Environmental Occurrence and Potential Health Impact of Phthalates: An Irish Perspective

Catherine Allen, BSc.

A thesis submitted for the award of PhD

School of Biotechnology

Dublin City University

Supervisors:

Dr. Jenny Lawler

Prof. Anthony Staines

Prof. Fiona Regan

July 2021

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: _____ ID No.: 16212003 Date: _____

Acknowledgements

Thank you to Jenny, Anthony and Fiona for their constant guidance and encouragement throughout the project. I couldn't have asked for better supervisors. I wish to acknowledge the EPA, Dublin City University, Arizona State University, the University of Cyprus and Agilent for funding and/or project support. In particular, the Agilent representatives Marcus and Ross who taught me maintenance and troubleshooting of the LC-MS. Thank you to Dr. Lisa Jones for being my mentor, for the laughs on sampling trips and for training me in all things analytical chemistry and life in general.

Thank you to all the scientists I've had the good fortune to work with, the coffee and chats helped me get through all those long lab days. Especially Burcu, Matthew, Brian and Imogen who had to put up with me the most. I'd also like to thank the undergraduate researchers who helped along the way; Katie, Chester, Aurelie, Rebecca and Louise, it was great to share some of my research experience with such curious and talented scientists. Thank you to the BRS for the all the fundraisers, events and nights out. They definitely kept me sane and I've made some lifelong friends along the way.

Finally, a huge thank you to my parents, brothers and Dónal for your endless support, positivity and belief in me. I wouldn't have been able to do it without you all.

Table of Contents

1 Introduction....1
1.1 Background....1
1.2 Physical Properties....4
1.3 Current Legislation....8
1.4 Environmental Occurrence of Phthalate Diesters....13
1.5 Phthalate Impacts on Human Health....14
1.5.1 *In-vivo* Animal Studies....16
1.5.2 *In-vitro* Studies....16
1.5.3 Epidemiological studies....17
1.5.4 Health Outcomes....19
1.6 Biomonitoring as a Tool to Assess Phthalate Exposure....26
1.6.2 Summary of Occurrence in Humans....31
1.7 Wastewater Based Epidemiology for the Analysis of Public Phthalate Body-Burden....37
1.7.1 Factors Affecting Phthalate Metabolite Levels Upstream of the WWTP 39
1.7.2 Factors Affecting Metabolite Levels at the Wastewater Treatment Plant 40
1.8 Analysis of Phthalates and Their Metabolites....42
1.8.1 Quality Control....42
1.8.2 Sample Pre-treatment....45
1.8.3 Sample Extraction....45
1.8.4 Instrumentation....48
1.9 Aims and Objectives of Thesis....51
2 Methods....52
2.1 Phthalate Diester Method Development....53
2.1.1 Sample collection....53
2.1.2 Quality control for the prevention of contamination and carry-over....56
2.1.3 Sample Extraction....59
2.1.4 Solutions and Standards Preparation....63
2.1.5 Mass Spectrometry Conditions....64
2.1.6 Liquid Chromatography Analysis....65
2.1.7 Raw Sample Data....71
2.1.8 Data Anaysis....80
2.2 Phthalate Monoester Method Development....81
2.2.1 Sample collection....81
2.2.2 Sample Extraction....82

2.2.3 Solutions and Standards Preparation 83
2.2.4 Mass Spectrometry Conditions 83
2.2.5 Raw Sample Data 87
2.2.6 Exposure Assessment 88
3 Occurrence of Phthalates in Ireland 90
3.1 Household Wastes and Leachate 91
3.2 Wastewater 97
3.2.1 Influent 99
3.2.2 Sludge 106
3.2.3 Effluent 113
3.2.4 Wastewater Treatment Fluctuation and Removal 120
3.3 Soil 123
3.4 Surface Water 134
3.5 Conclusion 147
4 Human Exposure to Phthalates in Ireland 149
4.1 Monoester Metabolite Levels in Ireland 150
4.2 Risk Assessment 156
4.2.1 Hazard Quotient 157
4.2.2 Odds Ratio 160
4.3 Feasibility of Wastewater as a Biomonitoring Tool 169
4.4 Conclusion 170
5 Conclusions and Recommendations 172
5.1.1 Reduction of Phthalate Burden 172
5.1.2 Feasibility of Wastewater Based Epidemiology for Compliance Monitoring 173
5.1.3 Future Research 175
6 Bibliography 176
7 Appendix 189

List of Figures

Figure 1: Phthalate structure and metabolic pathway 3
Figure 2: Plausible mechanism of action for phthalates and their metabolites on the male reproductive system as described by Benjamin et al., 2017 15
Figure 3: Confounders in wastewater based epidemiology 38
Figure 4: Process flow diagram for sample collection, management, extraction and storage 53
Figure 5: Pre-injection set up with bypassed degasser and delay column 56
Figure 6: Three consecutive injections of mobile phase (analytical blanks) on LC-MS 57
Figure 7: Comparison between analytical bank, procedural blank and sample comparison of surface water sample, procedural blank and analytical blank 58
Figure 8: SPE percentage recovery on Strata-X cartridges. 60
Figure 9: Difference in column packing between two trialled columns 65
Figure 10: Method development for the separation of phthalates on eclipse column using ms conditions in table 8, and various gradients described in table 10 66
Figure 11: Chromatogram for Optimized Method Targeting Phthalate Analytes 67
Figure 13: Quantifying and Qualifying MRM Transition for all Phthalate 68
Figure 12: Quantifying and Qualifying Transitions for Target Phthalates Using MS Conditions Described in Table 8 and Chromatographic Conditions Described in Table 11 68
Figure 14: Repeated injections diester standard (n=15), showing no deviation of above 5% RSD for retention time or peak area 69
Figure 15: Limits of detection DMP example, using MS conditions described in table 8 and chromatographic conditions described in table 11 70
Figure 16: Example scan of general waste (50-500 m/z, positive ionization, fragmentor voltage 50, collision energy 8) 72
Figure 17: Example scan of general waste within phthalate RT windows 73
Figure 18: common ion sim (149 m/z, positive ionization, fragmentor voltage 50, collision energy 8) of general waste, showing 74
Figure 19: SIM e.g. for general waste, showing detected phthalates using chromatographic conditions described in table 11 75
Figure 20: MRM e.g. of general waste peaks 76
Figure 21: MRM of general waste from different sample subsets, spiked with IS, tiled 76
Figure 22: Zoomed MRM example of general waste, spiked with IS. 77
Figure 23: Process flow diagram for sample analysis of phthalate monoesters 81
Figure 24: SPE Recovery Trials for Phthalate Monoester Metabolites 82
Figure 25: MRM of Phthalate Monoesters 85

Figure 26: Repeated Injections of Monoester Standard (n=15) 86
Figure 27: Comparison of spiked and un-spiked influent sample 87
Figure 28: Un-spiked suburban influent samples from September, December and February 87
Figure 29: Phthalate concentrations in household waste 94
Figure 30: Phthalate concentrations in landfill leachate 94
Figure 31: Phthalate concentrations in rural influent 101
Figure 32: Phthalate concentrations in suburban influent 101
Figure 33: Phthalate concentrations in urban influent 102
Figure 34: Phthalate contributors to variance between influent samples 103
Figure 35: Corrplot of phthalate concentrations in influent 104
Figure 36: Individual PCA of phthalates in influent 104
Figure 37: Concentrations of phthalates in all sludge samples (n=12, in triplicate) 108
Figure 38: Phthalate concentrations in rural sludge 108
Figure 39: Phthalate concentrations in suburban sludge 109
Figure 40: Phthalate contributors to variance between sludge samples 110
Figure 41: Corrplot of phthalates in sludge 111
Figure 42: Individual PCA of phthalates in sludge 111
Figure 43: Concentrations of phthalates in all effluent samples (n=12, in triplicate) 115
Figure 44: Phthalate concentrations in rural effluent 115
Figure 45: Phthalate concentrations in suburban effluent 116
Figure 46: Phthalate contributors to variance between effluent samples 117
Figure 47: Corrplot of phthalates in effluent 118
Figure 48: Individual PCA of effluent 118
Figure 49: Phthalate concentrations in g/person/day for influent, sludge and effluent (left-right). rural in red, suburban in green and urban in blue. 121
Figure 50: Phthalate concentrations in all soil samples (n=15, in triplicate) 127
Figure 51: Phthalate concentrations in farmland soil 127
Figure 52: Phthalate concentrations in urban soils 128
Figure 53: Phthalate contributions to variance in soils 129
Figure 54: Corrplot of phthalates in soil 130
Figure 55: Individual PCA of soil 130
Figure 56: Infographic on phthalate surface water contamination in Ireland 135
Figure 57: Phthalate concentrations in all surface water samples (n=16, in triplicate) 138
Figure 58: Phthalate concentrations in upstream surface water 139
Figure 59: Phthalate concentrations in receiving surface water 139
Figure 60: Phthalate concentrations in downstream suburban surface water 140

Figure 61: Phthalate concentrations in estuary surface water 140
Figure 62: Phthalate contributions to variance between surface water samples 142
Figure 63: Corrplot of phthalates in surface water 143
Figure 64: Individual PCA of phthalate concentrations in surface waters 143
Figure 65: Monoester concentrations at urban site 152
Figure 66: Monoester concentrations at suburban site 152
Figure 67: Monoester concentrations at rural site 152
Figure 68: Contributions of phthalate exposure to variance 154
Figure 69: Individual PCA of phthalate exposure 155
Figure 70: Hazard index of exposure to phthalates from this study compared to a small-scale irish biomonitoring study (Cullen et. Al 2017) and a large-scale international biomonitoring study (NHANES, 2013-2014) 159
Figure 71: HI of exposure to phthalates from this study compared to WBE studies from Spain (Gonzalez-Marino et al., 2017) and China (Du et al., 2018) 159
Figure 72: Association of phthalate exposure with male birth anomalies. 161
Figure 73: Association of phthalate exposure to adverse effects on pregnancy 164
Figure 75: Association of Phthalate Exposure to Endometriosis. 165
Figure 76: Association of phthalate exposure to decreased neurological development 168

List of Tables

Table 1: Chemical characteristics of phthalates investigated 5
Table 2: Characteristics of phthalate monoester metabolites 6
Table 3: Legislation and description of restrictions surrounding phthalates 10
Table 4: Limits of human phthalate consumption in relation to adverse health effect 12
Table 5: Occurrence of phthalate metabolites in humans 32
Table 6: Labwear preparation for the reduction of phthalate contamination 43
Table 7: Description of samples, sub-types, frequency of sampling and extraction methods 55
Table 8: Percentage recoveries for extraction trials for phthalates in soil 62
Table 9: Percentage recoveries for solid matrices using ultra-sonication method (30 g sample) 62
Table 10: Matrix effects on each matrix represented by percentage of ion suppression 63
Table 11: Optimized mass spectrometry conditions to target 11 phthalates 64
Table 12: Mass spectrometer source conditions 65
Table 13: Selection of separation methods on eclipse column targeting phthalates 66
Table 14: Chromatographic conditions for the separation of 11 target phthalates 67
Table 15: Detection limits, linearity and precision of response 70
Table 16: PNEC of phthalates for environmental matrices 80
Table 17: Optimized mass spectrometry source conditions for phthalate monoester metabolites 83
Table 18: Mass spectrometry conditions for the detection of phthalate monoester metabolites 84
Table 19: Optimized chromatographic conditions for phthalate monoester metabolites 85
Table 20: Method detection limits, linearity and sensitivity of response for phthalate monoesters 86
Table 21: International occurrence of phthalates in landfill leachate samples in µg/l 96
Table 22: Site descriptors for WWTPs studied, including capacities and flow rates 98
Table 23: Concentration of phthalates in WWTP influent (n=3, ±2SD) 100
Table 24: International comparison of phthalates in wastewater influent (µg/L) 105
Table 25: Concentrations of phthalates in sudge (n=3, ±2SD) as a source of contamination 107
Table 26: International comparison of phthalates in sludge reported in mg/kgdw 112
Table 27: Concentrations of phthalates in wastewater effluent (n=3, ±2SD) in µg/L 114
Table 28: International comparison of phthalates in WWTP effluent (µg/L) 119
Table 29: Removal of phthalates from the watered fraction at Irish WWTPs 122
Table 30: Characteristics of soil samples included in this study 124
Table 31: Concentrations of phthalates in soil (n=3, ±2SD) as an environmental fate 126
Table 32: International comparison of phthalates in soil (mg/kgdw) 131
Table 33: RQ for phthalate toxicity to soil organisms (±2SD) 133
Table 34: Concentrations of phthalates in surface water (n=3, ±2SD) as an environmental fate 136
Table 35: International comparison of phthalates in surface waters 145
Table 36: RQ for phthalate toxicity to freshwater and marine organisms 146
Table 37: Concentrations of phthalate monoester metabolites in WWTP influent (ng/L) 151
Table 38: Exposure to phthalate diesters in µg/inhabitant/day 153
Table 39: HQ of exposure to each phthalate 158

List of Abbreviations

Acronym	*Definition*
CAN	Acetonitrile
ANOVA	Analysis of variance
BBP	Benzylbutylphthalate
CHAP	Chronic Hazard Advisory Panel
CI	Confidence Interval
CPSC	Consumer Product Safety Commission
DBP	Dibutylphthalate
DEHP	Diethylhexylphthalate
DHP	Dihexylphthalate
DiBP	Diisobutylphthalate
DiDP	Diisodecylphthalate
DiNP	Diisononylphthalate
DiPP	Diisopentylphthalate
DMP	Dimethylphthalate
DnOP	Dioctylphthalate
DPP	Dipentylphthalate
EDC	Endocrine Disrupting Chemical
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EQS	Environmental Quality Standards
GC-MS	Gas Chromatography-Mass Spectrometry
HI	Hazard Index
HMW	High Molecular Weight
HQ	Hazard Quotient
IPA	Iso-Propyl Alcohol
LC-MS	Liquid Chromatography-Mass Spectrometry
LMW	Low Molecular Weight
LOD	Limit of Detection
$\log K_{ow}$	Octanol/Water Partitioning Co-efficient
LOQ	Limit of Quantification
MAX	Maximum Admissible Concentration
MBP	Monobutylphthalate
MBzP	Monobenzylphthalate
MCL	Maximum Contaminant Level
MEHP	Monoethylhexylphthalate
MiBP	Monoisobutylphthalate
MiNP	Monoisononylphthalate
MnOP	Monooctylphthalate
m/z	Mass-to-charge ratio
NHANES	National Health and Nutrition Examination Survey
OR	Odds Ratio

PSI	Pounds per Square Inch
PVC	Poly Vinyl Chloride
QuEChERS	quick, easy, cheap, effective, rugged, and safe
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RR	Relative Risk
RSD	Relative Standard Deviation
S/N	Signal/Noise Ratio
SD	Standard Deviation
SPE	Solid Phase Extraction
SVHC	Substances of Very High Concern
TDI	Tolerable Daily Intake
TTIP	Transatlantic Trade and Investment Partnership
USEPA	United States Environmental Protection Agency
WBE	Wastewater Based Epidemiology
WFD	Water Framework Directive
WWTP	Wastewater Treatment Plant

Abstract

Catherine Allen

Environmental Occurrence and Potential Health Impact of Phthalates: An Irish Perspective

Phthalates are synthetic chemicals used in many consumer products. They are not chemically bound, so they leach, and have become ubiquitous within the environment. Due to their high production and continuous release, humans are constantly exposed, with increasing evidence of detrimental health effects. The European Commission is preparing to amend the Authorisation List (Annex XIV to REACH), adding four phthalates as substances of very high concern with endocrine disrupting properties (ECHA, 2019). Other phthalates have yet to be studied extensively. This project assessed phthalates and their metabolites in an Irish context, including restricted and less well studied phthalates.

Sources and fates of phthalates were assessed for eleven phthalates. Soil was identified as a stressor for phthalates in Ireland, with higher incidences than other European countries. All surface water samples were below environmental health limits. Removal from effluent was efficient, with effluent passing surface water standards. However, this resulted in high sludge concentrations, a cause of concern as the primary means of sludge disposal in Ireland is land spreading. It is recommended that the effects of land spreading for a variety of endocrine disrupters is investigated.

Phthalate metabolites in wastewater influent were monitored and modelled to population exposure. Total exposure to phthalate ranged from 10.27 to 418.42 µg/inhabitant/day. Risk assessment using the Hazard Quotient (HQ) assessed the toxicological effect of phthalates in Ireland. All values were below reported levels of concern. This indicates that there is no phthalate induced risk in Ireland. However, only a selection of phthalate metabolites was monitored, and may not reflect the risk associated with all phthalates and plasticizers. It is recommended that periodic screening of wastewater for a wide range of metabolites related to contaminants of emerging concern could indicate temporal trends in exposure and give an early indication of priority area for research.

1 Introduction

1.1 Background

Phthalates are synthetic organic compounds, commonly used in plastic and particularly PVC products, with a wide range of end uses including food packaging, cosmetics and personal care products, medical devices, tubing and flooring. Due to the extensive presence and environmental persistence of phthalates, their effects on health have been frequently studied. It has been found that phthalates act as endocrine disruptors leading to a range of adverse effects including hypospadias, reduced anogenital distance, cryptorchidism, impaired neurological development in children and precocious puberty, with children and women at the highest risk of exposure (Kay et al., 2014). Due to these findings, certain phthalates have been legislated for including; Benzylbutylphthalate (BBP), Dibutylphthalate (DBP), Diethylhexylphthalate (DEHP), Di-n-octylphthalate (DNOP) and Diisodecylphthalate (DIDP). However, research is far from complete, and with new replacement phthalates having been introduced, it is vital that the health effects of these compounds are also assessed (US Consumer Product Safety Commission, 2014).

The ubiquitous nature of phthalates in the environment raises a valid concern for their effects on human health. Phthalates are colourless, odourless compounds that are liquid at room temperature. They are added to give a product flexibility and resilience due to their fluidity, stability, and low volatility. Phthalates are heavily used throughout PVC manufacturing with soft PVC containing up to 40% DEHP (Koch et al., 2006). The most common exposure of phthalates in humans identified in the literature is through food consumption (at least 67% of total exposure), but drinking water, air, dermal contact, and cosmetics all contribute to total exposure (Das et al., 2014). This project aims to investigate a number of matrices including influent from WWTPs, dry recyclables, drinking water sources and municipal solid wastes to define a link between the exposure roots of phthalates and their resulting impact on human health.

Phthalates including DMP, DEP, DnBP, BBP, DEHP, and DnOP have been banned or limited in manufacturing (in particular for items such as children's toys) and as a result these have typically been the most widely studied phthalates. Due to this legislation (Section 1.3) a number of higher molecular weight plasticizers were introduced as substitutes to reduce leaching from plastics. However, new research may indicate that

these substitute plasticizers have an equally negative impact on human health and this warrants further study. Current phthalate research is lacking in a comprehensive sample range of phthalates and there has yet to be conclusive evidence of phthalates' impact on human health. This research is timely as legislation on Endocrine Disrupting Chemicals (EDCs) is increasing (U.S. Environmental Protection Agency, 2014; Regulation (EU) 2018/2005). As there is relatively little information on the human health impacts of phthalates and the extent of phthalate contamination in Ireland, an investigation in this area is pertinent.

Humans readily metabolise phthalates, generally excreting the phthalate as a number of phthalate metabolites within 1-2 days (Anderson et al., 2011). This metabolism occurs in at least two steps; hydrolysis (Phase 1) and conjugation (Phase 2) (see **Figure 1**). Some of the phthalate metabolites will be excreted as a glucuronide-conjugate. These glucuronide conjugates are often broken down by enzymes contained in the matrix, however, all of the metabolites contained in urine will be glucuronide conjugates as there are no esterases in this matrix. For this reason, urine is the most widely studied matrix for human phthalate metabolite monitoring although many more (e.g. blood, sweat, breastmilk) have been studied. The simpler forms of phthalates such as DEP and DBP are usually excreted as their corresponding phthalate monoester, whereas highly branched phthalates undergo more extensive biological transformations (Saravanabhavan et al., 2012). These phthalate metabolites (or biomarkers) can be monitored to infer a subject's phthalate body burden. Knowledge of the metabolism of phthalates will be important for consideration in identifying biomarkers for phthalate body burden as only a small amount of phthalates are excreted in their unconjugated forms (Frederiksen et al., 2007). Using the metabolites can therefore give us a more accurate representation of the direct effects on humans. A large quantity of the research looks at monoesters due to the ease of analysis and the evidence it yields on human consumption. Although further oxidative metabolites may give more accurate information on the human consumption of phthalates, these more highly conjugated metabolite standards are very difficult to source (Koch et al., 2006).

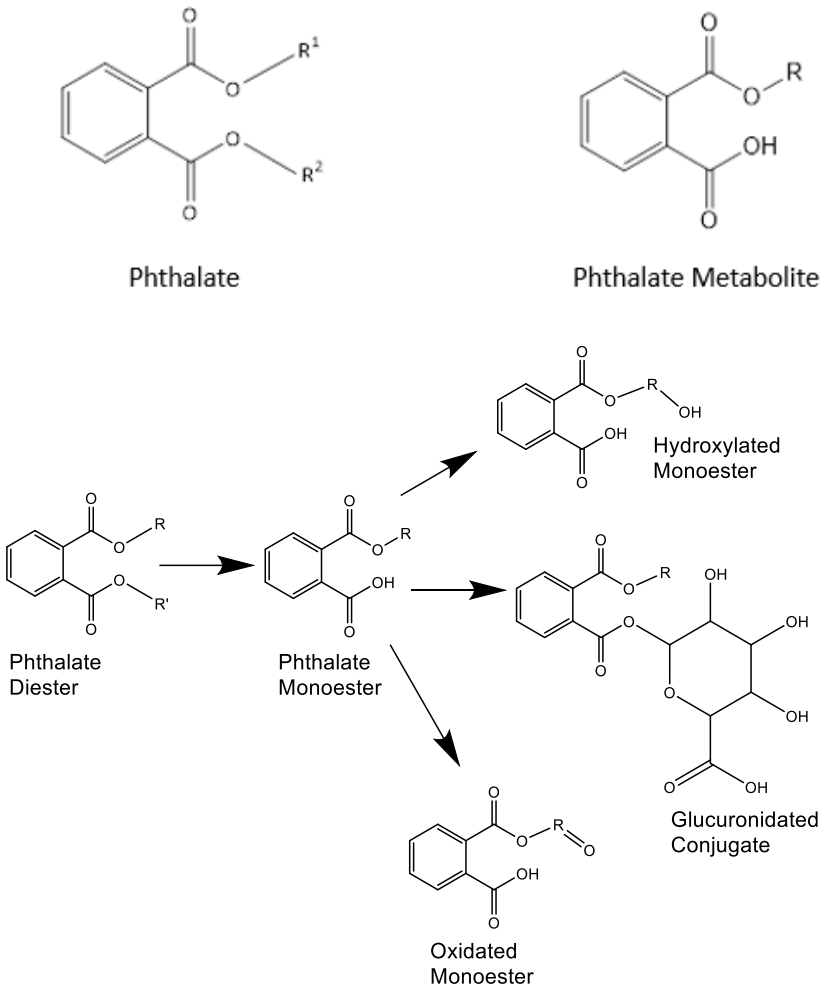


Figure 1: Phthalate structure and metabolic pathway

It has been determined that phthalates can produce a "cocktail effect" and can have additive effects. Overall, it has been found that when rats were exposed to a mixture of phthalates the resulting effect was stronger than if exposure was restricted to the most potent component (CHAP Report, 2014). Most studies involve focusing on the effects of isolated phthalates. This project monitored a diverse range of phthalates, aiming to give a greater understanding of how the Irish population is exposed. The physical properties of each phthalate and their metabolites will influence how it affects human health and how it is monitored (see **Table 1** and **Table 2**).

Previous studies on phthalates in the environment have focused solely on either occurrence or on human health effects. This project aims to offer a total view of phthalates to monitor the extent of environmental contamination, human exposure, and potential health impacts, monitoring restricted and some less studied phthalates.

1.2 Physical Properties

The physical properties of phthalates and their metabolites are important as they determine how they behave in a matrix and during analysis. This affects how they are monitored and how they act in the body. For both phthalate diesters (**Table 1**) and phthalate monoester metabolites (**Table 2**), log K_{ow} is important as it dictates the partitioning of the phthalate in a matrix by noting polarity. As such, higher molecular weight phthalates like DIDP will favour less polar matrices like soil, while lower molecular weight phthalates like DMP will favour more polar matrices like water (Gao and Wen, 2016). However, the pK_a is a more pertinent property in phthalate metabolites than in phthalate diesters, due to the readily ionisable carboxylic acid functional group. The value of the pK_a shows the point at which the ionisation of the group is at equilibrium, at equilibrium the compound is unstable and any chromatography of the analytes will have low resolution, so varying the pH will be an important step in the monitoring of phthalate monoesters (Ramesh Kumar and Sivaperumal, 2016).

The air-water distribution coefficient ($K_{a/w}$) can also influence how phthalates transfer in the environment. This describes the distribution of the compound between water and air. Although LMW phthalates have high volatilities, their uptake from water to air are actually lower than HMW phthalates, owing to HMW phthalates' low water solubility (Net et al., 2015). In the environment, however, surface waters contain large quantities of suspended solids, and high molecular weight phthalates tend to adsorb onto this material. This restricts their migration into the air drastically. Due to the higher polarity of phthalate monoesters, they largely remain in the aqueous phase and, once ionized, are completely solvated (Cousins and Mackay, 2000).

Table 1: Chemical characteristics of phthalates investigated

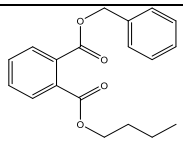
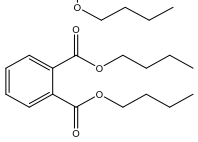
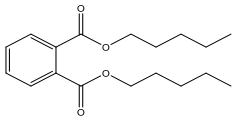
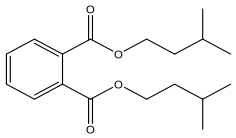
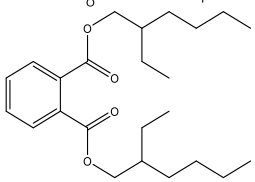
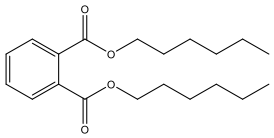
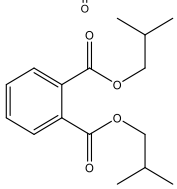
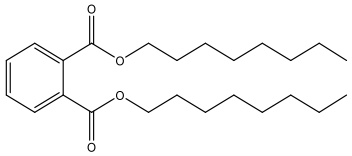
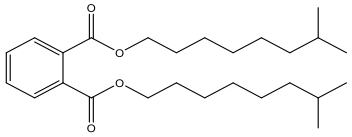
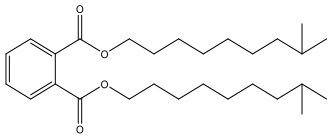
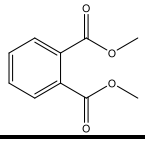
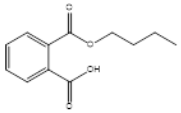
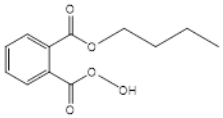
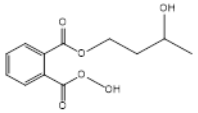
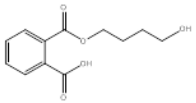
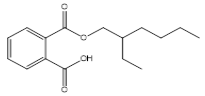
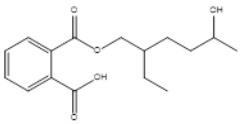
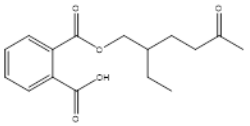
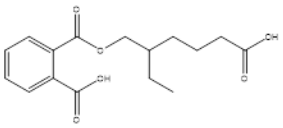
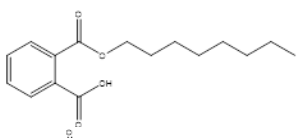
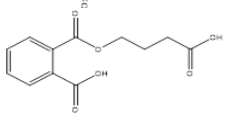
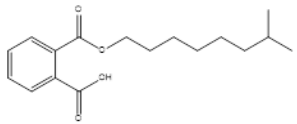
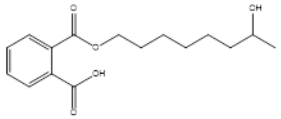
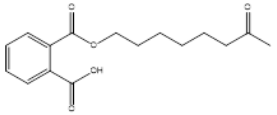
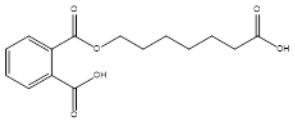
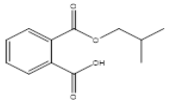
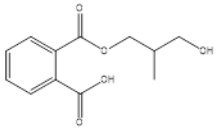
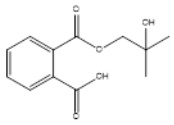
Compound	*CAS*	*Structure*	*Mw (g/mole)*	*Log K_{ow}*
Benzylbutyl phthalate (BBP)	85-68-7		312.36	4.65
Dibutyl phthalate (DBP)	84-74-2		278.34	4.16
Dipentyl phthalate (DNPP)	131-18-0		306.4	4.99
Diisopentyl phthalate (DIPP)	605-50-5		306.4	4.82
Diethylhexyl phthalate (DEHP)	117-81-7		390.56	7.5
Dihexyl phthalate (DHP)	84-75-3		334.46	6.8
Diisobutyl phthalate (DIBP)	84-69-5		278.35	4.12
Di-n-octyl phthalate (DNOP)	117-84-0		390.56	7.5
Diisononyl phthalate (DINP)	28553-12-0		418.62	8.16
Diisodecyl phthalate (DIDP)	26761-40-0		446.66	8.99
Dimethyl phthalate (DMP)	131-11-3		194.19	1.6

Table 2: Characteristics of phthalate monoester metabolites

Parent Phthalate	*Major Metabolite(s) Monoester*	*Cas No.*	*Structure*	*Molecular weight (g/mol)*	*Log K_{ow}*	*pK_a*
Benzylbutyl phthalate (BBP)	Mono-benzyl phthalate (MBzP)	2528-16-7		256.26	3.14	3.22
Dibutyl phthalate (DBP)	Monobutyl phthalate (MBP)	34-74-2		222.09	2.65	3.292
	Mono(3-hydroxybutyl) phthalate	57074-43-8		238.24	1.32	3.263
	Mono(4-hydroxybutyl) phthalate	17498-34-9		238.24	1.45	3.29
Diethylhexyl phthalate (DEHP)	Monoexylhexyl phthalate (MEHP)	4376-20-9		278.35	4.3	3.266
	Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	40321-99-1		294.35	3.01	3.266
	Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	40321-98-0		292.33	2.99	3.265
	Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	40809-41-4		308.33	3.9	3.266

Di-n-octyl phthalate (DNOP)	Mono-n-octyl phthalate (MNOP)	5393-19-1		278.35	4.32	3.29
	Mono-(3-Carboxypropyl) phthalate (MCPP)	66851-46-5		252.22	1.25	4.471
Diisononyl phthalate (DINP)	Monoisononyl phthalate (MINP)	68515-53-7		292.38	4.65	3.289
	Mono(hydroxyisononyl) phthalate (MHINP)	N/A		294.35	3.02	3.9
	Mono(oxoisononyl) phthalate (MOINP)	N/A		292.33	3.01	3.29
	Mono(carboxyisooctyl) phthalate (MCIOP)	N/A		294.3	3.5	3.29
Diisobutyl phthalate (DIBP)	Mono-iso-butyl phthalate (MIBP)	30833-53-5		222.24	3.263	3.278
	3OH-mono-iso-butyl phthalate (3OH-MIBP)	N/A		238.24	1.49	3.25
	2OH-mono-iso-butyl phthalate (2OH-MIBP)	N/A		238.24	1.43	3.121

1.3 Current Legislation

Total exposure by humans to phthalates has been estimated to be between 0.15 and 0.66 mg/kg/day. Phthalates are postulated to cause harm when they are found in humans above a certain concentration; regulation attempts to control for this and minimise the risk to human health. Present regulations surrounding phthalate manufacture and usage are summarised in **Table 3**.

Under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations, (European Commission, 2006), the following phthalates 'shall not be used as substances or as constituents of preparations, at concentration higher than 0.1% by mass of the plasticised material, in toys and childcare articles': DEHP, DBP, BBP, DINP, DIDP and DNOP. The listed phthalates were also categorized as toxic to reproduction (Regulation (EC) No 1907/2006). The phthalates classified as Category 1B reproductive agents, are restricted in cosmetic products under the Cosmetics Directive (European Commission, 2006).

Eleven LMW phthalates, classified as Category 1B reproductive agents, have been identified as Substances of Very High Concern (SVHC) and placed on the REACH Candidate List. Four of these substances, DEHP, DBP, BBP and DIBP, were placed on the Authorisation List, meaning, since February 2015, products are not placed on the EU market until authorization has been given to specific applicants (European Commission, 2006). As of 2018 fourteen phthalates are included on the authorization list, Annex XIV of REACH Regulation, including new additions monitored in this study (DPP and DHP) and also mixes of diesters and phthalic acid (Commission Directive 2018/2005).

Since July 22, 2019, concentrations of DEHP, DBP, BBP and DIBP in electrical material (excluding medical devices and monitoring instruments) must be less than 0.1%. From July 22, 2021, this also applies to medical devices and monitoring and control instruments (Directive (EU) 2015/863). However, on the 08 August 2019, a five-year exemption was granted for DEHP use in some rubber components of engine systems.

Internationally, six phthalates (DMP, DEP, DnBP, BBP, DEHP, and DnOP) have been classified as priority pollutants by the U.S. Environmental Protection Agency (U.S. EPA, 2011), and three of them (DEP, DnBP and DnOP) have been listed as priority pollutants

by the China State Environmental Protection Agency (Wang et al., 2018). The US EPA has also set the maximum admissible concentration (MAC) for DEHP in water systems at 6 mg L^{-1}.

In the USA, a congressional edict banned DBP, BBP and DEHP from children's articles owing to potential health risks. DINP, DNOP and DIDP were banned from children's toys that can be placed in a child's mouth, or children's toys smaller than 5 cm. The ban applies only to accessible parts of a toy. The Consumer Product Safety Commission (CSPC) advisory panel now recommends, from 2014, a permanent ban on DINP, but says the ban on DNOP and DIDP should be lifted.

In 2012, the USEPA developed an Action Plan for Phthalates based upon their toxicity, widespread use, and human exposure. Eight phthalates are included in the Action Plan: DBP, DIBP, BBP, DnPP, DEHP, DnOP, DINP, and DIDP. The USEPA states that it will 'coordinate with the CPSC and FDA on regulatory action to address the manufacturing, use, sale, and distribution of these compounds in the US.' (US-EPA, 2012). In 2017, the CPSC voted 3 to 2 to issue a final rule prohibiting children's toys and child care articles containing more than 0.1 percent of DiBP, DPP, DHP, DCHP or DiNP, in addition to those that were restricted in 2008. This legislation is also relevant given the current Transatlantic Trade and Investment Partnership (TTIP) negotiations, ongoing since 2013 (110th United States Congress, 2008).

Table 3: Legislation and description of restrictions surrounding phthalates

Legislation	*Description*
Directive 2005/84/EC of the European Parliament and of the council	placed restrictions on use of certain dangerous substances and preparations (phthalates in toys and childcare articles). This directive stated that 'use of certain phthalates in toys and childcare articles should be prohibited due to health risks, also any toys that can be put in the mouth.' This Directive highlights the phthalates: DEHP, DBP, BBP, DINP, DIDP, DNOP (European Parliament, 2005).
Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations; Cosmetics Directive; and EC Regulation No. 1907/2006.	DEHP, DBP, BBP, DINP, DIDP and DNOP 'shall not be used as substances or as constituents of preparations, at concentration higher than 0.1% by mass of the plasticised material, in toys and childcare articles. The listed phthalates were also categorized as toxic to reproduction (Regulation (EC) No 1907/2006). As of 2007 more than 50 plasticisers are currently registered under REACH. Eleven LMW orthophthalates, classified as Category 1B reproductive agents have been identified as Substances of Very High Concern (SVHC) and placed on the REACH Candidate List. Four of these substances, DEHP, DBP, BBP and DIBP, are on the Authorisation List (and the remaining seven are being proposed for the Candidate List) (European Commission, 2006). The phthalates classified as Category 1B reproductive agents, are also restricted in cosmetic products under the Cosmetics Directive (European Commission, 2006).
Regulation on Plant Protection Products (2009), the Regulation on Biocidal Products (2012)	10 phthalates listed.
Water Framework Directive (WFD)(2000/60/EC), Directive 2008/105/EC and 2013/39/EU	Identifies a list of priority and relevant pollutants. Lays down the environmental quality standards (EQS). DEHP is listed among the 33 priority substances listed in Directive 2008/105/EC, amended to classification as a priority hazardous substance in 2011 (2011/0429(COD)) Directive 2013/39/EU, in which the annual average concentration of DEHP in surface waters was recommended to be limited to 1.3 μg L^{-1}.
Commission Directive 2007/19/EC	Allows for the presence of DEHP in food production facilities, such as in conveyor belts, provided it does not exceed the substance migration limit of 1.5 mg kg^{-1} of food, although it is prohibited in the manufacture of single-use lips or caps.
Commission Delegated Directive 2015/863/EU, amending Annex II to Directive 2011/65/EU	Classes DEHP, BBP, DBP and DIBP as substances of very high concern (SVHC), reporting that the available evidence indicates that the SVHC phthalates, when used in electrical and electronic equipment (EEE), can have a negative impact on recycling and on human health and the environment during EEE waste management operations. The Directive also includes a clause to maintain the previous legislation on the restriction of DEHP,

	BBP and DBP in toys and in concentrations above 0.1% in plasticised material. (European Commission, 2015)
Commission Regulation (EU) N°10/2011	Restricts the quantities of substances able to migrate into the food on materials used for food packaging. Known as specific migration limits (SML) and are defined as "the maximum permitted amount of a given substance released from a material or article into food or food simulants" and expressed in mg substance per kg food (Pérez-Outeiral et al., 2016)
U.S. Environmental Protection Agency (U.S. EPA, 2011)	Six phthalates (DMP, DEP, DnBP, BBP, DEHP, and DnOP) have been classified as priority pollutants. In 2012, the USEPA developed an Action Plan for Phthalates based upon their toxicity, widespread use, and human exposure. Eight phthalates are included in the Action Plan: DBP, DIBP, BBP, DnPP, DEHP, DnOP, DINP, and DIDP. (US-EPA, 2012). A Significant New Use Rule has since been proposed for DnPP, which requires manufacturers or processors of the chemical to obtain USEPA approval (Environmental Protection Agency/ FDA, 2012).
China State Environmental Protection Agency	Lists DEP, DnBP and DnOP as priority pollutants. (Wang et al., 2018)
Consumer Product Safety Improvement Act (110th United States Congress, 2008)	DEHP, DnBP, and BBP were banned in the U.S. in children's toys and some child care articles.
Clean Drinking Water Act	Levels of DEHP in drinking water are regulated with a maximum contaminant level (MCL) of 0.006 mg L^{-1} for DEHP. DEHP and DBP are also listed as hazardous pollutants under the Clean Air Act (US-EPA, 2012). Several phthalates are listed among the risks to public health associated with PVC materials, and the American Public Health Association, which represents a broad array of public health professionals, urges federal and local governments to replace PVC when possible in medical care settings, schools, public housing, and building materials (APHA, 2011).
Commission Directive 2018/2005	Amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament, as regards to DiBP, DBP, BBP and DEHP

Toxicological studies have been conducted to determine the levels of exposure that are safe or associated with increased risk (EFSA, 2005a; EFSA, 2005b; EFSA, 2005c). Toxicological limits have been set for DEP, DBP, BBP, DEHP, DNOP, DINP and DIDP (***Table 4***) summarised in the most recent CHAP report on phthalates (Consumer Product Safety Commission, 2017). These values have helped shape legislation and include the Human Reference Dose (RfD), defined as the acceptable safety level for chronic non-

carcinogenic and developmental effects. The Lowest Observed Adverse Effect Limit (LOAEL) describes the lowest dose at which there was an observed toxic or adverse effect. The No Observed Adverse Effect Limit (NOAEL) is the highest dose in a toxicity study that showed no adverse effect. The Tolerable Daily Intake (TDI) is the degree of exposure that can be taken in daily over a lifetime without appreciable health risk. This data will be used to compare Irish exposure to adverse effect risk limits.

At present the toxicology of phthalate diesters is relatively well known. However, there seems to be some recent evidence that indicates phthalate monoester metabolites may have a similar toxicological effect (Engel et al., 2017; Pham et al., 2016). This only applies to the metabolite's free form, as glucuronide conjugates do not exhibit activity at site (Clewell et al., 2008). Humans are environmentally exposed to phthalate metabolites, through breakdown of phthalate parent compounds in consumer products and food. Currently, one study exists for the modelling of total exposure to phthalate diesters and their metabolites (Mittermeier et al., 2016). This used a single oral dose of labelled MBP and MEHP to determine their kinetics, so that monoester concentration can be measured and converted to diester using molecular ratios. This value can then be added to the calculated diester concentration in the same sample. In the future, if the toxicological effect of phthalate metabolites is found to be significant, the sum of parent compound and metabolite concentration in consumer product should be reported.

Table 4: Limits of human phthalate consumption in relation to adverse health effect

	RfD (mg/kg/day)	*LOAEL (mg/kg/day)*	*NOAEL (mg/kg/day)*	*TDI (mg/kg/day)*
DEP	0.8	3160	750	-
DBP	0.1	256	52	0.01
BBP	0.2	250	50	0.5
DEHP	0.002	-	19	0.05
DNOP	-	370	37	-
DINP	0.12	358	88	0.15
DIDP	-	75	15	0.15

1.4 Environmental Occurrence of Phthalate Diesters

Phthalates are relatively volatile, due to this, they have been frequently found in air, thus promoting exposure to phthalates through inhalation. With increased industrial manufacture of phthalates comes greater release into the environment; they are now being found to occur in atmospheric, terrestrial, and aquatic environments of populated regions, as well as repeatedly detected in various compartments of remote areas (Bubba et al., 2018). For example; DMP has been found in atmospheric particulate matter, fresh water, sediments, soil, and landfills at concentrations of N.D.–10.4 ng/m^3, N.D.–31.7 μg/L, N.D.–316 μg/kg dry weight, and N.D.–200 μg/kg dry weight, N.D.–43.27 μg/L, respectively (D.-W. Gao and Wen, 2016). Phthalates have been frequently monitored in environmental matrices in the research literature. The most studied phthalates in the literature are DMP, DBP, BBP, DEHP and DNOP and these are summarised and compared to results found in this study later in the text (Section 3).

BBP and DEHP have been reported on more frequently than other phthalates due to their inclusion in the toxic chemical release inventory since 1992, they are therefore the only two phthalates where data on general release into the environment can be found (US-EPA, 2012). According to the United States Environmental Protection Agency Toxic Chemical Release Inventory for 1987, 147000 kg butyl benzyl phthalate was released into the air, 860 kg was discharged into water, and 3900 kg was released onto the land from manufacturing and processing facilities in the United States. By 1993, 170000 kg was released into air, 620 kg was discharged into water, 38 kg was disposed of by underground injection, and 1200 kg was released onto the land (National Library of Medicine, 1998a). Butyl benzyl phthalate has been detected in surface water, groundwater and drinking water in many locations at levels generally well below 10 μg/L. Concentrations lower than 0.1 μg/m^3 have been found in indoor air due to release from products such as vinyl flooring, caulks and adhesives and carpets. It also has been detected at parts per million in a few foods (Solutia, 1998; National Library of Medicine, 1998b).

The Toxics Release Inventory reports the air emissions of DEHP in the US was 107 tonnes in 1997, compared to 27 tonnes Canada, according to the Canadian National Pollutant Release Inventory. DEHP concentrations of up to 790 ng/m^3 have been found in urban and polluted air, but usually the levels in ambient air are well below 100 ng/m^3.

DEHP emissions have been detected over the Atlantic and Pacific Oceans, suggesting that DEHP may be carried for long distances in the troposphere (National Centre for Environmental Assessment, 2011).

1.5 Phthalate Impacts on Human Health

The endocrine system is a collection of glands that secrete hormones to regulate a number of biological functions including metabolism, sexual function, growth, tissue function, and sleep. Phthalates disrupt the endocrine system by exerting strong anti-androgenic and weak estrogenic effects, leading to an association with a wide range of adverse health effects. Phthalates target a range of organs including the reproductive system, liver, and kidney, although the mechanism of this toxicity has yet to be described exactly (Asghari et al. 2015). Phthalates most likely induce toxicity though gene expressions or by affecting the antioxidant enzymes activity by increasing Reactive Oxygen Species (ROS). The most commonly studied receptors that elicit immune response from phthalate exposure are estrogen receptor (ER), estrogen related receptor (ERRs), Peroxisome Proliferator-activated Receptors (PPARs), Toll-like Receptors (TLRs), and NOD-like receptors **(**NLRs) (Hlisníková et al., 2020). Prenatal and perinatal windows of exposure are deemed to be the most critical due to their high sensitivity to hormonal dysregulation by EDCs (Latini et al., 2008). Different phthalates can cause a range of effects, with reproductive toxicity is related to the side chain length of phthalates (Hlisníková et al., 2020).

Taking phthalate toxicity in reproductive organs as an example mechanism, research has concluded that male reproductive function is impaired through an increase of ROS with the primary targets being Sertoli cells and Leydig cells (Benjamin et al., 2017) (**Figure 2**). This review of epidemiological and animal data determined that access may be mediated through the Hypothalamic–pituitary–gonadal axis (HPG axis) with multiple pathways at play. Sertoli cell functions are impaired through apoptosis during spermatogenesis. Leydig cell functions are reduced through suppressing insulin-like growth factor 3, therefore inhibiting testosterone production. Leydig cell hyperplasia was due to down-regulation of *c-kit* receptor.

Specific interactions of phthalates with receptor binding sites conducted *in vitro* can be difficult to ascertain due to tissue-specific expression and the different regulatory functions of these receptors *in vivo*. Research conducted through reporter gene assays has suggested that multiple phthalates activate and/or inhibit human ERα and ERβ. However, agonists of ERα induce cellular proliferation, while ERβ agonists have been shown to counteract ERα activity (Engel et al., 2017). Therefore, although phthalate exposure may result in an overall inhibition of the reproductive system, this effect cannot be attributed to a specific ER isoform. On the other hand, results for association of phthalate exposure to endocrine disruption *in vivo* can show conflicting results most likely due to variation in phthalate, dose type, and timing of the exposure (Hlisníková et al., 2020). This highlights the difficulty in differentiating the biochemical mechanism of action for phthalates and endocrine disruption.

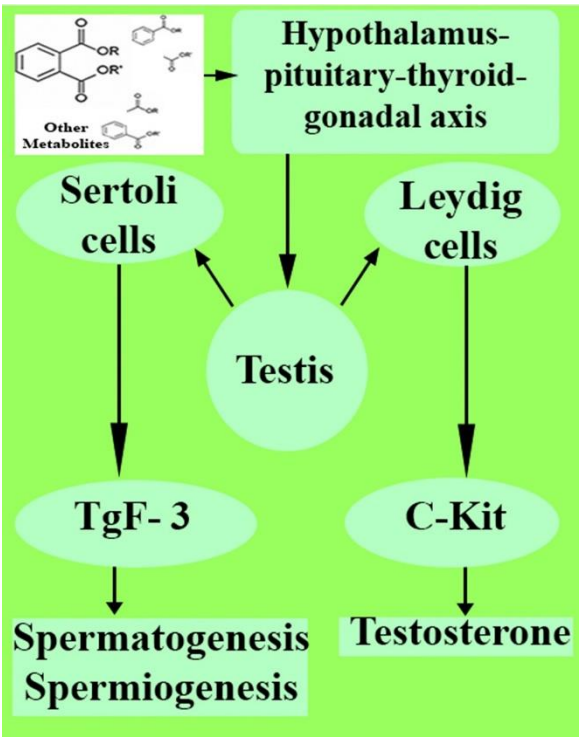


Figure 2: Plausible mechanism of action for phthalates and their metabolites on the male reproductive system as described by Benjamin et al., 2017

There are many complexities in determining the effect of phthalates on human health, leading to a lack of risk assessment data in humans. Many studies have been conducted in rats and other mammals however relating this risk to humans can be difficult due to inter-species variation. Some *in-vitro* studies have been conducted to determine the potency of specific phthalate's effects on binding sites, evaluating their estrogenic properties. Therefore, when human health risk assessment is carried out in this study, phthalate metabolite concentrations will be related to risk data generated from epidemiological studies. To do this, the literature needs to be assessed.

1.5.1 *In-vivo* Animal Studies

Animal studies can derive valuable toxicological data, enabling the researcher to derive data that would otherwise be unethical and dangerous to carry out in the human population. The health effects of phthalates on rats have been studied extensively, particularly in relation to male birth defects. Relating phthalate health effects in humans to a specific health effect in animals can present problems due to the variability between species. For example, the mechanism for testicular descent in humans and rats has several differences. In rats, transabdominal migration occurs just before birth whereas in humans it occurs at 10 to 15 weeks' gestation, while inguinoscrotal descent occurs after birth in rats and after 6 gestational weeks in humans (Bornehag et al., 2015). As there are differences in the length of time this process takes place between the two species it cannot be ascertained that the exact same effect would be observed in humans. Therefore, for the purposes of phthalate human health risk assessment in Ireland, animal studies will not be consulted for the risk of specific diseases.

1.5.2 *In-vitro* Studies

In-vitro studies examine effects of phthalates on binding sites through assays. This limits the need for animals, is inexpensive and the experiments are easily controlled. For the investigation of estrogenic activity of phthalates, binding assays and the ESCREEN method are generally used (Engel et al., 2017; Kratochvil et al., 2019; Soto et al., 2006; Zhang et al., 2015). There are some disadvantages to using this technique. The assays are conducted outside of their normal environments and therefore it cannot be fully ascertained that the same result would be found *in-vivo*, with "normal" blood and nutrient supply (Kinnings et al., 2009). It is not easy to mimic *in-vivo* exposures and metabolism. A review of the state of *in-vitro* analysis of endocrine disrupting properties stated that

while all the assays were adequate to determine estrogen or androgen agonist and antagonist activity, they were not equally sensitive. It was recommended that the potential causal role of endocrine disruptors in breast and prostate cancers, undescended testes, hypospadias and precocious puberty, be investigated through epidemiological studies (Soto et al., 2006). When looking to influence policy it is important to have evidence on data in humans, generally being able to ascertain specific endpoints and compounds is beneficial. Bioassays are extremely beneficial when looking to assess the endocrine disrupting effects of mixtures and assessing interventions. As research progresses on the possible toxicity of phthalate metabolites, however, *in-vitro* studies can be largely beneficial due to the challenge of determining the differential causation of toxicity from the metabolite itself.

1.5.3 Epidemiological studies

There are three main types of epidemiological studies; cohort, case-control and cross-sectional.

A cohort study is the optimal experimental design for determining the incidence and natural history of a condition (Mann, 2003). They are usually prospective or retrospective in nature. In prospective cohort studies, a population without the condition of interest is chosen. The researcher then investigates a variety of variables that might be relevant to the development of the condition. This population is then observed over time to see whether or not they develop the condition. In single cohort studies those people who do not develop the outcome of interest are used as internal controls. Where two cohorts are used, one group has been exposed to the agent of interest and the other has not, thereby acting as an external control (Porta, 2008). Alternatively, retrospective cohort studies use data already collected for other purposes. The cohort is "followed up" retrospectively. The study period may be many years but the time to complete the study is only as long as it takes to collate and analyse the data (Mann, 2003). The relative risk can be found from a cohort study (**Equation 1**). This is the ratio of the risk of occurrence of a disease among exposed people to that among the unexposed (Bonita, 2012). In the case of phthalates, all subjects will have been exposed but the group with the lowest level of exposure will be used as the referent.

$$RelativeRisk = \frac{Incidence\ Exposed}{Incidence\ Unexposed} \qquad \textbf{Equation 1}$$

Case-control studies examine the association between exposure and a health outcome by comparing individuals already ill with the disease of interest (i.e. exposed cases) and a control group who are a sample of the same population from which the cases were identified (i.e. exposed non-cases) (Mann, 2003). Case control studies are the least expensive studies to run as they intentionally select subjects with the condition of interest providing more cases. Case-control studies determine the relative importance of a predictor variable in relation to the presence or absence of the disease. Due to their retrospective nature they cannot be used to determine the relative risk. They can however be used to calculate odds ratios, which are estimates of the relative risk (Porta, 2008). Odds Ratio (OR) data forms the basis of association to health risk (**Equation 2**) An Odds Ratio value of above 1 associates that degree of exposure with an increased odds of developing that health outcome whereas an Odds Ratio of 1 has no association with the outcome (Szumilas, 2010). The Confidence Interval (CI) must be reported with the OR as it gives an estimate of the precision of the risk estimate. A 95% CI that reports values both below and above 1 is not significant as it crosses the "null value", implying that the exposure is both positively and negatively associated with the outcome of interest and therefore the risk estimate is not precise (Viera, 2008). Again, in the case of phthalates, all subjects will have been exposed but the group with the lowest level of exposure will be used as the referent.

$$OddsRatio(OR) = \frac{ExposedCases(n) \times UnexposedNon-cases(n)}{ExposedNon-cases(n) \times UnexposedCases(n)} \qquad \textbf{\textit{Equation 2}}$$

Cross-sectional studies are most frequently used to determine prevalence although they can also be used to determine causation (Bonita, 2012). Measurements are made at one point in time and the subjects are assessed to determine whether they have the exposure of interest and whether they have the outcome of interest. Some of the subjects will not have been exposed nor have the outcome of interest. Therefore, ethical difficulties are greatly reduced as subjects are not purposely exposed or treated, and treatment is not deliberately withheld. This type of study is relatively cheap as multiple outcomes can be monitored over only one group with minimal data collection labour (Porta, 2008).

Some epidemiological studies use surveys as a means to divide people into groups of magnitudes of exposure (Bonita, 2012). A hypothetical example would be subjects who used the most cosmetics being classed as the highest exposed group. However, as there

is very limited data available on the concentration of phthalates in most consumer products, this does not offer sufficient accuracy. Therefore, the risk data will only be analysed from epidemiological studies that use biomonitoring as their source of exposure, i.e. studies that have accurate exposure data derived from phthalate metabolite levels in a human biological sample. Odds ratio data seem to be the most prevalent in the literature so these values will be used in combination with any relative risk data to assess human health risk in Ireland.

1.5.4 Health Outcomes

1.5.4.1 Reproductive Health in Males

As phthalates are anti-androgens and as DEHP and BBP have been classified as toxic to reproduction, Category 2; R60-61, it is necessary that any association between phthalates and reproductive characteristics should be thoroughly investigated (IARC, 2010). Other phthalates have not been classified but may produce the same effects. Many studies have been conducted with rats that show the impact of phthalates on the male reproductive system now known as "rat phthalate syndrome". This comprises of incomplete masculinization of rats including cryptorchidism (undescended testes), retention of nipples/areolae, demasculinization (incomplete masculinization) of the perineum, resulting in reduced anogenital distance and through malformations of the epididymis, vas deferens, seminal vesicles, prostate and external genitalia (hypospadias) (Oehlmann et al., 2009). This direct effect of phthalates causing genital abnormalities may lead to further harmful effects like atypical sperm characteristics, which may in turn, develop into testicular cancer (Gray et al., 2001; Hauser, 2006).

Male infertility has become a prominent issue in recent years. Increased levels of cryptorchidism, reduced anogenital distance and sperm quality have contributed to this overall effect (Halden, 2010). At present there is inconclusive evidence that poor reproductive outcome is experienced by human males as a result of phthalate exposure; so this aspect needs to be elucidated.

1.5.4.2 Cryptorchidism

Cryptorchidism is a condition where the testes have not descended correctly. Testicular descent contains two phases; abdominal descent which occurs in the first trimester and inguinal scrotal descent which occurs in the third trimester and is highly dependent on testicular androgens. There is an increased risk of cancer associated with late descent. Multiple studies have associated high phthalate exposure to cryptorchidism (Kay et al., 2014; Swan et al., 2005; Wagner-Mahler et al., 2011; Virtanen and Adamsson, 2012). A greater than two-fold increase in the odds of incomplete testicular descent for a doubling in maternal urine MEHP has been found (OR = 2.38, adjusted for age and body weight percentile). No effects were indicated for MEP, MBP, MiBP, MMP, MBzP, or MCPP (Swan et al., 2005). A more recent study reported a higher prevalence of cryptorchidism in French children who had mothers with occupational phthalate exposure (OR=8.3) than among those who did not (OR=5) (Wagner-Mahler et al., 2011). However, there was a small sample size of children whose parents were occupationally exposed to phthalates, and exposure concentrations were not measured, using only an exposure matrix survey. Therefore, this study will not be included in the final risk data reported. There is a wealth of studies on cryptorchidism in rodents while the human data remains limited. The mechanisms of testicular descent vary significantly between humans and rats. However, there are sufficient epidemiological data to relate this adverse health effect to humans.

1.5.4.3 Hypospadias

Hypospadias results in incomplete closure of the urethra and is one of the most prominent urogenital congenital abnormalities found in baby boys. Urethral development is a testosterone dependent process that occurs between the 8th and 12th gestational weeks and as phthalates have anti-androgenic properties, an association between hypospadias and phthalate exposure has been hypothesised.

Three case-control studies and one proportional-morbidity study that considered a relationship between parental occupational exposure and hypospadias were reviewed (Kay et al., 2014). An increased risk for hypospadias with maternal occupational phthalate exposure (OR = 3.12), adjusted for income, birth weight, maternal smoking, and folate use during pregnancy was observed (Ormond et al., 2009). The study sample comprised 417 cases referred for surgical correction and 490 randomly selected controls,

all residing in South-Eastern England. However, only 14 cases and 4 controls were delivered to mothers with occupational exposure to phthalates. Occupational exposure was quantified through self-reported job history and a job exposure matrix that may have shown bias. In addition, capture of isolated defects referred for surgical correction excluded mild and syndromic cases. Vrijheid et al. 2003, found contradictory results in a registry-based birth defects study revealing no association between phthalate exposure and the incidence of hypospadias among all congenital anomalies, adjusted for social class, year of birth, region, and maternal age (OR = 0.90). All reported cases of hypospadias in England and Wales from 1980 to 1996 (3471) were included in the numerator and all defects reported by the National Congenital Anomalies System (*n* = 35,962) were included in the denominator. Maternal exposure was examined using a specially developed and validated job-exposure matrix.

1.5.4.4 Sperm Quality

Kay et al 2014 analysed the data on phthalates' effect on semen quality. Over a 50 year span, an overall decline in semen quality in relation to phthalate exposure was observed by Carlsen et al. 1992, leading to an interest in the topic. This was particularly controversial as semen quality is dependent on multiple variables including sperm concentration, count, and morphology. In Kay's systematic review studies following both general and clinical populations were analysed giving a complete picture on the subject. Studies in the general population were far less common than in clinical populations. However, studies conducted in the general population did show an association with phthalates and decreased sperm concentration.

Clinical studies have mostly been conducted in infertile subjects. In 2006 Hauser et al. followed the semen quality and phthalate metabolite concentrations of 443 men who had partners undergoing fertility treatment in the Massachusetts General Hospital. It was found that MBP posed the greatest risk for decreasing both sperm concentration and motility (Concentration OR 3.3, Motility OR 1.8).

The sheer quantity of data that records adverse effects of phthalates on male reproductive health makes it is clear that phthalates pose a risk to the male population. This occurs in terms of reduced AGD in male children, hypospadias, cryptorchidism and reduced sperm

quality. However, it is important to note that a large amount of the studies conducted were in animals and therefore the direct effect on humans needs to be investigated further.

1.5.4.5 Neurological Development of Children

Phthalates have been shown to reduce thyroid function and levels of Insulin Growth Factor 1 (Boas et al., 2010). As these factors hold an important role in the neurological development of children, the influence of phthalates on neurological development has been studied. Prenatal exposure to DBP and DEHP resulted in a negative association with Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) scores, scores that are used to monitor neurodevelopmental outcomes like cognitive and motor development in infants (Kim et al., 2009). A systematic review on the neurological outcomes of phthalate-exposed children found that exposure to phthalates resulted in reduced cognitive function in terms of lower IQ scores, poor social communication, reduced attention and hyperactivity. Low molecular phthalates were seen to have a significant impact (Ejaredar et al., 2015).

Assessing issues with neurological development can be more challenging. While medical history of Autism Spectrum Disorder (ASD) or Attention Deficit Disorder (ADD) are often used, there are some other behavioural tools that can be used for assessment at the time of study. In the literature several different techniques have been used. A cohort study of 417 Korean mother–infant pairs reported negative relationships between urinary concentrations of maternal MnBP and MiBP, and mental and physical developmental scores on the BSID-II at six months of age. When maternal IQ was controlled significant association remained (Kim et al., 2009). Higher urinary concentrations of MnBP and MiBP also correlated with poorer motor development at three years of age in a study of 296 mother–child pairs from New York City, USA and concluded that prenatal exposure to MnBP was associated with delayed mental development on the BSID-II in girls at three years of age (Whyatt et al., 2012). In addition, prenatal exposure to MnBP was associated with internalizing behavioural problems such as withdrawn symptoms in girls, based on maternal reports on the Children Behavior Checklist (CBCL) (Achenbach & Rescorla, 2000).

Due to the level of evidence supporting a link between phthalates and the reduction of children's neurodevelopment outcomes it is inferred that phthalates pose a risk to

children. As several studies have shown that even prenatal exposure to phthalates results in harmful health outcomes of children, it is imperative that the health impacts are clearly defined in order to progress legislation if needed. This justifies further investigation.

1.5.4.6 Cancer

The International Agency for Research on Cancer (IARC) has compiled reports on the phthalates DEHP and BBP, illustrating that high phthalate body burden of these phthalates is considered to pose a potential risk for the development of cancer. As less studies have been conducted on other phthalates it can reasonably be hypothesised that these phthalates could also possibly increase cancer risk. For DEHP there have been studies relating to hepatocellular tumours, pancreatic acinar-cell adenoma and testicular Leydig cell tumours in animals. Limited studies in humans exist.

Epidemiological studies associating phthalate exposure to cancer are rare. According to the IARC Monograph (2000) only one epidemiological study existed at time of reporting; a mortality study conducted by from 1940 and 1976 of 221 German employees of a DEHP production plant. There were some flaws in this plant-based study, the methods were not discussed thoroughly enough to ensure its accuracy, additionally, the majority of the study group were working after factory exposure levels had been greatly reduced. Different types of cancers have been studied in the plastic industry in relation to DEHP. From 1993 to 1997 a case-control study in Sweden followed 791 cases of germ cell tumours and 791 controls matched by 5-year age group (Hardell et al., 1997). A non-statistically significant increased risk was reported for exposure to soft plastics (containing plasticizer) but not for rigid plastics (with minimal plasticiser content). A population based case-control study evaluated the relationship between multiple myeloma and exposure among Danish men (Heineman et al., 1992). There were 1,098 cases and 4,169 control subjects matched by age. Non-statistically significant elevated odds ratios were found for exposure to phthalates and multiple myeloma development. Stratified analysis was conducted eliminate exposure to vinyl chloride, this showed a non-statistically significant increased risk.

IARC's research on BBP shows that it is also being investigated as a possible carcinogen. However, they report no human studies on cancer and BBP (IARC, 2010).

While DEHP and BBP are the most prevalent in the literature, further studies of the effects phthalates have on cancer growth have been conducted, although there have been few epidemiological studies. As breast cancer is hormone dependent this has been a focus of research into a possible connection between phthalates and cancer. A recent study into the effects of nine phthalates on breast cancer in Northern Mexico found that exposure to DEP is associated with a significant increased rate of breast cancer (López-Carrillo et al., 2010). The reported OR for DEP was 2.20. However, other phthalates analysis did not show an association and a significant negative association was found for MBzP and MCPP. Additionally, this study did not adjust for family history of breast cancer which could lead to some inconsistencies in data. Therefore, further research should be conducted to elucidate whether this DEP association is direct or if it is an environmental factor. This study was excluded from phthalate final risk tables (Section 4.2) for this reason.

As DEHP and BBP have been described in multiple IARC monographs, with other plasticisers being studied, there is scope for a wider range of phthalates to produce a similar risk. The epidemiological data is lacking in evidence but there have been associations drawn from *in-vivo* and *in-vitro* research. However, due to the nature of this study, focusing on human evidence, these studies will not be considered for human health risk.

1.5.4.7 Obesity and Insulin Resistance

Obesity has been rapidly growing in the past two decades and has contributed to a large burden on public health. Obesity, insulin resistance and type II diabetes are related in the fact that they indicate metabolic weaknesses. Emergent studies have indicated that phthalates may act as obesogens in humans. A systematic review of epidemiological data on associations of obesity with phthalate exposure concluded that the literature supports the hypothesis that phthalate exposure is associated with decreased lipid metabolism and metabolic changes (Goodman et al., 2014). Self-reported weight data could offer bias from subjects, however many of the studies used blood hormone/lipid levels and clinical measurement. Some of the studies examined were *in-vitro* assays and therefore would not give risk data (Polyzos et al., 2012; Zhang et al., 2015; Smerieri et al., 2015).

A limitation of the observational studies conducted is that it cannot be ascertained whether exposure to phthalates is the causative factor or whether the body's ability to metabolise phthalates is creating the adverse effect. There is a lot of interest in the effects of phthalates in obesity and insulin resistance and there is evidence supporting the hypothesis from study results. Therefore, it should be considered as a possible causative effect in this population study.

1.5.4.8 Puberty

Over the past number of decades, females have been observed to enter puberty at earlier ages (known as precocious puberty). Due to the association of phthalates with anti-androgenic properties, phthalates' impact on inducing precocious puberty in females has been studied. Although some journals reference the effect of phthalates on early pubertal development in girls there is conflicting evidence found in the literature. Research relating to increased kisspeptin activity shows a possible cause of precocious puberty when exposed to phthalates (Chen et al., 2013) with other studies supporting the possible association (Colón et al., 2000; Qiao et al., 2007). However, Lomenick et al. indicated that there was no relationship between phthalate exposure and precocious puberty in female children (Lomenick et al., 2010). Most research has shown negligible relationships. Therefore, there is no clear establishment of the effects of phthalates on puberty in females. As precocious puberty in females has most frequently produced ambiguous and insignificant results, this health aspect has been viewed as providing inconsequential risk and is not be a focus of this study.

From assessment of the literature, the most pertinent studies have been in pregnancy complications, genital anomalies in male infants, precocious puberty in females, decreased neurological development in children and obesity. The Odds Ratio data is discussed with the WBE study in Section **4.2.2**.

1.6 Biomonitoring as a Tool to Assess Phthalate Exposure

Biomonitoring measures the concentration of each phthalate or phthalate metabolite in human body fluids, e.g., urine, blood or sweat, using analytical techniques. The majority of biomonitoring studies focus on phthalate metabolites rather than parent compounds. This is due to increasing evidence that as little as 1% of phthalates are excreted unchanged (Frederiksen et al., 2007a). As there is limited data available on the exact metabolism of phthalates, the selection of an appropriate biomarker of exposure is subjective. There is an increasing level of evidence that shows selection of the more extensively metabolised analytes offers more accurate results (Saravanabhavan et al., 2012; Anderson et al., 2011). Monoesters have short half-lives resulting in lower concentrations in biological samples. Additionally, and particularly from the point of view of wastewater based epidemiology, monoesters may be produced through an abiotic process from parent phthalates of outside sources in some human samples that contain esterases (e.g. blood, faeces) but particularly in wastewater effluent as many enzymes are contained in this matrix (Hogberg et al., 2008; Gonzalez-Marino et al., 2017). However, preliminary data suggests that the percentage of monoesters formed abiotically is very low and mostly occurs near neutral pH. Therefore, if a matrix with esterases is analysed, overestimation of phthalate metabolite concentration should be negligible if pH is controlled (Gonzalez-Marino et al., 2017). Since the choice of metabolite may have a significant influence on the exposure estimate, literature exposure values may vary significantly when they are based on different metabolites (Calafat et al., 2010).

An issue that arises when modelling exposure is variability in phthalate exposure between populations. While this effect on the data may be small when a large population is studied, it may be significant when biomonitoring is performed on smaller samples (Dewalque et al., 2015). Skewing of the results by making measurements reflecting recent exposures might lead to an overestimate of average exposures and thus to the conclusion that risks are higher than they actually are. Variation in human metabolism could have an impact on the applicability of the model for exposure monitoring. Studies suggest that very little of the phthalates consumed are excreted unchanged. Therefore, in biomonitoring studies it is important to consider phthalate metabolism in the selection of biomarkers. Phthalate metabolism has been extensively described (Saravanabhavan et al., 2012)**.** The simpler forms of phthalates such as DEP and BPB are usually excreted as primary phthalate

monoesters. However, highly branched phthalates undergo more extensive biological transformations, signifying that the use of more extensively oxidised metabolites may prove to be more effective due to their higher selectivity and longer half-lives (Frederiksen et al., 2007b).

As phthalates are readily metabolized and change rapidly in concentration over time most phthalate monoesters have exhibited substantial within-subject variability. The time of day at which measurements are made may have a significant impact on the results because certain activities, such as use of personal care products, are likely to occur mainly at certain times of the day (Duty et al., 2005). In addition, ethnicity and socioeconomic variables have impacts on exposure (Koo et al., 2002). Of the factors that influence monoester levels in urine, sociodemographic and lifestyle factors (class, bodyweight, education) have been established as superior predictors of phthalate exposure when compared to food habits and cosmetic usage (Valvi et al., 2015). These factors are often not reflected in the data, which are generally summary values for particular age groups. In contrast, a heterogeneous population study found that monoester levels do not vary consistently by age or gender (Fromme et al., 2007).

Biomonitoring data are not generally available for some populations thought to be particularly at risk, i.e., infants and young children and most of the data available on conversion from body fluid concentrations to exposure levels are from adult based studies. Children have higher magnitudes of exposure to phthalates and including them in populations could cause a minor shift in the distribution (Dewalque et al., 2014). This is an important consideration to take into account when comparing data from wastewater epidemiology studies to conventional biomonitoring as these studies generally do not involve children.

Another significant limitation of biomonitoring is that it is usually based on a single sample at a particular date and time and so does not provide information about the time course of exposure in that individual or population. This is particularly true for compounds, like phthalates, that are readily metabolized and change rapidly in concentration over time (Anderson et al., 2001; Wittasek & Angerer, 2008).

The matrix examined should be strongly considered when conducting a biomonitoring study. Often the matrix will be preferential to certain phthalates or only give accurate information into long term or short term exposure. Each matrix will require a very specific means of sample pre-treatment. Some matrices e.g. blood will require a higher level of training for the analyst and be more invasive for the subject.

1.6.1.1 Urine

Urine is by far the most widely studied matrix for the determination of phthalate body burden (**Table 5**). Sample collection requires minimal training and is non-invasive. A benefit of urine analysis lies in the fact that urine does not contain the esterases that can break down phthalate parent compounds into metabolites, meaning that contamination is of little to no concern (Wang et al., 2019). Urine samples will often be adjusted for creatinine in order to find a more representative concentration as it controls for urinary dilution. Most studies use the same extraction method as developed by the CDC (Blount et al., 2000).

1.6.1.2 Blood and Serum

Data collected from blood and serum is more difficult to interpret than urine. More persistent phthalates may be found in higher concentrations in blood. An issue with this matrix is the presence of esterases which have the ability to hydrolyse contaminant parent phthalate compounds. Blood is considered the most invasive matrix proposed for the analysis of phthalate exposure. A reliable method to analyse phthalate metabolites in serum was developed by Jeong et al. using column switching LC-MS/MS. When urine was analysed through this method a lower level of variation was found than with serum (Jeong et al., 2011). Additionally, a stable isotope detection method was developed for detection of 6 phthalate metabolites in blood by ultra-performance liquid chromatography coupled with mass spectrometry (Fan et al., 2017). Serum was deemed an unreliable matrix when using less sensitive instrumentation due to many samples presenting below the Limit of Detection (LOD) for a variety of phthalates (Högberg et al., 2008; Hines et al., 2009). Sensitive results are possible, however there tends to be high variation in response when compared to other matrices. Therefore, at present data resulting from serum analysis should be examined with caution.

1.6.1.3 Semen

As male sexual health is one of the most prominent adverse health effects associated with phthalate exposure it is useful to measure metabolites in seminal fluid as it shows exposure at the target organ. The presence of phthalates in seminal fluid has confirmed that the male reproductive system is a target organ of these PAEs. Semen is much less frequently studied; this could be due to more complications regarding ethical approval. The data derived from this matrix offers information on phthalate exposure, this is an indicator of longer-term exposure than found with urine. Studies measure metabolite concentration and sperm quality from the same sample, offering reliable information on how the magnitude of exposure affects the target organ (Wang et al., 2016; You et al., 2015).

1.6.1.4 Breast Milk

As neonates and infants are at a higher risk of excessive phthalate body-burden, phthalate concentrations in breast milk have been studied mainly to assess the level of phthalates that are being passed on to infants through early stage diet. Collection of milk is considered invasive and may cause discomfort to the mothers. Preliminary results show that milk is a challenging matrix for determining phthalate body-burden, with limited research being conducted until recently. In lactating US women, most samples were found below the detection limits, while urine samples from these same women had detectable concentrations of oxidative and hydrolytic monoester phthalate metabolites (Hines et al., 2009). Therefore, measurements in other biological samples (i.e., urine, serum, saliva) do not accurately predict concentrations in milk from the same subject. In particular, high urinary metabolite levels do not predict detectable phthalates in human milk. It has been found in particular that most secondary and tertiary phthalate metabolites measured in this matrix present below the LOD (Hines et al., 2009; Högberg et al., 2008; Fromme et al., 2011). However, some studies of breast milk focussing solely on primary metabolites have higher rates of detection, suggesting that further oxidative metabolites do not favour this matrix (Kim et al., 2015; Kim et al., 2018; Main et al., 2006). Due to generally high limits of detection, analysis of breast milk relies on sensitive methods like tandem mass spectrometry. Alternatively, QuEChERs extraction methods could reduce the matrix effect, reducing the LOD and increasing detection (Fan et al.,

2019; An et al., 2020). In conclusion, breast milk is an analytically challenging matrix that can offer insight into infant exposure sources, but does not act as a proxy for overall phthalate exposure of the mother.

1.6.1.5 Hair and Nails

Hair provides a wider detection window than urine samples; offering an appropriate matrix for determining long term exposure. As hair is lipophilic, it is assumed that HMW phthalates will be found in greater abundance in this matrix. This study only investigated metabolites in terms of DEHP (Chang et al., 2013). It is possible that due to the high lipophilicity of hair it is possible that this matrix would not be suitable for biomonitoring studies involving LMW phthalates.

Nail analysis could also be a possible alternative for analysing long term phthalate exposure. Variability in phthalate metabolite concentrations found in nails are considerably lower than those in urine (Alves et al., 2016). This study only examined three subjects at two sampling points (with one subject not providing the first sample) so the experiment should be repeated to see if this is representative of a larger sample population. Metabolism of phthalates in the nail bed has been described using experimental data, the Lorber PK model and nail bond partitioning; allowing for informed biomarker selection (Bui et al., 2017). Hair and nails are not currently suitable matrices for the biomonitoring of phthalates but further investigation could yield improved methods for the determination of long term exposures.

1.6.2 Summary of Occurrence in Humans

As discussed phthalate metabolites are found in a variety of human samples due to the exposure humans face from the consumer products and the environment. Results from small to medium scale biomonitoring studies were summarised and are reported below (***Table 5***). These concentrations provide valuable data on the levels of phthalates expected to be seen in humans and can give an estimate of the global scale of phthalate body burden for multiple phthalates. The most common matrix is urine and the least frequently monitored matrices are hair and nails. From the data presented, there is expected variation in phthalate concentrations between studies. However, in urine all concentrations are within the same order of magnitude. This study aims to assess if similar exposure levels can be measured from wastewater influent as has been seen in urinary studies (**Table 5**). Larger sample sizes contributed by large scale governmental biomonitoring studies (e.g. National Health and Nutrition Examination Survey, NHANES) usually offer more data-rich sample and subject information with lower variation due to larger sampling numbers.

Table 5: Occurrence of phthalate metabolites in humans

Matrix	**Parent Phthalate(s)**	**Metabolite (s)**			**Mean Concentration**			**Location**	**Reference**
Urine	DBP	MnBuP			10.3		µg/L	Erlangen, Germany	(Koch et al., 2003b)
	BBP	MBzP			10.1				
	DEHP	MEHP			10.4				
		5OH-MEHP			9.0				
		5oxo-MEHP			10.8				
	DNOP	MNOP			7.1				
Urine, Breast Milk, Blood and Serum		**Urine:**	**Milk**:	**Serum:**	Urine (µg/L)	Milk (µg/L)	Serum: (ng/mL)	Lund, Sweden	(Högberg et al., 2008)
	DBP	MBP	MBP	MBP	56	1.2	1.8		
	BBP	MBZP	MBZP	-	20	0.64	-		
	DNOP	MCPP	-	-	2.4	-	-		
	DEHP	MEHHP	-	-	25	-	-		
		MEHP	MEHP	MEHP	18	1.3	0.77		
		MEOHP	-	-	18	-	-		
	DEP	MEP	-	MEP	101	-	1.2		
	DMP	MMP	-	-	2.5	-	-		
	DIBP	MIBP	-	MIBP	21	-	0.87		
Urine	DEP	MEP			9.0		µg/L	Erlangen, Germany	(Kato et al., 2004)
	DBP	MnBP			10.3				
	BBP	MBzP			10.1				
	DEHP	MEHP			10.4				
		5OH-MEHP			9.0				
		5oxo-MEHP			10.8				
	DNOP	MNOP			7.1				

Matrix	Parent Phthalate(s)	Metabolite(s)	Mean Concentration		Location	Reference
Urine	DEHP	MEHP	27.6	ng/mL	Boston, MA,USA	(Duty et al., 2005)
	DBP	MBP	76.2			
	BBP	MBzP	14.0			
	DMP	MMP	10.8			
Urine			**Pre-intervention (µg/g)**	**Post-intervention (µg/g)**	Boston, MA,USA	(Rudel et al., 2011)
	DMP	MMP	0.064	0.126		
	DEP	MEP	0.009	0.180		
	DBP	MBP	0.056	1.850		
	DEHP	MEHP	0.267	2.921		
	BBP	MBzP	0.100	0.172		
Urine			**Pre-intervention (µg/g)**	**Post Intervention (µg/g)**	Tainan, Taiwan	(Chen et al., 2015)
	DMP	MMP	10.4	4.54		
	DEP	MEP	58.6	16.4		
	DBP	MBP	123	84.7		
	BBP	MBzP	8.52	4.67		
	DEHP	MEHP	14.4	6.95		
		MEOHP	55.2	26.9		
		MEHHP	115	61.2		
		MECPP	124	52.9		
Urine	DEP	MEP	345	µg/g creatinine	Georgia, USA	(Blount et al., 2000)
	BBP	MBzP	20.2			
	DBP	MBP	36.9			
	DCHP	MCHP	0.3			
	DEHP	MEHP	3.0			
	DiNP	MNP	1.3			
	DnOP	MOP	0.5			

Matrix	**Parent Phthalate(s)**	**Metabolite(s)**	**Mean Concentration**		**Location**	**Reference**
Urine	DnOP	MCPP	5.8	ng/mL	Georgia, USA	(Kato et al., 2005)
	DMP	MMP	36.6			
	DMiP	MMiP	7.2			
	DEP	MEP	88.4			
	DiBP	MiBP	25.5			
	DBP	MBP	25.5			
	DEHP	MEHHP	13.8			
		MECPP	22.0			
		MEOHP	6.6			
	DCHP	MCHP	7.6			
	BBP	MBzP	28.4			
	DEHP	MEHP	12.3			
	DnOP	MOP	8.7			
	DiNP	MNP	11.7			
	DiDP	MDP	22.5			
Urine			**Males (**ng/mL)	**Females (**ng/mL)	Harbin, China	(Guo et al., 2011)
	DMP	MMP	21.3	26.2		
	DEP	MEP	61.9	77.4		
	DCPP	MCPP	1.8	2.2		
	DBP	MBP	107	102		
	DiBP	MiBP	108	86.9		
	DEHP	MECPP	61.6	55.1		
		MCMHP	46.6	40.7		
		MEHHP	36.2	20.4		
		MEOHP	22.0	12.3		
	BBP	MBzP	3.0	4.7		
	DEHP	MEHP	11.3	5.9		
	DnOP	MOP	0.1	-		

Matrix	**Parent Phthalate(s)**	**Metabolite(s)**	**Mean Concentration**		**Location**	
Urine	DEP	MEP	4.60	ng/mL	Wuhan, China	(Zhu et al., 2016)
	DBP	MBP	53.0			
	DEHP	MECPP	7.27			
		MEHHP	5.29			
		MEOHP	4.70			
		MEHP	1.73			
Urine, Nail Bed			**Nail** (ng/g):	**Urine (**μg/g crea.**):**	Nails: Belgium Urine: Oslo, Norway	(Bui et al., 2017)
	DEHP	MEHP	40.47	0.14		
		5-OHMEHP	6.98	0.55		
		5oxo-MEHP	1.40	0.36		
	DBP&DiBP	(MBP&MiBP)	46.13	1.98		
	BBP	MBzP	1.38	0.09		
	DEP	MEP	30.87	1.2		
Urine	DEP	MEP	180	ng/mL	Massachusetts, USA	(Hauser et al., 2006)
	BBP	MBzP	7.4			
	DBP	MBP	17.3			
	DMP	MMP	3.6			
	DEHP	MEHP	8.0			
		MEOHP	38.0			
		MEHHP	57.4			
Hair	DEHP	MEHP	44.87	pg/mg	Taichung, Taiwan	(Chang et al., 2013)
		MEHHP	5.66			
		MEOHP	9.17			
		5cx-MEPP	ND			

Matrix	Parent Phthalate(s)	Biomarker(s)	Mean Concentration		Location	Reference
Urine	DMP	MMP	6.99	Median (µg/L)	Madrid, Spain	(Herrero et al., 2015a)
	DEP	MEP	68.6			
	DiBP	MiBP	19.3			
	DBP	MBP	23.3			
	BBP	MBzP	2.55			
	DEHP	MEHP	4.40			
		5OH-MEHP	6.15			
		5oxo-MEHP	5.30			
		5cx-MEPP	22.5			
Urine	DEP	MEP	278	ng/mL	Brazil	(Rocha et al., 2017)
	DEHP	MECPP	106			
		MCMHP	26.3			
		MEOHP	36.6			
		MEHHP	53.8			
		MEHP	223			
	DiBP	MIBP	36.1			
	DBP	MBP	73.8			
	DMP	MMP	113			
	DHP	MCHP	10.4			
	BBP	MBZP	2.43			
	DnOP	MOP	3.83			
		MCHPP	1.66			
		MCPP	ND			
	DiNP	MINP	2.58			
		MCOP	ND			
	DiDP	MIDP	6.25			

"-" Denotes that the metabolite was not studied in that matrix. ND shows that the metabolite was studied but not detected.

1.7 Wastewater Based Epidemiology for the Analysis of Public Phthalate Body-Burden

Wastewater based epidemiology is an emerging method of gaining insight on human health and behaviour at a population level. This method utilizes human excretion products (metabolites/biomarkers) of certain compounds as they enter the wastewater system. Wastewater based epidemiology was first used as a means of assessing cocaine use in Italy (Zuccato et al., 2005). Since then it has grown to monitor a wide range of biomarkers for multiple xenobiotics, infectious agents, stress and cancer (Bicchi et al., 2009; Ryu et al., 2016; Yang et al., 2015; Bisseux et al., 2018). If the biomarker is considered stable in wastewater, then the calculated level can be attributed to human exposure. Phthalates are examined as a candidate for wastewater based epidemiology in this thesis. Human exposure to phthalates is unavoidable and as small study sizes are labour intensive and cannot capture the exposure of the general public, wastewater based epidemiology is an attractive method of analysis as it provides a cost-effective and unbiased means of determining human phthalate body-burden at the population level.

Analysing parent compounds (in this case phthalate diesters) to examine a population's exposure to phthalates is ineffective due to phthalates' extensive metabolism and the large quantity of phthalates that enter the sewage system through industrial disposal. The biomarkers used for phthalate exposure are their monoester metabolites (Frederiksen et al., 2007b). Attribution of phthalate body-burden in a population is calculated through multiplying the measured phthalate monoester metabolite concentrations in wastewater by the daily flow rates of that Wastewater Treatment Plant (WWTP) to find daily sewer loads. From this value, the total consumption of the phthalate is estimated by applying a specific correction factor, which considers the average excretion rate of a given phthalate and the molecular mass ratio of the parent phthalate diester to its monoester metabolite. Finally, the daily consumption can be found by dividing these daily values by the number of people served by the WWTP (Zuccato et al., 2008).

This current model for the analysis of down-the-drain chemicals is very basic and does not account for the in-transit and in-sewer transformations that can occur with unstable metabolite compounds. Down-the-drain metabolites can be lost through degradation or formed through enzymatic formation from their parent compounds in transit to the

WWTP. This in-transit and in-sewer transformation is a critical issue for the use of wastewater based epidemiology as a reliable estimator of population level exposure. At the treatment plant, there is an even greater likelihood of these transitions occurring due to the wastewater treatment process (**Figure 3**), which makes follow-on study of transmission of phthalate diesters and monoesters to the environment challenging. Although many studies examine the removal and stability of phthalate diesters in wastewater treatment plants, very little is known about their monoester metabolites in this environment with only one study to date that investigates phthalate monoesters from a wastewater epidemiology perspective (Gonzalez-Marino et al., 2017). There are significant knowledge gaps on the rate of enzymatic formation or removal of phthalate monoesters within the wastewater treatment plant, these variables have also not been accounted for with metabolites of other compounds. Any risk-based predictions for environmental exposure due to transmission from WWTPs determined through this method show an unknown degree of variation as a consequence.

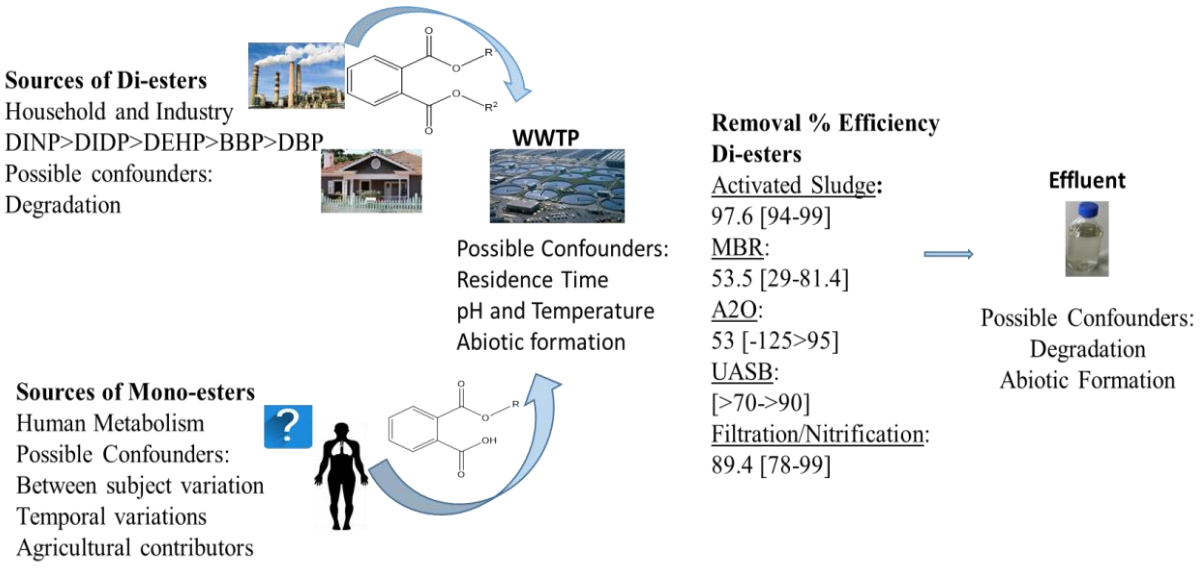


Figure 3: Confounders in wastewater based epidemiology; rational for not monitoring effluent

The available literature has illustrated that in-sewer transformation is compound-specific and influenced by environmental factors. Some compounds seen in the literature (e.g. MDMA, KET and MDPV) remain stable at neutral pH and temperatures up to 20°C. However, drugs like THCCOOH, fentanyl, mephedrone and cathinones have higher levels of variability (McCall et al., 2016). As the current model does not account for confounding factors, ideally, the data from influent should be assessed using a new model that accounts for the ratio of degradation and formation of metabolites, however, this would require detailed data of degradation kinetics for each compound and an advanced knowledge of piping systems and residence times for each WWTP. In order to compare results between different studies and environments, a standardised method with quality controls/correction factors for stability of compounds in addition to in-transit and in-sewer transformation should be developed. This would allow for a higher degree of accuracy when informing policy.

1.7.1 Factors Affecting Phthalate Metabolite Levels Upstream of the WWTP

The variation of metabolic rates between populations and individuals that affect all biomonitoring studies, as discussed previously, also apply to WBE. In-pipe transformation of phthalate monoesters in transit to the WWTP has not been investigated. Bio-films contained in the piping systems could possibly account for some in-sewer formation of monoester biomarkers from the degradation of their phthalate diester parent compounds. Additionally, some degradation could occur in transit as the degradation rates of monoesters in wastewater have not been studied. In humans the half-lives of phthalate monoesters have ranged from 4 to 8 hours for metabolites of DEHP and DINP, however lower molecular weight biomarkers may have shorter half-lives (Anderson et al., 2011).

The temporal variability of phthalate monoesters in wastewater is unknown, due to the evidence of within subject variability shown in urine it is estimated that a single grab sample of wastewater influent would not be sufficient for generating a reliable health risk assessment of that community. Further research needs to be conducted to elucidate this factor in the application of sewage epidemiology (Yang et al., 2015; Baz-Lomba et al., 2016; McCall et al., 2016). This suggests that for biomonitoring purposes more than a single sample should be analysed to account for variability. If one sample alone is taken then it should be a 24-hour composite sample, to get the most representative picture from

one time point as possible. A single grab sample only provides information from a particular date and time and so does not provide information about the time course of exposure in that individual or population.

1.7.2 Factors Affecting Metabolite Levels at the Wastewater Treatment Plant

Many studies have looked at the removal rate of phthalate diesters in WWTPs and the pathways by which they break down. All models of this degradation show transformation of the parent diester to a monoester, as would be seen in human metabolism (Huang et al., 2017; Vavilin, 2007; Liang et al., 2007). The concentration of monoesters formed during the degradation of these diesters is unknown. The rate of degradation of diesters will depend on the wastewater treatment process involved with low molecular weight phthalates degrading more readily (Liang et al., 2007).

Although individual microbes contribute to the degradation of phthalate esters, the rate at which phthalates degrade in wastewater is as a result of breakdown by a combination of many microbes. Phthalates in general have been found to degrade both aerobically and anaerobically with anaerobic degradation occurring at a slower rate (Liang et al., 2007). With average removal rates ranging from 53% in membrane bioreactors to 97.6% in activated sludge treatment, it is clear that some levels of monoesters are being formed in-sewer as opposed to in-human, although it is not known whether this will have a significant impact on results for all treatment plants (D.-W. Gao and Wen, 2016).

Sewage residence time is the length of time of which wastewater resides in a sewer system prior to being treated. Risk assessments based on wastewater data will have to take this factor into account as it can have a significant influence on predictions. There is no data available at present on how residence time affects phthalate monoester levels. However, information on monoester stability in storage can relate to time-related variation in monoester concentrations in-transit (Section 3.3.4).

No data are available on the effects of temperature on the degradation or formation of phthalate monoesters in wastewater although temperature has been long regarded an important variable in the interpretation of wastewater epidemiology data of other down the drain compounds.

The levels of analyte of interest found in the wastewater treatment plant are influenced by pH levels. No data exists on the effects of pH on phthalate monoesters in wastewater.

1.7.2.1 In-Storage Transformations

One existing wastewater-epidemiology study in phthalates looked at the effect of storage on non-human transformation of monoesters with respect to temperature and pH (Gonzalez-Marino et al., 2017). These results can be used primarily to prevent any formation of phthalate monoesters after sampling. However, this data can relate to in-transit degradation, not accounting for flow rates or biological transformation by biofilms. MnBP and MBzP were the only monoesters being formed above method qualification levels, but only at natural pH and at very low percentages of the parent compound concentrations after 24 h (Gonzalez-Marino et al., 2017). Metabolite stability experiments indicated that only MEP and MMP showed low levels of stability, concentrations dropped significantly (up to 35% and 23% of the initial concentration, respectively) after 48 h at room temperature and pH 2. At higher pH, the monoester is primarily found in its ionized form, making it more reactive to other agents in the matrix causing degradation of the original product. This could present a problem in the case of commonly used 24 h composite sampling where the sample spends an average time of 12 h in the sampling container, although modern composite samplers are refrigerated. To circumvent this Gonzalez-Marino et al. suggest to adjust samples to pH 2 as soon as received and store at 4 °C until extraction (performed within 8 h).

No long-term storage degradation studies have been carried out. However, best practice dictates that samples should be extracted and analysed as soon as possible. As discussed previously, when phthalates are metabolised, some are excreted in their free forms and some as glucuronide conjugates (Section **1.2**). Additionally, no studies were conducted to examine the level of glucuronide-conjugate metabolites in the influent for phthalates. However, previous studies on other compounds, using bench tests of free vs bound metabolites, have found that they exist mostly in the free form (Johns et al., 2015). It is predicted that the enzyme content in raw wastewater will also break down the phthalate metabolite conjugates, and that WWTP effluent will be affected at a higher rate owing to biological treatment (Gonzalez-Marino et al., 2017).

1.8 Analysis of Phthalates and their Metabolites

Examining the environmental and human burden of phthalates requires investigation into both parent phthalates and their monoester metabolites. These two groups offer different challenges analytically, with the reduction of laboratory contamination being of the highest priority. GC-MS analysis is highly favoured for phthalates due to fewer points of phthalate contamination within the instrument. LC-MS methods are favoured for their metabolites as no derivatization is required to reduce carboxylic acid shielding.

1.8.1 Quality Control

Stringent quality control is essential in any phthalate analysis due to the ubiquitous nature of phthalates in the environment posing a concern for sample contamination and therefore overestimation of results (Marega et al., 2013; Net et al., 2015).

The first step should be to carefully prepare glassware and materials for sampling and analysis. Minimal plastics should be used, replacing with glass, Teflon, aluminium, stainless steel, or polytetrafluoroethylene where possible (Net et al., 2015). Any unavoidable plastics will be incorporated into procedural blanks. All glassware should be cleaned prior to analysis according to the recommendations specified in U.S. EPA Method 506 to reduce phthalate contamination levels. Although glassware is recommended, it should also be noted that longer chain phthalate esters such as DNP, DNOP, and DHP can adsorb to the glassware. (Khan, 2014)

.

Table 6: Labware preparation for the reduction of phthalate contamination

Procedure	*Ref.*
Laboratory glassware was soaked overnight in K_2CrO_7/H_2SO_4 solution, washed with tap water and redistilled water, baked at 300 °C for 12 h, and then rinsed with acetone, DCM and Hex, respectively.	(Zhou et al., 2016)
All laboratory glassware was washed soaking the material in an alkaline solution for 48 h, rinse with purified water and then washed gently with methanol (super purity grade). Glassware was calcined at 450 °C overnight.	(Fernández-González et al., 2017)
Glassware was cleaned before use by repeatedly washing with hot methanolic potassium hydroxide, chromic and hot concentrated sulphuric acid mixture and purified water, and finally dried at 300 °C for 1 h. All glassware used to collect and extract milk samples was deactivated by rinsing with IPA before use.	(Bubba et al., 2018)
Glass bottles were washed with a detergent (Teepol), then rinsed with deionized water before being placed in an oven at 450 °C for 2 h.	(Deshayes et al., 2017)
All glassware was baked at 450 °C overnight and maintained at 120 °C in furnace.	(Jia et al., 2017)
Glassware was scrupulously cleaned by rinsing first with water and then with acetone and hexane.	(Khan, 2014)
All glassware used for the preparation of the phthalate standard solutions was washed with MTBE, two aliquots of cyclohexane and finally two times with methanol.	(Tienpont et al., 2005)
The equipment was washed with distilled water, acetone, methanol, and ethanol, then dried overnight at 70 °C.	(Kim et al., 2014)
Equipment was washed with different solvents (acetone, dichloromethane, hexane and methanol), heated to 400 °C for at least 2 h.	(Herrero et al., 2015b)
Equipment was washed with hot water and soap, rinsed ultrapure water and rinsed with acetone, before sealing with aluminium foil.	(Barciela-Alonso et al., 2017)

Organic solvents considered appropriate for laboratory glassware washing include: cyclohexane, n-hexane, isooctane, methanol or 2,2,4-trimethylpentane (Tienpont, 2004). Acidic solutions such as hydrochloric acid, sulfochromic or ammonium persulfate/sulfuric acid mixtures (Cincinelli et al., 2001; Dargnat et al., 2009; Mousa et al., 2013) or a potassium hydroxide solution can also be used. Glassware should be heated

overnight to remove organic materials and adsorbed PAEs (Marega et al., 2013; Net et al., 2015). The purity of the solvent should be chromatographic grade or above (Sun et al., 2014) and each new batch of solvent should be checked for phthalate contamination (Deshayes et al., 2017). Prepared glassware should be stored in aluminium foil to avoid air exposure and where possible the room should be over-pressurised so as to avoid air contamination from the outside (Bubba et al., 2018). Laboratory air has been reported to contain concentration ranges between 300 and 700 ng/m^3 for DiBP, DBP and DEHP, and in clean fume hoods these levels are 10 to 50 times higher (Tienpont et al., 2005), while an uncapped cyclohexane vial left uncapped over a 30 minute period was shown to accumulate DBP and DEHP at levels necessary for background subtraction – demonstrating the importance of minimising solvent contact time with the surrounding atmosphere.

Additional demonstration that reagents are free of contamination may be required because reagents may become contaminated during storage in the lab environment (EPA Method 8061a). The use of personal care products should be avoided during sample handling (Cousins et al., 2014; Net et al., 2015), and wearing latex or vinyl gloves should be avoided as they contain significant amounts of phthalate esters. Hands should be washed with hot water only and dried with paper tissue since the use of hand soaps or detergents can result in irreproducible background levels of mainly DBP and DEHP. Perfumes and deodorants affect the presence of phthalates in the surrounding atmosphere and the personnel performing the analytical work should be perfume-free (Tienpont et al., 2005).

A blank should be collected both in field, and at each stage of sample treatment. This should be demonstrated to be phthalate-free. Procedural blanks should be extracted together with each set of samples and measured in triplicate (Jia et al., 2017), and if any contamination above the LOQ is found, and is impossible to eliminate, it must be subtracted from the sample measurement (Net et al., 2015).

1.8.2 Sample Pre-treatment

Frequently in the literature enzymatic de-conjugation of biological samples must take place to cleave any metabolites (phthalate monoesters) excreted as glucuronyl conjugates (Saravanabhavan et al., 2012). A selective method for enzymatic de-conjugation of nine phthalate metabolites was first introduced by the CDC and has been widely accepted (Blount et al., 2000). Samples are buffered with ammonium acetate and then spiked with isotopically labelled and deconjugated internal standards. *E. Coli* β-glucuronidase is added and the samples are sealed and mixed. β-glucuronidase from *E. coli* source is used as this does not demonstrate lipase activity except in the case of its specificity to glucuronyl conjugates, therefore this prevents breakdown of phthalate parent compounds. An additional standard of 4-methylumbelliferone is added to monitor β-glucuronidase enzyme activity, as assessed by the levels of 4-methylumbelliferone released by internal standards. This has been adopted as gold standard, used in other studies with no deviation. (Kato et al., 2003; Kato et al., 2005; Jeong et al., 2011; Herrero et al., 2015).

However, it is thought that all metabolites contained in wastewater are in their free form due to the high microbial activity of the matrix (Li et al., 2018). Bench studies have shown that this is true for morphine and cocaine metabolites. Many researchers assume that their compounds of interest will undergo within matrix de-conjugation due to the behaviour exhibited for these few groups of metabolites. It has been confirmed that phthalates also occur readily in their free form.

1.8.3 Sample Extraction

Environmental samples need to be extracted before analysis due to the complexities of the mixtures and to avoid damaging sensitive analytical equipment. Liquid-Liquid Extraction (LLE) is very popular in phthalate analysis due to its simplicity. However, this method requires a large volume of solvents, such solvents must be phthalate free and the additional glassware and preparation steps are expensive and have a greater likelihood to introduce contaminants (Milojković et al., 2015). Due to this, more recent studies show an increased preference for solid phase extraction. Furthermore, for the specific analysis of phthalate metabolites, no use of LLE was found in the literature. This is most likely due to the increased sample preparation steps required for phthalate metabolite analysis.

The standardised EPA methods 506 and 525, based on LLE, have been replaced by the SPE (Net et al., 2015). Similar to LLE, SPE involves the partitioning of compounds between 2 phases. The analytes (in liquid form) are placed in a small cartridge containing an appropriate packing material where the analytes will have a stronger affinity for the solid packing than for the sample matrix and the liquid sample is passed through this cartridge either by suction or by positive pressure. The analytes are retained on the solid phase while many interferences are washed out; the analytes are subsequently removed by eluting with a solvent with a greater affinity for the analytes (Herrero et al., 2015a).

When analysing the samples, APHA guidelines recommend the use of automatic samplers, provided that they are shown not to contaminate the samples. They can eliminate human errors in manual sampling, can reduce labour costs, and may provide the means for more frequent sampling (APHA/AWWA/WEF, 2012). Amber vials should be used and sealed with Teflon coated caps or foil lids and stored in a freezer (-20 ^{o}C) (Kim et al., 2014). Syringes should be programmed for regular solvent washes and washing solvents should be replaced daily (Tienpont et al., 2005).

SPE cartridges appear to be the best method of sample extraction. This method offers better selectivity and recovery and uses much less solvent than LLE. This direct method cleans the sample effectively while reducing preparation steps and therefore offers less opportunity for phthalate contamination. This is also a much quicker method of extraction and it allows automation (Alzaga et al., 2003). Therefore, to the author's knowledge, using phthalate free SPE cartridges will result in a quicker analysis that may lead to more repeatable results. Acidifying phthalate samples prior to SPE extraction will promote equilibrium to the unionised form and therefore increase efficiency due to the acidity of the samples.

SPE extraction exists both in online and offline modes. Offline SPE methods generally use reversed phase hydrophobic-hydrophilic balanced polymeric sorbents giving them a wide range of polarities. These sorbents remain stable in a wide pH range, are non-effected by sorbent drying and contain no silanol reactions. The most popular sorbents include; polyamide, poly[n-vinylpyrrolidone-divinylbenzene (DVB)], methacrylate DVB, and hydroxylated polystyrene DVB, a particle size of 30-60 µm is recommended

to ensure the efficient passage of urine biomolecules (Ramesh Kumar and Sivaperumal, 2016).

Online SPE methods used in conjunction with HPLC increases efficiency as it is an automated and controlled process. SPE is coupled to HPLC through a small, 2–15-mm-long and 1–4.6-mm–id, precolumn, via a switching valve (Chen et al., 2012). The sorbent dictates parameters such as selectivity, affinity, and capacity. Generally, it is recommended that the sorbent used in the SPE column is similar to the analytical column material (Salazar-Beltrán et al., 2017). The SPE column needs to have a lower absorbance for the analytes of interest than for the analytical column, ensuring that the analyte band will refocus on the front of the analytical column. In order to avoid breakthrough effects, the sample should usually be aqueous and loaded on to the SPE with a non-eluting reverse phase solvent (aqueous solvent). Typical SPE–HPLC column pairs used for phthalate metabolites analysis are C_{18}-bonded silica (Hysphere-C_{18}HD: Intersil ODS-3) and monolithic-bonded silica pairs (Chromolith Flash RP-18e: BETASIL Phenyl) (Ramesh Kumar and Sivaperumal, 2016).

Both offline and online SPE methods have their own advantages and disadvantages. They provide similar LODs, accuracy, and reproducibility for phthalate diesters and metabolites, while online methods have greater sensitivity, offline methods use larger sample volumes (Ramesh Kumar and Sivaperumal, 2016). For online SPE, sample handling is minimized and, therefore, operator error and exposure to hazardous materials are reduced. This is especially useful in the analysis of phthalate metabolites which require extra preparation; derivatization, enzymatic de-conjugation, spiking internal standards, and dilution can be done online using a customized HPLC autosampler programme (Kato et al., 2005). In addition, the use of online SPE does not involve the evaporation and reconstitution of the sample extract, removing analyte losses by evaporation.

However, online SPE may introduce cross-contamination and column overloading due to unknown concentration ranges in samples. To reduce this, separate binary pumps for SPE and HPLC, and efficient cleaning is needed. This requires large investment and time commitment up front. It is therefore better suited to large-scale monitoring studies, or other high throughput methods.

1.8.4 Instrumentation

1.8.4.1 GC-MS

Diesters

Analysis of phthalate parent compounds frequently use GC-MS due to the lower background phthalate levels contributed by the instrument. GC–MS-based methods are simple, robust, do not create ion suppression effects and offer a high level of reproducibility, although run times tend to be much longer than those with LC-MS. EPA Methods 606 and 8106 contain a GC-electron capture detector (ECD) method to determine six phthalates in municipal/industrial wastewater and aqueous/solid matrices (including ground water, leachate, soil, sludge, and sediment), respectively, while EN ISO 18856:2005 outlines GC methods for aqueous matrices. MS based detectors have been widely applied for the phthalates determination by these two techniques, but less sensitive and more affordable techniques such as liquid chromatography with diode-array-detection (HPLC-DAD) and GC with flame ionisation detection (FID) have also been used (Pérez-Outeiral et al., 2016).

Monoesters

Analysing phthalate metabolites by GC-MS is considered a more challenging method of analysis in comparison to LC-MS due to the low volatility and polarity of the metabolites. The carboxylic acid group can cause shielding on the column providing poor chromatography. A common method of circumventing this problem is through derivatising this group. Common derivitising agents include silylating agents N,O Bis(trimethylsilyl) trifluoroacetamide (BSTFA), ethylating agent *N*-methyl-*N* trimethylsilyltrifluoroacetamide (MSTFA), alxoxylating agent 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), and methylating agents triethyloxonium tetrafluoroborate (TEOTFB), diazomethane, and trimethyl silyl diazomethane (TMSDM) (Martens and Martens, 2002; Dirven et al., 1993; Gries et al., 2012; Song et al., 2013).

The majority of phthalate metabolite GC analysis use nonpolar columns (e.g. DB-5, Ultra-2, HP-5 MS, and VF-5 MS). These are all Agilent columns but other manufacturers hae colmns of equivalent structures. However, intermediate polar columns (e.g. Rxi 17) can offer more effective separation for the secondary metabolites of some phthalates (Ramesh Kumar and Sivaperumal, 2016).

When analysing through GC-MS, electron Impact Ionization (EI) is preferred, in GC–HRMS (high resolution mass spectrometry), negative chemical ionization (NCI) is more commonly used. Quadrupole MS techniques are often operated in the selected ion monitoring (SIM) mode to improve selectivity. The LOQs reported are in the sub-μg L^{-1} to <5 μg L^{-1} range for different phthalate metabolites (Ramesh Kumar and Sivaperumal, 2016). Lower LOQs are achievable with other mass analyzers, such as HRMS, and quadrupole ion trap (QIT) mass spectrometers. GC–HRMS also allows for the quantitation of isomeric metabolites of DPHP and DiDP/DiNP, which co-elute using other instrumentation (Gries et al., 2012).

Derivatization is a major drawback of analysing monoesters through GC-MS. These reactions are labour intensive and highly specific, working only with water-free extracts. This step is also hazardous, often using toxic or potentially explosive compounds.

1.8.4.2 LC-MS

Diesters

LC has been emerging as a reliable and efficient method of determining levels of phthalates in a variety of matrices. The mobile phase(s) combination and choice, as well as the column sorbent, are critical in the successful separation of phthalate mixtures. Phthalates may arise in the sample due to contamination from the components of the HPLC instrument. One method of removing this interference is through adding a delay column before the injection port. However, a wide range in LODs for phthalate diester for various matrices has been reported in the literature, from 0.2 to 0.9 ng/mL for DMP in rain water using LC-DAD, to LODs for DMP, DEP, BBP, DnBP, DEHP, and DnOP of 13, 28, 6, 71, 15, and 11 mg L^{-1}, respectively, using UHPLC-DAD for soil samples (Wang et al., 2018). The separation of phthalates by HPLC (coupled or not coupled to IT-SPME) usually takes a long time or needs high flows. The use of a monolithic column allows for the improvement of chromatographic separation because it provides low pressures and very good efficiencies for its porous structure, however as more phthalates are included for analysis higher pressures, longer runtimes and more solvent are required (Fernández-Amado et al., 2017). An advantage of LC is that it provides superior resolution of isometric mixtures of phthalates when compared to GC.

Monoesters

The most commonly used method of analysis for phthalate metabolites is LC-MS. The tandem technique has been used for this purpose since 2000 (Blount et al., 2000). A lot of phthalate analysis in the literature is using GC-MS however; the monoester metabolites are less volatile so LC-MS is a more effective choice as it doesn't require derivitization steps. There are standardised methods available for metabolites of DEHP and BBP. The MS is typically used in multiple reaction monitoring (MRM) mode. Collision energy and mass-to-charge ratio transitions are selected from product ion scans and built into the MRM method to give an optimized signal. The most frequently used ionization technique for phthalate metabolites is ESI in negative mode. ESI is preferred to chemical ionization (CI), as metabolites containing two carboxylic acid groups do not readily ionize in CI. The LOQs are reported to range from sub-μgL^{-1} to <3 μgL^{-1} for different metabolites (Ramesh Kumar and Sivaperumal, 2016).

Betasil phenyl analytical column was used in the CDC methods. This column has increased sensitivity for polar analytes, offers a high level of peak separation and better peak shape for phthalate metabolites. The first method analysed eight phthalate monoesters using atmospheric pressure chemical ionization LC–MS/MS for quantitation. An isomeric pair of MEHP/MnOP was adequately resolved, but the structural isomeric pair of MBP/MiBP could not be resolved. However, using triple quad MS, once monitoring unique target transitions, can quantify without resolution. Additionally, this resolution has been improved by changing mobile-phase gradient, reducing the flow rate and increasing the run time (Blount et al., 2000).

1.9 Aims and Objectives of Thesis

Phthalates are ubiquitous in consumer products and have been found in all environments. This widespread environmental and human exposure is a cause for concern due to their endocrine disrupting properties and association with adverse health effects. The literature often focuses on limited research outcomes, often a narrow range of phthalates in one environmental matrix. This thesis has a broad scope, aiming to assess the environmental sources and fates of phthalates in Ireland to identify stressors and inform on policy, while also applying WBE to assess human exposure and risk.

This project aims to determine the extent of phthalate contamination within the Irish environment. Soil and surface water samples will be assessed for contamination with both well-known and less frequently studied phthalates. With greater knowledge of phthalate burden in Ireland, steps can be made to reduce and monitor these EDCs. Sources including wastewater influent, effluent, sludge, municipal waste and landfill leachate are examined to ascertain the flows of phthalates from waste streams to our environment (**Section 3**). The information found will inform on combative measures and prioritize future research in the area.

A major objective of this research is to develop sensitive and robust methods for the detection of phthalates in various environmental matrices. Compliance monitoring is a possible future outcome of increased legislation surrounding phthalates. Therefore, this project will provide method information to the Irish EPA for use in any future compliance work (**Section 2**). This method encompasses all phthalates listed in the US EPA phthalate action plan and all major phthalates included in the most recent amendment to REACH. The main challenge for phthalate analysis is laboratory contamination and, as such, methods for the reduction of phthalate contamination will be described in detail to facilitate efficient technology transfer.

Human exposure of phthalates will be investigated by applying WBE principles to Ireland for the first time (**Section 4.1**). There are limited opportunities for biomonitoring in Ireland this serves as a quick, affordable solution to gain insight on a wide population. The exposure rates are compared to literature risk data and the toxicological risk is also be determined (**Section 4.2**). This will constitute the first application of WBE in Ireland and form the basis of first stage human risk assessment of phthalates in Ireland.

2 Methods

Phthalate parent compounds and phthalate metabolites were measured separately. Upon defrosting the sample was split and extracted for phthalates and their metabolites separately. Two separate LC-MS methods were developed and will be presented separately in this text. This was mostly due to the necessity of buffers in the metabolite analysis. These buffers could not be used without contributing to a phthalate background in analytical blanks that were above the S/N. For ease of analysis and to reduce phthalate contamination, a separate, shorter column was used for monoester metabolites.

Sensitive and selective LC-MS methods were developed and validated to examine eleven phthalates and six monoester metabolites with the Agilent 6470 Triple Quad LC-MS. The targets under investigation were; Benzylbutylphthalate (BBP) and its metabolite Monobenzylphthalate (MBzP), Dibutylphthalate (DBP) and its metabolite Monobutylphthalate (MBP), Dipentylphthalate (DPP), Diisopentylphthalate (DiPP), Diethylhexylphthalate (DEHP) and its metabolite Monoethylhexylphthalate (MEHP), Dihexylphthalate (DHP), Diisobutylphthalate (DiBP) and its metabolite Monoisobutylphthalate (MiBP), Di-n-octylphthalate (DnOP) and its metabolite Monoisooctylphthalate (MnOP), Diisononylphthalate (DiNP) and its metabolite Monoisononylphthalate (MiNP), and Diisodecylphthalate (DiDP).

Efficient extraction methods for phthalate diesters were optimised for a variety of environmental matrices (surface water, wastewater, sludge, soil, leachate and municipal wastes). Solid samples use ultra-sonication paired with Solid Phase Extraction (SPE), with liquid samples using filtration and SPE. Phthalate monoester metabolites in influent were extracted using a standard addition approach to SPE. The resulting methods were robust and resistant to phthalate contamination.

2.1 Phthalate Diester Method Development

2.1.1 Sample collection

All samples were collected in the province of Leinster using methods to control for phthalate contamination. Sample bottles were triple rinsed with Optima LC-MS grade methanol, at sample site bottles were triple rinsed with sample before collection with minimal contact with air. Samples were transported in cooler boxes, adjusted to pH 2 and stored in the freezer (**Figure 4**).

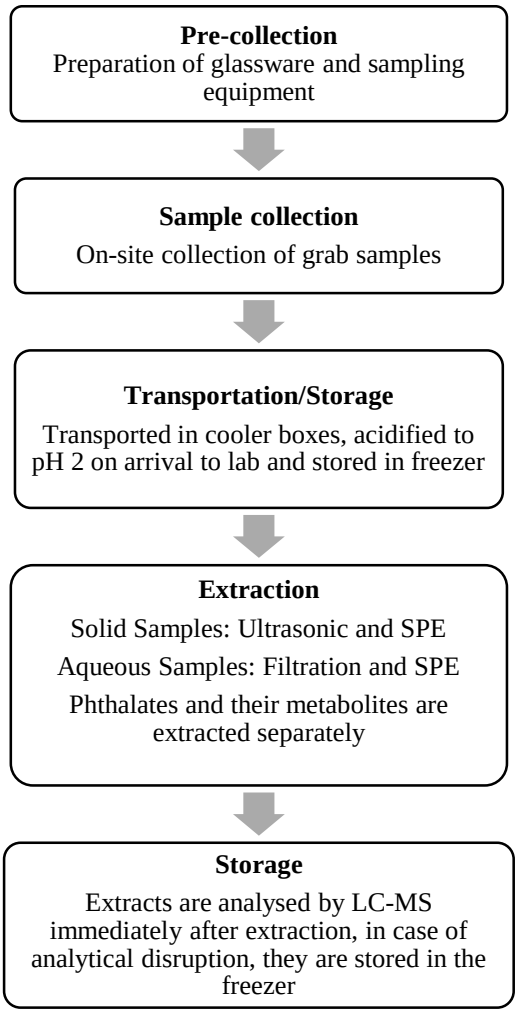


Figure 4: Process flow diagram for sample collection, management, extraction and storage

Samples were taken in a representative manner, sample types and frequency are summarised in **Table 7.**

Municipal waste was segregated into recyclables, food waste and general refuse. Staff of the waste centre took several fractions of each waste type to form one composite sample for recyclable, organic and general waste. In the lab, three subsamples from each of these types were formed. No industrial sized blender was available to the project and therefore the components of recyclable and general waste samples were cut into 1 cm by 1 cm squares. To obtain the most representative sample possible, 3 pieces were added for large items, 2 for medium items and 1 for small items. Food waste was processed in a blender after homogenising with a trowel. For all waste samples, three 30 g subsamples were extracted.

Leachate was sampled by staff of the waste site. Three 1 L fractions from both the lagoon and sump were take on one sample date. 100 mL from each were extracted to form averages for the lagoon and sump.

Wastewater was sampled by the staff of the WWTP, one representative 2 L sample was taken at three sites for influent and two sites for effluent and sludge and four sample dates were included. For influent and effluent 100 mL from each was extracted in triplicate. For sludge 30g from each site was extracted in triplicate.

Surface water sampling involved collecting four 250 mL bottles at each site, taken at mid depth, combined to form 1 L composite sample. Three 100 mL fractions from this composite sample were extracted for the average value at that sample point and date.

Soil was Topsoil (0-20 cm depth). From a site area (e.g. traditional farm, tillage), 100 g samples were collected from three points and homogenised into a 300 g composite sample, by trowel mixing after drying in desiccator. Three 30 g fractions from this composite sample were extracted for the average value at that sample point and date.

Table 7: Description of samples, sub-types, frequency of sampling and extraction methods

Sample Type	*Sample Description*	*Number of Samples*	*Extraction*
Household Waste	Large subsample from waste treatment site classed as food/organic, general, and recycling.	3 representative samples taken from each subsample type. One sampling date. (n=9)	Ultrasonication, SPE
Landfill Leachate	Lined landfill leachate from shut down landfill site	3 representative samples at lagoon and sump. 1 sampling date. (n=6)	Filtration, SPE
Influent	Urban, Suburban, and rural WWTPs	4 sampling dates at each site, one representative sample taken (n=12, extracted in triplicate).	Filtration, SPE
Effluent	Suburban, and rural WWTPs	4 sampling dates at each site, one representative sample taken (n=8, extracted in triplicate)	Filtration, SPE
Sludge	Suburban, and rural WWTPs	4 sampling dates at each site, one representative sample taken (n=8, extracted in triplicate)	Ultrasonication, SPE
Surface water	River samples (upstream, WWTP receiving waters and suburban downstream) seawater (river discharge point)	One representative sample taken at each site. 4 sampling dates (n=16, extracted in triplicate)	Filtration, SPE
Soil	Farm organic (plastic cover, open field), Farm traditional (tillage, pasture), urban parkland (lakeside, roadside, centre), urban business (roadside, centre, roadside)	Samples taken in triplicate from each subsite (e.g. farm traditional, tillage), 1 sampling date (n=10, extracted in triplicate)	Ultrasonication, SPE

2.1.2 Quality control for the prevention of contamination and carry-over

Reduction of contamination: Each new solvent bought in must be checked for phthalate contamination before use, even if the brand had passed this check before. The source was surface cleaned daily and deep cleaned weekly with a weekly flush of nebuliser.

Engineering controls: The instrument was retro fitted with stainless steel tubing and pump heads. The degasser was bypassed to prevent build-up of phthalates in the mobile phase. A delay column was installed after the mixer to push interfering phthalates from mobile phase and system into a different retention time window and a Dynamic Multi-Reaction Monitoring (DMRM) method was developed, removing any of these interferences from the analysis window. A multi-wash system was also used to remove any possible carry-over from the injector or needle seat.

Blank: A stronger solvent than the mobile phase was injected between runs to ensure no carry-over from the column (IPA:ACN 50:50).

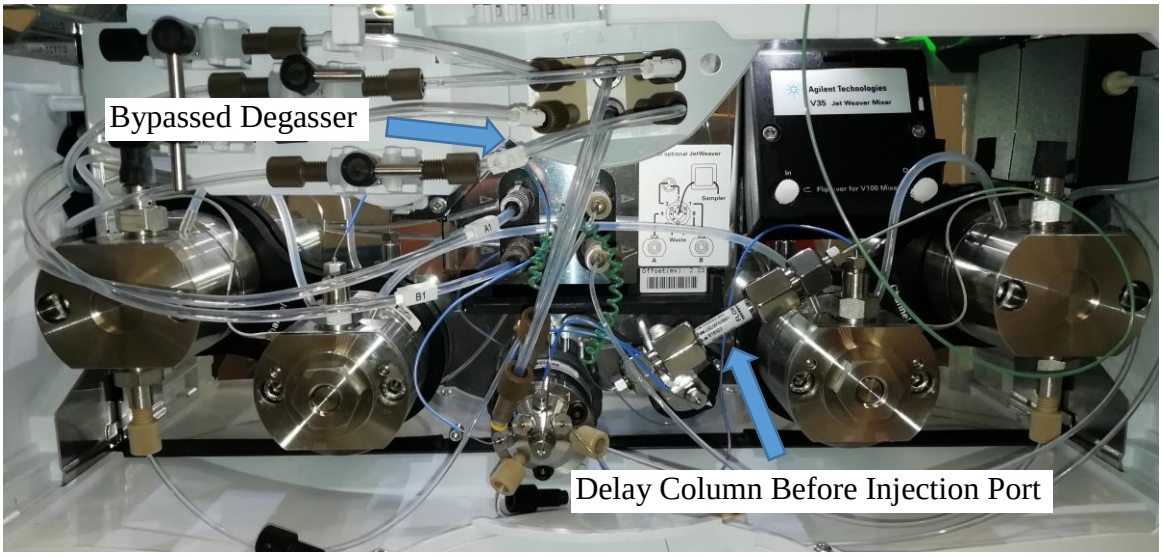


Figure 5: Pre-injection set up with bypassed degasser and delay column

The sourcing of solvents was heavily controlled and Thermo Fischer Optima^{TM} selected due to the lowest introduction of phthalate contamination. However, phthalate contamination was still present in the analytical blanks and it was hypothesised that the tubing and instrumental fittings were contributing to the phthalate background levels. This would explain why the vast majority of recent PAE research uses GC. In order to remove this factor a delay column was introduced, which causes the contaminant phthalates to elute at an alternate retention time.

After instrumental phthalate contamination was removed, phthalate concentration in analytical blanks needed to be reduced. It was found that injector and column carry over were a major source of phthalate contamination in the analytical blank as the delay column removes instrumental contamination. A multi-wash system was used to reduce carry over from the needle and three analytical blanks were run between samples to ensure carry over from the column was removed (**Figure 6**). No peaks above LOD were detected in analytical blanks throughout all sample analysis.

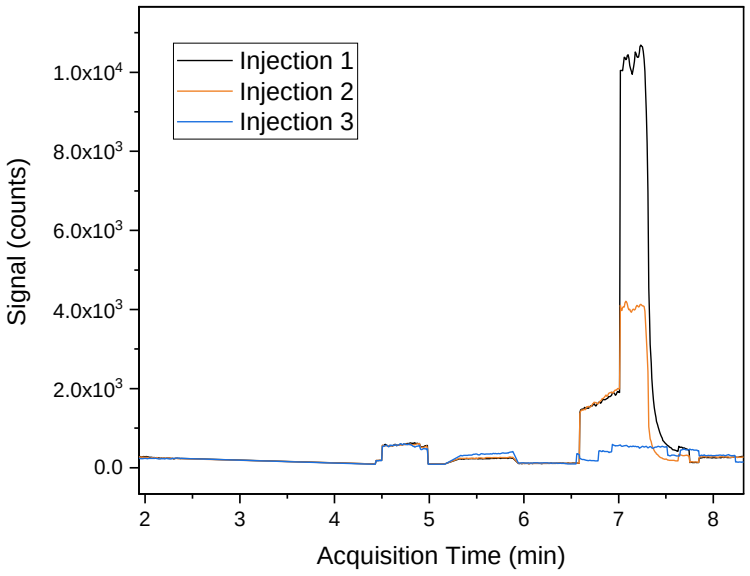


Figure 6: Three consecutive injections of mobile phase (analytical blanks) on LC-MS, the third analytical blank has no phthalate peaks demonstrating the effect of multi-wash programme and analytical blanks on the reduction of phthalate carry-over, using MS conditions described in table 8 and chromatographic conditions described in table 11

For each sample batch extracted by SPE, three procedural blanks were run to determine the amount of phthalate that was coming from background lab contamination. All procedural blanks were then subtracted from the sample to get the true environmental concentration. DBP, DiBP and DMP had the highest concentrations in the procedural

blanks of all samples. All phthalates were detected within the blanks over the course of analysis, although the newer replacement phthalates like DiPP, DPP and DHP were often not detected. **Figure 7** shows the sample peaks in relation to the analytical blank and the procedural blank. The sample in question was surface water from the WWTP discharge site, the analytical blank 100 % ACN and the procedural blank was spiked ultra-pure water extracted in the same sample batch as the surface water in question.

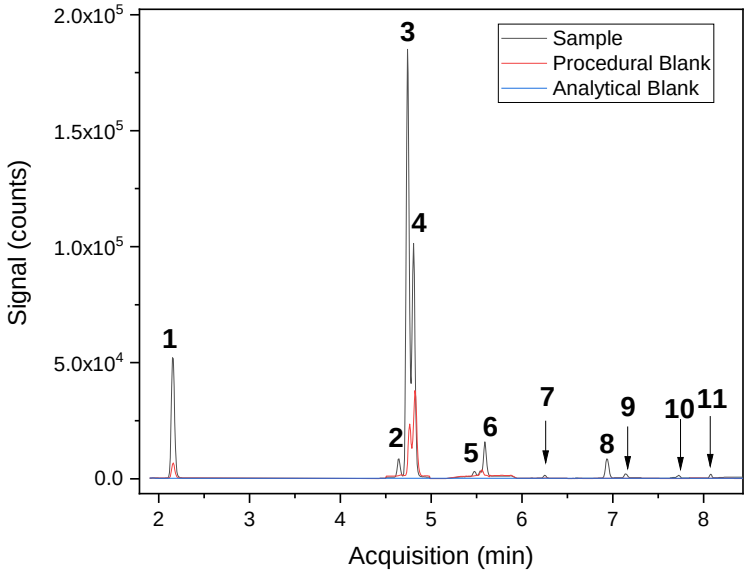


Figure 7: Comparison between analytical bank, procedural blank and sample comparison of surface water sample, procedural blank and analytical blank showing peaks (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DHP, 7 (DEHP), 8 (DnOP), 9 (DnOP), 10 (DiNP), and (11) DiDP, using MS conditions described in table 8 and chromatographic conditions described in table 11

2.1.3 Sample Extraction

All samples were extracted using Solid Phase Extraction (SPE). Solid samples required an extra clean up step prior to SPE due to the complexities of those matrices. Both soxhlet and ultra-sonication were trialled for the pre-extraction step for solid matrices.

2.1.3.1 Solid Phase Extraction (SPE)

Samples were initially filtered with 0.8 µm glass fibre filters followed by 0.45 µm nylon filters to remove suspended solids. Internal standards (Dibutylphthalate-3,4,5,6-d 4, and bis(2-ethylhexyl)phthalate-3,4,5,6-d, 50 µL, 1 ppb) were then added prior to extraction. The filtered samples were solid-phase extracted using reverse phase cartridges. As a validated method EPA 3535A exists for the extraction of phthalates, not many methods were trialled. The EPA method uses dichloromethane and acetone but the project was unable to source these solvents without gross phthalate contamination in the procedural blanks. Therefore, a modification of this method using ACN alone was investigated, and the percentage recoveries were sufficient, when using the same solvent to sample volume ratios and conditioning steps.

Strata-X cartridges (500 mg, 6 mL) were conditioned with methanol (LC-MS grade, 2 mL) followed by acetonitrile (LC-MS grade, 2 mL) and water (Milli-Q, 4 mL). Samples (100 mL, spiked with Dibutylphthalate-3,4,5,6-d4, and bis(2-ethylhexyl)phthalate-3,4,5,6-d, 0.5 ppb) were loaded under low vacuum, then washed with water (Milli-Q, 1 mL). Cartridges were dried under vacuum for 10 min and samples were eluted using acetonitrile (LC-MS grade, 4 mL). The extract was dried under nitrogen, then adjusted to a total volume of 1 mL with acetonitrile.

Strata-X cartridges purchased from Phenomenex. Cartridges were conditioned with methanol (LC-MS grade, 2 mL) followed by acetonitrile (LC-MS grade, 2 mL) and water (Milli-Q, 4 mL). Samples (100 mL) were loaded under low vacuum, then washed with water (Milli-Q, 1 mL). Cartridges were dried under vacuum for 10 min and samples were eluted using acetonitrile (LC-MS grade, 4 mL). The extract was dried under nitrogen, then adjusted to a total volume of 1 mL with acetonitrile. The percentage recovery for this method ranged from 70 to 98% (see ***Figure 8***).

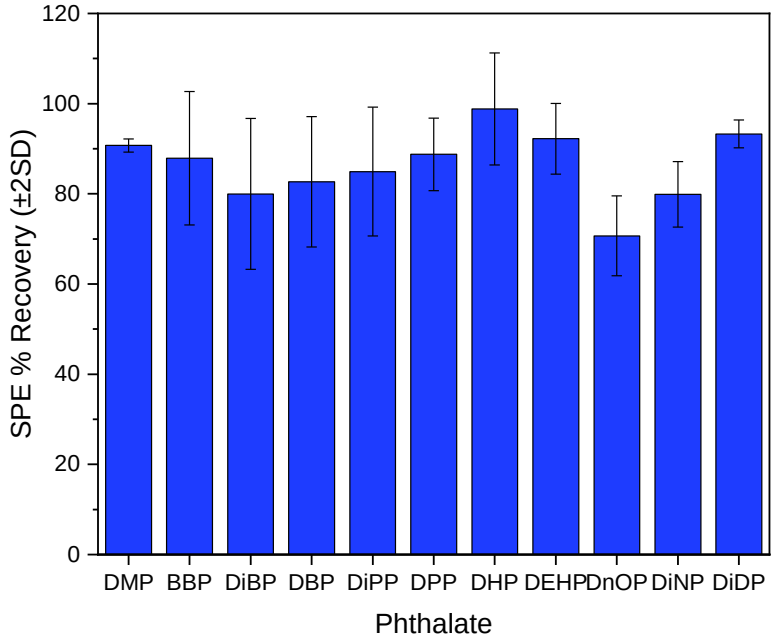


Figure 8: SPE percentage recovery on Strata-X cartridges (500mg, 6 mL) conditioned with methanol (LC-MS grade, 2 mL) followed by acetonitrile (LC-MS grade, 2 mL) and water (Milli-Q, 4 mL). Cartridge loaded with standard solution (1 mL) then washed with water (Milli-Q, 1 mL). Cartridges were dried under vacuum for 10 min and eluted using acetonitrile (LC-MS grade, 4 mL). The extract was dried under nitrogen, then adjusted to a total volume of 1 mL with acetonitrile.

2.1.3.2 Pre-extraction Steps for Solid Samples

For the extraction of solid samples, an additional extraction prior to SPE must be carried out. Phthalate analysis has mainly used soxhlet and ultrasonic extraction for these purposes and both were investigated for this project. The QuEChERS method is increasingly popular for solid matrix extraction but as this uses a high volume of plastics and additional reagents that are not phthalate free it was not investigated due to the probability of introducing high background contamination.

The extraction of phthalates used SPE for all aqueous samples, and solid samples used SPE coupled with ultra-sonication pre-extraction. As a validated method EPA 3535A exists for the extraction of phthalate, not many methods were trialled. The EPA method uses dichloromethane and acetone but the project was unable to source these solvents without gross phthalate contamination in the procedural blanks. Therefore, a modification of this method using ACN alone was investigated, and the percentage recoveries were sufficient, when using the same solvent to sample volume ratios and conditioning steps.

For solid matrices, acetonitrile (LC-MS grade, 100 g) was added to dried samples (30 g, spiked with Dibutylphthalate-3,4,5,6-d4, and bis(2-ethylhexyl)phthalate-3,4,5,6-d, 5ppb). This was placed in an ultra-sonicator, 1/2-inch below the surface of the solvent, but above the sediment layer. The sample was the extracted ultrasonically for 3 min, with output control knob set at 10, the mode set to pulse, and the percent-duty cycle knob set at 50%. The extract was decanted and filtered through Whatman No. 41 filter paper. This process was repeated twice with two additional 100 mL aliquots of clean solvent. On the final ultrasonic extraction, the entire sample was filtered through a Buchner funnel under low vacuum, and collect the solvent extract. This extract was then pre-concentrated by a factor of 10, using the same SPE method with modified load volume.

The soxhlet method trialled used a conventional apparatus setup. This comprised of a round-bottomed flask containing solvent in a heated water bath, attached to an adapter with a thimble containing sample, in line with a condenser. Soil sample (15 g, spiked, dried and homogenised) was placed in the thimble and DCM (150 mL, LC-MS grade) in the round bottomed flask. The water bath was equilibrated to 65 °C and the sample was extracted under reflux for 18 hours.

Once matrix was extracted through soxhlet/ultrasonication the same SPE method was applied to the extract, with modifications for sample volume loads. Spiked and un-spiked samples were compared to determine percentage recoveries that include any losses through both pre-extraction step and SPE. Three phthalates were selected for the spike, DMP, DEHP and DiDP, representing peak 1, 2 and 3 resectively. These were chosen as they covered the lowest, mid-range and highest logKow. Soxhlet was found to have a much lower percentage recovery, used solvents of increased eco-toxicity and is a highly water intensive method. The ultra-sonication method therefore offered increased efficiency and a greener method and was used for analysis of all solid samples.

Table 8: Percentage recoveries for soxhlet and ultra-sonication extraction trials for phthalates in soil

Extraction	*DMP*	*DEHP*	*DiDP*
Soxhlet	65.24 (±5.37)	56.47 (±7.63)	32.11 (±6.89)
Ultra-sonication	78.06 (±3.75)	88.83 (±3.29)	89.76 (±2.42)

Table 9: Percentage recoveries for solid matrices using ultra-sonication method (30 g sample)

Matrix	*DMP*	*DEHP*	*DiDP*
Soil	78.06 (±3.75)	88.83 (±3.29)	89.76 (±2.42)
Sludge	83.14 (±2.97)	91.53 (±2.83)	93.32 (±2.01)
Waste- Recyclable	89.92 (±2.75)	95.25 (±3.33)	97.70 (±1.82)
Waste-General	87.43 (±3.28)	88.25 (±3.09)	92.76 (±2.08)
Waste-Food	86.74 (±4.10)	90.48 (±3.12)	94.16 (±2.62)

The matrix effects for all matrices were examined. Spiked and blank samples were compared for triplicate samples. For the case of surface water, soil and wastewater samples three different sites were compared and averaged. Wastes and leachate came from one site so triplicates were analysed from the same site. The recovery of the target analyte was calculated, and the matrix effect expressed as percentage change (**Table 10*: Matrix effects***). All matrices showed a negative percentage change in phthalate analyte detection and therefore demonstrating ion suppression for all matrices. The strongest effects were noted for sludge, black and brown bin wastes. As the internal standard method was used all ion suppression is compensated for.

Table 10: Matrix effects on each matrix represented by percentage of ion suppression

	Recyclable Waste	*General Waste*	*Food Waste*	*Leachate*	*Influent*	*Sludge*	*Effluent*	*Soil*	*Surface Water*
DMP	4.09 (±0.10)	1.94 (±0.06)	18.81 (±0.56)	8.28 (±0.22)	8.69 (±0.35)	7.72 (±0.23)	7.92 (±0.24)	6.17 (±0.21)	0.33 (±0.01)
BBP	4.76 (±0.11)	9.52 (±0.30)	9.52 (±0.29)	7.93 (±0.21)	7.14 (±0.29)	7.14 (±0.24)	3.57 (±0.11)	3.57 (±0.12)	3.57 (±0.10)
DiBP	6.90 (±0.17)	12.88 (±0.40)	9.06 (±0.27)	9.61 (±0.26)	3.67 (±0.15)	4.96 (±0.15)	2.56 (±0.08)	2.34 (±0.08)	6.51 (±0.09)
DBP	0.19 (±0.01)	5.99 (±0.19)	16.29 (±0.49)	7.49 (±0.20)	9.13 (±0.37)	2.53 (±0.08)	6.60 (±0.20)	10.11 (±0.27)	1.40 (±0.04)
DiPP	2.15 (±0.05)	8.87 (±0.27)	7.53 (±0.23)	6.18 (±0.17)	7.66 (±0.31)	9.89 (±0.08)	4.44 (±0.13)	1.21 (±0.02)	4.84 (±0.14)
DPP	7.81 (±0.19)	12.50 (±0.39)	5.21 (±0.16)	8.51 (±0.23)	4.30 (±0.17)	1.17 (±0.08)	4.69 (±0.15)	7.03 (±0.24)	8.59 (±0.25)
DHP	9.26 (±0.22)	14.81 (±0.46)	5.56 (±0.17)	9.88 (±0.27)	4.17 (±0.17)	2.78 (±0.08)	2.78 (±0.35)	2.78 (±0.09)	6.94 (±0.20)
DEHP	1.34 (±0.03)	12.60 (±0.40)	4.99 (±0.15)	6.31 (±0.15)	7.73 (±0.31)	5.52 (±0.08)	0.86 (±0.35)	2.83 (±0.10)	5.17 (±0.16)
DnOP	0.49 (±0.01)	18.57 (±0.56)	6.10 (±0.18)	8.39 (±0.23)	1.22 (±0.05)	8.38 (±0.08)	3.61 (±0.35)	8.87 (±0.30)	0.69 (±0.02)
DiNP	2.99 (±0.07)	11.59 (±0.36)	4.65 (±0.14)	6.41 (±0.16)	12.42 (±0.50)	1.31 (±0.08)	0.71 (±0.35)	8.51 (±0.29)	7.29 (±0.21)
DiDP	1.34 (±0.03)	7.53 (±0.23)	3.04 (±0.09)	3.97 (±0.11)	6.18 (±0.25)	2.09 (±0.08)	1.85 (±0.35)	5.90 (±0.20)	4.82 (±0.13)

2.1.4 Solutions and Standards Preparation

All phthalate standards were purchased from Accustandard (New Haven, Connecticut, USA) as liquids. The standards were diluted in 50:50 (v/v) methanol:acetonitrile to obtain the concentrations required for the calibration standards. LC-MS grade methanol and acetonitrile were purchased from Thermo-Fischer (Waltham, Massachusetts, U.S.). Ultrapure water was sourced from a reverse osmosis system with 18.2 MΩ purity. Minimal standard preparation steps were taken, and all processes were carried out in a timely manner to avoid excess exposure to laboratory air. All glassware used was rinsed three times with LC-MS grade methanol after overnight bake-out at 200^{o}C. Although some literature suggests that mobile phase additives yield sharp peaks in LC-MS analysis (Chan and Shuang, n.d.; Schreiber et al., n.d.; Shah and Burgess, n.d), no buffer could be sourced that contained low enough levels of phthalates to incorporate into the method.

2.1.5 Mass Spectrometry Conditions

Full scans of all target analytes were run to assess precursor and possible product ions. The precursor ions were selected from the molecular weight of the empirical formula, +H, as the phthalate diesters favoured positive ionization. The Agilent Optimizer program was then used with these transitions to determine the optimal fragmentor voltages and collision energies for each compound. This could be done manually by running samples at varying fragmentor voltage and collision energy.

Table 11: Optimized mass spectrometry conditions to target 11 phthalates

Peak no	Analyte	Retention Time (Min)	Target Transitions (m/z)	Fragmentor (V)	Collision Energy
1	DMP	2.15	195.1-162.9	62	8
			195.1-77.0	62	40
2	BBP	4.65	313.2-148.9	77	12
			313.2-91.0	77	40
3	DiBP	4.75	279.2-205.1	90	4
			279.2-149.0	90	14
			279.2-57.1	90	14
4	DBP	4.82	279.2-205	50	4
			279.2-148.9	50	12
			279.2-120.9	50	40
5	DiPP	5.50	307.18-149.0	96	20
			307.18-71.1	96	12
6	DPP	5.60	307.2-219.0	96	4
			307.2-148.9	96	20
7	DHP	6.25	335.2-233.0	80	4
			335.2-148.9	80	12
8	DEHP	7.15	391.0-166.9	115	12
			391.0-148.9	115	28
9	DnOP	7.3	391.3-166.9	99	12
			391.3-148.9	99	32
			391.3-120.9	99	60
10	DiNP	7.5	419.31-148.9	96	24
			419.31-71.1	96	20
11	DiDP	8.2	447.3-141.1	99	8
			447.3-85.1	99	16

To optimize signal the source parameters need to be carefully controlled. Agilent Source Optimizer runs a variety of temperatures, flows, voltages and pressures to analyse which settings increase analyte detection. The parameters chosen gave the best average signal for all phthalates.

Table 12: Mass spectrometer source conditions

Parameter	*Value*
Mass Spectrometer System	G6470A
Ionization Mode	Positive
Gas Temperature	350
Gas Flow	10 L/min
Nebulizer	35 psi
Capillary	4000 V
Sheath Gas Temperature	400
Sheath Gas Flow	12 L/min
Nozzle Voltage	2000 V

2.1.6 Liquid Chromatography Analysis

Elimination of background phthalate contamination constituted the major analytical challenge within this project (Section 2.1.2). However, the eleven target analytes included three sets of isomeric pairs (DiBP and DBP, DiPP and DPP, and DEHP and DnOP) which required some chromatographic development. Two columns were trialled for the resolution of these compounds (**Figure 9**). The Eclipse column™ is a fully porous column that can provide both improved throughput and higher resolution.

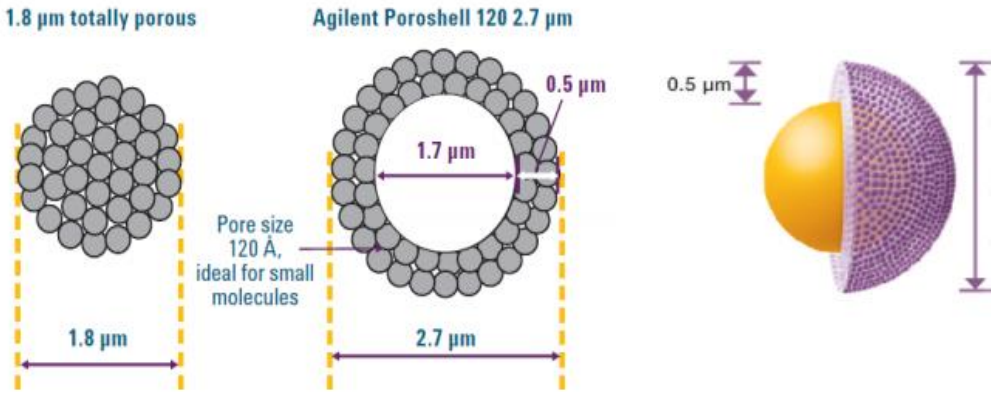


Figure 9: Difference in column packing between two trialled columns

Trials on the Eclipse column did not show sufficient resolution of the isomeric pairs at the optimal flow rate and mobile phase. BBP/DiBP/DBP co-eluted in addition to DiPP/DPP and DEHP/DnOP (**Figure 10**). Owing to these co-elutions, the longer and more porous column, "Poroshell" was chosen as it offered greater resolution.

Table 13: Selection of separation methods on eclipse column targeting phthalates

Trial	*Details*
Method 1	0 min 55%B, 3 min 80%B, 5.5 min 60%B, 5.51 60%B
Method 2	0 min 60%B, 3 min 90%B, 5.5 min 50%B, 5.51 50%B
Method 3	0 min 50%B, 2 min 80%B, 5 min 100%B, 5.5 100% B
Method 4	0 min 60%B, 1 min 100%B, 3 min 70%B, 5.5 min 60%B, 5.51 60%B
Method 5	0 min 60%B, 2 min 80%, 5 min 100%, 5.5 min 100%B, 5.51 60%B
Method 6	0 min 60%B, 5 min 80%B, 5.5 min 80%B, 5.51 60%B

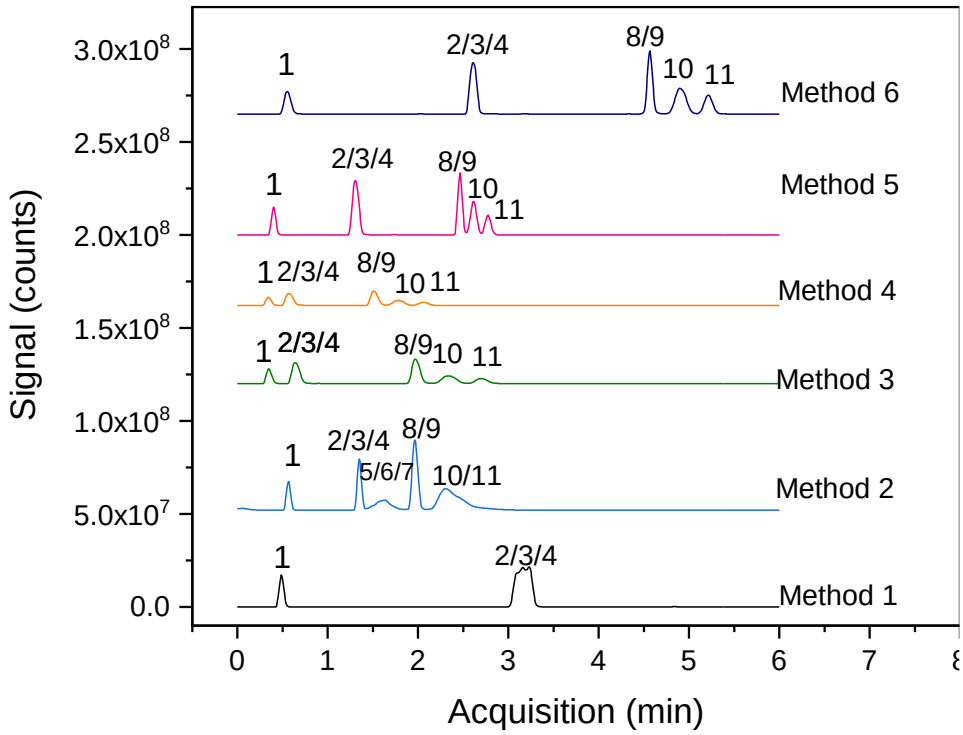


Figure 10: Method development for the separation of phthalates on eclipse column using ms conditions in table 8, and various gradients described in table 10

Sufficient resolution of phthalate isomeric pairs was achieved with the Poroshell™ column and conditions are shown in **Table 14**. The dMRM method locks transitions to a specific retention time. Compounds DiPP and DPP did not have unique transitions to distinguish each other but the differences in RT circumvented this issue. This can be seen in greater detail in **Figure 13**.

Table 14: Chromatographic conditions for the separation of 11 target phthalates

Parameter	*Value*
Delay Column	Eclipse Plus C18, 3.5 µm, 4.6 x 50 mm
Analytical Column	Poroshell 120 EC-C18, 2.7 µm, 2.1 x 150 mm
Injection Volume	2 µL
Column Temperature	50 °C
Mobile Phase	A) Water
	B) Methanol:ACN (50:50)
Gradient	0 min 60% B, 2 min 80% B, 5 min 100% B
Run Time	9 min
Post Time	2 min

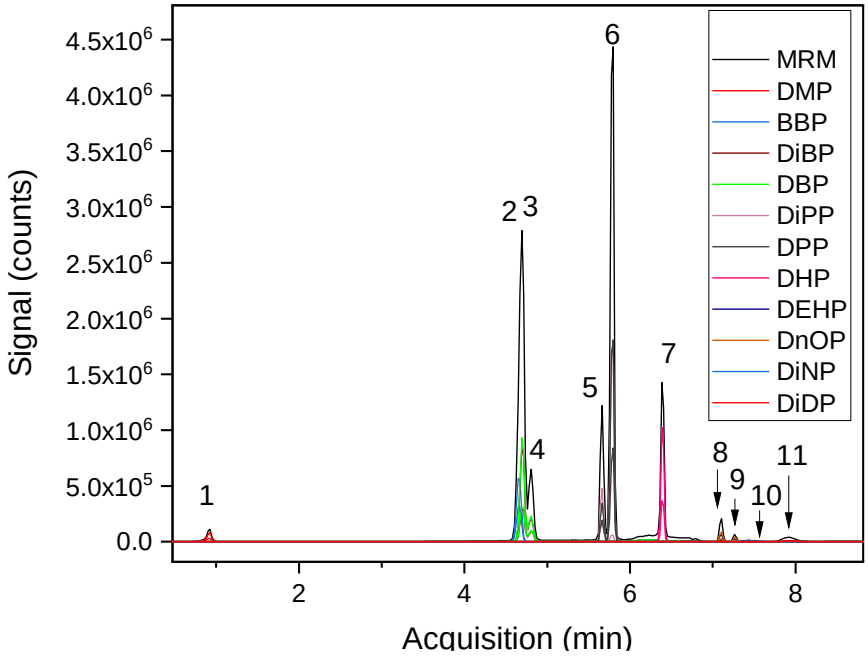


Figure 11: Chromatogram for Optimized Method Targeting Phthalate Analytes, Peaks (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP Using MS Conditions Described in Table 8 and Chromatographic Conditions Described in Table 11

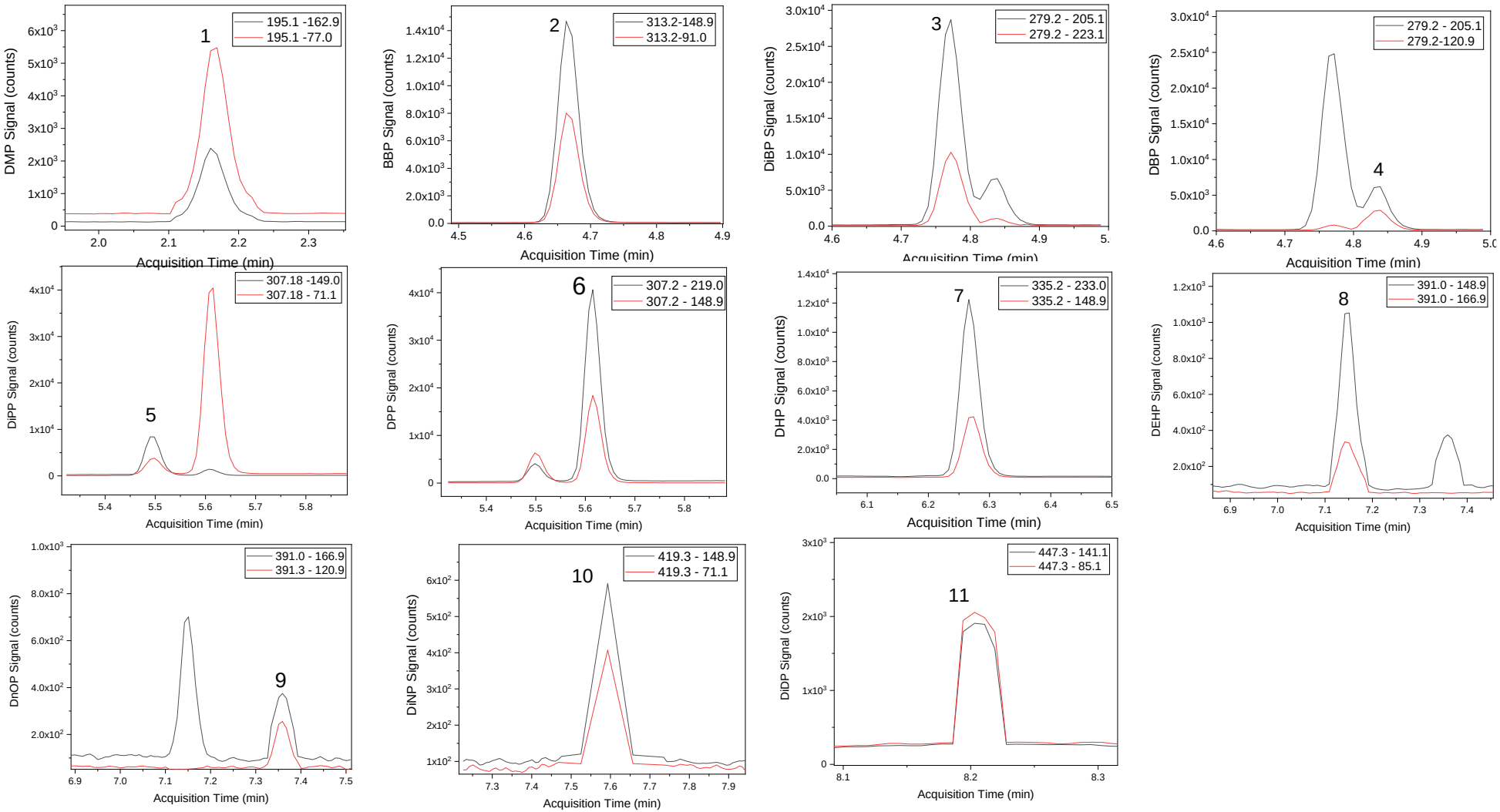


Figure 12: Quantifying and Qualifying Transitions for Target Phthalates Using MS Conditions Described in Table 8 and Chromatographic Conditions Described in Table 11

1.9.1.1 Method Validation

The method was found to be repeatable. Triplicate injections of reference standard (1ppb, ACN:MeOH) over the course of 0, 2, 4, 6, 8 hours were shown to be precise. The precision was investigated through % Relative Standard Deviation (%RSD), which is the relative percentage of the standard deviation to the mean, with <5 %RSD from the first sets of injections. To ensure that any variation was noted in the method, a calibration run was conducted at the start and end of an analytical batch, if there was a deviation of greater than 5% RSD the instrument was checked and samples re-run.

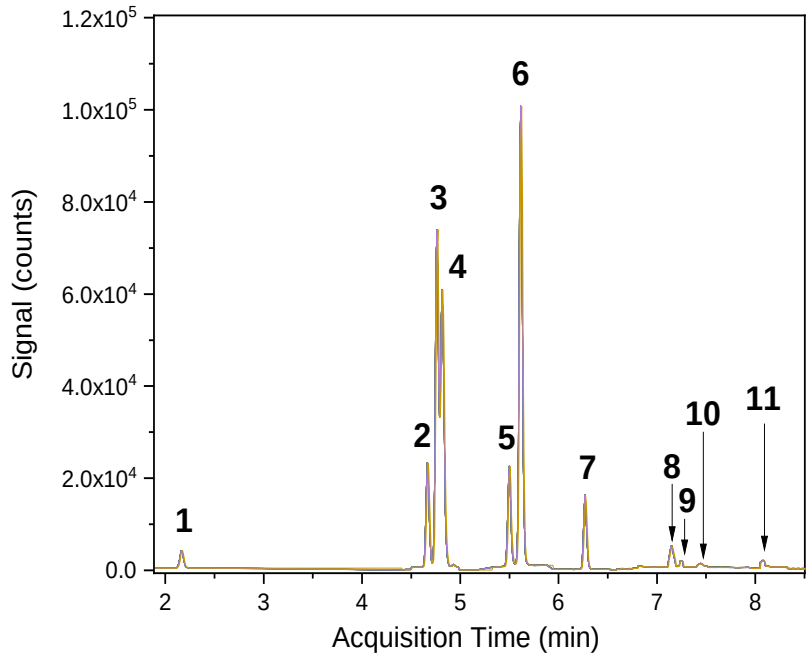


Figure 14: Repeated injections diester standard (n=15), showing no deviation of above 5% RSD for retention time or peak area, peaks are (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, AND (11) DiDP, using ms conditions described in table 8 and chromatographic conditions described in table 11

The linearity and sensitivity of response was determined through repeated calibration curve runs. The linearity of the phthalate monoesters was particularly important due to the use of standard addition for quantitation.

The linearity of phthalates was initially investigated by running calibration curves in triplicate between 0.001 and 10 ppb. The lowest calibration point (0.001 ppb) was found to be lower than the LOD for DiNP, DiDP DiPP and DPP, and higher than the LOD for DMP, DBP, and DHP. Therefore, further injections of low concentration standard were analysed to confirm the LOD and LOQ of each phthalate at 3 times the signal to noise

ratio and 10 times the signal to noise ratio respectively (example in **Figure 15**). As phthalate samples were pre-concentrated the resulting limits of detection were suitable for this study, as they would detect levels in line with literature. Sample calibrations were therefore run between (0.05 and 500 ppb). The LOD and the LOQ of each phthalate is presented in (**Table 15**) This was investigated through calibration lines of low concentration standards (**Figure A 1**).

Table 15: Detection limits, linearity and precision of response

	Compound	*R^2 (n=3)*	*RSD (%)*	*LOD (PPB)*	*LOQ (PPB)*
1	DMP	0.9989	0.15	0.0005	0.002
2	BBP	0.9883	0.39	0.001	0.005
3	DiBP	0.9910	0.68	0.002	0.01
4	DnBP	0.9927	0.28	0.0002	0.001
5	DiPP	0.9845	0.79	0.005	0.05
6	DPP	0.9786	1.80	0.005	0.01
7	DHP	0.9870	0.48	0.0001	0.005
8	DEHP	0.9851	1.72	0.001	0.005
9	DnOP	0.9797	1.50	0.001	0.005
10	DiNP	0.9924	0.31	0.002	0.005
11	DiDP	0.9828	1.04	0.01	0.05

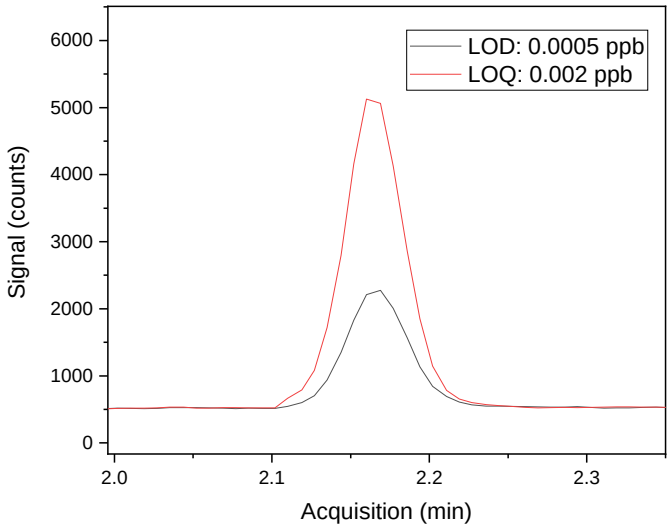


Figure 15: Limits of detection DMP example, using MS conditions described in table 8 and chromatographic conditions described in table 11

2.1.7 Raw Sample Data

All samples were analysed through MRM mode but raw data was also examined through MS2 scan and SIM modes. MS2 scans ranged from 50-500 m/z, and demonstrated the extent of sample clean-up.

SIM modes were run for the parent ions, additional monitoring using the characteristic ion of 149 m/z for phthalic anhydride which is common to all analytes was also implemented. This was investigated as a possible method to screen for all phthalates, including ones not included in this study. However, SIM was not sufficient for this high throughout method, and 149 m/z did not capture all peaks in the LMW zone so they are excluded from this study. An example of these monitoring methods are shown in general waste samples (**Figure 16**-**Figure 19**).

The results showed that at low concentrations, contamination peaks were at an intensity 2x fold higher than the low concentration standard injected (1 ppb). All contamination peaks elute prior to 1.5 min. The results show how vital the pre-column is for routine phthalate analysis, particularly with high-volume throughput on a shared instrument. Even with solvents running clean, the instrument itself can contribute high background levels of phthalates.

Agilent MassHunter software was used to quantitate the phthalate concentrations in sample (**Figure A 2)**. The ratio of phthalate to Internal Standard (IS) is above 100% for some phthalates due to high concentration in the procedural blank which was subtracted from the sample concentration.

2.1.7.1 Household Wastes

The general waste samples were taken from a representative sub-sample of a landfill, that segregated wastes. General waste came from household "black bins". Samples were extracted using ultra-sonic and solid-phase extraction, pre-concentrating by a factor of 4.

Scans showed a high volume mass of peaks at 0.5 min. This is suspected to be contamination and solvent peaks. All future scans of raw data will be presented within the RT window of phthalates.

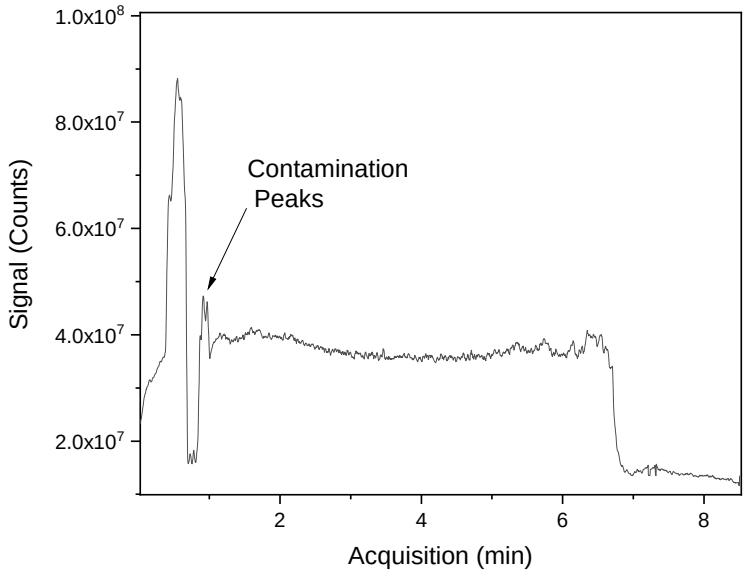


Figure 16: Example scan of general waste (50-500 m/z, positive ionization, fragmentor voltage 50, collision energy 8) with contamination peaks using chromatographic conditions described in table 11

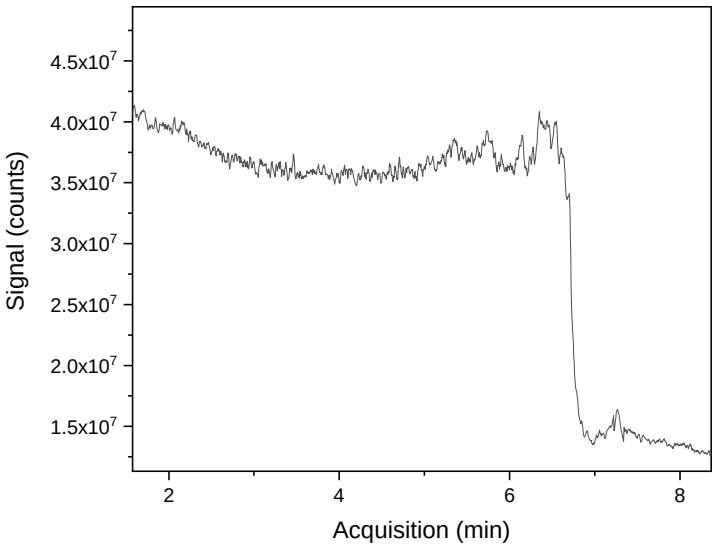


Figure 17: Example scan (50-500 m/z, positive ionization, fragmentor voltage 50, collision energy 8) of general waste within phthalate RT windows using chromatographic conditions as described in table 11

A common ion (149 m/z) SIM was run to identify whether all phthalates were detected through this ion and if you could identify whether other phthalates, external to this study, are contained in the sample (**Figure 18**). A peak, fully resolved from contamination peaks, was detected at 1.02 min. As DMP is the lowest molecular weight phthalate and was included in this study, eluting at 2.1 min, it is suspected that this early eluter is phthalic anhydride itself. Monitoring of the phthalic anhydride product ion did not detect most phthalates in this study. Only peaks 5, 6 and 7, namely DiPP, DPP and DHP were detected.

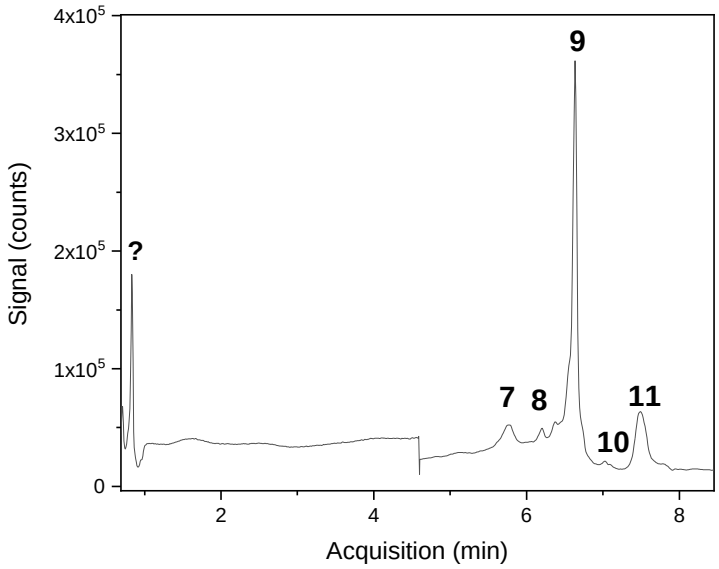


Figure 18: common ion sim (149 m/z, positive ionization, fragmentor voltage 50, collision energy 8) of general waste, showing (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, (11) DiDP. using chromatographic conditions described in table 11

A SIM was run to monitor the precursor ion of all the phthalates contained in the study. It is unsure why the LMW phthalates cannot be seen in the samples when using SIM. It could be due to the fact that the LMW elute within a very narrow window and the scan settings don't allow enough time to detect them. The HMW phthalates, although all but DiDP were detected, the peak shape and resolution of peaks were worse than the MRM method. Again, the contamination peaks prior to 2 min are high and are not shown here as they are not relevant to the sample. Improved signal could have been obtained by improving the MS scan time for each parent ion in the first half of the chromatogram, but considering that MRM samples allowed increased detection of all analytes, this was not investigated further.

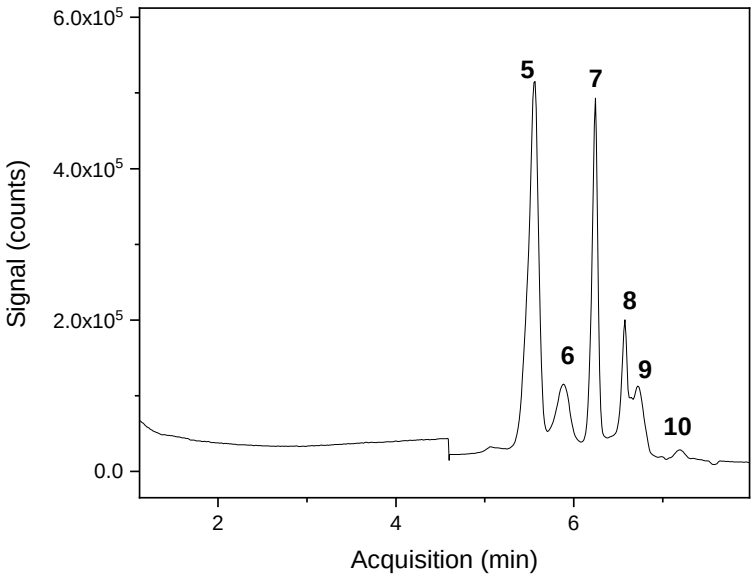


Figure 19: SIM e.g. for general waste, showing detected phthalates (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, using chromatographic conditions described in table 11

The MRM of general, black bin waste showed that all phthalates could be detected using this method (**Figure 20**). The MRM monitoring of each phthalate was locked to its characteristic retention time, selected from the initial MRM of reference standards. Three samples from this subsample were extracted and the resulting MRMs shown in **Figure 21**. The MassHunter software was used to extract the individual MRM peaks and compare them to the internal standard concentration. Samples analysis of general waste was considered to be precise. DnOP and DiDP were the only phthalates that were above 5 %RSD at 5.19 and 5.00 %RSD respectively. Procedural blanks run over the course of analysis showed high levels of DiBP, DBP and DnOP at 0.0009, 0.0008 and 0.002 µg/g respectively, averaged from triplicate blanks. All detected levels of phthalates in the procedural blanks were subtracted from the sample concentration. The detection frequency in general waste was 100%.

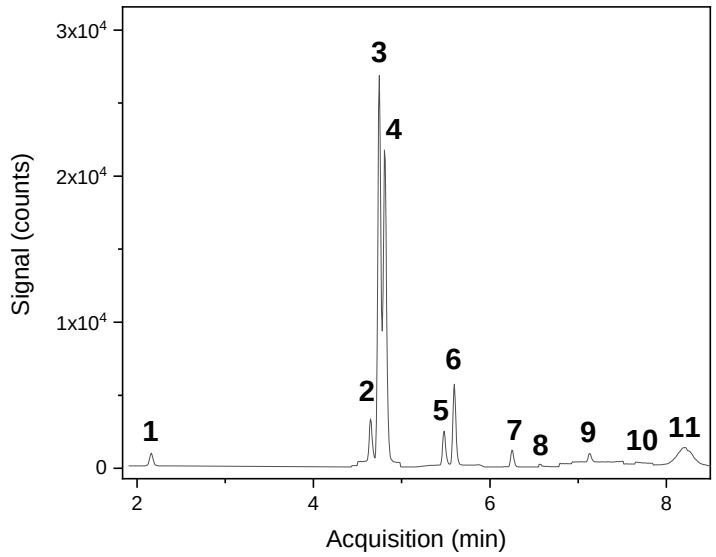


Figure 20: MRM e.g. of general waste spiked with is. peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

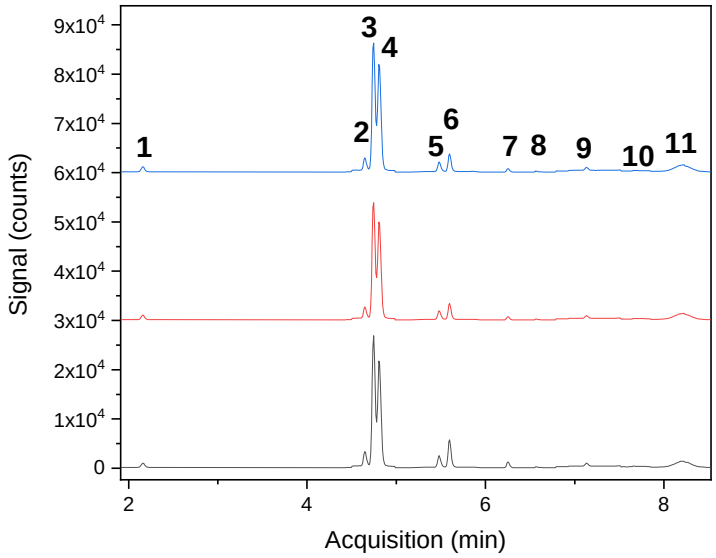


Figure 21: MRM of general waste from different sample subsets, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

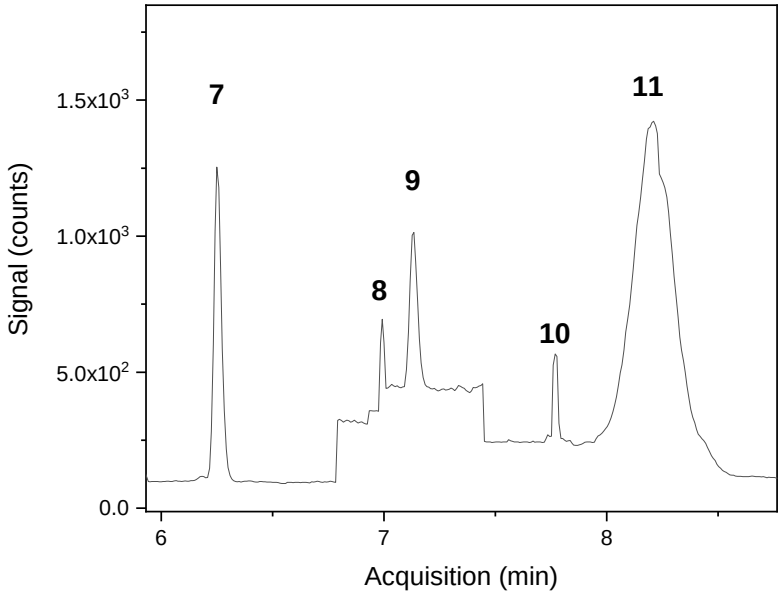


Figure 22: Zoomed MRM example of general waste, spiked with IS. Showing low intensity peaks (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

Recyclable waste was taken from the same segregated waste site and samples were prepared and extracted in the same manner as general waste. The MRM method detected all phthalates (**Figure A 4:**). The detection frequency in recyclable waste was 100%, and deviation of all samples was below 5 %RSD. The phthalates in highest concentration in the procedural blanks were DiBP, DBP, DEHP at 0.001, 0.0006, and 0.003 µg/g respectively. All detected levels were subtracted from the sample. DnOP. BBP, DiPP, DPP, DHP and DiDP were not detected in the procedural blank.

Food waste, from "brown bins" was analysed from the same site. This was a more homogenous sample so it was blended instead of cut. The MRM method detected all phthalates (**Figure A 6; Figure A *57***). The detection frequency in food waste was 100%, and had the lowest phthalate concentration out of all waste samples. Variation of all phthalates in food waste were below 5 %RSD apart from DMP, DiBP and DPP at 5.48, 5.58 and 5.08 %RSD respectively. The phthalates in highest concentration in the procedural blanks were DiBP, DBP, DEHP at 0.0007, 0.0005, and 0.0015 µg/g respectively. All detected levels were subtracted from the samples.

1.9.1.2 WWTP Influent

Influent was filtered and extracted through SPE, pre-concentrating by a factor of 100. MS2 scan of the data showed no discernible peaks with intensity of $x10^7$. MRM mode was used for all samples, and the detection frequency for all phthalates was 100% (**Figure A 8; Figure A 59**). 12 samples were analysed, 4 from each site. DnOP was above 5 %RSD for 6 samples, and BBP was above 5 %RSD for 1 sample, however, all of these deviations were below 10 %. All phthalates detected in the procedural blanks were subtracted from the samples.

1.9.1.3 Sludge

Sludge samples went through extensive clean-up through drying, ultra-sonication and SPE. Samples were dried at 130 °C to final weight. The percentage loss of water was recorded for population adjustment; as raw sludge volume is used to convert values to µg/inhabitant/day. As sludge concentrations were predicted to be high, pre-concentration by a factor of 4 was carried out to reduce column load. Scans of sludge samples had high intensities but no discernible peaks.

The MRM showed the highest rates of intra-sample variation by matrix (***Figure A 10; Figure A 11***). The highest %RSD found for a sludge sample was 13.1, and 2.0 the lowest, with 3 measurements above 10 %RSD. Higher standard deviation of the data was expected in sludge as it is the most complex matrix in this study. Some accredited methods for the detection of phenols and PAHs require 15 %RSD to ensure precision under Good Laboratory Practice (Environment Agency, 2018). The phthalates found in the highest concentrations in the procedural blank were found to be DBP, DiBP, DEHP and DnOP at 0.2 and 0.3. Concentrations were higher in this procedural blank batch most likely due to increased exposure to the air during the drying step, and longer analysis time compared to liquid samples due to ultra-sonication step.

1.9.1.4 WWTP Effluent

Effluent samples were extracted in the same method as influent samples, pre-concentrating by a factor of 100. All samples deviated by less than 10 %RSD. Three out of eight samples had one or more phthalates test above 5 %RSD, for these samples, phthalates not contained in the procedural blank were precise. The detection frequency for effluent samples was 100% for all phthalates (**Figure A 102; Figure A 113**).

1.9.1.5 Soil

Soil was extracted using the same method as sludge, with an adjustment to the drying step being carried out in a desiccator, as the soil samples were relatively drier. Soil was one of the most complex matrices that has a natural tendency to interact with different pollutants. All phthalate measurements in all soil samples were below 10 %RSD. Concentrations in the procedural blank were relatively high when compared to liquid matrices with DiBP, DBP, and DnOP being the highest. Detection frequency in soil was 100% (**Figure A 14**; **Figure A 15**).

1.9.1.6 Surface Water

Surface water was the cleanest matrix and therefore only used the 0.45 µm nylon filters prior to extraction and pre-concentration by a factor of 100. This was the only matrix to not have a detection frequency of 100% with two of the estuary samples below the limit of detection for DiNP, all other phthalates were detected in all samples (**Figure A 146; Figure A 157**) . Surface water contained the most precise sample results and had very low levels of phthalates in the analytical blank. This is thought to be due to the significantly decreased analysis time reducing contact of sample with air.

2.1.8 Data Analysis

Principle Component Analysis (PCA) was conducted for the speciation of phthalates, using R. The number of components to retain was based on score plot analysis and eigenvalue criteria. Orthogonal rotation to obtain a set of independent interpretable factors was used according to a factor loading >0.66. To confirm the statistical difference between samples, two-way ANOVA was performed in R.

To assess the effect of phthalates on eco-toxicity the Risk Quotient (RQ) was used (**Equation 3**). This compares the Measured Environmental Concentration (MEC) to the Predicted No Effect Concentration (PNEC) (Chen et al., 2019). A value of above 1 will indicate that the concentration is associated with an increased ecological risk. The PNEC values were obtained from their ECHA registration dossiers and are presented in **Table 16** (ECHA, 2020).

$$RQ = \frac{MEC}{PNEC}$$ **Equation 3**

Table 16: PNEC of phthalates for environmental matrices

	DMP	*BBP*	*DiBP*	*DBP*	*DEHP*	*DiNP*
Soil (mg/kg dw)	3.16	1.57	0.023	0.05	13	30
Freshwater (µg/L)	192	7.5	1	10	-	-
Marine (µg/L)	19.2	0.75	0.1	1	-	-

2.2 Phthalate Monoester Method Development

2.2.1 Sample collection

Six samples over six months were collected from a rural, suburban and urban WWTP. Sampling was conducted by WWTP staff using provided equipment. This included phthalate free shatter-proof sample bottles pre-washed with phthalate free methanol and rinsed with sample at site prior to filling. They were transported in cooler boxes, adjusted to pH 2 and stored at – 20 °C (**Figure 23***)*. One month of samples was omitted from analysis due to a freezer malfunction leading to degradation of sample.

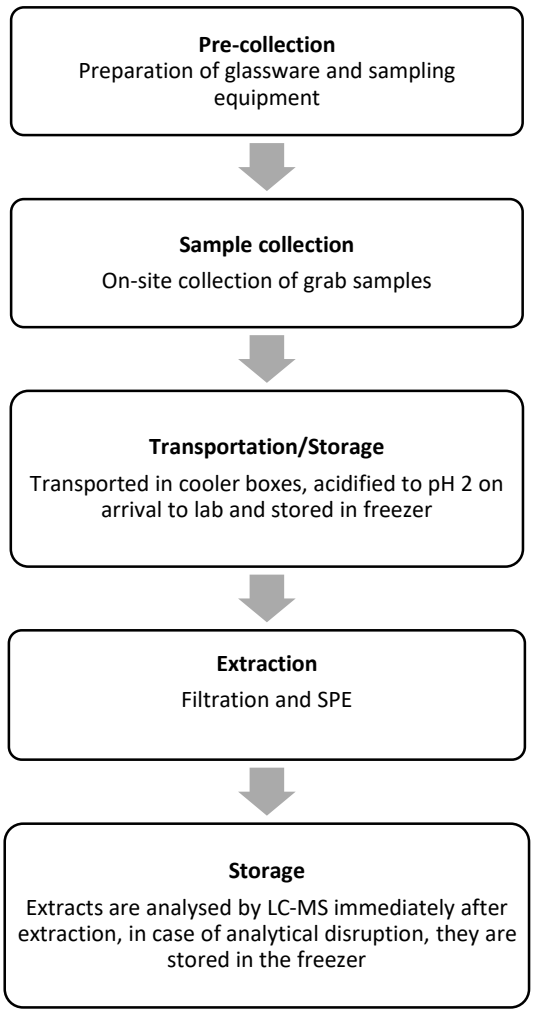


Figure 23: Process flow diagram for sample analysis of phthalate monoesters

2.2.2 Sample Extraction

Influent samples, for the analysis of phthalate monoester metabolites are adjusted to pH 2 with 37% HCl before filtration through glass fibre filters (0.8 μm) and nylon filters (0.45 μm). Extraction and quantification was carried out though standard addition. Three blank samples were analysed, with 5 calibration samples spiked with 0.001-1 ppb reference standard mix prior to extraction. Pre-concentration from 100 mL to 1 mL was performed on C18 cartridges. The cartridges were conditioned with acetonitrile (4 mL, 100%) and acidified water (4 mL, 100%) before loading sample (wastewater influent, 100 mL, pH 2), washing with deionized water (2 mL, 100%) and elution with acetonitrile (ACN) (1mL, 100%).

The extraction of the phthalate monoester metabolites was initially investigated through two solvents (MeOH and ACN) and two SPE cartridge types (C18 and Strata-X). The cartridge composition had more impact on the extraction, with Strata-X performing at a much lower extraction efficiency than the C18 (**Figure 24**). As there were no internal standards available for the extraction the matrix effects are unknown. However, losses through SPE and matrix effects are controlled for the standard addition method and by spiking prior to extraction.

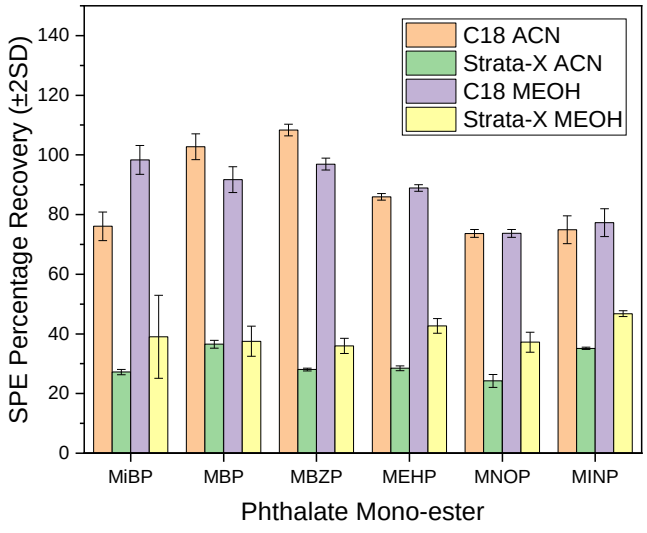


Figure 24: SPE recovery trials for phthalate monoester metabolites

2.2.3 Solutions and Standards Preparation

All phthalate monoester metabolite standards were procured from Accustandard (New Haven, Connecticut, USA) as solids. The standards were diluted in 50:50 (v/v) acetonitrile:water, buffered with ammonium acetate and glacial acetic acid to pH 5.5, to obtain the concentrations required for the calibration standards. LC/MS grade acetonitrile and methanol were purchased from Thermo-Fischer, while LC-MS grade ammonium acetate and glacial acetic acid were obtained from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was sourced from a RO system with 18.2 MΩ purity.

2.2.4 Mass Spectrometry Conditions

Mass spectrometry source conditions were assessed using MassHunter Optimizer software. The mass from the imperial formula, -H was used as a precursor ion as initial scans showed that the phthalate monoesters favoured negative ionization.

Table 17: Optimized mass spectrometry source conditions for the detection of 6 target phthalate monoester metabolites

Parameter	*Value*
Mass Spectrometer System	Agilent 6470 triple quad
Ionization Mode	Negative
Gas Temperature	350
Gas Flow	10 L/min
Nebulizer	35 psi
Capillary	2500 V
Sheath Gas Temperature	400
Sheath Gas Flow	12
Nozzle Voltage	2000V

Six phthalates monoesters were detected in wastewater influent in order to estimate population exposure to phthalate diesters in Ireland. The LC-MS method developed achieved separation of the monoester phthalates within 5 min. The selected analytes contained 2 sets of isomers MBP and MiBP, also MEHP and MnOP.

LC-MS characteristic transitions were determined through MS2 scans. Once target Multi Reaction Monitoring (MRM) transitions were selected, fragmentor voltage and collision

energy were optimized through Agilent Optimizer software. For the compound MBzP, peak 3, there was no qualitative transition above LOD.

Table 18: Mass spectrometry conditions for the detection of 6 target phthalate monoester metabolites

Peak	*Analyte*	*RT (min)*	*MRM Transitions (m/z)*	*Fragmentor (V)*	*Collision Energy*
1	MiBP	1.54	221.1-77.1	98	0
			221.1-141.9	98	16
2	MBP	1.60	221.1-77.1	82	20
			221.1-57.1	99	32
3	MBzP	1.84	255.1-77.1	88	20
4	MEHP	3.77	277.1-77.1	250	0
			277.1-163.5	103	8
5	MnOP	3.88	277.1-127.1	103	16
			277.1-134	103	16
			277.1-77.1	103	24
6	MiNP	3.94	291.2-141.1	109	20
			291.2-139.0	109	16
			291.2-121.0	109	20

The final Chromatographic conditions are presented in **Table 19**. Mobile phase was the most important variable for the detection of phthalate metabolites. As all monoesters contain an ionisable group, the pH should be controlled for optimal detection. The pka of is around 3.5, this method used 5mM ammonium acetate buffered to pH 5.5 with glacial acetic acid, resulting in the highest signal, with the analytes in the ionized form.

Table 19: Optimized chromatographic conditions for the separation of 6 target phthalate monoester metabolites

Parameter	*Value*	
System	Agilent 1290 Infinity II	
Analytical Column	EclipsePlus C18 RRHD, 2.1 x 50 mm, i.d 1.8 µm	
Injection Volume	5 µL	
Column Temperature	50 ^{o}C	
Mobile Phase	A) 5mM ammonium acetate buffer in water	
	B) 5mM ammonium acetate buffer in methanol:acetonitrile	
Flow Rate	0.4 mL/min	
Gradient	Time (min)	%B
	0	25
	3.5	65
Run Time	4.8 min	
Post Time	1 min	

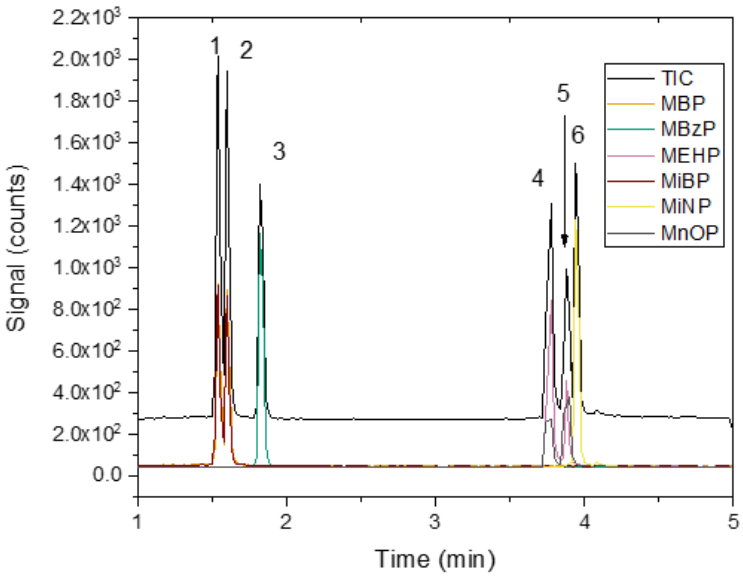


Figure 25: MRM of phthalate monoesters showing peaks (1) MBP, (2) MiBP, (3) MBzP, (4) MEHP, (5) MnOP and (6) MiNP for 0.5 ppb reference standards

The method was found to be repeatable. Triplicate injections of reference standard (1ppb, ACN:MeOH) over the course of 0, 2, 4, 6, 8 hours were shown to be precise, with under 5 %RSD when compared to the first sets of injections (**Figure 26**). To ensure that any variation was noted in the method, a calibration run was conducted at the start and end of an analytical batch, if there was a deviation of greater than 5% RSD between the two calibrations, the instrument was checked and samples re-run.

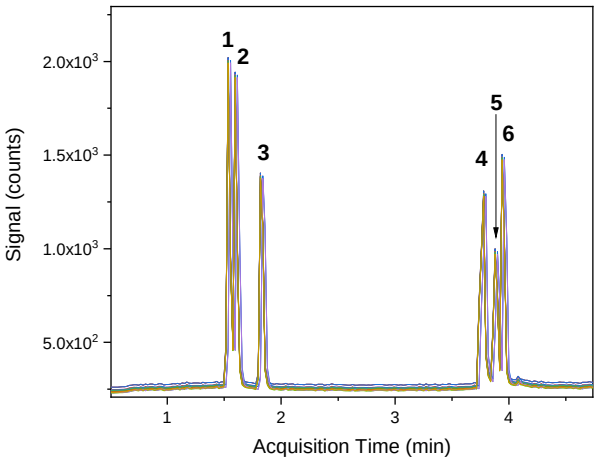


Figure 26: Repeated injections of monoester standard (n=15)

The linearity and sensitivity of response was determined through repeated calibration curve runs. The linearity of the phthalate monoesters was particularly important due to the use of standard addition for quantitation. All phthalates were found to be linear (with regression coefficients above 0.9) and low instrumental limits of detection (sample pre-concentrated by 100). Quantifying and Qualifying peaks shown in **Figure A 3**.

Table 20: Method detection limits, linearity and sensitivity of response for phthalate monoesters

	Analyte	*Range (ppb)*	*R^2 (n=3)*	*RSD (%)*	*LOD (ppb)*	*LOQ (ppb)*
1	MiBP	0.01-5	0.992	0.33	0.03	0.1
2	MBP	0.01-5	0.986	0.93	0.03	0.1
3	MBzP	0.01-5	0.984	0.84	0.0003	0.001
4	MEHP	0.01-5	0.987	2.12	0.0005	0.002
5	MnOP	0.01-5	0.982	1.80	0.01	0.03
6	MiNP	0.01-5	0.998	0.08	0.003	0.01

2.2.5 Raw Sample Data

Five months of samples were taken from three WWTPs, classed as rural, suburban and urban. Samples were spiked prior to extraction from 0.001 to 1 ppb, all calibration lines within sample were linear (R^2>0.9). Un-spiked sample was compared to spiked calibration to determine monoester concentration (**Figure 27**). All samples contained levels of phthalate metabolite above the LOD. The TIC from the MRM methods for each site over three months are shown as an example of detection in real samples.

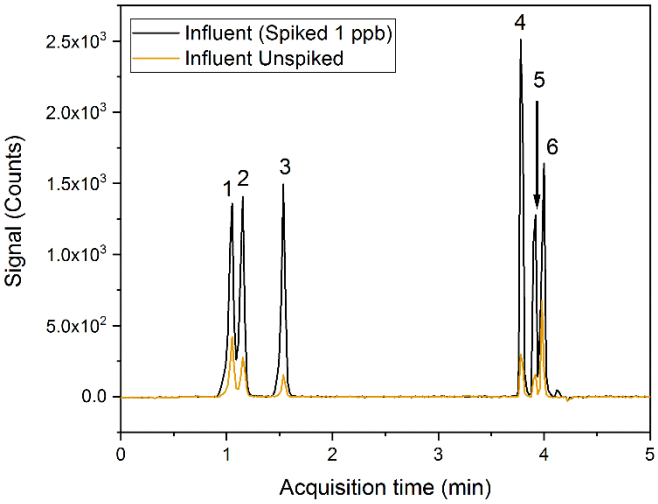


Figure 27: Comparison of spiked and un-spiked influent sample, showing peaks (1) MBP, (2) MiBP, (3) MBzP, (4) MEHP, (5) MnOP, (6) MiNP

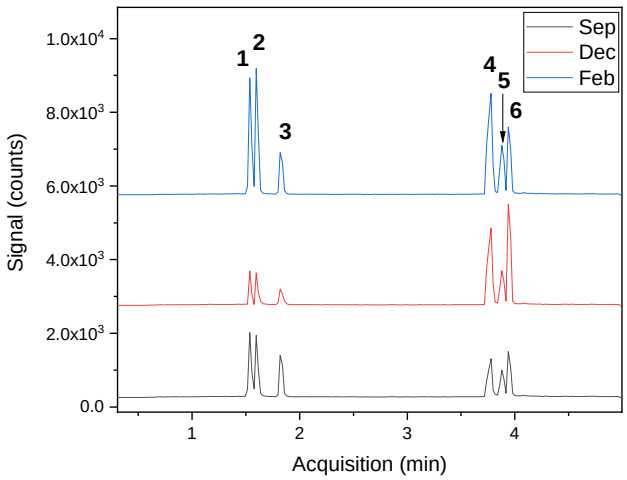


Figure 28: Un-spiked suburban influent samples from September, December and February, showing peaks (1) MBP, (2) MiBP, (3) MBzP, (4) MEHP, (5) MnOP, (6) MiNP

2.2.6 Exposure Assessment

For measuring the phthalate exposure per capita (Estimated Daily Intake, EDI) from metabolite concentration in influent, **Equation 4** was used (Zucatto et al., 2005). This uses wastewater flow rates, population served and phthalate excretion patterns to correct concentration.

$$EDI = \frac{Concentration \times Flow\ Rate \times Correction\ Factor}{Population} \qquad \textbf{Equation 4}$$

$$Correction\ Factor = \left(\frac{MWdiester}{MWmonoester} \times \frac{1}{\%Excreted\ as\ Monoester}\right)$$

For the estimation of exposure from urinary metabolite concentrations (µg/g creatinine) in NHANES, a different method was used. Relevant body weights and heights for each population group were extracted from the NHANES database.

An appropriate model was selected for the conversion of phthalate monoester levels to phthalate estimated daily intake. Models assessing EDI through urinary metabolite concentrations can be assessed using either urinary flow rate (Koch et al., 2003a) or smoothed creatinine (Kohn et al., 2000). Both of these methods of modelling have been consistently used throughout the literature up to the most recent studies using both methods (Dewalque et al., 2014; Chang et al., 2017). If concentration is expressed in µg/L then urinary flow rate is used (**Equation 5**).

$$EDI(mg/kg/day) = \frac{C_m \times V_u \times \frac{1}{F_{ue}} \times \frac{MW_d}{MW_m}}{BW} \qquad \textbf{Equation 5}$$

If concentration is expressed in µg/g creatinine, then smoothed creatinine excretion (CE) is used (**Equation 6**). This method is generally preferred for urine analysis as it controls for variations in subjects' urinary output. The creatinine excretion rate for each section of the population was standardised using data on excretion and pesticides (Mage et al., 2008).

$$EDI(mg/kg/day) = \frac{C_{pht} \times CE_{smoothed}}{F_{ue} \times 1000} \times \frac{MW_d}{MW_m} \qquad \textbf{Equation 6}$$

$$AdultMaleCE = 1.93 \times (140 - Age) \times BW^{1.5} \times ht^{0.5} \times 10^{-6}$$

$$AdultFemaleCE = 1.64 \times (140 - Age) \times BW^{1.5} \times ht^{0.5} \times 10^{-6}$$

Once the estimated daily intake of phthalate has been calculated in mg/kgbw per day the Hazard Quotient (HQ) can be calculated (**Equation 7**). This uses toxicological data collected from the EPA database to quantify a human health risk posed by phthalate exposure or; 'The ratio of the potential exposure to the substance and the level at which no adverse effects are expected' (US EPA, 2015). Any value above 1 is considered a hazard to human health (US EPA, 2011). However, lower HI values for phthalates (as low as 0.1 or 0.2) have been suggested to contribute to endocrine disruption when considering cocktail effect of all other endocrine disrupting chemicals within the environment (Apel et al., 2020). Using the HQ only gives reference to a phthalate's toxicity (Toxic to Reproduction Category 1B) and cannot relate to a specific health effect associated with exposure. In the event that a phthalate does not have a TDI set for e.g. DIBP, the TDI for a phthalate with a similar structure and chemical properties is used (in this case DBP).

$$HQ = \frac{EstimatedDailyIntake(EDI)(mg/kg/day)}{TolerableDailyIntake(TDI)(mg/kg/day)} \qquad \textbf{Equation 7}$$

3 Occurrence of Phthalates in Ireland

Phthalates are introduced to the environment from various sources like WWTP output, leaching, drainage, and atmospheric deposition. The knowledge of occurrence of phthalates in Ireland is relatively limited. A very limited study of environmental phthalate contamination was conducted in the Irish Midlands Shannon Catchment region during the winter of 2004/5 (Reid, 2009). Sediments, sludges and leachate were examined for DBP, DEHP, DINP and DIDP. In river sediment, levels of up to 24.4 mg kg^{-1} phthalate were found, while in leachate, sediments and in sludge, values of up to 49.8 mg kg^{-1} and 174 mg kg^{-1} were quantified. Further investigation of phthalates in Ireland is necessary due to eco-toxicity concerns.

The partitioning of phthalates depends on the polarity of the compound. LMW phthalates are more likely to be concentrated in aqueous matrices like surface waters and effluents, whereas HMW phthalates are more common in soils and sludges. This project examines multiple matrices to assess sources and fates of phthalates in the environment. Household waste, leachate and wastewater are quantified and hypothesised to be the major sources of phthalate contamination. Soil and surface waters are also analysed to assess the extent of phthalate contamination in the natural environment.

3.1 Household Wastes and Leachate

Household waste is a major contributor of phthalates to the environment. Phthalates are widely used in disposable products (e.g. food packaging) and are predominantly landfilled or incinerated after use. Landfilling remains one of the most common practices in the disposal of waste, as a result, many plastic products will migrate to municipal solid waste landfills. If landfills do not have adequate environmental protection systems installed (e.g. leachate collection systems) this can be detrimental to the ecosystem (Ghosh et al., 2015).

Ireland's relationship with waste management has changed dramatically in the last decade. Use of landfill has been reduced from 80% to 41% (EU average is 37%), with five landfills still accepting municipal waste for disposal. Two municipal waste incinerators have been installed since 2011 for increased energy recovery. Separate disposal measures for food waste from households have been legislated for since 2013 and the use of anaerobic digestion facilities has increased as a result. All residual and hazardous wastes depend on export markets (EPA, 2016).

Landfill leachate composition will depend on the quantity and type of wastes disposed in conjunction with the amount of precipitation. Leachate formation can cause groundwater pollution if not well controlled, posing environmental and human health risks from numerous hazardous substances (Abd El-Salam and I. Abu-Zuid, 2015). Once leachate is released to the groundwater environment, it will migrate down through the unsaturated zone to the saturated zone, where it will follow the hydraulic gradient of the groundwater system. When leachate migrates a number of factors can change its composition. Physical (filtration, sorption, advection, and dispersion), chemical (oxidation-reduction, precipitation-dissolution, adsorption-desorption, hydrolysis, and ion exchange), and biological (microbial degradation) forces all contribute to the degradation of the compounds within leachate. However, in some instances the breakdown products that are formed can be more toxic than the parent compounds; in the case of phthalates this has yet to be confirmed. Some evidence exists for improving the removal of phthalates from landfill, one study exhibited a higher rate of degradation in the landfill that utilized a methanogenic reactor when compared to the landfill with direct leachate discharge (Fang et al., 2009).

In this study, a representative sub-sample from landfill was analysed. Recyclable, general refuse and food wastes were analysed separately. 75.5% of total phthalates found in household waste was attributed to recyclable waste, 21% from general waste and 3.5% from food waste.

The predominant phthalates found in recycled waste are DiBP, DBP and DiDP. DiBP and DBP have been restricted in manufacturing of childcare articles. For green bin samples the concentration of BBP and DiDP in relation to the other phthalates was higher than seen in other matrices. BBP and DiDP have been limited in manufacturing and although this legislation may have reduced the concentration in environmental samples recycling may keep this compound in circulation for household goods. DPP was the fourth most predominant phthalate in recyclable waste, this phthalate was introduced to the market more recently. Phthalates in general waste followed the same pattern. In environmental samples seen later in this study, the levels of DPP are low in comparison to other phthalates.

The leachate examined in this study came from a lined municipal solid landfill and included samples from both the sump and the lagoon. This site was opened in the 1950's, vacated in 2010 and a remediation project has been underway since 2011. Leachate is collected on site and pumped via the leachate sump to the leachate lagoon. Leachate is discharged from the site via the leachate lagoon to the sewer. The leachate is conveyed in a rising main to a sewer discharge point before entering a gravity fed sewer that conveys it to a WWTP (EPA, 2016).

For leachate samples DnOP, DiNP and DiDP were present at relatively higher concentrations than in other matrices. Some novel phthalates were found in lower concentrations in this matrix due to the fact that the landfill associated with this leachate stopped accepting waste over 10 years ago, when replacements were relatively new on the market and new legislation surrounding other plasticizers had only recently been introduced.

Table 9: Concentrations of phthalates in household wastes and landfill leachates (n=3, ±2SD) as a source of contamination

	DMP	*BBP*	*DiBP*	*DBP*	*DiPP*	*DPP*	*DHP*	*DEHP*	*DnOP*	*DiNP*	*DiDP*
Food Waste (µg/g)											
Average	**0.62 (±0.06)**	2.09 (±0.14)	**6.05 (±0.68)**	1.00 (±0.10)	0.15 (±0.02)	**1.55 (±0.16)**	0.62 (±0.06)	0.30 (±0.02)	0.03 (±0.002)	0.25 (±0.02)	2.08 (±0.06)
General Waste (µg/g)											
Average	10.42 (±0.42)	8.59 (±0.24)	30.59 (±1.24)	11.80 (±1.38)	1.74 (±0.08)	10.23 (±0.08)	5.30 (±0.38)	0.93 (±0.02)	0.09 (±0.01)	1.52 (±0.04)	17.71 (±1.78)
Recyclable Waste (µg/g)											
Average	26.96 (±0.76)	29.16 (±1.04)	136.36 (±6.66)	81.36 (±1.90)	5.30 (±0.34)	32.59 (±2.04)	12.19 (±0.30)	4.24 (±0.22)	**0.25 (±0.01)**	14.74 (±0.42)	**40.31 (±1.42)**
Leachate (µg/L)											
Average Lagoon	2.42 (±0.18)	0.01 (±0.001)	6.36 (±0.12)	2.37 (±0.16)	0.02 (±0.002)	0.05 (±0.004)	0.00 (±0.000)	0.15 (±0.01)	5.43 (±0.18)	5.59 (±0.13)	15.16 (±0.25)
Average Sump	1.86 (±0.16)	0.01 (±0.001)	3.05 (±0.22)	1.17 (±0.08)	0.01 (±0.001)	0.01 (±0.001)	0.01 (±0.000)	0.08 (±0.002)	3.75 (±0.36)	3.19 (±0.24)	10.91 (±0.18)

Vales in bold represent data where the %RSD is ≥5

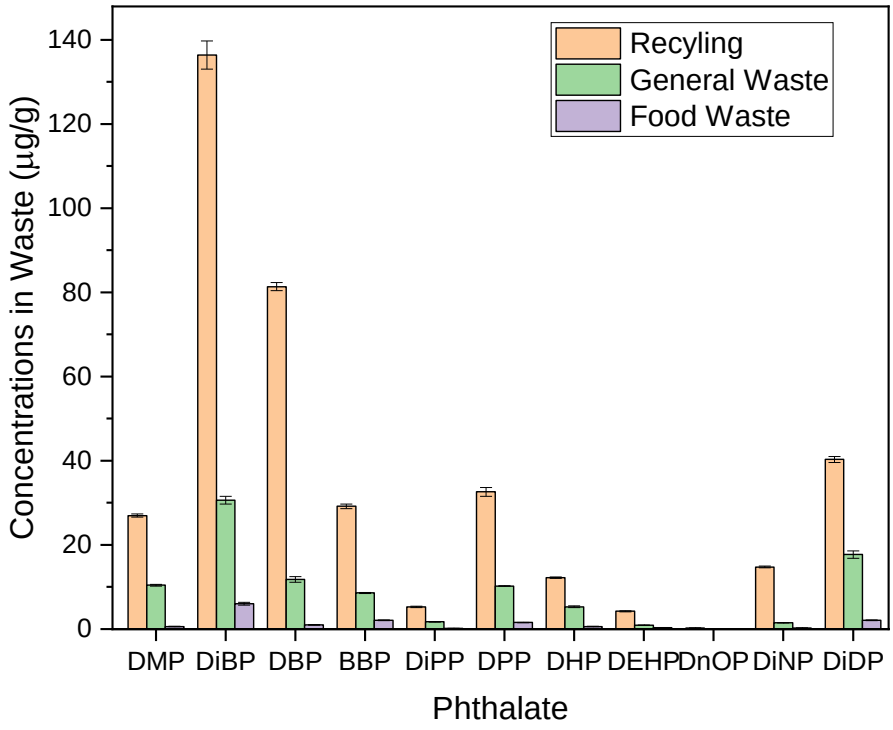


Figure 29: Phthalate concentrations in household waste

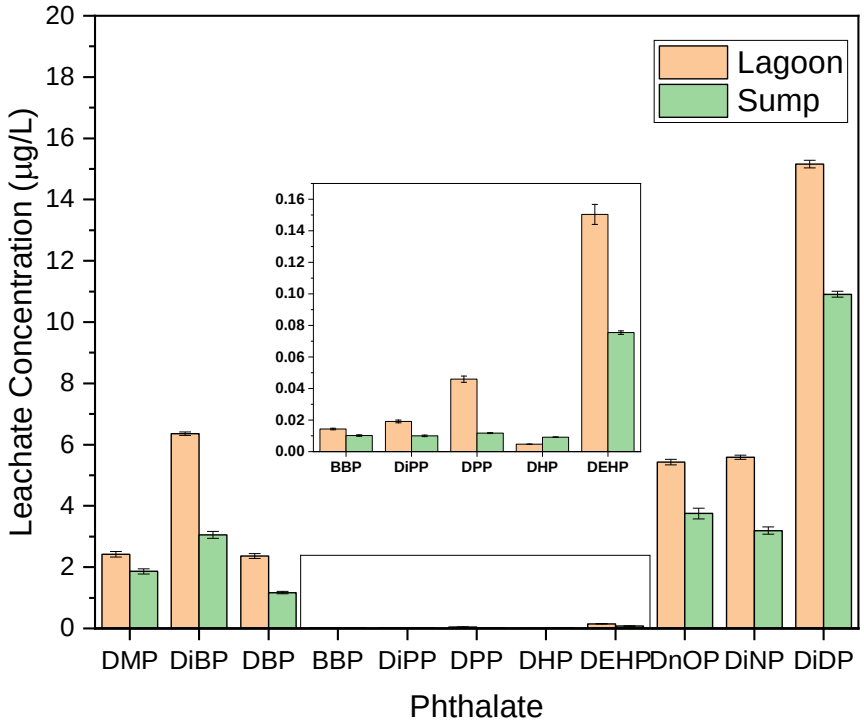


Figure 30: Phthalate concentrations in landfill leachate

The literature does not have any data on composite waste samples. To the author's knowledge there are only data on concentrations in specific consumer products. Therefore, leachate is used to compare waste levels in Ireland to international values. On review of the literature Ireland does not exceed concentrations expected in landfill leachate (See **Table 21**), adapted and updated from Gao and Wen, 2016). The levels of phthalate in Chinese leachates are generally higher than found in Europe. Although the highest values found in leachate came from Brazil, where DBP and DnOP were monitored at 17,900 and 93,300 µg/L respectively in 2004, no other studies were carried out in this country. If the landfill accepts electrical waste this would have an impact on the levels of phthalates found at that site. The Scandinavian countries Finland, Sweden and Denmark had the lowest levels, which could be due to the earlier introduction of incineration in these countries. No US data has been reported for the occurrence of phthalates in landfill leachate. In comparison, Irish leachates are within the lower range of European data. The longevity of phthalates within landfill sites and their respective leachates is not well described in the literature. Owing to this site's age the relatively higher concentrations of restricted phthalates like DnOP, DiNP and DiDP could be due to the lack of legislation at the time of opening. These phthalates being in higher concentration could also be due to the slower degradation of these compounds remaining in the landfill for a greater period and taking longer to leach.

Relevant recyclable materials should be carefully tested for restricted phthalates to lessen the risk of these materials being incorporated into toys and food contact materials.

Table 21: International occurrence of phthalates in landfill leachate samples in µg/l

Location	*DMP*	*DBP*	*BBP*	*DEHP*	*DNOP*	*Reference*
Sweden	< 0.10	< 0.05	< 0.05	< 0.05–0.18	< 0.05	(Kalmykova et al., 2014)
Finland	N.D.–< 1.00	N.D.–17.00	N.D.–1.00	N.D.–37.00	N.D.–<1	(Marttinen et al., 2003)
17 European Countries	N.D.	N.D.–29.00	N.D.–7.00	N.D.–460	–	(Jonsson et al., 2003)
Ireland	**1.75–2.49**	**1.12–2.49**	**0.01–0.02**	**0.07–0.16**	**3.54–5.54**	****This Study**
China	–	–	1.20–2.10	5.10–15	–	(Kadlec and Zmarthie, 2010)
Japan	–	3.00–15.00	0.70–7.80	9.60–49.00	–	(Asakura et al., 2004)
Wuhan, China	N.D.–43.27	7.27–15.43	N.D.	N.D.–7.24	N.D.	(Liu et al., 2010)
Hubei, China	N.D.–2.80	0.35–59.75	N.D.–21.80	1.61–232.50	N.D.–521.10	(Zhang and Wang, 2009)
Chennai, India	–	N.D.	–	–	56.80–495.30	(Swati et al., 2008)
Landfill leachate	–	–	–	88–460	–	(Zolfaghari et al., 2014)
Zhejiang, China	1.83	0.25	–	–	0.47	(He et al., 2015)
Brazil	–	17,900	–	–	93,300	(dos Santos et al., 2004)

N.D-Not Detected, "–" denotes that phthalate was not studied, *Denotes that the mean is reported, rather than the min-max reported for other studies.

3.2 Wastewater

The occurrence of phthalates in wastewater influent will give an indication of the degree of phthalate burden in a population. Down the drain disposal of phthalates is common due to the use of these plasticizers in personal care products, household cleaners etc. The presence of phthalate diesters will not serve to relate directly to human exposure as less than 1% of phthalates are excreted unchanged.

Sewage sludge is generated through the wastewater treatment process, all sites studied in this project used activated sludge. This consists of biosolids removed from wastewater during treatment as well as residual organic matter from the treatment process. 53,543 tonnes of sewage sludge were produced in Ireland in 2014. The majority of this sludge was treated and used for land spreading, as a fertiliser or soil enhancer (EPA, 2015b). Research has shown that capacity needs to be increased (EPA, 2014c). Irish Water issued a Draft National Wastewater Sludge Management Plan, detailing a standard approach for sludge management in the next 25 years (Irish Water, 2016). This plan assessed European alternatives and noted that incineration is the main viable option. Incineration has not become fully established in Ireland to take on the increased volumes required for sludge. As a result, land spreading will persist for the coming years. The report acknowledges that this will have the biggest potential to adversely affect soil sites. Land spreading will need to be fully assessed in terms of the potential to adversely affect the diversity of these sites but all plans focus on nutrient profiles and do not take into consideration any possible endocrine disrupting impacts that could arise from emerging contaminants.

Any remaining phthalate in wastewater treatment plant effluent will act as a source of phthalates to the aquatic environment. Wastewater effluent is not currently used as a source of crop irrigation in Ireland, as is the case in other European countries where this practice could further contribute to phthalate contamination in soil.

Three wastewater treatment plants were studied and identified as urban, suburban and rural. While these sites are all combined sewers and used activated sludge as tertiary treatment, the flows and capacities were very different (**Table 22**). Influent, effluent and sludge were analysed for suburban and rural sites, while influent alone was measured at the urban site due to site permissions.

Table 22: Site descriptors for WWTPs studied, including capacities and flow rates

Date	*Rural*	*Suburban*	*Urban*
Capacity (population equivalent)	500	130,000	1,640,000
September flow rate (m^3/day)	65	22,339	299,021
November flow rate (m^3/day)	251	29,473	503,342
December flow rate (m^3/day)	148	59,950	388,653
January flow rate (m^3/day)	89	26,685	361,730
February flow rate (m^3/day)	78	26,902	277,387

3.2.1 Influent

Influent samples over four months were analysed at an urban, suburban, and rural site. These were all tertiary treatment sites, using biological treatment but had very different site characteristics in terms of capacity, flows, residence times etc. It was assumed that phthalate concentration from the influent would partition into the sludge and be removed from the effluent during this treatment.

Influent concentrations were highest at the suburban site (**table 23**). Although the population equivalence is lower, higher rates at the suburban site could be due to proximity to farmland, among other factors. However, some months have shown differences in concentration. This indicates that there is some level of temporal variability in phthalate measurements at WWTPs. This could be largely due to the WWTP parameters at the time of sampling, including residence time and storm water overflows/capacity issues. There were spikes in November concentrations for the rural influent, and looking at wastewater flows for that date they were double those of the other months due to wet weather. The increase in wastewater influent volume at that site could indicate that contaminants in the influent were not being removed as efficiently during this sample time.

LMW phthalates were highest, in particular DMP, DBP and DiBP. DBP and DiBP were consistently high in most samples and were also found in all procedural blanks. Even though these phthalates are restricted in some consumer products they remain in circulation and are most likely contained in consumer products that do not fall under the legislative reach. The less well studied phthalates like DiPP, DPP and DHP were found in lower concentrations.

Table 23: Concentration of phthalates in WWTP influent (n=3, ±2SD)

	DMP	*BBP*	*DiBP*	*DBP*	*DiPP*	*DPP*	*DHP*	*DEHP*	*DnOP*	*DiNP*	*DiDP*
Rural Influent (µg/L)											
November	1.29	0.44	14.39	2.03	0.02	0.10	0.12	0.28	**0.39**	0.34	5.86
	(±0.04)	(±0.02)	(±0.34)	(±0.08)	(±0.002)	(±0.002)	(±0.02)	(±0.02)	**(±0.06)**	(±0.02)	(±0.48)
December	1.14	0.18	8.68	0.63	0.03	0.21	0.03	0.48	0.70	0.60	0.33
	(±0.004)	(±0.01)	(±0.28)	(±0.06)	(±0.002)	(±0.004)	(±0.002)	(±0.01)	(±0.058)	(±0.018)	(±0.026)
January	0.86	0.08	9.61	1.07	0.03	0.17	0.01	0.31	**0.05**	0.27	0.54
	(±0.06)	(±0.001)	(±0.48)	(±0.052)	(±0.002)	(±0.006)	(±0.000)	(±0.013)	**(±0.007**)	(±0.014)	(±0.026)
February	1.97	0.59	10.38	1.21	0.02	0.15	0.02	0.25	0.40	0.22	1.00
	(±0.02)	(±0.01)	(±0.06)	(±0.12)	(±0.002)	(±0.006)	(±0.002)	(±0.006)	(±0.05)	(±0.01)	(±0.07)
Suburban Influent (µg/L)											
November	30.51	0.08	53.13	54.26	0.01	0.08	0.05	0.20	**0.67**	2.65	0.21
	(±0.314)	(±0.004)	(±1.488)	(±4.768)	(±0.000)	(±0.002)	(±0.002)	(±0.006)	**(±0.088)**	(±0.252)	(±0.018)
December	70.07	0.40	141.63	75.66	0.05	0.23	0.07	0.38	1.78	5.42	3.66
	(±0.900)	(±0.004)	(±3.30)	(±5.184)	(±0.002)	(±0.008)	(±0.004)	(±0.012)	(±0.084)	(±0.234)	(±0.410)
January	82.05	**0.02**	47.77	43.92	0.07	0.07	0.02	0.36	**2.60**	9.02	11.44
	(±0.700)	**(±0.004)**	(±1.436)	(±1.764)	(±0.002)	(±0.002)	(±0.001)	(±0.010)	**(±0.324)**	(±0.328)	(±0.894)
February	19.75	0.14	95.21	25.97	0.02	0.10	0.04	0.31	1.26	8.75	6.03
	(±0.666)	(±0.006)	(±2.308)	(±2.180)	(±0.002)	(±0.002)	(±0.002)	(±0.026)	(±0.124)	(±0.276)	(±0.182)
Urban Influent (µg/L)											
November	50.57	0.02	34.44	16.42	0.03	0.04	0.01	0.17	**7.32**	11.46	11.96
	(±0.994)	(±0.002)	(±2.796)	(±0.770)	(±0.001)	(±0.004)	(±0.000)	(±0.004)	**(±1.098)**	(±0.562)	(±1.050)
December	71.46	0.04	30.58	32.32	0.01	0.05	0.01	0.03	**3.63**	5.52	10.43
	(±1.154)	(±0.004)	(±0.394)	(±2.942)	(±0.001)	(±0.002)	(±0.001)	(±0.002)	**(±0.558)**	(±0.392)	(±0.434)
January	94.05	0.03	28.36	1.70	0.03	0.06	0.03	0.16	4.75	7.58	14.38
	(±2.230)	(±0.004)	(±0.956)	(±0.064)	(±0.002)	(±0.002)	(±0.002)	(±0.012)	(±0.282)	(±0.162)	(±0.506)
February	17.51	0.02	51.70	3.82	0.02	0.03	0.01	0.20	2.73	3.06	4.09
	(±0.370)	(±0.002)	(±2.052)	(±0.098)	(±0.008)	(±0.002)	(±0.001)	(±0.016)	(±0.074)	(±0.112)	(±0.088)

Values in bold reflect samples that were ≥5%RSD, none were above 10% RSD

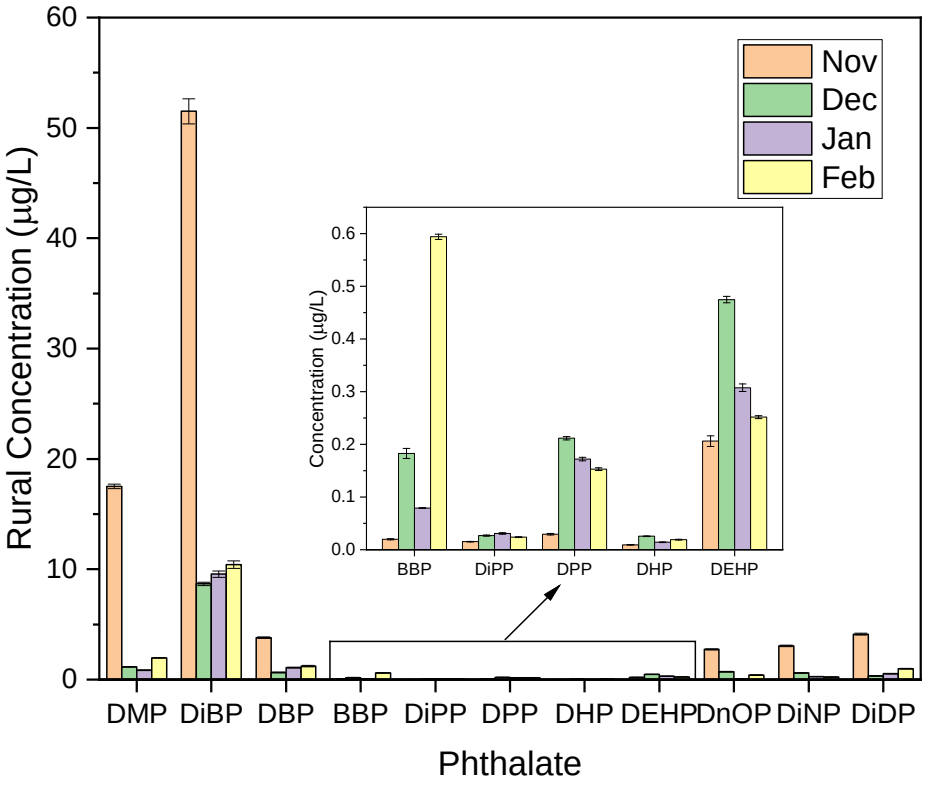


Figure 31: Phthalate concentrations in rural influent

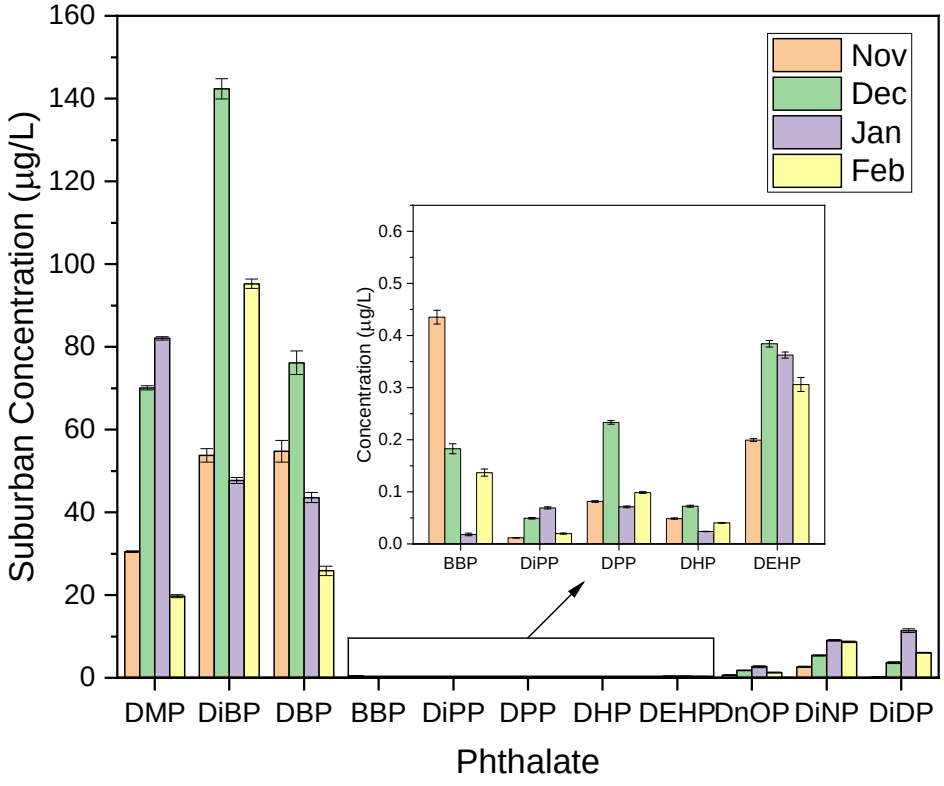


Figure 32: Phthalate concentrations in suburban influent

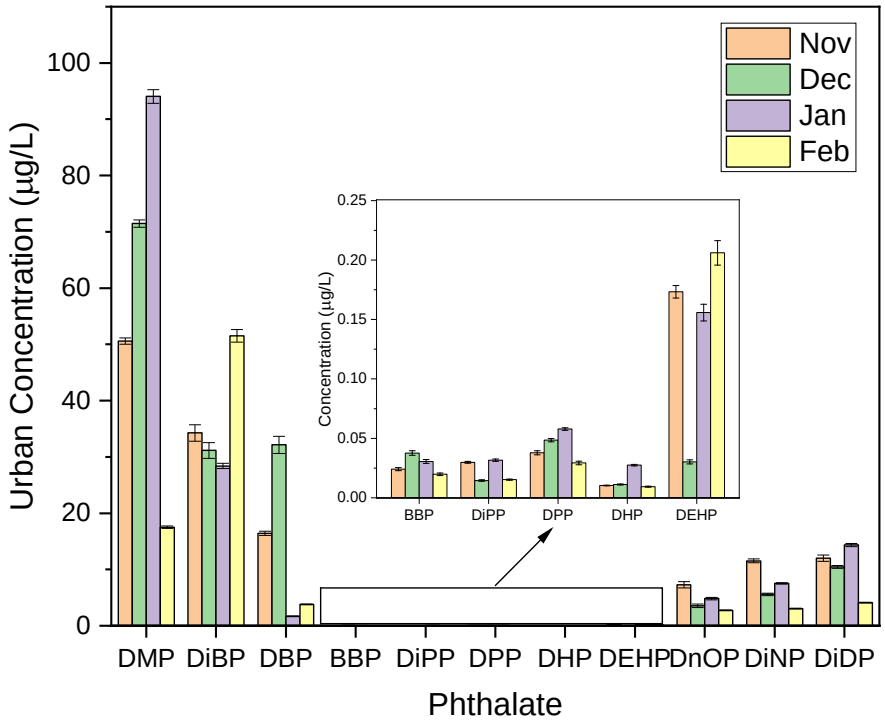


Figure 33: Phthalate concentrations in urban influent

To investigate the difference between samples PCA was carried out. For the purposes of PCA in this text, all data were log transformed, and all WWTP concentrations adjusted to µg/inhabitant/day. For influent the total variance of PC1 and PC2 accounted for the highest rate of variance, 67.2% and 19.1% respectively. The phthalates that showed the highest variances overall for dimension 1 were DMP, DBP, DnOP, DiDP, and DiNP, whereas BBP, DHP, and DPP contributed to the variance in dimension 2 (**Figure 34**). There was clear speciation of influent samples. The differences between all three sites were highly significant as confirmed through two-way ANOVA (**Figure 36**). Variation of phthalates could be due to many reasons, including agricultural, industrial activity, food sources and degree of consumer product use. Due to the limited amount of data available on these areas, no definite causative factors can be assigned for the spatial and temporal variation.

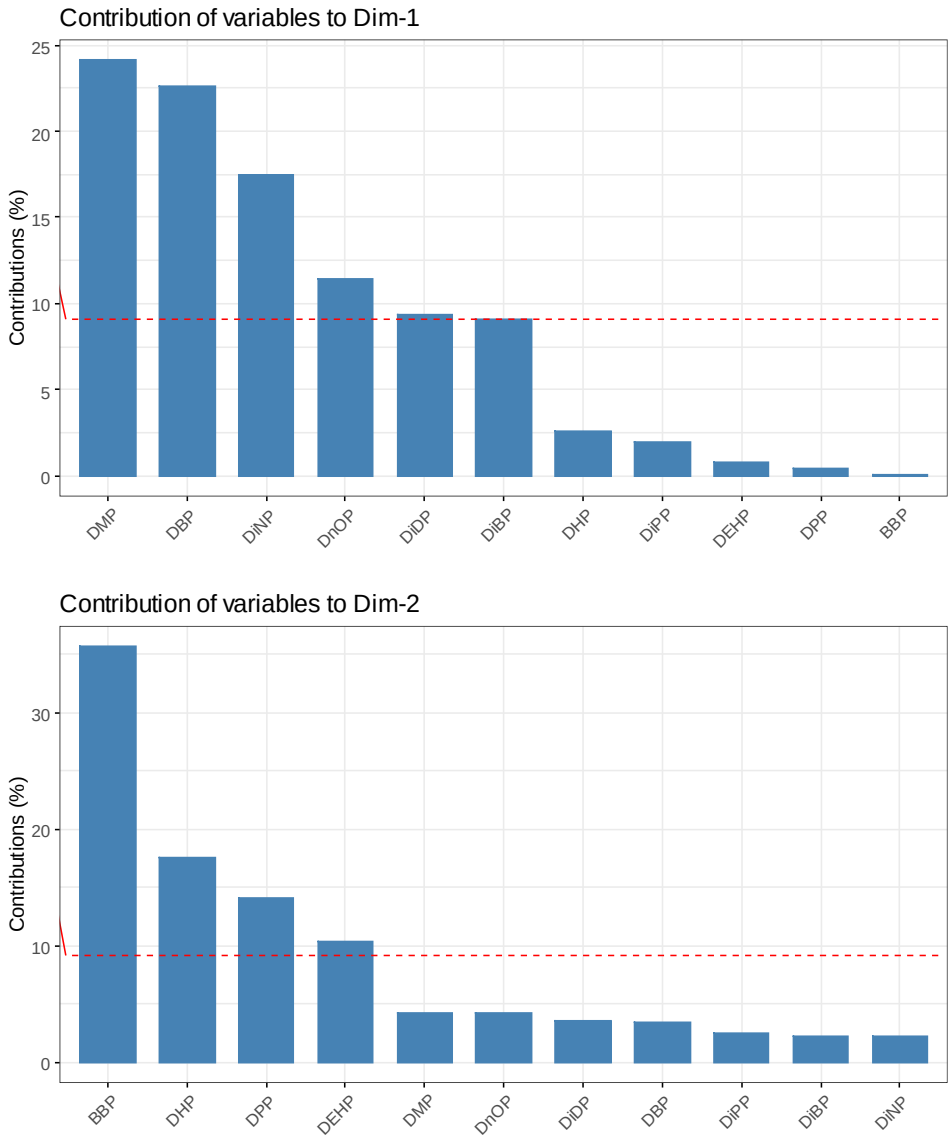


Figure 34: Phthalate contributors to variance between influent samples

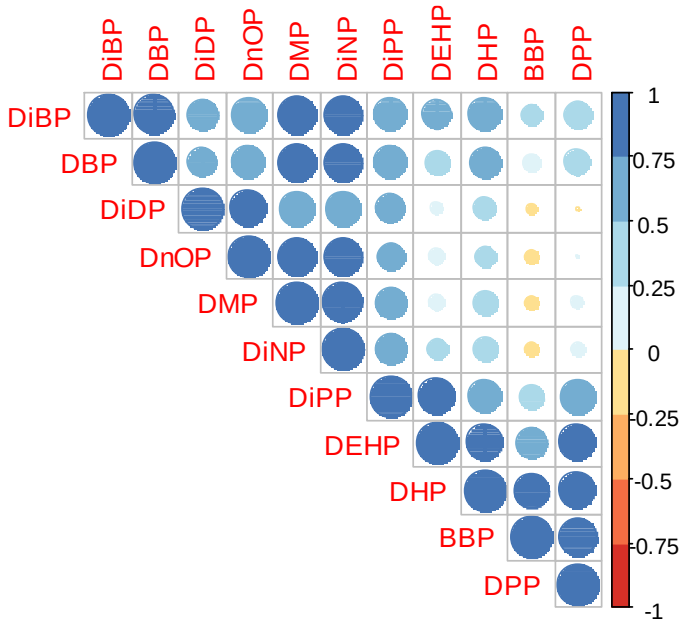
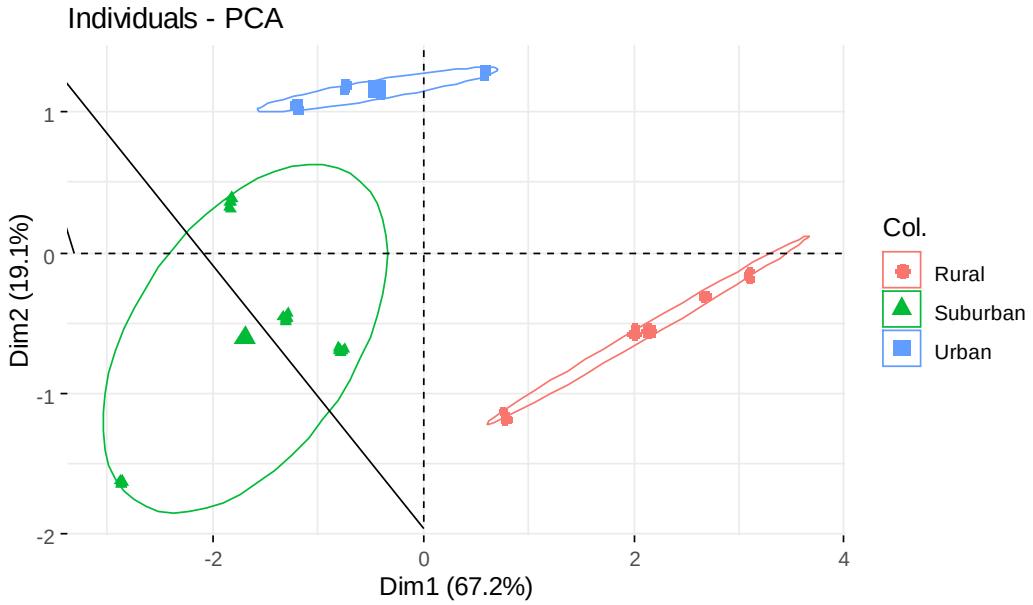


Figure 35: Corrplot of phthalate concentrations in influent



Linear Hypotheses:	Pr(>|t|)
Urban-Suburban ==0	< 1e-05 ***
Rural - Suburban == 0	< 1e-05 ***
Rural - Urban == 0	0.00011 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)

Figure 36: Individual PCA of phthalates in influent

The levels found in this study were compared to international levels (**Table 24**). Influent concentrations are not widely reported, often opting to report removal rates only. Ireland has high levels of phthalates in influent when compared to other European countries, apart from DEHP which is relatively low. Increased concentrations could be due to unknown increased down the drain deposal in this country, this could be connected to farming or industry but no causative factors could be determined due to the lack of knowledge in this area.

Table 24: International comparison of phthalates in wastewater influent (µg/L)

Location	*DMP*	*DBP*	*BBP*	*DEHP*	*DOP*	*Reference*
U.K*	0.26	2.54	1.46	23.6	0.11	(Oliver et al., 2005)
France*	1.5	4.1	4.0	33.3	0.7	(Tran et al., 2015)
Austria	N.D.–2.4	N.D.–8.7	0.31–3.2	3.4–34.0	N.D.–1.1	(Clara et al., 2010)
China	4.05–6.49	8.73–24.46	N.D.–5.67	2.42–30.99	4.63–12.84	(Gao et al., 2014)
South Africa*	–	0.92–18.26	N.D.–6.54	N.D.–53.21	–	(Gani and Kazmi, 2019)
Ireland	**0.80-95.76**	**0.58-78.60**	**0.01-0.60**	**0.03-0.48**	**0.08-7.85**	****This Study**
India	–	0.928–18.06	0.90–19.63	9.17–218.4	–	(Gani et al., 2016)
South Africa	0.89–24.51	3.12–2497	N.D. –52.25	6.16–96.18	3.08–67.37	(Salaudeen et al., 2018)
Puerto Rico*	520	13020	16920	7490	–	(Soler-Llavina et al., 2017)

N.D-Not Detected, "–" denotes that phthalate wasn't studied, *Denotes that the mean/mean range is reported, rather than the min-max reported for other studies.

3.2.2 Sludge

As sludge is the primary route of removal of EDCs from the WWTP it was expected that the levels of phthalates would be relatively higher in this matrix. Sludge also represents a more stable measure of phthalates in an environment owing to higher residence times relating to a longer time-weighted average. HMW phthalates tend to have higher residence times than LMW counterparts due to their higher $\log K_{ow}$ values, and are therefore often found in higher concentrations within sludge (Lee et al., 2019). Sludge was measured in microgram phthalate per gram dry weight sludge analysed.

Phthalates were found in higher concentrations in the rural sludge compared to the suburban sludge (***Table 25***). This could be due to WWTP capacities, and the ratio of sludge to influent. Similar to other matrices DBP and DiBP are the highest contributors to phthalate contamination. DEHP was in relatively higher concentration to other phthalates than was seen in influent. This could indicate that DEHP has longer residence times in sludge and can concentrate in this matrix.

Table 25: Concentrations of phthalates in sudge (n=3, ±2SD) as a source of contamination

	DMP	*BBP*	*DiBP*	*DBP*	*DiPP*	*DPP*	*DHP*	*DEHP*	*DnOP*	*DiNP*	*DiDP*
Rural Sludge (µg/g)											
November	6.76	3.22	48.84	34.33	0.51	4.06	1.51	14.78	0.22	12.40	6.23
	(±0.78)	(±0.44)	(±5.74)	(±4.60)	(±0.06)	(±0.56)	(±0.30)	(±0.70)	(±0.04)	(±0.01)	(±0.54)
December	90.84	39.48	286.46	300.85	10.04	37.76	2.63	18.29	1.49	14.83	7.34
	(±10.80)	(±3.76)	(±34.40)	(±30.30)	(±0.48)	(±6.00)	(±0.32)	(±2.30)	(0.14)	(±1.82)	(±1.16)
January	71.67	23.30	48.14	43.84	3.81	4.94	3.41	7.24	0.31	4.53	8.97
	(±5.28)	(±4.92)	(±5.68)	(±4.00)	(±0.44)	(±0.54)	(±0.32)	(±0.90)	(±0.04)	(±0.36)	(±0.80)
February	19.79	7.98	71.41	41.68	0.86	3.99	1.87	20.87	0.72	6.75	9.13
	(±1.62)	(±0.76)	(±5.48)	(±5.80)	(±0.08)	(±0.52)	(±0.22)	(±2.96)	(±0.10)	(±0.74)	(±1.38)
Suburban Sludge (µg/g)											
November	10.72	1.51	46.04	31.97	0.20	7.17	2.86	27.36	1.89	**7.03**	**2.03**
	(±0.48)	(±0.18)	(±6.72)	(±4.86)	(±0.02)	(±0.70)	(±0.60)	(±3.32)	(±0.34)	**(±0.98)**	**(±0.32)**
December	**15.89**	2.72	**21.09**	50.56	1.63	4.04	3.13	73.32	7.13	**13.78**	3.95
	(±2.20)	(±0.26)	**(±2.74)**	(±5.56)	(±0.22)	(±0.88)	(±0.50)	(±4.28)	(±0.62)	**(±1.90)**	(±0..36)
January	11.07	1.87	49.67	34.97	**1.53**	2.65	**2.19**	**21.72**	2.14	9.31	2.91
	(±0.44)	(±1.16)	(±5.46)	(±2.34)	**(±0.32)**	(±0.26**)**	**(±0.56)**	**(±3.84)**	(±0.14**)**	(±0.68)	(±0.28)
February	9.05	2.00	40.37	25.68	0.37	7.17	**2.14**	18.03	**1.23**	**6.92**	**2.27**
	(±0.82)	(±0.14)	(±3.32)	(±2.46)	(±0.04)	(±0.78)	**(±0.56)**	(±0.50)	**(±0.32)**	**(±1.08)**	**(±0.38)**

Values in Bold Represent Samples Where %RSD ≥5

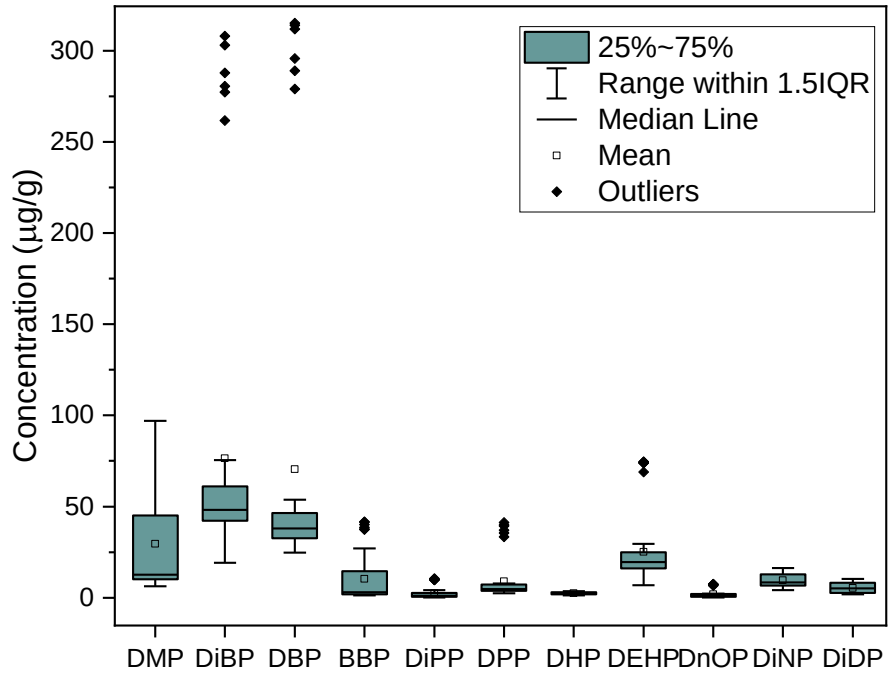


Figure 37: Concentrations of phthalates in all sludge samples (n=12, in triplicate)

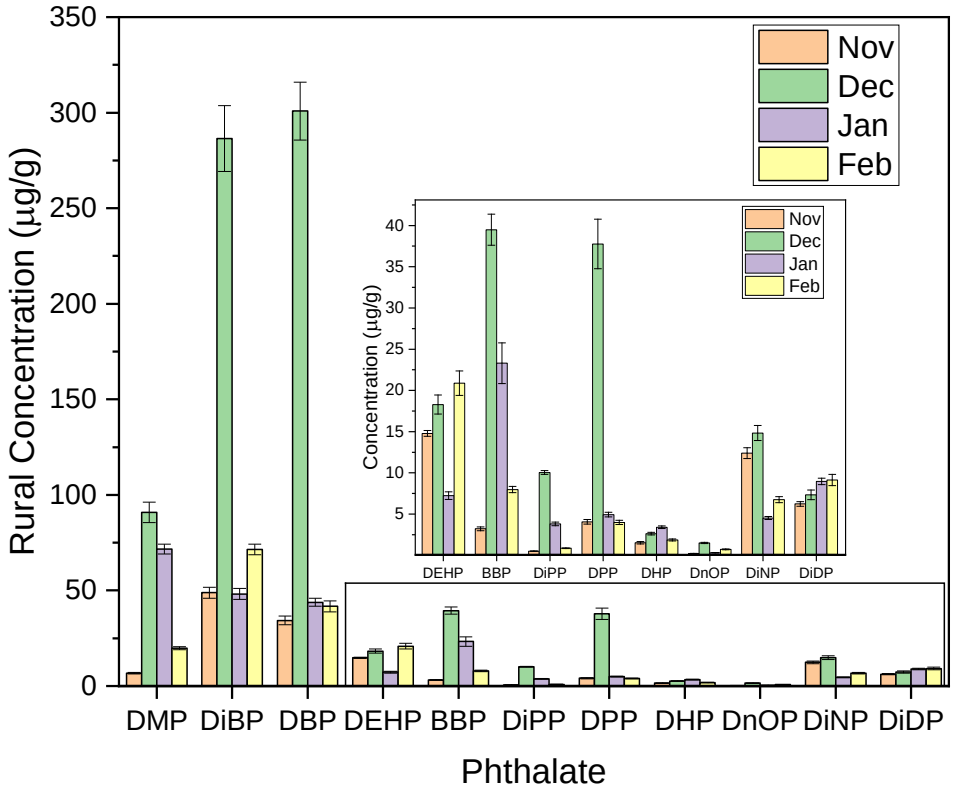


Figure 38: Phthalate concentrations in rural sludge

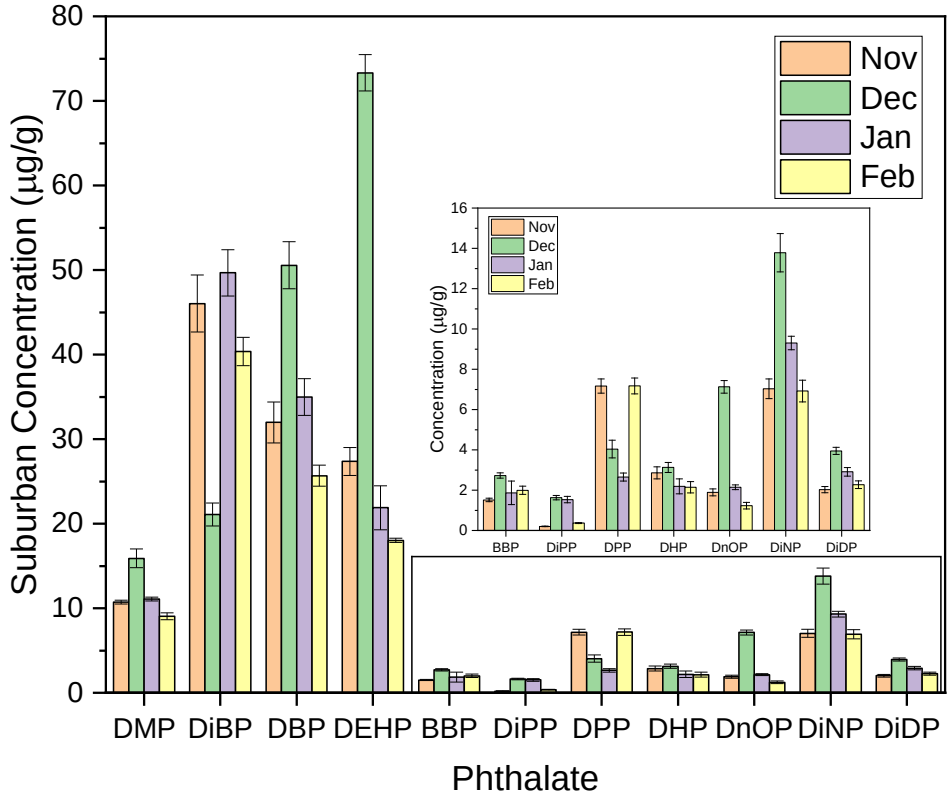


Figure 39: Phthalate concentrations in suburban sludge

PCA speciation was carried out on adjusted sludge data. This was µg/person/day, calculated using the volume of sludge at the WWTP on that day coupled with the population served. As all values were calculated in dry weight, the dewatering factor calculated as % weight loss at lab scale was also incorporated. This would remove the variances caused by treatment plant scale and only account for differences in occurrence at those sites/sample dates. The presence of all phthalates were positively correlated with the presence of all other phthalates (**Figure 41**). It was thought that there might be a greater affinity for HMW phthalates and that they would not correlate so strongly with the LMW phthalates due to the hydrophobicity of this matrix. Given that the LMW phthalates are still relatively hydrophobic in nature and share a similar structure, this is a logical result. The PCA conducted showed that dimension one (Dim-1) contributed 80.4% of known variance with DiPP, BBP, DMP and DBP contributing this variance, while dimension two (Dim-2) had 10.9% variance with DnOP, DEHP and BBP contributing most to this dimension (**Figure 40**). Samples clustered by site, within a confidence interval of 66% and sample sets from the two sites were found to be significantly different as confirmed by

2-way ANOVA (**Figure 42**). This illustrates that concentrations in sludge vary between sites irrespective of population size and sludge production rate. Sludge retains phthalates well at both sites, differences in sludge concentrations are most likely due to variation in biological activity and site capacity.

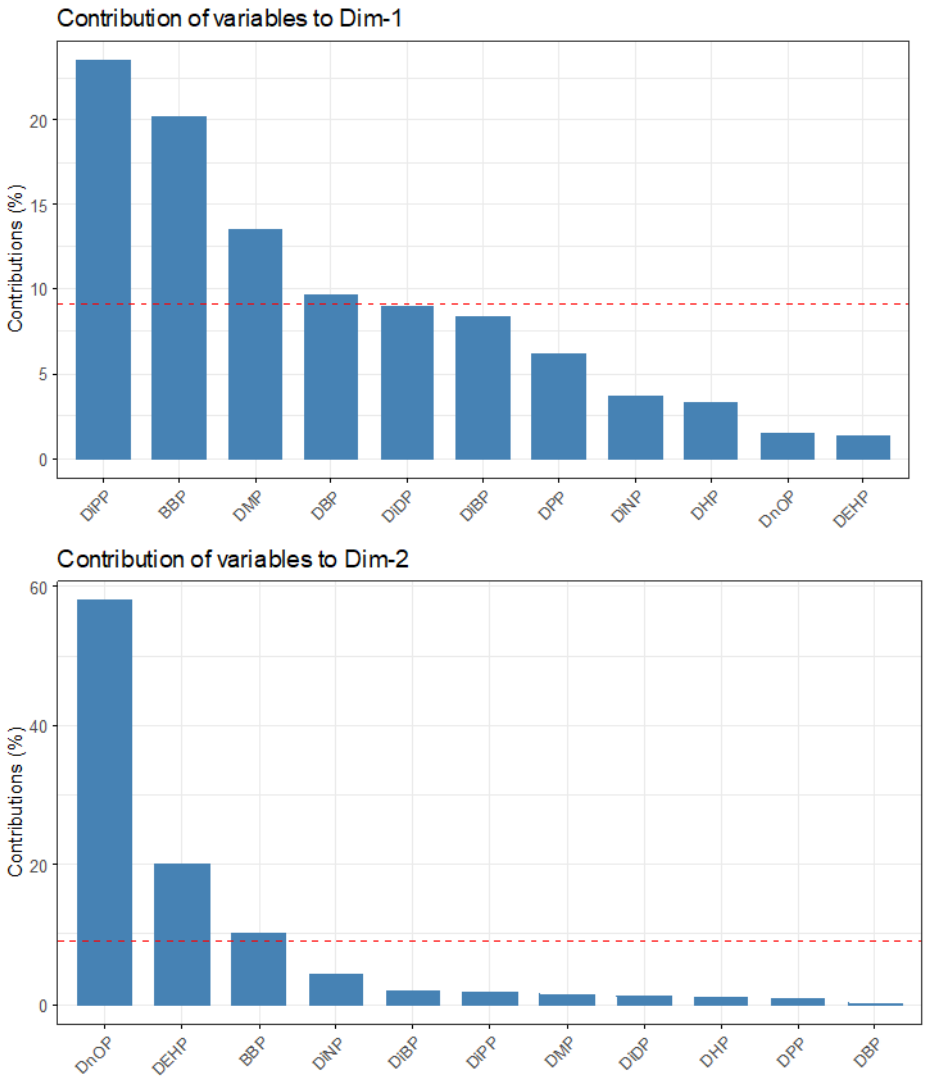


Figure 40: Phthalate contributors to variance between sludge samples

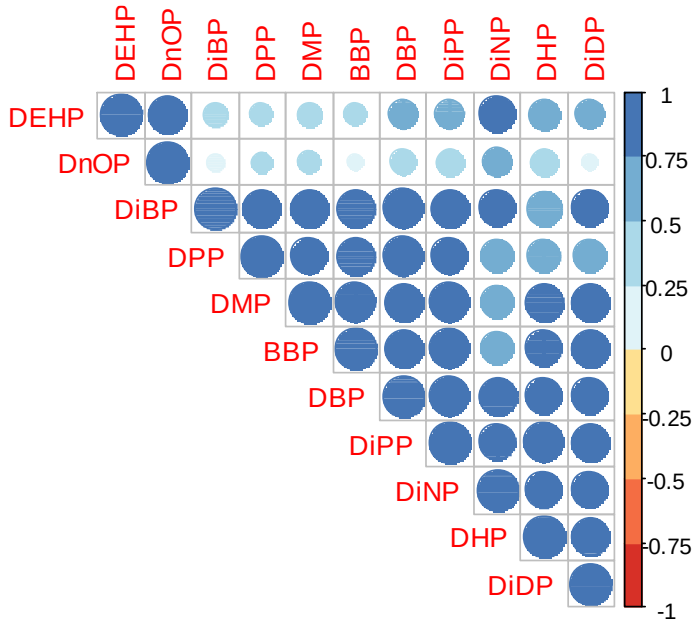
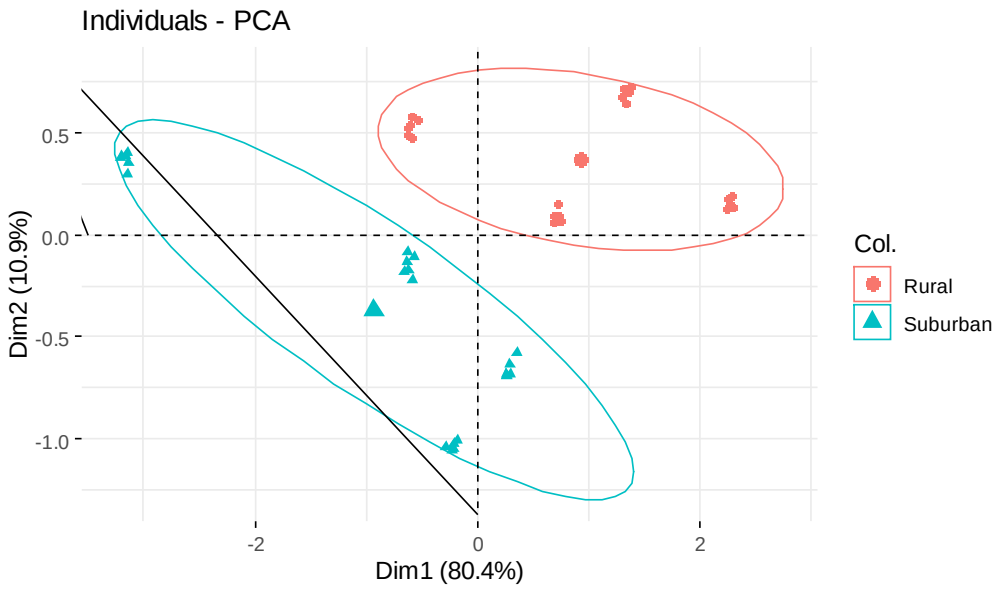


Figure 41: Corrplot of phthalates in sludge



Linear Hypothesis Pr(>|t|)
Rural - Suburban == 0 1.17e-12 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Figure 42: Individual PCA of phthalates in sludge

Phthalate contamination in sludge from this study were related to other countries. Ireland has higher levels of phthalate contamination in sludge than most other countries. Only Turkey and South Africa reported higher concentrations than this study. Ireland had lower levels of DEHP but higher levels of all other phthalates. This again could be partially attributed to the increased levels of DBP and DiBP in Ireland as DEHP levels and DnOP levels were closer to the min and max reported for other all countries. Studies from 2019 showed higher levels of DBP than older data but the dataset is small.

Table 26: International comparison of phthalates in sludge reported in mg/kgdw

Samples	*DMP*	*DBP*	*BBP*	*DEHP*	*DOP*	*Reference*
China	0.19–0.91	0.54–1.94	N.D.–6.89	1.85–9.41	1.11–8.09	(Gao et al., 2014)
U.K.*	0.04	0.97	1.45	30.2	0.14	(Oliver et al., 2005)
France*	2.7	0.09	0.37	72.1	1.9	(Dargnat et al., 2009)
South Africa	–	0.13–3.16	N.D.–10.21	N.D.–76.47	–	(Gani and Kazmi, 2019)
Ireland	**6.76-90.84**	**24.65-314.23**	**1.43-41.53**	**6.75-74.55**	**0.19-7.46**	****This Study**
Turkey	1.4-2.7	0.6-4.6	2.8-6.2	18-490	–	(Çifci et al., 2013)
South Africa	6.00-6.10	939-1248.6	277-621.8	271-352.7	71.9-94.9	(Salaudeen et al., 2018)
Korea	0.0024–17.00	0.58-59.00	N.D.-1.90	1.40-1000	–	(Lee et al., 2019)

*N.D-Not Detected, "–" denotes that phthalate was not studied, *Denotes that the mean is reported, rather than the min-max reported for majority of studies.*

3.2.3 Effluent

The levels of EDCs in effluent are often closely monitored as concentrations in this matrix are released into the aquatic environment. Some EDCs require compliance monitoring at this level to ensure removal at the WWTP is sufficient. Phthalates are not yet legislated against to this degree, but this could be in the pipeline as new evidence emerges on the environmental risks posed. It was found that levels in effluent are greatly reduced when compared to their influent concentrations from the same site and time. Although there is very little phthalate remaining, this still is higher than those concentrations found in surface waters and will contribute to overall phthalate burden in freshwater sites close to the WWTP.

LMW phthalates favoured this matrix with DMP occurring in the highest concentrations. DBP and DiBP remain relatively high, in comparison to concentrations of other phthalates. There was less visible difference between concentrations in effluents than other WWTP samples.

Table 27: Concentrations of phthalates in wastewater effluent (n=3, ±2SD) in µg/L

	DMP	*BBP*	*DiBP*	*DBP*	*DiPP*	*DPP*	*DHP*	*DEHP*	*DnOP*	*DiNP*	*DiDP*
Rural Effluent (µg/L)											
November	0.874 (±0.010)	**0.114 (±0.014)**	0.862 (±0.044)	0.676 (±0.020)	0.022 (±0.002)	0.029 (±0.002)	0.007 (±0.000)	0.011 (±0.001)	0.002 (±0.000)	0.015 (±0.000)	0.037 (±0.002)
December	0.965 (±0.016)	0.070 (±0.006)	0.938 (±0.030)	0.539 (±0.018)	0.028 (±0.004)	0.016 (±0.002)	0.006 (±0.000)	0.004 (±0.000)	**0.002 (±0.000)**	**0.006 (±0.001)**	**0.029 (±0.003)**
January	0.526 (±0.03)	0.028 (±0.004)	0.290 (±0.042)	0.612 (±0.058)	0.006 (±0.001)	0.010 (±0.001)	0.005 (±0.000)	0.010 (±0.000)	0.018 (±0.002)	**0.029 (±0.004)**	**0.056 (±0.002)**
February	1.474 (±0.012)	0.090 (±0.002)	0.546 (±0.032)	0.845 (±0.042)	0.013 (±0.002)	0.019 (±0.002)	0.007 (±0.001)	0.020 (±0.000)	0.005 (±0.001)	0.008 (±0.002)	0.020 (±0.000)
Suburban Effluent (µg/L)											
November	0.23 (±0.012)	0.02 (±0.002)	0.32 (±0.026)	0.46 (±0.012)	0.01 (±0.000)	0.01 (±0.000)	0.01 (±0.000)	0.02 (±0.002)	0.01 (±0.000)	0.01 (±0.002)	0.03 (±0.001)
December	0.24 (±0.010)	0.01 (±0.001)	**0.12 (±0.032)**	0.85 (±0.034)	0.01 (±0.001)	0.001 (±0.000)	0.01 (±0.000)	0.03 (±0.001)	**0.002 (±0.000)**	0.01 (±0.001)	0.01 (±0.000)
January	0.41 (±0.020)	0.02 (±0.002)	0.52 (±0.010)	0.49 (±0.017)	0.01 (±0.000)	0.01 (±0.000)	0.001 (±0.000)	0.018 (±0.000)	0.001 (±0.000)	0.008 (±0.001)	0.009 (±0.000)
February	1.443 (±0.036)	0.013 (±0.002)	0.566 (±0.016)	0.273 (±0.019)	**0.003 (±0.000)**	0.0004 (±0.000)	0.010 (±0.001)	**0.015 (±0.002)**	0.003 (±0.000)	**0.003 (±0.000)**	0.010 (±0.001)

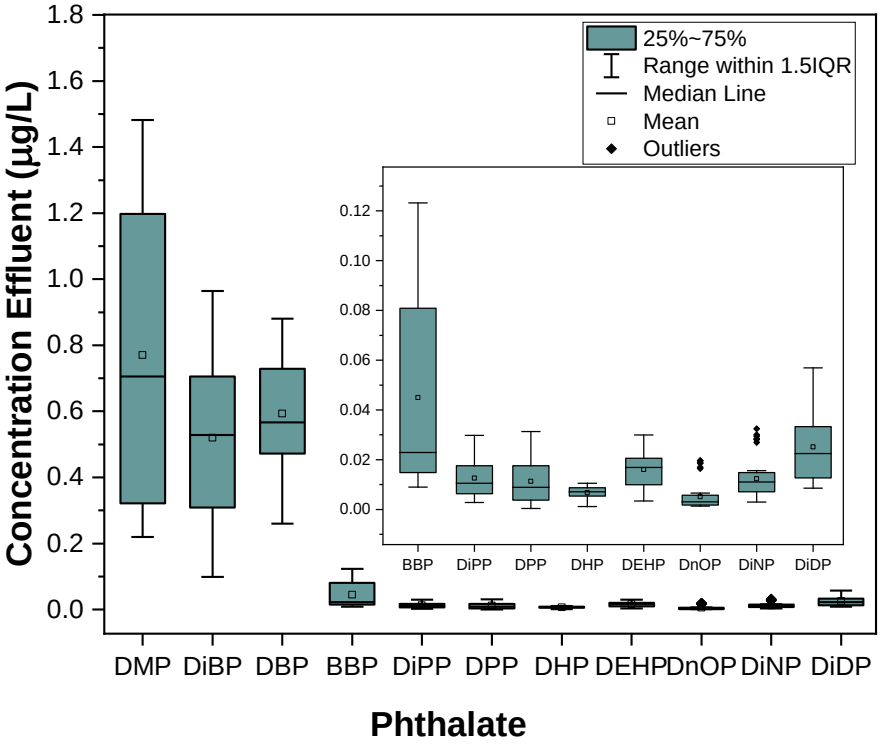


Figure 43: Concentrations of phthalates in all effluent samples (n=12, in triplicate)

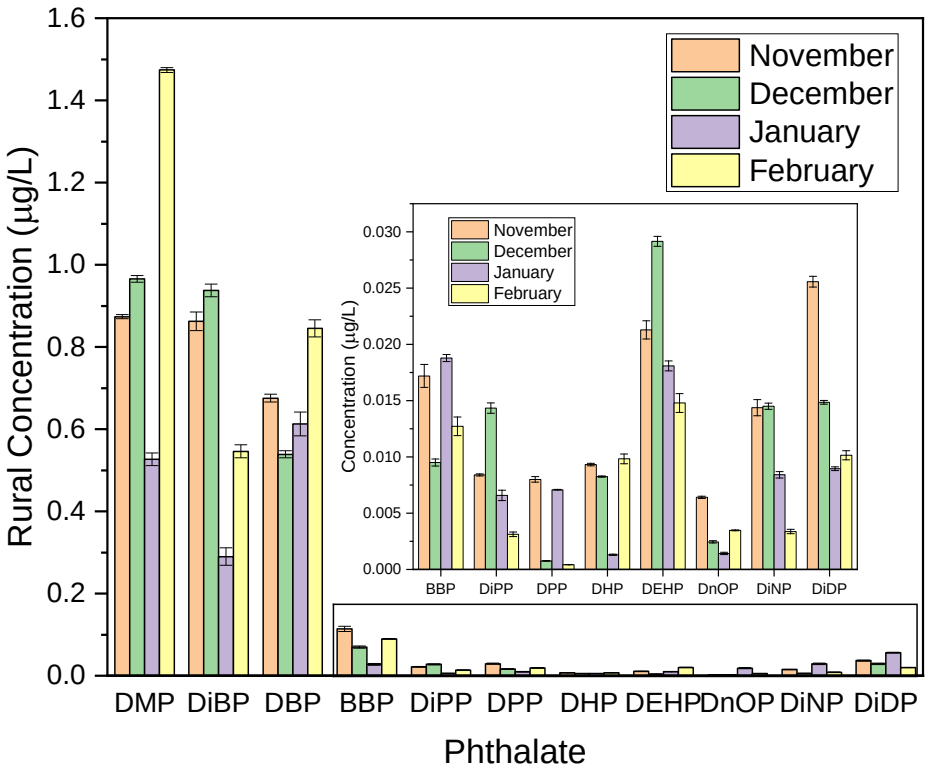


Figure 44: Phthalate concentrations in rural effluent

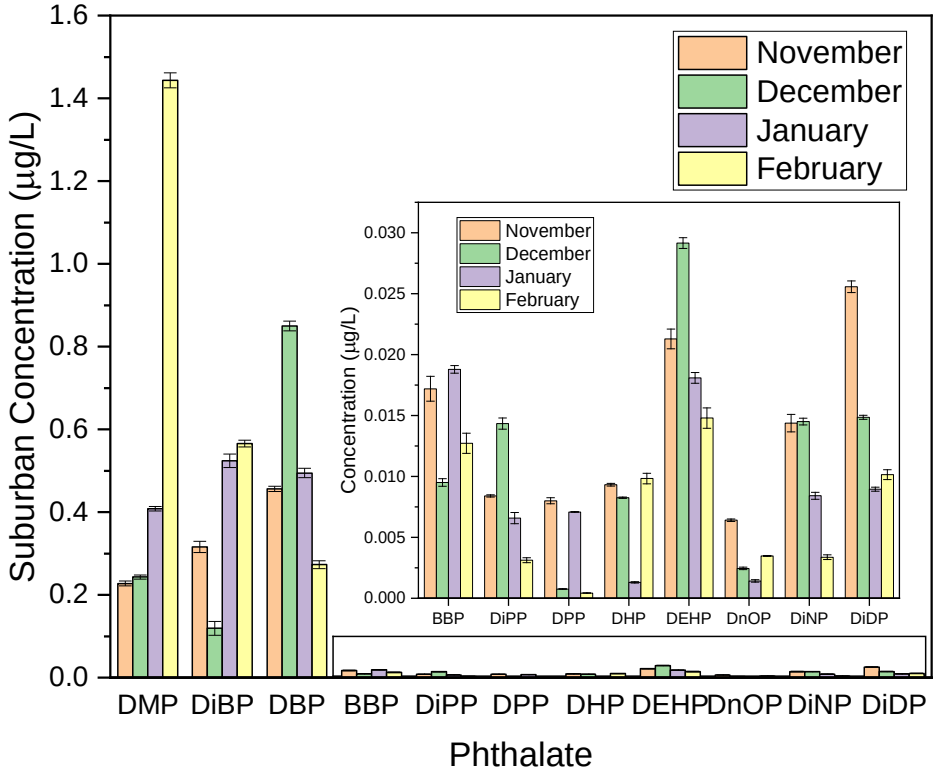


Figure 45: Phthalate concentrations in suburban effluent

Speciation of the samples from both the rural and suburban sites was carried out. There was less explained contribution to variance than with other samples (Dim 1 76.1%, Dim2 10.3%). The phthalates that did contribute most were DPP, BBP, DiPP, and DiDP in Dim1, and DnOP, DiNP, and DiBP in Dim 2 (**Figure 46**). Covariance of the phthalates in effluent showed that phthalates co-occurred in effluent (**Figure 47**). There was a weaker positive correlation when comparing LMW phthalates to HMW phthalates due to the differences in molecular weight affecting partitioning at WWTP. There was a significant difference between the overall phthalate levels at the two sites when population and flow rates were controlled for (**Figure 48**).

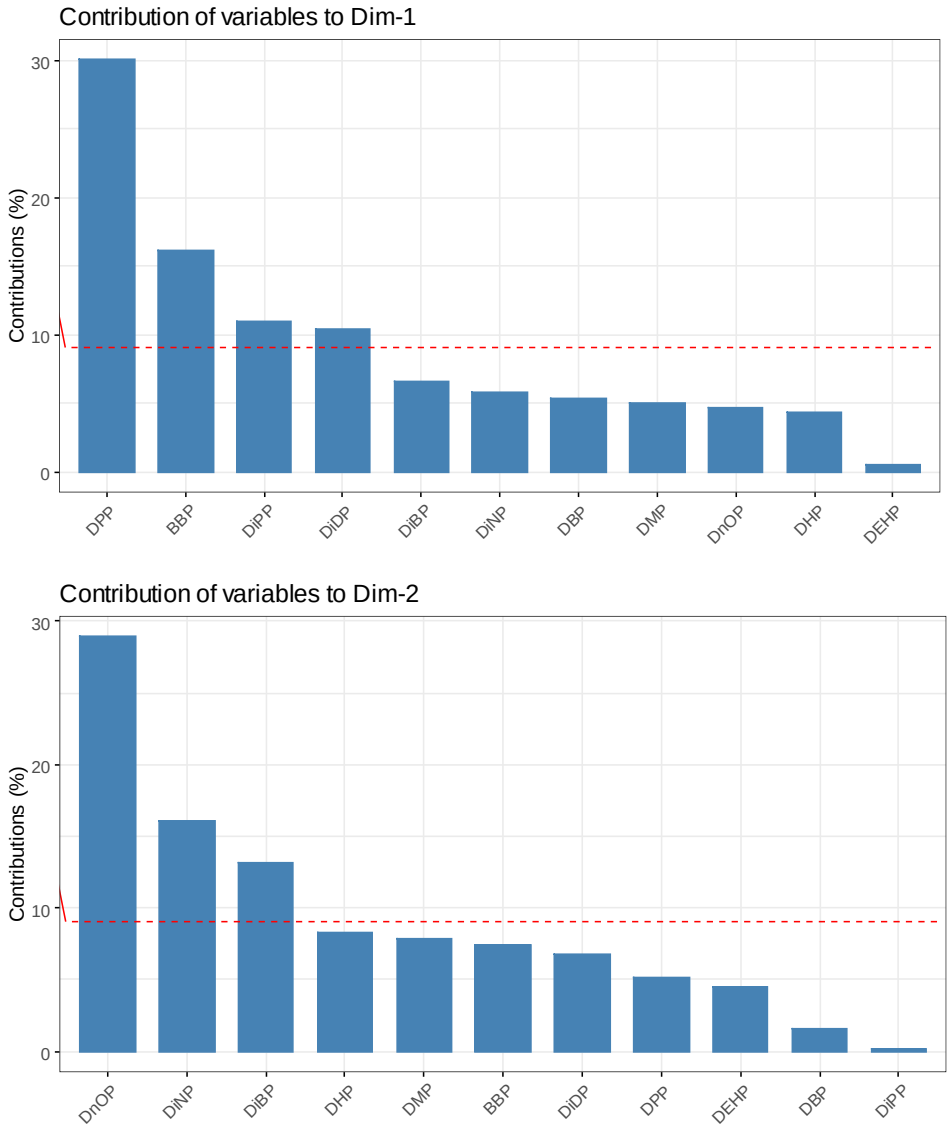


Figure 46: Phthalate contributors to variance between effluent samples

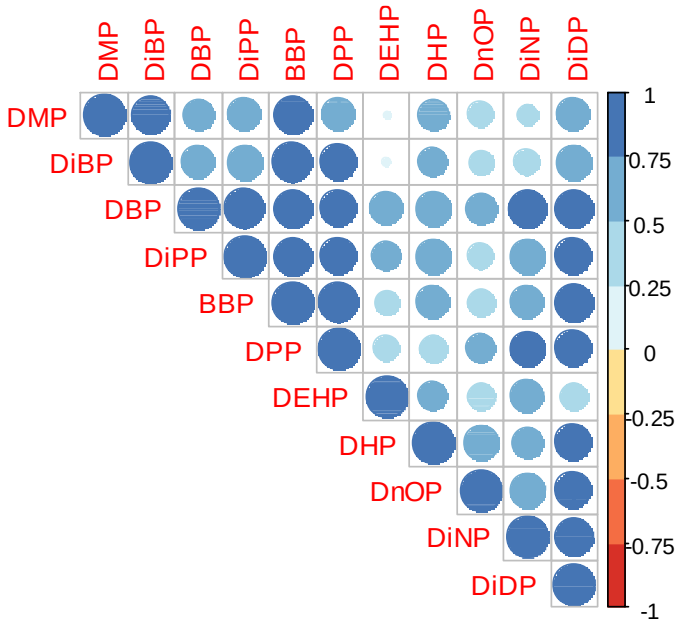
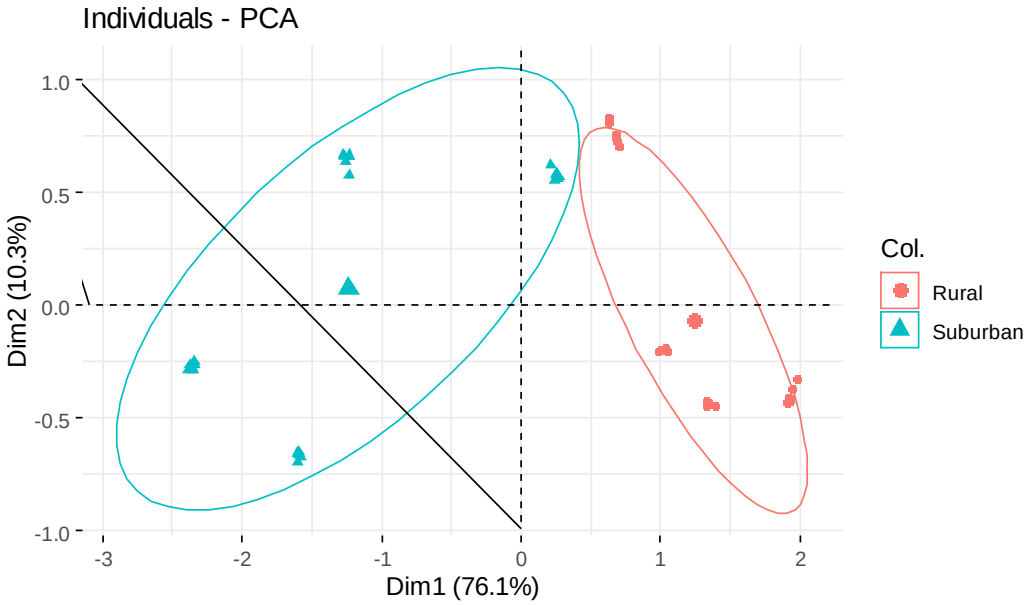


Figure 47: Corrplot of phthalates in effluent



Linear Hypotheses: Pr(>|t|)
Rural - Suburban == 0 <2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Figure 48: Individual PCA of effluent

Effluent concentrations from this study were compared to international levels. Ireland has low effluent levels when compared to other European countries, despite having relatively high influent concentrations.

Table 28: International comparison of phthalates in WWTP effluent (µg/L)

Location	*DMP*	*DBP*	*BBP*	*DEHP*	*DnOP*	*Reference*
Austria	N.D.	N.D.	N.D.	<0.2–1.3	N.D.	(Clara et al., 2010)
Ireland	**0.22-1.47**	**0.27–0.84**	**0.01–0.11**	**0.004–0.029**	**0.001–0.018**	****This Study**
France*	0.03	0.14	0.16	2.00	0.01	(Tran et al., 2015)
France*	N.D.	0.15	0.3	5.02	N.D.	(Dargnat et al., 2009)
Denmark	N.D.–0.237	1.83–2.73	1.99–4.33	2.08–9.93	–	(Roslev et al., 2007)
India	–	N.D.-8.134	N.D.-5.49	0.93-8.53	–	(Gani et al., 2016)
South Africa	0.34–4.87	1.23–24.19	0.28–8.95	1.84–15.13	N.D.–5.78	(Salaudeen et al., 2018)
China	N.D.–1.52	3.47–4.13	N.D.–17.03	1.70–25.4	1.11–14.15	(Gao et al., 2014)
India	–	0.91-3.94	0.08-1.48	1.30-5.88	–	(Gani et al., 2016)
France	–	0.60-3.31	0.46-3.91	39.40-160.00	0.49-2.97	(Burgé et al., 2014)
Puerto Rico*	290	1170	1290	650	–	(Soler-Llavina et al., 2017)

*N.D-Not Detected, "–" denotes that phthalate wasn't studied, *Denotes that the mean is reported, rather than the min-max reported for majority of studies.*

3.2.4 Wastewater Treatment Fluctuation and Removal

There appeared to be temporal variation of phthalates in wastewater. Concentrations were adjusted to g/inhabitant/day to take account any impact flows, sludge production rates and population size have on the concentration at that site. Although there are differences in concentrations between months, they tend to be uniform between phthalates and, generally, increased concentration in the influent shows corresponding increased concentration in the sludge (**Figure 49**).

At the urban site, however, temporal trend in most phthalates appeared more sporadic. Relatively little information is known on the catchment areas but there could be variations in exposure due to population fluctuations and behaviour at catchment site level. This should theoretically be most apparent at the urban site due to tourism, multiple third level education centres and a strong commuter base. However, some population fluctuations could also be seen at the suburban and rural catchment areas. One way of circumventing this for future work would be to use caffeine, ammonia or cotinine concentration in wastewater as a way to estimate population size instead of relying on antiquated and fixed catchment data (Rico et al., 2017; Zheng et al., 2017). This would give a better indication on whether concentration differences could be attributed to population or residence time fluctuations, rather than average human exposure fluctuations.

The temporal variation between sites tends to follow a similar pattern for all sites. There could possibly be an impact of residence times on concentrations at play here considering that operating at close to capacity will influence sewage treatment times. The differences in phthalate concentrations over time cannot be confirmed to be due to differences in human behaviour and phthalate use or population size fluctuations. From the literature there are no obvious activities driving seasonal differences for phthalate concentrations, unlike most pharmaceuticals and some pesticides. However, differences in concentrations between months and WWTPs show that in order to understand phthalate levels completely, multiple sites and sampling times are needed.

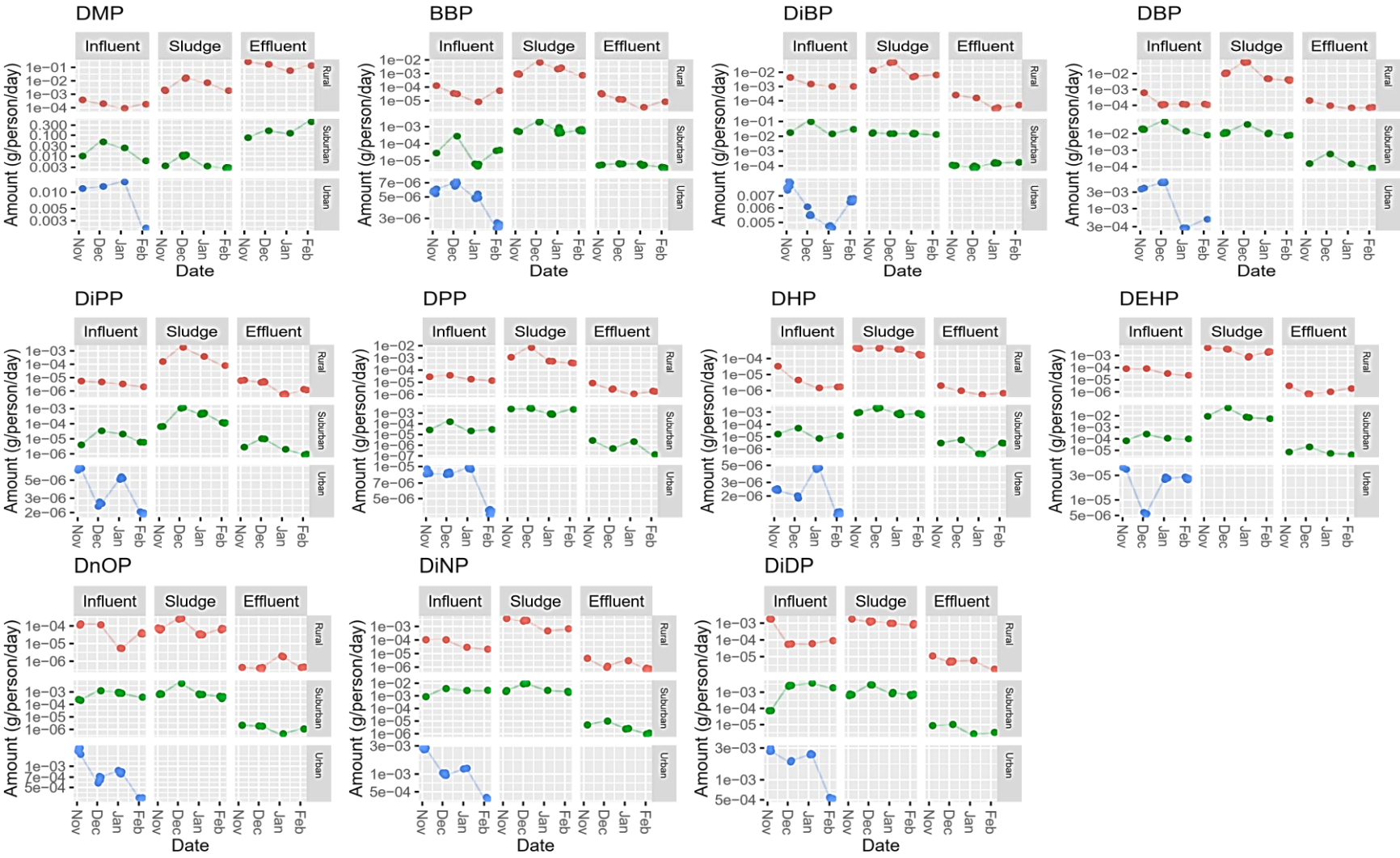


Figure 49: Phthalate concentrations in g/person/day for influent, sludge and effluent (left-right). rural in red, suburban in green and urban in blue.

Estimated removal of phthalates from the watered fraction of the WWTP ranged from 43.02 to 99.87 % (**Table 29**). Flow rates at the suburban site ranged from 22,339 to 59,950 m^3/day, whereas the rural site flow rate ranged from 65 to 251 m^3/day. The relatively small capacity at this site coupled to the large variation in flow rates may have contributed to a lower percentage removal on average due to higher ratio of influent loading to sludge present.

High removal rates may appear to represent a positive impact on phthalate contamination within the aqueous environment. However, while the levels in effluent (and thus surface waters) are reduced, levels in sludge are increased which may lead to higher phthalate-burden in soils in some agricultural areas that use land spreading of sludge. If there are elevated levels near water sources, transfer of phthalates into the wider environment would be likely.

Table 29: Removal of phthalates from the watered fraction at Irish WWTPs

	Suburban % Removal (n=4, ±2SD)	*Rural % Removal (n=4, ±2SD)*
DMP	95.59 (±30.13)	66.51 (±9.86)
BBP	60.77 (±9.17)	70.50 (±10.37)
DiBP	99.41 (±43.63)	93.64 (±3.28)
DBP	98.9 (±20.74)	69.64 (±21.72)
DiPP	81.93 (±9.86)	43.02 (±23.53)
DPP	96.43 (±8.08)	91.32 (±10.91)
DHP	86.27 (±2.16)	67.30 (±15.60)
DEHP	94.19 (±0.34)	95.99 (±3.03)
DnOP	99.84 (±1.64)	87.88 (±17.09)
DiNP	99.87 (±6.04)	94.86 (±4.21)
DiDP	99.78 (±8.72)	92.98 (±4.83)

3.3 Soil

Phthalates can concentrate in soils due to their hydrophobicity and can be introduced through various means e.g. ground water contamination, wet deposition and land treatment. Soil was therefore hypothesised to be one of the primary fates of phthalates in Ireland. Three soil samples in triplicate from various sites were analysed. Urban soils (urban parkland and industrial estate), and agricultural soils (an organic and a traditional farm) were investigated. It was expected that due to the hydrophobicity of soil, that high molecular weight phthalates would concentrate in this matrix. It was also expected that urban soils would have the highest concentrations of phthalates due to the association of phthalates with increased industry.

Different farming practices have been hypothesised to affect the concentrations of phthalates in soils, for example the use of plastic crop covers at organic farms. Elsewhere, many studies have looked at the introduction of EDC contamination though treatment of soils with reclaimed wastewater and bio-solids. Some studies have shown phthalate uptake in crops from soils, contaminating the food chain and putting humans at a higher exposure risk. Phthalates have been detected in leaves and fruits at the agricultural source in China (Ma et al., 2020). This uptake has been widely confirmed for other EDCs. It should be noted that in Ireland there is no use of reclaimed wastewater irrigation, however land spreading of bio-solids is in place at some sites in Ireland.

Some research suggests that the properties of soil may have an influence on the presence of contaminants. Therefore, small differences in concentrations may not relate directly to site activity but may however indicate soil quality differences. For example, the presence of heavy metals and organic contaminants is affected by multiple soil characteristics including; temperature, moisture, organic matter, mineral fractions, and microbial activities. The adsorption of DBP by soil can be divided into adsorption by mineral components and soil organic matter, with the latter playing a greater role. One study followed DBP residues in the three types of soil and found that concentrations in brown soil were higher than fluvo-aquic soil and cinnamon soil, this difference was mainly attributed to pH and not associated with organic matter content (Gao et al., 2019).

Soil characterisations for samples from this study were taken from the EPA soil survey Corine 2018, an update of the COPERNICUS European database, and pH measured using a soil pH probe. These properties are described in ***Table 30***.

Table 30: Characteristics of soil samples included in this study

	Classification	*Subsoils*	*Texture*	*Average pH*
Urban Industrial Estate	Urban	Limestone till (Carboniferous)	Variable	6.65
Urban Parkland	Artificial non-agricultural vegetated areas.	Limestone till (Carboniferous)	Variable	6.13
Organic Farmland	Heterogeneous agricultural areas	Sandstone and shale till (Cambrian/Precambrian)	Clayey	5.27
Traditional Farmland	Agricultural areas, pastures	Fine loamy drift with siliceous stones	Fine Loamy	5.45

Phthalates were found in highest concentrations in the farmland samples (see **Figure 51**). This was not as expected, but farming practices could be related to a higher contamination risk. Organic farmland uses plastic sheeting and tyres combined with poly-tunnels to protect soils and retain the farm's organic status. Traditional farms often use land spreading of treated wastewater sludge, or other fertilizers that could contain PAEs. As this land spreading causes direct contact of a phthalate contaminated material in a similar matrix this could cause increased migration of phthalates to soils than seen with covering of a plastic sheet due to the reduced surface area and more resilient material. Previous studies have shown that the use of bio-solids on land is associated with higher concentrations of organic contaminants. In China the use of phthalate-containing fertilizer has shown increased levels of phthalates in soils.

The farmland soils had lower pH than the urban soils. Soil pH influences chemical absorption by affecting the ionization of organic compounds and determining soil/sediment surface properties and organic matter configuration (Gschwend and Wu, 1985). It has been shown that PAH adsorption on humus decreases with increasing pH (Buergisser et al., 1993). If the same affect is observed for phthalates, as would be

expected, then the higher concentrations in these samples could be partially attributed to soil pH at these sites.

As seen with the sources of phthalates into the environment, DBP and DiBP were the most concentrated phthalates in soil. This could be due to higher use of these phthalates within Ireland. While HMW phthalates were high in the farmland samples as expected for this matrix, the urban soil samples did not follow this pattern.

Table 31: Concentrations of phthalates in soil (n=3, ±2SD) as an environmental fate

	DMP	*BBP*	*DiBP*	*DBP*	*DiPP*	*DPP*	*DHP*	*DEHP*	*DnOP*	*DiNP*	*DiDP*
Urban Parkland (µg/g)											
Area A	1.596	1.055	3.501	5.006	0.245	1.595	0.668	0.553	0.095	**0.271**	**0.143**
	(±0.145)	(±0.152)	(±0.325)	(±0.558)	(±0.019)	(±0.166)	(±0.051)	(±0.076)	(±0.008)	**(±0.042)**	**(±0.030)**
Area B	0.020	0.108	0.431	0.415	0.015	**0.044**	**0.030**	0.052	0.002	0.028	**0.007**
	(±0.010)	(±0.054)	(±0.216)	(±0.207)	(±0.008)	**(±0.022)**	**(±0.015)**	(±0.026)	(±0.001)	(±0.014)	**(±0.003)**
Area C	0.009	0.138	0.466	0.295	**0.007**	0.037	0.031	0.044	0.001	**0.036**	**0.011**
	(±0.004)	(±0.069)	(±0.233)	(±0.148)	**(±0.003)**	(±0.018)	(±0.016)	(±0.022)	(±0.000)	**(±0.018)**	**(±0.005)**
Urban Industry (µg/g)											
Area A	0.380	0.881	**6.111**	3.007	0.087	3.035	0.152	0.217	0.040	0.284	0.074
	(±0.022)	(±0.083)	**(±0.656)**	(±0.279)	(±0.006)	(±0.273)	(±0.014)	(±0.020)	(±0.003)	(±0.013)	(±0.003)
Area B	0.410	0.287	5.366	2.432	**0.125**	0.593	0.247	0.193	**0.012**	0.245	**0.051**
	(±0.008)	(±0.022)	(±0.622)	(±0.196)	**(±0.019)**	(±0.051)	(±0.019)	(±0.018)	**(±0.003**)	(±0.028)	**(±0.006)**
Area C	0.433	0.405	4.921	6.919	**0.163**	1.549	0.320	0.160	**0.011**	**0.225**	0.149
	(±0.008)	(±0.037)	(±0.311)	(±0.576)	**(±0.022)**	(±0.178)	(±0.037)	(±0.019)	**(±0.002)**	**(±0.036)**	(±0.009)
Farmland Organic (µg/g)											
PVC covered	3.110	2.697	6.596	6.682	0.299	2.936	1.738	0.377	0.041	0.289	0.260
	(±0.260)	(±0.229)	(±0.630)	(±0.574)	(±0.026)	(±0.259)	(±0.106)	(±0.034)	(±0.003)	(±0.027)	(±0.018)
Open	1.821	2.011	5.221	5.894	0.230	1.863	0.803	0.431	**0.042**	**0.201**	**0.237**
	(±0.165)	(±0.175)	(±0.405)	(±0.627)	(±0.022)	(±0.173)	(±0.64)	(±0.041)	**(±0.006)**	**(±0.038)**	**(±0.060)**
Farmland Traditional (µg/g)											
Tillage	1.642	3.324	7.563	8.456	0.224	2.115	0.846	0.416	0.041	0.260	0.196
	(±0.550)	(±0.970)	(±1.990)	(±2.530)	(±0.077)	(±1.001)	(±0.317)	(±0.150)	(±0.019)	(±0.105)	(±0.073)
Pasture	2.147	3.156	4.718	6.319	0.208	1.805	0.596	0.262	**0.039**	**0.232**	**0.401**
	(±0.689)	(±1.387)	(±1.666)	(±2.617)	(±0.064)	(±0.730)	(±0.218)	(±0.107)	**(±0.030)**	**(±0.192)**	**(±0261)**

Values in Bold Show samples that were ≥5%RSD

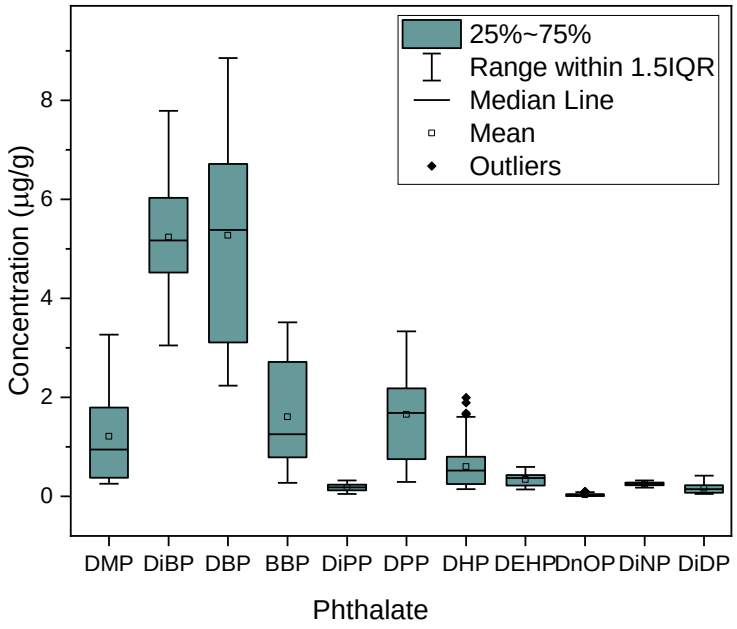


Figure 50: Phthalate concentrations in all soil samples (n=15, in triplicate)

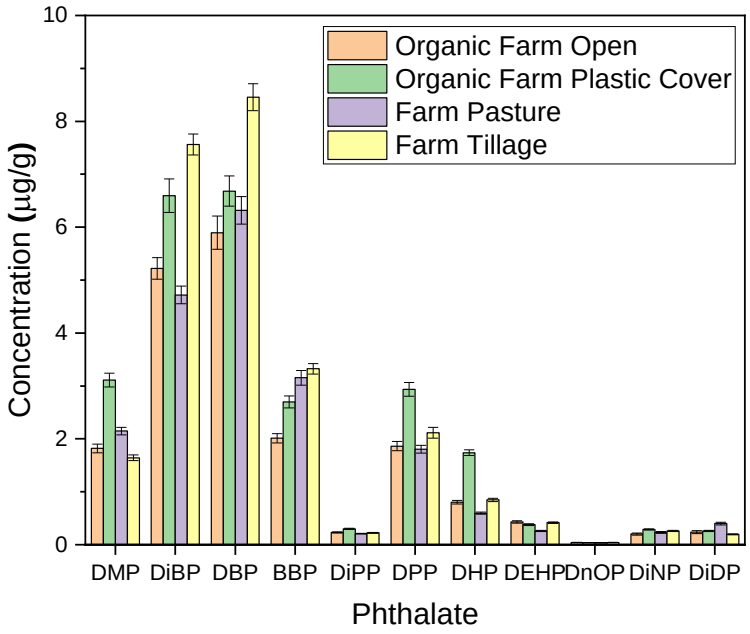


Figure 51: Phthalate concentrations in farmland soil

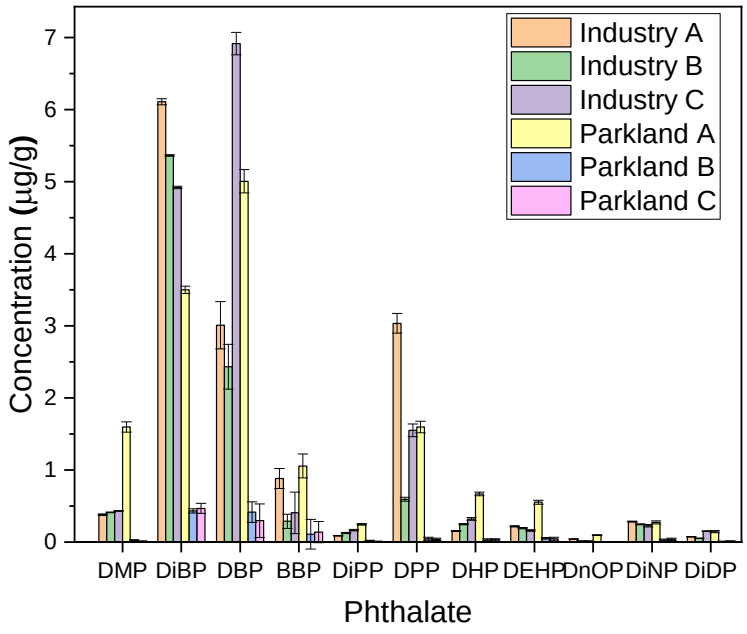


Figure 52: Phthalate concentrations in urban soils

Speciation of samples was determined through PCA, all concentrations were logged and no other adjustment of data was carried out. Dimension 1 accounted for 67.9% of all explained variance, and dimension 2 10.7%. DMP, BBP, DHP, DnOP and DiDP all contributed to over 10% of explained variance in dimension 1, whereas DPP, DnOP, DHP and BBP contributed most to dimension 2 (**Figure 53**). Based on this study, phthalates in soil tended to co-occur, with DiNP having the weakest correlation with other phthalates (***Figure 54***). Agricultural soils were significantly higher than urban soils. There was no intra-speciation of different urban and agricultural soils (**Figure 55**). This suggests that agricultural practices have an impact on phthalate concentrations. A degree of this speciation could also be attributed to the differences in the soil characteristics affecting how well the phthalates adsorb on site as both the farmland soils were pH 1 lower than the urban soils. This could be elucidated with further samples of varying pH from different site types.

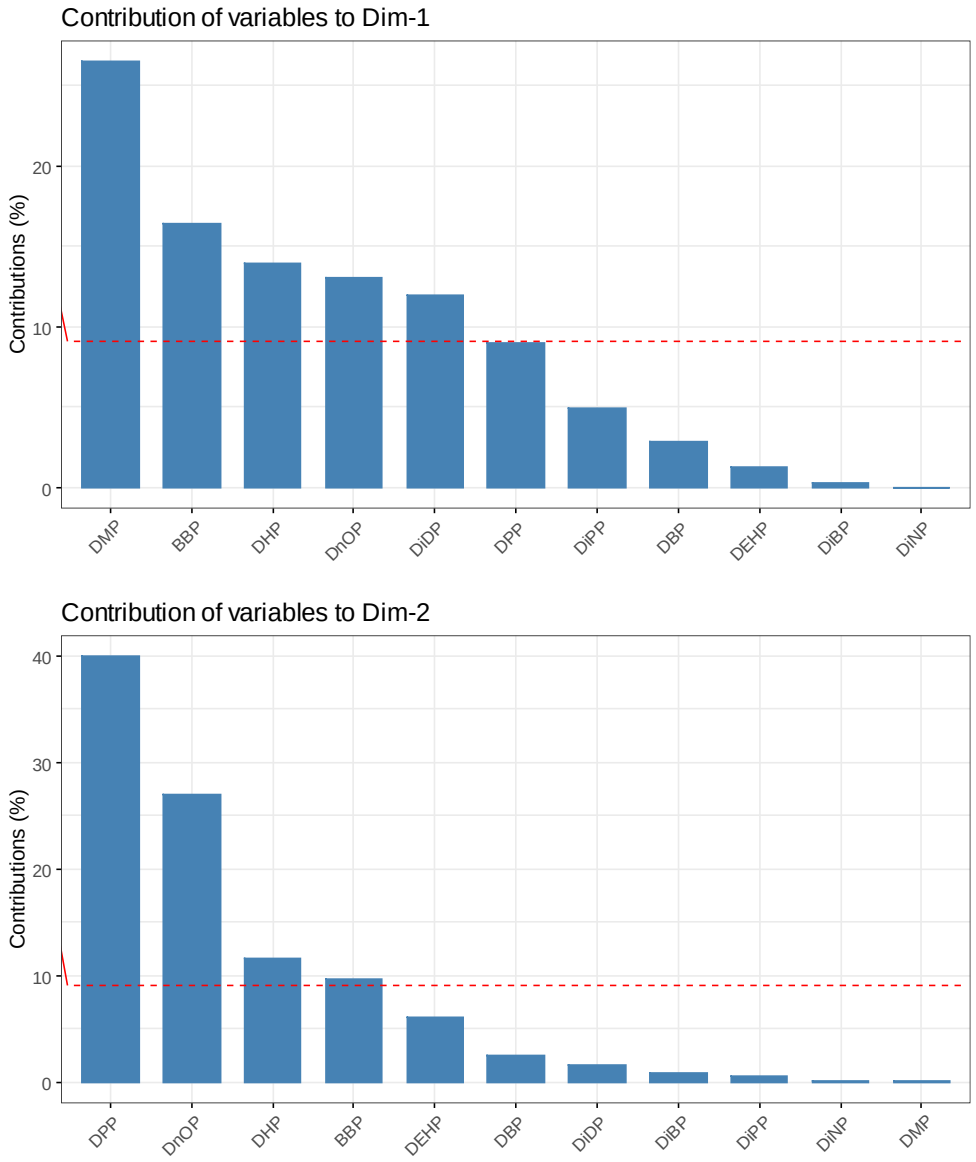


Figure 53: Phthalate contributions to variance in soils

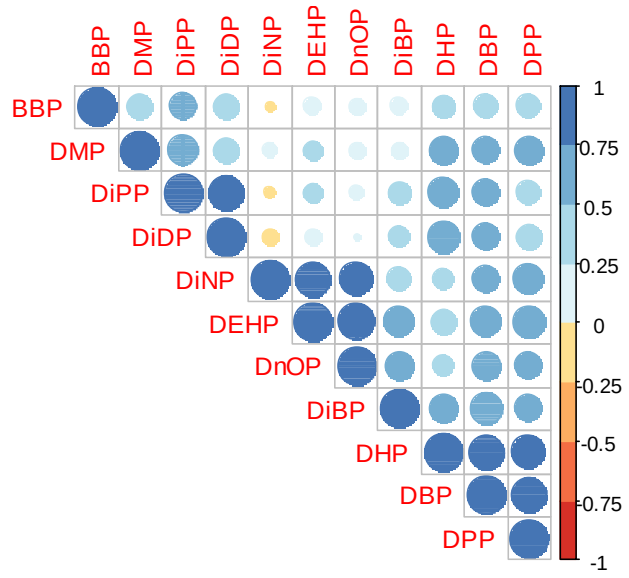
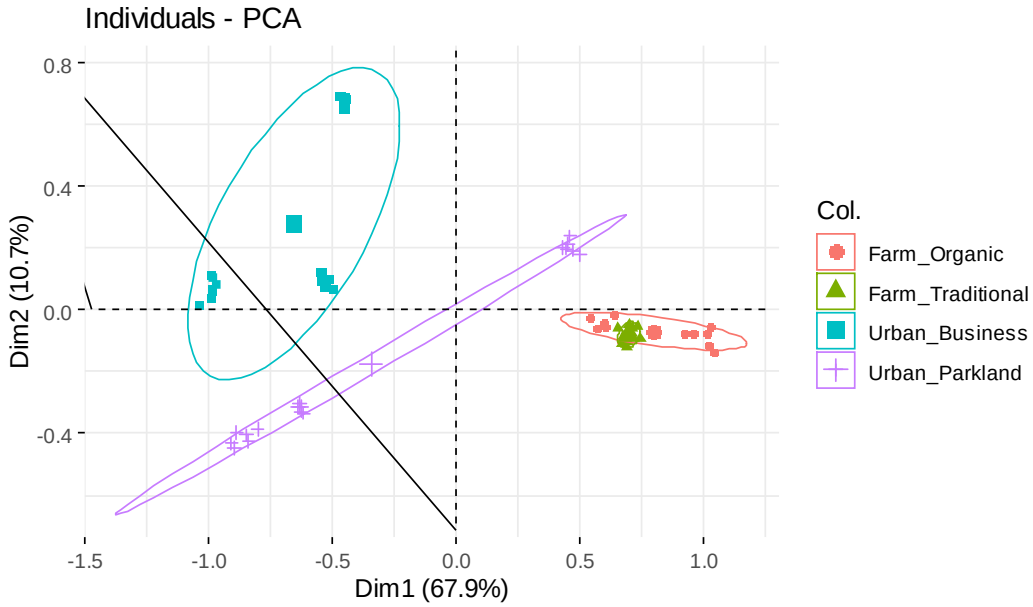


Figure 54: Corrplot of phthalates in soil



Linear Hypotheses:	Pr(>|t|)
Urban_Parkland - Urban_Business == 0	0.62080
Farm_Organic - Urban_Business == 0	< 0.001 ***
Farm_Traditional - Urban_Business == 0	< 0.001 ***
Farm_Organic - Urban_Parkland == 0	0.00109 **
Farm_Traditional - Urban_Parkland == 0	0.00330 **
Farm_Traditional - Farm_Organic == 0	0.98885

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Figure 55: Individual PCA of soil

Phthalate soil-burden was high compared to other countries (***Table 32***). Phthalate levels in soil in Ireland was relatively high, with only some soils from Russia and China having higher concentrations. This indicated that soil is a source of concern for phthalate contamination in Ireland.

Table 32: International comparison of phthalates in soil (mg/kgdw)

Locations	***DMP***	***DBP***	***BBP***	***DEHP***	***DnOP***	***Reference***
Denmark	–	0.0003–0.002	$1x10^{-5}$–0.003	0.01–0.04	0.0006–0.004	(Vikelsoe et al., 2002)
UK	0.0001-0.0001	0.008–0.008	0.0002–0.0008	0.02–0.076	0.012–0.014	(Gibson et al., 2005)
Netherlands*	–	0.006	–	0.318	–	(Peijnenburg and Struijs, 2006)
China	N.D.–0.014	0.002–0.15	N.D.	N.D.–0.43	N.D.	(Liao et al., 2010a)
China	0.0007–0.031	0.00015–2.58	–	0.0001–1.35	0.002–0.030	(Xia et al., 2011)
Spain	–	–	–	1–6.3	–	(Plaza-Bolanos et al., 2012)
China*	0.12	2.13	0.07	2.95	0.3	(Zhang et al., 2019)
China	N.D.-0.069	0.18–2.15	–	0.37–2.80	0.02–0.95	(Ma et al., 2019)
China	N.D.–0.20	N.D.–1.56	–	0.20–5.98	–	(Hu and Wen, 2003)
Ireland	**0.26–3.11**	**2.43–8.46**	**0.29–3.32**	**0.16–0.43**	**0.01–0.09**	****This Study**
China	0.002–0.10	0.007–0.285	N.D.–1.79	0.026–4.17	N.D.–9.78	(Kong et al., 2012)
China	0.63–0.68	2.53–3.96	N.D.–1.75	7.32–11.70	N.D.–3.46	(Lin et al., 2010)
Moscow, Russia	0.02–3.44	3.1–170.77	–	0.75–164.96	–	(Brodskiy et al., 2019)

*N.D-Not Detected, "–" denotes that the phthalate wasn't studied, *Denotes that the mean is reported, rather than the min-max reported for majority of studies.*

The toxicity of soil in this study was assessed by comparing the measured concentration to the PNEC. The PNEC of phthalates in soil have been estimated through published exposure-based studies. For example, a European risk assessment report reviewed the literature on DEHP, that indicated DEHP is not harmful to soil organisms or plants. There were some weaknesses in study design, however, with unrealistic exposure situations, or test concentrations above the solubility level. The European risk assessment report for DEHP only deemed the exposure situation relevant enough in four studies to derive a $PNEC_{soil}$. 130 mg/kg dwt (dry weight) was the lowest normalised NOEC from these studies, and an assessment factor of 10 was applied resulting in an estimated $PNEC_{soil}$ > 13 mg/kg dwt. REACH dossiers exist and have set limits for $PNEC_{soil}$ dry weight for DMP 3.16 mg/kg, DiBP at 0.023 mg/kg, DBP at 0.05 mg/kg, BBP at 1.57 mg/kg and DiNP 30 mg/kg (ECHA, 2020). All other phthalates in the study have no conclusive concentrations of no effect in soil.

The resulting ecological risk was highest for DiBP and DBP with all samples having a RQ of greater than one (**Table 33**). The $PNEC_{soil}$ for both of these phthalates was relatively lower than other phthalates due to the increased toxicity of these compounds. The farm samples were higher risk than urban samples due to the higher concentrations, the RQ of BBP was also greater than one for these sites. This illustrates that soil is a significant sink for phthalate in the Irish environment and could be posing a risk for soil organisms. If the PNEC of DEHP is applied to the other phthalates contained in this study, none pose an ecological risk.

Table 33: RQ for phthalate toxicity to soil organisms (±2SD)

	DMP	*BBP*	*DiBP*	*DBP*	*DEHP*	*DiNP*
Industrial Estate	0.12 (±0.007)	0.56 (±0.053)	265.70 (±28.51)	60.14 (±5.59)	0.02 (±0.002)	0.01 (±0.000)
Industrial Estate	0.13 (±0.002)	0.18 (±0.014)	233.29 (±27.02)	48.64 (±3.91)	0.01 (±0.001)	0.01 (±0.001)
Industrial Estate	0.14 (±0.002)	0.26 (±0.024)	213.94 (±13.53)	138.37 (±11.51)	0.01 (±0.001)	0.01 (±0.001)
Urban Park	0.51 (±0.046)	0.67 (±0.063)	152.22 (±14.13)	100.12 (±6.67)	0.04 (±0.004)	0.01 (±0.001)
Urban Park	0.11 (±0.003)	0.51 (±0.088)	145.99 (±20.25)	98.73 (±5.90)	0.03 (±0.003)	0.01 (±0.001)
Urban Park	0.08 (±0.035)	0.92 (±0.124)	218.55 (±17.30)	61.22 (±10.12)	0.03 (±0.002)	0.01 (±0.001)
Organic Farm	0.52 (±0.044)	2.12 (±0.177)	328.85 (±14.48)	169.12 (±10.47)	0.03 (±0.002)	0.01 (±0.001)
Organic Farm	0.68 (±0.052)	2.01 (±0.112)	205.13 (±17.60)	126.37 (±12.55)	0.02 (±0.003)	0.01 (±0.001)
Traditional Farm	0.58 (±0.082)	1.28 (±0.146)	226.98 (±27.38)	117.89 (±11.49)	0.03 (±0.003)	0.01 (±0.001)
Traditional Farm	0.98 (±0.075)	1.72 (±0.123)	286.77 (±24.01)	133.63 (±10.87)	0.03 (±0.001)	0.01 (±0.001)

The agricultural soil samples were higher than those at the urban site for all phthalates. This is most likely due to farming practices although differences in soil makeup could also have an impact on the degree of phthalate contamination in these areas. PVC covered soil was higher in phthalates than those in the open field at the organic farm, and tilled soil was higher in concentration than pasture at the traditional farm. However, neither of those differences were significant with a p value of 0.502 and 0.653 respectively. It is recommended that further soil monitoring for PAEs is carried out. Analysing the effect of land spreading would be of particular interest, in addition to investigating the rate of uptake in crops in Ireland as this would have a direct effect on the population.

3.4 Surface Water

Phthalate contamination can be introduced to fresh water through numerous routes. Effluent, surface runoff, industrial discharge, leachate, and atmospheric deposition are deemed to be the highest contributors (Wilkinson et al., 2017). Due to the hydrophobicity of phthalates, they tend to partition to sediments and suspended particulate matter. Sediments and soil therefore act as a source and sink of contamination in fresh waters through re-suspension of particulate matter (Fernández-González, Moscoso-Pérez, Muniategui-Lorenzo, López-Mahía, & Prada-Rodríguez, 2017). *Acinetobacter lwoffii* in river water causes complete degradation of DBP and 20% degradation of DEHP in 5 days. *Clostridium sp. NO9* and *Bacillus sp. NO11* in river sediments shows complete degradation of DEP and DBP in 28 days, with 99.2% and 91.7% DEHP degradation (Liang et al., 2008). Sediment concentrations were not assessed in this study.

DMP, DEP, DiBP, DMEP, DnBP, BBP, DEHP, and DnOP are among the most frequently detected in surface water. Rainfall is associated with the transfer of phthalates in the atmosphere to surface waters and soils, facilitating their accumulation in the wider environment with higher concentrations of phthalates found in short-term precipitation events (Fernández-Amado et al., 2017). This study aimed to investigate phthalate contamination at multiple points of a water source during both wet and dry periods to assess occurrences and concentration fluctuations.

Surface water grab samples were taken from four sites defined as: upstream, receiving waters (point of release from WWTP), downstream (25 km from WWTP, suburban area), and estuary (point where studied river meets sea). Samples from each of these sites were analysed from the months January, June, July, and November 2017. These months were chosen due to the high variation in precipitation. January and July were classed as dry months with and average rainfall of 0.2 and 0.5 mm/day respectively occurring over the 3 days leading up to sampling. High precipitation months were June and November were rainfall averaged 10.1, and 5.7 mm/day respectively over 3 days prior to sampling. Rainfall data was taken from a weather station at roughly equidistance (20 km) between the two furthest sites. It was expected that phthalates would be found in higher concentrations during the high precipitation months and at the wastewater effluent discharge site (receiving waters).

Phthalate concentrations at the receiving water site were higher than all other sites, as expected. HMW phthalates showed the highest increase at receiving water site (three-fold increase). Therefore, in Ireland wastewater output can contribute to phthalate contamination in surface waters, which was as expected. From the literature, phthalates levels are relatively high in wastewater and while removal rates are efficient, these phthalates have strong affinity to river sediments and can concentrate at discharge sites, continuously being re-suspended in particulate matter. Upstream concentrations had much lower concentrations for the HMW phthalates but still had detectable levels for all phthalates. Estuary samples were more saline and the only samples in the study to not have 100% detection frequency with two samples with DiNP concentrations below the LOD.

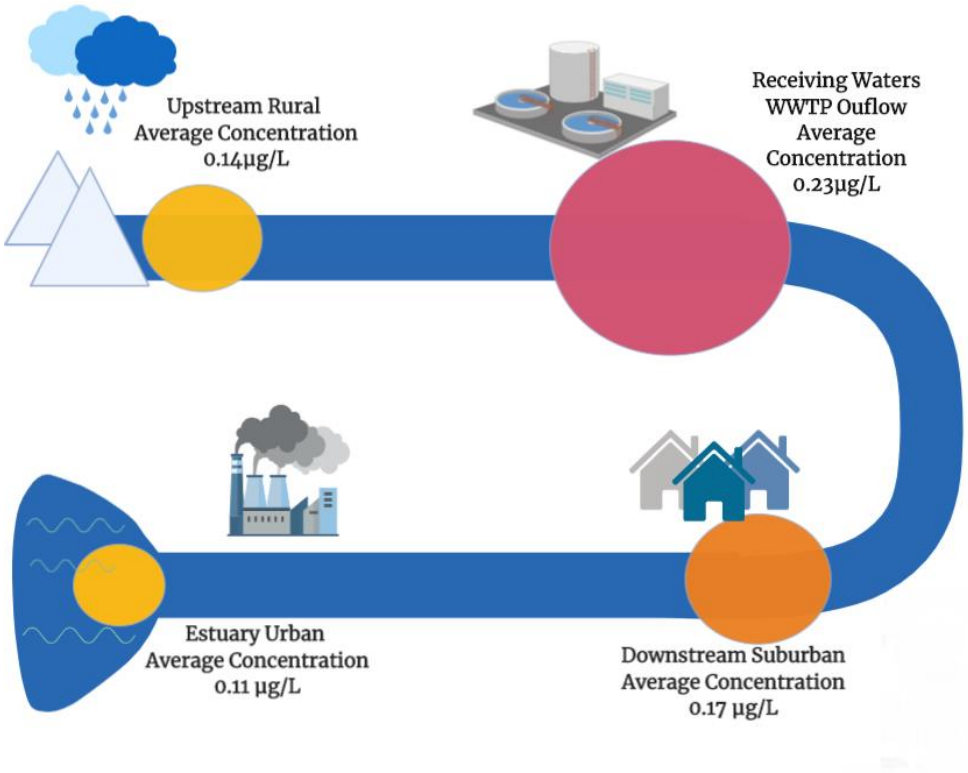


Figure 56: Infographic on phthalate surface water contamination in Ireland

The phthalates found in the highest concentrations were DMP, DiBP, DBP, DEHP and DnOP. There was temporal variation found at each site. This is most likely due to differences in precipitation and the amount of suspended solids in the sample (as samples were acidified, phthalates within suspended solids would enter the aqueous phase).

Table 34: Concentrations of phthalates in surface water (n=3, ±2SD) as an environmental fate

	DMP	BBP	DiBP	DBP	DiPP	DPP	DHP	DEHP	DnOP	DiNP	DiDP
Upstream (ng/L)											
January	117.35 (±1.20)	64.23 (±1.97)	252.75 (±2.89)	428.27 (±13.76)	12.82 (±0.30)	49.67 (±3.30)	10.84 (±0.80)	83.35 (±1.79)	3.86 (±0.05)	2.67 (±0.09)	49.83 (±2.21)
June	469.78 (±30.37)	63.59 (±5.96)	78.45 (±0.41)	772.12 (±16.07)	20.32 (±0.95)	34.27 (±1.61)	12.74 (±1.19)	31.28 (±2.95)	22.86 (±2.18)	1.61 (±0.06)	23.35 (±1.90)
July	87.04 (±3.72)	82.64 (±1.77)	809.14 (±16.07)	802.68 (±13.40)	16.03 (±0.45)	4.32 (±0.29)	5.99 (±0.18)	43.29 (±4.33)	31.27 (±2.54)	0.08 (±0.01)	24.16 (±2.14)
November	54.78 (±1.05)	44.53 (±2.09)	720.65 (±8.50)	725.01 (±6.87)	19.08 (±0.21)	6.71 (±0.11)	17.34 (±0.23)	58.35 (±2.67)	41.64 (±1.56)	1.70 (±0.03)	17.47 (±0.71)
WWTP Receiving Waters (ng/L)											
January	179.21 (±4.39)	137.58 (±2.48)	945.26 (±12.64)	941.70 (±12.85)	141.40 (±1.87)	27.27 (±0.64)	10.31 (±0.77)	178.96 (±6.09)	129.19 (±3.10)	1.01 (±0.05)	59.27 (±3.68)
June	330.52 (±12.30)	42.35 (±0.97)	853.48 (±16.31)	853.48 (±16.31)	122.52 (±1.29)	12.07 (±1.02)	12.05 (±1.09)	290.79 (±4.63)	210.65 (±5.92)	1.40 (±0.05)	89.78 (±3.28)
July	255.14 (±3.74)	104.86 (±3.27)	115.09 (±3.82)	1117.37 (±1.58)	137.16 (±3.00)	23.38 (±0.52)	16.38 (±0.45)	260.58 (±5.05)	191.56 (±3.38)	1.97 (±0.12)	71.86 (±4.95)
November	185.94 (±3.30)	73.91 (±1.63)	643.29 (±20.64)	645.27 (±7.80)	152.93 (±3.16)	14.22 (±0.28)	13.30 (±0.79)	321.62 (±3.92)	241.24 (±3.67)	1.43 (±0.04)	69.91 (±2.01)

	DMP	BBP	DiBP	DBP	DiPP	DPP	DHP	DEHP	DnOP	DiNP	DiDP
Downstream Suburban (ng/L)											
January	470.82 (±40.53)	69.96 (±4.48)	334.79 (±16.90)	346.70 (±83.30)	**20.09 (±2.57)**	**11.64 (±3.05)**	4.12 (±0.39)	545.35 (±31.43)	423.04 (±48.67)	30.51 (±2.53)	3.69 (±0.29)
June	118.82 (±9.71)	39.81 (±3.65)	509.49 (±34.50)	578.41 (±68.99)	1.09 (±0.22)	8.91 (±0.77)	6.46 (±0.63)	**300.75 (±38.40)**	**286.69 (±45.26)**	**19.64 (±3.13)**	**2.96 (±0.40)**
July	47.68 (±5.47)	30.35 (±2.36)	375.56 (±37.09)	349.58 (±13.41)	4.96 (±0.39)	5.07 (±0.44)	3.96 (±0.42)	250.85 (±16.78)	363.51 (±35.10)	20.64 (±2.05)	3.96 (±0.28)
November	30.19 (±2.30)	20.39 (±2.08)	183.52 (±6.84)	179.24 (±18.33)	0.50 (±0.04)	2.03 (±0.16)	0.91 (±0.09)	119.70 (±8.28)	149.63 (±6.79)	12.10 (±0.95)	1.01 (±0.11)
Estuary (ng/L)											
January	156.53 (±14.05)	18.61 (±1.32)	88.84 (±2.24)	88.84 (±2.24)	9.96 (±0.46)	0.25 (±0.005)	2.06 (±0.23)	1.20 (±0.05)	0.39 (±0.03)	N.D	5.65 (±0.16)
June	310.94 (±30.56)	94.24 (±6.75)	1200.53 (±19.73)	1197.31 (±13.98)	126.30 (±6.20)	13.72 (±0.18)	12.11 (±0.37)	401.46 (±4.82)	257.73 (±1.94)	4.91 (±0.24)	49.09 (±2.44)
July	70.13 (±6.40)	223.35 (±16.44)	56.80 (±2.39)	56.80 (±2.39)	9.17 (±0.44)	0.26 (±0.01)	0.92 (±0.04)	8.88 (±0.67)	7.67 (±0.35)	N.D	8.75 (±0.60)
November	31.23 (±2.23)	12.15 (±0.79)	62.02 (±2.00)	62.02 (±2.00)	10.53 (±0.40)	0.21 (±0.00)	1.22 (±0.06)	29.83 (±3.18)	19.23 (±1.76)	0.12 (±0.02)	15.11 (±0.49)

N.D "Not Detected"
Values in bold reflect samples that were ≥5%RSD, none were above 10% RSD

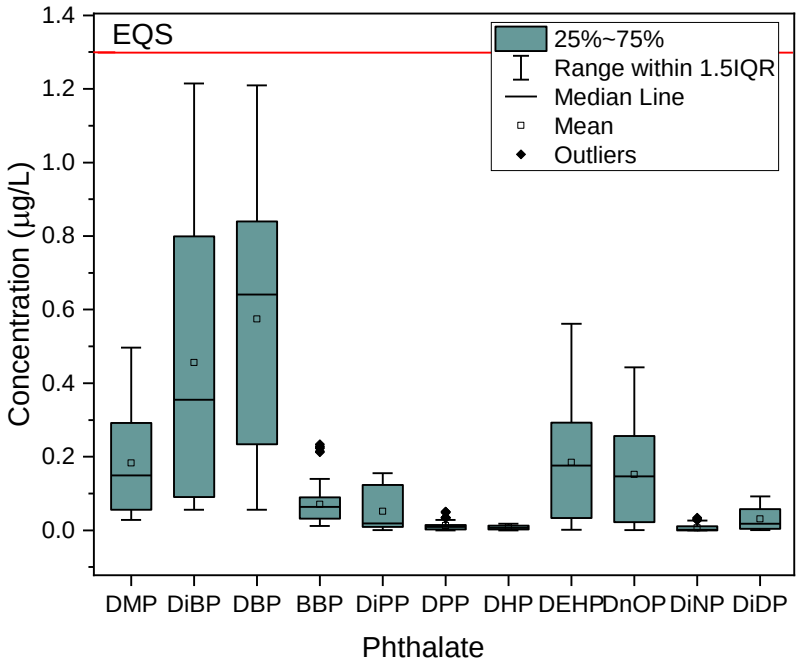


Figure 57: Phthalate concentrations in all surface water samples (n=16, in triplicate)

From the literature increased rainfall is associated with greater phthalate concentrations in surfaces waters possibly owing to runoff increasing the amount of suspended solids within the river. There was some temporal variability in the surface water concentrations from this study. However, only estuary samples showed a large increase in phthalate concentration in June which was during a very high rainfall event. This study does not have enough information on particulate concentrations, flow rates and other river characteristics to infer causation for temporal variability. Only proximity to WWTP and general population demographics of the area are known.

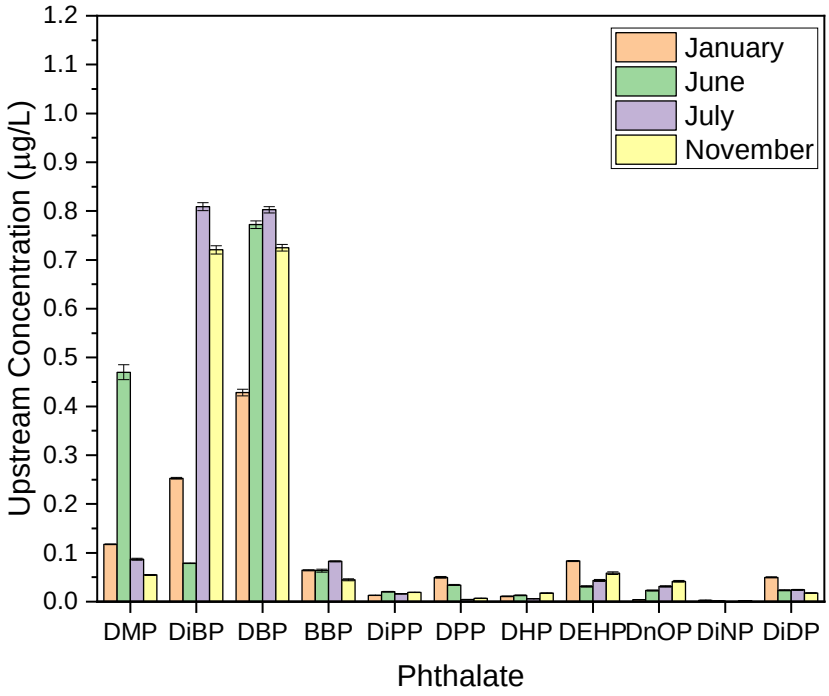


Figure 58: Phthalate concentrations in upstream surface water

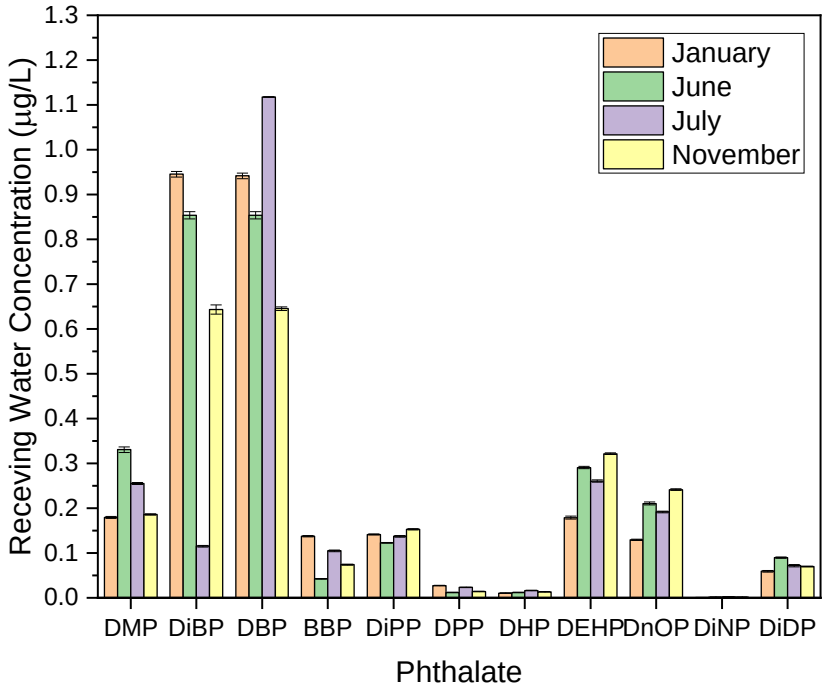


Figure 59: Phthalate concentrations in receiving surface water

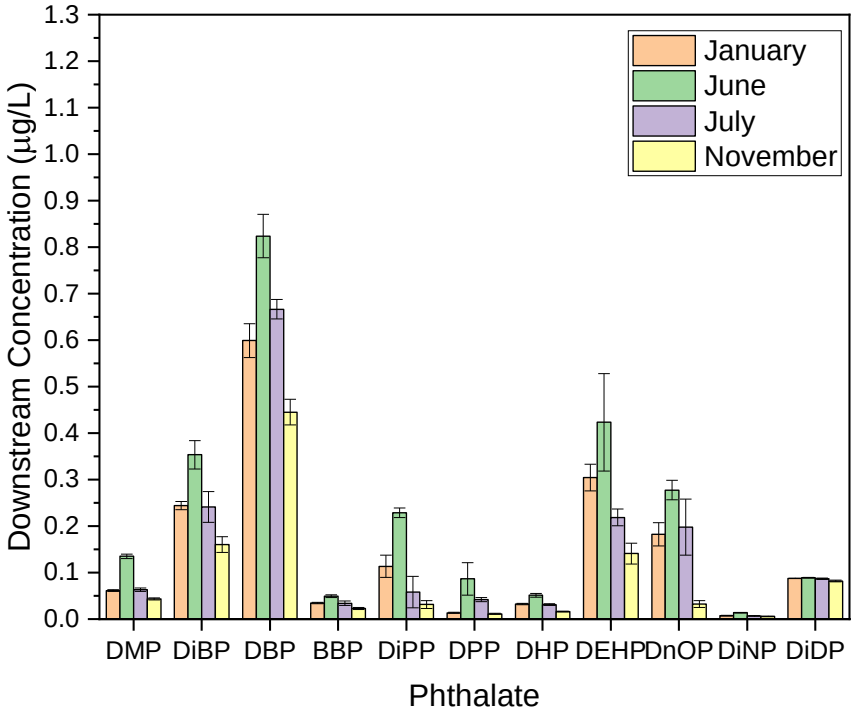


Figure 60: Phthalate concentrations in downstream suburban surface water

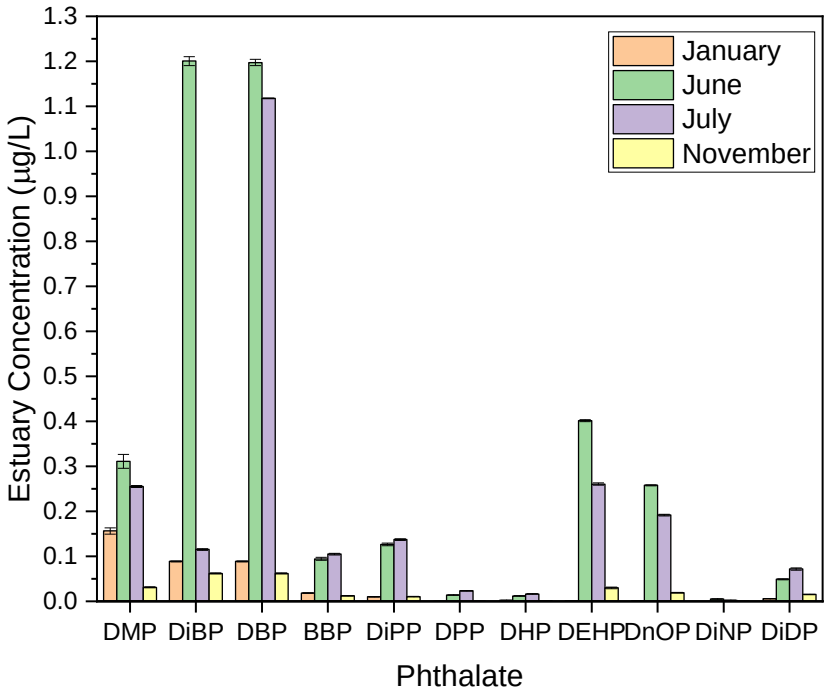


Figure 61: Phthalate concentrations in estuary surface water

PCA was conducted to speciate the surface water samples. PC1 and PC2 accounted for 66.5% and 17.1% of explained variance respectively. For dimension one DBP and DiBP contributed to over 10% of the variance and while DEHP, DMP and DBP contributed to variance in dimension 2 (***Figure 62***).

There was a significant difference between receiving waters and all other samples. Estuary and downstream samples were also significantly different. Receiving water (RW) samples showed the lowest amount of variation between months as all these samples formed a cluster, although estuary samples (E) from June clustered with them due to the spike in concentration for that month. June Samples had a higher rate of variation and cluster on their own, separately for Downstream (DS) and Upstream (US) and with another site for estuary as previously mentioned. As previously mentioned June was noted as a particularly high rainfall event and this indicates that high rainfall could possibly increase the rate of phthalate concentrations in surface waters. It was expected that estuary samples would show a high degree of distinction from others they are saline and the suspended solids profiles are very different. However, while estuary samples from January and November clustered they were similar to upstream samples, potentially due to dilution effects at the estuary. The individual PCA show greater separation of the sample sites but the partitioning clustering shows some overlap between sites.

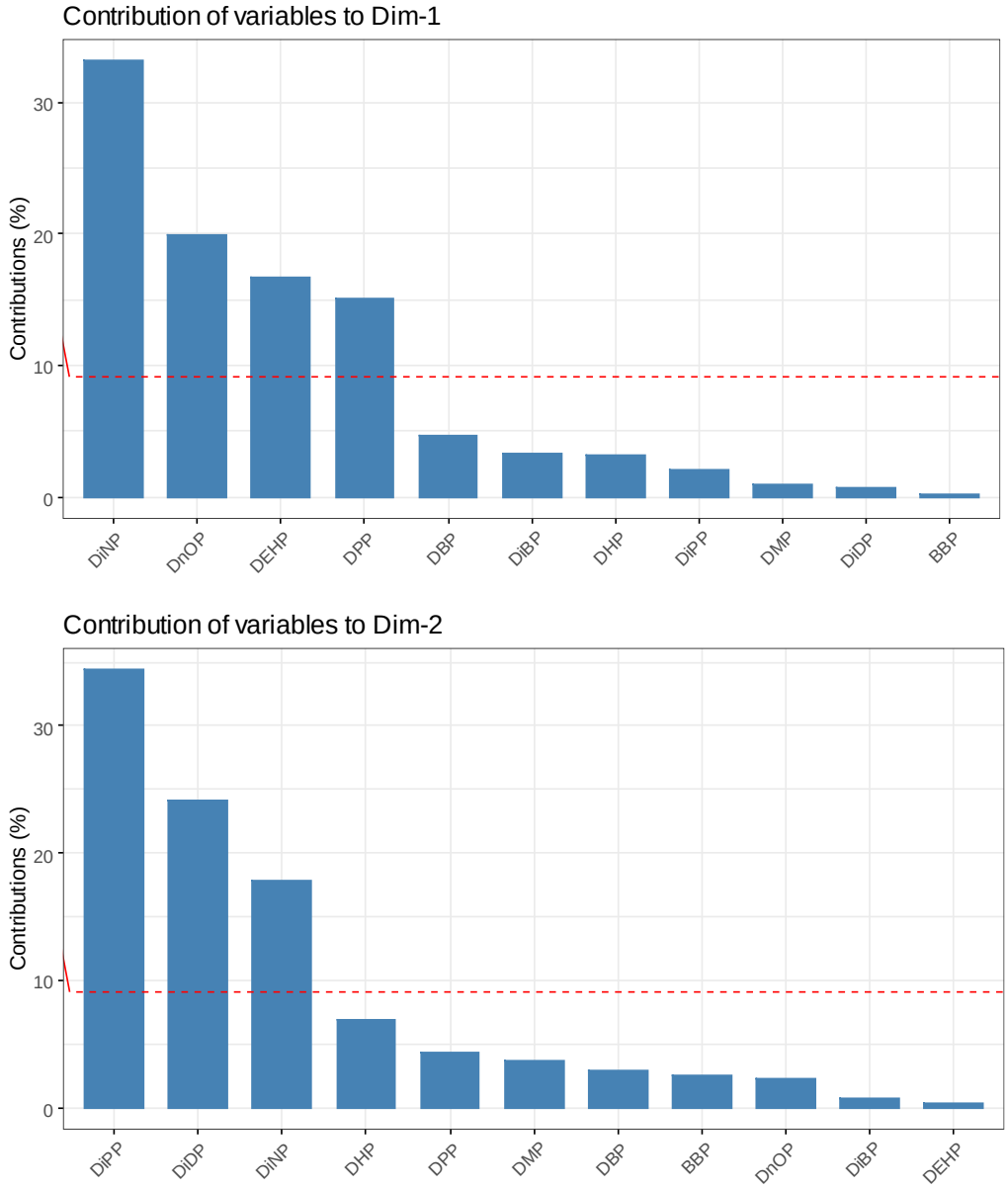


Figure 62: Phthalate contributions to variance between surface water samples

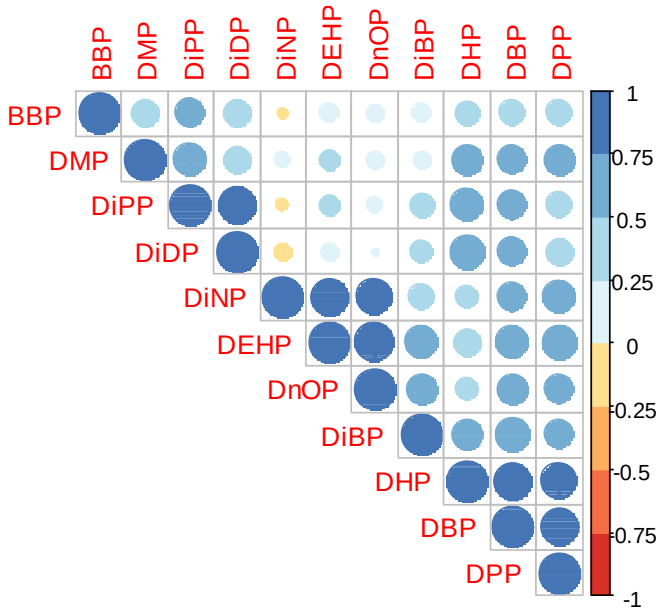
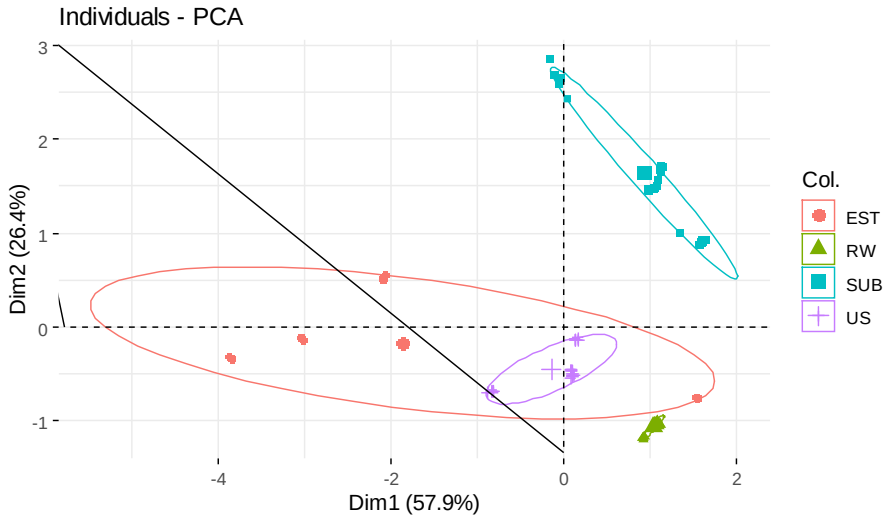


Figure 63: Corrplot of phthalates in surface water



Linear Hypotheses:	Pr(>|t|)
RW - US == 0	3.83e-05 ***
SUB - US == 0	0.999
EST - US == 0	< 1e-05 ***
SUB - RW == 0	1.18e-05 ***
EST - RW == 0	< 1e-05 ***
EST - SUB == 0	< 1e-05 ***

Figure 64: Individual PCA of phthalate concentrations in surface waters

Table 35 is adapted and updated from a previously published review (Gao and Wen, 2016). It was found that overall phthalate concentrations in surface waters were relatively low.

Samples from France, Belgium and South Africa were the highest, although other studies from France showed lower concentrations than Ireland.

The environmental quality standard (EQS) for DEHP in surface waters is set as 1.3 μg L^{-1}. All surface water samples remained well below this limit for DEHP. However, DBP and DiBP were found closer to this limit and the toxicity of these compounds appear to be higher. The PNEC of phthalates in freshwater and marine waters were taken from their respective ECHA registration dossiers (ECHA, 2020). The PNEC in freshwater for DMP, BBP, DiBP, and DBP respectively were 192, 7.5, 1, and 10 μg/L respectively. The PNECs for marine water were ten times lower than freshwater due to a higher assessment factor for this environment.

The ecological risk was highest at the receiving water site and the estuary site (**Table 36**). June is a significant spike for phthalate concentration, there was a sludge release into the bay at this time so this could have contributed to the relatively high concentrations. No data exists for PNEC of DEHP and DiNP even though values for these phthalates have been derived for soil. If all RQ values are summed, then estuary samples from January and June are deemed at risk in addition to January receiving water samples and November downstream samples. It is thought that the insolubility of HMW phthalates in the aquatic environment would decrease their toxic effects in the aquatic environment. Studies have been unable to derive a PNEC for DEHP and structural-activity relationship studies have suggested that these HMW phthalates would not pose a risk to aquatic life (Parkerton and Konkel, 2000). If the PNEC of DiBP is applied to the phthalates that have not been investigated for PNEC (DiPP, DPP, and DHP) then individually they do not pose a risk, however the cocktail effect of these chemicals is still relatively unknown.

Table 35: International comparison of phthalates in surface waters

Locations	*DMP*	*BBP*	*DBP*	*DEHP*	*DnOP*	*Reference*
Canada	0.002–0.005	0.002–0.006	0.05–0.244	0.17–0.44	0.005–0.04	(Mackintosh et al., 2006)
India	0.02	0.04	0.25	0.51	0.03	(Selvaraj et al., 2015)
France	0.03–0.18	N.D.	0.07–0.32	0.16–0.31	N.D.	(Dargnat et al., 2009a)
France	0.01–0.10	0.007–0.04	0.21–0.53	0.32–0.78	0.003–0.02	(Teil et al., 2007)
Iran	0.22-0.46	ND-0.16	0.07-0.25	0.17-0.87	ND-0.05	(Abtahi et al., 2019)
Spain	N.D.	N.D.	0.25–1.76	N.D.	–	(Dominguez-Morueco et al., 2014)
Ireland	**0.03-0.47**	**0.04-0.11**	**0.42-1.20**	**0.001-0.4**	**0.0004–0.26**	****This Study**
Spain	–	–	–	1.67	–	(López-Roldán et al., 2004)
Netherlands	–	–	0.14–1.80	0.08–2.35	–	(Peijnenburg and Struijs, 2006)
Japan	N.D.–0.09	N.D.–0.06	N.D.–0.54	N.D.–3.09	N.D.	(Suzuki et al., 2001)
Spain	–	N.D.–1.30	N.D.–1.30	N.D.–3.09	–	(Pina et al., 2005)
South Africa	0.04–0.56	–	0.79–3.65	0.49–5.58	–	(Sibali et al., 2013)
China	N.D.–0.04	N.D.	0.05–4.50	0.13–6.57	N.D.–0.45	(Liu et al., 2013)
China	0.98–4.12	N.D.–4.39	1.69–11.80	2.26–11.6	0.69–6.14	(Gao et al., 2014)
Korea	0.04–15.10	0.07	–	–	0.01–2.07	(Cho et al., 2014)
Taiwan	N.D.	N.D.	1.00–13.50	N.D.–18.50	N.D.	(Yuan et al., 2002)
France	0.02–0.25	N.D.	0.22–3.86	5.16–20.80	N.D.	(Net et al., 2014)
France and Belgium	1.3–5.6	0.4 – 34.7	N.D.–21.1	0.8–125.5	0.9– 3.3	(Net et al., 2015a)
South Africa	0.03–31.70	–	2.80–122	0.06–197	–	(Fatoki and Noma, 2002)
USA	∑ DEHP + DiBP + DBP = 0.40–34.80					(Liu et al., 2013)

*N.D-Not Detected, "–" denotes that phthalate wasn't studied, *Denotes that the mean is reported, rather than the min-max reported for majority of studies.*

Table 36: RQ for phthalate toxicity to freshwater and marine organisms

Site	*Month*	*DMP*	*BBP*	*DiBP*	*DBP*
Upstream	January	0.001	0.009	0.253	0.043
	June	0.002	0.008	0.078	0.077
	July	0.000	0.011	0.809	0.080
	November	0.000	0.006	0.721	0.073
Receiving Waters	January	0.001	0.018	0.945	0.094
	June	0.002	0.006	0.853	0.085
	July	0.001	0.014	0.115	0.112
	November	0.001	0.010	0.643	0.065
Downstream Suburban	January	0.002	0.009	0.335	0.035
	June	0.001	0.005	0.509	0.058
	July	0.000	0.004	0.376	0.035
	November	0.000	0.003	1.835	0.018
Estuary	January	0.008	0.025	0.888	0.089
	June	0.016	0.126	12.005	1.197
	July	0.004	0.298	0.568	0.057
	November	0.002	0.016	0.620	0.062

3.5 Conclusion

Phthalates occurred in all environmental samples within this study confirming that there is phthalate burden in Ireland.

There has been minimal research into the environmental and human burden of phthalates in Ireland. Environmental sources of phthalates found that wastewater sludge contained the highest concentration of phthalates ranging from 0.20 to 315.21 mg/kg dry weight. The primary method for sludge disposal in Ireland is land application of biosolids, so this could be a source of phthalates to soil in these regions. Wastewater treatment plant (WWTP) effluent contained low levels of phthalates, from 0.002 to 1.52 µg/L; for context the Environmental Quality Standard (EQS) set for DEHP in surface waters is 1.3 µg/L. The removal of phthalates from influent was efficient (however, phthalates are retained in sludge). Influent WWTP levels showed that phthalates are prevalent in Irish households and industry with down-the-drain disposal accounting for concentrations of 0.01 to 95 µg/L. Landfill remains one of the most common means of disposal of municipal waste in Ireland, though the introduction of incineration may have already helped to mitigate some phthalate contamination. Concentrations found in the leachate samples from a closed landfill ranged from 0.01 to 15.16 mg/kg, this was shown to be consistent with European literature but higher than some Scandinavian countries, possibly due to their earlier introduction of incineration practices. This was the only matrix in which DiDP had the highest concentration in comparison to other phthalates. As the landfill site has been shut down for some time this could indicate a temporal change in phthalate use, or that DiDP has a longer residence time in landfill owing to its high $\log K_{ow.}$ Composite samples of recyclable, general and food/garden household wastes were also examined. Recyclable wastes had the highest levels of phthalate, as expected, with concentrations ranging from 0.25 to 136.36 mg/kg. In all waste samples DiBP was found at the highest concentration, and DnOP the lowest. Further monitoring of phthalates in recyclable materials should be carried out to assess whether restricted phthalates are accumulating in plastics during the recycling process.

The aquatic environment displayed low level contamination in comparison with other European countries. Phthalate concentrations at the WWTP discharge site were significantly higher than other surface water sites, suggesting that wastewater treatment plant effluent plays a role in phthalate contamination at this site. Phthalates in soil were

found in higher concentrations than phthalates in surface waters (as expected due to hydrophobicity). Agricultural soil concentrations were significantly higher than urban samples in this study and had higher concentrations than other agricultural soils within Europe. Phthalate concentrations in agricultural soils ranged from 0.03-8.45 µg/g, while urban soils ranged from 0.01-5.02 µg/g. Similarly, to other matrices, DiBP and DBP contribute to the highest degree of contamination. This data suggests that despite legislation they remain prevalent in the environment. While most environmental samples are consistent with published European data, and meet environmental quality standards, further work should be undertaken to examine phthalate levels in soils with an emphasis on agricultural impact.

4 Human Exposure to Phthalates in Ireland

This project proposed Wastewater-Based Epidemiology (WBE) as an alternative to traditional biomonitoring. Concentrations of six phthalate monoester metabolites were monitored in wastewater influent. These phthalate monoesters were: monoisobutyl phthalate (biomarker of diisobutyl phthalate), monobutyl phthalate (biomarker of dibutyl phthalate), monobenzyl phthalate (biomarker of benzylbutyl phthalate), monoethylhexyl phthalate (biomarker of diethylhexyl phthalate), monooctyl phthalate (biomarker of dioctyl phthalate), and monoisononyl phthalate (biomarker of diisononyl phthalate). Concentrations of monoesters in wastewater ranged from (1.10 to 140.13) ng/L. All metabolite levels were converted into population averaged exposure levels for the respective parent phthalates. The feasibility of wastewater based epidemiology for the prioritisation of compounds of emerging concern is shown to be adequate for community assessment and the prioritization of emerging contaminants, although some aspects of the methods are prone to international variations.

4.1 Monoester Metabolite Levels in Ireland

The concentrations of phthalate monoester metabolites were measured in wastewater influent. Five months of grab samples were analysed at three different WWTPs. Each WWTP represented a rural, suburban or urban population and are labelled as such throughout. Grab samples were used due to limited amount of composite sample available, this could contribute slightly to monthly variations.

Concentrations of phthalate monoester metabolites were found ranging from 0.96 to 49.48 ng/L in wastewater influent (**Table 36**). All sample calibration curves were linear and all triplicate un-spiked samples had a sample variation below 5 %RSD, which indicates the precision of analysis. There is temporal and spatial variation for phthalate monoester metabolites in Ireland (**Figure 65-Figure 67**). Metabolite levels were highest at the suburban site, and all sites were significantly different from each other as confirmed by 2-way ANOVA. Although population levels and behaviour at each site are likely to fluctuate, temporal variation could be partly attributable to different degradation kinetics in transit to the wastewater treatment plants, or variations in residence times before sampling, allowing degradation of phthalate diester to the monoester metabolite in-sewer.

Concentrations at the suburban site were higher than at the suburban site which was not expected. This could be due to agricultural runoff contributing phthalate monoesters from animals or from degraded phthalate parent compounds in soils. There was a high level of hard surface runoff in this suburban area sampled which could indicate that concentrations from these types of area could over-estimate human exposure.

At present, there is no reference value for "background" phthalate exposure in the general population. Based on DEHP metabolite concentrations in controls of occupational studies, levels greater than 12 µg/L MEHP are considered to exceed background levels, and can therefore be used to investigate possible occupational exposures or excessive use of DEHP containing consumer products. No outlier values are found in this study as it is of the general population served by the WWTP.

Table 37: Concentrations of phthalate monoester metabolites in WWTP influent (ng/L)

Site	*Month*	*MiBP*	*MBP*	*MBzP*	*MEHP*	*MnOP*	*MiNP*	*Total*
Rural	**September**	**7.79**	**7.53**	**4.55**	**30.37**	**1.35**	**3.03**	54.61
	%RSD	2.55	0.84	1.87	2.22	1.15	3.59	
	November	**11.44**	**12.47**	**1.36**	**14.06**	**1.55**	**3.5**	44.37
	%RSD	4.11	2.25	1.86	4.23	2.35	2.67	
	December	**2.19**	**5**	**1.1**	**17.96**	**3.42**	**13.23**	42.91
	%RSD	2.76	1.4	2.1	1.39	4.46	4.04	
	January	**2.48**	**1.75**	**6.67**	**9.66**	**3.65**	**1.87**	26.08
	%RSD	1.11	2.16	3.88	2.9	1.1	1.12	
	February	**7.9**	**1.12**	**3.16**	**7.45**	**0.96**	**2.36**	22.96
	%RSD	0.5	9.28	3.28	2.67	2.26	2.11	
Suburban	**September**	**6.41**	**15.79**	**5.68**	**12.29**	**1.46**	**2.13**	43.78
	%RSD	1.1	1.01	3.49	2.76	1.5	2.17	
	November	**49.48**	**140.13**	**5.43**	**19.98**	**4.12**	**14.18**	233.33
	%RSD	4.04	1.44	4.7	4.23	4.73	5	
	December	**7.78**	**8.87**	**2.59**	**18.97**	**4.14**	**42.75**	85.1
	%RSD	0.81	1.93	2.32	1.81	2.97	4.32	
	January	**11.78**	**8.72**	**3.5**	**8.83**	**5.47**	**5.86**	44.16
	%RSD	2.22	1.76	3.03	1.22	2.6	2.22	
	February	**30.33**	**68.98**	**34.04**	**68.81**	**24.94**	**40.74**	267.84
	%RSD	3.61	4.03	4.95	1.7	4.08	3.23	
Urban	**September**	**9.51**	**27.99**	**12.95**	**42.31**	**5.72**	**5.6**	104.07
	%RSD	1.1	1.01	3.49	2.76	1.5	2.17	
	November	**3.88**	**14.1**	**4.85**	**5.46**	**2.98**	**3.69**	34.95
	%RSD	2.26	4.3	3.23	4.33	2.21	3.89	
	December	**7.91**	**6.25**	**2.22**	**39.27**	**19.6**	**58.28**	133.54
	%RSD	1.81	2.58	2.4	1.36	1.77	1.23	
	January	**16.42**	**14.58**	**7.45**	**30.5**	**20.69**	**15.48**	105.11
	%RSD	2.43	2.95	3.74	2.54	0.88	2.87	
	February	**42.34**	**36.48**	**5.09**	**29.28**	**6.66**	**6.21**	126.05
	%RSD	4.9	5.03	4.17	3.12	2.4	2.25	

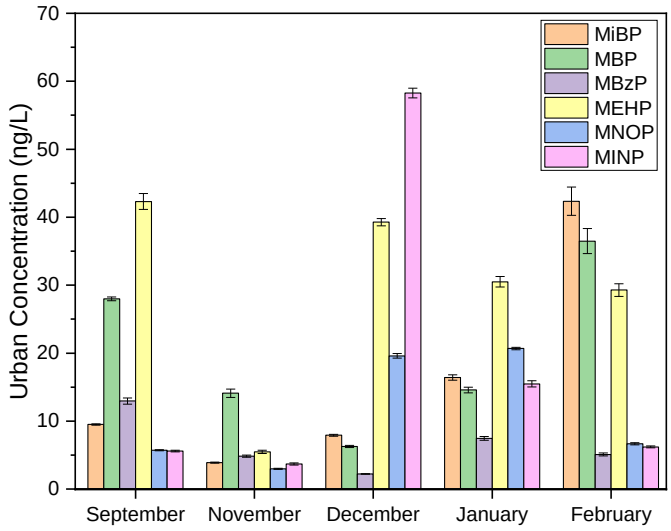


Figure 65: Monoester concentrations at urban site

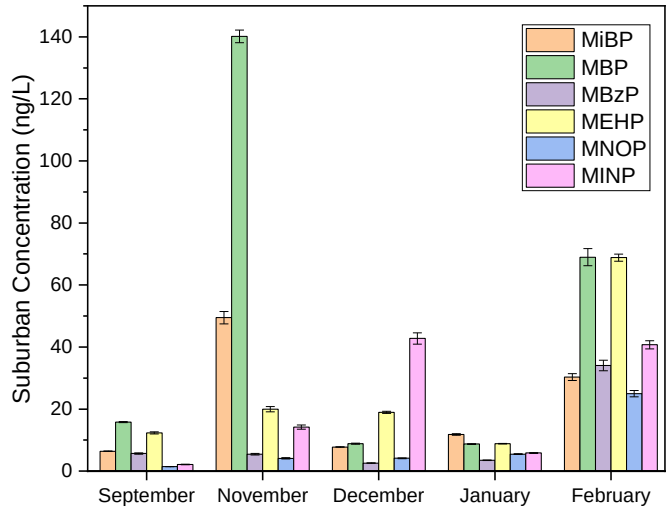


Figure 66: Monoester concentrations at suburban site

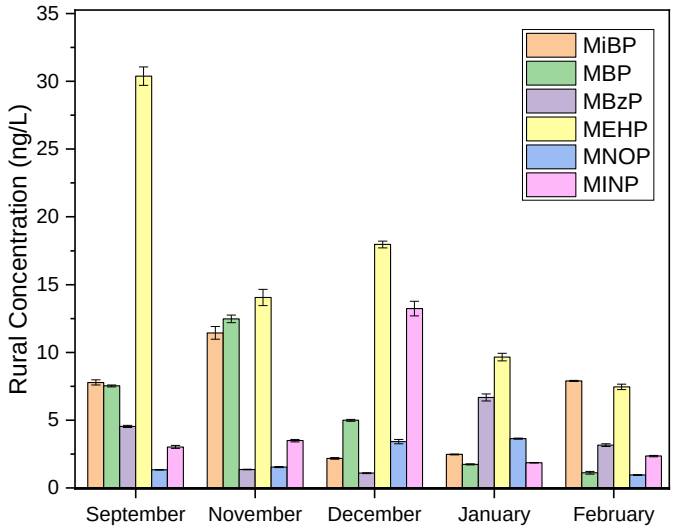


Figure 67: Monoester concentrations at rural site

Exposures to phthalate diesters were estimated from the monoester metabolite concentrations in influent using flow rates, population served and correction factors for phthalate metabolism. Levels were found to be between 0.32 to 277.25 µg/inhabitant/day (**Table 38**). All exposure levels were below the safe reference value (EFSA, 2005). From this study, on average, the phthalate that contributes the highest degree of exposure to humans is DEHP, followed by DiNP and DnOP. The LMW phthalates combine to form roughly 18% of total phthalate exposure. An analysis of NHANES biomonitoring data from 2001-2010 noted a decrease in DnBP, BBzP, and DEHP exposures while an increase in DiNP and DiBP was observed. This indicates a temporal trend, possibly associated with changes in legislation and consumer behaviour (Zota et al., 2014). This could explain the increased exposures to DnOP and DiNP, but as DEHP is also high, some of these HMW metabolites might be formed from their parent compounds prior to treatment.

Table 38: Exposure to phthalate diesters in µg/inhabitant/day as estimated from the wastewater influent metabolite concentration

Site	*Month*	*DIBP*	*DBP*	*BBP*	*DEHP*	*DNOP*	*DINP*	*Total*
Rural	September	1.25	1.21	0.68	22.84	1.01	2.28	29.27
	November	5.95	6.64	0.68	34.91	3.84	8.69	60.71
	December	0.67	1.57	0.32	26.30	5.01	19.38	53.25
	January	0.46	0.33	1.18	8.50	3.22	1.65	15.34
	February	1.28	0.19	0.49	5.75	0.74	1.82	10.27
Suburban	September	2.87	7.24	2.43	26.29	3.13	4.56	46.52
	November	29.25	84.74	3.07	56.39	11.63	40.03	225.11
	December	9.35	10.91	2.97	108.92	23.77	245.42	401.34
	January	6.31	4.77	1.79	22.57	31.42	33.66	100.52
	February	16.37	38.07	17.54	177.25	64.24	104.95	418.42
Urban	September	2.31	6.94	3.00	48.98	6.62	6.48	74.33
	November	1.58	5.89	1.89	10.64	5.80	7.19	32.99
	December	3.68	4.29	0.67	59.09	29.50	87.69	184.92
	January	4.82	4.37	2.09	42.72	28.97	21.68	104.65
	February	9.53	8.39	1.09	31.44	7.15	6.67	64.27

Speciation of overall phthalate exposure at each site was carried out with PCA of logged data. This compared Dim1 (76.1% explained variance) and Dim2 (12.1% explained variance). DiNP and DEHP contributed most to variance. It was found that exposure at all three sites was highly statistically significant, this represented both a temporal and spatial variation as illustrated by the dispersion of each site on the PCA. This confirms

that exposure to phthalates can vary between populations, temporal trends most likely indicate population fluctuations or instability of metabolite in-transit to the WWTP.

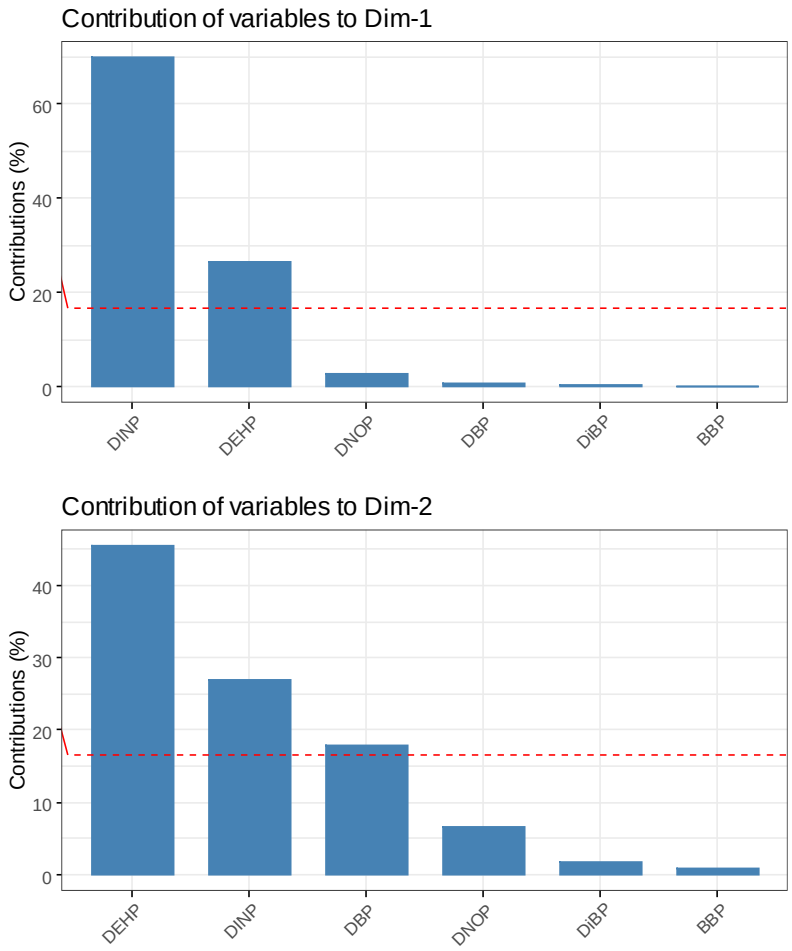


Figure 68: Contributions of phthalate exposure to variance

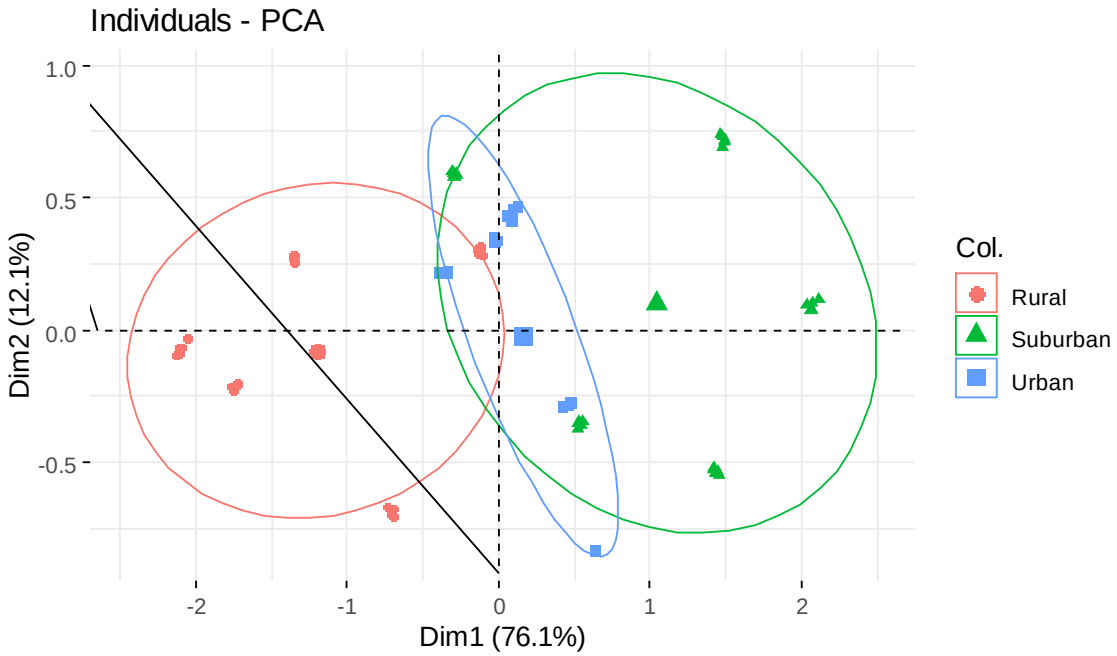


Figure 69: Individual PCA of phthalate exposure

```
Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
Rural - Suburban == 0 -0.90211  0.06616 -13.635 < 1e-07 ***
Urban - Suburban == 0 -0.35980  0.06616 -5.438 2.36e-07 ***
Urban - Rural == 0   0.54231  0.06616  8.197 < 1e-07 ***
---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

4.2 Risk Assessment

To carry out an assessment of the risk of phthalate exposure in Ireland, the results were compared to other studies conducted in this area. The hazard index was calculated for this study and compared to calculated and reported hazard indices from other biomonitoring studies. A literature review of odds ratio data was used to investigate which endpoints would be most likely from phthalate exposure.

One small scale biomonitoring study was conducted in Ireland that monitored metabolites of DEP, DiBP, DBP, BBP and DEHP, in mother-infant pairs (Cullen et al., 2017). All concentrations were calculated in µg/g creatinine; exposure was not reported in the paper but was calculated using the smoothed creatinine model shown in Section 2.2.6. The weight of the subjects was not contained in the text so the mean weight from NHANES was used to calculate risk. The urinary concentration from mothers (geometric mean, n=120) was compared to the mean measured exposure from this study. As the research conducted by Cullen et al. was carried out in Ireland with samples taken within two years of this study, it is considered the most relevant vector for accuracy of this WBE study.

The National Health and Nutrition Examination Survey (NHANES) is a program of studies designed to assess the health and nutritional status of adults and children in the United States (NHANES, 2018). The survey is unique in that it combines interviews and physical examinations, providing a wealth of data on each sample collected. As this is a large scale biomonitoring project it will form a basis of total population risk assessment that can be compared to the wastewater data collected in Ireland. NHANES has monitored phthalates since 1999, with the most recent data available coming from 2013-2014. The most recent phthalate data collected was used for comparison to the wastewater epidemiology study due to change in phthalate trends over time. The urinary concentrations were reported in µg/L and µg/g creatinine; the exposure was calculated using the smoothed creatinine so that it would be comparable to the Irish biomonitoring study.

There are two existing studies that follow phthalate metabolites in wastewater influent (Gonzalez-Marino et al., 2017; Du et al., 2018). These two studies used very similar analytical methods and the same modelling methods, including choice of weight data (mean weight from NHANES, as used in this study).

4.2.1 Hazard Quotient

The Hazard Quotient (HQ) and the sum of HQ (known as the Hazard Index, HI) were calculated. As discussed previously, this serves to relate a degree of exposure to an associated toxicological risk (Section 1.5). A HQ greater than 1 is deemed high risk for endocrine disruption and toxicological effects.

Individual hazard quotients for each phthalate were quite low for this study, as suggested by the lower level of exposure (see **Table 39**). Exposure to females was higher due to their lower weight on average. The phthalate that contributed the least to toxicological effects was BBP, this phthalate also has a relatively higher tolerable daily intake level. The phthalate with the highest health impact based on HQ was DiNP, with higher levels of risk associated with the HMW phthalates. HMW phthalates have higher tolerable daily intakes so this increased risk when compared to LMW is based on higher magnitudes of exposure. The literature suggests that populations should be less exposed to HMW phthalates due to their decreased leaching abilities. Perhaps changes in manufacturing practices have pushed for HMW phthalate production influencing exposure, or these metabolites are more stable/more likely to form in transit to the WWTP.

When an average HI value was compared to an Irish urinary biomonitoring study the exposure levels were within expected results, with our WBE values four times the risk level calculated from Cullen et al.'s work (**Figure 70**). The distribution is similar, although DEHP exposure seems slightly lower in the traditional biomonitoring, also, DnOP and DiNP were not investigated in that study. This suggests that overestimation of phthalate exposure may be increased for HMW phthalates. HI from NHANES are at a similar concentration to the Irish levels, exposure risk for females is lower in this model as creatinine excretion in females is lower. This shows the variations that can be introduced into data when different modelling methods are used, it would be beneficial to use harmonisation in modelling methods where possible. The Spanish WBE study had a HI roughly an order of magnitude higher than this study which showed no risk, while the Chinese HI was much higher, indicating high risk (***Figure 71***). Gonzalez-Marino et al. compared the levels found in their WBE study to other Spanish phthalate metabolite levels and found that they were roughly double the concentration. This demonstrates that WBE may overestimate phthalate exposure to degree, but the levels found are still within a degree of magnitude and would be useful for chemical prioritization.

From investigation of the HQ in Ireland there is no cause for concern for phthalate exposure from a toxicological endpoint. However, a number of phthalates and other plasticizers were not included in this study, so the cocktail effects of in Irish wastewater is unknown. It has been suggested that Hazard Indices for phthalates (as low as 0.1 or 0.2) could contribute to the combined impact of exposure to the wide spread of endocrine disruption compounds that exist (Apel et al., 2020). However, no regulations presently govern combined exposure to multiple chemicals with similar effects.

Table 39: HQ of exposure to each phthalate

		DiBP	*DBP*	*BBP*	*DEHP*	*DnOP*	*DiNP*
Male	Rural	0.0023 (0.005-0.0071)	0.0024 (0.002-0.0079)	No Risk	0.0047 (0.0014-0.0083)	0.0007 (0.0002-0.0083)	0.0005 (0.0001-0.0015)
	Suburban	0.0153 (0.0034-0.0348)	0.0347 (0.0057-0.1009)	0.0001 (0-0.004)	0.0186 (0.0054-0.0422)	0.0064 (0.0007-0.0153)	0.0068 (0.0004-0.0195)
	Urban	0.0052 (0.0019-0.0113)	0.0071 (0.0051-0.0100)	No Risk (0-0.001)	0.0092 (0.0025-0.0141)	0.0037 (0.0014-0.0070)	0.0021 (0.0005-0.0070)
Female	Rural	0.0028 (0.0007-0.0086)	0.0029 (0.0003-0.0096)	No Risk	0.0057 (0.0017-0.0101)	0.0008 (0.0002-0.0015)	0.0007 (0.0002-0.0019)
	Suburban	0.0186 (0.0042-0.0424)	0.0422 (0.0069-0.1228)	0.0002 (0.0001-0.0005)	0.0227 (0.0065-0.0514)	0.0078 (0.0009-0.0186)	0.0083 (0.0004-0.0237)
	Urban	0.0064 (0.0023-0.0138)	0.0087 (0.0062-0.0122)	0.0001 (0-0.0001)	0.0112 (0.0031-0.0171)	0.0045 (0.0017-0.0085)	0.0025 (0.0001-0.0085)

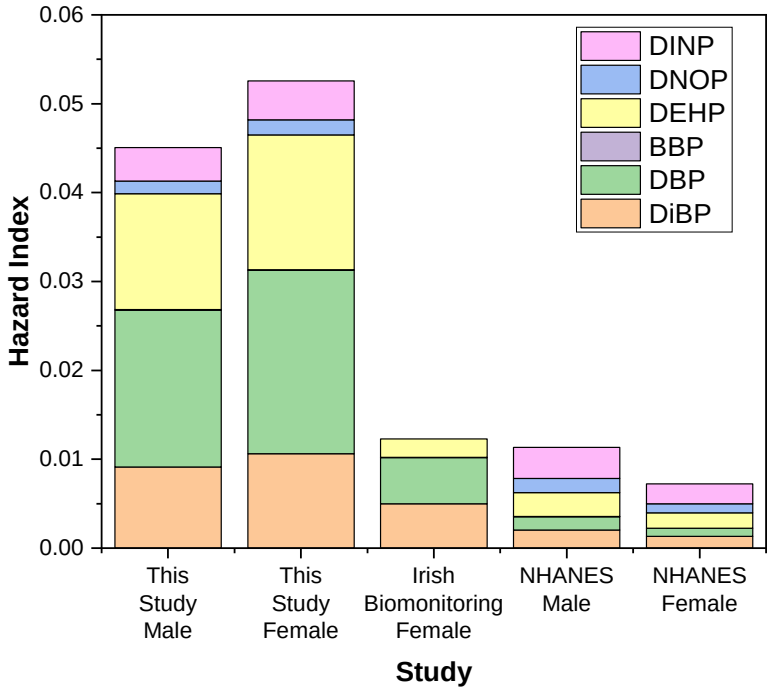


Figure 70: Hazard index of exposure to phthalates from this study compared to a small-scale irish biomonitoring study (Cullen et. Al 2017) and a large-scale international biomonitoring study (NHANES, 2013-2014)

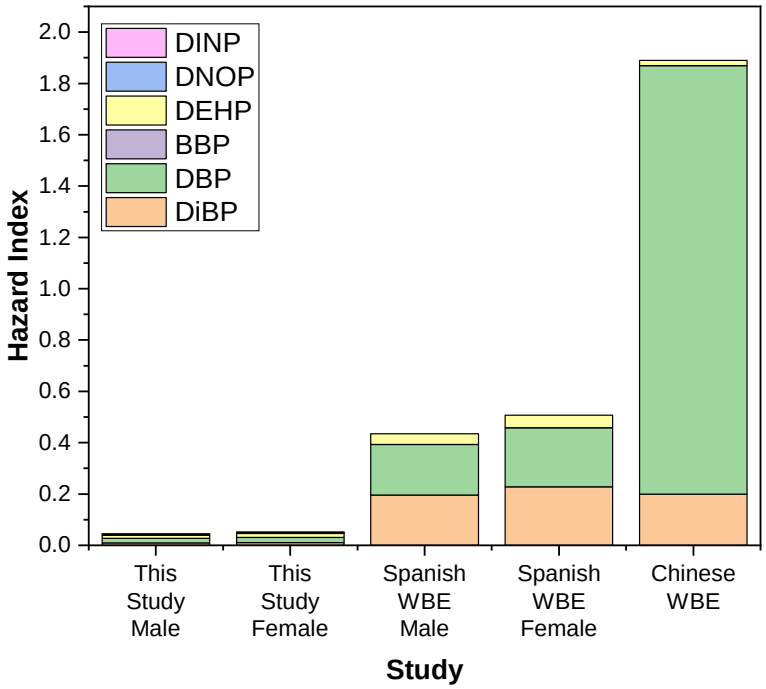


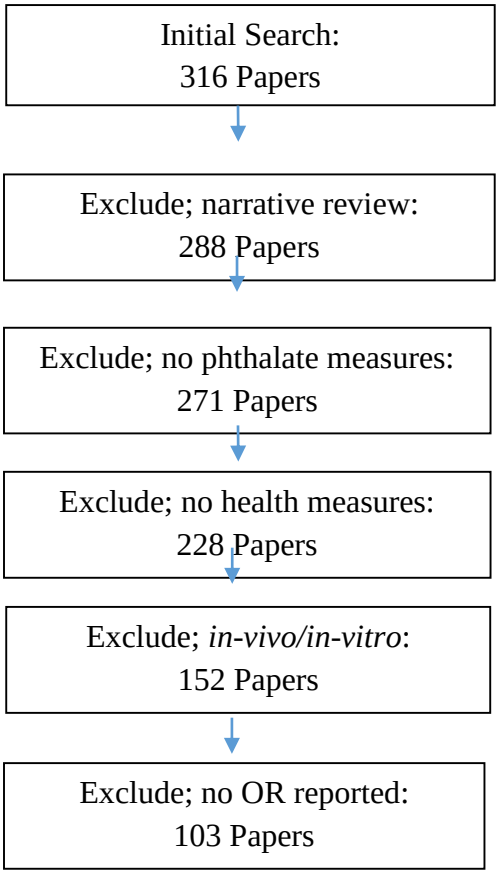
Figure 71: HI of exposure to phthalates from this study compared to WBE studies from Spain (Gonzalez-Marino et al., 2017) and China (Du et al., 2018)

4.2.2 Odds Ratio

As discussed in Section 1.5, the odds ratio conveys the degree of exposure to an associated risk. A detailed literature review surveyed the data with a particular focus on the following health outcomes: pregnancy outcomes, male birth defects, allergy, children's neurological development, precocious puberty in females, endometriosis and obesity. There has been some research surrounding the association of phthalate exposure to increased blood pressure in children but this was not included in this review of the literature. There were only two studies reported, neither statistically significant and both grouped all LMW phthalates and all HMW phthalates together.

The risk levels reported in the literature will indicate which health outcomes are the most pressing when populations are exposed to phthalates. This is an approximation and is not based on any health data from the population studied so it serves as an indication of possible risk only.

Human risk data was collected from the literature. The search parameters were (("Phthalic Acids/adverse effects") AND Humans)) NOT tha - PubMed – NCBI and studies were excluded as follows:



Some studies in this area report the logistic regression without the standard error in the text or supplemental data. This makes it impossible to calculate the OR from these studies, so only those with reported OR were included.

It was found that the most pertinent risk from human data in the literature was male birth anomalies (including decreased ano-genital distance, cryptorchidism and hypospadias). The average OR for all phthalates is 2.39 (**Figure 72**). DBP and DiBP had the greatest magnitude of exposure risk, although data from Swan et al., 2005 showed a very wide interquartile range that could indicate outliers in this data. Nevertheless, an average OR of 2.39 is a definitive association with effect, if phthalate exposure went above risk limits, this endpoint would be vital to monitor.

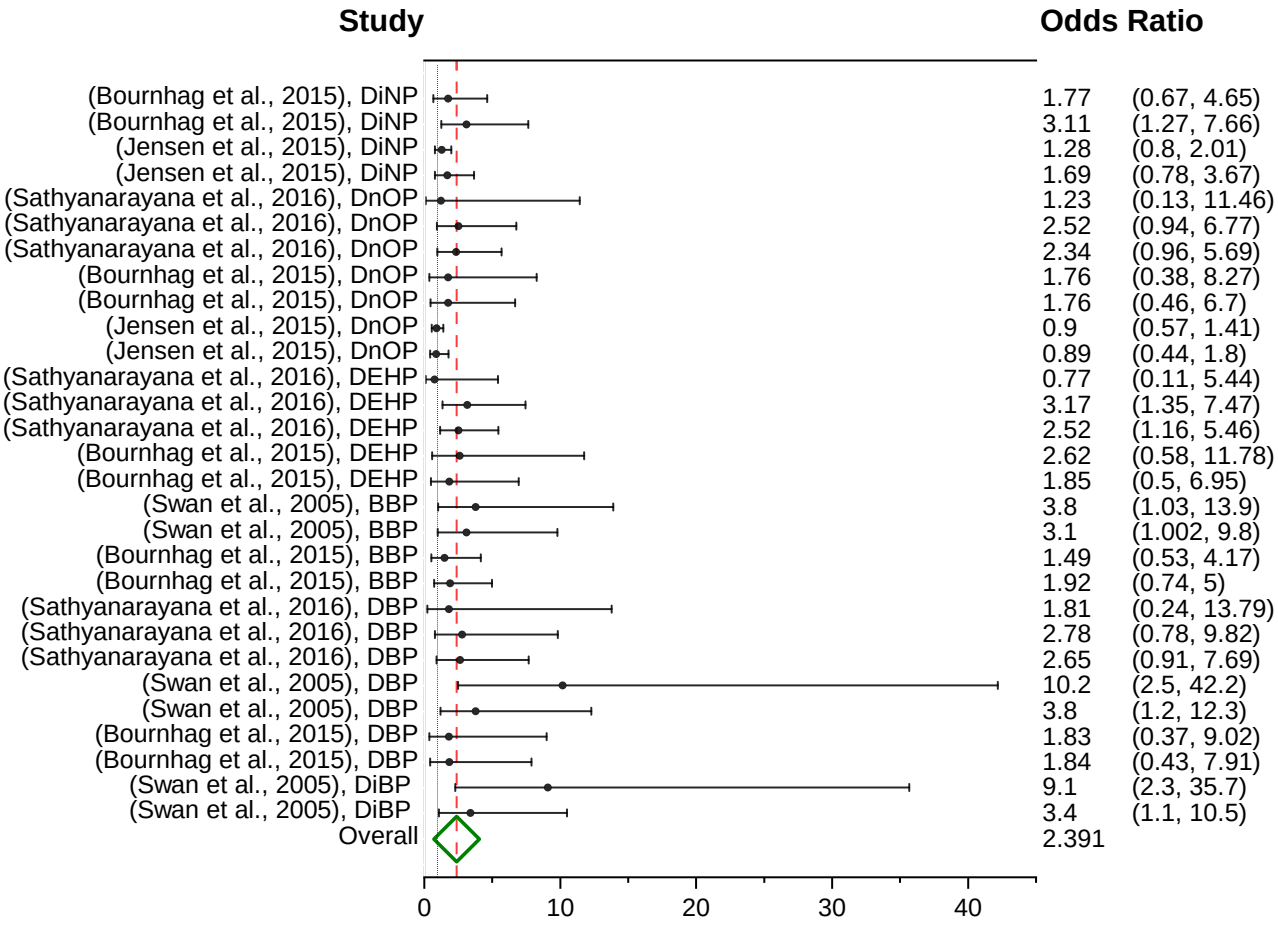


Figure 72: Association of phthalate exposure with male birth anomalies. OR is Presented as centre point, with upper and lower confidence interval. dashed blue line shows point of no effect OR=1, dashed red line shows mean OR from all studies

The impacts of phthalates on pregnancy is widely studied and includes prolonged time to pregnancy, pre-term birth and pregnancy loss. The overall OR from the published data is 1.33 meaning that the literature supports the theory that increased exposure to phthalates is associated with pregnancy complications (**Figure 73**). DEHP had the greatest impact on outcome with an average OR of 1.69, with DBP being the lowest at 1.05. However, data from Messelarian et al., 2016 had a wide confidence interval, which could suggest that the overall average risk assessment for study could be increased due to subjects with abnormally high phthalate exposure. Most of these studies were conducted in the US, and based on the small biomonitoring study conducted in Ireland, exposure levels are very similar. Therefore, there is possibly an increased risk of complications in pregnancy caused by phthalates in Ireland.

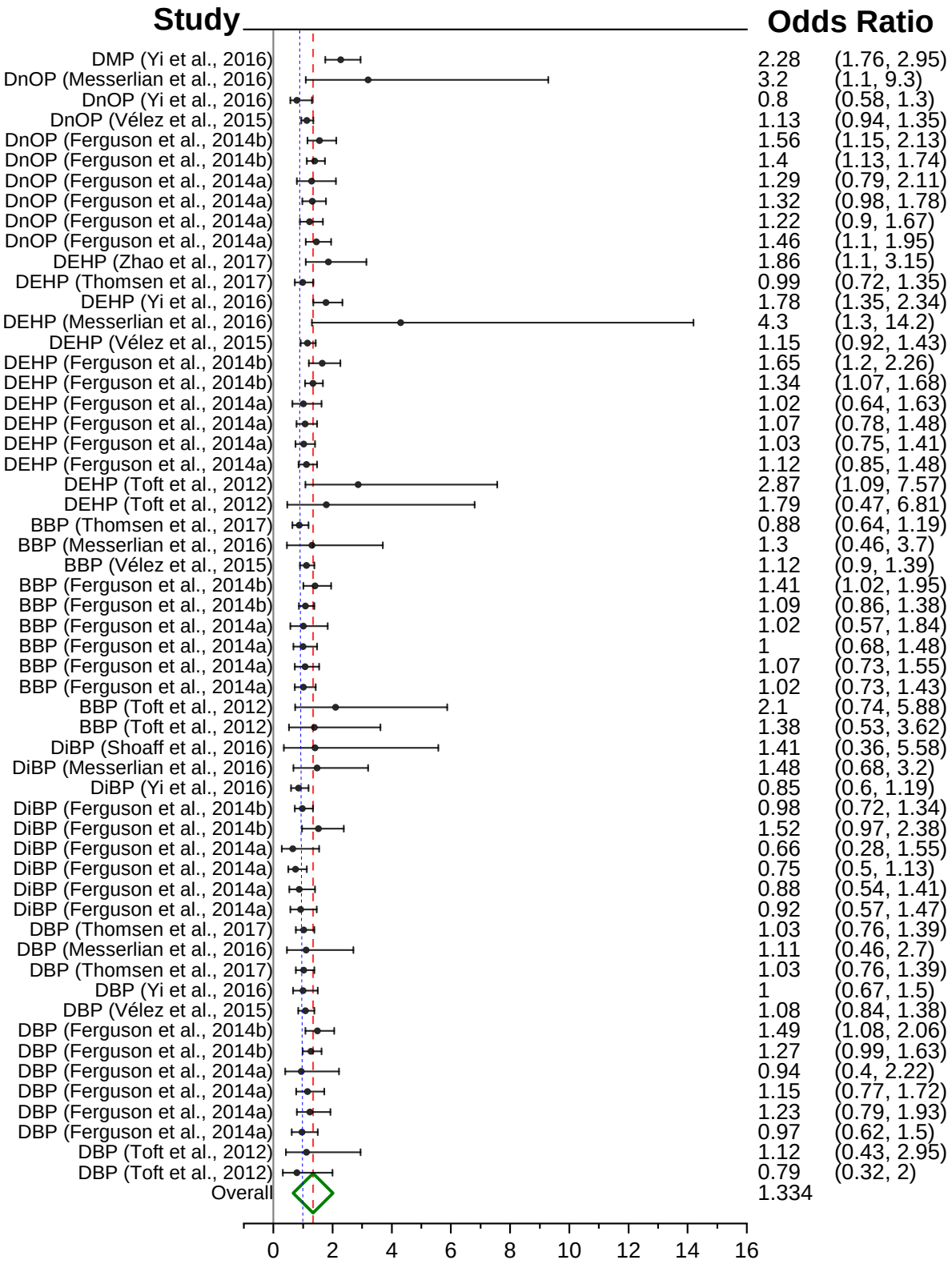


Figure 73: Association of phthalate exposure to adverse effects on pregnancy. OR is Presented as centre point, with upper and lower confidence interval. dashed blue line shows point of no effect OR=1, dashed red line shows mean OR from all studies

Allergenic type reaction including asthma and skin sensitisation was the third most widely studied epidemiological endpoint in the literature. For the studies that reported adjusted OR, the mean was 1.19 (**Figure 74**). This value, while it shows increased risk, is not as pertinent as male birth defects or complications in pregnancy, with many values falling below the line of no effect. Stelmach et al., 2015, showed a large interquartile range, however, this study was relatively smaller than the others (n=44) and therefore the weighted value did not increase the average to a large degree. It appears that LMW phthalates might have a slightly bigger impact on this endpoint but there are minimal studies conducted in HMW phthalates.

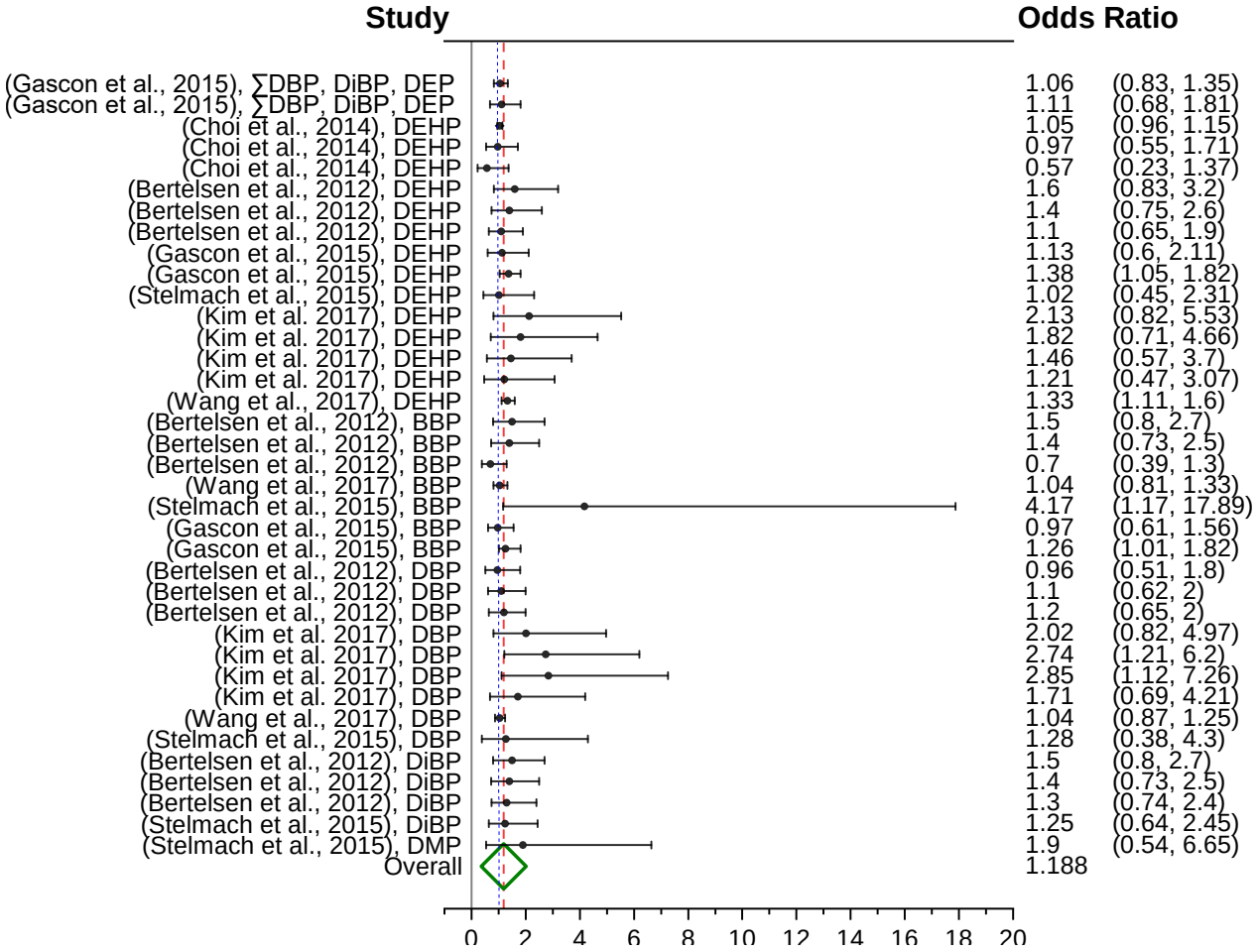


Figure 74: Association of phthalate exposure to allergy, described by atopy, atopic dermatitis and asthma. OR is presented as centre point, with upper and lower confidence interval. Dashed blue line shows mark of no effect OR=1, dashed red line shows mean OR from all studies

Endometriosis was investigated multiple times in the literature. The average OR was found to be 1.06 (**Figure 75**). Most of this data was statistically insignificant as the confidence intervals crossed the line of no effect (1). Therefore, there is no conclusive human evidence to support that phthalate exposure is associated with development of endometriosis.

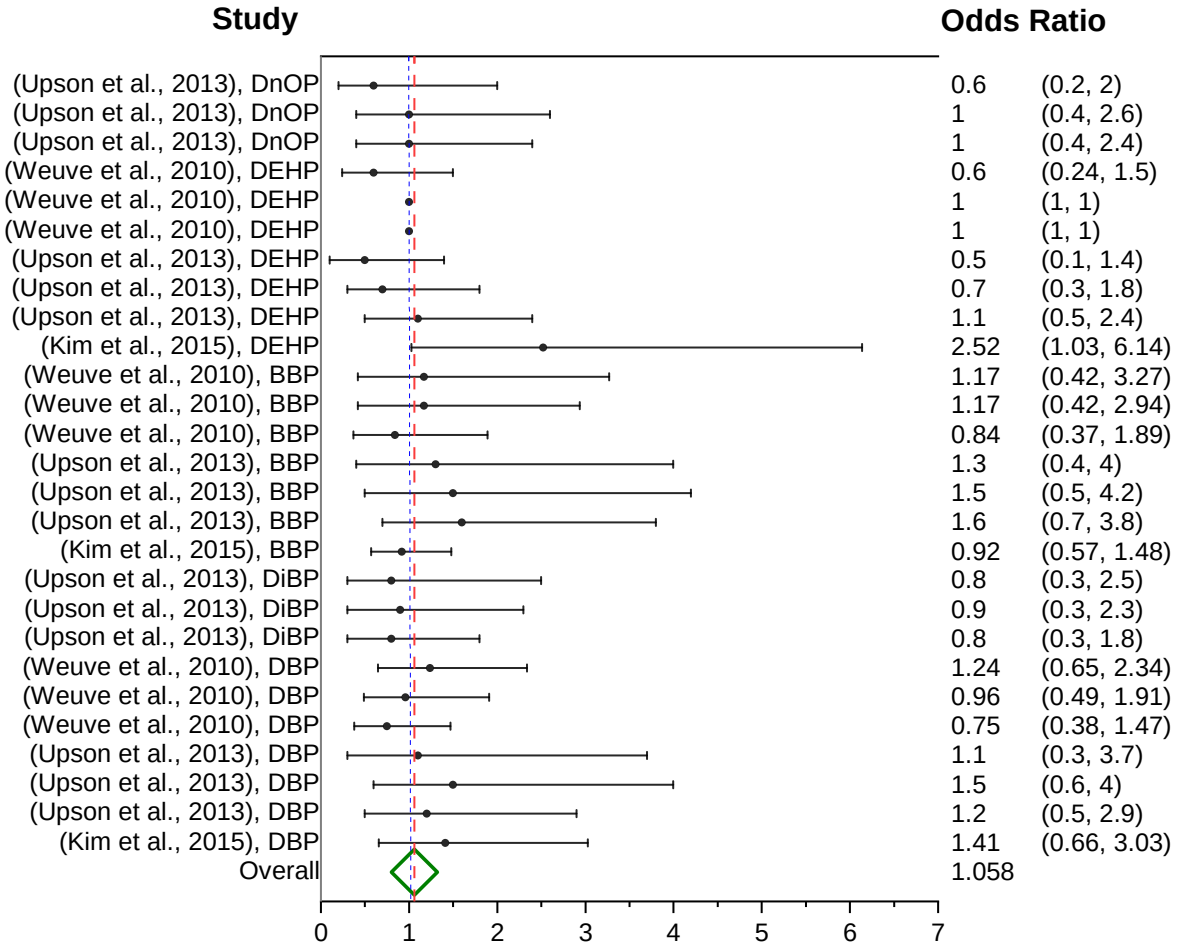


Figure 75: Association of Phthalate Exposure to Endometriosis. OR is Presented as Centre Point, with upper and lower confidence interval. dashed blue line shows point of no effect OR=1, dashed red line shows mean OR from all studies

Decreased neurological development is an endpoint that is more challenging to measure. These studies either used approved tests administered to children to assess neurological development, or medical history for autism spectrum disorders and attention deficit disorders were taken. The reviewed literature shows an average OR of 1.57 (**Figure 76**). The most exposed group in the Chopra et al., 2014 study was unreasonably high in comparison to the other studies, if this group is excluded, then the mean OR is 1.39. Therefore, the data supports the hypothesis that increased phthalate exposure is associated with negative neuro-developmental outcomes. There is no clear division between effects of HMW and LMW phthalates on neurological development although the study from Enel et al., 2009 showed increased risk for grouped LMW phthalates over grouped HMW phthalates.

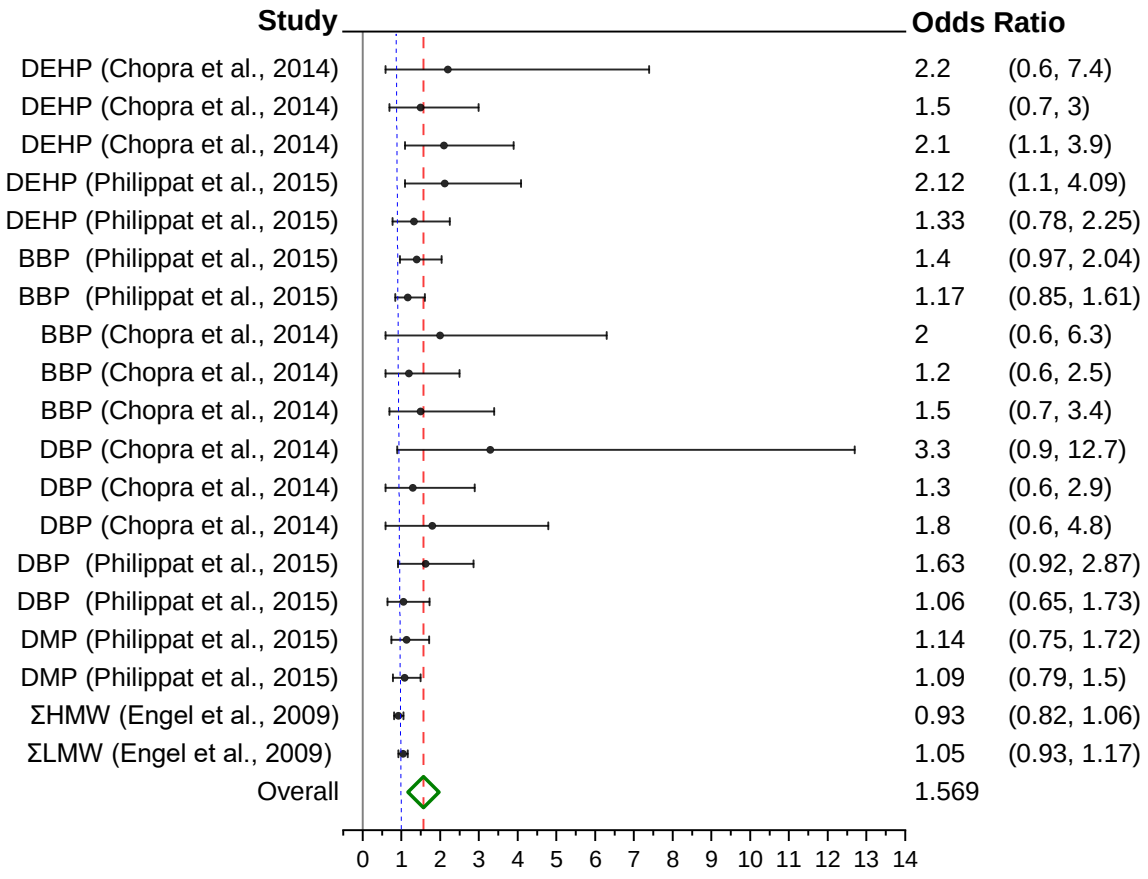


Figure 76: Association of phthalate exposure to decreased neurological development. OR is presented as centre point, with upper and lower confidence interval. Dashed blue line shows point of no effect OR=1, dashed red line shows mean OR from all studies

The mean OR for association of phthalate exposure to precocious puberty is 1.42 (**Figure 77**). The LMW phthalates appear to have a greater effect. A study by Cólon et al. reported adjusted OR values, however, it was excluded as there were some limitations in the methods used. The children used in the Colón study very young with a mean age of 2.58 years. This age premature breast tissue development is considered a benign condition with no known cause and cannot be classified as an indication to early entry into puberty (Colón et al., 2000).

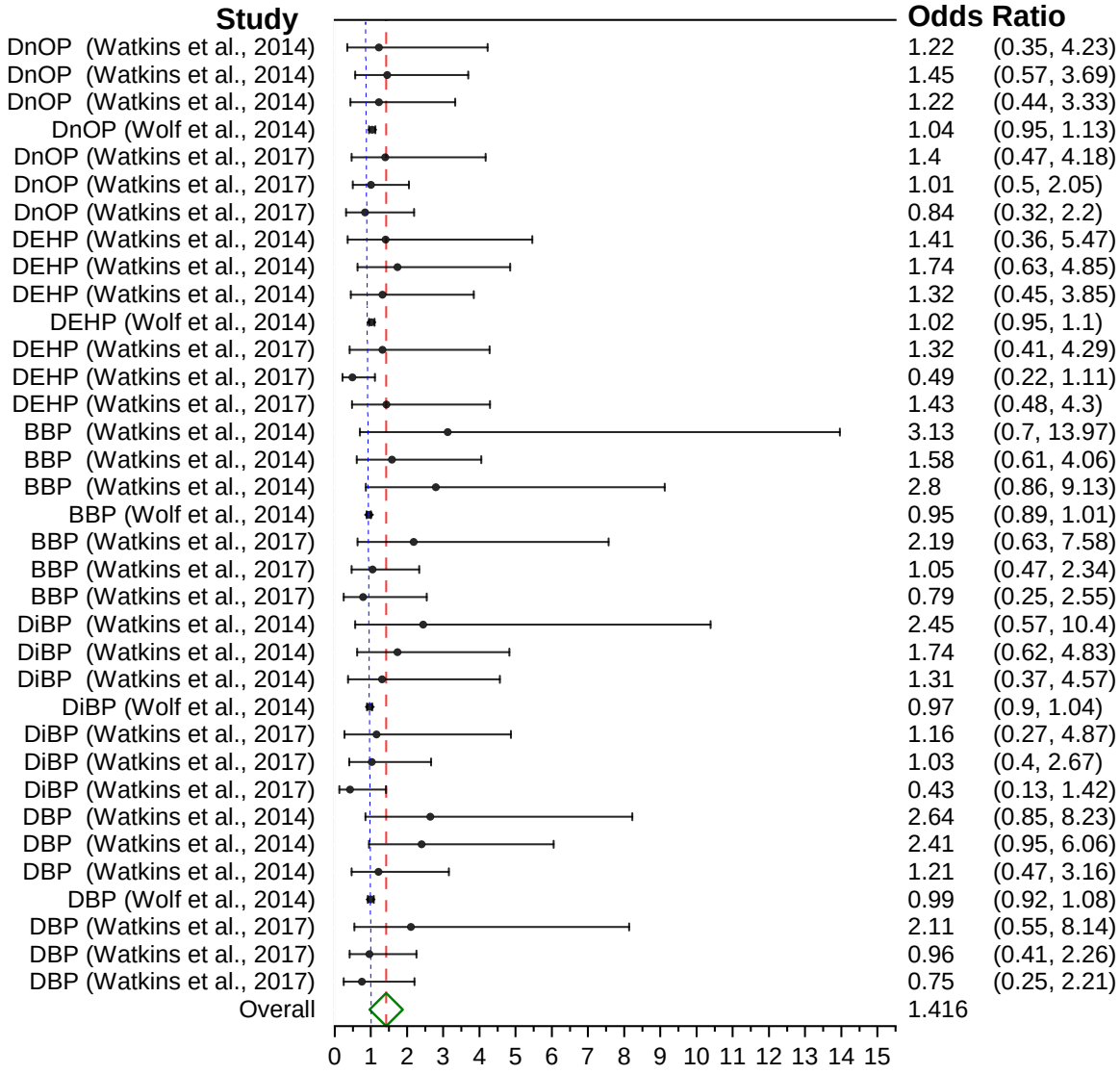


Figure 77: association of phthalate exposure to precocious puberty in females. OR is presented as centre point, with upper and lower confidence interval. Dashed blue line shows point of no effect OR=1, dashed red line shows mean OR from all studies

Phthalate exposure and incidence of obesity was studied due to the association of endocrine function with increased bodyweight. The OR values reported for reviewed human had an average OR of 1.10. This is a minor positive association but it is based off a small sample size with many crossing the line of no effect and DnOP showing a negative correlation. It is therefore believed that phthalate exposure is not associated with obesity and there would be no risk for phthalate-induced obesity in Ireland.

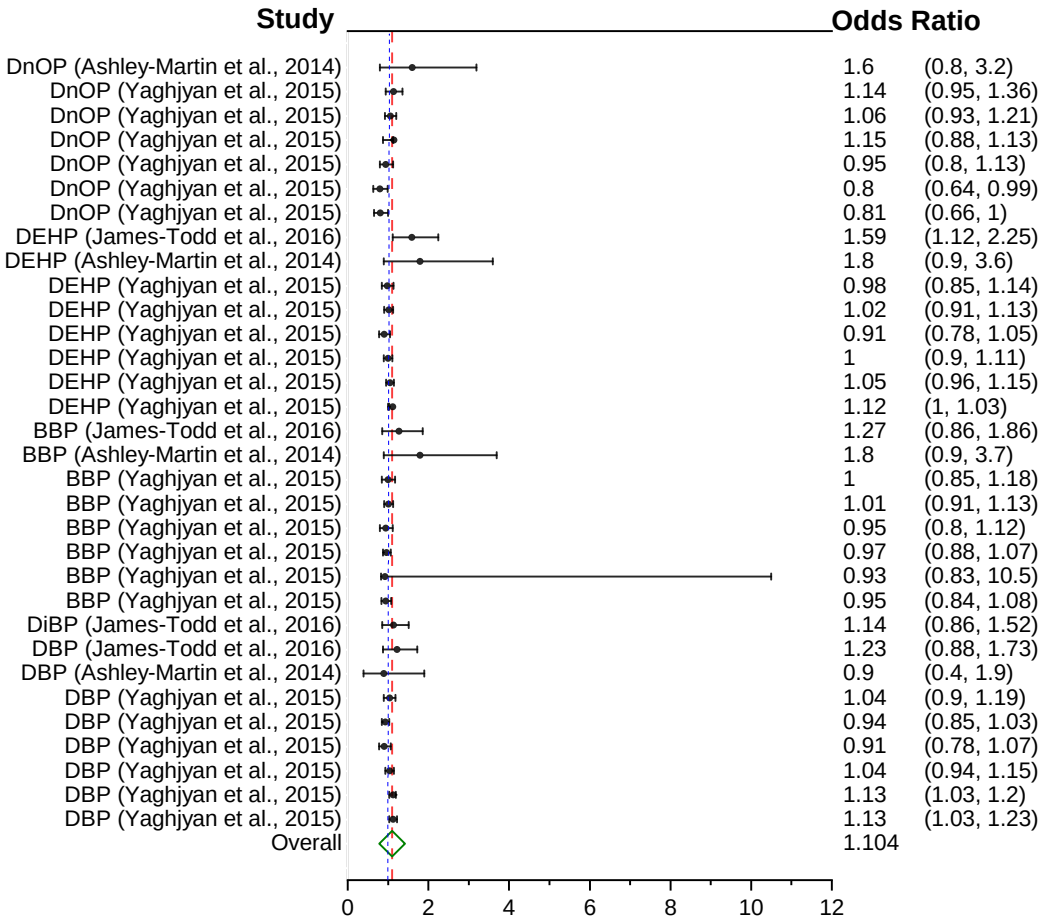


Figure 78: Association of phthalate exposure to obesity and metabolic disorders. OR is presented as centre point, with upper and lower confidence interval. Dashed blue line shows point of no effect OR=1, Dashed red line shows mean OR from all studies

From assessment of the OR, the most pertinent health endpoints are male birth anomalies, complications in pregnancy, decreased neurological development and female precocious puberty. The impact of phthalate depended on the endpoint, and no studies showed strong increases in affect based on molecular weight. It is thought that the level of exposure in Ireland is similar enough to NHANES that there is a slightly increased risk to male birth anomalies and complications in pregnancy but this cannot be proven.

4.3 Feasibility of Wastewater as a Biomonitoring Tool

There will be many catchment specific differences between influent samples at WWTPs that can affect the concentrations of metabolites used for exposure assessment. At present these variations in WWTP residence times could cause different in-transit transformations and therefore partially contribute to international variations in concentrations (Section 1.7.1). It is recommended that reference standards of more highly metabolised biomarkers are used for exposure assessment due to their increased resilience to biotic and abiotic formation outside the human body. However, these standards can be cost ineffective (for phthalates roughly 1000 times more expensive). This method would therefore not be attractive for governmental testing bodies due to the comparatively high cost, although this disparity may be less for other emerging contaminants. The assessment method could also be improved through determining the compound kinetics of degradation. If spiked analytes of interest are added to bioreactors and piping systems that simulate real-life sewer conditions, then accurate degradation rates could be obtained for a range of residence times. Once the kinetics of degradation are published then wastewater epidemiology could be improved through incorporating these into the existing exposure model.

WBE is an emerging method of analysis, and therefore impacts of this research for the future are relatively unknown. One discussion surrounding this analysis is the ethical implications that could arise from studying populations, mostly surrounding drug residue monitoring (Hall et al., 2012). As WBE does not involve individual participants and data is collected from public resources (WWTPs) it is generally considered unnecessary to have any ethical oversight. However, WBE studies have attracted a lot of media attention in the past and if marginalised communities are identified this could negatively impact the area's reputation. It is important if WBE is used in Ireland for the purposes of illicit drug (or other compound) monitoring, that a set of ethical guidelines be followed with reference to monitoring smaller areas like prisons, universities and workplaces (Prichard et al., 2014). This would prioritise anonymity of sites for publication and involve oversight from an ethical committee.

As it stands, the basic method for wastewater based epidemiology still serves as a useful diagnostic tool for the prioritization of contaminants of emerging concern. Within one community a variety of EDCs can be screened to assess the most critical compound for

body burden. Traditional biomonitoring studies are labour intensive, expensive and require patient involvement. With a first step assessment, these studies can be reserved for EDCs that are already known to cause body burden in that society. This evidence should increase the likelihood of funding these much needed biomonitoring studies for influencing policy change. It would however be recommended that a large scale biomonitoring study be carried out in Ireland in the future as richer data on a population can be obtained and we are far behind our European counterparts in this area of research. One large scale biomonitoring study could yield information on a wide range of EDCs and if proper sample management is maintained then retrospective studies on multiple chemicals of emerging concern could be carried out.

4.4 Conclusion

A comprehensive and critical review of the literature found that human exposure to phthalates is best assessed through metabolite concentration in biological samples. One study followed urinary phthalate metabolite concentration in Ireland, through DEMOCOPHES (Cullen et al., 2017). However, human biomonitoring studies are costly, and this publication served no relation to risk, i.e. reported concentrations but did not investigate possible risk associated with those exposure levels. As such, in the current study, the feasibility of Wastewater Based Epidemiology (WBE) for the assessment of phthalate exposure risk was examined, owing to its cost-effective nature and ready availability of data. Three WWTP influent streams were monitored for phthalate metabolite concentration and estimated population exposure was calculated using metabolic breakdown factors, flow rate and population served. Total exposure to phthalates ranged from 10.27 to 418.42 µg/inhabitant/day, with BBP accounting for the lowest body burden, and DINP the highest. Risk assessment involved using the Hazard Quotient (HQ) to assess the toxicological effect of phthalates in Ireland. In terms of HQ, BBP showed the lowest degree of endocrine disruption risk in the studied population, with DiBP deemed the highest risk phthalate, with an average HQ ranging from 0.0023 to 0.0186. All HQ values and the sum of all phthalate HQs, reported as the Hazard Index (HI), were below levels of concern. This indicates that there is no phthalate induced risk for the population studied. However, only a selection of phthalate metabolites was monitored, and may not reflect the risk associated with all phthalates and plasticizers. WBE techniques require improvement to give a more robust indication of population

exposure, however it is recommended that periodic screening of wastewater for a wide range of metabolites related to contaminants of emerging concern could indicate temporal trends in exposure and give an early indication of priority area for research or the need for a biomonitoring campaign.

5 Conclusions and Recommendations

This project aimed to examine the environmental sources, fates and body burden of phthalates in Ireland. To this end, the objectives of the research were to:

- Examine concentrations of phthalates in household wastes, leachates and wastewater, and to assess their contribution to environmental contamination.
- Develop methods for the detection of phthalates in wastewater process streams for future compliance monitoring.
- Assess human exposure through wastewater-based epidemiology and estimate a related risk.

Phthalates are pervasive in the Irish environment. The concentrations in this study have been consistent with other European countries and as such an increased cause for concern is not presented for the Irish population. Nevertheless, 100% detection frequency for 10 of the 11 phthalates studied in all samples suggests that further steps be taken to reduce this burden on the environment and prevent any further contamination in the ecosystem.

5.1.1 Reduction of Phthalate Burden

It is possible that restricted phthalates are remaining in consumer products due to the recycling process. If recyclable material contains more than the recommended levels for these phthalates, then recyclable materials should not be used for the manufacture of food contact materials or children's toys. If a toxicological concern is raised beyond this endpoint, then incineration of plastics should be considered until these compounds have been eliminated. As per EPA initiatives, landfill should be avoided and leachate systems need to be controlled and monitored.

Wastewater effluent was seen to contribute some degree of phthalate contamination to the wastewater discharge point (river system). The levels at the discharge point were significantly higher than at other points of the river. However, the levels in effluent are low, with all phthalates being under the EQS for surface water. No further action for reduction of phthalate in wastewater effluent needs to be taken.

The EPA notes that land spreading is the primary removal route of sludge in Ireland. Phthalates are well retained in sludge and literature has shown that the transfer of phthalates from fertilizer to soil and hence crops is high. Novel wastewater treatment

could be employed to remove these EDCs from sludge, some examples include chemical extraction, bioleaching, electroreclamation, and supercritical fluid extraction (SFE). This will not only reduce the level of phthalate, but heavy metals and other EDCs retained in sludge that could also be contributing to endocrine disrupting effects. These solutions are very expensive so if it is not financially viable to execute an improved removal from sludge then another method of sludge disposal should be promoted e.g. incineration.

5.1.2 Feasibility of Wastewater Based Epidemiology for Compliance Monitoring

Wastewater analysis is a feasible approach for compliance monitoring. Phthalate levels exhibit a level of temporal variation. The analytical method developed shows that once significant factors are identified and managed for the control of outside contamination, there is a simple and robust technique for determining the concentrations in this matrix. This is an SPE method which could be incorporated into analysis of other compounds once the correct control steps are put in place. The main recommendations for analysing phthalates by LC-MS are shown below.

- Check each new batch of solvent for phthalate contamination. Triple rinse all glassware with solvent after overnight bake-out, reduce solvent and sample contact with air and any plastic materials.
- Fischer Optima^{TM} solvents show the lowest phthalate contamination of all solvents studied.
- Use a delay column to remove instrument contamination.
- Conduct daily cleans of the instrument to remove residual phthalate and, if possible, use a multi-wash system to fully clean the needle.
- Include multiple analytical blanks to reduce column carry-over, ensure that all analytical blanks are clean before sample run, if not, subtract from sample. Include procedural blanks and subtract levels from samples.

If limits are set, this is a reliable method to determine levels at the WWTP and monitor emissions. At present, some phthalates (DEHP, DBP, BBP, DINP, DIDP and DNOP) are already legislated under REACH regulations; Cosmetics Directive; and EC Regulation No. 1907/2006. The European Commission has ammended Authorisation List (Annex XIV to REACH) with the addition of DEHP, DBP, BBP and DiBP as substances of very

high concern (SVHCs) due to their endocrine disrupting effects. This project has demonstrated that DiBP is one of the most prevalent phthalates contained in the Irish environment. As it is structurally similar to DBP, it is important that the addition of this phthalate is to the SVHC list be used as a catalyst to restrict this compound in manufacturing, in addition to further controlling phthalates on the SVHC list beyond 0.1 %w/w restriction in childcare articles when granting authorisation. The USEPA introduced a Significant New Use Rule for DPP in 2012, which requires manufacturers or processors of the chemical to obtain USEPA approval. No such EU legislation is in place. DPP, a novel phthalate, has been found at some of the lowest concentrations in environmental samples in Ireland, yet is more present in waste and sludge samples. This type of restriction could help prevent the further introduction of these new replacement phthalates to the environment.

Using WBE for phthalate health assessment is still a relatively novel methodology. A first step health risk assessment of phthalates was carried out by monitoring metabolites of 6 PAEs. These data were then converted to the daily intake rates of the parent phthalates. The average exposure levels found in Ireland were well below the levels of concern from a toxicological endpoint. The exposure rates were lower than found in the USA, China and Spain, but levels were within an order of magnitude so this can be attributed to population differences. The analytical method developed was robust and resistant to matrix effects, although time consuming due to the use of standard addition. This offers a cost-effective way to examine the impact an EDC has on a population, assuming that there are limited changes to the analyte of concern in transit to the WWTP, or that in-sewer transformation can be measured and incorporated into the model. This serves as a useful tool for estimating population exposure and prioritizing substances of emerging concern.

5.1.3 Future Research

As soils were deemed to have greater phthalate burden, an increased sample size soil survey should be carried out, including monitoring specific sites that utilize land application of bio-solids. This will establish if any land spreading practices pose a risk to soil ecology at those sites. It would be beneficial to also examine crop uptake of phthalates through these soils to determine the degree of phthalate contamination introduced to food prior to packaging.

Phthalate metabolites were only monitored in this study as a means to assess human exposure, but recent bio-assay research has suggested that the phthalate metabolites themselves exhibit similar levels of endocrine disrupting properties. If future work is carried out in the area of phthalate contamination in Ireland, phthalate metabolites should be considered to assess the total impact of phthalate in the environment.

The accuracy of WBE could be improved upon in further research. One method is by using secondary metabolites for greater selectivity and resistance to transformations in-transit to the WTWP. Another method would be to determine the degradation/abiotic formation kinetics of the primary metabolites using bioreactors and model piping systems. This kind of research, once published, would benefit the research community allowing basic WBE experiments to be carried out with kinetics incorporated into the exposure model to improve accuracy.

To determine the true human health impact of phthalates a full-scale biomonitoring study should be carried out, this will give detailed health information on the subjects and therefore give a targeted risk. A method could be developed to include monitoring of a wide-ranging suite of compounds of emerging concern to maximize the resources. If such a study was conducted, then there would be strong evidence to influence policy on emerging EDCs.

6 Bibliography

110th United States Congress, 2008. Consumer Product Safety Improvement Act of 2008 - Public Law 110-314.

Alves, A., Koppen, G., Vanermen, G., Covaci, A., Voorspoels, S., 2016. Long-term exposure assessment to phthalates: How do nail analyses compare to commonly used measurements in urine. J. Chromatogr. B 1036–1037, 124–135. https://doi.org/10.1016/j.jchromb.2016.09.039

Alzaga, R., Pena, A., Bayona, J.M., 2003. Determination of phthalic monoesters in aqueous and urine samples by solid-phase microextraction-diazomethane on-fibre derivatization-gas chromatography-mass spectrometry. J. Sep. Sci. 26, 87–96. https://doi.org/10.1002/jssc.200390020

Anderson, W.A.C., Castle, L., Hird, S., Jeffery, J., Scotter, M.J., 2011. A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. Food Chem. Toxicol. 49, 2022–2029. https://doi.org/10.1016/j.fct.2011.05.013

Apel, P., Kortenkamp, A., Koch, H.M., Vogel, N., Rüther, M., Kasper-Sonnenberg, M., Conrad,A., Brüning, T., Kolossa-Gehring, M., 2020. Time course of phthalate cumulative risks to male developmental health over a 27-year period: Biomonitoring samples of the German Environmental Specimen Bank. Environment International 137, 105467. https://doi.org/10.1016/j.envint.2020.105467

APHA, 2011. American Public Health Association: Reducing PVC in facilities with vulnerable populations. [WWW Document]. USA. URL https://www.apha.org/policies-and-advocacy/public-health-policy-statements/policy-database/2014/07/08/15/13/reducing-pvc-in-facilities-with-vulnerable-populations (accessed 7.20.08).

APHA/AWWA/WEF, 2012. Standard Methods for the Examination of Water and Wastewater. Stand. Methods 541. https://doi.org/ISBN 9780875532356

Asghari, M., Saeidnia, S. and Abdollahi, M., 2015. A Review on the Biochemical and Molecular Mechanisms of Phthalate-Induced Toxicity in Various Organs with a Focus on the Reproductive System. *International Journal of Pharmacology*, 11(2), pp.95-105.

Ashley-Martin, Jillian, et al. "A Birth Cohort Study to Investigate the Association between Prenatal Phthalate and Bisphenol A Exposures and Fetal Markers of Metabolic Dysfunction." *Environmental Health: A Global Access Science Source*, vol. 13, Oct. 2014, p. 84, doi:10/f6q7t3.

Barciela-Alonso, M.C., Otero-Lavandeira, N., Bermejo-Barrera, P., 2017. Solid phase extraction using molecular imprinted polymers for phthalate determination in water and wine samples by HPLC-ESI-MS. Microchem. J. 132, 233–237. https://doi.org/10.1016/j.microc.2017.02.007

Baz-Lomba, J.A., Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignano, E., Causanilles, A., Hernandez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., van Nuijs, A., Ort, C., Plosz, B.G., Ramin, P., Reid, M., Rousis, N.I., Ryu, Y., de Voogt, P., Bramness, J., Thomas, K., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. Bmc Public Health 16, 1035. https://doi.org/10.1186/s12889-016-3686-5

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R.C., Faisal, P.A., 2017.

Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. Journal of Hazardous Materials 340, 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036

Bertelsen, Randi J., et al. "Urinary Biomarkers for Phthalates Associated with Asthma in Norwegian Children." *Environmental Health Perspectives*, vol. 121, no. 2, Feb. 2013, pp. 251–56, doi:10/gd57b2.

Bicchi, C., Schilirò, T., Pignata, C., Fea, E., Cordero, C., Canale, F., Gilli, G., 2009. Analysis of environmental endocrine disrupting chemicals using the E-screen method and stir bar sorptive extraction in wastewater treatment plant effluents. Sci. Total Environ. 407, 1842–1851. https://doi.org/10.1016/j.scitotenv.2008.11.039

Bisseux, M., Colombet, J., Mirand, A., Roque-Afonso, A.-M., Abravanel, F., Izopet, J., Archimbaud, C., Peigue-Lafeuille, H., Debroas, D., Bailly, J.-L., Henquell, C., 2018. Monitoring human enteric viruses in wastewater and relevance to infections encountered in the clinical setting: a one-year experiment in central France, 2014 to 2015. Eurosurveillance 23, 16–26

Biswas, B., Qi, F., Biswas, J.K., Wijayawardena, A., Khan, M.A.I., Naidu, R., 2018. The Fate of Chemical Pollutants with Soil Properties and Processes in the Climate Change Paradigm—A Review. Soil Systems 2, 51. https://doi.org/10.3390/soilsystems2030051

Blount, B.C., Milgram, K.E., Silva, M.J., Malek, N.A., Reidy, J.A., Needham, L.L., Brock, J.W., 2000. Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS. Anal. Chem. 72, 4127–4134

Boas, M., Frederiksen, H., Feldt-Rasmussen, U., Skakkebæk, N.E., Hegedüs, L., Hilsted, L., Juul, A., Main, K.M., 2010. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ. Health Perspect. 118, 1458–1464. https://doi.org/10.1289/ehp.0901331

Bonita, R., 2012. Basic Epidemiology, 2nd ed. World Health Organisation.

Bornehag, C.-G., Carlstedt, F., Jönsson, B.A.G., Lindh, C.H., Jensen, T.K., Bodin, A., Jonsson, C., Janson, S., Swan, S.H., 2015. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ. Health Perspect. 123, 101–107. https://doi.org/10.1289/ehp.1408163

Bubba, M. Del, Ancillotti, C., Checchini, L., Fibbi, D., Rossini, D., Ciofi, L., Rivoira, L., Profeti, C., Orlandini, S., Furlanetto, S., 2018. Determination of phthalate diesters and monoesters in human milk and infant formula by fat extraction, size-exclusion chromatography clean-up and gas chromatography-mass spectrometry detection. J. Pharm. Biomed. Anal. J. Pharm. Biomed. 148, 6–16. https://doi.org/10.1016/j.jpba.2017.09.017

Buergisser, C.S., Cernik, M., Borkovec, M., Sticher, H., 1993. Determination of nonlinear adsorption isotherms from column experiments: an alternative to batch studies. Environ. Sci. Technol. 27, 943–948. https://doi.org/10.1021/es00042a018

Bui, T.T., Alves, A., Palm-Cousins, A., Voorspoels, S., Covaci, A., Cousins, I.T., 2017. Estimating uptake of phthalate ester metabolites into the human nail plate using pharmacokinetic modelling. Environ. Int. 100, 148–155. https://doi.org/10.1016/j.envint.2017.01.007

Calafat, A.M., Wong, L.-Y., Silva, M.J., Samandar, E., Preau, J.L., Jia, L.T., Needham, L.L., 2010. Selecting Adequate Exposure Biomarkers of Diisononyl and Diisodecyl

Phthalates: Data from the 2005–2006 National Health and Nutrition Examination Survey. Environ. Health Perspect. 119, 50–55. https://doi.org/10.1289/ehp.1002316

Cao, X.-L., 2008. Determination of phthalates and adipate in bottled water by headspace solid-phase microextraction and gas chromatography/mass spectrometry. J. Chromatogr. A 1178, 231–8. https://doi.org/10.1016/j.chroma.2007.11.095

Carrillo, J.D., Martínez, M.P., Tena, M.T., 2008. Determination of phthalates in wine by headspace solid-phase microextraction followed by gas chromatography-mass spectrometry. Use of deuterated phthalates as internal standards. J. Chromatogr. A 1181, 125–30. https://doi.org/10.1016/j.chroma.2007.12.053

Chan, J., Shuang, F., n.d. Rapid, Sensitive, and Robust Detection of Phthalates in Food Using GC/MS or LC/MS 14.

Chang, J.-W., Lee, C.-C., Pan, W.-H., Chou, W.-C., Huang, H.-B., Chiang, H.-C., Huang, P.-C., 2017. Estimated Daily Intake and Cumulative Risk Assessment of Phthalates in the General Taiwanese after the 2011 DEHP Food Scandal. Sci. Rep. 7. https://doi.org/10.1038/srep45009

Chang, Y.-J., Lin, K.-L., Chang, Y.-Z., 2013. Determination of Di-(2-ethylhexyl)phthalate (DEHP) metabolites in human hair using liquid chromatography–tandem mass spectrometry. Clin. Chim. Acta, Mass Spectrometry for Clinical Diagnosis 420, 155–159. https://doi.org/10.1016/j.cca.2012.10.009

Chen, C.-Y., Chou, Y.-Y., Wu, Y.-M., Lin, C.-C., Lin, S.-J., Lee, C.-C., 2013. Phthalates may promote female puberty by increasing kisspeptin activity. Hum. Reprod. Oxf. Engl. 28, 2765–2773. https://doi.org/10.1093/humrep/det325

Choi, W. J., et al. "Potential Nonmonotonous Association between Di(2-Ethylhexyl) Phthalate Exposure and Atopic Dermatitis in Korean Children." *The British Journal of Dermatology*, vol. 171, no. 4, Oct. 2014, pp. 854–60, doi:10/f6k58c.

Chopra, V., Harley, K., Lahiff, M., Eskenazi, B., 2014. Association between phthalates and attention deficit disorder and learning disability in U.S. children, 6-15 years. Environ. Res. 128, 64–69. https://doi.org/10.1016/j.envres.2013.10.004

Cincinelli, A., Stortini, A.M., Perugini, M., Checchini, L., Lepri, L., 2001. Organic pollutants in sea-surface microlayer and aerosol in the coastal environment of Leghorn—(Tyrrhenian Sea). Mar. Chem. 76, 77–98. https://doi.org/10.1016/S0304-4203(01)00049-4

Clewell, R.A., Kremer, J.J., Williams, C.C., Campbell, J.L., Andersen, M.E., Borghoff, S.J., 2008. Tissue Exposures to Free and Glucuronidated Monobutylyphthalate in the Pregnant and Fetal Rat following Exposure to Di-n-butylphthalate: Evaluation with a PBPK Model. Toxicological Sciences 103, 241–259. https://doi.org/10.1093/toxsci/kfn054

Colón, I., Caro, D., Bourdony, C.J., Rosario, O., 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ. Health Perspect. 108, 895–900.

Cousins, A.P., Holmgren, T., Remberger, M., 2014. Emissions of two phthalate esters and BDE 209 to indoor air and their impact on urban air quality. Sci. Total Environ. 470–471, 527–535. https://doi.org/10.1016/J.SCITOTENV.2013.10.023

Cousins, I., Mackay, D., 2000. Correlating the physical–chemical properties of phthalate esters using the `three solubility' approach. Chemosphere 41, 1389–1399. https://doi.org/10.1016/S0045-6535(00)00005-9

Cullen, E., Evans, D., Griffin, C., Burke, P., Mannion, R., Burns, D., Flanagan, A., Kellegher, A., Schoeters, G., Govarts, E., Biot, P., Casteleyn, L., Castaño, A., Kolossa-Gehring, M., Esteban, M., Schwedler, G., Koch, H.M., Angerer, J., Knudsen, L.E., Joas, R., Joas, A., Dumez, B., Sepai, O., Exley, K., Aerts, D., 2017. Urinary Phthalate Concentrations in Mothers and Their Children in Ireland: Results of the DEMOCOPHES Human Biomonitoring Study. International Journal of Environmental Research and Public Health 14, 1456. https://doi.org/10.3390/ijerph14121456

Dargnat, C., Teil, M.-J.J., Chevreuil, M., Blanchard, M., 2009. Phthalate removal throughout wastewater treatment plant. Case study of Marne Aval station (France). Sci. Total Environ. 407, 1235–44. https://doi.org/10.1016/j.scitotenv.2008.10.027

Del Carlo, M., Pepe, A., Sacchetti, G., Compagnone, D., Mastrocola, D., Cichelli, A., 2008. Determination of phthalate esters in wine using solid-phase extraction and gas chromatography-mass spectrometry. Food Chem. 111, 771–777. https://doi.org/10.1016/j.foodchem.2008.04.065

Deshayes, S., Eudes, V., Bigourie, M., Droguet, C., Moilleron, R., 2017. Alkylphenol and phthalate contamination of all sources of greywater from French households. Sci. Total Environ. 599–600, 883–890. https://doi.org/10.1016/j.scitotenv.2017.05.038

Dewalque, L., Charlier, C., Pirard, C., 2014. Estimated daily intake and cumulative risk assessment of phthalate diesters in a Belgian general population. Toxicol. Lett. 231, 161–168. https://doi.org/10.1016/j.toxlet.2014.06.028

Dewalque, L., Pirard, C., Vandepaer, S., Charlier, C., 2015. Temporal variability of urinary concentrations of phthalate metabolites, parabens and benzophenone-3 in a Belgian adult population. Environ. Res. 142, 414–423. https://doi.org/10.1016/j.envres.2015.07.015

Du, P., Zhou, Z., Huang, H., Han, S., Xu, Z., Bai, Y., Li, X., 2018. Estimating population exposure to phthalate esters in major Chinese cities through wastewater-based epidemiology. Sci. Total Environ. 643, 1602–1609. https://doi.org/10.1016/j.scitotenv.2018.06.325

Du, X., Li, X., Luo, T., Matsuur, N., Kadokami, K., Chen, J., 2013. Occurrence and Aquatic Ecological Risk Assessment of Typical Organic Pollutants in Water of Yangtze River Estuary. Procedia Environ. Sci. 18, 882–889. https://doi.org/10.1016/j.proenv.2013.04.119

ECHA, 2020. Benzylbutyl Phthalate - Registration Dossier - ECHA. [WWW document] URL https://echa.europa.eu/registration-dossier/-/registered-dossier/12721/6/1 (Accessed 8 May 2020).

ECHA, 2020. Dibutyl Phthalate - Registration Dossier - ECHA. [WWW document] URL https://echa.europa.eu/registration-dossier/-/registered-dossier/14862/6/1 (Accessed 8 May 2020).

ECHA, 2020. Diethylhexyl Phthalate - Registration Dossier - ECHA. [WWW document] URL https://echa.europa.eu/registration-dossier/-/registered-dossier/15358/6/1 (Accessed 8 May 2020).

ECHA, 2020. Diisobutyl Phthalate - Registration Dossier - ECHA. [WWW document] URL https://echa.europa.eu/registration-dossier/-/registered-dossier/13519/6/1 (Accessed 8 May 2020).

ECHA, 2020. Diisononyl Phthalate - Registration Dossier - ECHA. [WWW document] URL https://echa.europa.eu/registration-dossier/-/registered-dossier/14959/6/1 (Accessed 8 May 2020).

ECHA, 2019. Endocrine disrupting properties to be added for four phthalates in the Authorisation List - All news - ECHA [WWW Document]. URL https://echa.europa.eu/-/endocrine-disrupting-properties-to-be-added-for-four-phthalates-in-the-authorisation-list (accessed 28 January 2020).

Ejaredar, M., Nyanza, E.C., Ten Eycke, K., Dewey, D., 2015. Phthalate exposure and childrens neurodevelopment: A systematic review. Environ. Res. 142, 51–60. https://doi.org/10.1016/j.envres.2015.06.014

Engel, S.M., Zhu, C., Berkowitz, G.S., Calafat, A.M., Silva, M.J., Miodovnik, A., Wolff, M.S., 2009. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. Neurotoxicology 30, 522–528. https://doi.org/10.1016/j.neuro.2009.04.001

Engel, A., Buhrke, T., Imber, F., Jessel, S., Seidel, A., Völkel, W., Lampen, A., 2017. Agonistic and antagonistic effects of phthalates and their urinary metabolites on the steroid hormone receptors ERα, ERβ, and AR. Toxicology Letters 277, 54–63. https://doi.org/10.1016/j.toxlet.2017.05.028

Environment Agency, 2018. Performance standard for laboratories carrying out testing of samples from stack emissions monitoring, December 2018 Version 3 Environmental Protection Agency/ FDA, 2012. 40 CFR Part 721. Fed Reg 77, 15–18.

European Commission, 2018. Commission regulation (EU) 2018/2005 of 17 December 2018 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and diisobutyl phthalate (DIBP)

European Commission, 2015. Commision delegated directive (EU) 2015/863 of 31 March 2015 amending Annex II to Directive 2011/65/EU of the European Parliament and of the Council as regards the list of restricted substances. Off. J. Eur. Union L137, 10–12.

European Commission, 2006. Regulation (EC) 1907/2006 of the European Parliament and of teh Council of 18 December 2006 - REACH. Off. J. Eur. Union 396–849. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:396:0001:0849:EN:PDF

European Parliament, 2005. Directive 2005/84/EC Of The European Parliament And The Council. Off. J. Eur. Union 344, 40–43.

Fang, C. -r., Long, Y. -y., Shen, D. -s., 2009. Comparison on the removal of phthalic acid diesters in a bioreactor landfill and a conventional landfill. Bioresour. Technol. 100, 5664–5670. https://doi.org/10.1016/j.biortech.2009.06.039

Ferguson, K.K., McElrath, T.F., Ko, Y.-A., Mukherjee, B., Meeker, J.D., 2014. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. Environ. Int. 70, 118–124. https://doi.org/10.1016/j.envint.2014.05.016

Ferguson, Kelly K., et al. “Associations between Maternal Biomarkers of Phthalate Exposure and Inflammation Using Repeated Measurements across Pregnancy.” *PloS One*, vol. 10, no. 8, 2015, p. e0135601, doi:10/gg9q2v.

Fernández-Amado, M., Prieto-Blanco, M.C., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., 2017. A comparative study of extractant and chromatographic phases for the rapid and sensitive determination of six phthalates in rainwater samples. Chemosphere 175, 52–65. https://doi.org/10.1016/j.chemosphere.2017.02.001

Fernández-González, V., Moscoso-Pérez, C., Muniategui-Lorenzo, S., López-Mahía, P., Prada-Rodríguez, D., 2017. Reliable, rapid and simple method for the analysis of phthalates in sediments by ultrasonic solvent extraction followed by head space-solid phase microextraction gas chromatography mass spectrometry determination. Talanta 162, 648–653. https://doi.org/10.1016/j.talanta.2016.10.068

Frederiksen, H., Skakkebaek, N.E., Andersson, A.-M., 2007a. Metabolism of phthalates in humans. Mol. Nutr. Food Res. 51, 899–911. https://doi.org/10.1002/mnfr.200600243

Frederiksen, H., Skakkebaek, N.E., Andersson, A.-M., 2007b. Metabolism of phthalates in humans. Mol. Nutr. Food Res. 51, 899–911. https://doi.org/10.1002/mnfr.200600243

Fromme, H., Bolte, G., Koch, H.M., Angerer, J., Boehmer, S., Drexler, H., Mayer, R., Liebl, B., 2007. Occurrence and daily variation of phthalate metabolites in the urine of an adult population. Int. J. Hyg. Environ. Health 210, 21–33. https://doi.org/10.1016/j.ijheh.2006.09.005

Gao, M., Dong, Y., Zhang, Z., Song, Z., 2019. Metabolism and distribution of dibutyl phthalate in wheat grown on different soil types. Chemosphere 236, 124293. https://doi.org/10.1016/j.chemosphere.2019.07.024

Gao, D.-W., Wen, Z.-D., 2016. Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Sci. Total Environ. 541, 986–1001. https://doi.org/10.1016/j.scitotenv.2015.09.148

Gascon, Mireia, et al. "Prenatal Exposure to Bisphenol A and Phthalates and Childhood Respiratory Tract Infections and Allergy." *The Journal of Allergy and Clinical Immunology*, vol. 135, no. 2, Feb. 2015, pp. 370–78, doi:10/f25n6j.

Ghosh, P., Gupta, A., Thakur, I.S., 2015. Combined chemical and toxicological evaluation of leachate from municipal solid waste landfill sites of Delhi, India. Environ. Sci. Pollut. Res. Int. 22, 9148–9158. https://doi.org/10.1007/s11356-015-4077-7

Gonzalez-Marino, I., Rodil, R., Barrio, I., Cela, R., Quintana, J.B., 2017. Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.6b05612

Goodman, M., Lakind, J.S., Mattison, D.R., 2014. Do phthalates act as obesogens in humans? A systematic review of the epidemiological literature. Crit. Rev. Toxicol. 44, 151–175. https://doi.org/10.3109/10408444.2013.860076

Gray, L.E., Ostby, J., Furr, J., Wolf, C.J., Lambright, C., Parks, L., Veeramachaneni, D.N., Wilson, V., Price, M., Hotchkiss, A., Orlando, E., Guillette, L., 2001. Effects of environmental antiandrogens on reproductive development in experimental animals. Hum. Reprod. Update 7, 248–264.

Guidotti, M., Giovinazzo, R., Cedrone, O., Vitali, M., 2000. Determination of organic micropollutants in rain water for laboratory screening of air quality in urban environment. Environ. Int. 26, 23–8. https://doi.org/10.1016/S0160-4120(00)00074-X

Halden, R.U., 2010. Plastics and Health Risks. Annu. Rev. Public Health 31, 179–194. https://doi.org/10.1146/annurev.publhealth.012809.103714

Hauser, R., 2006. The environment and male fertility: recent research on emerging chemicals and semen quality. Semin. Reprod. Med. 24, 156–167. https://doi.org/10.1055/s-2006-944422

Herrero, L., Calvarro, S., Fernández, M.A., Quintanilla-López, J.E., González, M.J., Gómara, B., 2015a. Feasibility of ultra-high performance liquid and gas chromatography coupled to mass spectrometry for accurate determination of primary and secondary phthalate

metabolites in urine samples. Anal. Chim. Acta 853, 625–636. https://doi.org/10.1016/j.aca.2014.09.043

Herrero, L., Calvarro, S., Fernández, M.A., Quintanilla-López, J.E., González, M.J., Gómara, B., 2015b. Feasibility of ultra-high performance liquid and gas chromatography coupled to mass spectrometry for accurate determination of primary and secondary phthalate metabolites in urine samples. Anal. Chim. Acta 853, 625–636. https://doi.org/10.1016/j.aca.2014.09.043

Högberg, J., Hanberg, A., Berglund, M., Skerfving, S., Remberger, M., Calafat, A.M., Filipsson, A.F., Jansson, B., Johansson, N., Appelgren, M., Håkansson, H., 2008. Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. Environ. Health Perspect. 116, 334–339. https://doi.org/10.1289/ehp.10788

James-Todd, Tamarra M., et al. "The Association between Phthalates and Metabolic Syndrome: The National Health and Nutrition Examination Survey 2001-2010." *Environmental Health: A Global Access Science Source*, vol. 15, Apr. 2016, p. 52, doi:10/f92zn

Jensen, M.S., Anand-Ivell, R., Nørgaard-Pedersen, B., Jönsson, B.A.G., Bonde, J.P., Hougaard, D.M., Cohen, A., Lindh, C.H., Ivell, R., Toft, G., 2015. Amniotic fluid phthalate levels and male fetal gonad function. Epidemiol. Camb. Mass 26, 91–99. https://doi.org/10.1097/EDE.0000000000000198

Jeong, J.Y., Lee, J.H., Kim, E.Y., Kim, P.G., Kho, Y.L., 2011. Determination of Phthalate Metabolites in Human Serum and Urine as Biomarkers for Phthalate Exposure Using Column-Switching LC-MS/MS. Saf. Health Work 2, 57–64. https://doi.org/10.5491/SHAW.2011.2.1.57

Jia, L.L., Lou, X.Y., Guo, Y., Leung, K.S.Y., Zeng, E.Y., 2017. Occurrence of phthalate esters in over-the-counter medicines from China and its implications for human exposure. Environ. Int. 98, 137–142. https://doi.org/10.1016/j.envint.2016.10.025

Kato, K., Silva, M.J., Needham, L.L., Calafat, A.M., 2005. Determination of 16 phthalate metabolites in urine using automated sample preparation and on-line preconcentration/high-performance liquid chromatography/tandem mass spectrometry. Anal. Chem. 77, 2985–2991. https://doi.org/10.1021/ac0481248

Kay, V.R., Bloom, M.S., Foster, W.G., 2014. Reproductive and developmental effects of phthalate diesters in males. Crit. Rev. Toxicol. 44, 467–498. https://doi.org/10.3109/10408444.2013.875983

Khan, A.I., 2014. Determination of Phthalate Esters in Soft Drinks By GC-MS. Thermo Sci. Appl. Note.

Kim, B.-N., Cho, S.-C., Kim, Y., Shin, M.-S., Yoo, H.-J., Kim, J.-W., Yang, Y.H., Kim, H.-W., Bhang, S.-Y., Hong, Y.-C., 2009. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol. Psychiatry 66, 958–963. https://doi.org/10.1016/j.biopsych.2009.07.034

Kim, M., Song, N.R., Choi, J.-H., Lee, J., Pyo, H., 2014. Simultaneous analysis of urinary phthalate metabolites of residents in Korea using isotope dilution gas chromatography-mass spectrometry. Sci. Total Environ. 470–471, 1408–13. https://doi.org/10.1016/j.scitotenv.2013.07.037

Kim, Sunmi, Lee, J., Park, J., Kim, H.-J., Cho, G., Kim, G.-H., Eun, S.-H., Lee, J.J., Choi, G., Suh, E., Choi, S., Kim, Sungjoo, Kim, Y.D., Kim, S.K., Kim, S.Y., Kim, Seunghyo, Eom, S., Moon, H.-B., Kim, Sungkyoon, Choi, K., 2015. Concentrations of phthalate

metabolites in breast milk in Korea: Estimating exposure to phthalates and potential risks among breast-fed infants. Science of The Total Environment 508, 13–19. https://doi.org/10.1016/j.scitotenv.2014.11.019

Kim, Sunmi, Eom, S., Kim, H.-J., Lee, J.J., Choi, G., Choi, S., Kim, Sungjoo, Kim, S.Y., Cho,G., Kim, Y.D., Suh, E., Kim, S.K., Kim, Seunghyo, Kim, G.-H., Moon, H.-B., Park, J., Kim, Sungkyoon, Choi, K., Eun, S.-H., 2018. Association between maternal exposure to major phthalates, heavy metals, and persistent organic pollutants, and the neurodevelopmental performances of their children at 1 to 2years of age- CHECK cohort study. Science of The Total Environment 624, 377–384. https://doi.org/10.1016/j.scitotenv.2017.12.058

Kinnings, S.L., Liu, N., Buchmeier, N., Tonge, P.J., Xie, L., Bourne, P.E., 2009. Drug Discovery Using Chemical Systems Biology: Repositioning the Safe Medicine Comtan to Treat Multi-Drug and Extensively Drug Resistant Tuberculosis. PLoS Comput. Biol. 5, e1000423. https://doi.org/10.1371/journal.pcbi.1000423

Koch, H.M., Drexler, H., Angerer, J., 2003a. An estimation of the daily intake of di(2-ethylhexyl)phthalate (DEHP) and other phthalates in the general population. Int. J. Hyg. Environ. Health 206, 77–83. https://doi.org/10.1078/1438-4639-00205

Koch, H.M., Gonzalez-Reche, L.M., Angerer, J., 2003b. On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine. J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci. 784, 169–182

Kohn, M.C., Parham, F., Masten, S.A., Portier, C.J., Shelby, M.D., Brock, J.W., Needham, L.L., 2000. Human exposure estimates for phthalates. Environ. Health Perspect. 108, A440-442.

Kratochvil, I., Hofmann, T., Rother, S., Schlichting, R., Moretti, R., Scharnweber, D., Hintze, V., Escher, B.I., Meiler, J., Kalkhof, S., von Bergen, M., 2019. MEHP and MEOHP but not DEHP bind productively to the peroxisome proliferator-activated receptor γ. Rapid Commun Mass Spectrom 33, 75–85. https://doi.org/10.1002/rcm.8258

Latini, G., Scoditti, E., Verrotti, A., De Felice, C., Massaro, M., 2008. Peroxisome Proliferator-Activated Receptors as Mediators of Phthalate-Induced Effects in the Male and Female Reproductive Tract: Epidemiological and Experimental Evidence. PPAR Res 2008. https://doi.org/10.1155/2008/359267

Lee, Y.-S., Lee, S., Lim, J.-E., Moon, H.-B., 2019. Occurrence and emission of phthalates and non-phthalate plasticizers in sludge from wastewater treatment plants in Korea. Science of The Total Environment 692, 354–360. https://doi.org/10.1016/j.scitotenv.2019.07.301

Li, J., Gao, J., Thai, P.K., Sun, X., Mueller, J.F., Yuan, Z., Jiang, G., 2018. Stability of Illicit Drugs as Biomarkers in Sewers: From Lab to Reality. Environ. Sci. Technol. 52, 1561–1570. https://doi.org/10.1021/acs.est.7b05109

Li, X., Zhong, M., Xu, S., Sun, C., 2006. Determination of phthalates in water samples using polyaniline-based solid-phase microextraction coupled with gas chromatography. J. Chromatogr. A 1135, 101–8. https://doi.org/10.1016/j.chroma.2006.09.051

Liang, D.-W., Zhang, T., Fang, H.H.P., 2007. Anaerobic degradation of dimethyl phthalate in wastewater in a UASB reactor. Water Res. 41, 2879–2884. https://doi.org/10.1016/j.watres.2007.03.043

Lomenick, J.P., Calafat, A.M., Melguizo Castro, M.S., Mier, R., Stenger, P., Foster, M.B., Wintergerst, K.A., 2010. Phthalate exposure and precocious puberty in females. J. Pediatr. 156, 221–225. https://doi.org/10.1016/j.jpeds.2009.09.047

López-Carrillo, L., Hernández-Ramírez, R.U., Calafat, A.M., Torres-Sánchez, L., Galván-Portillo, M., Needham, L.L., Ruiz-Ramos, R., Cebrián, M.E., 2010. Exposure to phthalates and breast cancer risk in northern Mexico. Environ. Health Perspect. 118, 539–544. https://doi.org/10.1289/ehp.0901091

López-Roldán, P., López De Alda, M.J., Barceló, D., 2004. Simultaneous determination of selected endocrine disrupters (pesticides, phenols and phthalates) in water by in-field solid-phase extraction (SPE) using the prototype PROFEXS followed by on-line SPE (PROSPEKT) and analysis by liquid chromatography-atmosph. Anal. Bioanal. Chem. 378, 599–609. https://doi.org/10.1007/s00216-003-2187-4

Luks-Betlej, K., Popp, P., Janoszka, B., Paschke, H., 2001. Solid-phase microextraction of phthalates from water. J. Chromatogr. A 938, 93–101. https://doi.org/10.1016/S0021-9673(01)01363-2

Ma, T., Zhou, W., Chen, L., Li, Y., Luo, Y., Wu, P., 2020. Phthalate esters contamination in vegetable–soil system of facility greenhouses in Jingmen, central China and the assessment of health risk. Environ. Geochem. Health. https://doi.org/10.1007/s10653-019-00504-2

Mage, D.T., Allen, R.H., Kodali, A., 2008. Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. J. Expo. Sci. Environ. Epidemiol. 18, 360–368. https://doi.org/10.1038/sj.jes.7500614

Mann, C., 2003. Observational research methods. Research design II: cohort, cross sectional, and case-control studies. Emerg. Med. J. EMJ 20, 54–60. https://doi.org/10.1136/emj.20.1.54

Marega, M., Grob, K., Moret, S., Conte, L., 2013. Phthalate analysis by gas chromatography-mass spectrometry: Blank problems related to the syringe needle. J. Chromatogr. A 1273, 105–110. https://doi.org/10.1016/j.chroma.2012.11.076

Martens, F., Martens, M., 2002. [Determination of monoester metabolites of butylbenzyl phthalate (BBP) by GC-MS in the urine of exposed workers]. Acta Clin. Belg. 57 Suppl 1, 16–23.

Milojković, D.S., Anđelković, D.H., Kocić, G.M., Anđelković, T.D., 2015. Evaluation of a method for phthalate extraction from milk related to the milk dilution ratio. J Serb Chem Soc 80, 983–996. https://doi.org/10.2298/JSC141204028M

Mittermeier, A., Völkel, W., Fromme, H., 2016. Kinetics of the phthalate metabolites mono-2-ethylhexyl phthalate (MEHP) and mono-n-butyl phthalate (MnBP) in male subjects after a single oral dose. Toxicology Letters 252, 22–28. https://doi.org/10.1016/j.toxlet.2016.04.009

Mousa, A., Basheer, C., Rahman Al-Arfaj, A., 2013. Application of electro-enhanced solid-phase microextraction for determination of phthalate esters and bisphenol A in blood and seawater samples. Talanta 115, 308–313. https://doi.org/10.1016/J.TALANTA.2013.05.011

National Report on Human Exposure to Environmental Chemicals [WWW Document], 2018. URL https://www.cdc.gov/exposurereport/index.html (accessed 7.27.18).

Net, S., Delmont, A., Sempéré, R., Paluselli, A., Ouddane, 2015. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): A review. Sci. Total Environ. 515–516, 162–80. https://doi.org/10.1016/j.scitotenv.2015.02.013

Net, S., Sempéré, R., Delmont, A., Paluselli, A., Ouddane, B., 2015. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ.

Sci. Technol. 49, 4019–4035. https://doi.org/10.1021/es505233b

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2047–2062. https://doi.org/10.1098/rstb.2008.0242

Ormond, G., Nieuwenhuijsen, M.J., Nelson, P., Toledano, M.B., Iszatt, N., Geneletti, S., Elliott, P., 2009. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: case-control study. Environ. Health Perspect. 117, 303–307. https://doi.org/10.1289/ehp.11933

Parkerton, T.F., Konkel, W.J., 2000. Application of quantitative structure—activity relationships for assessing the aquatic toxicity of phthalate esters. Ecotoxicol. Environ. Saf. 45, 61–78. https://doi.org/10.1006/eesa.1999.1841

Peñalver, A., Pocurull, E., Borrull, F., Marcé, R.M., 2000. Determination of phthalate esters in water samples by solid-phase microextraction and gas chromatography with mass spectrometric detection. J. Chromatogr. A 872, 191–201. https://doi.org/10.1016/S0021-9673(99)01284-4

Pérez-Outeiral, J., Millán, E., Garcia-Arrona, R., 2016. Determination of phthalates in food simulants and liquid samples using ultrasound-assisted dispersive liquid-liquid microextraction followed by solidification of floating organic drop. Food Control 62, 171–177. https://doi.org/10.1016/j.foodcont.2015.10.016

Pham, N., Iyer, S., Hackett, E., Lock, B.H., Sandy, M., Zeise, L., Solomon, G., Marty, M., 2016. Using ToxCast to Explore Chemical Activities and Hazard Traits: A Case Study with Ortho-Phthalates. Toxicol Sci 151, 286–301.https://doi.org/10.1093/toxsci/kfw049

Philippat, C., Bennett, D.H., Krakowiak, P., Rose, M., Hwang, H.-M., Hertz-Picciotto, I., 2015. Phthalate concentrations in house dust in relation to autism spectrum disorder and developmental delay in the CHildhood Autism Risks from Genetics and the Environment (CHARGE) study. Environ. Health Glob. Access Sci. Source 14, 56. https://doi.org/10.1186/s12940-015-0024-9

Polyzos, S.A., Kountouras, J., Deretzi, G., Zavos, C., Mantzoros, C.S., 2012. The emerging role of endocrine disruptors in pathogenesis of insulin resistance: a concept implicating nonalcoholic fatty liver disease. Curr. Mol. Med. 12, 68–82.

Porta, M., 2008. A dictionary of Epidemiology, 5th ed. New York: Oxford University Press.

Ramesh Kumar, A., Sivaperumal, P., 2016. Analytical methods for the determination of biomarkers of exposure to phthalates in human urine samples. TrAC Trends Anal. Chem. 75, 151–161. https://doi.org/10.1016/j.trac.2015.06.008

Reid, A.M., Brougham, C.A., Fogarty, A.M., Roche, J.J., 2009. Accelerated solvent-based extraction and enrichment of selected plasticisers and 4-nonylphenol, and extraction of tin from organotin sources in sediments, sludges and leachate soils. Analytica Chimica Acta 634, 197–204. https://doi.org/10.1016/j.aca.2008.12.032

Roslev, P., Vorkamp, K., Aarup, J., Frederiksen, K., Nielsen, P.H.H., 2007. Degradation of phthalate esters in an activated sludge wastewater treatment plant. Water Res. 41, 969–76. https://doi.org/10.1016/j.watres.2006.11.049

Ryu, Y., Barcelo, D., Barron, L.P., Bijlsma, L., Castiglioni, S., de Voogt, P., Emke, E., Hernandez, F.,Lai, F.Y., Lopes, A., Lopez de Alda, M., Mastroianni, N., Munro, K., O'Brien, J., Ort, C., Plosz, B.G., Reid, M.J., Yargeau, V., Thomas, K.V., 2016. Comparative measurement and quantitative risk assessment of alcohol consumption

through wastewater-based epidemiology: An international study in 20 cities. Sci. Total Environ. 565, 977–983. https://doi.org/10.1016/j.scitotenv.2016.04.138

Saravanabhavan, G., Murray, J., Saravanabhavan, G., Murray, J., 2012. Human Biological Monitoring of Diisononyl Phthalate and Diisodecyl Phthalate: A Review, Human Biological Monitoring of Diisononyl Phthalate and Diisodecyl Phthalate: A Review. J. Environ. Public Health J. Environ. Public Health 2012, 2012, e810501. https://doi.org/10.1155/2012/810501, 10.1155/2012/810501

Soto, A.M., Maffini, M.V., Schaeberle, C.M., Sonnenschein, C., 2006. Strengths and weaknesses of *in-vitro* assays for estrogenic and androgenic activity. Best Pract. Res. Clin. Endocrinol. Metab., Endocrine Disrupters 20, 15–33. https://doi.org/10.1016/j.beem.2005.09.001

Sun, X., Wang, W., Technologies, A., Jing, W., Lu, B., District, C.Y., 2014. Determination of 19 Phthalic Acid Esters (PAEs) in Vegetables Using Modified QuEChERS and Gas Chromatography-Triple Quadrupole Mass Spectrometry (GC/MS/MS). Agil. Appl. Note.

Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., Study for Future Families Research Team, 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ. Health Perspect. 113, 1056–1061.

Szumilas, M., 2010. Explaining Odds Ratios. J. Can. Acad. Child Adolesc. Psychiatry 19, 227–229.

Tienpont, B., 2004. Determination of Phthalates in Environmental, Food and Biomatrices - An Analytical Challenge.

Tienpont, B., David, F., Dewulf, E., Sandra, P., 2005. Pitfalls and Solutions for the Trace Determination of Phthalates in Water Samples. Chromatographia 61, 365–370. https://doi.org/10.1365/s10337-005-0516-9

Toft, G., Jönsson, B.A.G., Lindh, C.H., Jensen, T.K., Hjollund, N.H., Vested, A., Bonde, J.P., 2012. Association between pregnancy loss and urinary phthalate levels around the time of conception. Environ. Health Perspect. 120, 458–463. https://doi.org/10.1289/ehp.1103552

Trasande, L., Sathyanarayana, S., Spanier, A.J., Trachtman, H., Attina, T.M., Urbina, E.M., 2013. Urinary phthalates are associated with higher blood pressure in childhood. J. Pediatr. 163, 747-753.e1. https://doi.org/10.1016/j.jpeds.2013.03.072

Upson, Kristen, et al. "Phthalates and Risk of Endometriosis." *Environmental Research*, vol. 126, Oct. 2013, pp. 91–97, doi:10/f5fv87

US EPA, O., 2015. NATA: Glossary of Terms [WWW Document]. US EPA. URL https://www.epa.gov/national-air-toxics-assessment/nata-glossary-terms (accessed 7.29.18).

US-EPA, 2012. United States Environmental Protection Agency. U Environ. Prot. Agency 1–16. U.S. Consumer Product Safety Commission, 2014. CHRONIC HAZARD ADVISORY PANEL ON PHTHALATES AND PHTHALATE ALTERNATIVES. 27-29.

Valvi, D., Monfort, N., Ventura, R., Casas, M., Casas, L., Sunyer, J., Vrijheid, M., 2015. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. Int. J. Hyg. Environ. Health 218, 220–231. https://doi.org/10.1016/j.ijheh.2014.11.003

Vélez, Maria P., et al. "Female Exposure to Phenols and Phthalates and Time to Pregnancy: The Maternal-Infant Research on Environmental Chemicals (MIREC) Study." *Fertility and Sterility*, vol. 103, no. 4, Apr. 2015, pp. 1011-1020.e2, doi:10/f262g7

Viera, A.J., 2008. Odds ratios and risk ratios: what's the difference and why does it matter? South. Med. J. 101, 730–734.

Virtanen, H.E., Adamsson, A., 2012. Cryptorchidism and endocrine disrupting chemicals. Mol. Cell. Endocrinol., Health Impacts Of Endocrine Disrupters 355, 208–220. https://doi.org/10.1016/j.mce.2011.11.015

Vrijheid, M., Armstrong, B., Dolk, H., van Tongeren, M., Botting, B., 2003. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med 60, 543–550. https://doi.org/10.1136/oem.60.8.543

Wagner-Mahler, K., Kurzenne, J.-Y., Delattre, I., Bérard, E., Mas, J.-C., Bornebush, L., Tommasi, C., Boda-Buccino, M., Ducot, B., Boullé, C., Ferrari, P., Azuar, P., Bongain, A., Fénichel, P., Brucker-Davis, F., n.d. Prospective study on the prevalence and associated risk factors of cryptorchidism in 6246 newborn boys from Nice area, France. Int. J. Androl. 34, e499–e510.

Wang, I. Jen, and Wilfried J. J. Karmaus. "Oxidative Stress-Related Genetic Variants May Modify Associations of Phthalate Exposures with Asthma." *International Journal of Environmental Research and Public Health*, vol. 14, no. 2, 08 2017, doi:10/f9xx6m.

Wang, Lijun, Liu, M., Tao, W., Zhang, W., Wang, Li, Shi, X., Lu, X., Li, X., 2018. Pollution characteristics and health risk assessment of phthalate esters in urban soil in the typical semi-arid city of Xi'an, Northwest China. Chemosphere 191, 467–476. https://doi.org/10.1016/j.chemosphere.2017.10.066

Wang, Y.-X., Zeng, Q., Sun, Y., Yang, P., Wang, P., Li, J., Huang, Z., You, L., Huang, Y.-H., Wang, C., Li, Y.-F., Lu, W.-Q., 2016. Semen phthalate metabolites, semen quality parameters and serum reproductive hormones: A cross-sectional study in China. Environ. Pollut. 211, 173–182.

Watkins, Deborah J., et al. "In Utero and Peripubertal Exposure to Phthalates and BPA in Relation to Female Sexual Maturation." *Environmental Research*, vol. 134, Oct. 2014, pp. 233–41, doi:10/f6tvbp

Watkins, Deborah J., et al. "Phthalate and Bisphenol A Exposure during in Utero Windows of Susceptibility in Relation to Reproductive Hormones and Pubertal Development in Girls." *Environmental Research*, vol. 159, 2017, pp. 143–51, doi:10/gchznp

Weuve, Jennifer, et al. "Association of Exposure to Phthalates with Endometriosis and Uterine Leiomyomata: Findings from NHANES, 1999-2004." *Environmental Health Perspectives*, vol. 118, no. 6, June 2010, pp. 825–32, doi:10/djvwvg

Whyatt, R.M., Liu, X., Rauh, V.A., Calafat, A.M., Just, A.C., Hoepner, L., Diaz, D., Quinn, J., Adibi, J., Perera, F.P., Factor-Litvak, P., 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ. Health Perspect. 120, 290–295. https://doi.org/10.1289/ehp.1103705

Wolff, M. S., et al. "Phthalate Exposure and Pubertal Development in a Longitudinal Study of US Girls." *Human Reproduction (Oxford, England)*, vol. 29, no. 7, July 2014, pp. 1558–66, doi:10/f58h4f

Yaghjyan, L., et al. "Associations of Urinary Phthalates with Body Mass Index, Waist Circumference and Serum Lipids among Females: National Health and Nutrition Examination Survey 1999-2004." *International Journal of Obesity (2005)*, vol. 39, no. 6, June 2015, pp. 994–1000, doi:10/f7fwtj

Yang, Z., d'Auriac, M.A., Goggins, S., Kasprzyk-Hordern, B., Thomas, K.V., Frost, C., Estrela, P., 2015. Sewage Biosensor to Identify Potential Population Biomarker for Monitoring of Public Health. Presented at the RSC Sensors-Bioanalytical Sensing Technologies.

Yi, H., et al. "A Pilot Study on Association between Phthalate Exposure and Missed Miscarriage." *European Review for Medical and Pharmacological Sciences*, vol. 20, no. 9, May 2016, pp. 1894–902.

You, L., Wang, Y.-X., Zeng, Q., Li, M., Huang, Y.-H., Hu, Y., Cao, W.-C., Liu, A.-L., Lu, W.-Q., 2015. Semen Phthalate Metabolites, Spermatozoa Apoptosis, and DNA Damage: A Cross-Sectional Study in China. Environ. Sci. Technol. 49, 3805–3812. https://doi.org/10.1021/acs.est.5b00588

Zhang, H., Zhang, Z., Nakanishi, T., Wan, Y., Hiromori, Y., Nagase, H., Hu, J., 2015. Structure-Dependent Activity of Phthalate Esters and Phthalate Monoesters Binding to Human Constitutive Androstane Receptor. Chem. Res. Toxicol. 28, 1196–1204. https://doi.org/10.1021/acs.chemrestox.5b00028

Zheng, Q.-D., Lin, J.-G., Pei, W., Guo, M.-X., Wang, Z., Wang, D.-G., 2017. Estimating nicotine consumption in eight cities using sewage epidemiology based on ammonia nitrogen equivalent population. Science of The Total Environment 590–591, 226–232. https://doi.org/10.1016/j.scitotenv.2017.02.214

Zhou, X., Cui, K., Zeng, F., Li, S., Zeng, Z., 2016. A simple and selective method for determination of phthalate biomarkers in vegetable samples by high pressure liquid chromatography-electrospray ionization-tandem mass spectrometry. Food Chem. 200, 336–342. https://doi.org/10.1016/j.foodchem.2016.01.008

Zhao, Ranran, et al. "The Risk of Missed Abortion Associated with the Levels of Tobacco, Heavy Metals and Phthalate in Hair of Pregnant Woman: A Case Control Study in Chinese Women." *Medicine*, vol. 96, no. 51, Dec. 2017, p. e9388, doi:10/ggkk4t

Zota, A.R., Calafat, A.M., Woodruff, T.J., 2014. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001-2010. Environ. Health Perspect. 122, 235–241. https://doi.org/10.1289/ehp.1306681

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating Community Drug Abuse by Wastewater Analysis. Environ. Health Perspect. 116, 1027–1032. https://doi.org/10.1289/ehp.11022

Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., Fanelli, R., 2005. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. Environ. Health 4, 14. https://doi.org/10.1186/1476-069X-4-14

Appendix A

Supplemental Method Development Figures

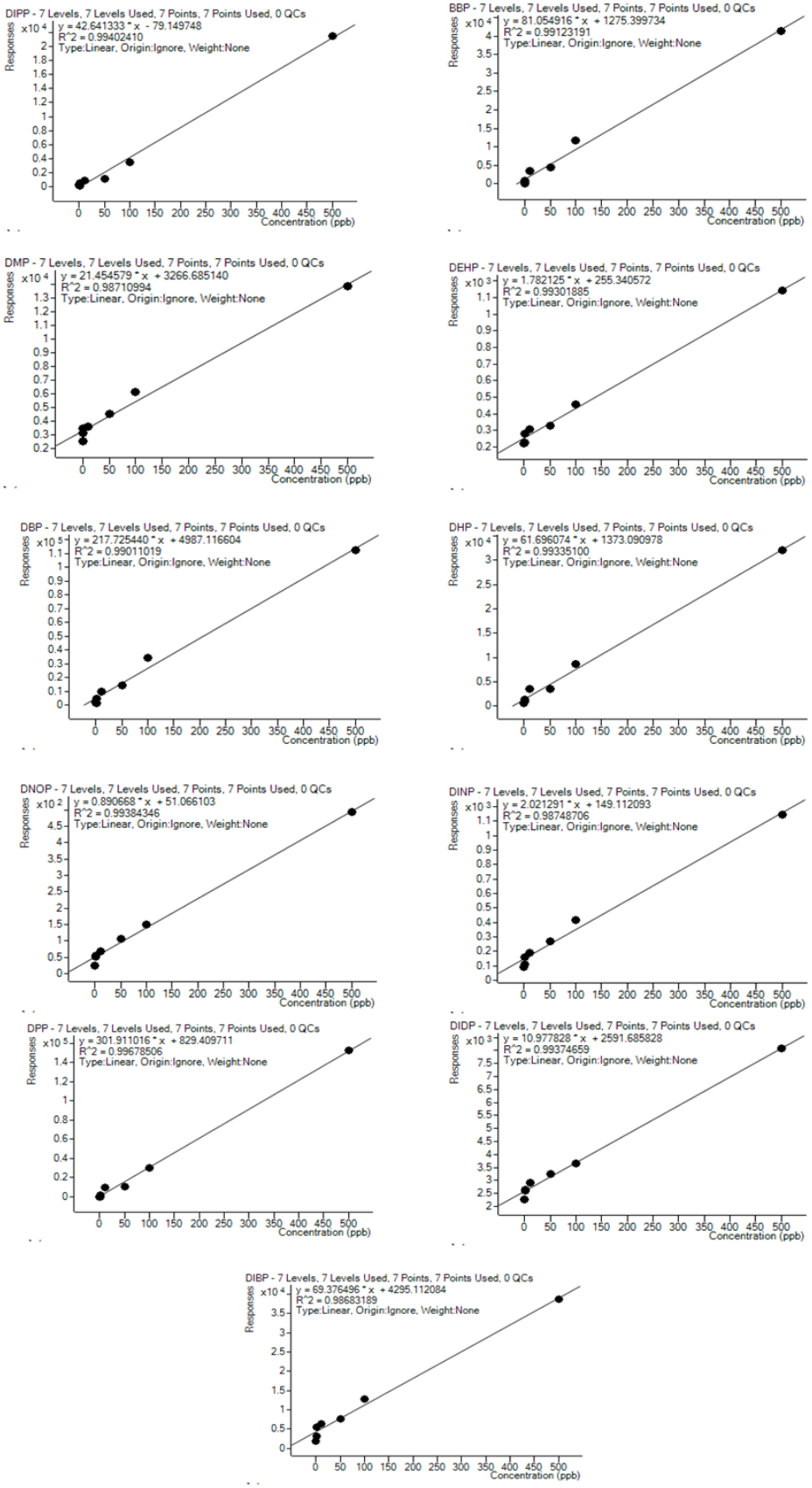


Figure A 1**: calibration lines of each phthalate in Masshunter software using MS conditions described in table 8 and chromatographic conditions described in table 11**

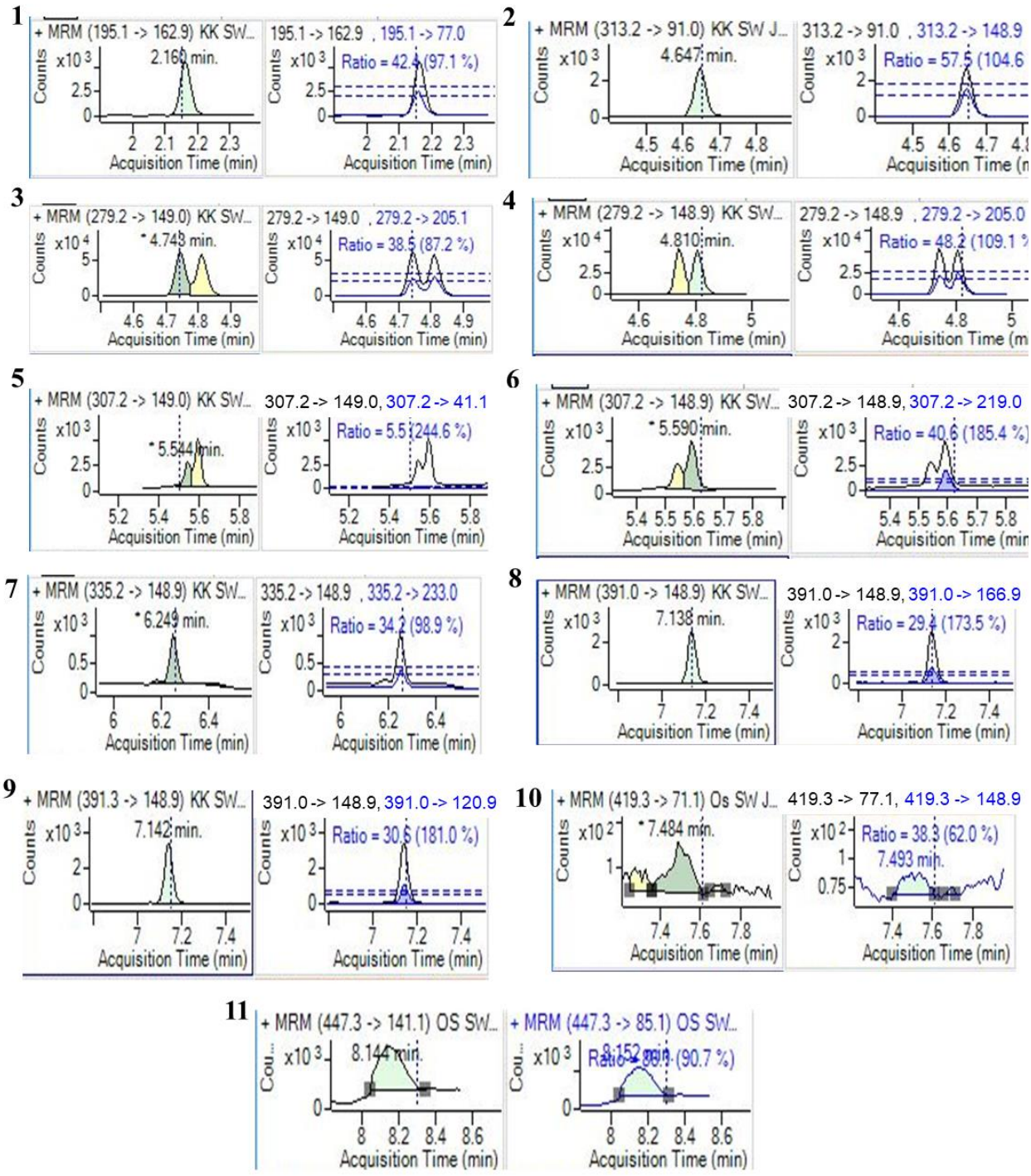


Figure A 2: Quantifying (left) and qualifying (right) transitions for each phthalate (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP in estuary surface water sample taken in January , spiked with 5 ppb internal standard. Refer to table 8

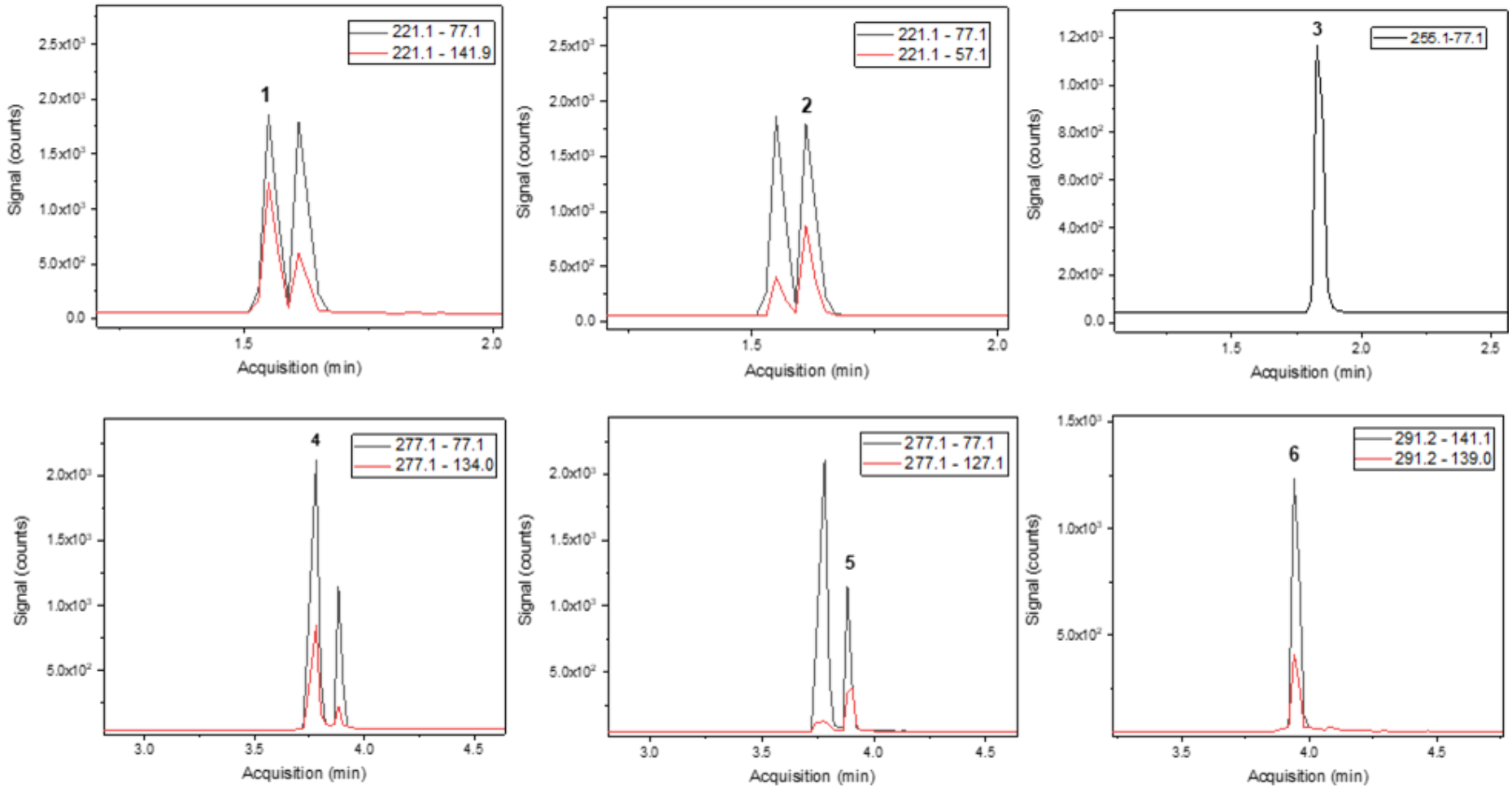


Figure A 3: Quantifying and qualifying transitions for each phthalate monoester (1) MBP, (2) MiBP, (3) MBzP, (4) MEHP, (5) MnOP and (6) MiNP

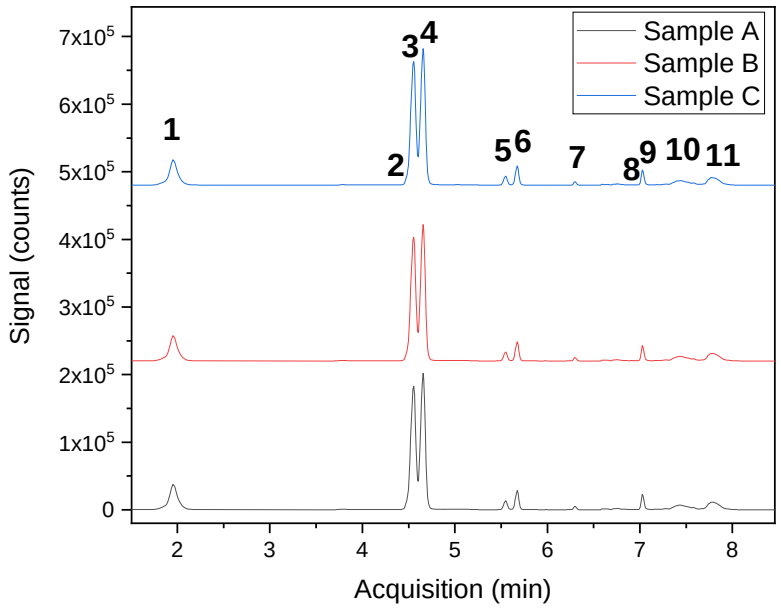


Figure A 4: MRM of recyclable waste for different sample sub-sets, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

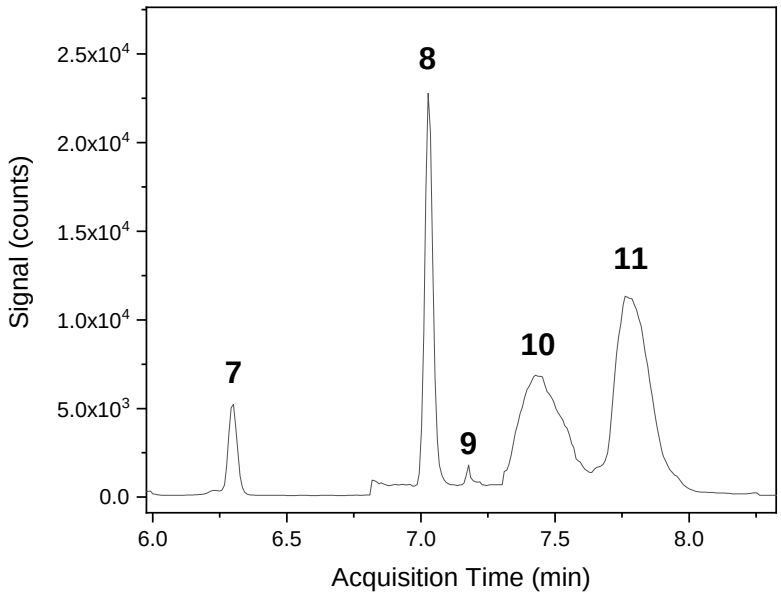


Figure A 5: Zoomed MRM of recyclable waste, spiked with IS. Showing low intensity peaks (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

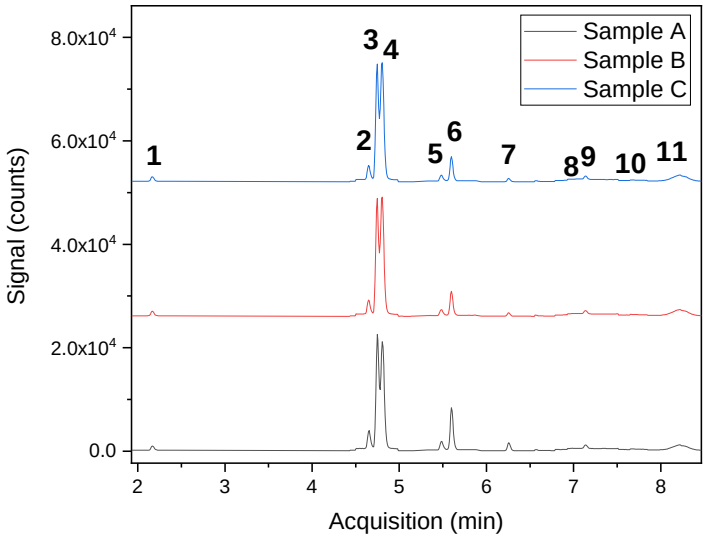


Figure A 6: MRM of food waste, from different sample sub-sets, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

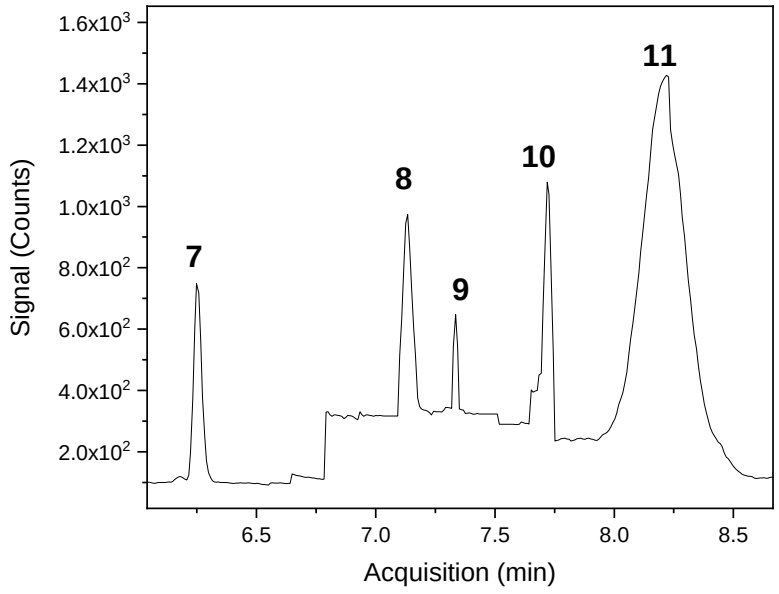


Figure A 7: Zoom of MRM of food waste, from different sample sub-sets, spiked with IS, tiled. Showing low intensity peaks (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

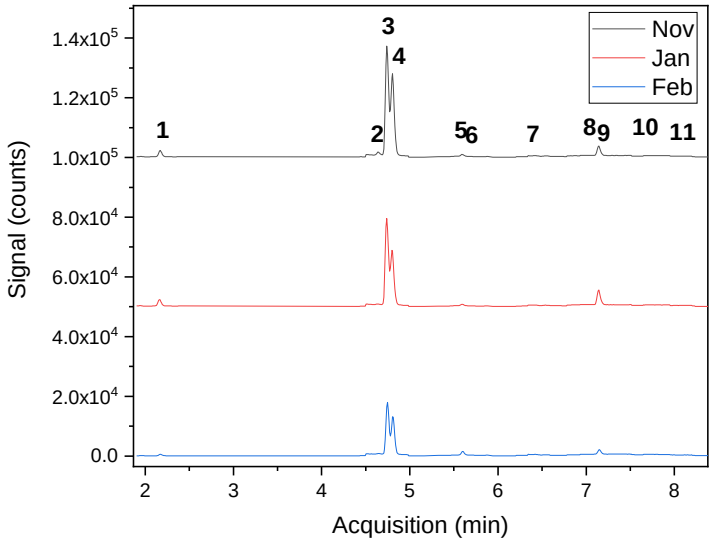


Figure A 8: MRM of different rural influent samples from November, January and February, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

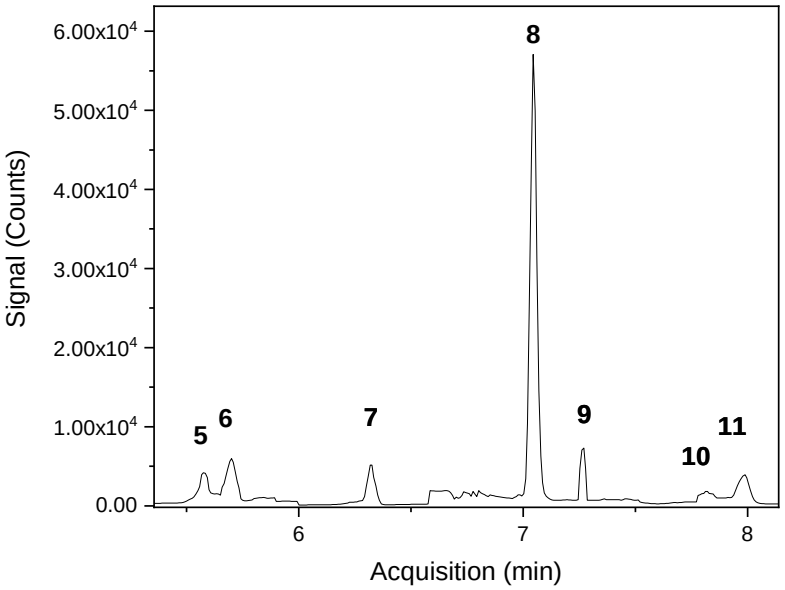


Figure A 9: Zoom MRM example of rural influent from November, spiked with IS. Showing low intensity peaks (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP, using MS conditions described in table 8 and chromatographic conditions described in table 11

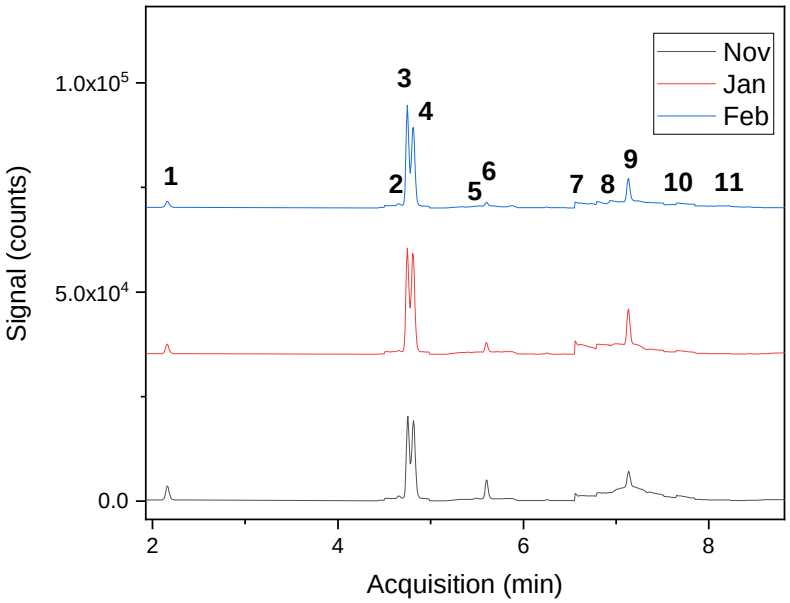


Figure A 10: MRM of different suburban sludge samples from November, January and February, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

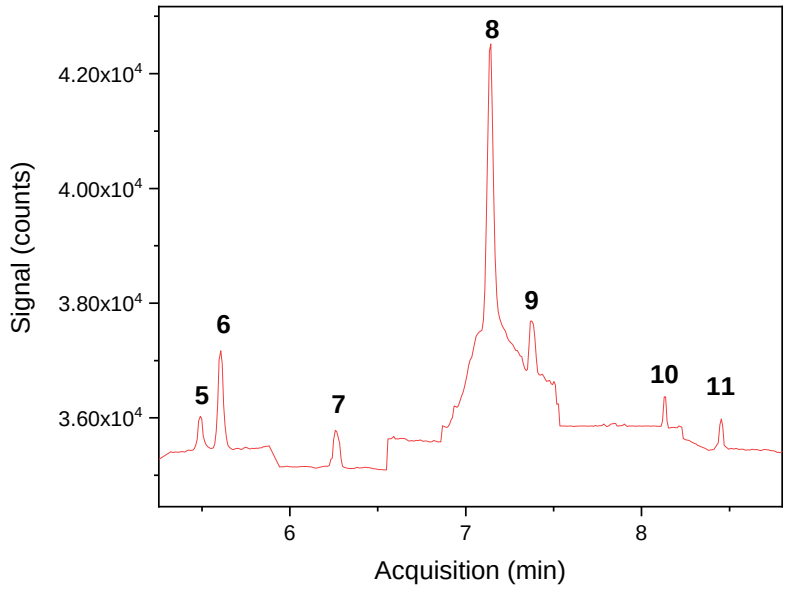


Figure A 11: Zoomed MRM example of suburban sludge from January, spiked with IS. Showing low intensity peaks (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

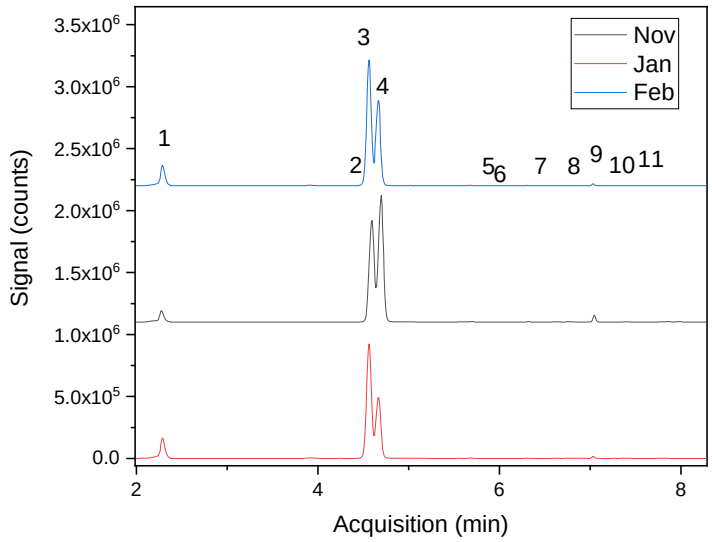


Figure A 12: MRM of different suburban effluent samples from November, January and February, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

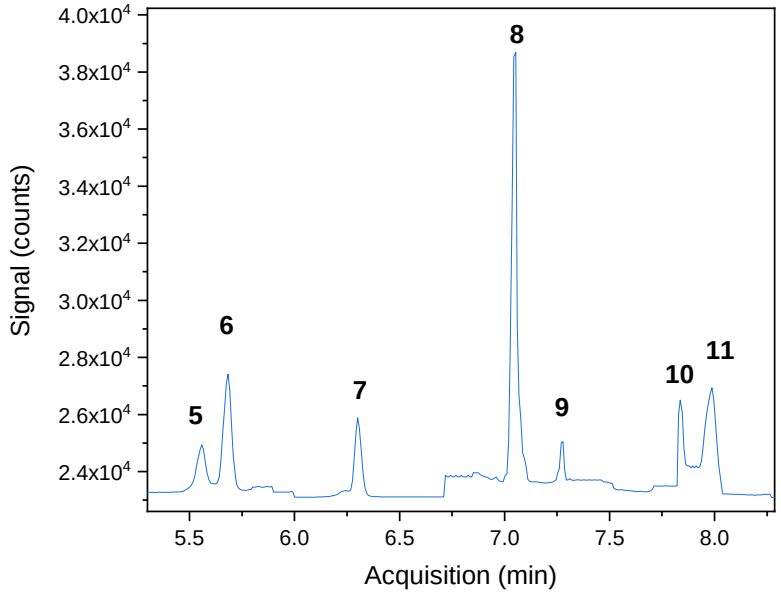


Figure A 13: Zoomed MRM of effluent for suburban samples from February, spiked with IS. Showing low intensity peaks (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

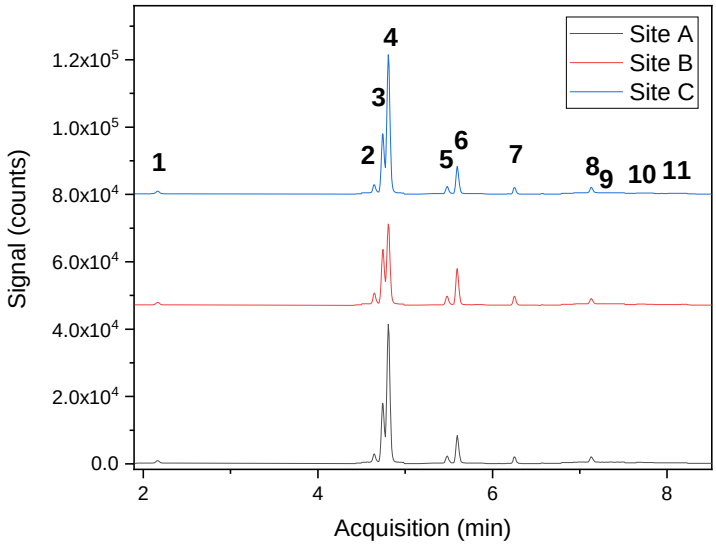


Figure A 14: MRM of three different urban parkland soil samples, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

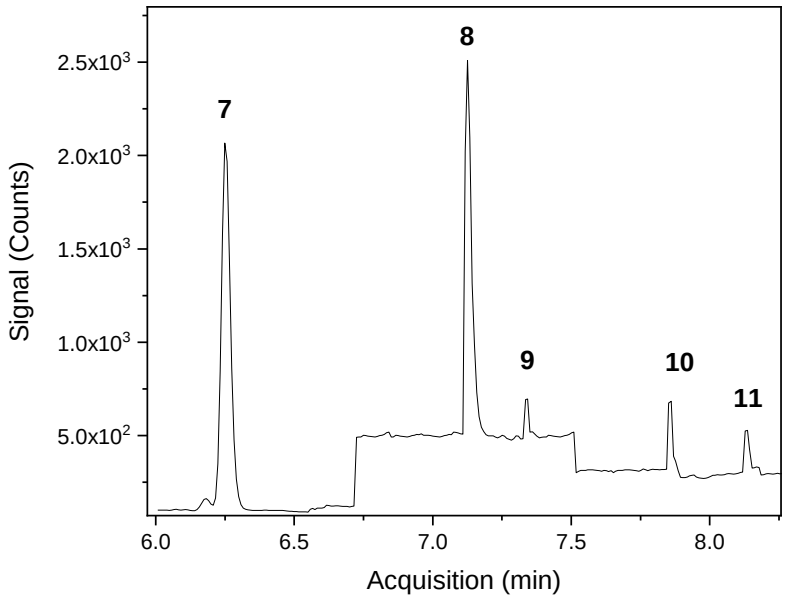


Figure A 15: Zoomed MRM of soil for urban parkland sample A, spiked with IS. Showing low intensity peaks, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

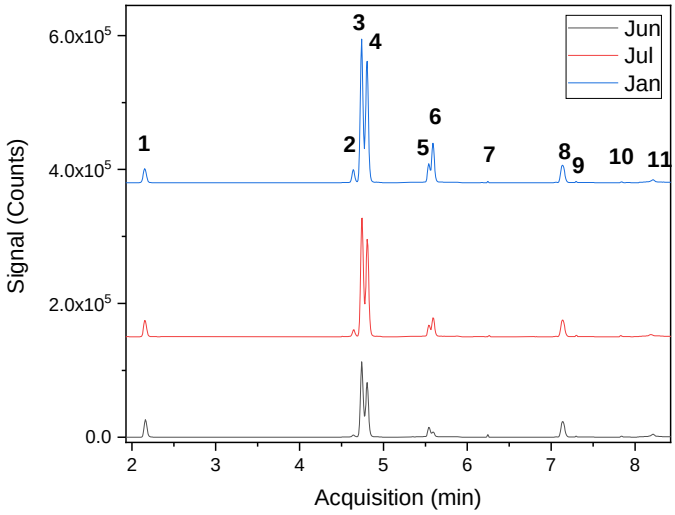


Figure A 16: MRM of different suburban surface water samples from June, July, and January, spiked with IS, tiled. Peaks showing (1) DMP, (2) BBP, (3) DiBP, (4) DBP, (5) DiPP, (6) DPP, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11

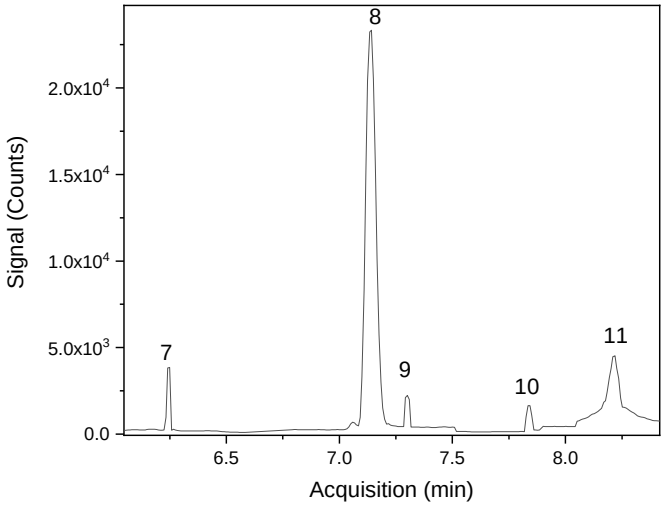


Figure A 17: Zoomed MRM of suburban surface water sample from June, spiked with IS, tiled. Showing example of low intensity peaks, (7) DHP, (8) DEHP, (9) DnOP, (10) DiNP, and (11) DiDP. Using MS conditions described in table 8 and chromatographic conditions described in table 11