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Environmental Toxicology and Chemistry, Vol. 31, No. 9, pp. 1948–1956, 2012

Printed in the USA
DOI: 10.1002/etc.1912

AN INTERLABORATORY COMPARISON STUDY FOR THE DETERMINATION OF DIALKYL PHTHALATE ESTERS IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

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(*Submitted* 22 *February* 2012; *Returned for Revision* 2 *April* 2012; *Accepted* 6 *May* 2012)

Abstract—A series of interlaboratory comparison exercises were conducted to assess the accuracy of dialkyl phthalate ester (DPE) concentration measurements in environmental and biological samples. Five laboratories participated in analyses to determine DPE concentrations in standard test solutions; marine sediments; three certified reference materials, including CARP-2 (fish muscle) and BCR-07 (fortified milk powder); and several livestock samples (sheep's milk, liver, and muscle). In addition, one laboratory determined DPE residue concentrations in 20 municipal sewage sludge samples, previously analyzed as part of the 2006/2007 U.S. Environmental Protection Agency's Targeted National Sewage Sludge Survey (TNSSS). The results showed relatively good interlaboratory agreement for analyses of di-ethylhexyl phthalate (DEHP). Three independent laboratories (Labs A, B, and C) reported concentrations of DEHP (ng/g wet wt) in fish muscle (CARP-2) of $1{,}550 \pm 148$, $1{,}410 \pm 193$, and $1{,}380 \pm 187$, respectively. Similarly, DEHP concentration measurements in sewage sludge samples showed good agreement with those reported in the 2006/2007 TNSSS report. Measured concentrations of individual DPEs and C6–C10 isomeric mixtures in these samples of municipal sewage sludge, which have not been previously reported, ranged between 1 and 200,000 ng/g dry weight. The results demonstrate that environmental monitoring of DPEs is often hampered by high method detection limits (MDLs), due to contamination of procedural blanks. It is important to note, however, that when background contamination is minimized (<10 ng/sample), relatively low MDLs (<0.1 ng/g) can be achieved, allowing for low-level quantification of DPEs in environmental and biological samples. Future efforts to develop better protocols to lower MDLs, as well to develop reference materials, would greatly benefit future DPE monitoring initiatives. Environ. Toxicol. Chem. 2012;31:1948–1956. © 2012 SETAC

Keywords—Dialkyl phthalate esters Quantification Interlaboratory Environmental Biological

INTRODUCTION

Dialkyl phthalate esters (DPEs) are high production volume chemicals used widely as commercial plasticizers and in various applications/products, including textiles, medical equipment, electronics, and personal care products [1]. Worldwide production of DPEs is estimated at approximately 6 million tons per year (http://www.umweltdaten.de/publikationen/fpdf-l/4263.pdf). Discharge of phthalates into the environment can occur via industrial, municipal, and household waste streams [1–4]. Environmental monitoring of DPEs is important, because elevated exposure can cause reproductive and developmental impacts in animals [5–7].

Commercially available DPEs vary in alkyl chain length and branching and range in molecular weight (MW) from 194 to more than 600 g/mol (Supplemental Data, Fig. S1). Dialkyl phthalate esters are commonly categorized into three MW classifications (groups I–III) [3]. Group I comprises low-MW DPEs, esterified with alcohols having straight-chain carbon backbones of ≤C3 including dimethyl phthalate (DMP) and diethyl phthalate (DEP). Group II is comprised of transitional MW phthalates esterified with alcohols having straight-chain carbon backbones of C4 to C6 including di-*n*-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-ethylhexyl phthalate (DEHP), di-iso-hexyl (C6), and di-iso-heptyl (C7) isomeric mixtures. Group III is comprised of high-MW phthalates esterified with alcohols having straight-chain carbon backbones of >C7 including di-*n*-octyl phthalate (DnOP) di-*n*-nonyl phthalate (DnNP), and also di-iso-octyl (C8), di-iso-nonyl (C9), and di-iso-decyl (C10) isomeric mixtures.

Monitoring DPE residues in environmental samples has been conducted for several decades, starting in the late 1970s and early 1980s [8–10]. Dialkyl phthalate ester concentration measurements have been previously reported in wastewater effluent [11,12] and various environmental media including air, water, and sediments [8–10,13–18], as well as fish and wildlife [8,17,19]. Tissue residue concentrations of phthalates in aquatic organisms have been reported between 1 and 30,000 ng/g lipid [8,17,19]. Dialkyl phthalate ester concentrations have also been monitored in human serum and breast milk to assess human exposure risks [20,21]. A recent field survey in the European Arctic demonstrated the presence of DPE residues at relatively low levels in samples of Arctic air and seawater [22], indicating that these compounds are widely distributed in the environment, including remote locations.

The most common approaches for trace residue analysis of DPEs include gas chromatography (GC)-based methods using flame ionization detection, electron capture detection, or single quadrupole mass spectrometry (GC-MS). Liquid chromatography–electrospray ionization tandem mass spectrometry has been employed to analyze isomeric mixtures [17]. However, the ubiquitous nature of DPEs in indoor environments results in pervasive contamination of laboratory air, glassware, and reagents [17,23]. For example, indoor air concentrations of widely used DPEs such as DnBP and DEHP can exceed >1,000 ng/m^3 [24,25]. High background contamination undoubtedly presents a challenge for precise and accurate

All Supplemental Data may be found in the online version of this article.

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Published online 15 June 2012 in Wiley Online Library (wileyonlinelibrary.com).

analytical determination of DPE residues in environmental samples, especially if present at relatively low levels.

Interlaboratory comparison studies to assess the reliability and accuracy of DPE concentration measurements in environmental and biological samples do not exist. The objective of the present study was to conduct a series of interlaboratory comparison studies that involved different analysts from multiple laboratories determining concentrations of DPEs in (1) sediment and fish tissue, (2) agricultural products (livestock and milk), and (3) sewage sludge. The present study aimed to critically evaluate these comparative analyses in terms of variability and reproducibility and provide insight to aid future DPE monitoring and assessment initiatives.

MATERIALS AND METHODS

Study 1: Test solutions, reference materials, and sediments

Study 1 involved several laboratories conducting comparable analyses of DPEs in standard test solutions, marine sediments, and two commercially available certified reference materials (CRMs) for fish and fortified milk powder. Specifically, 23 laboratories from 12 countries (Canada, United States, Japan, Netherlands, Sweden, Denmark, Spain, Italy, Norway, Germany, United Kingdom, and Belgium) were invited by the host laboratory (Fisheries and Oceans Canada, Institute of Ocean Sciences, Sidney, Canada) to participate in the study, which would require trace DPE analysis in various environmental and biological matrices. Selecting the laboratories invited to participate in the study was based on a literature search in which the aim was to identify laboratories that had produced DPE data for corresponding studies and whose results were published in the peer-review literature.

In addition to the host laboratory (Lab A), three other laboratories agreed to participate in various aspects of the study. The participating laboratories include one each from the United States (Lab B), Japan (Lab C), and Belgium (Lab D). A summary of the participating laboratories is given in Supplemental Data, Table S1. The relatively low degree of participation (i.e., $<15\%$ of invited laboratories) indicates that laboratories do not routinely monitor DPEs in environmental and biological samples, likely due to the inherent analytical challenges. Using their own methods of sample preparation and instrumental determination, each laboratory was asked to quantify concentrations of DPEs in standard solutions, as well as in three different matrices: sediment, fish mussel, and milk powder.

Test materials provided to each participating laboratory included (1) standard solutions containing DPEs, (2) samples of fish tissue, (3) samples of marine sediment, and (4) samples of fortified milk powder. Test materials were prepared and distributed by the host laboratory (Lab A) staff. Standard solutions consisted of seven individual DPE isomers (DMP, DEP, DiBP, DnBP, BBP, DEHP, and DnOP) dissolved in toluene. For the purpose of the present interlaboratory comparison study, this DPE mix solution was prepared at two different concentrations (i.e., high- and low-level standards). Specifically, solutions were prepared at 100 pg/μl and 5 pg/μl. Solutions were analyzed by GC-MS prior to distribution to other laboratories.

The fish samples were carp CRMs (CARP-2), which is a certified reference material for polychlorinated biphenyls and organochlorine pesticides, readily available from the National Research Council of Canada. Each sample was approximately 6 g. The CARP-2 matrix was selected for the purpose of evaluating this material as a potential reference material for future studies involving DPE analysis. Marine sediment samples were collected from an urbanized inlet (False Creek, Vancouver, Canada). Sediments were homogenized by manual shaking after collection. Subsamples (5 g) were transferred to solvent-rinsed glass vials prior to delivery. The fortified milk powder sample was also a CRM (BCR-607, European Community Bureau of Reference) and was supplied by Lab D (Belgium).

Each laboratory was asked to determine DPE concentrations in three replicate samples of the various supplied test materials. The laboratories were also instructed to provide details of their analysis including criteria used to confirm identity of the compound, method detection limits (MDLs), and a summary of the quality control procedures employed in the analysis and sample preparation, including procedural blanks. All laboratories reported blank levels and blank corrected DPE concentrations. Each laboratory was also requested to make gravimetric measurements of extractable lipid and/or moisture contents. It is important to note that the number of analytes quantified by each laboratory varied substantially. For example, Lab A and Lab C reported concentrations for all individual DPEs. Lab B reported concentrations of DnBP and DEHP. Lab D reported concentrations of DMP, DEP, DnBP, DEHP, di-isobutyl phthalate (DiBP), and DEHP.

Study 2: Measurements in livestock samples

Study 2 involved two laboratories conducting DPE measurements in livestock samples. Specifically, Lab A and a laboratory from the United Kingdom (Lab E) both analyzed samples of sheep muscle, liver tissue, and milk for the purpose of generating two independent DPE concentration data sets for those matrices. The samples of sheep tissues and milk were obtained from Lab E. Sheep milk samples were freeze-dried, whereas tissue samples were frozen. Lab A reported concentrations for all DPEs in sheep tissue and milk samples, whereas Lab E reported measurements of DEHP in sheep tissue samples, as well determination of total phthalate content (ΣDPEs) in sheep milk. Thus, only DEHP and DPE concentration data were evaluated in these livestock samples.

Study 3: Measurements in municipal sludge samples

In 2006/2007, the U.S. Environmental Protection Agency (U.S. EPA) conducted a Targeted National Sewage Sludge Survey (TNSSS) to determine which analytes (or chemicals) were present in sewage sludge and obtain national estimates of the concentrations of selected analytes [12]. The TNSSS Technical Report summarizes measured chemical concentration data for sewage sludge samples collected in 2006/2007 from 74 randomly selected, publicly owned treatment works in 35 U.S. states. Concentrations of 145 analytes were reported, including a variety of anions (nitrite/nitrate, fluoride, water-extractable phosphorus), metals, flame retardants, pharmaceuticals, steroids, hormones, polycyclic aromatic hydrocarbons, and semivolatile organics, including di(2-ethylhexyl) phthalate (DEHP). The municipality names corresponding to the sample ID and chemical concentration data are not provided.

At the request of the Phthalate Esters Panel (American Chemistry Council), a select number of these municipal biosolids were further analyzed for individual DPEs and commercial mixtures at the International Organization for Standardization laboratory in Sidney, British Columbia, Canada (Lab A). Specifically, 20 samples were analyzed for DEHP and seven other single-isomer DPEs (DMP, DEP, DiBP, DnBP,

BBP, DnOP, and DnNP), as well as C6 through C10 multi-isomer mixtures of branched chain alkyl phthalate esters.

Methods used by participating laboratories

The methods used by the various participating laboratories are summarized in Figure 1. Details of protocols and procedures employed by the host laboratory, Lab A (Institute of Ocean Sciences, Sidney, BC, Canada), for the extraction, cleanup, and analysis of DPEs in seawater, sediments, and biota by GC-MS (individual DPEs) and liquid chromatography–electrospray ionization mass spectrometry (isomeric mixtures) are described in detail by Lin et al. [17] and Mackintosh et al. [19]. Briefly, samples of sediment or tissue were weighed, spiked with a suite of mass-labeled surrogate internal standards (d_4-DMP, d_4-DnBP, and d_4-DnOP), blended with 15 to 20 g of prebaked Na_2SO_4, and ground to a free-flowing powder via mortar and pestle. The homogenates were then extracted by ultrasonic solvent extraction (i.e., sonication) with 50 ml of 1:1 (v/v) dichloromethane/hexane (DCM/Hex) using a Branson 5210 ultrasonic water-bath for 10 min, and shaken on a shaker table (Eberbach) for another 10 min. Once the suspended particles settled, the supernatant was removed. The extraction was repeated two more times with fresh solvent. The combined extracts were concentrated to approximately 5 ml, with a gentle stream of high-purity nitrogen. The concentrate was quantitatively transferred onto a 350 × 10 mm i.d. glass column packed with 15 g deactivated alumina (15% high-performance liquid chromatography water, w/w) and capped with 1 to 2 cm of anhydrous Na_2SO_4. The alumina column was eluted with three 30-ml fractions of (1) hexane; (2) 1:9 DCM/Hex; and (3) 1:1 DCM/Hex. The third fraction (1:1 DCM/Hex fraction) was concentrated to approximately 100 μl and spiked with isotope-labeled surrogate performance standards (d_4-DEP and d_4-BBP) before GC-MS analysis. The mass spectrometer was operated in the positive EI mode with electron energy of 70 eV. Data were acquired in the selective ion-monitoring mode (*m/z* 149 for all phthalates except 163 for DMP). Samples were processed in batches of seven, which included two procedural blanks, four real samples, and one DPE native-spiked sample. Procedural blanks consisted of 20 g of prebaked sodium sulfate, which were processed and analyzed the same as real samples. Results of method development experiments conducted by Lab A, showing mean blank levels and corresponding MDLs of selected DPEs in various matrices, are summarized in the Supplemental Data, Table S2. The criterion for positive detection of DPEs in samples was if observed peak was greater than the mean blank level + 3 SDs. As DPE concentrations in this study were all blank corrected (i.e., subtraction of mean blank level), the reported MDLs are therefore 3 × SD of the mean blanks. Method detection limits in ng/sample were converted to units of ng/g using the weight of the extracted sample.

The other participants in interlaboratory comparison Study 1 (Labs B, C, and D) used various GC-MS-based methods to measure DPEs in the test materials provided (Fig. 1). For example, Lab B extracted samples using acetonitrile by sonication, followed by liquid–liquid extraction into hexanes, followed by solid-phase extraction with 6 ml Supelclean LC-Si SPE glass body cartridges (Supelco). Lab C extracted samples in acetone using a Soxhlet apparatus, followed by gel permeation chromatography and florisil chromatography. Lab D extracted sediment samples by microwave-assisted extraction, whereas tissue and milk samples were extracted using sonication. Lab D employed similar cleanup techniques (gel permeation chromatography and solid-phase extraction) prior to GC-MS analysis. All laboratories employed the use of internal surrogate standards. Specific internal surrogate standards and spiking amounts were as follows: Lab A: 100 ng of mass labeled phthalates, DMP-d4, DnBP-d4, and DnOP-d4; Lab B: 2,500 to 5,000 ng of mass-labeled phthalates, DnBP-d4, DEHP-d4, and DnNP-d4; Lab C: 800 ng of native di-*n*-pentyl phthalate; Lab D: 2,000 ng of mass-labeled phthalates, DEHP-d4; and Lab E: 2,500 ng of mass-labeled phthalates, DEHP-d4. Laboratories generally reported good recoveries of internal surrogate compounds (60–120%).

In Study 2, Lab A and Lab E measured DPEs in several livestock samples, including sheep milk ($n = 5$), muscle ($n = 5$), and liver ($n = 5$). Lab A measured individual DPEs in milk and tissue samples using the same methods employed in Study 1. Lab E employed two different sample extraction procedures. Specifically, for sheep muscle and liver tissue, Lab E extracted

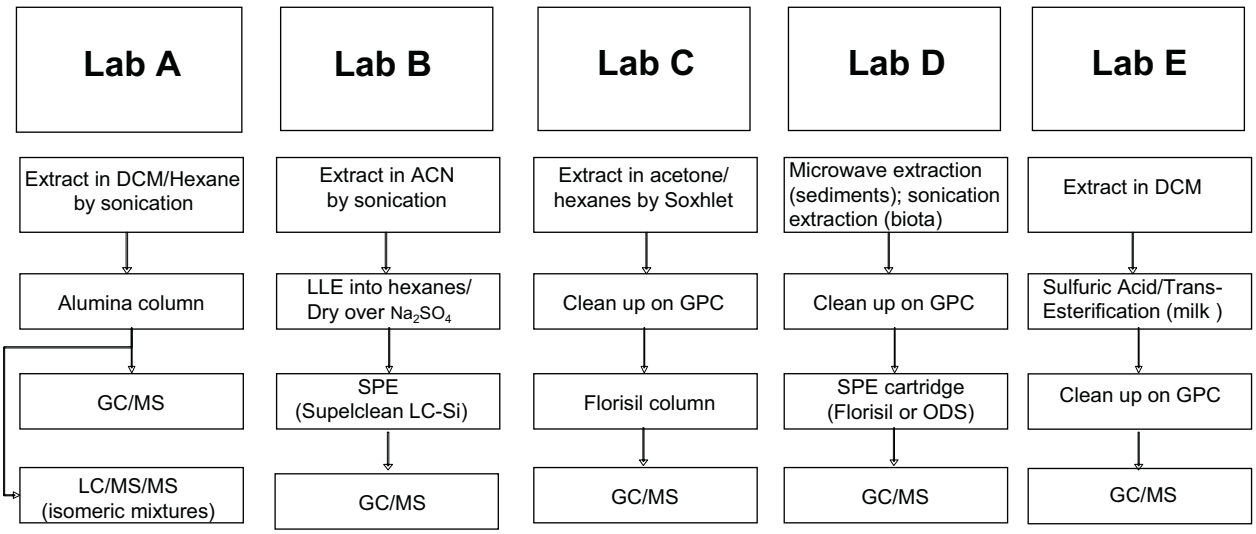

Fig. 1. Schematic showing different extraction, cleanup and analysis methods for determining dialkyl phthalate esters by participating laboratories, including Lab A (Canada), Lab B (USA) and Lab C (Japan), Lab D (Belgium) and Lab E (United Kingdom). DCM = dichloromethane; ACN = acetonitrile; LLE = liquid–liquid extraction; GPC = gel permeation chromatography; GC/MS = gas chromatography mass spectrometry; SPE = solid phase extraction; ODS = octyldecylsilane; LC/MS/MS = liquid chromatography tandem mass spectrometry.

samples using DCM refluxed for 2 h at 50°C. Sheep tissue extracts were further purified via gel permeation chromatography and analyzed for DEHP by GC-MS. For sheep milk samples, Lab E employed a method involving transesterification of phthalate diesters using sulfuric acid to methyl esters and partitioning into iso-octane. These extracts were cleaned up using gel permeation chromatography and analyzed by GC-MS to determine total DPE concentration (ΣDPEs).

In Study 3, DEHP data in the U.S. EPA TNSSS was generated by Columbia Analytical Services, which analyzed those samples for metals, anions, polycyclic aromatic hydrocarbons, and semivolatile organics, including DEHP. The U.S. EPA report provides details on the analytical methodologies used for the determination of DPEs in sewage sludge samples [12]. The analytical procedures employed by Lab A to process and analyze these same sludge samples were as follows. Approximately 2 g of wet sludge was weighed into a clean I-Chem vial. Then, 10 g of anhydrous sodium sulfate that had been baked in a muffle furnace overnight was added to the sample and mixed thoroughly. Each sample was spiked with 25 μl of 10 ng/μl internal standard mix described above. Then, 30 ml of 1:1 dichloromethane/redistilled hexane was measured and added to the vial. The vials were then covered with aluminum foil that had been hexane rinsed, baked at 350°C overnight, and then capped. The samples were shaken briefly by hand and place in an ultrasonic bath for 30 min.

Cleanup of sample extracts was performed by solid-phase dispersion using alumina as the sorbent. Neutral alumina sorbent (ICN Biomedical) was activated by baking it in an oven overnight to drive off the water; it was then cooled in a desiccator and deactivated by adding 15% w/w high-performance liquid chromatography grade water. After the sorbent was allowed to stand in a sealed container for 2 h, 5 g of sorbent was added to each vial. The vials were shaken and allowed to stand for 10 to 20 min as the sorbent settled. Approximately 500 μl of extract was removed from each vial and evaporated to 100 μl at which point 10 μl of an 8 ng/μl performance standard mix described above was added. Vials were then covered with aluminum foil and capped prior to GC-MS analysis. Instrumental analyses conditions used were exactly the same as those described for Lab A in Study 1.

Data analysis

Chemical concentration data were expressed as arithmetic means ± 1 SD. Concentrations are reported in units of pg/μl for standard solutions, ng/g dry weight for sediments and fortified milk, sheep milk, muscle, and liver, and ng/g wet weight for fish tissue. One-way analyses of variance (ANOVAs) and Tukey's Honestly Significant Difference tests were performed to evaluate differences in reported chemical concentrations among laboratories.

RESULTS

Quantification of DPEs in test solutions

Measured concentrations of DPEs in the high (100 pg/μl) concentration standard solution containing individual DPEs showed relatively good agreement between laboratories (Fig. 2A). For example, reported concentrations of DnBP in the high concentration solution (pg/μl), 87.2 (Lab A), 116 (Lab B), and 128 (Lab C), were ± 25% of the actual value of 109 pg/μl. Conversely, analyses of the low concentration standard were more variable. For example, reported concentrations of DEHP for Lab A (8.46) and Lab C (11.0) were approximately two

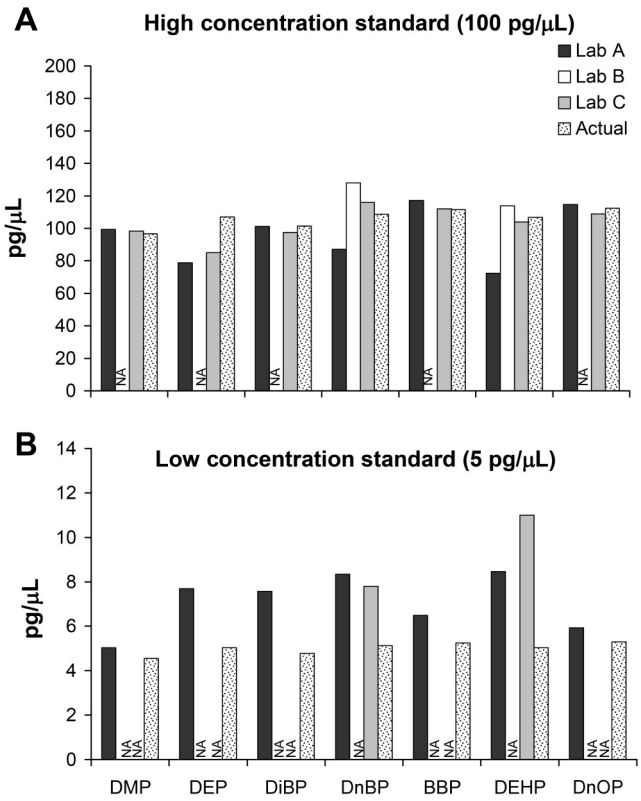

Fig. 2. Measured concentrations of dialkyl phthalate esters in (**A**) high concentration standard solution (100 pg/μL) and (**B**) low concentration standard solutions (5 pg/μL) reported by Labs A, B and C during interlaboratory comparison Study #1. The actual concentrations of the original solutions, prepared by Lab A, are shown for comparison. NA (not analyzed) indicates measurement not attempted by a given laboratory.

times higher than the actual concentration of 5.04 pg/μl (Fig. 2B). Lab B was not able to report measurements for the low concentration standard, due to detection limit limitations. The results also highlight that solution concentrations can vary over time. For example, Lab A, which prepared the low concentration standard, reported DPE concentrations that generally exceeded the original 5 pg/μl concentration. This may be due to contamination during storage or handling. It is important to note that the deviation from the actual values is greatest for the most ubiquitous DPEs, that is, DEP, DnBP, and DEHP. For the less frequently detected DPEs (DMP, BBP, and DnOP in particular), the measured concentrations are closer to the actual concentrations of these solutions. Considering that the replication of quantification for each of the seven DPE compounds measure was better than 15% for Lab A, these findings further suggest that the deviation observed (actual vs measured concentration) for the low concentration solution is due to laboratory contamination associated with sample handling and/or instrumental analysis.

Quantification of DPEs in certified reference materials and marine sediments

Blank corrected concentrations of DPEs in fish (CARP-2) and marine sediment samples reported by Labs A, B, C, and D are summarized in Supplemental Data, Table S2 and Table S3, respectively. The data show that in many cases, concentrations were deemed less than the MDL because of relatively high levels in procedural blanks. However, in some cases, good agreement was found between laboratories. For example, reported concentrations of DEHP (ng/g wet wt) in CARP-2

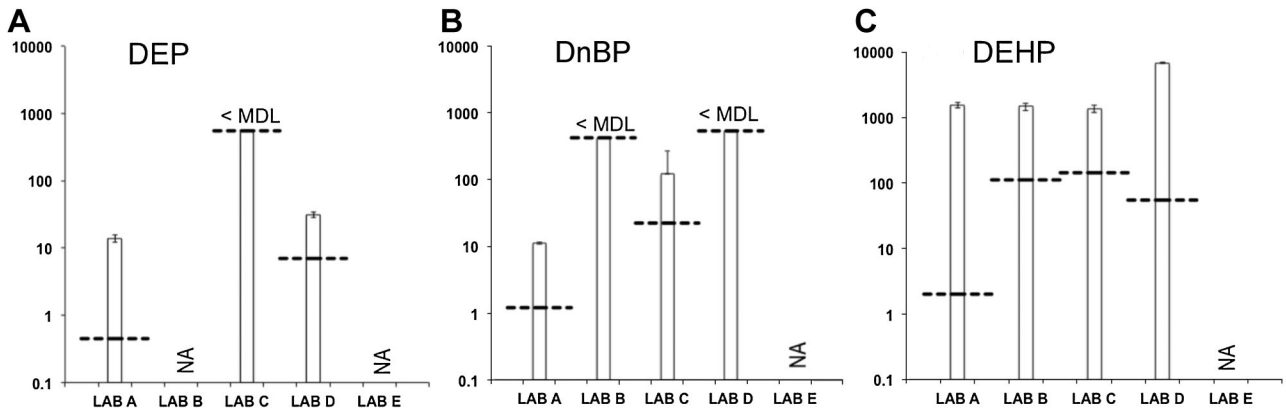

Fig. 3. Measured concentrations (ng/g wet wt.) of (**A**) diethyl phthalate (DEP), (**B**) di-*n*-butyl phthalate (DnBP) and (**C**) di-ethylhexyl phthalate (DEHP) in CARP-2 certified reference material ($n = 3$) reported by the four participating laboratories in interlaboratory comparison Study 1 (i.e., Labs A, B, C, and D). Bars represent blank corrected concentrations, shown as arithmetic means ± standard deviation on a logarithmic scale. NA (not analyzed) indicates no measurement was attempted. Method detection limits (MDLs) are shown as dashed lines and were determined as 3 × standard deviation of reported blanks.

samples from Labs A, B, and C were 1,550 ± 148, 1,410 ± 193, and 1,380 ± 187, respectively (Fig. 3). However, for this same matrix, Lab D reported a comparatively higher DEHP concentration (6,820 ± 94.4 ng/g wet wt), approximately five times above those reported by other laboratories. Measurements of other DPEs in CARP-2 samples varied substantially ($p < 0.05$) between different laboratories. For example, reported concentrations of DnBP in CARP-2 samples by Lab A and Lab C were 11.2 ± 0.40 and 121 ± 152, respectively (Fig. 3). Concentrations of DPEs in marine sediment were also not particularly consistent between reporting laboratories. For example, mean DnBP concentrations in sediment samples reported by participating laboratories, Labs A (26.5 ± 5.76), C (46.5 ± 16.1), and D (102 ± 12.2), were significantly different ($p < 0.05$), with the highest and lowest measurement differing by a factor of 3. Similarly, mean DEHP levels reported in sediment samples by these four laboratories, including Labs A (430 ± 46.6), B (1,720 ± 147), C (1,340 ± 208), and D (1,270 ± 28.8), were not consistent ($p < 0.05$), and varied by as much as a factor of 4.

Only two laboratories, Labs A and B, provided measurements of DPEs in fortified milk samples. Measurements of DEHP in fortified milk generated by Lab A (149 ± 13.7) and Lab B (257 ± 16.97) were within a factor of 2. The concentration of DnBP in the fortified milk reported by Lab B (222 ± 6.49) was three times higher than that reported by Lab A (67.2 ± 0.94).

The degree of background contamination observed in procedural blanks varied substantially between laboratories during these analyses (Fig. 4). The range of reported procedural blanks ranged between <1 and 2,500 ng/sample. Di-*n*-butyl phthalate, DEP, and DEHP were generally the most problematic DPEs in terms of background contamination, exhibiting the highest levels in procedural blanks. Lab A procedural blanks had the lowest background contamination, with mean blank levels ranging from 1.02 ng/sample for DEP to 11.4 ng/sample for DEHP. In many cases, high procedural blank levels of DPEs impeded the analyst's ability to report quantifiable concentrations. For example, high blank levels of DEP reported by Lab C during analysis of marine sediment samples resulted in a high MDL of 555 ng/g dry wt. In particular, DEP residue observed in procedural blanks (mean = 1,310 ± 416 ng/sample) exceeded the DEP residues extracted from the 10-g sediment sample. Lab C reported similar background contamination problems during quantification of BBP and DnOP in marine sediment samples.

Quantification of DPEs in livestock samples

Results from the comparative analyses of sheep milk and tissue samples (Labs A and Lab E) are illustrated in Figure 5. Blank corrected concentrations of ΣDPEs in sheep milk compared reasonably well between the two labs. Lab A reported a ΣDPE concentration equal to 1,930 ± 953 ng/g dry weight, whereas Lab E reported 3,650 ± 2,210 ng/g dry weight. It is important to note that ΣDPE concentrations reported by Lab A were derived from summation of individual DPE concentrations, whereas Lab E measurements of ΣDPE were determined via quantification of methyl esters.

Blank corrected concentrations of DEHP in sheep muscle and sheep liver reported by Lab E were significantly higher ($p < 0.05$) compared with those reported by Lab A, varying by orders of magnitude. Di-ethylhexyl phthalate levels in Lab E procedural blanks were 4,060 ± 2,530 and 426 ± 242 ng/sample for muscle and liver analyses, respectively. Di-ethylhexyl

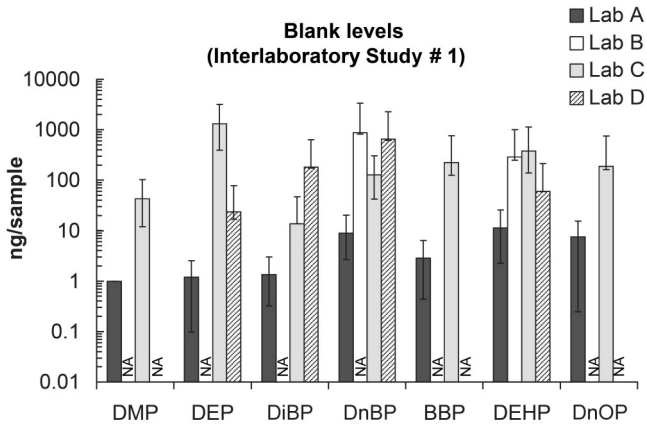

Fig. 4. Measured concentrations of dialkyl phthalate esters in procedural blanks (ng/sample) reported by participating laboratories in interlaboratory comparison Study 1, including Labs A ($n = 6$), B ($n = 3$), C ($n = 4$), and D ($n = 3$). Data are presented as arithmetic means ± standard deviation on a logarithmic scale. NA (not analyzed) indicates measurements not conducted by a given laboratory. DMP = dimethyl phthalate; DEP = diethyl phthalate; DiBP = di-iso-butyl phthalate; DnBP = di-*n*-butyl phthalate; BBP = butyl benzyl phthalate; DEHP = di-ethylhexyl phthalate; DnOP = di-*n*-octyl phthalate.

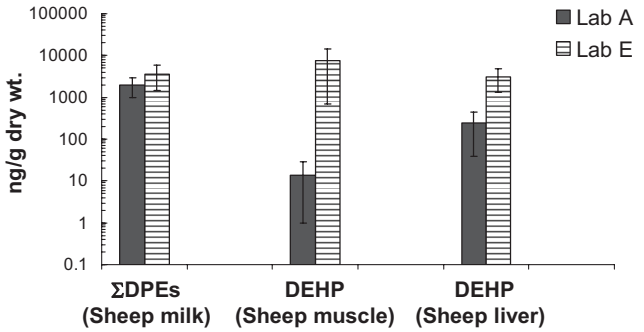

Fig. 5. Blank corrected concentrations (ng/g dry wt) of total dialkyl phthalate ester (ΣDPEs) in sheep milk ($n = 5$) as well as di-ethylhexyl phthalate (DEHP) concentrations in sheep muscle ($n = 5$) and liver ($n = 5$) reported by the two participating laboratories in interlaboratory Study 2 (i.e., Labs A and Lab E). Data for are presented as arithmetic means ± standard deviation on a logarithmic scale.

phthalate levels in Lab A procedural blanks were substantially lower than those reported by Lab E (Supplemental Data, Fig. S2).

Quantification of DPEs in municipal sludge

Lab A reported concentrations of individual DPEs and C6–C10 isomeric mixtures in samples of municipal sewage sludge ($n = 20$), ranging widely between approximately 1 to 200,000 ng/g dry weight (Table 1). Among individual DPEs, DEHP exhibited the highest concentrations in sludge (19,100 ± 43,200 ng/g dry wt). One sample (ID 68346) was found to have a relatively high DEHP concentration (187,000 ng/g dry wt). Dimethyl phthalate (15.1 ± 15.7 ng/g dry wt) and DEP (15.1 ± 15.7 ng/g dry wt) exhibited the lowest concentrations in sludge samples. Among the isomeric mixtures, C8 and C10 exhibited the highest mean concentrations in sludge samples, 1,940 ± 2,340 and 1,640 ± 1,970 ng/g dry weight, respectively. Concentrations of the C6 isomeric mixture were relatively low, often <MDL.

The degree of background contamination observed in Lab A procedural blanks during analyses of these municipal sludge were consistent with previous analyses, with DnBP, DEP, and DEHP exhibiting the highest levels in blanks compared with other DPEs (Supplemental Data, Fig. S3). In some cases, DPE residues varied substantially between blank replicates ($n = 5$). In particular, DEP levels in blanks ranged from 41.1 to 318 ng/sample. The relatively high levels of DEP observed in some blanks resulted in relatively high MDL, thus hampering positive detection of DEP residues in several of the analyzed sludge samples (Table 1).

Comparison of DEHP measurements in sludge samples conducted by Lab A with previous laboratory measurements of these same samples revealed relatively good agreement (Fig. 6). Mean DEHP concentrations reported by Lab A were not significantly different ($p > 0.05$) compared with the U.S. EPA data [12]. However, in some cases, DEHP concentrations were substantially different, varying by as much as 30 times between the two reporting laboratories. For example, Lab A reported 3,200 ng/g dry weight for DEHP in sample 68341, a value >30 times lower than the DEHP level reported in the U.S. EPA report (110,000 ng/g dry wt).

DISCUSSION

The results from interlaboratory comparison Studies 1 and 2 show relatively inconsistent reporting of DPE concentration measurements in marine sediments and biological tissues/fluids. In many cases, laboratories reported high background levels of DPEs in procedural blanks, especially DnBP, DEP, and DEHP. For example, DnBP residues reported in procedural blanks ranged from 10 to 2,500 ng/sample. While instrument detection limits for DPEs are typically in the low picogram range, MDLs of these compounds tend to be in the high ng/g range, due to high background levels in procedural blanks. Thus, positive detection of DPEs at ppb levels is possible, but extremely challenging.

Nevertheless, the various interlaboratory comparisons did provide some encouraging results. In particular, multiple participating laboratories reported relatively consistent concentrations of DEHP in CARP-2 samples. Similarly, DEHP measurements in sewage sludge samples were in good agreement with reported concentrations by the U.S. EPA. The data indicate that Lab A data and U.S. EPA analyses were generally successful at identifying samples with low DEHP levels (e.g., samples 68320, 68349, and 68359), as well as those samples with extremely high DEHP concentrations (e.g., samples 68340, 68345, and 68346) (Fig. 6). Some discrepancies between the two data sets were apparent. In some cases DEHP concentrations reported in the U.S. EPA TNSSS were 20 times higher than those levels reported by Lab A (e.g., samples 68315, 68321, and 68341) (Fig. 6). Background contamination during these original analyses may be the reason for the comparatively higher DEHP concentrations in these samples. Di(2-ethylhexyl) phthalate (DEHP) is a common target analyte in environmental monitoring programs. The relatively good agreement for DEHP concentration measurements in sewage sludge provides some degree of confidence regarding the accuracy of DPE concentrations from municipal wastewater effluent and sludge monitoring programs.

While the results indicate relatively consistent measures for DPEs in high-level samples such as sewage sludge, the accuracy of DPE measurements in relatively low-level environmental samples such as natural sediments and tissues can be significantly reduced, primarily because levels in procedural blanks approach levels in sample extracts. For example, DEHP amounts in blanks were relatively low (mean = 52 ng/sample) compared with levels in sludge (72,000 ± 3,720 ng/g). Analysis of replicate blank samples ($n = 5$) resulted in an MDL of 177 ng/g for DEHP in sludge. Thus, background contamination did not significantly affect the ability to detect DEHP in sludge. In contrast, positive detection of DPEs in less contaminated matrices (e.g., biota) is more challenging, as those samples may contain only slightly higher DPE residue amounts than procedural blanks. For example, Lab A reported concentrations of DEHP in fortified milk (149 ± 13.7), sediment (430 ± 46.6), and CARP-2 (1,550 ± 148), levels that are comparable to levels reported in procedural blanks. However, it is important to note that when background contamination was minimized (<10 ng/sample), relatively low MDLs of DPEs (<0.1 ng/g) were achieved, enabling low-level quantification of these compounds in environmental and biological samples.

The findings also clearly highlight the need for laboratories involved in low-level DPE residue analysis to mitigate background contamination. While reagents and sorbents can be contaminated with DPEs during manufacturing and packaging, laboratory air is undoubtedly a key factor influencing the degree of background contamination during DPE analysis. In particular, high DPE levels in laboratory air (gas-phase and/or particulate-bound residues) can contaminant sorbents and solvents. Airborne DPEs may also adsorb to glassware surfaces.

Table 1. Dialkyl phthalate ester concentration (ng/g dry wt) measured in individual municipal sewage sludge samples

			Individual dialkyl phthalate esters								Isomeric mixtures				
Sample ID	Moisture %	TOC %	DMP	DEP	DiBP	DnBP	BBP	DEHP	DnOP	DnNP	C6	C7	C8	C9	C10
68318	73.7	37.9	19.0	92.1	40.0	209	449	14,600	390	590	ND	708	4,950	194	4,540
68319	81.9	37.8	0.5	5.4	2.9	12.4	55.1	382	12.8	25.1	ND	49.9	831.0	32.9	735
68323	73.9	21.7	24.8	16.3	8.3	26.8	70.2	5,700	131	157	ND	76.9	2,720	107	2,660
68340	63.8	19.0	40.0	63.0	58.5	96.3	429	23,600	910	1,220	ND	858	5,420	236	4,510
68345	70.8	24.4	44.5	58.2	76.7	1,260	222	73,500	670	492	ND	2,170	6,600	263	5,200
68350	34.8	20.9	0.3	54.3	ND	ND	1.8	18,200	ND	ND	ND	60.5	2,020	79.2	2,470
68351	73.9	29.5	9.5	41.3	8.8	41.9	36.2	25,600	282	277	ND	341	4,710	152	3,200
68338	86.0	29.6	4.4	22.9	0.9	18.5	35.1	254	11.8	13.1	ND	167	513	47.5	1,010
68352	77.3	25.3	30.6	8.6	52.4	181	177	24,500	545	816	ND	908	5,980	269	5,240
68358	82.4	29.4	1.1	25.2	ND	1.7	7.5	305	6.3	10.2	ND	15.0	469	41.6	388
68315	68.9	31.1	ND	ND	23.4	12.8	8.7	1,190	36.7	82.4	4.7	20.5	232	345	148
68317	77.5	20.1	ND	225	ND	ND	6.1	243	2.5	8.2	0.8	0.5	63.5	63.3	8.3
68321	77.1	26.3	3.9	ND	ND	ND	39.6	739	12.9	39.2	4.6	11.9	146	194	60.2
68342	73.1	43.1	4.4	ND	12.6	267	693	3,530	82.0	ND	5.1	87.3	621	523	303
68346	59.0	34.5	27.5	ND	0.3	7.3	52.6	187,000	444	ND	0.1	120	1,300	1,390	564
68347	85.9	36.1	ND	ND	1.6	14.2	38.5	511	10.0	14.8	1.3	12.1	105.7	173	64.5
68320	38.0	17.3	0.5	ND	1.0	1.9	24.6	483	ND	1.9	–	–	–	–	–
68341	76.1	41.9	ND	ND	1.3	ND	30.9	767	23.6	39.4	3.5	32.9	196	409	123
68349	88.2	21.5	ND	163	ND	ND	4.3	60.4	3.7	7.1	1.0	6.3	1.4	37.9	2.6
68359	83.6	31.6	ND	ND	ND	ND	4.6	139	11.3	14.0	ND	6.9	26.7	430	23.5
Range	34.8–88.2	17.3–41.9	0.3–44.5	5.4–225	0.3–76.7	1.7–1,260	1.8–693	60.4–187,000	2.5–910	1.9–1,220	0.1–5.1	0.5–2,170	1.4–6,600	32.9–1,390	2.6–5,240
Median	75.0	29.5	6.95	47.8	8.55	22.6	37.4	978	30.2	39.2	2.39	60.5	621	194	563
Average	72.3	29.0	15.1	64.6	20.6	153	119	19,100	199	224	2.6	297	1,940	263	1,640
SD	14.3	7.77	15.7	66.8	25.7	330	189	43,200	278	355	2.1	540	2,340	310	1,970

TOC = total organic carbon; DMP = dimethyl phthalate; DEP = diethyl phthalate; DiBP = di-iso-butyl phthalate; DnBP = di-*n*-butyl phthalate; BBP = butyl benzyl phthalate; DEHP = di-ethylhexyl phthalate; DnOP = di-*n*-octyl phthalate; DnNP = di-*n*-nonyl phthalate; ND = not determined.

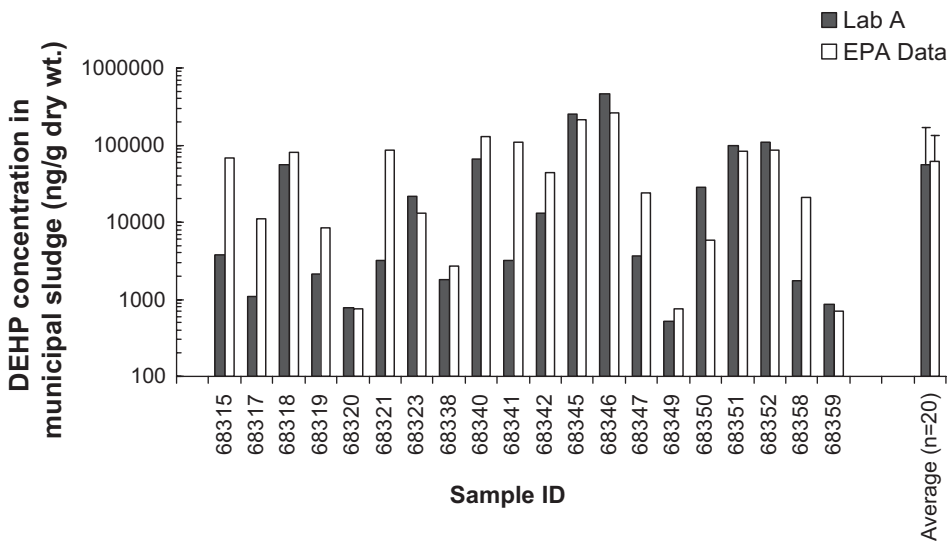

Fig. 6. Measured concentrations of di-ethylhexyl phthalate (DEHP) (ng/g dry wt) in municipal sewage sludge samples reported by Lab A and those reported by the U.S. Environmental Protection Agency. Data are presented on log scale. Arithmetic mean of all samples ($n = 20$) is shown along with standard deviation.

Lin et al. [17] previously demonstrated that DPE background contamination can be greatly reduced (by a factor of 2–3) by employing robust cleaning protocols for glassware and distilling solvents used for sample extraction and cleanup. In the present study, Lab A exhibited the lowest degree of background contamination, and thus lower detection limits. Following Lin et al. [17], Lab A uses extensive cleaning protocols and uses double-distilled hexane for extraction and alumina chromatography. Glassware and equipment were detergent washed, rinsed first with water and then acetone, double-distilled hexane, and dichloromethane, respectively, then baked at 400°C for at least 10 h, and stored in clean aluminum foil. Prior to use, glassware was rinsed again with acetone, double-distilled hexane, and dichloromethane. Mortar and pestles were cleaned using the same procedure as that for glassware but were baked at 150°C for 10 h. Alumina and sodium sulfate were baked at 200 and 450°C, respectively, for at least 24 h. Other laboratory items such as Teflon stoppers, GC vials, septa, and caps that decompose at elevated temperatures were washed extensively with 1:1 dichloromethane/hexane. Additional sample preparation procedures such as freeze-drying of samples may also be a source of DPE contamination. While one participating laboratory in the present study (Lab E) employed freeze-drying, the extent of DPE contamination originating from this approach could not be quantified in the present study.

CONCLUSIONS

The present study reports the results of several interlaboratory comparison studies to assess the accuracy of dialkyl phthalate ester concentration measurements in environmental and biological samples. The results demonstrate that environmental monitoring of DPEs is often hampered by high MDLs, due to high levels in procedural blanks. Interlaboratory comparisons of DPE analyses of two commercially available CRMs (CARP-2 and BCR-607) were conducted to evaluate the plausibility of establishing a CRM capable of assessing method performance and accuracy in future DPE analyses. Good interlaboratory agreement was found for DEHP measurements in CARP-2 samples. Similarly, DEHP measurements in sewage sludge samples were in good agreement with concentrations reported by the U.S. EPA. However, results were less consistent for other DPEs, primarily due to background contamination issues. The findings highlight the need for analysts to mitigate effects of background DPE contamination. Reagents, solvents, and sorbents can be contaminated with DPEs during manufacturing and packaging. In particular, use of double-distilled hexane for extraction and chromatography may greatly reduce DPE residues in procedural blanks. Also, laboratory air is undoubtedly a key factor influencing the degree of background contamination during DPE analysis. Thus, rigorous cleaning of laboratory glassware, equipment, and sorbents and minimizing sample handling during analysis are essential for trace residue analysis of DPEs in environmental samples. Use of clean rooms with positive-pressure/high-efficiency particulate filtered air for sample extraction/processing may also prove beneficial. Regardless, future efforts to develop better protocols to lower MDLs, as well develop reference materials, would greatly benefit future DPE monitoring initiatives.

SUPPLEMENTAL DATA

Table S1. Summary of laboratory participation.

Table S2. Summary of procedural blanks (ng/sample), method detection limits (MDL, ng/g wet wt) and observed concentrations (ng/g wet wt) of dialkyl phthalate in CARP-2 certified reference material reported by Lab A, B, C, and D.

Table S3. Summary of procedural blanks (ng/sample), method detection limits (MDL, ng/g dry wt) and observed concentrations (ng/g dry wt) of dialkyl phthalate in marine sediment reported by Lab A, B, C, and D.

Figure S1. Structural formula of dialkyl phthalate esters.

Figure S2. Measured blank levels (ng/sample) of ΣDPEs (sheep milk) and DEHP (sheep muscle and liver), reported by Lab A and Lab E during interlaboratory Study #2.

Figure S3. Measured blank levels (ng/sample) reported by Lab A during analyses of municipal sludge samples for individual DPEs (**A**) and C6-C10 isomeric mixtures (**B**). (205 KB DOC)

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