SSPI-CT-2003-502411

HORIZONTAL - ORG

HORIZONTAL STANDARDS ON ORGANIC MICRO-POLLUTANTS FOR IMPLEMENTATION OF EU DIRECTIVES ON SLUDGE, SOIL AND TREATED BIO-WASTE

Instrument: STREP

Thematic Priority: PRIORITY 8.1 STREP Topic 1.5
Environmental assessment

Deliverable D3.14 Phtalate ruggedness report

Due date of deliverable:

Actual submission date:

Start date of project: 1-10-2003

Duration: 3 years

Organisation name of lead contractor for this deliverable: UBA-D

Revision [draft, 1]

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

FINAL REPORT ON THE PROJECT „PHTHALATE“ IN THE FRAMEWORK OF THE EU-PROJECT HORIZONTAL-ORG

PART A: NORMATIVE WORK

PART B: PRE-NORMATIVE WORK

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Contract: SSPI-CT-2003-052411 Project Horizontal-Org March 2007

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PART A

Normative Work

A 1 INTRODUCTION

Plasticizers are used to improve or make possible the processing of plastics, and to increase flexibility or elasticity by decreasing the glass transition temperature of the respective polymer. They are liquid or solid compounds with low vapour pressure, mostly esters that do not react with the polymer but interact with it only physically to produce a homogeneous system. An ideal plasticiser should be odourless, colourless, resistant against water, light, cold and heat, neutral, not toxic and it should demonstrate low flammability and low volatility.

Plasticizers are found in plastics, varnishes, coatings, sealants, rubbers and adhesives and are often applied as mixtures in order to optimise the properties of the resulting plastic.

Chemically most of the compounds are esters, monomeric as well as polymeric, very often of dicarboxylic acids, but also of phosphoric acid. The use of polychlorinated biphenyls as plasticizers has been stopped (Römpp 2003).

Of the several hundreds of different plasticisers the most important group are the esters of 1,2-benzenedicarboxylic acid, the phthalic acid esters. The alcohol moiety consists mostly of linear or branched alkyl chains, usually saturated, and to a lesser extent also of phenyl, cycloalkyl, or alkoxy groups. The phthalates are mainly used as softeners in polyvinyl chloride (PVC) which can contain up to 60% plasticizer, preferably the mid- to high molecular weight esters. 87% of the total production and 95% of di (2-ethylhexyl) phthalate are applied for this (UK Marine SAC 2001). Di-n-butyl phthalate is mainly used in epoxy resins and in cellulose esters and as solvent for many purposes. Dimethyl and diethyl phthalate have similar applications (Staples et al. 1997).

The annual global production of phthalates in the 1990s was approximately 4 million tonnes (Lin et al. 2003) and about one million tonnes are produced each year in Western Europe, of which approximately 900,000 tonnes are used to plasticize PVC. The most common are: di (2-ethylhexyl) phthalate (DEHP, sometimes also referred to as DOP), diisodecyl phthalate (DIDP) and diisononyl phthalate (DINP) (ECPI, 2003 a) representing more than 85% of the total volume of phthalate esters produced in Western Europe (Ecobilan 2001). Of this amount di (2-ethyl hexyl) phthalate (CAS No 117-81-7) accounts for 50% of all plasticiser usage (ECPI, 2003 b).

As the plasticizers are only physically bound they are able to migrate within and leave the polymer and thus enter the environment.

The properties and the distribution of the phthalates in the environment are determined by their physical properties such as partition coefficients and vapour pressure/boiling point which are mainly determined by the length of the alkyl chain.

The better solubility and thus the higher availability of the phthalates with short alkyl chains lead to higher toxicity. Consequently they are susceptible to the influence of degrading micro-organisms and therefore not persistent. The compounds with higher molecular weight and a low solubility are strongly adsorbed to soil and to suspended particulate matter in water. Therefore they are not very accessible to biochemical processes leading to degradation.

The phthalates enter the environment during production and manufacture (minor pathway) and by leaching, migration and volatilisation (major pathway) during use and after disposal of the products. It is estimated that about 100 million tonnes of DEHP were present in the technosphere at the end of the 1990s (Furtmann 1994). Only two of the phthalates are regularly found in environmental samples: primarily DEHP and, to a much lesser degree, DBP.

There are not many data on those phthalates that are technical mixtures of isomers, mostly compounds with more than 8 C-atoms in the side chains. DiNP and DiDP in particular are economically significant, but their concentrations in environmental matrices

have only very rarely been determined. The quantification is difficult because the total amount is spread across several peaks, thus lowering the determination limit, and standards are not available (Lin et al. 2003).

As hazardous substances such as, for example, phthalates, are released into the environment through sewage sludge, composts and soil materials, future legal regulation is envisaged within the EU for organic harmful substances, the precise definition of which must be supported by analytical procedures that apply across the whole of Europe. There is therefore a need for the development of a standard procedure for the analysis of such harmful substances, which provides in its turn the basis for this development and research activity.

The following results were obtained in the context of the EU “Horizontal-Org” project, which was intended to provide a means of unifying the analysis of residues of various harmful organic substances, from sample preparation and processing up to the results of the instrumental analysis., For the soil, sludge and organic waste matrices, in addition to methods for some anorganic parameters, methods were to be worked out for the groups of polycyclic aromatic hydrocarbons (PAH), poly-chlorinated biphenyls (PCB), Adsorbable Organic Halides (AOH), linear alkylbenzol-sulphonates (LAS), nonylphenols and their associated mono- and diethoxylates (NP, NP1EO and NP2EO), as well as for phthalates.

Desk studies on individual substance groups, which preceded the analytical work, set out the current state of affairs for the various parameters. The overviews thus derived were presented and discussed at Horizontal meetings. The desk studies were made available to the wider specialist community and suggested amendments incorporated, as a result of which the studies were confirmed by the BT/TF 151.

The phthalate study investigated the relevance of individual phthalates, additionally collating and comparing the analytical methods that had hitherto been published. The literature under scrutiny was categorized and evaluated according to the following headings:

- Matrix (including amongst others sludge, soil, sediment, surface water, groundwater, waste water, household waste and milk)

- Phthalates investigated
- Extraction (including shaking, SPE, ultrasound, Soxhlet extraction)
- Solvents or extracting agents
- Clean-up (centrifugation, acids with silica gel, florisil, aluminium oxide)
- Separation and determination methods
- Recovery
- Detection and determination limit

A method suitable for more specific selection was supposed to fulfil the following criteria: minimal use of solvents, low solvent toxicity, simple and fast extraction procedure with the fewest possible steps, the use of the most widely available equipment, and good recovery rates with a good level of accuracy.

The evaluation of the data collated pointed already to a possible analytical procedure, which was to be compared with the others and validated for the matrices enumerated above.

After the incorporation of suggested additions and amendments, the phthalate study, along with the others, was published in the Internet under http://www.ecn.nl/horizontal/downloads/finaldeskstudies /index.php.

A total of 30 different methods were contrasted and evaluated. Details can be found in the desk study “Phthalate” by Heise and Litz (2004). Table A1 presents a contrast of the nine most important studies with their most significant work and procedural steps. In Annex I an overview table is reproduced of all the evaluated procedures, standardisations, and house methods that were itemized in the desk study.

Table A 1: Selected and evaluated methods, standards and house methods

author/ method	matrix	method	solvent	volume [mL]	time [h]	cleanup	measure-ment
Fauser(2003)	sludge	shake	DCM	100	4	-	GC/MS
Vikelsøe (2002)	soil	shake	DCM	100	1	-	GC/MS
Kolb (1997)	sludge	shake	EA	20	1,5	-	GC/MS
Berset (2001)	sludge	shake	EA	7	1	-	GC/MS
Chee (1996)	soil, sediment	microwave	ac/hx	30	0,2	-	GC/MS
Ventura (2000)	soil, sediment	ASE	hx	50	1,5	-	GC/MS
CEN/TC292	sludge	ultrasonic.	DCM	150	2	-	GC/MS
CEN/TC308	soil, sludge, sediment	shake	EA	20	0,3	alumina	GC/MS
Bauer (1997)	waste	ultrasonication	hx/ether	30		aumina	GC/MS

A2 NORMATIVE WORK

This includes all the activities dedicated to the establishment of a standard method for determining phthalates in solids, which is to be published as the Horizontal Standard “determination … etc.” These endeavors represented the significant activities of the whole project in terms of time and human resources invested in them.

The method is applicable for the determination of phthalates (see Table 2) in soil, sediment, sludge, waste, and, at the lowest mass content up to 0.1 mg/kg to 0.5 mg/kg, depending on the individual substance and the laboratory blank.

The principle of the procedure is to use samples dried by freeze drying or with sodium sulphate, and to conduct extraction with ethyl acetate on the shaking machine. An aliquot of the extract is cleaned with aluminium oxide (Al_20_3), followed by gas chromatographic

separation using capillary columns, and identification and quantification of the phthalates by mass spectrometry.

A 2.1 SELECTION OF THE PHTHALATES

The above-enumerated test conditions having been weighed up, recourse was had to a suggested procedure for the determination of various phthalates in water. The selection of 11 different phthalates was found to be in line with the experience of the State Environment Office of North Rhine Westphalia (Landesumweltamt Nordrhein-Westfalen – LUA NRW) in Düsseldorf, which had already declared them to be particularly significant phthalate species (see Table 2). They are sorted in decreasing order of importance and correspond to the following phthalates rarely found in water samples: DEHP (diethyloxylphthalate), DBP (dibutylphthalate), DEP (diethylphthalate), diisobutylphthalate (DIBP, also Di(methylpropyl).phthalate), dicyclohexylphthalate (DCHP), and benzylbutylphthalate (BBP). The remaining phthalates are dimethylphthalate (DMP), dipropylphthalate (DPP), di-n-octylphthalate (DOP), di-n-decylphthalate (DDcP), and Di-n-undecylphthalate (DUP). As the substances to be found in these compounds were expected to be found in the solids to be investigated, due to the volume of their production, these 11 phthalates were selected.

The isomeric mixture of phthalates with long side chains (diisooctylphthalate, diisononylphthalate and diisodecylphthalate), which, by virtue of their high production quantities are technically just as significant as, for example, DEHP, are however not included in the method. As the total quantity of these phthalates in one sample was found to be distributed over a numerous quantity of single peaks, which elute over a long retention period, it is difficult quantitatively to determine them, and the determination limits lie significantly higher than those of the defined individual connections (see example chromatogram in Annex I)

Table A 2: Phthalates determined by this method

No	Name	Formula	Abbrevia-tion	Molar mass g/mol	CAS [1])- No
1	Dimethylphthalate	$C_{10}H_{10}O_4$	DMP	194.2	00131-11-3
2	Diethylphthalate	$C_{12}H_{14}O_4$	DEP	222.24	00084-66-2
3	Dipropylphthalate	$C_{14}H_{18}O_4$	DPP	250.3	00131-16-8
4	Di-(2-methyl-propyl)phthalate	$C_{16}H_{22}O_4$	DIBP	278.4	00084-69-5
5	Dibutylphthalate	$C_{16}H_{22}O_4$	DBP	278.4	00084-74-2
6	Butylbenzylphthalate	$C_{19}H_{20}O_4$	BBzP	312.4	00085-68-7
7	Dicyclohexylphthalate	$C_{20}H_{26}O_4$	DCHP	330.4	00084-61-7
8	Di-(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	DEHP	390.6	00117-81-7
9	Dioctylphthalate	$C_{24}H_{38}O_4$	DOP	390.6	00117-84-0
10	Didecylphthalate	$C_{28}H_{46}O_4$	DDcP	446.7	00084-77-5
11	Diundecylphthalate	$C_{30}H_{50}O_4$	DUP	474.4	03648-20-2

[1]) CAS: Chemical Abstracts System

The toxicological evaluation of this group of phthalates is still the subject of heated debate; in the light of the large quantities produced, a validated procedure for quantitative determination is still nevertheless essential.

A 2.2 MATERIALS AND METHODS

A 2.2.1 SELECTION OF PLAYGROUND MATERIAL FOR NORMATIVE WORK

Two sorts of sewage sludge were taken as samples from the sewage works at Waßmannsdorf near Berlin: one a mixed sludge with a solid proportion of around 5%, the other a digested, dried sludge which also still contained precipitants, consisting of 94% solids.

The mixed sludge was freeze dried. The dried sludge was very fibrous and difficult to mill. In a cross hammer mill in Retsch it proved possible to break up the fibres to some extent, but pulverisation was impossible.

The dried sludge was treated with hot air at temperatures of up to 450°C, resulting in pellets of diameters between 0.5 and 3mm, which could readily be milled. It was sieved through a stainless steel sieve down to 0.2mm and subjected at this size to a ring test for the purpose of phthalate analysis (see below).

An additional advantage of this sludge was that it could be considered sterile.

Sludge sewage compost is no longer produced in the Berlin region, as the copper threshold value laid down in the Waste and Sewage Sludge by-law would be exceeded, rendering it unusable. Only small residual amounts were available. Oral accounts identified one sample as a sewage sludge/sand mixture (1+9), another as a compost with sewage sludge and a large quantity of structure material.

The composts were air-dried and sieved down to diameters of 2mm. Both contained very small quantities of phthalates (table A3). All figures relate to the dry mass (m_T) of the substrate under investigation.

Table A 3: Water contents, combustion loss, and phthalate concentration in (m_T) in sewage sludge compost (SSC), sewage sludge samples (SSS) and soils.

	DEP [mg/kg]	DIBP [mg/kg]	DBP [mg/kg]	DEHP [mg/kg]	Water content[a] [%]	Combus-tion loss [%][a]
KK Oegeln	<0.05	<0.05	<0.05	1.14		
KK Güterfelde	<0.05	<0.05	<0.05	1.02		
KS Waßm.	<0.05	1.61	0.59	37.3	9.7	83.0
KS Waßm. dried	<0.05	0.05	n.f.	34.4	6.3	66.8
Sandy soil, A	<0.05	0.34	0.23	0.74	10.5	12.8
Clay soil, B	<0.05	0.30	1.10	2.06	2.0	10.3
Humic soil C	<0.05	0.31	0.21	1.84	2.1	3.8

n.f. not found, Waßm. Waßmannsdorf near Berlin, site of a sewage works, [a] related in each case to the air-dried mass.

A 2.2.2 SPIKING OF THE SAMPLES

In the case of the spiking of the sample material with the analytes, it is to be ensured that these are evenly distributed and have sufficient time to reach adsorption points that are as similar as possible to those in “grown” samples.
In order to achieve homogeneity, the analytes were dissolved in some millilitres of solvent. The solution was dripped onto the surface until it was moist but not wet. The sample was then shaken to allow dry matrix material to come to the surface. This process was repeated until the spiking solution was fully used up. The solvent usually evaporated within 2 to 4 days, and the sample was repeatedly shaken and allowed to stand as a rule for at least one week, mostly two, so that a so-called “grown” sample was obtained.

A 2.2.3 SELECTION OF INTERNAL STANDARDS

Substances suitable for use as internal standards needed to behave as exactly as possible like the analytes themselves, both in clean-up and in detection phases. In the case of a mass selective detector, these conditions are well fulfilled by radio-labeled substances, which are however very expensive. By virtue of the lower concentrations used the costs per analysis are nevertheless low.

The literature designates two non radio-labeled phthalates as internal standards: di-allylphthalate (DAP) and Di-n-heptylphthalate (DHpP), which are both phthalates that are not used technically and are therefore not to be expected to occur in samples. DAP has a fairly short retention time, which is unfavourable for the determination of the most important phthalate, DEHP, which elutes significantly later. The retention time of DHpP is almost identical to that of DEHP, meaning that a chromatographic separation to the base line and under reasonable conditions cannot often be successfully undertaken, thus precluding correct quantification. All further investigations made use of radio-labeled phthalates (ring deuterated fourfold). At the start of the project, Ring-D4-DBP and Ring-D4-DOP were available, and Ring-D4-DEHP became available later. In line with retention times, D4-DBP was used for those phthalates up to and including DBP, D4-DEHP up to DEHP, and D4-DOP for the remaining phthalates (see sample chromatogram in Annex II).

A 2.2.4 EXTRACTION OF PHTHALATES FROM SOLID MATRICES

As a rule, a quantity of sample material was weighed out sufficient to allow for a ratio of sample mass (g) to volume of extraction agent (mL) of 1:40 to 1:80 in the case of sewage sludge and 1:10 to 1:20 in the case of soil and compost. Extraction agent was a solution in the proportion of 1µg /mL of the internal standard in ethyl acetate. The samples were extracted in 40 minutes on a horizontal shaker. The extracts were cleaned by a column with aluminium oxide (glass column, PFTE-frits, 1g $A1_20_3$).

A 2.2.5 MEASURES FOR THE REDUCTION OF BLANK VALUES

As a result of the experience derived from the method for phthalates in water mentioned above, all glass apparatus needs to be heated for several hours at a temperature of 400°C. The surface of the glass which is thus activated must be deactivated before use by rinsing it with solvent. We initially assumed that unused glass components such as GC vials and Pasteur pipettes were not contaminated by phthalates, which proved on the evidence of subsequent research into the sources of phthalate peaks in blank samples to be false. Heating and rinsing these components significantly reduced the blank values.

The solvents to be used must be tested before use for the presence of phthalates. As manufacturer's solvent batches vary, the solvent used must also always be taken into account as a possible source of increased blank values.

After heating, the apparatus were all either sealed (stoppers, aluminium foil) or kept in containers free of plastic. The aluminium foil used was also heated before use.

The vials for the GC were covered before being sealed with pieces of foil to prevent contact with the plastic material of the crimped cap. It was possible to reuse standard solutions a number of times, but always with new caps. Broached septs were a source of diethylphthalate.

A 2.2.6 EQUIPMENT AND MEASURING CONDITIONS FOR PHTHALATE DETERMINATION

A gas chromatograph mass spectrometer from ThermoFinnigan, a Trace GC, a Polaris Q Ion Trap Detector and the Autosampler AS3000 from ThermoFinnigan were used to determine the phthalates.

A 30 m long Rtx5-MS with a diameter of 0.25mm and a film thickness of 0.25µ was used as separating column; after 22.6.2006 an identical model with a 5m guard column was used.

The following injector conditions were given: split/splitless, 250°C, splitless time 2 minutes, splitless flow 50 mL/min.

Helium (1 mL/min, constant flow) was use as carrier gas, the transfer line was heated to 300°C and the ion source at 212°C. Ionisation was effected by electron bombardment (EI).

The temperature programme ran as follows: 70°C (3 min), 14°/min to 220°C, 4°/min to 260°C, 20°/min to 300°C (10 min). M/z 149 or 163 (for DMP only) and 153 (for D4 standards) were selected as target ions, qualifiers when needed according to Table 2 (Annex II).

A 2.3 SCREENING OF DIFFERENT METHOD STEPS

The desk study presented before the start of the project showed that only a very limited selection of solvents had proved to be suitable for the extraction from solid matrices. The extraction efficiency of the most important of them has been investigated. The methods were in principle derived from the literature but modified so as to enable the same clean-up to be carried out in all cases. As it was established that rotary evaporators were a source of contamination, these could not be used, even though such a use was described in the method derived from the literature.

Due to the high risk of contamination of samples with phthalates from the environment, the method should require the smallest possible number of steps in sample preparation. Of the methods investigated in the desk study only a few were able to fulfil this condition. The parameters of extraction method and solvent as well as various drying methods for samples were more closely investigated.

A freeze dried and spiked sewage sludge sample from Waßmannsdorf near Berlin was used.

The characteristics of these methods are summarised in Table A 4 and the results for sewage sludge extraction depicted in Table A 5.

Table A 4: Characteristics of selected methods of sample processing

solvents	dichloro-methane (DCM)	n-hexane/ acetone	n-hexane/ diethyl ether	dichloro-methane (DCM)	ethyl acetate
operation	shaking	shaking	sonfication (3 x)	sonification (3x)	shaking
	withdrawal of an aliquot	withdrawal of an aliquot	centrifugation (3)	centrifugation (3x)	
	evaporation of solvent	evaporation of solvent	evaporation of solvent	evaporation of solvent	
	cleanup on alumina	cleanup on alumina	cleanup on alumina	cleanup on alumina	cleanup on alumina
detection	GC/MS	GC/MS	GC/MS	GC/MS	GC/MS
resume	toxic,highly volatile, time consuming	time consuming	time consuming	toxic and highly volatile solvent, time consuming	less prone to contamination , minimum of operations

In line with the criteria mentioned in the introduction for the selection of a solvent ethyl acetate was chosen. Mixing with hexane gave rise to an insufficient extraction yield. Although Dichloromethane is comparable in terms of extraction efficiency to ethyl acetate, its toxicity has led to EU-wide efforts to limit its application. Its low boiling point and consequent high volatility are disadvantageous. The high standard deviations in the case of DCM and the mixture containing ethyl come about due to difficulties in practical work caused by the solvent’s high vapour pressure.

Table A 5: Comparison of the recovery (%) of various extraction agents (matrix: sewage sludge) (n=3)

solvent	mL solvent	% R	% std. Dev.	% WF	% std. dev.	% WF	% std. Dev..
		DIBP		DBP		DEHP	
Shaking with DCM	15	126.8	17.4	121.5	20.1	98.7	11.1
Shaking with Hx/Acetone	15	55.9	3.2	87.2	4.8	66.8	1.8
3x Ultrasound with Hx/Ether	60	70.2	2.6	78.2	3.9	53.2	13.3
3x Ultrasound with DCM	45	74.5	4.9	74.5	7.0	73.7	5.2
Shaking with ethyl acetate (IS)	15	114.3	4.6	90.6	4.3	93.9	4.5

Content (100%): R: recovery

A 2.3.1 COMPARISON OF EXTRACTION METHODS

The draft standard suggests a simple shaking method, which is remarkably straightforward due to its simplicity in respect of apparatus and of further operations, thus also to a great extent not susceptible to contamination by ubiquitous phthalates. The results of this method were compared to those obtained by Soxhlet extraction and ASE (Table A6).

Table A 6: Comparison of recovery in % of various methods of extraction with ethyl acetate.

conditions	volume of solvent		DBP %	DEHP %
soxhlet, 6 hrs.	60 mL EA	% recovery	98.0	85.7
		% std.dev. (n=3)	8.9	5.7
ASE, 120°C, 2 cycles	40 mL EA	% recovery	89.1	88.2
		% std.dev. (n=4)	5.2	5.9
shaking, 30 min	15 mL EA	% recovery	90.6	93.9
		% std.dev. (n=3)	4.3	4.5

Both the Soxhlet and raised pressure extraction methods require significantly more solvent than the shaking method. Both methods are also more susceptible to contamination. Simple cardboard cores for Soxhlet extraction need to be thoroughly extracted before use because they contain very large quantities of phthalate. The use of Teflon cores has not been tested, because this method not only requires high quantities of solvent but is also generally protracted.

The pressurized solvent extraction (PSE) only requires 15 to 20 minutes per sample, depending on the programme used, but the samples are processed consecutively. In contrast to this, several samples can be shaken at once, which means that low solvent use and minimal amount of time taken are also significant advantages. The shaking method was thus selected.

A 2.3.2 COMPARISON OF THE SHAKING TIMES FOR SAMPLE EXTRACTION

A further series of tests were conducted to compare various shaking times with ASE. The results depicted in Table A 7 are comparable, the rather low finds with ASE in the cases of aerosols and compost notwithstanding. These results therefore also favour the simple yet effective shaking method.

Table A 7: Comparative extraction of solid samples (m_T) using shaking and ASE (n=2)

	Sewage sludge 1 [mg/kg]	aerosols [mg/kg]	compost [mg/kg]
Shaking (40 min)	39.4	2.45	1.56
Shaking (24 h)	n.b.	2.14	1.69
ASE	42.0	1.31	1.37

In the further course of the work the volume used in shaking was raised for practical reasons to 20 mL. As is shown by work in the context of robustness, the volume of the extraction agent is not a factor within the investigated limits that might have influence on the results.

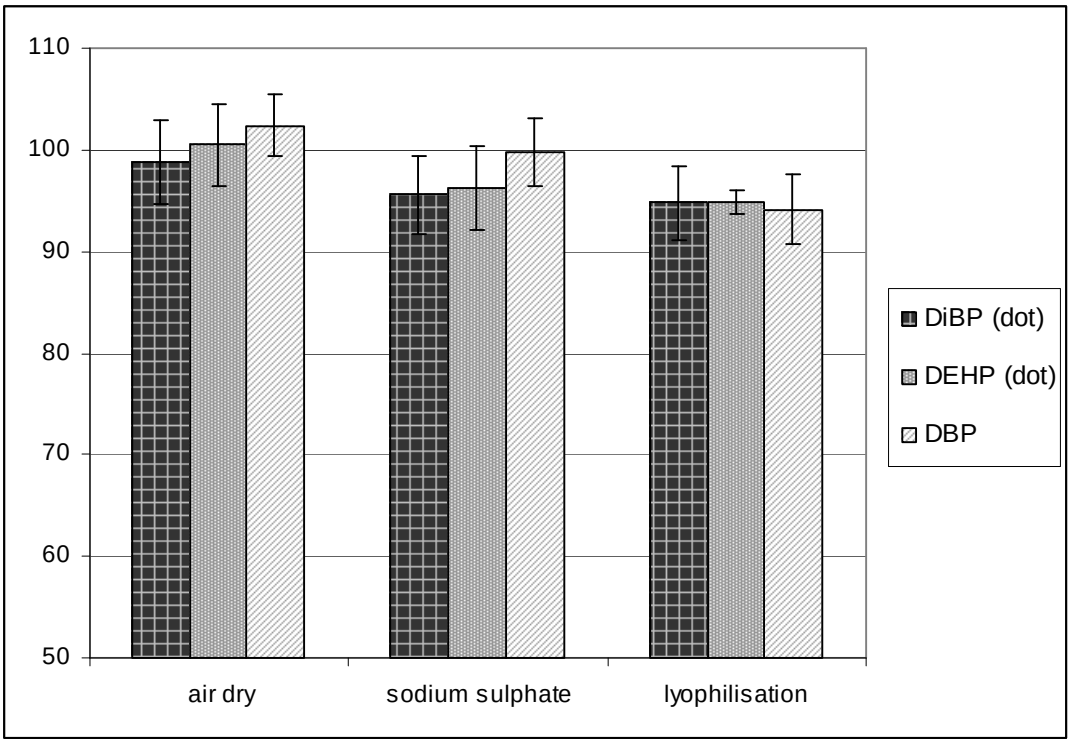
A 2.3.3 COMPARISON OF DIFFERENT METHODS USED FOR DRYING THE SAMPLES

The only suitable method for drying sewage sludge and other samples with a high water content is freeze drying. High temperature procedures were rejected because of concerns about analyte vapourisation or, at moderately raised temperatures, losses due to biological decomposition.

As well as freeze drying, sodium sulphate can also be used to dry damp samples. These two drying procedures, as well as air drying, were compared with one another. To this end, water was added to a clay soil spiked with DiBP and DEHP to a level of 30% by weight, and the sample was subsequently dried. The freeze drying took place overnight. For the sodium sulphate method, three times as much water was added. For the air drying

method, both samples were left until they were completely dry. The phthalate analysis results were compared with those of an untreated control sample. Figure A 1 shows the results for both of the spiked phthalates and for DBP, which, as a ubiquitous phthalate, was intended to act as an indicator of contamination by laboratory air. The sample is especially at risk of contamination in the case of air drying.

Fig. A 1: Comparison of drying methods with soil B, spiked with DiPB and DEHP, as matrix (n=4) in (m_T).

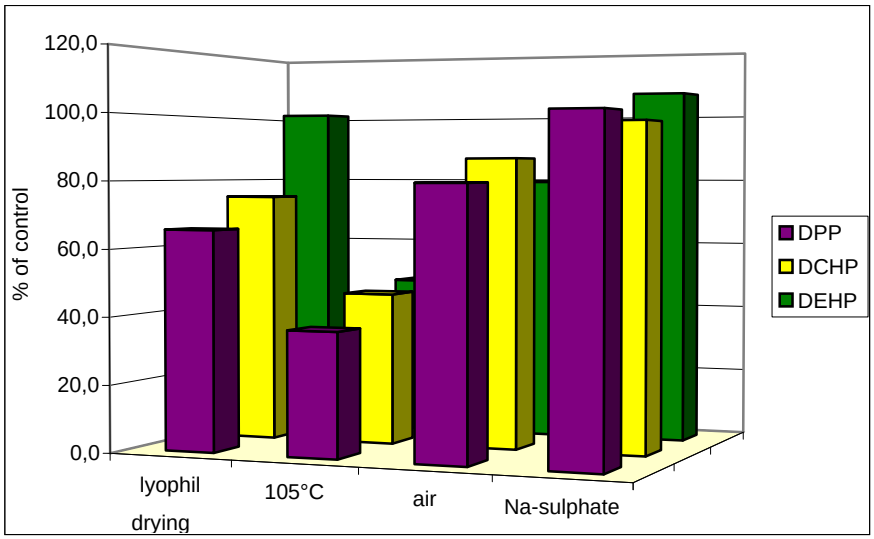


A sample of the spiked soil to which no water had been added served as a control. The differences between the results of the procedures tested are not very great and statistically insignificant. There was no traceable incursion of DBP.

Analogous experiments were carried out with sewage sludge compost (Fig. A 2), which had been spiked with DPP and DCHP. An additional drying method in the form of overnight drying at 105°C was also tested. The results are broadly similar with those obtained in the case of soil B, as figures A1 and A2 show. However, greater losses than in the control sample were observed, above all in the case of freeze drying. As it is above all the introduced phthalates which are affected by this, it is probable that the binding ratios are different from those in mature DEHP and they may be more volatile. What makes this

seem more likely is that DCHP, which is supposed to have a similar vapour pressure to DEHP, behaves just like DPP, whose vapour pressure is some 100 times greater (Staples 1997). For DEHP, the results of drying with sodium sulphate and freeze drying are comparably good, whereas significant losses occurred in the cases of air drying and, particularly, raised temperature drying.

Fig. A 2: Comparison of drying methods in the case of sewage sludge compost in (m_T).



A 2.4 VALIDATION

The parameters to be investigated in the context of validation include specificity, linearity, precision, correctness, reproducibility and determination or traceability limits. The specificity was given by the known retention time of known phthalates and by the quantification mass m/z 149. This mass trace showed only a few, mostly small peaks, of no significance for the quantification, which meant that there was no need to go back to the confirmation ions (qualifiers, see Annex II), as these are relatively small in comparison with the base peak.

A 2.4.1 THE LINEARITY AND MEASUREMENT RANGE OF THE STANDARD SOLUTIONS

The linearity of the concentration ranges (measurement ranges) comprehended in the standard solutions was tested using the standard solutions measured in conjunction with each series of samples. At the start of the project in particular, when no deuterium labelled DEHP was available, the regression line of this phthalate was only linear over a small concentration range. For the range of lower concentrations separate regression lines were worked out if necessary. The standard solutions comprehended a measurement range from around 10 ng/mL to around 10 µg/mL. A typical regression line for DEHP with D4-DEHP as internal standard is depicted in Annex II.

Precision was assured by carrying out regular three- or fourfold determination. Parameters such as correctness, traceability and determination limits, and reproducibility will be considered in the following section.

A 2.4.2 RECOVERY

Determining recovery serves the purposes of establishing the correctness of a method, although it must at the same time be borne in mind that extraction from spiked samples is not in all cases comparable with that from mature samples. Aging processes in the matrix can lead in the latter case to alterations in the binding conditions.

Recovery was investigated primarily using spiked samples of sewage sludge and soil. The most frequently occurring phthalates cannot thus directly be determined because most of the samples are contaminated with one or more of them. If the investigation of recovery reveals systematic deviations from the expected values e.g. if the samples are shown to have been previously exposed to the analytes, then the recovery can be determined with the aid of the recovery function. If the deviations are not dependent on concentration but are seen to be constant across the whole concentration range, then the recovery and the prior contamination of the matrix with the analytes can be determined using stockpiled

samples. This is a possible way of determining recovery for the ubiquitous substances DiBP, DBP, and DEHP. The recovery rates determined in the cases of some phthalates using this method are summarised in table A 8.

The gradient of the lines of the recovery function indicates the recovery values, and the y-intercept the amount of a particular component in the unspiked sample, which is only of relevance in the cases of DiBP, DBP, and DEHP. The values thus determined correlate closely to those measured directly. The relatively high value of the y-intercept for DMP and DUP are artefacts due to an increased spread of measurement values.

Table A 8: Recovery of some phthalates in a humic soil in %

	% WF	Recovery function
DMP	75.4	y = 0.754x + 0.1072
DEP	93.6	y = 0.9364x - 0.025
DPP	97.1	y = 0.971x - 0.0104
DiBP	96.8	y = 0.9681x + 0.2389
DBP	102.2	y = 1.0215x + 0.0871
BBP	104.4	y = 1.0437x - 0.007
DCHP	94.5	y = 0.9449x - 0.0079
DEHP	98.8	y = 0.9881x + 1.6727
DOP	83.2	y = 0.9321x + 0.0004
DDP	114.4	y = 1.1437x - 0.0099
DUP	103.6	y = 1.0356x - 0.0821

The results of an analogous experiment with spiked sewage sludge are depicted in Table A 9. In this matrix, the determination of some phthalates in small concentrations is disrupted by accompanying peaks, and the quantification of the very low spiked quantities of DEHP additional to the prior contamination of the matrix by this phthalate was only possible to a very limited extent. With the spiked concentration ranges, recovery was also not determinable for DMP, DDP, or DUP.

Table A 9: Recovery in sewage sludge in %

	% WF	Recovery function
DMP		n.d.
DEP	99.7	y = 0.997x + 0.1144
DPP	96.6	y = 0.9662x + 0.0466
DiBP	112.4	y = 1.1239x + 1.7387
DBP	87.9	y = 0.8786x + 2.6208
BBP	66.1	y = 0.6609x + 0.8274
DCHP	104.9	y = 1.0485x + 0.0229
DEHP	113.1	y = 1.1308x + 38.223
DOP	96.7	y = 0.9668x + 0.0135
DDP		n.d..
DUP		n.d..

n.d. no data

A 2.4.3 RECOVERY OF THE INTERNAL STANDARDS

In the course of discussions with other participants in the project “Horizontal Org” the question arose as to whether the internal standards were being adequately recovered in the extraction of samples. In order to answer this question, sewage sludge and soil C (humus) were spiked with the three internal standards and processed as usual. At the end, two PAHs – phenanthrene and fluoranthene - were added as recovery or injection standards. As this approach does not correspond to the actual conditions, instead representing a “worst case”, the PAH mixture was also added to further samples, which had as usual been extracted with a standard solution.

The spiking of soil with the deuterated phthalates differs fundamentally from the extraction of soil in the presence of these phthalates under the conditions of the method, because, in the first case, adsorption of the standards to the matrix can be assumed, which the large amounts of solvent almost completely excludes in the second case. The recovery was different for the three deuterated phthalates, but this was independent of the matrix and of

the PAH used as a reference. D4-DBP was thus recovered to around 85%, D4-DEHP to around 120%, and D4-DOP to around 125%.

In a second series of tests, a PAH solution was added as a recovery standard to a constant volume of the finished samples. The results were comparable to those of the first series (Table A 10). The early eluting phenanthrene was only usable as the standard for the similarly early eluting D4-DBP.

Table A 10: Recovery of the internal standards from soil and sewage sludge in m_T

Phthalate	PAH	% recovery of phthalates from soil after spiking *	% recovery of phthalates from soil sample**	% recovery of phthalates from sewage sludge after spiking*	% recovery of phthalates from sewage sludge sample**
D4-DBP	PHE	82	108	102	102
D4-DBP	FLA	79	93	91	96
D4-DEHP	FLA	96	120	124	118
D4-DOP	FLA	92	109	117	120

*: spiking of the matrix with I.S., extraction with pure solvent; ** extraction with I.S. solution

A 2.4.4 VARIOUS PROCEDURES FOR DETERMINING THE DETECTION LIMIT

Various procedures can be used to determine the detection limit. The Signal to Noise ration (SNR) is commonly used, in which case a SNR of 3 is considered to be the detection limit and a value of 10 the determination limit. The ratio can be determined using a straight edge on a printed chromatogram; alternatively, the software of the controlling computer for the GC can calculate the SNR value. This method is similar to the blank value method according to DIN 32645. The detection limit depends amongst other things on the matrix, the weighted sample, and the injected sample volumes.

Another method has as its basis the calibration curve method in DIN 32645. In this method, several samples are investigated in parallel which contain the analytes to be investigated in concentrations close to the limits to be determined. So that the concentrations in the spiked samples are known, they should be free from any impurity that may possibly only roughly be determined. This is generally not the case with the chiefly relevant phthalates such as DBP and DEHP. All the samples from solid matrices that have so far been obtained have contained at least one of these phthalates.

However, in order to make it possible to use the calibration curve method, if only for other phthalates, a sandy, humus-rich soil was spiked with DPP, BBP, DCGHP, and DOP in four concentrations both above and below the expected detection limits. The above named substances occur only very rarely in natural samples, and they appear in the gas chromatogram in proximity to the above mentioned DBP and DEHP. The values thus obtained for the detection and determination limits are summarised in Table A 11. The values are the result of the measurement of four levels of concentration; outliers were eliminated.

Table A 11: Detection and determination limits in soils after DIN 32645 related to dry mass (m_T).

Phthalate	DL [mg/kg]*	DEL [mg/kg]**	Corr. Coeff.
DPP	0.013	0.045	0.9993
BBP	0.035	0.117	0.9972
DCHP	0.021	0.071	0.9987
DOP	0.013	0.042	0.9995

* DL Detection limit, ** DEL Determination limit

The values for the detection limit estimated using the SNR ratio lie between 0.005 mg/kg and 0.05 mg/kg, depending on the matrix, sample mass, and phthalate.
The detection and determination limits were established for spiked sewage sludge using the same procedure (Table A 12). Due to a disruptive matrix peak with the same retention time as BBP, however, this phthalate could not be analysed in the same way. In the lowest spiking concentration the evaluation of the peaks of DPP was also disrupted.

Table A 12: Detection and determination limits in sewage sludge after DIN 32645 related to dry mass (m_T).

Phthalate	LOD [mg/kg] (m_T)	BG [mg/kg] (m_T)	corr. coeff.
DPP	0.208	0.687	0.9961
DCHP	0.133	0.444	0.9983
DOP	0.103	0.345	0.9988

A 2.5 ROBUSTNESS

In the context of a method validation it is also necessary to investigate the robustness of a method, to which end all those parameters need to be investigated which may influence the result of the analysis. This includes the sample preparation and the analysis itself. As, according to the standard method, sample preparation as a possible source of contamination does not apply in any meaningful way, there are only a very few variables in this area. The ratio of sample to extraction agent and the extraction time were varied in the course of the stages of sample preparation, and in the context of the analysis the numerous GC parameters and the volumes of samples were varied.

A 2.5.1 VARIATION OF GC CONDITIONS IN THE MEASUREMENT PROCESS

No influence of variations in the GC temperature programme was detected in the range tested as tables A 13 and A 14 show, as was to be expected, no deviations from the values of the standard programme occurred. What is significant here is that the pair DCHP-DEHP is adequately separated, also under the changed conditions. The type of carrier gas used (helium) and the column type (methyl silicon with 5% phenyl groups) were left unchanged in all cases.

Table A 13: Variation in GC conditions, part a (the given area ratio is that from analyte and internal standard peak areas for a standard solution)

Phthalate	DBP	DEHP		DBP	DEHP
Sample volumes	1 µL	1 µL		2 µL	2 µL
Standard programme	1.00	0.50		0.98	0.46
Starting temperature 65°C	1.01	0.48		1.00	0.46
Starting temperature. 75°C	1.04	0.46		1.03	0.47
13°/min	1.04	0.48		1.01	0.45
15°/min	1.01	0.49		0.97	0.46
1. Gradient to 210°C	1.01	0.48		1.01	0.46
1. Gradient to 230°C	1.03	0.48		0.98	0.47
2. Gradient to 250°C	1.04	0.51		0.97	0.48
2. Gradient to 270°C	1.04	0.51		0.98	0.49
Mean value	1.02	0.49		0.99	0.47
Std.dev.. (n=9)	0.02	0.02		0.02	0.01
% std. Dev..	1.7	3.1		2.1	2.3

Table A 14: Variation in GC conditions, part b (the area ratio given is that from analyte and internal standard peak areas for a soil extract)

Phthalate		DBP	DEHP
Gas flow	0.9 mL/min	0.809	0.499
	1.1 mL/min	0.826	0.504
Injector temp.			
	280°C	0.798	0.510
	220°C	0.789	0.555
Mean value		0.806	0.517
Std. Dev.. (n=16)		0.021	0.026
% Std. Dev..		2.6	5.1

The values indicated are the area ratios of phthalate to the corresponding internal standard and were obtained using standard solution. The mean values are those of all the results of the indicated variations. With the exception of the adduced values, the remaining GC parameters are those of the standard method.

Standard program: 70° (3 min), 14°/min up to 220°C, 4°/min up to 260°C, 20°/min up to 300°C (10 min)

The values in Table A13 were obtained at a constant rate of gas flow and constant injector temperature, as is illustrated in Table A 14 with the GC standard programme as stated under material and method with the exception of the variations mentioned.

The values indicated are the area ratios of phthalate to the corresponding internal standard and were obtained using standard solution. the values for DBP and DEHP are mean values from four measurements in each variant, the mean values and standard deviations cited in the table are derived from four values in each variant, i.e. from a total of 16 values.

A 2.5.2 INFLUENCE OF EXTRACTION CONDITIONS ON THE MEASURING PROCESS

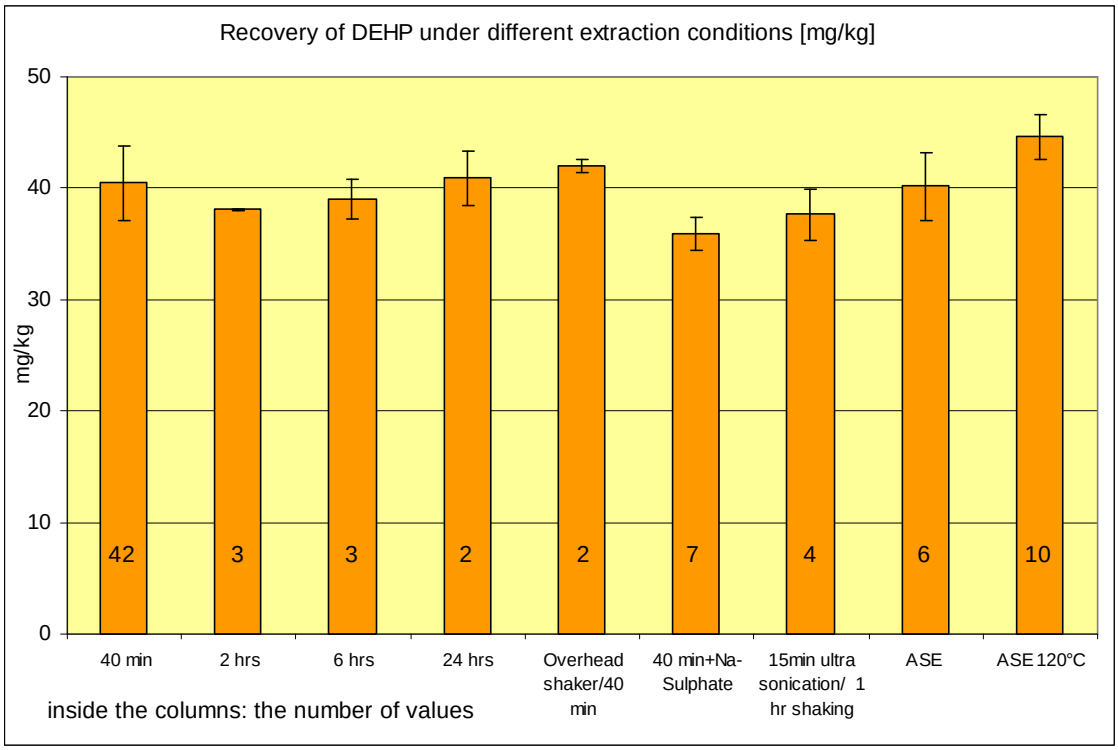
In the course of a series of experiments the extent to which the results depended on the extraction method was investigated. Table A 15 depicts the concentrations of DEHP in a sample of sewage sludge, as they were obtained over a longer period of time with the methods cited. The values derived from the ASE are, at least for the temperature of 120°C, also higher than those obtained by different means, whereby the differences are not statistically significant. In any case, the results after their investigation using single factor ANOVA (Duncan test) were, at around 0.05, not significant.

Table A 15: Comparison of extraction conditions, extraction of DEHP from sewage sludge using ethyl acetate (Waßmannsdorf)

	mg/kg (m_T)	Std. Dev..[mg/kg]	% std. Dev..	quantity of values
40 min shaking	40.46	3.38	8.4	42
2 h shaking	38.10	0.10	0.3	2
6 h shaking	39.05	1.82	4.7	7
24 h shaking	40.89	2.48	6.1	4
overhead shaker/40 min	41.99	0.65	1.6	3
40 min+Na-Sulfate	35.95	1.50	4.2	3
15 min ultrasound/1 h shaking	37.68	2.29	6.1	2
ASE 100°C	40.15	3.04	7.6	6
ASE 120°C	44.63	1.99	4.4	10

The mean value of all measurements is 40.6 mg/kg with a standard deviation of 8.3%. Figure A 3 depicts the results of Table A 15. The Soxhlet extraction has not been included here, having been discarded due to the overly high use of solvents and disproportionate amount of time needed.

Fig. A 3: Results for the extraction of DEHP in sewage sludge with ethyl acetate under different conditions in mg/kg m_T



Higher values are admittedly generally obtained using ASE, at least at an extraction temperature of 120°C, than by using the shaking method, but a significant disadvantage is the danger of contamination; DEP and DBP in particular showed particularly high blank values at certain points. No source of the impurities thus occurring could be found. One can thus assume that ASE, quite apart from the high degree of equipment complexity, is not a suitable method.

A 2.5.3 INFLUENCE OF THE QUANTITY RATIO OF INITIAL AND EXTRACTION VOLUMES

As the sample was shaken only with extraction agent, it can be seen that sufficient amounts of fluid must be present for a thorough mixing to take place. The extraction from a sample of material which is as far as possible free of solid material for cleaning using an aluminium oxide column also requires sufficient volumes for pipetting to take place. In the

context of the investigation of robustness some tests were carried out to determine the possible degree of influence of the ratio of sample mass to the volume of the extraction agent. To this end, different sample masses of clay soil with constant volumes of extraction agent were treated, whereby the volume [mL]/mass [g] ratio was 2-20. No difference could be detected within the context of normal result variations (Fig. A 4).

Fig. A 4: Influence of the volume-sample mass ratio on the amounts of DBP and DEHP found in a clay soil (n=4) in mg/kg m_T

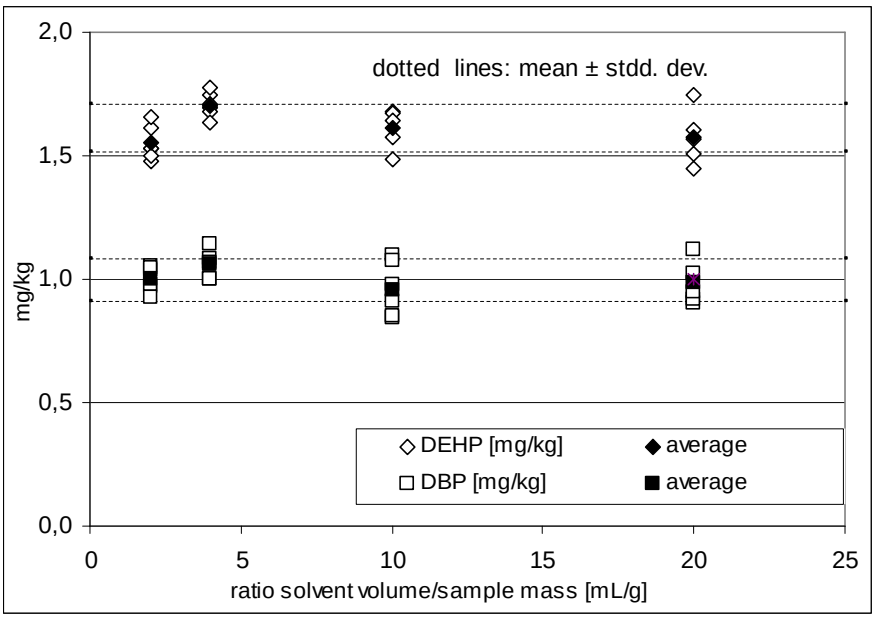
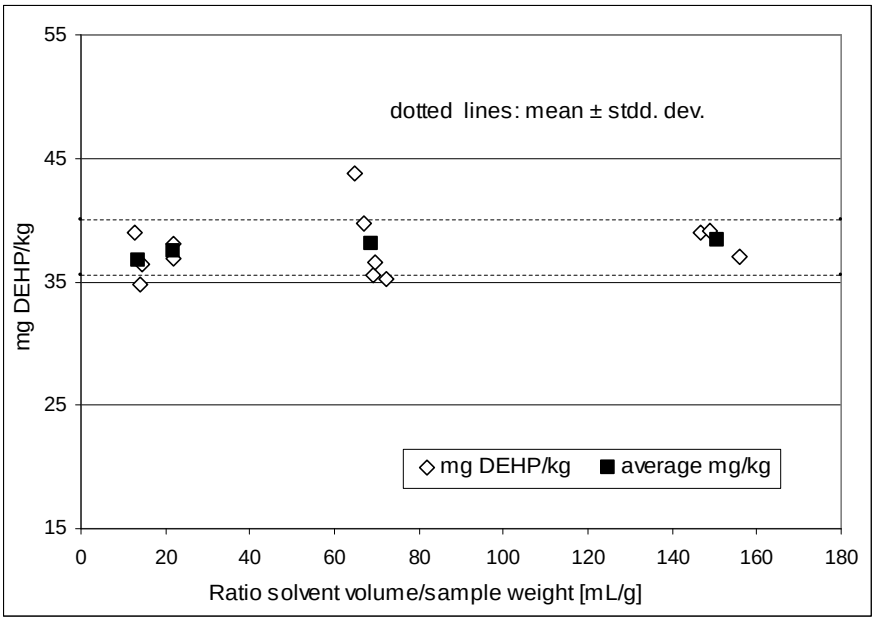


Figure A 5 shows the results of an analogous test with sewage sludge. The range under investigation here includes volume/mass ratios of between 10 and 150.

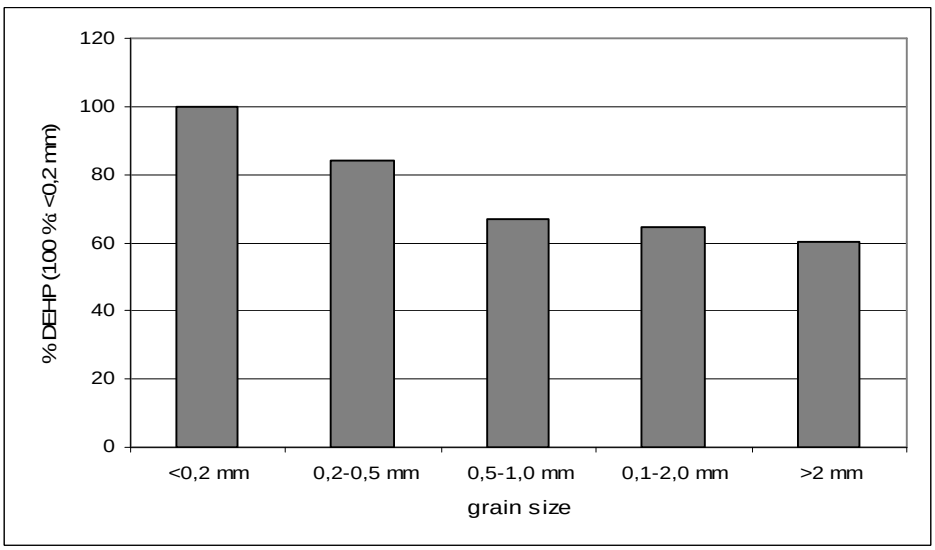
Fig. A 5: Influence of the volume/sample mass ratio on the amount of DEHP found in a sewage sludge sample (mean value per group, n=3 for ratio 15, n=2 for 22, n=5 for 70, and n=3 for 150) in mg/kg m_T



A 2.5.4 INFLUENCE OF GRAIN SIZE ON EXTRACTION

The grain size, thus size of the surface via which the exchange takes place between solid and liquid phase in an extraction has a significant role to play (Fig. A 6).

Fig. A 6: Dependency of the measured DEHP concentration in sewage sludge on grain size in %



For a test to this end, the sewage sludge was dried with hot air, partially milled, and sieved into grains of different sizes. As Figure A 6 shows, a maximum extraction yield was only obtained at the very finest level of milling, of under 0.2mm. The category between 0.2 and 0.5mm returned about 15% less.

A 2.5.5 INFLUENCE OF THE WATER CONTENT OF THE SAMPLES ON THE RECOVERY OF INDIVIDUAL PHTHALATES.

Air-dried sewage sludge contains 6-10% water, and air-dried humic soil (soil C) about 8%. This water content could influence the recovery of the analytes.

The analysis results for “grown” DEHP in air-dried sewage sludge were compared with those obtained on the basis of drying with sodium sulphate.

The water content in soil C was raised in two stages (6% and 12%) and the results for some selected phthalates compared with one another. The values have been converted to air-dried soil. Table A 16 shows a summary of the results. For both matrices the results for the air-dried samples were set at 100%.

Table A 16: Influence of water on the recovery of some phthalates (air-dried samples for comparison).

	% rec. DEP	% rec. DIBP	% rec. DBP	% rec. DEHP
air dry sewage sludge	n.i.	n.i.	n.i..	100
sodium sulphate dried sewage sludge	n.i.	n.i.	n.i..	102
air dry humic soil	100	100	100	100
air dry humic soil + 6 % water	98	97	96	95
air dry humic soil + 12 % water	100	106	104	110

n. i. not investigated

Te sewage sludge mentioned later used in the ring test in the project “sludge support” yielded a dry mass proportion of only 86% after drying and air contact, e.g., in the context of further processing. In order to investigate whether this relatively high proportion of water had an influence on the extraction some samples were levigated with water-free sodium sulphate and analysed jointly with untreated control samples. No difference could be determined (Table A 17).

Table A 17: Comparison of extraction from sewage sludge with and without sodium sulphate

	mg DEHP/kg (m_T)	mg DEHP/kg (m_T)
	with Na_2SO_4	without Na_2SO_4
Sample 1	37.5	34.7
Sample2	35.7	35.6
Sample 3	34.6	

A 2.6 SCREENING OF PHTHALATE SAMPLES FOR THE INTERNATIONAL RING TEST OF THE “HORIZONTAL-ORG” PROJECT

A 2.6.1 PLAYGROUND SAMPLES

In the initial phase of the “Horizontal” project the JRC (Joint Research Center) suggested in Ispra (Italy) making available to the participants in the project so-called playground samples, to help them prepare for the ring test to be conducted at a later date. The samples came from the collection of reference materials from the environment originally held by the “Institute for Environment and Sustainability” (IES) of the JRC. They have already been used for earlier ring tests, certifications, and similar activities. The sample material was manufactured in line with the earlier BCR approach for certified reference materials and can in respect of certain investigated parameters be regarded as homogeneous and stable. The analysis results obtained were to be used merely as information for the laboratories carrying out the work (Table A 18).The samples covered a

broad range of concentrations and were analysed according to the draft method chosen. The results are summarised in Table A 18.

Table A 18: Results of the playground samples related to dry mass (m_T)

Sample	Type of sample	mg/kg	DEP	DIBP	DBP	DEHP
CW1	Composted garbage		<0.05	0.12	0.67	18.81
CW5	Compost		n.d.	<0.05	0.07	6.54
S38	Chinese sediment		n.d.	0.20	n.d.	3.60
SL4	Sludge of domestic origin		<0.05	0.28	0.15	33.81
SL11	Sewage sludge, electronic industry		n.d.	0.21	27.28	58.37
SO1	Brown soil		n.d.	0.45	0.20	0.59
SO4	Terra rossa, <2mm		0.07	0.10	n.d.	0.12
SO7	Clay soil, milled		<0.05	0.08	n.d.	007
SO8	Rice soil		n.d.	<0.05	n.d.	<0.05
SO9	Mineralised soil		n.d.	5.69	n.d.	0.73
SO13	German soil		n.d.	n.d.	n.d.	0.14
SO16	Eurosoil 3R, <2mm		n.d.	n.d.	n.d.	n.d.

n.d. not detectable, no discernible peak, detection limits estimated:

The later stages of the work saw the introduction of DCHP into the measuring programme. After the necessary modifications to the GC temperature programme had been carried out, the remeasurement of some samples showed that sample SL4 contained this phthalate. Its concentration was around 10% of that of DEHP, with which it had originally been recorded.

In general, only DEP, DIBP, DBP, and DEHP were found in the samples. One sample indicated traces of BBP (SL 11), in another (SL 4, see above), DCHP was detectable. In a further sample there were indications of the presence of DOP, which was not however detectable with any certainty due to the overlapping of the peaks. SL 11 also contained significant amounts of isomer mixtures of octyl and nonyl phthalates, the precise quantity of which was estimated using a comparison with the peak areas of DEHP standard

solution. The amounts of isooctyl and isononyl phthalates could only be extrapolated using a concentrated DEHP solution (ca. 60µg/mL) as external standard. In this instance it needs to be taken into account that the relationship between the concentration of DEHP and the size of the GC detector signal is not linear across the whole concentration range. A rough estimate of around 800 mg/kg was made in the case of each isomer mixture.

A 2.6.2 SCREENING OF THE RING TEST SAMPLES OF THE "HORIZONTAL ORG" PROJECT

As a preparatory step ahead of the EU-wide ring test for Horizontal Org, a quantity of available samples was tested by the phthalates sub-project for this substance group (Table A 19). The samples were selected and prepared by IRMM in Geel (Belgium).

Table A 19: Screening samples (figures related to dry mass (m_T))

	DIBP	DBP	DCHP	DEHP	
	mg/kg m_T	mg/kg m_T	mg/kg m_T	mg/kg m_T	weight % water
Compost 1		0.058		1.426	5.57
Sewage sludge 1		0.135		41.474	3.85
Sewage sludge 2	0.538	0.034		30.634	5.47
Sewage sludge 3	0.184	0.037		31.399	1.46
Sewage sludge 4		0.354	1.528	6.678	2.29
Soil 1		0.045		0.263	17.65
Soil 2		0.015		0.183	11.38
Soil 3		0.032		0.119	6.69
Soil 4		0.011		0.302	0.55
Soil 5		0.005		0.011	1.54

A 2.7 IN-HOUSE RING TESTS

A 2.7.1 IN-HOUSE RING TESTS WITH TWO LABS

Ring tests are conducted to test a method, to determine the reproducibility, and of course also as a means of laboratory quality control. In the context of validation of the method to be investigated the laboratory took part in several laboratory comparisons or, as the case may be, in national and international ring tests. The national ring test was organized internally (See Table A 20).

Table A 20: Phthalate contents in mg/kg (m_T) of different substrates in the small laboratory comparative test between LUA and UBA

Sample/sample number	Phthalate	SEA mg/kg (m_T)	FEA mg/kg (m_T)
Suspended matter 1 (1281)	DMP	0.0116	n.d.
	DIBP	0.0531	0.079
	DBP	0.106	0.099
	BBP	0.335	n.d.
	DEHP	2.620	2.680
Dehydrated sludge	DEP	0.142	n.d.
(1546)	DIBP	0.302	0.275
	DBP	0.503	0.457
	DEHP	44.350	77.580
Suspended matter 2 (1681)	DMP	0.0295	n.d.
	DEP	0.0365	0.036
	DIBP	0.153	n.d.
	DBP	0.269	0.181
	BBP	0.120	0.260
	DCHP	0.393	n.d.
	DEHP	5.400	7.670
Compost (CW 5)	DEP	< 0.100	< 0.050
	DIBP	0.100	< 0.050

Cont. Table A 20			
	DBP	0.460	0.070
	DEHP	8.900	6.540
Terra rossa. <2mm (SO 4)	DEP	< 0.180	0.070
	DIBP	< 0.180	0.100
	DBP	< 0.180	< 0.050
	DEHP	< 0.180	0.120
Mineral soil (SO 9)	DEP	< 0.100	n.d.
	DIBP	7.400	5.690
	DBP	0.210	n.d.
	DEHP	1.700	0.730
Sludge of domestic origin (SL 4)	DEP	< 0.150	< 0.050
	DIBP	0.355	0.280
	DBP	0.405	0.150
	DCHP	1.050	n.dt.
	DEHP	30.800	33.800
Compost (CW 5)	DEP	< 0.100	< 0.050

n.d.: not detectable, n.dt.: not determined; SEA: State Environment Agency North Rhine Westphalia, FEA: phthalate laboratory of Horizontal Org at the Federal Environment Agency in Berlin.

As a control for the work of the laboratory, samples were exchanged with the State Environment Agency of North Rhine Westphalia (*Landesumweltamt Nordrhein-Westfalen – LUA-NRW*) in Düsseldorf, which had, especially in the case of water analysis but also in respect of solid matrices, carried out comprehensive preliminary work in phthalate analysis. The first samples were obtained very early, in fact at a time when, in some fields, insufficient experience had been gleaned.

Some low-concentration phthalates were not found, although it was not retrospectively investigated whether these results confirmed those obtained by the LUA. As a rule, however, the results did match quite satisfactorily. DCHP was not found in the Suspended Matter 2 sample because it was at that time not an ingredient in the standard cocktail, thus the GC method was not suitable for the separation of DEHP and DCHP. No explanation

was found for the relatively big differences in DEHP results in the case of dehydrated sewage sludge sample (Table A 20)

A 2.7.2 EXTENDED IN-HOUSE RING TEST WITH THREE DIFFERENT GERMAN LABS

For the purpose of the laboratory comparison the participants received four samples each: two sewage sludge samples, one Suspended Matter sample, and a compost sample. As in the “Horizontal Org” project, these were extracted using the ASE standard procedure. In contrast to the sewage sludge sample in the Sludge Support ring test, not significant differences emerged for DEHP, and the results for the other phthalates corresponded satisfactorily. The results are depicted in Table A 21.

Table A 21: Phthalate contents in mg/kg (m_T) of different substrates of the “small ring test”

	Suspended Matter			Compost		
	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3
	mg/kg(m_T)	mg/kg(m_T)	mg/kg	mg/kg(m_T)	mg/kg(m_T)	mg/kg(m_T)
DMP	< 0.293	0.005*		< 0.293	<0.10	
DEP	< 2.115	0.018*	<0.08	< 2.115	0.01*	<0.08
DPP	< 0.213	<0.10		< 0.213	<0.10	
DIBP	< 0.184	0.045*	<0.08	< 0.184	0.115	0.14
DBP	< 0.474	0.064*	0.080*	< 0.474	0.034*	0.1
BBP	< 0.476	0.040*		< 0.476	<0.20	
DCHP	< 0.364	0.059*	<0.20	< 0.364	0.024*	< 0.20
DEHP	2.415	2.45	2.45	1.2	1.55	1.56
DOP	< 1.254	0.075*		< 1.254	<0.20	
DDcP	< 0.281	0.140*		< 0.281	<0.50	
DUP	< 2.55	<500		< 2.55	<0.50	

Cont. Table A 21

	Sewage sludge 1			Sewage sludge 2		
	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3
	mg/kg(m_T)	mg/kg(m_T)	mg/kg(m_T)	mg/kg(m_T)	mg/kg(m_T)	mg/kg(m_T)
DMP	< 0.293	<0.40		< 0.293	< 0.20	
DEP	< 2.115	<0.40	0.98	< 2.115	< 0.20	0.57
DPP	< 0.213	< 0.40		< 0.213	< 0.20	
DIBP	< 0.184	0.12	< 0.10	< 0.184	0.088*	< 0.1
DBP	< 0.474	0.039*	< 0.10	< 0.474	0.40	0.26
BBP	< 0.476	< 0.70		< 0.476	< 0.40	
DCHP	< 0.364	n.dt.	< 0.20	< 0.364	0.865	1.89
DEHP	32.602	39.8	39.4	8.330	6.42	6.51
DOP	< 1.254	< 0.8		< 1.254	< 0.40	
DDcP	< 0.281	<1.9		< 0.281	< 1.0	
DUP	< 2.55	<1.9		< 2.55	< 1.0	

- below the determination limit, n.dt.: not determined

In the project laboratory the samples were additionally extracted using ASE. The results thus obtained correspond satisfactorily for DCHP and DEHP, but for DEP, DIBP, or, as the case may be, DBP, they are in some instances significantly above the values obtained by the shaking method. As in parallel samples in some cases a sample extracted with ASE gave no indications of the presence of a phthalate, whereas the other did show a clear signal, this indicates contamination in the extraction (results not shown).

For DEHP the following mean values related to dry mass (m_T) and standard deviations were obtained:

Suspended Matter:Mean value 2.44 mg/kg, Std.dev. 0.02 mg/kg, Corr. 0.83%
Compost: Mean value 1.44 mg/kg, Std.dev. 0.21 mg/kg, Corr. 14.3%
Sewage sludge 1: Mean value 37.3 mg/kg, Std.dev. 4.05 mg/kg, Corr. 10.9%
Sewage sludge 2: Mean value 7.09 mg/kg, Std.dev. 1.08 mg/kg, Corr. 15.2%
The standard deviations from all the results are acceptable according to the experience derived from ring tests, whereby it needs to be stated that the small number of participants naturally does not allow for correct statistical statements to be made. Laboratory 1, whose

results deviated in most cases from those of the other laboratories, had the least experience with the method to be investigated, whereas laboratories 2 and 3 had already been working with it for longer. This makes it clear that a certain level of experience is essential for the determination of phthalates.

A 3 VALIDATED ROUND ROBIN RING TESTS

A 3.1 NATIONAL ROUND ROBIN TEST GERMANY

For the first major ring test sewage sludge was used which had been dried with hot air at temperatures of up to 400°C and had left the drying plant at a temperature of 110 - 140°C (oral statement of the Berliner Wasserwerke, BWB). It could thus be considered to be sterile (see above). As it had originated in the sewage works and had been treated (milling, shaking), it was assumed that the sample was homogeneous.

Further samples were received by the SEA in Düsseldorf: a sediment, a compost, and a wood sample. The wood was meant to represent the area of waste, which plays no part in the “Horizontal” project.

The samples were divided up into individual vessels for the ring test participants according to a fixed scheme in the following sequence: reserve sample (RS) 1, samples 1,2, and 3, RS 2, samples 4,5, and 6, RS 3, samples 7,8, and 9, RS 4, samples 10, 11 and 12, RS 5, and sample 13. At the request of a participant, RS 2 was sent as further sample material, and the remaining reserve samples were later analysed as homogeneity controls. All participants received the same sample weight within a range of about ±0.2g, whereas the reserve samples were somewhat smaller.
The samples were dispatched at the beginning of April, which meant that they arrived in most cases by the middle of April. Another 11 analysis laboratories in Germany showed interest in participating in the ring test. Two laboratories had to withdraw after the samples were dispatched, meaning that 10 results of varying degrees of comprehensiveness were obtained. As far as is indicated, the analyses were completed by the beginning of June 2006.

Unfortunately, not all the participants had used the phthalate method that was in the process of being developed. For this reason and because of insufficient return of information two further labs were excluded from the evaluation, meaning that eight labs with more-or-less complete results on all four matrices could be included in the final analysis. Some of the labs had only carried out the analyses singly or with one repetition but were included anyway if the remaining results were complete.

The “ProLab” programme used for the evaluation can only work with actual numerical values and not with such figures as “<BG” or “<100mg/kg”. As the ring test samples consisted exclusively of “grown” rather spiked samples, only a few phthalates occurred in them in measurable quantities.

A total of 44 sets of data on 11 phthalates in four matrices were available, of which 24 consisted almost exclusively of <-values. A detailed breakdown can be found in Annex III.

Of the remaining 20 sets of data, at least four labs were able to give concentration figures for 11, whereby only the results for DEHP were complete, as shown in summary in Table A 22.

Table A 22: Mean values in mg/kg (m_T), reproducibility and repeatability of the determination of DEHP (calculated according to DIN 38402-A45)

Matrix	Mean [mg/kg]	Reprod. S.D.	% S.D.	Repeat. S.D.	% S.D.	Lab mean S.D.	% S.D.
Sewage sludge	40.51	10.74	26.5	1.47	3.6	7.54	18.6
Compost	1.84	0.55	29.9	0.21	11.3	0.39	21.4
Sediment	4.16	1.11	26.7	0.26	6.3	0.77	18.5
Wood	41.77	24.22	58.0	8.46	20.3	16.33	39.1

Apart from in the case of wood, values were given for DBP for some of the other samples, all of which were in any case within the determination range estimated by the other labs.

The wood sample indicated low concentrations of DMP and BBP. Measured values were given for other phthalates in individual cases, whereby these too were generally within the range of determination limits given by the participating labs. The wood sample was non-homogeneous, as can be seen in the high standard deviation of the laboratory mean value.

In the case of DEHP, the Z-scores could also be calculated for the individual laboratories for each matrix. In the evaluation of Z-scores all the submitted results were taken into account; however, the values used for the calculation of the overall mean value and its standard deviation were purged of outliers. Thus the Z-score is a measure of correspondence of the individually measured values with the total mean value, or, if known, with the correct or target value. Absolute Z-score values of below 1 or 2 can be considered good or satisfactory, and a ring test returning these values can be deemed to have been passed.

Out of the 31 absolute Z-score values (of each lab with evaluable results for sewage sludge, sediment, and wood, of 7 labs for compost), 24 are below 1, and 5 are between 1 and 2, meaning that 93.5% of them counted as passes. The results that did not fall within the required range both came from the same laboratory.
The individual values for dry-mass determination and the results for the test solution and the samples, as well as the Z-scores, are depicted in Annex II.

A 3.2 PARTICIPATION IN AN INTERNATIONAL ROUND ROBIN TEST FOR CERTIFIED REFERENCE MATERIALS

The period of this project saw the execution of a parallel international ring test (“sludge support”), in which the phthalate DEHP and further parameters were to be determined, the participation in which was intended to allow a qualitative comparison with other labs to be made.

In the framework of the EU-GROWTH supported FP5 project 'Feasibility study for certified reference materials for organic components in sewage sludge' (Sludge Support), two sludge materials (A and B) had to be characterised for their mass fractions of absorbable

organic halogens (AOX), Bisphenol A (BPA), brominated flame retardants (BFRs, including brominated diphenyl ethers BDEs and hexabromocyclododecane HBCD), diethylhexyl-phthalate (DEHP), nonylphenol and its mono- and diethoxylates (NP, NP1EO and NP1EO, respectively, or NPEs as a group), and polycyclic aromatic hydrocarbons (PAHs). AOX had to be characterised in material A, the remaining parameters in material B.

None of the six project partners could analyse all parameters, but as at least six different data sets should be available for the evaluation of the results, additional (external) laboratories had to be found that were willing and able to participate. The candidates were selected from the networks of the project partners and were invited to show their interest first and, after an inventory of the responses was made, to sign an agreement for participation next (Final report: Characterisation Report).

The sample material was produced for and made available to all the participants by the Federal Institute for Materials Research and Testing (*Bundesanstalt für Materialforschung und –prüfung: BAM*) in Berlin. The technical possibilities open to the BAM mean that it is safe to assume the homogeneity of the samples. The “Horizontal Org” project for phthalates took part in the tests for the DEHP and BPA parameters, and the determination of further phthalates was not foreseen. These were only detectable in very small quantities. The scope of the analyses was predetermined (three parallels for each on two different days, determination of a test solution, dry-mass determination, repeated extraction, blank samples), whereas the actual choice of method was left to the participants.

A 3.2.1 DETERMINATION OF NONYL PHENOL

A 3.2.1.1 EXTRACTION AND DERIVATISATION

The draft procedure developed by the Horizontal project was used for the quantitative determination of nonyl phenol, and nonyl phenolmono- and diethyloxylate. The same mass of water was added to 5 to 10 g of weighed-in soil and compost. 1-2g of dried sewage sludge was accurately weighed in and around twice the mass of water was added. 10 mL

of acetone and 10 mL of hexane, as well as 100 µL of 4-n-nonyl phenol (1.996 µg/mL in isooctane) were added as internal standard. The extraction was conducted in the horizontal shaker (2 h at 250/min for soil and compost, 1 h for sewage sludge). The organic phase was subsequently decanted into another vessel, washed with around 5mL water per mL of organic phase, decanted into a further vessel, and dried using dry Na_2SO_4.

1 mL of the dried solution (5 mL in the case of compost) was blown dry in a jet of nitrogen, whereupon 1 mL of 5% MSTFA solution was added. The solution was left to stand at room temperature for 15 minutes and was then decanted into a GC vial.

A 3.2.1.2 MEASUREMENT OF NONYL PHENOL

A gas chromatograph mass spectrometer from ThermoFinnigan, a Trace GC, a Polaris Q Ion trap detector and the ThermoFinnigan AS3000 autosampler were used to determine the nonyl phenols. An Rtx5-MS separating column with a length of 30m, a diameter of 0.25mm, a film thickness of 0.25 µm, and a 5m guard column was used.

The following injector conditions obtained: split/splitless, 250°C, splitless time 2 min, splitless flow 50 mL/min. The temperature programme ran as follows: 100°C (2 min), 10°/min up to 200°C (3 min), 10°/min up to 300°C (7 min). Helium was used as the carrier gas (0.9 mL/min, constant flow) with a transfer line 300°C, in EI mode, and an ion source 220°C. The following target ions were selected: 207, 221, 193 (NP und Qualifier), 179 (4-n-NP), 185 (^{13}C-4-n-NP), 251 (NP1EO), 295 (NP2EO).

A 3.2.2 DETERMINATION OF THE LINEAR ALKYLBENZOLSULPHONATE (LAS)

A 3.2.2.1 EXTRACTION

The draft Horizontal procedure was used for the quantitative determination of LAS. Sewage sludge: 2-3 g of dried sewage sludge was accurately weighed in, and 100 µL internal standard solution (4-octylbenzolsulfonate, 1 mg/mL) and 10 mL methanol were

added. The samples were extracted on a horizontal shaker (250Upm) over a 30 minute period. The samples were allowed to sediment, after which they were filtered through a 0.45 µm spray filter. Finally, 500µL of the solution was spiked with 500 µL 0.01 M ammonium acetate solution.

Soil, compost: 5-8 g dried soil or compost was accurately weighed in, and 25 µL of internal standard solution (4-octylbenzolsulphonic acid, I mg/mL) was added along with 25 mL methanol. The samples were extracted on a horizontal shaker (250/min) over a 60 min period. 5 mL of the extract was concentrated in the nitrogen jet to 0.5 ml and finally spiked with 0.5 mL of the 0.01 M ammonium acetate solution.

After the addition of the ammonium acetate solution, the mixture became cloudy, and, although the clouding could not be removed by means of filtration through 0.45 µm, this did not materially affect the determination by means of HPLC. The HPLC conditions were slightly altered from those in the “Horizontal” draft method.

A 3.2.2.2 MEASUREMENT OF LAS

A Waters 996Photodiode Array Detector and 470 Scanning Fluorescence Detector with Waters 717 Autosampler, plus S600 Controller and Waters 616 pump were used. Excitation was carried out at 230 nm, and the measurement was made at 310 nm. The gradient consisted of the mobile phase A: 0.01 M ammonium acetate in water and the mobile phase B: acetone nitril. The dimensions of the column were 125mm by 4mm, with a C18 filling of 5 µm particle diameter and a flow speed of 1 mL/min. The following programme was used: 55% A, 45% B, to 10% A/90% B in 20 min, 3 min constant, in 2 min to 55% A/45% B, degasification using 10 mL He/min.

A 3.2.3 DETERMINATION OF BISPHENOL A (BPA)

A 3.2.3.1 EXTRACTION AND DERIVATISATION

1-1.5 g sewage sludge was accurately weighed in and 5 mL 1 N HCl, 10 mL DCM and 5 µL internal standard (0.2 mg/mL D_6-BPA) was added. Extraction was conducted for 1 min on the vortex mixer, 10 min in the ultrasound bath, and 10 min on the horizontal shaker, and centrifugation was carried out for 10 min at 3500 rpm. The extraction with DCM was repeated twice; the DCM phases were amalgamated, dried with Na_2SO_4, and decanted into another flask. 0.4 mL MeOH, 25 mL diluted K_2CO_3 solution (10 g/L) and 1 mL acetate hydride were added to the extraction, shaken firmly by hand and then for 10 min on the horizontal shaker. 10 mL n-hexane was added, and the mixture firmly shaken by hand and extracted for 15 min on the horizontal shaker. The organic phase was separated using a separating funnel, dried with Na_2SO_4, and concentrated in the nitrogen jet to 1 mL.

A 3.2.3.2 MEASUREMENT OF BPA

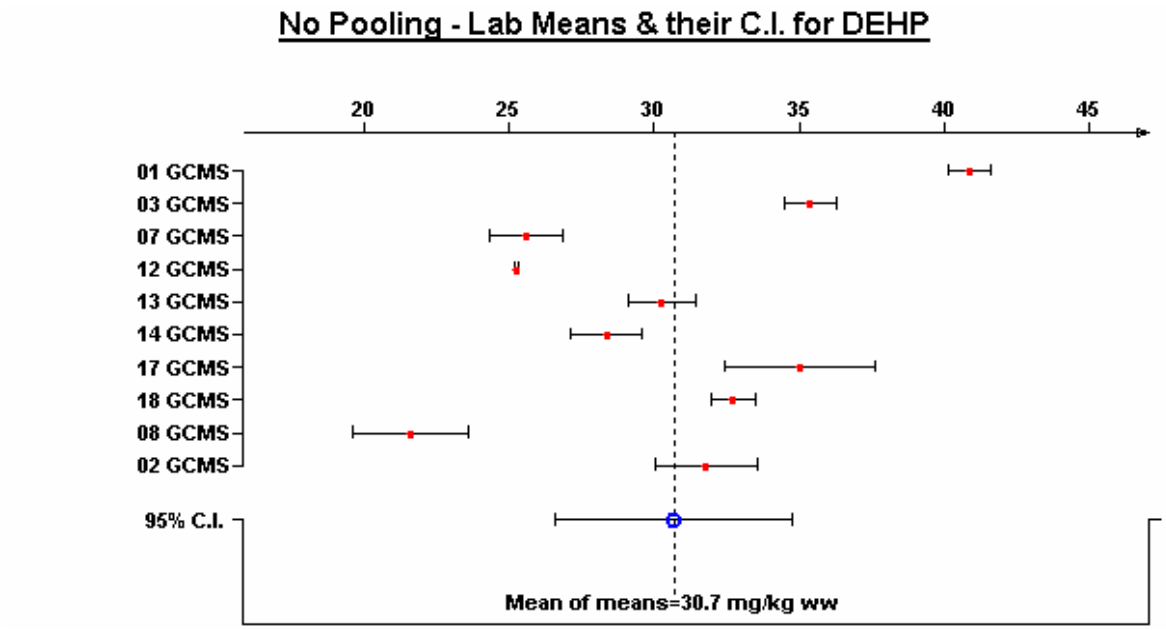
The GC conditions for BPA were the same as those described in A 2.2.6 for the phthalates. The internal standard was D_6-BPA. The target ions were m/z 213 (or, alternatively, m/z 216 with D_6-BPA) for BPA, 228 for BPA and 270 (alternatively, 276 with D_6-BPA) for the diacetate.

A 3.2.4 RESULTS OF THE INTERNATIONAL RING TEST “SLUDGE SUPPORT”, AMSTERDAM

A 3.2.4.1 PHTHALATES

It had been decided that only DEHP was to be determined. The phthalates were determined as described in A 2.2.6. the free choice of methods led, as can be seen in Figure A 7, to highly variable results, such that the values could only be compared to a limited extent.

Fig A 7: summary of the results of the “sludge support” ring test (IVM/Amsterdam) for DEHP in mg/kg (m_T)



The results were located within a range of 19.2 to 42.2 mg/kg, with a mean value of 30.7 mg/kg, which corresponds closely to the value of 30.7 mg/kg arrived at in previous homogeneity investigations (Table A 23)

Table A 23: Results in mg/kg (m_T) of the ring test certification “sludge support” for DEHP

	DEHP [mg/kg]
Day 1, Sample 1	25.6
Day 1, Sample 2	25.6
Day 1, Sample 3	24.4
Day 2, sample 1	27.5
Day 2, sample 2	26.2
Day 2, sample 3	24.3
Mean value	25.6
Standard deviation , (% dev..)	1.2 (4.7%)

The standard deviation for all the values is 5.7 mg/kg (18.6%); leaving out the two laboratories with the lowest and highest values respectively, the standard deviation amounts to only 2 mg/kg, which is also an indication of the homogeneity of the samples (see characterisation report).

The results from Table A 23 are subsumed under the laboratory number 07. The highest values were obtained using methods with a high contamination risk (Laboratory 01 worked with ASE, Laboratories 03 and 17 with Soxhlet extraction). If these results are disregarded, then those of the Horizontal Org phthalate laboratory are only a little below the mean value. Once all values have been included, the relative standard deviation lies at around 18.6%, which can be deemed acceptable. However, it was subsequently concluded that, although the data could not be rejected from a statistical point of view, the DEHP data did not overlap, which meant that it could not be recommended for CRM purposes.

The highest values were returned by the labs that used Soxhlet extraction or ASE, as these are both highly susceptible of sample contamination, as has already been seen. The results obtained by shaking with solvent and ultrasound treatment all lie, with one exception, within a similar range. The low ultrasound values returned by Lab 08 must thus be regarded as outliers, even though, statistically speaking, there are no grounds for singling these results out. The ring test results gave no indication of any influence of weighing-in size on the results submitted.

A test solution was sent with the solid samples. The Horizontal Org phthalate laboratory had found around 90% of the theoretical value and was therefore one of the four labs whose results demonstrated no serious deviation from it.

A 3.2.4.2 BISPHENOL A (BPA)

For the analysis of BPA, a search of the literature on the subject led to the selection of a method which seemed to promise easy manageability. Although BPA is directly detectable, in higher concentrations peak tailing could be observed, as is often the case with phenols. This could have been avoided using derivatisation. As, according to the

literature, derivatisation with silylisation reagents is more complicated and the products appear to be less stable, silylisation with acetane hydride was preferred, which did indeed prove both easy and reproducible. The results obtained were thus similar to those of the other ring test participants. The internal standard was a BPA that had been deuterated at both methyl groups (D_6-BPA). The results for the BPA ring test certification are reproduced in Table A 24. It can be seen that the findings are only subject to a very low standard deviation.

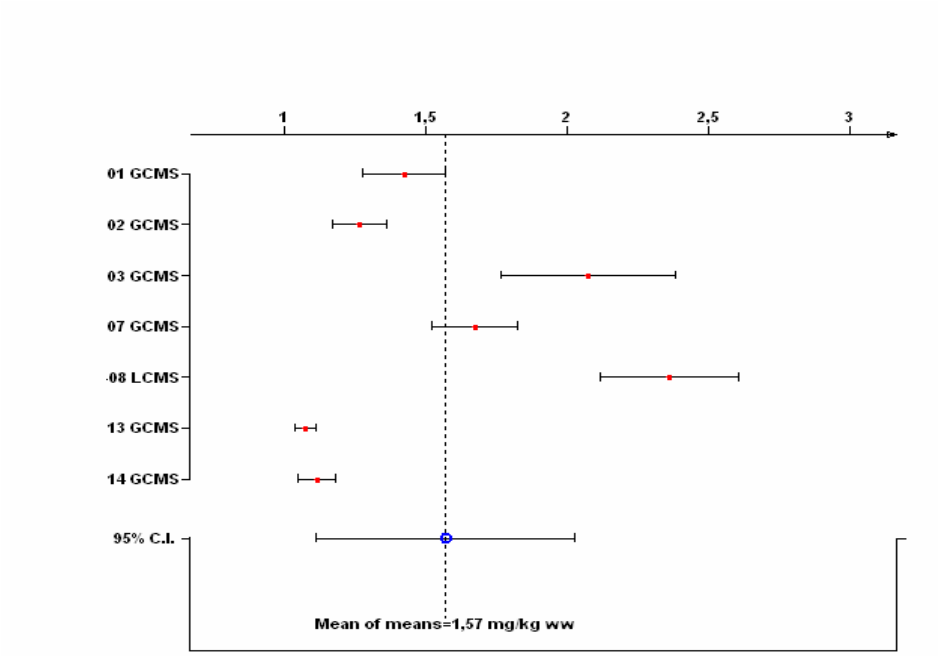
Table A 24: Results in mg/kg (m_T) of the ring test certification “sludge support”

	BPA [mg/kg]
Day 1, Sample 1	1.56
Day 1, Sample 2	1.74
Day 1, Sample 3	1.66
Day 2, Sample 1	1.55
Day 2, Sample 2	1.61
Day 2, Sample 3	1.93
Mean value	1.68
Standard deviation , (% dev.)	0.14 (8.6%)

Figure A 8 shows a graphic depiction of the results of all the participants in this part of the ring test. The phthalate laboratory was Lab 7, and the only BPA results are depicted in Table A 24.

The remaining labs included in Figure A 8 show an overlap of their 95 % confidence intervals, so that the sludge can be certified for the consensus value and the 95 % confidence interval: 1.6 +/- 0.5 $mg.kg^{-1}$ (on the basis of the characterisation only, uncertainties due to inhomogenity and instability should be added).

Fig A 8: Summary of the results in mg/kg (mT) of the “sludge support” ring test (IVM/Amsterdam) for BPA



As with DEHP a test solution had been sent with the solid sample. The test solution results were good. There was an overlap between the 95 % confidence intervals. The consensus value, the 95 % confidence interval of the mean of means (0.078 – 0.134 mg.kg^{1} overlapped with the target value, the theoretical amount (0.124 mg.kg^{-1}). Blank experiments showed no detectable BPA amounts or, at worst, amounts corresponding to 2 % of the BPA level in the sludge material which was the case also for the Horizontal phthalate lab.

In the final report of “Sludge Support” it was noted that, in order to collect reliable information on the extraction efficiency, the participants should use the prescribed proper experimental design, with deuterated BPA added to the extracts instead of the sample. With the addition of internal standard to the sample recovery tests are not possible. The lack of these tests was considered a shortcoming.
n the case of BPA, Labs 3 and 8 did not carry out a derivatisation, and Lab 8 was the only one to work with LC/MS. Participants 13 and 14 shook out with diethyl ether and desulfurised with copper, which are probably less suitable methods. The remaining three participants conducted different extractions (ASE, shaking with various solvents) and derivatisations (sylilation and acetalation) but arrived at similar results.

A 3.3 INTERNATIONAL RING TEST EXPERIMENT

A 3.3.1 PHTHALATE RING TEST

A 3.3.1.1 TYPE OF SAMPLES

As the closing stage in the validation of the methods for detecting phthalates and other substances or substance groups in various solid matrices, a Europe-wide ring test was conducted in the Horizontal Org project. After pretests, ring test samples were sent out in co-operation with the IRMM in Geel, Belgium, and the JRC in Ispra, Italy. The phthalates formed part of the so-called “Complexity Group 1”.

The three solid samples were:

a) a charged compost from Vienna, Austria (compost 1),
b) a mixed sewage sludge from Essen, Germany (sewage sludge 1), and
c) a soil spiked with sludge from Barcelona, Spain (soil 3).

Four jars were sent of each matrix, from each of which two independent samples were to be analysed. As could be seen later, this was reduced to two samples out of two jars respectively (for LAS and NP).

A 3.3.1.2 Own results of the International Round robin test for Phthalate

Of all the phthalates only DEHP was found; either no signal whatever was detected of the presence of the other phthalates or they were to be found within the range of blank values.

The results for sewage sludge demonstrated a standard deviation of only around 2%, whereas those for soils and compost demonstrated values of 10-15% (some compost samples were notable for values many times in excess of those found in the others, and these were consequently not used). The dry mass was determined by drying at 105°C until

a constant mass was achieved. Table A 25 shows a combined summary of the results without outliers.

Table A 25: Individual values from the analysis of the samples for phthalates in the context of the Horizontal ring test [in mg/kg (m_T)]

	sewage sludge	DEHP	
bottle 1	mg/kg	mean	stdd.dev.
KS 92a	18,6417		
KS 92a	18,9083	18,775	0,189
KS 92b	19,4678		
KS 92b	19,1850	19,326	0,200
bottle 2		mean	stdd.dev.
KS 93a	19,3332		
KS 93a	19,4801	19,407	0,104
KS 93b	19,9461		
KS 93b	19,9974	19,972	0,036
	compost	DEHP	
bottle 1	mg/kg	mean	stdd.dev.
Co 91 a	0,5355		
Co 91 a	0,5214	0,528	0,010
Cont. Table A 25			
Co 91 b	0,4842		
Co 91 b	0,4703	0,477	0,010
Cont. Table A 25			
bottle 2		mean	stdd.dev.
Co 92a	0,4400		
Co 92a	0,4549	0,447	0,011
Co 92b	0,5536		
Co 92b	0,5506	0,552	0,002
	soil	DEHP	
bottle 1	mg/kg	mean	stdd.dev.

Bo 108a	0,5089		
Bo 108a	0,4715	0,490	0,026
Bo 108b	0,4173		
Bo 108b	0,4437	0,431	0,019
bottle 2		mean	stdd.dev.
Bo 110a	0,4119		
Bo 110a	0,4124	0,412	0,0003
Bo 110b	0,4309		
Bo 110b	0,4314	0,431	0,0003

KS = sewage sludge, Co= Compost, Bo = Soil

A 3.3.1.3 Statistical evaluation of the results of the International round robin test for Phthalates

The results of the ringtest participants had been collected by the JRC in Ispra and their characteristics had been calculated there. The distribution of the results is represented by Figures A 9 to 11 for the three matrices for DEHP. The results were tested for outlyers by the Grubbs test and Cochran's criterium, for homogenity of variances by Mandel's test. For DEHP the repeatability within the labs is between 9 and 14%, the reproducibility lies for the three matrices at approx. 40%, which is quite high, but still acceptable. One of the lab seems to have had certain problems with the determination because all of its results are lying well below the results of the other labs.

Fig. A 9: Deviation from the mean value for DEHP in Soil 3 of different labs in the international round robin test.

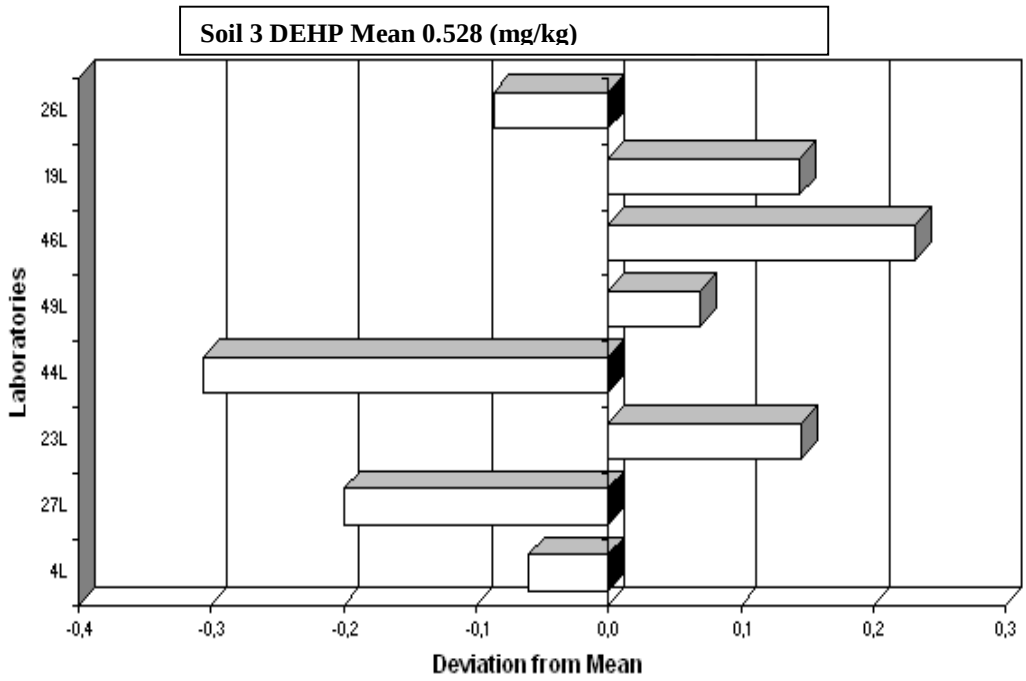


Fig. A 10: Deviation from the mean value for DEHP in Sludge 1 of different labs in the international round robin test.

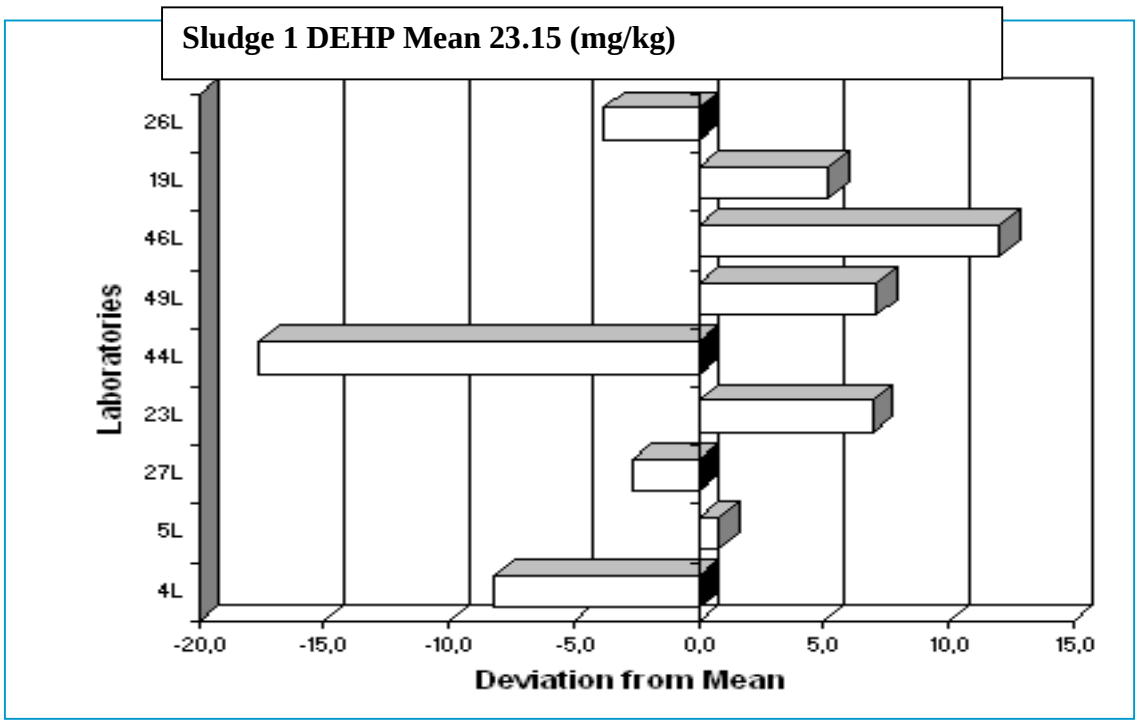


Fig. A 11: Deviation from the mean value for DEHP in Compost of different labs in the international round robin test.

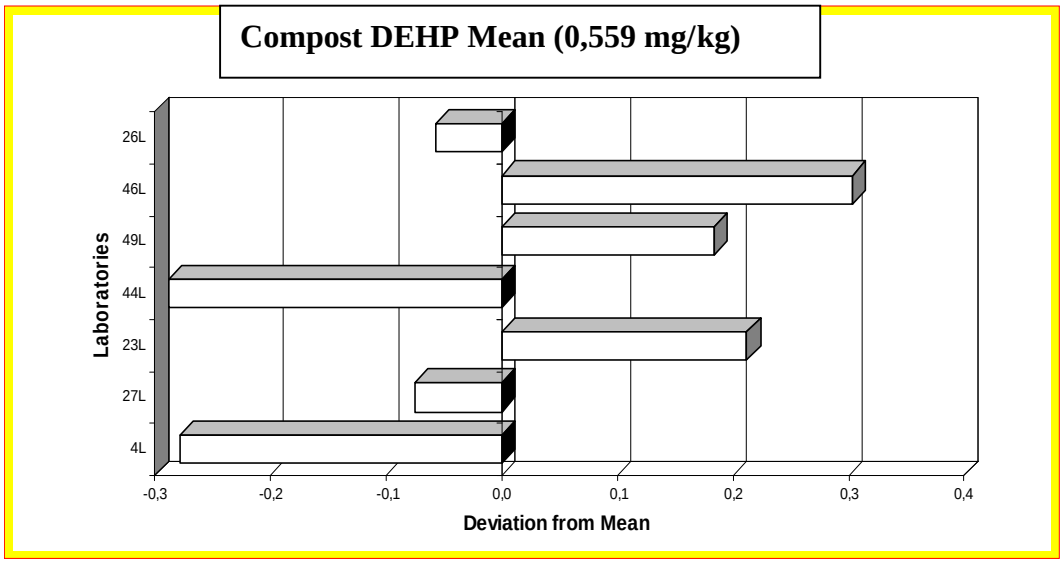
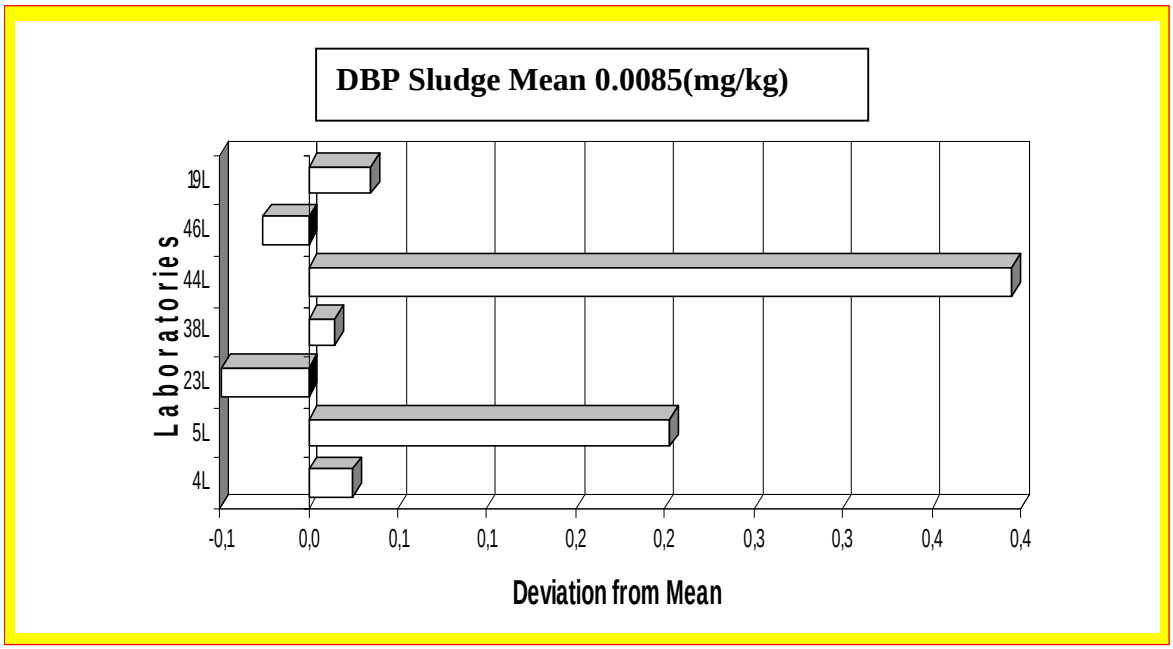


Fig. A 12: Deviation from the mean value for DBP in Compost of different labs in the international round robin test.



For DBP (Fig. A 12) the results of the phthalate lab had not been included in the evaluation, because they were below the limit of determination and “less than”-values could not be handled by the used program.Therefore only a small number of laboratories.

As with DEHP again one lab, the same had produced results which differed very much from those of the other labs. While the low DEHP results where not recognised as outliers, with DBP the results of that lab had been rejected. Only five labs remained for the statistical evaluation, which resulted in characteristics similar to those for DEHP in the case of sewage sludge.

As in the samples only DEHP was regularly found, the international round robin test was passed only for this compound with moderate success. A reason for this could be, that some of the participating laboratories didn't have sufficient experience with the analysis of phthalates, similar to the first small comparison with tree labs (see A 2.7.2). Better results, which we have got in the national round robin test, (see. A 3.1) gave us the certainty that this method is applicable when laboratories take more care to apply the method in the respective analytical steps very accurate.

A 3.3.2 LINEAR ALKYLBENZOLSULPHONATE (LAS) RING TEST

As a member of the "Horizontal Org" project association, the phthalate lab also took part in the validation of the draft method for the linear alkylbenzolsulphonate (LAS) and, to an extent, for nonyl phenol, investigating the same matrices. The ethoxy derivatives also recorded by the method for nonyl phenol were not analysed, because no suitable standard was available.

A 3.3.2.1 EXTRACTION

In line with the draft method, methanol was selected as the extraction agent for LAS. After shaking the samples with methanol, they were either measured directly after a 1:1 dilution using the aqueous of the two HPLC eluents, as was the case with sewage sludge, or concentrated and diluted with the eluent so that a fivefold concentration resulted, as was the case with soil and sediment.

A 3.3.2.2 MEASUREMENT OF LAS

After isomer separation on a C18 column, the individual components were quantitatively determined using a fluorescence detector. Octylbenzol sulphonic acid was used as the internal standard, and the regression lines for the individual LAS components of different chain lengths were obtained using standards of the same chain length (but as an isomer mixture). The isomers with C_{14} chains were not investigated, as they form less than 0.5% of the normal LAS mixtures. The determination of the C_{10} isomers in sewage sludge was prevented by a disruptive peak. For device parameters and further measuring details refer to 3.2.2.2.

A 3.3.2.3 RESULTS OF THE LAS RING TEST

The results for C10-C13-LAS of our Laboratory in soil, compost, and sewage sludge samples are given in Table A 26.

In dependence upon the contents, the standard deviations for both soil samples are, in the main, acceptable. With further decreases in contents in the compost samples come of course increases in standard deviation, because of the proximity to the determination limit. In the sewage sludge samples the standard deviations are very low.

Table A 26: Horizontal ring test, results for C10-C13-LAS in soil, compost, and sewage sludge

soil		mg/kg m_T	mg/kg m_T	mg/kg m_T	mg/kg m_T	mg/kg m_T
		C10	C11	C12	C13	Sum LAS
flask 1	MW	1.29	4,64	12.22	19.63	37.8
	Std. Dev.	0.19	0.55	2.22	3.92	
	% Std. Dev.	15.0	11.9	18.2	20.0	
flask 2	MW	1.57	4.08	12.72	18.94	37.3
	Std. Dev	0.20	0.60	1.34	1.89	
	% Std. Dev	12.9	14.7	10.5	10.0	

Compost		mg/kg m_T	mg/kg m_T	mg/kg m_T	mg/kg m_T	mg/kg m_T
		C10	C11	C12	C13	Sum LAS
flask 1	MW	< 1	1.75	1.78	< 1	3.53
	Std. Dev		0.36	0.21		
	% Std. Dev		20.5	11.6		
flask 2	MW	< 1	1.86	1.69	< 1	3.55
	Std. dev.		0.42	0.34		
	% Std. dev.		22.6	19.9		
Sewage sludge						
		C10	C11	C12	C13	Sum LAS
flask 1	MW	*	748.1	759.8	484.9	1993
	Std. dev.		30.6	26.7	14.9	
	% Std. dev.		4.1	3.5	3.1	
flask 2	MW	*	726.7	720.6	461.4	1909
	Std. dev.		15.3	23.4	5.6	
	% Std. dev.		2.1	3.3	1.2	

* Interfering peak

A 3.3.3 NONYL PHENOL RING TEST

A 3.3.3.1 EXTRACTION

After spiking the sample with water, the extraction was carried out using acetone/hexane. The extracts from soil and sewage sludge were derivatised without cleaning with MSTFA and measured directly; part of the compost extract was concentrated in the nitrogen jet and then derivatised. For more detail see 3.2.1.1

A 3.3.3.2 MEASUREMENT OF NONYL PHENOL

Nonyl phenol was determined according to the draft norm from “Horizontal Org” for this substance. The determination of mono and diethyl oxylate in nonyl phenol envisaged in the Horizontal Org ring test proved impossible because of the delay in preparation for the

ring test brought about by the delayed contract extension for the project's chemist. For further details on the measuring process see A 3.2.1.2.

A 3.3.3.3 RESULTS OF THE NONYL PHENOL RING TEST

The results of our laboratory are depicted in Table A 27. With the exception of soil sample 2, the standard deviations lie within acceptable limits.

Table A 27: Results in mg/kg (m_T) of the Horizontal Org ring test for nonyl phenol in soil, compost, and sewage sludge

soil	mg Nonyl phenol/kg (m_T)		
	Mean value	Std dev..	% Std. dev.
flask 1	2.203	0.178	8.1
flask 2	2.133	0.323	15.2
Compost	mg Nonyl phenol/kg TM		
	Mean value	Std. dev.	% Std. dev.
flask 1	0.111	0.008	7.6
flask 2	0.097	0.007	6.8
Sewage sludge	mg Nonyl phenol/kg TM		
	Mean value	Std. dev.	% Std. dev.
flask 1	40.51	3.40	8.4
flask 2	41.34	3.04	7.4

A 4 DISCUSSION

Several methods of phthalate analysis have been described, which mostly use GC, although some also use HPLC. The mass selective detector is preferred, because in excess of m/z 149, with just one exception, all phthalates can be detected and quantified with a high degree of sensitivity and specificity. An additional factor is the widespread use of GC/MS in analytical laboratories.

The ubiquitousness of phthalates entails a high risk of contamination of lightly charged samples, meaning that particular care needs to be taken in cleaning the equipment used, and that the method should encompass as few steps as possible. The shaking out of samples using a solvent proved to be suitable. Ethyl acetate was the solvent of choice, because it is moderately polar, and has not too low a boiling point and a low level of toxicity.

In the context of validation, the accuracy, recovery, detection and determination limits of some phthalates were tested, along with the linearity of the standard curves and repeatability. Participation in ring tests was used as a means of checking reproducibility.

The recovery from the spiked samples lay mostly in the range 90 – 110%. As expected, no adsorption to the solid matrix was found for the internal standard.
Detection and determination limits could be determined using various procedures. The method according to DIN 32465 yielded significantly higher values compared to the Signal/Noise Ratio (SNR). The former gave rise to detection limits for certain phthalates of around 0.03 mg/kg in soil and 0.2 mg/kg in sewage sludge; determination limits of ca. 0.1 mg/kg for soil and ca. 0.5 mg/kg for sewage sludge were arrived at. All the values were far below the threshold value for solid matrices, itself still the subject of debate.

The accuracy was good, with a standard deviation of around 5% in repeatability. The reproducibility tested in the German ring test was satisfactory, with a standard deviation in various matrices of around 25-30%, and, with one exception, all the labs in the ring test returned Z-score values which were deemed to have passed the test. This leads to the

conclusion that the method is suitable for the quantitative determination of phthalates in solid matrices.

The method only consists of a few steps in sample preparation, but only very few of the parameters could be changed. The extraction is of decisive importance. Once the solvent had been decided upon for the reasons mentioned above, an initial comparison between various extraction procedures needed to be made. As they all proved to be suitable within the context of measuring accuracy, the obvious choice was of the least complicated and costly procedure. In respect of this extraction procedure an investigation was undertaken as to whether the ratio of initial weight to volume of extraction agent could influence the results. This proved not to be the case in the range under investigation. A sample water content of up to around 10% has no influence on extraction, and higher quantities were not investigated.

Although the drying of the samples comes under the aegis of sample preparation, it still needed to be taken into account. Whereas samples with a moderate water content could be dried using sodium sulphate, sewage sludge could only be freeze dried. A low level of loss manifested in all cases, and spiked sludges showed that the higher level of volatility of phthalates with short side chains meant that greater losses were also possible. In this case it needs to be taken into account that one is talking about spiked samples rather than “mature” ones – spiked samples having other binding characteristics.

Varying the GC conditions had no influence on the separation. When separating the pair DCHP/DEHP, it is important to introduce a flatter gradient into the middle part of the temperature programme.

The method described here has proved to be straightforward reliable, as well as adequately robust.

A 5 SUMMARY

The following results were obtained:

- Ø the development of a simple detection method for various phthalates in different solids (soils, sewage sludge, compost etc.).
- Ø sensitive and specific detection by means of GC/MS with the mass m/z 149.
- Ø The recovery from spiked matrices lay mostly within the range 90 – 110%.
- Ø The detection limits are around 0.03 mg/kg in soil, and 0.2 mg/kg in sewage sludge. Determination limits of ca. 0.1 mg/kg for soil and ca. 0.5 mg/kg for sewage sludge were arrived at.
- Ø Simple extraction – the shaking out of solid samples with a solvent proved to be suitable. Ethyl acetate was the solvent of choice.
- Ø A good level of accuracy with a standard deviation of around 5% in terms of repeatability. Reproducibility was satisfactory, with a standard deviation of around 25-30%. With one exception, all the labs in the national ring test returned Z-score values which were deemed to have passed the test
- Ø Due to the small number of individual analytical steps involved, the risk of sample contamination is low.
- Ø It needs to be highlighted that “clean” working practices and the scrupulous adherence to working instructions are essential if sample contamination is to be avoided.
- Ø Participation in the international round robin test for Phthalates
- Ø Validation for DEHP in Sewage Sludge, Soil and Compost
- Ø Validation for DBP in Sewage Sludge
- Ø Participation in the international round robin test for LAS
- Ø Validation for LAS in Sewage Sludge
- Ø Participation in the international round robin test for Nonyl phenol
- Ø Validation for Nonyl phenol in Sewage Sludge

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PART B

PRE-NORMATIVE WORK

PRELIMINARY REMARKS

The pre-normative activities were, in the context of the whole project, only of secondary significance, because the aim was to develop a determination method for phthalates which could be used irrespective of medium for various solids, such as soil, soil auxiliaries, sewage sludges, compost, or sediment. For this reason, further investigations were undertaken only in respect of particular questions and, especially, of mobility in soils.

B 1 INVESTIGATIONS INTO PARTITION BETWEEN SOLID AND LIQUID PHASES

According to their characteristics, phthalates disperse in different ways in the solid soil and fluid water phases. Longer side chains reduce their hydrophilia, thereby also reducing the free availability in soil for biodegradation or transport. The octanol water partition coefficient can be seen as a parameter for this, representing as it does a measure of the hydrophobia of a compound, or the Freundlich Constant, which results from the adsorption isotherm at 1 µg/mL in the balance solution and a doubled logarithmic application of the yield of the test substance in the solid and fluid phases as a soil water partition coefficient, according to which the test substance could partition between the two phases.

The following shows the Freundlich Equation:

$$x/m = K_F C^{1/n} \qquad (1)$$

in which x= the quantity of the adsorbed material in mg, m the mass of the adsorbing soil amount in kg at the respective balance concentration C (mg/L) and n the gradient or, as the case may be, K_F is the Freundlich constant. By the application of logarithms one arrives at the linear form:

$$\log x/m = \log K_F + 1/n \log C \quad (2)$$

at a C of 1mg/L equation (2) becomes simplified (as 1/n*log 1 0 =) to:

$$\log x/m = \log K_F \quad (3)$$

the partition coefficient K_D describes the partition between sorbed quantity and that in solution according to the following equation:

$$K_D = C_a/C_e \quad (4)$$

Whereby K_D: = partition coefficient, C_a, = concentration in mg/kg adsorbens and C_e, = concentration in mg/L balance solution

At an equilibrium concentration of 1mg/L the K_D value is equivalent to the K_F value in the Freundlich Equation.

The Freundlich exponent is a measure for the non-linearity of the adsorption. The correlation is linear, if the exponent is one. An exponent less than one means that the adsorption capacity decreases at higher concentrations. The physical explanation is that adsorbed molecules are gradually blocking the access to other binding sites, so that fewer sites are available (antico-operative binding).In a situation where adsorbed molecules encourage the adsorption of further molecules, the Freundlich exponent will be above one (co-operative binding).

B 1.1 MATERIAL AND METHODS

B 1.1.1 EXTRACTION OF PHTHALATES FROM WATER

The water samples were extracted using a conditioned RP18 column and subsequently dried in a nitrogen jet. Elution was carried out using 2.5mL of internal standard solution in ethyl acetate. Further steps carried out were analogous to those in the procedure described in Part A.

B 1.1.2 SOIL WATER PARTITION EXPERIMENTS

The procedure using the OECD TG 106 method for determining adsorption and desorption in soils by means of the batch equilibrium method assumes dilute solutions of the substances under investigation. This was impossible in this case due to the low solubility of the phthalates, except in the case of DEHP, which would have required very low concentrations with the ensuing analytical difficulties. The approach selected here is appropriate to the situation after the application of, for example, sewage sludge containing phthalates onto farmland. The partition coefficients thus obtained are a measure of the mobility of a substance in soil and thus also of its downward transport into deeper strata.

The shaking time was limited to four hours, on the one hand to exclude the possibility of degradation processes, and, on the other, because it is largely in this time that sorption takes place.

The soils made available for experimentation were a non-humic sandy soil (soil A), a clay soil (soil B), and a humic sandy soil (soil C). the soil characteristics are detailed in Table B 1.

Table B 1: Characteristics of the different soil substrates.

Soil substrate		% clay	% silt	% sand	% Corg	pH
Sandy Soil	A	3	10	87	0.1	4.6
Clay rich Soil	B	67	11.8	11	0.01	7.1
Humic Soil	C	0.5	52.3	11	36.2	3.5

The selected soils varied in terms of their characteristics, so that the effect of higher humus contents could be measured just as accurately as high clay contents.

The phthalates used were DiBP (side chains with 4 C-atoms), DEHP (Side chains with 8 C-atoms) und Di-n-dodecylphthalat (DDoP, side chains with 12 C-atoms). After spiking,

the soils were generally stored for 2-3 days in the refrigerator before being used. This aging was intended at least to an extent to simulate sorption under natural conditions.

In order to determine the soil water partition coefficients, different spiked soils (Table B 1) were shaken with water in order to obtain a distribution balance. The phthalate content of the aqueous phase was determined analytically, and for the soil the content was used after spiking, as, out of the total phthalate yield, only negligible amounts were dissolved in water.

For Soil A, the distribution was repeated using 1.25mM $CaCl_2$ solution. This concentration was geared towards the calcium content of surface waters and was intended to test whether the mineral content of naturally occurring surface waters would lead to different phthalate partition characteristics. The values obtained for DiBP and DEHP are clustered around the same straight lines as those obtained from pure water (results not shown).

In order to stay within the measurement range of the analytical procedure, because of the high sorption rates expected, especially of those not easily water soluble phthalates, the spiking concentration was adapted to the soils (Table B 2).

Table B 2: Spiking of experimental soils with phthalates (in mg/kg (m_T))

	DiBP	DEHP	DDoP
Soil A	0.8/4/20/100/800	64/400/4000	10.4/104/1040
Soil B	4/20/100	800/4000/40000	1040/4160/10400
Soil C	4/20/100/800	160/800/4000	1040/4000/10400

50 mL liquid (ultra-pure water, $CaCl_2$ solution, LAS solution) was added to 10g spiked soil. The flasks were clamped in an overhead shaker and mixed for 4 hours at 20 rpm. The fluid phase was centrifuged at 3500 rpm, and 15 mL of the supernatant was put through a RP18 column and subsequently dried for 5 min with N_2. Elution was carried out using 2.5mL of a solution of the internal standard (see B 1.1.5) in ethyl acetate.

B 1.1.3 SYNTHESIS OF DIDOECYL PHTHALATE

0.15 moles of dodecanol-1 and 0.05 moles of phthalic acid anhydride were dissolved in 100 mL trichloromethane. After the addition of 0,5 mL conc. sulphuric acid the solution was heated and the water was removed by azeotropic distillation. After that the mixture was shaken with $NaHCO_3$ solution for neutralisation, the solvent was distilled off and the remainder was recrystallised twice from methanol with 5% ethyl acetate. Purity (GC) 95%, yield 80% (based on phthalic acid anhydride).

B 1.1.4 SORPTION OF DIBP AND DEHP IN THE PRESENCE IN THE SOIL OF LAS

50 mL liquid (ultra-pure water, $CaCl_2$ solution, LAS solution) was added to 10g spiked soil. The flasks were clamped in an overhead shaker and mixed for 4 hours at 20 rpm. The fluid phase was centrifuged at 3500 rpm, and 15 mL of the supernatant was put through a RP18 column and subsequently dried for 5 min with N_2. Elution was carried out using 2.5mL of a solution of the internal standard in ethyl acetate.

B 1.1.5 SOIL WATER PARTITION OF PHTHALATES IN THE PRESENCE OF DITHIONITE

5g spiked soil was weighed in, and 50mL water and 33.5mg solid N_a2SO_4 (0.25 mMol) or 43.5mg Na_2S20_4 (0.25 mMol) added as appropriate. The redox potential having been measured, the flasks were aerated for a short time with nitrogen and quickly sealed. After 4 h in an overhead shaker (20 rpm) the redox potential was re-measured and the fluid phase centrifuged at 3500 rpm. 15 mL of the supernatant was put through a RP18 column. Once the columns had been dried with nitrogen elution was carried out using 2.5 mL solution of 1 µg/mL D_4-DBP and of D_4-DOP respectively in ethyl acetate.

B 1.1.6 SOLUBILITY OF DIBP AND DEHP IN THE PRESENCE OF LAS

100mg of the phthalate was weighed into a flask, and 100mL LAS solution of the appropriate concentration, or water as control, was added carefully. The mixture was

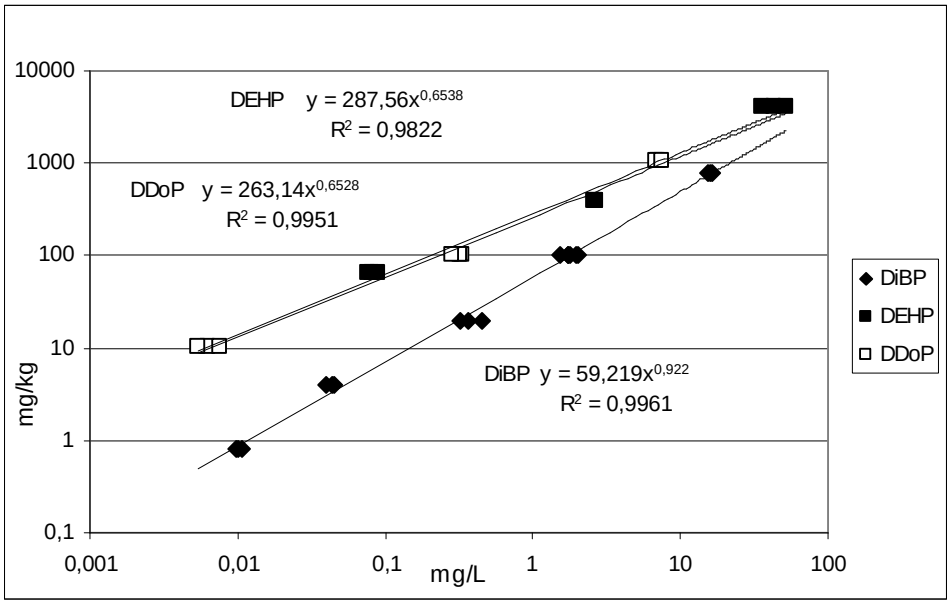
shaken for 4 hours in the horizontal shaker. Special attention was paid during this process to the avoidance of emulsification of the phthalate in water. Then 1 mL in the case of DiBP and 10 mL in the case of DEHP was further processed as in the section “extraction of phthalates from water”.

B 1.2 RESULTS OF SOIL WATER PARTITION OF PHTHALATES/SORPTION TO SOILS

Batch experiments with DEP, DEHP, and DDoP and various soils were carried out. As can be seen in Fig. B 1, DiBP is bonded at a K_D value of around 60, which is significantly lower than with DEHP and DDoP, which are sorbed at a K_D value of between 287 and 263. DiBP probably bonds less due to its higher level of water solubility. In contrast to clay soils with yet lower levels of humus, the higher proportion of organic substance is noticeable in the case of DiBP sorption. In the cases of DEHP and DDoP the rather higher proportion of organic mass (0.1% as opposed to 0.01%) is also relevant for the sorption process, as can be seen from the higher K_D values (Xu et al. 2005).

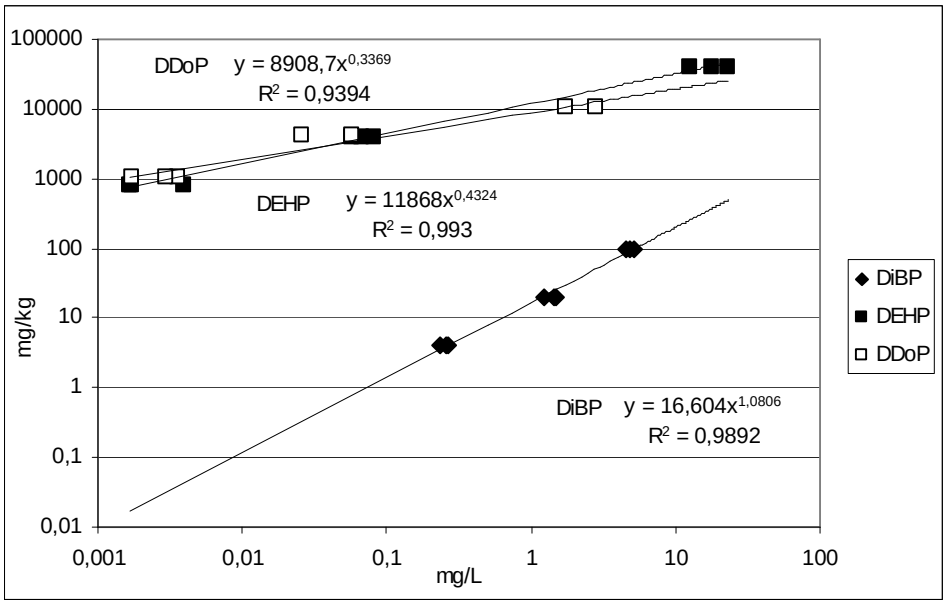
In general, however, it needs to be said that DEHP and DDoP were added to the sorption solution in higher quantities than the more water soluble DiBP for reasons of detection sensitivity, because a stronger sorption effect was expected in the former cases. It is well known that more hydrophobic phthalates like DDoP may easily form colloidal emulsions and thin films at the air-water interface as they are slightly less dense than water; this is especially true for the higher spiking concentrations and for extremely humic soil. This means that, in the case of DDoP, the possibility can not be excluded that, due to the abovementioned characteristics, higher than expected concentrations were determined in water.

Fig. B 1: Soil water partition of DiBP, DEHP, DDoP to soil A (sandy soil)



In clay-rich soil more sorption surface is available due to the higher proportion of fine particles, which can bring about a stronger sorption effect (Fig. B 2). This effect can be seen especially in the cases of the less soluble DEHP and DDoP, which bind to the fine material. This process is particularly supported by the surface-active characteristics of both substances and leads to KD values of 118,668 in the case of DEHP and 8,908 in that of DDoP. This effect is less pronounced in the case of DiBP, in which the lower organic carbon content comes into play. In addition, DiBP does not give rise to a surface-influenced sorption through the clay or silt contents.

Fig. B 2: Soil/water partition of DiBP, DEHP and DDoP to soil B (clay soil)



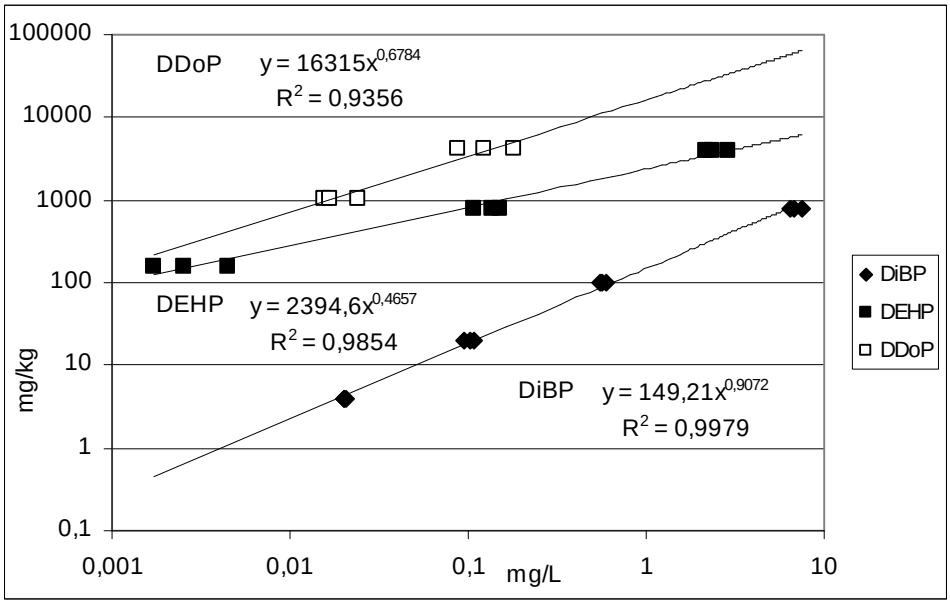
As can be readily recognized, the supply concentration in humic soil must be very high if measurable traces of DDoP or DEHP are to be found in groundwater (Fig. B3). This means that the sorption effect is stronger at the lower end of the concentration range than at the higher end. In the cases of Soil C and DDoP, the K_D value had to be extrapolated from only two values, because no invaluable results were obtained at the highest spiking concentration. The DDoP with longer chains and the DEHP probably bind very strongly to the humus matrix, it being the case that complex binding between the fulvo acids and the phthalates ensues (HAZDAT 2000). At the same time it is known that a higher DOC content can reduce sorption.

As far as DiBP is concerned it can also be assumed that the higher proportion of organic substance in comparison to sandy soil with 0.1% organic carbons leads to significantly higher sorption. The K_D values increase accordingly from 60 to 149.

In respect of the clay rich soil, the sorption of DEHP and DDoP increases by a relatively small amount compared to sandy soil, the presence of around 37% organic substance

notwithstanding. This effect will in particular be brought about by increased emulsification – increased surface-active characteristic – or, alternatively, by a higher DOC proportion in the dilute solution, and will itself lead to lower than expected sorption constants in the case of these compounds.

Fig B 3: Soil water partition of DiBP, DEHP, and DDoP in soil C (humic soil)



For the purposes of better comparison of the soils with one another, the K_D values obtained from the straight line equations based on the case where x=1 are related to the carbon content C_{org} of the soils. These K_{OC} values are depicted together with the K_D values in Table B 3.

The conversion of the K_D values to the content of organic carbon (K_{OC}) is carried out using the following equation:

$$K_{OC} = K_D \frac{100}{\%\ \text{organic carbon}} \qquad (5)$$

This conversion reflects the positive correlation between the high adsorption capacity of organic matter for organic compounds which is realized by a high content of phenoline and carbooxyline groups in the humus matrix.

Especial caution is needed if the K_{OC} values are to be calculated on the basis of extremely low carbon contents (e. g 0.01%), due to the danger of error multiplication. Such is the case with Soil B. Conversely, very low K_{OC} values are arrived at in the case of extremely high humus contents, as with Soil C (36.2%). This explains the broad spread of K_{OC} values that occasionally occurs in the specialist literature, in which they can vary by as much as a power of 10.

Table B 3: Log K_D values and Log K_{OC} values for Soils A, B, and C, and the phthalates DiBP, DEHP, and DDoP

	DiBP	DEHP	DDoP
Soil A			
K_D	1.78	2.46	2.42
K_{OC}	4.78	5.46	5.42
Soil B			
K_D	1.23	4.07	3.95
K_{OC}	5.23	6.07	7.95
Soil C			
K_D	2.16	3.38	4.21
K_{OC}	2.61	3.82	4.65

B 1.3 SOLUBILITY CONCENTRATION S OF DIBP AND DEHP IN WATER IN THE PRESENCE OF LAS

As has been demonstrated, the presence of surface-active substances has an influence on the solubility of hydrophobic substances in water. As both phthalates and tensides occur in wastewater and sewage processing, the solubility of the former should be measured in the various concentrations of a tenside (Fig. B 5). This was attempted with

DiBP and DEHP in the presence of the anionic tenside LAS To this end, dilute solutions of LAS at various levels of concentration were added to a surplus of phthalates. The mixtures were so shaken as to avoid the reduction in size of the large droplets of phthalate, in order to prevent emulsification from taking place. This binary system did not lead to the dissolution of the emulsions. The extent to which the phthalates came into contact with the LAS, which can in higher concentrations also form micelles, cannot be indicated.

The solubility in particular of DiBP, which is in any case soluble (ca. 10µg/mL), showed further significant increases. The relative rate of increase in the case of DiBP (factor of ca. 30) is, however, significantly lower than that of DEHP (factor of ca. 700). The solubility levels in pure water are within the range recorded by Staples et al. (1997). Staples gives “recommended” values for solubility. The determination methods having been taken into account, the figures given there are 20 µg/mL for DiBP, and 0.003 µg/mL for DEHP (in our case 10 µg/mL and 0.026µg/mL).

In the presence of LAS, the solubility increased significantly as was expected. This serves to make the results depicted in Fig. B 4 even less explicable. In any case, it was assumed that a very particular substrate was present in the case of Soil C, as, in addition to a very high humus content, iron oxide and allophane, which also influence the sorption characteristics, were probably also present. This was also not, however, along the same lines as the actual experimental findings, but rather the other way round.

B 1.4 SORPTION UNDER REDUCED CONDITIONS

Phthalates enter, for example, into water bodies via precipitation and via sorption into sediments, in which low oxygen depleted, or, in other words, reduced conditions obtain. It needs to be determined whether increased mobilization can be expected under such conditions. In order to simulate such reduced soil conditions, the tests with sewage sludge compost and DiBP or DEHP were repeated in the presence of the reduction agent sodium dithionite. In order that effects brought about by the additional presence of salt could be recognized, two further parallel tests were initiated using the same amount of sodium

sulphate. What also needed to be borne in mind was the fact that sewage sludge can by its very nature contain higher levels of salt.

In the case of the phthalates, the compounds were all within the category of moderately polar to non-polar, from which it can be assumed that changes in the pH value have no effect on adsorption. It is conceivable that the phthalate is forced out of the solution into the solid matrix by the salts, in other words, that a salting-out effect occurs. Table B 4 shows the redox potentials at the beginning and the end of

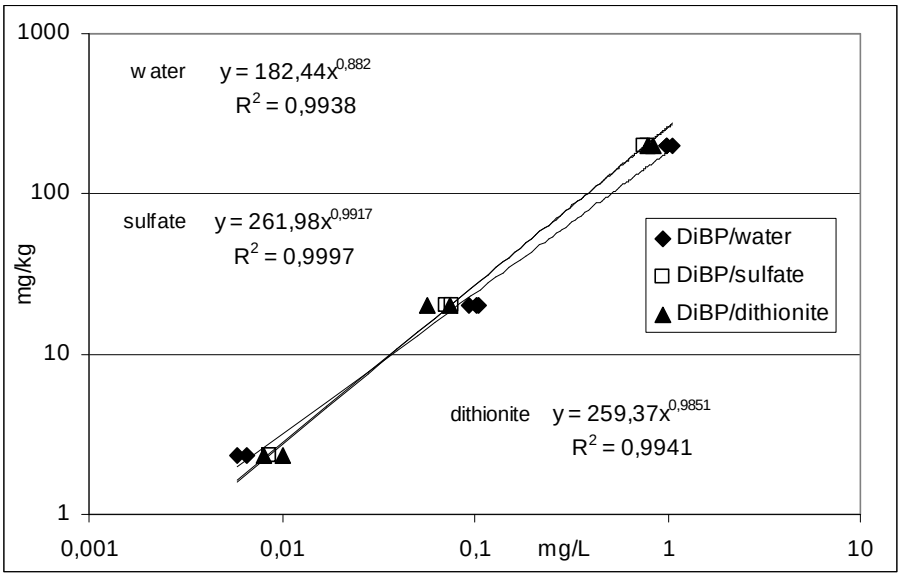
Table B 4: Redox potential after the addition of Na-dithionite in the experiment on partition between soil/sewage sludge compost mixture and fluid phase.

		Start		End	
	Spiking	Dithionite	Sulphate	Dithionite	Sulphate
	mg/kg	mV	mV	mV	mV
DEHP	23.74	-510	310	-70 to 230	200
	238.7	-480	240	100	200
	27201	-460	250	0	250
DiBP	2.304	-520	245	170	250
	20.16	150	260	150	200
	201.9	-480	200	200	170

the batch experiment for Na-dithionite and Na-Sulphate. At the end of the experiment, the redox potentials fell significantly due to the decomposition of the agent.

Fig. B 4 reproduces the results of sorption for DiBP in aqueous, sulphate, and dithionite solutions. The two straight lines obtained upon the addition of sulphate and of dithionite differ both in terms of gradient and of the Freundlich constants. In comparison to the sorption in aqueous solution with a K_D of 182, the K_D values in sulphate and dithionite solution are 262 and 259, representing a moderate increase. This is consistent with the effect of a slight salt out effect.

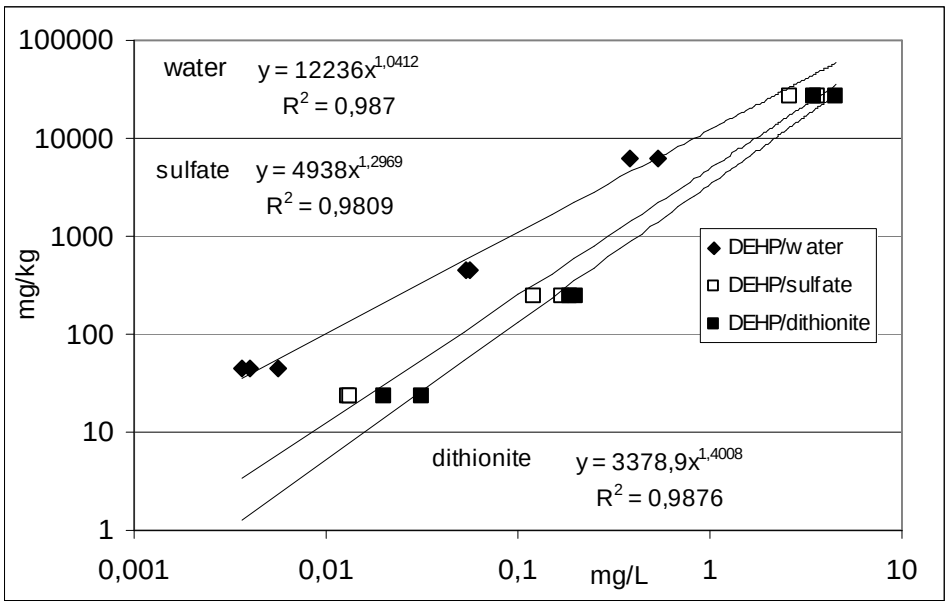
Fig. B 4: Determination of K_D according to Freundlich in sewage sludge compost/sand mixture for DiBP, under reduced conditions using sodium dithionite or in the presence of sodium sulphate



The results show that there is no redox effect; rather, that the slight increases show a salt effect (see also Brunk et al 1997, Turner 2003).

Also in the case of DEHP, the straight lines obtained in the presence of sulphate or dithionite are steeper than those in water (Fig. B 5). In comparison to the sorption in water, however, the sorption under sulphate conditions decreases. In the case of water-soluble DEHP the salt effect may be more pronounced, as is indicated by the results.

Fig. B 5: Determination of K_D for DEHP in sewage sludge compost/sand mixture according to Freundlich, under reduced conditions with sodium dithionite or in the presence of sodium sulphate



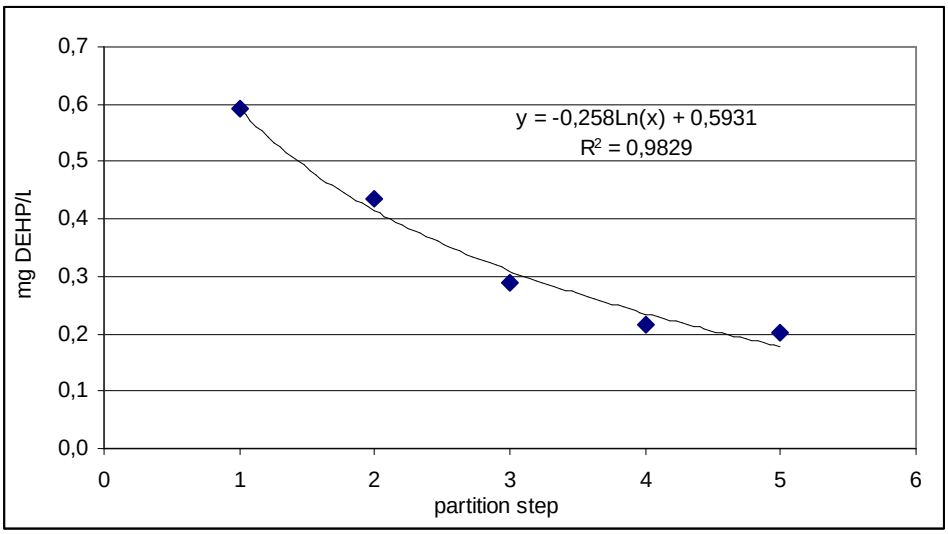
B 1.5 INVESTIGATION OF THE DESORPTION BEHAVIOUR OF DEHP

In order to check the desorption behaviour of a phthalate the same soil sample was shaken out successively with the same volume of water. The starting point was Soil A, which had been spiked with DEHP (Fig. B 6). The results of the section on soil water partition were taken as an indicator of the quantity of phthalate to be used for spiking. The phthalate concentration in the aqueous phase (at the point of the only or the first partition) should be around 1 µg/mL.

The concentration of 1 µg/mL given above only approximated to that in Soil A which was relatively lightly spiked. Soils B and C were significantly more heavily spiked. It is well known that phthalates can form micelles if present in appropriate quantities, which can lead to different results from those expected. Pressure of time made it impossible to

explain why such problems only occurred in the case of DDoP in simple partition experiments. After a fivefold partition with complete change of water in each case, 4.3% of the spiked DEHP (200 mg/kg) had been desorbed.

Fig. B 6: Decrease in the solution concentration of DEHP after fivefold exchanges of the fluid phase to determine desorption behaviour



The results support the hypothesis that DEHP is only desorbed slowly and, after the fourth desorption step, only to a very limited extent. Overall the concentration decreases from 0.6 mg/L to 0.2 mg/L, i.e. by around 2/3. In absolute terms this is very little. Under field conditions, in the case of DEHP in extremely high sewage sludge amounts, elutriations occurred in depths of up to 60cm (Carlsen et al. 2001).

B 2 LYSIMETER INVESTIGATIONS

B 2.1 LABORATORY LYSIMETERS

B 2.1.1 MATERIAL AND METHODS

B 2.1.1.1 SOIL CHARACTERISTICS

For the purposes of the laboratory lysimeters soils A and C were used (see section A). They represent less sorptive and very sorptive soils respectively. Soil A in particular can be roughly compared to that in the open land lysimeter, even though the experimental conditions differed from those in the laboratory lysimeter test.

The columns used for the laboratory lysimeter had a diameter of 9.7 cm and were filled according to soil type to a height of 16-17 cm. Care was taken when filling the columns to disperse the individual soil components homogeneously. Water was then slowly added from beneath until the soil was over-saturated, in order to expel the air. Before spiking, the columns were eluted with 700-800 mL water, so as to remove the high concentrations of soil components mobilized by the initial wetting process. As the laboratory tests were conducted initially with a very porous substrate, DEP, DiBP, DBP, and DEHP were comparatively tested. In the subsequent test with sorptive soil after the viewing of the findings, only DEP and DiBP were tested for mobility characteristics, because it was not expected that DEHP and DBP would be found in the eluate.

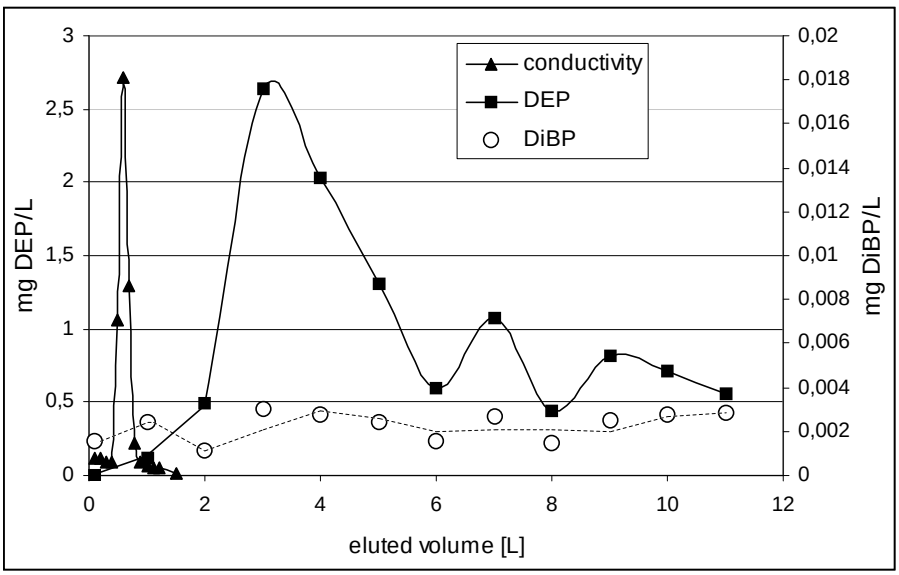
The columns were spiked with around 40 mg of each of the two above mentioned phthalates (using a thin layer of spiked sand as carrier) and then eluted with completely desalinated water. The eluates were captured in portions of 100 mL. The first sample and then, in each case, the last 100 mL before a full litre (Soil A), or a half litre (Soil C) were extracted using an RP18 solid phase column and measured after elution by GC.

B 2.1.2 YIELDS FROM LEACHATE IN THE SMALL LYSIMETERS

As a complement to the field lysimeters, tests were also carried out with DEP and DiBP using small lysimeters. Soils A and C were used for comparative purposes, in order to compare less sorptive and very sorptive materials. The lysimeter containing soil A was eluted with a total of 11 L over a period of around 10 hours. The conductivity was taken as a tracer for the water movement, and at the start confirmed a fast breakthrough of the waterfront.

Only DEP was found in the eluate (Fig. B 7), and it was significantly retarded in comparison to the water front. In total, somewhat more than 50% of the applied material migrated through the column. This corresponds to the investigations carried out by Russel and McDuffie in 1986 on a similar soil substrate.

Fig. B 7: Laboratory lysimeter with the sandy soil A, spiked with DEP and DiBP (conductivity without scale)

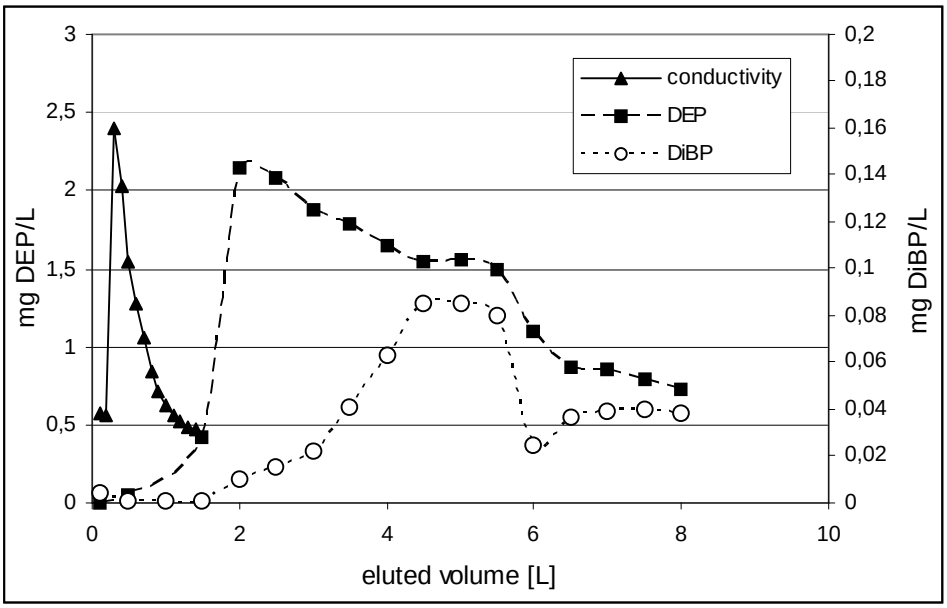


In the case of DiBP a very slight increase of concentration was found in the eluate, which only took place very slowly and had not reached the maximum by the end of the experiment. The overall absolute concentrations in the eluate were very low. In comparison to DEHP, both phthalates show a high level of mobility. As the investigations

on DEHP carried out by Jong et al. (2002) show, the concentration of DEHP reaches less than 1.0 µg/l in seepage and less than 0.5% of the applied. It was presumed that transport could only come about via the dissolved organic substance. The soils tested in those experiments, however, are not really comparable to the ones under investigation here, due either to the insufficient clay or humus contents in the soils in this study.

In a further test, DEP and DiBP were added as before to a small column with humic Soil C (Fig. B 8). Spiking was carried out as for Soil A, and the elution was performed using VE water. 6 L eluate were captured the same day, and a further 2 L after the column had stood without run through over night (about 10 h).

Fig. B 8: Laboratory lysimeter with the humic soil C, spiked with DEP and DiBP (conductivity without scale)



initially the water front migrated very quickly through the whole soil column. DEP achieved the maximum with a steep breakthrough after around 4 pore volumes, and further significant amounts were subsequently moved through the soil column. Both the height and the amount of the breakthrough concentration are comparable to those of sandy soil. This is only ostensibly unusual and has to do with the fact that the absolute soil quantity in

the soil columns in the case of Soil C was a third less, as was therefore also the absolute sorptive amount in the soil substrate. The somewhat higher mobility, also of DiBP, could perhaps be attributable to the higher DOC or allophane proportions probably present in the eluate.

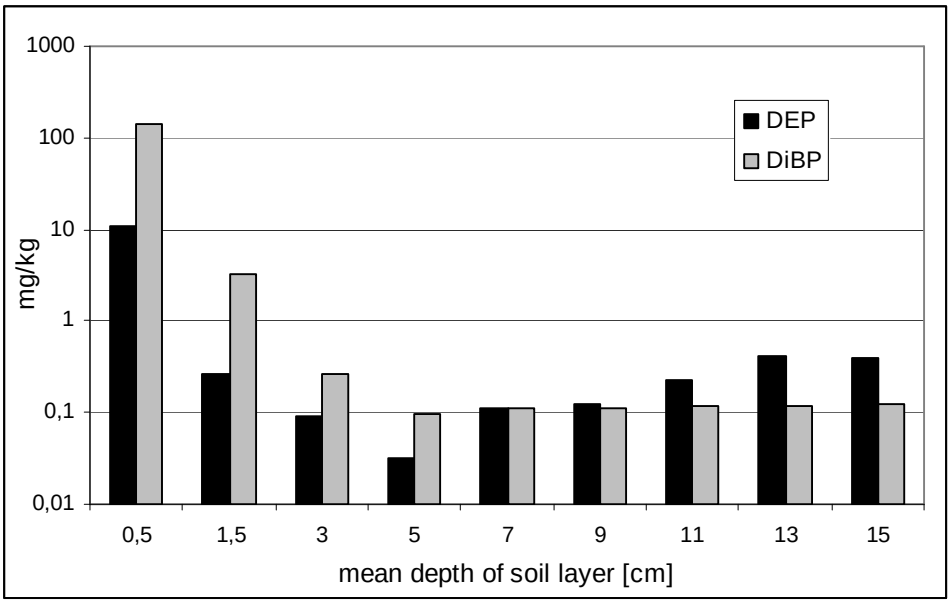
B 2.1.3 YIELDS IN DEPENDENCE ON THE SOIL DEPTH IN THE SMALL LYSIMETER

After the elution was complete, the soil in the column with Soil C was carefully removed in layers. The individual layers were allowed to air dry and were later tested according to the procedure described in Part A. It was recognized that residues of DEP were adsorbed in the uppermost layer, in subsequent strata the phthalate was present in much lower quantities, and by the lower end of the column, a further slight increase in concentration was detected, which possibly represented the remains of the concentration maximum which had previously migrated through the column.

DEP is probably somewhat more pronouncedly transferred than DiBP. This can be seen in the initially lower levels of accumulation in the first soil stratum, offset however by pronounced transfers in the deeper strata. DiBP is not only less water soluble than DEP, but is also more strongly adsorbed to the matrix. Consequently, it is to be found in much greater concentrations than DEP in the highest stratum of the Lysimeter

Figure B 9 shows the depth partition of DEP and DiBP in the small lysimeter substrate. It can be seem that the highest quantities of phthalates occur in the upper layers, but that remnants are also to be found in the further strata, which is in general similar to the pattern found in seepage.

Fig. B 9: Phthalate yields in dependence upon stratum depth in the laboratory lysimeter



B 2.2 OUTDOOR LYSIMETERS

B 2.2.1 MATERIALS AND METHODS

B 2.2.1.1 SOILS IN FIELD LYSIMETERS

In December 2004, four field lysimeters (Nos. 7, 9, 10 17 and 18)(isn't that 5?) with a surface area of 1m^2 and a depth of 2 m were spiked with a solution containing DEP and DiBP. The soils in question were brown earth of sandy soil type with 4% C_{org} and <5% clay in the topsoil, whereas the deeper soil consisted of sand with less than 5% clay, a field capacity of 12 and a pH of around 5.7 (Cambisol from boulder sand from grassland). At the time of spiking, the lysimeters were overgrown with grasses, and in the following spring herbaceous, perennial, deeper rooted plants started to grow. An increased quantity of water runthrough after three weeks pointed to rainfall throughout the period.

B 2.2.1.2 TEST CONDITIONS

For the purposes of spiking the lysimeters, sand was sieved to < 1 mm, washed with large quantities of water to remove fine material, heated overnight at 550°C and spiked with the test substances. DEP and DiBP were dissolved in 40 ml acetone and added in successive amounts of 2 ml, with shaking in between. The solvent was allowed to evaporate

overnight. Of the total amount of 1018 g sand left after the spiking, 509 g respectively was added as equally as possible to two lysimeter surfaces (Lysimeters 17 and 18) (Table B 5) and primed with 30 g KBr as tracer and 50 L water, corresponding to 50 mm precipitation. Natural precipitation was used in the further process of the experiment.

Table B 5: spiking of 1 kg sand

	DEP	DiBP
Initial weight/kg pure sand [g] (lysimeters 17 and 18)	6.00 g	6.00 g
Initial weight per lysimeter (all lysimeters)	3.00 g	3.00 g

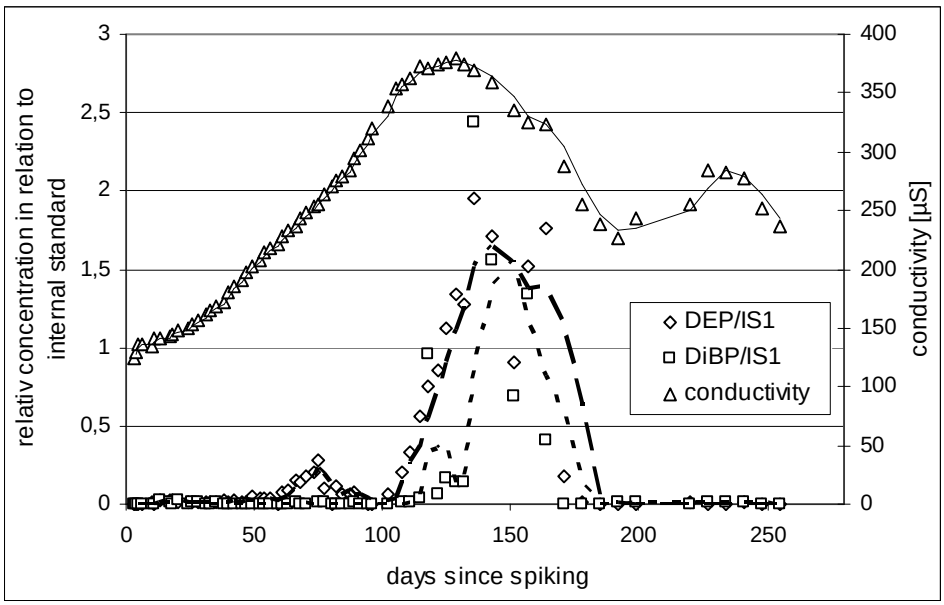
To record any possible incidence of test substance seepage past the soil pores through worm tunnels, root spaces or cracks in the ground, a seepage sample was extracted initially every 2 to 3 days to solid phases; the samples were later processed on a weekly basis. Lysimeters 17 and 18 mostly provided sufficient amounts of water, to the effect that, at the times the samples were taken, around 2 L were available. Every 1 L was put through a C18 and a SX column. The charged columns, which had been blow dried with nitrogen, were wrapped in aluminium foil and kept in the refrigerator until needed. The C18 columns were eluted with 2.5 mL ethyl acetate (with 2 IS) and analysed.

B 2.2.2 RESULTS OF PHTHALATE YIELD IN LEACHATE IN THE FIELD LYSIMETERS

Of the lysimeters used in the test, only lysimeter 18 delivered measurable concentrations of the applied substances (Fig. B 10). As no breakthrough occurred in the lysimeters used for comparative purposes during the investigative period it can be assumed that the findings in the leachate from lysimeter 18 were brought about by unfavourable hydrological conditions. These could for instance be favoured flowpaths left behind by deep dead plant roots or pathways bored by soil organisms etc. On the other hand, an inhibition in migration in lysimeters 7, 9, 10, and 17, which are all around 20 years old, could have been brought about by naturally occurring blockages, such as the formation of thin

secondary clay strata which reduce permeability. Such clay accumulation processes were observed in similar soils in the Berlin area.

Fig. B 10: Progression of DEP and DiBP concentrations and electrical conductivity in the outlet of lysimeter 18



Whereas the electrical conductivity of leachate increased from the start, the first small peak of DEP appeared only after around 50 days, 100 days being needed for the DEP mass to reach the lysimeter outlet. Russel and McDuffie (1986) investiga-ted the mobility of DEP using small glass columns and found only a low level of retardation from sorption to the soil, which was almost independent of the flow velocity of the leachate solution.

The simultaneously recorded DiBP migrated through the 2 m long flow section after around 130 days, with a maximum of 150 days. Once the maximum had been reached, the conductivity, which functioned as an indicator of the water front, de-creased, with the effect that the breakthrough of both substances was largely com-plete after 170-180 days. Of the applied quantities less than 0.1% in total was eluted.
Spiking with DEHP was generally not carried out, because no DEHP was expected to be found in the leachate within the period of the investigation. If DEHP was found in leachate, this was mostly only observed in connection with very high DEHP concentrations, for instance, in old waste deposits or in disposal sites. In the case of the DEHP concentrations

usually found in sewage sludge, no downward migration of any significance in depths of up to 2 m has hitherto been observed.

B 2.2.3 INVESTIGATION INTO THE FATE AND BEHAVIOUR OF PHTHALATES IN THE UPPER SOIL STRATA IN THE LYSIMETERS

Around 12 months after the insertion of the test substances, soil samples were extracted from the lysimeters used in the investigation, as well as from a control lysimeter, at seven different locations distributed over the surface of each, to a depth of around 5 cm. These were then combined to form one sample (dry mass 80 – 100 g). The soils were investigated for both phthalates. As can be seen in table B 5, DEP was in no case found in concentrations over the detection limit, whereas DiBP was found concentrations partly over and partly under the detection limit.

Table B 6: Concentrations of the applied phthalates in the lysimeter soils after one year

	DEP [mg/kg]	DiBP [mg/kg]
Lysimeter 9	<0.05	0.09
Lysimeter 10	<0.05	0.11
Lysimeter 17	<0.05	0.10
Lysimeter 18	<0.05	0.05
Lysimeter 7 (control)	<0.05	<0.05

B 3 DISCUSSION

Phthalates are substances which can be deemed ubiquitous. They are used world-wide as additives to prolong the life and plasticity of plastics. For example, 95% of DEHP produced goes into the production of PVC (Hazdat 2000, IARC 2000). In Europe alone, around 1 million tonnes of phthalates are manufactured annually. The most frequently occurring phthalates are DEP, DIDP, and DINP (diisononylphthalate). Building materials, floor coverings, and insulation and cable materials are just some of their diverse applications.

Phthalates escape into the environment via the air, or through lixiviation and thence via waste water. They are also detectable in water from precipitation, snow, surface water, and in sediments and soils, as well as in media that are not directly exposed to them. Phthalates also make their way onto the earth's surface through rain/snow, wash-out, particle and gas scavenging and become dispersed world-wide via the bio-, pedo-, and hydrospheres (Schiedeck 1996, Hofmann 2002).

One important dispersal route for phthalates throughout the environment is via the introduction of sewage sludges into soil (Leschber 2004), and via subsequent erosion into surface water bodies.

In the aquatic realm in particular, phthalates can have endocrine effects on animals. It is widely assumed that an anti-androgen activity induced effect on female reproductive systems can take place (NTP-CERHR 2000). In fish, DEHP inhibits the binding of estrogen to the receptor (Gies 1996). Endocrine effects are also ascribed to DBP.

A large number of hitherto unanswered questions concerning the dispersal and mobility behaviour of phthalates in the fluid phase, or, as the case may be, reciprocal effects in terrestrial and aquatic environments, need to be taken up and investigated in experimental conditions. In addition, those phthalates in particular that are less water-soluble need to be tested for their sorption and mobility behaviour.

Water solubility is of great importance in the quest for explanations into the behaviour of phthalates in respect of sorption and mobility. As Carlsen et al demonstrated with great

thoroughness (2001), large differences occur in the determination of water solubility or, as the case may be, of published values for the various phthalates (see also Rassmussen 1998). Experimentally derived data on water solubility show a connection between increasing water solubility and decreasing numbers of C atoms in the molecule. However, no visible change in water solubility is effected by numbers of C atoms in the chain in excess of 6. Differences in water solubility can be ascribed to the formation of emulsions, which leads with increased concentration to a reduction in surface tension (Thomsen et al 1999 and 2001). The formation of emulsions at concentrations in excess of the solubility of the substances and the decrease in surface tension are the subject of intense debate, especially as the experiments thus far conducted have not always led to clearly explicable results.

The water solubility and partition behaviour of phthalates are also subject to influence by the dissolved organic substance in the form of DOC, or by the presence and proportion of particular substances in the aquatic environment (Bauer and Herrmann 1998). Thus, for example, the solubility of DEHP increases in the presence of humic acid (Mitsunobu and Takahashi 2006). It also increases in a threefold system (DEHP/humic acid/particular substances). In this case, the decrease in hydrophobia of the humic acids was responsible for the partition of DEHP in the aqueous phase.

As is shown by the various investigations conducted into sorption and mobility behaviour, the picture emerges of a very complex set of events. In contrast to other contributions, an investigation was undertaken here into sorption and release, or desorption, and mobility behaviour using phthalates which had been sorbed to the substrates under investigation during a storage period of several days; the samples had thus been “aged”, meaning that a direct comparison with experiments carried out according to OECD 106 was not possible. Such “aged” substrates bind these substances in part in other ways e.g. through intramolecular sorption, as probably occurs in the usual batch experiments. A further difficulty is presented by the fact that, with the exception of DEHP, very little literature on the two other phthalates is available, which makes a comparative discussion more difficult.

A comparison of soil substrates with different characteristics and their sorption of different phthalates yielded results in line with expectations. It could be seen that water-soluble

DiBP was bound less strongly to the organic substance than the less water-soluble DEHP and DDoP, both of which were seen to bind in a similar order of magnitude. It could also be seen that clay minerals, dominant in Soil B, were less able to bind the investigated phthalates. This is especially true for the water-soluble DiBP. K_{OC} values from the literature need to be compared with those recorded in this study (Table B 6).

Table B 7: Comparison of K_{OC}-values of different studies summarised in Staples et al., (1997)

compound	K_{OC}	K_{OC}	this study
	soil/sediment	suspended particles	soil/sludge
DEP	69 – 1726	79400	
DiBP	1375 – 14 900	1230 – 158 500	149 – 170 000
DEHP	87 420 – 510 000	22 000 – 1 000 000	6613 – 11 868 000
DDoP	-	-	45 069 – 89 090 000

As can be seen, the K_{OC} values increase in direct proportion to the chain length of the compounds. The differences in the investigated substrates also become obvious. As the investigations in Part A into recovery in dependence upon particle size reveal, this value increases, which can be explained by increased sorption in dependence upon particle diameter. Thus the K_{OC} values of suspended particles are higher than those in soils and sediments. Investigations carried out by Germain and Langlois (1988) showed that, in surface water, a partition ratio in the case of DEHP of 53% particle bound to 47% dissolved was to be found. It can also be seen that colloids left over in the aqueous phase according to sample preparation also have a role to play in sorption.

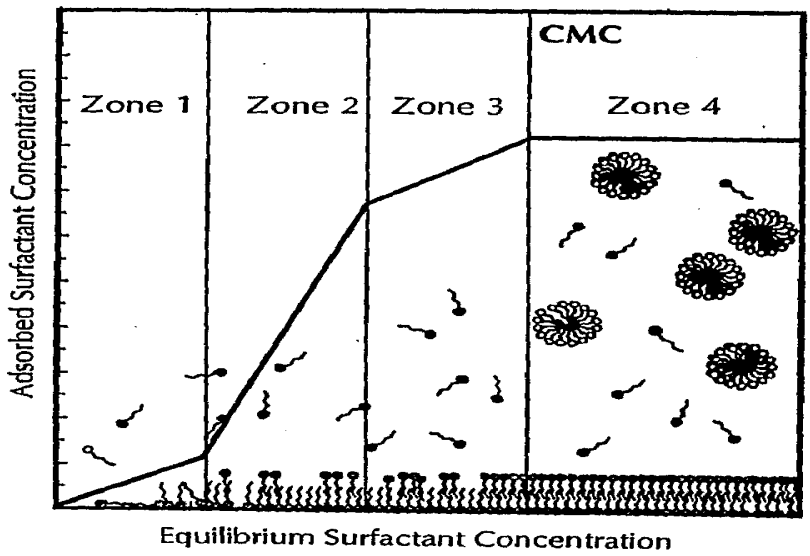
Of especial interest are those investigations, which are intended to simulate sorption under anoxic, that is, reduced, conditions. The findings available can lead one to assume that a slight influence on sorption behaviour occurs in the case of DiBP where sorption increased. In the case of DEHP, no salting-out effect such as that described by Zhou et al (2000) was observable. Brunk et al (1997) also found a salting-out effect in the case of phenanthrene. In this case, the critical micelle concentration (CMC) decreases in the

presence of electrolytes, such as, for example, sodium sulphate or sodium dithionite (Danzer 1999).

Investigations into the effect of solubilising or surface active substances such as LAS have shown that the solubility of DEHP increases in the presence of LAS, which would normally be equivalent to a decrease in sorption. If, however, this mixture is added to Soil C, the sorption shows a strong increase, or, to put it another way, the concentration in the aqueous phase decreases.

A binary system, such as a mixture of water and LAS in the presence of solids, demonstrates different behavioural characteristics to the purely aqueous system in respect of partition processes (Danzer 1999). The sorption of hydrophobic substances, such as phthalates with longer chains in binary tenside systems, depends on the proportion of adsorbed tensides and not on that of solids in general. There is a relationship between adsorbed tensides and hydrophobic substances, which can be termed adsolubilisation. In such a case, the adsorbed tenside phase is more important for the partition behaviour of such substances than the proportion of tensides found in the aqueous phase (Fig. 11).

Fig. B 11:Correlation between increase in equilibrium surfactant concentration and the quantity adsorbed (from West and Harwell 1992)



Thereby the proportion of adsorbed tensides increases up to the critical micelle concentration (CMC) (West and Harwell 1992). Under the experimental conditions used here, LAS was introduced below the critical micelle concentration, so as to avoid a decrease in sorption.

Residues bound to the soils can be desorbed by the step by step addition of aqueous solution. This does not represent a significant amount, although the soil solution concentration does fall from 0.6 to 0.2 mg/l after four desorption steps.

Leaching tests show the centrality of constant adsorption and desorption. So that residues in the leachate after passage through the lysimeters could be detected with greater constancy and accuracy, particularly mobile phthalates such as DEP and DiBP were selected. For DEP, KD values of 1.1 or, as appropriate, K_{OC} values of 69 (Russel and McDuffie 1986) were recorded. It can be seen, in dependence on water solubility, that DEP and traces of DiBP migrate after a long passage through the 2 m leaching section and can then be detected. It must be stated that, in similarly configured lysimeters no detectable traces were found, and that the breakthrough was probably brought about by fast draining pores.

Other phthalates, such as DEHP were only detected in small amounts in the leachate (Brown and Donnely 1998, Bauer and Herrmann 1997), and that only in domestic waste disposal sites etc. Schiedeck (1996) recorded mean DEHP concentrations of up to 15 µg/L in various surface water investigations, which could be ascribed to waste output from industrial areas.

B 4 SUMMARY

The following results were obtained:

- Phthalates bind in dependence on water solubility and the chain length to soil substrates the following sequence: DiBP>DEHP>DDoP
- Sorption increases in the sequence sand > clay and organic substance
- repeated changes of the aqueous phase in the batch test led to DEHP desorption. The solution concentration decreased from 0.6µ/L to 0.2 µ/l
- the addition of a reduction agent (sodium dithionide) to the sorption environment has no influence on the sorption of DiBP. In the case of DEHP sorption decreased.
- A salting-out effect caused by the addition of sodium sulphate to the sorption environment led to an increase in the solid phase only in the case of DiBP. DEHP bound comparatively less strongly under the conditions described
- DEHP bound more strongly in the presence of LAS and showed a concomitant decrease in the soil solution. In the case of DEP no clear influence could be established.
- Lysimeter experiments in the laboratory confirmed a migration of DEP and DiBP. The eluted amount remained <1% overall. DEHP was not detected in the eluate.
- outdoor studies using field lysimeters showed a migration of DEP and DiBP within 150 days of application. In this case as well the eluted amount was way below 1% of the applied.
- Investigations of the soil surface after 12 months revealed that applied DEP and DiBP could only be found in traces in the top 5 cm.

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ANNEX I

A I Table 1: Compilation of methods for the quantitative determination of phthalic acid esters

matrix	compounds	extraction	cleanup	Method	% recovery	Analytical quality	reference
MULTI METHODS							
-effluent, landfill leachate, sewage, - sludge	DMP, DEP, DBP, BBzP, DEHP,DOP, PAH, dinitro toluene	- DCM - sonication/DCM	Florisil	GC/MS HP-5	---	LOQ: 1 µg/L (phthalates)	Marttinen et al. (2003)
waste water, sludge	DPP, DBP, BBzP,DEHP, DnOP,DnNP, nonyl phenols, LAS	shaking with DCM/HCl	---	HRGC/MS DB-5MS		3-9 ng/L, DBP 30 ng/L, DEHP 25 ng/L	Fauser et al. (2003)
sewage sludge	anionic and non-ionic surfactants, BPA, DEP, DBP, DEHP	Ultrasonic extr. MeOH/DCM	concentrate, redissolve in water, SPE (C18)	LC-APCI-MS and LC-NI-ESI-MS, RP18 column	87%(DEP), 91%(DBP), 78%(DEHP)	LOD (instr.): 0.5 ng (DEP), 1,0 ng (DBP), 1.5 ng (DEHP) LOD (sludge): 15 ng/g (DEP), 25 ng/g (DBP), 50 ng/g (DEHP)	Petrovic and Barceló (2000)
sewage sludge, sediment	DBP,DEHP, fatty acids, non-ion. surfactants, carbohydrate derivatives	SPME (carbowax) from aqueous suspension of dried samples	---	HPLC/ESI/MS RP8 column	---	LOD 50 NG/ML (DEHP), 30 NG/ML (DBP)	Möder et al. (1998)
Air	DEP, DBP, BBzP, DCP, DEHP, Phos-phate esters	adsorption on charcoal	extraction with toluene/ultrasonication	GC/MS HP-5	68-100%	MDL: BETWEEN 0.11 µG (DEP) AND 0.51 µG (BBZP)	Otake et al. (2001)

A I Tab. 1 (continued)

matrix	compounds	Extraction	cleanup	method	% recovery	Analytical quality	reference
- water, STP influent, STP effluent, liqu. manure, - sewage sludge, sediment	BPA, BPF, DBP, BBzP, DEHP	- water: steam distillation/solvent extraction - dried sediment: soxhlet (chx/ethyl acetate)	- --- - GPC and silica column	GC/MS DB-XLB	82-110 % (WATER) 71-117% (SEDIMENT)	DETECTION LIMIT: 0.02-0.03 µG/L IN WATER, 0.02-0.05 MG/KG D.M. IN SEDIMENT	Fromme et al. (2002)
soil sludge	DBP, DPeP, BBzP,DEHP, DOP,DNP, DiNP, nonylphenol	shaking with DCM	---	HRGC/MS DB5-MS	DBP:ca.85 % BBzP:ca.80 % DEHP ca. 60%	lim. determ., low contam./ soil, 0.1-1µg/kg (DBP: 1.5µg/kg), high contam. 0.8-1 µg/kg (DBP 40 µg/kg, DiNP 10 µg/kg),sludge (low contam.): 8-60 µg/kg	Vikelsøe et al. (2002); NERI Report 268 (1999)
PHTHALATE METHODS							
surface water	DMP, DEP, DBP,BBzP, DEHP, DOP, DPP, DMPP, BMPP, DCHP	C18 cartridge	aluminium oxide (only when necessary)	GC/MS HP-1	91-108%	Detect. lim. 0.01-0.02 µg/L, 0.03 µg/L for BBzP Determ. lim. 0.02-0.03 µg/L, 0.04 µg/L for BBzP, 0.05 µg/L for DEHP	Furtmann (1994)
- aqueous solution - soil	DEP, DEHP	- shaking with n-hexane - ultrasonic extr. ethyl acetate	- none - centrifugation	- HPLC/UV C_{18} column - GC/FID BP5	-60%(DEP), 67%(DEHP) -94%(DEP), 73%(DEHP)	- detec. lim. 1 µg/mL - detec. lim. 0.1 µg/mL extract	Cartwright et al. (2000)

A I Tab. 1 (continued)

matrix	compounds	extraction	cleanup	method	% recovery	Analytical quality	reference
- water - suspended particulate matter (SPM)	DMP, DEP, DBP, BBzP, DEHP, DOP	- SPE C8 column - shaking with ac/water/hx	- none - centrifugation	GC/ECD GC/MS Sil-5	water (spike level 300-500 ng/L) 83-97% SPM: 82-93% except DEP (67%), DMP (26%)	detect. lim.: water: 0.01 µg/L (DMP, DEP, BBzP) 0.1 µg/mL (DBP, DEHP, DOP) ; SPM: 0.01-0.1 mg/kg, except DEHP: 1 mg/kg	R. Ritsema et al. (1989)
Bioreactor leachate, fractions of household wastes	DMP, DEP, DEHP, DBP, BBzP	Leachate: shaking with hx/diethyl ether Solids: ultrasound with diethyl ether/hx	Filtration through alumina	GC/MS DB-1			Bauer, Herrmann (1997)
Sewage sludge	DMP, DEP, DBP, BBzP, DEHP (separation from PCB, PAH, pesticides)	a) Ultrasonication (DCM) b) Soxhlet (DCM)	aluminium oxide followed by Florisil	GC/ECD	98-103%	---	Zurmühl (1990)
- soil - plants	DBP, BBzP, DEHP	- shaking with ac/hx - Ultrasonication (ac/hx)	- GPC - GPC, silica column	HPLC/UV C_{18} column	- 90-95% - 80-90%.	- detect. lim.:0.005 mg/kg - detect. lim.: 0.74 mg/kg d.m. (DEHP), 3.4 mg/kg d.m. (DBP), 1.0 mg/kg d.m. (BBzP)	Müller, Kördel (1993)
sewage sludge	DBP, BBzP, DEHP, DiNP, DiDP	SFE (CO_2), shaking with ethyl acetate	--- centrifugation	GC/MS HP5	85-100% for SFE and shaking	detect. lim. between 0.005 mg/kg d.m. (DBP) and 0.062 mg/kg d.m. (DiDP); LOQ: 0.015 and 0.212, resp.	Kolb et al. (1997)

A I Tab. 1 (continued)

matrix	compounds	extraction	cleanup	method	% recovery	Analytical quality	reference
sewage sludge	16 phthalates (same as EPA) 8061A)	Shaking of dried sample with ethyl acetate	centrifugation	GC/EI/MS GC/CI/MS (HT-8 column)		LOD: 0.08 µg/mL (DEHP, DiBP) to 3.8 µg/mL (bis(methoxyethyl)phthalate) for standards 10 µg/kg d.m. (DMP) to 632 µg/kg d.m. (BBzP) for sludge samples	Berset, Etter-Holzer (2001)
- water - sediment	DMP, DEP, DBP, DiBP, DEHP, DOP, DOiP	- DCM - multi step solvent extraction with DCM/petroleum ether, acetonitrile	Florisil	GC/FID SPB-608	- 60-80%, DMP 37% - 40-80%, DMP 26%	Detec.lim. (S/N 3/1): 0.01 ng (DMP, DEP, DBP), 0.05 ng (DOP), 0.1 ng (DEHP, DIBP), 0.4 ng dioctyl isophthalate	Tan (1995)
sediment, biota	DMP, DEP, BBzP, DBP, DEHP, DnOP, 5 isomeric mixtures	Dried samples: ultrasonication (DCM/hx)	Alumina Alumina and Florisil	HPLC/ESI/MS C_8 column GC/MS, DB-5	71-106% (individual compounds), 89-102% (mixtures)	0.3-1.1 ng/g (single comp./ GC), 3.3 ng/g (DEHP/GC), 0.5-4.2 ng/g (LC) no reproducible results for mixtures with GC, 0.5-3.0 ng/g with LC	Lin et al. (2003)
sediment	DEP, DBP, BBzP, DEHP, DiNP	SFE (CO_2)	Silica column, elution with DCM, change to hexane	GC/MS DB-5	85% (DBP), 88% (DEHP)	DEHP 0.81, DBP 0.30, DEP 0.18, BBzP 0.11, DiNP 0.09 [µg/g] for 0.2 g sample	McDowell, Metcalfe (2001)
- water leachate, sludge, soil -.sediment, l	16 phthalates	- separatory funnel, C8 and C18 membrane disks - Soxhlet (hx/ac) ultrasonication (DCM/ac)	Florisil, aluminium oxide (recommended), removal of sulphur	GC/ECD GC/FID; DB-5, DB-1701	a)	2-10 µg/L (1 L sample, 2 mL final volume), 6-60 µg/kg (30 g sample, 2 mL final volume)	Lopez-Avila et al. (1991)

A I Tab. 1 (continued)

matrix	compounds	extraction	cleanup	method	% recovery	Analytical quality	reference
- sewage sludge, - soil	DEHP	ac/petroleum ether/NaCl solution	- Florisil, aluminium oxide ---	GC/FID GC/ECD		LOQ: 1 mg/kg d.m (sludge), 0.1 mg/kg (soil)	Merkel, Appuhn (199
soil, sediment	DEP, DBP, BBzP, DEHP	ASE (hx)	---	GC/MS DB-5	"complete"	---	Ventura, Adam (200(
soil, sediment	DMP, DEP, DAP, DBP, BBP, DEHP	microwave assisted with ac/hx	centrifugation	GC/MS HP-5	70-91%	---	Chee et al. (1996)
blood products	DEHP (and mono-EHP)	SPE	---	HPLC (C_{18} column), UV	98-102%	---	Shintani (2000)
- parenteral nutrition, - plasma	DEHP	vortex with NaOH/hexane as above after protein precipitation	- centrifugation - centrifugation	HPLC (C_{18} column), UV		LOQ: 20 ng/mL for spiked samples	Kambia (2001)
milk and milk products	DEHP	Shaking with hx/MeOH/KOH	SEC on bio beads (fatty samples)	GC/MS CPSIL5CB	100±7%	---	Sharman et al. (1994
OFFICIAL METHODS OR DRAFTS							
water	11 phthalates	SPE (C18)	Alumina if necessary	GC/MS, 5% phenyl methyl siloxan	75-110%, 60-75% (DOP, DUP)		ISO 18856 (DRAFT) 20
sludge	DEHP, PAH, NPE, LAS	ultrasonic bath (DCM)	Dried and concentrated	GC/MS, HP-5	70 - 130 % (required)	for DEHP: 0.5 mg/kg d.m. (required)	NERI 2003

A I Tab. 1 (continued)

matrix	compounds	extraction	cleanup	method	% recovery	Analytical quality	reference
groundwater, leachate, sludge, soil, sediment,	16 phthalates	Shaking with DCM, or C18 disk DCM/ac	If necessary: methods 3610 (alumina), 3620 Florisil), 3640 (GPC), or 3660 (sulphur removal)	GC/ECD			EPA method 8061 A
waste water, solid waste, sediment, soil	semivolatile organics incl. 6 EPA phthalates	EPA methods 3510 (separatory funnel), 3520 (cont liq.-liq. Extraction), 3540/ 3541 (Soxhlet), 3550 (ultrason.), or 3580, (dilution with solvents)	If necessary: GPC (method 3640)	GC/FT-IR DB-5 GC/MS DB-5 or equivalent		Identification limit 2,5-5 µg/L Estimated quant. limits: 10 µg/l (ground water) 660 µg/kg (low contamination)	EPA method 8410 EPA method 8270
sludge, sediment, soil	11 phthalates	Shaking with ethyl acetate	alumina	GC/MS, 5% phenyl methyl siloxan			CEN/TC 308/WG 1/TG 4 N 0052

a) Water (spike level 10 µg/L): separation funnel: 73-110%; C_{18} disk 67-98%. Loamy sand (spike level 1 µg/g): soxhlet 54-135%, sonication 63-112%. For further recoveries from Florisil and alumina with and without interference see reference (Lopez-Avila et al. 1991).

ANNEX 2

A II Tab. 1: Summary of the investigated phthalates and their retention times

No. of the phthalate	Name	abbreviation	CAS-No.	RT
1	Dimethylphthalate	DMP	131-11-3	11.26 min
2	Diethylphthalate	DEP	84-66-2	12.50 min
3	Dipropylphthalate	DPP	131-16-8	13.95 min
4	Diisobutylphthalat, eDi(methylpropyl)phthalate	DiBP	84-69-5	14.71 min
5	Dibutylphthalate	DBP	84-74-2	15.55 min
6	Benzylbutylphthalate	BBP	85-68-7	20.22 min
7	Dicyclohexylphthalate	DCHP	84-61-7	22.68 min
8	Di(2-ethylhexyl)phthalate	DEHP	117-81-7	22.84 min
9	Dioctylphthalate	DOP	117-84-0	25.15 min
10	Didecylphthalate	DDP	84-77-5	28.65 min
11	Diundecylphthalate	DUP	3648-20-2	31.14 min

A II Tab. 2: Target and qualifier ions for phthalate determination (as per draft method)

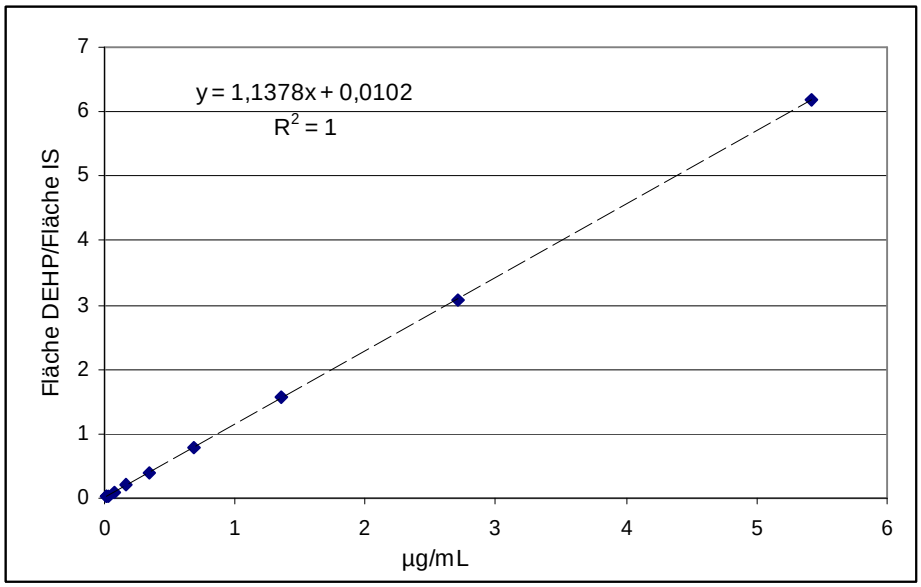
	Compound	Abbreviation	Specific monitored ions		
			Target ion M_1 (%)	Qualifier ion M_2 (%)	Qualifier ion M_3 (%)
1	Dimethylphthalate	DMP	163 (100)	194 (7.8)	135 (4.5)
2	Diethylphthalate	DEP	149 (100)	177 (23)	222 (1.6)
3	Dipropylphthalate	DPP	149 (100)	209 (5.9)	191 (6.9)
4	Di (2-methyl-propyl)phthalate	DiBP	149 (100)	223 (7.4)	205 (1.9)
5	Dibutylphthalate	DBP	149 (100)	223 (5.6)	278 (1.0)
6	Butylbenzylphthalate	BBzP	149 (100)	206 (22)	312 (1.0)
7	Dicyclohexylphthalate	DCHP	149 (100)	167 (32)	249 (5.5)
8	Di(2-ethylhexyl)phthalate	DEHP	149 (100)	167 (34)	279 (8.8)
9	Dioctylphthalate	DOP	149 (100)	279 (6.6)	207 (4.4)
10	Didecylphthalate	DDcP	149 (100)	307 (6.4)	---
11	Diundecylphthalate	DUP	149 (100)	321 (5.4)	---
	Internal standards				
12	D4-ring- Dibutylphthalate	D4-DBP	153 (100)	227 (5,7)	
13	D4-ring-Di(2-ethylhexyl)phthalate	D4-DEHP	153 (100)	171 (31)	283 (14)
14	D4-ring-Dioctylphtalate	D4-DOP	153 (100)	283 (17)	

Other compounds
BPA: Bisphenol A, 2,2-Bis-(4-hydroxyphenyl)propane, CAS-Nr. 80-05-7
LAS: linear Alkylbenzolsulfon acids, isomer mixture
NP: Nonylphenol, isomer mixture
4-n-NP: 4-n-Nonylphenol
NP1EO: Nonylphenolmonoethoxylate
NP2EO: Nonylphenoldiethoxylate
MSTFA: N-Methyl-N-(trimethylsilyl)-2,2,2-trifluoracetamid

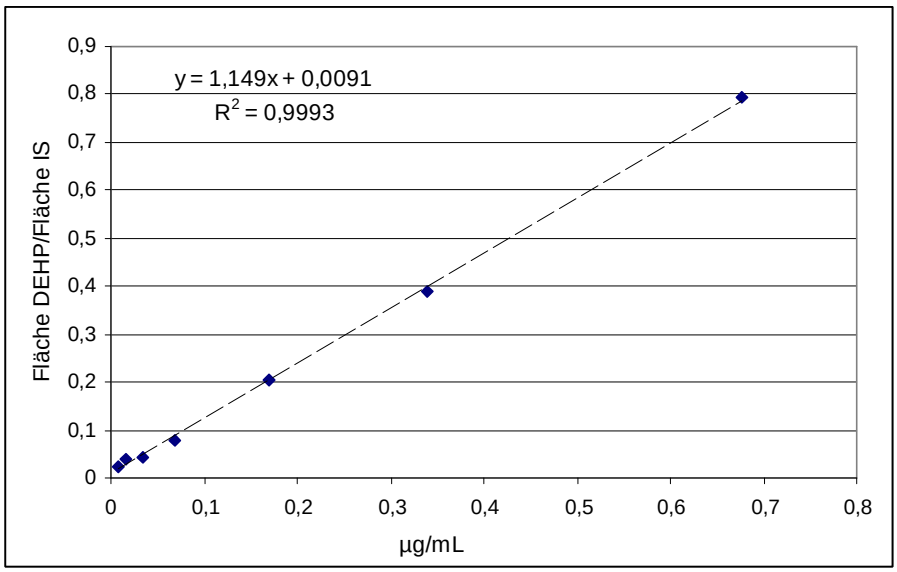
Further abbreviations: W: wood, se. sl.: sewage sludge, co: compost, se: sediment

A II Fig 1: Regression lines for DEHP für two concentration ranges

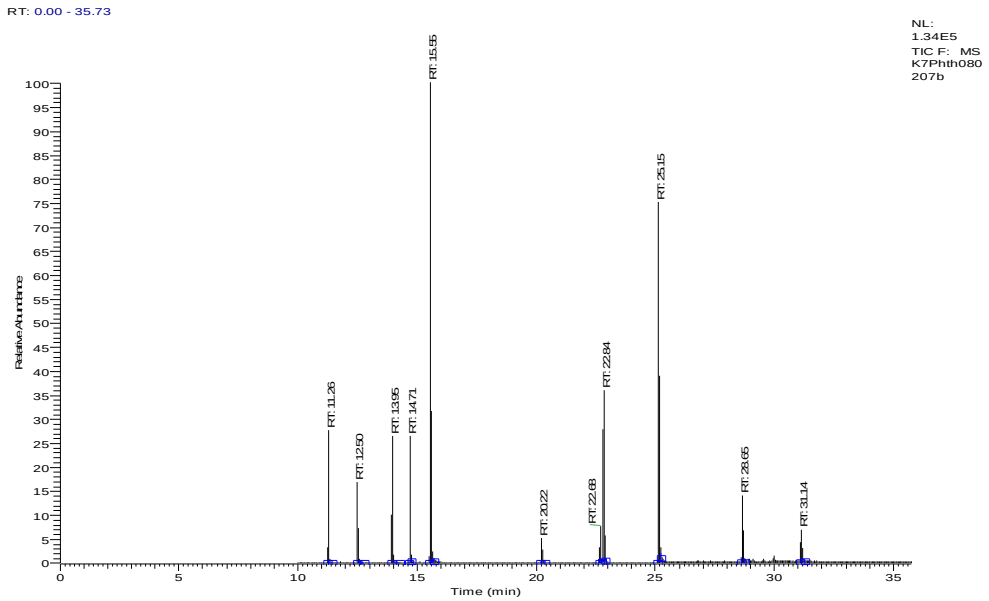
a) 0.008 µg/mL to 5.5 µg/mL



b) 0.008 µg/mL to 0.7 µg/mL

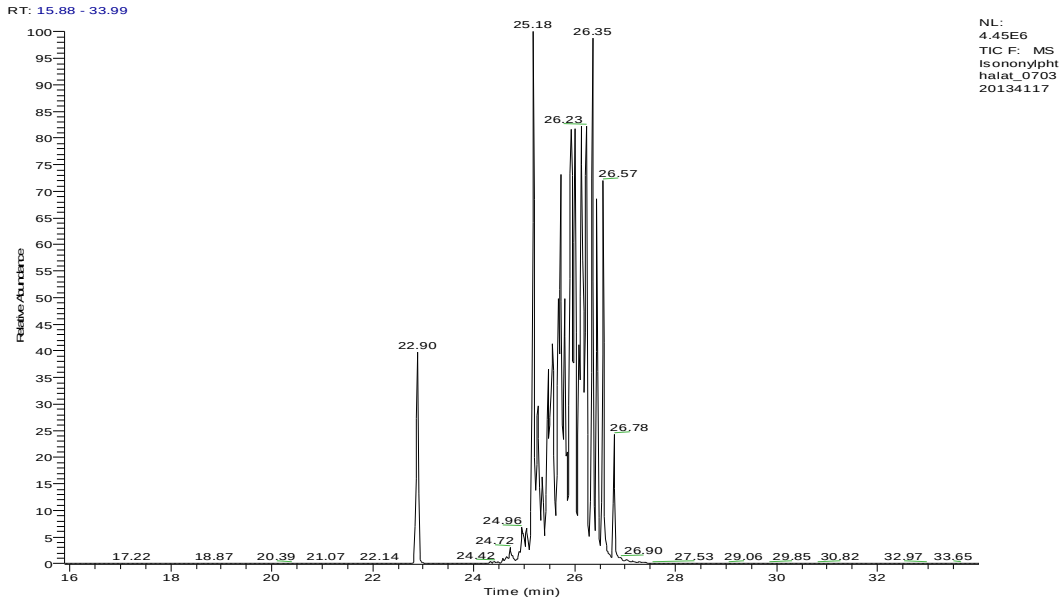


A II Fig. 2: Sample gas chromatogram of a phthalate standard mixture with ca. 0.35 µg/mL (for categorisation of substances to retention times see above)

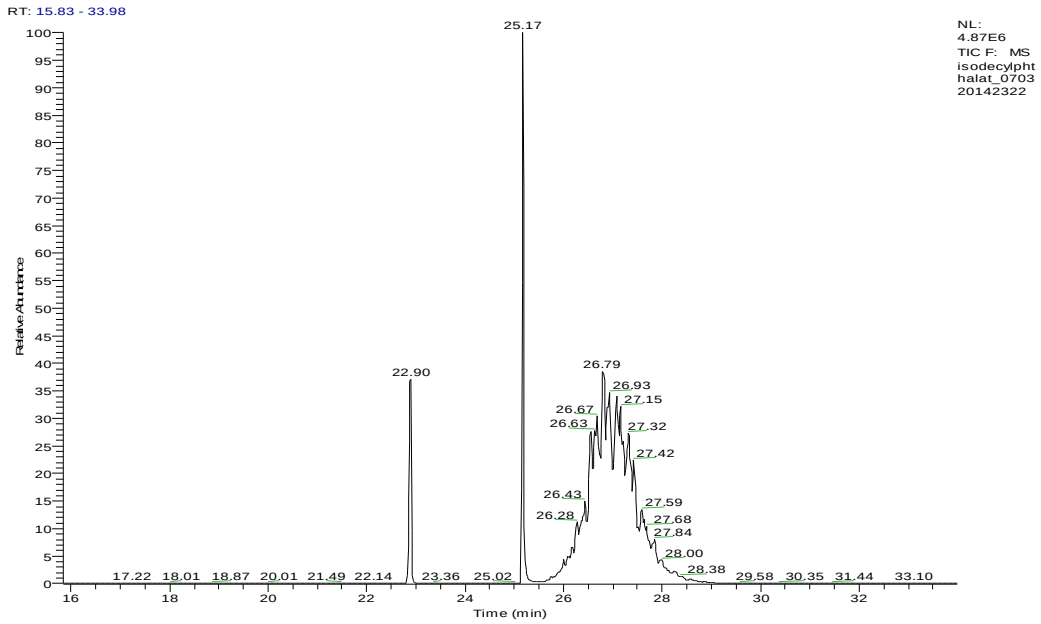


The corresponding isotope marked phthalates (1µg/mL) are also contained within the peaks at 15.55, 22.84, and 25.15 min, which accounts for the fact that they are larger than the others. DCHP/DEHP are separated to the base line.

A II Fig. 3a: Diisononylphthalate, DEHP (RT 22.90 min) and DOP (RT 22.18 min)



A II Fig. 3b: Diisodecylphthalate, DEHP (RT 22.90 min) and DOP (RT 22.17 min)


RT: 15.83 - 33.98
NL:
4.87E6
TIC F: MS
isodecylpht
halat_0703
20142322
Relative Abundance
Time (min)
25.17
22.90
26.79
26.93
27.15
26.67
26.63
27.32
27.42
26.43
26.28
27.59
27.68
27.84
28.00
28.38
17.22
18.01
18.87
20.01
21.49
22.14
23.36
25.02
29.58
30.35
31.44
33.10

ANNEX 3: GERMAN RING TEST

The laboratory of the “Horizontal” project in the German ring test was Laboratory 9
Anonymous raw data from the German phthalate ring test for test solution

A III Table 1: Z Scores for DEHP in sewage sludge, Sediment, compost and wood

Matrix	Laboratory	Z Score
sewage sludge	1	-3.176
	2	0.671
	3	0.802
	6	0.351
	7	-0.136
	8	0.333
	9	-0.452
	11	-0.550
Sediment	1	-3.207
	2	0.035
	3	-0.476
	6	0.302
	7	1.046
	8	0.553
	9	0.027
	11	-0.434
compost	2	-0.690
	3	0.739
	6	-0.181
	7	0.180
	8	0.397
	9	0.305
	11	-1.301
wood	1	-1.844
	2	1.082
	3	0.102
	6	-0.217
	7	0.384
	8	-0.347
	9	0.245
	11	-1.113

A III Tab. 2: Results of the national ring test

			sample 1: sediment		sample 2: sewage sludge			sample 3: compost		sample 4: wood		
	µg/kg TM	(mean values)										
		DMP	DEP	DPP	DiBP	DBP	BBP	DCHP	DEHP	DOP	DDP	DUP
lab 1	sample 1	<217,89	<404,42	<452,74	<477,69	276,45	<100,54	<160,96	853,75	<3,92	<196,52	<184,87
	sample 2	<231,35	<429,40	<480,71	<507,19	<293,52	<106,76	<170,91	10124	<4,165	<208,66	<196,30
	sample 3	<221,01	<410,20	<459,22	<484,52	<280,40	<101,98	<163,27	<163,27	<3,978	<199,33	<187,52
	sample 4	<237,80	<441,38	<494,12	<521,34	9655	4670	<175,67	12642	<4,281	<214,476	<201,771
lab 2	sample 1	<140	<140	<140	<140	<150	<250	<140	4080	<250	<690	<690
	sample 2	<150	<150	<150	<150	<180	<300	203	49300	<295	<740	165,8
	sample 3	<82	<82	<82	120	<140	<160	<82	1550	<160	<405	<405
	sample 4	1670	<610	<610	<610	14000	1730	<620	76500	<1300	<3000	<3000
lab 3	sample 1	<200	<200	<200	<200	<200	<200	<200	4588	252	553	
	sample 2	<200	<200	366	<200	<200	672	245	50912	473	<200	
	sample 3	<200	<200	<200	<200	<200	490	71	2375	<200	<200	
	sample 4	613	<200	<200	<200	10476	1180	490	44615	519	1121	
lab 5	sample 1			n.a.	n.a.	400		n.a.	3000		3000	n.u.
	sample 2			n.a.	n.a.			n.a.	15000		10000	n.u.
	sample 3			n.a.	n.a.	500		n.a.	1800		400	n.u.
	sample 4			n.a.	n.a.	11000	700	n.a.	13000			n.u.
lab 6	sample 1	<40	305	<40	950	92,1	110	<40	4420	<40	<40	<40
	sample 2	<40	<40	<40	<40	<40	<40	<40	45200	<40	<40	<40
	sample 3	<20	139	<20	246	99,1	<20	<20	1800	<20	<20	<20
	sample 4	919	<40	<40	<40	6540	598	<40	37700	<40	<40	<40
lab 7	sample 1	<100	<100	<100	<500	<500	<100	<500	5390	<500	<1000	<500
	sample 2	<100	<100	<100	<500	<500	<100	<500	39500	<500	<500	<500
	sample 3	<100	<100	<100	<500	<500	<100	<500	2010	<500	<500	<500
	sample 4	1250	<100	<100	599	16000	6740	1590	53800	2430	1460	<1000

Cont. A III Table 2		sample 1: sediment			sample 2: sewage sludge			sample 3: compost		sample 4: wood		
	µg/kg TM	(mean values)										
		DMP	DEP	DPP	DiBP	DBP	BBP	DCHP	DEHP	DOP	DDP	DUP
lab 8	sample 1	<50	<50	<50	<50	<50	<100	<50	4800	<100	<250	<250
	sample 2	<200	<200	<200	<200	<200	<400	<200	45000	<400	<1000	<1000
	sample 3	<50	<50	<50	<50	60	<100	170	2100	<100	<250	<250
	sample 4	<100	<100	<100	<100	4100	3000	<100	36000	1100	<500	<500
lab 9	sample 1	<170	<280	<160	<300	<140	<330	<140	4070	<180	<550	<600
	sample 2	<240	<400	<220	<430	<190	<460	<190	36500	<250	<800	<850
	sample 3	<160	<140	<80	<140	100	<150	<70	2090	<80	<260	<290
	sample 4	<340	<570	<320	<600	7060	<650	<270	49300	<350	<1100	<1250
lab 11	sample 1	<100	<100	<100	170	160	<100	<100	3600	<100	140	<100
	sample 2	<100	<100	<100	140	210	<100	<100	36000	<100	<100	<100
	sample 3	<100	<100	<100	210	250	<100	<100	1300	<100	<100	<100
	sample 4	270	<100	<100	510	9500	<100	<100	23000	<100	<100	<100
lab 12	sample 1	<10	20	<10	80	60	40	10	3440	50		
	sample 2	20	60	<10	150	90	30	590	39800	430		
	sample 3	<10	10	<10	100	60	<10	50	1550	<10		
	sample 4	350	60	<10	150	6060	310	40	20400	<10		

A III Tab 3: Results of the national ring test for phthalates (all results in µg/kg dm)

			sample 1: sediment		
		sample 1a	sample 1b	sample 1c	sample 1d
lab 1	Phth 1	<218	<218	<218	<218
	Phth 2	<404	<404	<404	<404
	Phth 3	<453	<453	<453	<453
	Phth 4	<478	<478	<478	<478
	Phth 5	276	276	276	276
	Phth 6	<101	<101	<101	<101
	Phth 7	<161	<161	<161	<161
	Phth 8	891	639	750	1135
	Phth 9	<3,9	<3,10	<3,11	<3,12
	Phth 10	<197	<197	<197	<197
	Phth 11	<185	<185	<185	<185
lab 2	Phth 1	<170	<140	<130	<110
	Phth 2	<170	<140	<130	<110
	Phth 3	<170	<140	<130	<110
	Phth 4	<170	<140	<130	<110
	Phth 5	210	160	<130	<110
	Phth 6	<340	<290	<250	<220
	Phth 7	<170	<140	<130	<110
	Phth 8	4000	4200	4000	4100
	Phth 9	<340	<290	<250	<220
	Phth 10	<840	<720	<630	<560
	Phth 11	<840	<720	<630	<560
lab 3	Phth 1	<200	<200	<200	<200
	Phth 2	<200	<200	<200	<200
	Phth 3	<200	<200	<200	<200
	Phth 4	<200	<200	<200	<200
	Phth 5	<200	<200	<200	<200
	Phth 6	<200	<200	<200	<200
	Phth 7	<200	<200	<200	<200
	Phth 8	4847	4002	4580	4924
	Phth 9	248	237	245	280
	Phth 10	550	562	549	553
	Phth 11	n.a.	n.a.	n.a.	n.a.
lab 5	Phth 1				
	Phth 2				
	Phth 3	n.a.			
	Phth 4	n.a.			
	Phth 5	400			
	Phth 6				
Cont. A III Table 3					

			sample 1: sediment		
		sample 1a	sample 1b	sample 1c	sample 1d
Lab 5	Phth 7	n.a.			
	Phth 8	3000			
	Phth 9				
	Phth 10	3000			
	Phth 11	n.a.			
lab 6	Phth 1	<40	<40	<40	<40
	Phth 2	257	296	268	399
	Phth 3	<40	<40	<40	<40
	Phth 4	916	1140	1020	720
	Phth 5	89,2	81,8	98,7	98,6
	Phth 6	67,5	128	120	124
	Phth 7	<40	<40	<40	<40
	Phth 8	3890	4440	4780	4580
	Phth 9	<40	<40	<40	<40
	Phth 10	<40	<40	<40	<40
	Phth 11	<40	<40	<40	<40
lab 7	Phth 1	<100	<100	<100	<100
	Phth 2	<100	<100	<100	<100
	Phth 3	<100	<100	<100	<100
	Phth 4	<100	<100	116	<100
	Phth 5	358	334	411	699
	Phth 6	<100	<100	<100	<100
	Phth 7	<500	<500	<500	<500
	Phth 8	5280	5000	5840	5450
	Phth 9	269	328	336	504
	Phth 10	520	547	618	928
	Phth 11	<500	<500	<500	<500
lab 8	Phth 1	<50	<50	<50	<50
	Phth 2	<50	<50	<50	<50
	Phth 3	<50	<50	<50	<50
	Phth 4	<50	<50	<50	<50
	Phth 5	<50	<50	<50	<50
	Phth 6	<100	<100	<100	<100
	Phth 7	<50	<50	<50	<50
	Phth 8	4700	4700	4800	4800
	Phth 9	<100	<100	<100	<100
	Phth 10	<250	<250	<250	<250
	Phth 11	<250	<250	<250	<250

Cont A III Table 3					
			sample 1: sediment		
		sample 1a	sample 1b	sample 1c	sample 1d
lab 9	Phth 1	<170	<170	<170	<170
	Phth 2	<280	<280	<280	<280
	Phth 3	<160	<160	<160	<160
	Phth 4	<300	<300	<300	<300
	Phth 5	<140	<140	<140	<140
	Phth 6	<330	<330	<330	<330
	Phth 7	<140	<140	<140	<140
	Phth 8	4150	4100	3820	4190
	Phth 9	<180	<180	<180	<180
	Phth 10	<550	<550	<550	<550
	Phth 11	<600	<600	<600	<600
lab 11	Phth 1	<100	<100	<100	
	Phth 2	<100	<100	<100	
	Phth 3	<100	<100	<100	
	Phth 4	130	250	140	
	Phth 5	110	250	130	
	Phth 6	<100	<100	<100	
	Phth 7	<100	<100	<100	
	Phth 8	3500	3800	3500	
	Phth 9	<100	<100	<100	
	Phth 10	120	150	150	
	Phth 11	<100	<100	<100	
				original results in mg/kg	
lab 12	Phth 1	<10			
	Phth 2	20			
	Phth 3	<10			
	Phth 4	80			
	Phth 5	60			
	Phth 6	40			
	Phth 7	10			
	Phth 8	3440			
	Phth 9	50			
	Phth 10				
	Phth 11				

Cont. A III Table 3

			sample 2: sewage sludge		
		sample 2a	sample 2b	sample 2c	sample 2d
lab 1	Phth 1	<231	<231	<231	<231
	Phth 2	<429	<429	<429	<429
	Phth 3	<481	<481	<481	<481
	Phth 4	<507	<507	<507	<507
	Phth 5	<294	<294	<294	<294
	Phth 6	<107	<107	<107	<107
	Phth 7	<171	<171	<171	<171
	Phth 8	11039	10090	7989	11378
	Phth 9	<4,2	<4,3	<4,4	<4,5
	Phth 10	<209	<209	<209	<209
	Phth 11	<196	<196	<196	<196
lab 2	Phth 1	<180	<150	<140	<120
	Phth 2	<180	<150	<140	<120
	Phth 3	<180	<150	<140	<120
	Phth 4	<180	<150	<140	<120
	Phth 5	<180	<150	170	210
	Phth 6	<350	<310	<270	<250
	Phth 7	200	210	200	200
	Phth 8	50000	49000	49000	49000
	Phth 9	<350	<310	<270	<250
	Phth 10	<880	<770	<680	<620
	Phth 11	4400	4200	4000	4000
lab 3	Phth 1	<200	<200	<200	<200
	Phth 2	<200	<200	<200	<200
	Phth 3	366	366	373	360
	Phth 4	<200	<200	<200	<200
	Phth 5	<200	<200	<200	<200
	Phth 6	651	616	735	687
	Phth 7	225	257	202	296
	Phth 8	56472	47651	48854	50650
	Phth 9	459	425	481	528
	Phth 10	<200	<200	<200	<200
	Phth 11	n.a.	n.a.	n.a.	n.a.
lab 5	Phth 1				
	Phth 2				
	Phth 3	n.a.			
	Phth 4	n.a.			
	Phth 5				
	Phth 6				
	Phth 7	n.a.			
	Phth 8	15000			

Cont. A III Table 3			sample 2: sewage sludge		
		sample 2a	sample 2b	sample 2c	sample 2d
	Phth 9				
	Phth 10	10000			
	Phth 11	n.a.			
lab 6	Phth 1	<40	<160	<40	<160
	Phth 2	<40	<160	<40	<160
	Phth 3	<40	<160	<40	<160
	Phth 4	<40	<160	<40	<160
	Phth 5	<40	<160	<40	<160
	Phth 6	<40	<160	<40	<160
	Phth 7	<40	<160	<40	<160
	Phth 8	41700	44500	43900	50800
	Phth 9	<40	<160	<40	<160
	Phth 10	<40	<160	<40	<160
	Phth 11	<40	<160	<40	<160
lab 7	Phth 1	<100	<100	<100	<100
	Phth 2	<100	<100	<100	<100
	Phth 3	<100	<100	<100	<100
	Phth 4	<100	<100	<100	<100
	Phth 5	416	406	572	537
	Phth 6	<100	<100	<100	<100
	Phth 7	<500	<500	<500	<500
	Phth 8	40200	39400	40600	37800
	Phth 9	<500	<500	<500	<500
	Phth 10	<500	<500	<500	<500
	Phth 11	<500	<500	<500	<500

Cont. A III Table 3

			sample 2: sewage sludge		
		sample 2a	sample 2b	sample 2c	sample 2d
lab 8	Phth 1	<200	<200	<200	<200
	Phth 2	<200	<200	<200	<200
	Phth 3	<200	<200	<200	<200
	Phth 4	<200	<200	<200	<200
	Phth 5	<200	<200	<200	<200
	Phth 6	<400	<400	<400	<400
	Phth 7	<200	<200	<200	<200
	Phth 8	45000	45000	45000	45000
	Phth 9	<400	<400	<400	<400
	Phth 10	<1000	<1000	<1000	<1000
	Phth 11	<1000	<1000	<1000	<1000
lab 9	Phth 1	<240	<240	<240	<240
	Phth 2	<400	<400	<400	<400
	Phth 3	<220	<220	<220	<220
	Phth 4	<430	<430	<430	<430
	Phth 5	<190	<190	<190	<190
	Phth 6	<460	<460	<460	<460
	Phth 7	<190	<190	<190	<190
	Phth 8	36900	37200	35400	36300
	Phth 9	<250	<250	<250	<250
	Phth 10	<800	<800	<800	<800
	Phth 11	<800	<800	<800	<800
lab 11	Phth 1	<100	<100		
	Phth 2	<100	<100		
	Phth 3	<100	<100		
	Phth 4	130	140		
	Phth 5	220	190		
	Phth 6	<100	<100		
	Phth 7	<100	<100		
	Phth 8	32000	39000		
	Phth 9	<100	<100		
	Phth 10	<100	<100		
	Phth 11				
lab 12	Phth 1	20			
	Phth 2	60			
	Phth 3	<10			
	Phth 4	150			
	Phth 5	90			
	Phth 6	30			
	Phth 7	590			
	Phth 8	39800			
	Phth 9	430			
	Phth 10				
	Phth 11				

Cont. A III Table 3

			sample 3: compost		
		sample 3a	sample 3b	sample 3c	sample 3d
lab 1	Phth 1	<221	<221	<221	<221
	Phth 2	<410	<410	<410	<410
	Phth 3	<459	<459	<459	<459
	Phth 4	<485	<485	<485	<485
	Phth 5	<280	<280	<280	<280
	Phth 6	<102	<102	<102	<102
	Phth 7	<163	<163	<163	<163
	Phth 8	<163	<163	<163	<163
	Phth 9	<4,0	<4,1	<4,2	<4,3
	Phth 10	<199	<199	<199	<199
	Phth 11	<188	<188	<188	<188
lab 2	Phth 1	<103	<85	<73	<64
	Phth 2	<103	<85	<73	65
	Phth 3	<103	<85	<73	<64
	Phth 4	110	140	120	110
	Phth 5	<103	220	120	120
	Phth 6	<205	<170	<150	<130
	Phth 7	<103	<85	<73	<64
	Phth 8	1400	1800	1600	1400
	Phth 9	<205	<170	<146	<130
	Phth 10	<510	<420	<370	<320
	Phth 11	<510	<420	<370	<320
lab 3	Phth 1	<200	<200	<200	<200
	Phth 2	<200	<200	<200	<200
	Phth 3	<200	<200	<200	<200
	Phth 4	<200	<200	<200	<200
	Phth 5	<200	<200	<200	<200
	Phth 6	575	498	461	431
	Phth 7	664	82	68	71
	Phth 8	1754	2502	3181	2065
	Phth 9	<200	<200	<200	<200
	Phth 10	<200	<200	<200	<200
	Phth 11	n.a.	n.a.	n.a.	n.a.
lab 5	Phth 1				
	Phth 2				
	Phth 3	n.a.			
	Phth 4	n.a.			
	Phth 5	500			
	Phth 6				
	Phth 7	n.a.			
	Phth 8	1800			
	Phth 10	400			
	Phth 11	n.a.			

Cont. A III Table 3			sample 3: compost	

		sample 3a	sample 3b	sample 3c	sample 3d
lab 6	Phth 1	<40	<40	<20	<20
	Phth 2	97,5	149	170	70,2
	Phth 3	<40	<40	<20	<20
	Phth 4	215	410	474	328
	Phth 5	92,4	118	102	84,8
	Phth 6	<40	<40	<20	<20
	Phth 7	<40	<40	<20	<20
	Phth 8	1670	2190	1640	1700
	Phth 9	<40	<40	<20	<20
	Phth 10	<40	<40	<20	<20
	Phth 11	<40	<40	<20	<20
lab 7	Phth 1	<100	<100	<100	<100
	Phth 2	<100	<100	<100	<100
	Phth 3	<100	<100	<100	<100
	Phth 4	<100	<100	<100	<100
	Phth 5	284	347	295	217
	Phth 6	<100	<100	<100	<100
	Phth 7	116	<100	104	<100
	Phth 8	2070	1940	2180	1840
	Phth 9	<500	<500	<500	<500
	Phth 10	<500	<500	<500	<500
	Phth 11	<500	<500	<500	<500
lab 8	Phth 1	<50	<50	<50	<50
	Phth 2	<50	<50	<50	<50
	Phth 3	<50	<50	<50	<50
	Phth 4	<50	<50	<50	<50
	Phth 5	60	56	70	55
	Phth 6	<100	<100	<100	<100
	Phth 7	170	170	170	180
	Phth 8	2200	2100	2200	2100
	Phth 9	<100	<100	<100	<100
	Phth 10	<250	<250	<250	<250
	Phth 11	<250	<250	<250	<250
lab 9	Phth 1	<160	<160	<160	<160
	Phth 2	<140	<140	<140	<140
	Phth 3	<80	<80	<80	<80
	Phth 4	<140	<140	<140	<140
	Phth 5	102	130	85	83,5
	Phth 6	<150	<150	<150	<150
	Phth 7	<70	<70	<70	<70
	Phth 8	1710	3090	2040	1520
	Phth 9	<80	<80	<80	<80
	Phth 10	<260	<260	<260	<260
	Phth 11	<290	<290	<290	<290

Cont. A III Table 3		sample 3: compost	

		sample 3a	sample 3b	sample 3c	sample 3d
lab 11	Phth 1	<100	<100		
	Phth 2	<100	<100		
	Phth 3	<100	<100		
	Phth 4	190	220		
	Phth 5	500	310		
	Phth 6	<100	<100		
	Phth 7	<100	<100		
	Phth 8	1300	1200		
	Phth 9	<100	<100		
	Phth 10	<100	<100		
	Phth 11	<100	<100		
lab 12	Phth 1	<10			
	Phth 2	10			
	Phth 3	<10			
	Phth 4	100			
	Phth 5	60			
	Phth 6	<10			
	Phth 7	50			
	Phth 8	1550			
	Phth 9	<10			
	Phth 10				
	Phth 11				
			sample 4: wood		
		sample 4a	sample 4b	sample 4c	sample 4d
lab 1	Phth 1	<238	<238	<238	<238
	Phth 2	<441	<441	<441	<441
	Phth 3	<494	<494	<494	<494
	Phth 4	<521	<521	<521	<521
	Phth 5	10865	11052	6372	10333
	Phth 6	4006	4614	5389	<110
	Phth 7	<176	<177	<178	<179
	Phth 8	12308	17386	8233	3752
	Phth 9	<4,3	<4,4	<4,5	<4,6
	Phth 10	<214	<214	<214	<214
	Phth 11	<202	<202	<202	<202
lab 2	Phth 1	2300	n.b.	1000	1700
	Phth 2	<800	<670	<570	<400
	Phth 3	<800	<670	<570	<400
	Phth 4	<800	<370	<570	<400
	Phth 5	16000	12000	14000	14000
	Phth 6	1900	1900	1400	n.b.
	Phth 7	n.b.	<670	<570	n.b.
	Phth 8	54000	79000	84000	89000
	Phth 9	<1600	<1300	<1100	<1300
	Phth 10	<4000	<3300	<2800	<2000
	Phth 11	<4000	<3300	<2800	<2000
Cont. A III Table 3			sample 4: wood		

		sample 4a	sample 4b	sample 4c	sample 4d
lab 3	Phth 1	466	751	673	553
	Phth 2	<200	<200	<200	<200
	Phth 3	<200	<200	<200	<200
	Phth 4	<200	<200	<200	<200
	Phth 5	11836	9976	9228	10864
	Phth 6	1279	937	1231	1273
	Phth 7	462	828	292	376
	Phth 8	53236	41536	39807	43881
	Phth 9	557	411	406	703
	Phth 10	1291	1081	1057	1056
	Phth 11	n.a.	n.a.	n.a.	n.a.
lab 5	Phth 1				
	Phth 2				
	Phth 3	n.a.			
	Phth 4	n.a.			
	Phth 5	11000			
	Phth 6	700			
	Phth 7	n.a.			
	Phth 8	13000			
	Phth 9				
	Phth 10				
	Phth 11	n.a.			
lab 6	Phth 1	977	7668	826	1104
	Phth 2	<40	<40	<400	<160
	Phth 3	<40	<40	<400	<160
	Phth 4	<40	<40	<400	<160
	Phth 5	8000	5760	6530	5860
	Phth 6	396	782	541	673
	Phth 7	<40	<40	<400	<160
	Phth 8	41500	38900	37600	32700
	Phth 9	<40	<40	<400	<160
	Phth 10	<40	<40	<400	<160
	Phth 11	<40	<40	<400	<160
lab 7	Phth 1	1040	1840	603	1500
	Phth 2	<100	<100	<100	<100
	Phth 3	<100	<100	<100	<100
	Phth 4	256	525	438	1180
	Phth 5	18200	8780	16000	20900
	Phth 6	2340	8690	13000	2980
	Phth 7	1280	2070	2480	513
	Phth 8	46300	53200	63500	52200
	Phth 9	2380	2390	4040	915
	Phth 10	1190	1120	2510	1040
	Phth 11	<1000	<1000	<1000	<1000
Cont. A III Table 3			sample 4: wood		

		sample 4a	sample 4b	sample 4c	sample 4d
lab 8	Phth 1	<100	<100	<100	<100
	Phth 2	<100	<100	<100	<100
	Phth 3	<100	<100	<100	<100
	Phth 4	<100	<100	<100	<100
	Phth 5	4200	4000	4300	4000
	Phth 6	3000	2900	3000	3000
	Phth 7	<100	<100	<100	<100
	Phth 8	39000	34000	35000	34000
	Phth 9	1100	1000	1100	1100
	Phth 10	<500	<500	<500	<500
	Phth 11	<500	<500	<500	<500
lab 9	Phth 1	<340	<340	<340	<340
	Phth 2	<570	<570	<570	<570
	Phth 3	<320	<320	<320	<320
	Phth 4	<600	<600	<600	<600
	Phth 5	5450	9670	4360	8740
	Phth 6	<650	<650	<650	<650
	Phth 7	<270	<270	<270	<270
	Phth 8	56400	24500	46300	69900
	Phth 9	<350	<350	<350	<350
	Phth 10	<1100	<1100	<1100	<1100
	Phth 11	<1250	<1250	<1250	<1250
lab 11	Phth 1	300	300	210	
	Phth 2	<100	<100	<100	
	Phth 3	<100	<100	<100	
	Phth 4	560	470	510	
	Phth 5	9900	9800	8900	
	Phth 6	<100	<100	<100	
	Phth 7	<100	<100	<100	
	Phth 8	3100	2400	1300	
	Phth 9	<100	<100	<100	
	Phth 10	<100	<100	<100	
	Phth 11	0,22	<100	<100	
lab 12	Phth 1	350			
	Phth 2	60			
	Phth 3	<10			
	Phth 4	150			
	Phth 5	6060			
	Phth 6	310			
	Phth 7	40			
	Phth 8	20400			
	Phth 9	<10			
	Phth 10				
	Phth 11				

A III: Tab. 4: Dry matter content of the samples.

lab	sample 1	sample 2	sample 3	sample 4
	% d_m	% d_m	% dm	% dm
1	97,78	92,13	96,42	89,6
2	98,8	80,8	97,6	
3	96,8	95,3	98,6	
5	n.d.	n.d.	n.d.	
6	98,68	93,79	98,46	
7	97,2	92,9	95,5	72,3
8	n.d.	n.d.	n.d.	
9	98,37	93,50	97,34	
11	n.d.	n.d.	n.d.	

n.d.: not determined

The dry matter content of wood was not to be determined because of easy decomposition of the material.

A III Tab. 5: Mean of dry matter content

	sample 1	sample 2	sample 3
mean	97,938	93,524	97,320
stdd.dev.	0,818	1,179	1,195
% stdd.dev.	0,84	1,26	1,23

For Information : Sample 2/lab 2: rejected as outlyer

A III: Tab. 6: Mean values of the single labs for the test solution [µg/mL]

	theor. conc.	lab 1	lab 2	lab 3	lab 5	lab 6	lab 7	lab 8	lab 9
DMP	475	561	860	693	n.d.	459	728	473	457
DEP	400	646	700	536	250	419	644	422	361
DPP	449	389	798	713	n.d.	395	696	450	422
DiBP	432	899	736	332	n.d.	417	666	454	393
DBP	381	1108	700	619	1000	439	688	434	242
BBP	461	566	760	829	420	461	790	455	426
DCHP	381		650	713	n.d.	366	770	384	351
DEHP	434	469	795	788	550	402	811	450	330
DOP	514	430	480	944	n.d.	375	712	388	350
DDcP	395	594	530	1206	2320	353	781	442	387
DUP	438	595	510		n.d.	385	771	327	400

n.d.: not determined
not all labs had submitted results for the test solution