DEPARTMENT OF TOXIC SUBSTANCES CONTROL

ENVIRONMENTAL CHEMISTRY LABORATORY

Mobile Laboratory Screening Method for Phthalate Esters in Children's Toys by Gas Chromatography/Mass Spectrometry

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Mobile Laboratory Screening Method for Phthalate Esters in Children's Toys by Gas Chromatography/Mass Spectrometry

Prepared By

Keh-Chuh Ting, Modan Gill and Orlando Garbin

Technical & Field Support Section
Environmental Chemistry Laboratory
California Dept. of Toxic Substances Control

2/11/09

Date

Bruce LaBelle, Ph.D.
Chief
Environmental Chemistry Laboratory
Department of Toxic Substances Control

EXECUTIVE SUMMARY

Phthalate esters are commonly added into PVC as softeners to make plastic material flexible. Phthalates are suspected cancer causing agents and possible teratogens which have been linked to liver and kidney damage, and the underdevelopment of reproductive organs in humans and animals. Public health concerns of human exposure to phthalates are on the rise as they do not chemically bond to PVC and may leach out over time.

Following in the European Union's and Japan's footsteps in restricting certain phthalates usage, a regulatory limit of 0.1% in children's toys was established by the California State Legislature (AB-1108). The Department of Toxic Substances Control (DTSC) has been delegated the role of lead agency for consumer product safety.

To support DTSC's Green Chemistry activities, ECL's Mobile Laboratory Team has developed an on-site screening method to monitor phthalates in children's toys. The method was first tested in a collaborative study with the San Francisco Department of the Environment who provided the samples. This method is simple, fast and effective with ample sensitivity to quantify the 6 phthalates in children's toys at 100 ppm (LOQ=100 ug/g) which is 10 times lower than the regulatory threshold of 0.1%. Additionally, this method has a high throughput capability and enables testing of approximately 6 to 10 samples per day depending on the complexity of the sample matrix and concentration. This method is designed to survey the 6 phthalates in children's toys and other consumer products for compliance with the threshold of 0.1% (1,000 ppm).

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ABBREVATIONS AND ACRONYMS

BBP = Benzyl butyl phthalate
DBP = Dibutyl phthalate
DEHP = Bis(2-ethylhexyl) phthalate
DIDP= (Di-isodecyl) phthalate
DINP = (Di-isononyl) phthalate
DNO = Di-n-octyl phthalate
GC/MS = Gas Chromatography/Mass Spectrometer
ISTD = internal standard
ML = Mobile Laboratory
PVC = Polyvinyl chloride [(CH2-CHCl)n]
STD = standard

LIST OF TABLES AND FIGURES

INTRODUCTION

Polyvinyl chloride or PVC [(CH2-CHCl)n] is widely used in toys, children's products and many other household items. PVC is generally hard and brittle, and different percentages of phthalate esters are commonly added to make the plastic material flexible. As a result, many soft PVC children's toys, chewing teethers, feeding and care products contain high concentrations of phthalate esters.

Due to public health concerns, the EU has set regulations to limit phthalates to 0.1% in toys (1). The City of San Francisco has also recently passed City Health Code Sections 34.1 to 34.6 to follow the EU standard in regulating children's products containing phthalates.

To support the Department of Toxic Substance Control (DTSC) in measurement of toxic chemicals in consumer products, this screening method was developed for use in the DTSC Mobile Laboratory (ML) to conduct on-site monitoring of toxic phthalates in consumer products. This method relies upon a streamlined sample preparation procedure, adjusted to accommodate smaller equipment, fewer apparatus and less manpower due to the ML's workspace limitations; however, the method is also suitable to be used in fixed laboratories.

Simple, quick and effective, this method enables the screening of phthalate esters in consumer products on site. The limit-of-detection (LOD) or detection limit (DL) is 100 ppm which is sensitive enough for screening purpose at the regulatory action level of 1,000 ppm or 0.1%.

MATERIALS AND METHODS

Materials and Reagents

1. Solvents: dichloromethane, n-hexane, pesticide grade, Thermo Fisher Scientific, Pittsburgh, PA).
2. Standards: dibutyl phthalate (DBP), di-n-octyl phthalate (DNOP) and diisodecyl phthalate (DIDP), Benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DINP), Restek Corporation, 110 Benner Circle, Bellefonte, PA 18823.
3. Internal Standard: bis(2-butoxyethyl) phthalate (DBEP), Restek Corporation, 110 Benner Circle, Bellefonte, PA 18823.
4. Surrogate: diethyl phthalate (DEP), Restek Corporation, 110 Benner Circle, Bellefonte, PA 18823.

Apparatus

The ML consists of one vehicle (Ford F-555), a satellite communication system and two gas chromatograph/mass spectrometers (GC/MS) specifically designed for detecting toxic industrial chemicals in air, soil and water. The advanced analytical instruments are complemented by a series of field instruments for the quick detection of volatile compounds, metals and other chemicals.

The GC/MS system used in this study was:

1. GC/MSD: Agilent 6850/5973 GC/MSD with ChemStation computer system.
2. Column: HP-5MS (5% phenyl methyl siloxane), 30m x 0.25mm x 0.25um.
3. Temperature program: rate 100°C/min., initial temperature 80°C for 1 min., temperature 250C° for 1 min., rate 10°C, temperature 320°C for 1 min., and run time 11.70 min. Injector temperature 250°C.
4. MSD setup: DFTPP tune, full scan mode (50-480 amu).
5. Valley-to-valley integration for DBEP (ISTD), DEP (surrogate), DBP, BBP, DNOP and DEHP and area-sum integration for DINP (8.5-9.9 min) and DIDP (9.6-10.6 min).

Standard and Sample Preparation

The following standards were prepared:

1. Internal standard – DBEP (Bis[2-butoxyethyl] phthalate), 1,000 ug/mL in dichloromethane.
2. Surrogate – DEP (Diethyl phthalate), 1,000 ug/mL in dichloromethane.
3. Standard mixture Group-1 – DBP (dibutyl phthalate), DNOP (di-n-octyl phthalate) and DIDP (diisodecyl phthalate), 1,000 ug/mL in dichloromethane.
4. Standard mixture Group-2 - BBP (Benzyl butyl phthalate), DEHP (bis(2-ethylhexyl) phthalate) and DINP (di-isononyl phthalate), 1,000 ug/mL in dichloromethane.
5. Standard linear curve quantitative levels –10, 50 100, 150 and 200 ug/mL in hexane.

Sample Preparation

Samples were ground or cut into pieces sized at less than 2 mm^2. Approximately 1g (0.9-1.1g) of the cut pieces were placed into a 40 mL VOC bottle and 8 mL of DCM (dichloromethane, CH2Cl2), 1mL of surrogate (DEP, conc. 1,000 ug/mL) and 1 mL of internal standard (DBEP, 1,000 ug/mL) were added. The sample bottle was shaken for 1 hour. Approximately 1 mL of the top layer of this extract was transferred into a vial for GC/MSD injection to **measure low concentration (0.1-0.2%) phthalates (1:10 dilution)**.

Alternatively, for anticipated high phthalate concentrations, 1 g of the cut pieces were placed into a 40 mL VOC bottle and 10 mL DCM were added into the bottle. After the sample bottle was shaken for 1 hour, 1 ml of the extract was transferred into another 40 mL VOC bottle and then,1 mL of internal standard (DBEP, 1,000 ug/mL), 1 mL surrogate and 7 mL DMC were added into the VOC bottle, mixing well. Approximately 1 mL of the top layer of this extract was transferred into a vial for GC/MSD injection to **measure high concentration (1-2%) phthalates (1:100 dilution)**. Further dilution may be needed to bring the sample concentration level into the calibration range (10 to 200 ug/mL) for some samples having very large content of phthalates (e.g. 45%).

RESULTS AND DISCUSSION

Phthalates in plastics are suspected cancer causing agents and possible teratogens which have been linked to liver and kidney damage, and the underdevelopment of reproductive organs in humans and animals (2). Public health concerns about the safety of human exposure to phthalates are on the rise. PVC and other types of plastics pollution in the California coastline, ocean and waterways are also increasingly prevalent. The Governor's ocean Protection Council adopted a new resolution to reduce and prevent marine debris on February 8, 2007 (3).

Following in he European Union's (1) and Japan's footsteps (4) in restricting phthalates usage in toys, the City and County of San Francisco passed an ordinance in April 2007 to regulate phthalates in toys, child care products and child feeding products. The health code limits six phthalates, including DBP (dibutyl phthalate), DNOP (di-n-octyl phthalate), DINP (di-isodecyl phthalate), BBP (benzylbutyl phthalate), DEHP (Bis [2-ethylhexyl] phthalate) and DINP (di-isononyl phthalate), to concentration levels of 0.1% or lower in all child-related products. Consequently, the DTSC has become increasingly interested in the matter due to its mission to protect public health and the environment from toxic harm. To this end, a quick and effective screening method was developed by the Mobile Laboratory Team to monitor the presence of toxic products on-site and to provide rapid information to the various government agencies (city, county and state) for necessary corrective action.

The structures of the 6 phthalates are presented in Figure 1. During method development two challenging aspects in testing the six phthalates were identified. The first unusual aspect encountered was that while DBP, BBP, DNOP and DEHP appeared as single peaks in the chromatogram, DIDP and DINP each appeared as multiple peaks due to isomeric congeners. The solution was to use traditional valley-to-valley integration to obtain area counts for quantitative calculation of the single-peak phthalates, and then subsequently change to area summation integration for the late eluting multi-peak phthalates. The second difficulty was that DEHP, DIDP and DINP eluted closely together and could not be easily separated by gas chromatograph temperature programming. The overlapping retention time presented challenges in identification and quantitation. Fortunately, these three phthalates are not commonly used together as mixture ingredients in a single product based on reported findings (2, 5). Using an innovative approach this method divided six phthalates into 2 groups and used 2 separate calibration schemes to process data consecutively after the GC/MSD data acquisition was completed. The autosampler data processing for 2 calibration programs was linked by a few macro commands in the method software. Chromatograms in Figure 2 present the 2 groups of phthalates.

The additional benefit of dividing the 6 phthalates into 2 groups was that the GC program time was reduced to less than 12 minutes per sample without sacrificing peak-to-peak resolution. Consequently, the throughput doubled that of other methods (5, 6). This improvement positively impacted turnaround time, enabling large sample loads to be rapidly analyzed in a short period of time.

Target ion selection for data processing is critical in quantitative analysis, with the base ion usually being the most favorable candidate to be chosen because it holds the highest abundance value in the spectrum. In this case, the predominant ion or base ion for all phthalates is m/z 149 ($C_8H_5O_3$), with S/N ratio approximately 10 to 100 fold higher than other ions in the spectrum. Furthermore, this ion is extremely sensitive. The stable m/z 149 ion, corresponding to a protonated phthalic anhydride, can be expected from the formation of any phthalate ester by charge of the two ester bonds during the ionization process (7). In order to eliminate matrix interferences from unwanted phthalates for quantitation, this method had to select less intense ions as application ions. Table 1 presents the selected target ion and two qualifying ions for each phthalate. Phthalate identification was confirmed by probability based match (PBM) logarithm with the NIST library data base, as all spectra were acquired in full scan mode by this method. Additionally, overlay target ion of sample peak and standard peak is a useful tool to identify and differentiate DINP and DIDP because both phthalates have slightly different retention times with distinguishing multiple peak shapes. Peak shape overlays are presented in Figure 5.

This method is simple, fast and effective; the recovery data and their statistics were based on a set of 5 replicates of PVC matrix spikes with 6 target phthalate esters and 1 surrogate at 100 ppm level, the recovery means, SD and RSD are presented in Table 2. The ranges of recovery mean, SD and RSD were 98%-108%, 6.42-15.58 and 5.92%-15.25% respectively. The chromatograms of PVC matrix blank and matrix spikes are presented in Figure 4. As a pilot trial, a total of 11 children's toy samples purchased by the City of San Francisco Department of the environment for this collaborative study were tested by this method, 54% were found to contain large amounts of the six regulated phthalate esters. Examples of 4 toy samples are presented in Figure 6. Duplicate results with relative percent difference (RPD) are presented in Table 3. The range of RPD was 0.32 to18.79%. All of these data demonstrate that this is a valid and effective method.

Conclusion

This screening method was developed for application in a space-limited mobile laboratory**;** however, the method is equally valid in all fixed laboratories. The method uses GC/MSD to screen six regulated phthalate esters with minimum sample preparation; as such, the reporting results could be lower than the actual amount in certain products (ca. 90-95%). If the amounts found are near the threshold level at 0.1%, longer extraction time (e.g. overnight) is recommended.

Simple, fast and effective, the method has a high throughput capability to meet the needs of providing information for on-site characterization purposes. This method is designed to survey the six phthalates in children's toys and other consumer products for compliance with the regulatory threshold of 0.1% (1,000 ppm).

Calculations and Reporting

The concentration of each phthalate (as ppm or percent by weight) in the test sample is calculated according to the following equation:

Phthalates (ug/g or ppm) = Area Spl/Area ISTD in Spl x Area ISTD in Std/Area Std x Std conc. (ug/mL)/ Spl W (g) x V (mL)

Where:
Spl = sample
ISTD = internal standard
STD = standard
W = weight
V = volume (dilution)

Calculation for percent recovery

% Rec. = (Amount of cpd. Recovered / Amount of cpd. spiked) x 100

Calculation for relative percent difference (RPD)

RPD = (Value A – Value B / Average of A+B) x 100

REFERENCES

(1) Suresh C. Rastogi, Gitte H. Jensen and Inge M. Worsoe, Analytical Chemical of Phthalates in Toys, NERI Technical Report, No. 404, National Environmental Research Institute, Ministry of the Environment, Denmark 2002.

(2) Ruth Stringer, Irina Labounskaia, David Santillo, Paul Johnston, John Siddorn and Angela Stephenson, Determination of the Composition and Quantity of Phthalate Ester Additives in PVC Children's Toys, Greenpeace Research laboratories Technical Note 06/97, September 1997.

(3) Charles Moore, Pelagic plastic, Algalitia Marine Research Foundation, April 9, (2007).

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(5) Health Canada, Determination of Phthalates in Polyvinyl Chloride Consumer Products, Product Safety Reference Manual, Test Methods Section, Method C-34, 2006.

(6) Semivolatile Organic Compounds by Gas Chromatography Mass Spectrometry, EPA Method 8270C, Revision 3, December 1996.

(7) F. W. Mclafferty, Interpretation of Mass Spectra, Third Edition, University Science Books, Mill Valley, California 1980.

ACKNOWLEDGMENTS

The authors thank Dr. Bruce La Belle (ECL Manager) and Dr. Myrto Petreas (Branch Chief) for their support and encouragement of this research project. The authors also thank Ms. Deborah O. Raphael (Manager of San Francisco Environment) for providing all toy samples.

Table 1: Phthalate esters, CAS number, quantitation ions and qualifiers.

* Group-1 targeted phthalates.
** Group-2 targeted phthalates.

Phthalate Esters	**CAS No.**	**Type**	**Quantitation Ion**	**Qualify Ion 1**	**Qualify Ion 2**
Bis(2-butoxyethyl) phthalate	000117-83-9	Internal std	193.1	249.2	294.2
DEP-Diethyl phthalate	000084-66-2	Surrogate	177.1	178.1	222.2
DBP-Dibutyl phthalate	000084-74-2	* Target	223.2	205.2	167.1
BBP-Benzyl butyl phthalate	000085-68-7	** Target	206.2	178.2	238.1
DEHP-Bis(2-ethylhexyl) phthalate	000117-81-7	**Target	167.1	279.2	390.3
DNOP-Di-n-octyl phthalate	000117-84-0	* Target	279.2	261.2	167.1
DINP (Di-isononyl) phthalate	020548-62-3	** Target	293.3	167.1	307.3
DIDP (Di-isodecyl) phthalate	026761-40-0	* Target	307.2	281.0	253.0

Table 2: Recovery of phthalate esters in PVC matrix.

Phthalate Esters	Spike Amount ppm or ug/g	Number of Replicates	Recovery Mean, %	SD	RSD
DEP-Diethyl phthalate (surrogate)	100	5	104	8.40	8.07
DBP-Dibutyl phthalate	100	5	107	8.61	8.04
BBP-Benzyl butyl phthalate	100	5	98	6.50	6.65
DEHP-Bis(2-ethylhexyl) phthalate	100	5	107	7.52	7.04
DNOP-Di-n-octyl phthalate	100	5	108	6.42	5.92
DINP (Di-isononyl) phthalate	100	5	103	7.69	7.44
DIDP (Di-isodecyl) phthalate	100	5	102	15.58	15.25

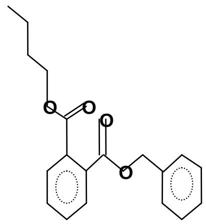
Table 3: Phthalate esters found in 11 children's toy samples purchased by the San Francisco Department of the Environment

ECL Lab No.	Product Description	Surrogate Recovery (%)	Phthalate found	Amount in Duplicate (% W/W)		RPD (%)
AR05613	White horse	102, 99.6	DINP	27.99	27.90	0.32
			DIDP	10.22	9.98	2.38
AR05614	Pink horse	85.3, 90.0	DINP	25.91	26.41	1.91
			DIDP	10.51	11.71	10.80
AR05615	Pink Doll	74.0, 64.6	ND	ND	ND	ND
AR04536	Yellow duck	101, 109	DINP	0.13	0.12	8.00
AR04538	Bath Jelly	170, 162a	DINP	44.50	44.23	0.61
AR04544	Plastic man	129, 121	ND	ND	ND	ND
AR04545	White doll	132, 140	ND	ND	ND	ND
AR00131	Squeez a bubble	104, 108	ND	ND	ND	ND
AR00132	Bendable pokey	163,168a	DINP	46.00	38.10	18.79
AR00133	My little pony	103, 103	ND	ND	ND	ND
AR00134	Bath sticker	160, 169a	DEHP	16.66	17.89	7.12
			DINP	7.96	7.86	1.26

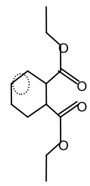
a. Matrix interference by high concentration of phthalates.

Figure 1: Phthalate structures, molecular weights and CAS numbers.

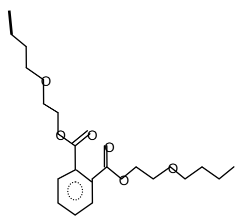
Benzylbutyl phthalate
M.Wt. 312.14, CAS 000085-68-7



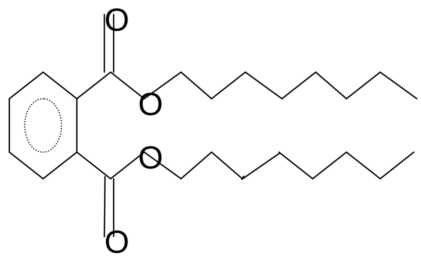
Diethyl phthalate
M.Wt. 222.09, CAS 000084-66-2



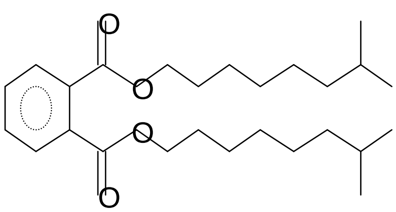
Bis [2-butoxyethyl] phthalate
M.Wt. 366.20, CAS 000117-83-9



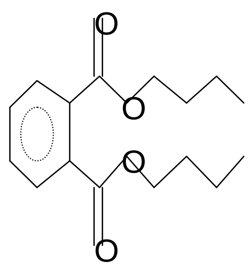
Di-n-ocytyl phthalate
M.Wt. 390.28, CAS 000117-84-0



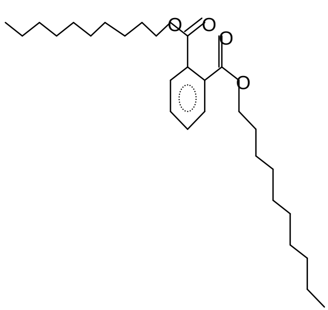
Di-isononyl phthalate
M.Wt. 418.31, CAS 020548-62-3



Dibutyl phthalate
M.Wt. 278.15, CAS 000084-74-2



Di-isodecyl phthalate
M.Wt. 446.34, CAS 026761-40-0



Bis[2-ethylhexyl] phthalate
Mt.Wt. 390.28, CAS 000117-81-7

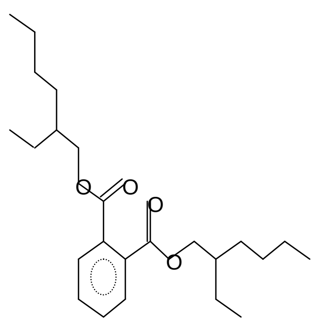


Figure 2: Group-1 phthalate esters including surrogate (diethyl phthalate), dibutyl phthalate, internal standard (bis [2-butoxyethyl] phthalate), di-n-octyl phthalate, and di-isodecyl phthalate.

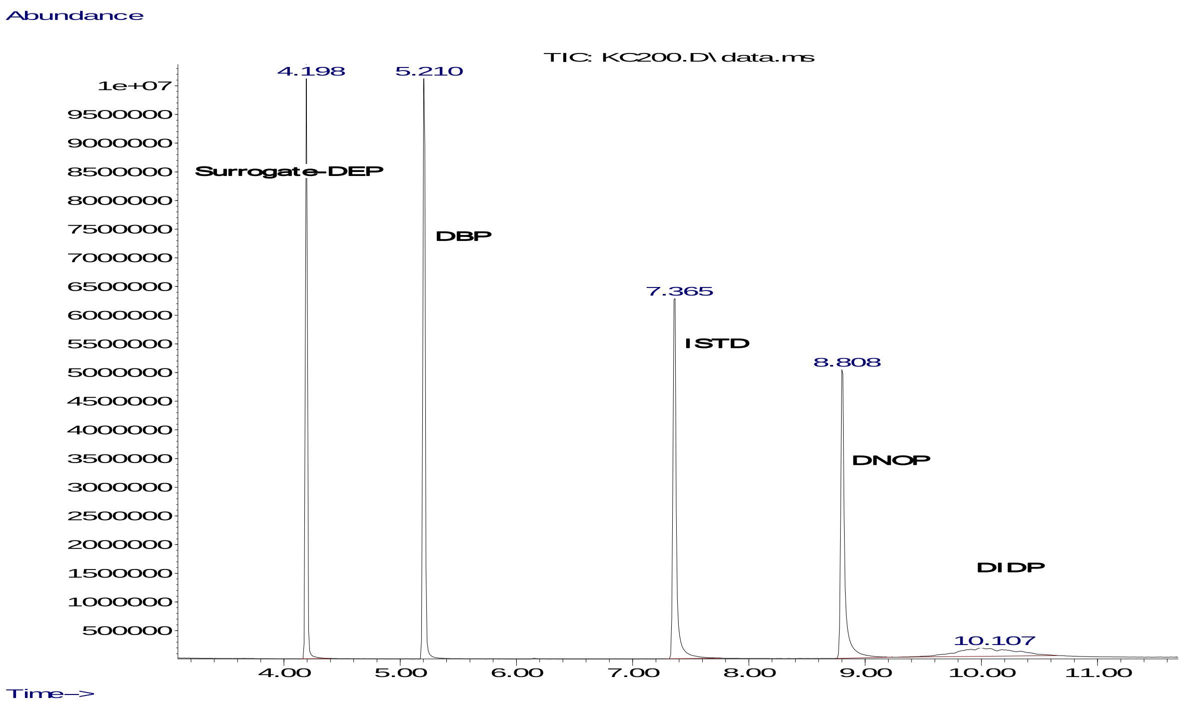


Figure 3: Group-2 phthalate esters including surrogate (diethyl phthalate), benzybutyl phthalate, internal standard (bis [2-butoxyethyl] phthalate), bis[2-ethylhexyl] phthalate, and di-isononyl phthalate.

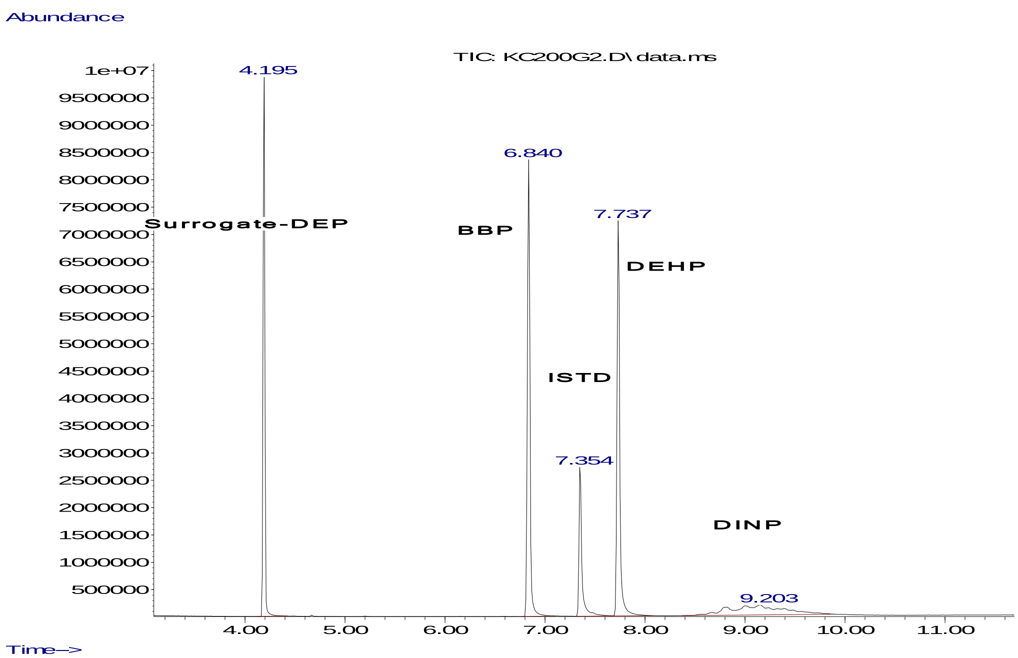
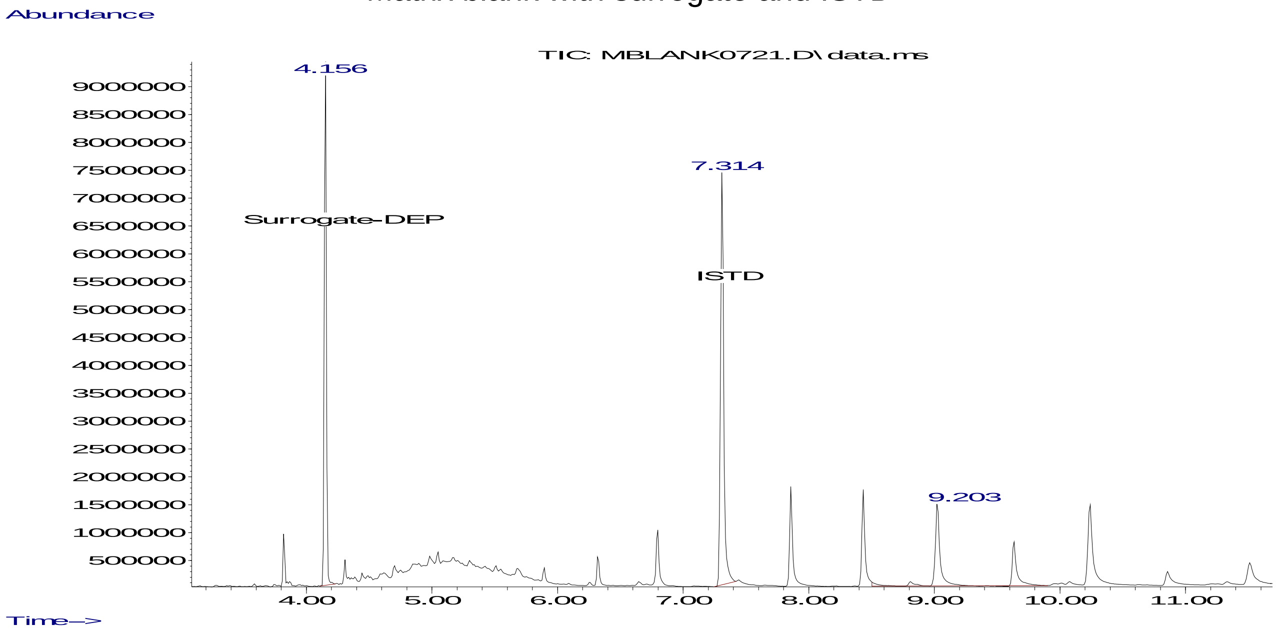
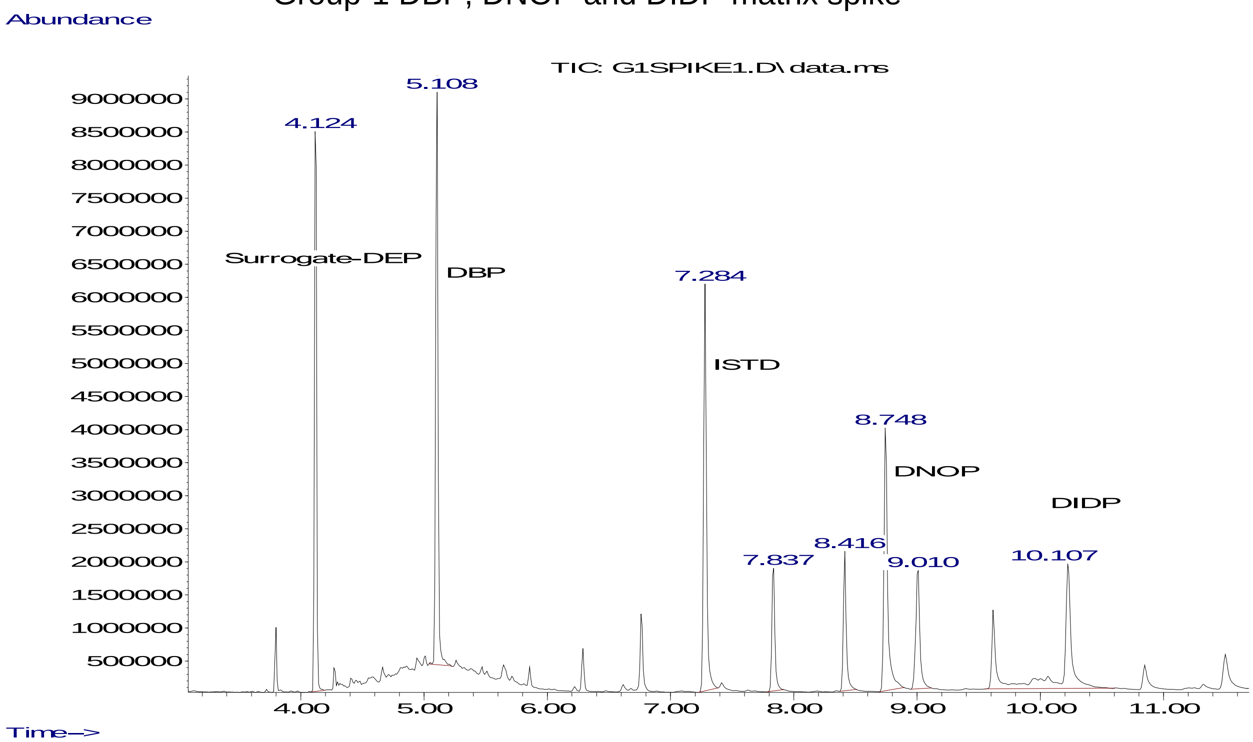


Figure 4: Chromatograms of PVC matrix blank, Group-1 phthalate matrix spike, and Group-2 phthalate matrix spike

Matrix blank with surrogate and ISTD



Group-1 DBP, DNOP and DIDP matrix spike



Group-2 BBP, DEHP and DINP matrix spike

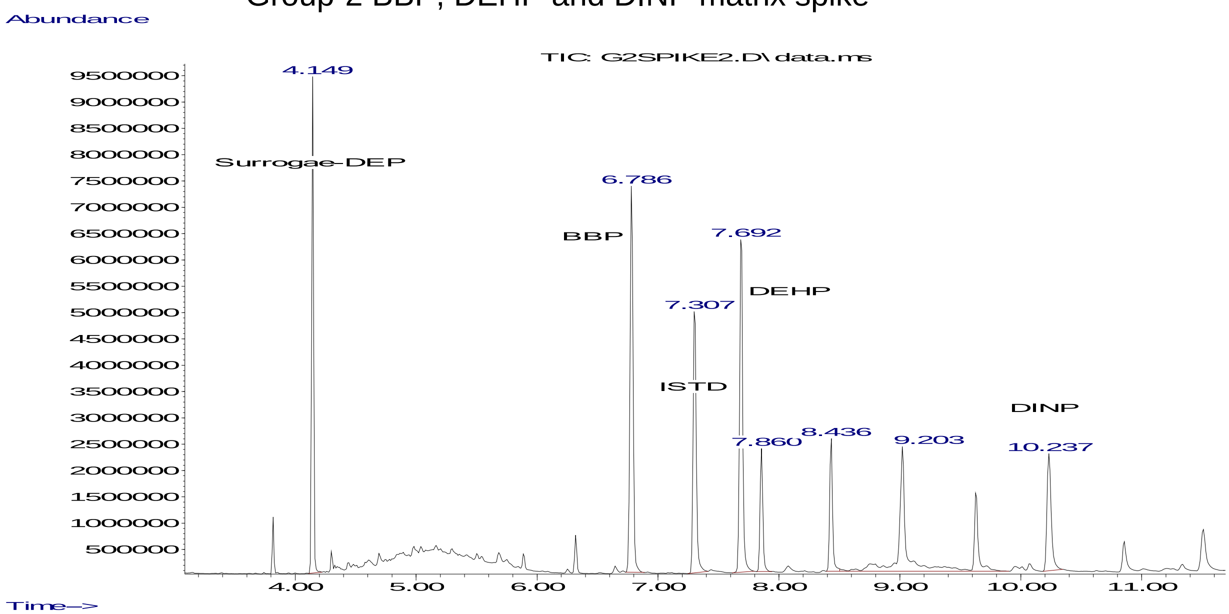
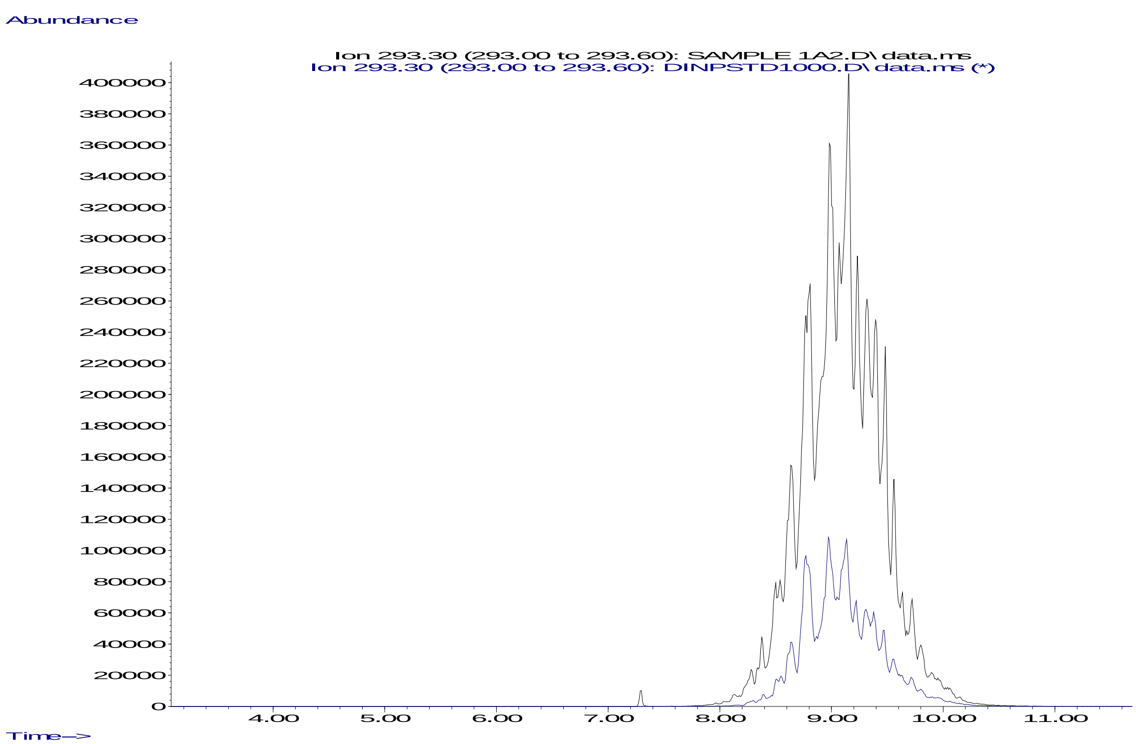
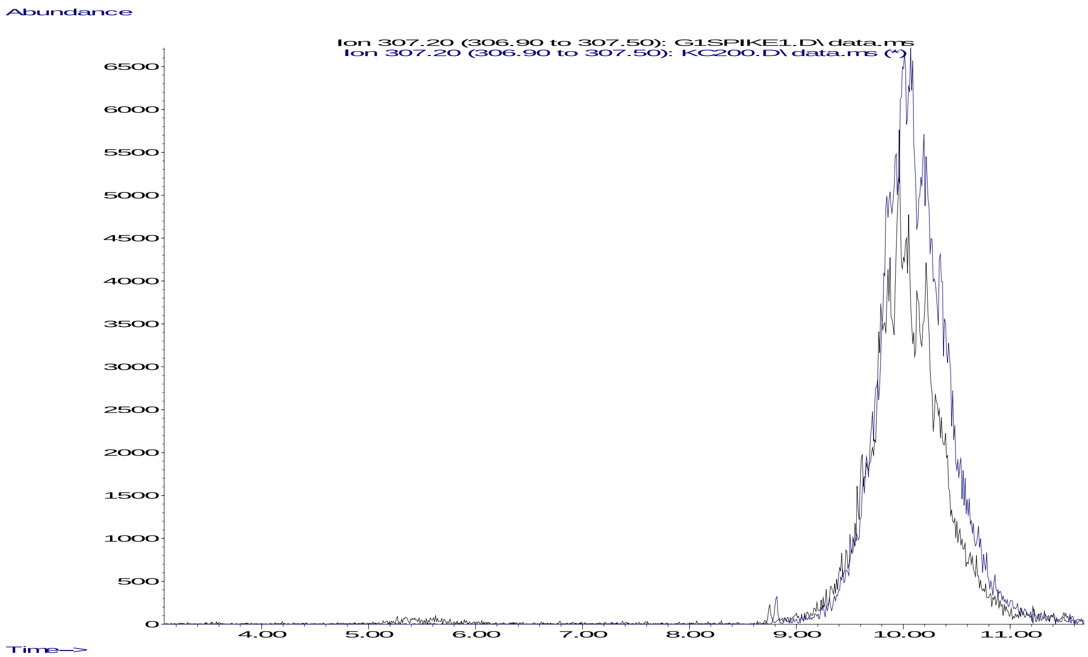


Figure 5: Sample and standard target ions overlay, a) DINP and b) DIDP

a) DINP target ion, m/z 293.3 overlay



b) DIDP target ion, m/z 307.2 overlay



Appendix – Sample Pictures and Reports
AR00131 to AR00134


PVC-19
PVC-18
PVC-16
PVC-17

DEPARTMENT OF TOXIC SUBSTANCE CONTROL
ENVIRONMENTAL CHEMISTRY LABORATORY-BERKELEY
700 HEINZ AVE., BERKELEY, CA 94710
TELEPHONE (510) 540-3003

ECL NO.	AR00131 to AR00134		DATE RECEIVED:	7/25/2008
REQUESTER:	Debbie Raphael		DATE SAMPLE PREPARED:	7/29/2008
SAMPLE LOCATION:	SF Environment 11 Grove St. San Francisco, CA 94102		DATE SAMPLE ANALYZED:	7/29/2008
METHOD(S):	Mobile Lab GCMS Screening Method For Phthalate Mobile Lab Phthalate Extracted By Methylene Chloride			

							QUANTITATION LIMIT*				
	ECL NO.	AR00131	AR00132	AR00133	AR00134	Meth	ECL NO.	AR00131	AR00132	AR00133	AR00134
	COL. NO.	PVC-16	PVC-17	PVC-18	PVC-19	Blank	Meth Blank				
	MATRIX	Soft Plastic	Soft Plastic	Soft Plastic	Soft Plastic	Methylene Chloride	Meth. Chloride				
ANALYTE	UNIT	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg		mg/Kg	mg/Kg	mg/Kg	mg/Kg
Di-n-buyl phthalate(DBP)		ND	ND	ND	ND	ND	10	100	100	100	100
Benzylbutyl phthalate(BBP)		ND	ND	ND	ND	ND	10	100	100	100	100
Bis-2-ethylhexyl) phthalate(DEHP)		ND	ND	ND	**179,000**	ND	10	100	100	100	10000*
Di-n-octyl phthalate (DNOP)		ND	ND	ND	ND	ND	10	100	100	100	100
Diisononyl phthalate(DINP)		ND	**460,000**	ND	**78,600**	ND	10	100	25000*	100	10000*
Diisodecyl phthalate(DIDP)		ND	ND	ND	ND	ND	10	100	100	100	100

NOTES: ND = NOT DETECTED   MG/KG=MILLIGRAM PER KILOGRAM
*= Multiply concentration of lowest std by dilution factor.

SAMPLE PREPARATION: | ANALYST: | SUPERVISING CHEMIST

Modan S. Gill 8.11.08 | Orlando Garbin 8/11/08 | K.C. Ting 8/11/08

Modan Gill DATE | Orlando Garbin DATE | KC Ting DATE

DEPARTMENT OF TOXIC SUBSTANCE CONTROL
ENVIRONMENTAL CHEMISTRY LABORATORY-BERKELEY
700 HEINZ AVE., BERKELEY, CA 94710
TELEPHONE (510) 540-3003

ECL NO.	AR00131 to AR00134	DATE RECEIVED:	7/25/2008
REQUESTER:	Debbie Raphael	DATE SAMPLE PREPARED:	7/29/2008
SAMPLE LOCATION:	SF Environment 11 Grove St.	DATE SAMPLE ANALYZED:	7/29/2008
METHOD(S):	Mobile Lab GCMS Screening Method For Phthalate Mobile Lab Phthalate Extracted By Methylene Chloride		

QC REPORT FOR

A: LABORATORY CONTROL SAMPLE
B: SAMPLE DUPLICATE ANALYSIS

	A	
	LABORATORY CONTROL SAMPLE	
	FOUND	Actual Amount
ANALYTE	MG/KG	MG/KG
Di-n-buyl phthalate(DBP)	116	100
Benzylbutyl phthalate(BBP)	90	100
Bis-2-ethylhexyl) phthalate(DEHP)	112	100
Di-n-octyl phthalate (DNOP)	125	100
Diisononyl phthalate(DINP)	80.2	100
Diisodecyl phthalate(DIDP)	125	100

B			
SAMPLE DUPLICATE ANALYSIS			
PERFORMED ON : 7/29/2008			
ECL NO. AR00134			
	RUN 1	RUN 2	RPD
ANALYTE	MG/KG	MG/KG	%
Di-n-butyl phthalate(DBP)	ND	ND	NA
Benzyl butyl phthalate(BBP)	ND	ND	NA
Di-(2-ethylhexyl) phthalate(DEHP)	166,600	178,900	7.12
Di-n-octyl phthalate (DNOP)	ND	ND	NA
Dilsononyl phthalate(DINP)	79,600	78,600	1.26
Dilsodecyl phthalate(DIDP)	ND	ND	NA

NOTES:

SAMPLE PREPARATION		ANALYST		SUPERVISOR	
Modan S. Gill	8.11.08	Orlando Garbin	08/11/08	K. C. Ting	8/11/08
Modan Gill	DATE	Orlando Garbin	DATE	KC Ting	DATE