Standard operation procedure for:

**Determination of release of phthalate plasticizers in saliva simulant**

**WARNING:** Phthalates are components 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 shall be avoided. All plastic materials are to be suspected for the presence of interfering components. Before using plastic equipment the occurrence of interfering substances shall be checked. Glassware shall be cleaned thoroughly before use.
Introduction

Various phthalates are used as plasticizer in PVC articles. Particularly PVC baby toys, such as teething rings, are manufactured using phthalate plasticizers. Diisononylphthalate (DINP) is most commonly used but others may be used in combinations or alone as well. The articles may contain up to 50% of plasticizer. Other plasticizers like diethylhexylphthalate (DEHP), diisodecylphthalate (DIDP), dibutylphthalate (DBP), butylbenzylphthalate (BBP) or di-n-octylphthalate (DNOP) may be used as well. During use of the articles by babies the plasticizer may leach into the saliva and as a consequence the baby may be exposed to phthalates. Considering the low body weight and the age of the babies the exposure to phthalates should not exceed a tolerable daily intake. The CSTEE committee established the following values:

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Name</th>
<th>TDI µg/kg b.w.</th>
<th>Guidance value mg/10 cm²/3h/8 kg b.w. g/min/10cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DINP</td>
<td>di-isononylphthalate</td>
<td>150</td>
<td>1.2</td>
</tr>
<tr>
<td>DNOP</td>
<td>di-n-octylphthalate</td>
<td>370</td>
<td>3.0</td>
</tr>
<tr>
<td>DEHP</td>
<td>bis(2ethylhexyl)phthalate</td>
<td>37</td>
<td>0.3</td>
</tr>
<tr>
<td>DIDP</td>
<td>di-isodecylphthalate</td>
<td>250</td>
<td>2.0</td>
</tr>
<tr>
<td>BBP</td>
<td>benzylobutylphthalate</td>
<td>200</td>
<td>1.6</td>
</tr>
<tr>
<td>DBP</td>
<td>di-n-butylphthalate</td>
<td>100</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The method described in this SOP allows for the determination of phthalates in artificial saliva after mechanical agitation with plasticized PVC.

The method described in this document has been validated by Joint Research Centre in Ispra, Italy. Only minor changes have been incorporated in order to reduce the amount of labour required for one analysis.
1 Scope

This method describes the mechanical extraction procedure suitable for the determination of the release of plasticizer from plasticized PVC articles such as baby toys and childcare articles and the analytical procedure to quantify the amount of phthalate released in simulated saliva. The method is appropriate for the quantitative determination of phthalate esters in saliva simulant. The HPLC system is not capable to discriminate between DINP, DIDP and DEHP. In case these esters are present at the same time then the release is quantified by GC/MS.

The mechanical procedure has been calibrated against data obtained from in-vivo studies with plasticized PVC samples. The method is appropriate for the quantitative determination of phthalate esters in approximate analyte concentration ranges of 0.1 to 5 mg/l of saliva simulant.

2 Principle

A test sample is mechanically treated with a salt solution intended to mimic saliva. After elapse of the mechanical treatment the test specimen is removed from the solution. The mechanical treatment is repeated using a fresh portion of saliva simulant. The collected simulated saliva solutions are extracted with cyclohexane in its initial container used for the mechanical treatment. The amount of phthalate ester in the cyclohexane solution is determined by straight phase HPLC using UV detection at 225 nm. Quantification is achieved using an external standard calibration procedure. Alternatively the quantification is performed by GC/MS using appropriate m/z values.

3 Reagents

Reagents and solvents shall be of analytical quality.

NOTE: Phthalates are wide spread components and may be present in many solvents of analytical quality as well as in water. Use of PVC tubing, pipetting balloons, rubber tubing etc. shall be avoided, whereas presence of interfering components from plastic equipment should be established before running analysis. Glassware should be cleaned thoroughly before use preferably with a suitable, pure organic solvent.

3.1 Analytes

3.1.1 Diisononylphthalate
Molecular weight: 418
Molecular formula: C_{26}H_{42}O_4

NOTE: Diisononylphthalate is a phthalate ester of isononyl alcohol. The isononyl alcohol is composed of a mixture of branched isomers and may contain in addition minor amounts of C_7 up to C_11 branched alcohols. As a consequence the phthalate ester is a complex mixture which may differ from the manufacturer.

3.1.2 Diisodecylphthalate
Molecular weight: 446
Molecular formula: C_{28}H_{46}O_4
NOTE: Diisononylphthalate is a phthalate ester of isodecyl alcohol. The isodecyl alcohol is composed of a mixture of branched isomers and may contain in addition minor amounts of C_9 up to C_11 branched alcohols. As a consequence the phthalate ester is a complex mixture which may differ from the manufacturer.

3.1.3 Dibutylphthalate
Molecular weight: 278
Molecular formula: C_{16}H_{22}O_4

3.1.4 Butylbenzylphthalate
Molecular weight: 298
Molecular formula: C_{18}H_{18}O_4

3.1.5 Diethylhexylphthalate
Molecular weight: 390
Molecular formula: C_{24}H_{38}O_4

3.1.6 Di-n-octylphthalate
Molecular weight: 390
Molecular formula: C_{24}H_{38}O_4

NOTE: Di-n-octylphthalate may be not available. A mixture of linear C7 – C11 may be used instead

3.2 Chemicals

3.2.1 Dioxane (Fluka, Cat nr.42500)

3.2.2 Iso-octane (Merck, Cat nr. 1.04718)

3.2.3 Iso propanol (Baker, Cat nr 8068)

3.2.4 Cyclohexane (Merck)

3.2.5 Water deionized (Milli Q quality)

3.2.6 Calcium chloride, dihydrate
CaCl_2 . 2 H_2O;  Mw = 147.02

3.2.7 Magnesium chloride, hexahydrate
MgCl_2 . 6 H_2O,  Mw 203.3

3.2.8 Potassium carbonate
K_2CO_3,  Mw 138.2

3.2.9 Potassium chloride
KCl,  Mw 74.55

3.2.10 Potassium phosphate, dibasic, trihydrate
K_3HPO_4, 3 H_2O,  Mw 228.2

3.3 Solutions
3.3.1 Standard stock solution of phthalate esters (3.1) in cyclohexane (approximate 5 mg/ml)

Weigh to the nearest 0.1 mg approximately 125 mg of phthalate ester (3.1) in a series of 25 ml volumetric flasks and fill to the mark with cyclohexane (3.2.4). Mix carefully.
Calculate the actual concentration in mg phthalate ester per ml solution.

Repeat the procedure to obtain a second standard stock solution.

NOTE: The two primary standard solutions of analyte shall be checked against one another. The response factor, i.e. detector response divided by concentration of analyte solution, of the two primary standard solutions (or dilutions of that) shall not differ more than 5%. If there is agreement within 5%, subsequent diluted standard solutions are made from only one of the primary standard solutions.

If the levels of the two independently prepared stock solutions do not correspond to within ±5% then both stock solutions shall be discarded, and new solutions shall be prepared.

3.3.2 Intermediate diluted standard solution of phthalate ester in cyclohexane (approximately 0.5 mg/ml)

Transfer by means of a volumetric glass pipette 5 ml of the standard stock solutions (3.3.1) into a series of 50 ml volumetric flasks and fill to the mark with cyclohexane (3.2.4). Mix carefully.
Calculate the actual concentration in mg phthalate ester per ml solution.

3.3.3 Diluted standard solution of phthalate ester in cyclohexane (approximately 0.05 mg/ml)

Transfer by means of a volumetric glass pipette 5.0 ml of the intermediate standard solutions (3.3.2) into a series of 50 ml volumetric flasks and fill to the mark with cyclohexane (3.2.4). Mix carefully.
Calculate the actual concentration in µg phthalate ester per ml solution.

3.3.4 Standard solution of phthalate ester in iso propanol (approximately 1 mg/ml)

Weigh to the nearest 0.1 mg approximately 50 mg of phthalate ester (3.1) in a series of 50 ml volumetric flasks and fill to the mark with iso propanol (3.2.3). Mix carefully.
Calculate the actual concentration in mg phthalate ester per ml solution.

3.3.5 Internal standard stock solution of BBP (1 mg/ml)

Weigh to the nearest 0.1 mg approximately 50 mg of butylbenzylphthalate (3.1.4) in 50 ml volumetric flasks and fill to the mark with cyclohexane (3.2.4). Mix carefully.
Calculate the actual concentration in mg BBP per ml solution.

3.3.6 Diluted internal standard solution of BBP (1µg/ml)

Transfer by means of a micro injection syringe 100 µl of the intermediate standard stock solution (3.3.5) into a 100 ml volumetric flasks and fill to the mark with cyclohexane (3.2.4). Mix carefully.
Calculate the actual concentration in µg BBP per ml solution.

3.3.7 Simulated saliva salt solution
Prepare a solution in water (3.2.5) of the following composition:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium chloride</td>
<td>MgCl₂</td>
<td>0.82</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>CaCl₂</td>
<td>1.0</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>K₂HPO₄</td>
<td>3.3</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>K₂CO₃</td>
<td>3.8</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>5.6</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>KCl</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Weigh the required amount of salts taking into account the presence of water of crystallisation. Dissolve the potassium and sodium salts in ca. 900 ml distilled water (3.2.5), then add the calcium and magnesium salts. Adjust the pH to 6.8 with hydrochloric acid. Transfer to a 1 liter volumetric flask and fill to the mark with distilled water. Store the solution protected from light.

**NOTE:** If the solutions should be used over a period longer than two weeks, it is advised to used water (3.2.5) that was boiled for 10 min and to store the solution in dark.

### 4 Apparatus

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

4.1 High performance liquid chromatograph 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. Detectors should be connected to an integrator.

4.2 HPLC column, capable of producing a symmetric peak of diisononylphthalate and capable to separate diisononylphthalate from peaks originating from sample matrix or extracting solvent.

For guidance, the parameters established with a column selected are given below.

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

4.2.1 Column: Stainless steel 150 mm x 3.0 mm, filled with cyanopropyl coated spherical silica gel 130 Å, 4% carbon loading, particle size 5 µm., Hypersil BDS CPS, obtained from Alltech.

<table>
<thead>
<tr>
<th>Column temperature</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent:</td>
<td>iso-octane (3.2.4)</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>0.5 ml/min</td>
</tr>
<tr>
<td>Injection volume:</td>
<td>20 µl</td>
</tr>
<tr>
<td>Detection:</td>
<td>UV/diode array</td>
</tr>
<tr>
<td>Wavelength:</td>
<td>200-400 nm, phthalate esters measured at 225 nm</td>
</tr>
</tbody>
</table>

**NOTE:** Retention time obtained for DINP is approx. 3.2 min

**NOTE:** 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 and...
dioxane (9:1) to elute strongly retained components from the column. After reconditioning of the column with iso-octane the samples analysis can be continued.

4.2.2 Alternative conditions of HPLC

Column: Stainless steel 150 mm x 4.6 mm, filled with spherical silica gel, particle size 5 µm., Spherisorb S5W, obtained from Waters Corporation, Part no PSS830113.

Column temperature: 30°C
Eluent: iso-octane : dioxane = 97 : 3
Flow rate: 1.0 ml/min
Injection volume : 20 µl
Detection : UV/diode array
Wavelength : 200-400 nm, phthalate esters measured at 225 nm

NOTE: Retention time obtained for DINP is approx. 4.5 min

NOTE: Depending on the number and type of samples injected and the purity of the samples it may be necessary to clean the column periodically by washing with a mixture of dioxane to elute strongly retained components from the column. For that purpose the a gradient should be run starting from the iso-octane/dioxane mixture to dioxane in about 5 min Cleaning with dioxane is maintained for 10 min and then following a linear gradient returned to the initial eluent. After reconditioning of the column with iso-octane/dioxane = 97/3 the sample analysis can be continued.

NOTE: The silica column is more conceivable to breakthrough of components then the cyanopropyl column. Peak shape is better on the cyanopropyl column, whereas selectivity is found better on the silica column.

4.3 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:

Column: 30 m x 0.25mm I.D. x 0.25µm df HP-5MS.
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 (52.6 kPa).
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 (3.2.4). The analysis is performed using the same conditions as for sample analysis. The MS is operated in SIM mode. On the obtained chromatogram, ions extracted are m/z 149 for DBP, BBP and DEHP, m/z 293 for DINP and m/z 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).

**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.

4.4 Mixer of Vortex type

4.5 10 ml glass tubes with screw cap with PTFE insert (Corning)

4.6 Injection vials suitable for HPLC and/or GC auto-sampler, and crimp caps with PTFE inlay

**NOTE:** Screw caps, new and re-used, as well as crimp caps, were found to be contaminated with interfering components. Contact of the lining of the crimp cap with the organic solution should be avoided at any time.

4.7 Micro injection syringe, 10 µl, 50 µl and 100 µl (Hamilton)

4.8 Micro pipette 500-2000 µl (Finnpipette Digital)
4.9 Head over heels rotator
Commercial available rotator or a home made apparatus capable to comply with following requirements.
The rotator shall be capable to hold 250 ml laboratory bottles (4.11). Rotation speed should be variable and capable to maintain the selected speed during the test period. The radius from the centre of the rotating spindle to the centre of the flask shall be 150 mm. (See figure 1).

4.10 Laboratory flask 250 ml with screw cap
Laboratory flask 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:** Shape and dimensions of the flask are of influence on the mechanical impact of the test sample. Deviation from the prescribed type of flask may result in unreliable results.

**NOTE:** The flask and the inside of the screw cap shall be free of interfering components. The flask and cap should be cleaned using a normal procedure with water and detergent and subsequent drying. Then flask and cap shall be rinsed with acetone and subsequently twice with 10 ml of iso-octane). Then flask and cap are dried for 1 h in an oven at 105 °C.

4.11 Punching press
Capable of producing 23mm+/-0.2mm diameter disks from 3mm thick PVC sheeting

5 Sample preparation

**NOTE:** From each test sample five test specimens shall be taken for analyses. If it is not possible to take five test specimens from one test sample then more test samples shall be taken to obtain five test specimens. Blank and recovery analysis shall be carried out in independent duplicate experiments.

5.1 Preparation and conditioning of test specimen
Select a flat as possible area of the article to be investigated and punch out five disks using the punching press (4.11). Measure the total 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. 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.

From massive samples a representative part shall be taken for analysis. The cutting edges should be as smooth as possible and loose particles should be removed beforehand.
5.2 Leaching of phthalate from test specimen

Rinse a disk by immersion for a few seconds in a beaker with saliva simulant (3.3.7) in order to remove adhering particles. Insert the test specimen disk in a flask (4.10) containing 50.0 ml of saliva simulant (3.3.7). Close the flask and place the flask in a head over heels rotator (4.9). Switch on the rotator fixed at 60 rpm and allows to rotate for 30 min. After this period, immediately remove the disk from the flask by means of a pair of tweezers. Transfer the disk into a second flask with 50 ml of saliva simulant and repeat the above procedure by rotating for 30 min at 60 rpm. After the rotation time remove the disk from the flask and add 50 ml of cyclohexane to the second flask. Shake vigorously for 30 second and immediately transfer the contents of the flask to the first flask. After closing the flask again shake vigorously for 30 seconds. Allow the phases to separate. Treat the extracts obtained above as described in section 5.5.

**NOTE:** If, for any reason, test specimens are examined with deviating surface area, then the ratio of sample area to simulant shall be maintained at 10 cm² 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.

5.3 Blank sample preparation

Pipette 50 ml of saliva simulant of the same batch as used in the leaching experiment into a 250 ml flask (4.10). Treat the solution as described in section 5.2, omitting the addition of a test specimen.

5.4 Recovery experiments

**NOTE:** Recovery of DINP from saliva simulant shall be established frequently.

Transfer 50 ml of saliva simulant (3.3.7) into a 250 ml flask (4.10). Add by means of a 50 µl injection syringe (4.7) 100 µl of a standard solution of phthalate ester in iso-propanol (3.3.4). 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 2 µg of DINP/ml saliva simulant. Close the flask and treat the mixture as described in section 5.2. Do not add any phthalate ester to the second flask.

5.5 Determination of phthalate ester in saliva simulant

Transfer 5.0 ml of the organic layer obtained in section 5.2 into a 10 ml glass tube. Evaporate the cyclohexane by means of a gentle stream of nitrogen. Add 1 ml of cyclohexane to the dry residue. If the quantification has to be performed by GC/MS then add 1 ml of the diluted internal standard solution (3.3.6) containing 1 µg BBP/ml. Swirl thoroughly to re-dissolve all residue. Transfer the solution into a suitable HPLC or GC vial (4.6) and close the vial with a crimp cap with PTFE-liner. Avoid any contact of the solution with the crimp cap.

**NOTE:** For samples giving a high release the concentration step can be omitted.

5.6 Calibration sample preparation

**NOTE:** Prepare standard solutions to cover the concentration range of 2-35 µg of phthalate ester/ml of cyclohexane.
Transfer into a series of seven 20 ml volumetric flasks 0, 1.0, 2.0, 4.0 6.0, 10.0 and 14.0 ml of the diluted standard solution (3.3.3). Fill the volumetric flasks up to the mark with cyclohexane (3.2.4) and mix thoroughly. The solutions thus obtained contain approximately 0, 2.5, 5.0, 10, 15, 25 and 35 µg of phthalate ester per ml cyclohexane. Use –if required- the diluted standard solution (3.3.3) as the standard solution containing 50 µg/ml. Calculate the actual concentration of phthalate ester in the solution in µg/ml. Transfer approximately 1ml of the standard solutions into a injection vial (4.5) and close the vial with a crimp cap with PTFE-liner. Avoid any contact of the solution with the crimp cap.

If the standard solution is used for GC/MS analysis then 20 µl of the internal standard stock solution (3.3.5) shall be added to each flask.

### 6 Procedure

#### 6.1 HPLC and/or GC/MS analysis

**NOTE:** When starting HPLC or GC/MS 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 instrument shall be maintained throughout the analysis (see sections 4.2 and 4.3) of all test samples and solutions as obtained in section 5.

#### 6.2 Sample treatment

Extracts of the saliva simulant samples and the calibration solutions prepared in section 5 are analysed by HPLC

#### 6.2.1 Calibration solutions

Inject each of the calibration samples (5.6) into the chromatographic column. Measure the peak height or area of the phthalate ester in the chromatogram obtained and plot that value against the concentration of phthalate ester in the calibration samples in µg per ml cyclohexane as calculated in section 5.6. Calculate the ratio of the peak area’s of the analyte /internal standard in case of GC/MS analysis and Plot that value against the concentration of phthalate ester in the calibration samples in µg per ml

**NOTE:** The calibration curve should be rectilinear and the correlation coefficient should be 0.996 or better. If either of the two requirements is not met, fresh standard solutions shall be prepared from the original stock solutions. Analysis of the solutions and construction of the calibration graph shall be repeated.

**NOTE:** Peak height was found to give better calibration curves in HPLC analysis. Peak area s should be used for GC/MS analysis
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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.

6.2.2 Test samples, blank and recovery solutions

Place the injection vials with the extraction solutions of saliva simulant, blanks and recovery samples obtained in section 5 in the auto sampler and run the analysis using the conditions set out in section 4.2 or 4.3. Measure the peak height or area of the phthalate ester peaks in the chromatograms obtained and calculate the ratios, if relevant.

6.3 Quantification

Graphical determination:

Using the peak height or area values or ratio values obtained from the test samples according to 6.2.2, read the phthalate ester concentration in the injected cyclohexane solution from the calibration graph (6.2.1) in µg/ml.

Calculation from the regression parameters:

Use the measured peak area as obtained in 6.2.2 in the following formula.

If the regression line equation is

\[ y = a \times x [\mu g/ml] + b \]

then the phthalate ester (PE) concentration in the cyclohexane solution \( C_{PE, cyclohexane} \) is

\[ C_{PE, cyclohexane} = \frac{y-b}{a} \]

Both procedures yield directly the PE concentration in the cyclohexane solution in µg/ml.

NOTE: The method applying calculation from the regression parameters should be the preferred one

6.4 Calculation of the PE release from the test specimen

The release of PE should be expressed in µg/min taking into account 10 cm² of surface area of the test specimen. Calculate the release as follows:

\[
\text{Release (µg/min)} = \frac{C_{PE, cyclohexane} [\mu g/ml] \times V_1 [ml] \times V_2 [ml] \times 10 [cm²]}{t[\text{min}] \times A[cm²] \times V_3 [ml]}
\]

In which:

\( C_{PE, cyclohexane} = \) concentration of PE in cyclohexane as calculated in section 6.3

\( A = \) area of test specimen (cm²)
V₁ = volume of extraction solvent cyclohexane (usually 50 ml)
V₂ = volume of cyclohexane taken from V₁ (usually 5.0 ml)
V₃ = volume of cyclohexane used to re-dissolve residue of V₂ (usually 1.0 ml)
t = time of rotation in minutes (usually 2*30 min)

7 PRECISION

7.1 Validation

This method was in house validated in 2001 by intra-laboratory precision experiments. The results are in line with the results obtained with the initial method as described in TNO report V99.598.

7.2 Repeatability

Evaluation (ISO 5725) of the within-laboratory precision experiments obtained for standard addition of phthalate esters at a level of 2 to 5 µg per ml of saliva simulant at the 95% probability level yielded the following performance characteristics:

Repeatability \( r = 2.83 \times Sd_{(n-1)} \)
- DINP = 7.7 %
- DBP = 5.0 %
- BBP = 14.2 %
- DIDP = 2.6 %

Repeatability was calculated from the DINP release of a standard PVC disk, which was determined, yielded the following performance characteristics at an average release level of 5.64 µg/min:

Repeatability \( r = 29.8 \%

8 TEST REPORT

The test report shall contain as a minimum, the following:

- an identification
- name of the laboratory
- name of the person responsible for the analysis
- date of report
- date of the analysis
- a reference to this method
- performance characteristics of the method
- sample details, such as:
  - origin and denotation of the sample
  - date and method of obtaining the laboratory sample
  - storage conditions
- results expressed in µg/min

Results should be reported as the average value from five or more determinations satisfying the repeatability criterion of (7.2)
- reasons for modifications introduced into the test method, if any.
References


Figure 1  
Ten position Head over Heels rotator

The flasks are fixed in laboratory clamps positioned on a central spindle. The spindle is instignated by a stepper motor. The rotation speed is controlled by the power controller.