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Hazard/Risk Assessment

# A 2-Tier Standard Method to Test the Toxicity of Microplastics in Marine Water Using *Paracentrotus lividus* and *Acartia clausi* Larvae

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**Abstract:** A 2-tier standardized protocol was designed to test the toxicity of microplastics to planktonic organisms. This approach uses sea urchin (*Paracentrotus lividus*) and copepod (*Acartia clausi*) larvae because they are common biological models in marine research, and standard methods for toxicity testing with regulatory applications are available. In Tier I, leachates obtained at a 100 to 1 liquid to solid ratio are tested, and toxic units are calculated using a probit dose–response model to quantify the toxicity of the plastics. In Tier II, which is conducted only if significant toxicity (> 1 toxic unit) is found in Tier I, particles less than 20 μm in size are tested at concentrations between 0.1 and 10 mg $L^{-1}$, and a toxicity threshold suitable for ranking materials according to their toxicity is obtained from the 10% effect concentration (EC10) values. Results point to chemical additives as being responsible for the toxicity found in certain plastic materials. This process is suitable for both a priori identification of the hazard posed by plastic objects in the aquatic environment, and a posteriori assessment of environmental risk caused by microplastic pollution. The method also provides a quantitative procedure appropriate for ranking plastic materials according to their toxicity to aquatic organisms. *Environ Toxicol Chem* 2019;38:630–637. © 2018 SETAC

**Keywords:** Marine plastics; Marine toxicity tests; Risk assessment; Leachates; Additives; Microplastics

## INTRODUCTION

Global plastic production has continuously increased during the past decades to reach the current 322 million tons per year (PlasticsEurope 2016); of this amount, between 4.8 and 12.7 million tons are estimated to end up in the oceans (Jambeck et al. 2015). In fact, between 60 and 80% of all marine litter consists of plastics (Derraik 2002). Although conventional polymers are not biodegradable, they are sensitive to photodegradation and physical abrasion that, on exposure to sunlight and the high energy hydrodynamics typical of coastal environments, lead to fragmentation into small pieces termed microplastics (Barnes et al. 2009).

Plastics are composed of a nonreactive polymeric matrix, inert and nonhazardous from a toxicological point of view (Sheftel 2000), and a broad range of chemical additives (plasticizers, stabilizers, ultraviolet-filters, and flame retardants) intended to give objects desired physical properties, or prepare them for use as processing aids. For example, the US Food and Drug Administration has identified more than 3000 substances that are considered indirect food additives because they migrate from the packaging into the food (US Food and Drug Administration 2011). Some chemicals used as additives are hazardous to human health and the aquatic environment; these include trace metals, Bisphenol A, alkylphenols, chlorinated hydrocarbons, and polybrominated compounds. Plastic additives are generally not bound to the polymer chains, and the parental molecules or degradation products readily leach into liquid matrices (see Brede et al. 2003 for Bisphenol A; Kim et al. 2006 for polybrominated flame retardants; Fernandes et al. 2008 for nonylphenol; Lithner et al. 2012 for metals). Among conventional polymers, polyvinyl chlorine (PVC) is the one carrying higher percentages of additives, particularly plasticizers that may account for more than 30% in weight of the final product (Organisation for Economic Co-operation and Development 2009). Bejgarn et al. (2015) tested the toxicity to copepods of leachates from 22 plastic products and found that PVC objects were toxic in most cases, whereas very few polyethylene objects showed toxicity. Polyvinyl chlorine was also found to be the most toxic polymer for barnacle larvae (Li et al. 2016). These studies revealed that chemicals contained

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in plastics have the potential to cause toxicity to aquatic organisms; therefore a robust standard method suitable for hazard identification of commercial plastic materials and risk assessment of derived environmental microplastics is needed.

The aim of the present study was to develop a 2-tier standardized protocol to test the toxicity of microplastics to marine plankton organisms. Tier I consists of a leachate test based on the hypothesis that chemical additives can leach from the polymeric matrix and cause waterborne toxicity. Tier II incorporates a test of microplastic particles within the size range the test species can ingest, and it is intended to assess the effects of the particles themselves. The evaluations employ *Paracentrotus lividus* and *Acartia clausi* planktonic larvae because they are common biological models in marine research, and standard methods for toxicity testing are available (International Organisation for Standardisation 1999; Beiras et al. 2012). These biological models have been previously used to assess the toxicity of plastic additives (Tato et al. 2018) and microplastics (Beiras et al. 2018), and showed similar sensitivity to both types of pollutants.

With the present study goal in mind, 2 sets of experiments were conducted. First, there were methodological bioassays intended to optimize the leachate test protocol in terms of liquid to solid ratio, particle size fraction used for leaching, and light conditions. Second, the established standard method was applied to microplastics obtained both from commercial plastic products and from plastics retrieved from the marine environment.

## MATERIALS AND METHODS

### Plastic materials

Plastic consumer products were bought from local shops, whereas in-kind micronized virgin plastic polymers were supplied by CETEC or purchased from Rotogal and Micro Powders. Environmental plastics were obtained from trawls conducted by fishing boats in the Northeast Atlantic, within the framework of the plastic recovery project REPESCAPLAS. The Centre for Scientific and Technological Support to Research at the University of Vigo in Spain identified any unknown polymer composition of the plastic objects by using Fourier-transform infrared spectroscopy (Thermo Scientific Nicolet 6700). Plastic products were ground by AIMPLAS utilizing a RETSCH ZM200 Ultra Centrifuge Mill after embrittlement with liquid $N_2$, and sieved through a 250 μm metallic mesh. Additional sieving, when necessary, was done at the University of Vigo with a 20 μm metallic mesh. The microplastics' size distribution of the $< 20$ μm fraction was recorded using a Multisizer 3 Coulter Counter (Beckman).

### Bioassays to optimize leachate conditions

For the leachate tests, the plastic powder was sieved by 250 μm and transferred into 50-mL glass bottles filled with artificial seawater (Lorenzo et al. 2002) with no head space. The highest concentration used was 100 g $L^{-1}$, a liquid to solid ratio of 10 to 1 proposed by European standards to test for leaching of waste materials with a particle size of less than 4 mm (CEN, European Committee for Standardization 2002), and previously applied to toxicity testing of plastic leachates (Lithner et al. 2012; Bejgarn et al. 2015). This is also similar to the 20 to 1 liquid to solid ratio set by the US Environmental Protection Agency in the standard method to test the toxicity of wastes (US Environmental Protection Agency 1992), and earlier used for toxicity testing of scrap tire leachates (Hartwell et al. 1998). However, artificial seawater replaced deionized water (CEN, European Committee for Standardization 2002) and acids (US Environmental Protection Agency 1992) to extract the leachates from the solids. Lower concentrations in geometric sequence were also tested. The bottles were incubated for 24 h at 20 °C using an overhead rotator (GFL 3040) at 1 rpm to keep the plastic particles in suspension. All incubations were conducted in darkness except for the light treatments of the dark versus light experiments, where continuous illumination using daylight fluorescent lamps was applied (600–700 lux, HT309). After incubation the leachates were filtrated by glass fiber filters (Whatman, GF/C) and geometric serial dilutions (×1 or undiluted, ×1/3, ×1/10, ×1/30, and ×1/100) were set up using artificial seawater.

Mature sea urchins (*P. lividus*) were collected by scuba divers in the outer part of the Ría de Vigo, Galicia, Spain, in the Northwest Iberian Peninsula. The sea urchin embryo test was performed according to the methods of Beiras et al. (2012), and the copepod test corresponded to the guidelines of the International Organisation for Standardisation (1999); both protocols were modified according to Beiras et al. (2018) to allow suspension of microplastics in the water column by means of 1-rpm rotatory shaking.

Fertilized sea urchin eggs were transferred into glass vials with airtight, Teflon-lined caps containing the experimental solutions, and the eggs were incubated at a density of 40 per mL, $20 \pm 1$ °C in the dark. Four replicates per treatment plus 8 artificial seawater controls were tested. After a 48 h incubation, the vials were fixed with a few drops of 40% formalin, and the length (maximum dimension) was recorded in 35 individuals per vial using a Leica DMI 4000B inverted microscope and Leica QWIN image analysis software Ver 3.

### Bioassays applying the method to commercial and environmental samples

Tier I experiments (leachate tests) were conducted as described in the *Bioassays to optimize leachate conditions* section. For Tier II trials (particulate tests) plastic powder sieved by 20 μm was added to a 250-mL glass bottle filled with artificial seawater at a final concentration of 10 mg $L^{-1}$. Three μL $L^{-1}$ of Tween 20, a value below the no-observed-effect concentration ($= 7$ μL $L^{-1}$; Beiras et al. 2018), were added to improve the dispersion. After shaking, geometric serial dilutions were prepared utilizing artificial seawater to obtain final plastic loads of 10, 3, 1, 0.3, and 0.1 mg $L^{-1}$.

Sea urchin embryos were gathered as discussed in the *Bioassays to optimize leachate conditions* section. For copepod tests, from 48 to 72 h before the start of the experiments *A. clausi*

 

mature adults from a laboratory stock maintained by ECIMAT at the University of Vigo were fed with cell suspension of *Rhodomonas lens* in an isothermal room at 20 °C with bubbling, filtered air. Using a 40-μm metallic mesh, ≤ 24-h-old nauplii were separated from the adults and delivered one by one, employing a glass pipette under binocular stereoscope in glass vials with airtight, Teflon-lined caps filled with the experimental solutions. A total of 10 individuals per vial were used. Copepod survival was recorded by checking mobility under a binocular stereoscope after a 48-h incubation in an overhead rotator (1 rpm) at 20 ± 1 °C during a 18:6-h light:dark photoperiod. In the case of sea urchins, larval growth was recorded as detailed in the *Bioassays to optimize leachate conditions* section but during the 48-h incubations vials were held in an overhead rotator at 1 rpm.

### Statistical analyses

Statistical analyses were conducted using IBM SPSS statistics software, Ver 24. Data departing significantly from normal distribution according to the Shapiro–Wilk test had been previously corrected using an angular (arcsine of the square root) transformation. The Dunnett's test was used to identify treatments significantly different from control (T Dunnet test when the variances were homogeneous and T3 Dunnett's test when the variances were heterogeneous).

Effect concentrations reducing larval growth (for *P. lividus*) or survival (for *A. clausi*) by 10% (EC10) and 50% (EC50), and their 95% confidence intervals (CIs), were calculated by fitting a probit dose–response model to the data. For these calculations, dilutions (proportion of leachate in artificial seawater) were used in the leachate test, and particle concentrations (mg $L^{-1}$) were employed in the particulate phase test. In the leachate test, toxic units were calculated as toxic unit = 1/EC50.

## RESULTS AND DISCUSSION

### Microplastic characterization

Table 1 shows the basic characteristics of the microplastics tested and the particle size distribution of the < 20-μm fraction used for the particulate phase test.

### Bioassays to optimize leachate conditions

***Liquid to solid ratio.*** The sea urchin test was used to compare the toxicity of leachates obtained using different liquid to solid ratios from 10 to 1 (100 g $L^{-1}$) down to 100 000 to 1 (0.01 g $L^{-1}$) in ×10 geometric sequence. For this experiment the most toxic material according to preliminary trials (PVC inflatable toy, BGUI) was used. As shown in Figure 1, the PVC resin used as reference material showed no toxicity (toxic unit < 1). With BGUI, larval growth inhibition at the 2 higher liquid to solid ratios of 0.01 and 0.1 g $L^{-1}$ did not exceed 50% even in the undiluted leachate; thus toxic units were also not calculable. These liquid to solid ratios were thought to be too high for routine testing. In contrast, 1, 10, and 100 g $L^{-1}$ did produce dose–response data suitable for toxic unit calculation: 1.46 (95% CI 1.35–1.57), 4.95 (95% CI 4.54–5.37), and 33.54 (95% CI 24.16–52.58) toxic units, respectively. As expected, leachates obtained at lower liquid to solid ratios were more toxic to the larvae. However, the increase in toxicity was not linear with the growth in particle concentration. On average, 10-fold increases in the loads of particles used to make up the leachate produced only a 4-fold escalation in toxic units. This suggests that at least some of the chemicals responsible for the toxicity are migrating to the liquid phase at concentrations reaching their limit of solubility, and higher particle loads fail to achieve a proportional growth in toxicity. This is consistent with the low solubility of most PVC plasticizers such as ortho-phthalates. In fact, diluting the leachates obtained at high particle loads reduced the toxicity more than making up the leachates employing the corresponding lower amount of particles. Thus the undiluted leachate made up with 1 g $L^{-1}$ of microplastics was more toxic than the 1/10 dilution of the 10 g $L^{-1}$ leachate, and this was more toxic than the 1/100 dilution of the 100 g $L^{-1}$ leachate (see Figure 1, hatched bars). The same pattern was found with copepod nauplii (Supplemental Data, Figure S1).

Higher concentrations of microplastics (i.e., lower liquid to solid ratios) have the advantage of boosting the sensitivity of the test, and the disadvantages of needing larger amounts of microplastics and further departing from environmentally relevant microplastic levels that were never reported to exceed 0.002 g $L^{-1}$ (Beiras 2018). Balancing sensitivity, feasibility, and environmental relevance, the intermediate liquid to solid ratio (100 to 1; i.e., 10 g $L^{-1}$) was selected for further testing.

***Effect of particle size.*** The sea urchin trial was used to measure the toxicities of leachates found utilizing different size ranges of particles obtained from the same material: a plastic fishing net. For particles larger than 1 mm, there was a trend

**TABLE 1:** Source and characteristics of plastic materials tested[a]

| ID | Material | Source | Polymer | Mean | Median | 10th percentile | 90th percentile |
|---|---|---|---|---|---|---|---|
| 635G | LDPE resin | Micro Powders | PE | 6.1 | 5.6 | 3.6 | 11.4 |
| PERE | PE resin | Rotogal | PE | | | Not used in Tier II | |
| PVCR | PVC resin | CETEC | PVC | 7.9 | 9.0 | 2.7 | 20.6 |
| BGUI | Inflatable toy | Local market | PVC | 7.9 | 7.9 | 2.5 | 26.0 |
| FISN | Fishing net | Seafloor | PE | 7.6 | 9.0 | 2.7 | 20.3 |
| FISC | Fishing cage | Seafloor | PE | | | Not used in Tier II | |
| PACK | Packaging sheet | Seafloor | PE | | | Not used in Tier II | |

[a]When the materials were used for the Tier II particulate phase test, particle size distribution of the < 20-μm fraction is shown.
LDPE = low-density polyethylene; PE = polyethylene; PVC = polyvinyl chlorine.

 

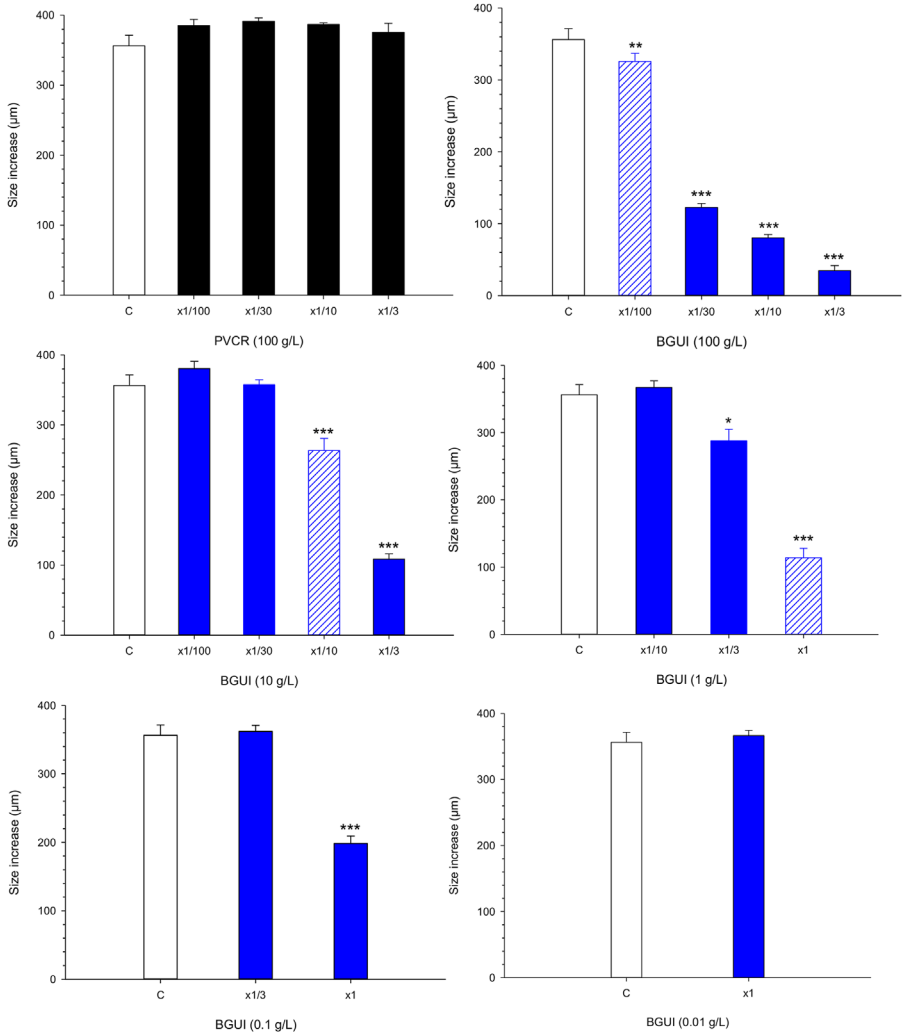


**FIGURE 1:** *Paracentrotus lividus* larval size increase in serial dilutions (×1/100, ×1/30, ×1/10, etc.) of leachates from polyvinyl chlorine resin (PVCR; reference material) and BGUI (PVC inflatable toy). The amount of plastic used per liter of artificial seawater to make up the leachate is indicated in each graph as g $L^{-1}$. Bars represent mean ± standard deviation ($n = 4$). Hatched bars denote equivalent treatments in terms of final liquid to solid ratio after dilution is taken into account. Asterisks refer to significant differences from control (C). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

toward an increase in the toxicity of the leachate as the particle size decreased (Figure 2). The > 1000 µm, 1000 to 500 µm, 500 to 250 µm, and < 250 µm fractions were 1.29 (95% CI 1.08–1.52), 0.87 (95% CI 0.62–1.08), 1.60 (95% CI 1.33–1.88), and 3.65 (95% CI 2.92–4.48) toxic units, respectively. Hence the largest toxicity was found with the smallest size fraction of < 250 µm, and this fraction was selected as the standard protocol. In terms of copepod survival, Lee et al. (2013) also detected higher chronic toxicity with the smallest polystyrene beads tested (0.05 µm), whereas reproduction was inhibited to a larger extent by the biggest particles tested (6 µm).

***Effect of light conditions.*** The sea urchin experiment was employed to analyze the toxicities of leachates obtained from BGUI and plastic fishing nets and tested under both continuous light and in darkness using virgin PVC and polyethylene resins as controls. Polymer resins in darkness displayed no toxicity (< 1 toxic unit), whereas leachates from plastic objects showed 10.6 (BGUI) and 1.6 (fishing net) toxic units (Table 2). Continuous exposure to light did not affect the toxicity of plastic products. In contrast, light did affect toxicity of the leachates from both virgin resins, causing an increase of up to 4.41 and 3.56 toxic units for polyethylene and PVC resins, respectively. No effect of light on the toxicity of commercial and environmental plastics was expected because they had been exposed to light during commercialization and use. The growth in toxicity of the polymer resins when exposed to light was more surprising, and the mechanisms underlying this finding deserve further research. For consistency with previously standardized methods (US Environmental Protection Agency 1992; Beiras et al. 2012), dark conditions were selected as the standard protocol.

## Bioassays applying the method to commercial and environmental samples

***Testing commercial plastic materials.*** The copepod test was used for application of the 2-tier approach to the assessment of the toxicity of a commercial plastic object, a soft PVC toy

 

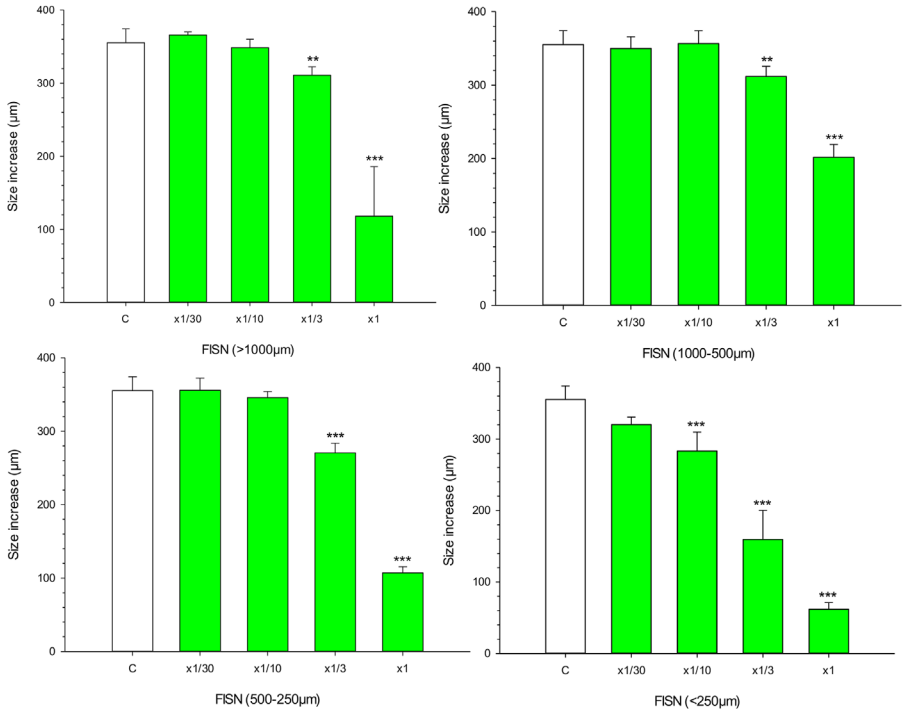


**FIGURE 2:** *Paracentrotus lividus* growth in larval size in serial dilutions of leachates from a plastic object collected from the seafloor (fishing net; FISN) using different particle size fractions. Refer to Figure 1 legend for explanation of further details.

(BGUI), using the virgin PVC resin as reference material. In the Tier I leachate test (Figure 3A), no toxicity of the PVC resin was detected even for the undiluted leachate. This supports the lack of relevant risk posed by both chemicals used as PVC polymerization aids and monomers or oxidation products potentially leaching from the polymeric matrix. In contrast, the PVC product (BGUI) induced remarkable larval mortality even after 10× dilution, resulting in a value of 8.0 toxic units for the leachate. This favors the hypothesis that chemical additives used after polymerization and during the compounding of the commercial object are responsible for the toxicity shown. Bejgarn et al. (2015) found that softer plastics tended to be more often toxic than rigid, and indicated that, in the case of PVC soft products, they contained large proportions of plasticizers. Lithner et al. (2009) reported that plasticized PVC and polyurethane were the only plastic types among 15 tested that displayed toxicity in the *Daphnia magna* immobilization test. Metals such as Zn and Pb are used in PVC as heat stabilizers, and Lithner et al. (2012) measured Zn concentrations in the leachates of PVC gloves high enough to explain at least in part their toxicity to *D. magna*. Finally, Hamlin et al. (2015) observed that 2 similar products (food-grade polyethylene) differed in the toxicity of their leachates to fish, and this was related to the differential leaching of nonylphenol into the seawater.

In the Tier II particle trial (Figure 3B), PVC resin particles $< 20\,\mu m$ were innocuous to the copepod larvae up to $10\,mg\,L^{-1}$, the maximum concentration tested. In contrast, the BGUI particles were again remarkably toxic, with $EC10 = 0.32\,mg\,L^{-1}$ and $EC50 = 4.32\,mg\,L^{-1}$.

**TABLE 2:** Toxicity of leachates from different plastic materials assessed using the *Paracentrotus lividus* embryo test[a]

| | | Toxic units (95% CI) | |
|---|---|---|---|
| ID | Material | Dark | Light |
| PERE | PE resin | < 1 | 4.41 (3.64–5.32) |
| PVC resin | PVC resin | < 1 | 3.56 (2.80–4.45) |
| BGUI | PVC toy | 10.56 (8.21–14.14) | 9.85 (7.26–14.17) |
| FISN | PE fishing net | 1.63 (0.24–3.40) | 1.63 (1.33–1.94) |

[a]Leaching and exposures were conducted either in darkness or under continuous light.
CI = confidence interval; PERE = polyethylene resin; PE = polyethylene; PVC = polyvinyl chlorine.

***Testing environmental sample.*** The sea urchin experiment was used to assess the toxicity of 3 polyethylene plastic objects recovered from the seafloor and thus exposed to environmental weathering. With that aim, leachates were obtained as described in the *Bioassays to optimize leachate conditions* section using a 100 to 1 liquid to solid ratio ($10\,g\,L^{-1}$). The results of these tests are shown in Figure 4. Polyethylene resin was used as reference material. Only one of the materials tested displayed toxic unit values for the leachate significantly higher than 1 (Supplemental Data, Table S1), the fishing net showed 2.82 (95% CI 1.03–4.78) toxic units. Consequently, only this material was used in the Tier II particle test. No toxicity was found in the Tier II particle test at any of the microplastic loads tested (Figure 5). This points again to the leaching of chemical additives, and not a mechanical effect on the larvae, as being responsible for the toxicity observed.

Lower toxicity of environmentally weathered compared with new polyethylene microplastics was also reported by Nobre et al. (2015). These findings support the hypothesis that chemical

 

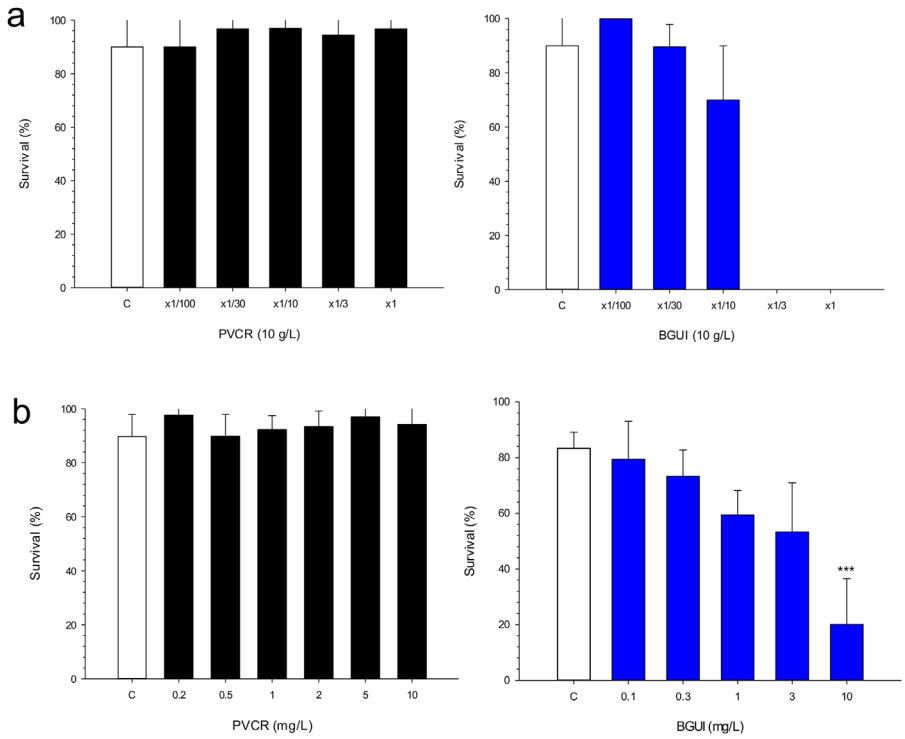


**FIGURE 3:** Survival of *Acartia clausi* larvae exposed to polyvinyl chlorine resin (PVCR) and PVC inflatable toy (BGUI). (**a**) Tier I leachate test. (**b**) Tier II particulate phase test. More information can be found in Figure 1 legend.

additives lost during the weathering process are at least partially responsible for the toxicity shown in new commercial plastics. In contrast, Gandara E Silva et al. (2016), by using polypropylene rather than polyethylene new microplastics, found greater toxicity in environmental pellets compared with virgin polypropylene pellets, and attributed that toxicity to water column chemicals adsorbed to the microplastic in the environment and leached during the test.

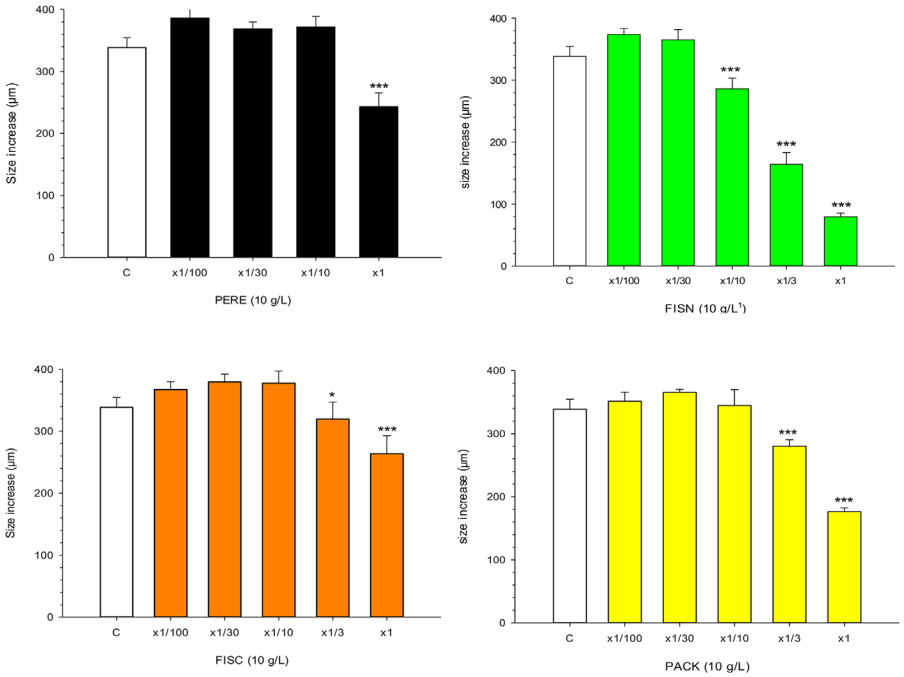


**FIGURE 4:** *Paracentrotus lividus* larval size increase using the Tier I leachate test for polyethylene resin (PERE; reference material) and for fishing net (FISN), fishing cage (FISC), and packaging sheet (PACK; plastic objects collected from the seafloor). Additional data are available in legend for Figure 1.

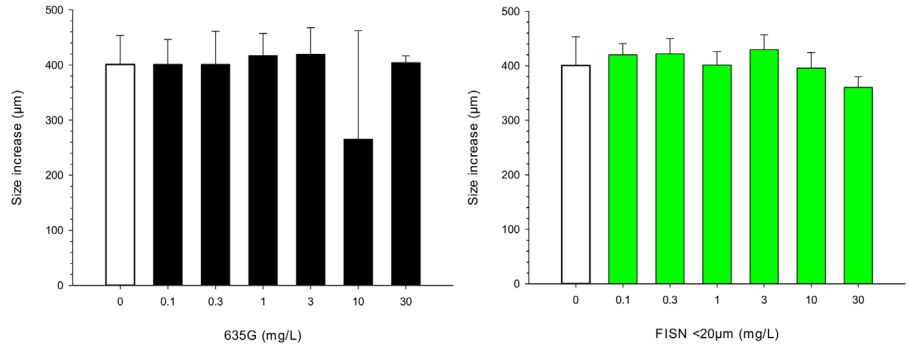


**FIGURE 5:** *Paracentrotus lividus* larval size increase using the Tier II particulate phase test for LDPE resin (635G, reference material) and fishing net (FISN; plastic object). No treatment was significantly different from control in any of the tests.

## *Derivation of a standard protocol*

We propose a 2-tier standard approach to test the toxicity of microplastics using the common marine models *P. lividus* and *A. clausi* larvae. This method consists first of a Tier I testing of the toxicity of leachates produced at a 100 to 1 liquid to solid ratio (i.e., 10 g $L^{-1}$). The Tier I output is a quantitative parameter, the toxic unit, suitable to identify mobilization of toxic organic and inorganic chemicals from the polymeric matrix, and to rank plastic materials according to their potential toxicity. After toxic effects are identified using an arbitrary threshold of toxic unit $> 1$, Tier II testing of the toxicity of the ingestible fraction of the microplastics ($< 20\,\mu m$) is conducted. Tier II outputs the EC10 and EC50 values; these are essential parameters in the toxicity assessment step of environmental risk assessment studies.

Thus this procedure combines the sensitivity of the leachate test, which uses very high plastic loads (10 g $L^{-1}$) particularly useful for hazard identification studies, with the environmental relevance of the particulate phase test, which uses lower particle loads (up to 10 mg $L^{-1}$) closer to those found in the aquatic environments, and takes into account potential additional mechanisms of toxicity derived from the ingestion of the microplastics by the organisms.

Acute toxicity of plastic leachates has already been proposed as a useful method to differentiate between toxic and nontoxic products (Lithner et al. 2009). Nevertheless, in contrast with previous approaches (Lithner et al. 2009, 2012; Bejgarn et al. 2015; Nobre et al. 2015; Gandara E Silva et al. 2016; Li et al. 2016), the procedure described in the present study includes a particle test conducted under more environmentally relevant conditions that facilitate continuous contact in the water column between microplastics and organisms throughout the exposure period. Thus an additional avenue of exposure not considered in earlier processes—ingestion of microplastic particles—is included in this evaluation. Utilizing this protocol permits more realistic environmental risk assessment studies in aquatic ecosystems.

In short, this 2-tier standard method can be used for characterization of plastic materials in terms of both a priori hazard identification and a posteriori assessment of the environmental risk caused by microplastic pollution in aquatic ecosystems. However, we must bear in mind that present techniques are intended to assess effects on the model species, representative of zooplankton organisms. Other life forms and feeding strategies may become affected through different mechanisms beyond the scope of the present study.

# CONCLUSIONS

Toxicity of microplastics is mainly caused by the leaching of chemical additives. This is supported by certain results. First, dilution of the leachates with clean seawater reduces the toxicity more than expected from the corresponding increase in the liquid to solid ratio. Second, leachates from smaller size ranges are more toxic than leachates from larger size ranges of the same material at the same liquid to solid ratio. The higher surface to volume ratios of smaller particles are expected to facilitate leaching of chemicals from the polymeric matrix not covalently bonded. Third, environmental plastics exposed to weathering tend to be less toxic than new commercial products.

The 2-tier method combines the sensitivity of the leachate test with the environmental relevance of the particulate phase test, and is useful in discriminating between toxic and nontoxic plastic materials.

*Supplemental Data*—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4326.

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*Data Accessibility*—Data, associated metadata, and calculation tools are accessible from the corresponding author (rbeiras@uvigo.es).

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