Aqueous Eluates of Foamed Plastic Consumer Products may Induce High Toxicity to Aquatic Biota

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Aqueous Eluates of Foamed Plastic Consumer Products may Induce High Toxicity to Aquatic Biota

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Abstract

Plastic pollution is a global challenge. Despite plastics being complex chemical mixtures, hazard research has focused on particulate forms and the risks of plastics additives, especially for environmental organisms, remain poorly understood. This is a significant knowledge gap considering ubiquitous organismal exposure to plastics and the associated 16 000+ additives. The aim of this study was to provide ecotoxicological characterization of aqueous eluates of foamed plastic consumer products and propose a test battery for toxicity screening. For that, hazard of eluates of six randomly selected foamed plastic products was evaluated using aquatic decomposers, autotrophs and heterotrophs (Vibrio fischeri, Raphidocelis subcapitata, Lemna minor, Thamnocephalus platyurus, Heterocypris incongruens, Daphnia magna). Alarmingly, all plastic eluates affected the organisms, though toxicity varied among materials and species. Results showed that short-term contact may underestimate plastic eluate toxicity. To increase environmental relevance of hazard assessment of foamed plastic eluates, harmonizing leachate preparation, using natural water and avoiding (excessive) filtration of eluates should be considered. OECD/ISO assays with R. subcapitata, H. incongruens and D. magna (96 h) can be recommended as a minimal sensitive battery for effective screening of plastic eluate toxicity.

Keywords: additives; leachate; polyethylene; polyurethane; ethylene vinyl acetate; *Raphidocelis subcapitata; Heterocypris incongruens; Daphnia magna*

1. Introduction

Plastic pollution has become a major environmental problem. To date, particulate plastics (often referred to as micro- or nanoplastics) has been the central research topic of plastic pollution and only recently concerns over plastic additives have also been expressed [1,2]. Plastic is a chemically diverse material, a blend of polymer(s) and additives, which total number mounts up to 16 000 [3]. Additives have the potential to migrate from the plastic matrix [4] and play a significant role in toxicity of plastics [5–7]. Not only additives but also the breakdown products such as nanoparticles and oligomers may also play a role in toxicity of a plastic material [8]. However, compared to microplastics, the risks related to leaching of additives from plastic materials and their consecutive uptake via water by biota are poorly studied [9–11] primarily addressing potential hazard to human health [12]. Significant knowledge gaps and challenges complicate environmental hazard assessment of plastic materials [13] as most of the available ecotoxicological data on plastic additives regard pure chemicals. At the same time, consumer products contain multitude of undisclosed additives [14]. Even if the information on additives was available, knowledge on chemical composition of plastics alone would not be sufficient for predicting the environmental hazard of plastic goods [15] due to limited knowledge on their mixture effects and bioavailability in the leachates [5,9,16]. Biological effects of plastics additives depend on their ability to migrate from plastic, persist in the environment and interact with other compounds and contaminants. Therefore, currently only experimental toxicity data can be relevant basis for environmental hazard prediction of plastics. Foamed plastics have wide applicability from (food) packaging to construction but at the end of life are mostly

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landfilled. Foamed plastics are lightweight, easily transported in the environment, and have been shown to constitute about 12% of beached plastic litter [17].

The aim of the current study was to i) evaluate the potential hazard of randomly selected foamed plastic consumer goods to aquatic biota and ii) propose a sensitive battery of aquatic bioassays for ecotoxicity screening of eluates of foamed plastic. In the study, no specific plastic additive (group) was targeted as the eluates typically contain a mixture of chemicals with different toxic potential.

2. Materials and Methods

2.1. Foamed Plastic Consumer Goods

In the current study, aquatic toxicity of six foamed plastic consumer products (**Table 1**) was studied. Five randomly selected consumer products were purchased from local retailers and one material originated from laboratory consumable packaging. We focused on homogenous foams (**Figure S1**) in order to minimize potential additional mixture effects due to e.g. adhesives and paints on the surface of product. All the tested items are commonly used, not recyclable and therefore end up in general mixed waste. Brand names are undisclosed, instead we provide general description of the product application. Among the tested materials, there were both 'open cells' and 'closed cell' polymeric foams (**Table 1**)

Table 1. Foamed plastic consumer products.

	Consumer product	Type of foam	Flexibility	Polymer[1]
KP-1	kneeling pad	closed cell	rigid	PE
KP-2	kneeling pad	closed cell	rigid	PE
KP-3	kneeling pad	closed cell	slightly flexible	PE
PACK	packaging foam	open cell	flexible	mixture
SPON	dish sponge	open cell	flexible	PUR
ISOL	isolation foam	closed cell	flexible	mixture (incl PUR, EVA) *

[1] Data of the product description (for SPON) or ATR-FTIR-analysis (**Figure S2**); [2]PE - polyethylene. *In ATR-FTIR, polyurethane (PUR) and ethylene vinyl acetate (EVA) spectra quality indexes were both 37%

2.2. Preparation of Eluates

Before the leaching procedure, foams were thoroughly rinsed with distilled water, dried at room temperature and cut into ~0.5 x 0.5 cm pieces. Leaching was performed according European standard EN 14735:2005 [18] except the recommended solid-to-liquid ratio (S/L) 1:10 was not applicable because of the very high volume/weight ratio (**Figure S3**) of the foams. Three eluates (solutions recovered from leaching procedures) of S/L 1:100, 1:1000 and 1:10000 were prepared just before conducting the toxicity testing. The eluates were prepared in the same test media (leachant) that was used for the toxicity assays. Upon 24 h shaking at 22 C°, the eluates were passed 0.4 mm filter before toxicity evaluation. In all the eluates, pH was within the valid range (6.6 - 8.5) for toxicity exposure. The conductivity of the eluates was marginally higher (less than 10%) than that of the test media used as the leachant.

2.3. Ecotoxicity Evaluation

Eight different exposure settings (**Table 2**) using 6 aquatic species from different food-web level: marine bacteria *Vibrio fischeri*, duckweed *Lemna minor*, microalgae *Raphidocelis subcapitata*, freshwater microcrustaceans pelagic *Daphnia magna* (water flea) and *Thamnocephalus platyurus* (fairy shrimp) and benthic *Heterocypris incongruens* were chosen for this study. All the tests were performed two-three times in several (n >2) technical parallels.

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Table 2. Test organisms and exposure settings of the toxicity assays.

Test species	Test conditions			Toxicity endpoint	Standard
	Duration	°C	Illumination		
Bacteria *Vibrio fischeri*	30 min	20 °C	continuous	bioluminescence inhibition	ISO 21338
Microalgae *Raphidocelis subcapitata*[1]	72 h	25 °C	continuous	growth inhibition	OECD 201
Duckweed *Lemna minor*	7 days	25±1 °C	continuous	growth inhibition	OECD 221
Crustaceans *Thamnocephalus platyurus*	24 h	25±1 °C	in dark	mortality	ISO 14380
Crustaceans *Heterocypris incongruens*	6 days	25±1 °C	in dark	mortality, growth inhibition	ISO 14371
Crustaceans *Daphnia magna*	48 h	21±1 °C	in dark	immobilization	OECD 202
Daphnia magna	96 h	21±1 °C	16h/8h light/dark	immobilization	OECD 202*
Daphnia magna	21 days	21±1 °C	16h/8h light/dark	mortality, reproduction	OECD 211

[1]previously Pseudokirchneriella subcapitata or Selenastrum capricornutum; *modified format.

In the 30-min bioluminescence inhibition assay with bacteria *V. fischeri*, the bacteria were suspended and the samples were tested in 2% NaCl solution. Reconstituted *V. fischeri* reagent (Aboatox, Turku, Finland) was used and bacterial bioluminescence measured by automated tube-luminometer 1251 (ThermoLabsystems, Finland), operated by Multiuse software (BioOrbit, Finland) according to modified Flash-assay protocol [19]. Inhibition of bacterial bioluminescence was calculated as a percentage of the unaffected control (2% NaCl). The 72 h algae *P. subcapitata* growth inhibition test was performed according to OECD 201 [20]. Algae were grown in the OECD medium and their biomass was determined using chlorophyll fluorescence. Growth inhibition was calculated as the ratio of the sample's biomass to the biomass of the negative control. The 7-day growth inhibition assay with duckweed *L. minor* was performed in artificial freshwater by OECD 221 [21]. Dry biomass of the plants was measured at the end of exposure. Inhibition of the growth rate was calculated as follows:

$$INH\% = \frac{Mc - Mt}{Mc} \times 100 \quad (1)$$

where:
- INH%: inhibition (%) of specific growth rate
- M_c: mean value in the control group
- M_t: mean value in the treatment group

For microcrustacean (*T. platyurus, H. incongruens, D. magna)* assays, dormant eggs (MicroBioTests Inc., Belgium) were used to hatch the test organisms. Two types of artificial freshwater (moderately hard synthetic freshwater [22] for *H. incongruens* and *T. platyurus*; ISO medium [23] for *D. magna)* and natural freshwater were used as the media for leaching and toxicity exposures. Natural water (**Table S1**) was collected from Lake Ülemiste (used for Tallinn water supply), passed 0.45 µm cellulose nitrate filter (Sartorius) and stored at 4 C° in the dark. In *T. platyurus* assay [24], organisms were exposed as <24 h larvae and mortality was assessed upon 24 h exposure.

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In *H. incongruens* assay [25], organisms were exposed as <4 h larvae and growth inhibition (relative to control) and mortality were assessed upon 6-day exposure. Acute 48 h *D. magna* assay was performed according OECD 202 but in addition, prolonged 96 h exposure was applied. In the 96 h format, after 48 h exposure, organisms were fed once with *P. subcapitata* at 0.1 mg C/*Daphnia*/day. The long-term impact of eluates on *D. magna* survival and reproduction was evaluated in 21-day exposure [26], conducted in natural freshwater.

2.4. Data Analysis

All the toxicity (EC_{50}) values (confidence interval CI 95%) were calculated from dose-response curves generated with the log-normal distribution model of the REGTOX software EV7.1.2 for Microsoft Excel™ [27]. To determine statistically significant differences ($p < 0.05$) between the EC_{50} values, one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test was performed in Microsoft Excel v2010.

3. Results and Discussion

Potential hazard of eluates of foamed plastic goods was evaluated using organisms from different trophic levels: producers/autotrophs (duckweed *L. minor* and microalgae *R. subcapitata*), heterotrophs (freshwater microcrustaceans *T. platyurus, D. magna* and *H. incongruens*) and decomposers bacteria *V. fischeri*. In real-life conditions, differently sized plastic particles enter aquatic ecosystems along with additives, leached from plastic goods [28], and their mixture effects pose a potential hazard to the biota [29]. To enhance environmental relevance of hazard evaluation but account for the small size of particle-ingesting test organisms, only larger (> 0.4 mm) fractions were removed from the eluates, i.e. mixture effects of the leached plastic additives and particles (**Figure S4**) were evaluated in the study. From the filtrate of KP-3 eluate (**Figure S4**) it was evident that heterogenous particulate matter (potentially originating also from fillers) was present in the eluate. Both artificial and natural freshwater may be used in aquatic crustacean assays [23,25,26]. Here, the same medium was used both for eluate preparation as well as for toxicity exposures. It is a common knowledge that chemical composition of the leachant may affect not only the leaching process but also bioavailability of the compounds. In our previous studies, modulating effects of natural freshwater, compared to AFW on the toxicity of both inorganic [30,31] and organic [32] compounds were demonstrated by the same acute bioassays as in the current study. Though here, for the eluates (S/L 1:100, 1:1000, 1:10000), no statistical difference between acute toxicity results from artificial vs natural freshwater exposures was detected neither for *D. magna* nor *H. incongruens*. Luo et al. [33] has demonstrated similar plastics leachate profiles in lake water and in tap water. Thus, for higher environmental relevance, the 21-day bioassays with *D. magna* (**Table 2**) were performed only in natural freshwater.

3.1. Toxicity of Eluates to Aquatic Biota

Results of the toxicity evaluation showed that eluates of all the foamed plastic products affected aquatic species but their toxicity potential differed (**Table 3**). The most concentrated eluates (S/L 1:100) of the two kneeling pads (KP-1 and KP-2) and dish sponge (SPON) showed slight toxicity to some test species. Eluates of kneeling pad KP-3, packaging foam (PACK) and isolation foam (ISOL) showed high hazard to the aquatic ecosystem. The most toxic eluate was of packaging foam (PACK) which showed (very) high toxicity even at S/L 1:10000. The kneeling pad (KP-3) and isolation foam (ISOL) were also inducing high hazard to some test species (microalga *P. subcapitata*, microcrustaceans *D. magna* and *H. incongruens*) in S/L 1:1000 eluates.

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Table 3. Toxicity of the eluates of foamed plastic consumer products to test organisms.

Sample	S/L	*Raphidocelis subcapitata* 72 h	*Lemna minor* 7 days	*Vibrio fischeri* 30 min	*Thamnocephalus platyurus* 24 h	*Daphnia magna* 48 h	*Daphnia magna* 96 h	*Daphnia magna* 21 days	*Heterocypris incongruens* 6 days
		growth inh. [%]		bioluminescence inh. [%]	mortality [%]				
KP-1	1:100								
KP-2	1:100	27 ± 18							25 ± 7
KP-3	1:100	100	73 ± 12	36 ± 2	40 ± 30	52 ± 3	100	n.a.	100
	1:1000	78 ± 5	44 ± 19				42 ± 29	40	54 ± 38
	1:10000								27 ± 6
PACK	1:100	99	41 ± 22				100	n.a.	100
	1:1000	99	37 ± 3			36 ± 17	90 ± 20	n.a.	83 ± 25
	1:10000	50 ± 16					88 ± 12	100	n.a.
ISOL	1:100	100	76 ± 6	43 ± 4	97 ± 4	80 ± 18	89 ± 23	100	100
	1:1000	79 ± 22	43 ± 16				73 ± 27	100	82 ± 31
	1:10000							40	n.a.
SPON	1:100	44 ± 23						n.a.	35 ± 5
	1:1000							n.a.	n.a.
	1:10000		n.a.					n.a.	n.a.
		< 20% - no hazard		< 50% - slight hazard		< 75% - moderate hazard		≥ 75% - hazard	

inh. – inhibition; n.a. – not available; KP-1, KP-2, KP-3: kneeling pads 1, 2, 3; PACK – packaging foam; ISOL – isolation foam; SPON – dish sponge. Data are presented as AVG±SD (n=2-3).

Sensitivity to eluate toxicity depended on test species as well as exposure durations. Acute exposures with marine bacteria *V. fischeri* (30 min), pelagic microcrustaceans *T. platyurus* (24 h) and *D. magna* (48 h) showed the lowest sensitivity (**Table 3**). Varying acute exposure durations in *D. magna* immobilization assay influenced the results significantly: upon standard 48 h [23] many non-hazardous eluates induced high hazard upon 96 h exposure (**Table 3**). Our results agreed with literature where toxicity of plastic eluates [34] and nanomaterials [35] for *D. magna* was recorded only upon extended exposure (from 48 h to 96 h). It is important to note that in the current study, the exposed daphnids were fed after 48 h to minimise likelihood of increased sensitivity due to starvation. Our results showed that standard 48 h exposure may lead to underestimation of the risks associated with plastics in aquatic ecosystems.

The 6-day bioassay with ostracod *H. incongruens was more sensitive than the acute tests. Since H. incongruens is a benthic microcrustacean, comparable sensitivity to* 96-h *D. magna assay* may be explained by enhanced contact with settled fractions. However, accumulation of (micro)particles (**Figure S4**) in the gut was visible in all the crustacean assays (**Figure S5**). Across the assays, the 6-day H. incongruens and the 96-h D. magna assays were the most sensitive ones along with the 72 h microalga R. subcapitata assay. The microalgal growth inhibition was in very good correlation (R^2 = 0.76) with 7-day L. minor growth inhibition but was more sensitive (**Table 3**) and resource-effective (**Table 2**). Importantly, the three most sensitive assays yielded comparable results to those of the 21-day chronic D. magna assay [26] that gives the most relevant data for environmental safety evaluation of plastics but is too resource-consuming to be used in the screening phase.

3.2. Uncertainties in the Environmental Hazard Assessment of Plastics Eluates

Currently, a variety of leaching procedures are used to prepare plastics eluates making it difficult to compare toxicity data across publications. The most crucial parameter is solid-to-liquid ratio (S/L) used in the leaching process. Generally, the toxicity of eluates is assessed by exposing the test organism to a dilution series of the initial eluate prepared at a single S/L ratio and toxicity (e.g., EC_{50}) values are expressed as a percentage of the undiluted initial eluate. At the same time, different S/L ratios 1:2 – 1:100 [9,36–39] are used for preparation of the initial eluate. Although, higher S/L ratio

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has been proposed a reliable proxy for eluate aqueous toxicity [40] however higher S/L ratio eluates may induce lower toxicity than lower ratio eluates due to saturation of leached compounds [32,41]. This was also shown in the current study for the most toxic samples (e.g. PACK) in case of which 10-fold dilutions of the eluate induced comparable toxicity (**Table 3**). Leaching duration [42], the leachant as well as the plastic particle size all affect potential toxicity of plastic eluates [40]. In some cases, leachant is other than the medium used in toxicity exposures [43].

From our perspective, applying S/L ratio of ≤1:100 and use of leachants other than the exposure medium for eluate preparation is not in line with the aims of relevant environmental risk assessment. Filtration (e.g. 0.45 μm) of the eluate prior to toxicity evaluation significantly increases both the duration and cost of the evaluation, especially for e.g. long-term toxicity tests that require large volumes of eluate.

4. Conclusions

This toxicity screening study raised concerns about environmental safety of commercially available plastic products intended for widespread consumer use. If frequently used products such as kneeling pads pose high hazard to aquatic biota, risks to human health cannot be excluded. By using aqueous eluates of foamed plastics, it was demonstrated that acute toxicity assays—such as the 30 min assay with *Vibrio fischeri* [19], the 24 h assay with *Thamnocephalus platyurus* [24], and the 48 h assay with *Daphnia magna* [23] may underestimate environmental hazards induced by these materials. However, extending the duration of *D. magna* assay from 48 h to 96 h increased its sensitivity significantly. Across the ecotoxicity evaluation panel of six aquatic species and eight exposure settings, we recommend the 72 h *Raphidocelis subcapitata* assay, the 96 h *D. magna* assay and the 6-day *Heterocypris incongruens* assay for screening the environmental safety of foamed plastics eluates. The study highlights the need for stronger regulation of additives in plastics and environmental hazard assessment of this complex material by harmonizing/standardizing methods for leachate preparation and ecotoxicity testing.

Supplementary Materials: Figure S1: Surface of the analysed foamed plastic materials., Figure S2: ATR-FTIR (PerkinElmer Spectrum Spotlight 400) spectra of the foamed plastics, analysed in SpectrumIMAGE software., Figure S3: Volume of the foamed plastic materials (kneeling pads) (green is KP-1 and black is KP-2) used for preparing eluate of SLR 1:100 (10 g per 1 L)., Figure S4: Filtrate of the eluate of the blue kneeling pad (KP-3)., Figure S5: Accumulation of the plastic particles in *Daphnia magna* upon 21-day exposure to KP-3 (blue kneeling pad) eluate (S/L 1:1000)., Table S1: The main physico-chemical parameters of natural freshwater from Lake Ülemiste (Tallinn, Estonia) used in this study.

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