Study Title

Determination of the Adsorption Coefficient (Koc) 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
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51368 Leverkusen
Germany

Laboratory Project Identification

Study No.: 2012/0061/06

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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
Process based inspection (2011/0032/13)	2012-07-25	2012-07-25
Final report review (draft)	2013-01-10	2013-01-10
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:	2012-11-14
End of experimental tests:	2013-01-04

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

2/2

6. SUMMARY

The adsorption coefficient (Koc) of Bis(2-ethylhexyl) tetrabromophthalate was determined according to the HPLC method at 40 °C. In order to comply with the guideline OECD 121 and considering environmental relevance, pH 6 was chosen as pH value for the buffer. The reference Koc-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 Koc- values of reference substances determined at 25 °C.

For the determination of the adsorption coefficient (Koc) five calibration standards with log Koc from 1.45 - 4.09 are established in the EG-test method. Additional a new reference substance 9,10-Dimethyl-1,2-benzanthracene (7,12-Dimethylbenzanthracene) was used in this test. The experimental value of log Koc = 5.37 for 9,10-Dimethyl-1,2-benzanthracene was published by Donald A. Bahnick and William J. Doucette in the “Chemosphere, Vol. 17, No. 9, pp 1703-1715, 1988”.

The chromatogram of the test item Bis(2-ethylhexyl) tetrabromophthalate showed 1 signal eluting at 5.2 minutes for the main component and 1 signals eluting at 4.3 minutes for a minor component.
No calibration substance with a Koc-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 (1.45 – 5.37), we consider the value as valid (measurement uncertainty +/- 0.5 log units).

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

log Koc = 7.3

7. EXPERIMENTAL PROCEDURE

7.1. Methods, guidelines and documents

REACH requirement EC/1907/2006

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

Currenta internal SOP 00193 Version 3, this SOP corresponds to the Council Regulations EC No 440/2008, Guideline Part C – Methods for the Determination of Ecotoxicity, C.19. “Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)” (adopted, May 2008) and to the OECD Guidelines for Testing of Chemicals, Section 1 – Physical-Chemical Properties No: 121 “Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)” (adopted, January 2001).

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

7.2. Principle of the test

The adsorption coefficient (K) is a partition coefficient and is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of a sorbent (soil or sewage sludge) and an aqueous phase:

$$\text{Koc} = \frac{C_{\text{soil (sludge)}}}{C_{water}}$$

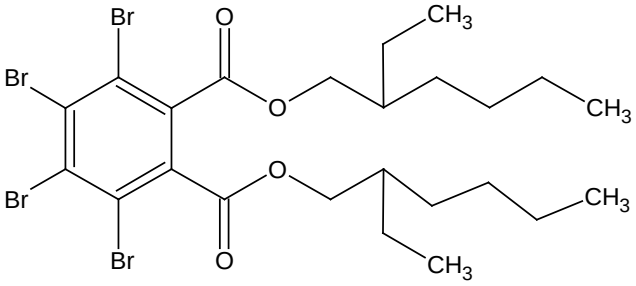
HPLC is performed on analytical columns packed with a commercially available cyano solid phase chemically bound onto silica. Chemicals injected onto such a column move along it by partitioning between the mobile solvent phase and the stationary cyano-phase. The chemicals are retained in proportion to their solid phase-water partition coefficient, with water-soluble chemicals eluted first and soil (sludge)-soluble chemicals last. This enables the relationship between the retention time on a cyano phase column and the soil(sludge/water adsorption coefficient to be established. The adsorption 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 adsorption coefficients to be estimated in the log Koc range between 1.3 and 5.4. pH has a significant influence on sorption behaviour in particular for polar substances. For agricultural soils or tanks of sewage treatment plants pH normally varies between pH 5.5 and 7.5. For this reason the test is performed at pH 6 (adding citrate buffer to the mobile phase). In case more than 10 % of the test item is dissociated at pH 6, a second test has to be performed at a pH where test item is undissociated.

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 the test item Bis(2-ethylhexyl) tetrabromophthalate exist between pH values of 0 – 14.

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

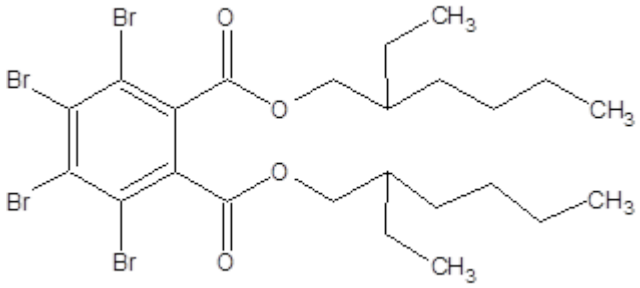
9. METHODS AND RESULTS

9.1. Determination of the adsorption coefficient Koc by the HPLC-method.

9.1.1. Log Koc Prediction.

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

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



Result: log Koc = 6.1

9.1.2. Test: Determination of the adsorption coefficient (soil(sludge) / water, log Koc) by HPLC.

Method no.: SOP 00193 Version 3

This procedure corresponds to the Council Regulations EC No 440/2008, Guideline Part C – Methods for the Determination of Ecotoxicity, C.19. “Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)” (adopted, May 2008) and to the OECD Guidelines for Testing of Chemicals, Section 1 – Physical-Chemical Properties No: 121 “Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)” (adopted, January 2001).

Supervisor: Joachim Leibold

9.1.2.1. Preparation of the test solutions.

The dead time marker sodium nitrate and the calibration substances were dissolved in acetonitrile / demineralized water 9:1 and diluted in acetonitrile. The test item was dissolved and diluted in acetonitrile.
The concentration for the calibration substances was approximately 0.02 to 0.06 mg/ml. The concentration for the test item was approximately 0.5 mg/ml.

9.1.2.2. Equipment and chromatographic conditions.

Chromatograph: High Performance Liquid Chromatograph, Agilent 1100

Chromatographic conditions:

Column type:	length: 250 mm, inner diameter: 4 mm
Stationary Phase:	LiChrospher 100 CN, particle diameter: 5 µm
Supplier:	Fa. Merck
Mobile Phase:	A: 832 ml demineralized water (Millipore) + 168 ml buffer pH 6 (Fluka, Art. No. 33545) B: Acetonitrile

Solvent program:

Time [*t*] = min	[φ (A)] = %	[φ (B)] = %
0	45	55
10.00	45	55
10.01	stop	

Flow rate:	1.0 ml/min
Column Temperature:	40 °C
Detection:	UV, 220 nm
Injection volume:	5 µl

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

Method no.:	SOP 00178 Version 2
Supervisor:	Rainer Jungheim

Result: 6.4

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 Adsorption Coefficient (Koc).

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 %
Sodium nitrate (dead time (t_0))	1.661	1.660	1.659	0.001	0.060
2-Nitrobenzamide	2.685	2.684	2.681	0.002	0.078
3,5-Dinitrobenzamide	2.782	2.781	2.777	0.003	0.095
Triazoxide	3.212	3.210	3.206	0.003	0.095
1,2,3-Trichlorobenzene	3.338	3.335	3.329	0.005	0.137
Phenanthrene	3.505	3.503	3.496	0.005	0.135
9,10-Dimethyl-1,2-benz-anthracene	3.929	3.926	3.915	0.007	0.188

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 Koc
Sodium nitrate (dead time (t_0))	1.660	----	----	----
2-Nitrobenzamide	2.683	0.616	- 0.210	1.45
3,5-Dinitrobenzamide	2.780	0.675	- 0.171	2.31
Triazoxide	3.209	0.933	- 0.030	2.44
1,2,3-Trichlorobenzene	3.334	1.009	0.004	3.16
Phenanthrene	3.501	1.109	0.045	4.09
9,10-Dimethyl-1,2-benz-anthracene	3.923	1.363	0.135	5.37

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

t_R = retention time of substances;
t_0 = dead time

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

Linear regression gives:
a = - 0.31028
b = 0.08679
r = 0.9332
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	5.192	5.181	5.164	0.0141	0.27

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 Koc
Bis(2-ethylhexyl) tetrabromophthalate	5.179	2.120	0.3267	7.34

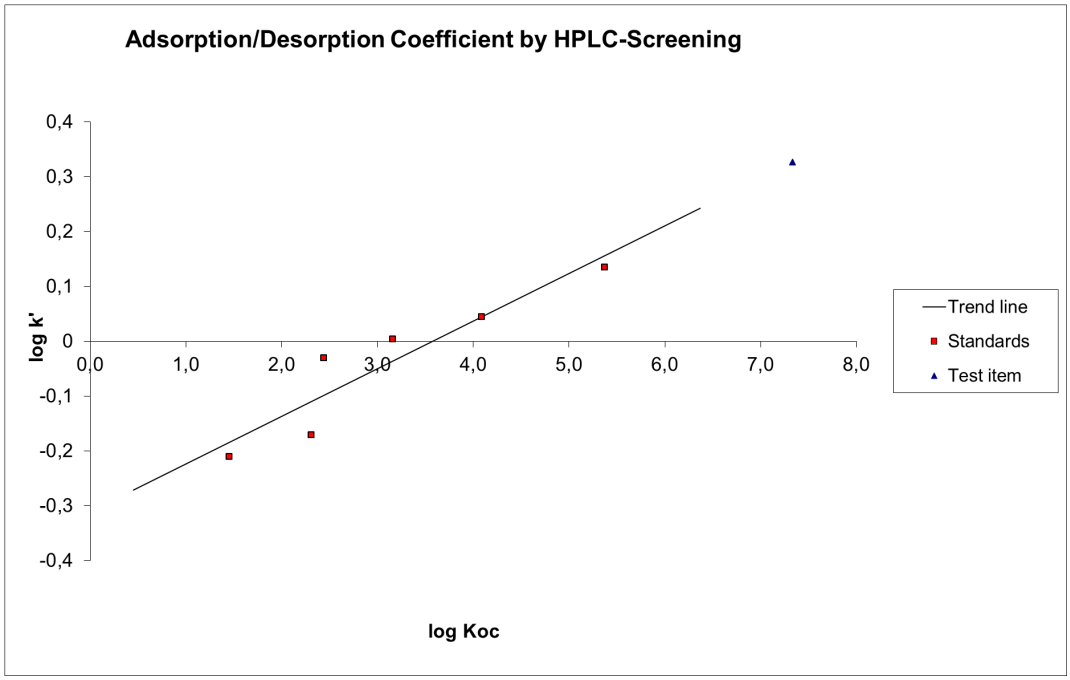


Figure 1: Graphic of the linear regression log k' / Log Koc
(for Log Koc = 7.34 of Bis(2-ethylhexyl) tetrabromophthalate)

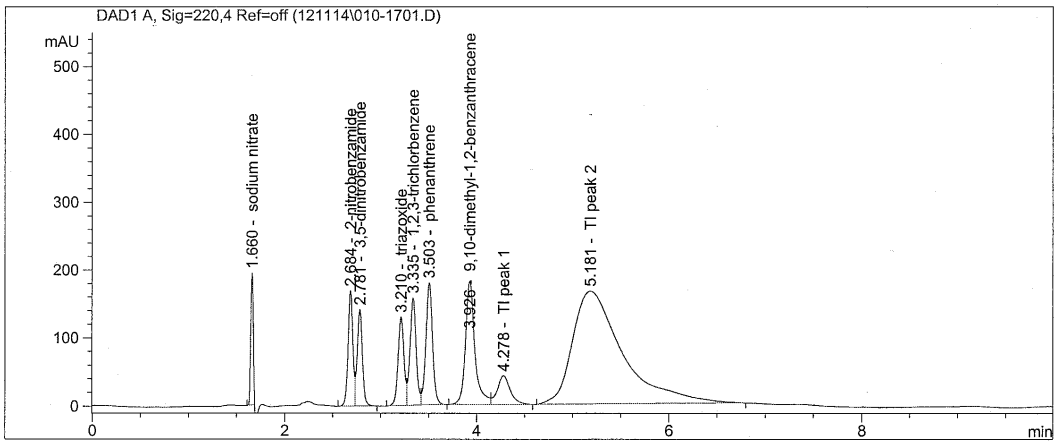


Figure 3: HPLC-chromatogram (adsorption coefficient) of the test item (peak 2) Bis(2-ethylhexyl) tetrabromophthalate and the calibration substances.

Result:

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

log Koc: = 7.3