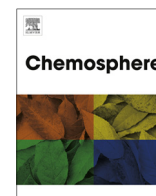


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## Toxicity of leachate from weathering plastics: An exploratory screening study with *Nitocra spinipes*

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## HIGHLIGHTS

- Plastics were repeatedly leached and irradiated with artificial sunlight.
- 8 of 21 plastics produced leachates causing acute toxicity to *Nitocra spinipes*.
- Both increases and decreases in toxicity were observed after irradiation.
- No consistent trend was seen among different materials.
- Chemical screening showed no evidence of PVC oligomers from the polymer backbone.

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## ABSTRACT

Between 60% and 80% of all marine litter is plastic. Leachate from plastics has previously been shown to cause acute toxicity in the freshwater species *Daphnia magna*. Here, we present an initial screening of the marine environmental hazard properties of leachates from weathering plastics to the marine harpacticoid copepod [Crustacea] *Nitocra spinipes*. Twenty-one plastic products made of different polymeric materials were leached and irradiated with artificial sunlight. Eight of the twenty-one plastics (38%) produced leachates that caused acute toxicity. Differences in toxicity were seen for different plastic products, and depending on the duration of irradiation. There was no consistent trend in how toxicity of leachate from plastics changed as a function of irradiation time. Leachate from four plastics became significantly more toxic after irradiation, two became significantly less toxic and two did not change significantly. Analysis of leachates from polyvinyl chloride (PVC) by liquid chromatography coupled to a full-scan high-resolution mass spectrometer showed that the leachates were a mixture of substances, but did not show evidence of degradation of the polymer backbone. This screening study demonstrates that leachates from different plastics differ in toxicity to *N. spinipes* and that the toxicity varies under simulated weathering.

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### 1. Introduction

Globally, approximately 288 million tons of plastics were produced in 2012 (PlasticsEurope, 2013), predominantly from petroleum and natural gas (Edshammar, 2002). Between 60% and 80% of all marine litter consist of plastics (Derraik, 2002), but the total amount of plastic litter in marine environments is uncertain and highly variable (Ryan et al., 2009; Lambert et al., 2014). The mass of plastic particles has been found to be approximately six times higher than of plankton in a study at the North Pacific gyre (Moore et al., 2001). Other estimations include 18000 pieces of

plastic litter floating on every square kilometer of ocean surface (UNEP, 2006). Recently, Cózar et al. (2014) confirmed the ubiquitous presence of plastic debris in the open ocean and identified a broad size distribution of floating plastic debris on a large scale. Although it is believed that only about 15% of marine debris is washed to shore, monitoring at Baltic Sea beaches found an average of 130 pieces of litter per 100 m of beachfront, whereof 56% was plastic (Marlin Baltic Marine Litter, 2014). Hundreds of animal species worldwide have been affected by entanglement or ingestion of plastics (Laist, 1997). Additionally, microplastics can transfer between trophic levels (Farrell and Nelson, 2013; Wright et al., 2013) and potentially modulate uptake of persistent organic pollutants (POPs) in marine food webs (Besseling et al., 2012).

The time required for plastics to degrade in the environment is estimated to be on the order of hundreds to thousands of years

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(Barnes et al., 2009). Photo-oxidation by ultraviolet (UV) light is the primary degradation pathway for plastics (Hammer et al., 2012). In marine environments, decreased sun exposure due to shading by water and fouling might increase the persistence of plastic (O'Brine and Thompson, 2010; Hammer et al., 2012). Furthermore, UV-stabilizers and other chemical additives are often added to plastics (Edshammar, 2002). Plastic additives have been shown to be released; for example, plasticizers can be found in leachate from landfills (Asakura et al., 2004).

The extent of leaching of additives from plastic and leachate composition is influenced by both the properties of the additive substance and the properties of the plastic polymer, such as the degree of amorphous or crystalline structure (Hansen et al., 2012). Also important is whether or not additives are chemically bound to the polymer (Bibi et al., 2012). Several studies on toxicity of individual plastic additives exist (Lambert et al., 2014), as well as studies concerning human health and migration into food or water from plastic materials (e.g. Wagner and Oehlmann, 2009; Cheng et al., 2010). Plastic leachate into freshwater has previously been found to cause acute toxicity to *Daphnia magna* (Lithner, 2011). However, we are not aware of any studies of the aquatic toxicity of leachates from plastic in marine environments during simulated weathering of plastics.

Here, we report an initial screening of the marine environmental hazard properties of leachates from weathering plastics. Plastics from commercially available products were repeatedly leached and weathered by artificial sunlight. Leachates were screened for acute toxicity with the micro-crustacean *Nitocra spinipes*. Our hypotheses were that leachates from common plastic materials would show different toxicity towards *N. spinipes*, and that the toxicity would change as the plastic material was weathered by artificial sunlight.

## 2. Material and methods

### 2.1. Materials

For this initial screening survey we selected plastics from products that are commercially available in department stores in Stockholm, Sweden, and that are likely to be common in Swedish households (Table 1). The range of selected plastics represent: (1) Examples of six common plastics that account for 80% of European plastic demand: Polyethylene (PE), Polypropylene (PP), Polyvinyl chloride (PVC), Polystyrene (PS), Polyethylene terephthalate (PET) and Polyurethane (PUR) (PlasticsEurope, 2012); (2) The largest application sector in Europe, i.e. packaging (PlasticsEurope, 2013); and (3) The common plastics found on the beaches of the Baltic Sea, i.e. packaging and short-term usage plastic (Marlin Baltic Marine Litter, 2014). Packaging with recycling labels was tested to ensure that different types of plastics were covered, however, some examples of unlabeled packaging and products were also selected for study.

In addition, three bioplastics were included. Bioplastics are biodegradable and/or made from renewable resources (European Bioplastics, 2014). Many of the selected plastics were thin films. Only one plastic, the toothbrush at 1.3 cm, was thicker than approximately 5 mm. For descriptions of each plastic material, see Supplementary Material (SM). Leachate from tires has been shown to be toxic in other studies (e.g. Gualtieri et al., 2005; Wik and Dave, 2006), hence pieces of a used (washed) car tire were included as a positive control.

### 2.2. Preparation of plastics for leaching and aging

All labels were removed from the plastic material and parts with glue residues were not used. Dust was wiped off, but the

plastic was only washed if necessary, e.g. for containers of soap and the used tire. These were washed with hand dishwashing liquid and rinsed in deionized water.

To facilitate leaching and increase the surface area available for exposure to artificial sunlight we used a variety of methods to grind the plastics into powders. Grinding techniques included mechanical cryogenic grinding, manual grinding in liquid nitrogen and grinding in a Blendtec HP3A blender. For the mechanical cryogenic grinding, a Retsch CryoMill with 50 mL cells and a 2 cm diameter steel ball were used. The cell was filled to  $\leq 1/3$  with plastic material that had been cut with clean stainless steel scissors or pliers. Each plastic was ground for approximately 2 min, with 4 min precooling. Several sets of grindings per sample were necessary to yield approximately 10 g of ground material. The cells were wiped clean with disposable cellulose tissues or washed between the different materials. Materials that resisted both types of cryogenic grinding were ground in the Blendtec blender, see SM. None of the grinding techniques were effective for PET, Bio-PET and the EN 13432 compliant biodegradable garbage bag, hence approximately 1 cm<sup>2</sup> pieces cut with scissors were used for leaching and weathering experiments. When necessary, the ground plastic materials were stored in sealed glass containers in a refrigerator at 4 °C.

The particle size of the ground plastics was assessed by sieving through two nets, with 1 and 0.3 mm mesh size, respectively. If sieving was not possible, the grain size was assessed using an optical microscope. For size distributions and pictures, see SM. One plastic product (PS-cups) was duplicated throughout the study.

### 2.3. Leaching

Leaching was performed according to the Swedish standard 12457:2003 "Characterization of waste – Leaching – Compliance test for leaching of granular waste materials and sludges – Part 2: One stage batch test at a liquid to solid ratio of 10 L kg<sup>-1</sup> for materials with particle size below 4 mm" (SIS, 2003), with some modifications. First, the leaching time was increased from 24 h to 72 h. Second, natural brackish water from the Baltic Sea was used. Third, no moisture content was determined since moisture content is generally very low in plastics (Bruder, 2011).

The leaching was accomplished by mixing approximately 10 g of each plastic with natural brackish water to obtain a liquid-to-solid-ratio (L/S) of 10. The salinity of the water was 7‰ (±0.2) and the pH 7.9 (±0.25). The water had previously been filtered (Munktell v5 filter) and heated to 80 °C, and been allowed to cool to room-temperature for at least 24 h. Samples 1–8 were leached in darkness in round bottom flasks on a rotary mixer (Labianco, model 256) at 6 rotations per minute (rpm). Due to a malfunction the first leaching (corresponding to 0 h of artificial sunlight) of samples 1–8 were not rotated during approximately 24 h, and were therefore left rotating an additional 24 h, making the total water exposure time 92 h. The mixer was replaced for samples 9–22 with a Heidolph elektro KG Kelheim type RZR E60 with a speed of 21 rpm.

The liquid and solids were separated by suction filtration, using a PALL 45 µm membrane filter (Supor 450 standard, 47 mm). Leachate pH was accepted within the range of 6–9 (which is the acceptable pH range for acute toxicity tests with *N. spinipes*), and was adjusted with sodium hydroxide or hydrochloric acid if necessary (for pH of leachates, see SM) to approximately 7–8. Filtrated leachates were stored in a refrigerator at 4 °C for a maximum of 72 h prior the ecotoxicological testing.

### 2.4. Test species and ecotoxicological test

The acute toxicity tests were performed according to the Swedish standard 028 106 "Determination of acute lethal toxicity

**Table 1**  
Plastic products included in the study, more details are available in the [Supplementary Material](#).

ID	Product	Polymer	Color	ID	Product	Polymer	Color
1	DVD case	Polypropylene (PP)	Transparent	12	Phone cover	Polyurethane (PUR)	Thermochromic (pink and yellow)
2	Cups	Polystyrene (PS)	Transparent	13	Wire harness connectors	Unknown	White
3	Q-tips box	Low Density Polyethylene (LDPE)	Transparent	14	Cable insulation	Polyvinyl Chloride (PVC)	Black
4	Garbage bags	High Density Polyethylene (HDPE)	Transparent, with white tone	15	Car dashboard	Unknown (possibly ABS/PC blend)	Grey, with brown tone
5	Costume packaging	Polyvinyl Chloride (PVC)	Transparent	16	Garden hose	Polyvinyl Chloride (PVC)	Green and black
6	Shoe soles packaging	Unknown	Transparent	17	Computer housing	Unknown (probably ABS/PC blend)	Black
7	Flyswatter packaging	Unknown (probably PVC)	Transparent	18 <sup>a</sup>	3D printer plastic	Polylactic Acid (PLA)	White
8	Toothbrush covers	Unknown	Transparent	19	Toy	Unknown	Several, mostly pink and yellow
9	Liquid soap bottle	Polyethylene Terephthalate (PET)	Transparent	20	Toothbrush	Mixed	White and Green
10 <sup>a</sup>	Soda bottle	Bio-PET (up to 22.5% plant based material)	Transparent	21	Shower curtain holders	Unknown	White
11 <sup>a</sup>	EN 13432 compliant biodegradable garbage bag	50% corn starch and 50% aliphatic polyester	Transparent, with green tone	22 <sup>b</sup>	Car tire	Polyisoprene Rubber	Black

<sup>a</sup> Bioplastics.

<sup>b</sup> Positive control.

of chemical substances and effluents to *N. spinipes* Boeck – Static procedure” (SIS, 1991). *N. spinipes* is a 0.6–0.8 mm harpacticoid copepod [Crustacea] that is widely distributed around the world, tolerating salinities between 1‰ and 35‰, temperatures between 0 to 30 °C and pH from 6 to 9 (SIS, 1991). In short, adult *N. spinipes* without egg sack (3–4 week old) were collected from the lab culture maintained at the Department of Environmental Science and Analytical Chemistry, Stockholm University, and exposed to different dilutions of the leachates at 20 °C (±1) in darkness. After 96 h the mortality in each test tube was noted. The same brackish water used to keep the lab cultures was also used as leachant and for dilution and in the controls of the tests.

Probit analysis was used to calculate the LC<sub>10</sub> and LC<sub>50</sub>, with the leachate (%) logged in XLSTAT (Addinsoft vers. 2014.2.03). Probit analysis is a regression model for binomial response variables (Finney, 1947). Natural mortality was disregarded as the mortality in the controls was under 3%, the validation criteria is that the control mortality shall be <10% (SIS, 2003). Differences between leachates over time were considered significant if the 95% fiducial limits (similar to confidence interval but for binominal data) for the LC<sub>50</sub>-values did not overlap, which is considered a conservative method (Payton et al., 2003).

## 2.5. Simulated weathering

The plastic materials were irradiated in a Suntest CPS Atlas system with a xenon lamp and a filter for simulation of natural sunlight (wavelengths 300–800 nm). The irradiance was set to 765 W m<sup>-2</sup>. Samples of the plastics were placed in 100 mL glass crystallizing dishes with an inner and outer diameter of 6.6 and 7 cm, respectively, and a height of 4.2 cm. Excess water was removed with a pipette. Four plastic materials were irradiated for 96 h at a time, covered with quartz glass discs. The temperature in the irradiation chamber was approximately 40 °C during irradiation. Eight plastics (ID: 1, 5, 7, 11, 15, 16, 17) were irradiated for an additional 96 h (total accumulated irradiation 192 h) after leaching and toxicity testing. Further, four of these (1, 5, 7, 11) were irradiated a third time (total accumulated irradiation 288 h). Leaching was started immediately after irradiation, with the exception of samples 1–4, which were stored in a refrigerator for 96 h before the first leaching.

The yearly-average insolation in Stockholm from 1961 to 1990 was 950 kW h m<sup>-2</sup> (SMHI, 2014). 96 h insolation with irradiance 765 W m<sup>-2</sup> corresponds to approximately 28 d of sun exposure in Stockholm, calculated as follows:  $765 \text{ W m}^{-2} * \frac{96 \text{ h}}{1000} = 73.44 \text{ kWh m}^{-2}$   $\frac{73.44}{950} * 365 = 28.2 \text{ d}$ .

## 2.6. Chemical analysis

Leachates from 0 h and 96 h irradiation from the PVC costume packaging (5), the biodegradable bag (11) and the flyswatter packaging (7) were screened for unknown chemical compounds. In parallel, two procedural blank samples (i.e. blank samples run through the analytical method) and one extraction blank sample (i.e. blank sample run through the extraction and clean-up procedure) consisting of Milli-Q water were also analyzed. Solid-phase extraction (SPE) with Oasis HLB cartridges (N-vinylpyrrolidone-divinylbenzene, 30 µm, 20 mg mL<sup>-1</sup>, Waters) was used to concentrate and clean-up samples. The columns were fitted into a vacuum manifold (Supelco Visiprep), and conditioned with 5 mL methanol and 5 mL Milli-Q water prior to loading of the sample. The leachates (14–90 mL) were loaded on the SPE cartridges and extracted with 5 mL of methanol. The volume of the extracts was then reduced to approximately 200 µL under a gentle stream of nitrogen.

For chemical screening, an ultra-performance liquid chromatography (UPLC) system (Waters) was used for injection of samples and delivering of the mobile phase. The column, Acquity HSS T3 (2.1 × 100 mm, dp 1.8 µm) (Waters), was kept at 65 °C and the sample vials were kept at 10 °C. Injection volume was 5 µL. The mobile phase consisted of 10 mM acetic acid in water (A) and 10 mM acetic acid in acetonitrile (B). A linear gradient, at a flow rate of 0.4 mL min<sup>-1</sup>, was used from 5% B to 100% B in 5 min, which was held for 3 min and thereafter returned to 5% B. For detection, a Micromass quadrupole time-of-flight high-resolution mass spectrometer (qTOF-HRMS) Premier (Waters) operated in electrospray ionization (ESI) positive and negative ion mode with the qTOF detector in 'V-mode', was used. The quadrupole was set to a wide pass mode and the collision energy was alternated between 2 and 20 eV, by using two full-scan HRMS functions. The scan time of each HRMS scan function was 0.08 s and the inter-scan time 0.002 s. The HRMS was run at a resolution of 10000 to scan the mass-to-charge range *m/z* of 75–1000 Da/e.

The following ion source settings were used: capillary voltage 3.0 kV; sampling cone voltage 35 V; extraction cone voltage 3.4 V; source temperature 100 °C, desolvation temperature 350 °C; cone gas (nitrogen) flow 50 L h<sup>-1</sup> and desolvation gas (nitrogen) flow 700 L h<sup>-1</sup>. External mass calibration was performed using a series of cluster ions formed from 0.05 M NaOH and 0.5% formic acid dissolved in 2-propanol/H<sub>2</sub>O (90:10).

### 3. Results

#### 3.1. Grinding and irradiation

Plastic grinding treatment and particle size proportions for the plastic samples are reported in the SM. Cryo-milling most often produced finely ground material. Grinding plastics in the blender allowed larger quantities to be treated and usually had some effect on all plastics, but the size reduction was in general not as efficient as with cryo-milling. Further, retrieval of the samples, especially the smallest fractions, was difficult due to static electricity and the lid design that was not fully sealed. Blending also heated the samples, e.g. pieces of the biodegradable garbage bag were burned and stuck around the blender-blade.

During and after irradiation a typical “plastic smell” became evident. Nearly all leachates from irradiated plastics emitted a noticeable scent, including those where no acute toxicity was observed. For example, the leached material from the PS cups had a sweet smell, which is typical for low concentrations of styrene (DHS, 1990). Irradiation caused most plastics to become brown or yellow at the surface, however, the most visually affected plastic (unknown packaging material, #6) did not induce any toxicity. The thermochromic phone cover turned permanently yellow after irradiation. No relation between visual differences after irradiation and toxicity could be seen. Coloring of the leachates was also noted, this was especially distinct for the tire (positive control), car dashboard and the computer housing leachates.

#### 3.2. Toxicity

Leachate from 62% (13 out of 21) of the plastic materials did not induce any acute toxicity towards *N. spinipes*. Leachate from the remaining 38% (8 out of 21) of the plastics did induce toxicity before and/or after irradiation. The probit statistical model is demonstrated in Fig. 1. For all dose–response curves as well as LC<sub>10</sub> and LC<sub>50</sub> values, see SM. The positive control (tire) produced the most toxic leachate. There was no common trend in how the toxicity changed as a function of irradiation time. Four plastics' leachates became significantly more toxic after irradiation (DVD-case, biodegradable bag, costume- and flyswatter packaging), two became significantly less toxic over time (computer housing and garden hose) and two showed no significant change in toxicity (car dashboard and phone cover). Leachates from two materials (DVD-case and flyswatter packaging) showed no toxicity before irradiation, but did afterwards.

The LC<sub>50</sub>-values for the costume and flyswatter packagings' leachates were significantly lower after 192 h and 96 h of artificial sun exposure, respectively, compared to 0 h of exposure (Fig. 2). The DVD-case leachates did not cause any acute toxicity until after 192 h of irradiation, and the leachate after 288 h was significantly more toxic (Fig. 2). The biodegradable bag leachates were significantly more toxic after irradiation than before (Fig. 2). The phone cover and the computer housing both induced toxicity in the first leachate but no or very minor in the following (Fig. 3). The car dashboard had no significant difference but a trend towards less toxicity (Fig. 3). The garden hose had a significant decrease in toxicity between the 192 h leachate and the previous two (Fig. 3).

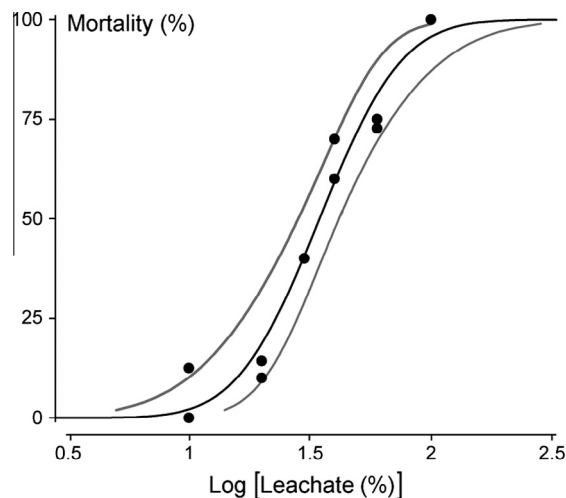


Fig. 1. Dose–response curve derived from the probit analysis, for the garden hose leachate before artificial sunlight exposure (0 h). Grey lines show the 95% fiducial limits, dots represent the individual data points.

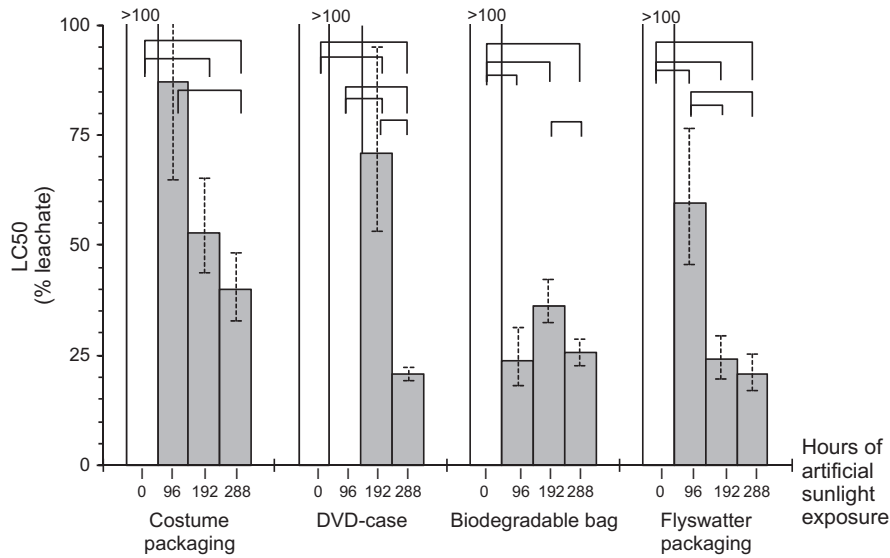
#### 3.3. Chemical analysis

No individual chemical structures could be identified in the broad chemical screening we applied. Generally, the chromatograms of the plastic leachates contained multiple signals and the corresponding mass spectra also had a complex composition. The chromatograms of the blank samples run in parallel to the leachate extracts contained hardly any signal. A comparison of the chemical screening of the leachates showed that some mass fragments were present before irradiation but not afterwards, whereas other mass fragments appeared only after irradiation. Fig. 4 shows the total ion chromatograms of the PVC costume packaging before (0 h) and after 96 h irradiation, as well as the chromatogram of the procedural blank sample. The leachate from the PVC costume packaging and the presumably PVC flyswatter packaging had many similarities in their mass chromatograms before and after irradiation, whereas the biodegradable bag showed some differences in the mass chromatogram. Mass fragments with a clearly distinguishable chlorine isotope pattern could not be identified in either of the leachates from PVC samples.

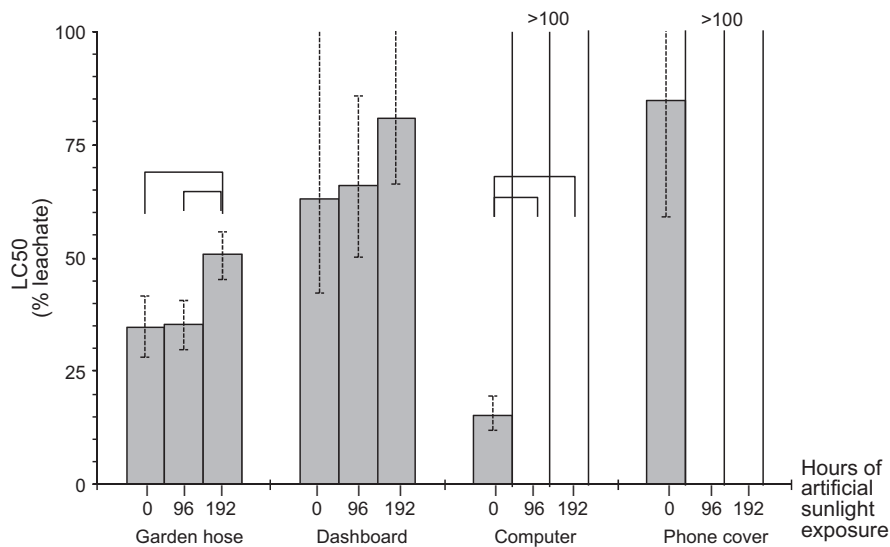
### 4. Discussion

The liquid-to-solid (*L/S*) ratio of 10 used in this study is a much higher concentration of plastic in water than typically found in nature (Lambert et al., 2014). The ratio was chosen since it is recommended by the standard for characterization of waste (SIS, 2003). Comparatively (Lithner, 2011) used an *L/S* ratio of 10 and 4 during 24 h or 72 h for leaching plastic. The results provide screening-level information about the intrinsic properties of the materials and should be viewed as such. Leachate from the majority of plastics did not induce any acute toxicity, but pH-changes and smell indicate that solutes were present in most of the leachates from irradiated plastics. Further experiments could explore longer irradiation times and different simulated weathering conditions, and a range of different endpoints.

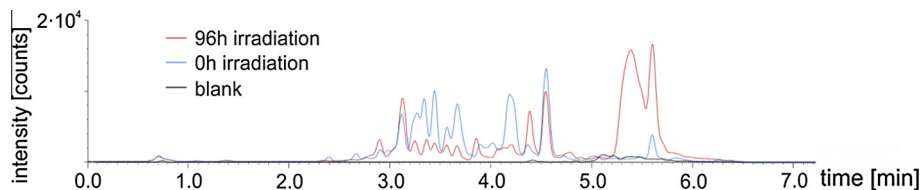
The different sizes of the ground materials introduce a limitation for comparing toxicity between the different plastics. The major aim of this study was to compare the toxicity of leachates from plastic after different periods of simulated weathering. Ensuring the same size distribution of plastic materials was not prioritized since each material was intended to be primarily compared to itself at different times rather than to other materials.



**Fig. 2.** LC<sub>50</sub>-values for plastics that produced more toxic leachates after exposure to artificial sunlight. All leachates shown in this figure had a LC<sub>50</sub> > 100% leachate before irradiation (0 h). Error bars show the 95% fiducial limits. The brackets show sample pairs with significant differences in LC<sub>50</sub>-values. White bars indicate that there was no toxicity or that the calculated LC<sub>50</sub> value was over 100% leachate.



**Fig. 3.** LC<sub>50</sub>-values for plastics that produced less toxic leachates after exposure to artificial sunlight. Error bars show the 95% fiducial limits. The brackets show significant differences between pairs of LC<sub>50</sub>-values. White bars indicate that there was no toxicity or that the LC<sub>50</sub> value was over 100% leachate.



**Fig. 4.** Base peak intensity total ion chromatogram of the PVC costume packaging before irradiation (blue line), after 96 h of irradiation (red line), and the procedural blank sample (black line). Note that the intensity of the peaks in the blank sample was so low that the black line is hardly visible in this plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Comparison among materials is further complicated by the different approaches for size reduction, where e.g. plastics grinded in the blender became heated in contrast to the plastics grinded in liquid nitrogen. This study provides a base for further research on leaching during weathering and degradation, where e.g. toxicity from

specific materials such as PVC or differences between types of polymers such as amorphous and crystalline could be systematically researched.

Although no chemical structures were positively identified with the chemical screening procedure, there was evidence that the



chemical composition of the leachate was different after irradiation. The non-detection of mass fragments with a chlorine isotope pattern indicates that the leachates from the PVC samples probably did not contain oligomers from the polymer backbone. However, differences between the plastics before and after the irradiation show an effect on the leaching. Hence testing leachates from one specific time does not necessarily give a representative picture of the whole leaching process during aging and weathering of the material in the environment.

Whether the polymers are amorphous or crystalline in structure seems to be of importance for leachate toxicity. The only (semi-)crystalline plastic that produced toxic leachates was the DVD-case (PP). However, leachates from the DVD-case were only toxic after a total of 192 h of irradiation of the plastic (corresponding to approximately 56 d of sunlight in Stockholm). We speculate that plastics made from other crystalline polymers might produce toxic leachates after sufficiently long periods of irradiation. The only leachates from known amorphous plastic that did not cause toxicity were from PS cups and the PVC cable. The PS cups are a food contact material, hence under legal obligations concerning migration of additives (European Commission, 2011). Migration can be prevented by e.g. using additives with high molecular weights (Hansen et al., 2012). Softer plastics also seemed to have a tendency to cause toxicity more often than rigid; 6 out of the 8 plastics causing toxicity were soft. Three of these soft plastics were PVC, most likely containing plasticizers. Two rigid plastics that are likely amorphous produced toxic leachates; the car dashboard and computer housing (see SM for details).

Weathering the PVC packaging materials in artificial sunlight increased toxicity of leachates, whereas the PVC garden hose became less toxic, and the PVC cable leachates showed no acute toxicity. These differences among the same material show that toxicity cannot be assessed based on the material alone, the composition and properties can differ for the same structural polymer. The toxicity of leachates determined in this study prior to simulated weathering are generally consistent with results of Lithner (2011), who found that e.g. PVC and PUR leachates caused toxicity to *D. magna* in most cases and PE leachates in very few cases.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2015.03.010>.

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