Study Title

Determination of the Partition Coefficient (Pow) by High Performance Liquid Chromatographie (HPLC) Method of Bis(2-ethylhexyl) tetrabromophthalate

Data requirements

REACH requirement EC/1907/2006

Author

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Study completion date

2013-01-10

Sponsor

LANXESS Deutschland GmbH
51369 Leverkusen
Germany

Testing facility

CURRENTA GmbH & Co. OHG
Analytik
51368 Leverkusen
Germany

Laboratory Project Identification

Study No.: 2012/0061/04

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CONTENTS

1. GLP DECLARATION

This study was conducted in compliance with the OECD principles of Good Laboratory Practice (1999) and with the Principles of Good Laboratory Practice according to Annex 1, German Chemical Law (2008).

Date / Signature

Study Director

2013-01-10

Mr. Jungheim

Date / Signature

Head of Test Facility

2013-01-10

Dr. Allmendinger / Dr. Kreiß

2. ARCHIVING

The original report, the study plan, and all raw data pertaining to this study are stored in the "GLP Archive, CURRENTA GmbH & Co. OHG, Analytik, CHEMPARK, Building Q 18, D-51368 Leverkusen". A sample of the test item is stored in "GLP-Sample Store, CURRENTA GmbH & Co. OHG, Analytik, CHEMPARK, Building DA 1, D-41538 Dormagen".

This page is intentionally left blank for the purpose of submitting administrative information that is required by regulations promulgated by various countries.

3. QUALITY ASSURANCE STATEMENT

This report was audited by the Quality Assurance Unit Currenta, Analytik, Quality Management at Currenta GmbH & Co. OHG and this statement confirms that the final report reflects the raw data.

The dates of Quality Assurance inspections and audits are given below.

Audits	Dates of QAU Inspections	Dates of Reports
Study plan review	2012-11-02	2012-11-02
Inspection of experimental phase	2012-11-19	2012-11-19
Final report review (draft)	2013-01-09	2013-01-09
Final report review	2013-01-10	2013-01-10

Date / Signature

2013-01-10 A. Senic

Ms. Senic / ~~Dr. Dörzbach-Lange / Dr. Neupert~~

4. STUDY TIME TABLE

Study initiation date:	2012-11-06
Study completion date:	2013-01-10
Start of experimental tests:	2012-11-19
End of experimental tests:	2013-01-03

5. GLP CERTIFICATE

Ministerium für Arbeit, Integration und Soziales
des Landes Nordrhein-Westfalen

Fürstenwall 25, 40219 Düsseldorf

Aktenzeichen III 5 – 31.11.58.06

Gute Laborpraxis/Good Laboratory Practice
GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

CURRENTA GmbH & Co. OHG
CHEMPARK, Geb. K 46
51368 Leverkusen

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	**Areas of Expertise** (according ChemVwV GLP Nr. 5.3/OECD guidance)
Kategorie 1	category 1
Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	physical-chemical testing
Kategorie 4	category 4
Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	environmental toxicity studies on aquatic and terrestrial organisms
Kategorie 5	category 5
Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	studies on behaviour in water, soil and air; bioaccumulation

Kategorie 6	category 6
Prüfungen zur Bestimmung von Rückständen	residue studies
Kategorie 8	category 8
Analytische Prüfungen an biologischen Materialien	analytical and clinical chemistry testing

Datum der Inspektion	Date of Inspection
11. November 2011	11th November 2011

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/ test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Düsseldorf, 23.03.2012
Im Auftrag

(Dr. Deden)

Dienstsiegel/official-seal

6. SUMMARY

The partition coefficient (Pow) of Bis(2-ethylhexyl) tetrabromophthalate was determined according to the HPLC method at 40 °C. In order to comply with the guideline OECD 117 and considering environmental relevance, demineralized water (pH 6) was chosen.
The reference Pow-values in the OECD-guideline presumably refer to 25 °C. Even though the column temperature was 40 °C, this temperature is without influence on the result of the determination. The calibration was done referring to Pow-values of reference substances determined at 25 °C.

For the determination of the partition coefficient (Koc) four calibration standards with log Koc from 1.9 - 5.7 are established in the EG-test method. Additional two new reference substances (bis(2-ethylhexyl)phthalate (dioctyl phthalate) and decachlorobiphenyl were used in this test. For both reference substances the log P_{ow} -values were determined by the slow stirring method.
The values of log P_{ow} = 7.5 for bis(2-ethylhexyl)phthalate and log P_{ow} = 8.3 for decachlorobiphenyl were published by Jack de Bruin, Frans Busser, Willem Seinen and Joop Hermens in the ‘Environmental Toxicology and Chemistry, Vol 8, pp 499-512, 1989.

The chromatogram of the test item Bis(2-ethylhexyl) tetrabromophthalate showed 1 signal eluting at 23.1 minutes for the main component and 2 signals eluting at 9.7 minutes and 19.0 minutes for minor components.
No calibration substance with a Pow-value above the value for the main signal of the test item Bis(2-ethylhexyl) tetrabromophthalate was available. For this reason the results for this signal was extrapolated.

Although the determined value does not exactly meet the range of application of the test guideline (1.9 – 8.3), we consider the value as valid (measurement uncertainty +/- 0.5 log units).

For partition coefficient of the test item Bis(2-ethylhexyl) tetrabromophthalate, determined by HPLC follows:

log Pow: 10.2

7. EXPERIMENTAL PROCEDURE

7.1. Methods, guidelines and documents

REACH requirement EC/1907/2006

The determination of the partition coefficient was based on the following guidelines:

Currenta internal SOP 00187 Version 3, this SOP corresponds to the Council Regulations EC No 440/2008, Guideline Part A – Methods for the Determination of the physical-chemical properties, A.8, “Partition Coefficient” (adopted, May 2008) and to the OECD guideline for testing of chemicals
No: 117 “Partition Coefficient (n-octanol/water)“ (adopted 13 April 2004).

Currenta internal SOP 00178 Version 2 for the determination of the pH-value.

7.2. Principle of the test

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$\text{Pow} = \frac{C_{\text{octanol}}}{C_{water}}$$

HPLC is performed on analytical columns packed with a commercially available solid phase containing long hydrocarbon chains (e.g. C8, C18) chemically bound onto silica. Chemicals injected onto such a column move along it by partitioning between the mobile solvent phase and the hydrocarbon stationary phase. The chemicals are retained in proportion to their hydrocarbon-water partition coefficient, with water-soluble chemicals eluted first and oil-soluble chemicals last. This enables the relationship between the retention time on a reverse phase column and the n-octanol/water partition coefficient to be established. The partition coefficient is deduced from the capacity factor (k), given by the expression where, t_R is the retention time of the test item, and t_0 is the dead-time, i.e. the average time a solvent molecule needs to pass the column. Quantitative analytical methods are not required; only the determination of retention times is necessary.

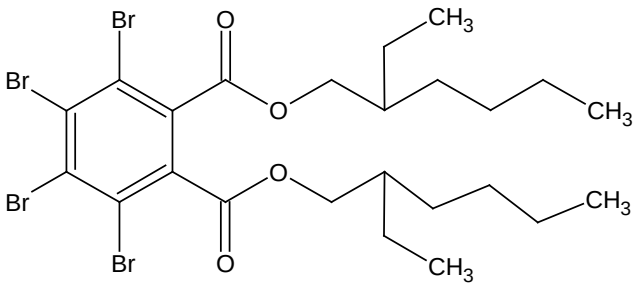
The HPLC method enables partition coefficients to be estimated in the log Pow range between 0 and 8.3. This method, however, is not applicable to strong acids and bases, metal complexes, substances which react with the eluent, or surface-active agents. Measurements should be made on ionisable substances in their nonionic from (free acid or free base), using only an appropriate buffer with a pH below the pK for a free acid or above the pK for a free base.

No pKa value of the test item Bis(2-ethylhexyl) tetrabromophthalate could be calculated because no ionizable centers are available (calculated with the software ACD/pKa v 7.0 at the structure of the test item). Therefore the undissociated form of Bis(2-ethylhexyl) tetrabromophthalate exist between pH values of 0 – 14. The test can be carried out in demineralized water (pH 6).

8. TEST MATERIAL

8.1. Sample description

Test item:	Bis(2-ethylhexyl) tetrabromophthalate
Chemical name:	3,4,5,6-Tetrabromo-1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl)ester
CAS name:	3,4,5,6-Tetrabromo-1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl)ester
CAS number:	26040-51-7
Empirical formula:	$C_{24}H_{34}Br_4O_4$
Molecular mass:	706.2 g/mol
Structural formula:	



Batch number:	12128E71
Purity:	90.6 %
Sample number:	1620
Date of receipt:	2012-06-21
Expiry date:	2015-05-09

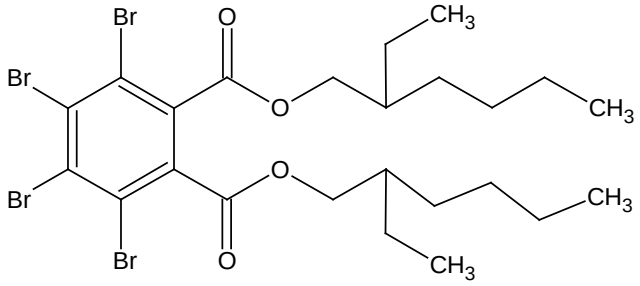
9. METHODS AND RESULTS

9.1. Determination of the partition coefficient Pow by the HPLC-method.

9.1.1. Log Pow Prediction.

The log Pow value was calculated with the software KOWWIN v1.66 assuming the nonionic form. KOWWIN is owned by the U.S.Environmental Protection Agency.

Structure of the test item Bis(2-ethylhexyl) tetrabromophthalate:



Result: log Pow = 12.0

9.1.2. Test: Determination of the partition coefficient (n-octanol / water, log Pow) by HPLC.

Method no.: SOP 00187 Version 3

This procedure corresponds to the Council Regulations EC No 440/2008, Guideline Part A – Methods for the Determination of the physical-chemical properties, A.8, “Partition Coefficient” (adopted, May 2008) and to the OECD guideline for testing of chemicals No: 117 “Partition Coefficient (n-octanol/water)“ (adopted 13 April 2004).

Supervisor.: Joachim Leibold

9.1.2.1. Preparation of the test solutions.

The test item, the dead time marker thiourea and the calibration substances (without decachlorobiphenyl) were dissolved in acetonitrile / demineralized water 9:1(v/v) and diluted in acetonitrile.
The calibration substance decachlorobiphenyl was dissolved and diluted in dimethyl formamide.
The concentration for the calibration substances was approximately 0.02 to 0.79 mg/ml. The concentration for the test item was approximately 0.20 mg/ml.

9.1.2.2. Equipment and chromatographic conditions.

Chromatograph: High Performance Liquid Chromatograph, HP 1100

Chromatographic conditions:

Column type:	length: 250 mm, inner diameter: 4 mm
Stationary Phase:	LiChrosorb RP8, particle diameter: 7 µm
Supplier:	Fa. Merck
Mobile Phase:	A: demineralized water (Millipore) B: acetonitrile

Solvent program:

Time [*t*] = min	[φ (A)] = %	[φ (B)] = %
0	25	75
30.00	25	75
30.01	stop	

Flow rate:	1.0 ml/min
Column Temperature:	40 °C
Detection:	UV, 224 nm
Injection volume:	5 µl for calibration and test item mixture

9.1.3. Test: pH determination (aqueous part of the mobile phase).

Method no.:	SOP 00178 Version 2
Supervisor:	Joachim Leibold

Result: 6.1

Procedure:
The pH was determined using a calibrated pH-meter with a single-rod glass electrode.

9.2. Results

9.2.1. Determination of the Partition Coefficient (Pow).

Table 1: Determined values of the calibration substances (single values).

Calibration items	Retention time [t_r] (min) Test 1	Retention time [t_r] (min) Test 2	Retention time [t_r] (min) Test 3	Standard deviation of the retention time [t_r] (min)	Relative standard deviation %
Thiourea (dead time (t_0))	2.288	2.288	2.287	0.001	0.025
Nitrobenzene	3.039	3.039	3.039	0.000	0.000
Benzophenone	3.423	3.423	3.423	0.000	0.000
1,2,4-Trichlorobenzene	3.969	3.969	3.970	0.001	0.015
Triphenylamine	4.845	4.844	4.848	0.002	0.043
Dioctyl phthalate	10.058	10.054	10.069	0.008	0.077
Decachlorobiphenyl	12.069	12.058	12.077	0.010	0.079

Table 2: Mean value of 3 single values of the calibration substances.

Calibration items	Retention time [t_r] = min	Capacity factor k'	Log k'	Log Pow
Thiourea (dead time (t_0))	2.288	----	----	----
Nitrobenzene	3.039	0.328	- 0.484	1.9
Benzophenone	3.423	0.496	- 0.305	3.2
1,2,4-Trichlorobenzene	3.969	0.735	- 0.134	4.2
Triphenylamine	4.846	1.118	0.048	5.7
Dioctyl phthalate	10.060	3.398	0.531	7.5
Decachlorobiphenyl	12.068	4.275	0.631	8.3

Capacity factor: $k' = \frac{t_R - t_0}{t_0}$

t_R = retention time of substances;
t_0 = dead time

Effective correlation: $\log k' = a + b \log Pow$,

Linear regression gives:
a = - 0.87229
b = 0.17930
r = 0.9918
where, a and b = regression factor and r = correlation factor

Table 3: Determined values of the test item Bis(2-ethylhexyl) tetrabromophthalate (single values)

Test item	Retention time [t_r] (min) Test 1	Retention time [t_r] (min) Test 2	Retention time [t_r] (min) Test 3	Standard deviation of the retention time [t_r] (min)	Relative standard deviation of the retention time [t_r] (%)
Bis(2-ethylhexyl) tetrabromophthalate	23.085	23.078	23.099	0.0107	0.05

Table 4: Mean value of 3 single values of the test item Bis(2-ethylhexyl) tetrabromophthalate

Test item	Retention time [t_r] = min	Capacity factor k'	Log k'	Log Pow
Bis(2-ethylhexyl) tetrabromophthalate	23.087	9.092	0.9587	10.21

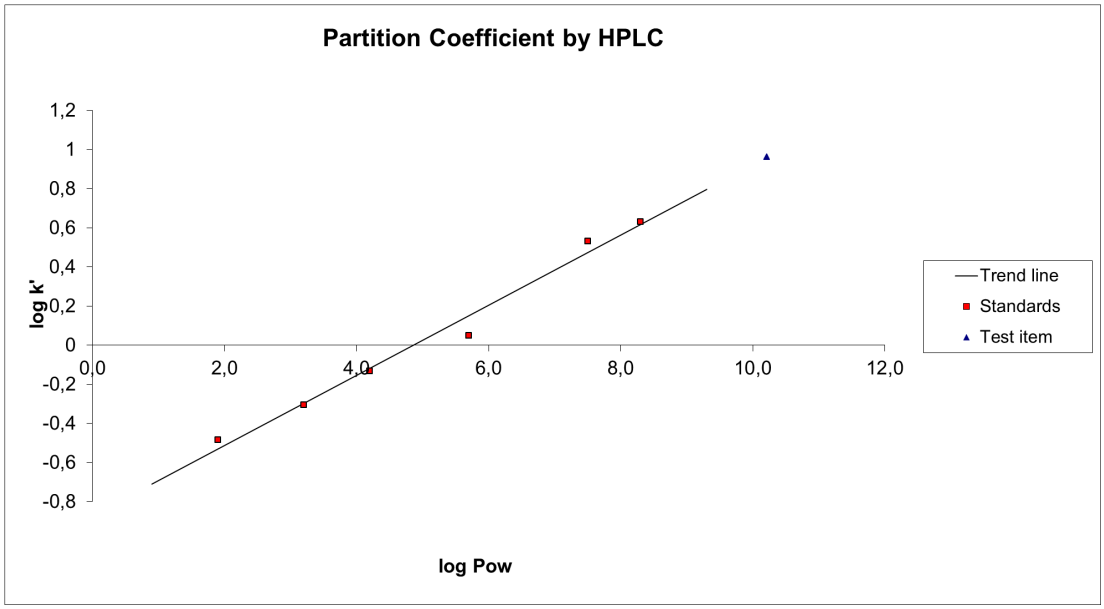


Figure 1: Graphic of the linear regression log k' / Log Pow
(for Log Pow = 10.21 of Bis(2-ethylhexyl) tetrabromophthalate).

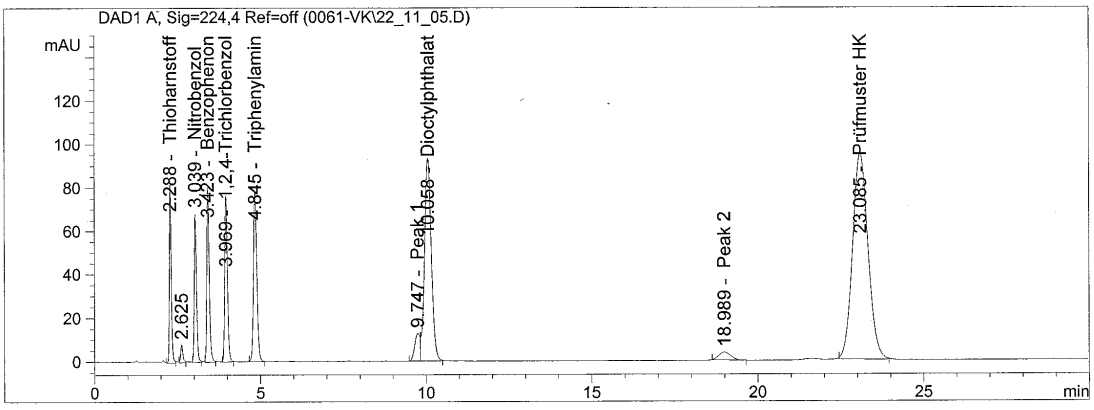


Figure 2: HPLC-chromatogram (partition coefficient) of the calibration substances (without decachlorobiphenyl) and the test item Bis(2-ethylhexyl) tetrabromophthalate dissolved in acetonitrile.

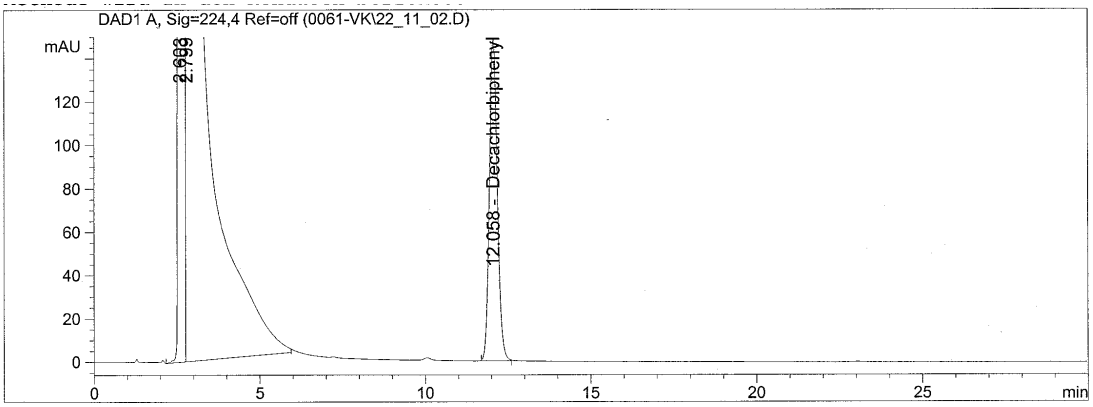


Figure 3: HPLC-chromatogram (partition coefficient) of the calibration substance decachlorobiphenyl dissolved in dimethyl formamide.

Result:

The partition coefficient of the test item Bis(2-ethylhexyl) tetrabromophthalate is:

log Pow: 10.2