Plasticizer metabolites in the environment

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Abstract

Earlier work with pure cultures had shown that the interaction of microbes with plasticizers leads to the formation of metabolites including 2-ethylhexanoic acid and 2-ethylhexanol that resist further degradation. The presence of these metabolites is now reported in a variety of environmental samples. Thus, even in a complex ecosystem, when plasticizers are degraded, the breakdown is not complete and significant amounts of 2-ethylhexanoic acid and 2-ethylhexanol are observed. These compounds have been shown to exhibit acute toxicity using Microtox, \textit{Daphnia}, rainbow trout and fathead minnow toxicity assays. Since it is already well established that plasticizers are ubiquitous in the environment, it is expected that their recalcitrant metabolites will also be ubiquitous. This is of concern because, while the plasticizers do not exhibit acute toxicity, their metabolites do.

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1. Introduction

Plasticizers are clear, colourless, oily liquids that are used to impart properties such as flexibility and workability to plastics. They are widely used in many products including medical equipment, food film, upholstery, flooring, moldings, gaskets, piping, rainwear, electrical wire insulation, auto trim and undercoating, pool and pond liners and roofing systems. Plasticizers are also used in other applications such as paints in which special coating properties are required. Polyvinyl chloride (PVC) is an excellent example of the magnitude of the industrial importance of plasticizers. The annual production of PVC is in the tens of millions of tons of which plasticizers can account for up to 40\% by weight (Mersiowsky et al., 2001). The most common plasticizer is di (2-ethylhexyl) phthalate (DEHP) whose production is estimated at 1.4 million tonnes per year (Bauer and Herrmann, 1997).

It has been demonstrated that plasticizers tend to leach from solid polymer matrices into the environment (Fromme et al., 2002). Numerous studies have confirmed the presence of plasticizers in air, soil and water samples to such an extent that they are now described as being ubiquitous in the environment (Fromme et al., 2002; Staples et al., 1997). Because DEHP is used so extensively, many studies have been conducted to evaluate its degradation within the environment using soil organisms and in a laboratory setting using pure and mixed cultures (Cartwright et al., 2000; Cassidy and Irvine, 1999; Ejlertsson et al., 1997; Gejlsbjerg et al., 2001; Marttinen et al., 2003; Roslev et al., 1998; Scholz et al., 1997). These earlier researchers had reported that

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DEHP is either recalcitrant or is mineralized (Cartwright et al., 2000; Marttinen et al., 2003). However, it has recently been shown that the exposure of common plasticizers including DEHP and di-2-ethylhexyl adipate (DEHA) to pure cultures of soil microorganisms under laboratory conditions resulted in the production and accumulation of toxic metabolites (Nalli et al., 2003, 2004). Because millions of tonnes of plasticizers are incorporated into plastics every year and are ultimately released to the environment, the objective of this work was to establish whether the toxic metabolites are also observed in the environment.

2. Experimental

2.1. Reagents

DEHP (99%), di (2-ethylhexyl) adipate (DEHA, 99%), 2-ethylhexanol (99% +) and 2-ethylhexanoic acid (99%) were purchased from Sigma Aldrich (St. Louis, MO). Lab grade acetone, HPLC grade water, HPLC grade chloroform and hydrochloric acid were purchased from Fischer Scientific (Montreal, QC).

2.2. Toxicity studies

The Daphnia (Daphnia magna) and the rainbow trout (Oncorhynchus mykiss) toxicity assays of reagent grade 2-ethylhexanol and 2-ethylhexanoic acid were conducted by Bodycote Material Testing Canada (Pointe-Claire, QC, Canada). The lethal concentrations for 50% mortality (LC50) were obtained by exposure to serial dilutions (100%, 50%, 25%, 12.5% and 6.25% by volume) of solutions of 300 mg/L 2-ethylhexanol or 1700 mg/L 2-ethylhexanoic acid. These solutions were prepared with the pure reagents in de-chlorinated municipal water. The LC50 was calculated using the mortality after a 48 h period for the Daphnia and a 96 h period for the rainbow trout.

The pure reagents were sent to Bodycote Material Testing Canada (Ste- Foy, QC, Canada) for the fathead minnow (Pimephales promelas) toxicity assay. Solutions of 15 mg/L 2-ethylhexanol and 86 mg/L 2-ethylhexanoic acid in carbon filtered municipal water with 88 mg/L CaCO3 were prepared. The same serial dilutions noted above were tested to obtain the 25% inhibition concentration (IC25) representing the concentration that would elicit a decrease in 25% of the organism’s body weight over the course of a 7-day study. The IC25 was estimated using linear interpolation.

Acute toxicity of these compounds based on the 5 min Microtox toxicity assay has been previously reported (Nalli et al., 2003). The assay involved the short-term incubation of the putative toxic compound with the luminescent marine bacterium Photobacterium phosphorum. The concentration of toxicant that caused a 50% decrease in light output is reported as the effective concentration, EC50, and is presented as percent by volume of the whole product mixture. The toxicity of a sample increases as the EC50 decreases.

2.3. Sample collection and preparation

Samples were selected to reflect a wide range of water quality from pure to highly contaminated. All the samples were collected in 10 L Pyrex® glass reagent bottles (Fisher Scientific, Montreal, QC). Before sampling, the bottles were rinsed three times with lab grade acetone, distilled water, and HPLC grade chloroform for a total of nine washes. Aluminum foil was used as a barrier between plastic screw tops and the samples to avoid potential contamination of samples with plasticizers. The bottles were stored at 4°C. Sediment and snow samples were collected using 200 mL glass bottles, rinsed as described above, and then transferred to 10 L reagent bottles. All samples were collected manually as grab samples.

Undisturbed snow was sampled from a green space in downtown Montreal within 5 h of falling. The St. Lawrence River site was at the downstream end of the island of Montreal. River samples, including sediment samples, were taken within 2 m of the shoreline. Water samples were drawn from a creek that drains an industrial area on the Island of Montreal and passes through parkland (Bois-de-Liesse Park, Montreal, QC). Landfill leachate was also collected from a landfill located inside the boundaries of the city of Montreal. The Miron landfill site was chosen to provide an example of the type of contamination that could be generated from typical urban waste. The sample was collected through a sampling port on a pipe that delivered the leachate that had been collected from the landfill to an aeration basin. A sample of HPLC-grade water from an unopened bottle was used as a control.

The pH of the samples was reduced to 1.5 using 10 M HCl and then the samples were extracted twice using 100 mL of HPLC-grade chloroform. The chloroform from the combined extracts was evaporated and the residue was re-dissolved in 1 mL of chloroform. Snow was melted and then extracted using the same procedure. The river sediment samples were extracted using 100 mL of HPLC grade chloroform in a Soxhlet extraction apparatus. The chloroform extract was prepared as above.

Tap water was sampled continuously from the Montreal water distribution system. A total of 44.3 L, at a flowrate of 1 L/h, was passed through 100 mL of HPLC grade chloroform. The chloroform extract was then prepared as above.
2.4. Gas chromatography/mass spectrometry

A Varian CP-3800 gas chromatograph with an FID detector was used to quantify the concentrations of DEHP and DEHA. The column used was the CP-Sil 5 CB 15 m × 0.53 mm (Varian, St-Laurent, QC). The settings on the GC were as follows: an injector temperature of 250 °C, an initial column temperature of 40 °C, a hold time 2 min, a first temperature ramp rate of 10 °C per minute to 150 °C and a second temperature ramp rate 20 °C per minute to a final temperature of 300 °C. The detector temperature was 300 °C.

The quantification of the metabolites 2-ethylhexanol and 2-ethylhexanoic acid was performed using a gas chromatograph/mass spectrometer (Thermo Quest model TRACE GC 2000/Finnigan POLARIS), because of its superior sensitivity to these compounds. The GC/MS contained an RTX-5 MS internal diameter 0.25 mm column (Resteek) and the settings on the GC were as follows: an injector temperature of 275 °C, an initial column temperature of 60 °C, a hold time of 1 min, a first ramp rate of 5 °C per minute to 100 °C and a second ramp rate of 20 °C per minute to a final temperature of 340 °C. Calibration curves were obtained for each compound. The 95% confidence limits on the analysis of DEHA, DEHP, 2-ethylhexanol and 2-ethylhexanoic acid were plus or minus 5, 4, 0.1 and 0.4 μg/L, respectively, based on six samples each.

Identification of the compounds was based on a comparison to the GC retention times and the fragmentation patterns from GC- mass spectrometry of plasticizer and metabolite standards.

3. Results and discussion

Plasticizers such as DEHP or DEHA can be degraded by a common soil bacterium in the presence of an easily used carbon source (Nalli et al., 2003). The degradation was not complete and resulted in the production of metabolites including 2-ethylhexanol, 2-ethylhexanoic acid and monoesters such as 2-ethylhexyl phthalate. A proposed pathway for the degradation of DEHP or DEHA is shown in Fig. 1. The final metabolite for both DEHP and DEHA was 2-ethylhexanoic acid, which was observed to be particularly resistant to further degradation.

Initial work using the Microtox assay showed that these metabolites were more toxic than the original plasticizers. Toxicity tests have now been extended to include assays based on various aquatic organisms including fathead minnows, rainbow trout and Daphnia. The results shown in Table 1 confirm that 2-ethylhexanoic acid and 2-ethylhexanol exhibit acute aquatic toxicity. Notably, previous reports have also linked the presence of 2-ethylhexanoic acid to peroxisome proliferation of liver cells in rats and mice, which resulted in tumour growth and death (Astill et al., 1996; Keith et al., 1992; Lhuguenot et al., 1985; Sundberg et al., 1994).

The above results could have important implications with respect to potential environmental impact. However, the production of metabolites by pure cultures would not necessarily lead to their accumulation in the environment if the whole ecology of a site results in further degradation of these compounds. To assess this possibility, a number of sites around the island of Montreal, Canada were sampled. All of the samples were found to contain the most common plasticizers (Table 2), which is consistent with earlier work in which plasticizers were found to be ubiquitous (Fromme et al., 2002; Staples et al., 1997). Neither plasticizers nor any of the possible metabolites were detected in the control sample.

Most of the environmental samples also contained one or more of the metabolites, which is consistent with recent studies of the degradation of these plasticizers using pure cultures (Nalli et al., 2003). This work had shown that 2-ethylhexanoic acid was a very intractable metabolite and, thus, it was not surprising to find appreciable amounts of it in the environment. However, it was surprising to find that these samples also contained significant amounts of the less stable metabolite, 2-ethylhexanol. This is important because the alcohol has a higher acute toxicity than the acid (Table 1). This was unexpected because biological systems have been shown to be able to easily oxidize alcohols including 2-ethylhexanol to the corresponding carboxylic acids (Cheung et al., 2003; French et al., 2002).

River sediments had the highest concentrations of the alcohol and acid. This is consistent with the fact that these compounds are relatively hydrophobic and thus likely to partition into the sediments. The concentrations of metabolites in the sediment were so large that it was even possible to observe a monoester, which is the first metabolite in the series shown in Fig. 1. This was surprising because the monoesters were seldom observed even in the laboratory experiments conducted with pure microorganisms and with high concentrations of plasticizers (Nalli et al., 2003).

The samples of snow and tap water were expected to have the lowest concentrations of the compounds of interest. In fact, even these were contaminated. The data for the sample of snow revealed that precipitation can act as a means of introducing plasticizers into surface waters. This sample also contained appreciable amounts of the most intractable metabolite. The tap water contained small amounts of the plasticizers and the acid metabolite. It is probable that this metabolite originated from the degradation of the plasticizers, but it is unknown whether this compound entered the water during processing or distribution or if it came from the water source (St. Lawrence River) and
The samples of the landfill leachate contained plasticizers in detectable amounts, but neither 2-ethylhexanol nor 2-ethylhexanoic acid were detected. Since the production of metabolites has only been observed during aerobic growth, the absence of these compounds in the leachate could be attributed to the anaerobic conditions of the landfill.

The ubiquity of these metabolites and their presence in significant concentrations indicate that these compounds...
compounds resist degradation in environments where mixed microbial populations would be present. There are several recent references, which briefly mention the observation of either the alcohol or the acid in studies involving analyses of samples of precipitation and indoor air. However, the sources of these compounds were not suggested (Balestrini and Polesello, 1999; Sartin et al., 2001; Sunesson et al., 1996). The presence of the 2-ethylhexanoic acid in these and in our own samples substantiates concerns about the fate of the plasticizers in the environment.

It must be acknowledged that there are other possible sources of the alcohol and acid besides the metabolism of plasticizers. For example, they could originate from plasticizer production processes, the use of lubricants and surfactants, as well as the pharmaceutical industry (Staples, 2001). However, it has been claimed that these sources are well contained and that the main sources of exposure would be due to accidental spills (Staples, 2001). These sources could not account for the quantities observed in our studies nor their presence in many different environmental samples. In addition, the mono-ester observed in the sediment sample could only come from the metabolism of the di-ester, since the half-life of DEHP degradation by abiotic alkaline hydrolysis has been reported to exceed 1000 years at neutral pH (Wolfe et al., 1980). Thus, these observations support the argument that the metabolites that were observed in the environment originated from the biodegradation of plasticizers.

4. Conclusions

As plasticizers themselves are ubiquitous and can be partially degraded by common soil bacteria, we conclude that plasticizer metabolites will also be widespread. While the levels of metabolites observed in this study may not result in acute aquatic toxicity, it is likely that their intractability will lead to a tendency for them to persist in the environment.

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