

ABSTRACT

Title of Document: QUANTITATIVE ASSESSMENT OF
BIS(2-ETHYLHEXYL) PHTHALATE IN
COMMONLY-CONSUMED FOODS AND
ELUCIDATION OF ITS NATURAL
COMPONENT IN STILTON CHEESE BY
COMPOUND-SPECIFIC CARBON
ISOTOPE ANALYSIS

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Bis(2-ethylhexyl) phthalate (DEHP) is classified as a “priority hazardous substance” by the European Union, a probable human carcinogen by the United States Environmental Protection Agency, and is a suspect human endocrine disruptor. This ubiquitous compound is measurable in many food matrices. Screening of nine fatty and processed foods commonly consumed in the United States for DEHP was performed with an internal standard addition method that utilized Gas Chromatography-Electron Impact Mass Spectrometry (GC-EIMS). Blank-adjusted average mass fractions in each food ranged from 0.18 mg/kg ($1 \sigma = 0.07$ mg/kg) to 1.57 mg/kg ($1 \sigma = 0.24$ mg/kg), with cheeses containing the largest. Organisms such as penicillium used in the production of Stilton cheese have been considered likely sources of naturally-occurring phthalate. While Anthropogenic DEHP is produced from petrogenic chemicals, biogenic DEHP is

likely produced by organisms utilizing atmosphere-equilibrated carbon containing a quantity of ^{14}C isotopes measurable by Accelerator Mass Spectrometry (AMS). The ^{14}C abundance of DEHP isolated from Stilton cheese allowed for the determination of its contemporary, and thus biogenic, fraction of carbon. Five $\approx 90\ \mu\text{g}$ quantities of DEHP were extracted from $\approx 12\ \text{kg}$ of cheese and isolated by silica gel, size exclusion, and high performance liquid chromatography (HPLC) for AMS. Sample masses were determined by GC-EIMS and combusted CO_2 manometric measurements. The purity of carbon as DEHP in each isolate ($87.2\ \% \pm 1.7\ \%$ to $94.0\ \% \pm 1.3\ \%$, $n=5$, 95 % C.I.) was determined by multivariate deconvolution of GC-EIMS fragmentation spectra. Concurrently processed isolation method blanks contained from $0.61\ \mu\text{g} \pm 0.04\ \mu\text{g}$ to $1.84\ \mu\text{g} \pm 0.09\ \mu\text{g}$ ($n=3$, 1σ uncertainty) DEHP per sample and significant quantities of extraneous carbon contamination. Measurements of $^{13}\text{C}/^{12}\text{C}$ isotope ratios were made to correct reported ^{14}C values for instrumental and natural fractionation. The mean ^{14}C -corrected contemporary carbon fraction of DEHP in all isolates was 0.242 ± 0.068 ($n=5$, $1\ \sigma$), revealing that the majority of DEHP at $75.8\ \% \pm 6.8\ \%$ in Stilton cheese is anthropogenic, but with a significant naturally-occurring component.

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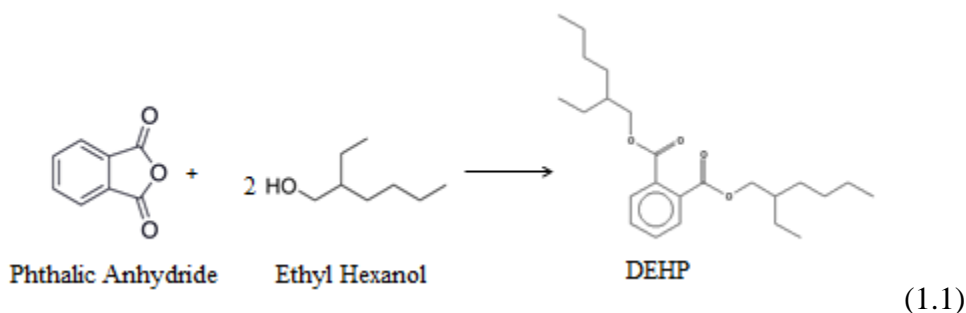
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Chapter 1: Introduction

Phthalic acid esters (PAE, i.e., 1,2-benzenedicarboxylic acids), also known as phthalates, are manufactured as additives for a myriad of commonly-consumed products. There is an estimated five million-metric ton annual worldwide production of phthalates that are used as, amongst other items, plasticizers in polymeric materials, solvents of lacquers and dyes, and fragrance-binding compounds (LCSP 2011). They therefore are integral components of countless items such as clothing, cosmetics, perfumes, adhesives, ink, paint, and a multitude of plastics (Cohen et al. 2007).

Products containing polyvinyl chloride (PVC) plastic may contain up to 50 % phthalate by weight, one of the most common being bis(2-ethylhexyl) phthalate (DEHP) (LCSP 2011), to provide them with a desired degree of flexibility. This is a viscous liquid that is produced by the esterification of phthalic anhydride with ethylhexanol.



Due to the fact that liquid phthalate additives are not chemically bound to polymeric materials (Heudorf et al. 2007), and that they are commonly used as solvents, it is estimated that nearly 2 % of their total annual production is released into the environment by leaching and volatilization (Huber et al. 1996).

Despite possessing a low acute toxicity (Jarosova 2006), DEHP has been classified as a probable human carcinogen by the U.S. Environmental Protection Agency (1997), a priority hazardous substance by the European Union (ECPI 2012), and is suspected to be a human endocrine disruptor that mimics estradiols at estrogen hormone receptor sites (Main et al. 2006). It is suggested that its potential for endocrine disruption may lead to irregular development and feminization in young boys and premature development of girls (Colón et al. 2000). Studies suggesting that DEHP reduces sperm counts in laboratory animals (Agarwal et al. 1986) raises concern for its capacity to decrease fertility in men. In-utero exposure and post-partum human milk consumption also raise concerns of the risk of DEHP exposure to mothers during times of important fetal and infant development (Zhu et al. 2006).

Though ubiquitously present in much of the environment, European studies infer that direct use of phthalate-containing consumer products and indoor environs provide the bulk of human exposure to most phthalates, with food having a particular proclivity to cache di-isobutyl (DIBP), diethylhexyl (DEHP), and dibutyl phthalates (DBP) (Wormuth et al. 2006). Phthalate absorption through human skin is minimal and human dietary consumption has been identified as the single most-likely route of exposure to the general populace (Fromme et al. 2004; Skakkebaek et al. 2006). Phthalates have a proclivity for leaching into fatty dietary consumables (Castle et al. 1990; Cavaliere et al. 2008), as was evidenced by their accumulation of 2 to 80 mg/kg in meats cold-stored with dioctylphthalate-plasticized PVC wrapping (Kondyli et al. 1992). As such, the EU has phased out the use of phthalates in food-contact materials (EC 2007) and its use in the U.S. has been severely curtailed as manufacturers have begun to use alternative

plasticizers such as bis(2-ethylhexyl) adipate or polymers that do not require plasticizer (U.S. FDA 2009). The American Plastics Council claims that phthalates are not “used in plastic food wrap, food containers, or any other type of plastic food packaging sold in the United States” (Enneking 2006), though the USFDA lists it as an Indirect Additive Used in Food Substances (USFDA, 2011) and an assessment of European food-contact materials conducted by the European Food Safety Authority, suggest that they are often present (ESFA, 2005), particularly in printed wrappings with phthalate-containing ink (Jarosova 2006). In addition, DEHP contamination of edible ingredients by PVC materials used for their harvesting, processing, and storage supports the need for analysis of several food types that potentially contain significant quantities of these trace contaminants (Castle et al. 1990; Petersen et al. 2010), especially as production processes and the equipment they utilize vary considerably amongst producers and distributors. This has been done for a wide range of food matrices in European and Asian nations, (Guo et al. 2012; Tomita et al. 1977; Wenzl 2009; Wormuth et al. 2006), however sparse data exists quantifying DEHP in food originating in the United States.

Of particular concern are fatty and highly-processed foods which have a higher propensity to leach fat-soluble plasticizers from contacting materials and have extended exposure to several synthetic surfaces during mechanized production (Tsumura et al. 2003). A comprehensive European study of DEHP in food (Wormuth et al. 2006) reported that average concentrations in non-dairy beverages were 0.01 mg/kg to 0.04 mg/kg, those in non-fatty foods such as fruit, vegetables, and grain products, were 0.01 mg/kg to 0.57 mg/kg, and those in fatty foods such as oils, dairy, animal, and nut

products were 0.22 mg/kg to 1.45 mg/kg. These concentrations were consistently greater than those observed of six other phthalates in the same food matrices.

In addition to food processing and storage, phthalate in the environment is believed to contribute to its presence in raw food items prior to their direct exposure to plastics used during their harvest and distribution. One such instance is suggested to be the accumulation of phthalate in meat and dairy products as a result of its presence in the soil of pastures used for grazing livestock (Rhind 2005). Given the propensity for phthalate to amass in dairy products, particularly those with a high lipid content, it is little surprise that European studies have found them to possess such relatively large mass fractions of DEHP (Sharman et al. 1994)

Aside from the aforementioned sources of industrially-produced DEHP, it has also come to light that several organisms, including marine algae and penicillium, have demonstrated the capacity to produce this phthalate naturally by, as yet unknown, inherent modes of biochemical synthesis (Amade et al. 1994; Chen 2004; Namikoshi et al. 2006; Sastry et al. 1995). Many of these organisms are often used as additives or supplements in commonly-consumed foods around the world. Blue cheese, including Stilton, is a food which typically contains some species of a microbial genus (*Penicillium*) evidenced to naturally produce phthalate (Amade 1994). Therefore, an assessment of the risk for phthalate exposure by such a food, and subsequent measures required to mitigate it, necessitate elucidation of the phthalate's origins. It is for this reason that the aims of this project were to quantify the presence of DEHP in several matrices and further examine the potential for this compound to exist in food as a naturally-produced ester. Accordingly, undertaken in this study was a screening and

quantitative analysis of DEHP in several domestically-produced food products by Gas Chromatography – Electron Impact Mass Spectrometry (GC-EIMS), as well as a quantitative determination of the contemporary, biogenic fraction of this phthalate in Stilton cheese via compound-specific ^{14}C isotope analysis by Accelerator Mass Spectrometry (AMS). These characterizations were made to provide additional, locally-applicable information concerning the prevalence of DEHP contamination in foods, as well as investigate the identity of its sources. To identify appropriate measures required to minimize exposure to this phthalate, it must first be determined if it is a potentially-preventable artifact of an anthropogenic process, or an inherent biological component of the food. Unlike many other phthalate-containing materials, such as cosmetics and certain plastic products, the consumption of food is a necessary and unavoidable route of human phthalate exposure. In the interest of public health, this makes recognition of the primary sources of dietary DEHP, as well as the elucidation of its origins, all the more pertinent.

Chapter 2: Screening of Fatty and Processed foods for DEHP

2.1 Background

Given that packaging materials and synthetic contact surfaces used during the production of food may impart DEHP contamination, highly-processed and lipidic foods have significant potential to accrue this phthalate by the time they reach the consumer (Castle et al. 1990; Kondyli et al. 1992; Tsumura et al. 2003). A need exists to assess phthalate contamination in the various products found in a typical American diet, especially considering that food-processing methods vary considerably from farm to table and ingredients travel from several geographically disparate sources. Herein, the mass fractions of DEHP, typically greater than those of any other phthalate, were assessed in several fatty or processed foods.

Many analytical laboratories contain polymers plasticized with DEHP. Aside from obvious sources such as plastic consumables and containers that may come into contact with a sample during preparation and analysis, ambient particles and various tubing and fittings embedded in gas lines, chromatographic systems, and ventilation systems, can also pose significant threats of contamination. Given the fact that only 0.1 mg/kg to 10 mg/kg of DEHP is present in most foods, it is imperative to minimize this contamination and fully account for its accruelement in samples during the analytical process. The physical similarity of DEHP to the many fatty acids and fatty acid esters often necessitates extensive purification of fatty samples prior to analysis, and thus increases a sample's exposure to potentially-contaminating surfaces. Methods

championing very diligent and minimal sample preparation, while achieving adequate detection sensitivity, are imperative for the screening of foods for DEHP.

In this study, selected ion monitoring by Gas Chromatography - Electron Impact Mass Spectrometry (GC-EIMS) provided adequate sensitivity for rapid analysis of small samples. Also, the addition of a fully-deuterated, d_{38} -DEHP internal standard (I.S.) to food samples prior to their extraction and the determination of a DEHP/ d_{38} -DEHP I.S. Relative Response Factor (RRF) helped to reconcile effects of reduced extraction efficiency, sample recovery, and instrumental inconsistencies. Two slightly different sample preparation techniques were used to assess DEHP levels in nine widely-available, domestically-produced (U.S.) foods. Dry food samples included supermarket brand snack crackers, chocolate chip cookies, and cornstarch. Higher-moisture foods included supermarket brand mayonnaise, vegetable shortening, cheddar cheese (sharp), American cheese (individually-wrapped slices), processed canned pork lunch meat, and brand-name canned chicken sausage, none of which were of a low-fat variety.

2.2 Sample Preparation

Food samples were purchased from a local (Gaithersburg, MD) supermarket and stored in their original wrapping at $-20\text{ }^{\circ}\text{C}$. Organic HPLC-grade n-hexane, acetonitrile, methylene chloride, and methanol (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) were used during sample preparation, calibration, and analyses. All glassware was baked at $450\text{ }^{\circ}\text{C}$ for eight hours, rinsed with acetone, and covered with baked aluminum foil to minimize phthalate contamination. Care was taken during transfer of samples to extraction vials and weighing to not touch them with any surface other than the original

food packaging and a stainless steel spatula that was cleaned by sonication in organic solvent.

Multiple 5 g – 10 g samples of each food were weighed (Appendix 1) into 50 mL glass extraction vials with a Mettler Toledo (Langacher Greifensee, Switzerland) Sartorius precision weighing balance. Samples were then spiked with aliquots of I.S. prepared with fully-deuterated (d_{38} , 98%) DEHP (Cambridge Isotope Laboratories, Andover, MA, USA) in acetonitrile (~ 63 $\mu\text{g/g}$). These samples were prepared during three sets of extractions, each with a corresponding set of sample preparation-method blanks (Table 2.1) comprised of extraction solvent spiked with I.S. and processed alongside samples.

Table 2.1 Food extraction sets and corresponding sample (g), Internal Standard (mg), and food matrix fat (%) masses.

	Food Type	Extracted Mass (g)	I.S. Spike Masses (mg)	n	% fat by mass ¹
Set A	Crackers	4.90 - 6.31	40.2 - 40.8	3	27
	Cornstarch	5.20 - 5.39	40.0 - 40.5	3	0
	Cookies	5.94 - 8.89	38.9 - 43.0	3	13
	A-Blanks	-	39.8 - 41.4	3	-
Set B	Mayonnaise	5.56 - 9.23	39.8 - 41.2	4	77
	Vegetable shortening	4.71 - 5.03	39.8 - 40.9	4	100
	Cheddar Cheese	5.56 - 7.92	39.2 - 41.4	3	32
	B-Blanks	-	39.8 - 43.3	4	-
Set C	American Cheese	4.17 - 6.46	38.4 - 40.7	4	26
	Chicken Sausage	7.63 - 8.86	39.1 - 41.9	3	20
	Processed Pork meat	6.81 - 8.04	42.7 - 44.4	4	29
	C-Blanks	-	35.6 - 41.5	4	-

¹Approximate, as determined from reported mass of fat on nutrition label (g)

Phthalates can be extracted from food by many organic solvents, though they are ideally extracted from *fatty* foods with acetonitrile (Wenzl 2009) due to the fact that they are readily partitioned, while many very non-polar lipids are not. Samples of extraction Set A, containing foods with very little water, were manually homogenized with a stainless steel spatula and extracted into 20 mL of acetonitrile at ~40 °C for 10 min and sonication for ~25 min. The extract vials were centrifuged for 5 min and their supernatants were decanted into clean secondary 50 mL glass extraction vials. The process was repeated with 15 mL of acetonitrile and both corresponding supernatants were combined. These acetonitrile extracts were shaken with 4 mL of acetonitrile-saturated hexane in a 60 mL separatory funnel and decanted into a 100 mL round bottom flask for concentration by vacuum rotary evaporation (Buchi, Flawil, Switzerland). All concentration was performed by rotary evaporation to minimize the potential for DEHP contamination. Concentration with a Turbovap (Biotage, Uppsala, Sweden) nitrogen gas vortex concentration system demonstrated markedly elevated levels of laboratory DEHP contamination in previously-assessed blanks.

The food matrices of extraction Set A and Set B were homogenized, spiked with internal standard, and extracted into 4-5 mL of hexanes. Similar to samples of extraction Set A, they were heated at ~40 °C and sonicated for ~25 min. Hexane extracted the lipidic components of these foods while limiting extraction of water and other polar constituents. These hexane extracts of foods and blanks were partitioned into 30 mL of hexane-saturated acetonitrile in a 60 mL separatory funnel. The solvents were allowed to stratify and the acetonitrile layer was decanted and reduced to ~1.25 mL for additional purification by preparatory size exclusion chromatography (SEC).

Size exclusion chromatography was performed with tandem 30 cm, 21 mm-I.D. Oligopore columns (Agilent Technologies, Santa Clara, CA) containing 6 μm particles with poly-dispersed pore diameters and conditioned with 100 % methylene chloride. These columns were preceded by a PLGel (Agilent) guard column containing 10 μm particles with 100-angstrom pore diameters. Mobile phase flow rate was 10 mL/min, delivered by a Varian 9012 pump system (Agilent). Samples (1.25 mL) were injected onto a 1.5 mL stainless steel sample injection loop. DEHP eluted from the column after ~ 19 min with mobile phase that eluted 189 mL – 199 mL after injection. These fractions were collected into clean 10 mL volumetric flasks, rotary evaporated to dryness in a 10 mL pear-shaped glass recovery flask, and reconstituted in ~ 0.5 mL of methanol for analysis by GC-EIMS. Samples were delivered into 2 mL glass amber (Agilent) auto-loading GC vials by glass pipette and tightly capped with polytetrafluoroethylene (PTFE)-lined polysiloxane septum screw-caps.

2.3 Quantification of DEHP Mass Fractions with GC-EIMS Calibration

Analyses of samples were performed in methanol due to its high phthalate solubility and suitability for gas chromatography. It also possesses a lower vapor pressure at room temperature than some other common GC solvents, such as methylene chloride and diethyl ether, which allowed for more accurate gravimetric DEHP calibrant mass fraction determinations and sample analyses (Appendix 1.3). GC-EIMS instrumentation consisted of an Agilent Technologies 6890N Network GC system, a 7683 Series Autosampler, and a 5973 *inert* quadrupole mass-selective detector (MSD). Sample, blank, and calibrant EI-MS measurements were made after 1 μL on-column injections to a 0.25 mm x 60 m DB-XLB (Agilent) 0.25 μm methyl polysiloxane

stationary phase column coupled to a 3 m deactivated fused-silica capillary retention gap. Helium carrier gas flow during analysis was 1.5 mL/min.

The GC temperature program was set at 63 °C for 3 min after injection, ramped 45 °C/min to 200 °C, then ramped 7.5 °C/min to 320 °C, where it held for 3 min. DEHP eluted at 20.1 min. The MSD performed continuous scans of ions with $m/z = 50$ to $m/z = 300$.

Eight calibrants were prepared from gravimetric dilutions of three separately-prepared stock solutions of bis(2-ethylhexyl) phthalate (99.8 % \pm 0.1 %, Supelco Analytical, Bellefonte, PA, USA) and I.S. in methanol to determine an appropriate relative response factor (RRF) for sample analysis (Appendix 1). GC-EIMS data acquisition was automated by Agilent MSD Chemstation® software and analyzed post-collection with its Enhanced Data Analysis feature. The selected ion integrated at 20.1 min. for quantification of DEHP was $m/z = 149$, and that for the d_{38} DEHP I.S. was $m/z = 154$. The I.S. EI-MS fragment of mass 154 has the same structure as that of DEHP fragment with mass 149 (Figure 2.1.a), though containing five deuterium rather than normal ^1H hydrogen atoms (Figure 2.1.b).

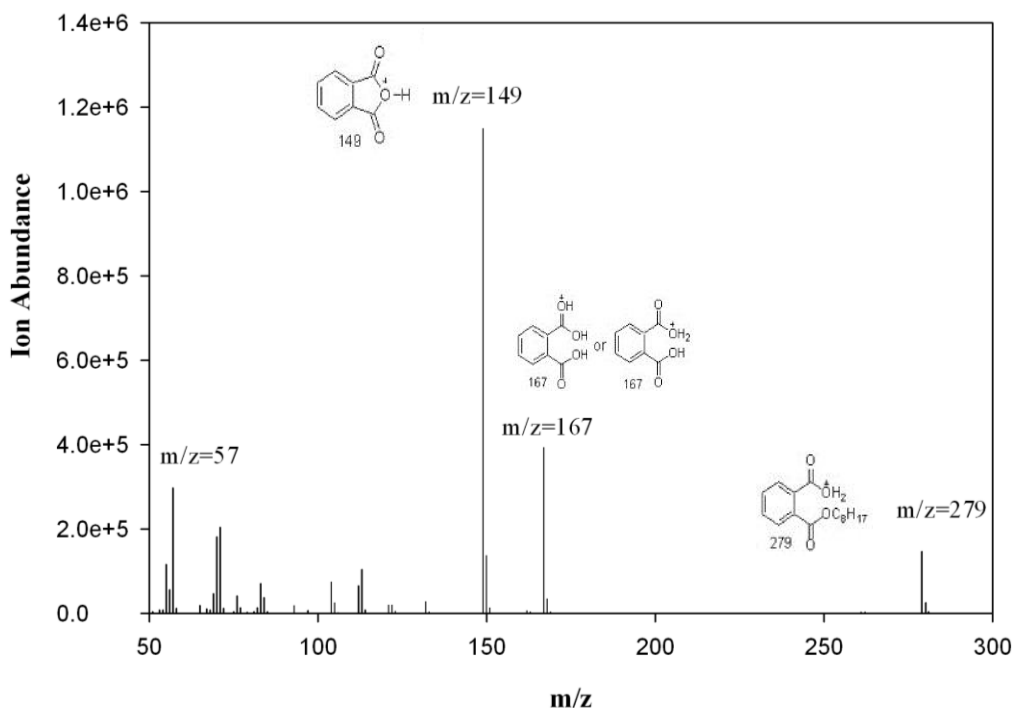


Figure 2.1.a EI-MS $m/z=50$ to $m/z=300$ spectrum of DEHP (0.3ng) and ion fragment structures.

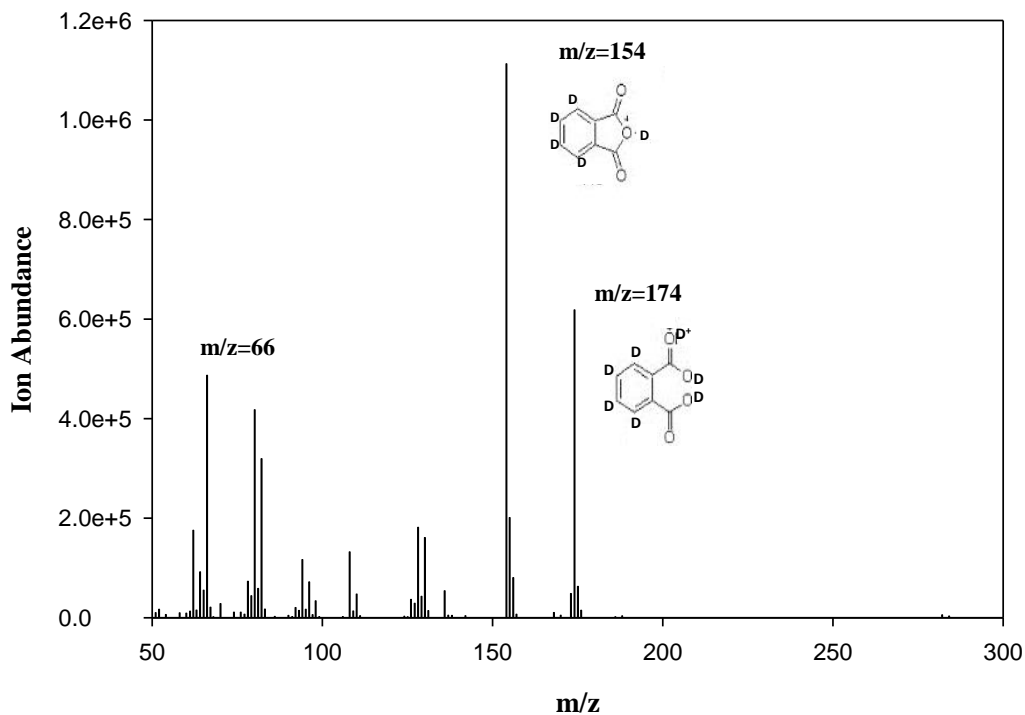


Figure 2.1.b EI-MS $m/z=50$ to $m/z=300$ spectrum of d_{38} -DEHP Internal Standard and structure of analyzed ion with $m/z=154$.

The mass fractions of unlabeled DEHP and I.S., along with their integrated ion relative abundances, are shown in Table 2.2.

Table 2.2 GC-EIMS calibrant DEHP($\mu\text{g/g}$) and I.S. mass fractions (g/g).

Original Stock Solution	Calibrant I.D.	[DEHP] ($\mu\text{g/g}$)	[IS] (g/g)	m/z=149:154 ratio	[DEHP]/[I.S.]
1	1-1	0.000 \pm -	0.00226 \pm 0.00002	0.0000	0.000
1	1-2	0.090 \pm 0.001	0.00157 \pm 0.00002	0.0760	57.44
2	2-3	0.179 \pm 0.001	0.00061 \pm 0.00001	0.3807	292.7
2	2-4	0.464 \pm 0.003	0.00160 \pm 0.00001	0.3935	289.9
2	2-5	0.430 \pm 0.003	0.00170 \pm 0.00001	0.3403	252.9
2	2-6	0.481 \pm 0.002	0.00219 \pm 0.00001	0.3153	219.3
3	3-7	0.421 \pm 0.007	0.00126 \pm 0.00002	0.3508	243.5
3	3-8	0.287 \pm 0.005	0.00173 \pm 0.00002	0.1255	99.92

The solutions were prepared such that the magnitude of their integrated m/z = 149 and m/z = 154 signals bracketed those observed in all signals and blanks (DEHP: 0 $\mu\text{g/g}$ to 0.68 $\mu\text{g/g}$, I.S.: 660 $\mu\text{g/g}$ to 2870 $\mu\text{g/g}$). The order of calibrant, blank, and sample GC-EIMS analyses was randomized to account for maximum uncertainty resulting from variations such as inconsistent EI ionization, quadrupole performance, detector drift, or memory effects.

Given that:

$$\left(\frac{A_{149}}{[DEHP]}\right)_{DEHP\ std} = RRF \times \left(\frac{A_{154}}{[I.S.]}\right)_{I.S.} \quad (2.1)$$

where A_{149} and A_{154} are the integrated peak areas obtained at ≈ 21 min. of the m/z=149 and m/z=154 chromatograms, and [DEHP] and [I.S.] are the mass fractions of DEHP and I.S. as $\mu\text{g/g}$ and g/g, respectively, then the relative response factor of DEHP and I.S. is determined by,

$$RRF = \frac{A_{149}}{A_{154}} \bigg/ \frac{[DEHP]}{[I.S.]} \quad (2.2)$$

The analysis and resultant eight-point linear least squares regression of calibrant detector responses (A_{149}/A_{154}) with respect to their DEHP and I.S. mass fractions was used to determine this response factor and its linearity in the appropriate range. Thus, the slope of the least squares calibration regression in Figure 2.2 ($R^2=0.9935$) is the determined response factor of DEHP: I.S. (RRF) and is equal to $1.368 \times 10^{-3} \pm 4.5 \times 10^{-5}$.

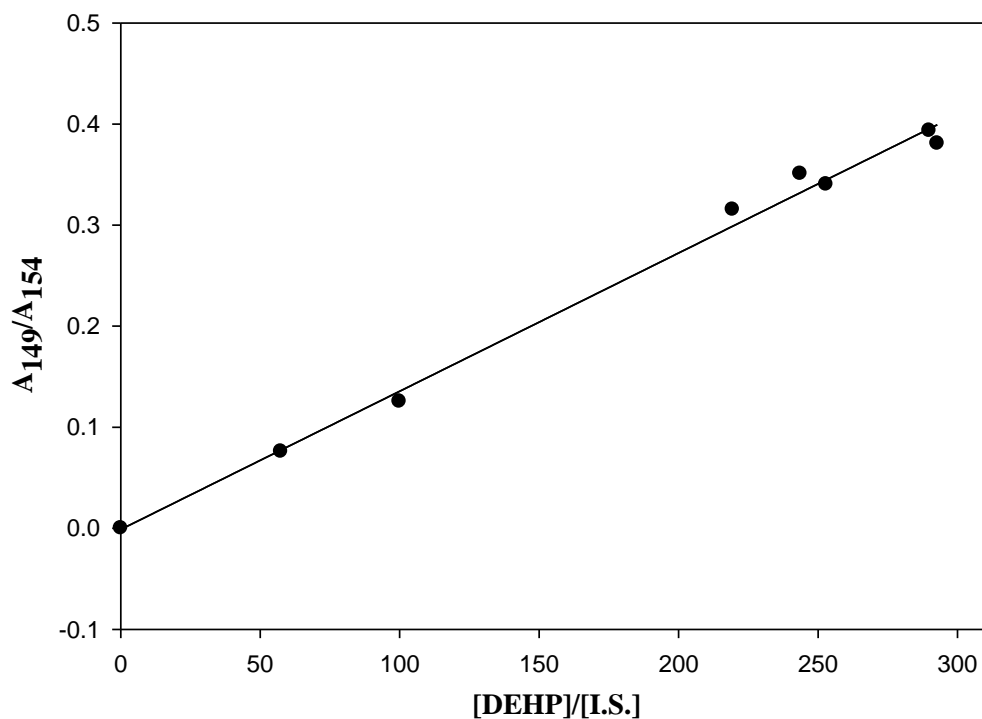


Figure 2.2 Linear least squares regression of GC-EIMS calibration. Slope of the regression ($1.368 \times 10^{-3} \pm 4.5 \times 10^{-5}$) is equal to the DEHP: I.S. GC-EIMS relative response factor.

The masses of DEHP accrued in the method blanks from all solvent and sample preparation steps were calculated by their [DEHP] ($\mu\text{g/g}$) determined from this calibration and their total mass in methanol by

$$M_{BLKDEHP} = M_{MeOH} \left(\frac{A_{149}}{RRF \times \left(\frac{A_{154}}{M_{IS}} \right)} \right), \quad (2.3)$$

where M_{IS} , M_{MeOH} , and $M_{BLKDEHP}$ are the masses of the internal standard solution added to the blank prior to processing (mg), mass of GC-EIMS-analyzed blank in methanol (g), and mass of estimated DEHP in method blank (μg), respectively. The values determined for $M_{BLKDEHP}$ are shown in Table 2.3.

Table 2.3 Mass estimates of DEHP in method blanks (μg).

Blank Set	Foods Co-processed with blank	(n)	Mass DEHP (μg) ¹		
A	Cookies, Crackers, Cornstarch	3	0.94	±	0.27
B	Mayonnaise, Vegetable Shortening, Cheddar Cheese	4	0.75	±	0.75
C	Pork Meat, Chicken Sausage, American Cheese	4	0.47	±	0.24

¹ $M_{BLKDEHP}$

The mass fractions of DEHP in each food sample ($[DEHP]_{food}$) were correspondingly adjusted for these blank masses and calculated by,

$$[DEHP]_{food} = \left(\frac{A_{149}}{RRF \times \left(\frac{A_{154}}{M_{IS}} \right)} - M_{BLKDEHP} \right) \times M_{sample} \quad (2.4)$$

Where M_{sample} is the mass of the whole food sample prior to extraction (g).

2.4 Screening Results

There was a degree of relative variability in the mass of contaminant DEHP in the blanks, particularly in Blank Set B. The variability likely results from the ubiquity of DEHP in indoor environments, and the fact that any incident contamination has a large relative effect on the small mass present in each blank. Many blanks had no detectable DEHP. The calculated estimates of blank-adjusted DEHP mass fractions in the food matrices and the standard deviation of n samples of each are shown in Table 2.4. These results are illustrated by box plots Figure 2.3.

Table 2.4 Results of GC-EIMS analyses of food samples: Relative DEHP and I.S. detector responses, masses of DEHP in extracts (μg), and blank-adjusted mass fractions (mg/kg) of DEHP in screened food.

	Food Type	$\frac{A_{149}}{A_{154}}$ Range	Mass of Extracted DEHP Range (μg)	Blank- Adjusted [DEHP] Range (mg/kg) ¹	Mean [DEHP] (mg/kg) ¹
Set A	Crackers	0.2745 to 0.2777	8.08 \pm 0.27 to 8.29 \pm 0.27	1.19 \pm 0.06 to 1.50 \pm 0.09	1.27 \pm 0.11
	Cookies	0.1100 to 0.1192	3.39 \pm 0.11 to 3.44 \pm 0.12	0.35 \pm 0.04 to 0.41 \pm 0.05	0.41 \pm 0.01
	Corn Starch	0.0577 to 0.0897	1.69 \pm 0.06 to 2.63 \pm 0.09	0.14 \pm 0.05 to 0.32 \pm 0.05	0.20 \pm 0.08
Set B	Mayonnaise	0.1001 to 0.1094	2.93 \pm 0.10 to 3.29 \pm 0.11	0.25 \pm 0.11 to 0.44 \pm 0.17	0.39 \pm 0.05
	Vegetable shortening	0.0398 to 0.0409	nd ²	nd ²	nd ² \pm n/a
	Cheddar Cheese	0.2812 to 0.4809	8.52 \pm 0.28 to 13.49 \pm 1.20	1.39 \pm 0.19 to 1.73 \pm 0.23	1.56 \pm 0.24
Set C	Processed Pork meat	0.0650 to 0.0822	2.03 \pm 0.07 to 2.64 \pm 0.09	0.23 \pm 0.04 to 0.30 \pm 0.04	0.25 \pm 0.03
	Chicken Sausage	0.0468 to 0.1241	1.34 \pm 0.04 to 3.62 \pm 0.12	0.11 \pm 0.03 to 0.36 \pm 0.03	0.25 \pm 0.13
	American Cheese	0.2451 to 0.2940	6.97 \pm 0.23 to 8.66 \pm 0.28	0.96 \pm 0.03 to 1.87 \pm 0.0.6	1.57 \pm 0.30

¹mass fraction in food

²not detected

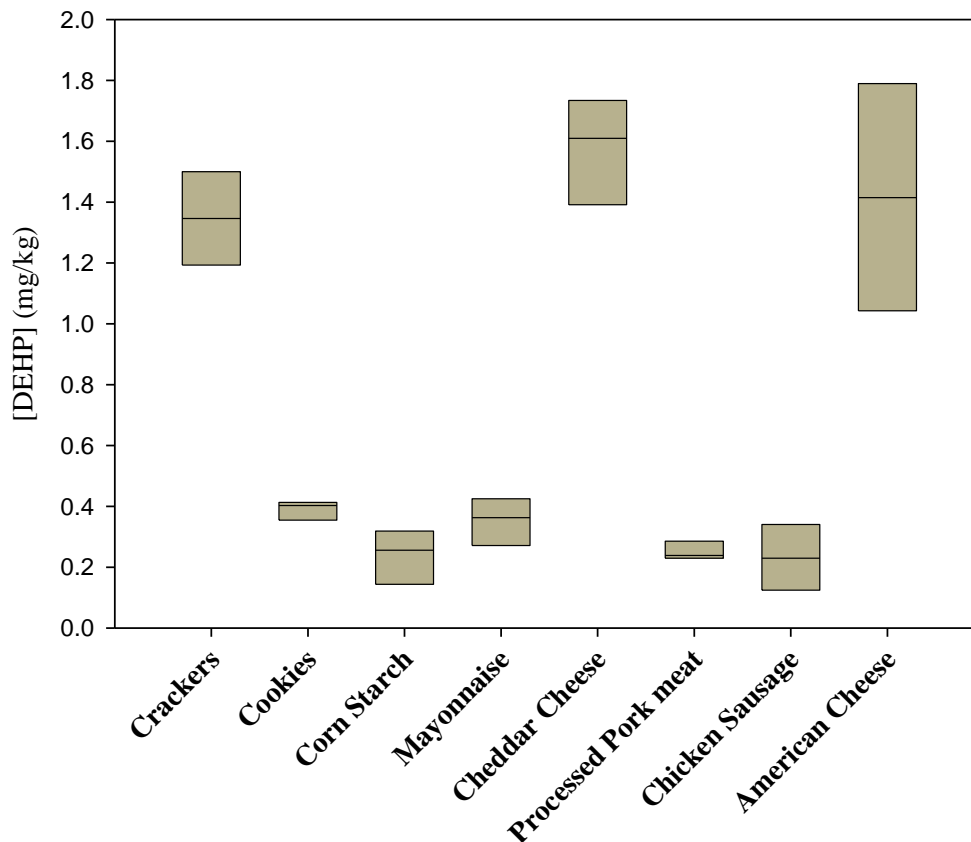


Figure 2.3 Box plots of blank-adjusted DEHP mass fractions in screened foods. The range of mass fractions calculated for each type of food is indicated by the width of its shaded box and the mean is indicated by the embedded horizontal black line.

Confidence in the identification of DEHP in a sample's chromatogram was supported by referencing its $m/z=50$ to $m/z=300$ mass spectrum at 21.0 min to the DEHP EIMS reference contained in the NIST/EPA/NIH Mass Spectral Database (NIST 11) with the NIST Mass Spectral Search Program (Version 2.0g).

The masses of DEHP extracted from all food samples were greater than those in the blanks, with the exception of samples from vegetable shortening. The $m/z=149$ and $m/z=154$ ion signals from these samples could not be discerned from their baselines at the expected time of DEHP elution and no quantification of DEHP could be made. The limits of detection for the mass of DEHP extracted from food matrices in extraction Sets

A, B, and C were 0.79 μg , 1.76 μg , and 0.56 μg , respectively. These were determined by multiplying the standard deviation of total DEHP masses estimated in corresponding method blanks by the appropriate one-sided t value for 95 % confidence. The errors reported are determined from the propagated errors of the response factor (1σ , as determined by the uncertainty of the slope in Figure 2.2) and mass measurement uncertainties used to calculate the estimated mass fraction of DEHP in food matrices. These estimates in most foods were comparable to those reported in recent years from various European studies (Wormuth et al. 2006) with mass fractions of DEHP typically below 2 mg/kg. This indicates that foods produced in the U.S. may not pose any greater risk of DEHP exposure than those produced in other industrialized nations. Determined mass fractions of DEHP in both cheeses, which are noted to be the highest of the food matrices analyzed in this study, were very similar, as were those in both types of processed meat (pork and chicken sausage). Based upon these results, the amount of DEHP in a given food was not observed to directly correlate with the relative amount of fat in the sample matrix. From this, it can be inferred that the inherent qualities of specific ingredients and the materials used during their production and handling are more influential to the levels of DEHP contamination in food.

Chapter 3: Natural Phthalate and Background

3.1. Evidence of Biogenic Phthalate

Evidence advocating the natural synthesis of phthalates as metabolites by species of brown algae (Sastry and Rao 1995) and marine fungi (Cui et al. 1996; Liberra et al. 1998) has been reported. In addition, DEHP was found to compose 2.3 % of the mass of ethanol-extracted residue from *Streptomyces* sp. (Uyeda et al. 1990) and 23 % of that extracted from laboratory-cultured marine fungus *penicillium olsonii* (Amade et al. 1994). MacKenzie et al. (2004) isolated DEHP from culture broths of *Monodictys pelagica*, a marine fungus collected off the coast of Prince Edward Island, Canada, however the authors were suspicious that the isolated phthalate was not necessarily a metabolite, but an artifact of the culturing and extraction procedures.

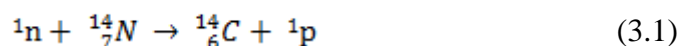
In 2004 Chen demonstrated further evidence for the biosynthesis of DEHP and di-n-butyl phthalate (DBP) by red alga *Bangia atropupurea*. This red alga was cultured in a seawater medium that had been spiked with $\text{NaH}^{14}\text{CO}_3$ (250 μCi and 6.8 mCi/mmole). The alga was harvested, extracted, and DEHP and DBP were isolated with high performance liquid chromatography (HPLC). A scintillation counter was used to determine that the radioactivity of GC-MS-verified DEHP and DBP isolates were 160 cpm and 4787 cpm, respectively, which were both significantly higher than the background radioactivity of 28 cpm. This was indicative that DEHP was synthesized by

the alga through fixation of the ^{14}C - labeled bicarbonate spike, rather than being a laboratory contaminant.

Some of these organisms are used in the production of foods, possibly contributing to the risk of human exposure to DEHP. One such food is Stilton cheese, to which penicillium roqueforti, of a genus evidenced to produce DEHP, is added to give this blue cheese its characteristic flavor, blue marbling, and strong odor. In addition, it contains bacterial cultures that were added to milk at the beginning of its production to induce curding by the conversion of lactose to lactic acid. Given the relatively large mass fractions of DEHP in cheese compared to several other foods that were screened and have been reported in literature (Wenzl 2009; Tomita 1977), as well as the use of microbial additives in specific varieties that have demonstrated a propensity for the synthesis of phthalate, the FDA Center for Food Safety and Applied Nutrition (CFSAN) Office of Food Additives and Safety chose Stilton cheese as a suitable source of DEHP to gain insight into its origins in food by carbon isotope analysis.

3. 2 Radiocarbon in Natural Sources

In 1946 it was demonstrated that when high-energy cosmic ray neutrons collide with atmospheric ^{14}N , they are absorbed by the atomic nuclei, causing them to emit a proton and yield radioactive ^{14}C (Libby 1946) according to the reaction,



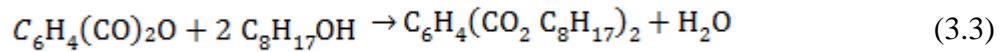
This isotope reacts with atmospheric oxygen to produce $^{14}\text{CO}_2$ that is incorporated into the atmospheric carbon cycle at a natural relative abundance of 1^{14}CO_2 : 10^{12}^{12}CO_2 (one part per trillion). Autotrophic organisms at the base of food chains, both terrestrial and marine, incorporate this radiocarbon produced in the atmosphere into the primary

components of their tissues, and thus the tissues of heterotrophic organisms which consume them. Natural DEHP synthesized by contemporary organisms is therefore expected to have a $^{14}\text{C}/^{12}\text{C}$ ratio that is comparable to these natural materials.

The death of an organism terminates the uptake of ^{14}C . Radiocarbon, with a half-life of 5730 years, beta decays according to,



Petroleum is formed from the hydrocarbon remains of decomposed organisms that have been buried and compressed by the deposition of thousands of meters of sedimentary layers over millions of years. These hydrocarbons have a $^{14}\text{C}/^{12}\text{C}$ ratio that has nearly completely decayed (Namikoshi et al. 2006). Anthropogenic, industrially-produced DEHP ($\text{C}_6\text{H}_4(\text{CO}_2 \text{C}_8\text{H}_{17})_2$) used to plasticize PVC materials is produced from a two-step alcoholysis of petrogenic phthalic anhydride with petrogenic ethylhexanol (ECPI 2013) according to the reaction,



and likewise contains a $^{14}\text{C}/^{12}\text{C}$ ratio that has nearly completely decayed.

The fraction of DEHP which is biogenic, or “contemporary”, can be determined by normalization of its $^{14}\text{C}/^{12}\text{C}$ ratio to that of a “contemporary material”:

$$f_{\text{C}_{\text{DEHP}}} = \frac{\left(\frac{\text{C}^{14}}{\text{C}^{12}}\right)_{\text{DEHP}}}{\left(\frac{\text{C}^{14}}{\text{C}^{12}}\right)_{\text{contemporary standard}}} \quad (3.4)$$

Where $f_{\text{C}_{\text{DEHP}}}$ is the fraction of contemporary carbon in a sample of DEHP, and therefore the fraction which is of biological origin.

3. 3 Accelerator Mass Spectrometry

For decades, radiocarbon measurements of biologically-derived materials were made with Geiger counters that continuously monitored ^{14}C decay events over several days in order to discern the material's age according to the radioactive decay law:

$$N_t = N_0 e^{-\lambda t} \quad (3.5)$$

where N_t is the number of radioactive atoms present at time, t , N_0 the initial number of radioisotopes present in the sample, $\lambda = \frac{\ln 2}{t_{1/2}}$, and $t_{1/2} = 5730$ years. Aside from lengthy analysis times, samples required up to a full gram of live carbon mass to obtain adequate counting statistics (Libby 1967).

The advent of AMS in the 1970's revolutionized radiocarbon analysis with the ability to detect attomole quantities of ^{14}C isotopes, as they exist, on sub-milligram masses of carbon (Ingalls et al. 2005). More recent technical improvements to ionization sources and ion deflectors have allowed for routine analyses of samples containing <100 μg of carbon (Pearson et al. 2001), and reliable measurements of samples as small as 10 μg (Uchida et al. 2004). This ability to produce reliable counting statistics from such small samples of carbon has made AMS the standard method for radiocarbon analysis and has opened the door for the ^{14}C characterization of rare and compound-specific sources (Ingalls et al. 2005). In particular, it has allowed for the practical carbon isotope characterization of trace-level compounds, such as DEHP in food.

At the most basic level, AMS broadly shares a common theory of operation with other mass spectrometers, but achieves an incredibly high degree of selectivity required to resolve a single atomic isotope due to the great velocity that ionized sample particles are passed through its series of mass selectors. All $^{14}\text{C}/^{13}\text{C}$ measurements in this study

were made by a 9 MV High Voltage Engineering Europa (HVEE) FN-class tandem electrostatic AMS system at the Lawrence Livermore National Laboratory Center for Accelerator Mass Spectrometry (LLNL CAMS) (Figure 3.1).

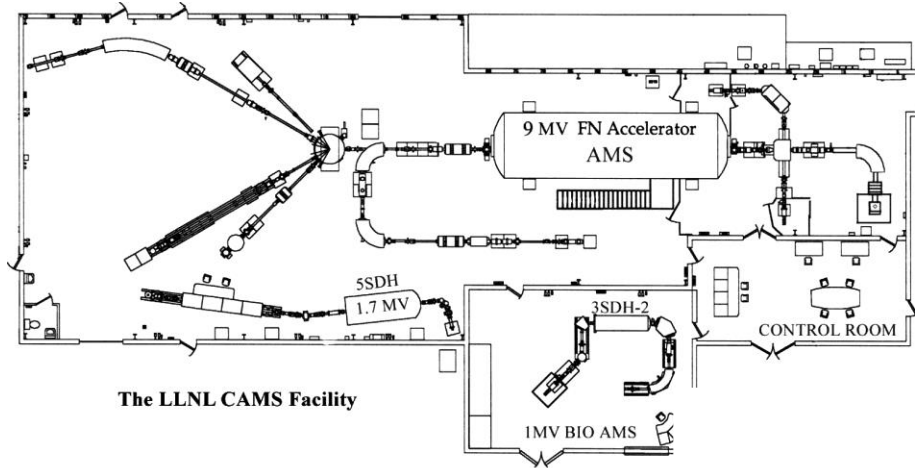


Figure 3.1 Floor plan of the Lawrence Liver National Laboratory Center for Accelerator Mass Spectrometry facility (Figure 1 from Tuniz, et al. 2008).

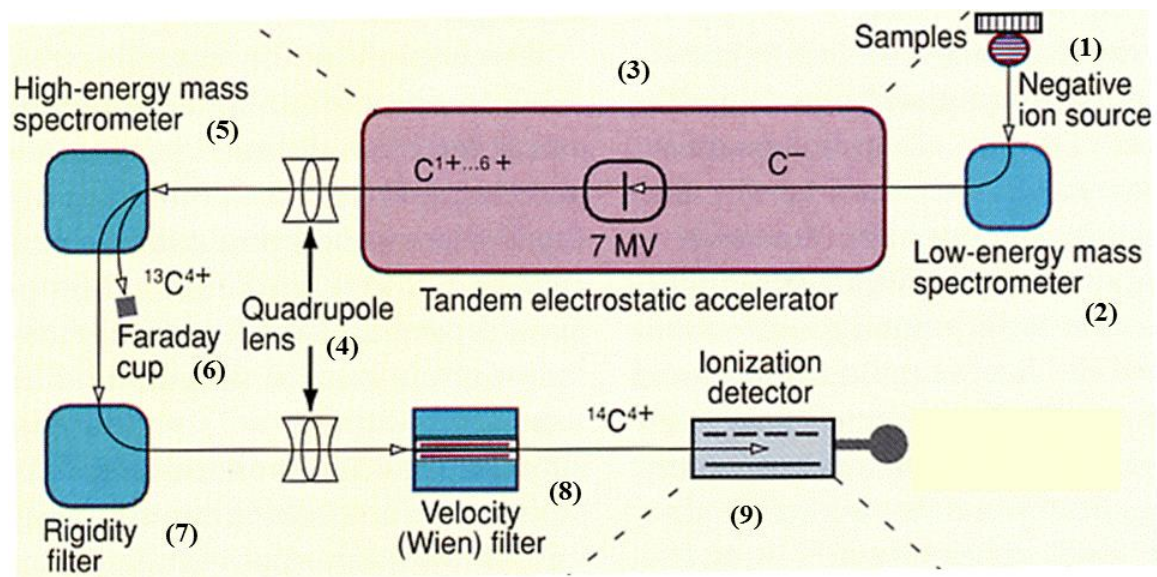


Figure 3.2 LLNL CAMS operational diagram (Figure 1, AMS System at Lawrence Livermore National Laboratory from Vogel, et al. 1995).

The following delineation of AMS operation at LLNL refers to the numbered elements in Figure 3. 2 and its description by Vogel et al. (1995). Samples to be analyzed at LLNL CAMS must first be graphitized and pressed into targets which typically contain a metallic powder as a binder and thermal conductor. These targets are bombarded with ions from a high energy cesium sputtering source (1) that impart electrons to incident sample atoms as they are knocked out of the pellet matrix, forming negative elemental ions. These ions are accelerated through a low-energy magnetic dipole (2) by the difference in electrostatic potential between ground and the magnet's positively-charged central vacuum chamber. The production of negative atomic ions is beneficial in ^{14}C analysis because ^{14}N , a ubiquitous atomic isobar, is not stable as a negative ion. The low-energy (20 – 100 keV/ion) magnetic mass spectrometer selects ions of 13 and 14 amu, though is unable to resolve molecular isobars, such as H^{13}C^- and CH_2^{2-} , from $^{14}\text{C}^-$. Remaining negative ions are then accelerated toward the positive + 9 MV terminal of the Tandem Van de Graaf electrostatic accelerator (3), where they pass through argon gas. The resulting collisions with this gas strip electrons from the ions, thus dissociating molecular isobars and making positive ions of various charge states that are then accelerated from the positive terminal back to ground potential. These ions reach energies of up to over 100 MeV. Now accelerated to a velocity that is a few percent the speed of light, the ion beam is focused (4) to a high-energy second magnetic dipole spectrometer (5) and switching magnet where small mass deviations of atomic isobars from $^{14}\text{C}^-$ cause them to be deflected to the spectrometer walls. $^{13}\text{C}^{4+}$ is deflected to an off-axis faraday cup (6) to monitor ^{13}C and serve as an isotopic ratio reference to ^{14}C

ions. The remaining ion beam passes through a magnetic dipole (7) to remove interfering ions of incorrect rigidity (momentum/charge), and an Electrostatic Analyzer (8) to remove those of incorrect velocity before entering a gas ionization detector (9), capable of measuring individual isotopes. The detector contains propane gas that decelerates the incident ion beam and ejects an electron each time a ^{14}C isotope is brought to rest. This provides a weak electronic signal to a metal plate in the detector that is then amplified. The nuclear charge of the ion, and thus confirmation of the identification of ^{14}C , is algorithmically deduced by the rate of energy loss during deceleration.

Contemporary and fossil-derived carbon sources have been differentiated by ^{14}C characterization of several natural materials, including aerosol particles (Jordan et al. 2006), lipid biomarkers (Pearson et al. 2001) and specific polycyclic aromatic hydrocarbons (Reddy et al. 2002). In 2006 Namikoshi et al. isolated DBP and DEHP from *Undaria pinnatifida* and *Laminaria japonica*, two edible species of brown algae, and *Ulva* sp., a green alga, by high performance liquid chromatography (HPLC). Radiocarbon contents of these isolates, along with those of industrially-derived petrochemical phthalate standards, were measured by ^{14}C Accelerator Mass Spectrometry (AMS; Center for Chronological Research at Nagoya University) to determine the fraction of each phthalate that was synthesized by these alga with carbon from the atmospheric CO_2 cycle, containing measureable quantities of ^{14}C . The amounts of ^{14}C in both petrogenic phthalate standards were below the detection limit. Isolates of DBP demonstrated radiocarbon levels that were well above those observed in the atmosphere (up to 281.2 ± 0.6 % live) and DEHP isolates from the same alga were found to contain relative ^{14}C abundances well above that of the petrogenic standard (49.8 ± 0.2 % to 87.2

± 0.2 % live carbon), indicating they were biologically synthesized. However, such elevated radioactivity in DBP extracts suggest that the alga were grown in a carbon reservoir with a high ^{14}C abundance, making measurements of the much lower modern carbon component observed in DEHP less conclusive. This is especially pertinent given that ^1H NMR and GC-MS-assessed purities of these isolates were 60 % and 70 %, respectively, for the *Ulva* sp. and *L. japonica* species, with the remainder being unsaturated fatty acids derived from this seemingly ^{14}C -enriched carbon source. Despite the degree of uncertainty of the results, they are indicative that phthalate, particularly DBP, is likely produced naturally by these algae species.

3.4 Carbon Isotopes in Natural Materials

It has been long known, even prior to the definitive discovery of ^{14}C in 1940 by Martin Kamen, that carbon isotope ratios vary in natural materials, as was evidenced by greater $^{13}\text{C}/^{12}\text{C}$ ratios in limestone formations compared to those detected in plant sources (Nier et al. 1939). In 1953 it was first established that specific biochemical processes directly influence these isotope ratios and that organisms in marine environs, primarily utilizing bicarbonate, had higher $^{13}\text{C}/^{12}\text{C}$ ratios than their terrestrial counterparts (Craig 1953; Smith 1972). It has since been revealed that carbon isotope ratios amongst biological materials are even more multifarious and that variations exist between individual species whose biochemical processes favor specific isotopes by varying degrees (Harkness et al. 1979). In turn, the cells of heterotrophic organisms can have a carbon constituency that not only has inter-species isotope variation, but a constant $^{13}\text{C}/^{12}\text{C}$ flux amongst like-populations and individuals that consume different dietary sources carbon (DeNiro et al. 1978; Smith 1972; Tieszen et al. 1983). These

$^{13}\text{C}/^{12}\text{C}$ variations are expressed as their *per mille* difference with respect to that in the standard for ^{13}C measurements, Vienna Pee Dee Belemnite (VPDB), a laboratory-produced remake of the original ^{13}C standard material consisting of limestone from the Pee Dee Belemnite formation in South Carolina, U.S. This difference is denoted as $\delta^{13}\text{C}$

$$\delta^{13}\text{C}_{\text{material VPDB}} = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{material}}}{(^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}} - 1 \right) \cdot 1000 \text{‰} \quad (3.6)$$

where, $(^{13}\text{C}/^{12}\text{C})_{\text{material}}$ is the corresponding isotope ratio of a particular carbon-containing material and $(^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}$ is that of Vienna Pee Dee Belemnite. Photosynthetic processes tend to favor lighter ^{12}C isotopes to ^{13}C (Harkness et al. 1979) and thus $\delta^{13}\text{C} < 0$ in most biological matter (Figure 3.3).

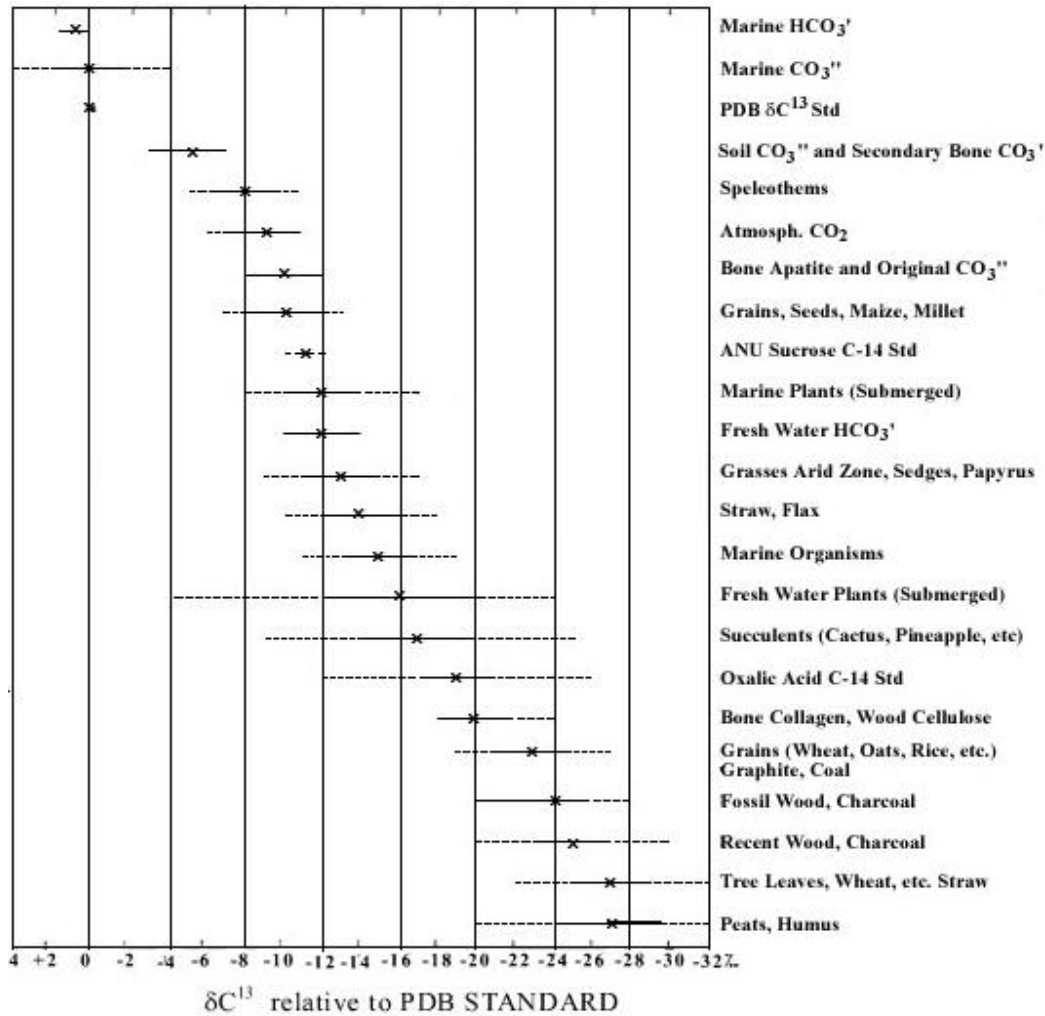


Figure 3.3 Stable Carbon Isotope characterization, as $\delta^{13}C$, in biological carbon sources. Figure 1 from Stuiver and Polach, 1977; *C* variation in nature.

Given that ^{14}C is heavier yet, its fractionation in biological processes is taken to be approximately twice that of ^{13}C in biogenic materials (Higham 1999).

Recent anthropogenic influences on the isotopic character of atmospheric carbon further contribute to those of contemporary biomasses. Particularly since the beginning of the 20th century, as the world at large has seen exponential industrial growth, huge amounts of fossil fuels have been burned. Given that these fuels come from ^{14}C -depleted organic carbon sources, it has had a diluting effect on the overall ^{14}C content of

atmospheric carbon available for use by biochemical processes. Conversely, there was a large increase in the $^{14}\text{C}/^{12}\text{C}$ ratio of atmospheric carbon in the late 1950s and early 1960's coinciding with the detonation of several nuclear weapons materials, known as the "bomb spike", which caused frequencies of atmospheric radiocarbon to nearly double (Reimer et al. 2004). Records of $^{14}\text{CO}_2$ maintained over the past few decades demonstrate that global ^{14}C distributions have not been nearly as uniform since the peak of this weapons usage (Levin et al. 1997; Nydal et al. 1983). All of these factors influencing isotopic composition complicate quantitative assessment of contemporary carbon fractions from natural materials. Therefore, measurements were appropriately adjusted to compensate for these factors and radiocarbon reporting technique was standardized and made uniform to eliminate ambiguity amongst the radiocarbon community as a whole.

3.5 Reporting Fraction of Modern (f_m) Carbon

In 1977, Minze Stuiver and Henry Polach set the field standard for reporting radiocarbon measurements. Their efforts were originally intent upon structuring radiometric dating by ^{14}C -beta decay, but their suggested approach inherently and directly transcends to the realm of ^{14}C atom-counting by AMS. It was already common consensus among the scientific community that all reported results be referenced to a standard with ^{14}C activity consistent with 95 percent of that in the National Bureau of Standards (now NIST) oxalic acid (SRM 4990 B, HOx1) in AD 1950, normalized to a $\delta^{13}\text{C} = -19$ per mille VPDB, though the techniques used by different labs were various and contingent upon individual interpretation. This standard definition is advantageous

because though the radiocarbon content of the oxalic standards (SRM 4990 B and SRM 4490 C, HOxII) decay over time, the $^{14}\text{C}/^{12}\text{C}$ deduced by this definition is fixed. It is equal to the measured activity of the absolute radiocarbon standard, “1890 AD wood”, as extrapolated to the year 1950 based on natural decay. “1890 A.D. Wood” serves as the absolute radiocarbon standard because it is representative of a carbon isotope ratio in terrestrial plant matter whose atmospheric carbon fixation ceased prior to extensive ^{14}C dilution by heavy fossil fuel combustion and the spike and in $^{14}\text{C}/^{12}\text{C}$ ratios resulting from nuclear weapons testing. The year 1950 was chosen to serve as the “modern carbon” reference only as an honorary nod to the first publication of dating results calculated from radiocarbon measurements in the closing days of 1949 (Davis 1988).

Given that ^{14}C fractionation is approximately twice that of ^{13}C , this aspect of the radiocarbon standard definition mitigates the variability in radiocarbon content related to reservoir effects in the isotopic composition of different natural materials and, in the case of AMS, instrumental fractionation.

The outlined approach to reporting of radiocarbon measurements is in accordance with that delineated in Stuiver and Polach’s seminal discussion published in Radiocarbon (1977).

The *absolute international standard activity* (A_{std}), or definition of “modern” carbon, is determined from measurement of an oxalic acid standard activity (A_{OxI}) by,

$$A_{\text{std}} = 95\% \cdot \left(A_{\text{OxI}} \cdot \left(1 - \frac{2 \cdot (19 + \delta^{13}\text{C})}{1000} \right) \right) \quad (3.7.1)$$

with $\delta^{13}\text{C}$ being that of the oxalic acid with respect to VPDB. The oxalic acid standard's measured value is normalized to $\delta^{13}\text{C} = -19$ to account for variability resulting from isotopic fractionation that occurs when certain carbon isotopes in a graphite target are preferentially ionized during AMS analysis.

Measurements of ^{14}C at LLNL CAMS are referenced to those of ^{13}C . A “modern” sample measurement by AMS is thus:

$$\frac{C^{14}}{C^{13}_{std}} = 95\% \cdot \left(\frac{C^{14}}{C^{13}_{OxI}} \cdot \left(1 - \frac{2 \cdot (19 + \delta^{13}\text{C})}{1000} \right) \right) \quad (3.7.2)$$

The $^{14}\text{C}/^{13}\text{C}$ values obtained by AMS analysis of a graphite target can be normalized to this modern definition to obtain a standardized “fraction of modern carbon”, f_m , present in the sample. Prior to normalization to this value, the AMS-derived $\frac{C^{14}}{C^{13}}$ ratios of samples are first adjusted for contaminant carbon mass, as determined by analysis of process blanks. In addition, adjustments are made for biological isotopic fractionation by normalization to $\delta^{13}\text{C} = -25$,

$$\frac{C^{14}}{C^{13}_{adj}} = \frac{C^{14}}{C^{13}_{sample}} \cdot \left(1 - \frac{2 \cdot (-25 + \delta^{13}\text{C})}{1000} \right) \quad (3.8)$$

The value of $\frac{C^{14}}{C^{13}_{sample}}$ is the measured isotope ratio after mass-based correction for contaminant carbon and $\delta^{13}\text{C} = -25$ is that of terrestrial wood. This adjusted value is then referenced to the absolute standard to determine its modern fraction by,

$$f_{m_{sample}} = \frac{\frac{C^{14}}{C^{13}}_{adj}}{\frac{C^{14}}{C^{13}}_{std}} \quad (3.9)$$

whereby a sample with a normalized $^{14}\text{C}/^{13}\text{C}$ ratio equal to that of the absolute standard has an $f_m=1$ and is considered 100% “modern” (Donahue et al. 1990).

Fossil fuel burning has been a significant diluent of ^{14}C carbon in the atmosphere, and although $^{14}\text{C}/^{12}\text{C}$ ratios have been on the decline since the 1960’s, the isotopic effects of the “bomb spike” have not been mitigated and the $^{14}\text{C}/^{12}\text{C}$ of contemporary atmospheric carbon is continually in flux. The biogenic DEHP present in Stilton cheese that is available for consumption is not from 1890 and its carbon isotope profile has been influenced by subsequent fluctuations of atmospheric ^{14}C . For this reason, it is necessary to reference standardized values of f_m to that of a “contemporary” material (Reddy et al. 2002) in order to determine the actual fraction of carbon in a sample that is from a coetaneous source. Biogenic DEHP in Stilton cheese is believed to be produced by constituent organisms which use carbon from the remainder of the contemporary, biological matter of the cheese matrix. Therefore, the perfect contemporary reference to determine the biogenic fraction of DEHP is Stilton cheese in its whole form. This reference, with appropriate corrections to account for isotopic fractionation of biogenic DEHP, is made by:

$$f_{C_{DEHP}} = \left(\frac{f_{m_{DEHP}}}{f_{m_{Stilton}}} \right) \left[\frac{\left(1 - 2 \frac{(\delta^{13}C_{VPDB})_{DEHP}}{1000} \right)}{\left(1 - 2 \frac{(\delta^{13}C_{VPDB})_{Stilton}}{1000} \right)} \right] \quad (3.10)$$

where $f_{C_{DEHP}}$ is the fraction of contemporary carbon in DEHP from Stilton cheese and

$f_{m_{DEHP}}$ and $f_{m_{Stilton}}$ are the fractions of “modern” carbon in DEHP from Stilton cheese and in Stilton cheese, respectively.

Chapter 4: Extraction, Isolation, and Preparation of DEHP for

¹⁴C AMS Analysis

4.1 Stilton Cheese and Affirmation of Laboratory Suitability

4.1.1 Stilton Cheese

Of distinctly English heritage dating to the 18th century, Stilton cheese has gained protected designation status (PDO), whereby production is legally bound to a strict code of operation and small area of geographical origin (Ilbery et al. 2000). As such, it is only produced in the U.K. counties of Derbyshire, Leicestershire, and Nottinghamshire from the pasteurized local milk of five licensed dairies (Stilton Cheesemakers' Association 2013). Starter bacterial cultures are added to milk to ripen it, followed by rennet induce curding, and the addition of penicillium roqueforti spores to later assist aging. After drainage of the whey, the curds are salted, molded, and washed in brine for development of a rind, and stored for 6 weeks. At this point the cheese is pierced with stainless steel needles to allow entry of air to its center and induce the growth of the penicillium roqueforti and development of its characteristic blue veins.



Figure 4.1 Photographed interior of bisected Colston Bassett Stilton cheese cylinder.

Two cylinders of Colston Bassett (Nottinghamshire, U.K.) Stilton cheese (~7.5 kg) were acquired from Neal's Yard Dairy (London, U.K.) distributor via Whole Foods Grocery Store (Gaithersburg, MD). These uncut cylinders were received on August 1, 2011 and February 9, 2012 in paper packaging, wrapped with aluminum foil, and stored at -20°C .

4.1.2 Laboratory Swipes and Aerosol Sampling

Although the histories of the laboratories in which DEHP from Stilton cheese was extracted and isolated were well-known, and no notable prior work with ^{14}C -enriched materials had taken place within them, very little ^{14}C contamination is needed to have a large relative impact on the $\sim 10^{-12}$ ^{14}C isotope abundances and AMS measurements of natural and contemporary materials. Therefore, contamination by trace levels of artificially ^{14}C -enriched substances that may be unknowingly introduced to a laboratory

via brief contact with materials or persons that have occupied spaces where such work has taken place are able to invalidate ^{14}C measurements of natural materials. To ensure that there was no potential for this to occur during the isolation of DEHP and sample preparation, a series of swipes and aerosol samples were taken from all laboratories at locations which would have frequent contact. These swipes and aerosols were analyzed by AMS to check for super-modern carbon.

A swipe kit was sent from LLNL CAMS that consisted of glass fiber swipe cloths and aluminum-foil-wrapped aerosol monitors that contained fullerene soot mixed with iron powder. The swipes were taken with a synthetic cloth that was wetted with isopropyl alcohol. The first swipe sample was a blank that had been wetted with alcohol and the others were used to sample approximately 100 cm^2 of several commonly-used surfaces, such as doorknobs, LC system parts, and lab bench tops (Table 4.1). These were rolled to fit in 4 mm I.D. glass vials, which were capped with PTFE-lined polymer caps. The aerosol monitors, placed, at various points throughout the labs, were left in their aluminum foil wrapping with two ends open to allow for incidental aerosol collection and allowed to sit for a week before placing in a sealable bag. These swipes and aerosol collectors were returned to LLNL CAMS for ^{14}C AMS analysis. The results of these swipes, presented as their fraction of modern carbon in Table 4.1 (f_m), are all well below one and indicative that there was no extensive ^{14}C -enriched carbon contamination of laboratory workspaces. Results of samples collected at UMCP and NIST were not significantly different.

Table 4.1 Laboratory swipe surfaces and aerosol sampling locations for ^{14}C contamination and their AMS measurements of f_m .

Date and Serial Number	Location (Inst./Bldg./Rm.) ¹	Location Description	Fraction of Modern Carbon
07-06-2011-001	UMCP 091 3110	Door Handle	0.205 ± 0.009
07-06-2011-002	UMCP 091 3110	Computer Keyboard	0.1776 ± 0.011
07-06-2011-003	UMCP 091 3110	Bench top near ICP-MS	0.1764 ± 0.008
07-06-2011-004	UMCP 091 3110	Bench top near sink	0.1812 ± 0.012
07-06-2011-005	UMCP 091 3110	Fume hood	0.1644 ± 0.011
07-06-2011-006	UMCP 091 3110	Balance	0.1741 ± 0.012
07-06-2011-007	UMCP 091 3110	Window	0.1528 ± 0.0010
07-06-2011-008	UMCP 091 3110	Blank	0.1624 ± 0.010
07-06-2011-009	UMCP 091 3107	Door Handle	0.1756 ± 0.011
07-06-2011-010	UMCP 091 3107	Keyboard	0.206 ± 0.012
07-06-2011-011	UMCP 091 3107	HPLC pump	0.1847 ± 0.007
07-06-2011-012	UMCP 091 3107	Detector	0.4022 ± 0.017
07-06-2011-013	UMCP 091 3107	Bench top	0.1557 ± 0.016
07-0-62011-014	UMCP 091 3107	Fume hood	0.143 ± 0.010
07-06-2011-015	UMCP 091 3107	Gas tank valve	0.1659 ± 0.009
07-06-2011-016	UMCP 091 3107	Blank	0.1456 ± 0.013
07-14-2011-001	NIST 227 A134	Blank	0.2247 ± 0.050
07-14-2011-002	NIST 227 A134	Door Handle	0.1381 ± 0.010
07-14-2011-003	NIST 227 A134	Fume hood	0.2031 ± 0.053
07-14-2011-004	NIST 227 A134	Oven	0.2267 ± 0.028
07-14-2011-005	NIST 227 A134	Bench top near sink	0.2138 ± 0.069
07-14-2011-006	NIST 227 A134	Refrigerator	0.3123 ± 0.025
07-14-2011-007	NIST 227 A134	Rotatory evaporator bench top I	0.144 ± 0.007
07-14-2011-008	NIST 227 A134	Rotatory evaporator bench top II	0.1557 ± 0.022
0-714-2011-009	NIST 227 B123	Balance room bench top	0.1466 ± 0.022
07-14-2011-010	NIST 227 B129	GPC room bench top	0.2019 ± 0.016
07-14-2011-011	NIST 227 A126	Centrifuge bench top	0.0936 ± 0.009
07-14-2011-012	NIST 227 A126	GC-MS bench top	0.1442 ± 0.010

¹UMCP : University of Maryland, College Park, MD 20742

NIST : National Institute of Standards and Technology, Gaithersburg, MD 20899

4.2 Extraction

Preliminary analyses of these specific cylinders indicated that they only contained approximately 0.11 mg/kg to 0.14 mg/kg DEHP, requiring nearly 7.5-million fold enrichment of DEHP to obtain a sample of adequate mass with a purity of 90 %. Due to the large mass of cheese that needed to be extracted in order to produce several DEHP isolates of requisite size, extractions were performed on multiple portions of this cheese during several occasions. This was due to practicalities concerning the size of glassware and volume of solvents that were readily and safely manageable at such a scale. Seven batches of cheese, whose masses are given in Table 4.2, were cut and extracted alongside four method blanks in a clean analytical chemistry aerosol Laboratory at the University of Maryland, College Park Chemistry Building. Two batches were extracted in the summer of 2011. The final isolate of one of these batches and a blank were sent as pilot samples to LLNL CAMS. This was done to assess the working method used to prepare samples and evaluate the quality of information it would provide before processing subsequent samples.

Table 4.2 Mass and date of extraction events, and identification of corresponding contemporaneously-processed method blanks.

Process Element	Batch 1	Batch 2	Batch 3 ¹	Batch 4	Batch 5	Batch 6	Batch 7
Cheese Wheel ID	1	1	2	2	2	2	2
Extraction Date	8/24/2011	9/26/2011	2/14/2012	2/27/2012	2/27/2012	4/3/2012	4/3/2012
Mass of Cheese							
Extracted (g)	2640	2622	1192	1633	1687	1247	1262
Blank ID	BL01	BL01	BL02	BL03	BL03	BL04	BL04
Column Pass							
Ratio No.;							
Columns	35/23	35/23	20/21	23/12	26/12	21/19	19/19
Isolate/No.							
Columns Blank ²							

¹Approximately ¼ of batch lost during isolation

²Ratio of number of chromatographic passes used to isolate DEHP from cheese and number of passes used for method blank (γ_{CP}).

Prior to extraction, the cheese cylinder was to allowed thaw. When partitioning and weighing samples, care was taken not to touch the cheese with any surfaces other than the original foil-lined wrapping or acetone-rinsed stainless steel spatula. Glassware used during the extraction was washed with Alkonox® Powdered Precision Cleaner detergent, baked at 250°C for 12 hours, rinsed with acetone prior to use, and kept covered with baked aluminum foil to minimize phthalate contamination. Hexane and acetonitrile solvents used during extractions were HPLC-grade (Mallinckrodt Baker Inc.). Cheese was weighed, transferred into 4 L beakers, and spiked with weighed aliquots (Appendix 2, Table A2.2) of d_{38} -bis(2-ethylhexyl) phthalate (98%) (Cambridge Isotope Laboratories) diluted in acetonitrile (~2540 µg/g). Method blanks were similarly

prepared and contemporaneously processed without cheese. Cheese batches were first homogenized with a stainless steel spatula. Along with method blanks, they were extracted into as much as 4500 mL hexane. The extracts were covered with foil and gently heated at 40 °C for up to 60 minutes, with periodic gentle stirring. This solvent was gently decanted by incremental pouring into a 1 L round bottom flask and concentrated via rotary evaporation. Each cheese batch was again extracted with up to 2250 mL of ~5:1 volume ratio acetone:hexanes. This extract was decanted and combined with the first extract, as was a subsequent third extract of up to 900 mL hexane. Combined extracts were concentrated by rotary evaporation and centrifuged at an acceleration of ~12,000 g for 10 min to remove any decanted or suspended solids before further processing.

The DEHP was next selectively partitioned from many non-polar species of the extract into hexane-saturated acetonitrile. Accordingly, each hexane extract was split into multiple 400 mL aliquots and extracted with acetonitrile using a 2 L glass separatory funnel. Aliquots were typically first partitioned into 1350 mL of hexane-extracted acetonitrile solution by vigorous shaking and allowing the two immiscible layers to stratify for 30 min before decanting the acetonitrile from the funnel. This was repeated once more on each aliquot with 800 mL of hexane-saturated acetonitrile. All decanted acetonitrile extracts were combined, concentrated by rotary evaporation, and stored at -20 °C in 1 L glass bottles. Chilling of the extracts caused some cheese matrix constituents to precipitate from acetonitrile solution. The acetonitrile extract was gravity filtered while still cold to remove this matter.

4.3 Liquid Chromatographic Isolation of DEHP

Three methods of chromatographic separation were used to adequately isolate DEHP for ^{14}C AMS measurement from Stilton cheese extracts.

4.3.1 Silica Gel “Flash” Chromatography

Preparatory silica gel “flash” chromatography was performed to isolate DEHP from much of the remaining polar and bulk cheese matrix components. This chromatography work was executed in a fume hood whose surfaces were covered with aluminum foil to minimize exposure to polymeric surfaces. Custom-made columns (Figure 4.2) were manufactured with 1.75 inch internal diameter, 15 inch glass tubes topped by 500 mL solvent reservoirs with 24/40 joint openings. Mobile phase flow through the column was induced by manually-applied pressure with a synthetic hand-powered pump bulb which was connected by a short air-tight segment of rubber hose to a size 24/40 male-jointed vacuum distilling adapter clamped to the opening of the solvent reservoir. Eluent was manually controlled with a PTFE stopcock at the column’s tapered bottom end.

A column was packed by first plugging the tapered end with a small wad of cotton fiber, followed by addition of a 1 cm-thick layer of sand (Mallinckrodt Baker). It was filled with 900 mL of 5 % acetone/ 95 % hexane volume fraction solution and 175 g of 32 μm to 63 μm “flash”-grade silica gel particles (Dynamic Adsorbents, Atlanta, GA). A 200 mL aliquot of this solution was eluted five times to minimize inconsistencies or pockets in the gel and another 1 cm sand layer topped the column.

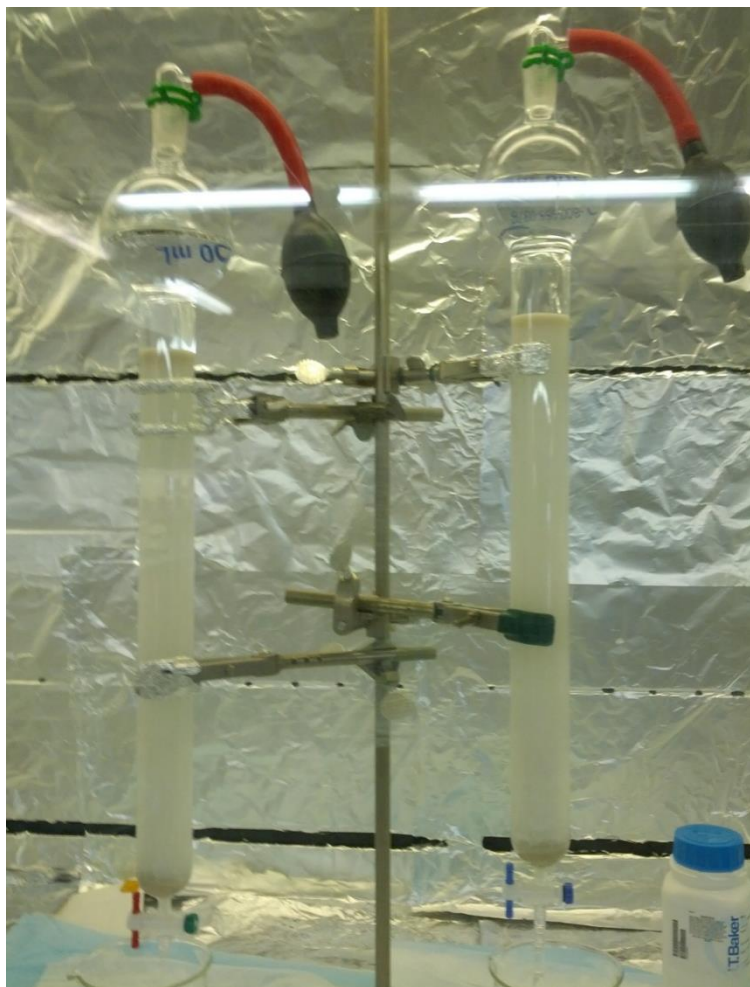


Figure 4.2 Photograph of silica gel “Flash” Chromatography columns.

Each column-packing was suitable for separation of an aliquot of extract representative of ~400 g of cheese. To remove contaminant DEHP from the silica particles, 400 mL of a 33 % acetone / 67 % hexane by volume solution was added to the reservoir and eluted through the column at ~ 5 mL/min. The column was re-conditioned for use by elution of 500 mL of 100 % hexanes (~ 5 mL/min), allowed to sit for 30 min, and flushed with another 600 mL of 100 % hexanes. An excess of 10 mL hexane was kept in the reservoir to keep the top of the column from drying.

After gravity filtration of acetonitrile extracts, solvent was totally removed from a ~250 mL aliquot by rotary evaporation. The sample residue was reconstituted in ~5 mL of hexane and carefully added to the top of a conditioned silica column with a glass Pasteur pipette. Solvent was eluted until the entire plug had descended below the sand and was in full contact with the silica stationary phase, noticeable as a yellow band (~2cm) on the white silica slurry.

To start each chromatographic separation, 200 mL of 100 % hexane was added to the column reservoir to elute compounds of very low polarity that were partitioned into the acetonitrile. Given that pressure was manually applied to the system with a hand-operated bulb, the flow rate had some variability but was targeted to remain consistent at ~ 15 - 20 mL/min. Mobile phase subsequently used had a 1.6 % acetone/98.4 % hexane composition and was added to the solvent reservoir in aliquots of 400 mL.

Six fractions of 100 mL were individually collected, beginning after the elution of 1000 mL of this mobile phase. These fractions were each rotary evaporated to \approx 3 mL and qualitatively checked for DEHP by GC-EIMS analysis, as identified by the presence of discernible peaks in the $m/z=149$ and $m/z=154$ chromatograms at ~21 min. Fractions from each silica column that were determined to contain DEHP were combined and rotary evaporated to 1.2 mL (Tables A2.3.a and A2.3.b in Appendix 2.3). The results of GC-EIMS analyses of 100 mL fractions concentrated to \approx 3 mL, and depiction of those fractions that were combined, are shown in Appendix 2.3, Tables A2.4.a to A2.4.n. A Total Ion chromatogram of the GC-EIMS analysis of a DEHP-containing fraction collected for Batch 2 is shown in Figure 4.3. Though well on the way to achieving a nearly 8-million-fold enrichment of DEHP, several fatty acid esters were large sources of

carbon mass in the sample after this stage of purification. Methyl 6,11-octadecadienoate and Methyl 8,11,14-eicosatrienoate were identified by referencing the $m/z=50$ to $m/z=300$ mass spectra at 15.6 and 18.0 min, respectively, to the NIST/EPA/NIH Mass Spectral Database (NIST 11) with the NIST Mass Spectral Search Program (Version 2.0g) and were present at each step of the purification. The integrated ion count of the DEHP peak is $\sim 1.5\%$ of all ions detected in the sample.

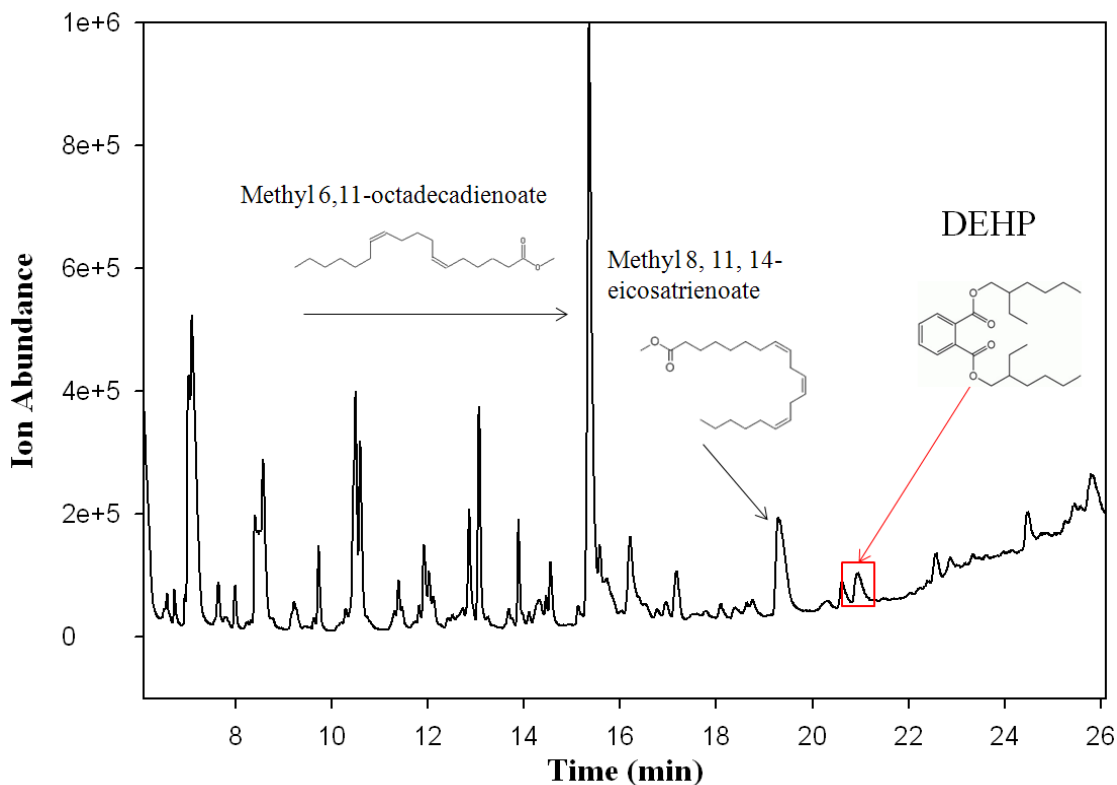


Figure 4.3 Total $m/z=50$ to $m/z=300$ GC-EIMS ion chromatogram of DEHP-containing flash chromatographic fraction (Batch 2) concentrated in ~ 3 mL hexane. The red box outlines the DEHP peak. Its integrated area contains $\sim 1.5\%$ of the total ion abundance of the chromatogram.

4.3.2 Size Exclusion Chromatography

To isolate DEHP from many other fatty acid esters and compounds of disparate size, the recovered DEHP fraction from each silica column was passed through tandem

30 cm, 21 mm-I.D. Oligopore columns (Agilent Technologies) containing 6 μm particles with poly-dispersed pore diameters, preceded by a PLGel (Agilent) guard column containing 10 μm particles with 100 angstrom pore diameters. These columns were first conditioned for use with 100 % methylene chloride from a Varian (Agilent) 9012 solvent delivery system.

The 1.2 mL DEHP-containing fractions from the silica chromatography runs were each injected onto this column at a flow rate of 10 mL/min. DEHP was eluted in 10 mL fractions that were collected in clean 10 mL volumetric flasks after prior elution of 189 mL of methylene chloride mobile phase. Details of the sample and blank passes made on these columns are found in Appendix 2.4 Tables A2.5.a and A2.5.b. Fractions corresponding to the same cheese extract batches were combined and concentrated to ~1.5 mL for GC-EIMS analysis. A Total Ion chromatograph of one of these analyses from Batch 2 is in Figure 4.4. The integrated ion abundance of the DEHP peak in this chromatogram is ~ 8.5 % of all ions detected from the sample, corresponding to a ~ 5.5-fold increase in enrichment of DEHP with respect to its concentration in the flash fraction (Figure 4.3). Samples were transferred to a 10 mL pear-shaped recovery flask in which all solvent was removed, and reconstituted in 3 mL of acetonitrile.

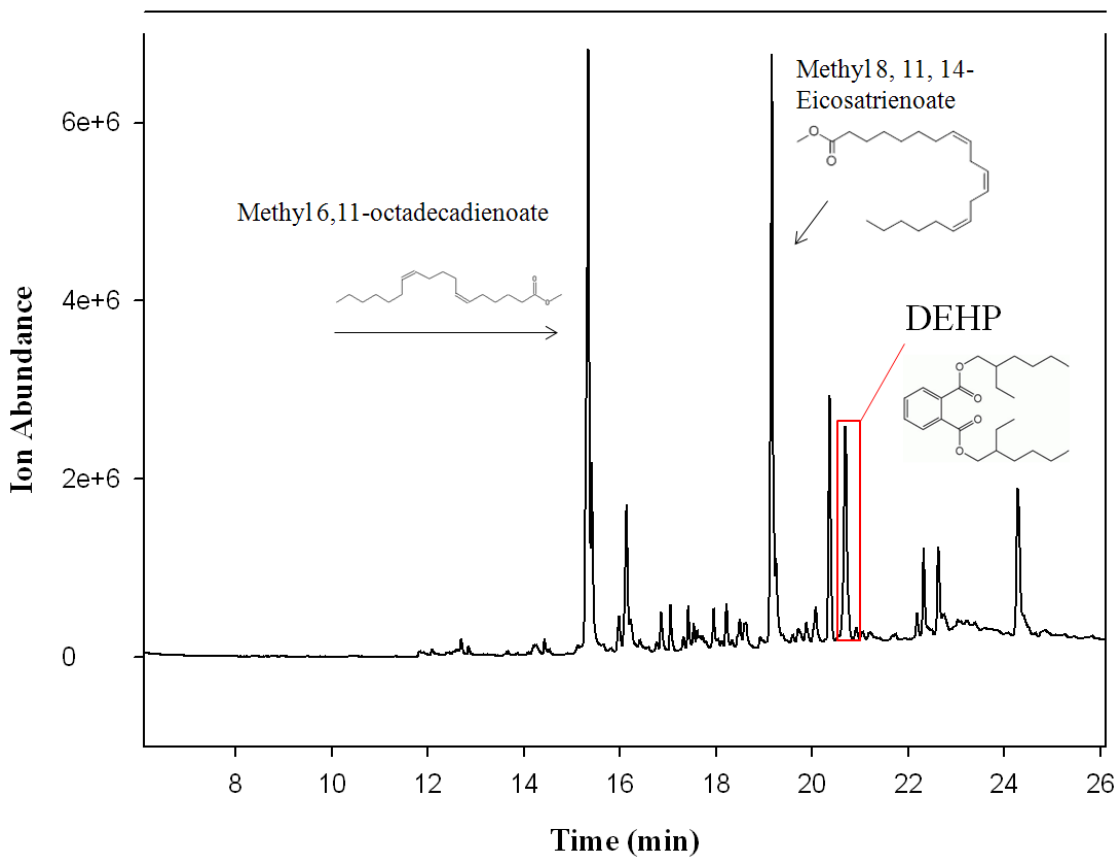


Figure 4.4 Total $m/z=50$ to $m/z=300$ GC-EIMS ion chromatogram of DEHP-containing size exclusion chromatographic fraction (Batch 2) concentrated in ~ 1.5 mL methylene chloride. The red box outlines the DEHP peak. Its integrated area contains $\sim 8.5\%$ of the total ion abundance of the chromatogram, a ~ 5.5 -fold enrichment of DEHP from the flash fraction.

Section 4.3.3 High Performance Liquid Chromatography

The final chromatographic purification of DEHP extracts was obtained by HPLC with a semi-preparatory 15 cm x 9.4 mm-I.D. Agilent Zorbax Eclipse C18 column, preceded by a C18 guard column, and coupled to a Spectroflow 757 UV/VIS Absorbance detector. Data acquisition from the detector response was automated with E-Lab software. Acetonitrile samples were purified by injections of 115 μL aliquots (≈ 15 injections/batch) on a 150 μL stainless steel sample injector loop. A 90 % acetonitrile/10

% water (volume ratio) initial mobile phase flow was set at 4 mL/min by a Dionex (Sunnyvale, CA) P580 solvent delivery system. Twenty minutes after sample injection, this mobile phase composition was brought to 95 % acetonitrile/5 % water. Elution of DEHP was detected by its UV absorbance at 254 nm, typically after ~33 min. d_{38} -DEHP, which typically eluted nearly 2 minutes prior to unlabeled DEHP, served as a chromatographic marker for anticipation of DEHP elution, and thus its collection time. The fractions of d_{38} -DEHP and DEHP were individually and manually collected from a short segment of LC tubing connected to the detector outlet in separate 40 mL borosilicate glass vials with PTFE-lined caps. These vials were kept tightly capped when DEHP fractions were not being collected. Each sample of DEHP contained a total volume of \approx 80 mL of HPLC eluent. This solvent was totally removed by rotary evaporation and samples were reconstituted in \approx 1 mL of methylene chloride. Details of individual injections are provided in Appendix 2.5, Tables A2.6 and A2.7a. to A2.7.c. Collection times and volumes of DEHP fractions for samples isolated in 2012 are in Appendix 2.5, Tables A2.8.a to A2.8.c. A Total Ion Chromatogram of the GC-EIMS analysis of a collected DEHP HPLC fraction is shown in Figure 4.5. The integrated ion abundance of the DEHP peak in this chromatogram is \sim 91 % of all ions detected from the sample, \sim 10.5-fold enrichment over DEHP in the size-exclusion fraction.

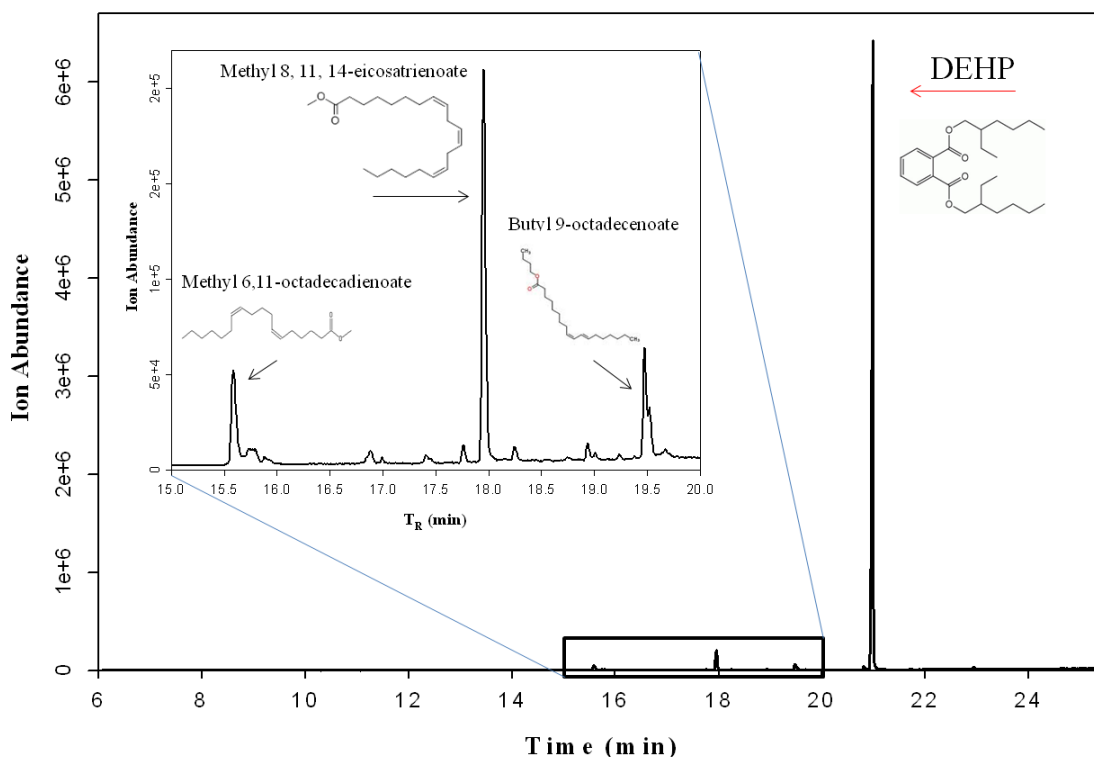


Figure 4.5 Total $m/z=50$ to $m/z=300$ GC-EIMS ion chromatogram of DEHP-containing HPLC chromatographic fraction (Batch 2) concentrated in ~ 1.5 mL methylene chloride. The integrated DEHP peak contains 91 % of the total ion abundance of the chromatogram and the inset is the chromatogram from 15 min to 20 min scaled on the y- (Ion Abundance) axis to better show resolution of compounds co-eluting with DEHP in the HPLC fraction.

4.4 Isolate Aggregation

Preliminary GC-EIMS assessment of DEHP masses isolated in each batch made it clear that some did not yield a mass of carbon that was adequate for AMS analysis. This was true for batches 3 through 7 (Table 4.3). These approximate estimations were made based on a previous calibration of the operative GC-EIMS for DEHP and the knowledge that DEHP is 73.8 % carbon by mass.

Table 4.3 Preliminary mass estimates of DEHP isolates (μg).

Batch	Preliminary DEHP Mass Estimate (μg)	Preliminary Carbon Mass Estimate (μg)
03	43	32
04	59	44
05	56	41
06	63	47
07	62	46

Batches 03 to 07, isolated in 2012, provided enough mass for a total of three AMS samples. The isolates of some extraction batches were therefore aggregated to obtain the requisite mass for ^{14}C analysis. Batch 04 was gravimetrically split into two aliquots. Table 4.4 denotes the mass fraction of each isolate that was a constituent of the newly aggregated and termed AMS samples ST01 through ST05.

Table 4.4 Mass fractions of batch isolates in five AMS samples (ST01 to ST05).

Sample ID	Batch ID						
	01	02	03	04	05	06	07
ST01	1						
ST02			1		1		
ST03				0.48		1	
ST04				0.52			1
ST05		1					

4.5. Quantification of DEHP in Samples and Method Blanks by GC-EIMS

The masses of DEHP in isolates and blanks were determined by calibrated GC-EIMS analysis using an Agilent Technologies 6890N Network GC system, with a 7683 Series Autosampler, and 5973 *inert* quadrupole mass-selective detector. Analyses were performed by on-column injection at 63 °C to a 0.25-mm x 60-m, DB-XLB (Agilent) polysiloxane (0.25 µm) wall-coated capillary column, preceded by a 5 m deactivated fused-silica capillary retention gap. Helium flow was 1.3 mL/min. The GC was programmed to elute DEHP at ~ 21.0 min. Its temperature was held at 60 °C for 3 min, ramped 45°C/min to 200 °C, followed by a 7.5 °C/min ramp to 320 °C, which was held for 3 min. DEHP in samples and GC-EIMS calibrants was quantified by integration of the ion fragment $m/z=149$ (Figure 2.1.a) relative ion abundance at 21.0 min after injection. Three calibrations were performed that coincided with the analysis of samples sent to LLNL for AMS analysis on 11/1/2011 (ST01), cheese-isolated samples sent 6/1/2012, and blanks sent 6/1/2012.

4.5.1 Pilot Samples

A stock solution of $1442.5 \mu\text{g/g} \pm 5.0 \mu\text{g/g}$ DEHP in methylene chloride was gravimetrically prepared in a baked (450 °C for 8 h) 10 mL volumetric flask by dilution of a Supelco (Bellefonte, PA) neat Bis(2-ethylhexyl)phthalate (99.8%) standard material (Appendix 3.1). Eight GC-EIMS calibrant solutions were gravimetrically prepared from this stock with a Mettler Toledo Sartorius precision weighing balance (Table 4.5). Three aliquots of each calibrant were delivered into separate 2 mL glass amber GC auto-loading vials (Agilent). Triplicate GC-EIMS analysis of 1.5 µL of each was performed to determine precision in injection volumes, consistency of DEHP electron impact

fragmentation, and linearity of detector response. The average relative standard deviation of $m/z=149$ ion counts measured by these triplicate calibrant analyses was 1.5 %. Sample ST01 was measured during calibration. The linear least squares regression from GC-EIMS analysis of these calibrants is shown in Figure 4.6.

Table 4.5 ST01 GC-EIMS Calibrant mass fractions of DEHP in methylene chloride ($\mu\text{g/g}$) and GC-EIMS detector responses.

[DEHP] in methylene chloride ($\mu\text{g/g}$)	Mean $m/z=149$ peak area ¹
13.2 \pm 1.0	7470000 \pm 35800
49.4 \pm 1.0	34859200 \pm 346800
65.1 \pm 1.0	52262600 \pm 709100
78.9 \pm 1.0	60023600 \pm 1305800
95.7 \pm 1.0	97892800 \pm 2083100
128.3 \pm 1.4	127759300 \pm 929200
147.8 \pm 1.6	136124200 ² \pm 5503700
161.7 \pm 1.7	155606600 \pm 707300

¹Area of ion relative abundances at 21 min. after injection

²Average of 2 injections

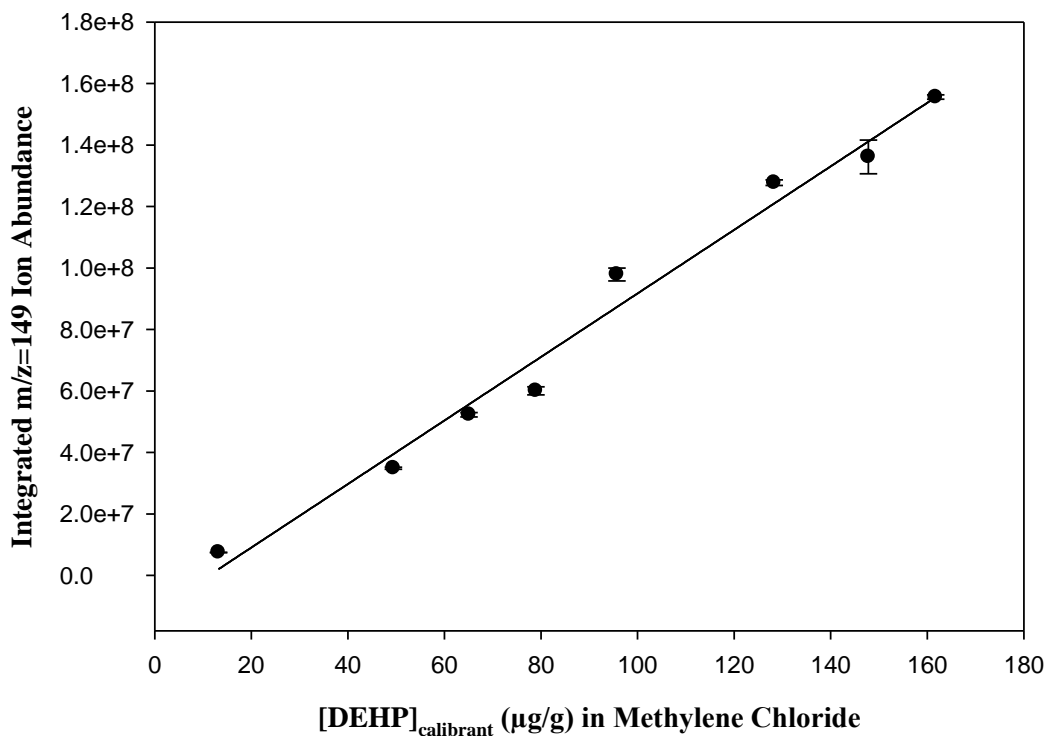


Figure 4.6 GC-EIMS calibration regression of integrated $m/z=149$ ion abundances at 21.0 min, with respect to DEHP mass fractions in methylene chloride for sample ST01. $y = 1,033,000x - 11,610,000$, with $R^2 = 0.983$.

The regression were fitted to the equation $y = 1,033,000x - 11,610,000$, with $R^2 = 0.983$. Its standard deviation of the slope is 5.4 % and standard error of regression (s_y) is 7571000. Sample ST01 had a mass of $1.28975 \text{ g} \pm 6 \times 10^{-5} \text{ g}$ in methylene chloride at the time of analysis and its DEHP mass fraction in methylene chloride was $113.3 \text{ } \mu\text{g/g} \pm 7.9 \text{ } \mu\text{g/g}$. This sample was therefore determined to contain $146.2 \text{ } \mu\text{g} \pm 10.2 \text{ } \mu\text{g}$ of DEHP ($107.9 \text{ } \mu\text{g} \pm 7.5 \text{ } \mu\text{g}$ of carbon as DEHP).

4.5.2 Primary Samples and Method Blanks.

Given the somewhat large 7 % relative uncertainty of the DEHP mass estimate obtained for sample ST01, more effort was applied to obtain precise mass estimates of Samples ST02, ST03, ST04, ST05, and their respective methods blanks. To do so, three

series of DEHP calibrants were gravimetrically prepared from dilutions of one of three stock solutions of DEHP standard (Supelco, 99.8 %) in methylene chloride (Table 4.5). The calibrants were gravimetrically prepared (Appendix 3.2) such that their DEHP mass fractions and m/z=149 relative ion abundances provided much tighter bracketing of corresponding values in the samples than calibrants prepared for quantification of ST01.

Table 4.6 GC-EIMS Stock solution mass fractions of DEHP in methylene choride ($\mu\text{g/g}$).

Stock Solution ID	[DEHP] ($\mu\text{g/g}$)
Stock 1	13411.1 \pm 3.0
Stock 2	14780.6 \pm 10.2
Stock 3	15283.5 \pm 8.7

From each stock solution, a series of 6 calibrants was gravimetrically prepared (Appendix 3.2) whose mass fractions of DEHP ranged from 50.5 $\mu\text{g/g}$ to 90.55 $\mu\text{g/g}$ (Table 4.7). Stock solutions were weighed just prior to dilution, as calibrants were just prior to analysis, to make adjustments to DEHP mass fractions resulting from methylene chloride evaporation. Solutions were transferred to 2 mL amber glass GC auto-sampler vials (Agilent) which were tightly capped with PTFE-lined polysiloxane-septum screw caps.

Table 4.7 GC-EIMS Calibrant Mass fractions of DEHP in methylene chloride ($\mu\text{g/g}$).

Diluted Stock Solution:	Calibrant ID	[DEHP] Calibrant Solution at time of GCMS Analysis ($\mu\text{g/g}$)
1	1-1	50.05 \pm 0.01
1	1-2	71.41 \pm 0.01
1	1-3	74.47 \pm 0.01
1	1-4	81.27 \pm 0.06
1	1-5	85.59 \pm 0.01
1	1-6	90.51 \pm 0.01
2	2-1	67.49 \pm 0.03
2	2-2	71.51 \pm 0.03
2	2-3	75.74 \pm 0.77
2	2-4	80.79 \pm 0.03
2	2-5	85.97 \pm 0.04
2	2-6	89.56 \pm 0.04
3	3-1	67.79 \pm 0.02
3	3-2	71.41 \pm 0.02
3	3-3	76.44 \pm 0.03
3	3-4	78.81 \pm 0.03
3	3-5	90.55 \pm 0.03
3	3-6	85.72 \pm 0.03

Injections of 1.0 μL of each of the eighteen calibrants and four samples were randomly analyzed to account for maximum calibration uncertainty resulting from inconsistent EI ionization and quadrupole performance, detector drift, and memory effects. The order by which these samples were run, along with the observed $m/z=149$ chromatogram peak areas at 21.0 min are listed in Appendix 3.2, Table A3.9.

Calibrants were prepared from three stock solutions in the event that the small mass of neat DEHP in any one of them was erroneously weighed or there were transfer inconsistencies during dilution. Signals produced from Stock 3 calibrants did not

demonstrate linearity consistent with those from Stock 1 and Stock 2. Given the randomization of analysis order this inconsistency was not resultant of variability in GC-EIMS performance, but rather in DEHP stock solution dilution or transfer. Analysis of calibrants prepared from Stock 1 and Stock 2 were thus used to fit a linear least-squares regression for the quantification of DEHP in the remaining samples. Though its signal was well-aligned with the regression, calibrant 1-1 was not included in the calibration since its mass fraction of DEHP was nearly 4σ less than the mean of the other calibrants and not necessary for calibration. This regression of integrated $m/z=149$ ion abundances at 21.0 min., with respect to DEHP mass fractions in methylene chloride (Figure 4.7), is shown in Figure 4.7 and fit according to the equation $y = 675800x - 11350000$, $R^2 = 0.974$.

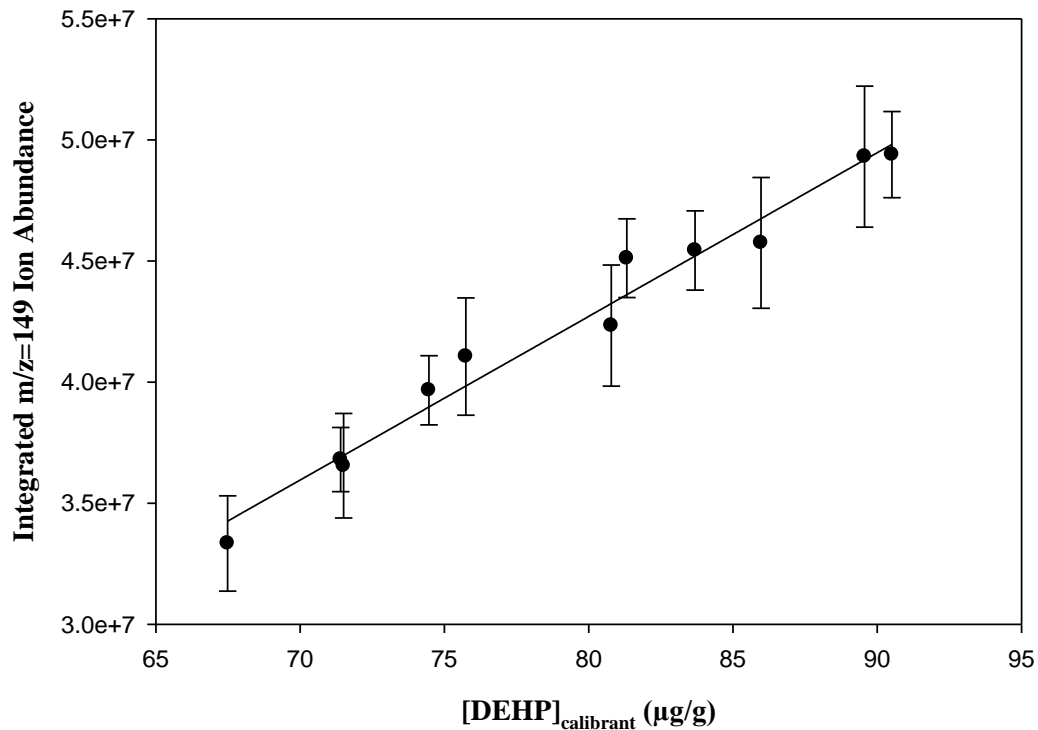


Figure 4.7 GC-EIMS calibration regression of integrated $m/z=149$ ion abundances at 21.0 min., with respect to DEHP mass fractions in methylene chloride for samples ST02, ST03, ST04, and ST05. $y = 675800x - 11350000$; $R^2 = 0.974$, 5.5 % standard error of the slope, and standard error of regression, $s_y = 904200$.

Methods blanks were similarly analyzed with calibrants containing much lower closely-bracketing DEHP mass fractions and $m/z=149$ ion abundances. Three stock solutions of DEHP standard were used to prepare 3 sets of calibrants. The two sets whose GC-EIMS analyses were most in agreement were used for calibration. The regression obtained from this calibration is depicted in Figure 4.8 and fit to the equation $y = 253300x - 70650$, $R^2 = 0.978$. Mass data and calculated mass fractions of calibrants, along with their measured $m/z=149$ ion abundances, are provided in Appendix 3.2, Table A3.10.

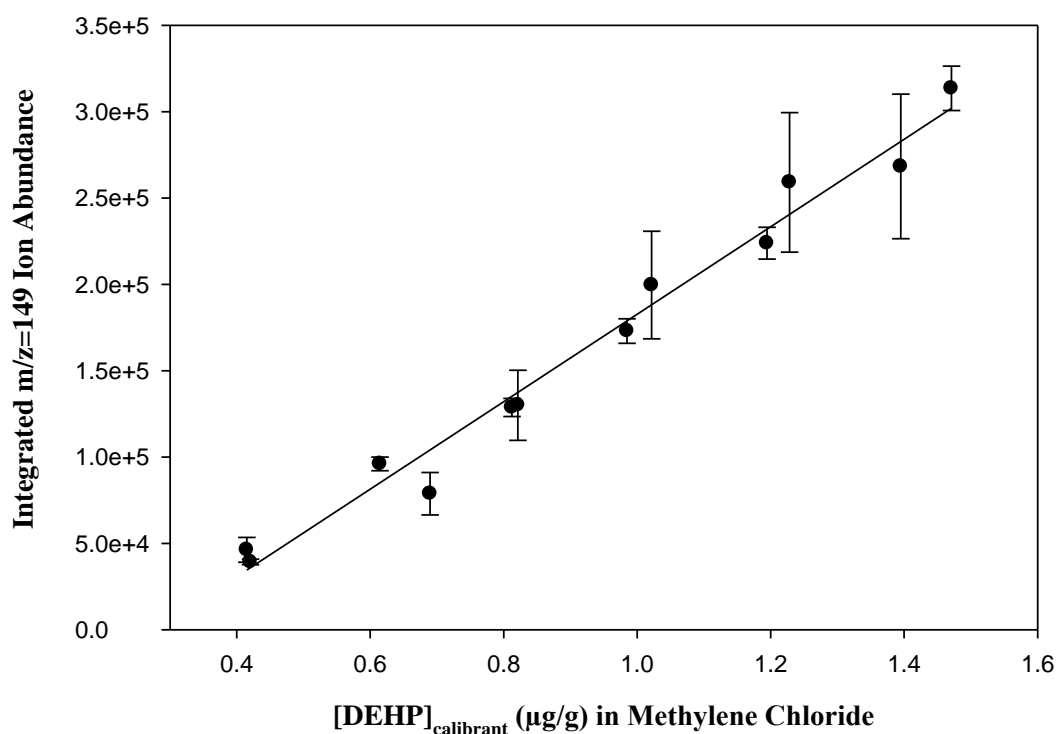


Figure 4.8 GC-EIMS calibration regression of integrated $m/z=149$ ion abundances at 21.0 min., with respect to DEHP mass fractions in methylene chloride for isolation method blanks BL02, BL03, and BL04. $y = 253300x - 70650$; $R^2 = 0.978$, 4.6 % standard deviation of the slope, and standard error of regression, $s_y = 13870$.

The uncertainties of DEHP mass fractions estimated from these regressions (s_x) were determined according to Equation 4.1.

$$s_x = \frac{s_y}{|m|} \sqrt{\frac{1}{n} + \frac{1}{k} + \frac{(y_{unk} - y_{avg})^2}{m^2 \sum (x_i - x_{avg})^2}}, \quad (4.1)$$

where m = slope, n = number of calibrant data points, y_{unk} = integrated ion abundance of $m/z = 149$ at 21.0 min in the DEHP sample or method blank, x_i is the estimated mass fraction of DEHP in methylene chloride ($\mu\text{g/g}$), and k = number of repeat measurements of the unknown. Also in this equation, y_{avg} and x_{avg} are, respectively, the mean integrated

m/z=149 relative ion abundances and mass fractions of DEHP ($\mu\text{g/g}$) in the calibration solutions.

4.5.3 GC-EIMS-measured DEHP masses

Prior to GC-EIMS analysis, the DEHP isolates and method blanks were concentrated in ~ 1 mL methylene chloride and weighed into 2 mL GC auto-sampler vials. These masses were used to determine the mass of DEHP in isolates and blanks from their measured DEHP mass fractions in methylene chloride. These values and the masses of carbon as DEHP in isolates and blanks, along with their propagated 1σ uncertainties, are listed in Table 4.8.

Table 4.8 Mass fractions of DEHP in methylene chloride ($\mu\text{g/g}$) and total DEHP masses (μg) in cheese-extracted samples and method blanks.

Sample/ Blank	Mass in MeCl_2 (g)	[DEHP] ($\mu\text{g/g}$)	DEHP mass estimate (μg)	Mass of Carbon as DEHP (μg)
ST01	1.28975 \pm 6.0E-05	113.3 \pm 7.9	146.2 \pm 10.2	107.9 \pm 7.5
ST02	1.31996 \pm 2.0E-05	72.67 \pm 1.44	95.87 \pm 1.91	70.75 \pm 1.41
ST03	1.32816 \pm 1.0E-05	69.93 \pm 1.49	92.88 \pm 1.97	68.55 \pm 1.16
ST04	1.32023 \pm 1.0E-05	72.76 \pm 1.44	96.07 \pm 1.91	70.90 \pm 1.40
ST05	1.50618 \pm 2.0E-05	78.06 \pm 1.40	116.10 \pm 2.11	87.16 \pm 1.56
BL01	0.33988 \pm 6.0E-05	4.4 \pm 1.7	1.5 \pm 0.6	1.1 \pm 0.5
SBL02	1.62083 \pm 3.0E-05	1.13 ¹ \pm 0.06	1.84 \pm 0.09	1.36 \pm 1.00
SBL03	0.72814 \pm 5.0E-05	0.83 ¹ \pm 0.06	0.61 \pm 0.04	0.45 \pm 0.33
SBL04	0.79032 \pm 2.0E-05	1.04 ¹ \pm 0.06	0.82 \pm 0.05	0.60 \pm 0.45

¹ m_{CB}

4.6 Assessment of carbon purity as DEHP in cheese-isolated samples

Chromatograms of GC-EIMS analyses of isolates collected after HPLC-processing indicated the presence of small quantities of several co-eluting compounds (Figure 4.6.), largely consisting of fatty acid esters, whose identifications were attempted via correlation of mass spectra observed during chromatographic peaks to reference spectra in the NIST/EPA/NIH Mass Spectral Database (NIST 11) by NIST Mass Spectral Search Program (Version 2.0g) (Table 4.9.). These compounds are present at much higher mass fractions in cheese than DEHP, and their physical similarity made total resolution by liquid chromatography very difficult and incomplete. Since graphitization of samples for ^{14}C AMS is non-selective and carbon isotope ratios are determined for entire samples regardless of their chemical source, these impurities contributed to the f_m of DEHP samples isolated from Stilton cheese. Being contemporary, biologically-synthesized, carbon-containing matter from the cheese matrix, they had measurable quantities of ^{14}C .

Purity assessments are often made by determining the integrated area of a given compound's measured signal peak relative to that of all other detectable compounds. However, a more rigorous approach was needed in order to accurately assess the purity of carbon as DEHP in isolates by GC-EIMS. The analysis must take into account the possibility that other compounds may elute simultaneously from the GC column, as there may be some impurities whose EIMS signals are indiscernible in a Total Ion Chromatogram. The analysis must also consider the fact that the measured ion

abundances are of EI-produced ion fragments with different masses and mass fractions of carbon.

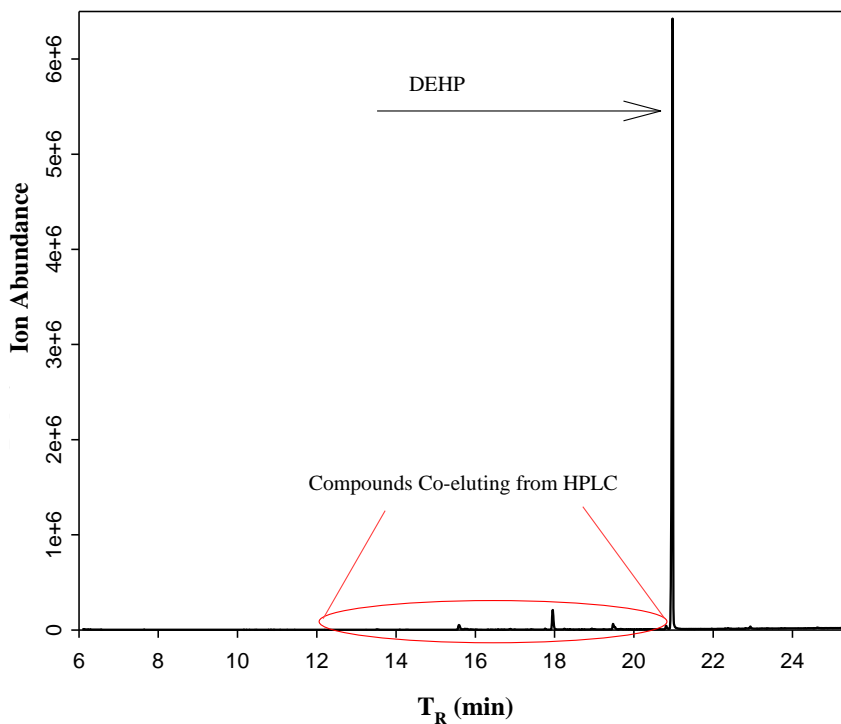


Figure 4.9 Sample ST05 total $m/z=50$ to $m/z=300$ GC-EIMS ion chromatogram.

4.6.1 Time-Resolved Mass Spectral Deconvolution

The purity of carbon as DEHP in each isolate was calculated according to,

$$Purity_c = 1 - \frac{(Counts_{carbon})_{co-eluted}}{(Counts_{carbon})_{sample}} \times 100 \quad (4.2)$$

where $(Counts_{carbon})_{co-eluted}$ is the amount of carbon as impurities from the cheese matrix that co-eluted from the HPLC column with DEHP, and $(Counts_{carbon})_{sample}$ is the amount of carbon in the entire sample. These amounts were determined with non-negative least-squares multivariate deconvolutions, programmed with Matlab®

computational software, of the time-resolved GC-EIMS fragmentation spectra of the samples and pure (99.8%) DEHP standard.

The GC-EIMS analysis of an isolate or standard produced a 3381 x 251 data matrix which contained the detected ion abundances of each $m/z=50$ to $m/z=300$ (251 ion masses) during each of the 3381 quadrupole scans collected at ~ 0.35 s intervals (2.9 scans/s), from 6.1 min to 25.6 min after sample injection to the GC column. Figure 4.10 illustrates the data matrix of ST05 from 10 min to 25 min, the time range of the chromatogram that compounds were detected in sample ST05. The inset is the same data matrix illustrated at a scale of ion abundance that more clearly allows the ion peaks from impurities to be discerned.

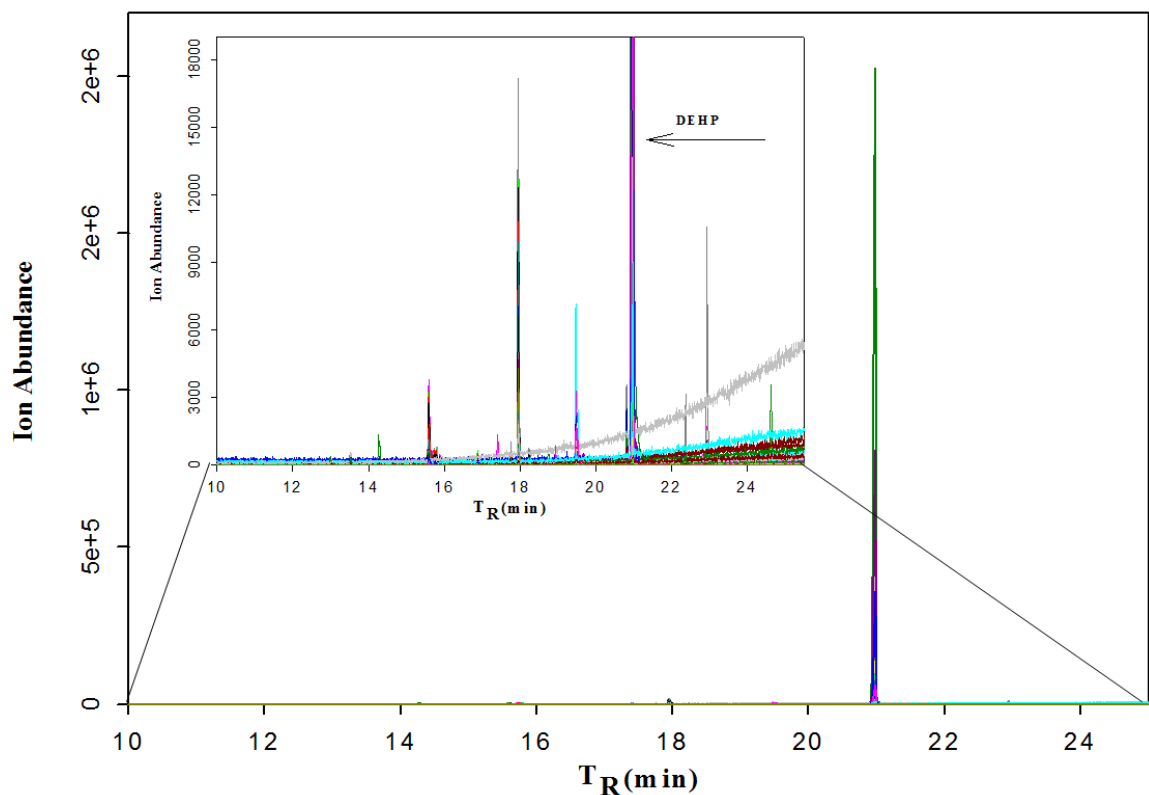


Figure 4.10 Sample ST05 GC-EIMS single ion chromatograms whose color gradient represents each measured $m/z=50$ to $m/z=300$.

Prior to deconvolution, each 3381 x 251 data matrix was “binned” with respect to time by summing the ion abundances of corresponding mass fragments from chronologically-adjacent quadrupole scans. This was performed to mitigate minor differences in the sample and standard spectra that resulted from variations in the algorithmic binning of detector responses during the continuous quadrupole scan. It was also utilized to increase signal to noise ratios for ion masses pertinent to the measured spectra of compounds in the isolate. Figure 4.11 represents the single ion chromatograms of ST05, at the ion abundance scale of the inset of Figure 4.10, after its time resolution was reduced by a factor of twelve (0.23 bins/s). Figure 4.12 is the binned spectra of the pure (99.8 % ± 0.1 %) DEHP standard, measured after 1 µL-injection of 76.6 µg/g DEHP in methylene chloride. A binning factor of 12 was determined to be the most suitable owing to its ability to provide sufficient reduction of noise without compromising the resolution of the data attributable to GC chromatography.

Deconvolutions were performed according to the notion that DEHP produces a GC-EIMS fragmentation spectrum that is consistent at all times, t , in the chromatograms of samples and standard. Therefore, the measured sample spectra ($S_{measured}$) is the sum of the DEHP spectral component, as determined from triplicate injections of 99.8 % ± 0.1 % pure DEHP standard ($S_{standard}$), and the spectral component of impurities ($S_{co-eluting}$) whereby;

$$S_{measured_t} = \gamma_t \cdot S_{standard_t} + S_{co-eluting_t} \quad (4.3)$$

and $\gamma_t = t^{th}$ DEHP mixing coefficient

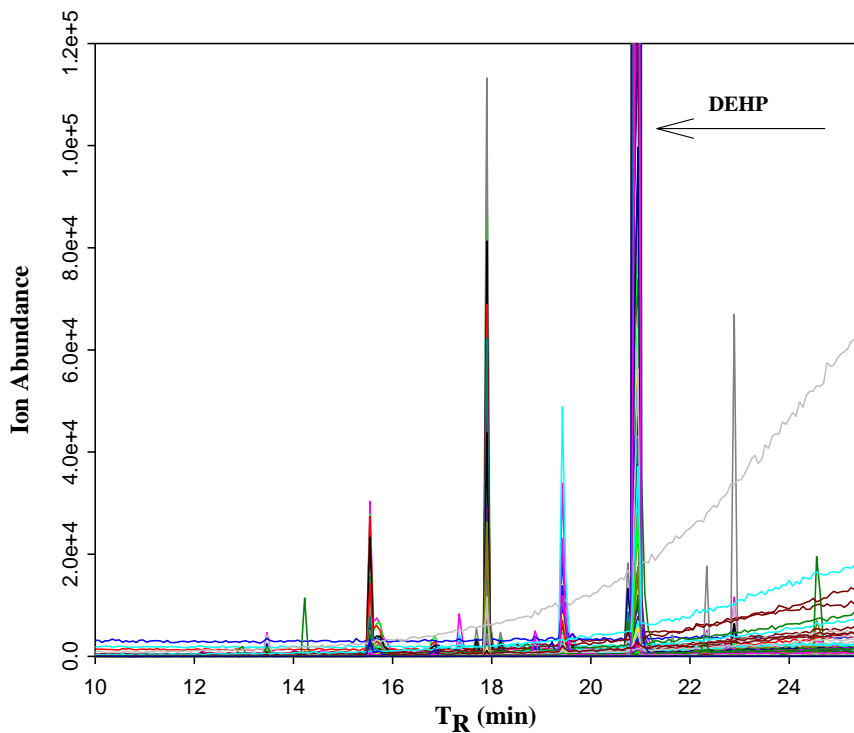


Figure 4.11 Sample ST05 GC-EIMS single ion chromatograms: 12 Scans/bin. The different colors represent fragmentation ions from $m/z=50$ to $m/z=300$.

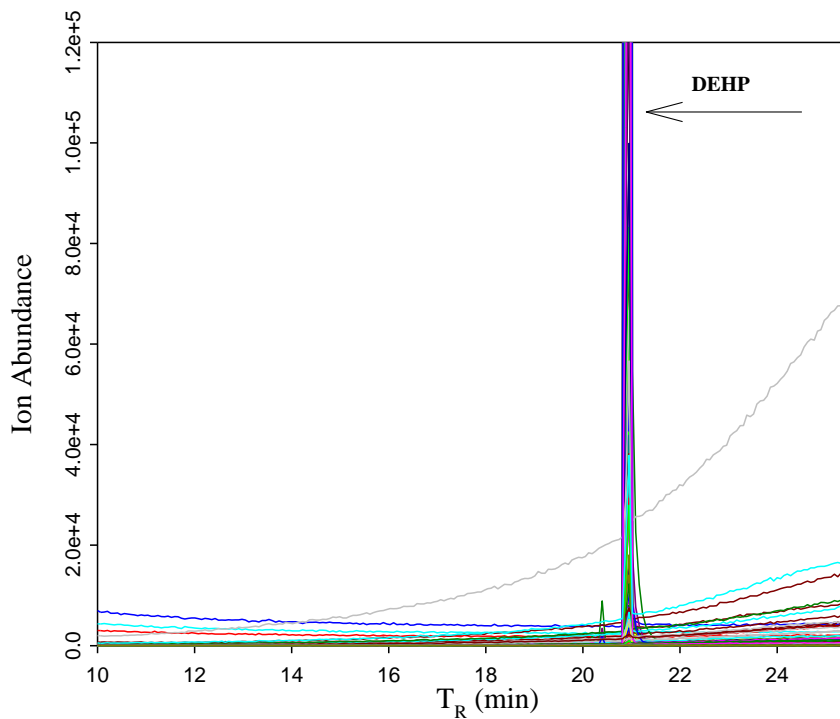


Figure 4.12 DEHP standard GC-EIMS single ion chromatograms: 12 scans/bin. The different colors represent fragmentation ions from $m/z=50$ to $m/z=300$.

The multiple non-linear least squares fitting criterion listed below (Equation 4.4) was used to remove the DEHP component of the measured sample spectra and produce a residual corresponding to the spectra of impurities.

$$\chi^2 = \sum_{i=1}^{tbin} \sum_{j=1}^{\frac{nm}{z}} \frac{(S_{measured_{ij}} - \gamma \cdot S_{standard_{ij}})^2}{\sigma_{i,j}^2} \quad (4.4)$$

In Equation 4.4, *tbin* is the number of 12-scan bins along the time axis of the chromatogram (281), $\frac{nm}{z}$ is the number of ions scanned from *m/z*=50 to *m/z*=300 (251), and σ is the combined uncertainty of the ion abundance of the measured sample and standard spectra. The residual of this least squares deconvolution is depicted in Figure 4.14.

The ions measured during the time of DEHP elution in the sample and standard were highly correlated. The residual in this region was only 0.04 % of the ion abundances in the total chromatogram, demonstrating that very little impurity co-eluted with DEHP from the analytical GC column.

The GC column bleed, distinguishable by the gently upward-sloping single ion chromatograms in Figure 4.10 and 4.11, is an artifact of the GC analysis and not actually present in the sample. It was removed with DEHP in the previous deconvolution because the DEHP standard spectra also had a GC bleed component. To determine the spectra of all compounds in the sample ($S_{isolate}$), this component ($S_{GC-Bleed}$), as determined with triplicate injections of methylene chloride, was removed from the measured spectra. This was accomplished with a nearly identical deconvolution whereby;

$$S_{measured_t} = \gamma_t \cdot S_{GC-Bleed_t} + S_{isolate_t} \quad (4.5)$$

$\gamma_t = t^{th}$ GC bleed mixing coefficient

The multiple non-linear least squares fitting criterion to determine the residual that corresponded to the spectra of all compounds in the sample ($S_{isolate}$) was,

$$\chi^2 = \sum_{i=1}^{tbin} \sum_{j=1}^{\frac{nm}{z}} \frac{(S_{measured_{i,j}} - S_{GC-Bleed_{i,j}})^2}{\sigma_{i,j}^2} \quad (4.6)$$

This residual for ST05 is in Figure 4.13.

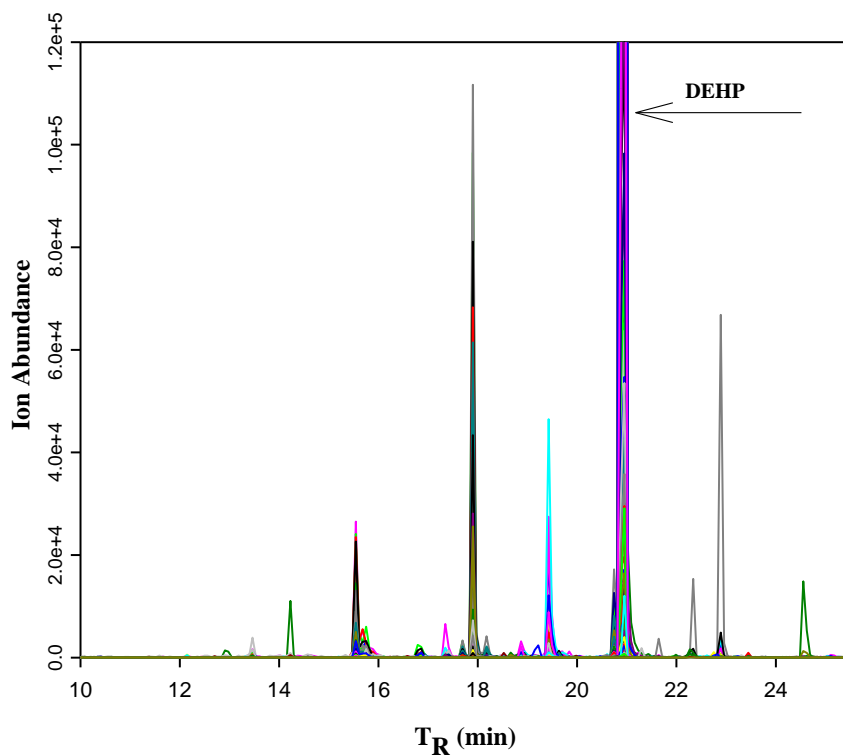


Figure 4.13 Multi-nonlinear least squares deconvolution residual illustrating the measured GC-EIMS spectrum of ST05 after removal of GC-Bleed.

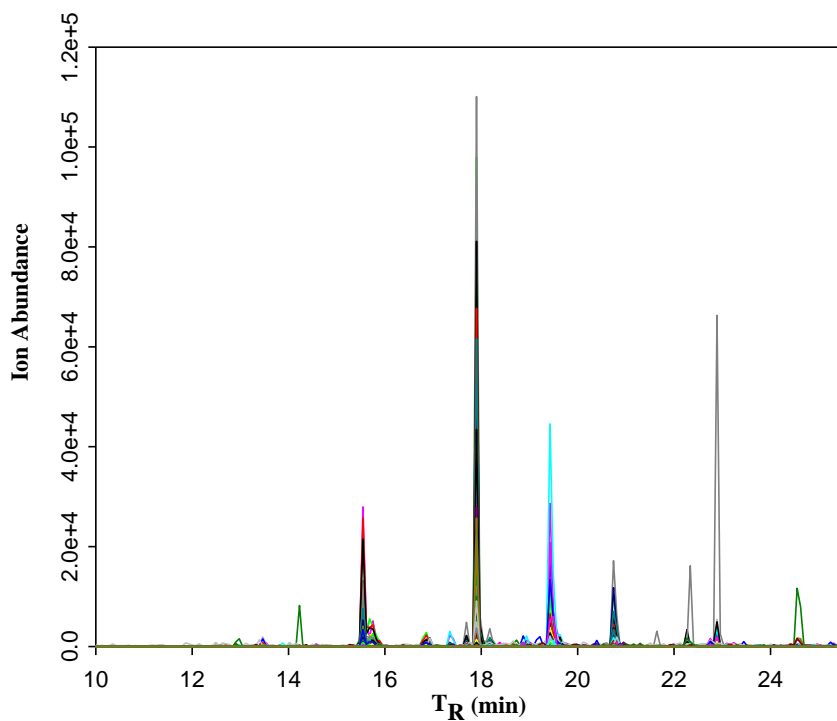


Figure 4.14 Multi-nonlinear least squares deconvolution residual illustrating the spectra of impurities in Sample ST05.

4.6.2 Purity of Carbon as DEHP

The ion abundances in the deconvolution residuals (illustrated for ST05 by Figure 4.13 and 4.14) were of many fragments with different masses and varying carbon mass fractions. As noted above, the impurities identified in the isolates were mostly fatty acid esters. These are listed in Table 4.9, along with their mass fractions of carbon. Also listed are estimates of the average total carbon mass fraction of impurities ($(Y_C)_{\text{coelute}}$) and that of all compounds ($(Y_C)_{\text{sample}}$) in the isolates. These were determined as the mean of the listed compounds' carbon mass fractions, weighted by the average integrated total ion abundance of their respective peaks in the GC-EIMS chromatograms (TIC) of the isolates.

Table 4.9 Mass fractions of co-eluting compounds identified in samples submitted for ^{14}C measurement by AMS.

Compound ¹	T _R (min)	Average % of sample TIC	Carbon Mass Ratio
hexadecanoic acid, ethyl ester	11.69	0.13	0.761
Butyl 9-tetradecenoate	13.20	0.08	0.765
i-Propyl 9-tetradecenoate	13.52	0.46	0.765
Butyl 9-tetradecenoate	14.08	0.07	0.765
Dibutyl Phthalate	14.27	0.05	0.961
Methyl 6,11-octadecadienoate	15.59	1.56	0.776
9,12-Octadecadienoic acid, methyl ester, (E,E)-	15.74	0.33	0.776
11,14-Octadecadienoic acid, methyl ester	15.80	0.38	0.776
10,13-Octadecadienoic acid, methyl ester	15.89	0.15	0.776
Methyl 12, 15 - octadecadienoate	15.94	0.09	0.776
i-Propyl 9,12,15-octadecatrienoate	16.58	0.08	0.788
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.88	0.54	0.776
Farnesol (E), methyl ether	17.00	0.05	0.814
n-Propyl 9,12,15-octadecatrienoate	17.76	0.15	0.788
methyl 8, 11, 14 - eicosatrienoate	17.96	4.10	0.788
Methyl 11,14,17-eicosatrienoate	18.25	0.23	0.788
Oxalic acid, decyl 2-phenylethyl ester	18.75	0.09	0.719
n-Propyl 9-octadecenoate	18.94	0.13	0.778
Hexanedioic acid, dioctyl ester	19.24	0.04	0.714
Butyl 9-octadecenoate	19.47	0.40	0.782
n-Propyl 11-octadecenoate	19.53	0.41	0.778
Octadecanoic acid, 2-propenyl ester	19.58	0.28	0.778
Oxiraneoctanoic acid	19.68	0.16	0.725
Butyl 5,8,11,14,17-eicosapentaenoate	20.81	0.71	0.801
Carbonic acid, hexadecyl phenyl ester	22.38	0.08	0.763
Oxalic acid, 2-phenylethyl tridecyl ester	22.52	0.08	0.734
i-Propyl 11-octadecenoate	22.83	0.08	0.778
Carbonic acid, octadecyl phenyl ester	22.93	0.25	0.770
Oxalic acid, dodecyl 2-phenylethyl ester	24.63	0.12	0.796
DEHP	20.98	89.43	0.738

¹ Identification by mass spectral reference to the NIST/EPA/NIH Mass Spectral Database (NIST 11), using NIST Mass Spectral Search Program (Version 2.0g), their mass fractions of carbon, their total relative ion abundances, and carbon mass fractions of HPLC-co-eluting compounds and the entire sample.

Table 4.9 (cont.) Mass fractions of co-eluting compounds identified in samples submitted for ^{14}C measurement by AMS.

	Carbon Mass Ratio
Weighted Mean Carbon mass ratio of total Sample - $(\gamma_{\text{C}})_{\text{sample}}$	0.743
Weighted Mean Carbon mass ratio of HPLC co-eluting compounds - $(\gamma_{\text{C}})_{\text{coelute}}$	0.781

The amount of carbon present ($\text{Counts}_{\text{carbon}}$) in each deconvolution residual (r), and thereby $(\text{Counts}_{\text{carbon}})_{\text{co-eluted}}$ and $(\text{Counts}_{\text{carbon}})_{\text{sample}}$ of ST01 to ST05, was determined from its appropriate weighted mean carbon mass ratio (γ_{C}) and the sum of all ion abundances ($\text{counts}_{\text{ion}}$) in the spectra normalized to 12 amu:

$$(\text{Counts}_{\text{carbon}})_r = \gamma_{\text{C}} \cdot \sum_{i=1}^{281 \text{ bins}} \sum_{j=1}^{251 \text{ ions}} \frac{(m/z)_j}{12} \cdot (\text{counts}_{\text{ion}})_{i,j} \quad (4.7)$$

where $(m/z)_j$ is the mass (amu) of j^{th} $m/z=50$ to $m/z=300$ scanned ion and.

The purity of carbon as DEHP in each sample (Purity_{C}) was then calculated according to Equation 4.2. The results of these calculations are listed in Table 4.10. The errors assigned to these estimates were based on the standard deviations of values estimated by assigning 3, 4, 6, 12, 17, and 34 quadrupole scans per data bin prior to performing the deconvolution.

Table 4.10 Estimated Purity (%) of carbon mass as DEHP in AMS samples.

Sample	Purity_{C}
ST01	94 ± 1.3
ST02	88.1 ± 1.7
ST03	87.2 ± 1.7
ST04	90.5 ± 1.7
ST05	92.3 ± 1.4

4.7 Measurement of $\delta^{13}\text{C}$ by Isotope Ratio Mass Spectrometry

The assessments of $^{13}\text{C}/^{12}\text{C}$ ratios in isolates, petrogenic DEHP standards, and Stilton cheese, needed to adjust f_m values for fractionation, were performed at the University of Maryland, College Park Department of Geology Gas-Source Mass Spectrometry II facility, supervised by Dr. Jay Kaufman and Dr. Michael Evans. Measurements to determine $\delta^{13}\text{C}$ were made by an Isoprime Continuous Flow (CF) Isotope Ratio Mass Spectrometer configured for carbon analysis of organic matter and equipped with a multicollector and high-temperature sample combustion oven. Samples and standards in solution were weighed into Costech (Valencia, CA) 3.5 x 5 mm Tin capsules for solid samples on a Mettler Toledo UMT2 Ultra-microscale balance. Solvent was allowed to evaporate before folding and sealing the capsules with clean, solvent-rinsed stainless steel tweezers. A total of three isolate samples were prepared for analysis; one entirely composed of ST01, one entirely of ST05, and one a combination of ST02, ST03, and ST04 isolates. Two sets of samples were prepared. The first set contained a sample of ST01, DEHP standards, and whole stilton cheese. They were analyzed prior to sending isolate ST01 and accompanying standards to LLNL CAMS for ^{14}C AMS analysis. The second, containing samples of aliquots of ST02, ST03, ST04, and ST05, as well as additional DEHP standards and lyophilized whole Stilton cheese, were analyzed after all isolates had been sent to CAMS. While awaiting analysis, these samples were kept in solution in glass borosilicate conical vials capped with PTFE-lined silica septum caps at 5 °C. Measurements were referenced to those of Urea standards

with an average measured $\delta^{13}\text{C}$ VPDB of -29.39 ± 0.03 . The results of $\delta^{13}\text{C}$ VPDB of each sample and the mean of like-samples are shown in Table 4.11.

Table 4.11 $\delta^{13}\text{C}$ VPDB of samples, DEHP standards, and Stilton cheese by IRMS.

Sample	Sample Description	Date	$\delta^{13}\text{C}$	Average $\delta^{13}\text{C}^1$	$1 \sigma^1$
ST01	DEHP ISOLATE	10/31/2011	-30.02	-30.02	0.04
ST02, ST03, ST04	DEHP ISOLATE	9/6/2013	-29.47		
ST05	DEHP ISOLATE	9/6/2013	-29.04	-29.26	0.30
FDS1	DEHP STD	10/31/2011	-29.04		
FDS2	DEHP STD	10/31/2011	-29.12		
FDS3	DEHP STD	10/31/2011	-29.11		
FDS4	DEHP STD	9/6/2013	-29.09		
FDS5	DEHP STD	9/6/2013	-29.41		
FDS6	DEHP STD	9/6/2013	-29.31	-29.18	0.14
IRS1	Whole Stilton Cheese	10/31/2011	-27.13		
IRS2	Whole Stilton Cheese	10/31/2011	-27.09		
IRS3	Whole Stilton Cheese	9/6/2013	-27.19		
IRS4	Whole Stilton Cheese	9/6/2013	-27.08		
IRS5	STILTON	9/6/2013	-27.35	-27.17	0.11

The mass of carbon remaining in each isolate to be analyzed at LLNL by AMS after the removal of these aliquots for $^{13}\text{C}/^{12}\text{C}$ analysis is listed in Table 4.12.

Table 4.12 Masses of carbon contributed by each isolate for IRMS measurement (μg) and masses remaining for ^{14}C AMS analysis (μg).

Isolate ID	IRMS Sample ID	Carbon Mass in DEHP Removed for IRMS (μg)	Total estimated Carbon Mass Removed (μg)	Carbon as DEHP Remaining for AMS (μg)	Estimated carbon mass for AMS (μg) ¹
ST01	ST01	20.7 \pm 1.3	22.0 \pm 1.4	92.6 \pm 6.5	98.5 \pm 6.9
ST02	IP2	3.50 \pm 0.09	3.98 \pm 0.09	66.32 \pm 1.41	76.60 \pm 1.60
ST03	IP2	5.04 \pm 0.13	5.79 \pm 0.13	62.36 \pm 1.46	71.49 \pm 1.64
ST04	IP2	5.44 \pm 0.13	6.02 \pm 0.14	64.29 \pm 1.41	71.89 \pm 1.55
ST05	IP1	18.03 \pm 0.34	19.64 \pm 0.34	68.28 \pm 1.59	75.10 \pm 1.73

¹Estimated from DEHP mass and carbon purity assessment

4.8 Sample Mass Determinations, Packaging, and Shipment to Lawrence Livermore National Laboratory's Center for Accelerator Mass Spectrometry

After the removal of aliquots of DEHP isolates for IRMS analyses, the remaining quantities of each of the isolates were transferred to previously-weighed 1 mL borosilicate glass conical vials with 9 mm screw-cap tops. It was noticed at this time, once transferred to transparent glass vials, that a small, nearly indiscernible transparent fiber was adhered to the side of the sample vial containing isolate ST02. The solvent was totally removed from this sample while it was still in the vial by vacuum rotary evaporation. It was then reconstituted with \sim 0.5 mL hexane, in which the particle adhered to the glass wall of the vial. The isolate was transferred to a newly-prepared vial with a solvent-cleaned stainless steel needle syringe, leaving the particle adhered to the previous vial. The interior of this vial was rinsed three times with 100 μL of hexane, which was added to ST02. The same process was performed with the other samples to remove particulates that were very difficult to discern with the eye.

Isolates and other materials sent to LLNL for AMS were constituted in ~ 200 μL of methylene chloride or hexane. This small volume minimized the amount of time required for solvent evaporation at LLNL. Concentration of samples from ~1 mL to ~200 μL was performed in the shipping vials to ensure that there was no loss of sample that would otherwise occur during the transfer of such a small volume to a new vial. Two adapters (Sigma Aldrich, St. Louis, MO), which included a special PTFE 9 mm screw-cap adaptor required to couple the conical sample vial to a second adaptor containing the male end of a 9 mm screw-cap joint and the female end of a 24/40 joint, provided an airtight connection between the conical vial and a 100 mL anti-splash guard of the rotary evaporator. The vacuum was applied at its lowest setting and the vial was rotated slowly at ~7 rad/s (~ 70 rotations/min) at a temperature of ~ 35 °C in order to gently evaporate organic solvent and prevent dispersion of sample DEHP through the evaporator system. After reduction to 200 μL , sample vials were capped with PTFE-lined silica septum, inert polypropylene caps (Sigma Aldrich) and weighed.

Sample Set 1, sent to LLNL CAMS on November 1, 2011, contained only ST01, its corresponding method blank (BL01), two samples of un-labeled DEHP standard prepared from a stock solution containing 1442 $\mu\text{g/g}$ DEHP in methylene chloride, two aliquots of d_{38} -DEHP internal standard in acetonitrile, and three samples of whole Stilton cheese. Sample Set 2, sent June 1, 2012, contained the remaining cheese isolates, spiked method blanks, three more unlabeled DEHP standards, and three more samples of whole Stilton cheese. Access to NIST (Gaithersburg, MD) lyophilizers to prepare whole Stilton cheese samples for AMS was provided courtesy of the Chemical Sciences Division and Biosystems and Biomaterials Division.

In the first shipment of samples for AMS, the combusted blank produced an insufficient mass of carbon for ^{14}C analysis. For this reason, each of the method blanks prepared and sent to LLNL CAMS (6/1/2012) with the second set of samples for analysis were spiked with weighed amounts of unlabeled DEHP standard. This allowed for the mass and $^{14}\text{C}/^{12}\text{C}$ composition of extraneous carbon in the method blanks to be determined, given that the masses of the DEHP standard spikes were known. In addition, blanks were split (by mass) for AMS analysis in order for the halves to be spiked separately with weighed aliquots of unlabeled petrogenic Supleco (99.8%) DEHP standard diluted in hexane ($241.53 \mu\text{g/g} \pm 0.54 \mu\text{g/g}$) and a ^{14}C -labeled $d38$ -DEHP standard diluted in neat unlabeled petrogenic DEHP to $f_m = 0.918$. The latter were sent separately and did not produce meaningful results that could be applied to blank-correct the samples. Along with the samples and spiked method blanks, shipping blanks that contained *only* the petrogenic DEHP spike material in hexane were also prepared and sent to LLNL. The latter were prepared to assess the amount contamination originating from the sample preparation after GC-EIMS analysis, shipping vials, and post-chromatographic transfer. Samples of lyophilized Stilton cheese were also sent for analysis with this shipment. The masses of samples contained in the two shipments to LLNL CAMS are given in Tables 4.13.a and Table 4.13.b.

Table 4.13.a Sample masses as methylene chloride solutions (g), DEHP (μg), and carbon in DEHP (μg) of isolates, blanks, and DEHP standards, as well as Stilton cheese (mg), shipped to LLNL CAMS 11/1/2011.

A. Masses of Isolate, Blank, and Prepared Standards

Sample ID	Sample Content	Mass of sample as MeCl solution (g) ¹	DEHP in sample ² (μg)	Mass of carbon from DEHP (μg)
ST01	DEHP from Cheese Isolate	0.31179	125.5 \pm 8.8	92.6 \pm 6.5
BI01	Isolation Method Blank	0.32138	1.5 \pm 0.6	1.1 \pm 0.5
STD01	Petrogenic Supelco DEHP Standard	0.27091	199.86 \pm 0.85	147.50 \pm 0.03
STD02	Petrogenic Supelco DEHP Standard	0.26489	193.77 \pm 0.83	143.00 \pm 0.61
D38DEHP01	Petrogenic D38 DEHP Standard	0.20266	201.24 \pm 1.50	135.44 \pm 1.11
D38DEHP02	Petrogenic D38 DEHP Standard	0.21703	197.00 \pm 1.47	132.58 \pm 1.08

B. Samples of Whole Lyophilized Stilton Cheese for AMS Analysis

Sample ID	Sample Content	Mass (mg)
RS01	Stilton Cheese	7.25 \pm 0.01
RS02	Stilton Cheese	11.07 \pm 0.01
RS03	Stilton Cheese	17.98 \pm 0.01

¹Determined by Mettler Toledo Balance

²GC-EIMS-estimated values in isolates and blanks prior to spiking. Spike Masses determined by weighing aliquots of Supelco standard DEHP in hexane solution and the manufacturer's purity determination (99.8 % \pm 0.1 %)

Table 4.13.b Sample masses as hexane solutions (g), DEHP (μg), and carbon in DEHP (μg) of isolates and blanks, as well as Stilton cheese (mg), shipped to LLNL CAMS 06/1/2012.

A. Masses of Isolates, Spiked Blanks, and Prepared Standards

Sample ID	Sample Content	Mass of sample as hexane solution (g) ¹	DEHP in sample (μg) ²	Mass of carbon from DEHP (μg) ³
ST02	DEHP from Cheese Isolate	0.2015	89.86 \pm 1.91	66.32 \pm 1.41
ST03	DEHP from Cheese Isolate	0.1961	84.50 \pm 1.97	62.36 \pm 1.46
ST04	DEHP from Cheese Isolate	0.1959	87.11 \pm 1.91	64.29 \pm 1.41
ST05	DEHP from Cheese Isolate	0.3484	92.52 \pm 2.15	68.28 \pm 1.59
SBL02 ³	Spiked Method Blank A	0.13060	62.4 \pm 0.08	46.74 \pm 0.07
SBL03 ³	Spiked Method Blank BC	0.16308	65.1 \pm 0.05	47.85 \pm 0.05
SBL04 ³	Spiked Method Blank D	0.26533	64.9 \pm 0.05	45.59 \pm 0.05
S1	petrogenic DEHP shipping blank	0.26533	64.1 \pm 0.1	47.30 \pm 0.04
S2	petrogenic DEHP shipping blank	0.26900	65.0 \pm 0.1	47.98 \pm 0.04
S3	petrogenic DEHP shipping blank	0.26777	64.74 \pm 0.06	47.78 \pm 0.04

B. Samples of Whole Lyophilized Stilton Cheese for AMS Analysis

Sample ID	Sample Content	Mass (mg)		
RS11	Stilton Cheese	13.290	\pm	0.022
RS12	Stilton Cheese	9.223	\pm	0.286
RS13	Stilton Cheese	11.290	\pm	0.037

¹Determined by Metler Toledo Ax105 Delta Range Balance

²GC-EIMS-estimated values in isolates and blanks prior to spiking. Spike Masses determined by weighing aliquots of Supelco standard DEHP in hexane solution and the manufacturer's purity measurements.

³Aggregate DEHP mass of GC-EIMS method contamination estimate and standard material spike.

4.9 Graphitization and QA/QC for AMS Analyses

Sample sets sent on 11/1/2011 and 06/1/2012 were express-delivered to Dr. Bruce Buchholz at LLNLS CAMS. Combustion and graphitization of samples in preparation for AMS and subsequent ^{14}C analyses were performed by Dr. Buchholz and LLNL CAMS staff according to the procedures described herein. Upon arrival of samples at LLNL, they were transferred to pre-combusted (900°C for 3.5 hours) quartz combustion tubes. These tubes were later heated overnight at 50 °C for complete removal of solvent. An excess of copper oxide was added to each quartz tube prior to evacuation with an oil free turbo pump and sealing with an acetylene torch. Samples in the sealed tubes were heated to 900 °C for 3.5 h to oxidize all carbon to CO_2 . The CO_2 was isolated from other products of combustion by cryogenic distillation. The carbon masses of the purified CO_2 samples were determined with a Baratron capacitance manometer to a precision of 1.5 % to 3.0 %. The CO_2 samples were graphitized with iron catalysts in individual reactors and pressed into pellets for AMS analysis.

All ^{14}C AMS data were normalized with identically-prepared NIST (SRM 4990C) Oxalic Acid standards to adjust for carbon isotope fractionation resulting from ionization of the graphite sample and to standardize their measurements to f_m . IAEA C-6, and TIRI wood served as secondary quality control standards to monitor spectrometer performance. Samples of ^{14}C -free coal were also combusted, graphitized, and analyzed with samples to allow mass-based adjustments to f_m that account for ^{14}C instrument background and contamination from the graphitization procedure. Corrections for

background contamination introduced during sample preparation at LLNL were made following standard procedures (Brown and Southon 1997).

Chapter 5: AMS Results and Data Interpretation

5.1 ^{14}C AMS Results

Results for AMS Sample Set 1 (containing ST01 and BL01 pilot samples) were received from LLNL CAMS on January 2, 2012, and those for Sample Set 2 on June 27, 2012. All of these reported results are presented in Table 5.1. All f_m 's are derived from measurements of $^{14}\text{C}/^{13}\text{C}$ in the sample, adjusted for ^{14}C contamination during the combustion and graphitization process using blanks containing ^{14}C -free coal, normalized to measurements of $^{14}\text{C}/^{13}\text{C}$ in the oxalic acid standard (as outlined in Section 3.5). The uncertainties reported with each determination of f_m are determined from ^{14}C counting statistics ($(\sqrt{n})/n$) of samples, standards, and ^{14}C -free coal blanks (Stuiver and Polach 1977). They also account for other experimental variations, including accelerator voltage, ionization efficiency, or temperature fluctuation through repeated measurements of a given target. Also included in Table 5.1 is each sample's total carbon mass (m_{LLNL}), measured after its combustion, as CO_2 at LLNL by a Baratron Capacitance manometer.

Table 5.1 Reported f_m and masses of samples, blanks, standards, and Stilton cheese (μg) by LLNL CAMS.

CAMS #	Sample	Sample Description	$\delta^{13}\text{C}$ VPDB ¹	f_m ^{2,3}	Carbon mass (μg) by LLNL, m_{LLNL} ⁴
155279	ST01	Isolate	-30.0±0.04	0.2829 ± 0.0042	145 ± 2
157755	ST02	Isolate	-29.26±0.30	0.2809 ± 0.0035	99 ± 2
157756	ST03	Isolate	-29.26±0.30	0.3526 ± 0.0030	127 ± 2
157757	ST04	Isolate	-29.26±0.30	0.3111 ± 0.0027	135 ± 2
157686	ST05	Isolate	-29.26±0.30	0.3335 ± 0.0031	114 ± 2
155280	STD01	DEHP Standard	-29.18±0.14	0.0018 ± 0.0042	135 ± 2
155281	STD02	DEHP Standard	-29.18±0.14	0.0000 ± 0.0044	130 ± 2
157687	S1	Shipping Blank	-29.18±0.14	0.0110 ± 0.0053	78 ± 2
157688	S2	Shipping Blank	-29.18±0.14	0.0076 ± 0.0053	81 ± 2
157689	S3	Shipping Blank	-29.18±0.14	0.0085 ± 0.0053	83 ± 2
	BL01 ⁵	Method Blank			
157690	SBL02	Spiked Blank	-29.18±0.14	0.0373 ± 0.0059	68 ± 2
157691	SBL03	Spiked Blank	-29.18±0.14	0.0224 ± 0.0046	88 ± 2
157692	SBL04	Spiked Blank	-29.18±0.14	0.0640 ± 0.0050	78 ± 2
	RS01 ⁶	Whole Cheese			
155284	RS02	Whole Cheese	-27.17±0.11	1.0443 ± 0.0048	- -
155285	RS03	Whole Cheese	-27.17±0.11	1.0401 ± 0.0039	- -
157693	RS11	Whole Cheese	-27.17±0.11	1.0440 ± 0.0058	535 ± 2
157694	RS12	Whole Cheese	-27.17±0.11	1.0437 ± 0.0031	831 ± 2
157695	RS13	Whole Cheese	-27.17±0.11	1.0477 ± 0.0029	158 ± 2
155282	D38DEHP01	D38 DEHP	-27.2	0.0000 ± 0.0042	- -
155283	D38DEHP02	D38 DEHP	-27.2	0.0030 ± 0.0041	- -

¹Measured by UMCP IRMS.

²Sample preparation backgrounds have been subtracted based on measurements of samples of ¹⁴C-free coal. Backgrounds were scaled relative to sample size.

³two-sigma limits as per Stuiver and Polach.

⁴Obtained at LLNL CAMS with Baratron capacitance manometer. Stated uncertainty: ~1 % to 2%. Mass uncertainties are shown as 1.5 σ .

⁵Mass of carbon not Sufficient for AMS.

⁶Quartz combustion tube broke during sample preparation and sample was not analyzed

5.2 Extraneous Carbon Corrections to f_m

It was immediately apparent that there was an obvious disparity between masses measured gravimetrically and with GC-EIMS at NIST and UMCP, and those determined manometrically at LLNL. These mass measurements and their disparities between the measurement laboratories (Δm_C) are shown in Table 5.2. A thorough assessment of this contamination, and all of its discernible sources, was needed in order to accurately deduce its effect on the measure f_m , and thus determination of the contemporary fraction of DEHP in Stilton cheese.

Table 5.2 Pre-shipment and post-combustion mass determinations and their total carbon mass disparities (ΔmC , μg).

Sample ID	Mass of DEHP (μg) ¹	Carbon Mass from DEHP (μg) ²	% DEHP Carbon purity ³	Total Carbon Mass Estimate (μg); UMCP, NIST ⁴	Total Carbon mass (μg) by LLNL, m_{LLNL} ⁵	ΔmC (μg)
ST01	125.5 ± 8.8	92.6 ± 6.5	94.0 ± 1.3	98.5 ± 7.0	109 ± 3	11 ± 8
ST02	91.12 ± 1.04	66.32 ± 1.41	88.1 ± 1.7	75.27 ± 2.16	99 ± 3	24 ± 4
ST03	85.92 ± 1.07	62.32 ± 1.46	87.2 ± 1.7	71.47 ± 2.17	127 ± 2	56 ± 3
ST04	88.64 ± 1.03	64.29 ± 1.41	90.5 ± 1.7	71.04 ± 2.05	135 ± 2	64 ± 3
ST05	93.62 ± 1.14	68.28 ± 1.59	92.3 ± 1.4	73.98 ± 2.06	114 ± 2	40 ± 3
BL01	1.5 ± 0.6	1.1 ± 0.5	- -	- -	- -	- -
SBL02	62.4 ± 0.08	46.74 ± 0.07	- -	46.74 ± 0.07	68 ± 2	21 ± 2
SBL03	65.1 ± 0.05	47.85 ± 0.05	- -	47.85 ± 0.05	88 ± 2	40 ± 2
SBL04	64.9 ± 0.05	45.59 ± 0.05	- -	45.59 ± 0.05	78 ± 2	32 ± 2
STD01	199.86 ± 0.85	147.50 ± 1.00	99.8 ⁶ ± 0.1	147.50 ± 1.00	135 ± 2	-13 ± 2
STD02	193.77 ± 0.83	143.00 ± 1.00	99.8 ⁶ ± 0.1	143.00 ± 1.00	130 ± 2	-13 ± 2
S1	64.1 ± 0.1	47.3 ± 0.04	99.8 ⁶ ± 0.1	47.3 ± 0.04	78 ± 2	31 ± 2
S2	65.0 ± 0.1	47.98 ± 0.04	99.8 ⁶ ± 0.1	47.98 ± 0.04	81 ± 2	33 ± 2
S3	64.74 ± 0.06	47.78 ± 0.04	99.8 ⁶ ± 0.1	47.78 ± 0.04	83 ± 2	35 ± 2

¹Method-blank adjusted and determined by analytical GC-EIMS for isolates and gravimetrically-prepared aliquots of standard. 1 σ combined uncertainty.

²Mass fraction of carbon in DEHP = 0.738

³Determined by deconvolution method; 1 σ uncertainty, n=5

⁴ Determined by analytical GCMS analyses and purity determinations. 1 σ combined uncertainty.

⁵ Determined by CO₂ pressure-volume manometry after combustion; 1 σ uncertainty; n=1.

⁶Average and 1 σ uncertainty of HPLC-UV and GC-FID determinations.

5.2.1 Sources of Extraneous Carbon

The total extraneous mass in the isolates (m_{TE}) was determined to contain three components, which consisted of i) carbon in the form of DEHP from the isolation method (m_{DMB}), ii) carbon from co-eluted impurities (m_{HCE}), and iii) carbon from all other sources of the isolation and shipping methods that was not identifiable by GC-EIMS analyses (ΔmC),

$$m_{TE} = m_{DMB} + m_{HCE} + m_{\Delta mC} \quad (5.1.1)$$

The mixing ratio of extraneous mass from each source is therefore:

$$Y_{DMB} = \frac{m_{DMB}}{m_{TE}}, Y_{HCE} = \frac{m_{HCE}}{m_{TE}}, \text{ and } Y_{\Delta mC} = \frac{\Delta mC}{m_{TE}} \quad (5.1.2, 5.1.3, 5.1.4)$$

As shown above, ΔmC was determined to be the disparity between the sum of all mass components measured by GC-EIMS and the total mass measured at LLNL (Table 5.2).

The value of m_{HCE} was determined in each isolate, also in Table 5.3, from its GC-EIMS-measured DEHP carbon mass (m_{DEHPGC} , μg , Table 5.2) and estimate of carbon purity ($Purity_C$, Table 4.10.) as,

$$m_{HCE} = \left(\frac{m_{DEHPGC}}{Purity_C} \right) \cdot (1 - Purity_C) \quad (5.2)$$

Since some samples were comprised of multiple batches of extract, and were thus represented by multiple blanks, the contribution of m_{DMB} to the total extraneous mass was determined individually for each sample. These determinations were made by scaling the masses of DEHP in the respective contemporaneously-processed method blanks (Table 4.8) to the number of liquid chromatographic column passes performed on the isolate, relative to the number of passes performed on the blanks (Y_{CP} ; Table 4.4) according to,

$$m_{DMB_{sample}} = \sum_{i=1}^7 Y_{batch_i} \cdot (Y_{CP_i} \cdot (m_{CB_i})) \quad (5.3)$$

where Y_{batch_i} is the mass mixing ratio of the i^{th} of 7 isolation batches constituting the sample (Table 4.4.), Y_{CP_i} is the liquid chromatographic processing ratio of the method

blank contemporaneously processed with the i^{th} extraction batch (Table 4.2), and m_{CB_i} is that method blank's mass of carbon as DEHP (Table 4.8). Each of these mass determinations was then normalized to the mass fraction of sample remaining after removal of an aliquot for IRMS analysis. The values of m_{DMB} and uncertainties (μg) are found in Table 5.3.

Table 5.3 Mass estimates of carbon in isolates from laboratory DEHP contamination, cheese-matrix impurities, sources not identified by GC-EIMS analysis, and the sum in all extraneous sources (μg).

Sample ID	m_{DMB} (μg) ¹	m_{HCE} (μg) ²	Δm_C (μg) ²	m_{TE} ($\mu\text{g C}$) ³
STO1	1.5 ± 0.6	5.9 ± 0.4	11 ± 7	18 ± 8
STO2	1.05 ± 0.6	8.96 ± 0.26	23 ± 3	34 ± 4
STO3	0.81 ± 0.06	9.12 ± 0.28	54 ± 3	66 ± 3
STO4	0.69 ± 0.05	6.75 ± 0.19	63 ± 3	71 ± 3
STO5	1.5 ± 0.6	5.70 ± 0.16	39 ± 2	46 ± 3

¹Adjusted for mass fraction removed as aliquot for IRMS analysis

²1 σ combined uncertainty.

³Total laboratory carbon blank calculated as the sum of the method, co-eluted, and total extraneous blank mass. 1 σ combined uncertainty.

5.2.2 Blank Corrections of f_m

The values of f_m reported for each of the isolates in Table 5.1 are linear combinations of the f_m of DEHP extracted from cheese ($f_{m_{DEHP}}$) and that from all extraneous sources of carbon ($f_{m_{TE}}$).

$$f_{m_{LLNL}} = f_{m_{DEHP}} \cdot \gamma_{DEHP} + f_{m_{TE}} \cdot \gamma_{TE} \quad (5.4.1)$$

where γ_{DEHP} and γ_{TE} are the respective mass mixing ratios of carbon from DEHP in cheese (m_{DEHP}) and all extraneous carbon (m_{TE}) contributing to the LLNL-measured mass (m_{LLNL});

$$\gamma_{DEHP} = \frac{m_{DEHP}}{m_{LLNL}}, \quad \gamma_{TE} = \frac{m_{LLNL} - m_{DEHP}}{m_{LLNL}} \quad (5.4.2, 5.4.3)$$

Equation 5.4.1 can also be expressed as,

$$f_{m_{DEHP}} = \frac{f_{m_{LLNL}} - f_{m_{TE}} \cdot \gamma_{TE}}{1 - \gamma_{TE}} \quad (5.5)$$

Since $f_{m_{LLNL}}$ is known and γ_{TE} is derived from the total sample mass measurements, $f_{m_{TE}}$ is the only remaining variable to be quantified in order to calculate $f_{m_{DEHP}}$.

Given the three identified sources of m_{TE} , $f_{m_{TE}}$ is a linear combination of the f_m of m_{DMB} ($f_{m_{DMB}}$), m_{HCE} ($f_{m_{HCE}}$), and ΔmC ($f_{m_{\Delta mC}}$),

$$f_{m_{TE}} = \gamma_{DMC} \cdot f_{m_{DMR}} + \gamma_{HCE} \cdot f_{m_{HCE}} + \gamma_{\Delta mC} \cdot f_{m_{\Delta mC}} \quad (5.6)$$

Laboratory DEHP contamination is likely petrogenic and $f_{m_{DMB}}$ was determined to be 0.0009 ± 0.001 , the mean and standard error of f_m reported for the pure DEHP standard material in STD01 and STD02. Since the compounds co-eluting with DEHP are fatty acid esters, and thus natural materials from the cheese matrix, $f_{m_{HCE}}$ was considered to be 1.045 ± 0.003 , the mean and standard deviation of f_m 's reported for lyophilized whole Stilton cheese. The only other variable in Equation 5.6 needed to calculate $f_{m_{TE}}$, is $f_{m_{\Delta mC}}$.

5.2.3 Determinations of $f_{m_{\Delta mC}}$ and $f_{m_{DEHP}}$

The mean reported f_m of the spiked method blanks (0.041 ± 0.021) was significantly higher than that of the shipping blanks prepared without any chromatographic processing (0.009 ± 0.001), which in turn was significantly higher than that of the petrogenic DEHP standard (0.0009 ± 0.001). This indicated that $f_{m_{\Delta mC}}$ of each isolate was effected by *modern* carbon contamination ($\Delta mMC_{isolate}$) imparted from both i) the shipping vials and subsequent transfers ($\Delta mMC_{shipping}$), and ii) the liquid chromatographic isolation method ($\Delta mMC_{LC-method}$).

$$\Delta mMC_{isolate} = \Delta mMC_{shipping} + \Delta mMC_{LC-method} \quad (5.7)$$

The shipping blanks delivered with Sample Set 2 to LLNL CAMS allowed estimation of $\Delta mMC_{shipping}$ in ST02, ST03, ST04, and ST05. This mass was determined to be the mean of modern extraneous carbon masses in each of the three shipping blanks (ΔmMC_{SB} , μg) calculated by,

$$\Delta mMC_{SB} = m_{LLNL-SB} \cdot f_{m_{SB}} - m_{DEHP-spike} \cdot f_{m_{DMB}} \quad (5.8)$$

where $m_{LLNL-SB}$ and $f_{m_{SB}}$ are their LLNL-measured mass and f_m , respectively, and $m_{C_{DEHP-spike}}$ is the corresponding mass of carbon that was added to each as pure (99.8 % \pm 0.1 %) DEHP. From this, $\Delta mMC_{shipping}$ was determined to be $0.68 \mu\text{g} \pm 0.12 \mu\text{g}$.

The extraneous modern mass from the isolation method, $\Delta mMC_{LC-method}$, was estimated from the modern extraneous masses in the spiked method blanks (ΔmMC_{MB}). These were calculated each for SBL02, SBL03, and SBL04 by,

(5.9)

$$\Delta mMC_{MB} = \frac{1}{\gamma_B} \cdot (m_{LLNL-MB} \cdot f_{m_{MB}} - m_{DEHP-spike} \cdot f_{m_{DMB}}) - 0.68 \mu g (\pm 0.12 \mu g)$$

where γ_B is the mass ratio of the blank analyzed by AMS (Table 5.4), and $m_{LLNL-MB}$ and $f_{m_{MB}}$ are LLNL-measured mass and f_m , respectively.

Table 5.4 Carbon mass determinations (μg) and f_m of spiked isolation method blanks.

ID	$m_{DEHP-spike}$ (μg) ¹	$m_{LLNL-MB}$ (μg) ²	$f_{m_{MB}}$	γ_B^3	ΔmMC_{MB} (μg) ⁴
SBL02	46.05 ± 0.06	68 ± 2	0.0373 ± 0.0059	0.52	3.48 ± 0.39
SBL03	48.04 ± 0.04	88 ± 2	0.0224 ± 0.0046	0.42	2.97 ± 0.57
SBL04	47.87 ± 0.04	78 ± 2	0.0640 ± 0.0050	0.50	8.54 ± 0.29

¹Determined from gravimetric addition of DEHP and its carbon mass ratio of 0.738.

²Determined by Baratron Capacitance Manometer at LLNL.

³Mass ratio of blank analyzed by AMS after splitting prior to addition of DEHP spike and shipment to LLNL CAMS (as outlined in Section 4.8).

⁴Derived with Equation 5.10.

Similar to the calculation of m_{DMB} by Equation 5.2, $\Delta mMC_{LC-method}$ was determined in each sample according to,

$$\Delta mMC_{LC-method} = \sum_{i=1}^7 \gamma_{batch_i} \cdot (\gamma_{CP_i} \cdot (\Delta mMC_{MB})_i) \quad (5.10)$$

where γ_{batch_i} is the mass mixing ratio of the i^{th} of 7 isolation batches constituting the sample (from Table 4.4.), and γ_{CP_i} and ΔmMC_{MB_i} are the liquid chromatographic processing ratio and modern extraneous mass of the method blank contemporaneously processed with the i^{th} extraction batch (Table 4.2., Table 5.4), respectively. Sample ST05 was apportioned a $\Delta mMC_{isolate}$ quantity that was the mean of those determined for ST02, ST03, and ST04, given that the ¹⁴C abundance of its contemporaneously-processed method blank, BL01, was not measured.

The $f_{m_{\Delta mC}}$ of ST02, ST03, ST04, and ST05 was thereby calculated according to,

$$f_{m_{\Delta mC}} = \frac{\Delta mMC_{isolate}}{\Delta mC} \quad (5.11)$$

As an exception, the mean of these values was designated as the $f_{m_{\Delta mC}}$ of sample ST01. This was also due to the fact that BL01 did not provide ^{14}C measurements by AMS to contribute to its derivation and because the mean of $\Delta mMC_{isolate}$ estimated in the other samples, as was apportioned to ST05, was larger than ΔmC of ST01.

Table 5.5 Modern masses (μg) and f_m of extraneous carbon from unidentified sources estimated in samples ST01 to ST05.

Sample	$\Delta mMC_{isolate}$ (μg) ¹	$f_{m_{\Delta mC}}$
STO1	2.72 ² \pm 0.74	0.259 \pm 0.070
STO2	9.70 \pm 2.17	0.410 \pm 0.111
STO3	16.6 \pm 3.2	0.299 \pm 0.061
STO4	15.7 \pm 3.1	0.246 \pm 0.049
STO5	14.0 ³ \pm 3.7	0.350 \pm 0.097

¹ Uncertainties are 1 σ propagated from mass and f_m of extraneous blank carbon. Uncertainty of ST01 is determined from its m_{NG} and the standard deviation in $f_{m_{NG}}$ of other four samples, for ST05 from standard deviation of m_{NGmH} ¹ in ST02, ST03, and ST04.

² Back-calculated from mean $f_{m_{NG}}$ and m_{NGmV} of other four samples and m_{NG} ST01.

³ Determined from mean values obtained for ST02, ST03, and ST04 and corrected for post-chromatographic carbon.

With the values of $f_{m_{\Delta mC}}$ in Table 5.5, $f_{m_{DMB}} = 0.0009$, and $f_{m_{HCE}} = 1.045$, $f_{m_{TE}}$ of each isolate was calculated by Equation 5.6 and listed in Table 5.6.

Table 5.6 Mass mixing ratios of extraneous carbon from different sources and the estimates of f_m of all extraneous carbon ($f_{m_{TE}}$).

Sample	Y_{DMB}	Y_{HCE}	Y_{AmC}	$f_{m_{TE}}$
STO1	0.056 ± 0.030	0.330 ± 0.143	0.614 ± 0.502	0.503 ± 0.250
STO2	0.031 ± 0.004	0.266 ± 0.030	0.703 ± 0.134	0.564 ± 0.091
STO3	0.012 ± 0.001	0.140 ± 0.007	0.848 ± 0.059	0.398 ± 0.053
STO4	0.010 ± 0.001	0.094 ± 0.005	0.896 ± 0.054	0.317 ± 0.045
STO5	0.016 ± 0.003	0.123 ± 0.008	0.862 ± 0.086	0.429 ± 0.086

The f_m of DEHP extracted from Stilton Cheese ($f_{m_{DEHP}}$) was therefore calculated for each isolate according to Equation 5.5, also using its derived $f_{m_{TE}}$, mass mixing ratio of total extraneous carbon (Y_{TE}) and reported f_m ($f_{m_{LLNL}}$). The results of these calculations are in Table 5.7.

5.3 Isotopic Fractionation Adjustments and Determination of Fraction of Contemporary Carbon in DEHP

For reasons outlined in Section 3.5 and in accordance with Equation 3.10, the derived values of $f_{m_{DEHP}}$ were referenced to the f_m of whole Stilton cheese ($f_{m_{WSC}} = 1.045 \pm 0.003$) by Equation 5.12 to determine the contemporary fraction of DEHP in Stilton Cheese ($f_{C_{DEHP}}$). This reference also made appropriate adjustments for biological fractionation.

$$f_{C_{DEHP}} = \left(\frac{f_{m_{DEHP}}}{f_{m_{WSC}}} \right) \left[\frac{\left(1 - 2 \frac{(\delta^{13}C_{VPDB})_{sample}}{1000} \right)}{\left(1 - 2 \frac{(\delta^{13}C_{VPDB})_{Stilton\ Cheese}}{1000} \right)} \right] \quad (5.12)$$

The contemporary fraction of DEHP from Stilton cheese determined for each sample and its respective uncertainty is given in Table 5.7. From the mean and standard deviation of these determinations, the DEHP in Stilton cheese was 24.0 % \pm 6.7 % contemporary, thereby 76.0 % \pm 6.7 % petrogenic.

Table 5.7 The fraction of contemporary DEHP in Stilton cheese ($f_{c_{DEHP}}$) determined from isolate extraneous mass mixing ratios (γ_{TE}), LLNL-reported f_m ($f_{m_{LLNL}}$), and the f_m of DEHP from Stilton cheese ($f_{m_{DEHP}}$).

Sample	γ_{TE}	$f_{m_{LLNL}}$	$f_{m_{DEHP}}$	$f_{c_{DEHP}}$
ST01	0.164 \pm 0.071	0.283 \pm 0.004	0.240 \pm 0.108	0.231 \pm 0.104
ST02	0.341 \pm 0.039	0.281 \pm 0.004	0.135 \pm 0.037	0.129 \pm 0.036
ST03	0.516 \pm 0.025	0.353 \pm 0.003	0.304 \pm 0.026	0.292 \pm 0.025
ST04	0.529 \pm 0.023	0.311 \pm 0.003	0.304 \pm 0.021	0.292 \pm 0.021
ST05	0.407 \pm 0.026	0.334 \pm 0.003	0.268 \pm 0.026	0.258 \pm 0.026
MEAN				0.240 \pm 0.067

Chapter 6: Uncertainty Analysis and Discussion

6.1 Monte Carlo Method

Equation 5.11 and supporting equations in Sections 5.2 and 5.3 were written into a Matlab® script for analysis by the Monte Carlo method. The values of 1 σ uncertainty associated with variables in Sections 5.2 and 5.3 were each individually derived by propagation of the 1 σ error associated with the parent mass estimates and reported f_m terms, except $f_{m_{DMB}}$ and $f_{m_{LLNL}}$, for which 2 σ uncertainties were utilized. The Monte Carlo method performs a specified number of model simulation iterations, whereby the base inputs are randomly selected from a domain of values that are appropriately distributed within a defined range of probability. The method employed herein to calculate $f_{c_{DEHP}}$ used base inputs that consisted of measured values obtained prior to and by AMS. For each base variable used in simulations performing i number of calculations of $f_{c_{DEHP}}$ in a given sample, a (1 x i) matrix of inputs was randomly populated with numbers selected from a normally-distributed value set centered around the mean of the given variable and with a standard deviation reflective of its 1 σ uncertainty.

Two separate functions were coded into the Monte Carlo program. One was used to first calculate contaminant carbon mass and its f_m with the base measurement inputs listed Table 6.1, obtained from analyses of the spiked method blanks and DEHP standard spiking solutions (all from AMS Sample Set 2 analyzed June, 2012). A second function calculated $f_{c_{DEHP}}$ with the contaminant masses derived by the first and base inputs pertinent to samples ST01, ST02, ST03, ST04, and ST05 (Table 6.2).

Table 6.1 Monte Carlo base input parameters for DEHP-spiked method blanks and DEHP spikes.

Parameter	Description
m_{LLNL}	Mass of carbon (μg) manometrically measured at LLNL
m_{CSDEHP}	Mass of carbon added as a DEHP spike (μg)
m_{DMB}	Mass of carbon from isolation method DEHP contamination (μg)
f_{mDMB}	Assigned f_m value obtained for petrogenic DEHP (0.0009 ± 0.001)
f_{mLLNL}	f_m of spiked method blank, as determined by LLNL
$\delta^{13}\text{C}_{DEHP}$	VPDB for DEHP standard

Table 6.2 Monte Carlo base input parameters of Samples ST01, ST02, ST03, ST04, and ST05.

Parameter	Description
m_{LLNL}	Mass of carbon (μg) manometrically measured at LLNL
m_{DEHP}	Mass of Carbon as DEHP isolated from cheese; determined by analytical GC-EIMS
m_{DMB}	Apportioned mass of carbon from isolation method DEHP contamination (μg)
f_{mHCE}	Fraction of modern carbon in HPLC co-eluted carbon; determined 1.045 ± 0.003 by AMS analysis of whole cheese
f_{mDMB}	Assigned the f_m value obtained for petrogenic DEHP (0.0009 ± 0.001)
$Purity_C$	Estimated percent purity of carbon in sample by GC-EIMS spectral deconvolution
f_{mLLNL}	f_m of spiked method blank, as determined by LLNL
f_{mWSC}	AMS-determined f_m of lyophilized Stilton Cheese
$\delta^{13}\text{C}_{sample}$	VPDB by IRMS for cheese-isolated sample
$\delta^{13}\text{C}_{Stilton\text{Cheese}}$	VPDB by IRMS for whole cheese

Listed in Tables A5.1 and A5.2 of Appendix 5 are all base input values for samples and spiked blanks, along with the respective uncertainties that define their Monte

Carlo distribution bounds. The distributions of the results of 100,000 calculations of f_{cDEHP} of each sample are represented in Figure 6.1 as five 100-bin histograms.

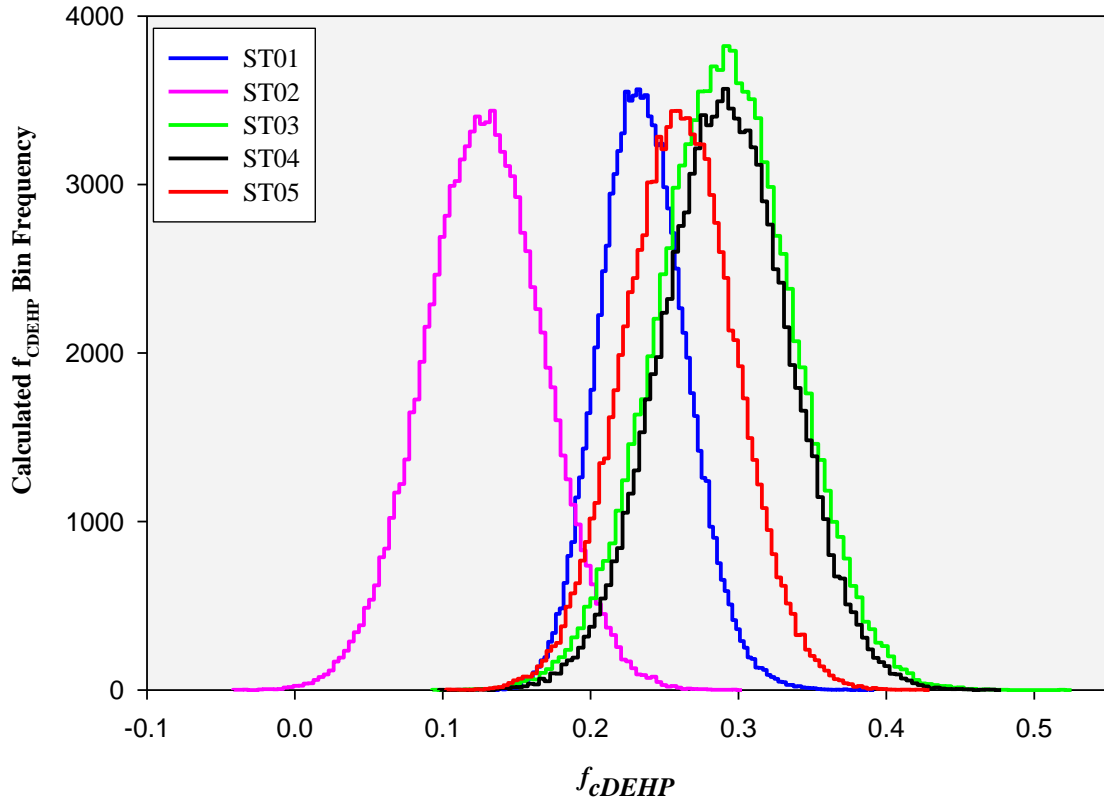


Figure 6.1 100-bin histograms of the distributions of the results of 100,000 calculations of f_{cDEHP} of 5 isolate samples (ST01 to ST05) calculated with the Monte Carlo method.

The mean and standard deviation of 100,000 Monte Carlo calculations of f_{cDEHP} in each of the five samples are in Table 6.3.

Table 6.3. Monte Carlo mean and standard deviation of 100,000 calculations of $f_{C_{DEHP}}$ in each isolate

Sample	$f_{C_{DEHP}}$	Std. Dev.
ST01	0.237	0.029
ST02	0.129	0.040
ST03	0.293	0.046
ST04	0.293	0.043
ST05	0.262	0.038
MEAN	0.243	0.068¹

¹Standard Deviation of $f_{C_{DEHP}}$ determined for all samples

6.2 Discussion

As evidenced by the results in Table 5.7 and Table 6.3, most of the DEHP present in Stilton Cheese, $75.8 \% \pm 6.8 \%$, is anthropogenic. The standard deviations of Monte Carlo results for each sample, with the exception of ST01, were larger than the propagated uncertainties in Sections 5.2 and 5.3. These Monte Carlo distributions more fully represent the effects of uncertainty in each of the derived variables and it is fitting that the standard deviations of results of ST03 and ST04 (0.046 and 0.043), which contained the greatest total extraneous carbon masses ($66 \mu\text{g} \pm 3 \mu\text{g}$ and $71 \mu\text{g} \pm 3 \mu\text{g}$), were the largest amongst individual samples. However, the standard deviation of the means of $f_{C_{DEHP}}$ of all samples (0.068) is 42 % to 67 % higher than the standard deviation of $f_{C_{DEHP}}$ of any individual sample, giving a more appropriate uncertainty of the fraction of contemporary DEHP in Stilton cheese determined by this study.

The mean of most Monte Carlo-derived variables agreed to within one to two percent of those derived in Sections 5.2 and 5.3. The greatest exception to this was

$f_{m_{\Delta mC}}$ of isolate ST01, which was listed in Table 5.5 as the mean of $f_{m_{\Delta mC}}$ estimated for ST02, ST03 ST04, and ST05 (0.259 ± 0.070). Rather than directly hardwiring this value as an input for ST01 in the Monte Carlo analysis, it was used in Equation 5.7, along with an $11 \mu\text{g } \Delta mC$ (Table 5.3), to back-calculate a $\Delta mC_{\text{isolate}}$ of $2.7 \pm 0.7 \mu\text{g}$. This mass and its uncertainty were then used as inputs to the appropriate Monte Carlo function to calculate $f_{m_{\Delta mC}}$. Perturbations of this mass input in equations supporting the derivation of $f_{m_{\Delta mC}}$ by this method allowed for a greater variability and more realistic assessment of its uncertainty. Upon recalculation with this input, the mean $f_{m_{\Delta mC}}$ was 0.109, which is 68% less than 0.259. However, the disparity between the two resultant derivations of $f_{C_{DEHP}}$ (0.237 ± 0.029 and 0.231 ± 0.108) was small and both were in agreement according to 1σ uncertainties. This indicated that the sensitivity of these derivations to the large relative uncertainty of $f_{m_{\Delta mC}}$ in ST01 was low due to the fact ΔmC in this isolate was fairly small ($11 \mu\text{g} \pm 8 \mu\text{g}$). It also engendered confidence in the value of $f_{C_{DEHP}}$ calculated for this isolate, despite the fact that $^{14}\text{C}/^{13}\text{C}$ in its method blank (BL01) was not assessed by AMS.

The mean Monte Carlo $f_{C_{DEHP}}$ determinations of ST01, ST03, ST04, and ST05 had a mean and standard deviation of 0.271 ± 0.027 (10 % relative standard deviation of fraction contemporary carbon, 4 % relative standard deviation of fraction of fossil carbon). According to the absolute differences of individual samples' mean Monte Carlo results and their respective uncertainties, these $f_{C_{DEHP}}$ determinations are in agreement with one another. Of these values, that for ST01 (0.237 ± 0.029) deviates the most from the mean of each of these samples (-0.034), though there is still considerable overlap of their 1σ distributions.

The f_{CDEHP} of sample ST02 was markedly low. Figure 6.2.a illustrates the mean and standard deviation of 100,000 Monte Carlo determinations of f_{CDEHP} for each sample and the mean of these five values.

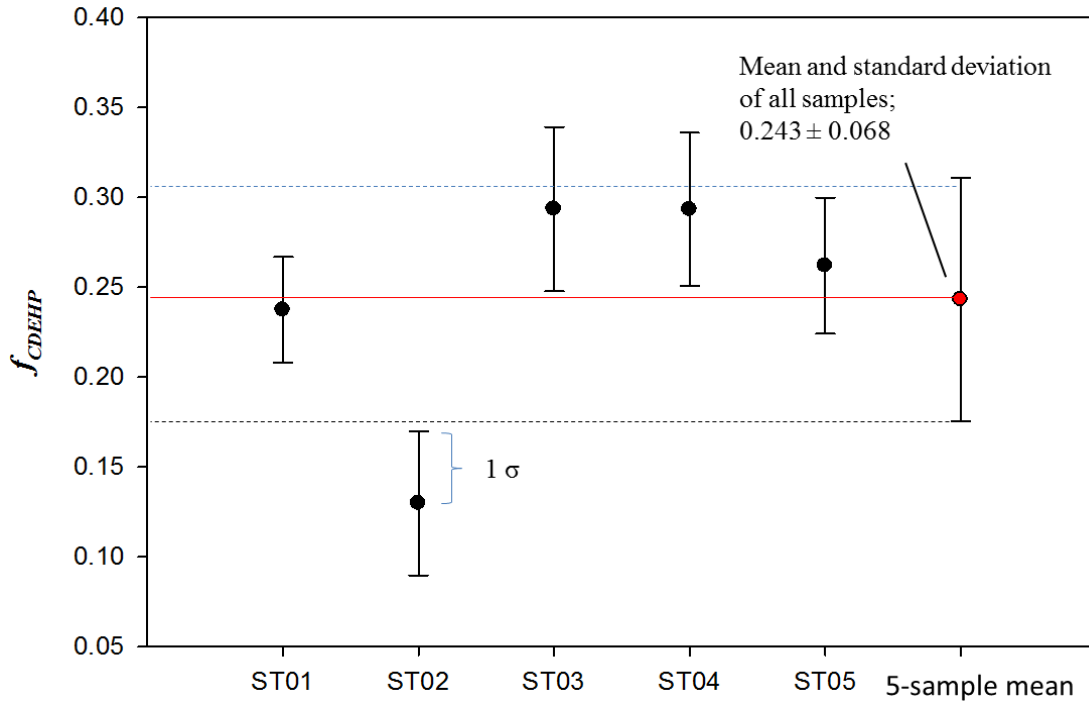


Figure 6.2.a Mean and standard deviation of 100,000 Monte Carlo determinations of f_{CDEHP} for each sample. Also graphed are the mean and standard deviation of the 5 Monte Carlo means of all samples (0.243 ± 0.068).

Despite the deviation of f_{CDEHP} determined for ST02 from the mean of all samples, the Dixon's Q-test and Grubbs' Statistical test for outliers of normally distributed data determine that this difference is not significant at a 95 % confidence level.

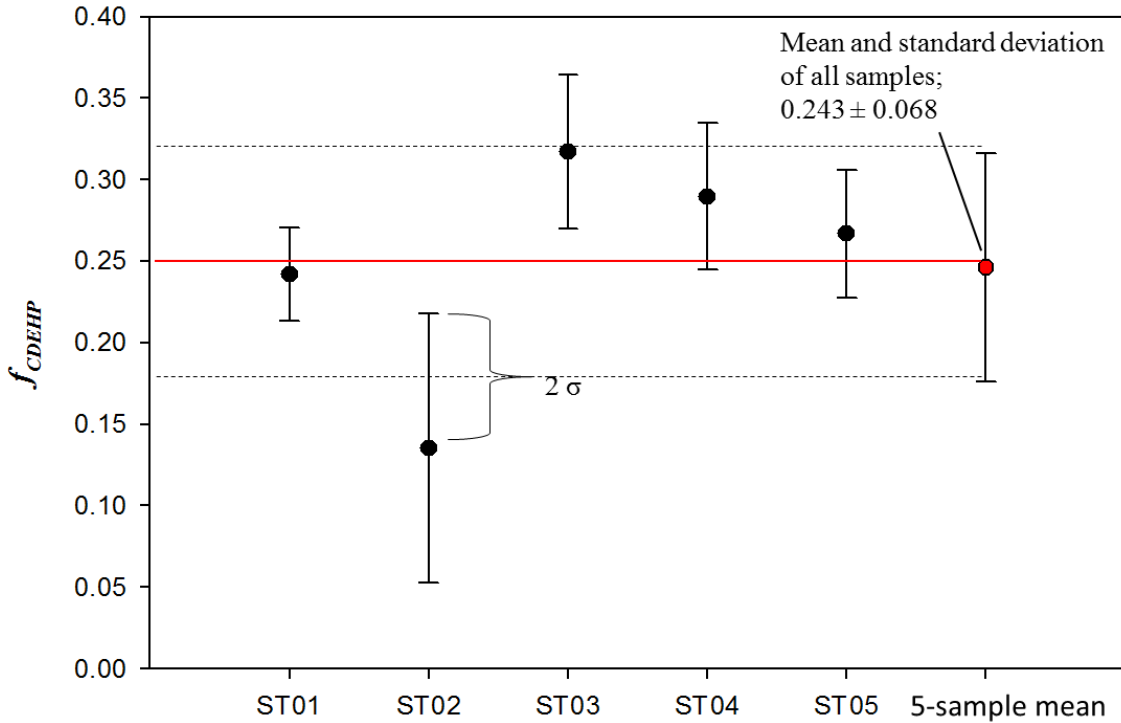


Figure 6.2.b Means and 1σ distributions of f_{CDEHP} values obtained by 100,000-iteration Monte Carlo analysis of ST01, ST03, ST04, ST05, and the mean and 2σ distribution of 100,000 Monte Carlo determinations of f_{CDEHP} of ST02. Also graphed are the mean and standard deviation of ST01, ST03, ST04, and ST05 Monte Carlo means (0.243 ± 0.068). The probability that the mean f_{CDEHP} of ST02 is significantly different from the mean of f_{CDEHP} of all samples is less than 95 % and it is not determined to be an outlier.

It is noted however, that comparison of the f_{CDEHP} determinations of ST02 to those of ST01, ST03, ST04, and ST05 (Figure 6.3.), suggest that this sample might have been affected by an additional variable.

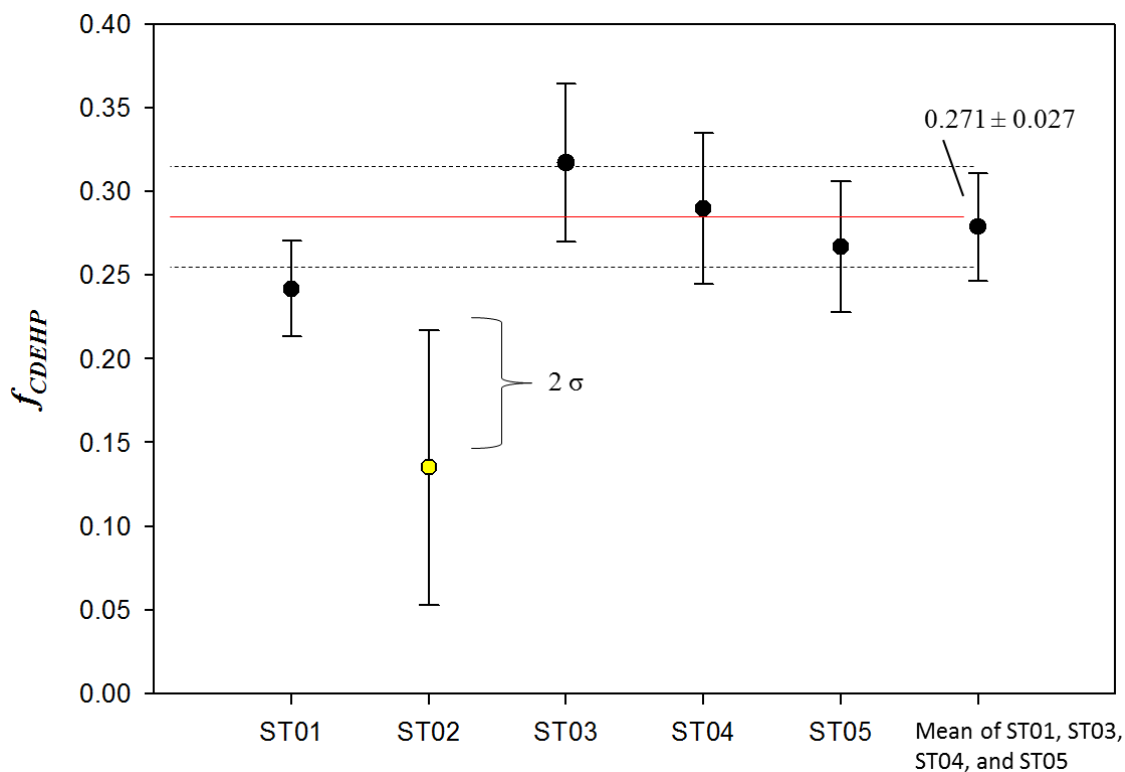


Figure 6.3 Means and standard deviations of 100,000 Monte Carlo determinations of f_{CDEHP} of each of samples ST01, ST03, ST04, ST05, the mean and 2σ distribution of 100,000 Monte Carlo determinations of f_{CDEHP} in ST02, and the mean and standard deviation of the Monte Carlo means of ST01, ST03, ST04 and, ST05 (0.271 ± 0.027).

Calculation of f_{CDEHP} is sensitive to a given sample's mass of extraneous carbon that was not discernible by GC-EIMS (Δm_C). In ST02, this mass was determined to be $23 \pm 3 \mu\text{g}$, well below the $53 \mu\text{g}$ mean of that in samples ST03, ST04, and ST05. Thus, sample ST02 either contained much less overall contamination than other samples and blanks, or some of the sample was lost during or after shipping to LLNL CAMS. If the former case were true, then the f_m of carbon in the sample attributable to contamination was over-estimated. However, this sample was treated with the same diligence used to prepare the others for June 2012 AMS analysis, and there was no deviation from the preparation method that suggested it contained less contamination. Thus, it appears that the entire sample was not combusted and graphitized for AMS analysis.

Also, LLNL-reported f_m values of samples are independent of total carbon mass, so long as their isotopic compositions are homogenous. As indicated in Table 5.1, f_m reported by LLNL for ST02 (0.281 ± 0.004) is more consistent with the mean of those reported for *all* samples (0.312 ± 0.031), which suggests that ST02 contained a similar proportion of extraneous carbon. The difference between ST02's manometrically-measured mass (99 μg) and a total mass adjusted to reflect a 53 μg average ΔmC of the other samples analyzed in June 2012 (139 μg), corresponds to a loss of $\sim 50 \mu\text{L}$ of sample solution prior to combustion. In this instance, again, the mass fraction of modern carbon contamination, as determined by the blanks, is over-estimated. Had Monte Carlo calculations of $f_{C_{DEHP}}$ for ST02 been made with a ΔmC of 53 μg , their mean would have been 0.250 ± 0.041 (Figure 6.4) and well in agreement with the mean of all samples (0.267 ± 0.025).

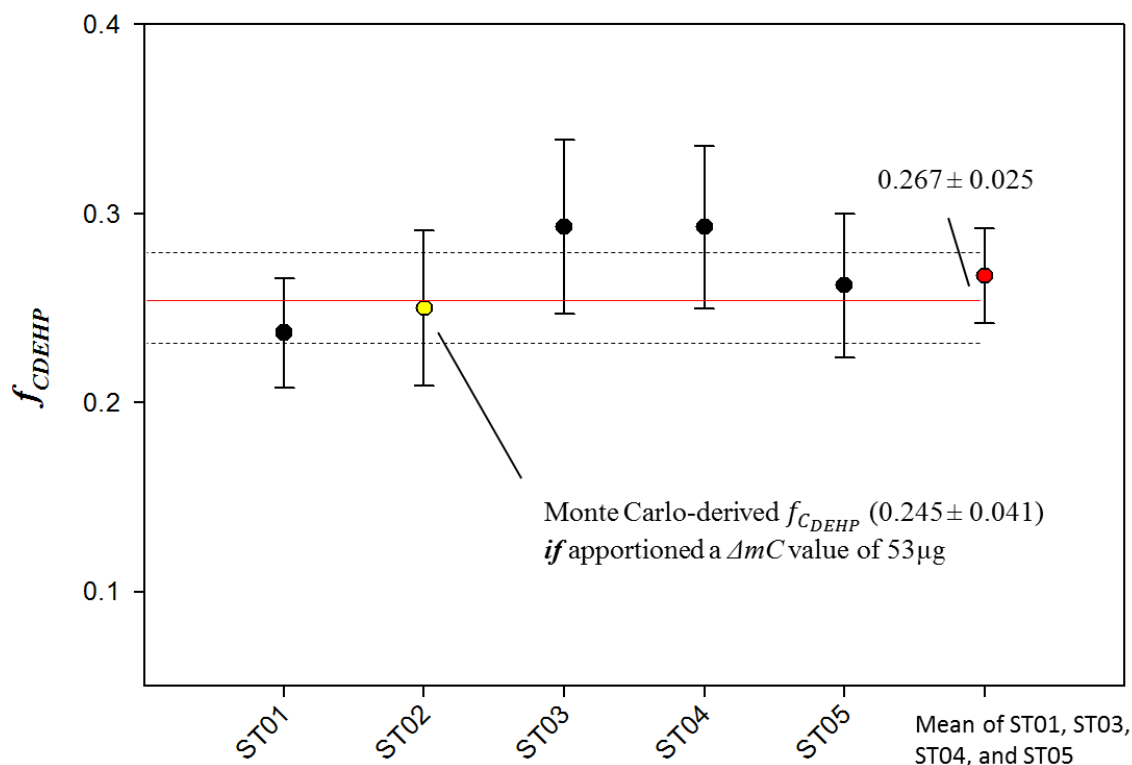


Figure 6.4 The means and standard deviations of 100,000 Monte Carlo determinations of f_{CDEHP} of all samples and the Monte Carlo means of ST01, ST03, ST04, and ST05 (0.271 ± 0.027). Sample ST02 Monte Carlo determinations were made with a 53 μg extraneous mass not discernible by GC-EIMS analysis (ΔmC), representative of its average in ST03 ST04, and ST05.

The substantial ΔmC in samples ST03, ST04, ST05, and the corresponding blanks was not predicted due to the fact that in ST01, isolated and analyzed prior to these, it was $11 \mu g \pm 8 \mu g$. The extraneous masses in the shipping blanks (S1, S2, and S3) demonstrated that $\sim 62\%$ of ΔmC in these samples ($33 \mu g \pm 2 \mu g$) is from residues in the shipping vials or subsequent transfers at LLNL. As determined with AMS and Equation 5.9, this extraneous carbon was only $2.1\% \pm 0.5\%$ modern. Given that the polymeric caps used with these shipping vials could not be baked, they are the likely source of this contamination. Hexadecamethyl heptasiloxane is a major component of these caps' septa

that contains petrogenic carbon. Though the PTFE liners of these septa were highly inert, repeated tightening of the cap may have exposed this leachable material to the enclosed solvent. It was qualitatively identified in some aliquots of solvent that were subsequently shaken in identically-cleaned vials, which were capped multiple times, via GC-EIMS analysis and mass spectral references to the NIST/EPA/NIH Mass Spectral Database (NIST 11) (Appendix 6).

The remaining 38 % of ΔmC can be attributed to the isolate preparation method prior to shipment to LLNL CAMS. Considering the sources of contamination, aside from co-eluted impurities, in the method blanks and their contemporaneously-prepared isolates to be homologous (68 % from shipping), the average f_m of this extraneous mass was estimated to be ~ 0.25 ($1 \sigma = 0.15$). This estimate was derived with the average f_m of all extraneous carbon in the spiked method blanks, which was calculated as 0.105 ± 0.053 from their reported total masses (m_{LLNL}) and AMS-measured f_m , and the notion that the isolate preparation method is the source of 38 % of all process contamination.

Alkonox® Powdered Precision Cleaner was used to wash glassware utilized by the sample preparation method, such as HPLC fraction-collection vials and recovery flasks, prior to baking and rinsing with acetone. This detergent was suspected to be a primary source of the extraneous modern carbon in the samples. It is 33 % to 43 % sodium bicarbonate and 0 % to 10 % sodium carbonate by weight. A 0.25 mg carbon sample from this powder was analyzed by AMS and determined to have an f_m of 0.289 ± 0.002 . This is similar to the f_m of ~ 0.25 estimated for contaminants in the spiked method blanks that were imparted during the isolation method and GC-EIMS analyses and lies well within the bounds of the standard deviation of Monte Carlo-derived $f_{m,\Delta mC}$

for all samples (0.285 ± 0.118), for all samples, excluding ST02 (0.251 ± 0.104), and for ST03, ST04, and ST05 (0.298 ± 0.053). These conclusions substantiate the hypothesis that detergent residuals are the primary source of modern carbon contamination in samples analyzed by AMS in June 2012.

Despite the presence of this extraneous carbon, the corrective adjustments applied to individual isolates were effective, particularly to ST01, ST03, ST04, and ST05, for which DEHP was determined to be $72.9 \% \pm 2.7 \%$ petrogenic. The relative uncertainty of this value is only 4 %, even though that of the average mass fraction of contaminate carbon (0.4) in these samples is 17 %. It is also noted that its absolute uncertainty (0.027) is very consistent with that of the LLNL-reported f_m of *all* isolates (0.031), indicating that isolate carbon compositions were homogenous and engendering confidence that the effects of the highly-varied masses of contamination were mitigated. Including the value of $f_{C_{DEHP}}$ determined for ST02, DEHP in Stilton cheese is $75.8 \% \pm 6.8 \%$ petrogenic (9 % relative 1 σ uncertainty), though as discussed above, this value in ST02 was deduced to be erroneously low due to incomplete sample recovery. In the end, all results show that DEHP in Stilton cheese is more than 62 % anthropogenic and at least 11 % biogenic at a confidence limit of 95%.

6.3 Future Investigations

Stilton cheese is only one of many foods which are prepared with organisms suspected of producing DEHP. Marine alga, evidenced to produce natural DEHP (Chen 2004; Namikosha 2006), are integral in the diets of many people, particularly in northern Europe and eastern Asia. They are also used in the production of agar and carrageenan,

which are very common emulsifiers used in foods around the world. Therefore, a multitude of foods, in addition to blue cheese, are known to contain ingredients that demonstrate a propensity to produce DEHP, or perhaps other phthalate esters. Additionally, the comprehensiveness of species which may similarly produce this phthalate is not known and other microbial processes used to produce food, such as various fermentation or culturing, may promote the accumulation of phthalate. As such, there is an extensive variety of edible matrices which should be assessed for the presence of a biogenic phthalate component. This includes DEHP, as well as other similar widely-effused esters such as di-n-butyl phthalate.

In addition, more clean sample preparation conditions in future radiocarbon characterizations of phthalate can help improve precision of results. Had the extraneous masses of samples analyzed by AMS in this study in June of 2012 been observed in the pilot sample ST01, improvements would have been subsequently made. These include practicalities such as direct transfer of samples to quartz combustion tubes, utilizing higher temperatures for the baking of glassware, and eliminating exposure to laboratory detergent. Additionally, preparative column gas chromatography (PCGC), whereby several repeat sample injections are used to selectively collect several fractions of gas chromatographic eluent directly from the column in liquid nitrogen-cooled traps, should be employed as a final step in isolating phthalate from complex matrices. This will likely purify phthalate isolates from other compounds in the food matrix to an even greater degree and isolate it from other components of carbon contamination from the isolation method.

It would also be very beneficial to future studies to have full access to combustion apparatuses and Baratron Capacitance manometers that are calibrated to a high degree of accuracy. This would allow for the development of methods to reduce contamination, as well as the ability to readily flag compromised samples prior to AMS analysis. It is evident that in order to gain a better understanding of the dietary phthalate exposure risk, and thus the efforts required to minimize it, a better understanding is needed of the origins of orally-consumed phthalates, particularly those of an inherently biological nature.

Chapter 7: Conclusion

Despite restrictions eliminating or greatly curtailing the use of bis(2-ethylhexyl) phthalate in food-contact materials, it is evident that it persists as a widespread, common food contaminant. Though the scope of the screening method employed herein was somewhat limited, it considered a variety of foods from different origins and demonstrated that DEHP presence in some similarly-processed products from separate manufacturers is consistent.

Compound-specific carbon isotope analysis by Accelerator Mass Spectrometry was utilized to determine the fraction of a common food contaminant, found at levels below 1 mg/kg in a fatty matrix, which is of biological origin. The use of compound-specific radiocarbon analysis to determine the origins of phthalates in food has been extremely limited, likely due to the fact that extensive preparations are required to isolate adequate masses of these low-level compounds from bulk matrices.

It is clear from this study that all extraneous mass from various sources must be quantified and characterized in ~100 µg samples to provide accurate quantitative isotope measurements by AMS. These assessments were made herein by the use of analytical GC-EIMS spectral deconvolution, manometric measurement of sample masses, target compound-spiked sample-preparation method blanks, and graphitization blanks. This is the first known study using AMS to directly deduce that organisms present in food contribute to the oral intake of DEHP. Assessment of DEHP in Stilton cheese has been demonstrated to be more intricate than that exclusively imparted by anthropogenic artifacts.

Appendices

Appendix 1: Supplemental Information and Data Procured During Screening Analysis

A1.1 Listing and Information of Foods screened for bis(2-ethylhexyl) phthalate.

DEHP analyses were made with foods purchased at a local (Gaithersburg, MD) supermarket.

9/28/2012

1. Vegetable Shortening (Guaranteed Value)
Ingredients: partially hydrogenated soybean and cottonseed oils with mono- and diglycerides

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949
S-22 0311
2. (Real) Mayonnaise (Giant Brand)
Ingredients: juice (from concentrate), oleoresin, paprika, natural flavors, calcium disodium EDTA Soybean oil, water, whole eggs and egg yolks, vinegar, salt, sugar, lemon

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949
S- 192 1009

3. (Old Fashioned) Chocolate Chip Cookies (Giant Brand)

Ingredients: Enriched bleached and unbleached flour (wheat flour, niacin, iron, thiamin mononitrate, riboflavin, folic acid), chocolate chips (sugar, chocolate liquor, cocoa butter, anhydrous dextrose, soy lecithin), sugar, vegetable shortening (partially hydrogenated soybean and/or cottonseed oils), high fructose corn syrup, water, corn syrup, emulsifier (water, sorbitan monostearate, polysorbate 60, mono and diglycerides, sodium propionate, phosphoric acid), molasses, wheat starch, leavening (baking soda, baking powder [sodium acid pyrophosphate, baking soda, corn starch, monocalcium phosphate]), dried eggs, dried whey salt, natural and artificial flavors, sorbitol, soy lecithin.

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949
S-159 0310

4. Sharp Cheddar Cheese (Giant Brand)

Ingredients: Pasteurized Milk, Cheese Cultures, salt, enzymes.

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949

5. American Cheese

Ingredients: American Cheese (Milk, cheese culture, salt, enzymes), water, milkfat, whey protein concentrate, whey, calcium phosphate, sodium citrate, salt, sorbic acid as preservative, annatto and oleoresin paprika.

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949

6. 100% Pure Corn Starch (Giant Brand)

Ingredient: Corn Starch

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949
0909 S-548

7. Classic Snack Crackers – Original: in plastic sleeve
Ingredients: Enriched flour (wheat flour, niacin, reduced iron, thiamin mononitrate, riboflavin, folic acid), vegetable oil (contains one or more of the following: palm, canola, soybean with TBHQ for freshness), sugar, salt, contains two percent or less of: high fructose corn syrup, leavening (baking soda, calcium phosphate), soy lecithin (emulsifier), sodium sulfite, natural flavor.

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949
©2011 Ahold Licensing, Sarl
0511 S-59

8. Chicken Vienna Sausage in Chicken Broth (Libby's)
Ingredients: Mechanically separated chicken, chicken broth, water, salt, less than 2% sugar, spices, sodium tripolyphosphate, sodium ascorbate, sodium nitrite, flavorings and paprika extract.

Distributed by ConAgra Foods™
P.O. Box 3768, DEPT. L,
Omaha, NE 68103-0768
32 4739587
08643-FGA 60375 H

9. Luncheon meat made with pork (Guaranteed Value)
Ingredients: Pork, salt, water, sugar, sodium nitrite

Distributed by Foodhold U.S.A., LLC
Landover, MD 20785 1-877-846-9949
EST199N F03152
S-152 1211

A1.2. Sample and Internal Standard Pre-Extraction Masses

Table A1.1 Measured masses of foods extracted and internal standard added

Table A1.1.a Cracker masses.

Sample	Dry Mass	Mass of I.S. Added (g)
CR1	4.89562	
	4.89564	
	4.89559	
Mean	4.89562	0.04082
Std. Dev.	0.00003	0.00004
CR2	5.30562	
	5.30570	
	5.30579	
Mean	5.30570	0.04029
Std. Dev.	0.00009	0.00002
CR3	6.30744	
	6.30748	
	6.30756	
Mean	6.30749	0.04022
Std. Dev.	0.00006	0.00002

Table A1.1.b Corn starch masses.

sample	Dry Mass	Mass of I.S. Added (g)
CS1	5.29167	
	5.29160	
	5.29167	
Mean	5.29165	0.04009
Std. Dev.	0.00004	0.00003
CS2	5.19819	
	5.19819	
	5.19824	
Mean	5.19821	0.04004
Std. Dev.	0.00003	0.00004
CS3	5.38955	
	5.38959	
	5.38963	
Mean	5.38959	0.04050
Std. Dev.	0.00004	0.00007

Table A1.1.c Cookie masses.

Sample	Dry Mass	Mass of I.S. Added (g)
CK1	6.89297	
	6.89308	
	6.89301	
Mean	6.89302	0.03889
Std. Dev.	0.00006	0.00005
CK2	6.21153	
	6.21154	
	6.21152	
Mean	6.21153	0.04061
Std. Dev.	0.00001	0.00004
CK3	5.93600	
	5.93600	
	5.93596	
Mean	5.93599	0.04210
Std. Dev.	0.00002	0.00003

Table A1.1.d Blank set A masses.

Sample	Mass of I.S. Added (g)
BL1	
Mean	0.03977
Std. Dev.	0.00002
BL2	
Mean	0.04147
Std. Dev.	0.00004
BL3	
Mean	0.04139
Std. Dev.	0.00005

Table A.1.1.e Mayonnaise masses.

Sample	Dry Mass	Mass of I.S. Added (g)
MA1	9.2292	
	9.22975	
	9.2299	
Mean	9.22961667	0.03984
Std. Dev.	0.00036856	0.00002
MA2	5.8444	
	5.8444	
	5.8437	
Mean	5.84416667	0.0412
Std. Dev.	0.00040415	0.00006
MA3	5.5639	
	5.5638	
	5.5631	
Mean	5.5636	0.04007
Std. Dev.	0.00043589	0.00006
MA4	6.88449	
	6.88423	
	6.88402	
Mean	6.88424667	0.040213333
Std. Dev.	0.00023544	0.00002

Table A1.1.f Vegetable shortening masses.

Sample	Dry Mass	Mass of I.S. Added (g)
VS1	5.03163	
	5.0169	
	5.03173	
Mean	5.026753	0.03977
Std. Dev.	0.008533	0.00001
VS2	4.98575	
	4.9857	
	4.98589	
Mean	4.98578	0.03982
Std. Dev.	9.85E-05	0.00002
VS3	4.91376	
	4.91373	
	4.91378	
Mean	4.913757	0.04087
Std. Dev.	2.52E-05	0.00002

Table A1.1.g Cheddar cheese masses.

Sample	Dry Mass	Mass of I.S. Added (g)
CC1	7.91755	
	7.91756	
	7.91749	
Mean	7.91753	0.03925
Std. Dev.	0.00004	0.00002
CC2	6.45252	
	6.45239	
	6.45227	
Mean	6.45239	0.03995
Std. Dev.	0.00013	0.00005
CC3	5.58530	
	5.58532	
	5.58531	
Mean	5.58531	0.04144
Std. Dev.	0.00001	0.00004

Table A1.1.h Blank set B masses. **Table A1.1.i** American cheese masses.

Sample	Mass of I.S. Added (g)
WB1	
Mean	0.03983
Std. Dev.	0.00004
WB2	
Mean	0.03982
Std. Dev.	0.00005
WB3	
Mean	0.04172
Std. Dev.	0.00005
WB4	
Mean	0.04332
Std. Dev.	0.00003

Sample	Dry Mass	Mass of I.S. Added (g)
AC1	6.45571	
	6.45573	
	6.45591	
Mean	6.45578	0.03895
Std. Dev.	0.00011	0.00002
AC2	4.89840	
	4.89810	
	4.89830	
Mean	4.89827	0.03837
Std. Dev.	0.00015	0.00002
AC3	4.16718	
	4.16707	
	4.16689	
Mean	4.16705	0.04080
Std. Dev.	0.00015	0.00003
AC4	5.08010	
	5.07980	
	5.09560	
Mean	5.08517	0.04028
Std. Dev.	0.00904	0.00004

Table A1.1.k Pork meat masses.

Sample	Dry Mass	Mass of I.S. Added (g)
LM1	7.98790	0.04284
	7.98720	
	7.98750	
Mean	7.98753	0.04284
Std. Dev.	0.00035	0.00026
LM2	7.26920	0.04390
	7.26950	
	7.26930	
Mean	7.26933	0.04390
Std. Dev.	0.00015	0.00031
LM3	8.03580	0.04436
	8.03570	
	8.03590	
Mean	8.03580	0.04436
Std. Dev.	0.00010	0.00018
LM4	6.80710	0.04269
	6.80740	
	6.80750	
Mean	6.80733	0.04269
Std. Dev.	0.00021	0.00002

Table A1.1.k Chicken sausage masses.

Sample	Dry Mass	Mass of I.S. Added (g)
PS1	6.86500	0.04206
	6.86540	
	6.86540	
Mean	6.86527	0.04206
Std. Dev.	0.00023	0.00004
PS2	7.93850	0.03915
	7.93860	
	7.93880	
Mean	7.93863	0.03915
Std. Dev.	0.00015	0.00002
PS3	8.86010	0.03994
	8.86080	
	8.86000	
Mean	8.86030	0.03994
Std. Dev.	0.00044	0.00002
PS4	7.62950	0.04194
	7.62960	
	7.62970	
Mean	7.62960	0.04194
Std. Dev.	0.00010	0.00003

Table A1.1.1 Blank set C masses.

Sample	Mass of I.S. Added (g)
MB1	
Mean	0.03556
Std. Dev.	0.00003
MB2	
Mean	0.03907
Std. Dev.	0.00003
MB3	
Mean	0.04152
Std. Dev.	0.00002
MB4	
Mean	0.03905
Std. Dev.	0.00002

A1.3 GC-EIMS Calibrant Solutions and Analyses

Table A1.2 shows the mass measurements made, in triplicate, of Internal Standard and diluting solvent during preparation of a working stock solution for subsequent addition to samples and preparation of calibrants.

Table A1.2 Mass measurements during preparation of I.S. in acetonitrile working solution.

	Mass of I.S. Stock (~2540 ppm)	Mass of Empty 10 mL vol. Flask	Mass I.S. after removal of Aliquot	Mass of I.S. stock added to Flask	Mass Flask and Dilute I.S. soln.	Mass of working solution (g)	Mass Fraction of I.S. in Working Solution
	3.45516	13.044	3.26175		20.84389		
	3.45519	13.04403	3.26177		20.84388		
	3.45508	13.044399	3.26178		20.84389		
Mean	3.45514	13.04414	3.26177	0.19338	20.84389	7.79974	0.02479
Uncertainty	0.00006	0.00022	0.00002	0.00006	0.00001	0.00023	8E-06

Three stock solutions of unlabeled DEHP, used GC-EIMS calibrants, were prepared from dilutions of neat bis(2-ethylhexyl) phthalate (Supelco, 99.8 ± 0.1 %) in methanol. To achieve the desired mass fractions of DEHP, two dilution were made to arrive at each stock solution. The masses of neat DEHP and methanol measured in triplicate during the first dilution of the DEHP standard are in Table A1.3, and those during the second in Table A1.4. These I.S. in acetonitrile and Stock Solutions 1B, 2B, and 3B were used to prepare GC-EIMS calibrants. The mass measurements made during dilution and resulting mass fractions of DEHP in each of these calibrants is listed in Table A1.5.

Table A1.3 Mass measurements of first Dilution of neat DEHP standard.

Stock ID	Mass of Empty 10 mL vol. Flask	Mass Flask and neat DEHP (g)	Mass of Flask and MeOH Solution (g)	Mass DEHP added (g)	Mass of solution (g)	[DEHP] (g/g)
1A	13.21285	13.22344	21.00401			
	13.21282	13.22349	21.00401			
	13.21285	13.22346	21.00495			
Mean	13.21284	13.223463	21.004323	0.01062	7.78086	0.0013653
Uncertainty	0.00002	0.00003	0.00054	0.00003	0.00054	4E-06
2A	13.31805	13.32711	21.15262			
	13.31804	13.32715	21.15259			
	13.31805	13.32712	21.15262			
Mean	13.31804667	13.327127	21.15261	0.00908	7.8254833	0.0011603
Uncertainty	0.00001	0.00002	0.00002	0.00002	0.00003	3E-06
3A	12.18309	12.19099	20.21881			
	12.18308	12.19093	20.2188			
	12.18312	12.19098	20.21881			
Mean	12.18309667	12.190967	20.218807	0.00787	8.02784	0.0009803
Uncertainty	0.00002	0.00003	0.00001	0.00004	0.00003	5E-06

Table A1.4 Mass measurements of second dilution of DEHP standard.

Stock ID	Mass of Empty 10 mL vol. Flask	Mass flask and Stock A (g)	Mass of Flask and MeOH Solution (g)	Mass of stock A Added (g)	Mass DEHP added (g)	Mass of Solution (g)	[DEHP] (ug/g)
1B	13.10189	13.89462	20.96317				
	13.10192	13.89471	20.96313				
	13.10186	13.89468	20.96313				
Mean	13.10189	13.89467	20.963143	0.79278	0.001082	7.86125	137.69
Uncertainty	0.00003	0.00005	0.00002	0.00006	3E-06	0.00004	0.40
2B	13.13831	13.96468	20.96336				
	13.13837	13.96472	20.96343				
	13.13841	13.9647	20.96337				
Mean	13.138363	13.9647	20.963387	0.8263367	0.000959	6.99869	137.00
Uncertainty	0.00005	0.00002	0.00004	0.00005	2E-06	0.00004	0.33
3B	13.78031	14.53338	21.63894				
	13.78025	14.53333	21.54074				
	13.78028	14.53333	21.54072				
Mean	13.78028	14.533347	21.54076	0.7530667	0.000738	7.00741	105.35
Uncertainty	0.00003	0.00003	0.056702	0.00004	4E-06	1.8E-02	0.99

Table A1.5 Mass measurements and fractions of I.S. and DEHP in GC-EIMS calibrants.

ID	mass of vial	Mass with I.S. working soln. (g)	Mass with Stock Solution B (g)	Mass in 10 mL (g)	Mass I.S. (g)	Mass Stock Solution B (g)	Mass soln. (g)	[I.S.] (g/g)	[DEHP] ($\mu\text{g/g}$)
1-1	12.58123	12.733	13.46721	20.33775					
	12.58123	12.732997	13.4616	20.33771					
	12.58124	12.73295	13.46713	20.33769					
Mean	12.58123	12.73298	13.46531	20.33772	0.15189	0.73233	7.75648	0.00226	0
Uncertainty	6E-06	3E-05	0.0032161	3 E-05	0.00083	0.00322	3E-05	2E-5	-
1-2	14.93769	15.0284	15.83536	22.63475					
	14.93765	15.02853	15.83528	22.63469					
	14.93759	15.02854	15.83525	22.6347					
Mean	14.937643	15.02849	15.83530	22.634713	0.09094	0.806811	7.69707	0.00157	0.090
Uncertainty	5E-05	8E-05	6E-05	3E-05	0.00080	0.00010	6E-05	3E-5	0.001
2-3	12.99264	13.03093	13.86039	20.86332					
	12.99261	13.03094	13.86029	20.86331					
	12.99262	13.03091	13.86022	20.86329					
Mean	12.99262	13.03093	13.8603	20.86331	0.03834	0.829373	7.87068	0.00061	0.178
Uncertainty	2E-05	2E-05	9E-05	2E-05	0.00018	8.8E-05	2E-05	1E-5	0.001
2-4	15.24743	15.31533	16.11278	20.70146					
	15.24747	15.31541	16.11272	20.70143					
	15.24743	15.31537	16.11267	20.7014					
Mean	15.24744	15.31537	16.11272	20.70143	0.06795	0.797353	5.45399	0.00160	0.464
Uncertainty	2E-05	4E-05	6E-05	3E-05	0.00044	7E-05	4E-05	1E-05	0.003
2-5	16.83123	16.93137	17.80908	24.74104					
	16.8313	16.93141	17.80902	24.74102					
	16.83124	16.93131	17.80902	24.74103					
Mean	16.83126	16.931363	17.80904	24.74103	0.10016	0.877677	7.90977	0.00170	0.43
Uncertainty	4E-05	5E-05	3E-05	1E-05	0.00072	6E-05	4E-05	1E-05	0.003
2-6	12.00015	12.13002	12.9585	19.74257					
	12.00006	12.13005	12.95848	19.74256					
	12.00005	12.13002	12.95847	19.74252					
Mean	12.00009	12.13003	12.95848	19.74255	0.13013	0.828453	7.74246	0.00219	0.481
Uncertainty	6E-05	2E-05	2E-05	3E-05	0.00058	3E-05	6E-05	1E-05	0.002
3-7	13.25838	13.3352	14.17011	21.00884					
	13.25837	13.33505	14.16998	21.0088					
	13.25833	13.3351	14.17011	21.00883					
Mean	13.25836	13.33512	14.17007	21.00882	0.07681	0.83495	7.75046	0.00126	0.513
Uncertainty	3E-05	8E-05	8E-05	2E-05	0.00067	1E-04	3E-05	2E-05	0.007
3-8	12.6179	12.72621	13.55272	20.47685					
	12.61792	12.72616	13.55283	20.47665					
	12.61789	12.72616	13.55281	20.47663					
Mean	12.61790	12.72618	13.55279	20.47671	0.10838	0.82661	7.85881	0.00173	0.421
Uncertainty	2E-05	3E-05	6E-05	0.00012	0.00060	7E-05	1E-04	12E-05	0.005

The GC-EIMS results of this calibration are listed in Table A1.6, where A_{149} and A_{154} are the integrated relative ion abundances of peaks in the single ion chromatograms for $m/z=149$ and $m/z=154$, respectively, at the time of DEHP elution (~21 min.).

Integrations were made manually with Agilent Chemstation® Enhanced Data Analysis Software.

Table A1.6 Data and results for screening GC-EIMS calibration for DEHP quantification.

ID	[DEHP]/[I.S.]	A_{149}^1	A_{154}^1	A_{149}/A_{154}
S1-1	0	0	7413544	0
S1-2	57.438	300953	3958369	0.0760295
S2-3	292.71	620314	1629252	0.3807355
S2-3	289.89	1528783	3884738	0.3935357
S2-5	252.88	1270793	3734013	0.340329
S2-6	219.37	1685766	5346624	0.3152954
S3-7	243.54	2116460	6032525	0.3508415
S3-8	99.917	1207220	9616116	0.1255413

¹Integrated Relative Ion Abundance of Peak During DEHP Elution

The A_{149} and A_{154} values and the resultant mass of DEHP estimates obtained from GC-EIMS analysis of screening method blanks and purified food extracts are shown in tables A1.7 and A1.8.a, and A1.8.b.

Table A1.7 Data and results for GC-EIMS analysis of DEHP in screening method blanks.

Sample	A ₁₄₉ ¹	A ₁₅₄ ¹	A ₁₄₉ /A ₁₅₄	Mass DEHP (µg)/I.S. (g)	DEHP mass (µg)	±
Blank A-1	6797124	174094005	0.0390428	28.54	1.13	0.04
Blank A-2	4198096	169622753	0.0247496	18.09	0.75	0.02
Blank A-3	2514361	144795655	0.0173649	12.69	0.53	0.02
Mean					0.94	
Std. Dev.					0.27	
Blank B-1	10069	121738	0.0827104	60.46	2.15	0.07
Blank B-2	2714	91853	0.0295472	21.60	0.84	0.03
Blank B-3	bd	122697	-	0.00	0.00	0.00
Blank B-4	bd	70503	-	0.00	0.00	0.00
Mean					0.75	
Std. Dev.					1.02	
Blank C-1	181312	11658426	0.015552	11.37	0.40	0.01
Blank C-2	69701	7427535	0.0093841	6.86	0.27	0.01
Blank C-3	184701	7638435	0.0241805	17.68	0.73	0.02
Blank C-4	bd	4389218	-	0.00	0.00	0.00
Mean					0.47	
Std. Dev.					0.24	

¹Integrated relative ion abundance of peak during DEHP Elution

Table A1.8a Data and results for GC-EIMS analysis of DEHP in purified food extracts.

Sample	A ₁₄₉ ¹	A ₁₅₄ ¹	A149/A154	Mass DEHP (µg)/I.S. (g)	Blank- corrected DEHP mass (µg)	±	[DEHP] (µg/g) in food	±
Cracker1	185923	669464	0.27772	203.01	7.34	0.08	1.50	0.08
Cracker2	262083	954791	0.27449	200.65	7.14	0.09	1.35	0.07
Cracker3	175506	638026	0.27508	201.08	7.23	0.08	1.19	0.06
Mean						0.07	1.27	
Std. Dev.						0.00	0.11	
Cookie1	352443	2957631	0.11916	87.11	2.44	0.29	0.35	0.04
Cookie3	225979	2049483	0.11026	80.60	2.45	0.29	0.41	0.05
Cookie4	253353	2313179	0.10953	80.06	2.50	0.30	0.40	0.05
Mean						0.30	0.41	
Std. Dev.						0.00	0.01	
CornStarch1	10251391	114236636	0.08974	65.60	1.69	0.29	0.32	0.05
Cornstarch2	6231114	107978722	0.05771	42.18	0.75	0.28	0.14	0.05
Cornstarch3	7658047	97682973	0.07840	57.31	1.38	0.28	0.26	0.05
Mean						0.28	0.20	
Std. Dev.						0.00	0.08	
² Mayo1	894017	8498155	0.10520	76.90	2.32	1.02	0.25	0.11
² Mayo2	669093	6118433	0.10936	79.94	2.55	1.02	0.44	0.17
² Mayo3	699860	6992746	0.10008	73.16	2.18	1.02	0.39	0.18
² Mayo4	788238	7635855	0.10323	75.46	2.29	1.02	0.33	0.15
Mean					2.34		0.39	
Std. Dev.					0.19		0.05	

¹Integrated relative ion abundance of peak during DEHP Elution

²Mayonnaise

Table A1.8.b. Data and results for GC-EIMS analysis of DEHP in foods.

Sample	m/z=149	m/z=154	A ₁₄₉ /A ₁₅₄	Mass DEHP (µg)/I.S. (g)	Blank-corrected DEHP mass (µg)	±	[DEHP] (µg/g) in food	±
Chicken Sausage1	170531	3241539	0.05261	38.46	1.15	0.25	0.17	0.04
Chicken Sausage2	107493	2295228	0.04683	34.23	0.87	0.24	0.11	0.03
Chicken Sausage3	189857	1529352	0.12414	90.75	3.16	0.27	0.36	0.03
Chicken Sausage4	191419	2177335	0.08791	64.26	2.23	0.26	0.29	0.03
Mean					2.08		0.25	
Std. Dev.					1.15		0.13	
Cheddar Cheese1	3001432	6241225	0.48090	343.75	12.74	1.58	1.61	0.20
Cheddar Cheese2	1959222	4686625	0.41805	298.82	11.19	1.47	1.73	0.23
Cheddar Cheese3	2437714	8669398	0.28119	205.55	7.77	1.05	1.39	0.19
Mean					9.48		1.56	
Std. Dev.					2.42		0.24	
Pork Meat1	232191	2983298	0.07783	56.89	1.97	0.25	0.25	0.03
Pork Meat2	151828	1846335	0.08223	60.11	2.17	0.26	0.30	0.04
Pork Meat3	223544	3117551	0.07171	52.42	1.86	0.25	0.23	0.03
Pork Meat4	209139	3217530	0.06500	47.51	1.56	0.25	0.23	0.04
Mean					1.89		0.25	
Std. Dev.					0.25		0.03	
American Cheese1	33129	135189	0.24506	179.14	6.98	0.67	0.96	0.03
American Cheese2	28353	113747	0.24926	182.21	6.99	0.66	1.27	0.04
American Cheese3	20635	72125	0.28610	209.14	8.53	0.70	1.87	0.06
American Cheese4	48592	165308	0.29395	214.87	8.66	0.69	1.55	0.04
Mean					7.79		1.57	
Std. Dev.					0.93		0.30	

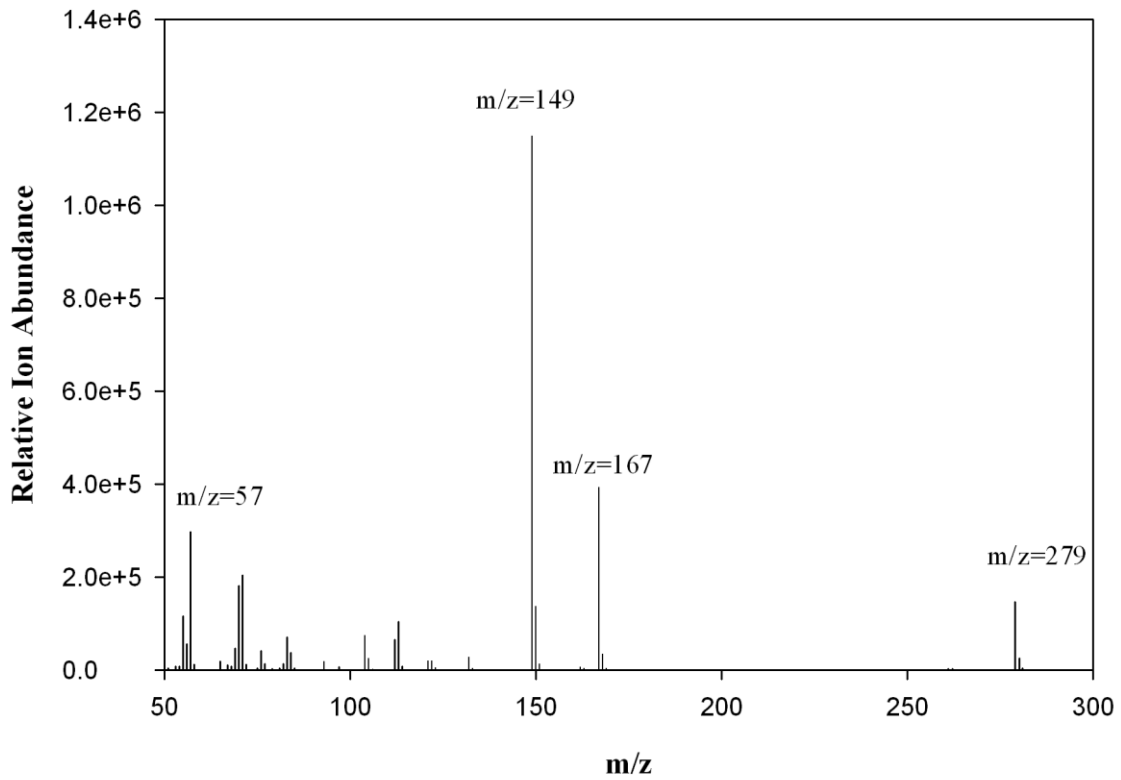


Figure A1.1 EI-MS $m/z=50$ to $m/z=300$ spectrum of DEHP (0.3ng).

Appendix 2: Extraction and Liquid Chromatographic Purification of DEHP in Stilton Cheese

A2.1 Composition of Stilton Cheese

The typical mass fraction of carbon in whole Stilton cheese is approximately 0.37. After the removal of water by lyophilizing the cheese, 95% of its mass is of contemporary carbon-containing species and 5% of its mass is non-carbonaceous salts. These are shown in Table A2.1.

Table A2.1 Typical composition of Stilton cheese

	% Mass
Water	38
Fat	35
Protein	23.7
Sodium	0.8
K, Ca, Mg, P, Zn, Cl ⁻ , Se, I	2.0
Lactose	0.1
Retinol	4.E-04
Carotine	2.E-04
Tryptophan	6.E-03
Folate	8.E-05
Pantothenate	9.E-04
Biotin	3.E-06
Vitament D	2.E-07
Vitamen E	6.E-04
Ribovlavin	5.E-04
Nicain	7.E-04
Vitament B6	1.E-04
Vitamin B12	1.E-03

Data in Table A2.1 is listed by Stilton Cheesemakers' Association (2013) from McCance & Widdowson's "The Composition of Foods - 6th Summary Edition" - Food Standards Agency, U.K.

A2.2 Stilton Cheese Extractions

Tables A2.2.a and A2.2.b lists details of the Stilton cheese extraction procedures of samples and method blanks. Each sample was extracted three times with the volumes listed and according to the procedures outlined in Section 4.2.

Table A2.2.a Stilton cheese and method blank extraction specifics.

Sample or Blank #	Date	Mass of Cheese (g)	Mass of D38 DEHP I.S. Stock Solution Added (g)	~Mass of D38 DEHP I.S. Added (μg)	Hot Plate Heat Number setting	Volume of solvent 1st Extraction (mL)	Heating Time and stirring interval
Batch - 1	8/24/2011 9/12/2011	3920	~0.540	1370	5	hexane - 4500	20 min; 3 min
SBL01	10/17/2012	-	0.30898	785	5	hexane - 3800	17 min; 3 min
Batch - 2	9/26/2011 10/5/2011	3287	0.31032	790	5	hexane - 3000	20 min; 3 min
Batch - 3	2/14/2012	1592	0.13979	355	5	hexane- 1200	20 min; 3 min
SBL02	2/14/2012	-	0.13735	350	5	hexane- 500	20 min; 3 min
Batch - 4	2/27/2012 2/28/2012	1632.6	0.13970	355	5	hexane- 1200	60 min; 3 min
Batch - 5	2/27/2012 2/28/2012	1687.3	0.13831	350	5	hexane- 1200	60 min; 3min
SBL03	2/27/2012 2/28/2012	-	0.13881	350	5	hexane- 500	60 min; 3 min
Batch - 6	4/3/2012	1246.5	0.08889	225	5	hexane - 900	40 min, 5 min
Batch - 7	4/3/2012	1261.5	0.08486	220	5	hexane - 900	40 min, 5 min
SBL04	4/3/2012	-	0.08841	225	5	hexane - 450	40 min, 5 min

Table A2.2.b Continued Stilton cheese and method blank extraction specifics.

Sample or Blank #	Date	Volume of solvent 2nd Extraction (mL)	Heating Time and stirring interval	Volume of solvent 3rd Extraction (mL)	Heating Time and Stirring Interval	Volume of extract after rotary evaporation (mL)
Batch - 1	8/24/2011	hexane – 2250 acetone-600	20 min; 5min	hexane-900	10 min; 5 min	2600
SBL01	10/17/2011	hexane-1200 acetone-300	15 min; 3 min	hexane-400	6 min/ 3 min	1000
Batch - 2	9/26/2011 10/5/2012	hexane – 1500 acetone-400	15 min; 3min	hexane-500	15 min; 3 min	2000
Batch - 3	2/14/2012	hexane-500 acetone-100	15 min; 3 min	hexane-400	6 min/ 3 min	1300
SBL02	2/14/2012	hexane-300 acetone-50	15 min; 3 min	hexane-200	6 min;3 min	500
Batch - 4	2/27/2012- 2/28/2012	hexane-500 acetone-100	15 min; 3 min	hexane-400	6 min/ 3 min	1300
Batch - 5	2/27/2012- 2/28/2012	hexane-500 acetone-100	15 min; 3 min	hexane-400	6 min/ 3 min	1300
SBL03	2/27/2012- 2/28/2012	hexane-300 acetone-10	15 min; 3 min	hexane-200	6 min;3 min	500
Batch - 6	4/3/2012	hexane – 400 acetone 50	20 min, 5 min	hexane-300	20 min; 5 min	1000
Batch - 7	4/3/2012	hexane – 400 acetone 50	20 min, 5 min	hexane 300	20 min; 5 min	1000
SBL04	4/3/2012	hexane – 200 acetone 25	20 min, 5 min	hexane150	20 min; 5 min	400

A2.3 Flash Chromatography

The dates and silica column loadings of the flash chromatography purification step are listed in table A2.3.a and A2.3.b. Flash chromatography fractions were successively collected from 800 mL to 1600 mL after initiation of 1.6 % acetone/98.4 % hexane mobile phase. These fractions were evaporated to ~3 mL each and qualitatively checked by noting those with discernible GC-EIMS $m/z=149$ and $m/z=154$ MSD signals

to confirm phthalate presence. Those fractions that contained DEHP and were eluted during the same column run were combined for further purification. Tables A2.4.a through A2.4. list the results of these qualitative analyses, with those fractions that were combined denoted by a gray-colored chart background fill.

Table A2.3.a Flash column loadings of AMS pilot sample Set 1 delivered to LLNL 11/2011.

Extraction Batch or Blank #	Date	Sample Subunit #	Volume of Sample Subunit	Volume of 35% acetone/n-hexane used to rinse and clean column (mL)	Volume of Hexane Used to Condition Column (mL)	Total Mobile Phase Eluted (mL)	Final Volume of Evaporated Fractions
Batch - 1	8/31/2011	1-1	200	400	1100	1700 mL	~3 mL
Batch - 1	8/31/2011	1-2	200	400	1100	1700 mL	~3 mL
Batch - 1	8/31/2011	1-3	200	400	1100	1700 mL	~3 mL
Batch - 1	8/31/2011	1-4	200	400	1100	1700 mL	~3 mL
Batch - 1	9/1/2011	1-5	200	400	1100	1700 mL	~3 mL
Batch - 1	9/6/2011	1-6	200	400	1100	1700 mL	~3 mL
Batch - 1	9/6/2011	1-7	200	400	1100	1700 mL	~3 mL
Batch - 1	9/14/2011	1-8	300	400	1100	1700 mL	~3 mL
Batch - 1	9/15/2011	1-9	300	400	1100	1700 mL	~3 mL
Batch - 1	9/16/2011	1-10	300	400	1100	1700 mL	~3 mL
Batch - 1	9/16/2011	1-11	300	400	1100	1700 mL	~3 mL
Batch - 2	9/28/2011	2-1	200	400	1100	1700 mL	~3 mL
Batch - 2	9/28/2011	2-2 ¹	200	400	1100	1700 mL	~3 mL
Batch - 2	9/30/2011	2-3	300	400	1100	1700 mL	~3 mL
Batch - 2	9/30/2011	2-4	300	400	1100	1700 mL	~3 mL
Batch - 2	10/3/2012	2-5	200	400	1100	1700 mL	~3 mL
Batch - 2	10/3/2011	2-6	200	400	1100	1700 mL	~3 mL
Batch - 2	10/4/2011	2-7	200	400	1100	1700 mL	~3 mL
Batch - 2	10/10/2011	2-8	200	400	1100	1700 mL	~3 mL
Batch - 2	10/10/2011	2-9	200	400	1100	1700 mL	~3 mL
SBL01	10/19/2011	BL1-1	250	400	1100	1700 mL	~3 mL
SBL01	10/19/2011	BL1-2	250	400	1100	1700 mL	~3 mL
SBL01	10/20/2011	BL1-3	250	400	1100	1700 mL	~3 mL
SBL01	10/20/2011	BL1-4	250	400	1100	1700 mL	~3 mL

¹Subunit lost to contamination

Table A2.3.b Flash column loadings of AMS primary sample Set 2 delivered to LLNL 06/2012.

Extraction Batch or Blank #	Date	Sample Subunit #	Volume of Sample Subunit	Volume of 35% acetone/n-hexane used to rinse and clean column (mL)	Volume of Hexane Used to Condition Column (mL)	Total Mobile Phase Eluted (mL)	Final Volume of Evaporated Fractions
Batch - 3	2/16/2012	3-1	250	400	1100	1700 mL	~3 mL
Batch - 3	2/16/2012	3-2	250	400	1100	1700 mL	~3 mL
Batch - 3	2/21/2012	3-3	250	400	1100	1700 mL	~3 mL
Batch - 3	2/21/2012	3-4	250	400	1100	1700 mL	~3 mL
SBL02	2/16/2012	B2-1	250	400	1100	1700 mL	~3 mL
SBL02	2/16/2012	B2-2	250	400	1100	1700 mL	~3 mL
SBL02	2/16/2012	B2-3	250	400	1100	1700 mL	~3 mL
SBL02	2/16/2012	B2-4	250	400	1100	1700 mL	~3 mL
Batch - 3	2/23/2012	3-5	300	400	1100	1700 mL	~3 mL
SBL03	3/2/2012	B3-1	230	400	1100	1700 mL	~3 mL
Batch - 4	3/4/2012	4-1	300	400	1100	1700 mL	~3 mL
Batch - 4	3/4/2012	4-2	300	400	1100	1700 mL	~3 mL
Batch - 4	3/6/2012	4-3	300	400	1100	1700 mL	~3 mL
SBL03	3/6/2012	B3-2	300	300	1100	1700 mL	~3 mL
Batch - 5	3/7/2012	5-1	300	300	1100	1700 mL	~3 mL
Batch - 5	3/7/2012	5-2	300	300	1100	1700 mL	~3 mL
Batch - 5	3/8/2012	5-3	300	300	1100	1700 mL	~3 mL
Batch - 4	3/12/2012	4-4	400	400	1100	1700 mL	~3 mL
Batch - 5	3/12/2012	5-4	400	400	1100	1700 mL	~3 mL
Batch - 6	4/5/2012	6-1	250	400	1100	1700 mL	~3 mL
SBL04	4/5/2012	B4-1	300	400	1100	1700 mL	~3 mL
Batch - 7	4/6/2012	7-1	250	400	1100	1700 mL	~3 mL
SBL04	4/10/2012	B4-2	300	400	1100	1700 mL	~3 mL
Batch - 7	4/10/2012	7-2	250	400	1100	1700 mL	~3 mL
Batch - 7	4/11/2012	7-3	250	400	1100	1700 mL	~3 mL
Batch - 6	4/11/2012	6-2	250	400	1100	1700 mL	~3 mL
SBL04	4/12/2012	B4-3	300	400	1100	1700 mL	~3 mL
Batch - 6	4/12/2012	6-3	300	400	1100	1700 mL	~3 mL

Table A2.4.a GC-EIMS analysis of Collected Flash chromatography fractions; batch 1.

Batch 1 Column 1					Batch 1 Column 2				
8/30/2011					8/31/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900		bd		bd	800-900		bd		bd
900-1000		bd		bd	900-1000		bd		bd
1000-1100		bd		bd	1000-1100		bd		bd
1100-1200	20.13	3322440	19.80	9445332	1100-1200	20.30	1420535	19.95	3798337
1200-1300	20.27	918134	19.91	4263191	1200-1300	20.40	554707	20.02	2466738
1300-1400	20.53	200342	20.20	740523	1300-1400	20.51	322048	20.16	983270
1400-1500	20.50	81713	20.20	370536	1400-1500	20.56	138719	20.27	454159
1500-1600	20.27	43330	20.36	65732	1500-1600	20.52	156783	20.19	442214
Batch 1 Column 3					Batch 1 S Column 4				
9/1/2011					9/1/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900		bd		bd	800-900		bd		bd
900-1000		bd		bd	900-1000		bd		bd
1000-1100		bd		bd	1000-1100		bd		bd
1100-1200		bd		bd	1100-1200		bd		bd
1200-1300		bd		bd	1200-1300	20.14	21805		bd
1300-1400	20.16	3778917	19.84	10384496	1300-1400	20.29	845381	19.95	1991416
1400-1500	20.34	401467	20.02	2038367	1400-1500	20.22	1813152	19.88	6816815
1500-1600	20.40	330816	20.06	1204948	1500-1600				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.b GC-EIMS analysis of collected Flash chromatography fractions; continued
Batch 1.

Batch 1 Column 5					Batch 1 Column 6				
9/2/2011					9/7/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900					800-900		bd		bd
900-1000					900-1000		bd		bd
1000-1100					1000-1100		bd		bd
1100-1200					1100-1200		bd		bd
1200-1300					1200-1300		bd		bd
1300-1400	20.23	1537978	19.90	5313653	1300-1400	20.28	2790402	19.95	7913800
1400-1500	20.31	784972	19.98	2444497	1400-1500	20.53	223726	20.23	1119067
1500-1600	20.36	162700	20.06	695221	1500-1600	20.84	37715	20.55	255885
Batch 1 Column 7					Batch 1 Column 8				
9/11/2011					9/15/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900		bd		bd	800-900		bd		bd
900-1000		bd		bd	900-1000		bd		bd
1000-1100		bd		bd	1000-1100		bd		bd
1100-1200		bd		bd	1100-1200		bd		bd
1200-1300		bd		bd	1200-1300				
1300-1400	20.36	567925	20.05	1232595	1300-1400	20.51	1331289	20.18	2183285
1400-1500	20.39	416064	20.06	1347846	1400-1500	20.56	517530	20.25	1001237
1500-1600	20.57	77762	20.24	254812	1500-1600				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.c GC-EIMS analysis of collected Flash chromatography fractions; continued Batch 1.

Batch 1 Column 9 9/19/2011					Batch 1 Column 10 9/20/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900	bd		bd	bd	800-900				
900-1000	bd		bd	bd	900-1000				
1000-1100	bd		bd	bd	1000-1100				
1100-1200	bd		bd	bd	1100-1200				
1200-1300	bd		bd	bd	1200-1300	20.50	2285532	20.14	3823089
1300-1400	20.42	3791913	20.09	6205648	1300-1400	20.64	413024	20.29	1030870
1400-1500	20.59	574870	20.27	1469898	1400-1500				
1500-1600					1500-1600				
Batch 1 Column 11 9/20/2011									
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area					
800-900									
900-1000									
1000-1100									
1100-1200	20.55	854445	20.22	854684					
1200-1300	20.44	3104152	20.10	5739769					
1300-1400									
1400-1500									
1500-1600									

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.d GC-EIMS analysis of collected Flash chromatography fractions; batch 2.

Batch 2 Column 3					Batch 1 Column 4				
10/4/2011					10/4/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900					800-900		bd		bd
900-1000					900-1000		bd		bd
1000-1100					1000-1100		bd		bd
1100-1200	20.84 3	2424902	20.51	1860789	1100-1200		bd		bd
1200-1300	20.84	1840937	20.51	2998096	1200-1300	20.94	1309112	20.612	1078933
1300-1400					1300-1400	20.87	1890139	20.549	2809494
1400-1500					1400-1500	20.95	685888	20.612	890583
1500-1600					1500-1600				
Batch 2 Column 5					Batch 2 Column 6				
10/4/2011					10/4/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900					800-900		bd		bd
900-1000					900-1000		bd		bd
1000-1100					1000-1100		bd		bd
1100-1200	21.06	387522	20.762	381857	1100-1200		bd		bd
1200-1300	20.87	1250933	20.549	1541135	1200-1300	20.826	1513512	20.502	1054514
1300-1400	20.87	588517	20.549	692450	1300-1400	20.785	2465375	20.456	3424673
1400-1500	20.84	469791	20.525	507225	1400-1500	20.866	402641	20.537	517108
1500-1600					1500-1600				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.e GC-EIMS analysis of collected Flash chromatography fractions; continued batch 2.

Batch 2 Column 7					Batch Column 8				
10/5/2011					10/11/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900		bd		bd	800-900		bd		bd
900-1000		bd		bd	900-1000		bd		bd
1000-1100		bd		bd	1000-1100		bd		bd
1100-1200		bd		bd	1100-1200		bd		bd
1200-1300	20.93	1233717	20.612	1244468	1200-1300	21.10	340021	20.76	87366
1300-1400	20.91	1394381	20.57	1869801	1300-1400	20.90	3089534	20.57	1704257
1400-1500	20.98	238967	20.65	310282	1400-1500	21.15	281127	20.81	212038
1500-1600					1500-1600				
Batch 2 Column 9					Batch 2 Column				
10/11/2011									
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area
800-900					800-900				
900-1000					900-1000				
1000-1100					1000-1100		bd		bd
1100-1200		bd		bd	1100-1200		bd		bd
1200-1300	20.89	2769340	20.57	1282618	1200-1300	20.51	2443530	20.19	3541562
1300-1400	20.98	1193914	20.65	726291	1300-1400	20.57	96553	20.82	206720
1400-1500	21.01	703306	20.70	439991	1400-1500				
1500-1600					1500-1600				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.f GC-EIMS analysis of collected Flash chromatography fractions; BL01.

BL01 Column 1					SBL01 Column 2				
10/20/2011					10/4/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900		bd		bd	800-900		bd		bd
900-1000		bd		bd	900-1000		bd		bd
1000-1100		bd		bd	1000-1100		bd		bd
1100-1200		bd		bd	1100-1200		bd		bd
1200-1300		bd		bd	1200-1300		bd	20.98	4002210
1300-1400		bd	20.92	4648134	1300-1400		bd	21.12	1916981
1400-1500		bd	21.68	94431	1400-1500		bd	21.51	227544
1500-1600		bd		bd	1500-1600				
SBL01 Column 3					SBL04 Column 4				
10/21/2011					10/21/2011				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z=149 Peak T _R	m/z=149 Peak Area ²	m/z=154 Peak T _R	m/z=154 Peak Area	Fraction Collection Time and Volume of Eluent	m/z=149 Peak T _R	m/z=149 Peak Area	m/z=154 Peak T _R	m/z=154 Peak Area
800-900					800-900		bd		bd
900-1000					900-1000		bd		bd
1000-1100					1000-1100		bd		bd
1100-1200					1100-1200		bd		bd
1200-1300		bd	21.31	636837	1200-1300		bd	20.94	4671725
1300-1400		bd	21.07	2345011	1300-1400		bd	21.30	703945
1400-1500		bd	21.36	437452	1400-1500		bd	21.79	60663
1500-1600					1500-1600				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.g GC-EIMS analysis of collected Flash chromatography fractions; batch 3.

Batch 3 Column 1 2/16/2012					Batch 3 Column 3 2/21/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
1000-1100	bd	bd			1000-1100				
1100-1200	bd	bd			1100-1200	19.55	27920	19.22	11533
1200-1300	19.64	6412	19.28	4701	1200-1300	19.57	60639	19.24	179648
1300-1400	19.57	42732	19.25	101363	1300-1400	19.61	9820	19.33	21323
1400-1500	19.6	16633	19.31	41736	1400-1500	19.73	3037	19.52	9186
1500-1600	19.73	7241	19.4	14165	1500-1600				
Batch 3 Column 2 2/21/2012					Batch 3 Column 4 2/21/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
1000-1100					1000-1100				
1100-1200					1100-1200	19.57	33332	19.24	68204
1200-1300	19.58	25789	19.24	76742	1200-1300	19.57	48608	19.24	122556
1300-1400	19.63	10421	19.28	26399	1300-1400				
1400-1500	19.69	6275	19.41	13560	1400-1500				
1500-1600	19.66	3906	19.42	7053	1500-1600				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.h GC-EIMS analysis of collected Flash chromatography fractions; SBL02.

SBL02 Column 1 2/16/2012					SBL02 Column 2 2/21/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
1000-1100					1000-1100				
1100-1200		bd		bd	1100-1200		bd		
1200-1300		bd	19.41	23202	1200-1300		bd	19.34	65399
1300-1400		bd	19.29	234129	1300-1400		bd	19.3	66470
1400-1500		bd	19.46	26389	1400-1500		bd	19.48	9755
1500-1600		bd	19.53	12408	1500-1600				
SBL02 Column 3 2/21/2012					SBL02 Column 4 2/21/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
1000-1100					1000-1100				
1100-1200					1100-1200				
1200-1300		bd	19.28	86375	1200-1300		bd	19.31	42581
1300-1400		bd	19.41	24811	1300-1400		bd	19.31	45800
1400-1500		bd	19.53	8676	1400-1500				
1500-1600					1500-1600				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.i GC-EIMS analysis of collected Flash chromatography fractions; batch 4.

Batch 4 Column 1 3/7/2012					Batch 4 Column 2 3/7/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
800-900					800-900				
900-1000					900-1000				
1000-1100		bd		bd	1000-1100	19.59	13114	19.262	9798
1100-1200	19.56	23845	19.23	25347	1100-1200	19.53	105685	19.192	226810
1200-1300	19.540	48726	19.21	127031	1200-1300	19.55	35570	19.221	104052
1300-1400	19.62	7688	19.30	18297	1300-1400	19.64	10571	19.262	31245
1400-1500					1400-1500				
Batch 4 Column 3 3/7/2012									
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area					
800-900									
900-1000									
1000-1100									
1100-1200		bd		bd					
1200-1300	19.52	1607	19.18	1407627					
1300-1400		bd	19.20	418406					
1400-1500									

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.j GC-EIMS analysis of collected Flash chromatography fractions; SBL03.

SB03 Column 1					SB03 Column 2				
3/7/2012					3/7/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
800-900					800-900				
900-1000					900-1000				
1000-1100					1000-1100		bd		bd
1100-1200		bd		bd	1100-1200		bd	19.204	280030
1200-1300	19.516	1607	19.181	1407627	1200-1300	19.5	1470	19.181	1523346
1300-1400		bd	19.198	418406	1300-1400		bd	19.193	393655
1400-1500					1400-1500				

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.k GC-EIMS analysis of collected Flash chromatography fractions; batch 5.

Batch 5 Column 1					Batch 5 Column 2				
3/12/2012					3/12/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
800-900					800-900				
900-1000					900-1000				
1000-1100	19.60	12386	19.27	11810	1000-1100	bd	bd	bd	bd
1100-1200	19.53	90117	19.20	226092	1100-1200	19.522	154261	19.19	264642
1200-1300	19.78	4632	19.33	11623	1200-1300	19.58	13840	19.27	49724
1300-1400	19.69	2773	7186	19.285	1300-1400	19.597	5766	19.30	16665
Batch 5 Column 3					Batch 5 Column 4				
3/12/2012					3/14/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
800-900					800-900				
900-1000					900-1000				
1000-1100	bd	bd	bd	bd	1000-1100	bd	bd	bd	bd
1100-1200	19.53	137680	19.192	238615	1100-1200	19.53	51216	19.20	45692
1200-1300	19.56	21130	19.233	88865	1200-1300	19.5	42569	19.22	122345
1300-1400	302	19.707	19.325	10510	1300-1400	19.56	8999	19.26	32981

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.1 GC-EIMS analysis of collected Flash chromatography fractions; batch 6.

Batch 6 Column 1					Batch 6 Column 2				
4/5/2012					4/5/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
1100-1200	21.109	84602	20.785	123558	1100-1200		bd		bd
1200-1300					1200-1300	21.109	122395	20.786	78947
1300-1400					1300-1400				
1400-1500	21.109	7023	20.791	16465	1400-1500				
1500-1600	21.132	5404	20.814	10546	1500-1600	21.103	30206	20.791	31913
Batch 6 Column 3									
4/9/2012									
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area					
1100-1200	21.109	17243	20.797	10552					
1200-1300	21.103	72450	20.785	120946					
1300-1400	21.103	31859	20.785	50991					
1400-1500	21.103	40346	20.78	63363					
1500-1600	21.115	20767	20.791	43581					

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.m GC-EIMS analysis of collected Flash chromatography fractions; batch 7.

Batch 7 Column 1 4/9/2012					Batch 7 Column 2 4/16/2012				
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area	Fraction Collection Time and Volume of Eluent ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area	m/z= 154 Peak T _R	m/z= 154 Peak Area
1000-1100					1000-1100				
1100-1200	21.138	2238	20.82	957	1100-1200	21.138	2568		bd
1200-1300	21.109	74606	20.785	101536	1200-1300	21.12	38299	20.797	28994
1300-1400	21.115	52746	20.791	100254	1300-1400	21.103	81790	20.791	155709
1400-1500	21.126	15450	20.809	26561	1400-1500	21.121	31163	20.797	49538
1500-1600	21.126	7012	20.814	11892	1500-1600	21.115	22597	20.797	37394
Batch 7 Column 3 4/17/2012									
Fraction Collection Time and Volume of Eluent (mL) ¹	m/z= 149 Peak T _R	m/z= 149 Peak Area ²	m/z= 154 Peak T _R	m/z= 154 Peak Area					
1000-1100									
1100-1200		bd		bd					
1200-1300	21.109	58175	20.791	32925					
1300-1400	21.109	88227	20.791	57295					
1400-1500	21.121	23960	20.814	16733					
1500-1600	21.138	6965	20.814	7520					

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

Table A2.4.n GC-EIMS analysis of collected Flash chromatography fractions; SBL04.

SBL04 - 1					SBL04 - 2				
4/5/2012					4/9/2012				
m/z=149 DEHP Peak Peak TR	m/z=149 DEHP Peak Area	m/z=154 D38 DEHP Peak TR	m/z=154 D38 DEHP Peak Area	m/z=154 D38 DEHP Peak Area	m/z=149 DEHP Peak TR	m/z=149 DEHP Peak Area	m/z=154 D38 DEHP Peak TR	m/z=154 D38 DEHP Peak Area	m/z=154 D38 DEHP Peak Area
1000-1100					1000-1100		bd		bd
1100-1200		bd		bd	1100-1200		1623	20.797	233069
1200-1300		bd	20.814	15169	1200-1300		bd	20.808	66200
1300-1400		bd	20.791	179934	1300-1400		bd		
1400-1500		bd		52409	1400-1500				
SBL04 - 3									
4/9/2012									
m/z=149 DEHP Peak Peak TR	m/z=149 DEHP Peak Area	m/z=154 D38 DEHP Peak TR	m/z=154 D38 DEHP Peak Area	m/z=154 D38 DEHP Peak Area					
1000-1100									
1100-1200		bd	20.814	95744					
1200-1300		bd	20.814	271095					
1300-1400		bd	20.814	124109					
1400-1500		bd	20.826	50326					

¹Volume eluted after initiation of 1.6 % acetone/98.4 % hexane mobile phase

²bd= below detection

A2.4 Size Exclusion Chromatography.

Size exclusion chromatography was performed with tandem 30 cm, 21 mm-I.D. Oligopore columns (Agilent Technologies, Palo Alto, CA) containing 6 μm particles, as outlined in Section 4.3.2. Table A2.5.a and A2.5.list injection volumes and collected fractions during this chromatographic step.

Table A2.5.a Oligopore injections; batch 1, batch 2, and BL01.

Sample or Blank #	Date	Sample Injection #	Volume of sample injected (hexane or MeCl_2)	Column Flow Rate (mL/min)	Fraction Collected ¹
Batch - 1	9/6/11	1	~0.8 mL	10	189-199
Batch - 1	9/6/11	2	~0.8 mL	10	189-199
Batch - 1	9/6/11	3	~0.8 mL	10	189-199
Batch - 1	9/15/11	4	~1 mL	10	189-199
Batch - 1	9/15/11	4	~1 mL	10	189-199
Batch - 1	9/20/11	5	~0.75 mL	10	189 - 199
Batch - 1	9/20/11	6	~0.75 mL	10	189 - 199
Batch - 1	9/20/11	7	~0.75 mL	10	189 - 199
Batch - 1	9/20/11	8	~0.75 mL	10	189 - 199
Batch - 1	9/30/11	9	~0.75 mL	10	189 - 199
BL01	10/17/11	1	~0.75 mL	10	189 - 199
BL01	10/17/11	2	~0.75 mL	10	189 - 199
BL01	10/17/11	3	~0.75 mL	10	189 - 199
BL01	10/17/11	4	~0.75 mL	10	189 - 199
Batch - 2	9/27/11	1	~0.75 mL	10	189 - 199
Batch - 2	10/6/11	2	~0.75 mL	10	189 - 188
Batch - 2	10/6/11	3	~0.75 mL	10	189 - 188
Batch - 2	10/6/11	4	~0.75 mL	10	189 - 188
Batch - 2	10/6/11	5	~0.75 mL	10	189 - 199
Batch - 2	10/11/11	6	~0.75 mL	10	189 - 199
Batch - 2	10/11/11	7	~0.75 mL	10	189 - 199
SBL02	2/22/2012	1	~1 mL	10	189-199
SBL02	2/22/2012	2	~1 mL	10	189-199
SBL02	2/22/2012	3	~1 mL	10	189-199
SBL02	2/22/2012	4	~1 mL	10	189-199

¹Volume eluted since sample injected

²Partial or total loss of subunit due to lowered recovery after HPLC fraction concentration and reconstitution.

Table A2.5.b Oligopore injections; batches 3, 4, 5, 6, 7, batch 2, SBL02, SBL03, and SBL04.

Sample or Blank #	Date	Sample Injection #	Volume of sample injected (hexane or MeCl ₂)	Column Flow Rate (mL/min)	Fraction Collected ¹
Batch - 3	2/22/2012	1	~1 mL	10	189-199
Batch - 3	2/22/2012	2	~1 mL	10	Subunit lost - sample leaked through injection port, repair made
Batch - 3	2/23/2012	3	~1 mL	10	189-199
Batch - 3	2/23/2012	4	~1 mL	10	189-199
Batch - 3 ²	2/23/2012	-	~1 mL	10	189-199
SBL03	3/7/2012	1	~1 mL	10	189-199
SBL03	3/7/2012	2	~1 mL	10	189-199
Batch - 4	3/7/2012	2	~1 mL	10	189-199
Batch - 4	3/7/2012	1	~1 mL	10	189-199
Batch - 4	3/7/2012	3	~1 mL	10	189-199
Batch - 4	3/14/2012	4	~1 mL	10	189-199
Batch - 5	3/12/2012	1	~1 mL	10	189-199
Batch - 5	3/14/2012	2	~1 mL	10	189-199
Batch - 5	3/14/2012	3	~1 mL	10	189-199
Batch - 5	3/14/2012	4	~1 mL	10	189-199
SBL04	4/17/2012	1	~1 mL	10	189-199
SBL04	4/17/2012	2	~1 mL	10	189-199
SBL04	4/17/2012	3	~1 mL	10	189-199
Batch - 6	4/17/2012	1	~1 mL	10	189-199
Batch - 6	4/17/2012	1	~1 mL	10	189-199
Batch - 6	4/17/2012	1	~1 mL	10	189-199
Batch - 7	4/17/2012	1	~1 mL	10	189-199
Batch - 7	4/17/2012	1	~1 mL	10	189-199
Batch - 7	4/18/2012	1	~1 mL	10	189-199

¹Volume eluted since sample injected

²post-oligopore fraction from subunit 1, 3, 4 eluted from 199-204 mL

A2.5 HPLC Purifications

The final liquid chromatographic step of DEHP purification was performed on a semi-preparatory 15 cm x 9.4 mm-I.D. Agilent Zorbax Eclipse C18 column and guard

column, coupled to a Spectroflow 757 UV/VIS Absorbance detector, as outlined in Section 4.3.3. Specifics of individual injections to the HPLC, system are listed in Table A2.6 and A2.7a to A2.7.c. The resolution achieved with each injection of a sample aliquot between the DEHP and d_{38} – DEHP absorbance peaks at $\lambda=254$ nm, which were used to monitor continuity of column performance, are listed in Tables A2.7.a to A2.7.c as well (where a DEHP peak was observed). The times which eluted mobile phase was collected from the system to recover DEHP, in addition to their volumes of 95 % acetonitrile/5 % water, are listed in Tables A2.8.a to A2.8.c.

Table A2.6.a HPLC injections; batch 1, batch 2, and BL01.

Date	Sample	Injection	Volume of Sample solution (ACN) Injected (µL)
9/6/2011	Batch - 1	1*	120
9/6/2011	Batch - 1	2*	120
9/6/2011	Batch - 1	3	120
9/6/2011	Batch - 1	4	120
9/7/2011	Batch - 1	5*	150
9/7/2011	Batch - 1	6*	150
9/7/2011	Batch - 1	7	150
9/7/2011	Batch - 1	8	150
9/7/2011	Batch - 1	9	150
9/8/2011	Batch - 1	10	150
9/8/2011	Batch - 1	11	150
9/8/2011	Batch - 1	12*	150
9/8/2011	Batch - 1	13*	150
9/13/2011	Batch - 1	14	400
9/13/2011	Batch - 1	15	320
9/13/2011	Batch - 1	16*	320
9/13/2011	Batch - 1	17*	320
9/13/2011	Batch - 1	18*	230
9/16/2011	Batch - 1	19	150
9/16/2011	Batch - 1	20	100
9/16/2011	Batch - 1	21	100
9/21/2011	Batch - 1	22	105
9/21/2011	Batch - 1	23	105
9/21/2011	Batch - 1	24	105
9/21/2011	Batch - 1	25	105
9/21/2011	Batch - 1	26	105
10/14/2011	Batch - 1	27	250
10/14/2011	Batch - 1	28	250
10/14/2011	Batch - 1	29	240

Date	Sample	Injection	Volume of Sample solution (ACN) Injected (µL)
9/28/2011	Batch - 2	1	95
9/28/2011	Batch - 2	2	95
9/28/2011	Batch - 2	3	95
9/28/2011	Batch - 2	4	95
10/7/2011	Batch - 2	5	100
10/7/2011	Batch - 2	6	40
10/7/2011	Batch - 2	7	20
10/7/2011	Batch - 2	8	100
10/7/2011	Batch - 2	9	100
10/10/2011	Batch - 2	10	100
10/10/2011	Batch - 2	11	100
10/10/2011	Batch - 2	12	100
10/10/2011	Batch - 2	13	100
10/12/2011	Batch - 2	14	100
10/12/2011	Batch - 2	15	100
10/12/2011	Batch - 2	16	100
10/12/2011	Batch - 2	17	100
10/12/2011	Batch - 2	18	100
10/12/2011	Batch - 2	19	100
10/17/2011	BL01	1	70
10/17/2011	BL01	2	70
10/17/2011	BL01	3	70
10/17/2011	BL01	4	70
10/17/2011	BL01	5	70
10/17/2011	BL01	6	70
10/17/2011	BL01	7	70
10/17/2011	BL01	8	70
10/17/2011	BL01	9	70
10/17/2011	BL01	10	70
10/17/2011	BL01	11	70
10/17/2011	BL01	12	70
10/17/2011	BL01	13	70
10/17/2011	BL01	14	70
10/17/2011	BL01	15	70

*Loss of sample – incomplete transfer from recovery flask after evaporation and reconstitution in methylene chloride; likely a result of incomplete evaporation of water from HPLC mobile phase.

Table A2.7.a Injections, peak properties, and resolution of DEHP by HPLC; batch 3, batch 5, and SBL02.

Date	Sample	Injection	volume sample solution injected (μL)	DEHP width at 254 nm (min)	DEHP T _R	¹ DEHP Peak Area	D38 DEHP T _R	D38 peak area	D38 DEHP peak width at 254 nm (s)	R ²
3/2/2012	Batch - 3	1	100	-	-					
3/2/2012	Batch - 3	2	100	0:47:00	32:49:00	11080	30:45:00	90400	1:02:00	2.28
3/5/2012	Batch - 3	3	120	0:49:00	34:07:00	13400	31:55:00	101000	1:06:00	2.30
3/5/2012	Batch - 3	4	130	0:51:00	33:23:00	16800	31:15:00	107000	1:07:00	2.17
3/5/2012	Batch - 3	5	130	0:51:00	33:18:00	14700	31:11:00	110000	1:07:00	2.15
3/5/2012	Batch - 3	6	130	1:01:00	33:07:00	13200	31:00:00	109000	1:06:00	2.00
3/5/2012	Batch - 3	7	130	0:54:00	33:21:00	16100	31:12:00	111000	1:09:00	2.10
3/5/2012	Batch - 3	8	130	0:48:00	33:50:00	16300	31:37:00	116000	1:03:00	2.40
3/6/2012	Batch - 3	9	130	0:50:00	34:19:00	13300	32:03:00	170000	1:12:00	2.23
3/6/2012	Batch - 3	10	130	0:55:00	34:16:00	13700	31:58:00	107000	1:04:00	2.32
3/6/2012	Batch - 3	11	130	0:46:00	33:47:00	15200	31:37:00	119000	1:09:00	2.26
3/6/2012	Batch - 3	12	180	0:54:00	33:12:00	8520	31:08:00	42600	0:55:00	2.28
3/6/2012	Batch - 3	13	340	0:52:00	31:58:00	11400	30:00:00	80800	1:03:00	2.05
3/9/2012	SBL02	1	120	-	-	bd	30:46:00	68800	1:00:00	-
3/9/2012	SBL02	2	120	-	-	bd	30:51:00	65600	0:54:00	-
3/9/2012	SBL02	3	120	-	-	bd	30:03:00	65000	0:56:00	-
3/9/2012	SBL02	4	120	-	-	bd	29:37:00	70500	0:55:00	-
3/9/2012	SBL02	5	120	-	-	bd	29:38:00	62200	0:51:00	-
3/9/2012	SBL02	6	120	-	-	bd	29:21:00	69740	0:56:00	-
3/13/2012	SBL02	7	120	-	-	bd	29:45:00	64500	0:55:00	-
3/13/2012	SBL02	8	120	-	-	bd	29:02:00	66400	0:53:00	-
3/13/2012	SBL02	9	120	-	-	bd	28:56:00	66200	0:53:00	-
3/13/2012	SBL02	10	120	-	-	bd	28:52:00	66100	0:52:00	-
3/13/2012	SBL02	11	120	-	-	bd	28:49:00	67700	0:54:00	-
3/13/2012	SBL02	12	120	-	-	bd	28:36:00	63000	0:53:00	-
3/13/2012	SBL02	13	100	-	-	bd				-
3/19/2012	Batch - 5	1	100	0:54:00	13:56:00	11700	11:34:00	73000	0:59:00	2.51
3/19/2012	Batch - 5	2	100	1:10:00	34:46:00	16600	32:25:00	72600	1:19:00	1.89
3/19/2012	Batch - 5	3	85	0:58:00	34:45:00	11600	32:16:00	62100	1:19:00	2.18
3/19/2012	Batch - 5	4	85	0:55:00	34:21:00	10700	32:03:00	61500	1:00:00	2.40
3/19/2012	Batch - 5	5	85	0:59:00	34:08:00	16000	31:52:00	64000	1:03:00	2.23
3/20/2012	Batch - 5	6	85	0:52:00	35:01:00	13300	32:38:00	57200	1:03:00	2.49
3/20/2012	Batch - 5	7	85	1:02:00	34:20:00	11700	32:04:00	60900	1:07:00	2.11
3/20/2012	Batch - 5	8	85	0:59:00	34:16:00	13800	31:57:00	65800	1:09:00	2.17
3/20/2012	Batch - 5	9	85	0:56:00	34:13:00	13000	31:55:00	67100	1:05:00	2.28
3/20/2012	Batch - 5	10	85	0:43:00	33:54:00	12400	31:41:00	66100	1:02:00	2.53
3/20/2012	Batch - 5	11	85	1:06:00	34:04:00	14800	31:47:00	66700	1:06:00	2.08
3/20/2012	Batch - 5	12	85	0:56:00	34:04:00	13600	31:47:00	65900	1:12:00	2.14
3/21/2012	Batch - 5	13	85	0:55:00	35:14:00	14200	32:51:00	63700	1:03:00	2.42
3/21/2012	Batch - 5	14	85	1:02:00	34:35:00	12800	32:17:00	69900	1:09:00	2.11
3/21/2012	Batch - 5	15	85	1:00:00	34:26:00	15600	32:10:00	71900	1:14:00	2.03
3/21/2012	Batch - 5	16	85	0:53:00	34:40:00	11200	32:20:00	64700	1:15:00	2.19
3/27/2012	Batch - 5	17	85	1:05:00	36:57:00	1.36E4	34:19:00	6.34E4	1:07:00	2.39
3/27/2012	Batch - 5	18	57	0:58:00	36:52:00	8.62E3	34:11:00	4.11E4	1:11:00	2.50

¹bd= below detection

²Resolution of d38-DEHP and unlabeled DEHP signals λ=254 nm.

Table A2.7.b Injections, peak properties, and resolution of DEHP by HPLC; batch 4, batch 7, and SBL03.

Date	Sample	Injection No.	volume sample solution injected (μL)	DEHP width at 254 nm (min)	DEHP T _R	¹ DEHP Peak Area	D38 DEHP T _R	D38 peak area	D38 DEHP peak width at 254 nm (s)	R ²
3/27/2012	Batch - 4	1	120	0:52:00	36:30:00	1.28E4	33:57:00	6.74E4	1:13:00	2.45
3/27/2012	Batch - 4	2	120	0:48:00	35:50:00	1.19E4	33:22:00	6.73E4	0:59:00	2.77
3/27/2012	Batch - 4	3	120	0:55:00	35:16:00	1.46E4	32:55:00	7.63E4	1:10:00	2.26
3/27/2012	Batch - 4	4	120	1:00:00	34:56:00	1.67E4	32:36:00	6.83E4	1:00:00	2.33
3/27/2012	Batch - 4	5	120	0:58:00	34:49:00	1.50E4	32:26:00	7.37E4	1:00:00	2.42
3/27/2012	Batch - 4	6	120	1:04:00	35:00:00	2.00E4	32:38:00	6.80E4	1:02:00	2.25
3/27/2012	Batch - 4	7	120	0:54:00	34:52:00	1.25E4	32:32:00	6.88E4	1:02:00	2.41
3/27/2012	Batch - 4	8	120	0:53:00	34:41:00	1.67E4	32:23:00	7.18E4	1:00:00	2.44
3/27/2012	Batch - 4	9	120	0:57:00	34:20:00	1.39E4	32:04:00	7.18E4	1:00:00	2.32
3/27/2012	Batch - 4	10	120	1:01:00	33:58:00	1.82E4	31:43:00	6.77E4	0:58:00	2.27
3/27/2012	Batch - 4	11	120	0:57:00	33:45:00	2.08E4	31:33:00	7.27E4	1:00:00	2.26
3/27/2012	Batch - 4	12	120	0:52:00	33:14:00	1.15E4	31:06:00	7.17E4	1:00:00	2.29
3/27/2012	Batch - 4	13	120	0:53:00	33:26:00	1.51E4	31:15:00	7.24E4	1:08:00	2.17
3/27/2012	Batch - 4	14	120	1:05:00	34:12:00	1.78E4	31:56:00	7.19E4	0:59:00	2.19
3/27/2012	Batch - 4	15	80	0:55:00	33:39:00	1.14E4	31:29:00	4.98E4	1:01:00	2.24
3/29/2012	SBL03	1	105	-	-	bd	30:43:00	9.05E4	1:00:00	-
3/29/2012	SBL03	1	105	-	-	bd	30:35:00	9.50E4	1:00:00	-
3/29/2012	SBL03	1	105	-	-	bd	31:28:00	9.06E4	1:00:00	-
3/29/2012	SBL03	1	105	-	-	bd	31:23:00	8.90E4	1:00:00	-
3/29/2012	SBL03	1	105	-	-	bd	31:27:00	9.50E4	1:02:00	-
3/29/2012	SBL03	1	105	-	-	bd	31:34:00	8.82E4	1:01:00	-
3/29/2012	SBL03	1	105	-	-	bd	31:19:00	9.65E4	0:59:00	-
3/29/2012	SBL03	1	110	-	-	bd	30:53:00	1.03E5	1:00:00	-
4/19/2012	Batch - 7	1	200	1:01:00	29:45:00	1.53E4	27:47:00	6.07E4	1:05:00	1.87
4/19/2012	Batch - 7	2	200	0:54:00	33:22:00	1.77E4	31:16:00	6.16E4	1:00:00	2.21
4/19/2012	Batch - 7	3	200	1:00:00	33:09:00	1.97E4	31:06:00	6.14E4	0:59:00	2.07
4/19/2012	Batch - 7	4	200	0:58:00	32:53:00	1.79E4	30:53:00	6.48E4	1:02:00	2.00
4/19/2012	Batch - 7	5	200	0:59:00	32:25:00	2.25E4	30:29:00	6.12E4	1:00:00	1.95
4/22/2012	Batch - 7	6	190	1:05:00	35:46:00	1.76E4	33:27:00	6.70E4	1:21:00	1.90
4/22/2012	Batch - 7	7	200	0:56:00	36:23:00	1.40E4	33:53:00	6.82E4	1:27:00	2.10
4/22/2012	Batch - 7	8	200	1:00:00	36:07:00	2.11E4	33:43:00	6.85E4	1:20:00	2.06
4/22/2012	Batch - 7	9	225	1:05:00	34:49:00	2.36E4	32:36:00	7.19E4	1:13:00	1.93
4/22/2012	Batch - 7	10	215	0:51:00	34:36:00	2.21E4	32:25:00	7.24E4	1:22:00	1.97
4/24/2012	Batch - 7	11	200	1:03:00	34:45:00	2.17E4	32:36:00	6.54E4	1:17:00	1.84
4/24/2012	Batch - 7	12	200	0:59:00	33:39:00	2.10E4	31:35:00	6.71E4	1:12:00	1.89
4/24/2012	Batch - 7	13	160	1:08:00	33:09:00	1.70E4	31:08:00	5.82E4	1:17:00	1.67

¹bd= below detection

²Resolution of d38-DEHP and unlabeled DEHP signals λ=254 nm.

Table A2.7.c Injections, peak properties, and resolution of DEHP by HPLC; batch 6 and SBL04.

Date	Sample	Injection No.	volume sample solution injected (μL)	DEHP width at 254 nm (min)	DEHP T _R	¹ DEHP Peak Area	D38 DEHP T _R	D38 peak area	D38 DEHP peak width at 254 nm (s)	R ²
4/25/2012	SBL04	1	175	-	-	bd	32:31:00	2.51E+04	0:56:00	-
4/25/2012	SBL04	2	175	-	-	bd	32:23:00	2.78E+04	0:53:00	-
4/25/2012	SBL04	3	175	-	-	bd	19:23:00	2.60E+04	0:56:00	-
4/25/2012	SBL04	4	175	-	-	bd	30:54:00	2.66E+04	0:56:00	-
4/25/2012	SBL04	5	175	-	-	bd	30:42:00	2.74E+04	0:57:00	-
4/25/2012	SBL04	6	175	-	-	bd	30:49:00	2.60E+04	0:57:00	-
4/25/2012	SBL04	7	175	-	-	bd	30:34:00	2.98E+04	0:59:00	-
4/25/2012	SBL04	8	175	-	-	bd	30:19:00	2.63E+04	0:58:00	-
4/25/2012	SBL04	9	175	-	-	bd	30:07:00	2.76E+04	0:54:00	-
4/25/2012	SBL04	10	150	-	-	bd	29:53:00	2.42E+04	0:55:00	-
4/25/2012	SBL04	11	150	-	-	bd	29:43:00	2.07E+04	0:47:00	-
4/26/2012	SBL04	12	150	-	-	bd	30:46:00	2.27E+04	0:53:00	-
4/26/2012	SBL04	13	130	-	-	bd	30:12:00	2.16E+04	0:54:00	-
4/26/2012	Batch - 6	1	250	0:55:00	32:02:00	2.82E4	30:09:00	1.20E5	1:08:00	1.84
4/26/2012	Batch - 6	2	200	0:49:00	32:12:00	1.55E4	30:19:00	9.93E4	1:04:00	2.00
4/26/2012	Batch - 6	3	215	0:58:00	32:03:00	2.14E4	30:10:00	1.04E5	1:04:00	1.85
4/26/2012	Batch - 6	4	215	0:55:00	31:52:00	1.76E4	30:00:00	1.05E5	1:03:00	1.90
4/26/2012	Batch - 6	5	215	0:55:00	31:40:00	2.01E4	29:48:00	1.07E5	1:05:00	1.87
4/26/2012	Batch - 6	6	215	1:00:00	31:40:00	2.11E4	29:49:00	1.01E5	1:06:00	1.76
4/27/2012	Batch - 6	7	215	0:51:00	34:23:00	1.67E4	32:13:00	1.08E5	1:08:00	2.18
4/27/2012	Batch - 6	8	215	0:56:00	32:26:00	2.31E4	30:32:00	1.11E5	1:08:00	1.84
4/27/2012	Batch - 6	9	215	1:02:00	32:06:00	2.26E4	30:14:00	1.07E5	1:05:00	1.76
4/27/2012	Batch - 6	10	215	0:50:00	31:52:00	1.79E4	30:00:00	1.07E5	1:05:00	1.95
4/30/2012	Batch - 6	11	215	0:54:00	34:02:00	1.93E4	31:59:00	1.03E5	1:09:00	2.00
4/30/2012	Batch - 6	12	200	0:57:00	33:38:00	1.87E4	31:34:00	1.02E5	1:07:00	2.00
4/30/2012	Batch - 6	13	200	0:58:00	33:28:00	1.75E4	31:24:00	9.68E4	1:06:00	2.00
4/30/2012	Batch - 6	14	210	0:54:00	33:07:00	1.97E4	31:07:00	1.11E5	1:12:00	1.90
4/30/2012	Batch - 6	15	200	0:48:00	33:05:00	1.64E4	31:05:00	1.09E5	0:51:00	2.42

¹bd= below detection

²Resolution of d38-DEHP and unlabeled DEHP signals $\lambda=254$ nm.

Table A2.8.a Time and HPLC eluent volume of DEHP fraction collections; batch 3, batch 5, and SBL02.

Date	Sample	Injection No.	flow rate (mL/min)	DEHP Fraction Collection Start Time	DEHP Fraction Collection End Time	DEHP Fraction Volume (mL)
3/2/2012	Batch - 3	1	4			
3/2/2012	Batch - 3	2	4	32:30:00	33:15:00	0.13
3/5/2012	Batch - 3	3	4	33:47:00	34:38:00	3.40
3/5/2012	Batch - 3	4	4	33:03:00	33:53:00	3.33
3/5/2012	Batch - 3	5	4	32:57:00	33:45:00	3.20
3/5/2012	Batch - 3	6	4	32:45:00	33:35:00	3.33
3/5/2012	Batch - 3	7	4	33:02:00	33:51:00	3.27
3/5/2012	Batch - 3	8	4	33:32:00	34:17:00	3.00
3/6/2012	Batch - 3	9	4	33:54:00	34:44:00	3.33
3/6/2012	Batch - 3	10	4	33:55:00	34:45:00	3.33
3/6/2012	Batch - 3	11	4	33:32:00	32:20:00	3.20
3/6/2012	Batch - 3	12	4	33:00:00	33:42:00	2.80
3/6/2012	Batch - 3	13	4	31:38:00	32:25:00	3.13
	Total					35.33
3/9/2012	SBL02	1	4	32:34:00	33:27:00	3.53
3/9/2012	SBL02	2	4	32:35:00	33:28:00	3.53
3/9/2012	SBL02	3	4	31:48:00	32:41:00	3.53
3/9/2012	SBL02	4	4	31:24:00	32:17:00	3.53
3/9/2012	SBL02	5	4	31:22:00	32:15:00	3.53
3/9/2012	SBL02	6	4	31:07:00	32:00:00	3.53
3/13/2012	SBL02	7	4	31:30:00	32:23:00	3.53
3/13/2012	SBL02	8	4	31:11:00	32:04:00	3.53
3/13/2012	SBL02	9	4	31:05:00	31:58:00	3.53
3/13/2012	SBL02	10	4	31:00:00	31:53:00	3.53
3/13/2012	SBL02	11	4	30:58:00	31:51:00	3.53
3/13/2012	SBL02	12	4	30:44:00	31:37:00	3.53
3/13/2012	SBL02	13	4	30:32:00	31:25:00	3.53
	Total					45.90
3/19/2012	Batch - 5	1	4	13:36:00	14:27:00	3.40
3/19/2012	Batch - 5	2	4	34:14:00	35:18:00	4.40
3/19/2012	Batch - 5	3	4	34:14:00	35:04:00	3.33
3/19/2012	Batch - 5	4	4	33:58:00	34:50:00	3.47
3/19/2012	Batch - 5	5	4	33:46:00	34:42:00	3.73
3/20/2012	Batch - 5	6	4	34:42:00	35:31:00	3.27
3/20/2012	Batch - 5	7	4	33:57:00	34:47:00	3.33
3/20/2012	Batch - 5	8	4	33:53:00	34:50:00	3.80
3/20/2012	Batch - 5	9	4	33:49:00	34:40:00	3.40
3/20/2012	Batch - 5	10	4	33:40:00	34:20:00	2.67
3/20/2012	Batch - 5	11	4	33:39:00	34:32:00	3.53
3/20/2012	Batch - 5	12	4	33:43:00	34:35:00	3.47
3/21/2012	Batch - 5	13	4	34:54:00	35:44:00	3.33
3/21/2012	Batch - 5	14	4	34:14:00	35:04:00	4.00
3/21/2012	Batch - 5	15	4	34:04:00	35:04:00	4.00
3/21/2012	Batch - 5	16	4	34:16:00	35:06:00	3.33
3/27/2012	Batch - 5	17	4	36:33:00	37:26:00	3.53
3/27/2012	Batch - 5	18	4	36:14:00	37:24:00	4.67
	Total					64.67

Table A2.8.b Time and HPLC eluent volume of DEHP fraction collections; batch 4, batch 7, and SBL04.

Date	Sample	Injection No.	flow rate (mL/min)	DEHP Fraction Collection Start Time	DEHP Fraction Collection End Time	DEHP Fraction Volume (mL)
3/27/2012	Batch - 4	1	4	36:07:00	36:57:00	3.33
3/27/2012	Batch - 4	2	4	35:27:00	36:22:00	3.67
3/27/2012	Batch - 4	3	4	34:54:00	35:46:00	3.47
3/27/2012	Batch - 4	4	4	34:33:00	35:30:00	3.80
3/27/2012	Batch - 4	5	4	34:24:00	35:20:00	3.73
3/27/2012	Batch - 4	6	4	34:34:00	35:35:00	4.07
3/27/2012	Batch - 4	7	4	34:29:00	34:21:00	3.47
3/27/2012	Batch - 4	8	4	34:26:00	35:15:00	3.27
3/27/2012	Batch - 4	9	4	34:02:00	34:50:00	3.20
3/27/2012	Batch - 4	10	4	33:40:00	33:38:00	3.87
3/27/2012	Batch - 4	11	4	33:22:00	34:20:00	3.87
3/27/2012	Batch - 4	12	4	32:57:00	33:47:00	3.33
3/27/2012	Batch - 4	13	4	33:05:00	33:54:00	3.27
3/27/2012	Batch - 4	14	4	33:49:00	34:45:00	3.73
3/27/2012	Batch - 4	15	4	33:19:00	34:09:00	3.33
	Total					53.40
3/29/2012	SBL03	1	4	32:36:00	33:33:00	3.80
3/29/2012	SBL03	1	4	32:27:00	33:24:00	3.80
3/29/2012	SBL03	1	4	33:20:00	34:17:00	3.80
3/29/2012	SBL03	1	4	33:15:00	34:12:00	3.80
3/29/2012	SBL03	1	4	33:21:00	34:18:00	3.80
3/29/2012	SBL03	1	4	33:26:00	34:23:00	3.80
3/29/2012	SBL03	1	4	33:10:00	34:07:00	3.80
3/29/2012	SBL03	1	4	32:46:00	33:43:00	3.80
	Total					30.40
4/19/2012	Batch - 7	1	4	29:37:00	30:18:00	2.73
4/19/2012	Batch - 7	2	4	33:00:00	33:53:00	3.53
4/19/2012	Batch - 7	3	4	32:48:00	33:45:00	3.80
4/19/2012	Batch - 7	4	4	32:35:00	33:25:00	3.33
4/19/2012	Batch - 7	5	4	32:06:00	33:03:00	3.80
4/22/2012	Batch - 7	6	4	35:23:00	36:18:00	3.67
4/22/2012	Batch - 7	7	4	36:00:00	36:57:00	3.80
4/22/2012	Batch - 7	8	4	35:43:00	35:43:00	4.00
4/22/2012	Batch - 7	9	4	34:23:00	35:26:00	4.20
4/22/2012	Batch - 7	10	4	34:13:00	35:13:00	4.00
4/24/2012	Batch - 7	11	4	34:19:00	35:19:00	4.00
4/24/2012	Batch - 7	12	4	33:15:00	34:14:00	3.93
4/24/2012	Batch - 7	13	4	32:44:00	33:44:00	4.00
	Total					48.79

Table A2.8.c Time and HPLC eluent volume of DEHP fraction collections; batch 4, batch 7, and SBL04.

Date	Sample	Injection No.	flow rate (mL/min)	DEHP Fraction Collection Start Time	DEHP Fraction Collection End Time	DEHP Fraction Volume (mL)
4/25/2012	SBL04	1	4	34:02:00	35:02:00	4.00
4/25/2012	SBL04	2	4	33:57:00	34:57:00	4.00
4/25/2012	SBL04	3	4	21:04:00	528:00:00	3.73
4/25/2012	SBL04	4	4	32:28:00	33:20:00	3.47
4/25/2012	SBL04	5	4	32:20:00	33:10:00	3.33
4/25/2012	SBL04	6	4	32:27:00	33:22:00	3.67
4/25/2012	SBL04	7	4	32:14:00	33:06:00	3.47
4/25/2012	SBL04	8	4	31:55:00	32:48:00	3.53
4/25/2012	SBL04	9	4	31:49:00	32:39:00	3.33
4/25/2012	SBL04	10	4	31:30:00	32:22:00	3.47
4/25/2012	SBL04	11	4	31:15:00	32:07:00	3.47
4/26/2012	SBL04	12	4	32:22:00	33:15:00	3.53
4/26/2012	SBL04	13	4	31:47:00	31:40:00	3.53
	Total					46.53
4/26/2012	Batch - 6	1	4	31:41:00	32:31:00	3.33
4/26/2012	Batch - 6	2	4	31:55:00	32:40:00	3.00
4/26/2012	Batch - 6	3	4	31:44:00	32:47:00	3.80
4/26/2012	Batch - 6	4	4	31:22:00	32:22:00	4.00
4/26/2012	Batch - 6	5	4	31:19:00	32:12:00	3.53
4/26/2012	Batch - 6	6	4	31:20:00	32:14:00	3.60
4/27/2012	Batch - 6	7	4	34:00:00	34:53:00	3.53
4/27/2012	Batch - 6	8	4	32:05:00	32:57:00	3.47
4/27/2012	Batch - 6	9	4	~31:56	32:45:00	3.27
4/27/2012	Batch - 6	10	4	31:29:00	32:19:00	3.33
4/30/2012	Batch - 6	11	4	33:40:00	34:31:00	3.40
4/30/2012	Batch - 6	12	4	33:20:00	34:15:00	3.67
4/30/2012	Batch - 6	13	4	33:04:00	34:02:00	3.87
4/30/2012	Batch - 6	14	4	32:49:00	33:39:00	3.33
4/30/2012	Batch - 6	15	4	32:49:00	33:36:00	3.13
	Total					52.27

Appendix 3: GC-EIMS Calibrant Preparation for Quantification of DEHP in AMS Samples

A3.1 Pilot Sample

Table A4.1 lists the masses of DEHP and dilution measured during the preparation of a stock standard solution to subsequently dilute as GC-EIMS calibrants for estimating DEHP mass in sample ST01. Those representing the Stock solution dilution during preparation of individual calibrants are listed in Table A4.2, along with their integrated $m/z=149$ integrations after analysis by GC-EIMS.

Table A4.1 Stock solution preparation for GC-EIMS calibration for DEHP quantification of ST01.

	Mass neat DEHP (g)	±	Mass of Solution Diluted with MeCl ₂ (g)	±	[DEHP] (per mil)	±	[DEHP] (µg/g)	±
Stock S1	0.01945	7E-05	1.31245	1.0E-04	14820	51	14820	51
	Mass of 10 mL Flask (g)	Mass of Dilution 1 added to Flask (g)	Mass of Flask and Solution Diluted with MeCl ₂ (g)	Mass of Solution in MeCl ₂ (g)	Mass DEHP added (µg)	±	[DEHP] (µg/g)	±
Stock SW1	12.5855	1.2257	25.17843	12.5929	18165	63	1442	5

Table A3.2 Calibration preparation and $m/z=149$ peak integrations for GC-EIMS quantification of sample ST01.

Calibrant ID #	Mass of Stock SW1 added (g)	Final Mass of Standard Solution (g)	[DEHP] ($\mu\text{g/g}$)	\pm	Mean $m/z=149$ peak area	Std. Dev.
1	0.01235	1.34809	13.2103198	1.0	7470055	35783
3	0.04292	1.25298	49.3947549	1.0	34859265	346777
4	0.05748	1.27369	65.0756149	1.0	52262559	709106
5	0.07206	1.31648	78.9305724	1.0	60023597	1305822
6	0.08461	1.27475	95.7110178	1.0	97892808	2083066
7	0.11335	1.27397	128.300274	1.4	1.28E+08	929237
8	0.12747	1.24355	147.812102	1.6	1.36E+08	55036692
9	0.14501	1.2929	161.732864	1.7	1.56E+08	707169

A3.2 June 2012 Sample Set

The masses measured during gravimetric dilution of DEHP in volumetric glassware to prepare a second set of stock solutions for GC-EIMS DEHP calibrants analyzed with the second set of AMS samples, along with their mass fractions of DEHP, are in Table A4.3. The masses measured during gravimetric preparation of GC-EIMS calibrants from each of these solutions and their mass fractions of DEHP are in Tables A4.4.a through A4.4.c.

Table A3.3 Mass measurements in triplicate during stock solution preparation for GC-EIMS calibration of DEHP in sample set 2.

	Mass of Flask (g)	Mass of Flask and Neat DEHP (g)	Mass of DEHP Added (mg)	Mass of Flask and Final Solution in Acetonitrile	Mass of Solution	[DEHP] ($\mu\text{g/g}$)
Stock 1	11.02435	11.07574		14.855892		
	11.02434	11.07574		14.85595		
	11.02436	11.07573		14.85593		
Mean	11.02435	11.075737	51.38667	14.855924	3.86287	13411.4
Uncertainty	1E-05	1E-05	0.01155	3E-05	3E-05	3.0
Stock 2	11.77956	11.83511		15.53828		
	11.77951	11.83511		15.53830		
	11.77954	11.83506		15.53831		
Mean	11.779537	11.835093	55.55667	15.53829	3.75876333	14780.6
Uncertainty	3E-05	3E-05	0.03830	1E-05	3E-05	10.2
Stock 3	12.15882	12.21779		16.01618		
	12.15879	12.21776		16.01613		
	12.15881	12.21773		16.01613		
Mean	12.158807	12.21776	58.95333	16.01614667	3.85732333	15283.5
Uncertainty	2E-05	3E-05	0.03367	3E-05	3E-05	8.7

Table A3.4.a Calibrants for GC-EIMS quantification of DEHP in ST02, ST03, ST04, and ST05.

Calibrants made with Dilute Stock DEHP Standard Solution 1 : 13411.4 (µg/g) ± 3.0 (µg/g)

Calibrant ID	Mass of Flask (g)	Mass of Flask and Stock Solution (g) after Addition to Calibrant	Mass of DEHP added (µg)	Mass of Flask and Final solution (g)	Mass of Final Solution (g)	[DEHP] (µg/g)
1-1	25.21281	3.69256		59.26446		
	25.2128	3.69258		59.26449		
	25.21282	3.69257		59.26439		
Mean	25.21281	3.69257	1704.27	59.26444667	34.05164	50.05
Uncertainty	1 E-05	1E-05	0.44	5E-05	5E-05	0.01
1-2	23.57358	3.51277		56.52659		
	23.5736	3.51278		56.52621		
	23.57361	3.51277		56.52626		
Mean	23.57360	3.51277	2411.32	56.52635333	32.95276	71.01
Uncertainty	2E-05	6E-06	0.56	2.E-4	2.1E-04	0.02
1-3	23.44281	3.32637		56.22476		
	23.44281	3.32635		56.22473		
	23.44284	3.32638		56.22472		
Mean	23.44282	3.32637	2500.00	56.22473667	32.78192	74.47
Uncertainty	2E-05	2E-05	0.60	2E-05	3E-05	0.02
1-4	24.69276	3.12884		57.26988		
	24.69276	3.12881		57.26988		
	24.6928	3.12883		57.26989		
Mean	24.69277	3.12883	2649.28	57.26988333	32.57711	81.32
Uncertainty	2E-05	2E-05	0.66	6E-06	2E-05	0.02
1-5	28.0569	2.91933		60.88524		
	28.05686	2.91931		60.88516		
	28.05689	2.91932		60.88519		
Mean	28.05688	2.91932	2809.77	60.88519667	32.82831	83.69
Uncertainty	2E-05	1E-05	0.677579	4.0E-05	5E-05	0.02
1-6	27.60081	2.69759		60.46029		
	27.60079	2.69756		60.46034		
	27.6074	2.697587		60.46027		
Mean	27.60300	2.69758	2973.85	60.4603	32.85730	90.51
Uncertainty	0.00381	2E-05	0.72	4E-05	3.8E-03	0.02

Table A3.4b Calibrants for GC-EIMS quantification of DEHP in ST02, ST03, ST04, and ST05

Calibrants made with dilute Stock DEHP Standard Solution 2 : 14780.6 (µg/g) ± 10.2 (µg/g)

Calibrant ID	Mass of Flask (g)	Mass of Flask and Stock Solution (g) after Addition to Calibrant	Mass of DEHP added (µg)	Mass of Flask and Final solution (g)	Mass of Final Solution (g)	[DEHP] (µg/g)
2-1	23.80610	3.75284		56.44077		
	23.80614	3.75280		56.44074		
	23.80614	3.75280		56.44068		
Mean	23.80613	3.75281	2202.50	56.44073	32.63460	67.49
Uncertainty	2.3E-05	2E-05	1.58	5E-05	5E-05	0.05
2-2	24.35995	3.59348		57.29347		
	24.35993	3.59348		57.29340		
	24.35994	3.59348		57.29337		
Mean	24.35994	3.59348	2355.04	57.29341	32.93347	71.51
Uncertainty	1.0E-05	1E-05	1.66	3E-05	3E-05	0.05
2-3	24.03625	3.42509		56.56331		
	24.03624	3.42506		56.56326		
	24.03626	3.42512		56.56321		
Mean	23.70292	3.42509	2488.90	56.56326	32.86034	75.74
Uncertainty	1E-05	3E-05	1.77	5E-05	6E-01	1.33
2-4	26.22019	3.24558		59.06598		
	26.22018	3.24555		59.06596		
	26.22019	3.24557		59.06585		
Mean	26.22019	3.24557	2653.46	59.06593	32.84574	80.79
Uncertainty	6E-06	2E-05	1.90	7E-05	7E-05	0.06
2-5	22.96494	3.05503		55.72726		
	22.96491	3.05498		55.72728		
	22.96489	3.05500		55.72725		
Mean	22.96491	3.05500	2816.63	55.72726	32.76235	85.97
Uncertainty	3E-05	3E-05	1.99	2E-05	3E-05	0.06
2-6	24.35445	2.85493		57.38009		
	24.36450	2.85491		57.38000		
	24.36448	2.85496		57.38001		
Mean	24.36114	2.85493	2957.15	57.38003	33.01889	89.56
Uncertainty	5.8E-03	3E-05	2.11	5E-05	6E-03	0.07

Table A3.4.c Calibrants for GC-EIMS quantification of DEHP in ST02, ST03, ST04, and ST05; not used for regression to calibrate GC-EIMS of samples.

Calibrants made with Dilute Stock DEHP Standard Solution 3 : 15283.5 (µg/g) ± 8.7 (µg/g)

Calibrant ID	Mass of Flask (g)	Mass of Flask and Stock Solution (g) after Addition to Calibrant	Mass of DEHP added (µg)	Mass of Flask and Final solution (g)	Mass of Final Solution (g)	[DEHP] (µg/g)
3-1	27.96410	3.60586		60.78922		
	27.96405	3.60585		60.78922		
	27.96403	3.60585		60.78919		
Mean	27.96406	3.60585	2225.28	60.78921	32.82515	67.79
Uncertainty	4E-05	1E-05	1.28	0.00002	4.E-05	0.04
3-2	24.90084	3.45050		60.78922		
	24.90080	3.45045		58.15417		
	24.90081	3.45051		58.15416		
Mean	24.90080	3.45049	2374.54	58.15411	33.25329	68.73
Uncertainty	2E-05	3E-05	1.45	3E-05	4E-05	0.04
3-3	22.76698	3.28578		55.69377		
	22.76698	3.28579		55.69375		
	22.76698	3.28579		55.69376		
Mean	22.76698	3.28579	2517.19	55.69376	32.92678	76.45
Uncertainty	1E-05	1E-05	1.51	5E-05	1.E-05	0.05
3-4	25.78110	3.11196		59.49361		
	25.78111	3.11192		59.49358		
	25.78115	3.11193		59.49368		
Mean	25.78112	3.11194	2657.03	59.43623	33.71250	78.81
Uncertainty	3E-05	2E-05	1.55	5E-05	6.E-05	0.05
3-5	23.51779	2.91852		56.16008		
	23.51780	2.91855		56.16002		
	23.51778	2.91853		56.16001		
Mean	2.4E+01	2.91853	2955.88	56.16004	32.64225	90.55
Uncertainty	1E-05	2E-05	1.73	4E-05	4.E-05	0.05
3-6	23.39063	2.73477		56.15775		
	23.39061	2.73476		56.15772		
	23.39059	2.73475		56.15766		
Mean	23.51779	2.73476	2808.70	56.15771	32.76710	85.72
Uncertainty	2E-05	1E-05	1.63	5E-05	5.E-05	0.05

The stock solutions used to prepare calibrants for analysis of the method blanks in the 2012 sample set were made from two dilutions of neat DEHP, whose masses and DEHP mass fractions are listed in Tables A4.5, A4.6, and A4.7. Table A4.6 shows adjustments to DEHP mass fractions that were made to account for concentration of DEHP by solvent evaporation while the stock solution was in storage after its previous dilution and prior to its secondary. Tables A4.8.a, A4.8.b, and A4.8.c contain the masses measured during stock dilution in each calibrant analyzed with the method blanks of the June 2012 sample set, along with their DEHP mass fractions.

Table A3.5 Mass measurements in triplicate during first dilution of primary stock solution preparation for GC-EIMS calibration of DEHP in method blanks of sample set 2.

	Mass of Flask	Mass of Flask and Neat DEHP (g)	Mass of DEHP Added (mg)	Mass of Flask and Final Solution in MeCl ₂	Mass of Solution	[DEHP] (µg/g)
Stock B1	12.43093	12.44107	10.19	18.96932	6.53844	1558.5
	12.43088	12.4411		18.96933		
	12.43090	12.44111		18.96937		
Mean	12.43090	12.44109	10.19	18.96934	6.53844	1558.5
Uncertainty	1E-05	1E-05	0.02	2E-05	2E-05	2.9
Stock B2	12.85205	12.86773	15.68	25.91220	13.06020	1200.6
	12.85204	12.8677		25.91229		
	12.85202	12.86772		25.91221		
Mean	12.85204	12.86772	15.68	25.91223	13.06020	1200.6
Uncertainty	1E-05	1E-05	0.01	3E-05	E-05	1.0

Table A3.6 Corrections to blank primary DEHP standard solutions' mass fractions for solvent evaporation.

a.

	5/3/2012 Mass of Solution	5/3/2012 Mass of soln.	5/3/2012 [DEHP] (µg/g)	5/14/2012 Mass of Solution	[DEHP] (µg/g)
Stock B1	6.50541	4.66	1566.4	4.37447	1667.1
Uncertainty	4E-05	12.43	2.9	3E-05	3.1

b.

	5/3/2012			5/4/2012				
	5/3/2012 Mass of Solution (g)	5/3/2012 Mass after Removal (g)	5/3/2012 [DEHP] (µg/g)	5/4/2012 Mass of Solution (g)	5/4/2012 Mass after Removal (g)	5/4/2012 [DEHP] (µg/g)	5/14/2012 2 Mass of Solution (g)	5/14/2012 2 [DEHP] (µg/g)
Stock B2	13.04316	10.38336	1201.42	10.37215	7.94155	1202.7	7.59637	1257.4
Uncertainty	6E-05	2E-05	0.96	7E-05	4E-05	1.0	2E-05	1.0

Table A3.7 Mass measurements in triplicate during secondary DEHP standard stock solution preparation for GC-EIMS calibration of DEHP in method blanks of sample set 2 on 5/14/2012.

	Mass of Flask	Mass of Flask and Primary Stock added (g)	Mass of DEHP Added (mg)	Mass of Flask and Final Solution in MeCl ₂	Mass of Solution	[DEHP] (µg/g)
Stock WB2: from Stock B1	23.78528	2.04531		56.59214		
	23.78525	2.04535		56.59209		
	23.78528	2.04534		56.59208		
Mean	23.78527	2.04533	3401	56.59210	32.80683	103.9
Uncertainty	1E-05	1E-05	6	2E-05	2E-05	0.2
Stock WB2: from Stock B2	24.13365	1.74846		56.96880		
	24.13364	1.74847		56.96879		
	24.13367	1.7485		56.96887		
Mean	24.13365	1.74848	2198	56.96882	32.83517	67.0
Uncertainty	1E-05	1E-05	2	3E-05	3E-05	0.1
Stock WB3: from Stock B2	22.62654	2.02457		56.02025		
	22.62655	2.0245		56.02027		
	22.62657	2.02455		56.02023		
Mean	22.62655	2.02454	2546	56.02025	33.39370	76.2
Uncertainty	1E-05	2E-05	2	1E-05	2E-05	0.1

Table A3.8 Masses measured in triplicate 5/14/2012 during preparation of calibrants for DEHP quantification in method blanks SBL02, SBL03, SBL04.

Table A4.8.a Calibration Set B1.

Calibrants made with Secondary Blank Calibrations Stock Solution Stock WB1:
103.9 µg/g ± 0.2 µg/g.

Calibrant ID	Mass of Flask (g)	Mass of Stock Solution (g) after Addition to Calibrant	Mass of DEHP added (ug)	Mass of Flask and Final solution (g)	Mass of Final Solution (g)	[DEHP] (µg/g)
B1-1	12.95685	4.07970		26.18740		
	12.95684	4.07967		26.18748		
	12.95686	4.07967		26.18735		
Mean	12.95685	4.07968	5.49	26.18741	13.23056	0.415
Uncertainty	1E-05	2E-05	0.01	7E-05	7E-05	2.1E-03
B1-2	12.05297	3.99515		24.80377		
	12.05296	3.99513		24.80379		
	12.05294	3.99509		24.80386		
Mean	12.05296	3.99512	8.79	24.80381	12.75085	0.690
Uncertainty	2E-05	3E-05	0.02	5E-05	5E-05	1.9E-03
B1-3	12.11683	3.89164		25.22540		
	12.11682	3.89165		25.22538		
	12.11682	3.89160		25.22538		
Mean	12.11682	3.89163	10.76	25.22539	13.10856	0.821
Uncertainty	1E-05	2E-05	0.02	1E-05	1E-05	1.9E-03
B1-4	13.04847	3.76594		25.84442		
	13.04851	3.76591		25.84441		
	13.04848	3.76590		25.84435		
Mean	13.04849	3.76592	13.07	25.84439	12.79591	1.022
Uncertainty	2E-05	2E-05	0.02	3.8E-05	4E-05	1.9E-03
B1-5	16.54173	3.61176		29.59445		
	16.54171	3.61171		29.59442		
	16.54172	3.61173		29.59439		
Mean	16.54172	3.61173	16.03	29.59442	13.05270	1.228
Uncertainty	1E-05	3E-05	0.03	3E-05	3E-05	1.9E-03
B1-6	13.29670	3.43634		26.37576		
	13.29679	3.43631		26.37566		
	13.29680	3.43628		26.37560		
Mean	13.29676	3.43631	18.24	26.37567	13.07891	1.395
Uncertainty	6E-05	3E-05	0.03	8E-05	10E-05	1.9E-03

Table A3.8.b Calibration Set B2.

Calibrants made with Secondary Blank Calibrations Stock Solution Stock WB2: 67.0 µg/g ± 0.05 µg/g.

Calibrant ID	Mass of Flask (g)	Mass of Stock Solution (g) after Addition to Calibrant	Mass of DEHP added (ug)	Mass of Flask and Final solution (g)	Mass of Final Solution (g)	[DEHP] (µg/g)
B2-1	12.30389	4.94097		25.35389		
	12.30387	4.94092		25.35391		
	12.30386	4.94093		25.35384		
Mean	12.30387	4.94094	5.49	25.35388	13.05001	0.421
Uncertainty	2E-05	3E-05	0.01	4E-05	4E-05	10E-04
B2-2	13.38233	4.82014		26.55735		
	13.38235	4.82009		26.55728		
	13.38232	4.82002		26.55729		
Mean	13.38233	4.82008	8.09	26.55731	13.17497	0.614
Uncertainty	2E-05	6E-05	0.01	4E-05	4E-05	10E-04
B2-3	16.39269	4.65144		29.64977		
	16.39265	4.65141		29.64973		
	16.39264	4.65128		29.64975		
Mean	16.39266	4.65138	10.77	29.64975	13.25709	0.812
Uncertainty	3E-05	9E-05	0.01	2E-05	3E-05	10E-04
B2-4	15.57497	4.45425		28.98224		
	15.57487	4.45426		28.98222		
	15.57488	4.45418		28.98218		
Mean	15.57491	4.45423	13.20	28.98221	13.40731	0.985
Uncertainty	2E-05	3E-05	0.01	3E-05	4E-05	10E-04
B2-5	12.79733	4.22393		25.71171		
	12.79728	4.22385		25.71168		
	12.79729	4.22386		25.71169		
Mean	12.79730	4.22388	15.43	25.71169	12.91439	1.195
Uncertainty	3E-05	4E-05	0.01	2E-05	3E-05	10E-04
B2-6	12.37800	3.94450		25.10248		
	12.37801	3.94443		25.10247		
	12.37800	3.94439		25.10247		
Mean	12.37800	3.94444	18.72	25.10247	12.72447	1.471
Uncertainty	1E-05	6E-05	0.02	1E-05	1E-05	10E-04

Table A3.8.c Calibration Set B2. Not used for final calibration to quantify DEHP in method blanks.

Calibrants made with Secondary Blank Calibrations Stock Solution Stock WB2: 67.0 µg/g ± 0.05 µg/g.

Calibrant ID	Mass of Flask (g)	Mass of Stock Solution (g) after Addition to Calibrant	Mass of DEHP added (ug)	Mass of Flask and Final solution (g)	Mass of Final Solution (g)	[DEHP] (µg/g)
B3-1	12.71534	4.97821		25.82819		
	12.71536	4.97818		25.82820		
	12.71533	4.97814		25.82815		
Mean	12.71534	4.97818	5.73	25.82818	13.11284	0.437
Uncertainty	2E-05	4E-05	0.01	3E-05	3E-05	10E-04
B3-2	12.38471	4.87214		25.64817		
	12.38473	4.87217		25.64823		
	12.38470	4.87215		25.64816		
Mean	12.38471	4.87215	8.08	25.64819	13.26347	0.610
Uncertainty	2E-05	2E-05	0.01	4E-05	4E-05	10E-04
B3-3	13.20154	4.73379		26.16925		
	13.20156	4.73373		26.16928		
	13.20149	4.73374		26.16920		
Mean	13.20153	4.73375	10.55	26.16924	12.96771	0.814
Uncertainty	4E-05	3E-05	0.01	2E-05	4E-05	10E-04
B3-4	13.56854	4.56259		26.57242		
	13.56855	4.56253		26.57236		
	13.56851	4.56252		26.57232		
Mean	13.56853	4.56255	13.06	26.57237	13.00383	1.004
Uncertainty	2E-05	4E-05	0.01	5E-05	5E-05	10E-04
B3-5	15.40680	4.35248		28.33189		
	15.40676	4.35241		28.33182		
	15.40676	4.35243		28.33184		
Mean	15.40677	4.35244	16.02	28.33185	12.92508	1.240
Uncertainty	2E-05	4E-05	0.01	4E-05	4E-05	10E-04
B3-6	13.45670	4.11140		26.56163		
	13.45658	4.11137		26.56156		
	13.45659	4.11138		26.56166		
Mean	13.45662	4.11138	18.00	26.56162	13.104993	1.374
Uncertainty	7E-05	2E-05	0.02	5E-05	8E-05	10E-04

Table A3.9 GC-EIMS calibration for quantification of DEHP in cheese isolate AMS sample; analysis sequence in descending order from row 1, and integrated $m/z=149$ relative ion abundance peaks at time of DEHP elution.

Calibrant or Sample ID	$m/z=149$ Peak Area
1-1	35706566
3-6	49307248
2-4	39418568
1-2	36473827
MeCl ₂	-
ST02	37946197.5
1-6	44666187
2-5	45429283
3-4	42332084
2-1	20285074
3-3	41054453
MeCl ₂	-
ST03	30669763.5
1-4	41493382
2-3	39661859
2-4	43578160
MeCl ₂	-
ST04	41620009.5
3-1	33336913
2-2	36800094
1-5	50825264
MeCl ₂	-
ST05	43812679
3-5	45744803
1-3	38324111
2-4	43150336

Table A3.10 GC-EIMS Calibration for quantification of DEHP in method blanks; integrated $m/z=149$ relative ion abundance peaks at time of DEHP elution.

Calibrant or Sample ID ¹	$m/z=149$ peak area
B1-1	46271
B1-2	78754
B1-3	129956
B1-4	199592
B1-5	259071
B1-6	268281
B2-1	39262
B2-2	96036
B2-3	128678
B2-4	172949
B2-5	223875
B2-6	313457
B3-1	44625
B3-2	79048
B3-3	116389
B3-4	146043
B3-5	225675
B3-6	271127
SB02	163015
SB03	78339
SB04	95397

¹Calibrants' and samples' $m/z=149$ integrated relative ion abundances not listed in order of analysis.

Appendix 4: Isotope Ratio Mass Spectrometry Data and Summary

The data obtained by $^{13}\text{C}/^{12}\text{C}$ analysis of samples, DEHP standards, lyophilized whole Stilton cheese, and standards are given in Tables A3.1, A3.2, and A3.3. Table A3.1 lists the data obtained in October 2011 of the pilot samples and first set of standards and cheese analyzed by AMS. Table A3.2 lists the data of samples and standards, containing small masses, sent for AMS analysis in June 2012. Table A3.3 lists that for analysis of larger masses of lyophilized whole Stilton cheese. The value $\delta^{13}\text{C}$ Std. Dev. listed are the standard deviations of all like-samples which were analyzed.

Table A4.1 IRMS stable isotope analysis batch results sheet, CO₂, for sample Set 1.

Name	Date	RT (Sec)	Height (nA)	¹³ C	¹⁸ O	^δ ¹⁸ O w.r.t. SMOW	Elemental Composition	^δ ¹³ C Corrected	^δ ¹³ C Mean	^δ ¹³ C Std. Dev.
Lantian STD_3	31/10/11 12:00	278.4	9.03	-32.22	-0.76	30.07	25.63			
Lantian 04d-2	31/10/11 12:09	278.8	4.79	-33.41	-1.67	29.14	0.15	-30.2		
Lantian 04d-3	31/10/11 12:19	279.0	3.62	-33.51	-1.66	29.15	0.10	-30.2		
Lantian 04d-4	31/10/11 12:28	278.8	5.44	-33.50	-1.87	28.93	0.14	-30.1		
Lantian 04d-5	31/10/11 12:37	278.8	3.60	-33.59	-1.86	28.94	0.11	-30.2		
Lantian 04d-6	31/10/11 12:46	278.9	4.95	-33.40	-2.18	28.61	0.15	-29.9		
Lantian 04d-7	31/10/11 12:56	278.7	4.79	-33.32	-2.30	28.49	0.15	-29.8		
Lantian 04d-8	31/10/11 13:05	279.0	4.53	-33.45	-2.61	28.17	0.11	-29.9		
Lantian 04d-9	31/10/11 13:14	279.2	4.97	-33.57	-2.83	27.95	0.13	-29.9		
Lantian 04a-3	31/10/11 13:24	279.1	3.09	-32.25	-2.75	28.02	0.11	-28.6		
Lantian STD_3	31/10/11 13:33	278.5	7.31	-33.11	-3.24	27.52	20.68			
Lantian STD_9	31/10/11 13:42	278.6	5.55	-33.03	-3.86	26.88	16.39			
IRS1	31/10/11 13:53	277.9	15.47	-30.80	-5.53	25.16	49.97	-27.1		
IRS2	31/10/11 14:02	278.1	13.01	-30.76	-6.32	24.35	41.96	-27.1		
IRS3	31/10/11 14:13	276.8	17.49	-35.45	-8.13	22.48	56.52	-31.8	-27.1	0.03
FDS1	31/10/11 14:23	278.6	4.78	-32.70	-7.72	22.91	15.43	-29.0		
FDS2	31/10/11 14:32	279.3	4.83	-32.78	-7.58	23.05	15.66	-29.1		
FDS3	31/10/11 14:41	280.1	5.27	-32.76	-7.71	22.91	17.16	-29.107	-29.1	0.04
ST01	31/10/11 15:18	282.3	1.23	-30.39	-7.75	22.87	4.01	-30.0	-30.0	¹
Lantian STD_11	31/10/11 15:37	281.0	2.80	-33.07	-7.33	23.30	9.14			
Lantian STD_12	31/10/11 15:46	281.8	3.83	-33.01	-7.49	23.14	12.49			
Lantian STD_13	31/10/11 15:55	281.7	3.07	-32.98	-7.66	22.97	10.02			
Lantian 04d-1-1	31/10/11 16:09	281.5	3.02	-32.15	-7.36	23.27	9.80			

¹Only one isolate analyzed in this set.

Table A4.2 IRMS stable isotope analysis batch results sheet, CO₂, for sample Set 2 – 150 μA run.

Name	Date	RT (Sec)	Height (nA)	¹³ C	¹⁸ O	δ ¹⁸ O w.r.t. SMO W	Elemental Composition	δ ¹³ C Corrected	δ ¹³ C Mean	δ ¹³ C Std. Dev.
urea-R1	9/6/2012 11:24	263.6	2.59021	-32.181	-2.298	28.491	17.291	-29.53	-29.4	0.07
urea-R2	9/6/2012 11:32	263.3	5.80938	-32.033	-3.117	27.647	19.922	-29.38		
urea-R3	9/6/2012 11:41	264.6	9.02207	-31.996	-3.537	27.214	22.506	-29.34		
urea-R4	9/6/2012 11:50	264.8	10.6243	-32.033	-4.374	26.351	18.893	-29.38		
urea-R5	9/6/2012 11:59	265.5	14.415	-32.102	-3.911	26.829	20.402	-29.45		
urea-R6	9/6/2012 12:07	265.2	14.788	-32.121	-3.406	27.348	20.197	-29.47		
ST0234	9/6/2012 12:23	265.5	2.77354	-32.122	-2.681	28.096	56.877	-29.47	-29.3	0.30
ST05	9/6/2012 12:32	266.5	2.24678	-31.698	-2.518	28.264	52.055	-29.04		
FDS4	9/6/2012 12:41	265.2	7.87318	-31.746	-2.615	28.165	35.360	-29.09	-29.3	0.16
FDS5	9/6/2012 12:50	265.4	14.095	-32.063	-2.592	28.188	126.986	-29.41		
FDS6	9/6/2012 12:59	265.3	12.4145	-31.962	-2.829	27.944	61.917	-29.31		
urea-R7	9/6/2012 13:52	265.5	8.85093	-31.880	-2.943	27.826	19.789	-29.23	-29.3	0.09
urea-R8	9/6/2012 14:01	265	11.1965	-32.011	-2.854	27.918	21.001	-29.36		

Table A4.3 IRMS stable isotope analysis batch results sheet, CO₂, for sample Set 2 – 100 μA run.

Name	Date	RT (Sec)	Height (nA)	¹³ C	¹⁸ O	δ ¹⁸ O w.r.t. SMO W	Elemental Composition	δ ¹³ C Corrected	δ ¹³ C Mean	δ ¹³ C Std. Dev.
urea-R1	9/6/2012 14:24	265.2	6.11	-31.96	-1.88	28.92	20.01	-29.31	-29.4	0.05
urea-R2	9/6/2012 14:33	264.6	7.31	-32.02	-1.32	29.5	19.87	-29.37		
urea-R3	9/6/2012 14:42	265.1	9.45	-32.06	-0.36	30.48	20.12	-29.41		
IRS4	9/6/2012 14:51	265	11.13	-29.84	1.08	31.98	63.76	-27.19	-27.2	0.136
IRS5	9/6/2012 14:59	265.1	14.45	-29.73	2.26	33.19	61.05	-27.08		
IRS6	9/6/2012 15:08	263.9	13.35	-30	2.62	33.57	62.53	-27.35		
urea-R4	9/6/2012 15:35	265.2	7.07656	-32.029	3.213	34.172	19.58	-29.37	-29.4	0.057
urea-R5	9/6/2012 15:52	265.6	10.2844	-32.109	3.947	34.929	20	-29.45		

Appendix 5: Monte Carlo Input and Derived Parameters

The Monte Carlo base input values and respective uncertainties of each for DEHP spike material and spiked method blanks are given in Table A4.1, as those for all cheese-isolated samples are shown in Table A4.2. The mean of 100,000 values of each of the variables that are eventually derived from these inputs during the Monte Carlo program and used to calculate $f_{m_{DEHP}}$ and $f_{C_{DEHP}}$, along with their standard deviation, are present in Table A4.3.

Table A5.1 Base input values of standard DEHP spikes and spiked method blanks for Matlab®-programmed Monte Carlo analysis.

Monte Carlo Base Input	S1	S2	S3	SBL02	SBL03	BL04
m_{LLNL}	78	81	83	68	88	78
Uncertainty	2	2	2	2	2	2
m_{DMB}	0	0	0	1.36	0.6	0.82
Uncertainty	0	0	0	0.05	0.03	0.03
m_{CSDEHP}	47.3	47.98	47.78	45.39	47.85	45.59
Uncertainty	0.04	0.04	0.04	0.04	0.04	0.04
$f_{m_{DMB}}$	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
Uncertainty	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
$f_{m_{LLNL}}$	0.011	0.008	0.008	0.037	0.022	0.064
Uncertainty	0.005	0.005	0.005	0.005	0.005	0.005
$\delta^{13}C_{DEHP}$	-29.1	-29.1	-29.1	-29.1	-29.1	-29.1
Uncertainty	0.04	0.04	0.04	0.04	0.04	0.04

Table A5.2 Base input values of cheese-isolated samples for Matlab®-programmed Monte Carlo analysis.

Monte Carlo Base Input	ST01	ST02	ST03	ST04	ST05
m_{LLNL}	109	99	127	135	114
Uncertainty	3	3	2	2	2
m_{DEHP}	92.6	66.32	62.32	64.29	68.28
Uncertainty	6.5	1.41	1.46	1.41	1.59
m_{DMB}	1.5	1.05	0.81	0.69	1.5
Uncertainty	0.6	0.04	0.06	0.05	0.6
$f_{m_{HCE}}$	1.04	1.04	1.04	1.04	1.04
Uncertainty	0.0081	0.0081	0.0081	0.0081	0.0081
$f_{m_{DMB}}$	0.0009	0.0009	0.0009	0.0009	0.0009
Uncertainty	0.009	0.009	0.009	0.009	0.009
$Purity_C$	0.94	0.881	0.872	0.905	0.923
Uncertainty	0.013	0.017	0.017	0.017	0.014
$f_{m_{LLNL}}$	0.2835	0.2809	0.3526	0.3111	0.3335
Uncertainty	0.0042	0.0035	0.003	0.0027	0.0031
$f_{m_{WSC}}$	1.0405	1.0405	1.0405	1.0405	1.0405
Uncertainty	0.002	0.002	0.002	0.002	0.002
$\delta^{13}C_{sample}$	-30	-29.3	-29.3	-29.3	-29.3
Uncertainty	0.043	0.3	0.3	0.3	0.3
$\delta^{13}C_{Stilton\ Cheese}$	-27.1	-27.1	-27.1	-27.1	-27.1
Uncertainty	0.03	0.03	0.03	0.03	0.03

Table A5.3 Monte Carlo-derived variables and 1σ uncertainties as mean and standard deviation of 100,000 determinations.

	ST01	1σ	ST02	1σ	ST03	1σ	ST04	1σ	ST05	1σ
m_{HCE}	5.924	1.427	8.985	1.466	9.175	1.410	6.764	1.347	5.717	1.131
Δm_C	10.500	7.660	23.697	3.697	55.507	2.957	63.936	2.867	40.012	2.873
m_{TE}	17.923	7.181	33.732	3.315	65.492	2.476	71.390	2.451	47.229	2.634
Y_{TE}	0.164	0.064	0.340	0.025	0.516	0.014	0.529	0.013	0.414	0.018
Y_{DMB}	0.095	2.907	0.031	0.003	0.012	0.001	0.010	0.001	0.032	0.013
Y_{HCE}	0.465	26.429	0.269	0.053	0.140	0.023	0.095	0.019	0.122	0.025
$Y_{\Delta m_C}$	0.439	28.486	0.699	0.055	0.847	0.023	0.895	0.019	0.847	0.028
$f_{\Delta m_C}$	0.109	36.280	0.420	0.114	0.299	0.045	0.245	0.037	0.351	0.059
$f_{m_{TE}}$	0.675	35.563	0.570	0.097	0.399	0.045	0.318	0.039	0.423	0.058

Appendix 6: Electron Impact Mass Spectrum of Hexadecamethyl heptasiloxane

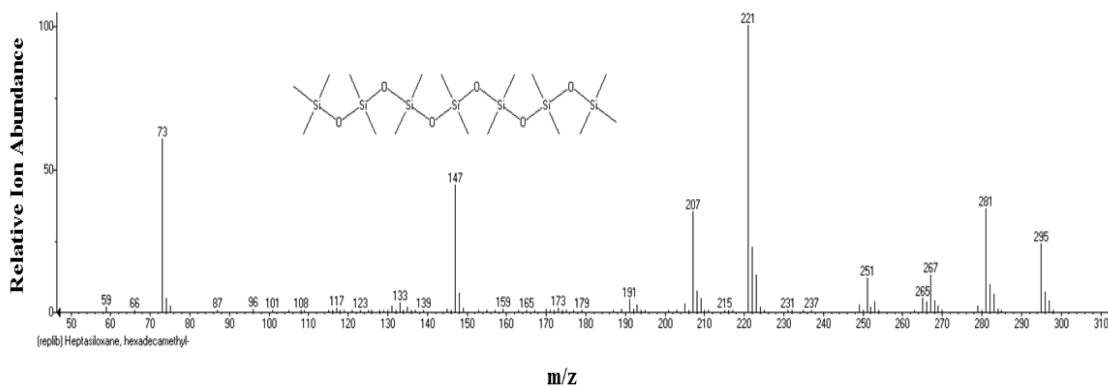


Figure A6.1 Electron impact mass spectrum of hexadecamethyl heptasiloxane in NIST/EPA/NIH Mass Spectral Database (NIST 11).

Bibliography

- Agarwal DK, Eustis S, Lamb JC. 1986. Effects of di (2-ethylhexyl) phthalate on the gonadal pathophysiology, sperm morphology, and reproductive performance of male rats. *Environmental Health Perspectives*. (65)343-350.
- Amade P, Mallea M, Bouaicha N. 1994. Isolation, Structural Identification and Biological-Activity of 2 Metabolites Produced by *Penicillium-Olsonii* Bainier and Sartory. *Journal of Antibiotics*. (47)2, 201-207.
- Brown, T. A., & Southon, J. R. 1997. Corrections for contamination background in AMS ¹⁴C measurements. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*. 123(1), 208-213.
- Castle L, Gilbert J, Eklund T. 1990. Migration of Plasticizer from Poly(Vinyl Chloride) Milk Tubing. *Food Additives and Contaminants*. (7)5, 591-596.
- Cavaliere B, Macchione B, Sindona G, Tagarelli A. 2008. Tandem mass spectrometry in food safety assessment: The determination of phthalates in olive oil. *Journal of Chromatography A*. (1205), 137-143.
- Chen CY. 2004. Biosynthesis of di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) from red alga-Bangia atropurpurea. *Water Research*. (38)4, 1014-1018.
- Cohen A, Janssen S, Solomon G. 2007. Clearing the Air: Hidden Hazards of Air Fresheners. Natural Resources Defense Council (NRDC) Issue Paper, September 2007.
- Colón I, Caro D, Bourdony CJ, Rosario O. 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. *Environmental Health Perspectives*. (108)9, 895-900.
- Craig H. 1953. The Geochemistry of the Stable Carbon Isotopes. *Geochimica et Cosmochimica Acta*. (3)2-3, 53-92.
- Cui CB, Ubukata M, Kakeya H, Onose R, Okada G, Takahashi I, Isono K, Osada H. 1996. Acetophthalidin, a novel inhibitor of mammalian cell cycle, produced by a fungus isolated from a sea sediment. *Journal of Antibiotics*. (49)2, 216-219.
- Davis EM. 1988. Radiocarbon dating: An Archaeological Perspective, by R.E. Taylor, 1987, Academic Press, Orlando, xii + 212 pp., *Geoarchaeology*. (3)2, 171-171.

- DeNiro MJ, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*. (42)5, 495-506.
- Donahue DJ, Linick TW, Jull AJT. 1990. Isotope-Ratio and Background Corrections for Accelerator Mass-Spectrometry Radiocarbon Measurements. *Radiocarbon*. (32)2, 135-142.
- Enneking PA. 2006. Phthalates not in plastic food packaging. *Environmental Health Perspectives*. (114)2, A89-A90.
- European Commission (EC). 2007. Commission Directive 2007/19/EC of 30 March 2007 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official Journal of the European Union*, 31(2007), 17-36.
- DEHP Information Center [Internet]. 2013. European Council for Plasticizers and Intermediates (ECPI): [cited 15 March 2013]. Available from: <http://www.dehp-facts.com/>.
- European Food Safety Authority (EFSA). 2005. Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission on the possibility of allocating a group-TDI for Butylbenzylphthalate (BBP), di-Butylphthalate (DBP), Bis(2-thylhexyl) phthalate (DEHP), di-Isononylphthalate (DINP) and di-Isodecylphthalate (DiDP). Minutes statement 28 June 2005, 12th Plenary Meeting, item 10. [Cited 15 March 2013]. Available from : <http://www.efsa.europa.eu/it/efsajournal/doc/747.pdf>.
- Fromme H, Lahrz T, Piloty M, Gebhart H, Oddoy A, Ruden H. 2004. Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). *Indoor Air*. (14)3, 188-195.
- Guo Y, Zhang Z, Liu L, Li Y, Ren N, Kannan K. 2012. Occurrence and Profiles of Phthalates in Foodstuffs from China and Their Implications for Human Exposure. *Journal of agricultural and food chemistry*. (60)27, 6913-6919.
- Harkness DD, Wilson HW. 1979. Scottish-Universities Research and Reactor Center Radiocarbon Measurements - Iii. *Radiocarbon*. (21)2, 203-256.
- Higham, Tom. 1999. Radiocarbon Web Info. Radiocarbon Laboratory. University of Waikato, NZ [Internet]. [Cited 15 March 2013]. Available from: <http://www.c14dating.com/>.
- Heudorf U, Mersch-Sundermann V, Angerer J+. 2007. Phthalates: Toxicology and exposure. *International Journal of Hygiene and Environmental Health*. (210)5, 623-634.

- Huber WW, GraslKraupp B, SchulteHermann R. 1996. Hepatocarcinogenic potential of di(2-ethylhexyl)phthalate in rodents and its implications on human risk. *Critical Reviews in Toxicology*. (26)4, 365-481.
- Ilbery B, Kneafsey M. 2000. Registering regional speciality food and drink products in the United Kingdom: the case of PDOs and PGI. *Area*. (32)3, 317-325.
- Ingalls AE, Pearson A. 2005. Ten Years of Compound-Specific Radiocarbon Analysis. *Oceanography*. (18)18-31.
- Jarosova A. 2006. Phthalic acid esters (PAEs) in the food chain. *Czech Journal of Food Sciences*. (24)5, 223-231.
- Jordan TB, Seen AJ, Jacobsen GE, Gras JL. 2006. Radiocarbon determination of woodsmoke contribution to air particulate matter in Launceston, Tasmania. *Atmospheric Environment*. (40)14, 2575-2582.
- Kondyli E, Demertzis PG, Kontominas MG. 1992. Migration of dioctylphthalate and dioctyladipate plasticizers from food-grade PVC films into ground-meat products. *Food chemistry*. (45)3, 163-168.
- Levin I, Kromer B. 1997. Twenty years of atmospheric (CO₂)-C-14 observations at Schauinsland station, Germany. *Radiocarbon*. (39)2, 205-218.
- Libby WF. 1946. Atmospheric Helium Three and Radiocarbon from Cosmic Radiation. *Physical Review*. (69)11-1, 671-672.
- Libby WF. 1967. History of radiocarbon dating. *Radioactive Dating and Methods of Low-Level Counting. Proceedings of a Symposium, Monaco*. (3-25).
- Liberra K, Jansen R, Lindequist U. 1998. Corollosporine, a new phthalide derivative from the marine fungus *Corollospora maritima* Werderm. 1069. *Pharmazie*. (53)8, 578-581.
- Lowell Center for Sustainable Production (LCSP). 2011. Phthalates and Their Alternatives: Health and Environmental Concerns. Technical Briefing, University of Massachusetts Lowell, Lowell, MA, January 2011. Available from: <http://www.sustainableproduction.org/downloads/PhthalateAlternatives-January2011.pdf>
- MacKenzie SE, Gurusamy GS, Piorko A, Strongman DB, Hu TM, Wright JLC. 2004. Isolation of sterols from the marine fungus *Corollospora lacera*. *Canadian Journal of Microbiology*. (50)12, 1069-1072.
- Namikoshi M, Fujiwara T, Nishikawa T, Ukai K. 2006. Natural abundance C-14 content of dibutyl phthalate (DBP) from three marine algae. *Marine Drugs*. (4)4, 290-297.

- Nier AO, Gulbransen EA. 1939. Variations in the relative abundance of the carbon isotopes. *Journal of the American Chemical Society*. (61)697-698.
- Nydal R, Lovseth K. 1983. Tracing Bomb C-14 in the Atmosphere 1962-1980. *Journal of Geophysical Research-Oceans and Atmospheres*. (88)NC6, 3621-3642.
- Pearson A, McNichol AP, Benitez-Nelson BC, Hayes JM, Eglinton TI. 2001. Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific Delta C-14 analysis. *Geochimica et Cosmochimica Acta*. (65)18, 3123-3137.
- Petersen JH, Jensen LK. 2010. Phthalates and food-contact materials: enforcing the 2008 European Union plastics legislation. *Food Additives and Contaminants*. (27)11, 1608-1616.
- Reddy CM, Pearson A, Xu L, McNichol AP, Benner BA, Wise SA, Klouda GA, Currie LA, Eglinton TI. 2002. Radiocarbon as a tool to apportion the sources of polycyclic aromatic hydrocarbons and black carbon in environmental samples. *Environmental Science & Technology*. (36)8, 1774-1782.
- Reimer PJ, Brown TA, Reimer RW. 2004. Discussion: Reporting and calibration of post-bomb C-14 data. *Radiocarbon*. (46)3, 1299-1304.
- Rhind SM. 2005. Are Endocrine Disrupting Compounds a Threat to Farm Animal Health, Welfare and Productivity? *Reproduction in Domestic Animals*. (40)4, 282-290.
- Sastry VMVS, Rao GRK. 1995. Dioctyl Phthalate, and Antibacterial Compound from the Marine Brown Alga - *Sargassum Wightii*. *Journal of Applied Phycology*. (7)2, 185-186.
- Sharman M, Read WA, Castle L, Gilbert J. 1994. Levels of Di-(2-Ethylhexyl)Phthalate and Total Phthalate-Esters in Milk, Cream, Butter and Cheese. *Food Additives and Contaminants*. (11)3, 375-385.
- Skakkebaek N, Schettler T, de Kretser D, Leffers H. 2006. Human exposure to phthalates via consumer products - Discussion. *International Journal of Andrology*. (29)1, 139-139.
- Smith BN. 1972. Natural abundance of the stable isotopes of carbon in biological systems. *BioScience*. 226-231.
- Stuiver, M., & Polach, H. A. 1977. Reporting of C-14 data—discussion. *Radiocarbon*, 19(3), 355-363.
- Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia*. (57)1-2, 32-37.

- Tomita I, Nakamura Y, Yagi Y. 1977. Phthalic acid esters in various foodstuffs and biological materials. *Ecotoxicology and Environmental Safety*. (1)2, 275-287.
- Tsumura Y, Ishimitsu S, Saito I, Sakai H, Tsuchida Y, Tonogai Y. 2003. Estimated daily intake of plasticizers in 1-week duplicate diet samples following regulation of DEHP-containing PVC gloves in Japan. *Food Additives and Contaminants*. (20)4, 317-324.
- Tuniz C, Norton G. 2008. Accelerator mass spectrometry: New trends and applications. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*. (266)8, 1837-1845.
- Uchida M, Shibata Y, Yoneda M, Kobayashi T, Morita M. 2004. Technical progress in AMS microscale radiocarbon analysis. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms*. (223)313-317.
- U.S. Environmental Protection Agency (US EPA) Integrated Risk Information System Database [Internet]. 1997 - Di(2-ethylhexyl)phthalate (DEHP) - CASRN 117-81-7. [Cited 2012 Sept 14]. Available from: <http://www.epa.gov/IRIS/subst/0014.htm>.
- U.S. Food and Drug Administration (US FDA) [Internet]. 2009. Bisphenol-A (BPA), Statement of Norris Alderson, Ph.D., Congressional Testimony before the Subcommittee on Commerce, Trade and Consumer protection House Committee on Energy and Commerce, June 10, 2008. [Cited 15 March 2013]. Available from: <http://www.fda.gov/NewsEvents/Testimony/ucm115235.htm>.
- U.S. Food and Drug Administration (US FDA) List of Indirect Additives Used in Food Contact Database [Internet]. 2011 - Di(2-ethylhexyl) phthalate, Doc No. 7065 [cited 15 March, 2013]. Available from: <http://www.accessdata.fda.gov/scripts/fcn/fcnavigation.cfm?rpt=ialisting&displayAll=true>
- Uyeda M, Suzuki K, Shibata M. 1990. 3315-Af2, A Cell-Aggregation Factor Produced by *Streptomyces* Sp Strain No-A-3315. *Agricultural and Biological Chemistry*. (54)1, 251-252.
- Vogel JS, Turteltaub KW, Finkel R, Nelson DE. 1995. Accelerator Mass Spectrometry. *Analytical Chemistry*. (67)11, 353A-359A.
- Wenzl T. 2009. Methods for the Determination of Phthalates in Food. Methods for the Determination of Phthalates in Food, JRC Scientific and Technical Reports, Institute for Reference Materials and Measurements, Joint Research Centre, European commission, EUR23682 EN, Luxembourg, 2009.

Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Analysis*. (26)3, 803-824.

Zhu JP, Phillips SP, Feng YL, Yang XF. 2006. Phthalate esters in human milk: Concentration variations over a 6-month postpartum time. *Environmental Science & Technology*. (40)17, 5276-5281.