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Methods for the determination of phthalates in food

Outcome of a survey conducted among European food control laboratories

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Contents

Abbreviations

Abbreviation	Common name
DMP*	Dimethyl phthalate
DEP*	Diethyl phthalate
DPrP	Dipropyl phthalate
DBP*	Dibutyl phthalate
DIBP*	Diisobutyl phthalate
DPeP	Dipentyl phthalate
DHXP*	Dihexyl phthalate
DNOP*	Di-n-octyl phthalate
DIOP*	Diisooctyl phthalate
DNP*	Di-n-nonyl phthalate
DINP*	Diisononyl phthalate
DIDP*	Disodecyl phthalate
DAP	Diallyl phthalate
DEHP**	Bis(2-ethylhexyl) phthalate
BBP*	Benzylbutyl phthalate
DCHP*	Dicyclohexyl phthalate
BMPP	Bis(4-ethyl-2-pentyl) phthalate
DMEP*	Bis(2-methoxyethyl) phthalate
BEEP	Bis(2-ethoxyetyl) phthalate

* Abbreviations according to EN ISO 1043-3:1999 D

** The abbreviation according to EN ISO 1043-3 is DOP. However DEHP will be applied in this report for referring to bis(2-ethylhexyl) phthalate due to its wider spread within the analytical community.

Rational

The issue of phthalates in food was raised in 2007 in meetings of the Experts Group on Industrial and Environmental Contaminants, organised by the Directorate General for Health and Consumers (DG SANCO). The experts considered it necessary to evaluate the status of measurement capabilities of official food control laboratories in EU prior taking any further action.

In response to this, the Institute for Reference Materials which is part of the European Commission's Joint Research Centre (JRC-IRMM) was requested to conduct a survey among European food control laboratories on analytical methods applied for the determination of phthalates in food. The survey was conducted in order to evaluate comparability of the analysis protocols, to highlight potential pitfalls and as a follow up to provide support to laboratories that are new in that field.

Introduction

1,2-Benzenedicarboxylic acid esters, which are commonly denoted as phthalates, form a group of compounds that is mainly used as plasticisers for polymers such as polyvinylchloride (PVC). Other areas of application are adhesives, paints, films, glues, cosmetics, and so forth. The number of potential different phthalates is infinite. Despite only a few phthalates are produced at the industrial scale, the annual production of phthalates was estimated by the World Health Organisation (WHO) to approach 8 million tons [1]. The most important congeners are in that respect DEHP, which accounts for about 50 % of the world production of phthalates, DIDP, and DINP. Due to their widespread application phthalates have become ubiquitous in the environment, e.g. Hubert et al. estimated the release of DEHP to the environment to about 1.8 % of the annual production [2]. In addition phthalates are stable in solution and are able to resist high temperature [3]. They degrade under exposure to sunlight and are readily metabolised under aerobic microbial activity.

Humans are exposed to phthalates via food, the air, water and other sources such as cosmetics or pharmaceutical products.

This report focuses on the analysis of phthalates from food products. Food might be contaminated through the migration from packaging materials, via different kinds of environmental sources, or during processing. Fatty and oily foods are primarily contaminated with phthalates due to their lipophilic character. A number of papers dealt with the analysis of phthalates in different kinds of food [4-5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. An overview of the phthalates investigated, food matrices studies, and analytical methods applied in a selected number of papers is given in Table 1 to Table 21 of the Annex 1. The published information is scattered in terms of analytes and food matrices studied. Also the number of samples analysed varies strongly, and information on the representativeness of sampling is hardly given. Some authors investigated the occurrence of phthalates in food from the respective country. Page et al. [5], and Pfordt [6] published surveys of selected phthalates in different foods covering different geographical areas. Other surveys covering individual food items, or total diet samples were initiated by National Authorities of Austria [16], Germany (data not published), Denmark [10], Japan [8], and the UK [17]. The largest survey in that respect was conducted by Germany, covering in total more than 3400 samples analysed between the years 2000 and 2006. A summary of the data was presented to the European Commission in 2007. Most of these samples (2745) were tested for contamination with DBP, but only 2.3 % gave positive results. More significant was the contamination of food with DEHP (31 % of 264

samples), and DINP (23.4 % of 175 samples). More than 59 % of samples tested were positive for DIBP, a phthalate whose toxicity has not been evaluated yet by EFSA. However, the number of both samples (32) and food categories tested (2 – cereals, and cereal products) was limited. In general the given relative figures should be treated with caution, since they might be strongly influenced by the selection of the food matrices investigated, which seems to differ for all analytes.

However, occurrence data of one country cannot be easily extrapolated to another country, since the contamination of food with phthalates depend very much of the predominant pathway of phthalate input into food. This was reported by Sharman et al. [4] who investigated milk samples from Norway and the UK concluding that milk samples from Norway showed higher DEHP levels than those form the UK. However the contrary was found for retail cream and cheese samples. The authors interpreted this additional contamination of Norwegian milk by input during the production process and/or from food packaging, which was different to the UK.

This example highlights the potentially different food contamination levels that can be expected in different geographical regions/countries. The history of a particular food sample has big influence on the phthalate content too. Frankhauser-Noti et al. [15] found large differences of the phthalate contents of food samples of the same type of oily food and concluded that the extent of contact between the fatty food and the food packaging, which is influenced by the way of handling of the food during its shelf life, in other words the history of the particular food sample, plays an important role for the level of contamination.

With regard to the mentioned facts, a number of conditions have to be fulfilled to provide data for a reliable assessment of the exposure of EU citizens to phthalates from food. These are in particular:

- Application of appropriate analysis methods to achieve comparability of data.
- Monitoring of the phthalate content levels in food in all EU Member States due to the potential influence of geography on contamination levels.
- Analysis of a representative number of samples to diminish the influence of the history of a particular food sample on the average phthalate content of the particular food type.

This report focuses on the first point, by summarising information on analysis methods for the determination of phthalates from food that was questioned from official food control laboratories of EU Member States. This information is complemented by details of analysis procedures intended for this purpose that were taken from literature.

Overview on survey

A questionnaire on details of the analysis methods applied for the determination of phthalates from food was set up in spring 2007. It contained questions regarding the analytes covered, food matrices tested, extraction and clean-up of samples, applied measurement technique, as well as a series of questions on details of quality control, and precautionary measures to prevent high blank levels including questions on the design of the laboratories used for performing phthalate analysis. The point's quality control, back ground levels, and precautionary measures to prevent blanks were considered especially important due to the ubiquity of some phthalates, which in some cases is the limiting factor for method performance parameters such as the limit of quantitation (LOQ). Hence the focus of the questionnaire was put on these issues.

The questionnaire was distributed by DG SANCO to the Competent Authorities in the EU Member States as well as by the Community Reference Laboratory for Food Contact Materials to the network of respective National Reference Laboratories. The deadline for returning the filled questionnaire was extended twice due to the initially low number of replies.

The laboratories were requested to submit details on different analysis procedures separately. In total 26 questionnaires were received from food control laboratories of 12 countries. Seven laboratories stated that they do not analyse food but only aqueous food simulants for the phthalate content. Hence these methods were not considered in this report. Another six laboratories stated that they are not at all active in this field.

The German Federal Ministry of Food, Agriculture and Consumer Protection kindly supplied the results of an own survey on analysis methods for the determination of phthalates in food, which was conducted among German official food control laboratories. However, these data are listed separately since the level of detail of the German questionnaire was different to that set up by the JRC.

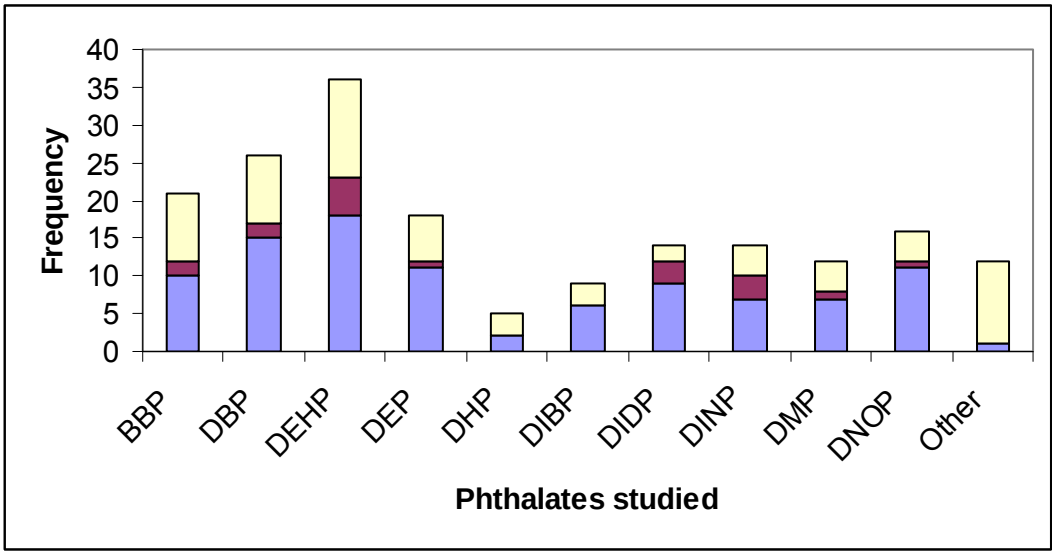
The information given by the food control laboratories is completed by information from scientific literature. It must however be stressed that some providers of data to the JRC are also authors of published papers that are considered in this report.

Phthalates studied

An overview of the frequency at which the individual phthalates are determined in the respective laboratories is given in Figure 1. It combines the responses to the JRC survey (19) and to the German survey (8). In addition information was extracted from 13 published papers and merged with that retrieved from the surveys, resulting in 40 individual data sets.

The most frequently determined congener is DEHP, which is not surprising, since it accounts for about 50 % of the world production of phthalates. It is also the most frequently detected phthalate in food. Page et al. [5] found traces of DEHP in the entire 99 total diet samples they analysed. DBP and BBP are the second and third most frequently analysed phthalates. DIBP, which was found in the German survey at the highest relative rate, has not yet become a routine analyte. It is considered only in about a third of the analysis methods. DIDP and DINP, which are both complex mixtures of different substances generated from the respective technical mixtures of isomeric alcohols, are currently determined by less than 50 % of the laboratories. Other phthalates than the ten listed were included in some studies, but the content of the analysed food was mostly below LOQ [5, 16].

Figure 1: Frequency of analysis of individual phthalates in food

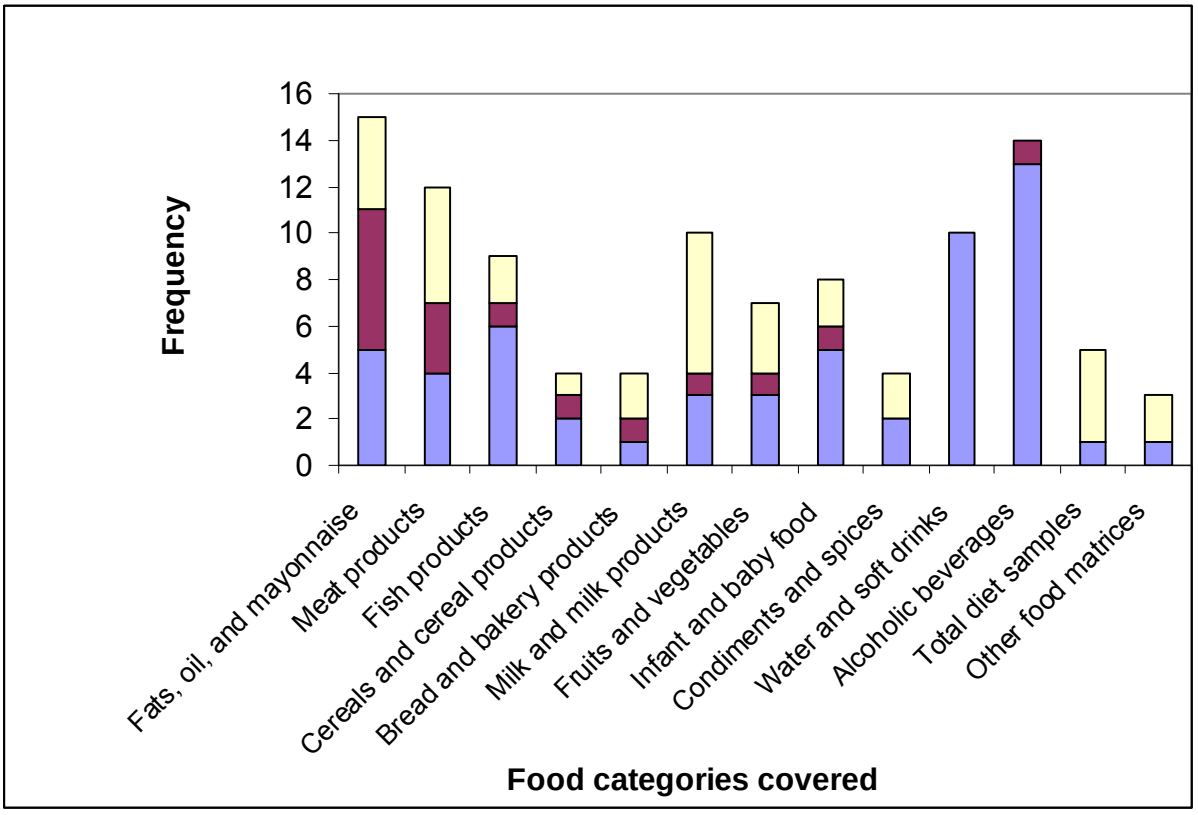


Blue: Compiled results of JRC survey
Purple: Compiled results of German survey
Yellow: Compilation of data from literature

Food matrices investigated

Figure 2 gives an overview on the food matrices that are tested in the respective laboratories. The pattern of the data gathered by the JRC is remarkably different to those of both the German official food control laboratories and the literature data. Table 2 of the Annex 1 lists the food matrices covered by the individual analysis methods that were reported to the JRC. As can be seen the majority of laboratories participating in the survey focuses only on the determination of phthalates in beverages. This is different to the German official food control laboratories, which focus primarily on edible oils and fats. Details are given in Table 15 of the Annex 1. Among the reported methods, covering one or more food categories, are a few that can also be applied for the analysis of total diet samples, which are with respect to matrix composition frequently more complex than individual food commodities. The advantage of these methods is their broad applicability to all kinds of food, as was confirmed by one participant in the JRC survey (Annex 1, Table 2, Method 10).

Figure 2: Food matrices covered by the analysis methods



Blue: Compiled results of JRC survey
Purple: Compiled results of German survey
Yellow: Compilation of data from literature

Sample storage, homogenisation and extraction

In phthalate analysis special attention has to be given to sources of contamination. A potential source of contamination are sampling containers, which for reasons of convenience (tight, unbreakable, resistant to low temperature etc) are frequently made of plastics. Even glassware and aluminium foil might be contaminated with phthalates and might therefore bias analysis results. Hence the original food packaging should be used for sample storage if possible. This principle was explicitly stated by many laboratories participating in the JRC survey (see Annex 1, Table 3). As most food samples, also those intended for phthalate analysis are stored cooled.

Samples have to be homogenised prior to sub-sampling and extraction. This can be achieved by shaking, stirring or mixing. However, many laboratories analysing liquids assume homogeneity of the samples (Annex 1, Table 4) and do not foresee any treatment to homogenise them. In case of solid samples, mixers are used to pulpify them, which is frequently enhanced by the addition of distilled water or polar organic solvents.

Phthalates are extracted from non-fatty liquid samples with unpolar organic solvents and frequently measured without any additional clean up. This is particularly the case for water and soft drinks, and alcoholic beverages (see Table 4) for which most laboratories apply liquid-liquid (L/L) extraction procedures for the isolation of phthalates from the matrix. The solvents employed are chloroform, n-hexane, n-heptane, or isooctane. One laboratory applies solid phase extraction (SPE) for that purpose, but did not give any details of the SPE protocol.

Non-fatty solid foods are frequently extracted with acetonitrile or mixtures of acetonitrile and water. The latter serves for swelling of the matrix respectively lowering of the viscosity of the sample.

Two strategies are applied in case of solid fatty food. Phthalates are extracted from the matrix either together with the fat by application of solvents such as dichloromethane, mixtures of dichloromethane with cyclohexane, n-hexane, and mixtures of n-hexane with acetone, or acetonitrile is used for a more selective extraction of phthalates from the food, which is based on the weak solubility of fat in acetonitrile. The latter procedure was applied for the analysis of total diet samples [8, 9]. The extraction is mostly accomplished by simply shaking of the sample extractant mixture. However the application of ultra-sonic extraction (Annex 1, Table 4, Laboratories 2 and 19), and microwave assisted extraction (MAE) (Annex 1, Table 4, Laboratory 6) were reported as well.

Test portion sizes of 0.1 g to 5.0 g were reported in case of solid samples and up to 300 mL for liquid samples. Other analysis procedures specify test portion sizes of 10 g [4, 16], which however are adapted inversely proportional to the fat content of the sample [6].

Sample clean up

Mainly two techniques are applied for the clean up of the food extracts. These are liquid-liquid partitioning and gel permeation chromatography (GPC).

Most authors of the published papers discussed in this report (Annex 1, Tables 19 to 21) and the majority of the laboratories participating in the German survey (Annex 1, Tables 15 to 18) employed GPC for the clean up of their extracts which, depending on the nature of the sample, contained large amounts of fat. Biobeads® S-X3 (Bio-Rad Laboratories, Hercules, CA, USA) was used in all cases as the filling material of the GPC columns. The dimensions of the applied columns were lengths 30 cm to 50 cm and internal diameter 1.5 cm to 2.5 cm. Mixtures of dichloromethane and cyclohexane (1/1), or cyclohexane and ethyl acetate (1/1) were applied as mobile phase. The dichloromethane mixture provides elution of the analytes in a smaller volume compared to the ethyl acetate mixture. However there is a clear tendency towards the application of the less toxic and with regard to disposal costs cheaper ethyl acetate cyclohexane mixture.

Preparative liquid chromatography on silica columns was used by one German laboratory (Annex 1, Table 16, Laboratory DE06) as an alternative to GPC.

Liquid-liquid partitioning is the preferred extraction technique for non-fatty liquid samples such as soft drinks or alcoholic beverages. Further clean up is not required for these samples. However, Tsumura et al. [8, 9] applied L/L partitioning also for the clean up of extracts of total diet samples. Unpolar, co-extracted interferences were removed by them from the acetonitrile solution by partitioning into n-hexane. Pfannhauser et al. [16] applied dichloromethane for the isolation of the lipid fraction from aqueous acetone extracts of total diet samples and different food items. The lipid fraction was evaporated, reconstituted in cyclohexane/ethyl acetate (1/1) and further cleaned up by GPC on Biobeads® S-X3. Also mixtures of n-hexane and dichloromethane were applied for clean up by L/L partitioning [5].

One laboratory (Annex 1, Table 5, Laboratory 6) applied fractionation on Florisil® columns for the clean up of extracts of fatty food. The eluent was a mixture of diethyl ether (20 %) and n-hexane (80 %).

Florisil was also employed for trapping of the analytes in the sweep co-distillation of extracts of fatty food. This technique is characterised by the transfer of substances released from a heated non-volatile matrix in a stream of inert gas to an adsorbent, on which they are trapped and successively extracted with an organic solvent. However, this technique is used since long in the determination of pesticides from food and rather rarely for phthalate analysis.

Measurement techniques

The major technique for the measurement of phthalates is gas chromatography with mass spectrometric detection. Gas chromatography with flame ionisation detection (GC-FID), or electron capture detection (GC-ECD) are alternatives to mass spectrometry, but are of less importance with regard to frequency of application. Thirteen participants from the JRC survey analyse the sample extracts by GC-MS, whereas only four laboratories apply GC-ECD for that purpose. One laboratory employs both techniques. GC-FID respectively GC-ECD were not applied by any of the surveyed German laboratories (Annex 1, Table 17) and only in two of the papers from literature [5, 14]. Usually columns of low polarity containing stationary phases of the type 5 % phenyl methylpolysiloxane are applied for chromatographic separation of the analytes. The temperature programs vary depending of the complexity of the separation task. Electron ionisation and single ion monitoring mode are commonly applied for GC-MS measurements. A few laboratories operate the mass spectrometer in scan mode, covering a mass-to-charge range of 50 to 350 or even higher (Annex 1, Table 7). After electron ionisation at 70 eV, the major fragment ion of all phthalates but DMP is represented by a mass-to-charge ratio of 149, which is formed by the protonated phthalic acid anhydride ion. This is usually the ion used for quantitation of the analyte content. Despite their low abundance, the majority of laboratories recorded additional ions, which were applied for confirmation of peak identity.

Positive chemical ionisation (PCI) is an alternative to electron ionisation. PCI applying both methane and ammonia as reagent gas produces significantly different mass spectra, which contain more abundant peaks of the molecular ions of the individual phthalates, allowing better identification of the chromatographic peaks as well as differentiation of different phthalates [18]. This is especially advantageous in the analysis of complex mixtures of different isomeric phthalates. However, none of the surveyed laboratories applies chemical ionisation (CI) for the mass spectrometric determination of phthalates.

Two laboratories applied high performance liquid chromatography (HPLC) for the separation of the analytes, one in combination with UV detection (HPLC-DAD), the other with tandem quadrupole mass spectrometry (HPLC-MS/MS) in selected reaction monitoring mode (SRM). Two transitions were recorded for each of the four analytes (Annex 1, Table 17, Laboratory DE03).

Details on chromatographic and mass spectrometric operating conditions are given in Annex 1 in the Tables 7, 17, and 20.

Internal standardisation with isotope labelled standards is not generally applied by all laboratories using GC-MS for the measurement of the sample extracts. Some laboratories employ DAP, DHXP, or BBP as internal standard, and many laboratories participating in the JRC survey used external calibration. The latter was not expected considering potential losses during the specified extraction and clean up procedures.

Method performance

The precision of analysis and recovery were questioned for characterisation of the performance of the analysis method. Other method performance parameters such as the LOD and LOQ were omitted in the JRC survey on purpose, because the laboratories usually do not apply a uniform approach for estimating them. Hence the parameter values are not directly comparable, which might lead to wrong conclusions. LOD and LOQ depend for some ubiquitous phthalates strongly on blank levels and are much higher than the LOD respectively LOQ that could be deducted from the analysis of standard solutions only. However background levels were questioned separately and will be discussed later.

The surveyed laboratories were asked to express the (intermediate) precision of analysis as relative standard deviation. Values between 0.5 % and 28 % were reported in the JRC survey, which is consistent with data from the German survey. Very high relative standard deviations were reported by the laboratory DE03 (Annex 1, Table 18) for the determination of DBP from wine (30 %) and spirits (47 %). An explanation for the high variability was not given by the laboratory. However, the achievable precision is strongly influenced by the food matrix/analyte combination and the analyte content level. Frankhauser-Noti and Grob [15] specified a precision value of 8 % to 11 % for the determination of DIDP in edible oil at a level of 15 mg/kg, whereas the precision improves to 2 % to 5 % at higher concentrations (Annex 1, Table 20). The authors reasoned the lower precision at lower content levels with

bigger problems with integration of the hump formed by the unresolved isomers of DIDP [15]. Precision of analysis might also be affected by background levels which are for some analytes almost unavoidable and difficult to control. However this will be discussed in the next chapter.

Most laboratories correct their results for recovery. Recovery is usually estimated from spiking experiments and is specified for the individual analytes mostly between 80 % and 110 % (Annex 1, Tables 10, 18, and 21). Substantially higher values were reported for DIOP and DINP [8]. The authors reasoned the overestimation of recovery by discrimination between the recovery of the native analytes and the isotopic labelled compounds (D_4-DOP and D_4-DNP). It is also remarkable that recovery of DEHP could not be estimated in two food items due to the high level of naturally incurred DEHP, despite spiking levels of 80 µg/kg respectively 160 µg/kg.

Quality assurance and blank values

The ubiquity of some phthalates causes severe problems in the determination of their content in food. Special measures are required to keep the background levels low. Hence a series of questions were included in the JRC survey targeting quality assurance issues, determination of blank levels, and correction of blank levels. The participants in the survey were also asked about measures applied to keep background contamination low. The results are presented in Annex 1 in the Tables 11 to 14 and Table 21, the latter containing respective information from literature.

Blank values are defined as "a reading or result originating from the matrix, reagent and any residual bias in the measurement device or process, which contributes to the value obtained for the quantity in the analytical procedure" [19]. A variety of different sources, potentially contributing to the blank values of DEHP, and DBP as well as measures to keep them low, were described by Frankhauser-Noti and Grob [20]. Blank values are hardly constant. Therefore they need to be well controlled. The surveyed laboratories include at least one blank sample in each sequence, mostly at the beginning of the sequence. Some laboratories run additional blank samples at the end of the sequence. One laboratory analyses a blank sample with each food sample, another laboratory after each series of nine samples. However, in case blank correction becomes significant, the same attention shall be given to the determination of blank values as it is given to the determination of the analyte in the test

sample. Quality control charts shall be applied for evaluating and monitoring of blank values, which allow the estimation of the mean value of the blank value as well as the corresponding standard deviation [21]. The number of determinations of the blank value in relation to the determinations of the test sample is important with regard to the uncertainty of the blank-value-corrected-result of the test sample, which is equal to the combined uncertainty of the determinations of the analyte contents of both the test sample and the blank value. The magnitude of both the uncertainties of the results for the test sample and the blank value are influenced by the number of replicate analyses. It decreases with increasing numbers of replicate determinations. This is reflected in the analysis procedure of Pfordt [6], who analyses each sample in triplicate and includes two blank determinations in each analysis sequence (Annex 1, Table 21).

Although not explicitly stated, the application of quality control charts for blank correction can be assumed for some studies described in literature [8, 9, 11]. Blank correction is also applied by the majority of the participants in the JRC survey. All laboratories that provided information on typical background levels of the individual phthalates specified blank values for DEHP and DBP ranging between a few µg/kg and 1000 µg/kg,. The majority of the blank values for these two compounds were within the range of 18 µg/kg and 50 µg/kg. This is consistent with information provided in literature (Annex 1, Table 21) [10, 16]. Seven laboratories provided information on maximum tolerable background levels for their analyses, which vary between "blank values are not tolerated at all", "level must not exceed LOQ" and maximum "30 % of the analyte content of the test sample".

However the goal must be to keep blank values low. Frankhauser-Noti and Grob [20] found that, if the use of phthalates containing plastic materials such as PVC is avoided during sample preparation, blank values result mainly from input via the air and particulates. A special design of the laboratories, which focuses on omitting the use of PVC for e.g. coating of the floor, construction of cable ducts etc., could minimise the indoor air contamination with phthalates and hence reduce blank values of certain phthalates. However, only about half of the participants in the JRC survey perform phthalate analysis in laboratories without PVC floors (Annex 1, Table 13). The number of laboratories without other PVC containing items used for construction purposes is even lower. Therefore saturation of the indoor air with phthalates should be avoided by appropriate ventilation, which is applied in about half of the laboratories participating in the JRC survey.

Other measures to prevent high blank values consist of checking solvents and chemicals for contamination before use, distillation respectively clean up of solvents on aluminium oxide prior to use, heating of glass ware in a furnace, rinsing of glass ware with solvents,

exchanging frequently wash solvents, and performing runs without injection to clean the instrument. Their application by the participants of the JRC survey is presented in Table 14 of Annex 1. Frankhauser-Noti and Grob [20] investigated the efficiency of these measures and found that storing apolar solvents, such as n-hexane, over thermally pre-cleaned aluminium oxide is more efficient than redistilling of solvents. The addition of aluminium oxide to wash solvent bottles makes also frequent exchange dispensable. They recommended to heat out glass ware for 2 h at 400°C and store it until use in a desiccator containing aluminium oxide. This procedure was found more efficient than solely rinsing it with solvents [20].

Instrument blanks can be reduced by installing a charcoal filter into the gas supply of the gas chromatograph. Frequently heating out of the injector is also recommended. However attention has to be paid to the temperature of the injector head, which must be sufficiently high to release potentially adsorbed phthalates [20].

Summary

This report summarises details of 19 methods of analysis for the determination of phthalates in food as reported by European food control laboratories to the JRC. This information is completed by information from a survey on the same topic conducted among German official food control laboratories, and data retrieved from scientific publications.

The scopes of the methods range from simple matrices such as beverages to complex total diet samples, and from the determination of single phthalates to a broad range of different phthalates including complex isomeric mixtures. However, bis(2-ethylhexyl) phthalate (DEHP) makes part of the set of analytes in most laboratories. The next most frequently determined phthalate is dibutyl phthalate (DBP). Diisobutyl phthalate (DIBP) whose occurrence in food was recently discussed among risk managers is targeted in only about a quarter of the described analysis procedures.

The analysis procedures are mostly composed of rather simple extraction procedures followed by sample clean up based on either liquid/liquid partitioning or gel permeation chromatography. Separation and detection of the analytes is mainly performed by gas chromatography mass spectrometry, and only rarely by gas chromatography with flame ionisation detection respectively electron capture detection.

The major difficulty in the analysis of phthalates is provided by the ubiquitous presence of the, with respect to potential food contamination, most important members of this class of

compounds. The analyst has continuously to deal with blank problems and has to give special consideration both to minimise them and to keep them under control. The application of plastic materials for sample handling and sample preparation has to be avoided in phthalate analysis. Additional measures such as thermal treatment of glass ware, redistillation of organic solvents, or rinsing of glassware with solvents aim to reduce them as well. The application of thermally cleaned aluminium oxide was found very efficient for cleaning-up of apolar solvents. However, blank problems might also be caused by the analytical instrument. Carry-over respectively input via the carrier gas has to be considered in that respect.

Results of analysis have to be corrected for blank levels since they are hardly avoidable. From the information received from the laboratories it seems that there is no uniform approach to do so. The reported blank levels are as scattered as the frequency of their determination. Hence potential bias cannot be excluded. The validation of analytical methods for determination of phthalates in food is additionally hampered by the unavailability of suitable certified matrix reference materials. Therefore special importance has to be given to the participation in inter-laboratory comparison tests, in order to evaluate the comparability of the results of analysis.

Acknowledgement

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Annex 1: Details of the individual methods

Table 1: JRC survey - Phthalates covered

	Method																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
BBP	Yes	-	Yes	-	Yes	Yes	-	-	-	Yes	Yes	-	Yes	Yes	-	Yes	-	-	Yes
DBP	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	-	Yes
DEHP	Yes	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
DEP	Yes	Yes	Yes	Yes	Yes	Yes	-	-	-	-	Yes	-	Yes	Yes	-	Yes	-	-	Yes
DHXP	-	Yes	-	-	-	Yes	-	-	-	-	-	-	-	-	-	-	-	-	-
DIBP	-	-	-	-	-	Yes	-	-	-	Yes	Yes	Yes	Yes	-	-	-	-	-	Yes
DIDP	-	-	-	Yes	-	Yes	-	-	Yes	Yes	Yes	Yes	-	Yes	Yes	-	-	Yes	-
DINP	-	-	-	-	-	Yes	-	-	Yes	Yes	Yes	Yes	-	-	Yes	-	-	Yes	-
DMP	Yes	-	Yes	-	Yes	Yes	-	-	-	-	-	-	Yes	-	-	Yes	-	-	Yes
DNOP	Yes	-	Yes	Yes	Yes	Yes	-	-	-	Yes	Yes	-	Yes	Yes	-	Yes	-	-	Yes
Other	-	-	-	-	-	-	-	-	-	-	-	Yes[1]	-	-	-	-	-	-	-

[1]: only if detected

BBP	Butylbenzyl phthalate	DIBP	Diisobutyl phthalate
DBP	Dibutyl phthalate	DIDP	Diisodecyl phthalate
DEHP	Bis(2-ethylhexyl)phthalate	DINP	Diisononyl phthalate
DEP	Diethyl phthalate	DMP	Dimethyl phthalate
DHXP	Dihexyl phthalate	DNOP	Di-n-octyl phthalate

Table 2: JRC survey – Food matrices covered

	Method																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Fats, oil, and mayonnaise	-	-	-	-	-	Yes[2]	-	-	Yes[5]	Yes[6]	-	Yes	-	-	-	-	-	Yes[9]	-
Meat products	-	-	-	-	-	-	-	-	Yes[5]	Yes[6]	-	Yes	-	-	-	-	-	Yes[9]	-
Fish products	-	-	-	-	-	Yes[2]	-	-	Yes[5]	Yes[6]	-	Yes	-	-	-	-	-	Yes[9]	Yes
Cereals and cereal products	-	-	-	-	-	-	-	-	-	Yes[6]	-	Yes	-	-	-	-	-	-	-
Bread and bakery products	-	-	-	-	-	-	-	-	-	Yes[6]	-	-	-	-	-	-	-	-	-
Milk and milk products	-	-	-	-	-	Yes[2]	-	-	-	Yes[6]	-	Yes	-	-	-	-	-	-	-
Fruits and vegetables	-	-	-	-	-	Yes[2]	-	-	-	Yes[6]	-	Yes	-	-	-	-	-	-	-
Infant and baby food	-	-	-	-	-	Yes[2]	-	-	Yes[5]	Yes[6]	-	Yes	-	-	Yes[8]	-	-	-	-
Condiments and spices	-	-	-	-	-	-	-	-	-	-	-	Yes	-	-	Yes[8]	-	-	-	-
Water and soft drinks	Yes	Yes	Yes	-	Yes	Yes[2]	Yes[3]	-	-	-	-	-	Yes	Yes[7]	Yes[8]	-	Yes	-	-
Alcoholic beverages	Yes	Yes	Yes	Yes[1]	Yes	Yes[2]	-	Yes[4]	-	-	Yes	-	Yes	Yes[7]	Yes[8]	Yes	Yes	-	-
Other food matrices	-	-	-	-	-	-	-	-	-	Yes[6]		-	-	-	-	-	-	-	-

[1]: Brandy, plum brandy, whisky, cognac, egg-whisky
[2]: Fruits: peach, sherry, plum, pear; Water: drinking water, bottled water; Alcoholic beverages: distillates, fruit distillates, wine; Infant food: food made from various components/meet, rice, flour; Milk and baby foods: powder milk with fruits
[3]: Soft drinks, juice, bottled water
[4]: Spirits, beer, wine
[5]: Pesto, mustard, mayonnaise, duck pie
[6]: Different types of foodstuffs like milk, baby food and total diet samples
[7]: Mineral water, wine, alcohol
[8]: Foodstuffs (paste) Rajah
[9]: Canned meat, preparations of meat, animal fat, liver; canned fish

Table 3: JRC survey - Sample storage: storage temperature and material of storage containers

	Method																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
below 0°C[1]	-	-	-	-	-	-	-	-	Yes	-	-	-	-	-	-	-	-	Yes	Yes
0 to 10°C[1]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	-	Yes	-	Yes	-	-	-	Yes	-	-
above 10°C[1]	-	-	-	-	-	-	-	-	-	-	-	Yes	-	Yes	Yes	Yes	-	-	-
Glass[2]	-	Yes[3]	Yes	Yes	Yes	Yes	Yes[3]	Yes[3]	Yes	-	Yes[3]	Yes	Yes	Yes[3]	Yes[3]	Yes	-	Yes[3]	Yes
Metal[2]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Yes[3]	-	-	-	-
Plastic[2]	-	-	-	-	-	-	Yes[3]	Yes[3]	-	-	-	-	-	-	Yes[3]	-	-	-	-

[1]: Storage temperature
[2]: Material of storage container
[3]: Samples stored in original packaging

Table 4: JRC survey - Sample homogenisation and extraction

		Sample homogenisation	Sample intake	Sample extraction
Method	**1**		10 g	Liquid extraction
	2	For alcoholic beverages not important	2 g	Ultrasonic extraction with 1 mL of n-hexane in small tube (carefully cleaned)
	3	Shaking before analysis	250 mL	SPE
	4	Samples are usually homogeneous	5 g	L/L partitioning into n-hexane, centrifugation, drying with sodium sulphate
	5	Agitation for 30 min in an ultrasonic bath	200 g	200 mL (g) of sample + 2 x 2 mL chloroform, agitation for 2 min, volume of extract is reduced to 0,5 mL
	6	NR	0.2-5.0 g	Water and soft drinks: aliquot of sample extracted for 30 min with 1 mL of n-hexane or isooctane; Vegetables and fruits: aliquot of water added to test portion, shaking for 5 minutes, then addition of 4 mL of isooctane and shaking for 5 minutes; Fat, milk, infant food, oil, fish: adding Na_2SO_4 without water and levigate with sample, then addition of water, methanol and 60 mL of n-hexane:acetone=1:1, microwave assisted extraction applying Microdigest 3.6 (Prolabo, France) conditions: 30% power, 15 minutes
	7	Shaking before analysis	300 mL	Shaking the sample for 3 min after addition of 2 mL of isooctane
	8	Shaking before analysis	2 g	Shaking the sample for 3 min with addition of 20 mL of tap water, 5 mL of saturated solution of NaCl and 2 mL of isooctane
	9	Mixing for 15 minutes at ambient temperature	0.5 g	Liquid/liquid partitioning
	10	NR	NR	NR
	11	NR	10 mL	Add 5mL of aqueous NaCl-solution (5%) + 5mL isooctane; shake for 2 min
	12	NR	0.1-1.0 g	Humid samples homogenised with ethanol, centrifuged, mixed with n-hexane and then water added to split the phases.
	13	NR	2 mL	Water and soft drinks: sample transferred into vial, mixed with 20mL distilled water and centrifuged. Alcoholic beverages: If clean sample then direct injection on column
	14	No homogenisation (liquid sample)	25 g	Extraction of alcoholic beverages with n-hexane
	15	Liquid matrices: without homogenisation; other matrices: homogenisation at room temperature	<1 g	Extraction with n-hexane
	16			Extraction with n-hexane
	17	No homogenisation needed	25 mL	Solid phase extraction on C-18 columns
	18	Homogenise with Büchi mixer B 400, with glass tub, ceramic knife and titan rotor at room temperature, max. 30 sec.	1- 2 g	Extraction with acetone/water mixture, homogenisation with Ultra Turrax 1 min., separation from matrix by filtration, addition of dichloromethane, organic phase evaporated to 1mL, drying under N2-stream, reconstitution in cyclohexane, then addition of ethyl acetate to cyclohexane:ethyl acetate = 1:1
	19	Sample is lyophilised	1.0 g	The freeze-dried sample is extracted with n-heptane in an ultrasonic bath.

Table 5: JRC survey - Sample clean up

		Liquid/liquid partition	Details on liquid/liquid partition	GPC	Details on GPC	Other clean up	Remarks
Method	1	NR	NR	NR	NR	NR	-
	2	-	-	-	-	-	No clean up
	3	-	-	-	-	-	No additional clean up to SPE
	4	-	See extraction	-	-	-	-
	5	-	-	-	-	-	No clean up
	6	-	-	-	-	Yes	Only for oil, fat, milk, milk products, infant food, and fish: clean up on florisil column with 20% diethyl ether in n-hexane
	7	-	See extraction	-	-	-	-
	8	-	See extraction	-	-	-	-
	9	Yes	25 ml of methanol	-	-	Yes	Filtration with 0.45 µm pore diameter membrane filters

Table 5: JRC survey - continued

		Liquid/liquid partition	Details on liquid/liquid partition	GPC	Details on GPC	Other clean up	Remarks
Method	**10**	-	-	Yes	Bio-Beads S-X3, eluent: cyclohexane / ethyl acetate = 1/1. If needed centrifugation before GPC	-	-
	11	NR	NR	NR	NR	NR	-
	12	-	-	-	-	-	no clean-up. Separation from the edible oil occurs in the injector.
	13	-	-	-	-	Yes	SPE, elution - with ethyl acetate
	14	NR	NR	NR	NR	NR	-
	15	-	-	-	-	Yes	Without cleaning but with a pre-column
	16	-	-	-	-	-	-
	17	-	-	-	-	-	-
	18	-	-	Yes	Bio-Beads S-X3, 300 mm lengths, 25 mm i.d.; eluent = cyclohexane / ethyl acetate = 1/1	-	-
	19	NR	NR	NR	NR	NR	-

Table 6: JRC survey – Working range and calibration

	Method																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Working range lower limit (mg/kg)	0.2	1	0.5	0.1	0.1	0.01	0.05	0.02	5	NR	5	10	200	20	10	100	5	0.2	0.1
Working range upper limit (mg/kg)	5	100	1000	10	10	4.0	20	5.0	2500	NR	5000	1000	2000	5000	1000	2000	2000	200	10
External calibration	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	Yes	-	Yes	Yes	-	Yes	Yes	Yes[4]	Yes
Standard addition	-	-	-	Yes	-	Yes	-	-	-	-	-	-	-	-	-	-	-	-	-
Internal standardisation	-	-	-	-	-	-	-	-	-	Yes[1]	-	Yes[2]	-	-	Yes[3]	-	-	-	-
Amount ISTD (mg/kg)	-	-	-	-	-	-	-	-	-	NR[5]	-	1 - 100	-	-	50	-	-	-	-
ISTD added prior extraction	-	-	-	-	-	-	-	-	-	-	-	Yes	-	-	Yes	-	-	Yes	-
ISTD added after extraction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

NR: not reported
[1]: D_4-DBP and D_4-DEHP
[2]: DHXP as main internal standard. Diundecyl-, dihexyl- and dipentylphthalate for verification of thermal desorption and checking linearity of the MS.
[3]: Benzyl-n-butylphthalate
[4]: External calibration, but D_4-DNOP added for quality control purposes
[5]: ISTD added at appropriate level

Table 7: JRC survey – Instrument configuration and instrument parameters

		GC-MS	LC-MS/MS	Other technique	Specify other technique	Column type	Column dimensions (l x i.d. x df)	Temperature programme / Solvent gradient	Measured m/z ratios
Method	**1**	-	-	Yes	GC-ECD	Ultra 2	30 m x 0.2 mm x 0.33 µm	50 °C (1 min) - 25 °C/min - 170 °C - 4°C/min - 270°C	NR
	2	-	-	Yes	GC-ECD	RTX 5	60 m x 0.32 mm x 2.5 µm	50 °C - 180 °C at 30 °C/min, 180 °C - 270 °C at 5 °C/min	NR
	3	Yes	-	-	-	DB-5	NR	50-300 °C	50-350
	4	Yes	-	-	-	MDN - 5S	30 m x 0.25 mm x 0.2 µm	Confidential information	Confidential information
	5	Yes	-	-	-	HP - 1	30 m x 0.25 mm x 0.25 µm	150 °C, hold for 0,5 min., 150 °C to 220 °C at 5 °C/min, 220 °C to 275 °C at 3 °C/min, final temperature 275 °C for 15 min	NR
	6	Yes	-	Yes	GC-ECD	HP 5 MS	30 m x 0.25 mm x 0.25 µm	60 °C/1min - 20 °C/min - 200 °C - 5 °C/min - 280 °C - 12min - 10 °C/min - 300 °C	149-163-206-223-279-293-307-177-194-99-155-251
	7	-	-	Yes	GC-ECD	Ultra 2	50 m × 0.2 mm × 0.33 µm	100 °C (hold 1.0 min), 20 °C/min up to 280 °C (hold 15 min.)	-
	8	-	-	Yes	GC-ECD	Ultra 2	50 m × 0.2 mm × 0.33 µm	100 °C (hold 1.0 min), 20 °C/min up to 280 °C (hold 15 min.)	-
	9	Yes	-	-	-	Varian VF-5MS	30.0 m x 0.25 mm x 0.30 µm	Initial temp: 80°C, t(2min)=80°C, t(15 min)=280°C, t(25,33 min)=280°C	149 for DEHP, 293 for DINP, 307 for DIDP
	10	Yes	-	-	-	DB5-MS	e.g 30 m x 0.32 mm x 0,25µm	Dependent of exact dimension of the column. 2-3 gradients e.g. 90°C in 2 min , ramp 1: 30°C/min to 180°C, ramp 2: 7°C/min to 250°C, ramp 3: 30°C/min to 330°C kept as long as needed. Helium, flow 44 cm/sec.	At least one verification ion is to be used beside the quantification ion (two are better)
	11	Yes	-	-	-	DB-5MS	30m x 0.25mm x 0.25 µm	50°C (0.8 min), 50-280°C (180°C / min), 280°C (23min)	50 - 500

l: lenghts
i.d.: internal diameter
df: film thickness
NR: not reported
m/z: mass charge ratio

Table 7: JRC survey - continued

		GC-MS	LC-MS/MS	Other technique	Specify other technique	Column type	Column dimensions (l x i.d. x df)	Temperature programme / Solvent gradient	Measured m/z ratios
Method	**12**	Yes	-	-	-	Home-made OV-225 with short OV-61	15-20 m x 0.25 mm i.d.	90-300 °C at varied rates (adjusted to obtain best selectivity)	149 (occasionally in scan)
	13	-	-	Yes	HPLC - DAD	Lichrospher 100RP-18 with pre-column	4 mm i.d.	-	-
	14	Yes	-	-	-	DB5	30 m length	Initial temp. 90°C, rate 8°C/min to 250°C, 4°C/min to 280°C, 15min at 280°C, 10°C/min to 300°C, 15min to 300°C	NR
	15	Yes	-	-	-	DB5-MS with precolumn	30m;0,25mm;0,25µm	60°C(1 min) -> 300 °C (7 °C/min)	DEHP: m/z 149; DINP: m/z 293; DIDP: m/z 307; Internal Standard: m/z 149.
	16	Yes	-	-	-	HP-5MS	30 m x 0.25 mm x 0.25 µm	70°C 0,3 min; 30°C/min 220°C; 5°C/min 250°C; 5 min	NR
	17	Yes	-	-	-	HP-5	30m x 0.53 mm x 1.5um	Initial temp 80°C (1min), 30°C/min to 180°C (0min), 6°C/min to 205°C (4min), 20°C/min to 290°C (4min)	NR
	18	Yes	-	-	-	HP 5 ms	30 m x 0.25 mm x 25 µm	50 °C (1 min)- 12 °C/min- 130 °C (0°C/min)- 5 °C/min- 290 °C (15 min)	DEHA: 129, 112, 241, 259; DEHP: 149, 167, 279, DINP: 149, 167, 127; DIDP: 149, 141, 293
	19	Yes	-	-	-	CP SIL 8 CB	0.25 mm i.d.	1.5 min 100 °C, 10 °C/min to 270 °C 6.5 min	GC/MS/MS: DMP - 163 --133 m/z, DBP, DEHP, DIBP, DEP, BBP, DNOP - 149 -- 121 m/z

l: lenghts
i.d.: internal diameter
df: film thickness
NR: not reported
m/z: mass charge ratio

Table 8: JRC survey - Quality control

		Application of QC materials	Specification of QC materials	Application of QC charts
Method	**1**	NR	NR	NR
	2	No	-	Yes
	3	Yes	QC samples of water (spiking of distilled water)	Yes
	4	Yes	Alcohol	Yes
	5	Yes	Phthalic esters mix	No
	6	Yes	Comparison of two standards against each other from different providers - for example Supelco or Fluka; Spiking of samples to appropriate level	Yes
	7	Yes	Mixture of spirits, DBP 0.97 mg/kg, DEHP 0.39 mg/kg used as in-house QC material	NR
	8	Yes	Mixture of spirits, DBP 0.97 mg/kg, DEHP 0.39 mg/kg used as in-house QC material	Yes
	9	Yes	Spiked samples	Yes
	10	Yes	Materials from earlier FAPAS PTs (phthalate mixtures in oil)	NR
	11	Yes	Phthalate esters Mix 1 (Dr. Eherenstorfer, Germany) containing benzyl butyl ester, bis-butyl ester, bis-ethyl ester, bis-2-ethylhexyl ester, bis-methyl ester, and bis-1-octyl ester of phthalic acid, final conc. 2000.00 mg/l; diisobutyl phthalate (>97% Merck); diisononyl phthalate (Fluka); diisodecyl phthalate (>99%, Merck)	No
	12	Yes	Samples spiked with phthalates	Yes
	13	No	-	No
	14	Yes	Phthalates are added to the matrix ; similar concentration like in sample	Yes
	15	No	-	No
	16	Yes	NR	Yes
	17	Yes	DNB and DEHP in ethanol or in methanol as matrix; provider: AccuStandards, USA	Yes
	18	Yes	sample material spike by us	Yes
	19	No	-	No

QC: Quality control
NR: not reported

Table 9: JRC survey - Precision of analyses (expressed as relative standard deviation (%))

		BBP	DBP	DEHP	DEP	DHXP	DIBP	DIDP	DINP	DMP	DNOP	Remarks
Method	**1**	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-
	3	3.5	2.9	2.7	3.0	-	-	-	-	4.1	2.3	-
	4	-	20	20	20	-	-	20	-	-	20	-
	5	15	15	15	15	-	-	-	-	15	15	-
	6	15-20	15-20	15-20	15-20	15-20	15-20	20-25	20-25	15-20	15-20	-
	7	-	5	5.4	-	-	-	-	-	-	-	-
	8	-	5	5.4	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	Determined for each matrix
	10	-	-	-	-	-	-	-	-	-	-	Dependent of the concentration level
	11	5	5	6	5	-		-	-	-	6	-
	12	-	-	-	-	-	-	-	-	-	-	Maximum uncertainty estimated as 25 %
	13	18	18	4	4	-	18	-	-	4	5	-
	14	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-	-	-
	16	-	22	28	-	-	-	-	-	-	-	-
	17	-	0.5	0.5	-	-	-	-	-	-	-	-
	18	-		-	-	-	-	-	-	-	-	Number of data too small
	19	10	8	12	14	-	13	-	-	15	11	-

Table 10: JRC survey - Recovery

		BBP	DBP	DEHP	DEP	DHXP	DIBP	DIDP	DINP	DMP	DNOP	Remarks	Recovery correction
Method	**1**	-	-	-	-	-	-	-	-	-	-	-	No
	2	-	-	-	10	10	-	-	-	-	-	-	NR
	3	85.6	79.8	96.2	72.1	-	-	-	-	85.9	78.3	-	Yes
	4	-	80-100	80-100	80-100	-	-	80-100	-	-	80-100	-	Yes
	5	95 - 100	95 - 100	95 - 100	95 - 100	-	-	-	-	95 - 100	95 - 100	-	Yes
	6	90-100	90-100	90-100	50-60	80-90	80-90	70-90	70-90	50-60	90-100	-	Yes
	7	-	104+/-5	96+/-5	-	-	-	-	-	-	-	-	Yes
	8	-	104+/-5	96+/-5	-	-	-	-	-	-	-	-	Yes
	9	-	-	-	-	-	-	-	-	-	-	Determined for each matrix	Yes
	10	-	-	-	-	-	-	-	-	-	-	Dependent of the concentration level	NR
	11	97.7	90.9	95.9	98.9	-	-	-	-	-	100.6	-	No
	12	-	-	-	-	-	-	-	-	-	-	Matched by internal standard	No
	13	104	91	86	103	-	98	-	-	94	86	-	Yes
	14	-	-	-	-	-	-	-	-	-	-	-	No
	15	-	-	-	-	-	-	-	-	-	-	-	No
	16	-	94	129	-	-	-	-	-	-	-	-	No
	17	-	94 - 105	95 - 105	-	-	-	-	-	-	-	-	No
	18	-	-	-	-	-	-	-	-	-	-	Number of data too small	No
	19	106	101	95	113	-	105	-	-	105	107	-	Yes

NR: not reported

Table 11: JRC survey - Blank levels and background correction

		System blank samples	Typical background level (µg/kg)										Background correction	Description of background correction
			BBP	DBP	DEHP	DEP	DHXP	DIBP	DIDP	DINP	DMP	DNOP		
Method	**1**	Once	-	-	-	-	-	-	-	-	-	-	Yes	-
	2	Every 1	-	-	-	0.1-0.5	0.1-0.5	-	-	-	-	-	No	-
	3	Once	-	-	-	-	-	-	-	-	-	-	NR	-
	4	Every 10	-	50	50	50	-	-	50	-	-	50	Yes	Subtraction of blank signal/area
	5	Begin	-	-	-	-	-	-	-	-	-	-	Yes	Subtraction of blank concentration from sample concentration
	6	Once	5-20	20-30	30-50	5-10	10-20	10-20	0-5	0-5	5-10	5-10	Yes	Subtraction of blank concentration from sample and also subtraction of blank signal/area from sample signal/area
	7	Begin/End	-	0.03	0.16	-	-	-	-	-	-	-	Yes	Determination of average level from 2 blank samples (at the begin and the end of sequence) and subtraction of blank signal from sample and standard areas
	8	Begin	-	20	24	-	-	-	-	-	-	-	Yes	Determination of average level from 2 blank samples (at the begin and the end of sequence) and subtraction of blank signal from sample and standard areas
	9	Begin	-	-	-	-	-	-	-	-	-	-	Yes	Subtraction of blank concentration from sample concentration

Once: once per sequence; Every: every … samples; Begin: at the begin of the sequence; Begin/End: at the begin and at the end of the sequence
NR: not reported

Table 11: JRC survey - continued

		System blank samples	Typical background level (µg/kg)										Background correction	Description of background correction
			BBP	DBP	DEHP	DEP	DHXP	DIBP	DIDP	DINP	DMP	DNOP		
Method	**10**	Begin	-	-	-	-	-	-	-	-	-	-	Yes	Usually blank concentration from sample concentration
	11	Once	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	-	<LOQ	No	-
	12	Every 10	-	10	70	-	-	15	<LOD	<LOD	-	-	No	-
	13	Once	-	-	-	-	-	-	-	-	-	-	No	-
	14	Every 1	-	-	-	-	-	-	-	-	-	-	Yes	Subtraction of blank concentration from sample concentration
	15	Once	-	-	1000	-	-	-	3000	3000	-	-	No	-
	16	-	-	-	-	-	-	-	-	-	-	-	Yes	Subtraction of blank concentration from sample concentration
	17	-	-	10	10	-	-	-	-	-	-	-	No	-
	18	Regularly	-	-	-	-	-	-	-	-	-	-	Yes	Subtraction of blank concentration from sample concentration
	19	Begin	-	5.6	4.3	-	-	9.7	-	-	-	-	Yes	Subtraction of blank concentration from sample concentration

Once: once per sequence; Every: every … samples; Begin: at the begin of the sequence;
NR: not reported
LOD: limit of detection
LOQ: limit of quantitation

Table 12: JRC survey - Maximum tolerable background levels

		Maximum limits for tolerable background levels set	Specification of maximum limits for tolerable background levels	Special procedure when changing instrument from another task to phthalate analysis	Specification of special procedure
Method	**1**	NR	-	No	Instrumentation dedicated to phthalates
	2	No	-	No	-
	3	NR	-	Yes	Blank runs
	4	Yes	10% of analyte content of sample	Yes	Validation of methods
	5	No	-	NR	-
	6	Yes	30% of analyte content of sample	Yes	Blank run; checking used solvents; Analysing blank samples
	7	Yes	Background levels must not exceed LOQ	Yes	Exchange of column, liner, septum; performing blank runs till response of phthalates is below background level
	8	Yes	Background levels must not exceed LOQ	Yes	Exchange of column, liner, septum; performing blank runs till response of phthalates is below background level
	9	Yes	Background level must not exceed the LOQ	Yes	Analyses of blanks
	10	No	-	Yes	Blank runs until satisfactory performance
	11	No	-	NR	-
	12	No	Depends of the purpose of the measurement, but so far the blank was always far below the corrective action limit.	Yes	Heat out the PTV injector at 400 °C
	13	No	-	No	-
	14	NR	-	NR	-
	15	No	-	NR	-
	16	NR	-	No	-
	17	NR	-	NR	-
	18	Yes	In general we don't accept background signals of blank runs	Yes	Rinsing, heating, cleaning, blank runs until there isn't any artefact
	19	NR	-	No	-

NR: not reported

Table 13: JRC survey - Laboratory environment

		Laboratory specially designed for phthalate analysis	PVC floors in laboratory	Other PVC items in laboratory	Air conditioning of laboratory	Permanent ventilation of laboratory
Method	**1**	No	No	No	Yes	No
	2	No	No	No	Yes	Yes
	3	No	No	Yes	No	No
	4	No	No	No	Yes	Yes
	5	No	Yes	NR	No	No
	6	No	Yes	No	Yes	Yes
	7	No	Yes	Yes	Yes	No
	8	No	Yes	Yes	Yes	No
	9	No	No	Yes	Yes	Yes
	10	No	Yes	Yes	No	No
	11	No	No	No	No	No
	12	No	Yes	Yes	Yes	Yes
	13	No	No	Yes	Yes	Yes
	14	-	Yes	Yes	Yes	Yes
	15	No	Yes	Yes	Yes	Yes
	16	No	Yes	Yes	NR	Yes
	17	No	No	No	Yes	No
	18	No	No	Yes	No	No
	19	No	No	Yes	Yes	Yes

NR: not reported

Table 14: JRC survey – Precautionary measures to reduce blank levels

		Check of solvents and chemicals	Treatment of solvents prior to use		Treatment of glass ware prior to use					Check for carry over	Reduction of background stemming from instrument		
			Distillation	Clean up on alox	Heating	Heating time (min)	Heating temperature (°C)	Rinsing with solvent	Type of solvent used for rinsing		Filtering of (carrier) gas(es)	Runs without injection	Frequent exchange of wash solvents
Method	**1**	Yes	No	No	NR	-	-	Yes	n-Hexane	NR	Yes	No	Yes
	2	Yes	Yes	-	Yes	120	550	No	-	NR	Yes	No	Yes
	3	Yes	No	No	No	-	-	Yes	NR	Yes	Yes	Yes	Yes
	4	Yes	No	No	Yes	20	420	Yes	Acetone, n-hexane	Yes	NR	NR	Yes
	5	Yes	-	Yes	Yes	120	200	NR	-	NR	Yes	Yes	Yes
	6	Yes	No	No	Yes	120	300	Yes	n-Hexane	Yes	Yes	Yes	Yes
	7	Yes	No	No	No	-	-	Yes	Tap water, ethanol	No	No	No	Yes
	8	Yes	No	No	No	-	-	Yes	Tap water, ethanol	No	No	No	Yes
	9	Yes	No	No	NR	-	-	Yes	The extraction solvent	Yes	No	Yes	No
	10	Yes	No	No	Yes	240	450	NR	NR	NR	Yes	Yes	Yes
	11	Yes	No	No	No	-	-	Yes	Isooctane	No	Yes	No	No
	12	No	No	Yes	Yes	60	350	Yes	Only septum caps with n-hexane over alox (also the vial with wash solvent contains active alox)	Yes	Yes	No	No
	13	Yes	Yes	-	Yes	120	180	No	-	NR	NR	NR	Yes
	14	Yes	No	No	No	-	-	Yes	Acetone and n-hexane	Yes	Yes	Yes	NR
	15	Yes	No	No	No	-	-	No	-	NR	Yes	Yes	Yes
	16	Yes	No	No	No	-	-	No	-	NR	Yes	Yes	Yes
	17	Yes	Yes	No	No	-	-	Yes	Methanol	Yes	Yes	Yes	Yes
	18	Yes	No	No	Yes	240	400	Yes	Acetone	Yes	No	Yes	Yes
	19	Yes	Yes	-	Yes	120	180	No	-	NR	Yes	NR	NR

NR: not reported
alox: Aluminium oxide

Table 15: German survey - Overview on phthalates and food matrices analysed by German official food control laboratories

		DE01	DE02	DE03	DE04	DE05	DE06	DE07	DE08
Analytes	BBP	Yes	-	-	-	-	Yes	-	-
	DBP	Yes	-	Yes	-	-	-	-	-
	DEHP	Yes	-	Yes	Yes	-	Yes	Yes	-
	DEP	Yes	-	-	-	-	-	-	-
	DHXP	-	-	-	-	-	-	-	-
	DIBP	-	-	-	-	-	-	-	-
	DIDP	-	-	Yes	-	-	Yes	Yes	-
	DINP	-	-	Yes	-	-	Yes	Yes	-
	DMP	Yes	-	-	-	-	-	-	-
	DNOP	Yes	-	-	-	-	-	-	-
	Other	-	-	-	plus others	various	-	-	various
Food matrices	Fats, oil s, and mayonnaise	Yes	Yes	-	Yes	Yes	Yes	-	Yes
	Meat products	-	Yes	-	-	-	Yes	Yes	-
	Fish products	-	-	-	-	-	-	Yes	-
	Cereals and cereal products	-	Yes	-	-	-	-	-	-
	Bread and bakery products	-	Yes	-	-	-	-	-	-
	Milk and milk products	-	Yes	-	-	-	-	-	-
	Fruits and vegetables	-	Yes	-	-	-	-	-	-
	Infant and baby food	-	Yes	-	-	-	-	-	-
	Condiments and spices	-	-	-	-	-	-	-	-
	Water and soft drinks	-	-	-	-	-	-	-	-
	Alcoholic beverages	-	-	Yes	-	-	-	-	-
	Total diet samples	-	-	-	-	-	-	-	-
	Other food matrices	-	-	-	-	-	-	-	-

Table 16: German survey – Sample extraction and sample clean up

		DE01	DE02	DE03	DE04	DE05	DE06	DE07	DE08
	Sample intake	-	-	-	-	-	-	1 g to 10 g	-
	Sample extraction	-	Mixing of samples with water and acetone; addition of isotope labelled internal standard; homogenisation and filtration; extraction with dichloromethane	No extraction	Extraction of fat with diethyl ether	Dilution in ethyl acetate/cyclohexane=1/1	Extraction of fat	Extraction with water/acetone mixture	-
Sample clean up	Liquid/liquid partition	-	-	-	Multiple extraction of the isolated fat with acetonitrile	-	-	L/L partitioning into dichloromethane, evaporation and reconstitution in cyclohexane	-
	GPC / details	Yes / Biobeads S-X3	Yes / -	-	-	Yes / -	-	-	Yes / -
	Other sample preparation	-	-	No clean up	-	-	Isolation of phthalates from fat by preparative liquid chromatography on silica column	-	-

Table 17: German survey – Instrument calibration, instrument configuration and instrument parameters

	DE01	DE02	DE03	DE04	DE05	DE06	DE07	DE08
External calibration	-	-	-	-	-	-	-	-
Standard addition	-	-	-	-	-	-	-	-
Internal standardisation	-	Yes[1]	Yes[2]	Yes[3]	-	-	Yes[4]	-
Working range lower limit (mg/kg)	0.1	-	-	0.2	1	-	0.1	1
Working range upper limit (mg/kg)	10	-	-	-	-	-	200	50
GC-MS	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes
LC-MS/MS	-	-	Yes	-	-	-	-	-
Column type	-	-	Thermo, Betasil Pheny-Hexyl	-	-	-	-	-
Column dimensions	-	-	150 mm x 2.1 mm i.d.; 3 µm particle size	-	-	-	-	-
Temperature/gradient	-	-	Eluent A: acetonitrile + 10 % water + 1 % formic acid + 5 mmol ammonium acetate; Eluent B: water + 1 % formic acid + 5 mmol ammonium acetate: Time 0 min: A/B=65/35; time 6.0 min: A/B=95/5; time: 19.0 min: end of run	-	-	-	-	-
m/z ratios	-	-	ESI+: D_4-DEHP: 395>171, 395>153; DBP: 279>149, 279>205; DEHP: 391>149, 391>121; DIDP: 447>141, 447>149; DINP: 419>127, 419>149	-	-	-	-	-

[1]: Isotopically labelled phthalate, details are missing
[2]: D_4-DEHP
[3]: Diallyl phthalate (DAP)
[4]: D_4-DNOP

Table 18: German survey – Precision of analyses, and recovery

		DE01	DE02	DE03	DE04	DE05	DE06	DE07	DE08
Precision of analyses	BBP	-	-	-	-	-	-	-	-
	DBP	-	-	30 % - 47 %	-	-	-	-	-
	DEHP	-	-	26 % - 31 %	9.5 %	-	-	-	-
	DEP	-	-	-	-	-	-	-	-
	DHXP	-	-	-	-	-	-	-	-
	DIBP	-	-	-	-	-	-	-	-
	DIDP	-	-	13 % - 56 %	-	-	-	-	-
	DINP	-	-	8 % - 17 %	-	-	-	-	-
	DMP	-	-	-	-	-	-	-	-
	DNOP	-	-	-	-	-	-	-	-
	Remarks	7 % -18 % for target analytes	-	-	-	3 % - 10 % (at 5 mg/kg)	-	-	-
Recovery	BBP	-	-	-	-	-	84 %	-	-
	DBP	-	-	75 % - 97 %	-	-	-	-	-
	DEHP	-	-	80 % - 100 %	108 %	-	97 %	-	-
	DEP	-	-	-	-	-	-	-	-
	DHXP	-	-	-	-	-	-	-	-
	DIBP	-	-	-	-	-	-	-	-
	DIDP	-	-	96 % - 103 %	-	-	95 %	-	-
	DINP	-	-	96 % - 98 %	-	-	99 %	-	-
	DMP	-	-	-	-	-	-	-	-
	DNOP	-	-	-	-	-	-	-	-
	Remarks	65% - 95% for target analytes	-	-	-	37 % - 137 % (at 5 mg/kg)	-	-	60 % - 100 %

Table 19: Literature data – Phthalates, and food matrices covered, sample extraction, and sample clean up

(Numbers refer to the respective reference in the references section)

#	Phthalates	Food matrices	Extraction	Clean up
4	DEHP	Mill. Butter, cream, cheese	Extraction into n-hexane	GPC on Biobeads S-X3 column (40 cm x 1.5 cm) after solvent exchange to dichloromethane/cyclohexane (1:1)
5	DMP, DEP, DIBP, DBP, BBP, DCHP, DEHP, DNOP	Various fatty and non-fatty foods	Non-fatty foods: extraction with acetonitrile (+ water) Fatty foods: blended with sodium sulphate and extracted with dichloromethane	Non fatty foods: partitioning into n-hexane/ dichloromethane (10+1) Fatty foods: sweep co-distillation with Florisil trapping and clean-up
6	DEHP, DBP	Bread, cheese, minced meat, ham sausage, hazelnuts, breast milk	Blending of sample with water and acetone followed by L/L-partition into dichloromethane	GPC
7	BBP, DBP, DCHP, DEP, DEHP, DIBP	Jelly, gummy candy, bacon biscuit, egg custard roll	Non-fatty foods: addition of water and partition into cyclohexane/dichloromethane (1/1) Fatty foods: extracted with cyclohexane/dichloromethane (1/1) followed by GPC	GPC on Biobeads S-X3 column (50 cm x 2.5 cm) after solvent exchange to dichloromethane/cyclohexane (1:1)
8	DEP, DPrP, DBP, DPeP, DHXP, BBP, DCHP, DEHP, DIOP, DOP, DINP	Total diet samples	Extraction of sample with acetonitrile	Partition of interferences into n-hexane, evaporation of acetonitrile and reconditioning of extract in n-hexane, clean up on dual layer column (Florisil and Bondesil PSA), elution of analytes with 5 % of acetone in n-hexane
9	DPrP, DBP, DPeP, DHXP, BBP, DCHP, DEHP, DINP	Total diet samples	Extraction of sample with acetonitrile	Partition of interferences into n-hexane, evaporation of acetonitrile and reconditioning of extract in n-hexane, clean up on dual layer column (Florisil (2g) and Bondesil PSA (0.5 g)), elution of analytes with 5 % of acetone in n-hexane
10	DBP, BBP, DEHP	Total diet samples, infant formulae, baby food	Sample mixed with n-pentane	Aliquot of n-pentane extract evaporated applying a Kuderna-Danish evaporator and reconstituted in cyclohexane/ethyl acetate (1/1) prior to GPC clean up on Biobeads S-X3 column (42 cm x 1.5 cm)

Table 19: Literature data – continued (Numbers refer to the respective reference in the references section)

#	Phthalates	Food matrices	Extraction	Clean up
11	DEHP, DIDP, DINP, BBP	Pesto sauce, tomato sauce, olive oil	Extraction with THF/n-hexane (1/1), drying extract with anhydrous sodium sulphate, evaporation and reconstitution in THF	GPC on Biobeads S-X3 column (50 cm x 2.5 cm) after solvent exchange to dichloromethane/cyclohexane (1:1)
12	DEP, DBP, BBP, DEHP, DNOP, DMP,	Ham, sausage, minced meat, milk, cream, margarine, edible oil, trout filets	Liquid food: addition of diethyl ether/n-hexane/methanol (5/4/1) Solid food: addition of acetone / n-hexane / methanol (50/25/25) shaking for 2 h	Filtration of sample extract, evaporation and reconstitution in toluene
13	DMP, DEP, DBP, BBP, DEHP	Whole milk	Samples diluted with methanol and water	Fractionation on C18 cartridge (0.5 g) followed by SPE on Florisil cartridge (5 g)
14	DEHP	Whole milk	Mixing of samples with same volume of ethanol and 0.1 g potassium oxalate, and partitioning lipophilic fraction into diethyl ether/n-pentane (1/1); re-extraction of organic phase with aqueous sodium chloride solution	GPC on Biobeads S-X3 column (26 cm x 2.5 cm) after solvent exchange to dichloromethane/cyclohexane (1:1)
15	DEHP, DIDP, DINP, DHXP, DUP	Edible oil	Thermo desorption in the GC injector	-
16	DMP, DEP, DIBP, DBP, BMPP, BMEP, DAP, BEEP, HEHP, DHXP, BBP, BBEP, DCHP, DEHP, DNOP, DNP	Total diet samples, milk, milk products, fat, oil, margarine, meat, sausage, fish, eggs, bread, cereals, cereal products, infant food, fruits, vegetables, nuts, spices, snacks, and sweets	Addition of distilled water, acetone and internal standards to sample, homogenisation and filtration	L/L partition between aliquot of filtered extract and dichloromethane, isolation and evaporation of dichloromethane phase, reconstitution of residue in cyclohexane/ethyl acetate (1/1) followed by GPC on Biobeads S-X3

Table 20: Literature data - Instrument configuration, instrument parameters, and precision of analyses

(Numbers refer to the respective reference in the references section)

#	Measurement	Column type	Column dimensions	Temperature programme	Measured m/z ratios	Precision of analyses
4	GC-MS in selected ion monitoring mode; splitless injection	CPSIL 5CB	17 m x 0.25 mm x 0.12 µm	100 °C (3 min) - 25 °C/min - 280 °C	DEHP: 149, 167; D_4-DEHP: 153, 171	Milk: 9-21 % (at 0.1 mg/kg); cream and cheese: consistently 10 % and 15 % (at 1.4 mg/kg respectively 0.6 mg/kg)
5	GC-FID; on-column injection	DB-5	15 m x 0.30 mm x 0.25 µm	60 °C (1 min) - 30 °C/min - 120 °C (0 min) - 10 °C/min - 250 °C (3 min) - 50 °C/min - 295 °C (12 min)	-	Depending on analyte/matrix combination: between about 2 % and 20 %
6	Isotope dilution GC-MS	-	-	-	-	-
7	GC-iontrap-MS in selected ion monitoring mode; splitless injection	DB-5	30 m x 0.23 mm i.d.	100 °C (5 min) - 15 °C/min - 300 °C (10 min)	149	Estimated from spiked bread sample (spiking level of 300 µg/kg): 3.6 % to 7.5 % depending on analyte
8	Isotope dilution GC-MS in selected ion monitoring mode; splitless injection	DB-5MS	30 m x 0.25 mm x 0.25 µm	50 °C (1 min) - 10 °C/min - 270 °C (27 min)	-	-
9	Isotope dilution GC-MS in selected ion monitoring mode; splitless injection	DB-5MS	30 m x 0.25 mm x 0.25 µm	50 °C (1 min) - 10 °C/min - 270 °C (27 min)	149, and 293 for quantitation; 191, 209, 104, 150, 237, 251, 206, 167, 167, 279 for confirmation	1.4 % to 3.5 %
10	Isotope dilution GC-MS in selected ion monitoring mode; splitless injection	Restek XTI-5	30 m x 0.25 mm x 0.25 µm	90 °C (1 min) - 8 °C/min - 250 °C (0 min) - 4 °C/min - 280 °C (5 min)	149 for phthalates and 153 for isotopic labelled analogues	DEHP: 9.4 % at level of 0.2 mg/kg

Table 20: Literature data – continued (Numbers refer to the respective reference in the references section)

#	Measurement	Column type	Column dimensions	Temperature programme	Measured m/z ratios	Precision of analyses
11	GC-MS in selected ion monitoring mode; splitless injection, internal standardisation with BBP	DB-17HT	30 m x 0.25 mm x 0.15 µm	60 °C (1 min) - 7 °C/min - 300 °C	-	-
12	GC-MS in selected ion monitoring mode; splitless injection, internal standardisation with DAP	DB-5HT	28 m x 0.32 µm	70 °C (0 min) - 13 °C/min - 280 °C (5 min)	-	-
13	GC-MS in selected ion monitoring mode; splitless injection, internal standardisation with D_4-DEHP (added at the end of sample preparation procedure)	HP-5MS	30 m x 0.25 mm x 0.25 µm	60 °C (0 min) - 6 °C/min - 175 °C (1 min) - 3°C/min - 280°C (0 min) - 7 °C/min - 300 °C	149, and 163 for quantitation; 77, 135, 105, 177, 76, 223, 91, 206, 167, and 279 for confirmation	Milk (spiked to 8 µg/kg): BBP and DBP: 3 % to 5 %; DMP, DEP and DEHP: 18 % to 21 %
14	GC equipped with 2 ECDs; splitless injection; internal standard: BBP (added before injection, after performing test run to check for interferences)	Column 1: CP Sil 5 CB Column 2: CP SIL 19 CB	Column 1: 10 m x 0.25 mm x 0.12 µm; Column 2: 10 m x 0.32 mm x 0.19 µm	90 °C (0 min) - 10 °C/min - 250 °C (0 min)	-	-
15	Injector-internal desorption GC-MS with backflush; selected ion monitoring mode	Column 1: PS 089 Column 2: OV 225, both self prepared	Column 1: 25 cm x 0.5 mm x 0.04 µm; Column 2: 30 m x 0.25 mm x 0.15 µm	120 °C (4 min) - 15 °C/min - 300 °C (3 min)	149	DIDP (15 mg/kg): 8 % - 11 %; at higher concentrations: 2 % - 5 %
16	GC-MS in selected ion monitoring mode; splitless injection, internal standardisation with isotope labelled phthalates	HP-5MS	0.25 mm i.d, x 0.25 µm	110 °C (10 min) - 20 °C/min -150 °C (0 min) - 4°C/min - 250°C (0 min) - 15 °C/min - 300 °C (5 min)	149 and 163 for quantitation; 150, 154, 167, 176, 177, 193, 206, 237, 251 279 for confirmation; isotope labelled compounds 153 and 167	-

Table 21: Literature data – Recovery, working range, precautionary measures to reduce blank levels, and quality control (Numbers refer to the respective reference in the references section)

#	Recovery	Precautionary measures	Working range	Background levels	Quality control
4	100 % ± 7 %	All glass ware, septa, caps, and sample vials rinsed twice with methanol and n-hexane	Milk: about 15 µg/kg to 200 µg/kg; dairy products: about 100 µg/kg to 1500 µg/kg	-	-
5	Depending on analyte/matrix combination: between about 75 % and 122 %	Al glass ware rinsed directly before use with methanol, acetone, and n-hexane	0.25 µg/ml to 10 µg/mL	-	-
6	Breast milk sample spiked to 0.05 mg/kg and 0.1 mg/kg; DEHP: 109 % and 110 %; DBP: 115 % and 116 %; DIBP: 105 % and 106 %-	Heating of glass ware for 24 h to 400 °C, Soxhlet extraction of filter papers,	-	-	Triplicate analyses of each sample, two system blank samples per analysis series, subtraction of system blank contents from results for food samples
7	95 % to 106 % depending on analyte and matrix	All glass ware rinsed twice with distilled dichloromethane	0.02 mg/L to 25 mg/L	-	-
8	DEHP: 67 % to 82 %; DEP, DPrP, DBP, DPeP, DHXP, BBP: 90 % to 105 %; DOP and DCHP: 62 % to 96 %; DIOP and DINP: 96 % to 143 %	All glass ware and items made of stainless steel heated for 2 h at 200 °C followed by rinsing with n-hexane prior to use	-	-	Analysis of system blank sample daily, content values of all system blank samples averaged and subtracted from analyte content of food samples
9	Croquette (fortified to 40 µg/kg to 400 µg/kg): 89 % to 113 % after correction with isotopic labelled standard	-	-	-	Analysis of system blank sample daily, content values of all system blank samples averaged and subtracted from analyte content of food samples
10	At content level of 0.2 mg/kg (9 replicate analyses): DBP : 87 % to 128 %; BBP: 93 % to 101 %; DEHP: 76 % to 116 %	All glass ware rinsed several times with isooctane and methanol	-	Average: DBP: 0.11 mg/kg; BBP: 0.003 mg/kg; DEHP: 0.09 mg/kg	Each analysis batch contained up to 9 single determinations of real food samples, three reagent blanks, two recovery experiments with spiked samples at different levels, and one duplicate determination of a real sample

Table 21: Literature data – continued (Numbers refer to the respective reference in the references section)

#	Recovery	Precautionary measures	Working range	Background levels	Quality control
11					
12	79 % to 90 % depending on analyte	Phthalate free solvents used, all glass ware heated prior to use	-	-	Frequent analysis of system blank samples, content values of all system blank samples averaged and subtracted from analyte content of food samples
13	Spiked milk (8 µg/kg): 86 % to 111 % depending on analyte	Thorough rinsing of SPE cartridges and sorbents	-	-	Analysis of system blank samples
14	Gradual increase of recovery throughout study from about 50 % to above 90 %	All solvents (HPLC grade) and chemicals checked for contamination; removal of potential DEHP sources from the laboratory; all glass ware and equipment rinsed with n-pentane prior to use	-	-	Repetition of analyses when difference between duplicate determination exceeded repeatability limit; analysis of spiked milk sample and system blank sample before and after each food sample; Results corrected with recovery of spiked sample
15	DEHP, DIDP, DINP, and DHXP: >95 % DUP: 88 %	Redistillation of solvent (n-butyl acetate)	-	-	-
16	-	Rinsing of all glass ware with acetone and heating for at least 4 h at 400 °C, heating of sodium sulphate and sodium chloride for 12 h at 400 °C, Soxhlet extraction of paper filter for 24 h with acetone,	-	DEP: 0.003 ±0.001 mg/kg DIBP: 0.012 ±0.005 mg/kg DNBP: 0.030 ±0.011 mg/kg DEHP: 0.023 ±0.004 mg/kg	-

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Abstract

This report summarises details of 19 methods of analysis for the determination of phthalates in food as reported by European food control laboratories to the JRC. This information is completed by information from a survey on the same topic conducted among German official food control laboratories, and data retrieved from scientific publications.

The scopes of the methods range from simple matrices such as beverages to complex total diet samples, and from the determination of single phthalates to a broad range of different phthalates including complex isomeric mixtures. However, bis(2-ethylhexyl) phthalate (DEHP) makes part of the set of analytes in most laboratories. The next most frequently determined phthalate is dibutyl phthalate (DBP). Diisobutyl phthalate (DIBP) whose occurrence in food was recently discussed among risk managers is considered in only about a quarter of the described analysis procedures.

The analysis procedures are mostly composed of rather simple extraction procedures followed by sample clean up based on either liquid/liquid partitioning or gel permeation chromatography. Separation and detection of the analytes is mainly performed by gas chromatography mass spectrometry, and only rarely by gas chromatography with flame ionisation detection respectively electron capture detection.

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