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Leaching of residual monomers, oligomers and additives from polyethylene, polypropylene, polyvinyl chloride, high-density polyethylene and polystyrene virgin plastics

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Abstract

Plastic debris are accumulating in our oceans and are degraded into smaller pieces which eventually becomes small enough to be available to lower trophic level organisms. Microplastics, commonly defined as plastic particles <5 mm, are globally distributed and found at remote locations far away from industrialized and populated areas. The effects of macro sized plastics is well understood whilst the effects of microplastics is hard to predict. It is known that microplastics act as transfer vectors for a wide range of toxic chemicals into organisms, and it is also known that the particle itself can cause toxic responses such as increased immune response and endocrine disruption. Researchers utilize virgin plastic pellets in order to determine the toxicological effect of the plastic particle itself, but recent research suggest that these virgin plastics may release chemicals that contribute to the toxic response and thus complicates the interpretation of the results. In present study, five different virgin plastics were allowed to leach in artificial seawater under conditions that mimic those used in particle toxicity studies. Plastics included were polyethylene, polypropylene, polyvinyl chloride, high-density polyethylene and polystyrene. Leachable monomers and oligomers were found in three of the five plastics tested: polyvinyl chloride, high-density polyethylene and polystyrene. Leached compounds from polyvinyl chloride were not identified due to time limitations. Aliphatic hydrocarbons in the size C₁₄-C₂₂ were leached out from high-density polyethylene in the concentration range $0.47 \times 10^{-3} - 1.13 \times 10^{-3} \mu\text{g ml}^{-1}$ within 24 hours. Polystyrene was found to leach styrene monomer which reached a concentration of $0.17 \mu\text{g ml}^{-1}$ within 24 hours.

Keywords: Microplastics; Virgin plastic pellets; Residual monomers; Particle toxicity; GC-MS

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1. Introduction

Plastics have been widely used and produced since the mid-19th century (SSNC 2014) and the global annual production continues to increase (PlasticsEurope 2014). Plastics have not only contributed to the development of our modern society, but also to the pollution of our planet, including the oceans. Plastics accumulate in the oceans and it can be assumed because of its durability that all plastics that have ever reached the oceans are still present today. In december 2014, Eriksen et al., estimated that the oceans contains more than 5 trillion floating plastic pieces of different sizes weighing over 250,000 tonnes together (Eriksen et al 2014).

Plastic consumer products are not always recycled and tend to end up in landfills where the route for many plastics to the oceans begins. Once in the ocean, the buoyancy allows for long-distance distribution of plastics depending on the wind, wave, tidal and oceanic currents. Plastic pellets have been found in remote locations far away from populated areas (Gregory 1999). The primarily polymers are made from petroleum and broken down when exposed to UV radiation resulting in degradation into smaller fragments (Andrady 2011, Webb et al. 2013), which eventually becomes small enough to be available to lower trophic organisms (Wright et al. 2013).

Plastic pollution is now recognized as one of the major problems concerning the world's oceans (Andrady 2011, Cole et al. 2011), and is associated with entanglement of marine mammals, seabirds and other species (NOAA Marine Debris Program 2014) as well as with ingestion by a wide range of marine organisms including fish (Rochman et al 2014), mussels (Browne et al. 2008), and seabirds (Yamashita et al. 2011). Harm to marine life caused by macro sized plastic (>5 mm) is primarily mechanical due to ingestion and entanglement, whilst the threats caused by micro sized (<5 mm) plastic debris depend on a variety of factors and are thus hard to predict.

Ingested microplastics have been shown to migrate through tissue, translocate to the circulatory system and produce an increased immune response in the blue mussel *Mytilus edulis* (Browne et al. 2008). The plastic particles persisted in the circulatory system for up to 48 days suggesting a potential for trophic level transfer, which have later been confirmed to take place from mussels to crabs (Farrell & Nelson 2013) and from mesozooplankton to macrozooplankton (Setälä et al. 2014). Rossi et al. 2013 showed that nanosized polystyrene (PS) easily penetrates cellular membranes and alter their structure. Alteration of cellular membranes might affect the function of membrane associated proteins and can thus cause severe alternations in cellular functions. Furthermore, nanosized plastic beads have been shown to adsorb to algae and prevent photosynthesis and increase the production of reactive oxygen species (Bhattacharya et al. 2010).

Microplastics are known to act as transfer vectors for not only persistent organic pollutants, but also additives and residual monomers into organisms followed ingestion (Browne et al. 2013, Teuten et al. 2009). Many of these chemicals are known to cause adverse effects including endocrine disruption and increased immune response and it is often suggested that ingestion of microplastics increases the exposure to the associated chemicals and thereby the risk of developing adverse responses (Browne et al. 2013, Teuten et al. 2009). Recent research suggests

that the exposure to nonylphenol (NP) and bisphenol A (BPA) contributed by ingestion of microplastics is negligible (Koelmans et al. 2014). There are however other chemicals associated with plastics that might pose a risk of developing adverse responses in living organisms.

The toxicological responses resulting from ingestion of microplastics may thus be a combination between particle- and chemical toxicity.

Particle toxicity studies include *in vivo* tests based on exposure to virgin plastic pellets, which are supposed to be free from any additives and/or residual monomers. There are however discussions going on whether these virgin plastic pellets really are free from leaching compounds or if toxic chemicals could be released from the polymer during the experiments and contribute to the resulted toxic response. In order to determine if the particles themselves causes toxic responses, it is required to eliminate all possible mixed effects due to leaching of residual monomers and/or additives that may have been trapped during the polymerization process. The objective with this study is therefore to determine if virgin plastic pellets used in particle toxicity studies contain residual monomers, oligomers, and/or additives that might leach out and contribute to the resulted toxic response.

In a recent study not yet published, fluorescent polystyrene microspheres were shown to have a toxic effect on the fertilization rate of sea urchin eggs as well as on normal larval development and growth. However, limited interactions between the polystyrene microspheres and eggs were observed, and ingestion of microspheres was absent (Martinez-Gómez et al.). This suggests that residual monomers, oligomers and/or additives may be leaching out and contribute to the toxic response. Present study therefore focuses on fluorescent polystyrene microspheres and the possible leaching of residual monomers, oligomers and additives.

2. Method

2.1. Total extraction of additives and residual monomers

Additives and/or residual monomers from five different virgin plastic polymers/polymer mixtures were extracted by shaking in an organic solvent. Virgin plastic pellets included were polyethylene (PE) (\varnothing ~5.0 mm), polypropylene (PP) (\varnothing ~5.0 mm, Sigma Aldrich, St. Louis, USA), polyvinyl chloride (PVC) (\varnothing not defined, Sigma Aldrich, St. Louis, USA), high-density polyethylene (HDPE) (\varnothing 0-80 μ m) and polystyrene (PS) (fluoresbrite polychromatic red, \varnothing 5.5 μ m, Polysciences, Inc. Warrington, PA).

Approximately 0.1 g and 0.5 g virgin plastic pellets of PE, PP, PVC, and HDPE were extracted with 15 ml n-hexane (Picograde®, LGC Standards, Wesel, Germany) by shaking for 24 h. The higher amount plastic (0.5 g) was necessary to meet the sensitivity requirements of the GC-MS. A blank was prepared by addition of n-hexane to an empty glass vial. Extraction of PS were performed by transferring 20 μ l and 50 μ l fluoresbrite polychromatic red solution (5% PS microspheres in water) into 20 ml glass vials, giving concentrations of approximately 8×10^5 and

19×10^5 microspheres ml^{-1} respectively (for calculations see Appendix A). The microspheres were then extracted by shaking in 15 ml n-hexane for 24 h.

After extraction, 2 ml of the extracts from 0.1 g plastic was transferred to 2 ml glass vials and evaporated to near dryness under a gentle stream of N_2 . Before the n-hexane were completely evaporated, 1 ml methanol (MeOH) (HPLC Gradient Grade, J.T. Baker®, Deventer, The Netherlands) was added. The remaining n-hexane was then allowed to evaporate and the vials were sealed and stored in a refrigerator (4°C) until analysis with LC-TOF. Samples for GC-MS analysis were prepared by transferring 10 ml extract from 0.5 g plastic to 15 ml glass tubes. The glass tubes were placed in a 30°C water bath to allow the n-hexane to evaporate under a gentle stream of N_2 . Before the n-hexane was completely evaporated, 200 μl isooctane (SupraSolv®, Merck Millipore, Darmstadt, Germany) were added and the extract were transferred to 200 μl inserts in 2 ml glass vials. The remaining n-hexane was evaporated under a stream of N_2 and the vials were sealed and stored in refrigerator (4°C) until analysis.

2.2. *Leaching of the bioavailable fraction of additives and residual monomers*

The bioavailable fraction of additives and/or residual monomers from approximately 1.0 g virgin PE, PP, PVC, and HDPE plastic pellets were allowed to leach in 50 ml artificial sea water (3.5% NaCl, pH 7.8) for 24 h. Only the virgin plastic pellets where extractable compounds were detected in previous step were carried through the leaching experiment. For the PS, 50 μl fluoresbrite polychromatic red solution were allowed to leach in 50 ml artificial sea water for 24 h, giving a concentration of approximately 6×10^5 microspheres ml^{-1} (Appendix A). Blanks were prepared by addition of 50 ml artificial salt water to empty glass vials. The leaching were performed in a cold chamber (16°C) to mimic the conditions used during microplastic toxicity experiments with blue mussels (*Mytilus edulis* (L.)) (Browne et al. 2008, Moos et al. 2012).

After leaching, the PE, PP, and HDPE virgin plastic pellets were removed with a metal spoon and the leachate stored in a refrigerator (4°C) until further processing. The samples containing PVC were centrifuged to precipitate solids before the leachate were transferred to new glass vials. Some of the PVC content remained floating and were thus transferred along with the leachate. The PS leachate were filtered with sterile filters, pore size 0.45 μm (Millex®, Millipore, Molsheim, France). Any additives and/or residual monomers were then extracted from the leachate by liquid-liquid extraction (LLE) (shaking for 10 minutes) with 3×4 ml n-hexane. The solvent was evaporated to near dryness and 200 μl isooctane were added to each sample. The remaining n-hexane was then allowed to evaporate and the vials were sealed and stored in a refrigerator (4°C) until analysis. Unfortunately, no leachates were prepared for LC-TOF analysis due to limited availability of equipment.

2.3. *LC-TOF analysis*

Samples prepared in MeOH were analyzed with a 1290 infinity LC (Agilent Technologies) coupled with a micrOTOF II (Bruker Daltonics). A sample size of 3.0 μl was injected onto a

50x2.1 mm symmetry C18 column with 5.0 μm particle size (Waters). The flow rate were 0.3 ml min^{-1} and the initial solvent composition were water:MeOH 60:40 which were held for 1.5 minutes and then gradually changed during 13.5 minutes to water:MeOH 2:98 and held for 10 minutes. The composition were then changed back to water:MeOH 60:40 and held for 10 minutes. Electrospray ionization was used as ionization source and the samples were analyzed in both positive and negative mode.

Data analysis was performed using Bruker compass data analysis version 4.1 in combination with chemspider database.

2.4. GC-MS analysis

Samples prepared in isooctane were analyzed with a 5973N GC-MS (Agilent Technologies) with a 30 m HP-5MS capillary column with i.d. 0.25 mm and 0.25 μm film thickness (Agilent, Santa Clara, USA). Samples were injected with pulse splitless mode at an inlet temperature of 300°C. Helium was used as carrier gas with a constant flow of 0.9 ml min^{-1} . Electron impact was used as ionization source. The source and quadrupole temperature were 230°C and 150°C respectively.

The PE, PP, PVC, HDPE, and PS extracts and leachates were analyzed in full scan mode at m/z 35-500 along with a styrene standard (1.0 $\mu\text{g ml}^{-1}$ in isooctane) and a standard containing C₇-C₃₀ alkanes (1.0 $\mu\text{g ml}^{-1}$ in isooctane). The initial oven temperature was 60°C, held for 2 minutes followed by a 5°C min^{-1} increase to 250°C. The temperature was then increased to 300°C at 20°C min^{-1} and held for 3 minutes.

Standard solutions containing C₇-C₃₀ alkanes was prepared in isooctane in a concentration range of 0.05-10.0 $\mu\text{g ml}^{-1}$. A process standard for determination of extraction efficiency was prepared by addition of 100 μl C₇-C₃₀ alkanes standard solution (10.0 $\mu\text{g ml}^{-1}$ in isooctane) to 50 ml artificial seawater. The process standard was put through the same process as the leachates of PE and HDPE and dissolved in 1 ml isooctane after evaporation of the n-hexane used in the LLE. The alkane standards were analyzed in full scan mode at m/z 40-422 along with the PE and HDPE extracts and leachates. The initial oven temperature was 60°C, held for 3 minutes followed by a 5°C min^{-1} increase to 150°C. The temperature was then increased to 300°C at 20°C min^{-1} and held for 3 minutes.

Standard solutions of styrene monomer in concentration range 0.05-10.0 $\mu\text{g ml}^{-1}$ was prepared as well as two process standards in order to determine the efficiency of the filtration step and the extraction with n-hexane. The process standards were prepared by addition of 100 μl styrene standard solution (10.0 $\mu\text{g ml}^{-1}$ in isooctane) to 50 ml artificial sea water and treated in the same way as the PS leachate. One standard were filtered prior to LLE with n-hexane and the extracts were then evaporated and dissolved in 1 ml isooctane. The styrene standards were analyzed at SIM mode along with the PS extract and leachate. The initial oven temperature was 60°C, held for 2 minutes followed by a 5°C min^{-1} ramp to 100°C. The temperature was then increased to 250°C at 20°C min^{-1} and held for 3 minutes. The samples were scanned at m/z 51, 77, 78, 103,

104, 208, and 312 representing the major fragments and the molecular ions from the styrene monomer (104), dimer (208), and trimer (312). Other possible impurities in PS include isopropyl benzene, toluene, benzene, p-xylene, and 2-phenyl propene, but the concentrations are reported to be <0.1% (EU 2002) why only the monomer, dimer and trimer are scanned in this study.

Data analysis of the GC-MS output was performed using the Agilent MSD productivity chemstation for GC and GC/MS systems in combination with NIST mass spectral library version 2.0.

2.5. *Identification and quantification*

Identification and quantification of styrene monomer in PS extract and leachate and aliphatic hydrocarbons in PE and HDPE extracts and leachates were performed by using external standards. Identification of styrene in PS or C₉-C₃₀ aliphatics PE and HDPE were performed by comparison of retention times of the standards with those obtained in analyzed samples. Associated mass spectra were used for confirmation. Standard solutions in concentration range 0.05-10.0 µg ml⁻¹ were analyzed and the peak area in relation to concentration were used to calculate the concentration styrene monomer in the PS extract and leachate. Standard solutions in concentration range 0.1-10.0 µg ml⁻¹ were analyzed and the peak area in relation to concentration were used to calculate the concentration aliphatic hydrocarbons monomer in the PE and HDPE extracts as well as the HDPE leachate.

3. Results

3.1. *Extractable compounds*

No residual monomers, oligomers or additives could be detected in the plastics extracted with n-hexane using LC-TOF in negative mode. With positive mode, extractable compounds could be detected in PE, PS, and PVC plastics. However, none of the detected compounds could be identified as additives commonly used in polymers.

Fullscan at m/z 35-500 indicated presence of extractable compounds in all plastics included in this study. However, due to time- and material limitations, only styrene monomer in PS and aliphatic hydrocarbons in PE and HDPE could be identified. Analysis of the PS extract and comparison of obtained mass spectrum with NIST mass spectral library suggested extraction of styrene monomer, which could be confirmed by comparing the retention time with a standard. Analysis of the PE and HDPE extracts indicated presence of primarily aliphatic hydrocarbons. Analysis of the PP extract resulted in poor separation, comparison with NIST mass spectral library however suggests extraction of a complex mixture of branched alkanes and alkenes. Extractable compounds in PVC were detected but could not be identified.

Fullscan of PE and HDPE extracts at m/z 40-422 indicate extraction of a homologous serie of aliphatic hydrocarbons (figure 1-3). The total ion chromatogram of a standard containing C_7 - C_{30} alkanes in concentration $5.0 \mu\text{g ml}^{-1}$ in figure 1 shows the retention time for respective alkane. C_7 and C_8 alkanes could not be detected due to the choice of isooctane as solvent. Figure 2 shows the total ion chromatogram of the PE extract. The chromatogram indicates presence of residual oligomers in the range C_{10} - C_{30} and the associated mass spectra for respective peak confirm extraction of aliphatics. Figure 3 shows the total ion chromatogram of the HDPE extract. The chromatogram indicates presence of residual oligomers in the range C_{12} - C_{30} and the associated mass spectra for respective peak confirm extraction of aliphatics. Extracted concentrations are given in table 1.

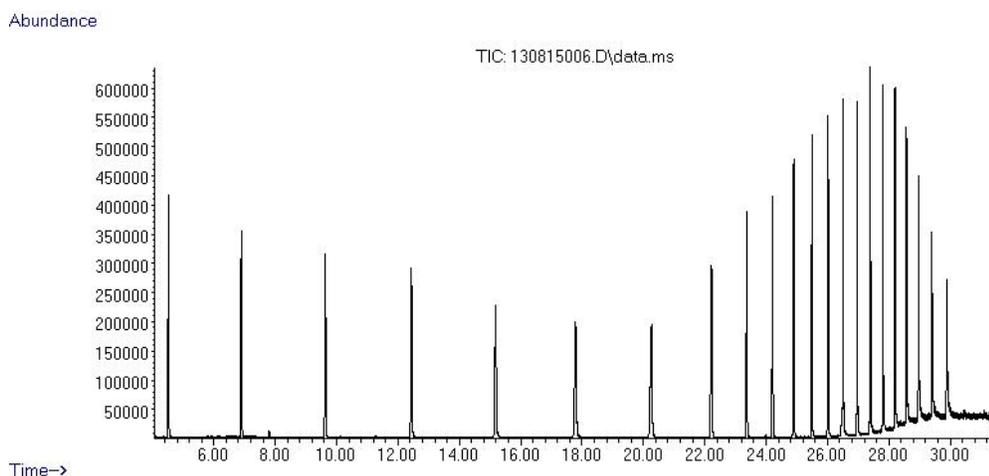


Figure 1. Total ion chromatogram of C_9 - C_{30} alkanes at concentration $5.0 \mu\text{g ml}^{-1}$. The chromatogram shows the retention time of respective alkane.

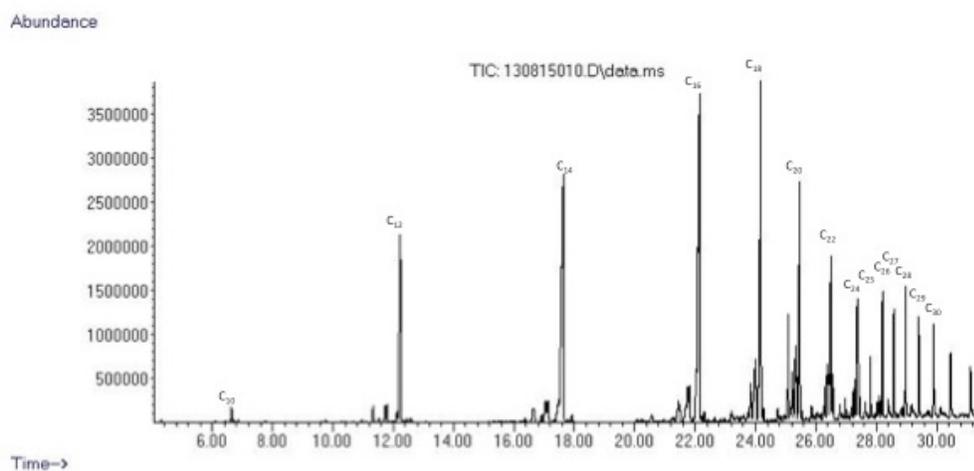


Figure 2. Total ion chromatogram of PE extract. The chromatogram indicate presence of C_{10} , C_{12} , C_{14} , C_{16} , C_{18} , C_{20} , C_{22} , C_{24} , C_{25} , C_{27} , C_{28} , C_{29} , and C_{30} residual oligomers.

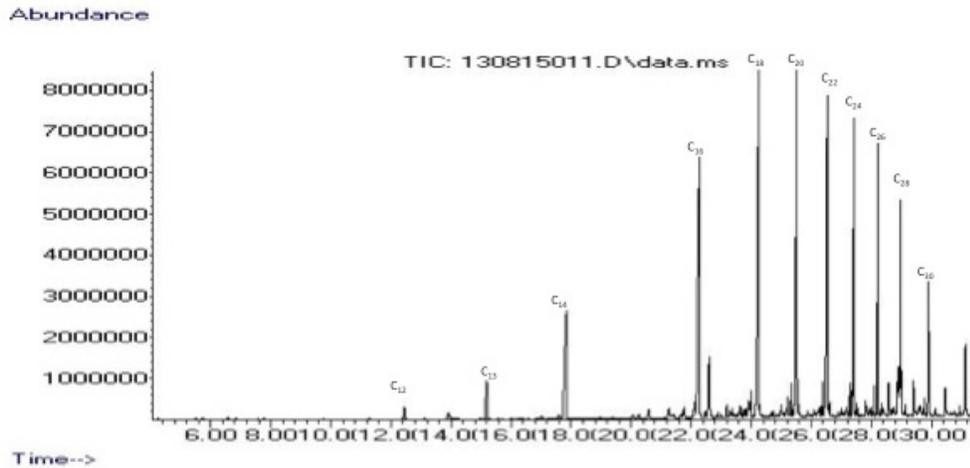


Figure 3. Total ion chromatogram for HDPE extract. The chromatogram indicates presence of C₁₂, C₁₃, C₁₄, C₁₆, C₁₈, C₂₀, C₂₂, C₂₄, C₂₆, C₂₈, and C₃₀ residual oligomers.

Table 1. Extracted concentrations ($\mu\text{g ml}^{-1}$) of aliphatic hydrocarbons from polyethylene and high-density polyethylene.

	PE extract	HDPE extract
C ₁₀	0.130	-
C ₁₂	2.101	0.264
C ₁₃	-	1.036
C ₁₄	3.792	3.401
C ₁₆	3.566	5.828
C ₁₈	2.839	5.839
C ₂₀	1.941	6.184
C ₂₂	2.650	8.720
C ₂₄	2.676	17.797
C ₂₅	2.831	-
C ₂₆	1.550	7.623
C ₂₇	1.260	-
C ₂₈	1.340	4.669
C ₂₉	0.800	-
C ₃₀	1.533	4.661

Analysis with GC-MS of PS extract in SIM mode at m/z 51, 77, 78, 103, 104, 208, and 312 confirmed presence of extractable residual styrene in the tested polystyrene microspheres as already indicated by fullscan at m/z 35-500. The extracted concentration in 15 ml n-hexane was calculated to $0.85 \mu\text{g ml}^{-1}$. Chromatogram and associated mass spectrum are shown in figure 4.

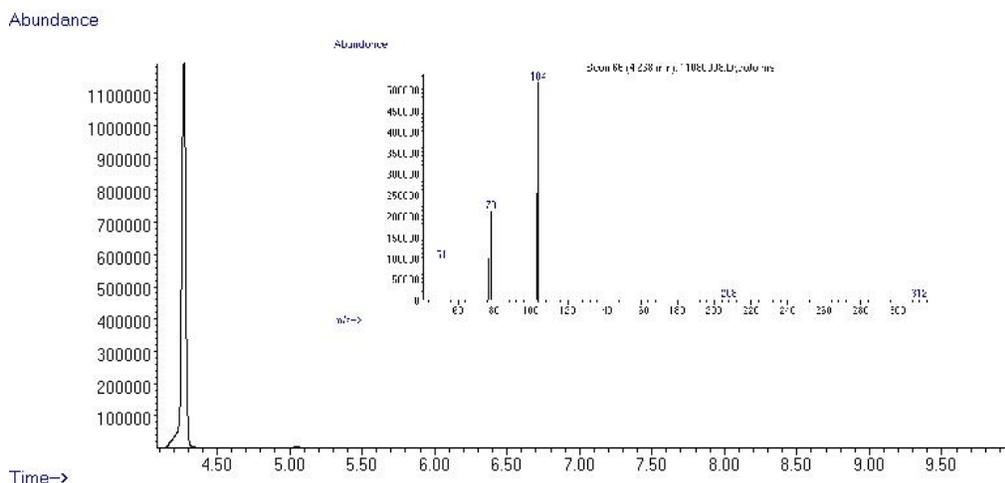


Figure 4. Chromatogram and associated mass spectrum of PS extract. The chromatogram shows elution of an extracted compound after 4.2 minutes. Comparison of associated mass spectra with NIST mass spectral library suggests extraction of styrene.

3.2. Leachable compounds

Fullscan at m/z 35-500 with GC-MS indicate leaching of styrene monomer from PS and aliphatics from HDPE. Analysis of the PE extract indicated presence of extractable residual monomers. However, no residual monomers could be detected in the leachate. Analysis of the PP extract resulted in unresolved peaks hard to identify but the use of NIST database suggest presence of extractable residual monomers, however, no aliphatics could be detected in the PP leachate. Leachable compounds in PVC were detected but could not be identified due to time- and material limitations. Chromatograms for each analyzed leachate are given in Appedix E.

3.2.1. Leaching of styrene monomer in polystyrene

Styrene monomer was detected in both the extracted samples and the leachates when analyzed with GC-MS. The concentrations of extracted and leached styrene were calculated to $0.85 \mu\text{g ml}^{-1}$ and $0.17 \mu\text{g ml}^{-1}$ respectively (compensated for method recovery).

3.2.2. Leaching of aliphatic hydrocarbons in polyethylene, polypropylene, and high-density polyethylene

No residual oligomers were detected in the PE or PP leachates. The total ion chromatogram of the HDPE leachate (fig 5) indicates leaching of C_{14} , C_{16} , C_{18} , C_{20} , and C_{22} aliphatics and the associated mass spectrum for each peak confirm leaching of mentioned aliphatics. The concentrations of respective hydrocarbon in the leachates are given in table 2.

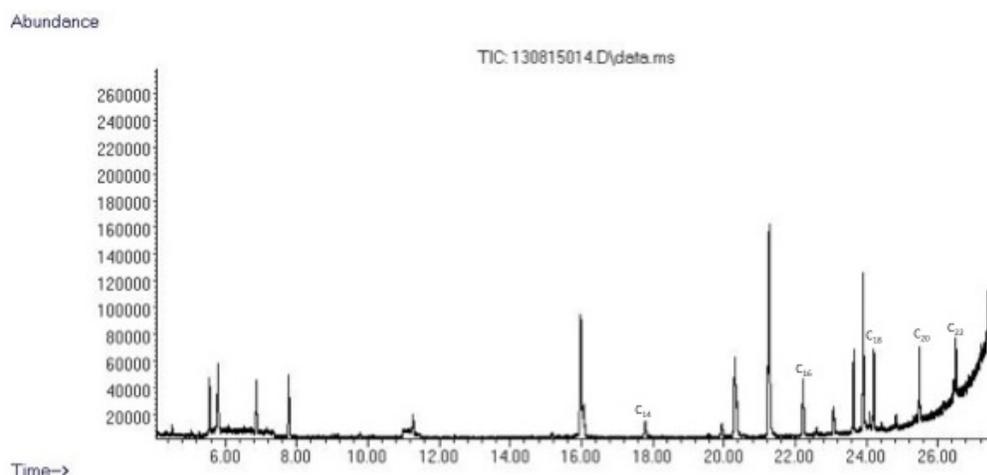


Figure 5. Total ion chromatogram of HDPE leachate. The chromatogram indicates presence of leachable aliphatic hydrocarbons C₁₄, C₁₆, C₁₈, C₂₀, and C₂₂.

Table 2. Concentrations of leached aliphatics from 1.0 g HDPE in 50 ml artificial seawater.

	Concentration ($\mu\text{g ml}^{-1}$)
C ₁₄	0.60×10^{-3}
C ₁₆	1.10×10^{-3}
C ₁₈	1.13×10^{-3}
C ₂₀	1.02×10^{-3}
C ₂₂	0.47×10^{-3}

4. Discussion and Conclusions

Commonly used exposure concentrations in particle toxicity studies are 0.50-2.50 mg ml⁻¹ (Browne et al. 2008, von Moos et al. 2012) or if reported in microspheres per volume water it ranges from only 10 microspheres ml⁻¹ to 10⁵ microspheres ml⁻¹ depending on size of the microspheres and also the aim with the study. In the present study, approximately 500 mg of PE, PP, PVC and HDPE virgin plastic pellets were allowed to leach in 15 ml solvent, giving a concentration of approximately 33 mg ml⁻¹. The concentration PS microspheres leached in present study was approximately 5.7×10⁵ microspheres ml⁻¹. This suggests that possible concentration of leachable compounds during particle toxicity studies will not reach concentrations equal to or higher than the concentrations leached in this study as long as the used concentrations microplastics do not exceed concentrations used in this study.

The lowest reported EC₅₀ in the environmental protection agency (EPA) database for styrene monomer is 0.56 $\mu\text{g ml}^{-1}$ for the green algae *Pseudokirchneriella subcapitata* and 4.7 $\mu\text{g ml}^{-1}$ for the water flea *Daphnia magna* (2 days exposure) (Cushman et al. 1997). Measured concentration of styrene monomer leached from PS microspheres after 24 h in artificial seawater at 16°C in this study was 0.17 $\mu\text{g ml}^{-1}$, suggesting that using a concentration equal to or lower than 6×10⁵ PS

microspheres ml^{-1} would not lead to a toxic response caused by leached styrene monomer in the green algae *Pseudokirchneriella subcapitata* or in the water flea *Daphnia magna*. However, this does not mean that a concentration of 6×10^5 PS microspheres ml^{-1} will not cause a toxic response in other organisms as a result of leaching styrene monomer. The measured leached concentration styrene monomer is close to the reported lowest effect concentration (LOEC) for the blue mussel *Mytilus edulis* is $0.2 \mu\text{g ml}^{-1}$ when exposed for 7 days (Mamaca et al. 2005). The PS microspheres in this study were only allowed to leach for 24 h and it is not clear yet whether the PS microspheres would continue to leach and reach higher concentrations if they were left in the artificial seawater for a longer time. Further studies is required in order to determine what concentration that could be reached within 7 days, and if the leaching rate decreases with time.

The method recovery of styrene was however only 22.19%, making the results less reliable than if the recovery would have been within the universal acceptable range of 70-120%. The extraction recovery was 71.49%, suggesting the filtration step caused a loss of approximately 50%. Method improvement is needed for future studies in order to obtain more accurate and reliable results.

There are a limited number of toxicity tests with aliphatic hydrocarbons available. The toxicity and bioaccumulation for aliphatic hydrocarbons is however correlated with $\log K_{ow}$ and can thus be estimated based on equilibrium partitioning (MADEP 2007). Regression of available toxicity data versus $\log K_{ow}$ produces a straight line from which toxicity data for other hydrocarbons can be estimated. A chronic toxicity value (LC_{50}) that are protective of most aquatic organisms have been estimated for aliphatic hydrocarbons divided into fractions of C_9-C_{12} , $C_{13}-C_{18}$, and $C_{19}-C_{36}$ and are listed in table 3. Leaching of HDPE in artificial seawater resulted in measurable concentrations of 6 aliphatic hydrocarbons: C_{14} , C_{16} , C_{18} , C_{20} , and C_{22} . The concentrations are listed summarized in fractions in table 3 for comparison with the estimated chronic toxicity values. The measured concentration for each of the leached aliphatics is listed in table 4 with comparison with estimated acute toxicity values.

Table 3. Estimated chronic toxicity values (LC_{50}) for saturated aliphatic hydrocarbon fractions compared to measured concentrations leached from HDPE.

Aliphatic hydrocarbon fraction	Chronic toxicity value ($\mu\text{g ml}^{-1}$)	Measured concentration ($\mu\text{g ml}^{-1}$)
C_9-C_{12}	6.30×10^{-3}	-
$C_{13}-C_{18}$	0.05×10^{-3}	2.83×10^{-3}
$C_{19}-C_{36}$	0.0001×10^{-3}	1.49×10^{-3}

Table 4. Estimated acute toxicity values (LC_{50}) for saturated aliphatic hydrocarbons compared to measured concentrations leached from HDPE.

Aliphatic hydrocarbon	Acute toxicity value ($\mu\text{g ml}^{-1}$)	Measured concentration ($\mu\text{g ml}^{-1}$)
C ₁₄	12.4×10^{-3}	0.60×10^{-3}
C ₁₆	1.6×10^{-3}	1.10×10^{-3}
C ₁₈	0.2×10^{-3}	1.13×10^{-3}
C ₂₀	0.4×10^{-5}	1.02×10^{-3}
C ₂₂	-	0.47×10^{-3}
C ₂₄	1.17×10^{-7}	-

This suggests that the use of 500 mg HDPE in 50 ml artificial seawater leaches concentrations of aliphatic hydrocarbons that might cause a chronic toxic response in aquatic organisms. However, measured and estimated acute toxicity data suggest that 1-tetradecene and higher alpha olefins are not likely to cause acute toxicity in aquatic organisms due to the low solubility in water (UNEP 2000). Estimated acute aquatic toxicity values (LC_{50}) for saturated, unbranched hydrocarbons ranges from $1.17 \times 10^{-7} \mu\text{g ml}^{-1}$ for tetracosane (C₂₄H₅₀) to $12.4 \times 10^{-3} \mu\text{g ml}^{-1}$ for tetradecane (C₁₄H₃₀) and are listed in table 4 (MADEP 2007). This suggest that the higher saturated aliphatic hydrocarbons (C₁₈-C₂₀) might leach out in concentrations high enough to cause harm to aquatic organisms during particle toxicity studies with HDPE, whilst the lower (C₁₄ and C₁₆) are below the estimated acute toxicity concentration. However, the measured method recoveries ranged between 136-267%, also here outside the universal acceptable range of 70-120% for each of the detected aliphatics in the leachate. The reason why such high recoveries were obtained is not clear, there was however some problems with contamination with aliphatics that could have contributed to the high recoveries. Method improvement is therefore required in order to obtain more reliable results.

Lower aliphatic hydrocarbons C₉-C₁₃ were not detected in the HDPE leachate which would be expected since C₁₄ was detected and decrease in chain length also leads to increased aqueous solubility. However, the extracted concentrations of C₉-C₁₃ from HDPE were lower than for the aliphatics detected in the HDPE leachate, which could explain why the lower aliphatics were not detected in the leachate (table 1, section 3.1.). The reason why higher aliphatics than C₂₂ were not detected in the HDPE leachate is probably because of low aqueous solubility. It can also be seen from table 1 in section 3.1. that the concentration aliphatic hydrocarbons in the PE extract is lower than in the HDPE extract, which might explain why no aliphatics were detected in the PE leachate.

GC-MS analysis of the PP extract resulted in absence of resolution making it hard to identify any of the extracted compounds. However, data analysis suggests presence of a complex mixture of branched alkanes and alkenes, which is common for PP due to the branched structure of the polymer. An analysis of the leachate was carried out even though no extracted compounds could be identified in order to determine if the extractable compounds could also be leached out in

conditions used during particle toxicity studies. The analysis of the PP leachate suggest no or limited leaching of residual monomers and/or oligomers.

Fullscan at m/z 35-500 with GC-MS of the PVC extract indicate presence of extractable compounds. Also here, a leachate was analyzed in order to determine if extracted compounds could also be leached out. Analysis of the PVC leachate indicates presence of leachable compounds but unfortunately, no of the detected compounds could be identified due to time- and material limitations.

Extracted compounds detected with LC-TOF could not be identified due to time- and material limitations. An analysis of the leachates would have given an indication of whether the extracted compounds were leached out in artificial seawater or not. Unfortunately, no leachates were prepared for LC-TOF analysis due to limited availability of equipment.

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