Ecotoxicity of organic UV-filters to freshwater benthic invertebrates

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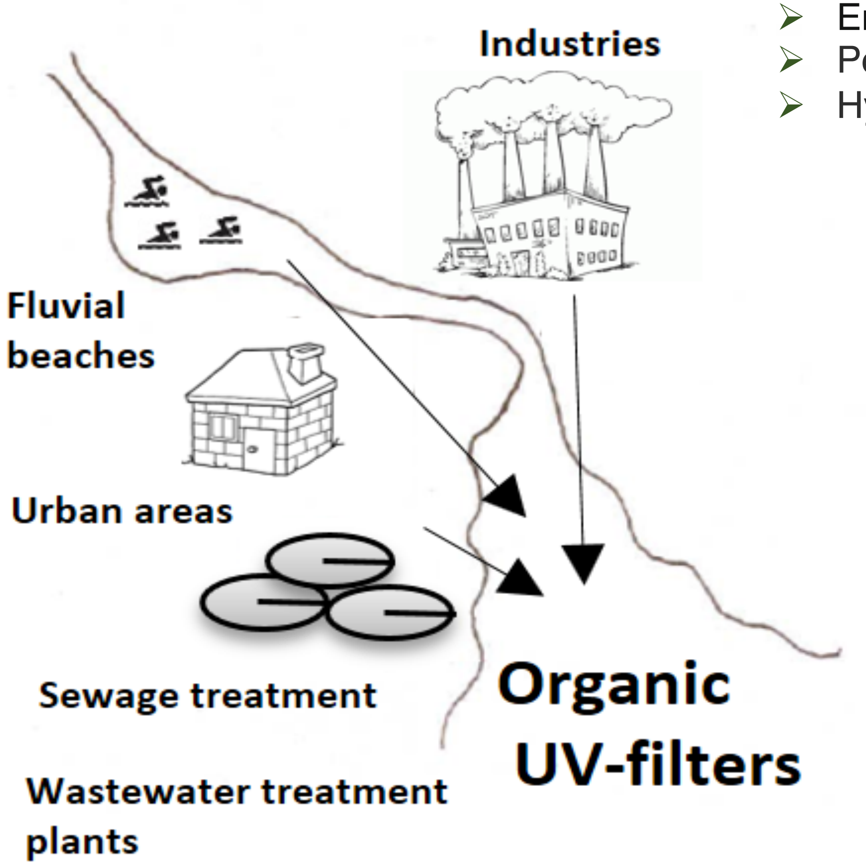
Webinar: Environmental Impact of Currently Marketed Sunscreens and Potential Human Impacts of Changes in Sunscreen Usage

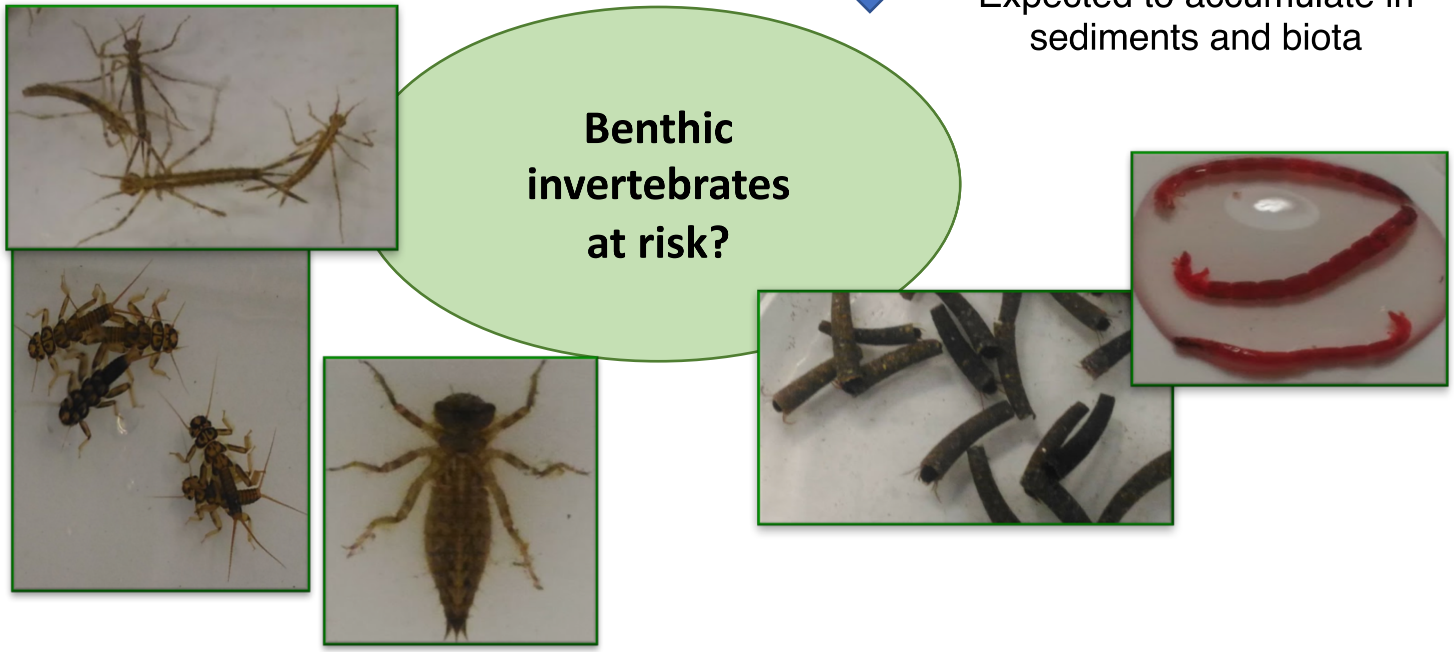
August 5th, 2021

Organic Ultraviolet filters in the environment



- Emerging environmental pollutants (Massive production and usage)
- Poor removal efficiency by WWTPs
- Hydrophobic coumpounds

Expected to accumulate in sediments and biota



River sediments: 2.4 mg/kg (Gago-Ferrero et al., 2011)
Stream sediments: 0.6 mg/kg (Kameda et al., 2011)
Lake sediments: 0.09 mg/kg (Rodil and Moeder, 2008)
Sewage sludge: 27 mg/kg (Plagellat et al., 2006)

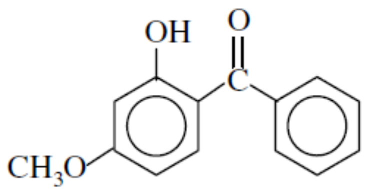
Diana Campos PhD work (2014-2019)

"Assessment of the ecological effects of UV-filters in freshwaters"

Assessment of the ecological effects of UV-filters in freshwaters

three different classes and most commonly detected in aquatic ecosystems

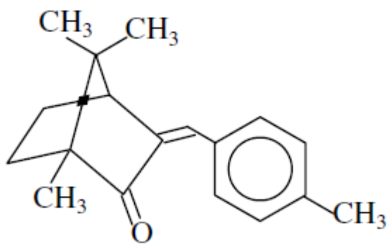
BP3 : Benzophenone 3



Benzophenones derivates
Protect against UVA & UVB
Allowed in: Europe, United States and Japan
Concentration in freshwater sediments: 0.051 mg/kg (Mizukawa et al., 2017)

CAS No Purchased (Sigma-Aldrich)	Molecular weight (g/mol)	Log K_{ow}	Water solubility (g/L)
131-57-7	228.24	3.79	0.10

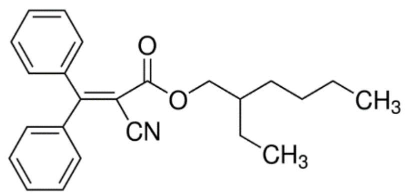
4-MBC : 3-(4-methylbenzylidene)camphor



Camphor family
Protect against: UVB
Allowed in: Europe, Australia, Canada; not allowed/approved in United States
Concentration in freshwater sediments: 1.4 mg/kg (Días-Cruz et al., 2019)

CAS No Purchased (Sigma-Aldrich)	Molecular weight (g/mol)	Log K_{ow}	Water solubility (g/L)
36861-47-9	254.37	4.95	0.017

OC : Octocrylene



Crylene family
Protect against: UVB
Allowed in: Europe, Japan, United States
Concentration in freshwater sediments: 2.4 mg/kg (Gago-Ferrero et al., 2011)

CAS No Purchased (Sigma-Aldrich)	Molecular weight (g/mol)	Log K_{ow}	Water solubility (g/L)
6197-30-4	361.48	6.88	3.6×10^{-4}

Approach

1- **Sub-lethal toxicity** : laboratory toxicity assays using benthic invertebrates

Scarce information existed, being mostly based from limited number of aquatic species. No acute toxicity- LC50 values higher than 100 mg /kg

2- **Multigenerational exposures** : evaluation of long term effects of organic UV-filters

Short term toxicity may underestimate the toxicity of UV-filters especially since these compounds are persistent and have shown to act as endocrine disruptors. Multigenerational studies allow to predict population level effects

3- **Mesocosms experiments**: effects UV-filters on natural benthic invertebrate communities and ecosystem functioning

Facilitates extrapolation of effects to ecosystems by including a range of habitat conditions and biological interactions (that can modify toxicity) Allow the assessment of direct and indirect effects of contaminants by focusing on community and ecosystem level responses. No data about effects of UV-filters in natural freshwater benthic communities

4- **Mixture toxicity assessment :** Mixture of UV-filters and of UV-filters with other contaminants

In natural ecosystems, a cocktail of contaminants are frequently found. Commercial sunscreens with combination of different UV-filters to improve adequate protection against UV-radiation. Other personal care products are also used simultaneously with UV-filters. Few studies have been performed to assess effects of mixtures of different organic UV-filters in aquatic organisms

1- **Sub-lethal toxicity** : laboratory toxicity assays using benthic invertebrates- aquatic insects

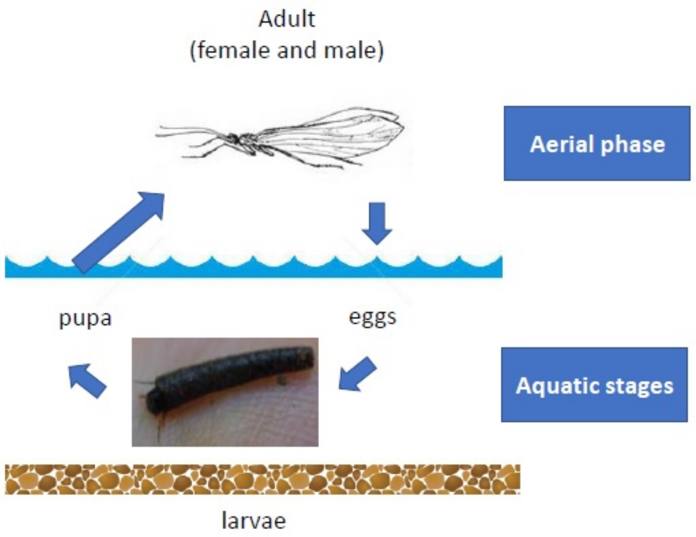
Endpoints

- Life history traits (feeding, growth, development)

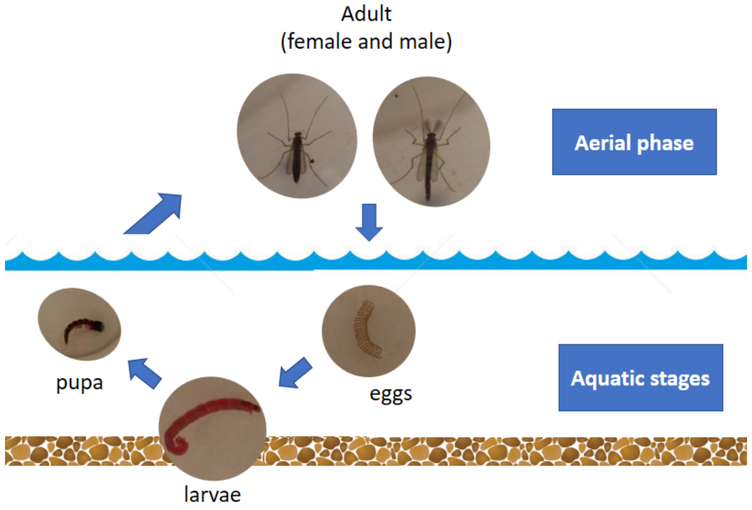
- Sub-cellular responses:battery of biochemical markers associated with essential physiological functions; xenobiotic metabolism, cellular toxicity, energy metabolism:
  - ✓ Oxidative stress and damage: lipid peroxidation (LPO)
  - ✓ Antioxidant and detoxification mechanisms: Catalase (CAT), Gluthatione – *S*-transferase (GST) activities ; total glutathione (tGSH)
  - ✓ Neurotoxicity : Acetylcholinesterase(AChE) activity
  - ✓ Energy metabolism (Aerobic energy production –electron transport system and energy reserves – lipids, carbohydrates and protein content

1- Sub-lethal toxicity : Laboratory ecotoxicity assays with aquatic insects

Sericostoma vittatum (caddisfly)



***Chironomus riparius* (Midge)**



- Insecta
- Live in close contact with sediments (aquatic stages)
- Complex life cycles
- Detritivores

laboratory conditions : (20 ± 1 °C and light-dark cycle of 16:8 h)

Spiked sediment

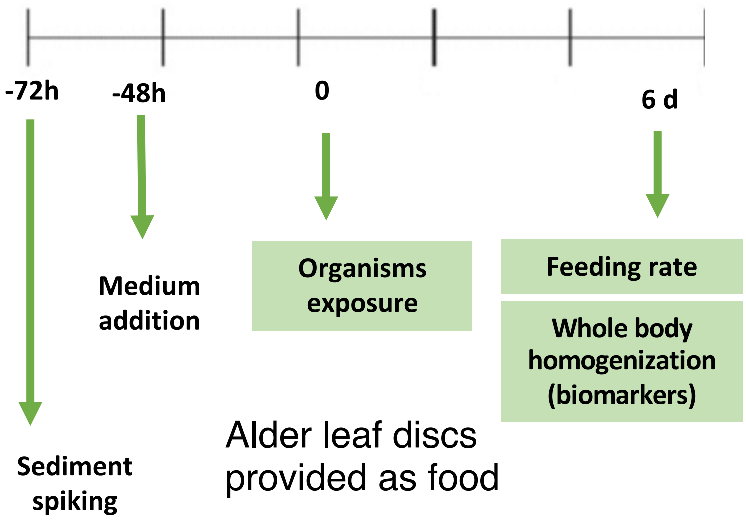
Stock solutions in ethanol

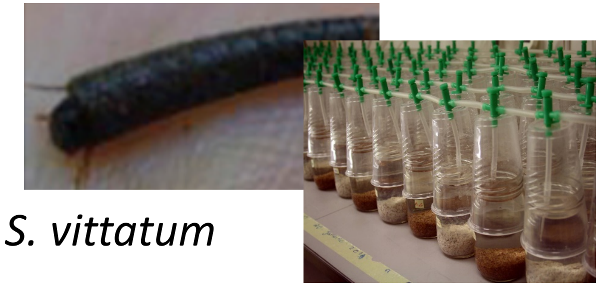
Medium: ASTM reconstituted hard water

Artificial sediment OECD 218 – Sediment- Water Chironomid Toxicity Test Using Spiked sediment

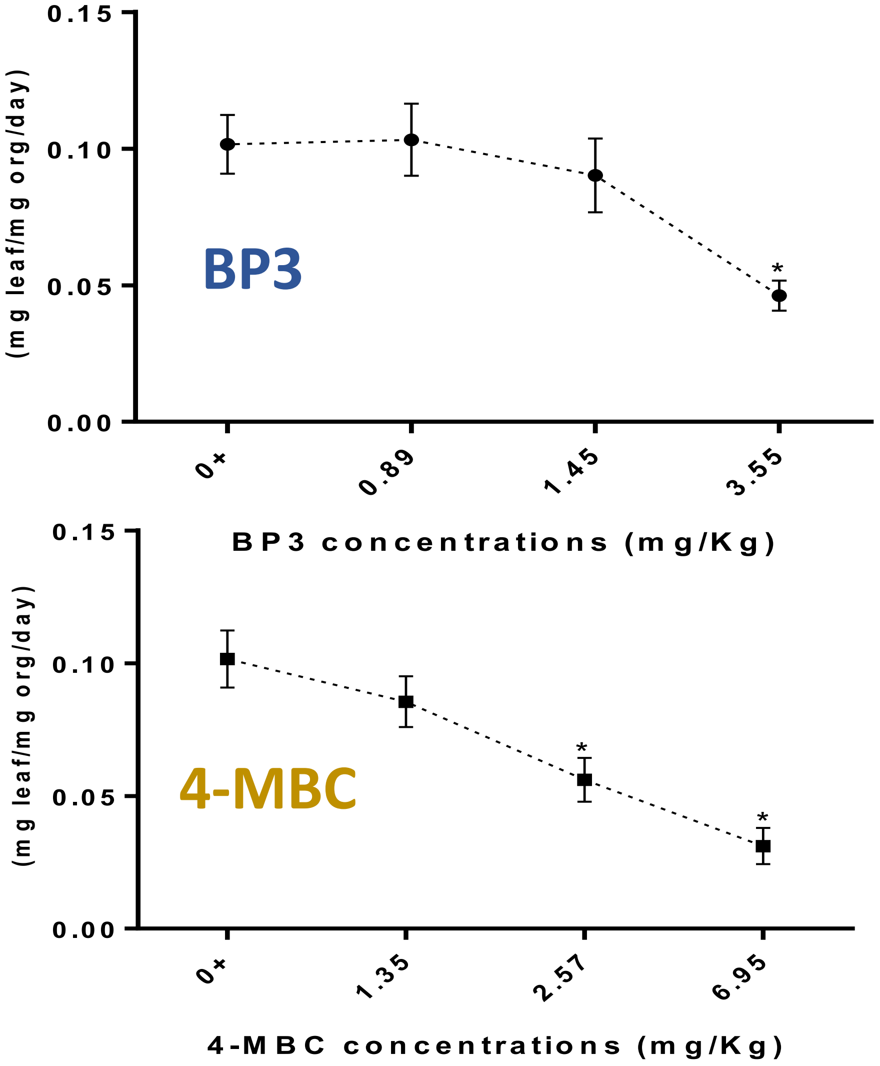
75% inorganic fine sediment (river sand)
20% kaolin
5% α- cellulose
0.1% calcium carbonate

1- Sub-lethal toxicity : Results – *S. vittatum* assay





S. vittatum



Feeding inhibition

- may lead to deleterious effects in growth and development
- alter population dynamics
- compromise organic matter processing in streams
- reduce performance of other detritivores species

Alterations in AChE activity have been linked with behavioural responses such as feeding and locomotion activity

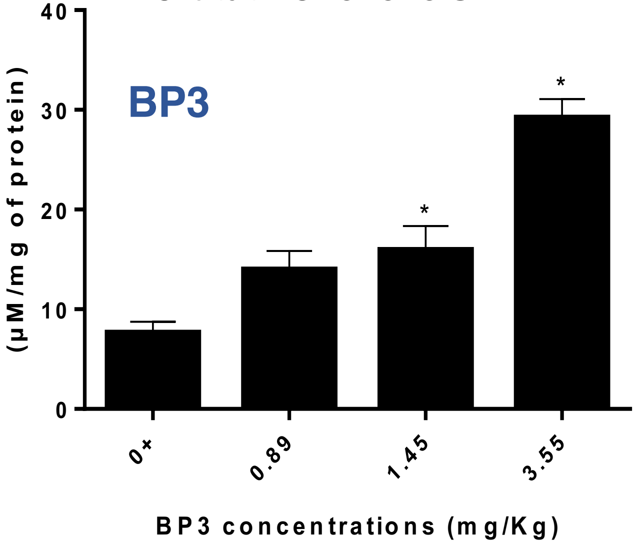
However, NO alterations were observed in AChE activity

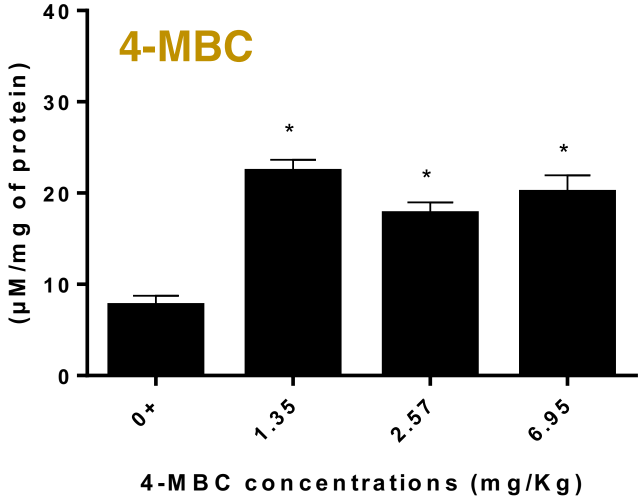
Concentrations of BP3 and 4-MBC in sediments at the end of the exposure period (6 days) were up to 71% and 48.6% lower than nominal concentrations

Campos, D., Gravato, C., Fedorova, G., Burkina, V., Soares, A. M. V. M., & Pestana, J. L. T. (2017). Ecotoxicity of two organic UV-filters to the freshwater caddisfly Sericostoma vittatum. Environmental Pollution , 228, 370–377. http://doi.org/10.1016/j.envpol.2017.05.021

1- Sub-lethal toxicity : Results – *S. vittatum* assay

Glutathione levels





Increase in Glutathione

reduction in carbohydrates

maintain the cellular redox status neutralizing ROS and free radicals

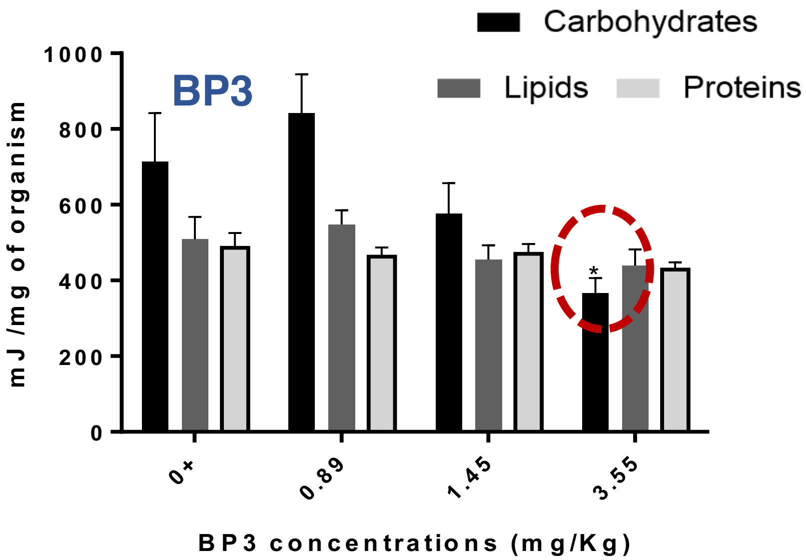
No observable oxidative damage in lipids (no effects in LPO)

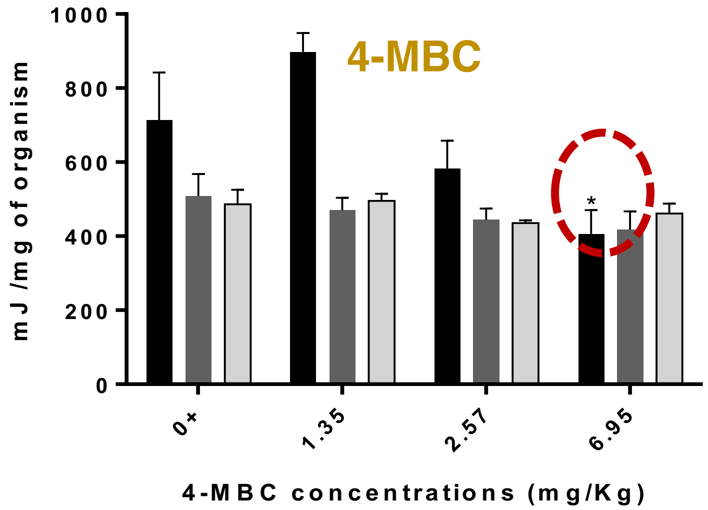
Synthesis/ recycling of tGSH may be responsible for the decrease in energy reserves (carbohydrates)

Decrease in carbohydrates could also be due to feeding inhibition and energy requirements related to other detoxification mechanisms

No significant effects observed for CAT, GST AChE and ETS activity

Energy reserves





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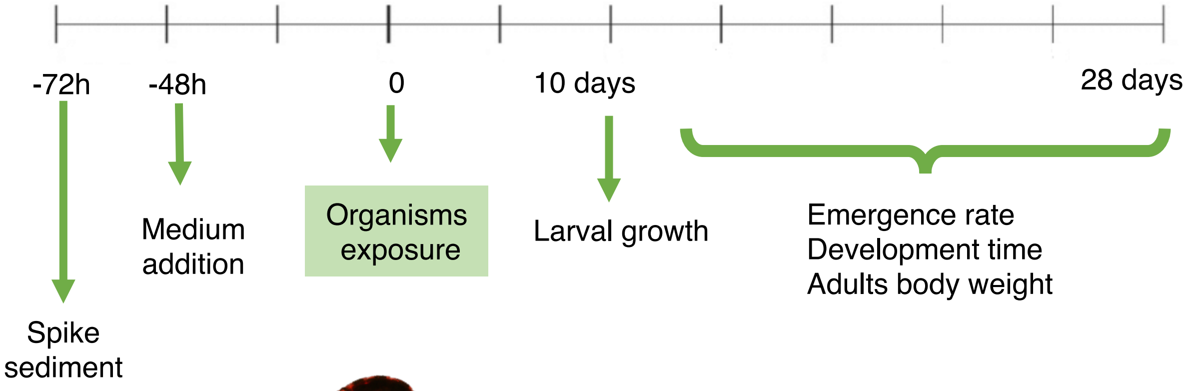
1- Sub-lethal toxicity : Results – *C. riparius* assay

OECD 218 – Sediment- Water Chironomid Toxicity Test Using Spiked sediment

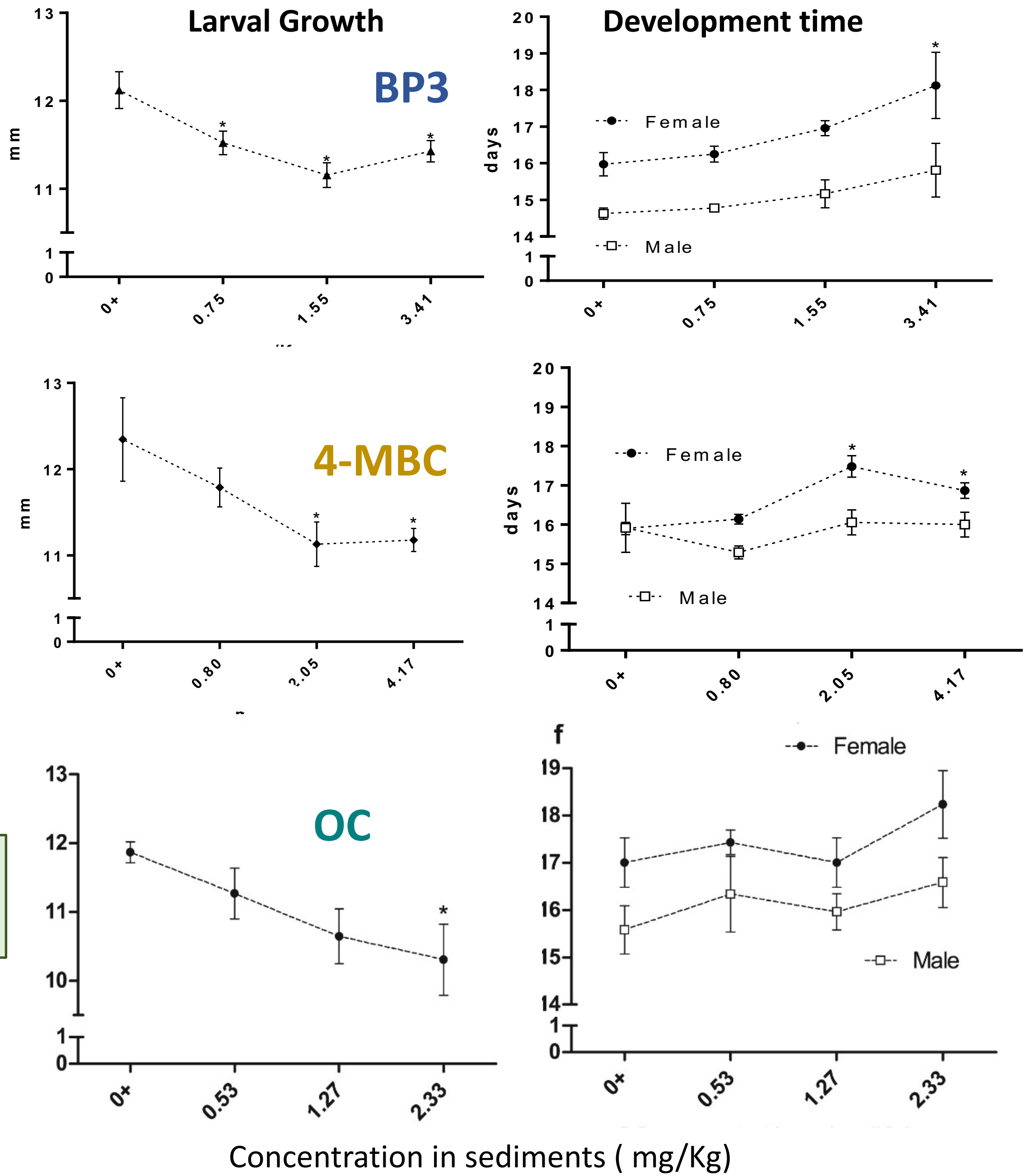


C. riparius
< 24h (1st instar)

Decrease growth and delayed emergence

decreased body weight of male imagoes induced by BP3 and 4-MBC (data not shown) suggests that *C. riparius* reproduction might be affected (flying performance and total number of gametes produced)

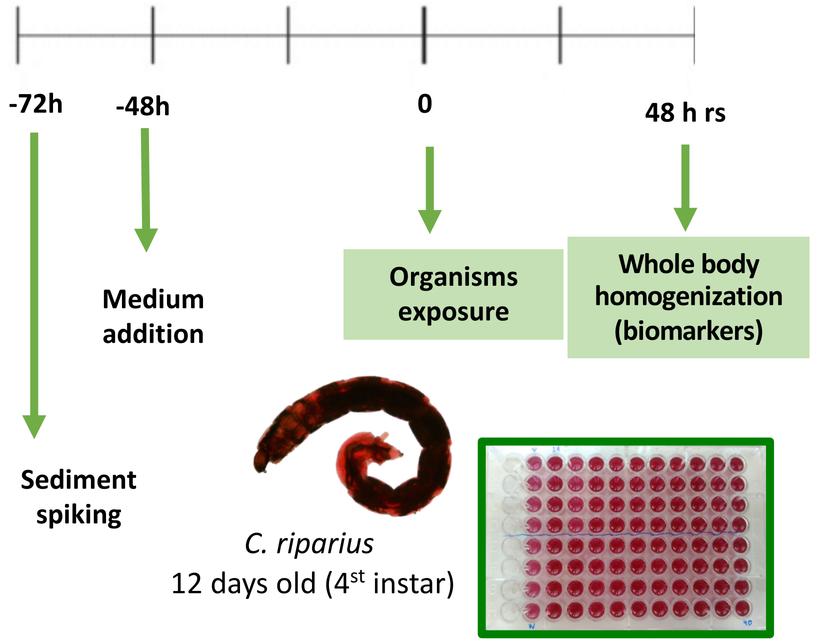
Suggests **reproductive impairments** caused by UV-filters (effects on fertility and fecundity?) and **Population level effects**



measured concentrations in sediments after 10 days of exposure were up to 70%, 68% and 79% lower than nominal concentrations for BP3, 4-MBC and OC.

Campos, D., Gravato, C., Quintaneiro, C., Golovko, O., Zlabek, V., Soares, A. M. V. M., & Pestana, J. L. T. (2017). Toxicity of organic UV-filters to the aquatic midge Chironomus riparius. Ecotoxicology and Environmental Safety, 143, 210–216. http://doi.org/10.1016/j.ecoenv.2017.05.005

1- Sub-lethal toxicity : Results – *C. riparius* assay



- NO alterations were observed in AChE activity (no neurotoxicity)
- NO suggestion of oxidative damage in lipids (LPO)
- **Dysregulation of the antioxidant system** suggesting longer exposures might lead to oxidative stress

BP3 Inhibition of CAT activity *(non significant)*

4-MBC Inhibition of CAT activity, induction of GST activity and increased levels of total glutathione *(non significant)*

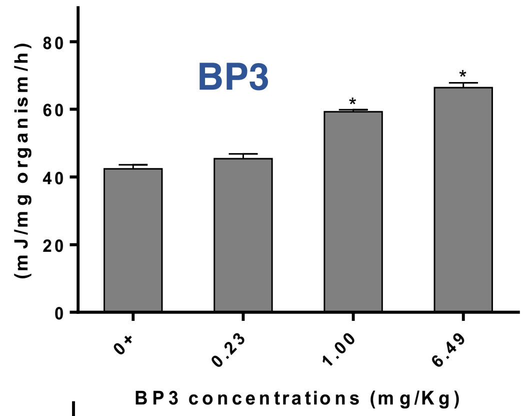
OC Increased levels of total glutathione

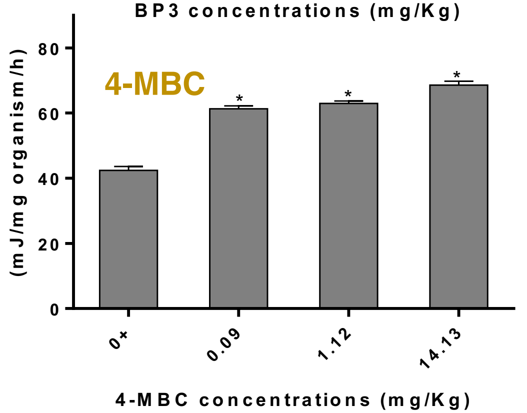
measured concentrations in sediments after 2 days of exposure were 74%, 64% and 27% lower than nominal concentrations for BP3, 4-MBC and OC respectively.

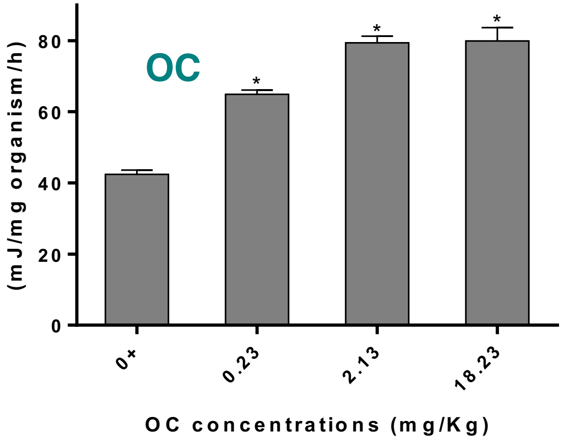
Campos, D., Gravato, C., Quintaneiro, C., Golovko, O., Zlabek, V., Soares, A. M. V. M., & Pestana, J. L. T. (2017). Toxicity of organic UV-filters to the aquatic midge Chironomus riparius. Ecotoxicology and Environmental Safety, 143, 210–216. http://doi.org/10.1016/j.ecoenv.2017.05.005

1- Sub-lethal toxicity : Results – *C. riparius* assay

ETS activity







C. riparius
12 days old (4st instar)

Increase in the Electron Transport System activity
(aerobic energy production)

Reflects an increase of energy requirements for detoxification and might compromise the allocation of energy for growth and reproduction

Metabolic costs of the stress response were probably responsible, together with other behavioural and physiological responses (e.g., feeding and assimilation), for the reduced growth and development of *C. riparius* exposed to BP3, 4-MBC and OC

measured concentrations in sediments after 2 days of exposure were 74%, 64% and 27% lower than nominal concentrations for BP3, 4-MBC and OC respectively.

Campos, D., Gravato, C., Quintaneiro, C., Golovko, O., Zlabek, V., Soares, A. M. V. M., & Pestana, J. L. T. (2017). Toxicity of organic UV-filters to the aquatic midge Chironomus riparius. Ecotoxicology and Environmental Safety, 143, 210–216. http://doi.org/10.1016/j.ecoenv.2017.05.005

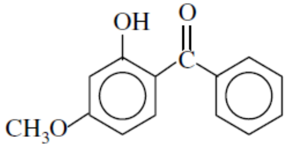
2- Multigenerational exposures : Two-generational assay w/ *C. riparius*

Effects on fertility and fecundity?
Population level effects?

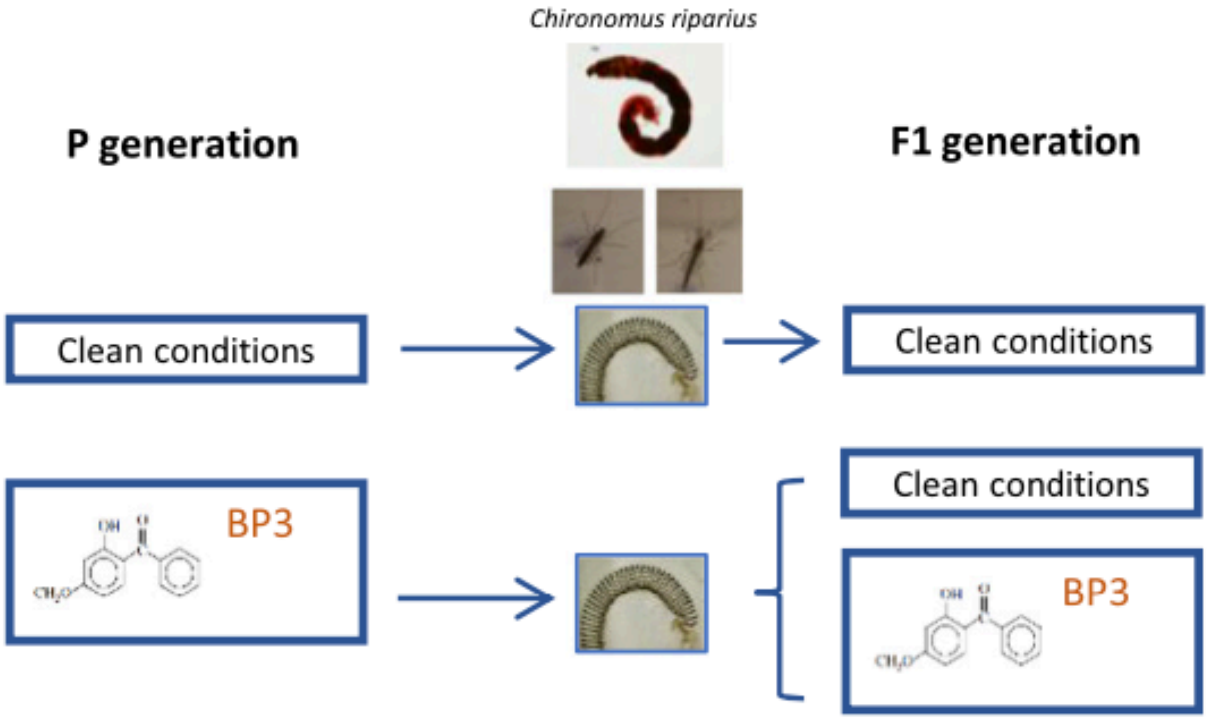
- Partial life-cycle tests may underestimate the toxicity of UV-filters especially since these compounds are persistent and have shown to act as endocrine disruptors. inclusion of complete life cycles (relevant life stages - eggs, larvae, pupae, imagoes) and endpoints controlled by hormones (fertility, development, and emergence patterns)

- Multigenerational ecotoxicity studies are of extreme importance to understand how continuous exposure to contaminants can compromise fitness of populations.

BP3



OECD 233 – Sediment-Water Chironomid Life-Cycle Toxicity Test



P generation:

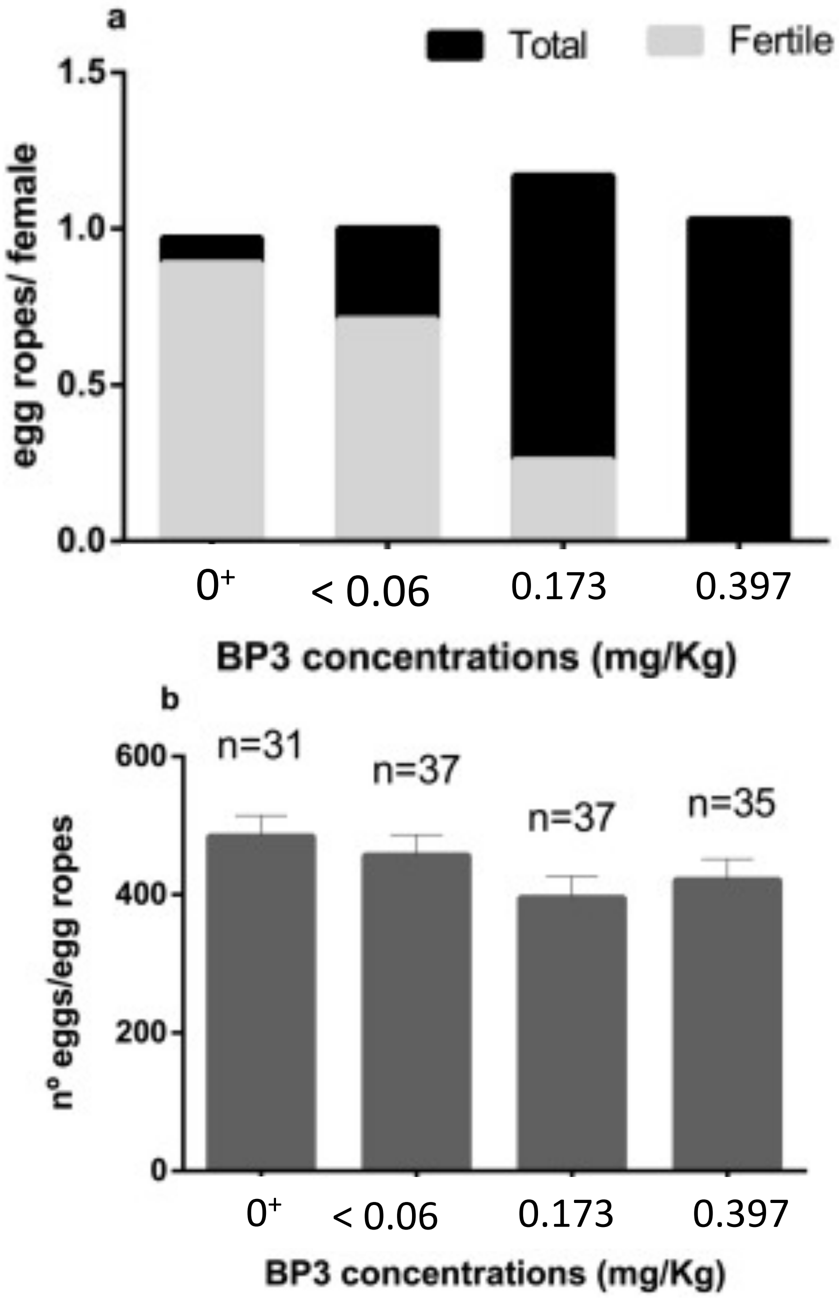
- Emergence rate
- Fertility/ Fecundity

F1 generation:

- Emergence rate

Campos, D., Silva, A. R. R., Loureiro, S., Grabicova, K., Staňová, A. V., Soares, A. M. V. M., & Pestana, J. L. T. (2019). Two-generational effects of Benzophenone-3 on the aquatic midge Chironomus riparius. The Science of the Total Environment, 669, 983–990. http://doi.org/10.1016/j.scitotenv.2019.03.023

2- Multigenerational exposures : Two-generational assay w/ *C. riparius*



Fecundity and Fertility (Parental generation)

Fertility was strongly affected
with none of the egg ropes hatching at the highest concentration

These effects occurred without significant changes regarding developmental time, fecundity (number of egg ropes produced by females) and number of eggs per egg rope in P generation

- Energetic costs related to defence mechanisms and detoxification can lead to detrimental effects in terms of embryonic development and hatching, likely due to less energy allocated into reproduction

- endocrine disruption ?

- Exposure to BP-3 had a direct impact the endocrine system (ecdysone-responsive genes); of on *C. riparius* larvae and embryos, altering embryo development Ozáez et al., 2016; Ozáez et al., 2013
- Alterations in the levels of these hormones may affect the development and the reproduction of *C. riparius* Planelló et al., 2015

Campos, D., Silva, A. R. R., Loureiro, S., Grabicova, K., Staňová, A. V., Soares, A. M. V. M., & Pestana, J. L. T. (2019). Two-generational effects of Benzophenone-3 on the aquatic midge Chironomus riparius. The Science of the Total Environment, 669, 983–990. http://doi.org/10.1016/j.scitotenv.2019.03.023

2- Multigenerational exposures : Two-generational assay w/ *C. riparius*

No effects on emergence rate of parental generation (in accordance with previous partial life cycle test)

Emergence rates and of F1 generation were altered even when organisms were not exposed (but whose parents were previously exposed to BP-3), implying carry-over effects of BP-3.

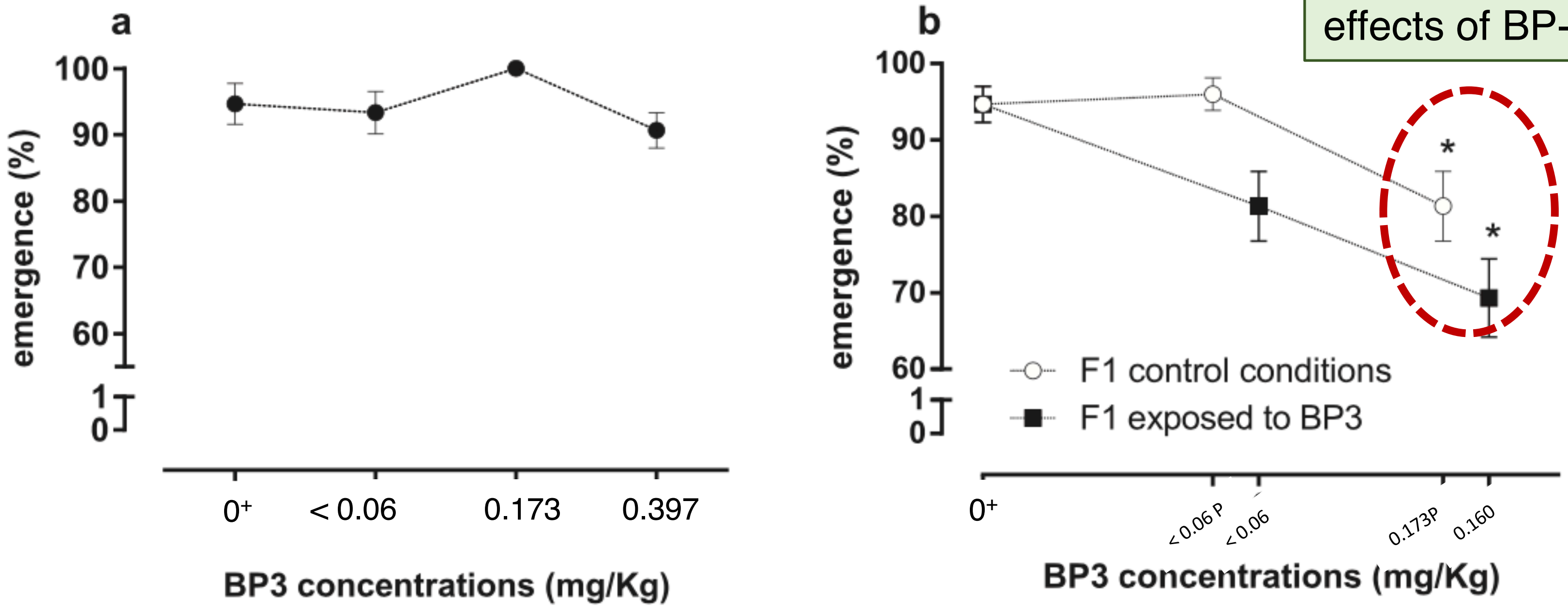


Fig. 2. Effects of BP3 on *Chironomus riparius* emergence (%; mean ± SE) during two consecutive generations. a) P generation and b) F1 generation. Open circles represent F1 generation larvae exposed under control conditions whose P generation was exposed to BP3. Black squares represent F1 generation larvae exposed to the same treatments as the P generation. Asterisks denote a significant difference compared with control treatment (0).

Results show higher sensitivity of the F1 generation in comparison with the P generation thus suggesting carry-over, parental effects induced by organic UV-filters that would not be detected using standard partial life cycle assays

Campos, D., Silva, A. R. R., Loureiro, S., Grabicova, K., Staňová, A. V., Soares, A. M. V. M., & Pestana, J. L. T. (2019). Two-generational effects of Benzophenone-3 on the aquatic midge Chironomus riparius. The Science of the Total Environment, 669, 983–990. http://doi.org/10.1016/j.scitotenv.2019.03.023

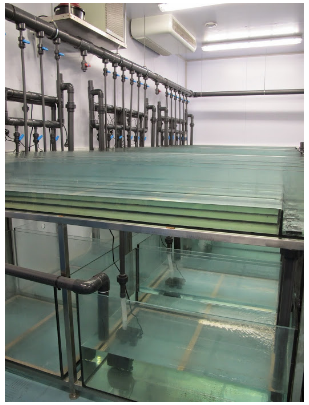
3- Mesocosms experiments: Short term effects of 4-MBC

Effects on structure and functioning of ecosystems?

- Simulating natural field conditions can provide ecological relevant data.
- Benthic invertebrates are prone to exposure to organic UV-filters that accumulate in sediments.
- The assessment of direct and indirect effects of organic UV-filters is needed

Mau River – Sever do Vouga, Aveiro

mesocosm facility



36, 2m-long indoor artificial streams

Replication
Water sediment systems
Freshwater / saltwater
Pulse/press contamination
Manipulation of flow, temperature, etc
Community and Ecosystem level endpoints

3- Mesocosms experiments: Short term effects of 4-MBC

Organisms collected in summer from a reference stream

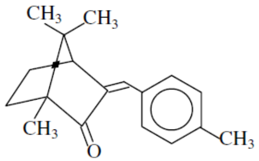
macroinvertebrate sampling

Endpoints:

- community structure
- Leaf litter degradation
- Primary production

- Indoor artificial streams (9)
- 200 cm x 30 cm x 22,5 cm
- recirculating water (APW enriched) approx. 280 L
- 7 kg of sediment (99% sand + 1% grinded alder leaves)
- 15ºC

159 organisms/ stream → 15 different taxa



4-MBC

Concentrations of 4-MBC in sediment (mg/kg) after 24 h and 7 days of organisms' exposure (mean ± SD) in artificial streams (n = 3).

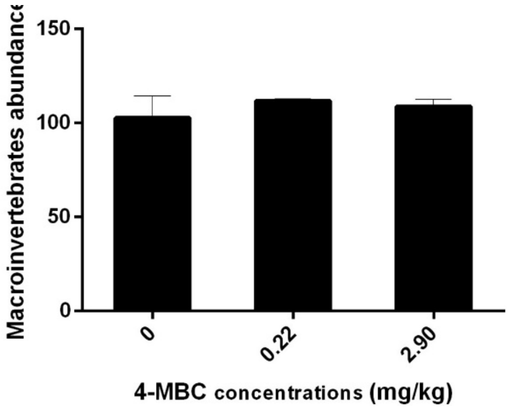
		Measured concentration (mg/kg)	
		24 h	7d
Sediment	Low	0.22 ± 0.02	0.21 ± 0.04
	High	2.90 ± 0.44	2.07 ± 0.51

In the test systems the measured concentrations of 4-MBC in sediments, after 7 days of exposure, were 30% lower than initial concentrations

Fig. 1. Detailed view of a) artificial stream; b) *Alnus glutinosa* leaf packs; c) ceramic tiles with periphyton and d) artificial stream system (overview) used in the present study.

Campos, D., et al. (2020). Effects of the organic UV-filter, 3-(4-methylbenzylidene) camphor, on benthic invertebrates and ecosystem function in artificial streams. Environmental Pollution 260,. http://doi.org/10.1016/j.envpol.2020.

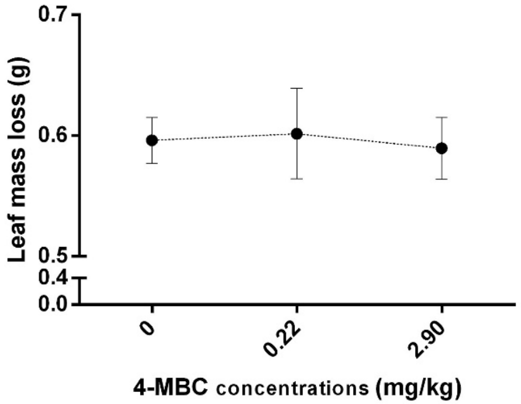
3- Mesocosms experiments: Short term effects of 4-MBC



Macroinvertebrate community

- No effects on macroinvertebrates community structure

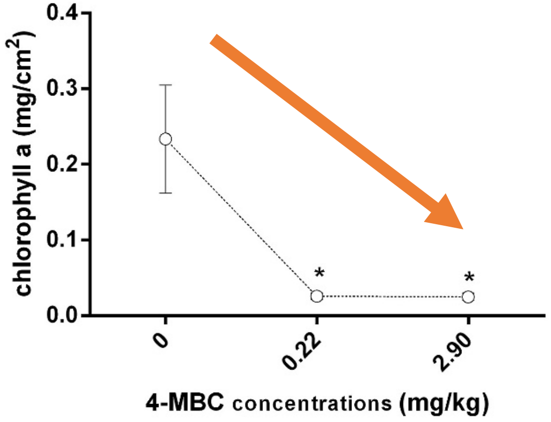
Expected since sub-lethal concentrations were used
Preliminary studies: LC 50> 100 mg/kg *C. riparius* and *S. vittatum*



Leaf decomposition

- Exposure to 4-MBC did not elicit density-mediated effects (density of shredders) nor trait-mediated effects (alteration of feeding behaviour)
- Mismatch between laboratory tests and mesocosms experiments

Organisms may have sought refuge within leaf packs, reducing their direct contact with the contaminated sediment
Tests conducted at 15 ºC , Temperature-dependent toxicity



Primary production

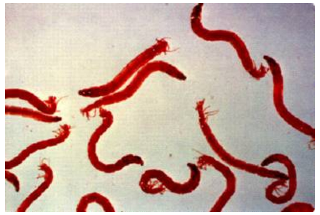
- Strongly affected

No effects were observed in grazers → **Direct effects on periphyton**
4-MBC could have fluxed out of the sediments into the experimental tiles due to bioturbation or flow within our experimental streams being trapped in the periphyton biofilm - effects on algae?

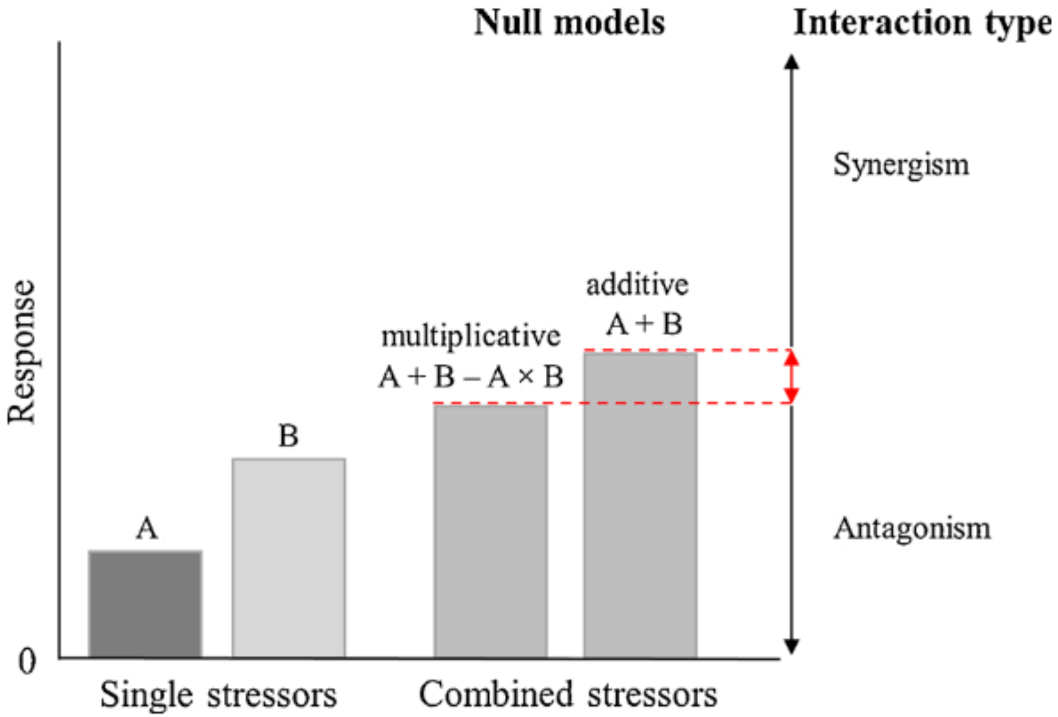
Campos, D., et al. (2020). Effects of the organic UV-filter, 3-(4-methylbenzylidene) camphor, on benthic invertebrates and ecosystem function in artificial streams. Environmental Pollution 260,. http://doi.org/10.1016/j.envpol.2020.

Mixture toxicity assessment

- Commercial sunscreens have in their composition a combination of different UV-filters to improve adequate protection against UV-radiation
- Few studies have been performed to assess the effects of mixture of different organic UV-filters in aquatic organisms and research show evidences of both synergisms and antagonisms in different species / different mixtures



C. riparius



The combined effect of stressors A and B can be additive (no interaction), **synergistic (stronger effect than predicted by a null model)** or antagonistic (weaker effect than predicted by a null model).

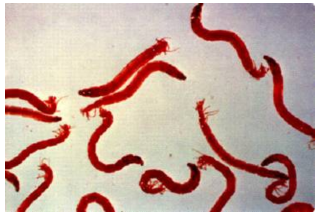
Independent action (IA) is a multiplicative null model

Independent Action Model

$$E_{mix} = 1 - \prod^{i} (1 - E_i)$$

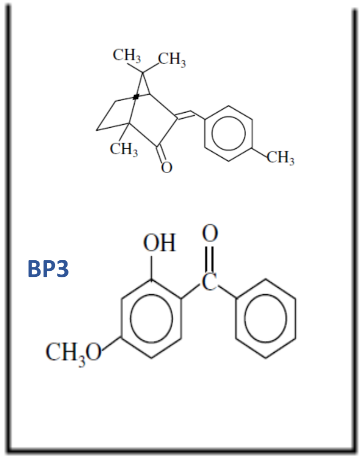
$$E_i = \frac{(e_i - e_{control})}{(e_{max} - e_{control})}$$

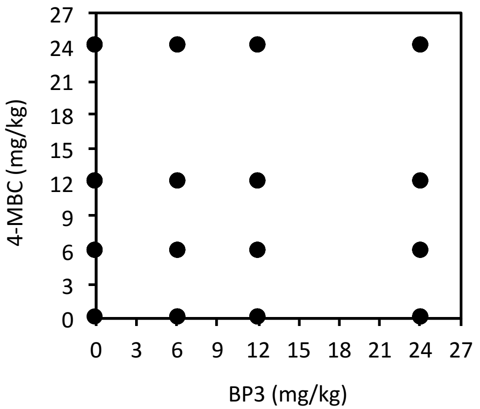
4- Mixture toxicity assessment : *C. riparius* exposed to combination of different UV-filters



C. riparius

4-MBC

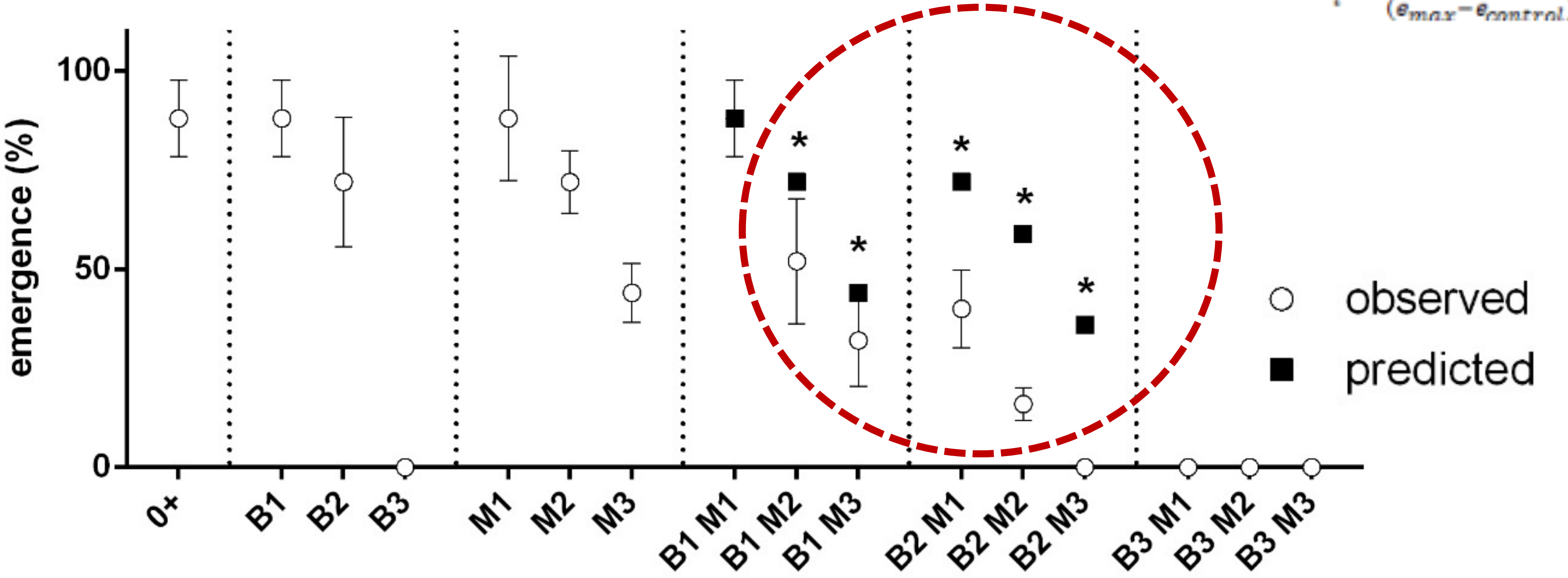




BP-3 / 4-MBC

Emergence rate

SYNERGISM



Independent Action Model

$$E_{mix} = 1 - \prod^{i} (1 - E_i)$$

$$E_i = \frac{(e_i - e_{control})}{(e_{max} - e_{control})}$$

- Individual chemical testing can underestimate toxicity of organic UV-filters
- Synergism in other endpoints (e.g. development time) observed for combination of UV filters (BP-3) and DEET (an insect repellent)

Main Conclusions

- **BP3, 4-MBC and OC are toxic to benthic invertebrates** such as *C. riparius* and *S. vittatum* at environmentally relevant concentrations
- **Multigenerational effects of BP-3** have been shown in *C. riparius* and need a closer look
- **The potential for synergism** is suggested and toxicity of complex mixtures containing UV-filters needs evaluation
- **Ecological effects** on benthic invertebrate communities and on ecosystem functional endpoints were not accurately predicted by single species assays and deserve further investigation (effects of temperature / direct effects of 4-MBC on primary producers)

Still many knowledge gaps

- ***In situ* monitoring of UV filters in freshwater sediments** (e.g. hotspots such as downstream effluents and swimming/recreational areas)
- **Influence** of temperature, salinity, organic matter, etc., in the toxicity of UV-filters
- **Mixture toxicity –** combination of different UV filters, w/ other contaminants
- **Bioaccumulation** and transport of organic UV-filters along food webs

Thank you for your attention!

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