

UV-filters in the aquatic environment: contamination in Swiss rivers, hormonal effects in fish and tentative risk assessment

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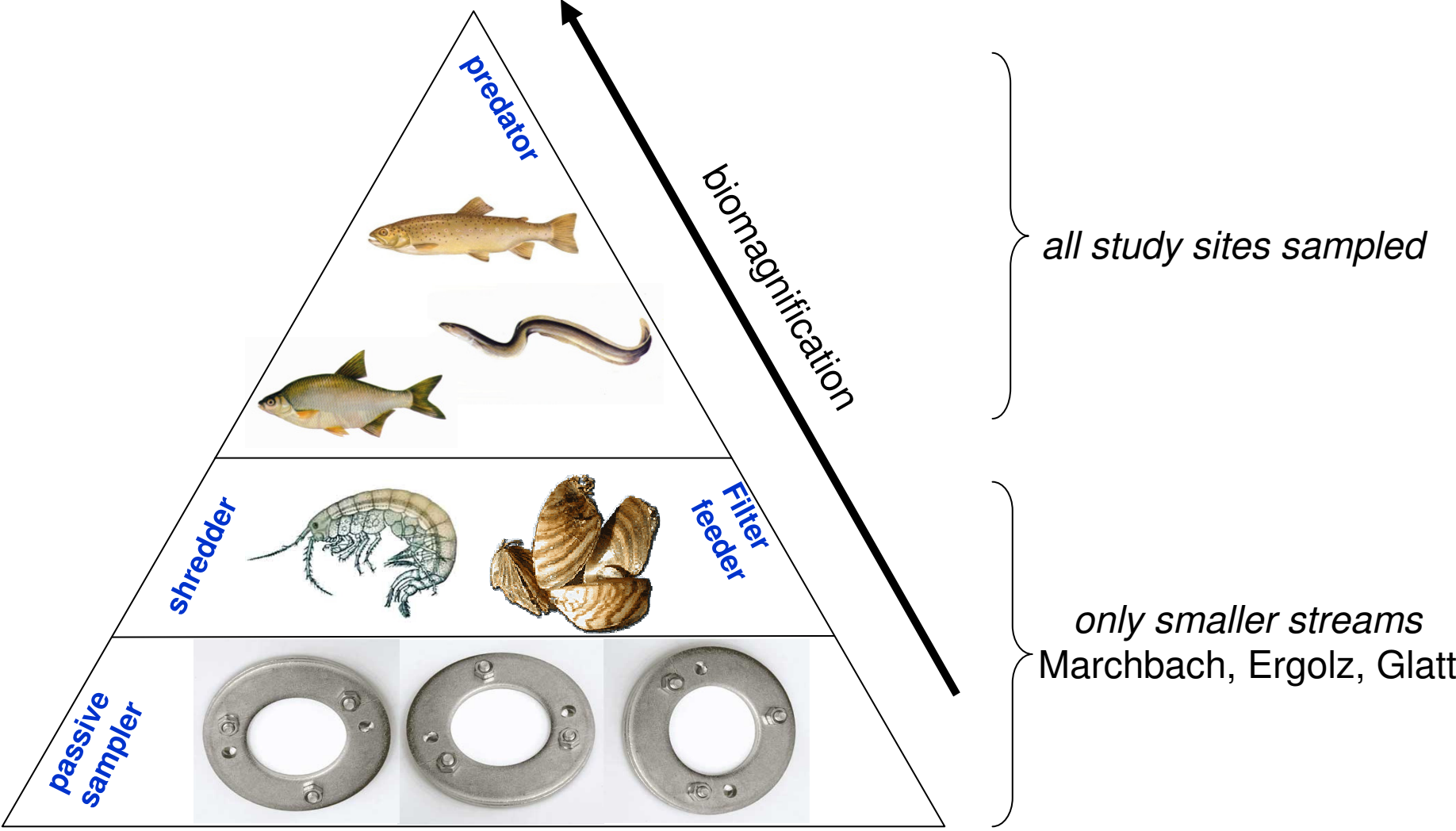
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Exposure analysis in Swiss rivers

Forthcoming and extended analysis to previous data by
M. Balmer, T. Poiger, M. Müller and H.R. Buser
(Agroscope Wädenswil)

- Which endocrine active UV-filters occur in water and biota?
 - What are the levels?
 - Is there a biomagnification in food web?
 - Sources?
-
- Analysis of 9 proven endocrine active UV filters
 - Focus on river Glatt (relation to other NFP50-analyses)
 - Analysis of river water 2006 and summer 2007 using passive samplers (POCIS)
 - Sampling of representative aquatic biota from different trophic levels

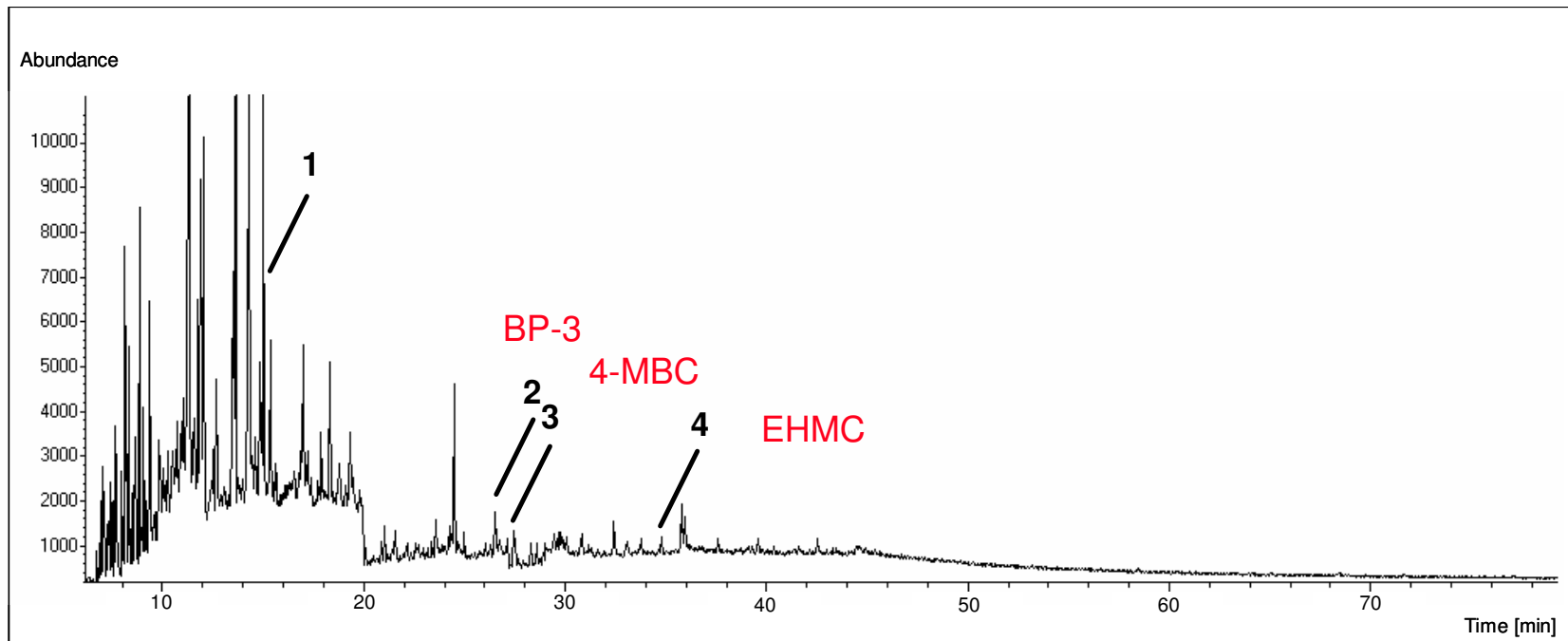
Samples for assessing biomagnification



POCIS (pesticide)

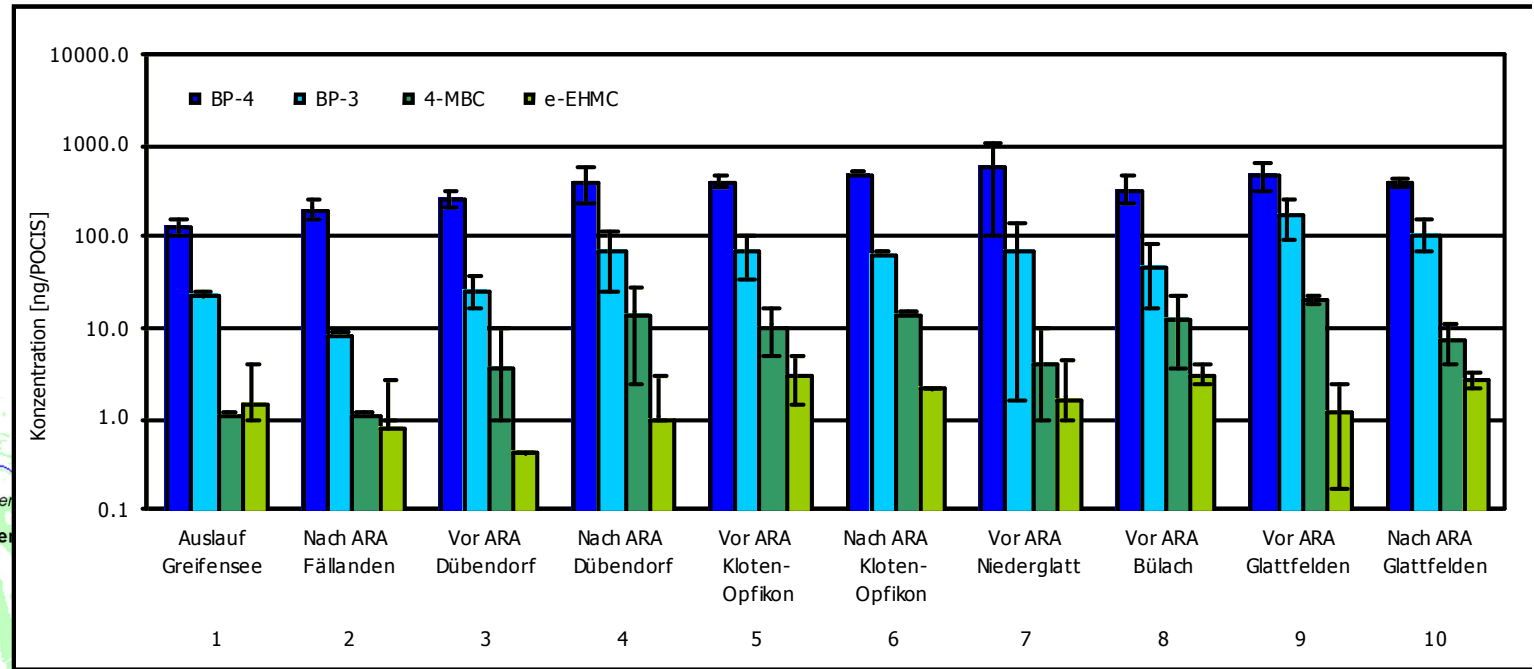
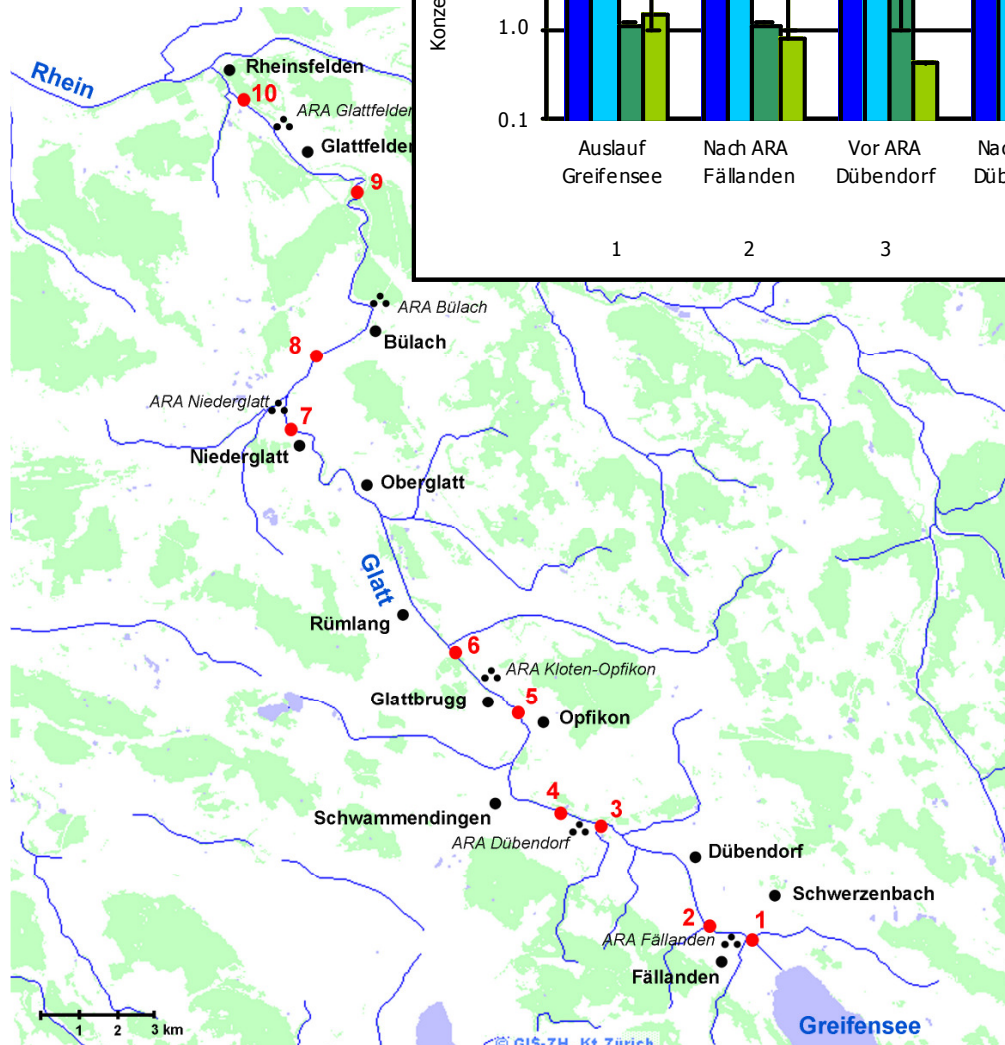
- Polar organic chemical integrative samplers (POCIS)
- Passive integrative sampling devices
- Membranes deployed in river water for 3-4 weeks
- Extracts analysed by LC-MS (polar compounds) and GC-MSMS (lipophilic compounds)
- Analysis for estrogenic BP-1, BP-2, BP-3, BP-4, 4-DHB, EAB, EHMC, 4-MBC, 3-BC

UV filters in Glatt river water: BP-3, BP-4, 4-MBC, EHMC



POCIS sample from river Glatt upstream the STP Dübendorf in 2006.
1 = BP-d10, 2 = BP-3, 3 = 4-MBC, 4 = EHMC.

Glatt



Estrogenic UV filters

Polar: BP4 and BP3

Lipophilic: 4MBC and EHMC

POCIS show that wastewater is a significant source

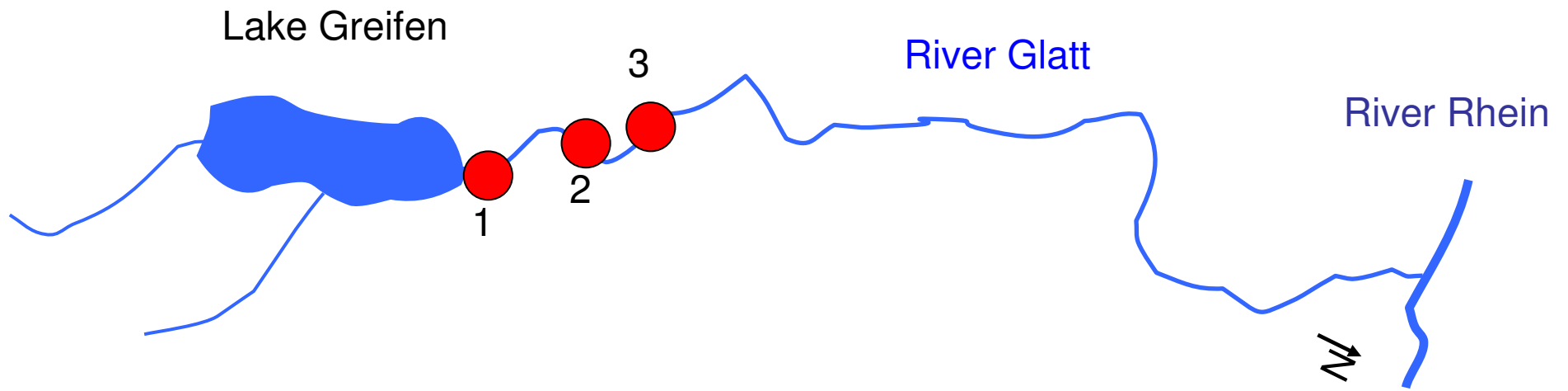
Levels in river Glatt samples (2006)

POCIS

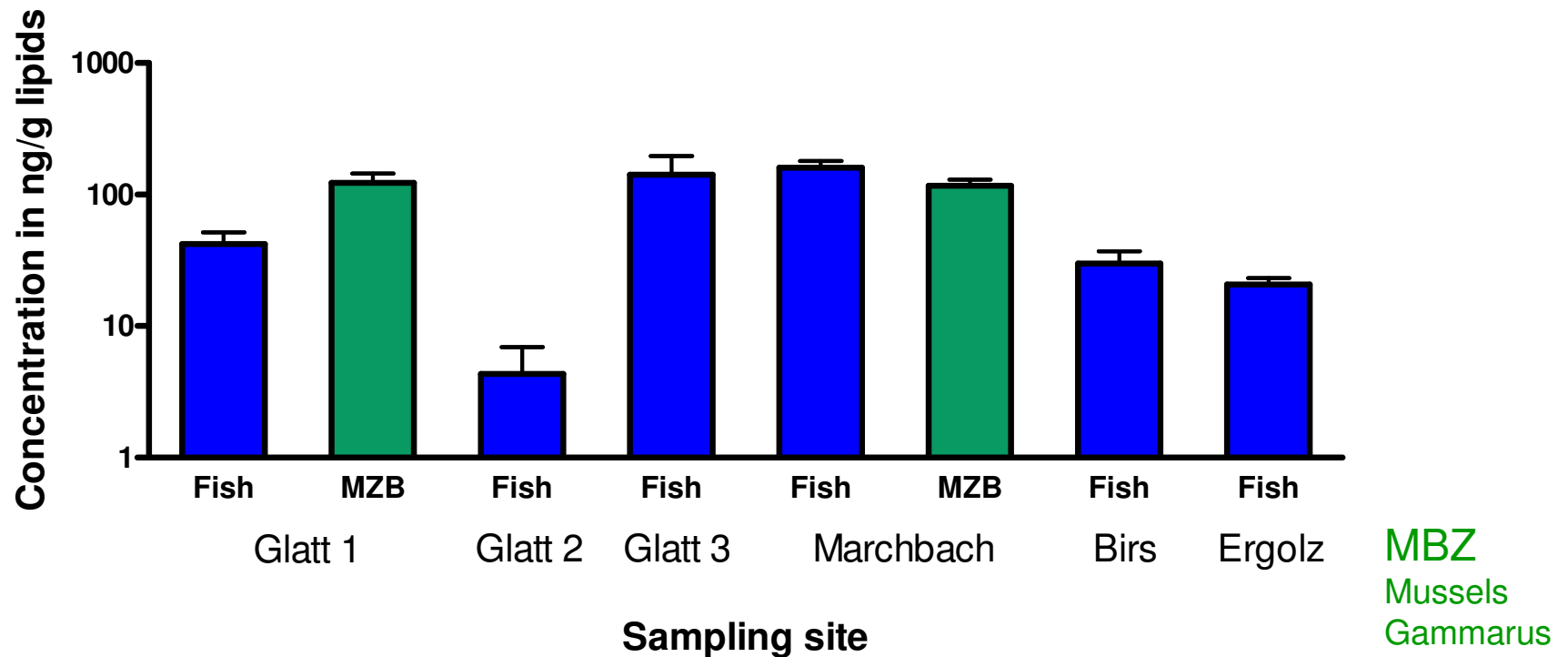
BP-4	(log P = 0.89)	up to	1344 ng
BP-3	(log P = 3.64)	up to	96 ng
4-MBC	(log P = 4.95)	up to	64 ng
EHMC	(log P = 5.66)	up to	27 ng

Fish and macroinvertebrates

BP-4	not detected
BP-3	not detected
4-MBC	not detected
EHMC	up to 142 ng/g lipids



Lipophilic EHMC in fish and invertebrates (2006)



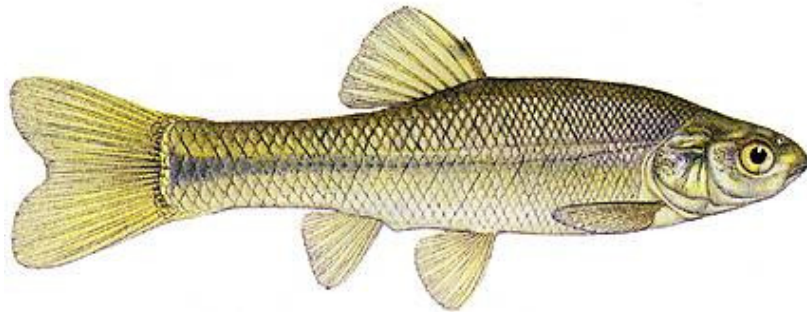
Analysis of endocrine effects

- *In vitro* hormonal activity: 4 different activities
(Kunz & Fent 2006, *Aquat. Toxicol.* 79: 305-324)
 - Estrogenicity, anti-estrogenicity
 - Androgenicity, anti-androgenicity
- *In vivo* estrogenicity in fish: 4 compounds
(Kunz et al. 2006, *Tox. Sci.* 90: 349-361)
 - BP-1, BP-2, 3-BC, Et-PABA
- *In vivo* reproduction effects
(Kunz et al. 2006, *Tox. Sci.* 93:311-321; Weisbrod et al. 2007, *Tox. Appl. Pharmacol.* 225: 255-266)
 - 3-BC, BP-2
- Mixture activity *in vitro* (Kunz & Fent 2006, *Tox. Appl. Pharmacol.* 217: 86-99) and *in vivo* in fish
- Environmental risk assessment
(Fent et al. 2008, *Chimia* 62:368-375)

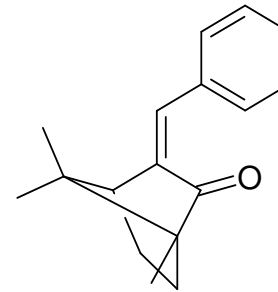
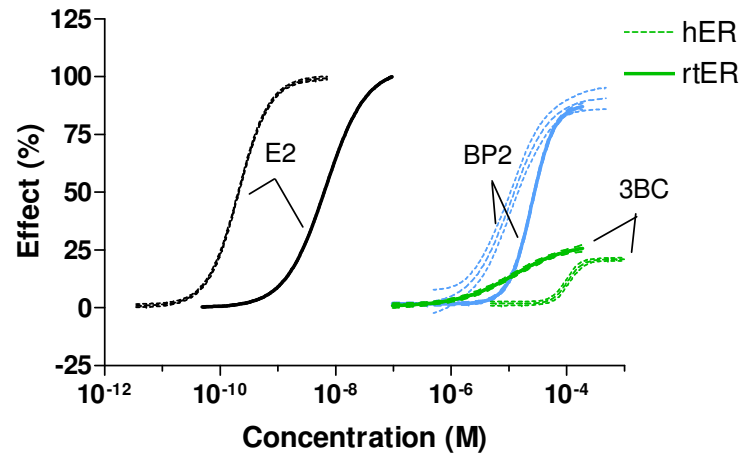
All 18 UV filters show hormonal activity *in vitro*

UV-Filter	Estrogenic 9	Antiestrogenic 14	Androgenic 6	Antiandrogenic 10
Benzophenone-1	+++	-	-	+++
Benzophenone-2	+++	-	+++	+
4,4'-Dihydroxybenzophenone	+++	-	-	+++
Ethyl-4-aminobenzoate	+++	-	-	+
Benzophenone-3 (BP-3)	+	+++	-	nd
Benzophenone-4 (BP-4)	+	+++	-	nd
Benzylsalicylate (BS)	+	+++	-	nd
Phenylsalicylate (PS)	+	+++	-	nd
3-Benzylidene camphor (3-BC)	+	+++	-	+++
Ethylhexyl methoxy cinnamate (EHMC = OMC)	-	+++	++	+++
Octyl salicylate (OSC)	-	+++	+++	+++
4-Methylbenzylidene camphor (4-MBC)	-	+++	-	+++
Homosalate (HMS)	-	+++	+++	+++
Isopentyl-4-methylcinnamate (IMC)	-	+++	++	+++
Octocrylene (OC)	-	+++	+	nd
4-Aminobenzoate (PABA)	-	+++	-	nd
(N,N')-dimethyl-octyl-4-aminobenzoate (OD-PABA)	-	+++	-	nd
Ethyl 4-aminobenzoate (Peg25 PABA)	-	+	-	nd

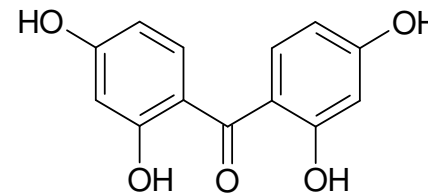
Estrogenic activity (vitellogenin induction) and effects on fertility and reproduction in fathead minnows



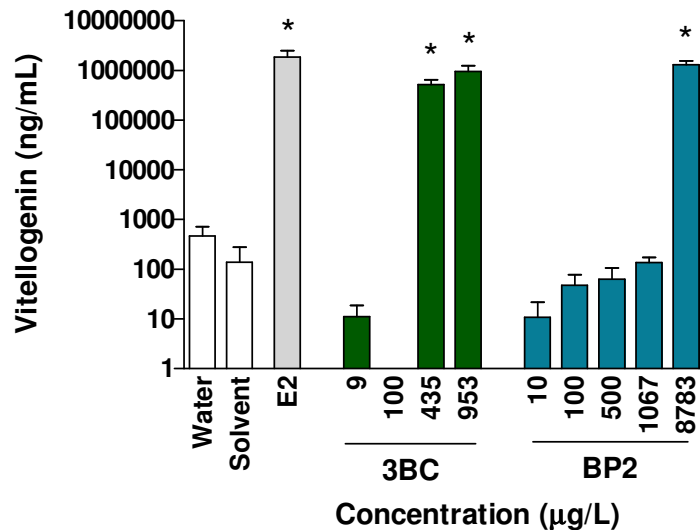
Effects on fecundity and reproduction: 3BC and BP2



3BC (3-Benzylidene camphor)



BP2 (Benzophenone-2)



	Yeast <i>in vitro</i>	Fish <i>in vivo</i>
BP2	estrogenic (full agonist)	estrogenic (VTG induction)
3BC	estrogenic (partial agonist)	estrogenic (VTG induction)

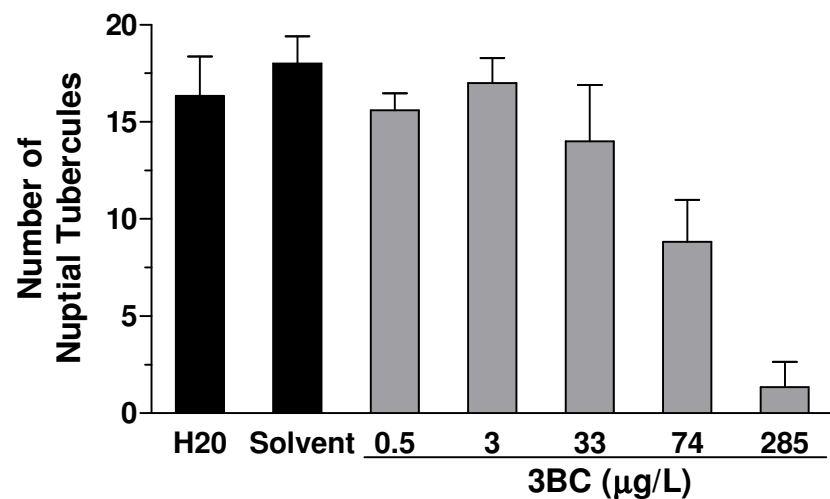
Kunz et al. (2006). *Toxicol.Sci.* 90 (2): 349-361.

Kunz & Fent (2006). *Aquat. Toxicol.* 79: 305-324.

Secondary sexual characteristics in males

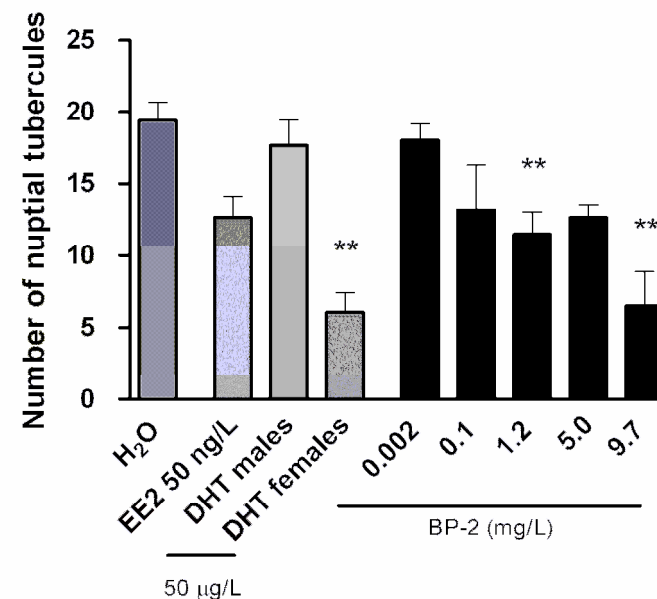
Dose-dependent feminization and effects on fertility and
Reproduction: LOEC for 3BC: 3 ug/L, for BP-2: 1.2 mg/L

3-Benzylidene camphor

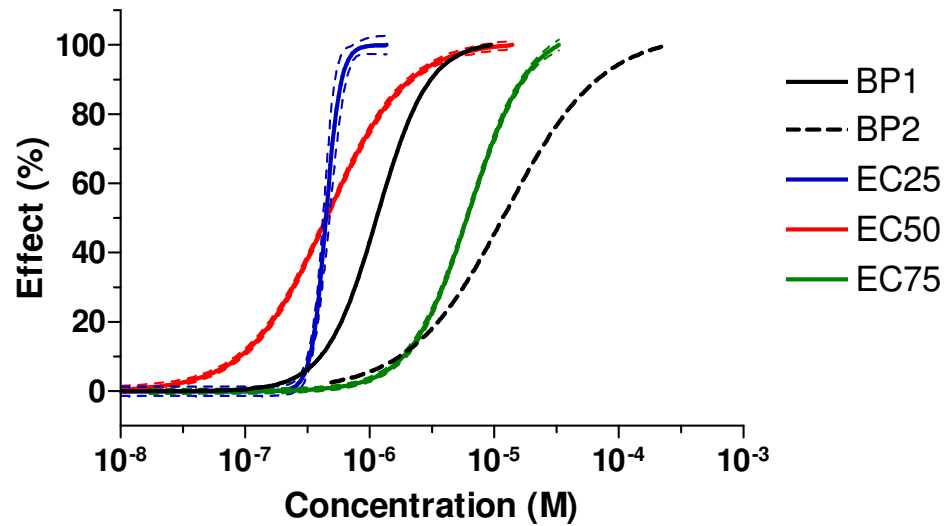


Benzophenone-2

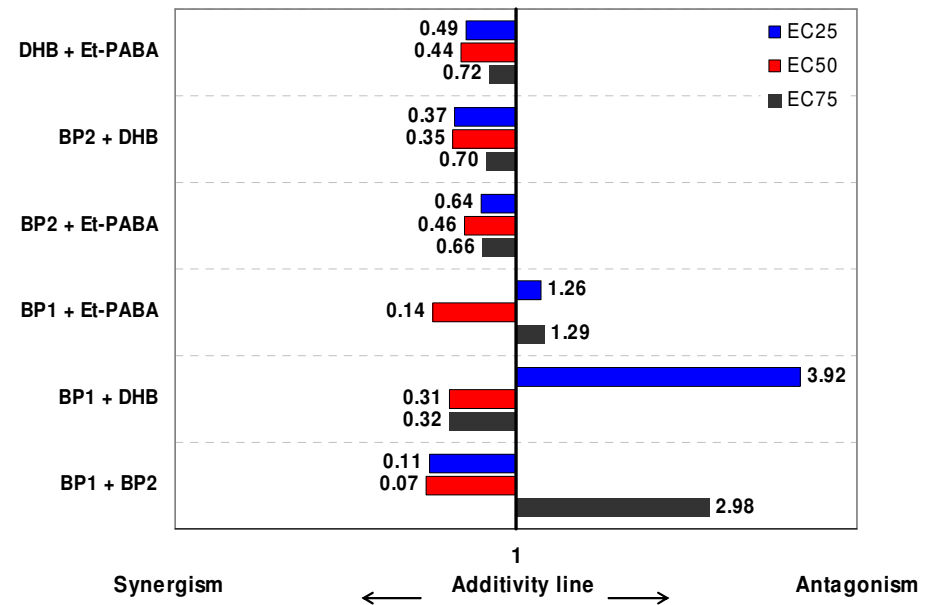
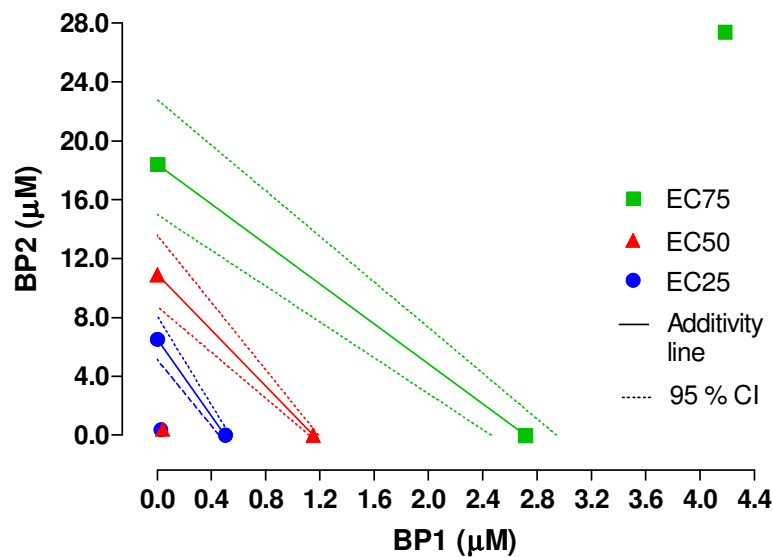
Nuptial Tubercles



Mixtures of 2 UV filters show mainly synergistic activity *in vitro*

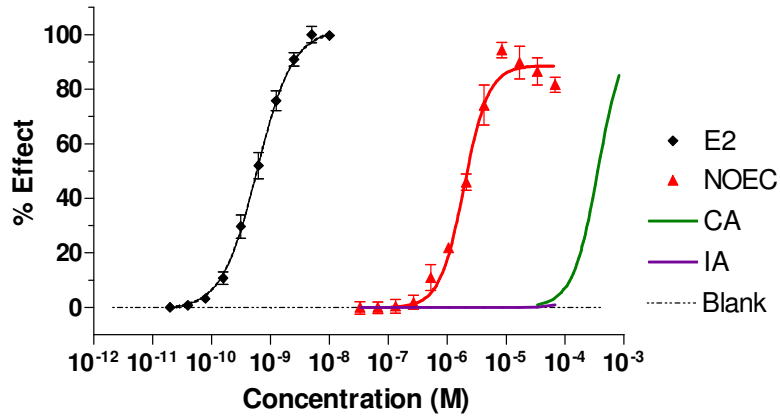


Recombinant yeast YES

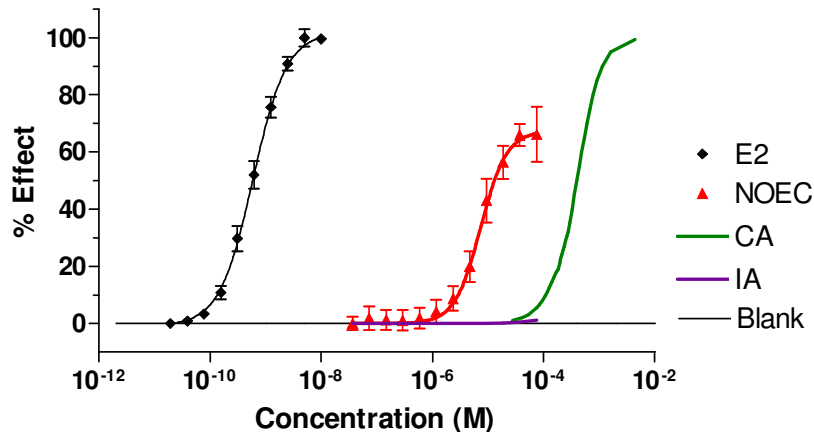


Four and eighth UV filters show marked activity at NOEC They act synergistically *in vitro*

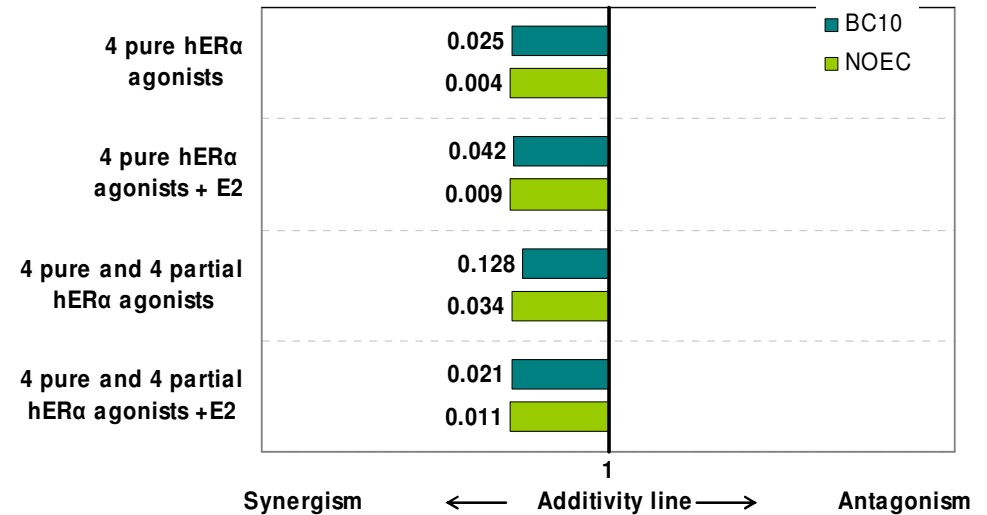
Mixture of 4 UV filters at NOEC



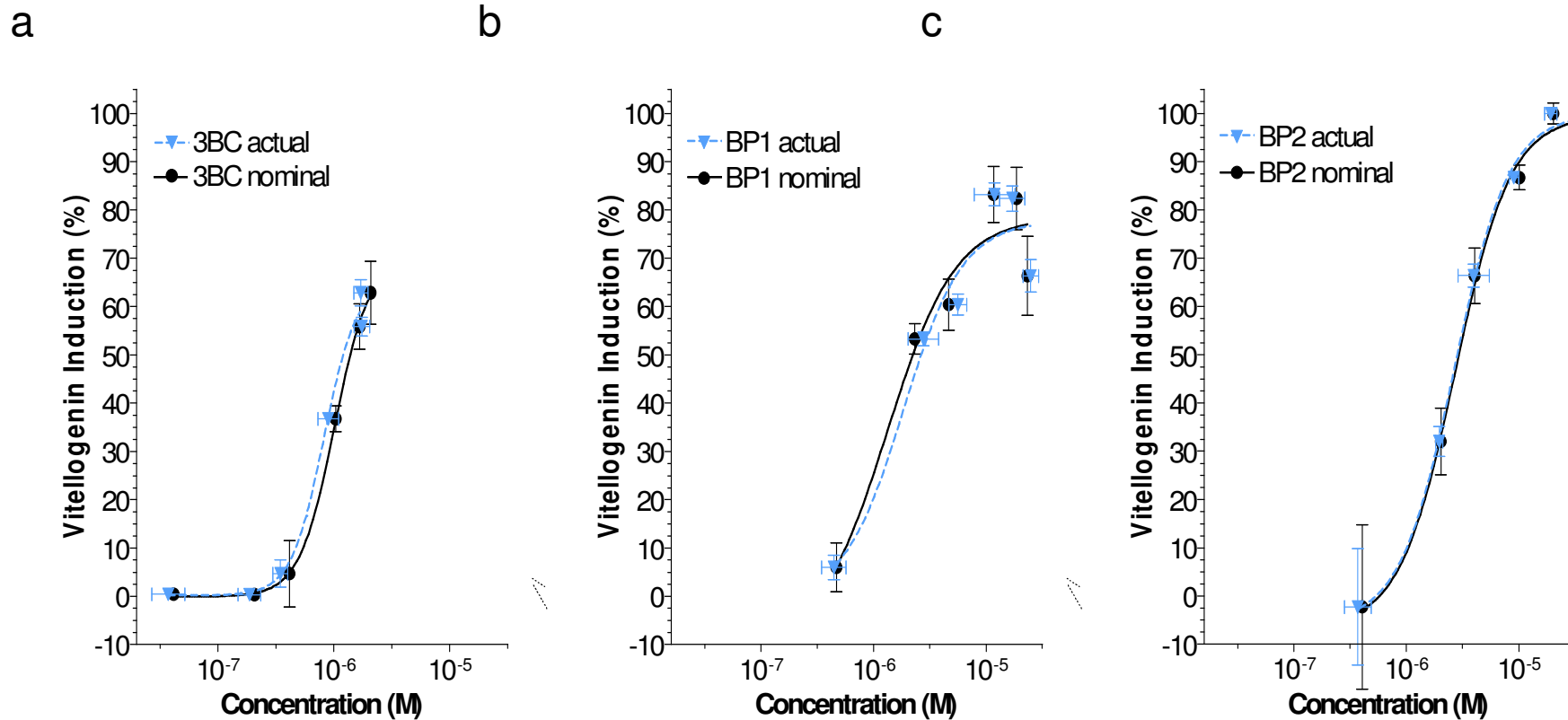
Mixture of 8 UV filters at NOEC



Recombinant yeast YES

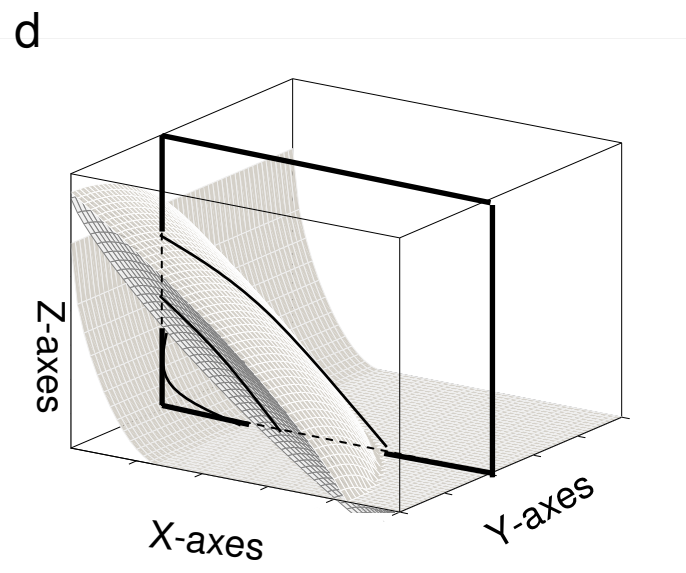
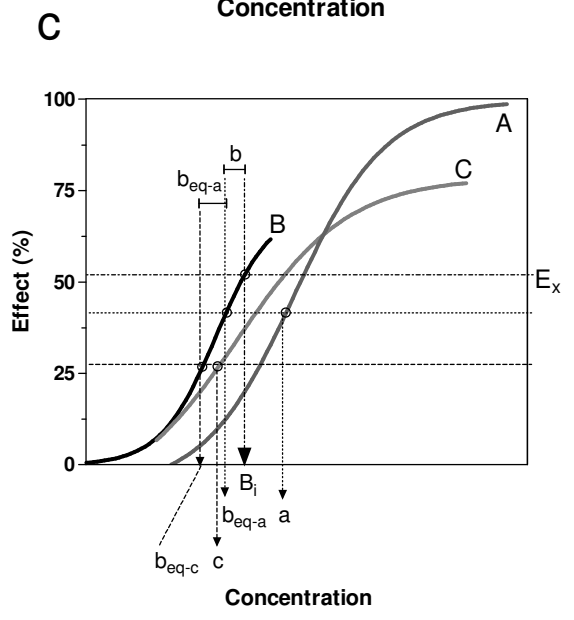
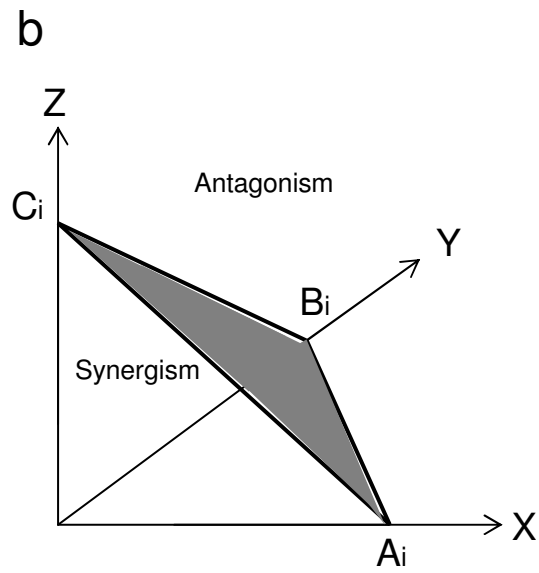
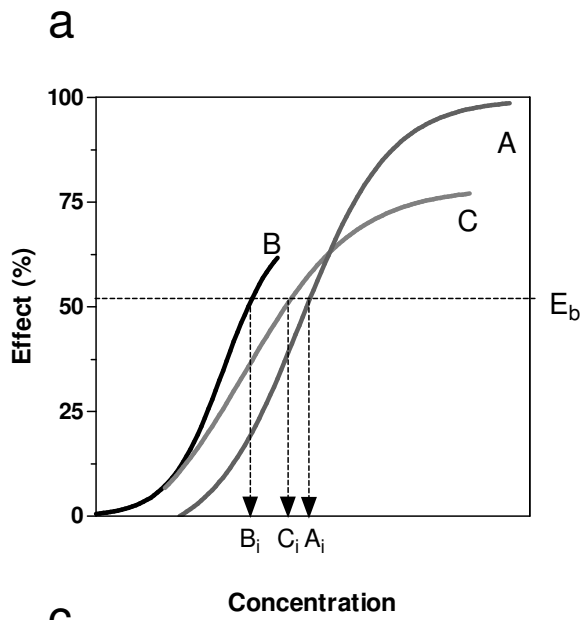


Estrogenic activity of single UV filters BP1, BP2, 3BC after 14 d exposure in juvenile fathead minnows: dose-dependent VTG-induction



Nominal and measured concentration well correlated

Estrogenic effects of ternary UV filters in fish



Estrogenic activity of ternary mixture of UV filters BP1, BP2
3BC in fathead minnows (VTG induction):

Comparison with concentration addition concept

1. Concentration addition at medium to high mixture levels
2. Antagonistic activity at low mixture levels

Summary: Mixture activity of 3 UV filters

- Mixture activity: a challenging task
- *In vitro* activity in recombinant yeast shows often synergistic activity
- *In vitro* synergism is not paralleled *in vivo*:
 - *Mostly concentration addition in vivo. At low concentration antagonistic interaction (NOEC, EC1, EC5)*
 - *In vitro* synergism based on recombinant yeast system: interaction of UV filters with cofactors and reporter gene expression
- *Concentration addition should be considered for risk assessment of endocrine disrupters*

Environmental risk assessment for estrogenic UV filters

see also Fent et al. 2008, *Chimia* 62:368-375 for 3BC, BP1, BP2, Et-PABA

Measured environmental conc. (MEC)

(Langford & Thomas 2007)

4MBC: 0.488 ug/L

EHMC: 0.238 ug/L

BP3: 0.268 ug/L

3BC: n.d.

BP1, BP2: n.d.

Et-PABA: n.d.

Effect concentration (PNEC)

Safety factor (SF) 1000 (acute toxicity)

Reference: fish acute toxicity: manufacturer information, SciFinder

4MBC: 0.74 ug/L

EHMC: 100 ug/L

BP3: 3.88 ug/L

3BC: 30 ng/L (Kunz et al. 2006) (SF 100)

Et-PABA: 439 ng/L (Kunz & Fent 2006) (SF100)

MEC / PNEC

<1: environmental risk not probable for one single compound

For 3BC and BP2: Risk probable, however not yet detected in environment

However, when mixtures of 4MBC, EHMC, BP3 and BP4 present in water are regarded: Environmental risk cannot be not excluded

Conclusion and research needs

- Contamination of 4MBC decreased
- EHMC always present in aquatic biota
- BP3, BP4, EHMC and 4MBC present in water
- Endocrine potential and chronic effects of EHMC, BP3, BP4 and OC not known
- **Research needs:**
 - Chronic effects and effects on fertility and reproduction of EHMC, BP4, BP3 and OC in fish and *Daphnia magna*
 - Effects of environmentally relevant mixtures *in vivo* in *Daphnia* and fish