TOXICITY ASSESSMENT
WP3 Organism Level WP4 Cellular and Molecular levels
FISH

Partners involved in fish experiments:

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Several fish species with different characteristics

	*Dicentrarchus labrax* (seabass)	Marine	Long-lived	Juveniles	Temperate
	*Pomatoschistus microps* (sand goby)	Marine	Short-lived	Juveniles	Temperate
	*Oryzias melastigma* (marine medaka)	Marine/brackish	Short-lived	All stages	Tropical
	*Danio rerio* (zebrafish)	Freshwater	Short-lived	All stages	Tropical

General objectives

The general objectives of these WP are to evaluate the physiological consequences of an exposure to MPs at individual (WP3) and molecular (WP4) levels.

For this purpose, several approaches have been used including short waterborne exposures (acute toxicity test) to either MPs or extracts and longer dietary exposures.

Embryo-larval exposure

Embryo-larval exposures have been performed according to Fish Embryotoxicity Test FET) procedure described in OECD TG236. FET using zebrafish has proven to be a sensitive method to evaluate acute toxicity.

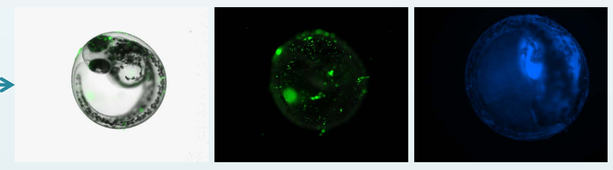
In zebrafish, the FET procedure includes ~2days of exposure post-hatch. To follow a similar procedure of exposure with marine medaka, it has been performed during 13 days (hatching between 10-11 days post fertilisation).

The strict FET endpoints are lethal endpoints. In some cases, sub-lethal endpoints such as morphological (e.g. body deformities, vascular system anomalies, oedema) or behavioural defects have also been analysed.

→ The main conclusion is that the short (4d) FET performed with zebrafish did not reveal acute toxicity. On the contrary preliminary results obtained with marine medaka which corresponding stages last 13d revealed some acute and sub-acute toxicity.

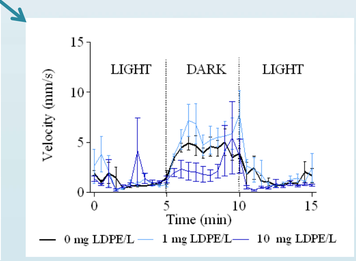
MPs	Species	Exposure route	Concentration range mg/L	Effects				POP transfer
				AT	Morph.	Biom.	Behav.	
PE-BaP	zf	WB (96h)	1-10	No	No			Yes
LDPE-BaP	zf	WB & WB-E (96h)	10 & equivalent to 10	No	No			-
LDPE-PFOS	zf	WB & WB-E (96h)	10 & equivalent to 10	No	No			-
PVC-BaP	zf	WB & WB-E (96h)	10 & equivalent to 10	No	No			-
PVC-PFOS	zf	WB & WB-E (96h)	10 & equivalent to 10	No	No			-
LDPE	mm	WB (13d)	1-10	No	**1, 10**	**1,10**	**1,10**	-
LDPE-BaP	mm	WB (13d)	1-10	No	No	No	No	-
LDPE-BP3	mm	WB (13d)	1-10	**1, 10**	No	No	**10**	-
LDPE-PFOS	mm	WB (13d)	10	**10**	No	**10**	No	-
LDPE	mm	WB-E (13d)	Equivalent to 1-10	No	No	No	**eq. 1**	-
LDPE-BaP	mm	WB-E (13d)	Equivalent to 1-10	**eq. 1**	No	No	**eq. 1**	-
LDPE-BP3	mm	WB-E (13d)	Equivalent to 1-10	No	No	No	**eq. 1**	-
LDPE-PFOS	mm	WB-E (13d)	Equivalent to 1-10	No	No	No	**eq. 1**	-

zf: zebrafish; mm: marine medaka; WB: waterborne; WB-E: waterborne to MPs extract; AT: acute toxicity; Morph.: morphological defects; Biom.: growth; Behav.: behaviour;-: not measured



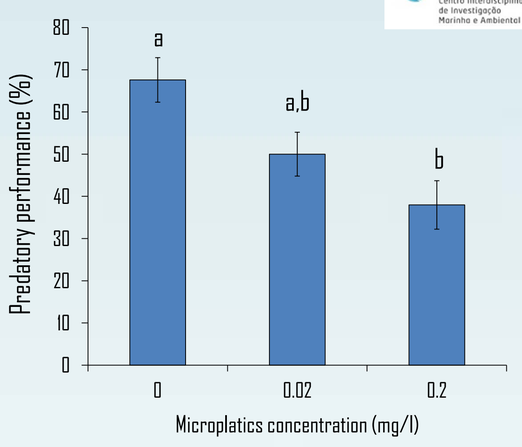
Zebrafish embryo (48hpf) exposed to fluorescent PE MPs (1-5 µm) visible + fluorescence channels (left) or fluorescence only (right)

Evidence for BaP transfer in zebrafish embryo (48hpf) after exposure to MP-BaP (blue fluorescence in the yolk).



Disruption of behaviour during a larval photomotor response test with marine medaka exposed to LDPE at either 1 or 10 mg/mL of MPs.

Waterborne juvenile exposure



Waterborne exposure of *P. microps* juveniles to PE fluorescent micropsheres (1-5 µm) during 96h.

→ Significant decrease of predatory performance.

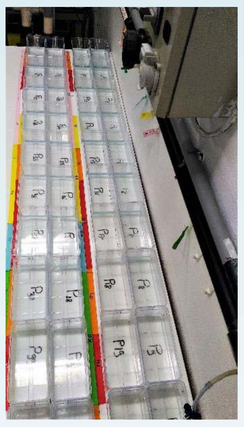
Dietary exposures

In order to mimic trophic exposure, regular diet has been spiked with MPs. Concentration of MPs has been set at 0.1 and 1%. Diet has then been distributed to fish according to regular feeding regimes.

Short-term exposure of marine medaka have been performed using diet spiked with the whole range of ORU MPs (spiking at 0.1 and 1% of diet). Exposure started just after hatching and lasted 10 days. Survival, growth and behaviour have been monitored as well as EROD activity and *cypla* expression.

→ No clear effect has been observed.

A long-term exposure (3 mo) has been initiated with only 1% MPs and the highest POP concentrations.



Seabass juveniles have been exposed to dietary MPs in two trials. First exposure has been performed over 30 days using virgin MPs (PE, PVC; 100 or 500 mg MPs/ kg diet) while the second exposure has been performed over 21 days with virgin PE MPs (100 mg MPs/ kg diet) as well as MPs spiked with PFOS (100 mg MPs and 4.8 µg PFOs/kg diet) and PFOS (4.8 µg PFOs/kg diet).
The expression of a set of genes in several organs (head kidney, liver and gut) to evaluate inflammation, oxidative status, apoptosis as well as cellular and physiological stress was determined.

MPs	Exposure route	Immunity	Toxicity	Oxidative stress
Virgin PE/PVC	Diet (30 d)	↑		Very low
PE	Diet (21 d)	↑		
PFOS	Diet (21 d)	↓	↑	
PE-PFOS	Diet (21 d)	↓	↑ ↑	Exacerbation of oxidative stress

→ Virgin PE and PVC triggered immune response
→ PFOS induced a decrease of immune response and toxicity (molecular response) which appeared to be amplified when PFOS is spiked to PE MPs.

General conclusions

→ Classical embryo-larval tests used in eco/toxicology, such as zebrafish FET, which are suitable to evaluate acute toxicity did not reveal toxicity after exposure to MPs or MPs spiked with pollutants, even at concentrations much above environmental concentrations.
→ Embryo-larval assay performed using marine medaka (and so longer exposure duration) indicated toxic effects of some MPs or MPs spiked with pollutants. Difference of sensitivity between embryo-larvae zebrafish or medaka could also be due to difference in salinity (which could be evaluated with medaka since this species can spawn and be raised in both).
→ Behaviour appeared to be a sensitive endpoint (WB exposure or *P. microps* and marine medaka embryo-larval exposure).
→ Dietary exposure of seabass juveniles resulted in changes in immune response and cellular toxicity (in particular oxidative stress) while no effects have been observed after 10 days of exposure of marine medaka larvae. The difference may be explained by the longer exposure in seabass and/or the amount of plastic eaten (which is minimal for marine medaka larvae). In this last case a longer exposure is in progress.

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