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Effect of the nature and concentration of phthalates on their migration from PVC materials under dynamic simulated conditions of mouthing

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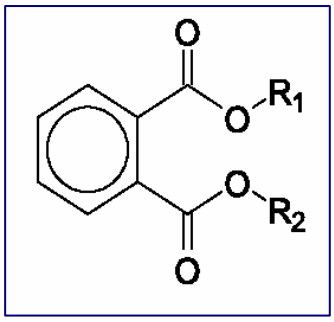
D. Sarigiannis, ed.



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EXECUTIVE SUMMARY

Polyvinylchloride (PVC) has been a very common material for the production of toys. It generally is manufactured with an addition of additives such as plasticisers to improve its functionality and facilitate its production process. PVC that can contain up to 50% by weight of plasticisers (Simoneau et al., 2001). Among plasticisers, 90% of the most commonly used to soften polymers for toys production are constituted by phthalates, such as diisononyl phthalate (DINP).

The purpose of this study was to investigate the influence of the relative concentration of percentage of phthalates and nature of phthalates on their release in standard conditions. To obtain a suitable but rapid method of analysis, experiments were performed to study the effects on the modification of a standard operation procedure (SOP) previously validated at the EU level by JRC Ispra. A single extraction with a sufficient large volume of cyclohexane thus could be used instead of two extractions with a smaller volume of cyclohexane and resulted in more rapid yet reliable method. The final method is described in Annex 1 of this report.

Samples of soft PVC were industrially produced especially for this study.
Standard PVC disks with various percentages of di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP), di-ethyl-hexyll phthalate (DEHP), benzylbutyl phthalate (BBP) dibutylphthalate (DBP) or a binary mixture DINP/DBP in various proportions were prepared. 30 different types of disk were produced and tested. The disks were analysed for contents, homogeneity and sets were subjected to migration experiments of the various phthalates under dynamic conditions using the previously validated SOP with some modifications.

The release from samples with a systematic manufacturing process and containing different phthalates at different concentrations showed correlations to their concentrations. Since previous studies using commercial toys had no showed such specific trends, these results suggest that the production process of toys may be an important issue with respect to release properties. The release of DEHP BBP and DBP tended to show a more linear correlation to the concentration, whereas for DINP, DIDP, DEHP and release the plasticiser showed non linear tendencies and saturation of release for high formulation contents.

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GLOSSARY

DINP: di-isononyl phthalate

DIDP: di-isodecyl phthalate

DEHP: di-ethyl-hexyll phthalate

BBP: benzylbutyl phthalate

HPLC: high performance liquid chromatography

GC-MS: gas chromatography mass spectrometry

SIM: Single ion monitoring

SOP: Standard operating procedure

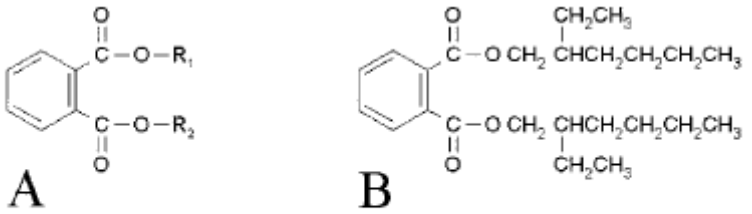


FIG. 1. (A) General structure of a phthalate ester. The diesters of the phthalic acid are often symmetric ($R_1 = R_2$) like, e.g., DEHP; (B) structure of di(2-ethylhexyl) phthalate, commonly known as DEHP.

SCOPE

Systematic approaches to examine the migration behaviour of the above mentioned phthalates and their mixtures is not known. In order to obtain data on the release behaviour of relevant phthalates, the JRC initiated a study to 1) establish the suitability of existing in vitro methods for the determination of the release of phthalates 2) investigate the effect of the nature of the phthalate on their release in saliva simulant under foreseen human conditions, and 3) investigate the effect of the percentage of different phthalates in PVC materials on their release in saliva simulant.

Thus the study was aimed to provide insight into the relation of phthalate concentration and nature versus release.

The results are now reported in the context of the Chem-Test project on behalf of DG SANCO.

MATERIALS AND METHODS

Samples

Based on the requirements of the project, test samples were requested for production by the industry. Sheets made to specific technical specifications were produced by industry. The European Council for Plasticisers and Intermediates (ECPI) and Toys Industries of Europe (TIE) as well as their members provided chemical standards, and PVC materials necessary to the manufacture of the test samples. The samples were manufactured at Solvay.

PVC samples were manufactured into especially prepared disks with either individual phthalates or binary mixtures of phthalates; The samples were produced as strips to be punched as disks for sampling purposes. The strips produced also represented increasing concentrations of phthalates or mixtures of phthalates. The study included DINP DIDP. DEHP, BBP, and DBP. The investigation was applied to various concentrations of the phthalates. The results were also compared again to a standard reference strip reproducing the one used in the validation test at EU level conducted successfully in 2001.

The disks were made according to the formulation and manufacture used in the in-vivo study led by the Dutch Consensus Group (CCG) in 1998. PVC standard material was processed in sheets or strips, which could then be punched into disks with a specifically designed press prior to experiments.

The industrial descriptions of the samples are summarised in table 1 below (the full descriptions are reported in Annex); as the disks were produced sequentially, the samples codes represent respectively the disks produced in a first phase (phase 1, numbers 1 to 13) and second phase (phase 2, numbers 2.1 to 2.8).

For all disks the common characteristics were the production process, as well as the remaining additives and components, where the only variating component was the nature and or the percentage of the phthalates (expressed in %). All disks had a diameter of 2.3 cm (punched), thickness of 3mm, and weight ca 12g. The base composition was 100 Phr of PVC, 3 Phr of Epoxidised Soybean Oil (ESBO), and 3 Phr of Ca-Zn. Strips produced with a binary mixture of DINP and DBP were made to have a ration of 4 for the proportions of DINP:DBP (based on the Phr).

Sample code	phthalate	intended Conc. (%)
Strip 1	DINP	15.20
Strip 2	DINP	24.80
Strip REF	DINP	38.00
Strip 3	DINP	45.10
Strip 2.1	DIDP	24.80
Strip 4	DIDP	38.00
Strip 2.5	DIDP	45.10
Strip 2.2	DEHP	24.80
Strip 5	DEHP	38.00
Strip 2.6	DEHP	45.10
Strip 2.3	BBP	24.80
Strip 6	BBP	38.00
Strip 2.7	BBP	45.10
Strip 2.4	DBP	24.80
Strip 11	DBP	34.50
Strip 7	DBP	38.00
Strip 12	**DINP**/DBP	12.16 / 3.04
Strip 2.9	**DINP**/DBP	19.84 / 4.96
Strip 13	**DINP**/DBP	30.40 / 7.60
Strip 2.10	**DINP**/DBP	36.08 / 9.02

Table 1: compositions of the different formulations produced

The PVC strips and disks are shown on figure 1.

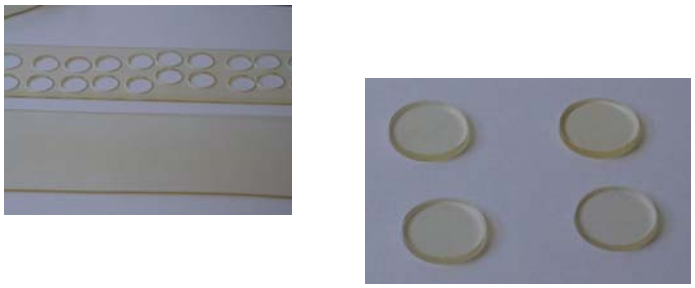


Figure 1: PVC standard material before and after punching

Homogeneity testing study

JRC performed a homogeneity study on the standard material (disks) to estimate the variations of the reference materials themselves. Samples used in release experiments were in this case extracted and analysed for the quantity of the phthalate plasticizer present.

Sampling

Homogeneity was performed by JRC on 5% of total population, i.e. 10 disks.

This number was therefore far superior to the actual number used in the "release" study. The population was divided into strips (from the manufacturing process). A stratified sampling was applied, which could test both the homogeneity within strips but also homogeneity between strips. Therefore a percentage of each strip

subpopulation, proportional to the size of disks punched from the strip, was taken at random (computer generated) for testing.

Furthermore each disk was cut in half coded part A and B respectively. This design also allowed the testing of homogeneity within disks. Homogeneity was performed independently on both.

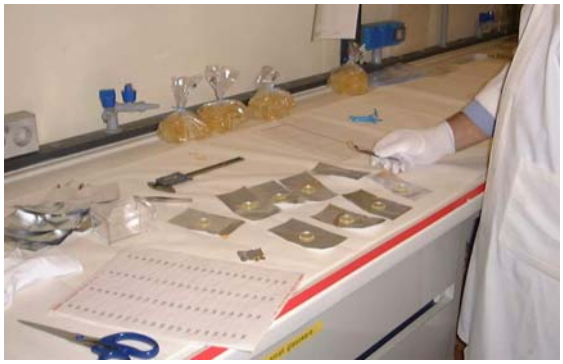


Figure 2: sampling of disks for homogeneity testing

Analysis – extraction-identification -quantification

The homogeneity testing was conducted by the dissolution-precipitation method previously used.

A disk (diameter 23 mm) was weighed and cut in two pieces. The weight of one piece was determined. From this piece, 100 mg ± 5 mg of the sample was weighed accurately and dissolved in 10 ml of tetrahydrofuran (THF), overnight at 20 ± 2 C. The polymer was then precipitated with 20 ml hexane. The solution was filtered through a 0.45µm disposable filter (e.g. nylon, polypropylene), and the portion of the clear THF/hexane solution was transferred into a capped vial, diluted to 1/1000 and injected in GC-MS.

A total ion current spectrum was then recorded, and the obtained GC-MS spectrum was compared to known spectra or phthalate ester standards to allow qualitative identification of phthalate ester plasticisers.

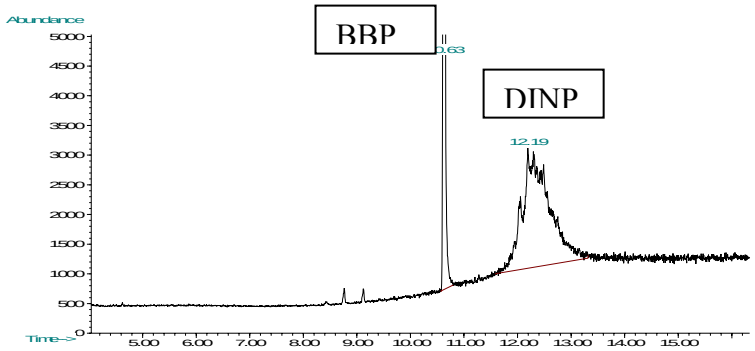


Figure 3: typical chromatogram (internal standard BBP) compared to the multipeak isomeric phthalate DINP.

The quantification of the plasticisers was done on external calibration curves of ratios of specific ions of reference phthalates vs. an internal standard.

For confirmation of the quantification of the plasticiser a 1 ml aliquot of THF/hexane solution was diluted in isooctane and analysed by HPLC.

For both, butylbenzylphthalate (BBP) was used as internal standard.

For calibration, quantification was obtained using standard solutions of the relevant phthalates of known concentration. From the chromatograms obtained and the calibration curves the amount of phthalate in PVC was calculated. In addition mean values and standard deviations were calculated.

Each sample was analysed at least in 5 replicates.

Determination of weight and thickness

In order to allow any interpretation or correction of the release results it was also considered that thickness and weight of the test specimens could influence its release. Therefore the weight of each disk, diameter 23 mm, used for quantification of the phthalates was determined. In addition the thickness was determined using a micrometer. As thickness may vary at different places of a disk, the thickness was determined in the middle of the disk and at three places at 3 mm from the outside of the disk. Average values and standard deviations were calculated.

Migration methodology and Standard operating procedure (SOP) for extraction identification and quantification of the released phthalates

The core of the study was the investigation of the release of phthalates. The disks were subjected to migration testing followed by extraction and analysis. The Standard Operating Procedure (SOP) used in the current study was based on the validated SOPs previously published (EUR 19899 EN, 2001; EUR 19826 EN, 2001).

The tests were conducted on 6 disks, 2 fortified samples for recoveries (spikes) and 2 blanks for each material tested. The disks were punched with the dedicated punching press previously described (EUR 19826 EN, 2001).

The head over heels method previously validated at the European level under co-ordination of JRC was used to provoke the release of phthalates. The released phthalates were subsequently extracted from the saliva simulant, identified and quantified by both HPLC and GC/MS. These methods were both validated at JRC and appeared to be the most reliable methods.

Migration experiments

The method used was the method validated by JRC and that became the source to an EN standard. The validation was is based on dynamic migration of a phthalate containing PVC sample into artificial saliva via mechanical agitation using a head over heels device (developed by the Nutrition Research Institute, TNO, The Netherlands)

Figure 3: head over heels device

The sample was placed in a glass bottle containing 50 ml of saliva simulant. Th composition of the saliva simulant was the one that had been developed from comparative investigations towards the validation study as well as a final consensus for the participants of the EU validation (Simoneau et al, 2001).

Compound	Formula	mmol/l	mg/l
Magnesium chloride	$MgCl_2$	0.82	166.7
Calcium chloride	$CaCl_2$	1.0	147.0
Dipotassium hydrogen phosphate	K_2HPO_4	3.3	753.1
Potassium carbonate	K_2CO_3	3.8	525.2
Sodium chloride	NaCl	5.6	327.3
Potassium chloride	KCl	10.0	745.5

Table 2: composition and amounts to be weighted for 1 l of solution of saliva simulant

The table shows the amounts taking into account the water of crystallisation. The potassium and sodium salts are dissolved first in ca. 900 ml distilled water, then the calcium and magnesium salts are added.

The pH is then adjusted to 6.8 with diluted hydrochloric acid. The solutions is then transferred to a 1 litre volumetric flask and filled to the mark with distilled water.

The solution should be stored in the dark and should not be used for more than two weeks. After that time, a fresh solution should be prepared

In the migration bottles of 100 ml were used, filled with 50ml of saliva simulant and the punched disk were placed inside (see SOP in Annex). The bottles were rotated in the head over heels rotator at 60 rpm for 30 min at room temperature. After that period the simulant was transferred into a separation funnel. A fresh 50 ml aliquot of saliva simulant was added to the flask and rotated for a second period of 30 min.

Extraction and quantification from the saliva simulant

In these experiments the JRC SOP was followed accurately, which includes the following: The simulant was added to the separation funnel. The flask was washed with 20 ml of cyclohexane, which was transferred to the separation funnel. The

mixture was shaken thoroughly for 1 min. After separation of the phases the cyclohexane layer was transferred into a 50 ml measuring flask. Then the flask was washed again with 20 ml cyclohexane and the cyclohexane was added to the saliva simulant, which was extracted a second time with the 20 ml of cyclohexane. The cyclohexane layers were combined and the flask was filled to the mark. Then approximately 1 g of water free sodium sulfate was added to remove any dissolved water. An aliquot of 5 ml was taken and evaporated to dryness and the residue was dissolved in 1 ml cyclohexane. From this solution 20 µl was injected onto the HPLC column.

A simplification was made to the SOP by reducing the number of extraction and avoiding the concentration step, and the change was validated in-house. The simplified procedure is depicted in Figure 4.

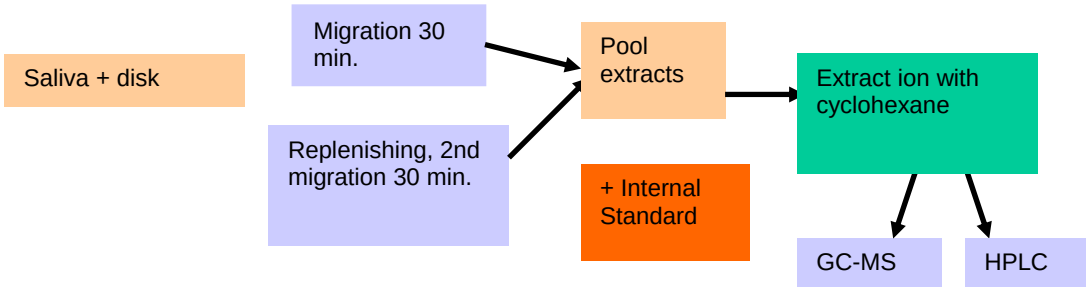


Figure 4: simplified SOP

Quantification of the migrated DINP was then done by chromatography using both high performance liquid chromatography (HPLC) and confirmation by gas chromatography with mass spectrometer (GC-MS).

In GC-MS all quantification was done in selected ion monitoring (SIM), which allows quantification based on the presence of specific ions unique to each substance. The following ions were used: DINP: 293; di-isodecyl phthalate (DIDP):307; BBP: 149. Non-linearity of response of isomeric phthalates (particularly DINP) occurs in GC-MS. The sensitivity of the response is also affected by the spread of multiple smaller peaks which cannot be integrated consistently among low and higher concentrations.

HPLC quantification was done without a ratio to the internal standard (only based on external DINP calibration). The integration was based on peak height not peak area.

To establish the reliability of the method, standard additions of the relevant phthalates were used. The standard additions were made at the level varying from 10 - 23 ug/ml of cyclohexane as injected in the HPLC column or in GC-MS. Converted to the release in saliva simulant and an agitation period of 60 minutes, this meant a standard addition of 1.8 - 3.95 μg/min. The amount of phthalate recovered was expressed as percentage of the standard addition.

Blank saliva simulant was taken through the whole procedure and the amount of phthalates was determined frequently, in order to establish the presence of any interfering substance or contamination of phthalates.

RESULTS

Homogeneity of test materials

The table below represents the results obtained for the homogeneity study; this part of the investigation allows to verify the homogeneity of the material, as well as the efficiency of the analytical method (extraction –quantification) to accurately quantify the substances sought in the materials.

Sample code	phthalate	intended Conc. (%)	specimen code	Sample weight (g)	Quantity of DINP/100 g of sample (%)	stdev	RSD %
Strip 1	DINP	15.2	15DINP	0.79	15.9	0.1	0.4
Strip 2	DINP	24.8	25DINP	0.67	26.0	0.2	0.7
Strip REF	DINP	38.0	38DINP	0.66	39.2	0.5	1.2
Strip 3	DINP	45.1	45DINP	0.64	47.0	1.6	3.4
Strip 2.1	DIDP	24.8	25DIDP	0.84	24.2	1.2	4.9
Strip 4	DIDP	38.0	38DIDP	0.78	37.5	0.6	1.7
Strip 8	DIDP	38.9	39DIDP	0.69	38.7	1.2	3.1
Strip 2.5	DIDP	45.1	45DIDP	0.84	52.6	1.6	3.1
Strip 2.2	DEHP	24.8	25DEHP	0.78	25.6	1.0	3.8
Strip 9	DEHP	36.6	37DEHP	0.57	39.4	1.1	2.7
Strip 5	DEHP	38.0	38DEHP	0.79	40.0	0.5	1.2
Strip 2.6	DEHP	45.1	45DEHP	0.73	50.8	0.8	1.7
Strip 2.3	BBP	24.8	25BBP	0.79	23.0	1.3	5.8
Strip 10	BBP	34.8	35BBP	0.77	34.4	1.2	2.6
Strip 6	BBP	38.0	38BBP	0.80	45.5	2.2	4.9
Strip 2.7	BBP	45.1	45BBP	0.90	42.6	2.1	5.0
Strip 2.4	DBP	24.8	25DBP	0.88	20.6	0.3	1.5
Strip 11	DBP	34.5	35DBP	0.65	36.8	1.2	3.2
Strip 7	DBP	38.0	38DBP	0.78	41.1	1.1	2.6
Strip 2.8	DBP	45.1	45DBP	0.86	36.6	0.5	1.4
Strip 12	DINP/DBP	12.2	15BIN	0.73	11.7	0.5	4.6
Strip 12	DINP/DBP	3.0	15BIN	0.74	4.2	na	na
Strip 2.9	DINP/DBP	19.8	25BIN	0.90	19.9	1.2	6.2
Strip 2.9	DINP/DBP	5.0	25BIN	0.90	4.9	0.3	6.1
Strip 2.1	DINP/DBP	36.1	45BIN	0.82	36.5	0.7	1.7
Strip 2.1	DINP/DBP	9.0	45BIN	0.82	9.3	0.8	8.8

Table 3: homogeneity and measurements of the phthalates in the PVC test material

The overall results showed values for coefficients of variation ranging from 0.4 to 9%, so where in the range that was quite acceptable. 10 blanks were run to insure the absence of detectable peak for each specific phthalate analysed.

The measurements were generally overestimating with respect to the intended concentration as seen by the regressions, but were found to be linear for 3 phthalates. For DINP, there was little difference whether DINP was measured alone or when measured in a PVC where another smaller phthalate DBP was also present (figure 5). The presence of a second did not seem to influence the capacity to measure analytically a majority phthalate such as DINP (as could have been the case in presence of specific interactions).

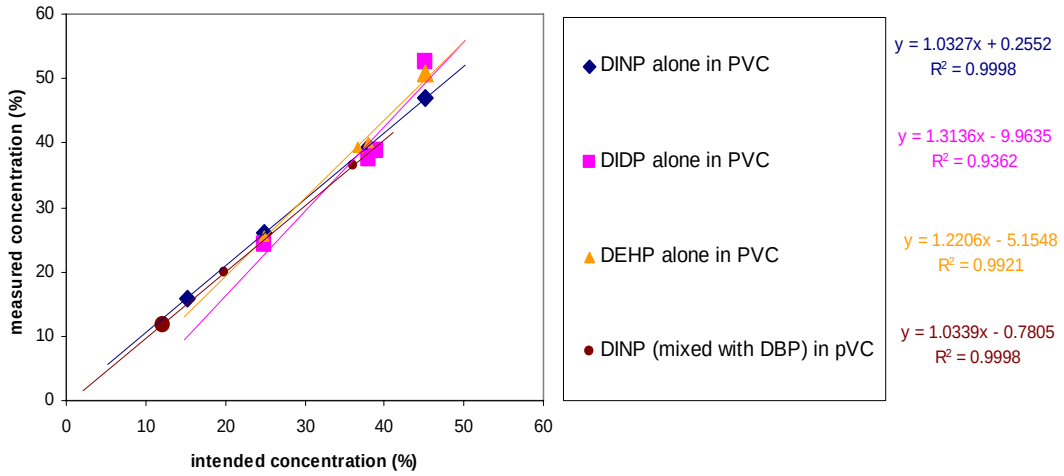


Figure 5: intended vs. measured concentrations fort he different phthalates alone or in mixtures in PVC strips

For smaller molecular weight phthalates, the measured values were less representative of the intended concentration (figure 6), suggesting that

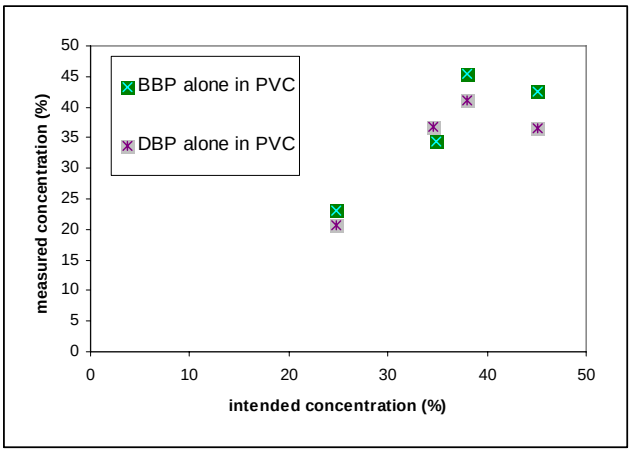


Figure 6: intended vs. measured concentrations fort he different phthalates alone or in mixtures in PVC strips

Values from GC-MS and HPLC were also performed for all analyses and compared for each phthalates. The results suggested that GC-MS is a more adequate (more accurate) when measuring larger molecular weights phthalates such as DEHP, DIDP DINP and HPLC a more accurate analytical technique when measuring smaller weights phthalates such as DBP and BBP.

Release of phthalates from PVC materials

The release was measured and the results are summarised in the following table. The values shown are the averages of 5 replicates samples for each measurement.

Sample Info						GC-MS release				HPLC release		
Sample code	phthalate	**intended Conc. (%)**	**AV DINP g/100g**	stdev		AV Release (ug/min)	stdev	RSD %		AV Release (ug/min)	stdev	RSD %
Strip 1	DINP	**15.2**	**15.9**	0.1		0.2	0.0	0.0		0.1	0.0	19.1
Strip 2	DINP	**24.8**	**26.0**	0.2		1.1	0.0	4.2		1.3	0.2	16.6
Strip REF	DINP	**38.0**	**39.2**	0.5		3.9	0.3	7.3		4.7	0.3	6.7
Strip 3	DINP	**45.1**	**47.0**	1.6		4.8	0.5	8.1		5.0	0.2	3.5
Strip 2.1	DIDP	**24.8**	**24.2**	1.2		1.0	0.1	6.8		1.1	0.1	4.8
Strip 8	DIDP	**38.9**	**38.7**	1.2		2.0	0.1	6.4		2.5	0.2	7.0
Strip 2.5	DIDP	**45.1**	**52.6**	1.6		2.6	0.3	9.6		3.2	0.3	8.1
Strip 2.2	DEHP	**24.8**	**25.6**	1.0		1.2	0.1	6.7		1.6	0.2	9.9
Strip 9	DEHP	**36.6**	**39.4**	1.1		2.7	0.6	44.1		3.4	0.2	5.8
Strip 5	DEHP	**38.0**	**40.0**	0.5		2.7	0.1	5.7		3.3	0.2	5.3
Strip 2.6	DEHP	**45.1**	**50.8**	0.8		3.9	0.1	3.6		4.9	0.2	3.8
Strip 2.3	BBP	**24.8**	**23.0**	1.0		1.6	0.0	1.7		1.5	0.0	1.9
Strip 10	BBP	**34.8**	**34.4**	1.5		3.4	0.3	7.3		3.9	0.3	7.3
Strip 6	BBP	**38.0**	**45.5**	3.1		4.6	0.3	7.8		5.2	0.3	6.2
Strip 2.7	BBP	**45.1**	**42.6**	6.0		11.0	0.7	6.3		7.1	0.4	5.6
Strip 2.4	DBP	**24.8**	**20.6**	2.3		11.3	0.3	2.6		5.6	0.0	0.8
Strip 11	DBP	**34.5**	**36.8**	2.3		11.8	0.6	5.5		10.5	0.5	4.8
Strip 7	DBP	**38.0**	**36.6**	1.0		14.5	0.9	5.9		12.1	0.4	3.0
Strip 2.8	DBP	**45.1**	**41.1**	4.0		32.7	0.9	2.7		14.5	0.3	2.4
Strip 12	DINP/DBP	**12.2**	**11.7**	0.5		0.4	0.1	11.9		0.0	0.0	27.5
Strip 12	DINP/DBP	**3.0**	**4.2**	**na**		0.3	0.0	19.1		0.1	0.0	17.8
Strip 2.9	DINP/DBP	**19.8**	**19.9**	1.2		1.2	0.1	6.5		1.3	0.1	8.8
Strip 2.9	DINP/DBP	**5.0**	**4.9**	**0.3**		1.9	0.1	6.5		1.0	0.1	8.9
Strip 13	DINP/DBP	**30.4**	**32.2**	6.5		2.4	0.1	5.5		2.9	0.1	5.2
Strip 13	DINP/DBP	**7.6**	**8.5**	**1.8**		3.3	0.1	3.7		2.2	0.1	4.3
Strip 2.10	DINP/DBP	**36.1**	**36.5**	0.7		3.9	0.4	9.4		4.5	0.4	7.1
Strip 2.10	DINP/DBP	**9.0**	**9.3**	**0.8**		5.4	0.2	4.0		2.8	0.1	4.3

Table 4: phthalate contents and corresponding release measured under dynamic conditions.

Detailed graphs (figures 7-8) showed that the results are fairly comparable for DINP, DIDP and DEHP between the formulation content and the measured content as well as performing analysis of the release using GC-MS or HPLC (figure 7 a,b,c). However, for smaller molecular weights phthalates, the correlation is much less reliable, due to the lack of reliability of the measured value of contents, which is a very common problem for these lighter environmental phthalates where ubiquitous contaminations often occurs. However it should be noted that the release values are still reliable both by HPLC and GC-MS giving similar values Figure 8, a and b)

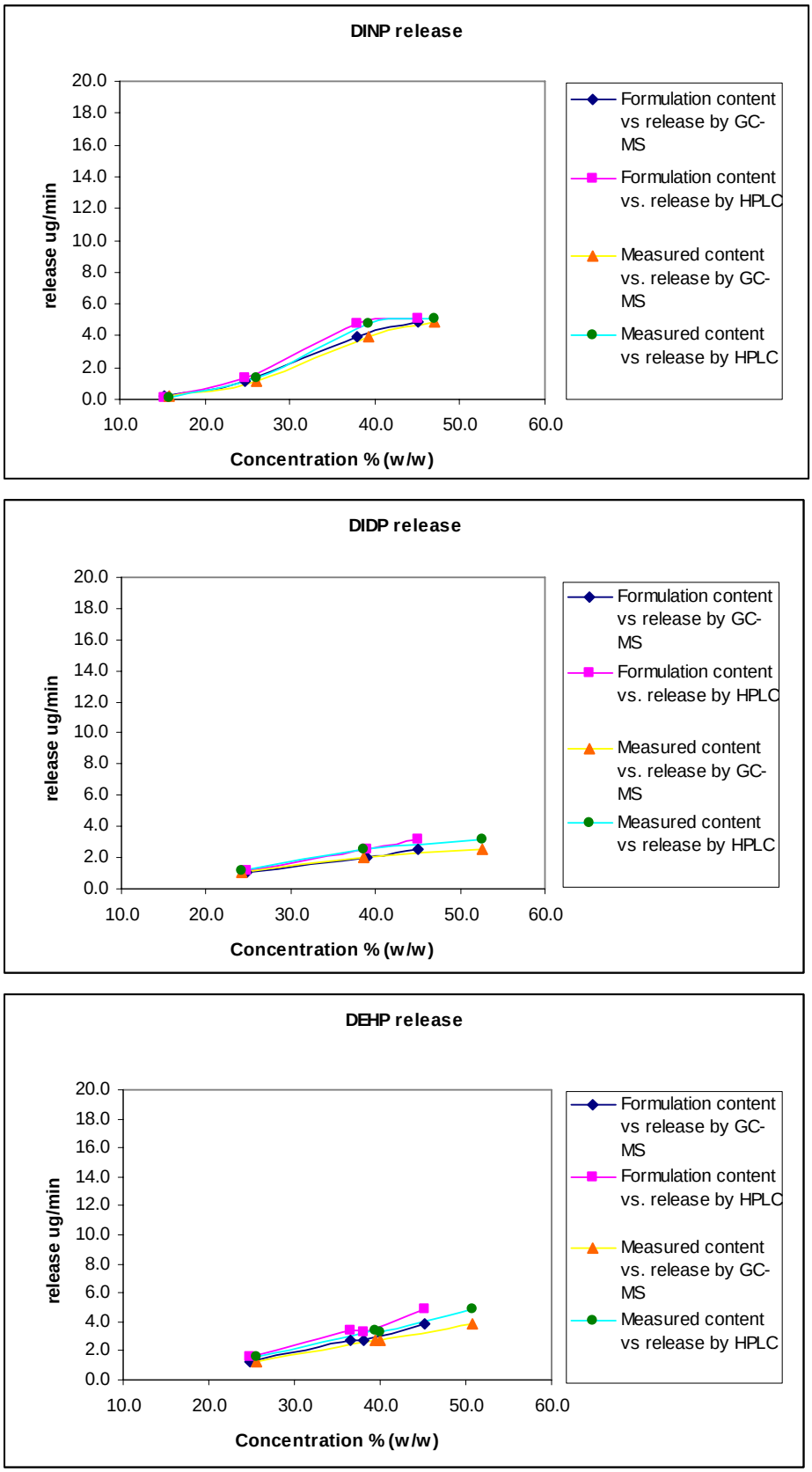


Figure 7: concentrations (both formulation or measured contents) vs. release under dynamic conditions measured either by GC-MS or HPLC a) for DINP, b) for DIDP, c) for DEHP

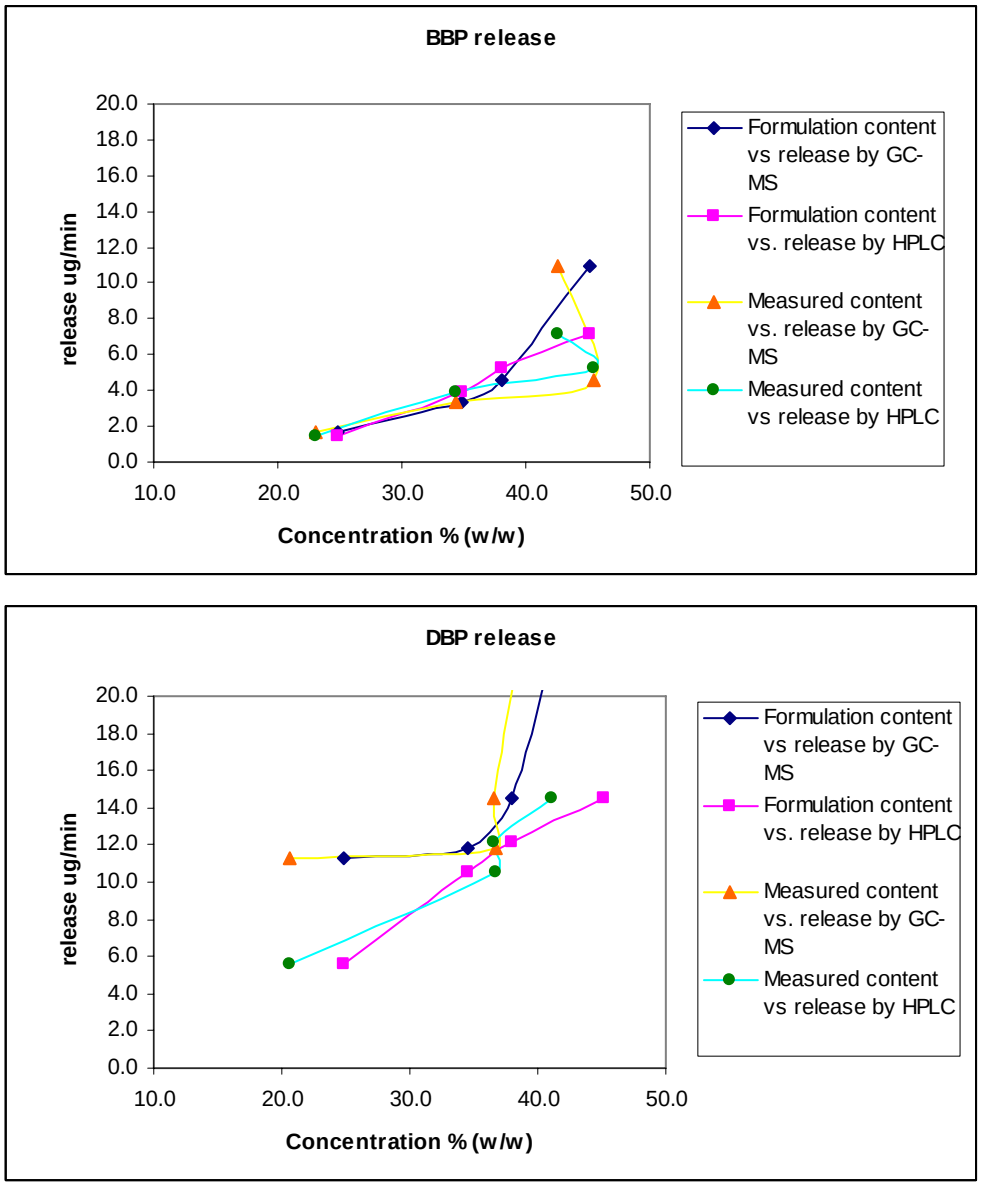


Figure 8: concentrations (both formulation or measured contents) vs. release under dynamic conditions measured either by GC-MS or HPLC a) for BBP, b) for DBP

The results of the release as a function of phthalate nature and concentration were then plotted concurrently, as shown in figure 9-10.

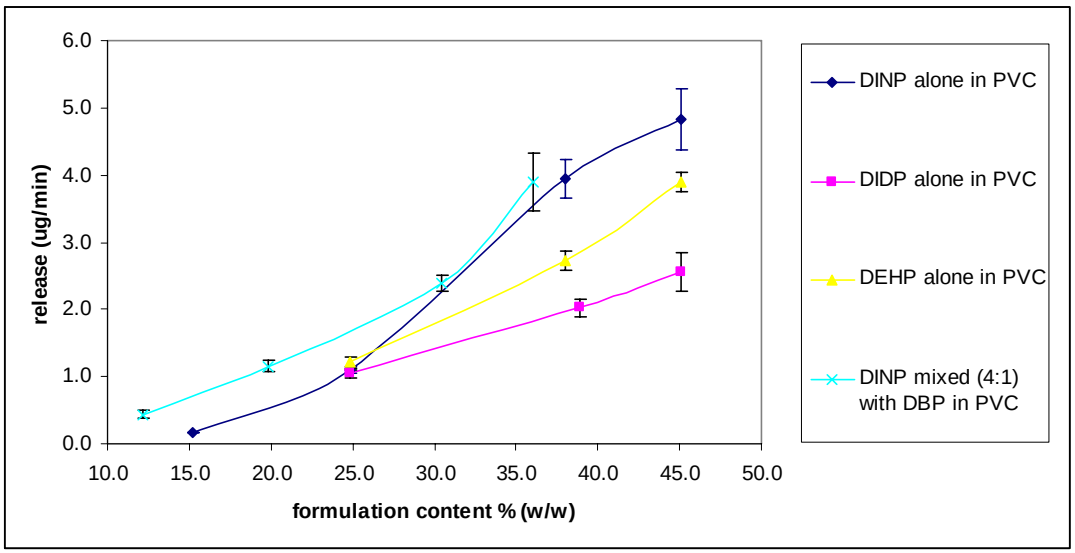


Figure 9: formulation content vs. release for the larger molecular weights phthalates

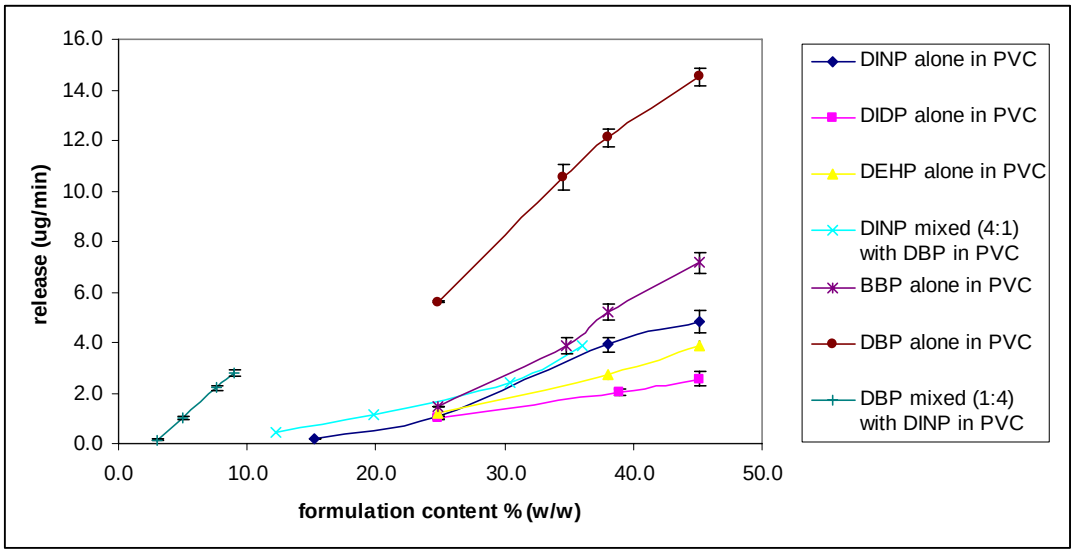
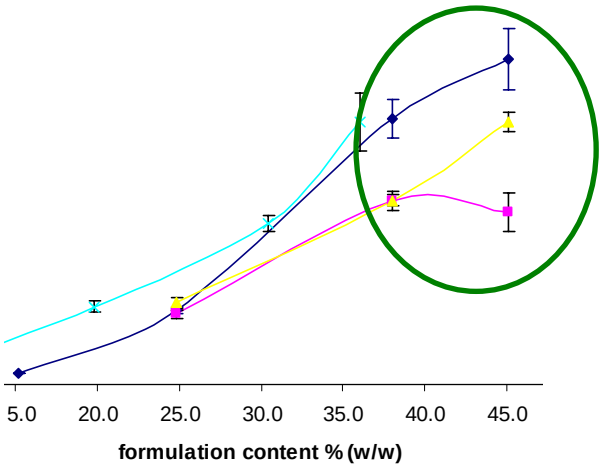


Figure 10: formulation content vs. release for all phthalates including the smaller molecular weights

The results suggest a number of relevant interpretations:

- The limitations of the extent of the release seems a combination of larger molecular weight and lack of volatility; Indeed, DIDP is the phthalate with the

larger molecular weight, longer chain length (figure xx), presents a release that is generally lower and shows signs of saturation (another experiment with another batch of strips showed a potential confirmation of the trend (figure xx). This can be explained by the lowest solubility of DIDP, followed by DINP, DEHP.



- The smaller molecular weights phthalates have higher solubility in water, as shown in the table below both by the solubility and by the partition coefficient n-octanol-water (Log Kow), which is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature. This explains the patterns of release seen that tend to be more linear without this tendency to reach a plateau of limit of release for larger phthalate concentrations in the PVC strips (figure above)

Chemical Name	CAS Number	Log K_{ow} "Slow-Stir"	Log K_{ow} SPARC	Water Solubility (SPARC), mg/L
Dimethyl phthalate	131-11-3	1.60 ± 0.04 (n=12)	1.48	3.3E3
Diethyl phthalate	84-66-2	2.42 ± 0.04 (n=9)	2.51	4.0E2
Dibutyl phthalate	84-74-2	4.50 ± 0.03 (n=9)	4.63	4.9E0
Butylbenzyl phthalate	85-68-7	4.73 ± 0.06 (n=6)	4.77	2.4E0
Diamyl phthalate	131-18-0	5.62 ± 0.04 (n=6)	5.66	4.9E-1
Dihexyl phthalate	84-75-3	6.82 ± 0.10 (n=5)	6.67	4.9E-2
Bis(2-ethylhexyl) phthalate	117-81-7	7.27 ± 0.04 (n=3)	7.54	2.6E-3
Dioctyl phthalate	117-84-0	8.10 ± 0.11 (n=3)	8.30	4.6E-4

Table 5: Octenol-water partition coefficient for eight phthalate esters (source: Ellington and Floyd, US-EPA Environmental Research Brief, EPA/600/S/006, September 1996)

- There an effect of having DINP alone vs. DINP mixed with DBP in PVC materials; it would seem that the presence of the 25% of DBP in the mix of DBP:DINP formation tends to increase the propensity of DINP to be released under dynamic conditions; This is seen mostly for lower formulation contents: for the range of 10-30% of DINP in a strip made of DINP:DBP mixture in (4:1) – i.e. up to a mix of 38 % total phthalate content), the release of DINP seen is higher than the release of DINP of a strip made of the same content of DINP but in alone in the PVC strip. This could be inferred when considering the release of DBP shows greater value; the results can be again be explained by the larger solubility of the smaller molecular weights phthalates such as DNP in saliva (an aqueous media). The greater release of DINP of DINP when in mix with DBP compared to alone is thus likely due to a synergy from the DBP; One hypothesis from a more fundamental standpoint could be the formation of micellar structures where the smaller phthalate act as a carrier to the larger one, therefore overcoming the limit of solubility that the larger one would have if alone. These

assemblies have been mentioned in the literature (Lodge et al, 2004; waters et al, 2005). This is however an hypothesis that would be limited to lower concentration values of DINP which suggest the extent of this role by DBP would be limited to a saturation level by increasing levels of DINP.

CONCLUSIONS

The investigation was able to demonstrate that the release of phthalates under dynamic conditions simulating mouthing is related to their content in PVC polymer strips; This is of relevance, since it suggests that if a limit of migration could be envisioned,

- it would be possible to measure this migration with an accepted method (the principle of the method used here was validated at EU level and went to be taken as a basis in the corresponding EN-71 CEN standards.
- The measurements of the release are fairly consistent and can be measured with two techniques (for example for screening and confirmation purposes)
- The existence of relationships between contents in materials and migration/release indicates that measuring phthalate concentration (similar to the notion of quantity in materials [QM] in the field of food contact materials) could provide also a means to estimate potential migration.

Currently in the EU the food contact materials legislation has a very recent legislation (Directive 2007/19/EC amending Dir. 2002/72/EC on plastics materials). In this new Directive which will apply as of April 2008, the new specific migration

limits (SMLs) and Quantity in Materials (QMs) for phthalates are as follows (table 6). This means SML and QM are allowed in the EU legislation for non fatty foods for all 5 phthalates, ranging for 0.3 mg/kg to 30 mg/kg (exception to single –use article for DEHP and DBP for which the QM applies).

		SML	Qm	Parameter to control in single use Food Contact Material			Parameter to control in repeated use Food Contact Material			Limit in fatty food simulant
PM-no	Substance	(mg/kg food simulant)	(% in the plastic)	Fatty food	Infant food	Non-fatty food	Fatty food	Non-fatty food	Infant food (non-fatty)	(mg/kg simulant D)
74560	Phthalic acid, benzyl butyl ester (BBP)	30	0.1	Qm		SML	SML			30-150
74640	Phthalic acid, bis(2-ethylhexyl)ester (DEHP)	1.5	0.1	Qm			Qm	SML		Not of relevance
74880	Phthalic acid, dibutyl ester (DBP)	0.3	0.05	Qm			Qm	SML		Not of relevance
75100	Phthalic acid, diester with C8-C10 (DiNP)	9 (SML(T) incl. DiDP)	0.1	Qm		SML	SML			9-45
75105	Phthalic acid, diester with C9-C11 (DiDP)	9 (SML(T) incl. DiNP)	0.1	Qm		SML	SML			9-45

Table 6: Regulation of "classical" phthalates in the fourth amendment to the plastics directive: Survey of the critical parameters to control in enforcement work.

Consequently it is relevant to further investigations into the release of phthalates from polymeric materials to anticipate future alignment regarding consumer products. Future work should be directed to confirmation of such data with other common manufacturing process for toys aside from the rotocast mode which was the process used in this study (the most common one).

REFERENCES

J. Tickner- Department of Work Environment, University of Massachusetts at Lowell, USA, 1999, A Review of the Availability of Plastic Substitutes for Soft PVC in Toys; Danish Environmental Protection agency, 1993, PVC and Alternative Materials, and Danish Technical Institute, 1995, Environmental Aspects of PVC; J. Edenbaum, 1996, Plastics additives and modifiers Handbook , Chapman and Hall Ed., revised edition).

M. Baccaredda Boy,1976, Materie Plastiche ed Elastomeriche. Ed. Ambrosiana Milano

Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on Di(2-ethylexyl) Phtalate (DEHP), September, 1985; Kluwe,W.M.; Haseman,J.K.; Huff,J.E., 1983, The carcinogenicity of di(2-ethylhexyl) phthalate (DEHP) in perspective. Journal of Toxicology and Environmental Health, vol 12, n. 1, pp 159-169; Kluwe,W.M., 1986, Carcinogenic potential of phthalic acid esters and related compounds: structure-activity relationships. Environmental Health Perspectives, vol 65, pp 271,278

Bouma, K.; Schakel, D.J., Migration of phthalates from PVC toys into saliva simulant by dynamic extraction, 2002. Food Addititives and Contaminants, vol. 19, n. 6, pg. 602-610; Wilkinson, C. F., Lamb, J. C. IV., 1999, The potential health effects of phthalates esters in children's toys: a review and risk assessment. Regulatory Toxicology and Pharmacology, vol. 30, pp. 140-155

Scientific Committee on Toxicity, Ecotoxicity and the Environment, 27 November 1998, Opinion on phthalate migration from soft PVC toys and children articles

Directive 1999/815/CE temporarily banned the use of phthalates in soft PVC toys intended to be used by children younger than 3. Directive 2002/372/CE

Scientific Committee on Toxicity and Ecotoxicity and the Environment, 28 September 1999, Opinion on the toxicological characteristics and the risks of certain citrates and adipates used as a substitute for phthalates in certain soft PVC products

Bouma, K., Douwes, B. A., Schakel, D. J., 2000, Diisononylphthalate release from PVC toys. De Ware(n) Chemicus, vol. 30, pp. 96-104; Gill, U. S., Lalonde, P. J., Chantal, P.D., Subramanian, K. S., 1999, Analyses of diisononylphthalate in PVC

consumer products used by children. International Journal for Consumer and Product Safety, vol. 6, pp. 223-234.

Simoneau C. and R. Rijk – "Standard Operation Procedure for the determination of release of di-isononyl phthalate (DINP) in saliva simulant from toys and childcare articles using a head over heels dynamic agitation device". EUR technical report EUR 19899 EN (2001)

Simoneau C., H. Geiss, Roncari, A., Zocchi, P., Hannaert, P. – "Validation of methodologies for the release of di-isononyl phthalate in saliva simulant from toys" EUR technical report EUR 19826 EN (2001)

Annexes

Industrial composition of the samples

Phase 1

Different weight %

	Ref		Form 1		Form 2		Form 3	
	Phr	Weight %	Phr	Weight %	Phr	Weight %	Phr	Weight %
PVC	100		100		100		100	
ESBO	3		3		3		3	
Ca-Zn	3		3		3		3	
J-DINP	65	38.0%	19	15.2%	35	24.8%	87	45.1%
	171		125		141		193	

Different plasticizers - same weight %

	Form 4		Form 5		Form 6		Form 7	
	Phr	Weight %	Phr	Weight %	Phr	Weight %	Phr	Weight %
PVC	100		100		100		100	
ESBO	3		3		3		3	
Ca-Zn	3		3		3		3	
J-DIDP	65	38.0%						
DEHP			65	38.0%				
BBP					65	38.0%		
DBP							65	38.0%
	171		171		171		171	

Combination of two plasticizers - 38 weight %

	Form 12		ratio	Form 13		ratio
	Phr	Weight %	Dinp/Dbp	Phr	Weight %	Dinp/Dbp
PVC	100			100		
ESBO	3			3		
Ca-Zn	3			3		
DINP	15.2		4	52		4
DBP	3.8			13		
		15.2%			38.0%	
	125			171		

Phase 2

Different plasticizers at 25 weight %

	Form 2.1		Form 2.2		Form 2.3		Form 2.4	
	Phr	Weight %	Phr	Weight %	Phr	Weight %	Phr	Weight %
PVC	100		100		100		100	
ESBO	3		3		3		3	
Ca-Zn	3		3		3		3	
J-DIDP	35	24.8%						
DEHP			35	24.8%				
BBP					35	24.8%		
DBP							35	24.8%
	141		141		141		141	

Different plasticizers at 45 weight %

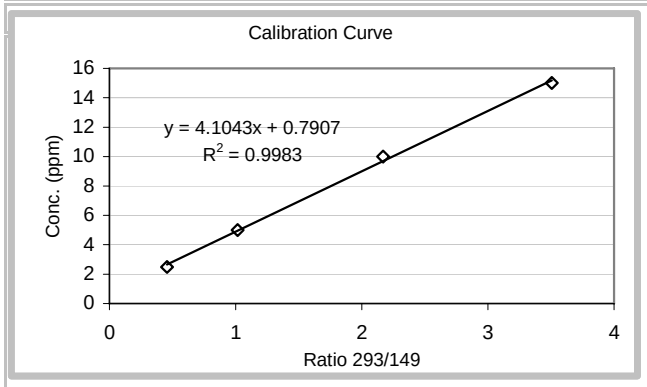
	Form 2.5		Form 2.6		Form 2.7		Form 2.8	
	Phr	Weight %	Phr	Weight %	Phr	Weight %	Phr	Weight %
PVC	100		100		100		100	
ESBO	3		3		3		3	
Ca-Zn	3		3		3		3	
DIDP	87	45.1%						
DEHP			87	45.1%				
BBP					87	45.1%		
DBP							87	45.1%
	193		193		193		193	

Combination of two plasticizers - at 25 and 45 weight %

	Form 2.9		ratio	Form 2.10		ratio
	Phr	Weight %	Dinp/Dbp	Phr	Weight %	Dinp/Dbp
PVC	100			100		
ESBO	3			3		
Ca-Zn	3			3		
DINP	28		4	69.6		4
DBP	7			17.4		
		24.8%			45.1%	
	141			193		

Calibration and Homogeneity punched disk (reference strip) – raw data

Sample		Area 149	Area 293	Area 307	293/149	conc. (ppm)
punched disk	01A	740780	1574865	392568	2.125955074	9.516257412
punched disk	01B	698368	1455483	396160	2.084120406	9.344555384
punched disk	02A	736107	1397015	359529	1.89784230	8.580014141
punched disk	02B	747461	1705113	450278	2.281206645	10.15345643
punched disk	03A	894641	1460379	380629	1.632363149	7.490408073
punched disk	03B	754607	1757347	479057	2.328824143	10.34889293
punched disk	04A	712725	1487716	413446	2.087363289	9.357865146
punched disk	04B	755284	1532560	401410	2.029117524	9.118807054
punched disk	05A	812067	1614089	421166	1.987630331	8.948531168
punched disk	05B	677036	1605792	414991	2.371797068	10.52526671
punched disk	06A	814210	1756295	481856	2.157054077	9.643897048
punched disk	06B	720856	1478471	394804	2.050993541	9.208592790
punched disk	07A	865956	1647478	443414	1.90249620	8.599115157
punched disk	07B	798462	1565844	420879	1.961075167	8.839540808
punched disk	08A	802579	1340121	361984	1.669768334	7.643930175
punched disk	08B	678550	1707231	462028	2.515998821	11.11711396
punched disk	09A	750722	1602163	427371	2.134162846	9.549944568
punched disk	09B	779330	1640025	443756	2.104403783	9.427804445
punched disk	10A	732854	1273667	297324	1.737954627	7.923787174
punched disk	10B	770119	1763880	433701	2.290399276	10.19118575
Blank		808969				



Calibration curve			
Conc. (ppm)	Area 149	Area 293	Ratio
0	728901		0
2.5	815080	372296	0.45676
5	686466	697914	1.016677
10	880218	1908726	2.16847
15	778961	2730998	3.50595
y=4.1043x + 0.7907			

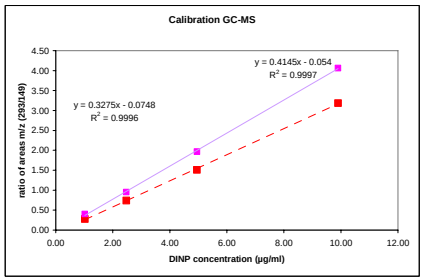
Homogeneity of rotocast strips for different phthalates

GC-MS analyses

DINP

Calibration curve						
Target DINP conc. (µg/ml)	Exact DINP conc. (µg/ml), Xi	Exact BBP conc. (~1 µg/ml)	Area DINP (293)	Area DIDP (307)	Area BBP (149)	ratio m/z DINP/BBP
1	1.02	1	53296	14993	192816	0.28
2.5	2.48	1	136020	36731	195814	0.74
5	4.95	1	289449	83736	191957	1.51
10	9.90	1	583922	176462	183430	3.18
1	1.02	1	25734	7050	63956	0.40
2.5	2.48	1	59489	17794	62475	0.95
5	4.95	1	137551	44153	69889	1.97
10	9.90	1	288786	91752	71035	4.07

Calibration graph 1			Statistic calculations	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration points	4.00
1.04	0.08	0.28	Sum X i	18.35
6.13	0.55	1.84	Sum X i ^2	31.67
24.50	2.27	7.46	Sum Y i	5.71
			Sum Yi^2	2.90
			Sum X i * Y i	9.58
			Mean value X	4.59
			Mean value Y	1.43
			Slope B	0.32
			Intercept of blank	-0.02
			Sum (X i - X) ^2	-52.48
			Sum (X i - X) * (Y i - Y)	-16.60
			Sum (Y i -Y) ^2	-5.25



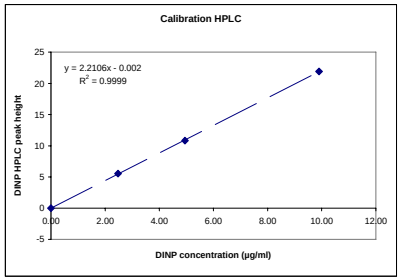
Calibration graph 2			Statistic calculations	Curve 2
X i ^2	Y i ^2	X i * Y i	Calibration points	4.00
1.04	0.16	0.41	Sum X i	18.35
6.13	0.91	2.36	Sum X i ^2	129.68
24.50	3.87	9.74	Sum Y i	7.39
98.01	16.53	40.25	Sum Yi^2	21.47
0.00	0.00	0.00	Sum X i * Y i	52.76
0.00	0.00	0.00	Mean value X	4.59
0.00	0.00	0.00	Mean value Y	1.85
0.00	0.00	0.00	Slope B	0.41
			Intercept of blank	-0.05
			Sum (X i - X) ^2	45.53
			Sum (X i - X) * (Y i - Y)	18.87
			Sum (Y i -Y) ^2	7.82

HPLC analyses

DINP

Calibration curve		
Target DINP conc. (µg/ml)	Exact DINP conc. Xi (µg/ml)	Height DINP, Yi
0	0.00	0
1		
2.5	2.48	5.54
5	4.95	10.83
10	9.90	21.92

Calibration graph 1			Statistic calculation	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration points	4.00
0.00	0.00	0.00	Sum X i	17.33
0.00	0.00	0.00	Sum X i ^2	128.64
6.13	30.69	13.71	Sum Y i	38.29
24.50	117.29	53.61	Sum Yi^2	628.47
98.01	480.49	217.01	Sum X i * Y i	284.33
0.00	0.00	0.00	Mean value X	4.33
0.00	0.00	0.00	Mean value Y	9.57
0.00	0.00	0.00	Slope B	2.21
			Intercept of blank	0.00
			Sum (X i - X) ^2	53.60
			Sum (X i - X) * (Y i	118.48
			Sum (Y i -Y) ^2	261.94



GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Area DINP (293)	Area DIDP (307)	Area BBP (149)	ratio m/z DINP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantity of DINP (g) for sample	Quantity of DINP/100 g of sample (%)
		strip REF	38DINP1A		0.66	329127	92104	77595	4.24	10.36	0.03	0.26	40.04
		strip REF	38DINP1B		0.66	312221	87207	74700	4.18	10.21	0.03	0.26	39.47
		strip REF	38DINP2A		0.66	300051	81062	72659	4.13	10.09	0.03	0.26	39.00
		strip REF	38DINP2B		0.63	279201	78751	71440	3.91	9.56	0.03	0.24	38.69
		strip REF	38DINP3A		0.60	267159	76157	71114	3.76	9.19	0.03	0.23	39.08
		strip REF	38DINP3B		0.63	276199	75700	70025	3.94	9.65	0.03	0.25	39.05
		Strip 1	15DINP1A		0.79	337230	86649	218891	1.54	4.94	0.03	0.13	15.95
		Strip 1	15DINP1B		0.68	272401	66818	207156	1.31	4.23	0.03	0.11	15.87
		Strip 2	25DINP1A		0.67	440113	113247	208543	2.11	6.75	0.03	0.17	25.81
		Strip 2	25DINP1B		0.86	581404	153011	212299	2.74	8.73	0.03	0.22	25.86
		Strip 2	25DINP2A		0.80	518866	138666	202902	2.56	8.16	0.03	0.21	26.00
		Strip 2	25DINP2B		0.72	504289	130420	217132	2.32	7.42	0.03	0.19	26.27
		Strip 2	25DINP3A		0.65	425382	111713	203453	2.09	6.68	0.03	0.17	26.22
		Strip 2	25DINP3B		0.85	553786	145060	203791	2.72	8.66	0.03	0.22	25.99
		Strip 3	45DINP1A		0.64	822801	224057	220280	3.74	11.88	0.03	0.30	47.54
		Strip 3	45DINP1B		0.58	738503	200098	227059	3.25	10.36	0.03	0.26	45.53
		Strip 3	45DINP2A		0.67	860533	233765	233483	3.69	11.72	0.03	0.30	44.62
		Strip 3	45DINP2B		0.71	910020	247196	219274	4.15	13.19	0.03	0.34	47.38
		Strip 3	45DINP3A		0.66	852637	230339	214799	3.97	12.62	0.03	0.32	48.76
		Strip 3	45DINP3B		0.68	864157	232545	214228	4.03	12.82	0.03	0.33	48.09

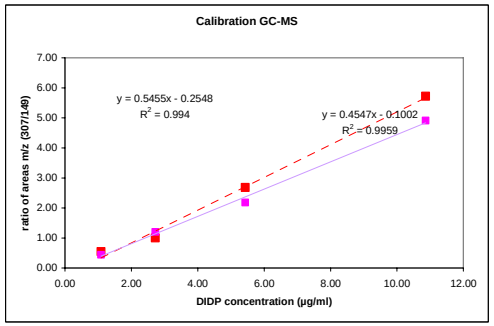
HPLC analyses Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height DINP	Solution conc. (µg/ml)	Conc. factor	Quantity of DINP (g) for sample	Quantity of DINP/100 g of sample (%)
		strip REF	38DINP1A		0.66	25.76	11.65	0.03	0.30	45.03
		strip REF	38DINP1B		0.66	25.41	11.50	0.03	0.29	44.42
		strip REF	38DINP2A		0.66	25.49	11.53	0.03	0.29	44.54
		strip REF	38DINP2B		0.63	24.95	11.29	0.03	0.29	45.69
		strip REF	38DINP3A		0.60	23.28	10.53	0.03	0.27	44.76
		strip REF	38DINP3B		0.63	24.30	10.99	0.03	0.28	44.50
		Strip 1	15DINP1A		0.79	13.13	5.94	0.03	0.15	19.16
		Strip 1	15DINP1B		0.68	10.89	4.93	0.03	0.13	18.48
		Strip 2	25DINP1A		0.67	17.21	7.79	0.03	0.20	29.79
		Strip 2	25DINP1B		0.86	22.32	10.10	0.03	0.26	29.91
		Strip 2	25DINP2A		0.80	19.68	8.90	0.03	0.23	28.37
		Strip 2	25DINP2B		0.72	19.18	8.68	0.03	0.22	30.72
		Strip 2	25DINP3A		0.65	16.50	7.47	0.03	0.19	29.29
		Strip 2	25DINP3B		0.85	21.42	9.69	0.03	0.25	29.07
		Strip 3	45DINP1A		0.64	29.51	13.35	0.03	0.34	53.42
		Strip 3	45DINP1B		0.58	27.27	12.34	0.03	0.31	54.24
		Strip 3	45DINP2A		0.67	31.97	14.46	0.03	0.37	55.05
		Strip 3	45DINP2B		0.71	33.58	15.19	0.03	0.39	54.56
		Strip 3	45DINP3A		0.66	31.07	14.06	0.03	0.36	54.31
		Strip 3	45DINP3B		0.68	31.59	14.29	0.03	0.36	53.59

GC-MS analyses

DIDP

Calibration curve						
Target DINP conc. (µg/ml)	Exact DIDP conc. (µg/ml), Xi	Exact BBP conc. (−1 µg/ml)	Area DINP (293)	Area DIDP (307)	Area BBP (149)	ratio m/z DIDP/BBP
1	1.09	1		48053	88267	0.54
2.5	2.72	1		85004	85004	1.00
5	5.43	1		236493	88168	2.68
10	10.86	1		494987	86539	5.72
	1.09	1		88923	200347	0.44
	2.72	1		222111	184601	1.20
	5.43	1		436357	200017	2.18
	10.86	1		967780	197097	4.91



Calibration graph 1			Statistic calculations	Curve 1
X i ^2	Y i ^2	X i * Y i		
1.18	0.30	0.59	Calibration points	4.00
7.42	1.00	2.72	Sum X i	20.10
29.48	7.19	14.56	Sum X i ^2	38.09
			Sum Y i	9.95
			Sum Yi^2	8.49
			Sum X i * Y i	17.88
			Mean value X	5.03
			Mean value Y	2.49
			Slope B	0.51
			Intercept of blank	-0.08
			Sum (X i - X) ^2	-62.92
			Sum (X i - X) * (Y i - Y)	-32.10
			Sum (Y i -Y) ^2	-16.24

Calibration graph 2			Statistic calculations	Curve 2
X i ^2	Y i ^2	X i * Y i		
1.18	0.20	0.48	Calibration points	4.00
7.42	1.45	3.28	Sum X i	20.10
29.48	4.76	11.85	Sum X i ^2	156.03
117.94	24.11	53.32	Sum Y i	8.74
0.00	0.00	0.00	Sum Yi^2	30.51
0.00	0.00	0.00	Sum X i * Y i	68.93
0.00	0.00	0.00	Mean value X	5.03
0.00	0.00	0.00	Mean value Y	2.18
			Slope B	0.45
			Intercept of blank	-0.10
			Sum (X i - X) ^2	55.02
			Sum (X i - X) * (Y i - Y)	25.02
			Sum (Y i -Y) ^2	11.42

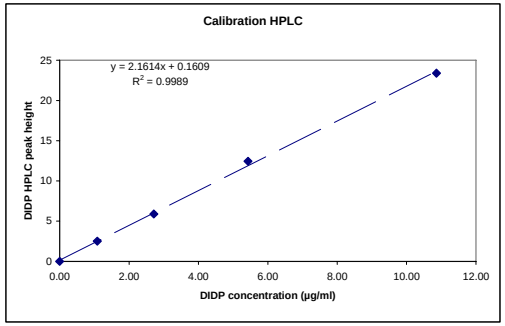
used first calibration

HPLC analyses

DIDP

Calibration curve		
Target DINP conc. (µg/ml)	Exact DIDP conc. Xi (µg/ml)	Height DIDP. Yi
0	0.00	0
1	1.09	2.51
2.5	2.72	5.88
5	5.43	12.44
10	10.86	23.4

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i		
0.00	0.00	0.00	Calibration po	5.00
1.18	6.30	2.73	Sum X i	20.09
7.37	34.57	15.96	Sum X i ^2	155.98
29.48	154.75	67.55	Sum Y i	44.23
117.94	547.56	254.12	Sum Yi^2	743.19
0.00	0.00	0.00	Sum X i * Y	340.36
0.00	0.00	0.00	Mean value	4.02
0.00	0.00	0.00	Mean value	8.85
			Slope B	2.16
			Intercept of bl	0.16
			Sum (X i - X)	75.25
			Sum (X i - X)	162.64
			Sum (Y i -Y) ^	351.93



GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Area DINP (293)	Area DIDP (307)	Area BBP (149)	ratio m/z DIDP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantity of DIDP (g) for sample	Quantity of DIDP/100 g of sample
		Strip 4	38DIDP1A		0.78	49050	1110929	223903	4.96	11.13	0.03	0.28	36.39
		Strip 4	38DIDP1B		0.77	48763	1065016	206766	5.15	11.55	0.03	0.29	38.24
		Strip 4	38DIDP2A		0.71	45832	1014360	216842	4.68	10.51	0.03	0.27	37.74
		Strip 4	38DIDP2B		0.70	44727	1049840	229198	4.58	10.29	0.03	0.26	37.50
		Strip 4	38DIDP3A		0.75	45157	1065861	218534	4.88	10.95	0.03	0.28	37.22
		Strip 4	38DIDP3B		0.67	41194	900079	204448	4.40	9.90	0.03	0.25	37.69
		Strip 8	39DIDP1A		0.69	14298	550723	102005	5.40	10.73	0.03	0.27	39.67
		Strip 8	39DIDP1B		0.65	12086	458271	94632	4.84	9.64	0.03	0.25	37.83
		Strip 8	39DIDP2A		0.68	16394	541324	99811	5.42	10.78	0.03	0.27	40.43
		Strip 8	39DIDP2B		0.71	16389	568312	104545	5.44	10.81	0.03	0.28	38.81
		Strip 8	39DIDP3A		0.78	18175	582250	101819	5.72	11.36	0.03	0.29	37.14
		Strip 8	39DIDP3B		0.75	13635	567817	100193	5.67	11.26	0.03	0.29	38.28

HPLC analyses Homogeneity

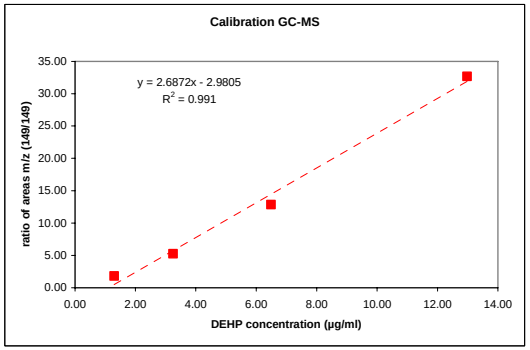
		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height DIDP	Solution conc. (µg/ml)	Conc. factor	Quantity of DIDP (g) for sample	Quantity of DIDP/100 g of sample (%)
		Strip 4	38DIDP1A		0.78	27.24	12.53	0.03	0.32	40.96
		Strip 4	38DIDP1B		0.77	?	#VALUE!	0.03	#VALUE!	
		Strip 4	38DIDP2A		0.71	25.11	11.54	0.03	0.29	41.46
		Strip 4	38DIDP2B		0.70	24.87	11.43	0.03	0.29	41.64
		Strip 4	38DIDP3A		0.75	26.34	12.11	0.03	0.31	41.18
		Strip 4	38DIDP3B		0.67	?	#VALUE!	0.03	#VALUE!	
		Strip 8	39DIDP1A		0.69	25.66	11.80	0.03	0.30	43.60
		Strip 8	39DIDP1B		0.65	22.22	10.21	0.03	0.26	40.04
		Strip 8	39DIDP2A		0.68	24.06	11.06	0.03	0.28	41.46
		Strip 8	39DIDP2B		0.71	25.40	11.68	0.03	0.30	41.94
		Strip 8	39DIDP3A		0.78	27.37	12.59	0.03	0.32	41.15
		Strip 8	39DIDP3B		0.75	26.59	12.23	0.03	0.31	41.57

GC-MS analyses

DEHP

Calibration curve						
Target DINP conc. (µg/ml)	Exact DEHP conc. (µg/ml), Xi	Exact BBP conc. (~1 µg/ml)		Area DEHP (149)	Area BBP (149)	ratio m/z DEHP/BBP
1	1.30	1		271372	150200	1.81
2.5	3.25	1		763711	145401	5.25
5	6.49	1		1961237	152614	12.85
10	12.98	1		4890641	149584	32.69

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	4.00
0.00	0.00	0.00	Sum X i	24.01
1.68	3.26	2.35	Sum X i ^2	222.82
10.53	27.59	17.04	Sum Y i	52.61
42.12	165.15	83.40	Sum Yi^2	1264.96
168.48	1068.96	424.38	Sum X i * Y	527.17
0.00	0.00	0.00	Mean value	6.00
0.00	0.00	0.00	Mean value	13.15
0.00	0.00	0.00	Slope B	2.69
			Intercept of bl	-2.98
			Sum (X i - X)	78.66
			Sum (X i - X)	211.37
			Sum (Y i -Y)	573.14

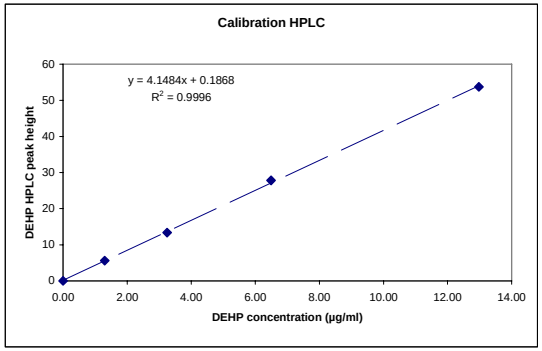


HPLC analyses

DEHP

Calibration curve		
Target DINP conc. (µg/ml)	Exact DEHP conc. Xi (µg/ml)	Height DEHP, Yi
0	0.00	0
1	1.30	5.6
2.5	3.25	13.39
5	6.49	27.82
10	12.98	53.74

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	5.00
0.00	0.00	0.00	Sum X i	24.01
1.68	31.36	7.27	Sum X i ^2	222.82
10.53	179.29	43.45	Sum Y i	100.55
42.12	773.95	180.55	Sum Yi^2	3872.59
168.48	2887.99	697.55	Sum X i * Y	928.82
0.00	0.00	0.00	Mean value	4.80
0.00	0.00	0.00	Mean value	20.11
0.00	0.00	0.00	Slope B	4.15
			Intercept of bl	0.19
			Sum (X i - X)	107.49
			Sum (X i - X)	445.91
			Sum (Y i -Y)	1850.53



GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)		Area DEHP (149)	Area BBP (149)	ratio m/z DEHP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantity of DEHP (g) for sample	Quantity of DEHP/100 g of sample
		strip 5	38DEHP1A		0.79		4682071	156168	29.98	12.27	0.03	0.31	39.59
		strip 5	38DEHP1B		0.81		5174006	164068	31.54	12.84	0.03	0.33	40.44
		strip 5	38DEHP2A		0.75		4796500	169759	28.25	11.62	0.03	0.30	39.52
		strip 5	38DEHP2B		0.79		4914782	163060	30.14	12.33	0.03	0.31	39.79
		strip 5	38DEHP3A		0.85		5730512	170840	33.54	13.59	0.03	0.35	40.78
		strip 5	38DEHP3B		0.85		5602774	170920	32.78	13.31	0.03	0.34	39.92
		strip 9	37DEHP1A		0.57		3412838	168987	20.20	8.62	0.03	0.22	38.58
		strip 9	37DEHP1B		0.73		4479527	158739	28.22	11.61	0.03	0.30	40.56
		strip 9	37DEHP2A		0.58		3340464	164943	20.25	8.65	0.03	0.22	38.01
		strip 9	37DEHP2B		0.65		3727978	157212	23.71	9.93	0.03	0.25	38.97
		strip 9	37DEHP3A		0.66		3810736	155635	24.49	10.22	0.03	0.26	39.49
		strip 9	37DEHP3B		0.69		4195946	158144	26.53	10.98	0.03	0.28	40.59

HPLC analyses - Homogeneity

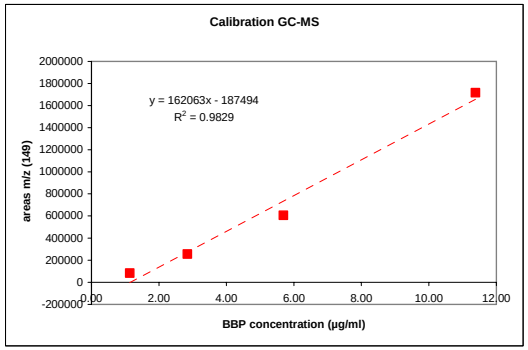
		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height DEHP	Solution conc. (µg/ml)	Conc. factor	Quantity of DEHP (g)	Quantity of DEHP /100 g of sample
		strip 5	38DEHP1A		0.79	53.13	12.76	0.03	0.33	41.19
		strip 5	38DEHP1B		0.81	54.68	13.14	0.03	0.33	41.35
		strip 5	38DEHP2A		0.75	52.16	12.53	0.03	0.32	42.60
		strip 5	38DEHP2B		0.79	51.67	12.41	0.03	0.32	40.06
		strip 5	38DEHP3A		0.85	56.21	13.50	0.03	0.34	40.51
		strip 5	38DEHP3B		0.85	56.88	13.67	0.03	0.35	41.00
		strip 9	37DEHP1A		0.57	39.54	9.49	0.03	0.24	42.44
		strip 9	37DEHP1B		0.73	48.69	11.69	0.03	0.30	40.84
		strip 9	37DEHP2A		0.58	40.26	9.66	0.03	0.25	42.47
		strip 9	37DEHP2B		0.65	44.36	10.65	0.03	0.27	41.77
		strip 9	37DEHP3A		0.66	44.52	10.69	0.03	0.27	41.29
		strip 9	37DEHP3B		0.69	48.80	11.72	0.03	0.30	43.31

GC-MS analyses

BBP

Calibration curve						
Target DINP conc. (µg/ml)		Exact BBP conc. (~1 µg/ml)			Area BBP (149)	
0						
1		1.14			83162	
2.5		2.85			254975	
5		5.69			607332	
10		11.38			1716465	

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	4.00
0.00	0.00	0.00	Sum X i	21.05
1.30	6.92E+09	9.46E+04	Sum X i ^2	171.27
8.09	6.50E+10	7.25E+05	Sum Y i	2.66E+06
32.38	3.69E+11	3.46E+06	Sum Yi^2	3.39E+12
129.50	2.95E+12	1.95E+07	Sum X i * Y	2.38E+07
0.00	0.00	0.00	Mean value	5.26
0.00	0.00	0.00	Mean value	665483.50
0.00	0.00	0.00	Slope B	162062.94
			Intercept of b	-1.87E+05
			Sum (X i - X)	60.46
			Sum (X i - X)	9.80E+06
			Sum (Y i -Y)	1.62E+12

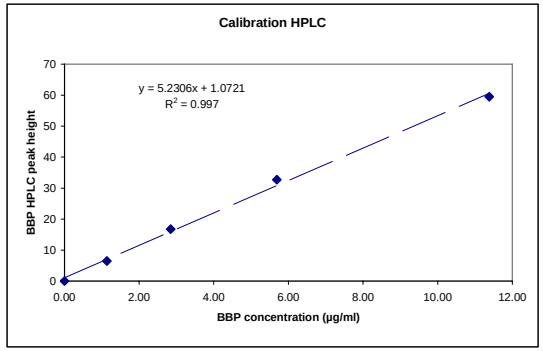


HPLC analyses

BBP

Calibration curve		
Target DINP conc. (µg/ml)	Exact BBP conc. Xi (µg/ml)	Height BBP, Yi
0	0.00	0
1	1.14	6.49
2.5	2.85	16.76
5	5.69	32.73
10	11.38	59.5

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	5.00
0.00	0.00	0.00	Sum X i	21.05
1.30	42.12	7.39	Sum X i ^2	171.27
8.09	280.90	47.68	Sum Y i	115.48
32.38	1071.25	186.23	Sum Yi^2	4934.52
129.50	3540.25	677.11	Sum X i * Y	918.41
0.00	0.00	0.00	Mean value	4.21
0.00	0.00	0.00	Mean value	23.10
0.00	0.00	0.00	Slope B	5.23
			Intercept of b	1.07
			Sum (X i - X)	82.62
			Sum (X i - X)	432.17
			Sum (Y i -Y)	2267.39



GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)				Area BBP (149)	Solution conc. (µg/ml)	Conc. factor	Quantity of BBP (g) for sample	Quantity of BBP/100 g of sample (%)
		strip 6	38BBP1A		0.80				2165773	14.52	0.03	0.37	46.28
		strip 6	38BBP1B		0.70				1665537	11.43	0.03	0.29	41.65
		strip 6	38BBP2A		0.78				2044050	13.77	0.03	0.35	45.02
		strip 6	38BBP2B		0.75				1621006	11.16	0.03	0.28	37.94
		strip 6	38BBP3A		0.78				2067848	13.92	0.03	0.35	45.50
		strip 6	38BBP3B		0.77				1965550	13.29	0.03	0.34	44.00
		strip 10	35BBP1A		0.77				2007105	13.54	0.03	0.35	44.85
		strip 10	35BBP1B		0.83				1958790	13.24	0.03	0.34	40.69
		strip 10	35BBP2A		0.88				2187066	14.65	0.03	0.37	42.50
		strip 10	35BBP2B		0.77				1824396	12.41	0.03	0.32	41.11
		strip 10	35BBP3A		0.80				1914736	12.97	0.03	0.33	41.35
		strip 10	35BBP3B		0.76				1828406	12.44	0.03	0.32	41.74

HPLC analyses Homogeneity

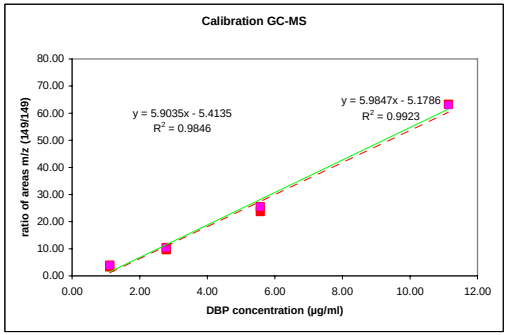
		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height BBP	Solution conc. (µg/ml)	Conc. factor	Quantity ofBBP (g) for sample	Quantity of BBP/100 g of sample (%)
		strip 6	38BBP1A		0.80	76.42	14.41	0.03	0.37	45.92
		strip 6	38BBP1B		0.70	65.12	12.24	0.03	0.31	44.61
		strip 6	38BBP2A		0.78	74.52	14.04	0.03	0.36	45.91
		strip 6	38BBP2B		0.75	66.37	12.48	0.03	0.32	42.45
		strip 6	38BBP3A		0.78	78.67	14.84	0.03	0.38	48.50
		strip 6	38BBP3B		0.77	?	#VALUE!	0.03	#VALUE!	
		strip 10	35BBP1A		0.77	74.83	14.10	0.03	0.36	46.70
		strip 10	35BBP1B		0.83	75.14	14.16	0.03	0.36	43.51
		strip 10	35BBP2A		0.88	79.65	15.02	0.03	0.38	43.57
		strip 10	35BBP2B		0.77	70.97	13.36	0.03	0.34	44.26
		strip 10	35BBP3A		0.80	73.54	13.85	0.03	0.35	44.16
		strip 10	35BBP3B		0.76	70.28	13.23	0.03	0.34	44.39

GC-MS analyses

DBP

Calibration curve1						
Target DINP conc. (µg/ml)	Exact DBP conc. (µg/ml), Xi	Exact BBP conc. (~1 µg/ml)	Area DBP (149)		Area BBP (149)	ratio m/z DBP/BBP
1	1.11	1	226361		68458	3.31
2.5	2.79	1	648077		67087	9.66
5	5.57	1	1693458		71466	23.70
10	11.14	1	4554483		72013	63.25
1	1.11	1	553616		142208	3.89
2.5	2.79	1	1552176		149452	10.39
5	5.57	1	3864053		151635	25.48
10	11.14	1	9662975		153714	62.86

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	4.00
1.24	10.93	3.68	Sum X i	20.61
7.76	93.32	26.90	Sum X i ^2	164.12
31.02	561.50	131.99	Sum Y i	99.91
124.10	3999.97	704.55	Sum Yi^2	4665.72
			Sum X i * Y	867.13
			Mean value	5.15
			Mean value	24.98
			Slope B	6.08
			Intercept of b	-6.36
			Sum (X i - X)	57.94
			Sum (X i - X)	352.38
			Sum (Y i -Y)	2170.31



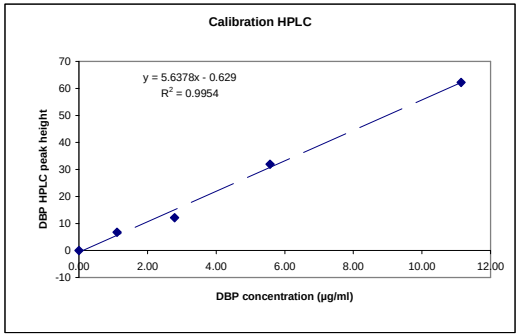
Calibration graph 2			Statistic calcu	Curve 2
X i ^2	Y i ^2	X i * Y i	Calibration pc	4.00
1.24	15.16	4.34	Sum X i	20.61
7.76	107.86	28.92	Sum X i ^2	164.12
31.02	649.36	141.94	Sum Y i	102.62
124.10	3951.80	700.30	Sum Yi^2	4724.18
0.00	0.00	0.00	Sum X i * Y	875.50
0.00	0.00	0.00	Mean value	5.15
0.00	0.00	0.00	Mean value	25.66
0.00	0.00	0.00	Slope B	5.98
			Intercept of b	-5.18
			Sum (X i - X)	57.94
			Sum (X i - X)	346.75
			Sum (Y i -Y)	2091.22

HPLC analyses

DBP

Calibration curve		
Target DINP conc. (µg/ml)	Exact DBP conc. Xi (µg/ml)	Height DBP. Yi
0	0.00	0
1	1.11	6.75
2.5	2.79	12.15
5	5.57	31.92
10	11.14	62.23

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	5.00
0.00	0.00	0.00	Sum X i	20.61
1.23	45.56	7.49	Sum X i ^2	164.14
7.78	147.62	33.90	Sum Y i	113.05
31.02	1018.89	177.79	Sum Yi^2	5084.64
124.10	3872.57	693.24	Sum X i * Y	912.43
0.00	0.00	0.00	Mean value	4.12
0.00	0.00	0.00	Mean value	22.61
0.00	0.00	0.00	Slope B	5.64
			Intercept of b	-0.63
			Sum (X i - X)	79.19
			Sum (X i - X)	446.44
			Sum (Y i -Y)	2528.58



GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Area DBP (149)		Area BBP (149)	ratio m/z DBP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantiy of DBP (g)	Quantity of DBP/100 g
		strip 7	38DBP1A		0.78	11527200		154589	74.57	13.32	0.03	0.34	43.56
		strip 7	38DBP1B		0.73	10680907		159935	66.78	12.02	0.03	0.31	42.00
		strip 7	38DBP2A		0.80	12061369		161935	74.48	13.31	0.03	0.34	42.43
		strip 7	38DBP2B		0.73	11011882		167696	65.67	11.84	0.03	0.30	41.35
		strip 7	38DBP3A		0.71	9925510		157415	63.05	11.40	0.03	0.29	40.95
		strip 7	38DBP3B		0.74	10873783		163685	66.43	11.97	0.03	0.31	41.23
		strip 11	35DBP1A		0.65	4024035		75684	53.17	9.79	0.03	0.25	38.40
		strip 11	35DBP1B		0.67	3479629		73678	47.23	8.81	0.03	0.22	33.53
		strip 11	35DBP2A		0.59	3109189		73623	42.23	7.99	0.03	0.20	34.53
		strip 11	35DBP2B		0.66	3586114		73371	48.88	9.08	0.03	0.23	35.09
		strip 11	35DBP3A		0.70	4198873		70885	59.24	10.79	0.03	0.28	39.29
		strip 11	35DBP3B		0.58	3074106		73453	41.85	7.93	0.03	0.20	34.85
		strip 12	15BIN1A		0.73	233966		72867	3.21	1.57	0.03	0.04	5.50
		strip 12	15BIN1B		0.66	197652		71483	2.77	1.50	0.03	0.04	5.80
		strip 13	38BIN1A		0.69	533120		69879	7.63	2.30	0.03	0.06	8.50
		strip 13	38BIN1B		0.78	453123		79802	5.68	1.98	0.03	0.05	6.47
		strip 13	38BIN2A		0.71	578861		78642	7.36	2.26	0.03	0.06	8.10
		strip 13	38BIN2B		0.57	612831		74982	8.17	2.39	0.03	0.06	10.69
		strip 13	38BIN3A		0.73	505590		69868	7.24	2.24	0.03	0.06	7.81
		strip 13	38BIN3B		0.75	595664		72115	8.26	2.40	0.03	0.06	8.17

HPLC analyses Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height DBP	Solution conc. (µg/ml)	Conc. factor	Quantiy of DBP (g)	Quantity of DBP/100 g
		strip 7	38DBP1A		0.78	68.49	12.26	0.03	0.31	40.08
		strip 7	38DBP1B		0.73	66.57	11.92	0.03	0.30	41.64
		strip 7	38DBP2A		0.80	74.87	13.39	0.03	0.34	42.69
		strip 7	38DBP2B		0.73	65.79	11.78	0.03	0.30	41.15
		strip 7	38DBP3A		0.71	62.43	11.19	0.03	0.29	40.17
		strip 7	38DBP3B		0.74	64.05	11.47	0.03	0.29	39.53
		strip 11	35DBP1A		0.65	54.00	9.69	0.03	0.25	38.01
		strip 11	35DBP1B		0.67	52.34	9.40	0.03	0.24	35.76
		strip 11	35DBP2A		0.59	45.72	8.22	0.03	0.21	35.53
		strip 11	35DBP2B		0.66	52.74	9.47	0.03	0.24	36.57
		strip 11	35DBP3A		0.70	58.00	10.40	0.03	0.27	37.88
		strip 11	35DBP3B		0.58	46.18	8.30	0.03	0.21	36.50
		strip 12	15BIN1A		0.73	6.20	1.21	0.03	0.03	4.23
		strip 12	15BIN1B		0.66	5.51	1.09	0.03	0.03	4.21
		strip 13	38BIN1A		0.69	12.38	2.31	0.03	0.06	8.53
		strip 13	38BIN1B		0.78	10.48	1.97	0.03	0.05	6.44
		strip 13	38BIN2A		0.71	13.28	2.47	0.03	0.06	8.86
		strip 13	38BIN2B		0.57	13.69	2.54	0.03	0.06	11.36
		strip 13	38BIN3A		0.73	11.53	2.16	0.03	0.05	7.53
		strip 13	38BIN3B		0.75	13.51	2.51	0.03	0.06	8.53

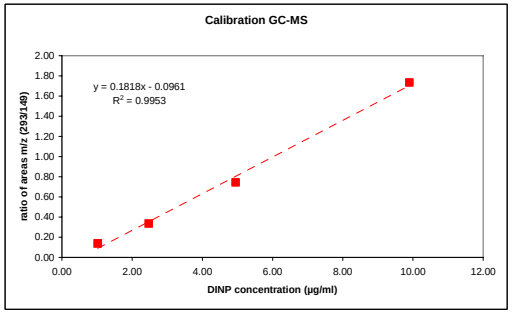
Calibration and homogeneity from phase 2 – samples.

GC-MS analyses

DINP

Calibration curve					
Target DINP conc. (µg/ml)	Exact DINP conc. (µg/ml), Xi	Exact BBP conc. (~1 µg/ml)	Area DINP (293)	Area BBP (149)	ratio m/z DINP/BBP
1	1.02	1	8016	58206	0.14
2.5	2.48	1	23838	70797	0.34
5	4.95	1	51933	69816	0.74
10	9.90	1	93186	53756	1.73

Calibration graph 1			Statistic calculations	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration points	4.00
1.04	0.02	0.14	Sum X i	18.35
6.13	0.11	0.83	Sum X i ^2	129.68
24.50	0.55	3.68	Sum Y i	2.95
98.01	3.01	17.16	Sum Yi^2	3.69
			Sum X i * Y i	21.82
			Mean value X	4.59
			Mean value Y	0.74
			Slope B	0.18
			Intercept of blank	-0.10
			Sum (X i - X) ^2	45.53
			Sum (X i - X) * (Y i - Y)	8.28
			Sum (Y i -Y) ^2	1.51

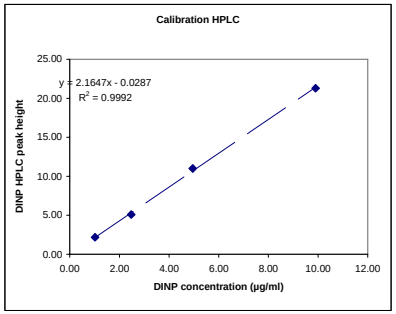


HPLC analyses

DINP

Calibration curve		
Target DINP conc. (µg/ml)	Exact DINP conc. Xi (µg/ml)	Height DINP, Yi
1	1.02	2.20
2.5	2.48	5.10
5	4.95	11.00
10	9.90	21.30

Calibration graph 1			Statistic calculati	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration points	4.00
1.04	4.84	2.25	Sum X i	18.35
6.13	26.01	12.62	Sum X i ^2	129.68
24.50	121.00	54.45	Sum Y i	39.60
98.01	453.69	210.87	Sum Yi^2	605.54
			Sum X i * Y i	280.19
			Mean value X	4.59
			Mean value Y	9.90
			Slope B	2.16
			Intercept of blank	-0.03
			Sum (X i - X) ^2	45.53
			Sum (X i - X) * (Y	98.56
			Sum (Y i -Y) ^2	213.50



GC-MS analyses - Homogeneity

	Sample code	specimen code	Sample weight (g)	Area DINP (293)	Area DIDP (307)	Area BBP (149)	ratio m/z DINP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantity of DINP (g) for sample	Quantity of DINP/100 g of sample (%)
	strip REF	38DINP1A	0.6657	105088	24254	55779	1.88	10.89	0.03	0.28	41.71
	strip REF	38DINP1B	0.6364	86082	20852	46724	1.84	10.66	0.03	0.27	42.72
	strip REF	38DINP2A	0.7637	105150	23890	50333	2.09	12.02	0.03	0.31	40.13
	strip REF	38DINP2B	0.7647	144964	35277	60714	2.39	13.66	0.03	0.35	45.55
	strip REF	38DINP3A	0.7634	141887	34735	57505	2.47	14.10	0.03	0.36	47.09
	strip REF	38DINP3B	0.5960	86832	20706	51631	1.68	9.78	0.03	0.25	41.83
2.9	25BIN1A	0.9025	75563	20593	61641	1.23	7.27	0.03	0.19	20.54
2.9	25BIN1B	0.7948	72503	18061	72431	1.00	6.03	0.03	0.15	19.36
2.9	25BIN2A	0.8431	51861	13423	46253	1.12	6.69	0.03	0.17	20.25
2.9	25BIN2B	0.8468	48710	12644	50113	0.97	5.87	0.03	0.15	17.69
2.9	25BIN3A	0.8756	73919	18668	60618	1.22	7.23	0.03	0.18	21.07
2.9	25BIN3B	0.8115	91668	25967	83693	1.10	6.55	0.03	0.17	20.59
2.10	45BIN1A	0.8182	150025	39142	63340	2.37	13.55	0.03	0.35	42.24
2.10	45BIN1B	0.7794	151138	39704	69071	2.19	12.56	0.03	0.32	41.10
2.10	45BIN2A	0.8647	169787	44139	68775	2.47	14.11	0.03	0.36	41.60
2.10	45BIN2B	0.8303	155659	39602	66837	2.33	13.34	0.03	0.34	40.96
2.10	45BIN3A	0.9244	128702	34282	47816	2.69	15.33	0.03	0.39	42.29
2.10	45BIN3B	0.8138	132579	33723	58667	2.26	12.96	0.03	0.33	40.60

HPLC analyses Homogeneity

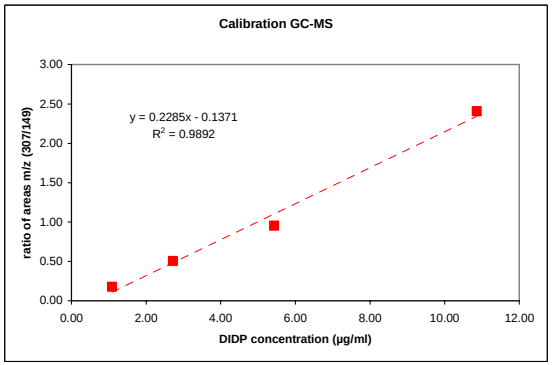
Sample code	specimen code	Sample weight (g)	Height DINP	Solution conc. (µg/ml)	Conc. factor	Quantity of DINP (g) for sample	Quantity of DINP/100 g of sample (%)
strip REF	38DINP1A	0.6657	23.80	11.01	0.03	0.28	42.17
strip REF	38DINP1B	0.6364	22.00	10.18	0.03	0.26	40.78
strip REF	38DINP2A	0.7637	26.20	12.12	0.03	0.31	40.46
strip REF	38DINP2B	0.7647	26.20	12.12	0.03	0.31	40.41
strip REF	38DINP3A	0.7634	27.30	12.62	0.03	0.32	42.17
strip REF	38DINP3B	0.5960	22.30	10.32	0.03	0.26	44.13
2.9	25BIN1A	0.9025	16.60	7.68	0.03	0.20	21.71
2.9	25BIN1B	0.7948	16.10	7.45	0.03	0.19	23.91
2.9	25BIN2A	0.8431	15.80	7.31	0.03	0.19	22.12
2.9	25BIN2B	0.8468	15.95	7.38	0.03	0.19	22.22
2.9	25BIN3A	0.8756	15.60	7.22	0.03	0.18	21.03
2.9	25BIN3B	0.8115	15.30	7.08	0.03	0.18	22.25
2.10	45BIN1A	0.8182	26.10	12.07	0.03	0.31	37.62
2.10	45BIN1B	0.7794	24.90	11.52	0.03	0.29	37.68
2.10	45BIN2A	0.8647	32.50	15.03	0.03	0.38	44.32
2.10	45BIN2B	0.8303	25.40	11.75	0.03	0.30	36.08
2.10	45BIN3A	0.9244	31.50	14.57	0.03	0.37	40.18
2.10	45BIN3B	0.8138	28.80	13.32	0.03	0.34	41.73

GC-MS analyses

DIDP

Calibration curve						
Target DINP conc. (µg/ml)	Exact DIDP conc. (µg/ml), Xi	Exact BBP conc. (~1 µg/ml)	Area DINP (293)	Area DIDP (307)	Area BBP (149)	ratio m/z DIDP/BBP
1	1.09	1.00		10319	57864	0.18
2.5	2.72	1.00		22533	44667	0.50
5	5.43	1.00		64952	68089	0.95
10	10.86	1.00		163271	67818	2.41

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	4.00
1.18	0.03	0.19	Sum X i	20.10
7.42	0.25	1.37	Sum X i ^2	156.03
29.48	0.91	5.18	Sum Y i	4.04
117.94	5.80	26.15	Sum Yi^2	6.99
			Sum X i * Y	32.89
			Mean value	5.03
			Mean value	1.01
			Slope B	0.23
			Intercept of bi	-0.14
			Sum (X i - X)	55.02
			Sum (X i - X)	12.57
			Sum (Y i -Y)	2.90

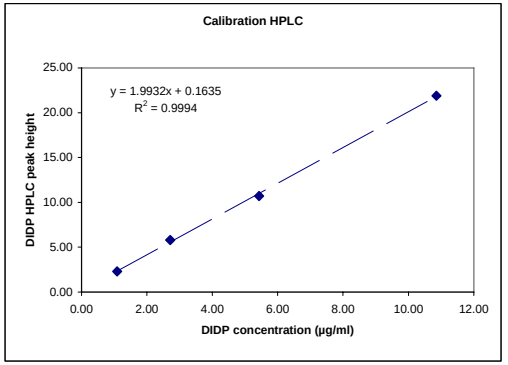


HPLC analyses

DIDP

Calibration curve		
Target DINP conc. (µg/ml)	Exact DIDP conc. Xi (µg/ml)	Height DIDP, Yi
0	0.00	
1	1.09	2.30
2.5	2.72	5.80
5	5.43	10.70
10	10.86	21.90

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration pc	4.00
0.00	0.00	0.00	Sum X i	20.09
1.18	5.29	2.50	Sum X i ^2	155.98
7.37	33.64	15.75	Sum Y i	40.70
29.48	114.49	58.10	Sum Yi^2	633.03
117.94	479.61	237.83	Sum X i * Y	314.18
0.00	0.00	0.00	Mean value	5.02
0.00	0.00	0.00	Mean value	10.18
0.00	0.00	0.00	Slope B	1.99
			Intercept of bi	0.16
			Sum (X i - X)	55.06
			Sum (X i - X)	109.75
			Sum (Y i -Y)	218.91



GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample weight (g)	Area DINP (293)	Area DIDP (307)	Area BBP (149)	ratio m/z DIDP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantity of DIDP (g) for sample	Quantity of DIDP/100 g of sample (%)
		2.1	25DIDP1A	0.8415		74726	47219	1.58	7.53	0.03	0.19	22.81
		2.1	25DIDP1B	0.8076		108935	67073	1.62	7.71	0.03	0.20	24.34
		2.1	25DIDP2A	0.8879		154121	80797	1.91	8.95	0.03	0.23	25.70
		2.1	25DIDP2B	0.7546		90362	63489	1.42	6.83	0.03	0.17	23.08
		2.1	25DIDP3A	0.8087		104375	65250	1.60	7.60	0.03	0.19	23.97
		2.1	25DIDP3B	0.8403		106780	59962	1.78	8.39	0.03	0.21	25.47
		2.5	45DIDP1A	0.8419		222210	58768	3.78	17.15	0.03	0.44	51.94
		2.5	45DIDP1B	0.7308		372651	112129	3.32	15.15	0.03	0.39	52.85
		2.5	45DIDP2A	0.8218		335006	84791	3.95	17.89	0.03	0.46	55.52
		2.5	45DIDP2B	0.8262		254616	68245	3.73	16.93	0.03	0.43	52.25
		2.5	45DIDP3A	0.8023		255212	72888	3.50	15.93	0.03	0.41	50.62
		2.5	45DIDP3B	0.7851		293819	82855	3.55	16.12	0.03	0.41	52.36

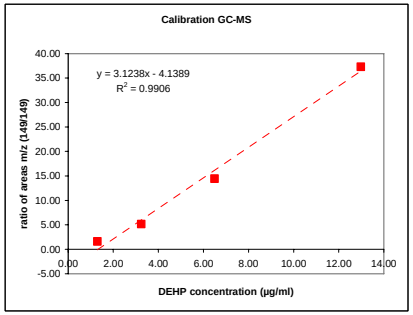
HPLC analyses Homogeneity

	Sample code	specimen code	Sample weight (g)	Height DIDP	Solution conc. (µg/ml)	Conc. factor	Quantity of DIDP (g) for sample	Quantity of DIDP/100 g of sample (%)
	2.1	25DIDP1A	0.8415	16.10	8.00	0.03	0.20	24.23
	2.1	25DIDP1B	0.8076	16.06	7.98	0.03	0.20	25.19
	2.1	25DIDP2A	0.8879	20.00	9.95	0.03	0.25	28.58
	2.1	25DIDP2B	0.7546	17.60	8.75	0.03	0.22	29.56
	2.1	25DIDP3A	0.8087	16.00	7.95	0.03	0.20	25.05
	2.1	25DIDP3B	0.8403	16.90	8.40	0.03	0.21	25.48
	2.5	45DIDP1A	0.8419	43.70	21.84	0.03	0.56	66.16
	2.5	45DIDP1B	0.7308	26.70	13.31	0.03	0.34	46.45
	2.5	45DIDP2A	0.8218	25.53	12.73	0.03	0.32	39.49
	2.5	45DIDP2B	0.8262	44.70	22.34	0.03	0.57	68.96
	2.5	45DIDP3A	0.8023	27.70	13.81	0.03	0.35	43.91
	2.5	45DIDP3B	0.7851	28.00	13.97	0.03	0.36	45.36

GC-MS analyses

DEHP

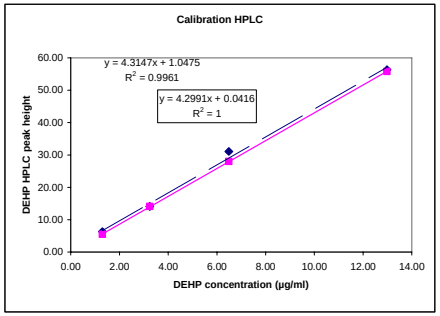
Calibration curve							Calibration graph 1			Statistic calcu	Curve 1
Target DINP conc. (µg/ml)	Exact DEHP conc. (µg/ml), Xi	Exact BBP conc. (~1 µg/ml)		Area DEHP (149)	Area BBP (149)	ratio m/z DEHP/BBP	X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
										Sum X i	24.01
1	1.30	1.00		77189	48771	1.58	1.68	2.50	2.05	Sum X i ^2	222.82
2.5	3.25	1.00		260853	50722	5.14	10.53	26.45	16.69	Sum Y i	58.46
5	6.49	1.00		772235	53567	14.42	42.12	207.83	93.56	Sum Yi^2	1629.09
10	12.98	1.00		1561241	41841	37.31	168.48	1392.31	484.33	Sum X i * Y	596.64
										Mean value	6.00
										Mean value	14.61
										Slope B	3.12
										Intercept of bi	-4.14
										Sum (X i - X)	78.66
										Sum (X i - X)	245.71
										Sum (Y i -Y)	774.83



HPLC analyses

DEHP

Calibration curve			Calibration graph 1			Statistic calcu	Curve 1
Target DINP conc. (µg/ml)	Exact DEHP conc. Xi (µg/ml)	Height DEHP, Yi	X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
0	0.00		0.00	0.00	0.00	Sum X i	24.01
1	1.30	6.30	1.68	39.69	8.18	Sum X i ^2	222.82
2.5	3.25	14.10	10.53	198.81	45.75	Sum Y i	107.80
5	6.49	31.10	42.12	967.21	201.84	Sum Yi^2	4375.40
10	12.98	56.30	168.48	3169.69	730.77	Sum X i * Y	986.54
			0.00	0.00	0.00	Mean value	6.00
			0.00	0.00	0.00	Mean value	26.95
			0.00	0.00	0.00	Slope B	4.31
						Intercept of bi	1.05
						Sum (X i - X)	78.66
						Sum (X i - X)	339.39
						Sum (Y i -Y)	1470.19



Calibration graph 2			Statistic calcu	Curve 2
X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
0.00	0.00	0.00	Sum X i	24.01
1.68	30.25	7.14	Sum X i ^2	222.82
10.53	198.81	45.75	Sum Y i	103.40
42.12	784.00	181.72	Sum Yi^2	4126.70
168.48	3113.64	724.28	Sum X i * Y	958.90
0.00	0.00	0.00	Mean value	6.00
0.00	0.00	0.00	Mean value	25.85
0.00	0.00	0.00	Slope B	4.30
			Intercept of bi	0.04
			Sum (X i - X)	78.66
			Sum (X i - X)	338.16
			Sum (Y i -Y)	1453.81

Calibration curve		
Target DINP	Exact DEHP	Height
0	0.00	
1	1.30	5.50
2.5	3.25	14.10
5	6.49	28.00
10	12.98	55.80

GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample weight (g)	Area DEHP (149)	Area BBP (149)	ratio m/z DEHP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantity of DEHP (g) for sample	Quantity of DEHP/100 g of sample (%)
		2.2	25DEHP1A	0.7819	1191928	60840	19.59	7.60	0.03	0.19	24.77
		2.2	25DEHP1B	0.9203	1321197	56252	23.49	8.84	0.03	0.23	24.50
		2.2	25DEHP2A	0.8227	1435672	67520	21.26	8.13	0.03	0.21	25.20
		2.2	25DEHP2B	0.8664	1461728	61222	23.88	8.97	0.03	0.23	26.40
		2.2	25DEHP3A	0.7917	1981585	95069	20.84	8.00	0.03	0.20	25.76
		2.2	25DEHP3B	0.8995	2867871	111990	25.61	9.52	0.03	0.24	27.00
		2.6	45DEHP1A	0.7259	1858583	45428	40.91	14.42	0.03	0.37	50.66
		2.6	45DEHP1B	0.8775	2712241	52211	51.95	17.95	0.03	0.46	52.18
		2.6	45DEHP2A	0.8157	2472911	52659	46.96	16.36	0.03	0.42	51.14
		2.6	45DEHP2B	0.8618	1855364	37617	49.32	17.11	0.03	0.44	50.64
		2.6	45DEHP3A	0.8205	1865891	40776	45.76	15.97	0.03	0.41	49.64

HPLC analyses - Homogeneity

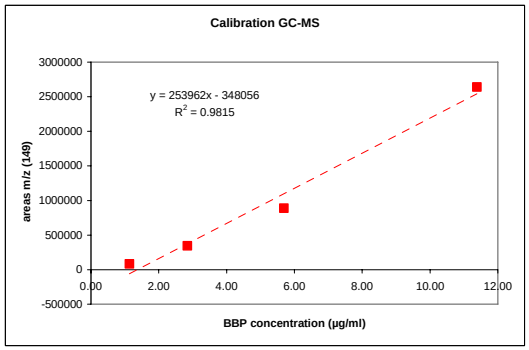
		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height DEHP	Solution conc. (µg/ml)	Conc. factor	Quantity of DEHP (g)	Quantity of DEHP /100 g of sample
		2.2	25DEHP1A		0.78	32.50	7.29	0.03	0.19	23.77
		2.2	25DEHP1B		0.92	37.10	8.36	0.03	0.21	23.15
		2.2	25DEHP2A		0.82	32.50	7.29	0.03	0.19	22.59
		2.2	25DEHP2B		0.87	37.80	8.52	0.03	0.22	25.07
		2.2	25DEHP3A		0.79	33.90	7.61	0.03	0.19	24.52
		2.2	25DEHP3B		0.90	36.60	8.24	0.03	0.21	23.36
		2.6	45DEHP1A		0.73	47.40	11.02	0.03	0.28	38.70
		2.6	45DEHP1B		0.88	57.50	13.37	0.03	0.34	38.84
		2.6	45DEHP2A		0.82	54.90	12.76	0.03	0.33	39.89
		2.6	45DEHP2B		0.86	56.10	13.04	0.03	0.33	38.58
		2.6	45DEHP3A		0.82	53.20	12.37	0.03	0.32	38.43

GC-MS analyses

BBP

Calibration curve						
Target DINP conc. (µg/ml)		Exact BBP conc. (~1 µg/ml)			Area BBP (149)	
0						
1		1.14			81757	
2.5		2.85			344166	
5		5.69			888997	
10		11.38			2639529	

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
0.00	0.00	0.00	Sum X i	21.05
1.30	6.68E+09	9.30E+04	Sum X i ^2	171.27
8.09	6.97E+12	7.51E+06	Sum Y i	3.95E+06
32.38	7.90E+11	5.06E+06	Sum Yi^2	1.47E+13
129.50	6.97E+12	3.00E+07	Sum X i * Y	4.27E+07
0.00	0.00	0.00	Mean value	5.26
0.00	0.00	0.00	Mean value	988612.25
0.00	0.00	0.00	Slope B	361968.61
			Intercept of b	-9.17E+05
			Sum (X i - X)	60.46
			Sum (X i - X)	2.19E+07
			Sum (Y i -Y)	1.08E+13

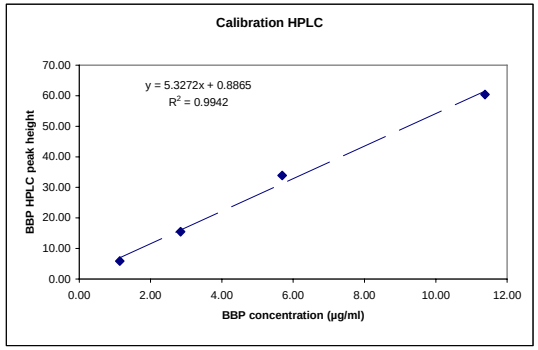


HPLC analyses

BBP

Calibration curve			
Target DINP conc. (µg/ml)	Exact BBP conc. Xi (µg/ml)	Height BBP, Yi	
0	0.00		
1	1.14	5.90	
2.5	2.85	15.50	
5	5.69	33.90	
10	11.38	60.40	

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
0.00	0.00	0.00	Sum X i	21.05
1.30	34.81	6.71	Sum X i ^2	171.27
8.09	240.25	44.10	Sum Y i	115.70
32.38	1149.21	192.89	Sum Yi^2	5072.43
129.50	3648.16	687.35	Sum X i * Y	931.05
0.00	0.00	0.00	Mean value	5.26
0.00	0.00	0.00	Mean value	28.93
0.00	0.00	0.00	Slope B	5.33
			Intercept of b	0.89
			Sum (X i - X)	60.46
			Sum (X i - X)	322.10
			Sum (Y i -Y)	1725.81



GC-MS analyses - Homogeneity

		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)				Area BBP (149)	Solution conc. (µg/ml)	Conc. factor	Quantity of BBP (g) for sample	Quantity of BBP/100 g of sample (%)
		2.3	25BBP1A		0.7947				940692	5.13	0.03	0.13	16.46
		2.3	25BBP1B		0.7834				919330	5.07	0.03	0.13	16.51
		2.3	25BBP2A		0.6926				591127	4.17	0.03	0.11	15.34
		2.3	25BBP2B		0.8482				793505	4.72	0.03	0.12	14.20
		2.3	25BBP3A		0.7107				628107	4.27	0.03	0.11	15.31
		2.3	25BBP3B		0.8391				802071	4.75	0.03	0.12	14.43
		2.7	45BBP1A		0.8958				2049807	8.19	0.03	0.21	23.33
		2.7	45BBP1B		0.8384				1528467	6.75	0.03	0.17	20.54
		2.7	45BBP2A		0.8987				3804441	13.04	0.03	0.33	37.01
		2.7	45BBP2B		0.8147				1568979	6.87	0.03	0.18	21.49
		2.7	45BBP3A		0.8358				2087042	8.30	0.03	0.21	25.32
		2.7	45BBP3B		0.8784				2492742	9.42	0.03	0.24	27.34

HPLC analyses Homogeneity

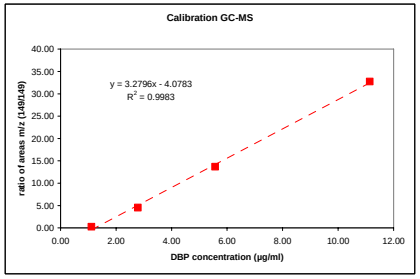
		Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height BBP	Solution conc. (µg/ml)	Conc. factor	Quantity ofBBP (g) for sample	Quantity of BBP/100 g of sample (%)
		2.3	25BBP1A		0.79	39.80	7.30	0.03	0.19	23.44
		2.3	25BBP1B		0.78	40.10	7.36	0.03	0.19	23.96
		2.3	25BBP2A		0.69	34.70	6.35	0.03	0.16	23.37
		2.3	25BBP2B		0.85	42.90	7.89	0.03	0.20	23.71
		2.3	25BBP3A		0.71	31.10	5.67	0.03	0.14	20.35
		2.3	25BBP3B		0.84	41.40	7.60	0.03	0.19	23.11
		2.7	45BBP1A		0.90	73.50	13.63	0.03	0.35	38.80
		2.7	45BBP1B		0.84	79.30	14.72	0.03	0.38	44.77
		2.7	45BBP2A		0.90	79.20	14.70	0.03	0.37	41.71
		2.7	45BBP2B		0.81	75.90	14.08	0.03	0.36	44.07
		2.7	45BBP3A		0.84	76.90	14.27	0.03	0.36	43.53
		2.7	45BBP3B		0.88	79.20	14.70	0.03	0.37	42.68

GC-MS analyses

DBP

Calibration curve1						
Target DINP conc. (µg/ml)	Exact DBP conc. (µg/ml), Xi	Exact BBP conc. (~1 µg/ml)	Area DBP (149)		Area BBP (149)	ratio m/z DBP/BBP
1	1.11	1.00	21906		81085	0.27
2.5	2.79	1.00	351194		77209	4.55
5	5.57	1.00	997517		72820	13.70
10	11.14	1.00	2836904		86599	32.76

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
1.24	0.07	0.30	Sum X i	20.61
7.76	20.69	12.67	Sum X i ^2	164.12
31.02	187.65	76.30	Sum Y i	51.28
124.10	1073.16	364.94	Sum Yi^2	1281.57
			Sum X i * Y	454.21
			Mean value	5.15
			Mean value	12.82
			Slope B	3.28
			Intercept of b	-4.08
			Sum (X i - X)	57.94
			Sum (X i - X)	190.02
			Sum (Y i -Y)	624.25

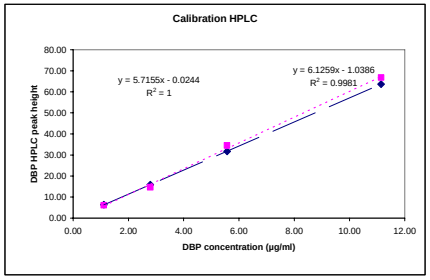


HPLC analyses

DBP

Calibration curve		
Target DINP conc. (µg/ml)	Exact DBP conc. Xi (µg/ml)	Height DBP, Yi
1	1.11	6.40
2.5	2.79	15.90
5	5.57	31.70
10	11.14	63.70

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
1.23	40.96	7.10	Sum X i	20.61
7.78	252.81	44.36	Sum X i ^2	164.14
31.02	1004.89	176.57	Sum Y i	117.70
124.10	4057.69	709.62	Sum Yi^2	5356.35
			Sum X i * Y	937.65
			Mean value	5.15
			Mean value	29.43
			Slope B	5.72
			Intercept of b	-0.02
			Sum (X i - X)	57.95
			Sum (X i - X)	331.20
			Sum (Y i -Y)	1893.03



Calibration curve		
Target DINP	Exact DBP	Height DBP
1	1.11	6.10
2.5	2.79	14.70
5	5.57	34.50
10	11.14	66.80

Calibration graph 1			Statistic calcu	Curve 1
X i ^2	Y i ^2	X i * Y i	Calibration po	4.00
1.23	37.21	6.77	Sum X i	20.61
7.78	216.09	41.01	Sum X i ^2	164.14
31.02	1190.25	192.17	Sum Y i	122.10
124.10	4462.24	744.15	Sum Yi^2	5905.79
			Sum X i * Y	984.10
			Mean value	5.15
			Mean value	30.53
			Slope B	6.13
			Intercept of b	-1.04
			Sum (X i - X)	57.95
			Sum (X i - X)	354.98
			Sum (Y i -Y)	2178.69

GC-MS analyses - Homogeneity

	Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Area DBP (149)		Area BBP (149)	ratio m/z DBP/BBP	Solution conc. (µg/ml)	Conc. factor	Quantiy of DBP (g)	Quantiy of DBP/100 g
	2.4	25DBP1A		0.8776	5939427		107544	55.23	18.08	0.03	0.46	52.54
	2.4	25DBP1B		0.8352	3917661		72664	53.91	17.68	0.03	0.45	53.99
	2.4	25DBP2A		0.7976	3371777		66017	51.07	16.82	0.03	0.43	53.77
	2.4	25DBP2B		0.8410	4063753		80954	50.20	16.55	0.03	0.42	50.18
	2.4	25DBP3A		0.8129	4297189		89069	48.25	15.95	0.03	0.41	50.05
	2.4	25DBP3B		0.8337	4585228		96151	47.69	15.78	0.03	0.40	48.28
	2.8	45DBP1A		0.8550	7922841		65490	120.98	38.13	0.03	0.97	113.73
	2.8	45DBP1B		0.7570	6574128		65657	100.13	31.77	0.03	0.81	107.03
	2.8	45DBP2A		0.7778	10186433		103061	98.84	31.38	0.03	0.80	102.88
	2.8	45DBP2B		0.8328	8839855		80816	109.38	34.60	0.03	0.88	105.93
	2.8	45DBP3A		0.8089	8800395		83099	105.90	33.53	0.03	0.86	105.72
	2.8	45DBP3B		0.8298	9531537		90007	105.90	33.53	0.03	0.86	103.05
	2.9	25BIN1A		0.90	430022		61641	6.98	3.37	0.03	0.09	9.52
	2.9	25BIN1B		0.79	448221		72431	6.19	3.13	0.03	0.08	10.04
	2.9	25BIN2A		0.84	354730		46253	7.67	3.58	0.03	0.09	10.83
	2.9	25BIN2B		0.85	409037		50113	8.16	3.73	0.03	0.10	11.24
	2.9	25BIN3A		0.88	466925		60618	7.70	3.59	0.03	0.09	10.46
	2.9	25BIN3B		0.81	595479		83693	7.12	3.41	0.03	0.09	10.72
	2.10	45BIN1A		0.82	912758		63340	14.41	5.64	0.03	0.14	17.57
	2.10	45BIN1B		0.78	1004257		69071	14.54	5.68	0.03	0.14	18.57
	2.10	45BIN2A		0.86	1105491		68775	16.07	6.14	0.03	0.16	18.12
	2.10	45BIN2B		0.83	1051246		66837	15.73	6.04	0.03	0.15	18.55
	2.10	45BIN3A		0.92	860923		47816	18.00	6.73	0.03	0.17	18.57
	2.10	45BIN3B		0.81	896581		58667	15.28	5.90	0.03	0.15	18.50

HPLC analyses Homogeneity

	Sample code	specimen code	Sample dimension (D*h)	Sample weight (g)	Height DBP	Solution conc. (µg/ml)	Conc. factor	Quantiy of DBP (g)	Quantiy of DBP/100 g
	2.4	25DBP1A		0.88	41.70	6.98	0.03	0.18	20.27
	2.4	25DBP1B		0.84	40.40	6.76	0.03	0.17	20.65
	2.4	25DBP2A		0.80	39.40	6.60	0.03	0.17	21.10
	2.4	25DBP2B		0.84	40.20	6.73	0.03	0.17	20.41
	2.4	25DBP3A		0.81	39.40	6.60	0.03	0.17	20.71
	2.4	25DBP3B		0.83	40.60	6.80	0.03	0.17	20.79
	2.8	45DBP1A		0.86	73.70	12.20	0.03	0.31	36.39
	2.8	45DBP1B		0.76	65.20	10.81	0.03	0.28	36.42
	2.8	45DBP2A		0.78	67.50	11.19	0.03	0.29	36.66
	2.8	45DBP2B		0.83	71.40	11.83	0.03	0.30	36.21
	2.8	45DBP3A		0.81	71.80	11.89	0.03	0.30	37.46
	2.8	45DBP3B		0.83	72.90	12.07	0.03	0.31	37.09
	2.9	25BIN1A		0.90	9.30	1.63	0.03	0.04	4.61
	2.9	25BIN1B		0.79	9.00	1.58	0.03	0.04	5.07
	2.9	25BIN2A		0.84	9.60	1.68	0.03	0.04	5.09
	2.9	25BIN2B		0.85	9.84	1.73	0.03	0.04	5.20
	2.9	25BIN3A		0.88	8.90	1.56	0.03	0.04	4.55
	2.9	25BIN3B		0.81	8.80	1.54	0.03	0.04	4.85
	2.10	45BIN1A		0.82	16.10	2.82	0.03	0.07	8.79
	2.10	45BIN1B		0.78	15.20	2.66	0.03	0.07	8.71
	2.10	45BIN2A		0.86	20.20	3.54	0.03	0.09	10.43
	2.10	45BIN2B		0.83	15.70	2.75	0.03	0.07	8.42
	2.10	45BIN3A		0.92	19.30	3.38	0.03	0.09	9.33
	2.10	45BIN3B		0.81	18.50	3.24	0.03	0.08	10.10

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Unit J02 : Food Products

Standard Operation Procedure for the Determination of Release of Di-Isononylphthalate (DINP) in Saliva Simulant from Toys and Childcare Articles using a Head Over Heels Dynamic Agitation Device

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Institute for Health and Consumer Protection - IHCP

Unit J02 : Food Products

Standard Operation Procedure for the Determination of Release of Di-Isononylphthalate (DINP) in Saliva Simulant from Toys and Childcare Articles using a Head Over Heels Dynamic Agitation Device

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WARNING:

Phthalates are widely spread in the environment. They may be present in many solvents of analytical quality as well as in water. To avoid contamination the use of PVC tubing, pipeting balloons, rubber tubing etc. should be avoided. All plastic materials are to be suspected for the presence of interfering components, therefore the absence of potential interferences should be established before running analysis (using blank runs).
Glassware should be thoroughly cleaned before use, preferably with a suitable, pure organic solvent.

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0 Introduction

Phthalates are commonly used as plasticisers in soft PVC articles such as toys and childcare articles, which may contain up to 50% of them. During use of the articles by babies, these plasticisers may leach into the saliva and consequently expose them to phthalates. Considering the low body weight and the age of the babies, the exposure to phthalates should not exceed a tolerable daily intake. Previous in-vivo studies on human volunteers (Dutch Consensus Group) using standard PVC disks containing di-isononylphthalate (DINP) as plasticiser have indicated the range of release that could be expected in-vivo for DINP on standard materials. Thus there is now a need to develop in-vitro mechanically based methodologies to test toys and childcare articles which will be able to simulate the range of releases observed in vivo.

The methods allow the identification and quantification of DINP in artificial saliva after mechanical agitation with plasticised PVC. A “head over heels” device

was developed based on a rotational agitation principle originating in the Netherlands (Nutrition and Food Research Institute, TNO). The method was optimised by the European Commission Joint Research Centre (JRC) and validated with Member States' laboratories. DINP was taken as model since it is currently the most commonly used phthalate in PVC toys and childcare articles.

1 Scope

This SOP describes a mechanical extraction procedure that is suitable for the determination of the release of plasticisers from baby toys using 2 analytical procedures to quantify the amount of phthalate released in simulated saliva. The methods described in this SOP allow the identification and quantification of DINP in artificial saliva after mechanical agitation with plasticised PVC.

The mechanical procedure was calibrated against data obtained from in-vivo studies with plasticised PVC samples. The conditions of shaking were defined to obtain values above the mean migration of DINP of the Dutch Consensus in-vivo study.

The analytical part of the method (extraction and analysis) used both HPLC and GC-MS. The GC-MS system allows for simultaneous identification of phthalate esters and their quantification as long as calibration curves have been established for the phthalates considered. An analysis on HPLC system was used to verify the quantification, since the system not capable at this point to discriminate between DINP and 2 other phthalate esters, namely di-isodecyl phthalate (DIDP) and di-ethylhexylphthalate (DEHP).

2 Principle

The test sample is mechanically treated with a saliva simulant solution made of an aqueous salt solution. After 30 min. of mechanical dynamic treatment have elapsed, the saliva extract is transferred to be extracted, while the flask and specimen

are replenished with fresh saliva simulant, and subjected to another migration treatment. The pooled simulant solution extracts are subjected to two successive extractions using cyclohexane in a separatory funnel. The amount of DINP in the cylohexane solution is determined on GC-MS using a non-polar column, and by normal phase HPLC using UV detection at 225 nm. Quantification is achieved by using an external standard calibration procedure as well as an internal standard.

3 Reagents

WARNING: Phthalates are widely spread in the environment. They may be present in many solvents of analytical quality as well as in water. To avoid contamination the use of PVC tubing, pipeting balloons, rubber tubing etc. should be avoided. All plastic materials are to be suspected for the presence of interfering components, therefore the absence of potential interferences should be established before running analysis (using blank runs).

Glassware should be thoroughly cleaned before use, preferably with a suitable, pure organic solvent.

3.1 Analytes

3.1.1 Di-isononyl-phthalate (DINP)

Molecular weight: 418g/mol, Molecular formula: $C_{26}H_{42}O_4$

NOTE: DINP is a phthalate ester of isononyl alcohol, which is composed of a mixture of branched isomers and may contain in addition minor amounts of C7 up to C11 branched alcohols. Thus it will appear as a complex mixture and may differ between different manufacturers.

3.1.2 Benzylbutyl phthalate, BBP (as internal standard)

3.2 Solvents

3.2.1 Iso-octane (HPLC grade)

3.2.2 Iso-propanol (analytical grade)

3.2.3 Cyclohexane (analytical grade)

3.2.4 Dioxane, HPLC grade (for flushing / cleaning of HPLC column)

3.2.5 Acetone (for pre-rinsing glassware)

3.2.6 Tetrahydrofuran (analytical grade)

3.2.7 Hexane (analytical grade)

3.3 Stock standard DINP solutions

3.3.1 Concentrated stock solution (ca. 5000 µg/ml)

Weigh to the nearest 0.1 mg approximately 100 mg of DINP (3.1.1) in a 20 ml volumetric flask and fill to the mark with cyclohexane (3.2.3). Mix carefully.

Calculate the actual concentration in mg DINP per ml solution.

Repeat the procedure to obtain a second standard stock solution.

3.3.2 Standard stock solution (ca. 500 µg/ml)

Transfer by means of a volumetric glass pipette 5 ml of the concentrated standard solution at 5000 µg/ml (3.3.1) into a 50 ml volumetric and fill to the mark with cyclohexane (3.2.3). Mix carefully and thoroughly.

Calculate the actual concentration in mg DINP per ml solution.

3.3.3 Dilute stock solution (ca. 50 µg/ml)

Transfer by means of a volumetric glass pipette 5 ml of the standard solution at 500 µg/ml (3.3.2) into a 50 ml volumetric and fill to the mark with cyclohexane (3.2.3). Mix carefully and thoroughly.

Calculate the actual concentration in mg DINP per ml solution.

3.4 Calibration samples of DINP for analysis (HPLC, GC-MS) in cyclohexane

Transfer by means of a volumetric glass pipette the volume of the dilute standard solution (3.3.3) into volumetric flasks as indicated in the table below and fill to the mark with cyclohexane (3.2.3). Mix carefully and thoroughly. A high concentration of 50 ppm (directly from 3.3.3) may be used if it is proven to not bring interferences to the GC-MSD system by overloading.

Calculate the actual concentration in mg DINP per ml solution. The solutions thus obtained contain approximately the amount in g of DINP per ml outlined in the "resulting concentration" column of the table.

Add a constant concentration of 1ppm of Internal standard solution of BBP (3.5, solution at 1000µg/ml), the volume to attain this constant concentration for the various dilutions is shown on the right column in the table below.

ml of	solution (µg/ml)	in flask vol*	resulting sol (ug/ml)	µl of Internal Std (3.5) to add
14	50	20	35	20µl
10	50	20	25.0	20µl
6	50	20	15	20µl
4	50	20	10.0	20µl
2	50	20	5.0	20µl
1	50	20	2.5	20µl

NOTE: The above table is an example for preparing the solutions. By taking X ml (first column) of a solution of concentration Y (second column) into a flask of volume Z (third column) and filling to the mark with cyclohexane, one can obtain a resulting solution of V µg/ml (last column) The solutions may be prepared slightly differently, provided the amount of error on the glass pipette used is always minimised and all calibration concentrations come from one dilute standard to trace potential deviations. If the volume of the flasks for dilutions differ from above, the amount of internal standard should be adapted accordingly to remain at 1ppm final concentration in all dilutions.

Transfer approximately 1ml of the standard solutions into an HPLC vial and 1 ml of the standard solution into a GC vials (4.6).

Close the vials with a crimp cap with PTFE-liner.

Avoid any contact of the solution with the crimp cap.

3.5 Internal Standard solution of BBP (ca. 1000 µg/ml and 250µg/ml)

A solution of BBP (3.1.2) is prepared in cyclohexane at 1000µg/ml for spiking into the calibration solutions

A one fourth dilution of the solution above is prepared to obtain a solution at 250µg/ml to be used as internal standard for spiking the saliva samples and blanks post-migration.

3.6 Standard solution of DINP in iso-propanol (ca. 1000 µg/ml) for recovery check

Solutions are prepared in iso-propanol (3.2.2) for spiking purposes in saliva.

Weigh to the nearest 0.1 mg approximately 50 mg of DINP (3.1.1) in a 50 ml volumetric flask and fill to the mark with iso-propanol (3.2.2). Mix carefully.

Calculate the actual concentration in mg DINP per ml solution.

3.7 Saliva simulant chemicals

3.7.1 Water deionised (Milli Q quality)

3.7.2 Calcium chloride, dihydrate, $CaCl_2 .2H_2O$; Mw = 147.02 (e.g Aldrich, 22,350-6*)

3.7.3 Magnesium chloride, hexahydrate, $MgCl_2 .6H_2O$, Mw 203.3 (e.g. Sigma M-9272*)

3.7.4 Potassium carbonate, K_2CO_3, Mw 138.2 (e.g. Sigma P-4879*)

3.7.5 Potassium chloride, KCl, Mw 74.55 (e.g. Sigma P-3911*)

3.7.6 Potassium phosphate, dibasic, trihydrate, $K_2HPO_4.3H_2O$, Mw 228.2 (e.g. Sigma P-5504*)

3.7.7 Sodium chloride, NaCl, Mw 58.44 (e.g. Sigma S-9888*)

3.7.8 Hydrochloric acid, HCl, , dilute solution (e.g. 3M)

3.8 Simulated saliva salt solution

Prepare a solution in water (3.7.1) of the following composition:

Compound	Formula	mmol/l	mg/l
Magnesium chloride	$MgCl_2$	0.82	166.7
Calcium chloride	$CaCl_2$	1.0	147.0
Dipotassium hydrogen phosphate	K_2HPO_4	3.3	753.1
Potassium carbonate	K_2CO_3	3.8	525.2
Sodium chloride	NaCl	5.6	327.3
Potassium chloride	KCl	10.0	745.5

Weigh the required amount of salts taking into account the presence of water of crystallisation.

NOTE: The above table shows the amounts to be weighed for one litre taking into account the water of crystallisation.

Dissolve the potassium and sodium salts in ca. 900 ml distilled water (3.7.1), then add the calcium and magnesium salts.
Adjust the pH to 6.8 with diluted hydrochloric acid (3.7.8). Transfer to a 1 litre volumetric flask and fill to the mark with distilled water (3.7.1).
Store the solution in the dark.

NOTE: The solution should not be used for more than two weeks. After that time, a fresh solution should be prepared.

3.9 Sodium sulphate, anhydrous (as moisture trap)

4 Apparatus

NOTE: An item is listed only where it is special or made to a particular specification, the usual laboratory glassware and equipment being assumed to be available.

NOTE: Screw caps both new and re-used as well as crimp caps may contain interfering components. Contact of the lining of the crimp cap with the organic solution should be avoided at any time.

NOTE: Cleaning of metal implements: metal implements should be thoroughly cleaned, washed with acetone, and rinsed with iso octane before use and left to dry thoroughly. Examples of metal implements (not exhaustive): hollow punching press, stainless steel balls, tweezers, paper clips.

4.1 Metal stainless steel tweezers

4.2 Punching press

Capable of producing 23mm+/-0.2mm diameter disks from 1mm thick PVC sheeting

4.3 Conical glass separatory funnel of 250ml with glass stopper and Teflon faucet

NOTE: Separatory funnels using a glass faucet which requires the use of silicone and an O rubber ring should be avoided when possible, unless it can be proven there are no interferences.

4.4 General volumetric glassware (grade A) and glass stoppers (grade A)

4.5 Metal paper clip for weighing thin toy samples (e.g bath books)

The weight of the metal clip should be approximately of 0.5g

4.6 Glass vials (2ml) suitable for chromatographic auto-sampler, and crimp caps with PTFE inlay

4.7 Micro injection syringe, 20 µl, 50µl and 250 µl (e.g. Hamilton)

4.8 Rotary evaporator or Kuderna Danish (KD) device

4.9 Pear or round bottom flask, 50 ml or less (for rotary evaporation) or dedicated glass containers (for KD device)

4.10 Volumetric flask of 50 ml

4.11 10 ml screw cap vials and caps with PTFE inlay

4.12 Analytical balance

4.13 Migration glassware

NOTE: Cleaning of glassware: All glassware (laboratory glassware and migration glassware including both flask and caps) should be thoroughly cleaned, thoroughly rinsed several times with distilled water then rinsed with acetone and subsequently twice with 10 ml of cyclohexane. Then flask and cap should be dried for 1 h in a highly clean oven at 105 °C.

Laboratory bottles 250 ml with flat bottom, and a screw neck, provided with screw cap with PTFE lined rubber septum.

Dimensions:	outside diameter: 70 mm total height of bottle: 138 mm height bottom to start of neck: 75 mm inside neck opening: 30 mm
Supplier: Schott Duran	flask: Cat. nr.2180136 screw cap: Cat. nr 2924028

NOTE: New glassware must be used since the effect of ageing and increased porosity are unknown and under investigation. Shape and dimensions of the flasks influence the mechanical impact of the test sample. Deviation from the prescribed type of flask may result in unreliable results. In the experiments, mild conditions should be used first, whereas stringent conditions should be kept for last.

NOTE: The flask and the inside of the screw cap must be free of interfering components. The flask and cap must be thoroughly cleaned following the protocol outlined in the “warning” section.

4.14 Dynamic migration device (Head over heel rotator)

Requirements:

- Must be capable of holding 250 ml bottles (4.13)
- Speed must be variable and able to maintain during the test period
- Radius from the centre of the rotating axis to the centre of the flask must be 150 mm

4.15 Analytical Determination

4.15.1 Gas chromatography with mass detector (GC-MS)

Preferably with an automatic on-column injector and a mass detector, or alternatively with a PTV or splitless injector. In any case however, the absence of interference or contamination must be established. A mass detector should be used capable of scan range 50 amu to 500 amu. The detector should be connected to an integrator.

The gas chromatographic capillary column must be capable of producing a symmetric peak of DINP and capable to separate DINP from peaks originating from sample matrix or extracting solvent.

NOTE: Depending on the type of equipment used for the determination, the appropriate operating conditions are to be established.

The following conditions and parameters are recommended:

Injection:	cool on-column, splitless, 1 µl, oven track mode.
Oven:	50°C, 1 min., 30°C/min to 280°C, 15°C/min to 320°C, 3 min.
Carrier:	helium, 1 ml/min (36.2 cm/s), constant flow.
Transfer-line:	325°C.
Detection:	MS in SIM mode.

The following ions may be monitored, and DEHP DINP and DIDP should be monitored. Before starting a sequence of analyses, the instrument is checked by the injection of 1 µl cyclohexane (3.2.3). The analysis is performed using the same conditions as for sample analysis. The MS is operated in SIM mode. On the obtained chromatogram, ion extracted are m/e 149 for DBP, BBP and DEHP, m/e 293 for DINP and m/e 307 for DIDP. Record the peak areas and calculate the LOD (limit of detection) and LOQ (limit of quantification) for a signal to noise of (3:1).

solute	DBP	BBP	DEHP	**DINP**	DIDP
primary ion	149	149	149	**149**	149
secondary ion	223	91	279	**293**	307

NOTE: Using an on-column injector, the column and mass detector are very sensitive to overloads. 50ppm has been shown to be able to overload the MSD lens and repeller. Thus it is recommended to lower the calibration curve to 10 or maximum 25 ppm maximum value in this case

Using a splitless injector, it is advised to choose one of a volume as small as possible, preferably like a straight splitless liner of 2mm diameter 250 µl volume (e.g. for HP, part# 18740-80220) or even 1.5mm diameter 150 µl volume. Avoid the generic split/splitless liner of 4mm due to lack of sensitivity.

The following conditions have been found optimised in splitless using the 1.5mm 150µl volume splitless straight narrow “direct liner” (e.g. HP#18740-80200 or equivalent) . In addition, the settings were the following (whenever possible on the brand of GC used):

Mode:	Pulsed splitless
Initial temperature:	290°C
Pressure:	7.64 psi
Pulse pressure:	35.0 psi
Pulse time	0.50 min.
Purge flow:	20.0 ml/min.
Purge time	2.0 min.
Total flow:	23.05 ml/min
Gas saver:	on
Saver flow:	20.0 ml/min
Saver time:	2.5 min.

4.15.2 High performance liquid chromatography (HPLC)

Preferably with an automatic injector or injection loop (20 or 100 µl), and a variable UV detector, set to 225 nm, or a photodiode array detector scanning from 200-400 nm. The detector should be connected to an integrator.

The HPLC column must be capable of producing a symmetric peak of DINP and be able to separate DINP from peaks originating from sample matrix or extracting solvent.

NOTE: Depending on the type of equipment used for the determination, the appropriate operating conditions are to be established.

The following parameters are recommended:

Column temperature:	30°C
Eluent:	iso-octane (3.2.1)
Flow rate:	1ml/min
Injection volume:	20 µl
Detection:	UV/diode array
Wavelength:	200-400 nm, DINP measured at 225 nm

NOTE: Retention time obtained for DINP is approx. 4.5 min. Depending on the number of samples injected and the purity of the samples it may be necessary to clean the column periodically by washing with a mixture of iso-octane (3.2.1) and dioxane (9:1) to elute strongly retained components from the column. After reconditioning of the column with iso-octane (3.2.1) the samples analysis can be continued.

5 Procedures

5.1 Rapid identification of plasticisers in samples

Approximately 100 mg 5 mg of the sample is weighed accurately and dissolved in 10 ml of tetrahydrofuran (THF) (3.2.6), by vortexing 30 5 seconds

The polymer is precipitated with 20 ml hexane (3.2.7)

The solution is filtered through a 0.45µm disposable filter (e.g. nylon, polypropylene)

The portion of the clear THF/hexane solution is transferred into a capped vial and injected in GC-MS (see section 4.15.1)

A total ion current spectrum is recorded

The obtained GC-MS spectrum is compared to known spectra or phthalate ester standards to allow qualitative identification of phthalate ester plasticisers. The identity of the plasticisers is established based on retention time and reference phthalates.

For confirmation of the quantification of the plasticiser a 50 µl aliquot of THF/hexane solution is diluted in isooctane and analysed by HPLC (4.15.2)

5.2 Sample preparation and conditioning

Five replicate test specimens should be analysed per material (toy) investigated, together with one blank and two recovery spikes. If it is not possible to take five test specimens from one test sample then more test samples should be taken to obtain five test specimens.

5.2.1 Cutting of test specimens

Select a flat as possible area of the article to be investigated and punch out five disks of approximately 10 cm2 surface area using a 23 mm hollow punching press or cork borer.

NOTE: Use a Teflon surface to cut on, which allows for easy punching and “inert” surface.

Measure and record accurately the total surface area of each disk, taking both sides of the test specimen into account. If the thickness of the punched disk is greater than 1 mm then also the cutting edges should be taken into account.
A representative part should be taken from massive samples for analysis. The cutting edges should be as smooth as possible and loose particles should be removed beforehand.
Test specimens cut from thin materials may stick to the wall of the extraction container during rotation in the head over heals rotator. To prevent sticking, the test specimen should be supplied with some extra weight. For that purpose it is considered sufficient to pierce a little hole in the test specimen and to fix a metal paper clip (4.5) through the hole.

5.2.2 Conditioning of the test specimens

Store the disks in a glass container and allow the disks to recover from the mechanical treatment for at least 24 hours. The disks should not be stacked.

5.2.3 Preparation of test specimens

Rinse the cut disks by immersion for a few seconds in a beaker using tweezers with dionised water (3.7.1) and saliva simulant (3.7) in order to remove adhering particles.
Insert the test specimen disk in a flask (4.13) containing 50.0 ml of saliva simulant (3.8).

5.3 Dynamic migration testing

Blanks and recovery samples should be analysed daily or ideally with each series of samples, as described below.

NOTE: If, for any reason, test specimens are examined with deviating surface area, then the ratio of sample area to simulant should be maintained at 10 cm^2 to 50 ml. Although no analytical data are available it is considered that the total volume

of simulant should not exceed 50 ml. Otherwise a larger size of extraction flask should be used.

WARNING:It is necessary to observe the samples during the agitation period because a test specimen may stick to the walls of the container.

5.3.1 Sample specimens

Place the 10 cm2 disk (5.2) into the 50 ml saliva (3.7) at 20ºC 2ºC using metal tweezers (4.1).

Close the flask and place the flask in a head over heels rotator (4.14).

Switch on the rotator, fixed at 60 5 rpm, and allow to rotate for 30 1 min.

After this period, immediately remove the disk from the flask (4.13) by means of a pair of tweezers (4.1).

Transfer the content of the flask into a closed separatory funnel (4.3)

Add fresh saliva simulant (3.7) to the flask (4.13)

Add the disk previously treated to the flask

Close the flask and place the flask in a head over heels rotator (4.14).

Switch on the rotator, fixed at 60 5 rpm, and allow to rotate for 30 1 min.

After this period, immediately remove the disk from the flask (4.13) by means of a pair of tweezers (4.1).

Add the content of the flask into the separatory funnel (4.3) already containing the first extract, and proceed to the extraction.

NOTE: Always place an even number of flasks in the head over heels rotator to avoid unbalanced rotation.

5.3.2 Blanks

Pipette 50 ml of fresh saliva simulant into a 250 ml flask or bottle (4.13). Treat the solution as described in the previous section, omitting the addition of a test specimen but following the second 30 minute replenishment extraction procedure.

Use the blank to correct the sample result if necessary

5.3.3 Recovery samples

Transfer 50 ml of fresh saliva simulant (3.7) into a 250 ml flask or bottle (4.13)

Add by means of a 250 µl injection syringe (4.7) 150 µl of a standard solution of DINP at 1000 µg/ml in iso-propanol (3.6).

Assure that the tip of the syringe is submerged in the saliva simulant, and swirl before retracting the syringe from the saliva simulant. The saliva simulant thus obtained contains approximately 150 µg of DINP.

Close the flask and treat the mixture as described in the previous sections, omitting the addition of a test specimen but following the second 30 minute replenishment extraction procedure (no standard need be added again).

NOTE: The concentration of the spike DINP was made to be 15 µg/ml

5.4 Extraction from the saliva

NOTE: This SOP was designed for GC-MS and HPLC limits of quantification at 0.5 ppm for DINP and 0.01 ppm for BBP, and allowing quantification of releases from 1 to 10 µg/min even considering the integration of multiple peaks for DINP.

Add 20 ml of cyclohexane (3.2.3) to the bottle (4.13) used for migration which have been emptied of their simulant.

Close the bottle and shake vigourously to dissolve any absorbed phthalate. Remove the cap.

Add to the 250 ml separatory funnel (4.3) containing the 100 ml of saliva simulant.

Add by means of a 50 µl injection syringe (4.7) 40 µl of a standard solution of BBP in cyclohexane at 250 µg/ml (3.5) (except for blank sample)

Assure that the tip of the syringe is submerged in the solution, and swirl before retracting the syringe. The extract contains 10 µg of BBP in the saliva and solvent mixture.

Close the funnel and shake, vigorously, the stoppered 250 ml separatory funnel for approximately 2 0.5 min., releasing pressure periodically (ca every 20 sec.).

Allow the phases to separate for 20 min.

Pour out the lower saliva simulant layer into a flask or bottle used for migration (4.13), by excess.

Pour the upper solvent layer into a 50 ml volumetric flask (4.10)

Transfer the saliva simulant from the migration glassware (4.13) back into the separatory funnel (4.5) for a second extraction.

Repeat the extraction procedure by adding another 20 ml of cyclohexane (3.2.3) to the bottle (4.13) used for migration which have been emptied of their simulant.

Swirl the solvent to absorb any phthalates on the sides of the glass vessel.

Add to the 250 ml separatory funnel (4.3) containing the 100 ml of saliva simulant.

Close the funnel and shake, vigorously, the stoppered 250 ml separatory funnel for approximately 2 0.5 min., releasing pressure periodically (ca every 20 sec.).

Allow the phases to separate for 20 min.

Pour out the lower saliva simulant layer into a waste flask

Pour the upper solvent layer into the 50 ml volumetric flask (4.10) previously used for the first extraction. Fill up to the 50 ml mark with cyclohexane.

Add 1-2 g sodium sulphate (3.9) to the 50 ml volumetric flask (4.10).

NOTE: The sodium sulphate allows to trap potential moisture remaining from the extraction

Transfer a 20 ml aliquot of the cyclohexane extract to a pear round bottom flask or a KD flask (4.8).

NOTE: Care should be taken to avoid any transfer and contamination by the sodium sulphate

Transfer the flask (4.8) to a rotary evaporator or alternatively a Kuderna Danish (KD) apparatus and evaporate down to approximately 2 ml. Mild temperature conditions should be used (max. 40ºC.) using rotary evaporation.

NOTE: Care should be taken to avoid cross-contamination of the evaporating unit, therefore frequent solvent blanks should be run.

Remove from the concentrating unit and gently evaporate the remaining solvent to dryness using a flow of nitrogen at mild temperatures (<40°C) mild flow.
Add by means of a glass pipette 4.0 ml of cyclohexane (3.2.3), swirl thoroughly for approximately 30 seconds to entirely re-dissolve the phthalate ester including any which could be on the sides of the glass container.
Transfer 1ml of the solution into a vial for GC/MS and 1 ml into a vial for HPLC.
Close the vial with a crimp cap with PTFE-liner, ensuring caps are tightly crimped. Avoid any contact of the solution with the crimp cap.
Retain 8 ml of the remaining unconcentrated solution for potential re-analysis in screw-cap vials (4.11).

NOTE: The concentration of the internal standard was made to be 1µg/ml. If necessary the solution may require dilution to meet the limits of the calibration curve for unknown samples for DINP. The amount of internal standard must then be adjusted to remain constant at 1 µg/ml.

For blanks and recoveries, proceed as above without the disk on saliva alone for the blank and on fortified samples for recoveries. Determine and record recoveries

5.5 Analytical determination

NOTE: When starting analyses, baseline stability and response linearity of the instrument should be examined.

NOTE: Each vial should be injected only once as it was found that interferences occurred upon a second injection from one and the same vial. If for any reason the analysis has to be repeated then a new vial should be filled and closed with a new crimp cap.

The same operating conditions of the GC-MS and HPLC system should be maintained throughout the analysis of all test samples and solutions.

5.5.1 Calibration samples

Prepare the standard solutions for calibration as described in section 3.4.

Transfer approximately 1ml of the standard solutions into a HPLC vial and 1 ml of the standard solution into a GC vials (4.6).

Close the vials with a crimp cap with PTFE-liner.

Avoid any contact of the solution with the crimp cap.

Inject each of the calibration samples (3.4) one into the GC-MS column (4.15.1), and its duplicate in the HPLC column (4.15.2).

Measure the peak heights or areas of DINP and of the Internal Standard in the chromatogram obtained.

NOTE: For GC-MS take m/z 149 for BBP and 293 for DINP

NOTE: For HPLC analysis, the use of the internal standard may be omitted as injection in HPLC is sufficiently repeatable.

Calculate the peak area ratio (PAR) for each of the calibration standards, by dividing the DINP peak area by the internal standard area.

Construct the calibration curve by plotting the peak area ratio against the concentration of DINP in the calibration samples in g per ml cyclohexane

Repeat with the second set of dilutions (duplicate set).

NOTE: The calibration curve should be rectilinear and the correlation coefficient should be 0.990 or better. If either of the two requirements is not met, fresh standard solutions should be prepared from the original stock solutions. Analysis of the solutions and construction of the calibration graph should be repeated.

NOTE: The calibration curves obtained in GC-MS tend to have a quadratic tendency, however, because of the concentration step placing the quantification in the middle of the curve, a linear regression should be used.

NOTE: Peak height was found to give better calibration curves for HPLC. Peak Areas should be used for GC-MS analyses

NOTE: Calibration solutions should be injected frequently during the analysis of saliva simulant samples. At least 1 calibration solution should be injected on every ten saliva simulant samples.

5.5.2 Test samples, blank and recovery solutions

Extracts of the saliva simulant samples, blanks and recoveries are analysed.

Place the vials with the extraction solutions run the analysis using the conditions set out in section 4.15.1 (GC-MS) and 4.15.2 (HPLC).

Measure the peak areas (GC-MS) or peak heights (HPLC) of the DINP peak and Internal Standard peaks respectively in the chromatograms obtained.

Calculate the ratio of the peaks (GC-MS) by dividing the DINP peak area by the internal standard area.

Use the measured peak ratio –GC-MS (height, HPLC) as obtained above in the following formula.

If the regression line equation is

y (PAR) = a * x [g/ml] + b

then the DINP concentration in cyclohexane (µg/ml) is CDINP,solvent = (y-b)/a

5.5.3 Calculation of the DINP release from the test specimen

The release of DINP should be expressed in µg/min taking into account 10 cm² of surface area of the test specimen.

Calculate the release as follows:

$$\text{Release [µg/min]} = \frac{C_{DINP,solvent}\,[\mu g/ml] \times V_{extract}\,[ml] \times 10\,[cm^2]}{t\,[min] \times A\,[cm^2]} \times F$$

In which:

$C_{DINP,solvent}$:	concentration of DINP in cyclohexane
$V_{extract}$:	volume of extraction (ml): 50ml.
T:	time of experiment (60 minutes)
A:	area of test specimen (cm²)
F:	Factor for concentration step F= 0.2

5.5.4 Calculation of recoveries

$$\text{Recovery (\%)} = \frac{C_{DINP,solvent}\,[\mu g/ml] \times V_{extract}\,[ml] \times 100}{\text{Concentration of recovery solution}\,[\mu g/ml] \times 1\,[ml]} \times F$$

6 Reporting

The report should contain the reference to this method, and all information necessary for the complete identification of each sample, including the code number, description of the sample, area taken and weight. It should also contain the sample arrival date or sampling date, the method of sampling, the date of analysis together with note on any intervening storage conditions.

The results from GC-MS analysis on the sample, reference material and recovery solution should be transposed to a spreadsheet for calculating and reporting.

The results should then be reported in µg/min/10cm2, reporting all individual results as well as the mean of the five determination satisfaying the repeatability criterion in section 7.

The report should mention any deviations from the method description and reasons for variations, and relevant comment on the test results.

Should results be unexpected and subject to doubt the analyses should be performed using a standard reference disks which can then be used as a calibrant to the performance of the method and/or operator.

The report should also identify the laboratory conducting the test and the name of the analyst.

The responsibilities of the officer in charge are to explain the work and tests to be performed, and supply a written experimental design showing the chronology and

requirements during the experiments. The officer is also responsible for performing a visual observation during one experiment in order to observe the proper execution of the tests. The officer is then responsible for providing the electronic data sheet to input the results, and to interpret, perform statistical analysis when necessary and write the report.

The responsibilities of the technician(s) responsible for the analysis are to execute the experiments, check the functioning of instruments and availability of consumables, state of materials needed, receive and store the samples and standards, check the chromatograms obtained and input the data into the proformat provided.

7 Repeatability and reproducibility

The method was validated at the European Level on 14 laboratories using both PVC reference material and 5 toys. The maximum repeatability relative standard deviation (RSDr) was found to be 10%. Since the determination cannot be done on several real toy samples, the repeatability is expressed for the 5 replicates from one sample within laboratories (value for all participating labs, according to the definition of ISO 5725).

In addition standard PVC reference samples (n= 10) of known homogeneity were analysed in 2 various days, and the maximum RSDr was found to be 8%.

The reproducibility relative deviation (RSDR) was found to be 30% using a reference PVC material containing 38% of DINP.

8 Safety

General safety instructions should be followed at all times; if in doubt, advice must be sought from the officer in charge. The following specific hazards must be taken into consideration: use of sharp blades, use of solvents

All appropriate protective safety equipment should be worn and a fume cupboard must be used.

9 References

C. Simoneau, H. Geiss, A. Roncari, P. Zocchi, P. Hannaert, 2001. “Validation of methodologies for the release of di-isononylphthalate (DINP) in saliva simulant from toys”. EUR 19826 EN .

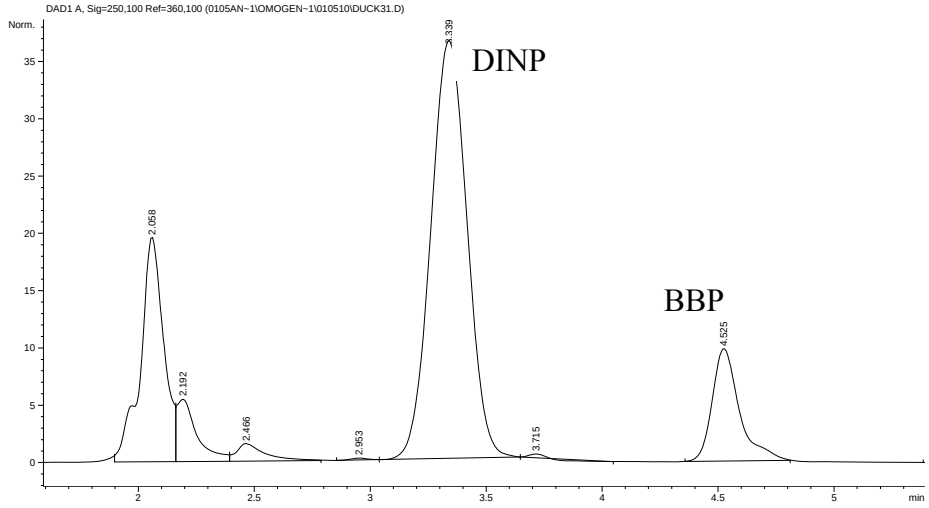
Rijk, R. & Ehlert, K., May 28, 1999. “Validation of the method determination of diisononylphthalate in saliva simulant”. TNO report V99.598.

Scientific Committee on Toxicity, Ecotoxicity and the Environment, 27 November 1998, Opinion on phthalate migration from soft PVC toys and childcare articles – data made available since the 16th of June 1998. Brussels, 6th plenary meeting.

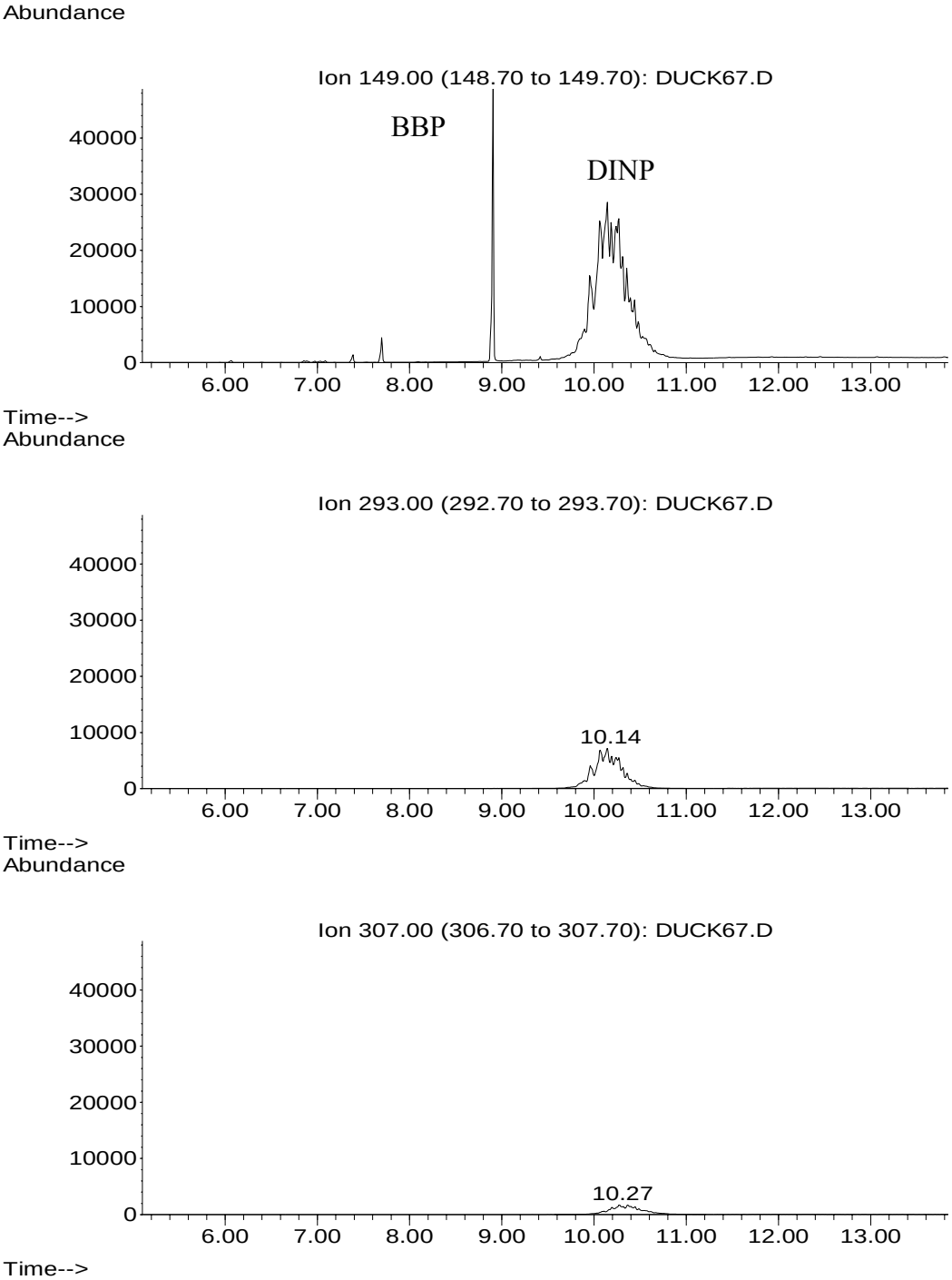
10 ANNEXES

10.1 Annex 1 – Example of a prototype head over heels apparatus

10.2 Annex 2 – typical HPLC chromatogram showing the peak of DINP and that of the internal standard



10.3 Annex 2 – typical GC-MS chromatogram showing the extraction of ion 293, 307 and 149.



European Commission

EUR 23813 EN – Joint Research Centre – Institute for Health and Consumer Protection
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Abstract
The purpose of this study was to investigate the influence of the relative concentration of percentage of phthalates and nature of phthalates on their release in standard conditions. To obtain a suitable but rapid method of analysis, experiments were performed to study the effects on the modification of a standard operation procedure (SOP) previously validated at the EU level by JRC Ispra.
Standard PVC disks with various percentages of di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP), di-ethyl-hexyll phthalate (DEHP), benzylbutyl phthalate (BBP) dibutylphthalate (DBP) or a binary mixture DINP/DBP in various proportions were prepared. 30 different types of disk were produced and tested. The disks were analysed for contents, homogeneity and sets were subjected to migration experiments of the various phthalates under dynamic conditions using the previously validated SOP with some modifications.
The release from samples with a systematic manufacturing process and containing different phthalates at different concentrations showed correlations to their concentrations. Since previous studies using commercial toys had no showed such specific trends, these results suggest that the production process of toys may be an important issue with respect to release properties. The release of DEHP BBP and DBP tended to show a more linear correlation to the concentration, whereas for DINP, DIDP, DEHP and release the plasticiser showed non linear tendencies and saturation of release for high formulation contents.

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