Coalition for Safer Food Processing & Packaging

About the "Final Report: Analysis of Selected Phthalates in Food Samples"

Attached is the report from VITO, an independent laboratory contracted by the Coalition to test cheese products in order to determine the concentrations of 13 *ortho*-phthalates. The report describes the methods used and details the results. The report is as received from the laboratory, except that we have blacked out references to specific product and brand names, and to non-cheese products run in the same batches.

VITO, located in Mol, Belgium was selected based on their directly applicable experience. Their researchers have carried out several studies on phthalates in food, including dairy products. VITO carried out the instrumental analysis for the following studies:

1. Fierens et al, Transfer of eight phthalates through the milk chain — A case study. *Environment International* 51 (2013) 1–7.
2. Fierens et al, Analysis of phthalates in food products and packaging materials sold on the Belgian market. *Food and Chemical Toxicology* 50 (2012) 2575–2583
3. Fierens et al, Modelling the environmental transfer of phthalates and polychlorinated dibenzo-p-dioxins and dibenzofurans into agricultural products: The EN-forc model. *Environmental Research* 133 (2014) 282–293
4. Fierens et al, Effect of cooking at home on the levels of eight phthalates in foods. *Food and Chemical Toxicology* 50 (2012) 4428–4435
5. Van Holderbeke et al, Determination of contamination pathways of phthalates in food products sold on the Belgian market. *Environmental Research* Volume 134, October 2014, Pages 345–352
6. Fierens et al, Phthalates in Belgian cow's milk and the role of feed and other contamination pathways at farm level. *Food Chem Toxicol*. Vol 50(8), 2012, 2945-53

Background phthalate contamination is ubiquitous in indoor environments, including laboratories, due to the use of phthalates in many products and manufacturing processes. To measure parts-per-billion levels in food without significant background contamination, special laboratory cleaning and extensive quality control measures must be carried out. To preserve a low phthalate background level, the laboratory must be dedicated to the project for its duration. Few labs are equipped to meet these requirements.

VITO follows ISO 17025, a standard that specifies procedures for testing and calibration laboratories, to establish and monitor appropriate laboratory quality control methods and equipment. ISO 17025 does not include specifications for phthalates in food, but VITO's researchers work and operate within an ISO 17025 environment.

Our findings indicate that further research is needed to determine the extent to which phthalates are a food industry-wide problem. Since only a single item for each product was tested by the laboratory, there is not enough data to tell which products or brands are higher or lower by comparison. There are enough data, however, to say that on average the phthalate levels in the 10 mac & cheese powders we tested were **more than four times higher**, on a fat basis, than in the 15 "natural" cheeses we tested (block cheese, string cheese, cottage cheese, and shredded cheese).

We are calling on Kraft to lead the industry to address the challenge posed by phthalates because Kraft is, by the far, the largest brand, and therefore has the power to push for industry-wide change. If Kraft commits to identifying and eliminating any sources of phthalates that end up in food, we believe the entire industry will follow suit. We're asking consumers to sign our petition to Kraft and learn more at: http://kleanupkraft.org/.

Distribution: Restricted

Final report

Analysis of selected phthalates in food samples

Voorspoels Stefan, Nady Hufkens, Jo Lievens, Guido Vanermen

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VITO NV
Boeretang 200 - 2400 MOL - BELGIE
Tel. + 32 14 33 55 11 - Fax + 32 14 33 55 99
vito@vito.be - www.vito.be

BTW BE-0244.195.916 RPR (Turnhout)
Bank 375-1117354-90 ING
BE34 3751 1173 5490 - BBRUBEBB

DISTRIBUTION LIST

Jeff Gearhart
Gillian Z. Miller
Lauren Olson
VITO

SUMMARY

By order of Ecology Center a selected set of dairy food samples were analysed for phthalate content. After extraction, samples were further treated to facilitate instrumental measurement by means of gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS). Mass-labeled internal standards and authentic reference standards were used for all target analytes. The method used was previously validated. Quality was assured by means of analysis of procedural blanks, duplicate analysis and spiked control samples.

In total 51 samples were measured. Blanks were low, under control and used to determine the LOQ to minimize false positive results. Quality control samples, such as duplicates and in-house reference material were of sufficient quality. The reported results are considered of good quality. All results should be interpretated in relation to the associated measurement uncertainty.

Most relevant compounds within the sample set were DEP, DiBP, DnBP, and DEHP, with a prevalence ranging from 63 to 92 %. DAP and DCHP were never found above the LOQ, and DMP only in 2 samples. Prevalence of DnHP, BBP, DNOP, DiDP/DPHP and DiNP ranged between 12 and 22 %.

Data interpretation and risk assessment was not part of this project.

The work was performed according to ISO 17025 guidelines in a BELAC accredited laboratory.

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CHAPTER 1 INTRODUCTION

In this study the occurrence 13 phthalate esters in 51 food samples were investigated by VITO. The phthalates that were quantitatively determined are: dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), dicyclohexyl phthalate (DCHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), di-propylheptyl phthalate (DPHP), diallyl phthalate (DAP) and di-n-hexyl phthalate (DnHP).

The food samples under study were individually packed samples purchased on the US market. Samples were delivered in unopened commercial packaging as sold in stores. Sample integrity was thus assured.

A short description of the analytical procedures is described below (Work package 1). All necessary measures were taken to avoid contamination by the laboratory environment. Suitable extraction and clean up techniques were applied depending on the type of food, namely high-fat foods. Interfering fat was removed from the extract by means of gel permeation chromatography (GPC). The instrumental analysis was performed by means of gas chromatography-low resolution-mass spectrometry with electron ionisation (GC-EI-MS). Quantification was done by an internal standard method, making use of isotope labelled phthalate esters. An interference-free and separate determination of DiNP, DiDP and DPHP was not possible with GC-MS. These phthalates were therefore determined with LC-MS. For fatty samples an underestimation of the DiNP and DiDP concentration is however possible due to signal suppression. Because of the non-availability of labelled internal standards a correction for recovery of these phthalates was made by applying the standard addition method.

Each analytical series was composed of a maximum of 8 samples, calibration solutions and QC samples. Method performance characteristics are given below.

The results of the analyses were compiled in this report. Phthalate concentrations are reported both in ug/kg fresh weight and in µg/kg fat (using the fat content either mentioned on the packaging or determined experimentally).

CHAPTER 2 SAMPLE LIST

A total of 20 cheese, [redacted] and 10 cheese sauce mix powder samples were delivered by courier on December 7th 2016. [redacted] All samples were stored at 4 °C in their unopened packaging until analysis. An overview of all samples including coding is given in Table 1. Figure 1, Figure 2 and Figure 3 show the samples upon arrival and registration. Sample 170208-0013 [redacted] was compromised as the packaging was opened during transport. This sample was therefore not measured. Samples 170208-0016 and 170208-0017 [redacted] and 170208-0018 and 170208-0019 [redacted] were duplicate to ensure sufficient sample amount. As one sample container was sufficient, only 170208-0016 and 170208-0018 were analysed.

Table 1. Samples.

VITO-code	Brand	Type
161208-0087	[redacted]	Cheese
161208-0088		Cheese
161208-0089		Cheese
161208-0090		Cheese
161208-0091		Cheese
161208-0092		Cheese
161208-0093		Cheese
161208-0094		Cheese
161208-0095		Cheese
161208-0096		Cheese
161208-0097		Cheese
161208-0098		Cheese
161208-0099		Cheese
161208-0100		Cheese
161208-0101		Cheese
161208-0102		Cheese
161208-0103		Cheese
161208-0104		Cheese
161208-0105		Cheese
161208-0106		Cheese
161208-0107		cheese sauce mix-powder
161208-0108		cheese sauce mix-powder
161208-0109		cheese sauce mix-powder
161208-0110		cheese sauce mix-powder
161208-0111		cheese sauce mix-powder
161208-0112		cheese sauce mix-powder
161208-0113		cheese sauce mix-powder
161208-0114		cheese sauce mix-powder
161208-0115		cheese sauce mix-powder
161208-0116		cheese sauce mix-powder
161208-0117		[redacted]

161208-0118	[redacted]
161208-0119	
161208-0120	
161208-0121	
161208-0122	
161208-0123	
161208-0124	
161208-0125	
161208-0126	
170208-0007	
170208-0008	
170208-0009	
170208-0010	
170208-0011	
170208-0012	
170208-0013	
170208-0014	
170208-0015	
170208-0016	
170208-0017	
170208-0018	
170208-0019	
170208-0020	

[redacted]

Figure 1. Cheese samples

Figure 2. [redacted] *macaroni-and-cheese samples*

Figure 3. [redacted]

CHAPTER 3 ANALYSIS

3.1. QUALITY

The work was performed according to ISO 17025 guidelines in a BELAC accredited laboratory. Phthalate analysis did not fall not within the scope of ISO 17025 accreditation, but people, methods and equipment are working and/or operated within an ISO 17025 environment.

3.2. TARGET ANALYTES

Thirteen phthalates were determined. They are listed in Table 2.

Table 2. Target analytes.

Trivial name	Abbreviation	CAS-number
dimethyl phthalate	DMP	131-11-3
diethyl phthalate	DEP	84-66-2
diisobutyl phthalate	DiBP	84-69-5
di-n-butyl phthalate	DnBP	84-74-2
benzylbutyl phthalate	BBP	85-68-7
di(2-ethylhexyl) phthalate	DEHP	117-81-7
dicyclohexyl phthalate	DCHP	84-61-7
di-n-octyl phthalate	DnOP	117-84-0
diisononyl phthalate	DiNP	28553-12-0
diisodecyl phthalate	DiDP	26761-40-0
di-propylheptyl phthalate	DPHP	53306-54-0
diallyl phthalate	DAP	131-17-9
di-n-hexyl phthalate	DnHP	84-75-3

3.3. ANALYTICAL METHODS

3.3.1. SAMPLE PREPARATION

→ Cheese

In order to minimize the risk of contamination, sample manipulation was minimized. As a result, the whole sample was not homogenized but a representative transversal cut in the middle of the block of the edible part was taken. The sample was weighted (approx. 5 g), dried using sodium sulfate and doped with internal standard. The sample was then column extracted using aceton:hexane (1:2; *v/v*). The extract was evaporated to constant weight. An aliquot of 0.5 g of fat was weighted and dissolved in 2 mL of DCM. The extract was then cleaned using gel-permeation chromatography (GPC) using an Envirogel column with DCM as mobile phase. GPC is necessary to remove interfering fats and other co-extracted interfering compounds from the measurement extract. The fraction containing the analytes of interest was collected and concentrated to 1 mL. The fraction

containing the phthalates was transferred to an appropriate solvent and analyzed using GC-MS and LC-MS.

→ **Cheese sauce-mix powder**

The powders were considered homogeneous. The sample was weighted (approx. 5 g) in a glass vial, doped with internal standard and 20 mL of aceton:hexane (1:2; *v/v*) was added. The mixture was homogenized and sonicated for 30 min. The supernatant was collected and the procedure was repeated 3 times. All collected extracts were combined. The extract was evaporated to constant weight. An aliquot of 0.5 g of fat was weighted and dissolved in 2 mL of DCM. The extract was then cleaned using gel-permeation chromatography (GPC) using an Envirogel column with DCM as mobile phase. GPC is necessary to remove interfering fats and other co-extracted interfering compounds from the measurement extract. The fraction containing the analytes of interest was collected and concentrated to 1 mL. The fraction containing the phthalates was transferred to an appropriate solvent and analyzed using GC-MS and LC-MS.

3.3.2. GC-ANALYSIS

All except 3 target anaytes (DiNP, DiDP and DPHP) were analyzed by GC. The instrumental analysis of phthalates was performed by gas chromatography-low resolution-mass spectrometry with electron ionisation (GC-EI-MS). One µL of the sample extract was injected at 250 °C in splitless mode. Phthalates were separated on a 30 m x 0.25 mm id x 0.25 µm DB-XLB column with a suitable temperature program from 60 to 340°C. The MS was operated in Selected Ion Monitoring (SIM) mode. **Quantification was done by the internal standard method, making use of isotopically labeled phthalate esters.** For each compound, beit a target analyte or internal standard, a target and a qualifier ion were used (**Table 3**). Criterion for a positive identification are detection of peaks at appropriate *m/z*, retention time match and a ratio target/qualifier within 20 % of that observed in a standard solution.

Table 3.Target and qualifier ions of the different compounds under study.

Compound	Abbreviation	Target ion	Qualifier ion
dimethylphtalate	DMP	163	194
Diethylphtalate	DEP	149	177
diallyl ftalate	DAP	149	189
di isobutylftalate	DiBP	149	223
di n-butyl ftalate	DnBP	149	223
di n-hexylftalate	DnHP	149	251
benzylbutylftalate	BBP	149	206
di (2-ethylhexyl) ftalate	DCHP	149	167
dicyclohexyl ftalate	DEHP	149	167
di-n-octylftalate	DnOP	149	279
Dimethylphtalate-d4	DMP-d4	167	198
Diethylphtalate-d4	DEP-d4	153	181
di isobutylftalate-d4	DiBP-d4	153	227
di n-butyl ftalate-d4	DnBP-d4	153	227
benzylbutylftalate-d4	BBP-d4	153	210
di n-hexylftalate-d4	DnHP-d4	153	255
di (2-ethylhexyl) ftalate-d4	DCHP-d4	153	171
di-n-octylftalate-d4	DnOP-d4	153	283

3.3.3. LC-ANALYSIS

An interference free and separate determination of DiNP, DiDP and DPHP cannot be achieved by GC-MS. Therefore these phthalates were determined by means of ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Five µL of extract was injected on an Acquity UPLC BEH C18 column (2.1 x 100 mm, 1,7µm) and the phthalates were separated with a water/acetonitrile/methanol gradient with ammonium acetate buffer. The separated phthalates were ionised by means of atmospheric pressure chemical ionization (APCI). For each phthalate specific precursor ion and product ions were monitored. Because signal suppression for fatty samples (> 10 % fat content) could lead to underestimation of the concentration of DiNP and DiDP, quantification was based on the standard addition approach since labeled internal standards for those compounds are not available. Positive identification of the phthalates DiNP and DiDP was based on LC retention time match and their specific MRM transitions (Table 4).

Table 4. Compounds analyzed by LC-MS: Parent and fragment mass.

Compound	Abbreviation	Parent mass	Fragment mass
diisononyl phthalate	DiNP	419.2589	85.0876
		419.2589	149.0283
		419.2589	275.2249
diisodecyl phthalate	DiDP	447.3089	85.0876
		447.3089	149.035
		447.3089	289.2142
di-propylheptyl phthalate	DPHP	447.3089	85.0876
		447.3089	149.035
		447.3089	289.2142

→ **Limitation**

DPHP and DiDP have the same MRM transition and retention time (Figure 4). This is not unexpected as they are isomers. We hypothesize that DiDP consists mainly of DPHP. The DPHP standard itself appears not to be very pure on its own, judging from its peak shape. For those reasons, DiDP and DPHP are reported as a sum.

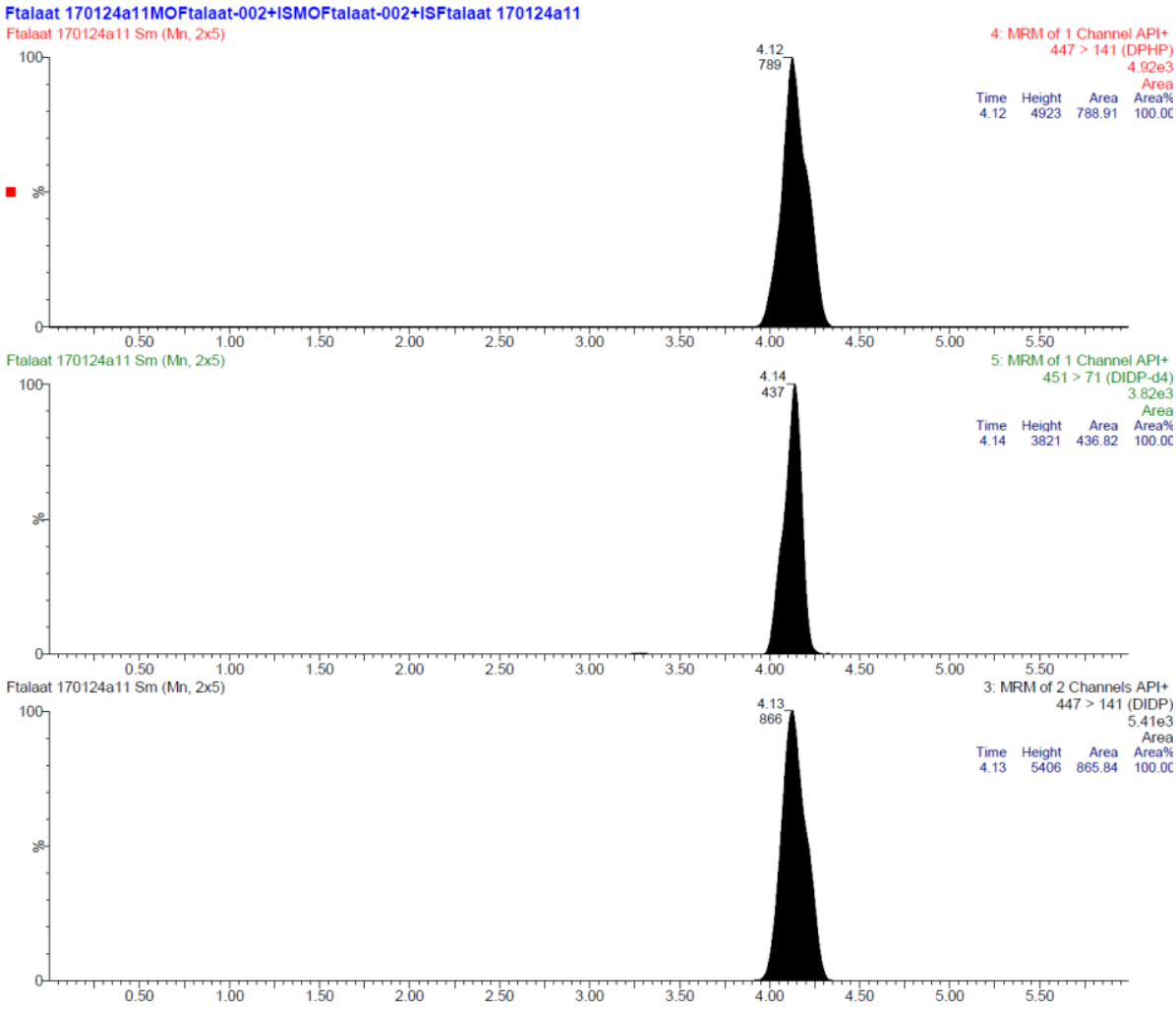

Figure 4. MRM trace of DPHP (upper), DiDp-d4 (middle) and DiDP (lower).

3.3.4. METHOD VALIDATION AND QUALITY CONTROL

The methods used were previously in-house validated. To ensure good method performance, a quality assurance scheme is in place. Each analytical sequence was composed of two procedural blanks (also called method blanks), calibration solutions, solvent blanks, a spiked reference sample, the study samples and a 1 or 2 duplicates. Sunflower oil fortified with a known amount of phthalates was used as control sample. Results of the quality control measures are reported in the results section.

→ Blanks

Due to the omnipresence of phthalates in each (laboratory) environment, sample contamination can occur in every stage of the analytical procedure. Therefore, control of the blank levels is a prerequisite to perform reliable and sensitive analysis of phthalates. A list of guidelines has been set up to reduce the risk of contamination during sample preparation and analysis. For instance, all glassware was heated at 450 °C for at least 4 hours and was covererd with aluminiumfoil prior to use. Furthermore, all glassware, syringes, spatula, etc. were rinsed carefully with dichloromethane prior to use. No laboratory gloves were used during sample preparation.To assess the exposure to phthalates and the possible contamination during the handling of thesamples, petri dishes were

placed at different locations in the laboratories, i.e. on the table and inthe fume hood. These dishes were filled with *n*-hexane and left in place for 8 hours with occasionally refilling the dish with solvent. After 8 hours, the residue in the petri dish was redissolved in dichloromethane and the mixture was brought into a vial. The mixture was evaporated to 1 mL and the internal standards were added, followed by GC-MS analysis. These experiments confirmed absence of significant phthalate contamination in the lab environment before even unpacking the samples. Based on these results, it can be concluded that phthalate exposure during sample handling, homogenization and analysis is under control. Blank measurements during the actual analyses are discussed further on (4.2.1 Method blanks).

→ **Method LOQs**

The levels of blanks that could be measured were used to estimate the method limit of quantification (LOQ) for those compounds for which blank levels were measurable. In the other case, instrumental performance was used to calculate the LOQ.
Raw data was corrected for blank contribution: the average blank per batch was subtracted (see also 4.2.1). The LOQ of each individual phthalate compound was set to three times the standard deviation of replicate procedural blank measurements under intermediate precision conditions (each replicate determination was obtained from an independent extraction). Standard deviation was calculated per batch, and the highest value throughout all series was used to calculate the LOQ. This conservative approach ensures a 99 % certainty that a reported value is not a blank induced artefact. Fresh weight based LOQs are calculated based on the fat content of each sample. As an indication a range is used for the fresh weight based LOQs. LOQs are listed in Table 5 and Table 6.

Table 5. LOQs - partim 1.

µg/Kg	DMP	DEP	DAP	DiBP	DnBP	DnHP
LOQ in fat	3.0	9.0	0.2	10	4.0	0.9
LOQ in product (< 2.5 % fat)	0.1	0.3	0.01	0.3	0.1	0.02
LOQ in product (7.5 % fat)	0.3	0.7	0.02	0.8	0.3	0.06
LOQ in product (15% fat)	0.5	1.4	0.03	1.5	0.6	0.13
LOQ in product (30 % fat)	0.9	2.7	0.06	3.0	1.2	0.26

Table 6. LOQs - partim 2.

µg/Kg	BBP	DEHP	DCHP	DNOP	DiDP+DPHP	DiNP
LOQ in fat	8.5	55	0.02	0.02	20	20
LOQ in product (< 2.5 % fat)	0.2	1.4	0.01	0.01	0.5	0.5
LOQ in product (7.5 % fat)	0.7	4.1	0.01	0.01	1.5	1.5
LOQ in product (15% fat)	1.3	8.3	0.01	0.01	3.0	3.0

LOQ in product (30 % fat)	2.6	17	0.01	0.01	6.0	6.0

3.3.5. MEASUREMENT UNCERTAINTY

The estimates of the measurement uncertainty for the determination of phthalates are summarized in Table 7. The measurement uncertainty is based on previous method validation using cheese and milk. The same estimate was used for the cheese sauce powders [redacted] The expanded measurement uncertainty was estimated using a coverage factor of 2 (k=2). In general, U varies between 20 and 40 percent.

Table 7. Estimated measurement uncertainty U (%) for the analysis of phthalates (k = 2).

Compound	Abbreviation	U (%)
dimethyl phthalate	DMP	25
diethyl phthalate	DEP	25
diallyl phthalate	DAP	30
di isobutyl phthalate	DiBP	30
di n-butyl phthalate	DnBP	25
di n-hexyl phthalate	DnHP	30
benzylbutyl phthalate	BBP	25
dicyclohexyl phthalate	DCHP	25
di (2-ethylhexyl) phthalate	DEHP	40
di-n-octyl phthalate	DnOP	20
diisononyl phthalate	DiNP	30
diisodecyl phthalate + di-propylheptyl phthalate	DiDP + DPHP	30

CHAPTER 4 RESULTS

4.1. ANALYTICAL RESULTS

All results are presented in Table 8 (product basis) and Table 9 (lipid weight basis). Results are corrected for blanks. Results are rounded according to the associated measurement uncertainty. The fat content used for calculation is reported alongside the lipid based results (Table 9). Fat content was taken from the food labels (cheese, [redacted]) or determined experimentally (macaroni-and-cheese powder; fat content of the cheese powder in the macaroni-and-cheese products was not available on the food labels). Product based results (Table 8) of the macaroni-and-cheese products might therefore be less accurate than for the other products. This was not considered in the analytical method uncertainty.
All samples except one could be measured [redacted] spoiled during transport). In total 5 out of 612 results could not be reported because of disturbances related to matrix interferences (denoted as "NQ" in the tables). An electronic excel worksheet file containing all results is attached to this report.

Table 8. Results on product basis (µg/Kg).

Sample Name	Customer ID (short)	DMP	DEP	DAP	DiBP	DnBP	DnHP	BBP	DEHP	DCHP	DNOP	DiDP+DPHP	DiNP
		µg/Kg product											
161208-0087	[redacted]	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
161208-0088		< LOQ	4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	25	< LOQ	3.5	< LOQ	< LOQ
161208-0089		< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	39	< LOQ	< LOQ	< LOQ	< LOQ
161208-0090		< LOQ	3.4	< LOQ	< LOQ	4	< LOQ	< LOQ	18	< LOQ	3.1	< LOQ	< LOQ
161208-0091		< LOQ	< LOQ	< LOQ	3.1	1.3	< LOQ	< LOQ	51	< LOQ	2.1	< LOQ	< LOQ
161208-0092		< LOQ	5	< LOQ	3	0.8	< LOQ	< LOQ	19	< LOQ	2.2	< LOQ	< LOQ
161208-0093		< LOQ	6	< LOQ	5	1.2	< LOQ	< LOQ	25	< LOQ	2.2	< LOQ	< LOQ
161208-0094		< LOQ	6	< LOQ	3.2	< LOQ	< LOQ	< LOQ	24	< LOQ	< LOQ	< LOQ	< LOQ
161208-0095		< LOQ	9	< LOQ	5	17	< LOQ	< LOQ	60	< LOQ	7	< LOQ	< LOQ
161208-0096		< LOQ	6	< LOQ	4	5	< LOQ	< LOQ	< LOQ	< LOQ	5	< LOQ	< LOQ
161208-0097		< LOQ	11	< LOQ	< LOQ	1.4	< LOQ	< LOQ	20	< LOQ	5	< LOQ	< LOQ
161208-0098		< LOQ	29	< LOQ	8	6	< LOQ	5	22	< LOQ	< LOQ	< LOQ	< LOQ
161208-0099		< LOQ	13	< LOQ	3.3	1.5	< LOQ	< LOQ	38	< LOQ	< LOQ	< LOQ	31
161208-0100		< LOQ	51	< LOQ	4	2.2	< LOQ	< LOQ	61	< LOQ	< LOQ	< LOQ	< LOQ
161208-0101		2.4	23	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	102	< LOQ	89	< LOQ	< LOQ
161208-0102		< LOQ	18	< LOQ	9	6	< LOQ	< LOQ	165	< LOQ	5	< LOQ	< LOQ
161208-0103		< LOQ	14	< LOQ	7	5	< LOQ	< LOQ	90	< LOQ	< LOQ	< LOQ	< LOQ
161208-0104		< LOQ	12	< LOQ	3.2	1.2	4	< LOQ	23	< LOQ	< LOQ	< LOQ	5
161208-0105		< LOQ	13	< LOQ	< LOQ	2.4	< LOQ	< LOQ	18	< LOQ	< LOQ	13	< LOQ
161208-0106		< LOQ	0.6	< LOQ	0.9	0.5	< LOQ	2.0	4	< LOQ	< LOQ	< LOQ	< LOQ
161208-0107		< LOQ	11	< LOQ	2.6	< LOQ	< LOQ	< LOQ	17	< LOQ	< LOQ	4	< LOQ
161208-0108		< LOQ	5	< LOQ	15	< LOQ	< LOQ	< LOQ	43	< LOQ	< LOQ	6	< LOQ
161208-0109		< LOQ	8	< LOQ	2.7	< LOQ	< LOQ	< LOQ	127	< LOQ	< LOQ	< LOQ	< LOQ
161208-0110		< LOQ	7	< LOQ	4	< LOQ	< LOQ	< LOQ	90	< LOQ	15	< LOQ	15
161208-0111		0.5	7	< LOQ	5	< LOQ	< LOQ	1.4	44	< LOQ	< LOQ	< LOQ	36
161208-0112		< LOQ	13	< LOQ	4	1.4	< LOQ	< LOQ	19	< LOQ	< LOQ	< LOQ	27
161208-0113		< LOQ	15	< LOQ	2.9	4	< LOQ	< LOQ	106	< LOQ	< LOQ	< LOQ	< LOQ
161208-0114		< LOQ	5	< LOQ	2.5	< LOQ	< LOQ	51	19	< LOQ	< LOQ	< LOQ	< LOQ
161208-0115		< LOQ	9	< LOQ	5	< LOQ	< LOQ	< LOQ	157	< LOQ	< LOQ	47	< LOQ
161208-0116		< LOQ	9	< LOQ	7	10.7	< LOQ	< LOQ	78	< LOQ	< LOQ	5	4
161208-0117		[redacted]											
161208-0118													
161208-0119													
161208-0120													
161208-0121													
161208-0122													
161208-0123													
161208-0124													
161208-0125													
161208-0126													
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170208-0015													
170208-0016													
170208-0018													
170208-0020													

Table 9. Results on lipid basis (µg/Kg).

Sample Name	Customer ID (short)	Fat %	DMP	DEP	DAP	DiBP	DnBP	DnHP	BBP	DEHP	DCHP	DNOP	DiDP+DPHP	DiNP
			µg/Kg lipid											
161208-0087	[redacted]	35.7%	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
161208-0088		32.1%	< LOQ	13	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	77	< LOQ	11	< LOQ	< LOQ
161208-0089		33.3%	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	116	< LOQ	< LOQ	< LOQ	< LOQ
161208-0090		32.1%	< LOQ	11	< LOQ	< LOQ	13	< LOQ	< LOQ	57	< LOQ	10	< LOQ	< LOQ
161208-0091		21.4%	< LOQ	< LOQ	< LOQ	15	6	< LOQ	< LOQ	238	< LOQ	10	< LOQ	< LOQ
161208-0092		32.1%	< LOQ	15	< LOQ	11	< LOQ	< LOQ	< LOQ	58	< LOQ	7	< LOQ	< LOQ
161208-0093		19.0%	< LOQ	33	< LOQ	24	6	< LOQ	< LOQ	132	< LOQ	11	< LOQ	< LOQ
161208-0094		32.1%	< LOQ	19	< LOQ	10	< LOQ	< LOQ	< LOQ	75	< LOQ	< LOQ	< LOQ	< LOQ
161208-0095		32.1%	< LOQ	28	< LOQ	16	51	< LOQ	< LOQ	186	< LOQ	22	< LOQ	< LOQ
161208-0096		32.1%	< LOQ	20	< LOQ	12	14	< LOQ	< LOQ	< LOQ	< LOQ	16	< LOQ	< LOQ
161208-0097		21.1%	< LOQ	54	< LOQ	< LOQ	7	< LOQ	< LOQ	96	< LOQ	24	< LOQ	< LOQ
161208-0098		9.5%	< LOQ	305	< LOQ	89	58	< LOQ	53	230	< LOQ	< LOQ	< LOQ	< LOQ
161208-0099		31.6%	< LOQ	40	< LOQ	10	5	< LOQ	< LOQ	119	< LOQ	< LOQ	< LOQ	98
161208-0100		20.8%	< LOQ	245	< LOQ	19	11	< LOQ	< LOQ	291	< LOQ	< LOQ	< LOQ	< LOQ
161208-0101		17.6%	14	132	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	578	< LOQ	506	< LOQ	< LOQ
161208-0102		35.7%	< LOQ	49	< LOQ	24	18	< LOQ	< LOQ	462	< LOQ	14	< LOQ	< LOQ
161208-0103		26.3%	< LOQ	53	< LOQ	25	18	< LOQ	< LOQ	341	< LOQ	< LOQ	< LOQ	< LOQ
161208-0104		21.4%	< LOQ	54	< LOQ	15	6	18	< LOQ	108	< LOQ	< LOQ	< LOQ	26
161208-0105		32.1%	< LOQ	40	< LOQ	< LOQ	7	< LOQ	< LOQ	55	< LOQ	< LOQ	40	< LOQ
161208-0106		2.1%	< LOQ	28	< LOQ	41	23	< LOQ	96	208	< LOQ	< LOQ	< LOQ	< LOQ
161208-0107		9.3%	< LOQ	119	< LOQ	28	< LOQ	< LOQ	< LOQ	180	< LOQ	< LOQ	38	< LOQ
161208-0108		13.2%	< LOQ	40	< LOQ	116	< LOQ	< LOQ	< LOQ	326	< LOQ	< LOQ	43	< LOQ
161208-0109		24.9%	< LOQ	33	< LOQ	11	< LOQ	< LOQ	< LOQ	511	< LOQ	< LOQ	< LOQ	< LOQ
161208-0110		20.2%	< LOQ	35	< LOQ	21	< LOQ	< LOQ	< LOQ	444	< LOQ	77	< LOQ	75
161208-0111		7.5%	7	89	< LOQ	67	< LOQ	< LOQ	19	583	< LOQ	< LOQ	< LOQ	481
161208-0112		8.0%	< LOQ	166	< LOQ	51	18	< LOQ	< LOQ	240	< LOQ	< LOQ	< LOQ	336
161208-0113		21.6%	< LOQ	70	< LOQ	13	19	< LOQ	< LOQ	492	< LOQ	< LOQ	< LOQ	< LOQ
161208-0114		5.6%	< LOQ	82	< LOQ	45	< LOQ	< LOQ	912	338	< LOQ	< LOQ	< LOQ	< LOQ
161208-0115		8.6%	< LOQ	102	< LOQ	58	< LOQ	< LOQ	< LOQ	1816	< LOQ	< LOQ	546	< LOQ
161208-0116		14.5%	< LOQ	64	< LOQ	45	74	< LOQ	< LOQ	538	< LOQ	< LOQ	32	30
161208-0117														
161208-0118														
161208-0119														
161208-0120														
161208-0121														
161208-0122														
161208-0123														
161208-0124														
161208-0125														
161208-0126														
170208-0007														
170208-0008														
170208-0009														
170208-0010														
170208-0011														
170208-0012														
170208-0014														
170208-0015														
170208-0016														
170208-0018														
170208-0020														

4.2. QUALITY CONTROL

4.2.1. METHOD BLANKS

Method blanks were analysed alongside test samples. Each batch around 4 method blanks were processed. As said before, analytes were found in those samples, albeit at low and controllable levels. Those levels were used to estimate the method LOQ.
All raw sample results were blank subtracted. Therefore the mean of the blank contribution of each batch was used.
In 2 procedural blank samples the response was significantly elevated. This potential problem was already observed during analysis as the (blank) extracts were left unattended after clean-up in the GPC equipment. These blanks were therefore not used for blank correction. These events highlight the importance of strictly adhering to the procedure to avoid blank interference. This also happened to one of the control samples. None of the actual samples were left standing longer than necessary during the analysis so such *contamination* is unlikely to have occurred for any of the samples. An overview of all blank measurements that were used for blank correction is given in Table 10. In this table N/F signifies “not found above limit of detection (LOD)”. LOD was set at a signal-to-noise of 3.

Table 10. Method blank results, expressed in µg/Kg fat.

Sample Name	Filename	DMP	DEP	DAP	DIBP	DBP	DnHexP	BBP	DEHP	DCHP	DNOP	DiDP+DPHP	DiNP
BL 170109 A	17011110	N/F	4.1	N/F	4.8	2.7	N/F	N/F	16	N/F	N/F		
BL 170109 B	17011111	N/F	4.7	N/F	4.1	2.2	N/F	N/F	13	N/F	N/F		
BL 170109 C	17011112	0.7	5.0	N/F	5.6	3.4	1.1	3.0	14	N/F	N/F		
BL 170109 D	17011113	1.2	5.1	N/F	5.9	2.7	N/F	N/F	50	N/F	N/F		
BL 170112 A	17011309	0.5	2.9	N/F	3.1	2.1	N/F	N/F	15	N/F	N/F		
BL 170112 B	17011310	0.5	4.3	N/F	4.4	2.5	N/F	N/F	17	N/F	N/F		
BL 170112 C	17011311	0.6	3.6	N/F	4.2	2.5	N/F	N/F	46	N/F	N/F		
BL 170116 A	17011711	0.5	3.5	N/F	3.7	1.4	1.0	N/F	17	N/F	N/F		
BL 170116 B	17011712	0.7	2.9	N/F	3.6	1.8	1.1	N/F	27	N/F	N/F		
BL 170116 C	17011713	N/F	3.6	N/F	3.6	1.9	0.7	N/F	19	N/F	N/F		
BL 170118 A	17011911	N/F	3.0	N/F	3.4	1.8	N/F	2.3	14	N/F	N/F		
BL 170118 B	17011912	N/F	4.9	N/F	5.1	3.2	0.5	2.7	11	N/F	N/F		
BL 170118 C	17011913	N/F	4.5	N/F	4.9	2.9	N/F	3.3	13	N/F	N/F		
BL 170118 D	17011914	N/F	6.4	N/F	6.5	3.4	N/F	2.6	9	N/F	N/F		
BL 170120 A	17012609	1.9	11.4	N/F	10.6	7.1	2.12	N/F	13	N/F	N/F		
BL 170120B	17012610	3.1	11.1	N/F	11.4	7.5	1.74	N/F	15	N/F	N/F		
BL 170120 C	17012611	2.0	8.1	N/F	9.5	7.1	2.4	N/F	22	N/F	N/F		
BL 170120 D	17012612	1.7	9.3	N/F	9.8	6.6	2.18	N/F	23	N/F	N/F		
BL 170123 A	17012635	1.3	9.3	N/F	12.5	7.1	N/F	N/F	17	N/F	N/F		
BL 170123 B	17012636	1.8	10.5	N/F	14.4	8.3	N/F	N/F	36	N/F	N/F		
BL 170123 C	17012637	2.0	9.1	N/F	11.6	7.5	N/F	N/F	N/F	N/F	N/F		
BL 170123 D	17012638	2.4	11.5	N/F	9.9	7.0	N/F	N/F	N/F	N/F	N/F		
BL 170119 A	17012659	3.5	9.8	N/F	10.8	7.3	N/F	N/F	N/F	N/F	N/F		
BL 170119 B	17012660	2.0	9.5	N/F	11.1	6.3	N/F	5.1	13	N/F	N/F		
BL 170119 C	17012661	1.3	7.7	N/F	6.6	5.5	N/F	N/F	12	N/F	N/F		
BL 170119 D	17012662	1.6	14.6	N/F	14.3	8.4	N/F	4.2	20	N/F	N/F		
BL 170213 A	17022219	2.2	6.1	N/F	3.6	4.1	2.34	2.1	11	N/F	N/F		
BL 170213 B	17022220	1.8	6.2	N/F	3.6	3.3	N/F	2.5	16	N/F	N/F		
BL 170213 C	17022221	2.3	6.1	N/F	3.2	3.7	1.74	1.8	15	N/F	N/F		
BL 170213 D	17022222	2.9	7.7	N/F	5.9	4.5	2.44	2.5	36	N/F	N/F		
proc bl 1	Ftalaat 170123a13											N/F	N/F
proc bl 2	Ftalaat 170123a14											N/F	N/F
proc bl 3	Ftalaat 170123a15											N/F	N/F
proc bl 4	Ftalaat 170123a16											N/F	N/F
proc bl 5	Ftalaat 170124a13											N/F	N/F
proc bl 6	Ftalaat 170124a14											N/F	N/F
proc bl 7	Ftalaat 170124a15											N/F	N/F
proc bl 8	Ftalaat 170124a16											N/F	N/F
proc bl 9	Ftalaat 170126a13											N/F	N/F
proc bl 10	Ftalaat 170126a14											N/F	N/F
proc bl 11	Ftalaat 170126a15											N/F	N/F
proc bl 12	Ftalaat 170126a16											N/F	N/F
proc bl 170213A	Ftalaat 170214a14											N/F	N/F
proc bl 170213B	Ftalaat 170214a15											N/F	N/F
proc bl 170213C	Ftalaat 170214a16											N/F	N/F
proc bl 170213D	Ftalaat 170214a17											N/F	N/F

4.2.2. SPIKED SAMPLES

Spiked oil and matrix (food) samples were analysed between the samples among each batch. Results are listed in Table 12 and Table 13. No exceptional deviations were noted and performance was within the method's specifications.

4.2.3. REPLICATE ANALYSIS

As additional quality control, food samples were analyzed in duplicate in each analytical series. The results of these duplicate analyses are summarized in Table 11. Taking into account the measurement uncertainty, it was checked if the results of the duplicate analysis are in agreement. The confidence interval to check agreement in thas particular case is defined by 2 × U (because U is equal for both measurements). Results of all duplicate analyses were in agreement. The first result is always reported, no results were averaged. Duplicate analysis on LC-amenable compounds was done on samples below LOQ. All results were in agreement (details not shown).
For all except one analysis, results were in agreement. The sample that failed the duplicate analysis showed a result close to the LOQ of the method. We consider the performace acceptable for the levels reported.

Table 11. Results of duplicate analysis of GC amenable compounds.

Filename	Sample Name	DMP amount in product µg/kg	DEP amount in product µg/kg	DAP amount in product µg/kg	DiBP amount in product µg/kg	DnBP amount in product µg/kg	DnHP amount in product µg/kg	BBP amount in product µg/kg	DEHP amount in product µg/kg	DCHP amount in product µg/kg	DnOP amount in product µg/kg	DiDP+DPHP amount in product µg/kg	DINP amount in product µg/kg
	U (%)	25	25	30	30	25	30	25	40	25	20	30	30
17011131	161208-0094 A	< LOQ	6.25	< LOQ	3.25	< LOQ	< LOQ	< LOQ	24.01	< LOQ	< LOQ	< LOQ	< LOQ
17011133	161208-0094 B	< LOQ	5.71	< LOQ	3.74	2.35	< LOQ	< LOQ	24.83	< LOQ	< LOQ	n.m.	n.m.
	% delta	<LOQ	9%	<LOQ	-14%	-81%	<LOQ	<LOQ	-3%	<LOQ	<LOQ	n.a.	n.a.
	Score	PASS	PASS	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	PASS	n.a.	n.a.
17011327	161208-0101 A	2.44	23.21	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	101.97	< LOQ	89.23	< LOQ	< LOQ
17011329	161208-0101 B	2.14	17.17	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	102.74	< LOQ	92.72	n.m.	n.m.
	% delta	13%	30%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	-1%	<LOQ	-4%	n.a.	n.a.
	Score	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	n.a.	n.a.
17011725	161208-0105 A	< LOQ	12.83	< LOQ	< LOQ	2.35	< LOQ	< LOQ	17.71	< LOQ	< LOQ	12.80	< LOQ
17011727	161208-0105 B	< LOQ	12.73	< LOQ	< LOQ	1.67	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	n.m.	n.m.
	% delta	<LOQ	1%	<LOQ	<LOQ	34%	<LOQ	<LOQ	34%	<LOQ	<LOQ	n.a.	n.a.
	Score	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	n.a.	n.a.
17011924	161208-0110 A	< LOQ	7.14	< LOQ	4.30	< LOQ	< LOQ	< LOQ	89.82	< LOQ	15.48	< LOQ	15.23
17011926	161208-0110 B	< LOQ	8.78	< LOQ	3.24	< LOQ	< LOQ	< LOQ	89.15	< LOQ	16.84	n.m.	n.m.
	% delta	<LOQ	-21%	<LOQ	28%	<LOQ	<LOQ	<LOQ	1%	<LOQ	-8%	n.a.	n.a.
	Score	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	n.a.	n.a.
17012674	161208-0116 A	< LOQ	9.30	< LOQ	6.58	10.75	< LOQ	< LOQ	78.15	< LOQ	< LOQ	4.62	4.30
17012676	161208-0116 B	< LOQ	8.63	< LOQ	6.28	12.19	< LOQ	< LOQ	67.58	< LOQ	< LOQ	n.m.	n.m.
	% delta	<LOQ	7%	<LOQ	5%	-13%	<LOQ	<LOQ	14%	<LOQ	<LOQ	n.a.	n.a.
	Score	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	n.a.	n.a.
17012620	161208-0119 A												
17012622	161208-0119 B												
	% delta												
	Score												
17012647	161208-0124 A												
17012649	161208-0124 B												
	% delta												
	Score												
17022233	170208-0010 A												
17022235	170208-0010 B												
	% delta												
	Score												

n.m. - not measured; n.a. - not applicable; % delta calculated based on difference between 2 measurements and average; theoretical confidence interval is defined by 2 × U.

Table 12. Results of analysis of spiked control samples for GC-amendable compounds.

			DMP	DEP	DAP	DIBP	DBP	DnHexP	BBP	DEHP	DCHP	DNOP
Target value (arbitrary units)			60	65	60	59	59	53	52	61	54	59
Batch	**Date**	**ID**	**Measured value**									
1	9/01/17	Ref olie 170109	58	59	68	58	57	57	47	57	41	64
		Delta target %	-3%	-9%	14%	-2%	-3%	6%	-10%	-6%	-24%	8%
2	12/01/17	Ref olie 170112	60	62	71	60	58	69	52	56	46	64
		Delta target %	-1%	-4%	18%	2%	-1%	29%	1%	-7%	-13%	8%
2	16/01/17	Ref olie 170116	57	64	66	62	60	69	55	57	45	64
		Delta target %	-5%	-1%	10%	4%	2%	28%	7%	-6%	-17%	8%
3	18/01/17	Ref olie 170118	53	61	64	57	56	66	47	54	42	75
		Delta target %	-12%	-5%	7%	-4%	-5%	23%	-8%	-11%	-22%	27%
3	19/01/17	Ref olie 170119	56	64	70	63	60	59	58	60	52	63
		Delta target %	-7%	-1%	16%	7%	2%	10%	13%	-1%	-2%	7%
4	20/01/17	Ref olie 170120	57	60	68	58	59	61	51	68	55	70
		Delta target %	-6%	-8%	13%	-2%	1%	15%	-1%	13%	3%	20%
4	23/01/17	Ref olie 170123	53	62	68	61	63	59	55	66	57	66
		Delta target %	-12%	-4%	14%	4%	7%	11%	6%	9%	6%	13%
5	17/02/17	Ref olie 170213A	56	60	76	60	55	67	52	48	52	55
		Delta target %	-7%	-7%	27%	2%	-6%	26%	1%	-22%	-3%	-6%
5	17/02/17	Ref olie 170213B	56	70	74	63	57	67	53	882 *	47	56
		Delta target %	-7%	8%	23%	7%	-3%	25%	2%	1353 %	-12%	-5%

* Sample was labelled as faulty; error noted during work-up; left overnight in GPC apparatus

Table 13. Results of analysis of spiked matrix samples for LC-amendable compounds.

			DiDP+DPHP	DiNP
Target value (arbitrary units)			1300	628
Batch	**Date**	**ID**	**Measured value**	
1	23/01/17	Ftalaat 170123a42	1008	482
			-22%	-23%
2	23/01/17	Ftalaat 170123a43	1199	448
			-8%	-29%
2	24/01/17	Ftalaat 170124a41	1248	573
			-4%	-9%
2	24/01/17	Ftalaat 170124a42	763	733
			-41%	17%
2	24/01/17	Ftalaat 170124a43	939	647
			-28%	3%
3	26/01/17	Ftalaat 170126a32	1281	604
			-1%	-4%
3	26/01/17	Ftalaat 170126a33	1182	615
			-9%	-2%
4	14/02/17	Ftalaat 170214a37	573	553
			-56%	-12%
4	14/02/17	Ftalaat 170214a38	496*	636
			-8%	1%

* Was spiked with 538 instead of 1300

CHAPTER 5 CONCLUDING REMARKS

In total 51 samples were measured. Blanks were low, under control and used to determine the LOQ to minimize false positive results. Quality control samples, such as duplicates and in-house reference material were of sufficient quality. The reported results are considered of good quality. All results should be interpretated in relation to the associated measurement uncertainty.

Most relevant compounds within the sample set were DEP, DiBP, DnBP, and DEHP, with a prevalence ranging from 63 to 92 %. DAP and DCHP were never found above the LOQ, and DMP only in 2 samples. Prevalence of DnHP, BBP, DNOP, DiDP/DPHP and DiNP ranged between 12 and 22 %.

Data interpretation and risk assessment was not part of this project and will not be discussed.

ANNEX A

Ecology Center_13988_Annex_All results.xlsx