

# Analysis of PCBs in Sewage sludge for the characterization study of ERM-CC392

Peter Korytár and Quy Dao

Report number C136/07



Institute for Marine Resources and Ecosystem Studies

# Wageningen *IMARES*

Location IJmuiden

Client: European Commission  
Joint Research Centre  
Institute for Reference Materials and Measurements  
Retieseweg 111  
B-2440 Geel  
Belgium

Publication Date: 14 December 2007

- Wageningen **IMARES** conducts research providing knowledge necessary for the protection, harvest and usage of marine and coastal areas.
- Wageningen **IMARES** is a knowledge and research partner for governmental authorities, private industry and social organisations for which marine habitat and resources are of interest.
- Wageningen **IMARES** provides strategic and applied ecological investigation related to ecological and economic developments.

© 2007 Wageningen **IMARES**

Wageningen IMARES is a cooperative research organisation formed by Wageningen UR en TNO. We are registered in the Dutch trade record Amsterdam nr. 34135929, BTW nr. NL 811383696B04.

The Management of IMARES is not responsible for resulting damage, as well as for damage resulting from the application of results or research obtained by IMARES, its clients or any claims related to the application of information found within its research. This report has been made on the request of the client and is wholly the client's property. This report may not be reproduced and/or published partially or in its entirety without the express written consent of the client.



A\_4\_3\_2-V4

# Contents

|   |    |
|---|----|
| Introduction.....   | 4  |
| Samples and their storage .....   | 4  |
| Study design .....  | 4  |
| PCB method.....   | 5  |
| Quality Assurance .....   | 6  |
| Uncertainty calculation .....   | 6  |
| Results .....   | 7  |
| Referees and Authors .....  | 8  |
| Appendix A. Results of the measurements in the supplied MS Excel format .....   | 9  |
| Appendix B. Raw data sheets with chromatograms and integration lines for samples of Series 1 on CP-Sil 8 column.....                  | 10 |
| Appendix C. Raw data sheets with chromatograms and integration lines for samples of Series 2 on CP-Sil 8 column.....                  | 11 |
| Appendix D. Zoom in visualization of the most important chromatograms for samples of Series 1 and 2 obtained on CP-Sil 8 column.....  | 12 |
| Appendix E. Raw data sheets with chromatograms and integration lines for samples of Series 1 on CP-Sil 19 column.....                 | 13 |
| Appendix F. Raw data sheets with chromatograms and integration lines for samples of Series 2 on CP-Sil 19 column.....                 | 14 |
| Appendix G. Zoom in visualization of the most important chromatograms for samples of Series 1 and 2 obtained on CP-Sil 19 column..... | 15 |

## Introduction

The aim of this study was measurement of PCB congeners (28, 52, 101, 105, 118, 138, 153, 156, 170, 180) in a sewage sludge material ERM – CC392, in a control sample and in ampoule of the “Beltest” solution as part of the characterization study of ERM – CC392 “PCBs in Sewage sludge” organized by JRC-IRMM, Geel, Belgium. This document provides detailed description of the sample handling, measurement method used, study design applied, quality control measures and results of the measurements.

## Samples and their storage

The samples were delivered to Wageningen IMARES on 9.11.2007. The samples were received in good conditions and the cooling elements were still cold. Wageningen IMARES received 2 bottles (nr. 0658 and nr. 0092) with the dried sewage sludge sample ERM – CC392, control sample No. 7 and two ampoules (No. 07 and No. 03) containing a PCB solution known as “Beltest”. LIMS number was assigned after the delivery for each sample. The overview is given in the following table:

| LIMS no.  | Sample description                                    |
|-----------|---|
| 2007/1057 | “Beltest” solution No. 7                              |
| 2007/1058 | “Beltest” solution No. 3                              |
| 2007/1059 | Control sample No. 7                                  |
| 2007/1060 | Dried sewage sludge sample ERM-CC392, bottle No. 0658 |
| 2007/1061 | Dried sewage sludge sample ERM-CC392, bottle No. 0092 |

Samples until their analysis were stored as was indicated on the sample packaging, i.e. 2 bottles with the dried sewage sludge sample ERM – CC392 were stored in darkness at -15°C, and control sample and two ampoules with the “Beltest” solution were stored in darkness at laboratory temperature (ca. 20°C).

## Study design

Study design and technical specifications prepared and requested by the JRC-IRMM for characterization of ERM-CC392 were followed. Analysis of the samples was performed between 13.11.2007 and 4.12.2007. The samples were analyzed in two independent series in two different days. Fresh calibration standards were used for each series. The details of the design followed are given in the following tables:

### Extraction and clean-up

| <b>Day 1</b>                        |                | <b>Day 2</b>                        |                |
|-------------------------------------|----------------|-------------------------------------|----------------|
| Description                         | Code           | Description                         | Code           |
| 1 x extraction ERM-CC392, No. 0658  | 2007/1060-S1.1 | 1 x extraction ERM-CC392, No. 0658  | 2007/1060-S2   |
| 1 x extraction ERM-CC392, No. 0658  | 2007/1060-S1.2 | 1 x extraction ERM-CC392, No. 0092  | 2007/1061-S2.1 |
| 1 x extraction ERM-CC392, No. 0092  | 2007/1061-S1   | 1 x extraction ERM-CC392, No. 0092  | 2007/1061-S2.2 |
| 1 x extraction control sample No. 7 | 2007/1059-S1   | 1 x extraction control sample No. 7 | 2007/1059-S2   |
| 1 x blank                           | BL-S1          | 1 x blank                           | BL-S2          |
| 1 x recovery standard               | REC-S1         | 1 x recovery standard               | REC-S2         |
| 1 x CRM1944                         | CRM-S1         | 1 x CRM1944                         | CRM-S2         |

## GC-ECD determination (in order of injection)

| <b>Series 1</b>             |          | <b>Series 2</b>             |          |
|-----------------------------|----------|-----------------------------|----------|
| Description                 | Code     | Description                 | Code     |
| Blank                       | BLS1     | Blank                       | BLS2     |
| Cal. point 1/500            | PCD500S1 | Cal. point 1/500            | PCD500S2 |
| Cal. point 1/100            | PCD100S1 | Cal. point 1/100            | PCD100S2 |
| Extract control sample No.7 | 1059S1   | Extract control sample No.7 | 1059S2   |
| Cal. point 1/5              | PCD5S1   | Cal. point 1/5              | PCD5S2   |
| Extract ERM-CC392, No.0658  | 1060S11H | Extract ERM-CC392, No.0658  | 1060S2   |
| Extract ERM-CC392, No.0658  | 1060S12  | Cal. point 1/10             | PCD10S2  |
| Cal. point 1/10             | PCD10S1  | Extract ERM-CC392, No.0092  | 1061S21  |
| Extract ERM-CC392, No.0092  | 1061S1   | Extract ERM-CC392, No.0092  | 1061S22  |
| Cal. point 1/25             | PCD25S1  | Cal. point 1/25             | PCD25S2  |
| "Beltest" solution No. 7    | 1057S1   | "Beltest" solution No. 3    | 1058S2   |
| Cal. point 1/50             | PCD50S1  | Cal. point 1/50             | PCD50S2  |
| CRM1944                     | CRMS1    | CRM1944                     | CRMS2    |
| Recovery standard           | RECS1    | Recovery standard           | RECS2    |

## PCB method

GC-ECD determination on two column system after Soxhlet extraction and clean-up on alumina and silica gel column was applied. More detailed description of the method is as follow.

After addition of 200 µL of CB 29 (0.4 µg/ml in iso-octane) as an internal standard, sample mixed with sodium sulphate was Soxhlet extracted for 6.5 hours with 140 ml of pentane/dichloromethane (1:1). 2 ml of iso-octane was added to the extract as a keeper and the extract was concentrated to about 2 ml in a rotary evaporator. 2-propanol and tetrabutylammonium sulphate solution were added to the extract for sulphur removal and mixed for 1 min with hand and vortex. After addition of 50 ml of water and 25 ml of n-pentane, organic phase was recovered in a separation funnel. Water was released from the separation funnel and the organic phase was dried by passing through the glass funnel filled with sodium sulphate. The water phase was extracted once more by addition of 25 ml of n-pentane and organic phase was recovered and dried as in the previous step. Finally, separation funnel was flushed with 25 mL of n-pentane, which was then dried by passing through the glass funnel filled with sodium sulphate and all three organic phases were combined. The extract was then concentrated to about 2 ml using a rotary evaporator and quantitatively transferred to a measuring cylinder with ca. 18 ml of n-pentane. Further clean-up and fractionation was then performed by putting the extract on top of a 15 g alumina · 8% H<sub>2</sub>O column and eluting with 100 mL n-pentane to collect the PCB fraction. The eluate was then concentrated to about 2 ml in a rotary evaporator, quantitatively transferred to a test tube with ca. 10 ml of n-pentane and concentrated to ca. 2 ml by Turbovap. The concentrate was transferred to a silica column (1.8 g silica · 1.5% H<sub>2</sub>O) and eluted with 11 ml of iso-octane into the volumetric flask to collect PCB fraction. The eluate was then concentrated to 10 ml by gentle stream of nitrogen. Ca. 0.5 ml of the extract was transferred to autosampler vial and test determination by GC-ECD was then performed to estimate concentration of the analytes in the eluate. The levels of PCBs were found to be within the calibration range and no dilution/concentration was necessary. 1 ml of the extract was then transferred to a test tube, 1 ml of internal standard CB 112 (0.04 µg/ml in iso-octane) was added and concentrated back to ca. 1 mL by Turbovap. The final extract was transferred to an autosampler vial and injected on GC-ECD.

Final determination was performed by gas chromatography (GC) with electron capture detection (ECD) using two column system: Cp-Sil 8 (50 m long with internal diameter of 0.15 mm and film thickness of 0.20 µm) and Cp-Sil 19 (50 m long with internal diameter of 0.15 mm and film thickness of 0.20 µm). Agilent 6890 gas chromatograph used was equipped with one split/splitless injector and two ECD detectors. Both columns were connected to one split/splitless injector operating at 250°C in a splitless mode with splitless time of 1 min. 2-µL samples were injected by Agilent autosampler 7683. ECD detectors were operated at 300°C with nitrogen make-up gas of 60 ml/min and nitrogen anode gas flow of 6 ml/min. Hydrogen was used as carrier gas at constant

pressure of 40 psi. Temperature programme was: 90°C (6 min), then 20°C/min to 215°C (30 min), then 5°C/min to 270°C (20 min) and then 5°C/min to 300°C (5 min).

PCB concentrations in each sample were calculated using data from both columns. Reported concentration was always the one with lower value. Quantification was based on peak height using 6 point calibration curve and a quadratic model. Recovery standard was analysed in each serie and the results for the samples were corrected for the determined recoveries. CB 112 was used as syringe standard. Internal standard CB 29 was used only as a check up and was not included in the calculations.

## Quality Assurance

IMARES utilises an ISO 9001:2000 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 December 2009. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. The last certification inspection was held the 16-22 of May 2007. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2009 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation, with the last inspection being held on the 12<sup>th</sup> of June 2007.

IMARES' PCB method is ISO 17025:2005 accredited for food (fish, fishery products) and environmental (sediment) matrices. The sewage sludge matrix is outside the accreditation scope and no extra validation was performed for this matrix. The sewage sludge samples in this study were treated as sediment matrix and analysed according to the sediment accredited procedure. Quality control measures applied included analysis of one blank, one recovery standard and one sediment certified reference material NIST-1944 in each series.

## Uncertainty calculation

Expanded measurement uncertainty (U) was estimated from the within-laboratory reproducibility and the bias as a % difference from the certified value.

The uncertainty of interlaboratory reproducibility,  $u(Rw)$ , was estimated as a relative standard deviation of 11 measurements of sediment internal reference material:

$$u(Rw) = \text{rsd}(\%) \text{ of 11 IRM measurements}$$

The uncertainty of the bias,  $u(bias)$ , was estimated from the last 6 measurements of sediment certified reference material NIST-1499. The following formulas were used:

$$u(bias) = \sqrt{bias^2 + \left(\frac{s(bias)}{\sqrt{n}}\right)^2 + u(cref)^2}$$

where  $bias = \frac{|x - cref|}{cref} \cdot 100$ ;

$$s(bias) = \text{rsd}(\%) \text{ of 6 CRM measurements};$$

$$u(cref) = \frac{s(cref)}{1.96} \cdot \frac{100}{c(ref)}$$

Combined standard uncertainty was calculated as:  $uc = \sqrt{u(Rw)^2 + u(bias)^2}$

Expanded standard uncertainty was calculated as:  $U = 2 \cdot uc$

Raw data used for calculations and calculations are given in the supplied MS excel file in the added sheet "Uncertainty calculation". Uncertainty for PCB 170 was not calculated because was not present in the used CRM.

## Results

Results of the measurements together with other required information such as uncertainty and method information were filled into the supplied MS excel file named 'Reporting PCB sewage sludge\_IMARES'. The file was sent by e-mail to [penka.shegunova@ec.europa.eu](mailto:penka.shegunova@ec.europa.eu) on December 14, 2007. The printed version of the excel file is attached as Annex A.

As explained in the method description, samples were measured on two columns and lower (i.e. free of interferences) value were reported. The following table shows which column was used to report the data. In addition, comments on (possible) co-elutions are added.

| PCB congener | Data reported from column | Comments                                       |
|--------------|---------------------------|--|
| PCB 28       | Sil 8                     |  |
| PCB 52       | Sil 19                    | co-elution on Sil 8                            |
| PCB 101      | Sil 19                    | possible co-elution on Sil 8 (101+84)          |
| PCB 105      | Sil 8                     | co-elution on Sil 19                           |
| PCB 118      | Sil 8                     |  |
| PCB 138      | Sil 19                    | <b>co-elution of CB138+163 on both columns</b> |
| PCB 149      | Sil 8                     |  |
| PCB 153      | Sil 8                     |  |
| PCB 156      | Sil 19                    | <b>incomplete resolution of CB156-202</b>      |
| PCB 170      | Sil 8                     | co-elution on Sil 19                           |
| PCB 180      | Sil 8                     | co-elution on Sil 19                           |

Copies of original chromatograms with assigned peaks, integration lines and the corresponding raw data sheets are given in Annexes B-G for all calibration, recovery and sample measurements.

Since there was a co-elution of CB 138 and CB 163 on both columns and we have just implemented in our laboratory determination by GC-MS, the samples were analysed also on GC-MS instrument using HT-8 column. This column allowed separation of CB 138 and CB 163. The same series were ran on GC-MS as on GC-ECD, i.e. including all samples, CRM, blank and recovery standard. The concentrations calculated from these GC-MS measurements are given in the supplied MS excel file in the 'Remarks' section.

In case of interest, detail on GC-MS determination can be provided. However, one should keep in mind that GC-MS determination is outside of our quality system.

# Referees and Authors

Rapport C136/07

Project Number: 439.51014.01

This report has been professionally prepared by Wageningen IMARES. The scientific validity of this report has been internally tested and verified by another researcher and evaluated by the Scientific Team at Wageningen IMARES.

Approved: Drs. J. Schobben  
Head of Department Environment

Signature:

Date: 18 December 2007

Number of copies: 5  
Number of pages: 8  
Number of tables: 4  
Number of graphs:  
Number of appendix attachments: 7



## Appendix A. Results of the measurements in the supplied MS Excel format

Appendix B. Raw data sheets with chromatograms  
and integration lines for samples of Series 1 on  
CP-Sil 8 column

Appendix C. Raw data sheets with chromatograms  
and integration lines for samples of Series 2 on  
CP-Sil 8 column

Appendix D. Zoom in visualization of the most important chromatograms for samples of Series 1 and 2 obtained on CP-Sil 8 column

Appendix E. Raw data sheets with chromatograms  
and integration lines for samples of Series 1 on  
CP-Sil 19 column

Appendix F. Raw data sheets with chromatograms  
and integration lines for samples of Series 2 on  
CP-Sil 19 column

Appendix G. Zoom in visualization of the most important chromatograms for samples of Series 1 and 2 obtained on CP-Sil 19 column