metformin-and-other-high-consumption-pharmaceuticals-in-wastewater-and-regional-surface-water-based-on-sales-data.pdf)

### [Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data](https://www.sciencedirect.com/science/article/pii/S0048969712013289?via%3Dihub)

Oosterhuis, Sacher & ter Laak (2013)

* Predicted emissions of metformin, metoprolol, sotalol, losartan, valsartan, irbesartan, hydrochlorothiazide, diclofenac and carbazepine in the environment
* Compared to WW concentrations, removal in WWTPs, and recovery in region SW
* Various people have done sales based risk prediction better than me, but the authors believe their paper is novel in that they make predictions and then “follow” then through the WW system
* Top-50 sales were extracted for a village (7000 people) and a city (160,000 people), in DDD/year
  + Hospital pharmacy contribution was excluded, justified as it being negligible
  + Kg/yr emissions to both WWTPs were predicted, and a human excretion factor used to approximate the percentage of API entering the environment
* These guys know far more about WWTPs than me
* Water was sampled in September/October/December in 2010
* HPLC/MS–MS analysis was done
* Predicted concentrations were generally slightly higher than measured concentrations, although “the consumption based prediction is rather accurate”
* Lots of details, including underestimates, overestimates and removal rates
* Recovery of pharmaceuticals in surface water after 4 days retention was high (71-187%), though this was in December

### [Pharmaceuticals in Northern environments; what, where and how much?](https://pub.norden.org/temanord2020-502/temanord2020-502.pdf)

A whole bunch of NIVA guys (2020)

## Estrogens

* “development and regulation of female reproductive system and secondary sex characteristics”
* 3 major endogenous estrogens: estrone (E1), estradiol (E2), and estriol (E3) + estetrol (E4) only produced during pregnancy
* Conserved in all verts and some inverts
  + Loooads of effects in humans
* Lots of salts, which we probably can’t justify excluding at this point

Big list of sex hormones in Norway:

* 9 substances (tox data for 7), of which:
  + 2 antagonists
  + 3 selective receptor modulators (?)
  + 6 substances with estrogenic effects

|  |  |  |  |
| --- | --- | --- | --- |
| API\_Name | Desc\_Long | Ecotox data? | FASS PNEC (mg/L) |
| anastrozole | estrogen agonist for breast cancer | Chronic [Carp LOECs, CompTox](https://comptox.epa.gov/dashboard/chemical/hazard/DTXSID9022607) |  |
| bazedoxifene | selective estrogen receptor modulator for postmenopausal osteoporosis | [Full ERA with some EC50s, FASS](https://www.fass.se/LIF/product?userType=2&nplId=20120802000016&docType=78&scrollPosition=0) | 1.4E+3 mg/L (AF = 10) |
| clomifene | selective estrogen receptor modulator for infertility | No FASS/CompTox/Norman |  |
| estradiol | estrogen for menopause symptoms, ovarian failure, some cancers | [Full ERA of estradiol and metabolites, SSD used to generate PNEC (but not published), FASS](https://www.fass.se/LIF/product?userType=2&nplId=19980306000165&docType=78&scrollPosition=204)  Abundant NOECs/LOECs, somewhat fewer EC50s, Norman  CompTox has mammal only | 4.0E-7 mg/L (AA-EQS) |
| estriol | estrogen for menopause symptoms, and, in dogs, urinary incontinence | [No ERA on estriol-only medications, but assessed under estradiol, FASS](https://www.fass.se/LIF/product?userType=2&nplId=19980306000165&docType=78&scrollPosition=204)  Loads of EPA test EC50s on Norman | 4.7E-6 mg/L (AF = 10) |
| ethinylestradiol | estrogen widely used in combination birth control pills | [Full ERA with some EC50s, FASS](https://www.fass.se/LIF/product?userType=2&nplId=20090611000029&docType=78&scrollPosition=102)  Great abundance of EC50s on Norman | 3.0E-8 mg/L (AF = 10) |
| norethisterone | progestin for birth control, menopause, gynecological disorders with weak estrogenic and androgenic effects | [Some tests but insufficient for full ERA, FASS](https://www.fass.se/LIF/product?userType=2&nplId=19980306000165&docType=78&scrollPosition=0)  Some NOECs/LOECs on Norman (as norethindrone), still no algae |  |
| prasterone | androgen and estrogen for various gynecological disorders and applications | Rat/mouse data only |  |
| raloxifene | selective estrogen receptor modulator for osteoporosis after menopause | None found (haven’t checked lit) |  |
| tibolone | progestogen, estrogen, androgen used in hormone therapy | None found (haven’t checked lit) |  |
| Fulvestrant | antiestrogen | Daphnia and fish tests on Norman  [Full ERA (FASS)](https://www.fass.se/LIF/product?userType=0&nplId=20200226000011&docType=78&scrollPosition=408) | 5.7e-7 mg/L (AF = 10) |

### [Derivation of an Aquatic Predicted No-Effect Concentration for the Synthetic Hormone, 17α-Ethinyl Estradiol](https://pubs-acs-org.ezproxy.uio.no/doi/10.1021/es800633q)

Caldwell et al (2008)

* Chart

  Description automatically generatedPulled data from 39 (!) studies in 26 species 1994-2007 to construct an SSD from reproduction NOECs
* The TGD AF of 1000 is insufficient because EE2 is specially designed to impede normal reproductive function at dosed levels
  + Fish have estrogen receptors, algae don’t, inverts might (?)
* Studies were Klimsch ranked for Reliability, Relevance and Adequacy
* NOECs ranged from 0.3 – 500,000 ng/L, with verts being most sensitive
* Full life cycle fish studies are, apparently, especially critical
* Goodness-of-fit statistics were used to select an appropriate distribution, leading to the selection of a Weibull distribution
* A PNEC was calculated from the HC5 – hazardous concentration at which 5% of all species are effected, then a 50% CI slapped on for safety (?) – HC5,50
  + Lemna species were excluded from this calculation due to the lack of a proposed MoA
* The actual output NOEC varies a little depending on a slightly ambiguous NOEC from one of the constituent studies, but the authors went with the value 0.35 ng/L as it fit well with the NOECs in said studies
* The authors raise the steepness of DRCs in fish studies as a potential issue with EE2 NOECs, but since they did an SSD it shouldn’t be a problem!
* The authors conclude **0.35 ng/L** fits with other field/population/etc. studies, and conclude it’s a decent value, especially given the present difficulties of working at such low levels

### [Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol](https://setac-onlinelibrary-wiley-com.ezproxy.uio.no/doi/10.1002/etc.1825)

Caldwell et al (2012)

* Builds on 2008 paper
* Not enough data to construct an SSD for estrone (E1), 17β-estradiol (E2)

### [Conversion of Estrone to 17β-Estradiol: A Potential Confounding Factor in Assessing Risks of Environmental Estrogens to Fish](https://setac-onlinelibrary-wiley-com.ezproxy.uio.no/doi/full/10.1002/etc.4828)

Tapper et al (2020)

* Everyone’s talking about fish feminisation
* Estrone (E1) may be in the environment at higher concentrations than other estrogens, but its potency is reported as being far lower (but there’s a considerable range reported)?
* Estrone can, of cause, be converted back into 17β-estradiol (E2) in the fish
  + According to the authors small-sample-size-study, these variations may be down to sex and reproductive status (in rainbow trout)

### [Assessing combined toxicity of estrogen receptor agonists in a primary culture of rainbow trout (Oncorhynchus mykiss) hepatocytes](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0166445X10004017?via%3Dihub)

Petersen & Tollefsen (2011)

* Primary hepatocytes from fish are a well-characterised high-throughput screening tool
  + Vitellogenin is used as an estrogenic biomarker (KET has worked previously on this)
* Vtg genes are usually silent in males, but can be induced by exposure to ER-agonists
* CA models built, fish killed, juvenile hepatocytes extracted
* Sigmoidal DRCs constructed for single chemicals and mixtures
  + Mixtures: four highly potent agonists (estradiol, estrone, estriol, diethylstilbestrol)
  + Plus 5-compound, 7-compound and 9-compound mixtures including various non-API Eds
* I don’t understand the results
* Only slight differences between CA and IA predictions were seen
* Less-than-additive effects were seen at the highest concentrations of all mixtures
  + This may be due to cytotoxicity at higher concentrations, perhaps driven by BPA and 4-t-octylphenol
* The Funnel Hypothesis (Warne and Hawker, 1995) states that as you add chemicals to a mixture, deviation from the additive model decreases
* Authors say: CA and IA are both adequate for predicting mixture effects of ER agonists (in fish livers)
* How to square this with Hutchinson et al.’s suggestion that Vtg induction is best used as a signpost, but not to use to determine toxicity?

### [The consequences of exposure to mixtures of chemicals: Something from ‘nothing’ and ‘a lot from a little’ when fish are exposed to steroid hormones](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S004896971733139X?via%3Dihub)

Thrupp et al. (2017) – John P. Sumpter’s Brunel Buddies

* Single exposures of fish to five different synthetic steroids (EE2, trenbolone, beclomethasone dipropionate, desogestrel & levonorgestrel)
  + egg production in breeding pairs of Pimephales promelas (fathead minow)
  + DRCs constructed
* Then 5-component mixture of APIs at fixed EC10 ratio applied at 3 different concentrations (insignificant, medium & high)
  + Repeated once, to make it reproducible
  + Three APIs (EE2, levonorgestrel & trenbolone) were measured on days 0, 7, 14 and 21
* No evidence of acute toxicity
* IA and CA predicted mixture effects reasonably well, although I don’t really understand the details
* Egg production was supressed partially by a mixture of APIs at insignificant levels, and fully at EC18-40 levels (I really don’t understand this second part)
* Some janky writing in places

### [European demonstration program on the effect-based and chemical identification and monitoring of organic pollutants in European surface waters](https://www.sciencedirect.com/science/article/pii/S0048969717314365)

Tousova et al (2017)

* 50L of surface water sampled from 18 sites in four European river basins
* Big old battery of effect-based analysis (EDA) (algal, fish embryo, amphibian toxicity, ED screening), target analysis (151 organic micropollutants), + non-target screening
  + But this study was cheaper and many EDAs
* Most pronounced effects were estrogenicity, algal toxicity & fish embryo toxicity
* Most bioassays, especially androgenicity, glucocorticoid activity and fish embryo toxicity couldn’t be explained by target compounds
* Estrone & nonylphenoxyacetic acid drove estrogenicity
* Risk Assessment & Prioritisation based on MEC95 / Norman PNEC

### [Risk of endocrine disruption to fish in the Yellow River catchment in China assessed using a spatially explicit model](https://doi-org.ezproxy.uio.no/10.1002/etc.3133)

Liu et al (2015)

* Measuring estrogens across the whole of China would take a lot of work, so let’s model concentrations
* E1 and E2 concentrations across surface water in the Yellow River catchment were modelled using a “spatially explicit model” – global water availability assessment (GWAVA)
* The river was modelled in GWAVA, which estimated gridded water flows
* Gridded pollution load releases were then added to the model
* Loss by water transfer, abstraction & biodegradation were modelled
* Sources of estrogen were assumed to be STP-treated effluent (78.1%), untreated sewer effluent (5.5%) and rural effluent (5%)
* Population and WWTP and river locations were related to each other, somehow
* Conversion of E2 to E1 (50%) in sewers was also modelled
* Best, worst and middle case scenarios were used to bin variety in estrogen removal performance
* Measured and estimated river flow were compared – and seemed to agree
* Modelled E1 levels along the river under various scenarios were compared to point measurements:

Diagram

Description automatically generated

* Combined effects were modelled using estradiol equivalent concentrations (EEQ) based on UK Environment Agency concepts. This was used to make a nice map

A picture containing shape

Description automatically generated

### [Natural Variations in Flow Are Critical in Determining Concentrations of Point Source Contaminants in Rivers: An Estrogen Example](https://pubs-acs-org.ezproxy.uio.no/doi/10.1021/es101799j)

### [Do Concentrations of Ethinylestradiol, Estradiol, and Diclofenac in European Rivers Exceed Proposed EU Environmental Quality Standards?](https://pubs-acs-org.ezproxy.uio.no/doi/abs/10.1021/es4030035)

### [Putting pharmaceuticals into the wider context of challenges to fish populations in rivers](https://royalsocietypublishing-org.ezproxy.uio.no/doi/pdf/10.1098/rstb.2013.0581)

### [Predicting concentrations of the cytostatic drugs cyclophosphamide, carboplatin, 5-fluorouracil, and capecitabine throughout the sewage effluents and surface waters of Europe](https://setac-onlinelibrary-wiley-com.ezproxy.uio.no/doi/full/10.1002/etc.2311)

### [Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0048969714017574?via%3Dihub)

### [Ecological risk assessment of fifty pharmaceuticals and personal care products (PPCPs) in Chinese surface waters: A proposed multiple-level system](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0160412019335731?via%3Dihub)

### [A national risk assessment for intersex in fish arising from steroid estrogens](https://setac-onlinelibrary-wiley-com.ezproxy.uio.no/doi/full/10.1897/08-047.1)

### [Worldwide estimation of river concentrations of any chemical originating from sewage-treatment plants using dilution factors](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4253128/)

### [Comparing predicted against measured steroid estrogen concentrations and the associated risk in two United Kingdom river catchments](https://doi-org.ezproxy.uio.no/10.1002/etc.1756)

Williams et al. (2012)

* Measuring toxicants effective at very low concentrations is difficult and expensive
* A number of geographically referenced water quality models have been developed to predict concentrations of “down the drain chemicals” in catchments
* These take modelled rivers and measured WWTP parameters
  + At the time of writing, measurement data for the UK’s WWTPs wasn’t great, but the authors have “state of the art” analysis of two UK rivers, and set out to do a comparison
* The rivers Avon and Erewash were sampled repeatedly between 2007 and 2009, as were WWTPs along the studied stretches
* Samples were analysed for E1, E2 and EE2 on the day of sampling
* Complicated Liquid Chromatography-tandem mass spectrometry was performed
* Mixtures of steroid estrogens have been shown to act additively in effects on fish, so effects of E1 and EE2 were expressed relative to E2 (E2eqv)
* Overall, although the model and measurement’s didn’t always agree, they both classified the majority of both rivers as having an E2eqv concentration between 1 – 10 ng/L, and therefor being at risk
  + This was largely driven by EE2, which the model did a good job of predicting
  + That said, fate of chemicals in STPs is still tricky, and work needs to be done to figure out variance between and variability at STPs

### [From single chemicals to mixtures—Reproductive effects of levonorgestrel and ethinylestradiol on the fathead minnow](https://www.sciencedirect.com/science/article/pii/S0166445X15300680#!)

Runnalls et al., (2015) – John P. Sumpter, OBE

* Fathead minnow, 21 day pair-breeding test with a variety of endpoints, + plasma concentration prediction (& measurement for levonorgestrel)
* All empirical evidence suggests CA is the best approach, especially (but not only) with same MoAs
* EE2 is an estrogen, and Levonorgestrel a progestogen and androgen, so the assumptions for IA are arguably met, but both chemicals are expected to supress egg production
* 3 sets of studies conducted – individual stressors + mixture (fixed ratio)
* Concentrations maintained in a flow-through system by peristaltic pump, eggs counted daily
* Finally, fish were anesthetised and measured for various growth & sexual characteristics
  + Plasma also analysed for male/female sex hormones, VTG and plasma levonorgestrel
  + Human therapeutic plasma concentrations of EE2 and levonorgestrel used to hypothesise point at which hormones would suppress egg production in fish (Fish Plasma Model)
* Cumulative number of eggs produced was extremely variable, seemingly driven by biological variation both pre and post exposure
* A picture containing diagram

  Description automatically generatedCA:
* Chart, scatter chart

  Description automatically generatedMeasured tank EE2 concentrations were between 52 & 74% of nominal values, levonorgestrel between 63 & 105%
* Graph show relationship between nominal concentration and reproductive performance (cumulative number of eggs produced by fish after three weeks exposure, compared to pre-exposure)
* Broadly, levonorgestrel had pronounced effect on females, EE2 on males
* A mixture of levonorgestrel and EE2 showed no significant deviation from additivity, although the experimental design was in any case unable to detect effects at the lowest concentrations
* Binary mixtures are apparently not sufficient to judge whether CA or IA is better at explaining the observed mixture effects
  + Ultimately, all you can do is make sweeping statements about modes of action
  + That said, the actual mixture toxicity should fall between the two – “prediction window”

## Progestogens

* Includes progestogens/progestogens/gestagens/gestogens, of which progestins are a synthetic subset
* Controls male and female reproductive system in humans
* Not endocrine disruptors, though?
* Used for birth control, hormone therapies
* Some crossover with estrogens, androgens (e.g. tibolone)
  + Also a mixed bag of effects on other systems (glucocorticoid, etc.)
* Affinity for various receptors generally well-characterised
* 19 substances with known effect (+ or -) on progestogen receptors, full ERAs on FASS for 6 (partial data for a further 5)
  + Haven’t checked for EC50s for all taxa

|  |  |  |  |
| --- | --- | --- | --- |
| API\_Name | Desc | Ecotoxicity | RQ (FASS) |
| aglepristone | antiprogestogen used as abortifacient in animals | None found (haven’t checked lit) |  |
| altrenogest | veterinary birth control, progestin | None found (haven’t checked lit) |  |
| cyproterone | antiandrogen and progestin for birth control, androgen conditions, feminising hormone therapy | [ERA, Daphnia acute tox only](https://www.fass.se/LIF/product?userType=2&nplId=19780414000015&docType=78&scrollPosition=0) (FASS) |  |
| desogestrel | progestin used for birth control and menopausal hormone therapy, often with an estrogen | [Full ERA](https://www.fass.se/LIF/product?userType=2&nplId=20100621000022&docType=78&scrollPosition=0) (FASS) | 2 |
| dienogest | progestin and antiandrogen for birth control, menopausal hormone therapy, endometriosis | [Full ERA](https://www.fass.se/LIF/product?userType=2&nplId=20160803000042&docType=78&scrollPosition=0) (FASS) | 0.24 |
| drospirenone | progestin birth control | [Full ERA](https://www.fass.se/LIF/product?userType=2&nplId=20031114000017&docType=78&scrollPosition=0) (FASS) |  |
| dydrogesterone | progestin for various reproductive and gynecological disorders | None found (haven’t checked lit) |  |
| etonogestrel | progestin used in birth control implants | [Full ERA](https://www.fass.se/LIF/product?userType=2&nplId=19990226000145&docType=78&scrollPosition=0) (FASS) | 2.8 |
| levonorgestrel | progestin for birth control including monthly, implanted and emergency | [Full ERA](https://www.fass.se/LIF/product?userType=2&nplId=20090611000029&docType=78&scrollPosition=0) (FASS) | 141 |
| medroxyprogesterone acetate | progestin for birth control and various hormone therapy | One Daphnia exposure, NOEC only (Norman) |  |
| megestrol acetate | progestin appetite stimulator, birth control, antineoplastic | None found (haven’t checked lit) |  |
| mifepristone | progestin abortifacient and treatment for hyperglycemia with Cushing's | Fish acute NOECs/LOECs (Norman) |  |
| nomegestrol | progestin for birth control, menopause, gynecological disorders | [Full ERA, but only algal EC50](https://www.fass.se/LIF/product?userType=2&nplId=20090917000020&docType=78&scrollPosition=0) (FASS) |  |
| norelgestromin | progestin for birth control | [Full ERA](https://www.fass.se/LIF/product?userType=2&nplId=20020822000037&docType=78&scrollPosition=0) (FASS) | 0.0011 |
| norethisterone | progestin for birth control, menopause, gynecological disorders with weak estrogenic and androgenic effects | [Full ERA](https://www.fass.se/LIF/product?userType=2&nplId=19980306000097&docType=78&scrollPosition=0) (FASS)  [Partial ERA](https://www.fass.se/LIF/product?userType=2&nplId=19980306000165&docType=78&scrollPosition=0) (FASS) | 4 |
| osaterone acetate | antiandrogen and progestin for enlarged canine prostates | None found (haven’t checked lit) |  |
| progesterone | progestin for hormone replacement therapy | Extensive Fish LC50s, Crustacean NOECs (Norman) |  |
| tibolone | progestogen, estrogen, androgen used in hormone therapy | None found (haven’t checked lit) |  |
| Ulipristal (acetate) | progesterone receptor modulator for emergency birth control | None found (haven’t checked lit) |  |

### [Effects of the Gestagen Levonorgestrel in a Life Cycle Test with Zebrafish (Danio rerio)](https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5008#.YipWo1Dlako.twitter)

Teigler et al. (2021)

* Long story short, at higher (>1.64 ng/L) concentrations levonorgestrel shifts the sex ratio male
* At the highest concentration measured (5.45 ng/L) all fish were male

### [Synthetic Progestins in Waste and Surface Waters: Concentrations, Impacts and Ecological Risk](https://www.mdpi.com/2305-6304/10/4/163/htm)

Rocha & Rocha (2022)

## Statins

Statins sold in Norway:

* Atorvastatin, Fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin
* Come in acid and lactone forms, acids are the active form
* “Statins act by competitively inhibiting HMG-CoA reductase, the rate-limiting enzyme of the mevalonate pathway”
  + Very conserved, used to manufacture wide range of biomolecules in cholesterol, vitamin K, all steroid hormones
  + Also targeted by bisphosphonates (treatment for bone-degenerative diseases)
* Gonna call this good enough for the time being, though there’s plenty more to look at. I was wrong, there’s a whole bunch of EC50s for statins
  + PNECs are rarer, but maybe that’s a good thing

Search terms: “statin”, categories: “environmental sciences”, “plant sciences”, “zoology”, “marine freshwater biology”, “entomology”, “ecology”, “water resources”

*This excluded loads of relevant papers, though…*

96 results

|  |  |  |
| --- | --- | --- |
| Source | Summary | Toxicity Endpoint |
| [Brain et al. (2006)](https://pubs-acs-org.ezproxy.uio.no/doi/10.1021/es0600274) | *Lemna gibba* exposed to atorvastatin and lovastatin for 7 days | Fresh Weight  Atorvastatin EC50: **135 ± 27.1 μg/L**  Lovastatin EC50: **106 ± 8.27 μg/L** |
| [Pasha & Moon (2017)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S1382668917300996?via%3Dihub) | Zebrafish larvae exposed to atorvastatin | Mortality  Atorvastatin EC50: **3.3 mg/L** |
| [Fulton et al. (2009)](https://www.tandfonline.com/doi/full/10.1080/03601230902801083) | Estuarine fish *Fundulus heteroclitus* exposed to simvastatin | Mortality  Simvastatin EC50: **2.68 mg/L** |
| [Ellesat et al. (2011)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0141113611001097?via%3Dihub)  (also, Ketil H) | Hepatocytes of plaice, long rough dab, Atlantic cod exposed to Atorvastatin and simvastatin for 24 hours (acids & lactones) (Lowest value of 3 species) | Metabolic Activity  Atorvastatin acid EC50: **395** **mg/L**  Atorvastatin lactone EC50: **6** **± 4 mg/L**  Simvastatin acid EC50: **298** **± 138 mg/L**  Simvastatin lactone EC50: **14** **± 1 mg/L** |
| [Ellesat et al. (2010)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0887233310001487)  (also, Ketil H) | Hepatocytes of rainbow trout exposed to atorvastatin and simvastatin (24/48/72h) | Toxicity not directly stated |
| [Richards & Cole (2006)](https://link-springer-com.ezproxy.uio.no/article/10.1007/s10646-006-0102-4) | *Xenopus laevis* larvae exposed to atorvastatin and simvastatin (96h) | Teratogenesis  Atorvastatin EC50: **23.1 mg/l**  Lovastatin EC50: **20.5 mg/l** |
| [Barros et al. (2018)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0166445X18301619?via%3Dihub) | Chronic (90 day) exposure of zebrafish (reproduction at 70 days) to 8 – 1000 ng/L simvastatin | Some effects on survival, development, reproductive parameters seen at **1** **μg/L** |
| [Dahl, Gorokhova & Breitholtz (2006)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0166445X06000440) | simvastatin + copepods |  |
| [Ribeiro et al. (2015)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0147651315000093) | simvastatin + sea urchin embryos |  |
| [Neuparth et al. (2014)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0166445X14002458) | simvastatin + amphipods |  |
| [Meng et al (2020)](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0048969719346947?via%3Dihub) | Lovastatin/simvastatin/pravastatin + zebrafish embryos |  |

CompTox Search: Atorvastatin, Fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin

|  |  |  |
| --- | --- | --- |
| Substance |  | Toxicity |
| Atorvastatin | EC50s | Acute plants, fungi, frogs, inverts |
| Fluvastatin |  | None found |
| lovastatin |  | Mouse/rat only |
| pravastatin |  | None found |
| rosuvastatin |  | None found |
| simvastatin |  | Rat only |

CompTox not working today, try again tomorrow

Felleskatalogen/FASS

|  |  |
| --- | --- |
| Substance | PNECs |
| Atorvastatin |  |
| Fluvastatin |  |
| lovastatin |  |
| pravastatin |  |
| rosuvastatin | [FASS](https://www.fass.se/LIF/product?userType=0&nplId=20040916001474&docType=78&scrollPosition=697) |
| simvastatin | [FASS](https://www.fass.se/LIF/product?userType=0&nplId=20040607006269&docType=78&scrollPosition=814) |

## COX Inhibitors

* In humans, the enzyme cyclooxygenase-2 or Prostaglandin-endoperoxide synthase 2 forms a key part of the prostacyclin synthesis pathway, which is upregulated during inflammation
* NSAIDs non-selectively inhibit COX-1 (which make cause gastrointestinal adverse effects) and COX-2
* Inhibiting COX-2 is the mode of action of a number of newish (developed around the turn of the century) NSAIDs, but significant cardio adverse effects have been found, and most of these substances have been banned, but some were still sold in Norway 2016-19
* In Norway, we recorded 26 different analgesics with a COX-inhibiting mode of action, 7 of which have toxicity data available on FASS/CompTox/Norman
* COX-1 & COX-2 are found in many vertebrates, while isoforms have been found in invertebrates (Järving *et al.*, 2004)

|  |  |  |  |
| --- | --- | --- | --- |
| API | Receptors | Description | Ecotoxicity? |
| cimicoxib | COX-2 inhibitor | coxib NSAID used for arthritis and pain management in dogs | No data found in databases |
| mavacoxib | COX-2 inhibitor | veterinary COX-2 inhibitor for degenerative joint disease | No data found in databases |
| parecoxib | COX-2 inhibitor | COX-2 selective inhibitor for pain management | No data found in databases |
| bromfenac | COX-2 inhibitor,  some COX-1 | NSAID for eye inflammation for cataract surgery | No data found in databases |
| carprofen | COX inhibitor | NSAID veterinary analgesic for joint and post-operative paint | No data found in databases |
| celecoxib | COX-2 inhibitor | NSAID for arthritis, ankylosing spondylitis, menstrual pain | Daphnia chronic & acute tests, Norman |
| cimicoxib | COX-2 inhibitor | coxib NSAID used for arthritis and pain management in dogs | No data found in databases |
| dexketoprofen | COX inhibitor | NSAID for mild to moderate pain relief | No data found in databases |
| diclofenac | COX inhibitor | NSAID for mild-moderate pain, arthritis, etc. | [Full ERA (FASS)](https://www.fass.se/LIF/product?userType=0&nplId=19811218000090&docType=78&scrollPosition=172.8000030517578) |
| etoricoxib | COX-2 inhibitor | NSAID for arthritis and related conditions | [Full ERA (FASS)](https://www.fass.se/LIF/product?userType=0&nplId=20151015000036&docType=78&scrollPosition=510) |
| firocoxib | COX-2 inhibitor | NSAID for pain management in dogs, horses | No data found in databases |
| flunixin | COX inhibitor | veterinary NSAID | No data found in databases |
| flurbiprofen | COX inhibitor | NSAID for arthritis | No data found in databases |
| ibuprofen | COX inhibitor | NSAID for many minor pains | No data found in databases |
| ketoprofen | COX inhibitor | NSAID for mild-moderate pain, arthritis | [Full ERA (FASS)](https://www.fass.se/LIF/product?userType=0&nplId=19951018000026&docType=78&scrollPosition=306) |
| ketorolac | COX inhibitor | NSAID for moderate-severe pain | No data found in databases |
| meloxicam | COX-2 inhibitor,  some COX-1 | NSAID for arthritis | No data found in databases |
| nabumetone | COX-2 inhibitor,  some COX-1 | NSAID for arthritis | No data found in databases |
| naproxen | COX inhibitor | NSAID for arthritis, gout, etc. | [Full ERA (FASS)](https://www.fass.se/LIF/product?userType=0&nplId=19871211000206&docType=78&scrollPosition=408) |
| nepafenac | COX inhibitor | NSAID for ophthalmic pain | No data found in databases |
| phenylbutazone | COX inhibitor | NSAID for ankylosing spondylitis | No data found in databases |
| piroxicam | COX inhibitor | NSAID for arthritis | No data found in databases |
| suxibuzone | COX inhibitor | NSAID for equine joint and muscle pain | No data found in databases |
| vedaprofen | COX-2 inhibitor,  some COX-1 | veterinary NSAID | No data found in databases |
| paracetamol | COX inhibitor | ubiquitous painkiller | [Full ERA (FASS)](https://www.fass.se/LIF/product?userType=0&nplId=19880205000177&docType=78&scrollPosition=294) |
| naproxen | COX inhibitor | NSAID for arthritis, gout, etc. | [Full ERA (FASS)](https://www.fass.se/LIF/product?userType=0&nplId=20091015000059&docType=78&scrollPosition=159) |

## Apropos of nothing, the Thyroid

### [ERGO: Breaking Down the Wall between Human Health and Environmental Testing of Endocrine Disrupters](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7215679/)

Holbech et al. (2020)

* ED isn’t part of REACH
* ERGO aims to improve cell tests, fish & amphibian assays, plus, of course, models, to improve/reduce in vivo/whole organism tests
* All vertebrates have thyroid systems (even fish!), and they do various vital developmental things
* A sickeningly fast project paper compared to ECORISK2050
  + No PhDs?

## Meeting with ADL on Endocrine Disruptors

* Big project funded by Norwegian Department for Climate (KLD), etc.
* Initial prioritisation effort based on ~60,000 substances
  + Filtered down to remove known EDs, substances outside of various physico-chemical parameters
* Consortia called ERGO – European cluster under the umbrella of GOLIATH – looking at thyroid effects, Henrick Holbeck, Denmark
* Ana Caterina did PhD on mixtures of pesticides
* Read about IMI project: Premier
* EATS modality – Estrogen, Androgen, Thyroid & Steroids
* Worth considering mixtures with different binding mechanisms

# Thomas Bayes’ Incredible Networks

Hierarchical modelling of EC50s/PNECs

Object-Oriented something?

## Some of Jannicke’s Papers over the last few years

### Development of a hybrid Bayesian network model for predicting acute fish toxicity using multiple lines of evidence

Moe (2020)

Diagram

Description automatically generated

* Fish Embryo Tests can’t replace AFT on their own, but maybe with our WoE BN they can?
* Got a biiiig dataset of EC50s from Procter and Gamble (Threshold Database)
  + Also whatever QSARs were available, which were then averaged?
* Anders/Raoul made a slick interactive online tool that uses the BN to predict stuff
* CPTs appear to be separate excel files – is this something I can do?
* Lots of rough weighting and low/medium/high kinda characterisation
* Daphnia:algae toxicity – where value is between 0.5 – 2, shared mode of toxicity and therefore probably the same mode of tox to fish?

### Weight of evidence tools in the prediction of acute fish toxicity

Moe (2022)

* How can acute fish tox tests be replaced with MoA assessment, chemical knowledge, algal/daphnia toxicity, and in vitro fish cell toxicity approaches
* AFT tests (OECD 203) are a key driver in the Big Fish Poisoning Endeavour
  + Efforts have been going on to replace them for 30 years, but doing so without compromising statistics are tricky
  + AFT tests have never been fully validated (lmao)
* Different Lines of Evidence (physical-chemical properties, mode of action, QSARs, etc.) are integrated into a BN
* BN was tested on two substances (triclosan and tetradecyl sulfate) and where 3+ LoEs were available, the BN was able to predict toxicity

Diagram

Description automatically generated

### Development of a Bayesian network for probabilistic risk assessment of pesticides

Diagram, engineering drawing

Description automatically generatedMentzel (2021)

* Basic PEC/PNEC = RQ, replacing various components with probability distributions
* SSDs are nice if you can get ‘em
* You can quantify a lot of uncertainty in your BN, but not all (for instance, choice of distribution?)

### A Bayesian Approach to Incorporating Spatiotemporal Variation and Uncertainty Limits into Modelling of Predicted Environmental Concentrations from Chemical Monitoring Campaigns

Wolf and Tollefsen (2021)

* With PECs from monitoring campaigns, data with <LOD and <LOQ measurements needs special treatment, such as distributional regression models
* Brms/Stan used to do some kind of Monte Carlo thing to predict PEC distributions from MECs including censored data
* Interesting but not relevant to anything I’m doing

# Increased Use of Bayesian Network Models Has Improved Environmental Risk Assessments

Moe et al (2021)

* Why is it when Americans assess the risks a substance poses to the environment, they do it like “ecological risk assessment”, but Europeans, when Europeans assess the risks, they’re like “environmental risk assessment”
  + it’s true, we’re so lame
* Other than that, the intro to a special series

# Bayesian Networks in Environmental Risk Assessment: A Review

Kaikkonen et al (2020)

* Systematic review of ERAs AND BN in the literature (497 articles)
* Used CADIMA, an online OA tool for systematic mapping/reviewing
* Boiled 497 down to 72 articles, 2004 – 2019
* Largely ERAs of substances, some of activities
* ERA split to three components
  + Risk Identification
  + Risk Evaluation (aka mitigation prioritisation)
  + Risk Analysis – most papers focused on this
* Most models were based on literature, rather than structural learning
* Most models focused on inference of values of interest by inputting new evidence
* Only 18 BNs included decision analytical elements
* End users were often not specified
* BNs that predict risk but don’t call it risk are necessarily excluded
* On the whole, papers rarely referred to the phases of ERA or specific frameworks (regulatory?)
* Not enough cumulative risk assessment?
* Not enough spatial/temporal consideration
* Not enough being explicit about variable discretisation
  + It should be avoided whenever possible?
* Expert opinion protocols aren’t good enough/don’t exuist
* People talk about how good BNs are for public participation, but no-one actually involves the public
* Pros of BNs in ERA:
  + Good for adding all sorts of things
  + Fit well into continuous learning processes
  + Easy on the yes
* Cons:
  + Acyclic
  + Lack of temporal scale (typically)
  + Expert elicitation is still hard

# Good practice in Bayesian network modelling

<https://www.sciencedirect.com/science/article/pii/S1364815212001041?via%3Dihub>

Chen & Pollino (2012)

# Developing best-practice Bayesian Belief Networks in ecological risk assessments for freshwater and estuarine ecosystems: A quantitative review

<https://www.sciencedirect.com/science/article/pii/S0301479715001061?via%3Dihub>

McDonald, Ryder & Tighe (2015)

# [The Origin, Development, Application, Lessons Learned, and Future Regarding the Bayesian Network Relative Risk Model for Ecological Risk Assessment](https://doi.org/10.1002/ieam.4351)

Landis (2020)

* Don’t understand/ not super relevant to my work

# Integrating Metapopulation Dynamics into a Bayesian Network Relative Risk Model: Assessing Risk of Pesticides to Chinook Salmon (*Oncorhynchus tshawytscha*) in an Ecological Context

<https://setac.onlinelibrary.wiley.com/doi/10.1002/ieam.4357>

Mitchell et al (2020)

# Meeting with KET

* Look closer at Miljødirektorat data on fresh and salt water estrogen concentrations
* ED has two components
  + Adverse effects relevant to regulation – consequence of modulation of reproduction
  + Endocrine Modulators affect endocrine system without adverse effects
  + Need evidence of binding to receptor and downstream endpoints
    - Need to consider different effects in males & females
    - Antiestrogenic & androgen effects make more sense in females
* Might see, for instance, VTG effects before reproduction – can use B
* Look at the endpoints used for reproductive effects
* Consider your assumptions
  + Focus on the most relevant endpoints (e.g. fecundity in females, ovo-testis development in males)
* If you want to make the most out of this with the least effort, see what you can do with NOECs rather than just PNECs
* Cumulative RA uses a two-tiered system – sum RQ, then TUmix
* Try to focus on NOECs which are directly comparable to PNECs, whereas EC50s aren’t
* Have a think about whether to ask KET for VTG data from RaDB

## Follow-up Email

Definition of and EDC:

*"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations"* ['Community strategy for endocrine disruptors'](https://ec.europa.eu/environment/chemicals/endocrine/documents/index_en.htm)

Requirement is thus that Functions = Mode of Action (MOA) and Adversity = effects of higher level of biological organization both need to be documented. As reproduction in itself is not necessary a good indicator for estrogens (as can be caused by other MOA) considering teaming up data on adversity that is clearly relevant for EDC may be required. I have pulled data for endpoints in RAdb of relevance for this (see below), where you may want to consider whether most relevant to focus on the feminization of males by the estrogen agonists for your current assembly of chemicals. Alterative strategy would be to focus on masculinization of females (androgen agonists and/or estrogens antagonists),

I would suggest considering a tiered approach consistent with recent suggestions for cumulative risk assessment using BN for your mixtures:

Tier 1: use PNECs (se paper below for PNECs) to identify if you have risk scenario, using adverse endpoints of largest relevance (Reproduction or sexual development, in green below), derive SRQ and develop initial BN for the 4 estrogens. This will likely generate a risk scenario of both magnitude and probability of exceedance of SRQ =1 although potentially being an overestimation of risk if chemicals selected have multiple MoAs.

Tier 2: Use NOEC and Sum of TU for MoA endpoints (markers for estrogenic endocrine action: vitellogenin, activation of the ER, steroid levels etc.) and most relevant adversity (e.g. sex ratio, sperm development). These data can give rise to two BNs – one for the MOA and one for the Adversity. If both provide a STU that in magnitude and probability of exceedance of STU=1 exist, you should be able to document that combined effect of the 4 estrogens is a risk and of ecological relevance.

Your predictions could be aligned against some mixture effect assessments done with whole fish for both MOA and adversity, there are likely some papers that can assist this effort, but do not presently have an overview (remember that some studies were undertaken about 10years ago).

Here are some of the toxicity targets of relevance that we have full (all chemicals, minimum one study) or partial data for in RAdb (the mot relevant combo of MOA and Adversity indicated in yellow):

**Target (MoA)**

Estrogen receptor alpha mRNA

Vitellogenin

Estrogen receptor beta mRNA

Estrogen receptor beta1 protein mRNA

Estrogen receptor beta2 protein mRNA

alpha vitelline envelope protein mRNA

Estrogen receptor protein

Choriogenin L-mRNA

Zona pellucida protein2 mRNA

Estrogen mRNA

Zona radiata mRNA

Zona pellucida glycoprotein3 mRNA

Estradiol receptor sites

Vitellogenin:protein ratio

17beta-Estradiol:Testosterone ratio

**Target (Adversity)**

Hatch

Fertility

Reproduction, general

Hatch

Spermatocytes

Sex ratio

Fertilization

Spawning frequency

Imposex, intersex conditions

Sperm cell counts

Fecundity

Sexual development

Spermatigonia

Pregnant, Paris or Gravid

Number spawning

Gamete production

Germ cell count

Infertile

Time to spawn

Mean spawns per female

Vitellogenesis

There are also some papers on deriving PNECs for fish that may be useful – see :

Environ Toxicol Chem

. 2012 Jun;31(6):1396-406. doi: 10.1002/etc.1825. Epub 2012 Apr 27.

Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol

Daniel J Caldwell 1, Frank Mastrocco, Paul D Anderson, Reinhard Länge, John P Sumpter

Affiliations expand

PMID: 22488680 DOI: 10.1002/etc.1825

Anyway, leave it with you to consider your options.

# Methods

## Super Conceptual Model

## Bayesian Network / Hugin

## Statistics/R

Built by Jannicke, because I am not a clever man.

Fit a linear mixed-effects model (LMM) to sales data

lmer(log(Sales\_Weight\_g) ~ -1 + API + (1 | Year), data = estrogen\_example\_data)

* Some points from a video I watched
  + Year could do with more variables
  + Residual vs fitted diagnostic plot should be more or less randomly distributed (it definitely isn’t)
  + ???

## SimpleTreat / WWTP Modelling

FASS has modelled removal for at least [Novo Nordisk’s estradiol](https://www.fass.se/LIF/product?userType=2&nplId=19980306000165&docType=78&scrollPosition=204):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Product | Conjugation | % Excreted | % Removal | % Used |
| **17β-estradiol** |  | 501 | 402 | 402 |
|  | glucuronides | 501 | 6-8? |
|  | sulphates |
| estrone |  | 421 | 191 | 82 |
|  | ??? | 581 |
| estriol |  |  |  | 0? |
|  | ??? |  |  |
|  |  |  |  |  |
| 17α-ethinylestradiol |  | 6 (urine)3  9 (faeces)3 |  |  |
|  | glucuronides | 80 (urine)3 |  |  |
|  | sulphates | 8-10 (urine)3 |  |  |
|  |  |  |  |  |

1. The source for a lot of the measured percentages is [Adler, Steger-Hartmann & Kalbfus](https://onlinelibrary.wiley.com/doi/10.1002/1521-401X(200111)29:4%3C227::AID-AHEH227%3E3.0.CO;2-R) (2001), a paper which is both a) not easily accessible and b) in German 😠.
2. SimpleTreat 3.1 via FASS
3. [Stanczyk, Archer & Bhavnani](https://www.sciencedirect.com/science/article/pii/S0010782412010797?via%3Dihub) (2013) – but not original source

* WWTP extraction to sludge – include in overall removal %
* State of Norwegian WWTPs
* Kow vs Log Kow

## Methods of Predicting Mixture Toxicity

### What’s it all for?

* Regulatory risk assessment is built on the assumption of a maximum safe level of toxicant in the environment – as long as the PEC is below the PNEC, all species (most sensitive test species, + an AF to cover everyone else) are being protected
* API ERA is purely descriptive currently, but might move to a prescriptive model in the future (as is the case in REACH), where manufacturers are required to reduce exposure (or refine toxicity) until RQ > 1, and therefore all species are protected from the API
* However, as many APIs share modes of action (and may also do so with non-pharmaceutical pollutants), the overall risk of an endpoint happening to wildlife is (assuming additivity) higher than predicted, and therefore protective measures may yet be insufficient
* By assessing a mixture of everything that acts via a given MoA, we can correctly assess the risk of a relevant endpoint, and thus (if the law allowed) protect against that endpoint happening
* If we continue to set our goal as “protect all species from endpoint”, we can then provide the recommendation that to reduce (for instance) fish feminisation to acceptable levels
  + levels of estrogens entering the environment must be reduced, or
  + estrogen toxicity must be refined such that it can be shown that a higher PNEC would still protect fish
* Alternatively, we may find that the current, AF-based approach is sufficiently protective – although assessment factors for estrogens are often fairly low (estradiol = 2, estrone = 10, estriol = 10, ethinylestradiol = 10)
* Ultimately, mechanistically, APIs are only a small part of the risk landscape and observed ecosystem collapse. We can’t (not as risk assessors, anyway) tease the contribution of APIs or specific groups of APIs to observed effects out
* Demonstrating that AFs don’t protect against mixture effects feels like a bit of a farce, as API AFs already don’t protect anything, but maybe it’s reasonable to write under the pretence that RQ < 1 legislation for APIs may be introduced in the future

### Concentration Addition – Same MoA – Assumes Additivity

#### SumRQ

* Can use probabilistic RQs, but not required
* Crude but extremely easy way of assessing mixture toxicity, suitable as a first-tier approach
* Detects cases where mixture toxicity may be an issue, but may be over-conservative due to summing many AFs
* Can use as a flag (SumRQ > 1) for higher-tier mixture assessment

#### MAF

* Prevent the possibility of mixture toxicity every becoming an issue by adding an additional AF of 100 to all APIs
* Intended to protect against, but not assess mixture toxicity

#### Sum of Toxic Units

* Less conservative than SumRQ, as no AFs built in
* For a given endpoint/species/duration combination, allows comparison of different APIs
* Let’s say TUestrogens = 16
  + What does this mean? Is it a useful piece of information by itself?
  + Are we just going to be demonstrating that the sum of several numbers is greater than each individual number?
  + Is there a conventional threshold for STU, like for RQ?

### Independent Action

P(RQgroup > 1)

* Requires probabilistic RQs
* For a group of APIs for which P(RQindiv > 1) is low, what is the probability that at least one exceeds this threshold
* Useful for making the point that if you have a large number of APIs with low risk, the chance that one of them will fall on the right side of the risk distribution will still be significant
* Makes no assumptions about the interactions of stressors or even stresses

# Wastewater Treatment Plants

### [Occurrence and removal of selected organic micropollutants at mechanical, chemical and advanced wastewater treatment plants in Norway](https://www-sciencedirect-com.ezproxy.uio.no/science/article/pii/S0043135406004271)

Vogelsang, Grung, Jantsch, Tollefsen & Liltved (2006)

* Don’t get your hopes up, none of the micropollutants in question are APIs
* Mechanical treatment in the North is preferred due to its lower sensitivity to temperature?

### [Worldwide estimation of river concentrations of any chemical originating from sewage-treatment plants using dilution factors](https://doi-org.ezproxy.uio.no/10.1002/etc.2441)

Keller et al. (2013)

* Seasonal river flow is a Big Deal in assessing PPCP risk
* Internationally, data on rivers and WWTPs is scarce
* However, a 2010 paper called *Global threats to human water security and river biodiversity* demonstrated availability of sufficient data to predict DFs?
* The authors calculated on a gridded scale (0.5° × 0.5°), the domestic wastewater effluent generated in a catchment, and the river flow (from globally distributed runoff estimates)
* Behold the diagram:

A picture containing map

Description automatically generated

Extract from **Table S2**. Statistics describing predicted values of national annual median dilution factor (DF)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Country | 5thile | 25thile | Median | 75thile | 95thile | Cell Count |
| Norway | 358.89 | 1217.54 | 2453.29 | 3598.47 | 8679.58 | 98 |

### [An Integrated Addition and Interaction Model for Assessing Toxicity of Chemical Mixtures](https://academic.oup.com/toxsci/article/87/2/520/1730749)

### [A Model To Estimate Influent and Effluent Concentrations of Estradiol, Estrone, and Ethinylestradiol at Sewage Treatment Works](https://pubs-acs-org.ezproxy.uio.no/doi/10.1021/es035342u)

### [Influence of Conjugation on the Fate of Pharmaceuticals and Hormones in Canadian Wastewater Treatment Plants](https://pubs.acs.org/doi/10.1021/acsestwater.1c00376)

Gewurtz et al (2022)

### Notes from Combined toxicity assessment of organic pollutants in small-scale fish bioassays

Petersen (2012)

* Estrogenic mixture effects were mainly well predicted by IA and CA
  + Both models overestimated effects at higher concentrations
  + More adverse endpoints were better predicted (additivity)

## Seasonal Variation

### Investigation of pharmaceuticals in a conventional wastewater treatment plant: Removal efficiency, seasonal variation and impact of a nearby hospital

Bijlsma et al (2021)

* In Spain: not really

### Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China

Sun et al (2014)

* Beijing (10-14C in winter, 25-27C in summer), and Xiamen (20-27C year-round)
* Seasonal variation in both PPCP occurrence and removal, possibly due to less waste water use in cold seasons
  + Seasonal variation in removal due to temperature effect on microbial activity of activated sludge and effects of water consumption on retention time in WWTP
* Some PPCPs had consistent removal times (paracetamol, caffeine, fenoprofen), while propyl paraben, ketoprofen, diclofenac, codeine, indomethacin, and crotamiton from activated sludge treatment “showed a marginal positive correlation with sewage temperature”