RESEARCH ARTICLE

Comparative acute toxicity of leachates from plastic products made of polypropylene, polyethylene, PVC, acrylonitrile–butadiene–styrene, and epoxy to *Daphnia magna*

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Abstract

Purpose The large global production of plastics and their presence everywhere in the society and the environment create a need for assessing chemical hazards and risks associated with plastic products. The aims of this study were to determine and compare the toxicity of leachates from plastic products made of five plastics types and to identify the class of compounds that is causing the toxicity.
Methods Selected plastic types were those with the largest global annual production, that is, polypropylene, polyethylene, and polyvinyl chloride (PVC), or those composed of hazardous monomers (e.g., PVC, acrylonitrile–butadiene–styrene [ABS], and epoxy). Altogether 26 plastic products were leached in deionized water (3 days at 50°C), and the water phases were tested for acute toxicity to *Daphnia magna*. Initial Toxicity Identification Evaluations (C18 filtration and EDTA addition) were performed on six leachates.
Results For eleven leachates (42%) 48-h EC50s (i.e the concentration that causes effect in 50 percent of the test organisms) were below the highest test concentration, 250 g plastic/L. All leachates from plasticized PVC (5/5) and epoxy (5/5) products were toxic (48-h EC50s ranging from 2 to 235 g plastic/L). None of the leachates from polypropylene (5/5), ABS (5/5), and rigid PVC (1/1) products showed toxicity, but one of the five tested HDPE leachates was toxic (48-h EC50 17–24 g plastic/L). Toxicity Identification Evaluations indicated that mainly hydrophobic organics were causing the toxicity and that metals were the main cause for one leachate (metal release was also confirmed by chemical analysis).
Conclusions Toxic chemicals leached even during the short-term leaching in water, mainly from plasticized PVC and epoxy products.

Keywords Plastics · Plastic products · Leachates · Toxicity · *Daphnia magna* · PVC · Epoxy

1 Introduction

Plastic materials are extremely versatile and are used in countless number of applications, but dominate in packaging, followed by building and construction, automotive, electrical and electronic sectors, and other sectors such as medical and leisure (PlasticsEurope 2009). The global annual production of plastics has doubled in 15 years, reaching 245 million tons in 2008 (calculated from PlasticsEurope 2009). In Western Europe and the North American Free Trade Agreement countries, the annual plastic material consumption per capita was 100 kg in 2005, which was 10 times as much as in African countries and 5 times as much as in Asian countries (excluding Japan; PlasticsEurope 2009). Plastic products are made from plastic polymers to which additives are added to enable processing and/or to give certain desired properties for a specific application (OECD 2004). There are many different plastic polymers and several thousand different additives. Combining these will create a large variation in chemical composition of plastic products.

The large global consumption, low recycling rates, extensive littering, and persistence of plastic materials

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cause accumulation of plastic products, debris, and microparticles in the terrestrial and marine environments (Barnes et al. 2009). Many substances that are used to produce plastic products are hazardous to human health and the environment, and these, or their degradation products, may be released during the life cycle of a plastic product. The plastic polymer is made by polymerizing monomers and is, because of its large molecular size, not regarded as hazardous. However, several nonpolymeric substances present in the plastic product are hazardous, are of low molecular weight, and are either weakly bound or not bound at all to the polymeric macromolecules and may be released from the plastic product (Crompton 2007; OECD 2004). These include residual monomers, oligomers, low-molecular-weight fragments, catalyst remnants, polymerization solvents, and a wide range of additives (Crompton 2007).

Release of hazardous substances, for example, phthalates, brominated flame retardants, bisphenol A, formaldehyde, acetaldehyde, 4-nonylphenol, and many volatile organic compounds, from plastic products has been shown by chemical analysis (e.g., Tønning et al. 2010; Kim et al. 2006; Brede et al. 2003; Mutsuga et al. 2006; Fernandes et al. 2008; Henneuse-Boxus and Pacary 2003). Usually, these studies are made with extraction tests using extraction fluids to simulate environmental conditions or show potential leaching, or migration tests showing release by diffusion into contact media, or leaching tests showing release into water, or emission tests showing release to air. Toxicity studies mainly of rodents exposed to plastic materials by oral route have been compiled by Sheftel (2000). Few leaching tests with toxicity tests have been performed on plastic products. Previously, Lithner et al. (2009) studied toxicity of leachates from 32 plastic products and found acute toxicity to *Daphnia magna* in the plasticized PVC and polyurethane leachates. Wagner and Oehlmann (2009) cultivated mudsnails in polyethylene terephthalate (PET) mineral water bottles and found evident endocrine disrupting effects, a more than doubled reproductive output compared with cultivation in borosilicate Erlenmeyer flasks.

The aims of this study were to determine and compare the acute toxicity of leachates from plastic products made of plastic types which have the highest global annual production, or are composed of hazardous monomers, and to identify which class of compounds (cationic metals or hydrophobic organics) that is causing the toxicity.

A screening approach with short-term leaching and acute toxicity test was chosen to be able to screen many different products of varying chemical composition with unknown and varying toxic responses. *D. magna* was chosen as a test organism because it is a sensitive species, is suitable for laboratory testing and screening purposes, and has been and is widely used in regulatory testing of chemicals and because there are reliable standardized methods available.

The studied plastic types, their global annual production, and main applications (Gnanou and Fontanille 2008; calculated from PlasticsEurope MRG 2008; Platt 2003; Chanda and Roy 2008; Fink 2005; Vasile and Pascu 2005) were as follows:

- Polypropylene (PP; 45 million tons) used as fibers and filaments, packaging film, and in medical applications,
- High-density polyethylene (HDPE; 32 million tons) used as pipes and pipe fittings for water and petroleum tanks, cable insulation, bottles, and wrapping materials for food, containers, and toys,
- Polyvinyl chloride (PVC; 36 million tons) used as pipes and fittings (rigid PVC) and cable insulation, films, and sheets (plasticized PVC),
- Acrylonitrile–butadiene–styrene (ABS), terpolymer (5–8 million tons), used in automotive instrument panels and consoles, as well as in housings for consumer products (e.g., TVs, audio equipment, and vacuum cleaners),
- Epoxy (0.8 million tons) coatings for heavy corrosion protection and chemical resistance, constituent in paints, white ware, and automotive and naval sectors.

2 Methods

The plastic products were selected to be made of (1) plastic types with the largest global annual production, that is, PP, polyethylene (e.g., HDPE) and PVC, or (2) plastic types composed of hazardous monomers, for instance, PVC, ABS, and epoxy. PVC fits into both criteria. A total of 26 newly bought plastic products were studied, 5 each of the five plastic types and 1 rigid PVC type (Table 1).

The plastic products were cut into 2×2-cm pieces, except for the epoxy products that consisted of an epoxy resin and a curing agent mixture. The resin and curing agent were blended in the specified quantities and were cured as thick (0.3–1.4 cm) layers onto washed plain microscope slides made of pure glass. No washing of the products was made prior to testing. Duplicates were made for each product and were placed in glass vessels (borosilicate beakers or flasks). Deionized water (pH 7, ultrapure water; Elgastat Maxima, HPLC; Elga Ltd, High Wycombe Bucks HP14 3HJ England) was added to give a leachate concentration of 250 g plastic per liter of deionized water, equivalent to a liquid-to-solid ratio (L/S) of 4. Plastics with lower density than water were immersed by a washed microscope glass slide. The vessels were covered with glass Petri dishes and were incubated in darkness at 50°C for 3 days. An alternative batch leaching method, previously described by Lithner et al. (2009), was used in five of the products. In this method, the bottles were placed on a horizontal shaker table (Gerhardt Schüttelmaschine RO 20; Gerhardt GmbH, Bonn). Shaking (90 rpm), to

enable the plastic pieces to move freely in the water, and leaching was performed for 24 h at room temperature (20°C ±2°C) in darkness. Two of the products were tested using both leaching methods.

The plastic pieces were removed from the water phase prior to toxicity testing. The acute toxicity of the leachates was determined for *D. magna* according to ISO (1996). For each sample, 50 mL leachate was added to a Petri dish, and appropriate amounts of stock solutions for ISO standard reference water (SRW) were added to give a hardness of 250 mg CaCO₃/L and a pH of 8. After 1-h equilibration, 20 newborn (0–24 h) *D. magna* were added, and immobility was recorded after 24 and 48 h. For leachates with >50% immobility, dilution series were prepared and used for toxicity testing. Effective concentration 50% (EC50) is the concentration which causes effect in 50 % of the test organisms. The 24- and 48-h EC50s for *D. magna* were determined according to Peltier and Weber (1985).

For the toxic leachates, initial Toxicity Identification Evaluations (TIE), phase I, based on US EPA (1991) methods were conducted to characterize classes of toxicants. This was made by solid-phase extraction with C18 cartridges to remove hydrophobic organics and by EDTA addition (10 μL 0.01 M to 10 mL; final concentration 10 μM) to complex bind cationic metals. The C18 cartridges (Sep. Pak®Plus from Waters; Milford, MA, USA) were conditioned with 15 mL methanol, followed by 15 mL of deionized water and 15 mL of standard reference water. Then, 10 mL SRW was passed though the cartridge and was used as a negative control. Finally, 10 mL of leachate was passed through the cartridge, and this filtered leachate, as well as the negative control, was tested for toxicity. Toxicity tests were performed as described above, but with immobility only recorded after 24 h and with only 10 daphnids exposed in smaller glass vessels.

Parallel with every test run, a positive control test with the reference compound potassium dichromate (K₂Cr₂O₇) was performed. In addition, every test run was accompanied with three to five negative controls with deionized water fortified with ISO (1996) SRW stock solutions. After termination of toxicity tests, pH was determined in the leachates.

A new leachate of the PVC gloves, made on a new set of gloves from the same package (250 g laboratory gloves/L deionized water), and a control containing deionized water were incubated in glass bottles for 3 days at 50°C. The bottles had been treated for 3 days with hydrochloric acid and rinsed with deionized water prior to incubation. The gloves were removed, and the leachate and control were sent for metal analysis by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) to Eurofins Environment AB, Lidköping, Sweden.

3 Results and discussion

The observed toxicity of leachates from plastic products is presented below and is discussed and interpreted in relation to the results from TIE analyses and/or available information on chemical composition and hazardous properties.

3.1 Acute toxicity

The 24-h EC50s for potassium dichromate for *D. magna* ranged from 0.8 to 0.9 mg/L, and measured pH values ranged from 7.4 to 8.0, in accordance with ISO (1996). No immobility over 10% was observed in the negative controls with standard reference water, with one minor exception. In one of the test runs, the immobility in the controls was slightly elevated, at most 20%. However, because the immobility in 30 leachate dilutions made simultaneously in this test run were below 10%, the results from this test run were considered valid. These results confirm that the tests were valid according to ISO (1996), with the negligible exception.

All concentrations presented refer to the solid (plastic)-to-liquid ratios during the leaching period, prior to removal of plastic products and toxicity tests. The concentrations for the leachates are expressed as a solid to liquid ratio×1,000, which means that 250 g plastic/L is actually 250 g plastic material in 1 L of deionized water (i.e., S/L=0.25 or L/S=4).

Eleven (42%) of 26 plastic products had leachates with 48-h EC50s below 250 g plastic/L. For the remaining 15 products, no acute toxicity was seen at the highest test concentration (48-h EC50 >250 g plastic/L). All tested products with their 48-h EC50s are presented in Table 1. The toxic products were in descending order of toxicity: laboratory gloves, super epoxy, marine epoxy, a boat fender, a watering can, quick epoxy, arm pads for floating, a rain poncho (batch test), laminating epoxy, epoxy putty, the same rain poncho (diffusion test), and a wet room wall carpet. All plasticized PVC and epoxy products were toxic and had 48-h EC50s between 2 and 235 g plastic/L. One rigid PVC product (a window frame) was tested but did not show any toxicity. Among the PVC products, the laboratory gloves and the boat fender were most toxic, and among the epoxy products, the super epoxy and the marine epoxy were most toxic. None of the tested products of PP and ABS products showed acute toxicity at the highest test concentration (250 g plastic/L), and only one of the five tested HDPE products was toxic (48-h EC50 17–24 g plastic/L).

The surface area exposed during leaching may be of great importance for the outcome of leaching of chemical substances, since leaching mainly occurs from the surface. Toxicity can be related to surface area. However, the very diverse shapes, thickness, and densities, as well as the cutting of the plastic products, made it difficult to obtain

Table 1 Tested plastic products, plastic types, and their 24-h and 48-h EC50s with confidence intervals

No.	Plastic product	Plastic type	Surface area (≈cm²/L)	24-h EC50 (g/L) (95% confidence interval)		48-h EC50 (g/L) (95% confidence interval)	
				Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	Bucket (5 L)	PP	M	>250	>250	>250	>250
2[a]	Food container for micro-oven	PP	M	>250	>250	>250	>250
3	Plastic bag clips	PP	M	>250	>250	>250	>250
4[a]	Plate cover for micro-oven	PP	M	>250	>250	>250	>250
5	Toolbox	PP	M	>250	>250	>250	>250
6	Watering can	HDPE	1450	111 (96–∞)[b]	103 (0–∞)[b]	**17** (0–83)	**24** (0–84)
7	Oil measuring jug (1 L)	HDPE	M	>250	>250	>250	>250
8[a]	Sports bottle	HDPE	M	>250	>250	>250	>250
9[a]	Tarpaulin	HDPE	L	>250	>250	>250	>250
9	Tarpaulin	HDPE	L	>250	>250	>250	>250
10	Winter sport slide patch	HDPE	M	>250	>250	>250	>250
11	Window frame	Rigid PVC	M	>250	>250	>250	>250
12	Laboratory gloves	Plasticized PVC	350	5 (4–6)	7 (5–9)	**2** (1–3)	**4** (3–5)
13	Boat fender	Plasticized PVC	M	97 (69–149)	67 (49–95)	**21** (0–120)	**11** (0–60)
14	Arm pads for floating	Plasticized PVC	3560	180 (138–270)	≈270	**79** (53–109)	**70** (49–94)
15[a]	Rain poncho	Plasticized PVC	29,300	≈250	225 (0–∞)	**52** (0–182)[b]	**81** (0–225)
15	Rain poncho	Plasticized PVC	29,300	≈260	≈260	**172** (123–201)	**160** (0–∞)
16	Wet room wall carpet	Plasticized PVC	3,410	>250	>250	**235** (182–597)	**219** (175–398)
17	Camping light casing	ABS	M	>250	>250	>250	>250
18	Fishing rod holder	ABS	M	>250	>250	>250	>250
19	Flashlight	ABS	M	>250	>250	>250	>250
20	Lego bricks	ABS	M	>250	>250	>250	>250
21	Measuring-tape casing	ABS	M	>250	>250	>250	>250
22	Super epoxy (filler)[c]	Epoxy	5	15 (12–17)	3 (2–4)	**10** (8–13)	**2** (0–4)
23	Marine epoxy (filler)[c]	Epoxy	20	13 (10–16)	13 (10–17)	**9** (7–11)	**6** (5–8)
24	Quick epoxy glue[c]	Epoxy	325	>90 (25% effect)	>70 (25% effect)	**44** (36–60)	**39** (30–55)
25	Laminating epoxy[c]	Epoxy	190	>80 (15% effect)	≈80	> **80** (25% effect)	**27** (21–33)
26	Epoxy putty (sealant)[c]	Epoxy	50	225 (56–∞)	181 (136–318)	**114** (98–134)	**99** (74–127)

No superscript lowercase letter, in the No.-column, means diffusion leaching procedure, 3 days at 50°C in darkness. Values in bold indicate products with 48-h EC50s below the highest test concentration (250 g/L). Surface area: S, small; M, medium; L, large

[a] Batch leaching procedure with 24-h shaking in darkness

[b] Uncertain values, very varying

[c] Cured epoxy resin

accurate measurements and calculations of the surface area. No adjustments were made to give equal surface areas during leaching. The products with no EC50s at the highest test concentration were roughly classified into small, medium, or large surface area, as shown in Table 1. The toxicity was mainly related to weight as shown in Table 1.

If the toxicity is related to surface area to show how many square centimeters of the product in 1 L of water that causes 50% immobility for *D. magna*, the order of toxicity for the toxic products is changed. Roughly estimated, super epoxy was the product where toxicity was caused by the smallest surface area, that is, 5 cm^2/L at the 48-h EC50 concentration, followed by marine epoxy (20 cm^2), epoxy putty (50 cm^2), fender (110 cm^2), laminating epoxy (190 cm^2), quick epoxy (325 cm^2), laboratory gloves (350 cm^2), watering can (1,450 cm^2), wet room wall carpet (3,410 cm^2), arm pads for floating (3,560 cm^2), and rain poncho (29,300 cm^2) (Table 1).

For the majority of the toxic leachates, toxicity increased with increasing exposure time (24 h compared with 48 h; see Table 1), but the 24-h/48-h EC50 ratios varied greatly between the products (ranging from 1.1 to 6.5), which suggests that the responsible toxicants had different accumulative properties (log P values).

In most cases, the replicates showed consistent results, with replicate 48-h EC50s within a factor of 2 (Table 1). This is less consistent than for tests with water soluble toxicants, but quite acceptable considering the possible heterogeneity of a plastic product where nonpolymeric substances may be unevenly distributed in the product and that the replicate was made on the product (not the leachate). The difference between replicates was larger for laminating epoxy, and super epoxy (Table 1), and may be caused by variability in the curing process.

3.2 TIE

TIEs were conducted with four PVC leachates and two epoxy leachates. The results are shown in Fig. 1. Due to lack of leachate water, not all of the toxic leachates could be tested, and the number of manipulations had to be limited to two, that is, C18 and EDTA. For all four PVC products, there was a decrease in toxicity from both manipulations, meaning that both hydrophobic organics and cationic metals may have caused toxicity. For the arm pads, the toxicity was completely eliminated after C18 filtration, indicating that hydrophobic organics were the main cause of toxicity. Also, for the boat fender, the C18 filtration reduced the toxicity significantly. For the rain poncho, there was a slight reduction after C18 filtration and a significant reduction for one of the replicates after the EDTA addition. For the laboratory gloves, the EDTA treatment was responsible for the major reduction in

toxicity, indicating that cationic metals were the primary cause of toxicity. EDTA typically forms relatively nontoxic complexes with Al^{3+}, Ba^{2+} Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sr^{2+}, and Zn^{2+} (US EPA 1991).

For both epoxy products, the toxicity was completely eliminated after C18 filtration, which means that hydrophobic organics are the likely cause of toxicity. For the super epoxy leachates, there was also a slight reduction after the EDTA addition, indicating that metals may have been responsible for some of the toxicity. Since not enough leachate was available for super epoxy (replicate 2), a lower concentration had to be used.

When performing the EDTA chelation test, the EDTA concentration should be optimal, high enough to complex bind metals but low enough not to cause toxicity (US EPA 1991). In this test, the final test concentration of EDTA may have been too low (only 10 μM), and the toxicity caused by cationic metals may have been underestimated. Further TIE studies and chemical analyses are needed to determine which toxicants are involved.

3.3 Chemical composition, hazardous substances, and chemical analysis

For plastic products in general, there is no declaration of composition, and most commonly, not even the type of plastic is specified. The composition of the plastic product is usually only known to the supplier to some extent. Even for the epoxy products, which were uncured chemical products and, consequently, had safety data sheets, only around 50% of the ingredients were specified, and even if there is information on ingredients, the accuracy is not always optimal. For instance, 4-nonylphenol was declared on the product labels as an ingredient for super epoxy and quick epoxy, but not on the safety data sheets. According to the supplier, 4-nonylphenol had been substituted several years ago, but the printed product labels had not been updated. The known compositions for the tested products, as well as environmental and health hazard information associated with the plastic types and ingredients, are presented in more detail in Electronic Supplementary Material.

3.3.1 PVC products

PVC is made from vinyl chloride and is the plastic type that requires, by far, the most additives, accounting for 73% of the world production of additives by volume (Murphy 2001). Heat stabilizers are required to prevent the polymer from degrading during processing (Jennings and Starnes 2005). In plasticized PVC, plasticizers in amounts up to 60 percentages by weight can be used, but most commonly, 35–40 wt% is used (Navarro et al. 2010). The plasticizers are not bound chemically to the polymer
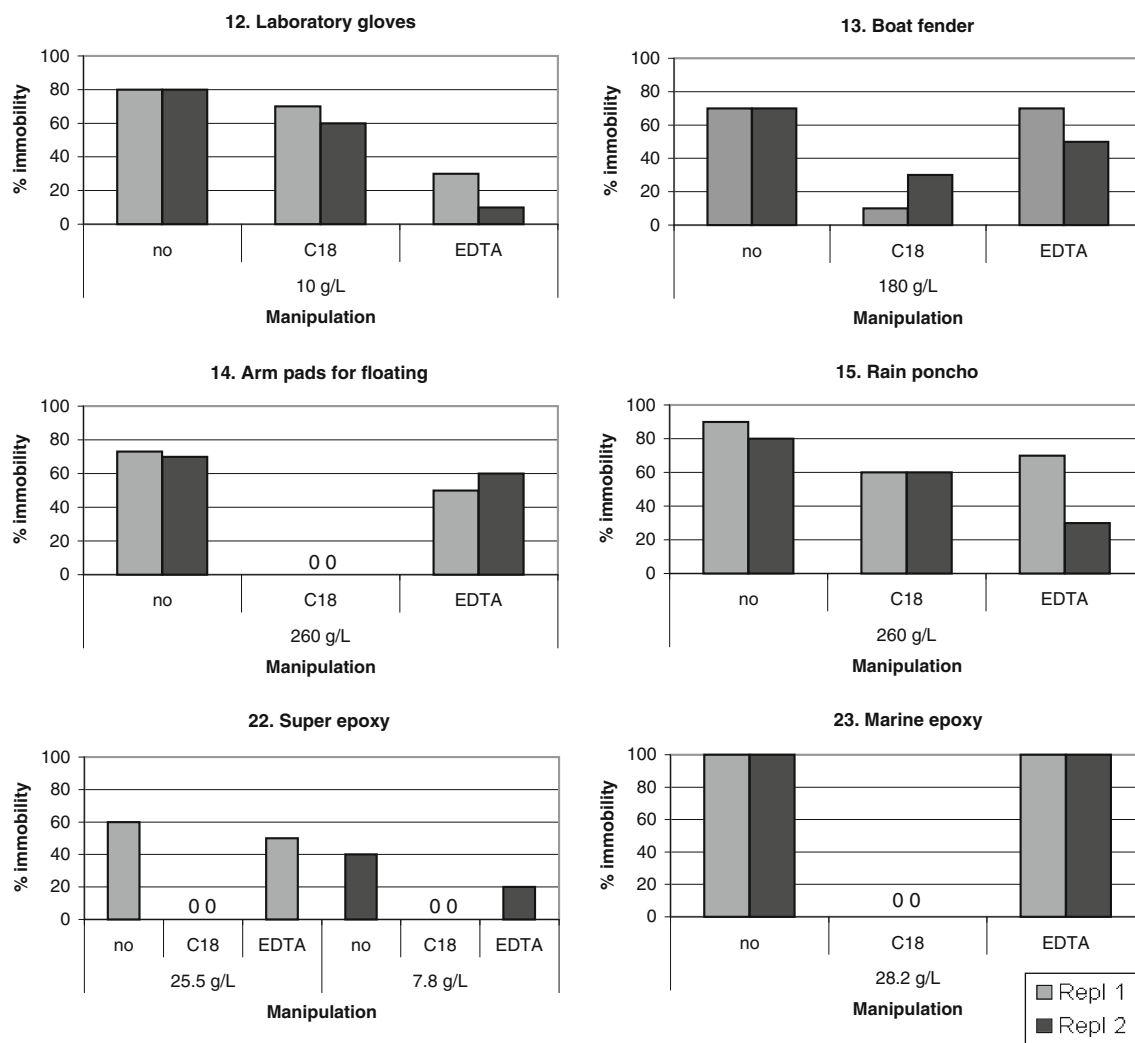
Fig. 1 TIE on leachates from laboratory gloves, boat fender, arm pads for floating, rain poncho, super epoxy, and marine epoxy. Manipulations include filtrations through C18 resin (removing hydrophobic organics) and EDTA addition (cationic metal complexation), respectively. Immobility (%) of *D. magna* after a 24-h exposure is presented for replicated leachates with and without manipulations

and are capable of migrating from the plastic material (Patrick 2005).

According to the manufacturer, the PVC laboratory gloves were plasticized with diisononyl phthalate (DINP) and calcium/zinc stabilizers were used as heat stabilizers (personal communication). The results from the metal analysis of the reproduced leachate from the gloves are shown in Table 2. Only the elements that were higher in the leachate than in the control are presented in the table. Those substances that increased the most compared with the control were Ca, Zn, S, Sn, and Na. It is likely that the Ca and Zn came from the heat stabilizers. Of the metals released, especially Zn could explain part of the toxicity seen. Zinc is very toxic to aquatic organisms. The lowest EC50 for *D. magna* reported for zinc is 70 μg/L, according to the OECD (2005) SIDS Initial Assessment Profile, and the highest test concentration (250 g glove/L) contained

1,600±480 μg Zn/L (Table 2). In the EC50 concentration, the leachate was diluted (a factor 0.016), so the resulting Zn concentration does not alone explain the toxicity in the EC50 concentration but may do so in the higher concentrations. In leaching and toxicity test performed on rubber products, that is, tires (Wik et al. 2009) and shoe soles (Ingre-Khans et al. 2010), zinc was detected as a major inorganic toxicant. Some of the other metals, especially tin (Sn), released from the gloves may also have contributed to the toxicity (Table 2). The TIE also indicated that hydrophobic organics were responsible for some of the toxicity. DINP is a likely candidate with a 48-h EC50 to *D. magna* reported to be ≥0.088, ≥1, >74, and ≥500 mg/L (European Commission 2003). There are far more hazardous alternatives to use for PVC than zinc/calcium stabilizers and DINP plasticizers, which are considered to be among the least hazardous ones.

Table 2 Concentrations of elements in laboratory glove leachate (250 g/L) that were higher than the corresponding control (deionized water)

Ag, Be, Fe, K, Li, Mg, Sb, Se, Ti, Tl, and U were under detection limit for both leachate and control. Values in bold indicates elements with >100 times higher concentration in leachate compared with control

Element	Leachate (μg/L)	Control (μg/L)	Measurement uncertainty (±%)	Factor of increase (leachate/control)	Method
Al	19	<1	20	>19	ICP-MS
As	0.76	<0.2	15	>3.8	ICP-MS
Ba	2.1	<1	25	>2.1	ICP-MS
B	73	5.4	25	13.5	ICP-MS
Ca	4,400	<0.2	10	**>22,000**	ICP-AES
Cd	0.054	<0.02	15	>2.7	ICP-MS
Cr	0.42	<0.2	10	>2.1	ICP-MS
Cu	4.3	<0.2	25	>21.5	ICP-MS
Mn	1.6	<0.2	10	>8	ICP-MS
Mo	2.8	<0.5	25	>5.6	ICP-MS
Na	55,000	<500	15	**>110**	ICP-AES
Ni	0.54	<0.2	15	>2.7	ICP-MS
Pb	0.44	<0.05	10	>8.8	ICP-MS
Si	17,000	<500	10	>34	ICP-AES
Sn	**18**	<0.16	15	**>112.5**	ICP-MS
Sr	1.8	<1	15	>1.8	ICP-MS
S	**47,000**	<100	15	**>470**	ICP-AES
V	0.71	<0.2	20	>3.6	ICP-MS
Zn	**1,600**	<1	30	**>1,600**	ICP-AES

For the PVC wet room wall carpet, DINP was used as plasticizer, and barium–zinc soaps, and epoxidized soya bean oil were used as stabilizers (Supplier, personal communication). The arm pads for floating should, according to the supplier, be free of phthalates, but not further specified.

3.3.2 Epoxy products

Epoxy products are made by curing an epoxy resin, usually a reaction product of bisphenol A and epichlorohydrin (DGEBPA epoxy resin), with a hardener, most commonly aliphatic or aromatic amines (Gnanou and Fontanille 2008). The chemical composition of the tested epoxy products has been double checked with the suppliers (personal communication). All of the five epoxy products are made of a reaction product of bisphenol A and epichlorohydrin (DGEBPA epoxy resin). Bisphenol, A has in other studies, been shown to leach into canned food from the epoxy lining in the cans (Sajiki et al. 2007; Geens et al. 2010). The curing agent mixture that was blended with the DGEBPA resin to get the cured resin was specific for each product (see Electronic Supplementary Material, Table A). Uncured residuals of resins and curing agents are especially likely in "do-it-yourself" products, since it is difficult to obtain proper mixing and exact proportions of resin and curing agent. Uncured residuals are a possible cause of the toxicity seen in the epoxy product leachates.

3.3.3 PP, polyethylene, and ABS products

Neither of the monomers (propylene and ethylene) used to produce PP and polyethylene are hazardous. Which type of substance that caused the toxicity in the watering can (made of HDPE) is unknown. However, the present study shows that also plastic products based on polymers made from nontoxic or low toxic monomers (e.g., ethylene) can leach enough additives to cause acute toxicity. The monomers needed to produce ABS are acrylonitrile, butadiene, and styrene. ABS products may release both toxic residual monomers and/or additives, but in this study, possible releases were not high enough to cause acute toxicity.

3.4 Factors increasing release of nonpolymeric substances

The migration (or diffusion) potential of substances from a plastic product is controlled by many factors besides the content of nonpolymeric substances. These factors include: permeability of the polymer matrix; the size of gaps between polymer molecules compared with size of migrant; the size, solubility and volatility of migrant; temperature; and the type of surrounding medium (Brydson 1999; Sheftel 2000). The size of the gaps depends on whether the polymer's physical state is glassy, rubbery (amorphous), or crystalline (Brydson 1999). A more rubbery polymer has larger gaps than a crystalline polymer, which has a greater degree of molecular packing (Brydson 1999). In a crystalline polymer, migration

only occurs from the amorphous zones or zones of imperfection, and the migration potential is lower (Brydson 1999). In plasticized PVC, the gaps between the polymer chains are large, which favors migration (Godwin and Krauskopf 2008). In addition, the concentration of possible migrants is also higher in plasticized PVC compared with other plastic types, meaning that larger amounts of nonbound substances may leach. This was also seen in this study and the previous study by Lithner et al. (2009), where the toxic leachates came from plasticized PVC and flexible polyurethane (artificial leather), and not from rigid PVC window frame (this study) and rigid PVC pipes (previous study).

3.5 The significance of sample preparation and leaching method

The influence of the leaching procedure (temperature, mechanical shaking, and time) was studied for two products, the rain poncho and the tarpaulin, and there was some difference in the results. The higher EC50 for the rain poncho at a longer leaching time (72 h versus 24 h) and higher temperature may be due to slower release or increased volatilization and/or hydrolysis of toxic compounds (Table 1). This implies that the leaching procedure with diffusion for 3 days at 50°C compared with shaking for 24 h in room temperature may slightly underestimate leachate toxicity for some compounds, whereas the opposite could be true for other compounds, and that the choice of leaching procedure is important for the outcome. These leaching methods with only deionized water do not show the potentially leachable fraction, as extraction tests do, but show the test specific release. Under real environmental conditions, the different pH and ionic compositions in different environments affect the leaching and toxicity. For example, a lower pH in a landfill may increase leaching for some substances, for example, metals. The pH of the deionized water was 7.0 (resistivity 18.2 Ωcm equal to a conductivity of 0.055 μS/cm), which could represent the pH of a lake or river (that is neither acidic nor alkaline), and the pH of the leachate during the toxicity test, 8.0, could represent the pH in sea water (which has an average around pH 8; Garrison 2007). Because of the mild leaching conditions (as opposed to, e.g., extraction tests), release of chemicals was expected to predominate from the surface layer. By not washing the plastic products prior to leaching, the effect from chemicals released from the plastic surface layer could be measured. However, with this approach, there is a slight risk that contaminants not related to the plastic product, which may have been present, could also have been released. To investigate leaching over time, sequential leaching tests would be valuable.

The major reason for using 3-day leaching was that it was convenient; leaching can be initiated on Friday, and toxicity testing (screening, EC50 determination, and TIE) can be performed Monday to Friday. The leaching temperature of 50°C was chosen because it was suspected that leaching might be increased if the temperature was increased and because it was regarded as a realistic surface temperature that plastic products may have in some applications. During outdoor exposure to sunlight, the surface temperature of a dark plastic material may be as much as 60°C higher than the surrounding air, and a comparison between black and white PVC foils showed a 15°C −23°C higher surface temperature for the black foils (Wypych 1999). The diffusion procedure allowed more flexibility in the shape and size of leaching beakers compared with the batch leaching procedure.

Only laboratory ware made of glass was used during the leaching and toxicity tests in order to minimize the risk of adsorption of hydrophobic substances. Many of the non-polymeric substances are volatile organic compounds (VOCs), and evaporative loss of VOCs is difficult to prevent during the leaching test (especially in tests using shaking methods) and during the preparation of dilution series for the toxicity tests. Emission studies have shown that a wide range of VOCs are emitted to air from plastic products (Henneuse-Boxus and Pacary 2003).

3.6 Acute toxicity compared with other toxic effects

This study shows the acute toxicity from plastic products leached in water, and this end point requires that large amounts of chemicals are leached during a short period of time. Immobility often occurs as a consequence of toxic substances affecting the nervous system. Many other toxic effects, for example, carcinogenicity, mutagenicity, reproductive toxicity, sensitization, and chronic aquatic toxicity, as well as endocrine disruption, are highly relevant and even more common for many of the chemicals used for producing plastic polymers (Lithner et al. 2011). Leaching of chemical substances from plastic materials is also likely to occur in low concentrations under a prolonged time period and, in many cases, also under the influence of degradation. However, acute toxicity tests can be used for screening purposes to identify some of the toxic products but should not be used alone to conclude that a certain plastic polymer type is nontoxic.

The study, however, shows that such large amounts of toxic compounds may leach from some products to cause acute toxicity, even during short term leaching with deionized water (pH 7). The findings of this study also corresponds with the findings from the previous study by Lithner et al. (2009) that plasticized PVC product leachates were among the most toxic leachates, and that the toxicity from the products was mainly caused by hydrophobic organics and to a lesser extent by metals. Based on these studies acute toxic leachates seems to be more frequent

from products of plasticized PVC and epoxy. From these results it is not possible to assess the risks for human exposure, since that would require chemical analyses in order to know which substances and quantities that were emitted, and which risks that would be expected.

4 Conclusions

Even during short-term (24–72 h) leaching in water plastic products can leach toxic substances that cause acute toxicity. All plasticized PVC (5/5) and epoxy (5/5) products leached chemicals that were acutely toxic to *D. magna*. The toxicity characterization (TIE, phase 1) suggested that the toxicity for four of the toxic products were mainly caused by hydrophobic organics and that cationic metals were the main cause of toxicity for one product for which metal release also was confirmed by chemical analysis. For PP (5/5), ABS (5/5), and rigid PVC (1/1) plastics, no leachate toxicity was seen at the highest test concentration (250 g plastic/L), whereas the leachates from one of the five HDPE product leachates caused toxicity. Additional chronic studies are needed to study other effects of released chemicals from plastic products.

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