An investigation into possible sources of phthalate contamination in the environmental analytical laboratory

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A study of common laboratory equipment and components was performed in order to identify sources of contamination of phthalates prior to testing environmental samples for such compounds. A screening study revealed significant leaching from plastic syringes, pipette tips released maximum leachings of 0.36 μg cm^{-2} diethylhexyl phthalate (DEHP) and 0.86 μg cm^{-2} diisononyl phthalate (DINP), plastic filter holders produced maximum leachings of 2.49 μg cm^{-2} dibutyl phthalate (DBP) from polytetrafluoroethylene (PTFE), 0.61μg cm^{-2} DBP from regenerated cellulose and 5.85 μg cm^{-2} dimethyl phthalate (DMP) from cellulose acetate and Parafilm® leaching levels up to 0.50 μg cm^{-2} DEHP. In addition, a high temperature bake-out process was found necessary to eliminate quite high levels of two phthalates present in a commercial bulking agent for pressurised liquid extraction (PLE).

Keywords: Phthalates; Contamination; HPLC

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Introduction

According to Harris *et al*. [1], Europe produces an estimated 500,000 tonnes of phthalates per annum, with DEHP being the most commonly used. DMP and DBP have lower molecular weights than DEHP and will partition more from the polymer matrix so are consequently used to a lesser extent. These lower members of the phthalate series, however, tend to be more soluble in water so we would still expect to see significant environmental concentrations of these compounds. An estimated 90% of plasticisers manufactured are used in PVC and a flexible PVC product will contain between 20% and 50% plasticiser. DEHP accounts for 90% of all plasticiser usage in Europe[2]. Martinnen *et al*. [3] found DEHP to be the most abundant occurring pollutant in landfill leachate, with levels of up to 122 $\mu g\ L^{-1}$ DEHP being found and concentrations of other phthalates below 17 $\mu g\ L^{-1}$. Some phthalates have been identified as possible xeno-oestrogens, making them potentially harmful to human reproductive health and possibly playing a role for the development of breast cancer in humans[4].

Exposure to these agents is not confined to landfill run-offs, for instance, Kambia *et al.* [5], demonstrated that a DEHP quantity of 122.95 ± 33.94 mg was leached from PVC tubing during a single dialysis session. In a later study, between 0.8 mg and 2 mg of DEHP was found to leach from PVC bags containing medical fluids[6]. This lead to the suspicion that phthalates could also be leaching from laboratory equipment typically used in environmental analysis. Detections of phthalates have also been reported in diverse media such as reagent bottle liners[7] and in air[8]. Such occurrences have led to the questioning of historical data on phthalate levels by industry trade representatives[9], leading some toxicology researchers to confining their determinations to the metabolites of phthalates[10].

At the ultra-trace level typical of modern environmental analysis the potential for corruption of sample matrices is exacerbated. Furtmann[11] reported a solid-phase extraction (SPE) method prior to analysis by gas chromatography mass selective (GC/MS) detection. In our case we wished to avoid concerns about the blockade of the C_{18} reverse-phase (RP) pores in the extraction material so we used an organic modifier with the twin objective of desorbing phthalates from particulate or suspended matter and also ensuring sample preservation. Subsequent to removal of particulates using HPLC filters we then carried out analysis on the narrow-bore system described below. EPA Method 8061A[12] recommends GC with electron capture detection (GC/ECD) for analysis of phthalates, however, our objective was to develop and validate a sensitive yet simple isocratic HPLC method for determination of trace levels of phthalates without using any sophisticated or expensive equipment such as a mass spectrometer. To date most studies have been carried out using GC/MS although there have been a few gradient methods examined. Using an RP gradient HPLC method combined with SPE for environmental analysis, Jen *et al.* [13], developed a method for the separation of dimethyl-, diethyl- and dibutyl phthalate with respective limits of detection of 12.2, 7.0 and 15.7 µg L^{-1}. Another method developed by De Orsi *et al*. [14], for analysis of phthalates in cosmetics showed LOD values for DBP and DEHP and DINP of 0.4, 0.5 and 0.6 µg mL^{-1} respectively. However, we have developed a method which delivers detection limits following a pre-concentration protocol (magnification factor equal to 2,500) of 19.2, 22.0, 28.4, 52.8, and 51.2 ng L^{-1} for DMP, DBP, DEHP, DINP and DIDP, respectively.

As no prior testing of waterways had been carried out in the Irish Midlands Shannon Catchment preceding this study, a method was developed to quantify levels of phthalates in the nanogramme per litre range. Before testing environmental samples, however, a battery of tests

were run to identify the possible sources of contamination in the analytical laboratory so that these sources could be avoided when carrying out sampling, enrichment techniques and analytical detection. There is a lack of quality control data available on this type of work to date, despite an increasing interest in oestrogenic type contaminants in the environment, and so this paper aims to make the reader aware of some potential pitfalls in analysing for certain types of organic substances.

Experimental

Five phthalates; - 1, 2-benzenedicarboxylic acid dimethyl ester CASRN 131-11-3 (dimethyl phthalate; DMP); 1, 2-benzenedicarboxylic acid dibutyl ester CASRN 84-74-2 (dibutyl phthalate; DBP); 1, 2-benzenedicarboxylic acid bis (2-ethylhexyl) ester CASRN 117-81-7 (diethylhexyl phthalate; DEHP); 1, 2-benzenedicarboxylic acid bis(3, 5, 5-trimethylhexyl) ester CASRN 28553-12-0 (diisononyl phthalate; DINP); and 1, 2-benzenedicarboxylic acid diisodecyl ester CASRN 26761-40-0 (diisodecyl phthalate; DIDP), all >99%, were selected for analysis based on their extensive usage. All were analytical grade and were sourced from Sigma-Aldrich. Solubility studies were carried out to determine the respective solubilities according to the OECD[15] shake flask method and the results over various temperatures showed the solubilities in the following order; DMP > DBP > DEHP > DINP > DIDP.

All chromatographic measurements were performed on a modular liquid chromatographic system consisting of Waters® Autosampler 717, Waters® Pump 510, and Shimadzu LC-6AD Detector. The column used was a Pinnacle™ II Phenyl (150 x 2.1mm, 5 μm) and an equivalent guard column was also purchased from Restek, Ireland. A Dionex 100 Accelerated Solvent Extractor (purchased from Dionex, UK) with 66 mL extraction cells is being used to perform pressurised liquid extractions of solid samples in our laboratory and its

extraction process was also screened for leachables. An isocratic method of elution using an acetonitrile-water (70:30) mix pumped at 0.2 mL min^{-1} and ultraviolet detection at 226 nm was devised. The main laboratory components which were tested are shown in Table 1. SPE cartridges and syringe barrels, which were considered initially for the extraction of environmental samples, were also examined for as possible sources of contamination.

[insert table 1 about here]

Results and Discussion

Triplicate blank injections of mobile phase in a vial with no lid and another with a lid in place were run, the purpose being to see if the plastic lid was causing any contamination. This experiment would also have demonstrated the purity and integrity of the selected solvent. No contamination was observed. Another set of injections of mobile phase, which had only been in contact with glass, was carried out and no peaks were observed from this, eliminating the autosampler as a possible source of contamination. Further investigations of plastic syringes of the type commonly used for sample handling or transfer and indeed as holders for various phases in SPE work, were carried out at room temperature for a period of 30 min. Mobile phase was transferred in triplicate from the same syringe into three separate vials. Another aliquot of mobile phase was transferred into a vial using a glass syringe for comparison. Results showed definite contamination arising from the plastic syringes with the phthalates DMP, DBP and traces of DEHP being identified. Mobile phase held in glass syringes showed no contamination by comparison.

Three different types of filters were analysed; one brand made from polytetrafluoroethylene (PTFE), another from regenerated cellulose and another from cellulose acetate. For each different filter, two types of comparisons were made; unfiltered mobile phase with filtered mobile phase and an unfiltered standard mix (5µg mL^{-1} of each phthalate) with a filtered one. An unfiltered standard was run in triplicate and this was used to compare with the filtered standards. This analysis illustrated two types of problems; the first one was the presence of interferences in the filtered mobile phase i.e., unwanted peaks probably due to leaching of unknown components in the filter casing, and the second problem being that the filters themselves appeared to be retaining some of the target analytes. The cellulose acetate filters showed the greatest amount of leaching from the filter casing, whilst the greatest retention (of the standard; data not shown here) occurred with those made from PTFE. The results in Table 2 show that the PTFE casing leaches the most in terms of variety, whilst the cellulose acetate leaches the highest overall amount in terms of concentration. The regenerated cellulose appears to leach the least amount. The decision was thus made to avoid final sample clarification prior to injection and to rely on a guard column to prevent fouling of the analytical column.

[insert table 2 about here]

Micropipette usage would be put to use during the reconstitution steps of SPE and so, analysis of two types of micropipette tips (from the same supplier) of the type historically used in our laboratory was carried out. One type was size A (2-100 µL) and the other size B (50-1000 µL). Mobile phase was transferred into vials using a single pipette tip to pipette 200 µL five times into one vial. The analysis showed mild contamination from both pipette types so their

usage was avoided and instead a glass 0.2mL pipette was used. The levels of contaminant found (Figure 1) were as follows: - size A tips leached more into acetonitrile than methanol but overall, the size B pipette tips exhibited more leaching than the size A tips and the amount of leaching was dependent on the surface area and the solvent that it came into contact with. Methanol leached more from the size B tips and acetonitrile by comparison appeared to leach less. These two solvents were selected due to their prevalence in reverse-phase HPLC. These differences were slightly surprising, subjectively we found the larger tips to be somewhat more flexible to handle.

[insert figure 1 about here]

Parafilm® (a piece weighing 0.50 g), the SPE frit (8.36 g), solvent Winchester lids (20.75 g) and closures (0.93 g), rubber tubing sectioned from a nitrogen blow-down and drying apparatus (a piece weighing 5.00 g) and stir bars (2.37 g) were subsequently tested. Each component was sonicated in a beaker containing 15 mL of methanol for 30 min and this 15 mL was then dried down under nitrogen at 37 ^{0}C and reconstituted with 0.2 mL of solvent. The results were expressed as μg cm^{-2} exposed to the 15 mL of solvent (Figure 2) and we can see from the results that DEHP is by far the most omnipresent contaminant in all components.

[insert figure 2 about here]

Further investigations were carried out on the bulking agents used for packing the extraction cells for pressurised liquid extractions of solid environmental samples such as

sediments and sludges. A dispersing agent is required in this scenario in order to prevent aggregation of sample particles and also acts as a filler to reduce the volume of solvent in the final extract. Initial studies were carried out using acid purified analytical grade sand purchased from Sigma-Aldrich. The use of sand proved to be unsuitable for trace analysis as there was severe discolouration of extracts leading to poor chromatographic results. Further experimental work showed that there was contamination arising from Chem Tube-Hydromatrix, which was purchased from Varian through JVA Analytical (Ireland) as an alternative to the sand. It was not possible to obtain this material in a glass container and so, a 'bake-out process' was required to remove the detected contaminants, DEHP and DINP. This involved cremating portions of the hydromatrix in crucibles in the furnace at a range of test temperatures to completely eradicate interfering compounds. Bakings at 400, 500, 600, 700, 750, 800 and 850 ^{0}C were carried out and chromatographic results showed that a temperature of 850 ^{0}C for 24 hours was the optimum cremation temperature and time consistent with retaining accurate recoveries of analyte in excess of 85 % (Figure 3). This further increased the duration of experimental procedures but improved chromatographic results dramatically.

[insert figure 3 about here]

As a final investigation, environmental samples were taken of river waters from the facing bank of an old, disused landfill facility adjacent to the river (Athlone Lock) and one at a downstream tributary location from a lined and managed facility (Ballydonagh). Samples from each location had contact either with glass only or solely with plastic. The following table (Table 3) contrasts the handling of each. DBP, DEHP and DIDP were identified in the sample

matrices. The results (Table 4) showed that using plastic laboratory components greatly augmented the levels observed. These results illustrate the extreme importance in eliminating potential contamination sources from experimental work as we see up to 1.94 (6.22 minus 4.28) $\mu g\ L^{-1}$ DEHP contributed from contact of the sample with plastic during analysis.

[insert tables 3 and 4 about here]

One must consider at this point, however, previous studies, which have been carried out. EPA method 8061A4[12] used GC/ECD analysis and similar to this method, we also used an octadecyl- silica bonded membrane disk, as we found contamination when using cartridges or syringe barrels and although cartridge/barrel decontamination may be carried out, it was not plausible for our analysis as it would have significantly increased the extraction and analysis times and is found to be only moderately successful[16]. Furthermore a much greater volume of sample liquid may be analysed using disks thereby improving analyte detection limits. We also found ethyl acetate to be a better elution solvent with improved recoveries since the commonly used alternative, acetonitrile, eluted excess humic material and caused discolouration of the extracts. Blount *et al.* [17], analysed for the monoester metabolites of phthalates. In our case, we wished to monitor and quantify the levels of phthalates entering aquatic environments from landfill leachate and sewage effluents at source before their subsequent ingestion by aquatic organisms as opposed to a human reference where metabolism occurred. Furthermore, Blount *et al.* [17], whilst analysing for the metabolite, avoided contamination from the parent compound but found difficulty when analysing for DINP, which is a technical mixture containing a mix of

isomers. In this scenario, they were only able to choose a monoester metabolite of a single isomer, hence presenting a result, which was likely to be an underestimate of DINP exposure.

Another study, carried out by Tienpont *et al*. [16], utilised a liquid-liquid extraction and an automated large volume injection GC/MS analysis at the μg L^{-1} range. Similar to our study, they carefully selected tools, glassware and reagents and carried out frequent blank checks. The isocratic HPLC method in this study had very similar LOD values to Tienpont *et al*. [16], who had method detection limits of 6.0, 80.0, 30.0, 45.0 and 45.0 ng L^{-1} for DMP, DBP, DEHP, DINP and DIDP respectively, but did not require the use of any technologically advanced and expensive equipment. Furthermore, in the aforementioned study, filtration of wastewater samples to remove particulate matter was carried out and they then applied thermal desorption GC/MS to isolated particulate which had been removed. In our study, we added an organic modifier to wastewater samples, which was compatible with the SPE procedure and had a double function of desorbing analytes from particulate matter and inhibited microbial activity in the sample thus preventing metabolism of our analytes. Consequently, our overall aim, which was to develop a simple isocratic method of HPLC detection for ultra trace levels is consequently vindicated.

Conclusions

From the study carried out, significant quantities of phthalates were found to leach from various components commonly found in the environmental analytical laboratory. Items such as plastic syringes, pipette tips, plastic filters and Parafilm® were thus completely avoided and glass was used instead. Although the micropipette tips are made from polypropylene, which is supposed to be phthalate free, phthalates were identified possibly from the plastic packaging or

from the plastic pipette box. Nylon filters used in the filtration of mobile phase were shown to be contamination free. Tinfoil was shown to have no contamination and was used instead of Parafilm®. For drying down samples under nitrogen, rubber tubing was eliminated due to significant levels of DBP and DEHP leaching. Glass pipettes replaced plastic ones and bulking agents for accelerated solvent extraction required intensive pre-treatment prior to sample extraction and consequently, all contaminants were eliminated in subsequent experimental work, which was carried out on real environmental samples.

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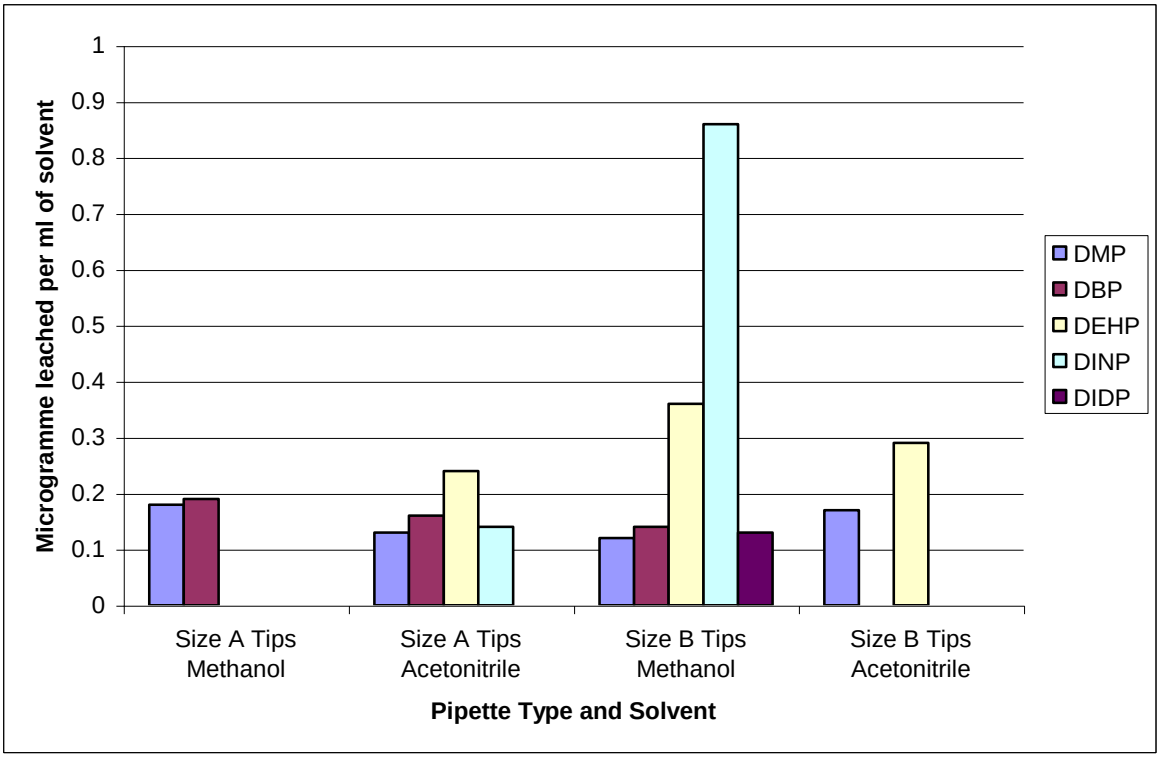

Figure 1. Levels and types of phthalate leaching from the two pipette tip types and the differences in amounts leached using different solvents.

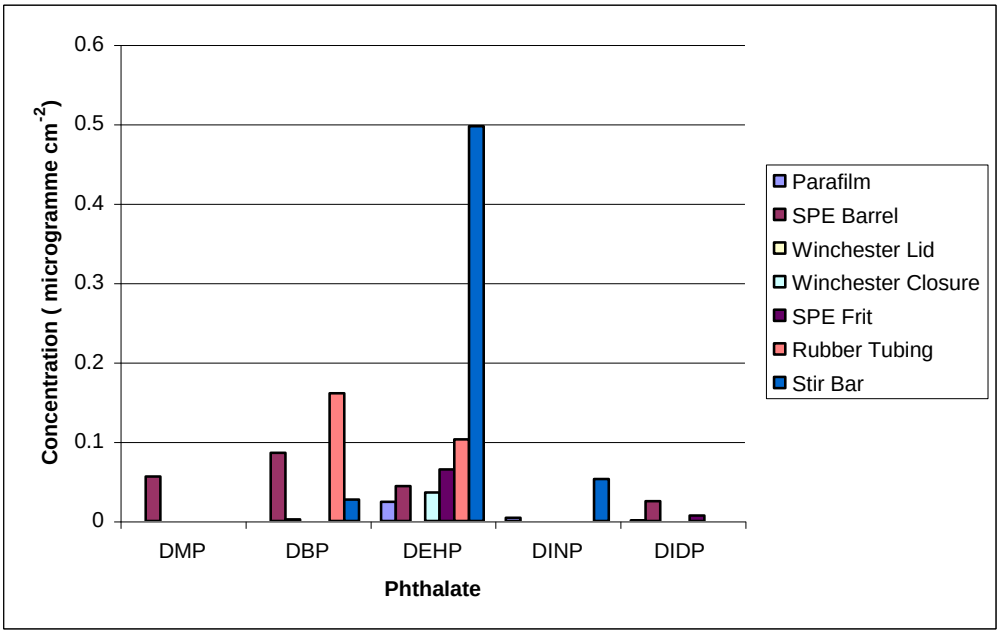

Figure 2. Amounts and type of phthalates leaching from various laboratory components.

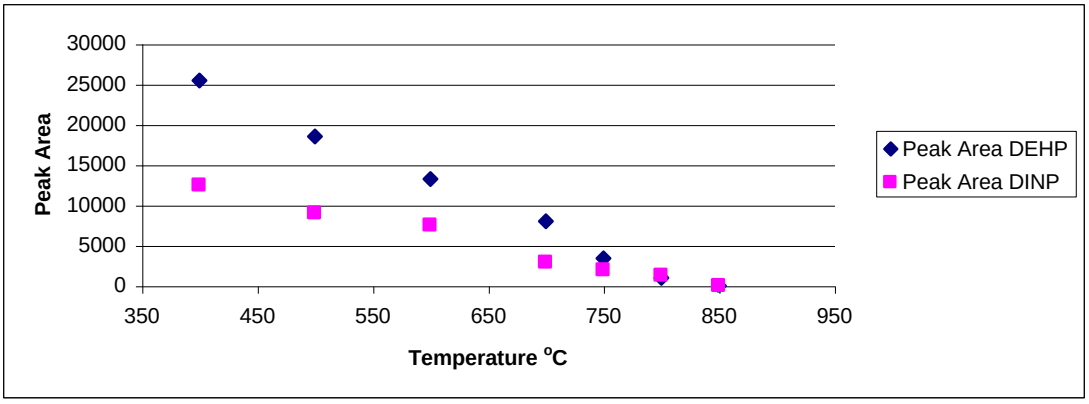

Figure 3. Decreasing levels of phthalate in Hydromatrix with increasing temperature in bake-out.

Table 1. Test materials

Test Material	**Packaging**	**Supplier**
HPLC vial caps (polyethylene)	Plastic box	Waters®
HPLC autosampler	None	Waters®
Plastic syringes	Plastic bag	Omnifix® Braun (Germany)
Filter holder (3 types made from various casings)	Plastic bag	Sartorious Millex® SR Millipore Schleicher & Schuell
Pipette tips (polypropylene)	Plastic bag	Plastibrand®
Stir bars	Plastic bag	VWR international
Winchester lids	n/a	Labscan Analytical Ltd.
Parafilm®	Cardboard	Pechiney Plastic Packaging
Tinfoil	Cardboard	AGB
Chem Tube-Hydromatrix	High density polyethylene	Varian

Table 2. Amount (µg) of phthalate leached per mL of solvent from the filter casings into mobile phase.

Phthalate	SPE recovery quantified (%)	Detection limit (absolute)	Quantitation limit	PTFE	Regenerated cellulose	Cellulose acetate
DMP	60.4	0.048	≤ 0.1	1.39	0.57	5.85
DBP	73.0	0.055	≤ 0.1	2.49	0.61	3.30
DEHP	83.6	0.071	≤ 0.1	0.12	<LOD	<LOD
DINP	114.8	0.132	1.00	0.28	<LOD	<LOD
DIDP	84.0	0.128	2.00	0.20	<LOD	<LOD

Table 3. Comparison and contrast of the handling of the environmental sample.

Sample handling	
500 mL aliquots taken at aforementioned location. 5 % methanol organic modifier/preservative added to both. Samples stored on ice and transported immediately to the lab for analysis. Primary filtration followed by SPE carried out using 47 mm C_{18} Empore™ disks (JVA Analytical Ltd., Ireland). Extracts were dried under a stream of nitrogen at 37 ^{0}C, and then reconstituted into 200 μL of acetonitrile for subsequent chromatographic analysis.	
Sample in contact with glass only	**Sample in contact with plastic**
Pre-cleaned (with acetone) amber glass bottle, rinsed with sample water from the site before filling.	Pre-cleaned (with acetone) plastic bottle, rinsed with sample water from the site before filling.
Taken using inert, stainless steel telescopic sampling pole.	Taken using a plastic bottle attachment on the telescopic rod.
Filled into glass sample bottle.	Filled into a plastic sample bottle.
Glass pipettes used.	Plastic pipettes used.
No syringes used.	Syringes used.
No filters used.	Filters used.
Tinfoil used.	Parafilm® used.
Teflon tubing used.	Rubber tubing used.
Glass rod used.	Stir bar used.
Glass sample collection tube.	Plastic sample collection tube.

Table 4. Results from environmental samples (μg L^{-1}) where n = 3.

Ballydonagh	**Without Plastic**			**With Plastic**		
Leachate	**DBP**	**DEHP**	**DINP**	**DBP**	**DEHP**	**DINP**
Amount found	3.84	6.24	0.49	4.32	7.97	0.75
RSD (%)	3	3	16	13	5	22
Athlone Lock	**Without Plastic**			**With Plastic**		
River Water	**DBP**	**DEHP**	**DINP**	**DBP**	**DEHP**	**DINP**
Amount found	1.02	4.28	0.25	1.22	6.22	0.59
RSD (%)	13	2	29	8	11	23