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Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
Attn: TSCA Section 8(e) Coordinator  
Ariel Rios Building  
1200 Pennsylvania Avenue, NW  
Washington, DC 20004



Re: TSCA Section 8(e) Notification of Substantial Risk:  
Correction to the submission mailed on December 3, 2009 which had an incorrect date of December 13, 2009. Octylmethylcyclotetrasiloxane, Decamethylcyclopentasiloxane and Dodecamethylcyclohexasiloxane

Dear TSCA Section 8(e) Coordinator:

In accordance with the provisions of Section 8(e) of the Toxic Substances and Control Act (TSCA), as interpreted in the TSCA Section 8(e) Policy Statement and Guidance, Fed. Reg. 33129 (June 3, 2003) and other Agency guidance, Dow Corning is submitting enclosed copy of the following report: Cyclic volatile methylsiloxane materials (D3, D4, D5, and D6) in Atlantic cod (*Gadus morhua*) from Oslofjord, Norway. Comparison and assessment of analytical methods utilized by Dow Corning Corporation, Evonik Goldschmidt, and the Norwegian Institute for Air Research. Dow Corning has not made a determination at this time that any significant risk of injury to human health or the environment is presented by these findings.

### Chemical Substances

541-05-9	Hexamethylcyclotrisiloxane
556-67-2	Octylmethylcyclotetrasiloxane
541-02-6	Decamethylcyclopentasiloxane
540-97-6	Dodecamethylcyclohexasiloxane



### Study

An inter-laboratory comparison of processing and analytical procedures for analysis of cyclic volatile methylsiloxane (cVMS) materials in fish liver obtained from Atlantic cod collected from the inner Oslofjord.

### Summary of Results

When comparing the results from the 3 laboratories, it was necessary to control for the differences in the size of the livers analyzed by each laboratory. A correlation analyses between fish characteristics using the four different

correlation measurements (Pearson, Spearman, Kendall, and Hoeffding correlation coefficients) were used. The correlation analyses between fish characteristics and concentrations of the cVMS materials in liver were statistically significant ( $p < 0.05$ ) for liver mass and liver condition index but were mixed (i.e., the correlations may or may not be significant, depending on correlation coefficient used) or not significant for condition factor, weight, or length. Consequently, the fish characteristics of weight, liver mass, liver condition index, and condition factor were included as random effects in the mixed ANOVA model used to test for differences between the three laboratories. However, it should be recognized that concentrations of the lipophilic cVMS materials in the cod livers were likely related to lipid content of the liver rather than to mass of the liver. One laboratory, determined lipid content of the livers that they harvested ( $n=6$ ). Correlation analyses (Pearson product moment correlation coefficient only) based on this limited data set (degrees of freedom = 5) indicated that liver mass was marginally correlated ( $p=0.06$ ) with lipid content of the liver. Although liver mass and liver condition index are reasonable surrogates for lipid content of the liver (Lambert and Dutil 1997), incorporation of measured lipid content as a random effect in the mixed ANOVA model would likely have been much more effective at controlling variability in cVMS concentrations.

It is well documented that Atlantic cod may contain concentrations of contaminants, particularly in the liver, which because of its very high lipid content, tends to accumulate persistent lipophilic compounds (Schneider et al. 2000; Falandysz 2003; Schnell et al. 2008). The cytochrome P450 system plays an important role in metabolism of contaminants in fish and is known to be induced by exposure to certain materials, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dioxins, and furans (Stagg and McIntosh 1998). Exposure to these materials from highly contaminated areas, such as the Inner Oslofjord is a confounding factor that any interpretation of increased liver size difficult.

A description of all the results is available in the enclosed reports. Dow Corning has not made a determination at this time that any significant risk of injury to human health or the environment is presented by these findings.

Sincerely,



Kathleen P. Plotzke, Ph.D.  
Director, Health and Environmental Sciences  
(989) 496-8046

---

Study Title: Cyclic volatile methylsiloxane materials (D3, D4, D5, and D6) in livers of Atlantic cod (*Gadus morhua*) from Oslofjord, Norway. Comparison and assessment of analytical methods utilized by Dow Corning Corporation, Evonik Goldschmidt, and the Norwegian Institute for Air Research.

HES Study No: 10922-108

Test Substances: Hexamethylcyclotrisiloxane (D<sub>3</sub>; CAS No. 541-05-9)  
Octamethylcyclotetrasiloxane (D<sub>4</sub>; CAS No. 556-67-2)  
Decamethylcyclopentasiloxane (D<sub>5</sub>; CAS No. 541-02-6)  
Dodecamethylcyclohexasiloxane (D<sub>6</sub>; CAS No. 540-97-6)

Author: David E. Powell, Dow Corning Corporation

Contributing Scientists: Jeremy Durham, Dow Corning Corporation  
Darren Huff, Dow Corning Corporation  
Thomas Böhmer, Evonik Goldschmidt GmbH  
Reinhard Gerhards, Evonik Goldschmidt GmbH  
Martin Koerner, Evonik Goldschmidt GmbH  
Regina Unthan, Evonik Goldschmidt GmbH  
Henriette Leknes, Norwegian Institute for Air Research  
Martin Schlabach, Norwegian Institute for Air Research  
Norman Green, Norwegian Institute for Water Research  
Merete Schøyen, Norwegian Institute for Water Research

Sponsor: Centre Européen des Silicones (CES)

Testing Facility: Health and Environmental Sciences  
Dow Corning Corporation  
2200 West Salzburg Road  
Auburn, MI 48611

HES Group Manager: Roy A. Campbell

Date: 13 July 2009

## Abstract

An inter-laboratory comparison of processing and analytical procedures for analysis of cyclic volatile methylsiloxane (cVMS) materials in fish liver was performed across three separate laboratories: Norwegian Institute for Air Research (NILU), Evonik Goldschmidt GmbH, and Dow Corning Corporation. Whole Atlantic cod (*Gadus morhua*) used for the inter-laboratory comparison were collected from Inner Oslofjord, Norway by the Norwegian Institute for Water Research (NIVA). Each Laboratory received five or six whole frozen fish, which were processed according to each laboratory's protocol. Each laboratory was responsible for harvesting livers that were free of adipose and mesenteric tissue from their assigned fish, homogenizing the liver samples, and for providing samples of the homogenized livers to the other two laboratories for analysis. Livers from individual fish were harvested and processed following laboratory-specific protocols. Similarly, each laboratory analyzed the homogenized liver samples following laboratory-specific protocols for hexamethylcyclotrisiloxane (D<sub>3</sub>), octamethylcyclotetrasiloxane (D<sub>4</sub>), decamethylcyclopentasiloxane (D<sub>5</sub>) and dodecamethylcyclohexasiloxane (D<sub>6</sub>) by GC-MS. No attempt was made to standardize sample collection, sample processing, or sample analysis across the three laboratories. Quality control procedures, sample processing, analytical methods, and measured concentrations of the cVMS materials were compared for consistency across the three laboratories. Methods of processing, extraction, and analysis were variable across the three laboratories, which was attributed to the lack of standard procedures. Although concentrations of the cVMS materials measured in the cod livers were similar, there were statistically significant differences that were not related to fish characteristics or to processing of the fish. Based on these differences recommendations are provided for collection, processing, and analysis of samples for cVMS materials.

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## Signatures and Approval

This report consists of 47 pages with 3 Appendices of 41 pages for a total of 88 pages.  
The undersigned have read and approved this report.

Author: David E. Powell  
David E. Powell, Ph.D.  
Chemistry and Environmental Sciences  
Dow Corning Corporation

13-Jul-2009  
Date

Management: Roy A. Campbell  
Roy A. Campbell, B.S.  
Chemistry and Environmental Sciences  
Dow Corning Corporation

13-July-2009  
Date

## Introduction

Cyclic volatile methylsiloxane (cVMS) materials, specifically octamethylcyclotetra-siloxane (D<sub>4</sub>; CAS No. 556-67-2), decamethylcyclopentasiloxane (D<sub>5</sub>; CAS No. 541-02-6), and dodecamethylcyclohexasiloxane (D<sub>6</sub>; CAS No. 540-97-6), are widely used in industrial, personal care, and household applications (Hori and Kannan 2008), and the wastewater stream represents a major post-use disposal route. Generally, cVMS materials have relatively low molecular weight (297 to 445 amu), are volatile (vapor pressure 4.7 to 132 Pa at 25°C), have very low water solubility (5 to 56 µg/L), and are very lipophilic (Log K<sub>OW</sub> 6.5 to 9.1). These properties are a consequence of the weak dispersion interactions in the neat liquid due to the low polarizability of the methyl siloxane materials, as reflected by their low molar refractivity, and their relatively large molecular size (Hirner et al. 2003; Ahmed et al. 2007). Because of these properties, cVMS materials occupy a somewhat unique chemical space, which makes analysis of these materials in environmental matrices challenging. In addition, as a result of the widespread use of the cVMS materials it may be difficult to collect, process, and analyze environmental matrices that are free of contamination.

Relatively little data is currently available on the behavior of cVMS materials in the environment (reviewed by Brooke et al. 2009a;b;c). Cyclic volatile methylsiloxane materials have been measured in wastewater effluents (reviewed by Hirner et al. 2003; Brooke et al. 2009a,b,c). A Nordic survey (Kaj et al. 2005) found concentrations of cVMS materials ranging from < 0.1-6 µg/L in wastewater effluents with D5 predominating. However, the cVMS materials were not detected in urban or background surface waters. Urban sediments in Scandinavia had concentrations of cVMS materials ranging from <1 to 2200 ng/g dry weight (dw) while they were not detected at background sites. Schlabach et al. (2007) measured D4, D5, and D6 in wastewater effluent, surface water, sediment and biota from Inner Oslofjord. Concentrations of cVMS materials ranged from < 0.02-12 µg/L in wastewater and from < 4-920 ng/g dw in sediment, with D5 predominating, but were not detected in surface waters. Concentrations of cVMS materials in biota were 1.3-8.7 ng/g wet weight (ww) in mussels, 0.9-27 ng/g ww in livers of flounder, 70-2200 ng/g ww in livers of cod. Kaj et al. (2004, 2005) analyzed fish, seabird eggs, and marine mammals from Norway and Iceland for cVMS materials, finding D4, D5 and D6 in livers of fish (flounder, sculpin, dab) from urban areas and from several "background" marine locations. The cVMS materials were also detected in freshwater fish (pike, vendace) from Finland but not in fish from background sites (arctic char and brown trout, Faroe Islands). Cyclic volatile methylsiloxane materials were not detected in seabird eggs (fulmar, herring gull). However, cVMS materials were reported at low ng/g concentrations in blubber of harbor seals from Denmark and pilot whale from the Faroe Islands. Knudsen et al. (2007) measured D5 residues ranging from 32.2-68.8 ng/g (wet weight; ww) in livers of dead or dying glaucous gulls from Bear Island, located midway between the mainland of Norway and the southern tip of Svalbard Archipelago. Kierkegaard et al. (2008) measured D5 residues averaging 10-12 ng/g ww in Arctic char taken from Lake Vattern, a large lake in Sweden receiving both industrial and wastewater discharge, while residues in a remote Swedish lake were detectable but below the limit of quantitation of 1 ng/g ww. A

Nordic survey (Evenset et al. 2009) on Svalbard Archipelago measured cVMS residues in livers of Arctic fish (polar cod and Atlantic cod) ranging from below detection (< 4.3 ng/g ww) to 10.4 ng/g ww for D3, from 2.6 to 9.2 ng/g ww for D4, from 2.7 to 19.1 ng/g ww for D5, and from below detection (< 9.7 ng/g ww) to 10.7 ng/g ww for D6. Concentrations in whole-body polar cod ranged from 3.6-9.9 ng/g ww for D3, from 3.6 to 7.8 ng/g ww for D4, from 2.2 to 5.1 ng/g ww for D5, and from 2.2 to 3.8 ng/g ww for D6. Low levels of the cVMS materials were measured in livers of sea birds (kittiwake and eider) that ranged from below detection (< 3.1 ng/g ww) to 3.8 ng/g ww, but were also detected in the field blanks suggesting that samples may have been contaminated during collection and processing. While these limited measurements appear to suggest that cVMS materials may accumulate in top predator fish and marine mammals and birds, it is unclear whether these results represent actual food web biomagnification or are simply a result of continuous exposure and rapid elimination or contamination of samples during collection, processing and analysis.

The objective of the project described in this report was to compare and contrast analytical results and laboratory quality control measures for analysis of hexamethylcyclotrisiloxane (D<sub>3</sub>; CAS No. 541-05-9), D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub> in livers of Atlantic cod (*Gadus morhua*) collected from Inner Oslofjord, Norway. This report does not evaluate field quality control measures or potential contamination of samples that may have occurred during collection. Field contamination of the samples was considered unlikely because only livers of the codfish were analyzed and the fish were kept intact until the livers were collected in the laboratory. The laboratories selected for this inter-laboratory comparison were actively involved with analysis of cVMS materials in environmental matrices and included Dow Corning Corporation (DCC, located in Auburn, Michigan, USA), Evonik Goldschmidt GmbH (Evonik, located in Essen, Germany) and the Norwegian Institute for Air Research (NILU, located in Kjeller, Norway).

## Materials and Methods

### Sample Collection

A total of 17 Atlantic cod (*Gadus morhua*) were used for this project (Table 1) and were collected by trawl from Inner Oslofjord on 10 December 2007 (Fig. 1). Cod were collected from aboard the *F/F Trygve Braarud* by the Norwegian Institute of Water Research (NIVA), measured for total length (cm) and fresh weight (g) in the field, and individually frozen in plastic bags for distribution to the three analytical laboratories for processing and analysis.

Before being distributed to the laboratories for processing the 17 cod were first separated into two size classes to control for possible confounding effects of size and age on concentrations of cVMS. The age-growth relationship for Atlantic cod is dependant upon location and water temperature. Age and growth relationships reported for Atlantic cod (Brander 1995) collected from the North Sea (T=8.6°C) and off the West Coast of Scotland (T=10°C) suggested that Oslofjord cod less than 1000 g

fresh weight were likely age-1 individuals (n=11) and cod greater than 1000 g fresh weight were likely age-2 individuals (n=6).

The two size classes of cod were allocated and randomly distributed across the three analytical laboratories for processing following a randomized block design (blocked on fresh weight). Each laboratory was provided with two fish greater than 1000 g fresh weight (presumably age-2 fish) and with three or four fish less than 1000 g fresh weight (presumably age-1 fish). Each laboratory was then responsible for harvesting the livers from their assigned fish (Table 1), and for providing samples of the homogenized livers to the other two laboratories for analysis.

A summary of the sample Chain-of-Custody after collection of the fish by NIVA is provided in Table 2. Briefly, frozen whole fish were transported from NIVA (Oslo, Norway) to Norwegian Institute for Air Research (NILU; Kjeller, Norway) on 25 Jan 2008 and stored at -18 °C, where they were held until processed. Frozen whole fish packed under dry ice were shipped from NIVA (Oslo, Norway) to Evonik Goldschmidt (Evonik; Essen, Germany) on 11 Feb 2008, received by Evonik in frozen condition on 13 Feb, and stored at -20°C where they were held until processed. Frozen whole fish packed under dry ice were shipped from NIVA (Oslo, Norway) to Dow Corning Corporation (DCC; Auburn, Michigan) on 11 Feb 2008, received by DCC in frozen condition on 14 Feb 2008 and stored at -80°C where they were held until processed. All bags containing the fish were intact with most of the dry ice still in place.

Quality control samples were prepared using livers from commercially obtained fish. Dow Corning obtained control livers from rainbow trout (*Oncorhynchus mykiss*) that were purchased live from Rainbow Ranch Trout Farm located in Tawas City, Michigan. Evonik obtained a control liver from a wolffish (*Anarhichas* sp.) that was purchased from a commercial supplier. NILU obtained control liver from an Atlantic cod (*Gadus morhua*) that was purchased through a local supermarket.

### **Sample Preparation (Table 2)**

Each laboratory was responsible for harvesting livers that were free of adipose and mesenteric tissue from their assigned fish, homogenizing the liver samples, and for providing samples of the homogenized livers to the other two laboratories for analysis. Livers from individual fish were harvested and processed following laboratory-specific protocols. No attempt was made to standardize sample collection or processing across the three laboratories. After collection individual livers were weighed, homogenized, and dispensed to pre-cleaned storage containers that were provided by each laboratory with instructions specifying how the homogenized liver samples were to be stored and shipped.

DCC (Appendix 1): Livers from the 6 cod sent to Dow Corning were harvested from the thawed fish using a scalpel on 23 April 2008. The harvested livers were processed by cutting the samples into small pieces using scissors that were cleaned with solvent between each liver sample. The finely-cut liver samples were then dispensed to the appropriate containers and stored at -80°C. Frozen

liver samples from the 6 cod were shipped under dry ice from Dow Corning (Auburn, Michigan) to Evonik (Essen, Germany) on 06 May 2008, where the samples were received in frozen condition on 08 May 2008 and stored at -20°C. Frozen liver samples from the 6 cod were shipped under dry ice from Dow Corning (Auburn, Michigan) to NILU (Kjeller, Norway) on 06 May 2008, where the samples were received in frozen condition on 09 May 2008 and stored at -18°C.

Evonik (Appendix 2): Livers from the 5 cod sent to Evonik were harvested from the thawed fish on 24 April 2008. The harvested livers were processed using an Ultra-Turrax tissue homogenizer that was cleaned between each liver sample. The liver homogenates were then dispensed to the appropriate containers and stored at -20°C. Frozen homogenized liver samples from the 5 cod were shipped under dry ice from Evonik (Essen, Germany) to Dow Corning Corporation (Auburn, Michigan) on 28 April 2008, where the samples were received in frozen condition on 30 April 2008 and stored at -80°C. Frozen homogenized liver samples from the 5 cod were shipped under dry ice from Evonik (Essen, Germany) to NILU (Kjeller, Norway) on 05 May 2008, where the samples were received in frozen condition on 07 May 2008 and stored at -18°C.

NILU (Appendix 3): Livers from the 6 cod sent to NILU were harvested from the thawed fish using a scalpel on 12 May 2008 (Table 2). The harvested livers were processed using an Ultra-Turrax tissue homogenizer that was cleaned with several rinses of hexane between each liver sample. The liver homogenates were then dispensed to the appropriate containers and stored at -18°C. Frozen homogenized liver samples from the 6 cod were shipped under dry ice from NILU (Kjeller, Norway) to Evonik (Essen, Germany) on 14 May 2008, where the samples were received in unfrozen condition on 16 May 2008 and stored at -20°C. Frozen homogenized liver samples from the 6 cod were initially shipped under dry ice from NILU (Kjeller, Norway) to Dow Corning Corporation (Auburn, Michigan) on 13 May 2008, but the samples were never received. A second set of frozen homogenized liver samples from 4 of the 6 cod (sufficient mass was not available for 2 of 6 cod) were shipped under dry ice from NILU to Dow Corning Corporation on 03 June 2008, where the samples were received in frozen condition on 05 June 2008 and stored at -80°C.

Indices used to describe the general condition of the cod were the Fulton condition factor (K) and the liver condition index (LCI; also known as the hepatosomatic index, or HSI, when based on somatic tissue mass). The Fulton condition factor was calculated as:

$$K = \frac{W_{fish}}{L^3} \times 100$$

where:  $K$  = condition factor of fish  
 $W_{fish}$  = fresh weight of fish (in g)  
 $L$  = total length of fish (in cm)

The liver condition index was calculated as:

$$LCI = \frac{W_{liver}}{W_{fish}} \times 100$$

where:  $LCI$  = condition factor of liver  
 $W_{liver}$  = fresh weight of liver (in g)  
 $W_{fish}$  = fresh weight of fish (in g)

### **Analytical Methods**

Each laboratory was responsible for extracting and analyzing the homogenized liver samples following laboratory-specific protocols and quality control procedures. There was no attempt to standardize sample extraction or sample analysis across the three labs, including methods for determination of limits of detection and quantification. The experimental design for this project was developed so that each lab would measure concentrations of  $D_3$ ,  $D_4$ ,  $D_5$ , and  $D_6$  in 17 homogenized liver samples. Because of samples being lost in shipment, DCC was able to analyze only 15 of 17 homogenized liver samples. Evonik did not report concentrations of  $D_3$  in the homogenized liver samples because of poor reproducibility during the analysis of blanks and calibration standards.

#### **Sample Extraction (Table 3; Fig. 2)**

DCC (Appendix 1): Except for one sample from NILU, liver homogenates (ca. 1.5 g) were extracted in duplicate or triplicate using a 2:1 solvent-to-sample mass ratio of tetrahydrofuran (THF) containing known concentrations of  $M_4Q$  and isotopically-labeled  $^{13}C-D_4$ ,  $^{13}C-D_5$ , and  $^{13}C-D_6$  to serve as internal standard surrogates. Samples were placed on a vortex mixer for 30 min, the solids separated by centrifugation for 5 min, and the THF layer transferred to a separate vial. The extraction process was repeated with a second portion of THF without the internal standards and the THF layer was combined with the THF layer from the first extraction. The combined THF was dried over anhydrous magnesium sulfate (ca. 0.25 g) and transferred to a crimp-cap vial for GC-MS analysis.

Evonik (Appendix 2): Liver homogenates (ca. 0.4 g) were extracted in duplicate using a 10:1 solvent-to-sample mass ratio of pentane containing known concentrations of isotopically-labeled  $^{13}C-D_4$ ,  $^{13}C-D_5$ , and  $^{13}C-D_6$  to serve as internal standard surrogates. Samples were homogenized for 1 min using an Ultra-Turrax, 1 mL of water was added and the homogenization was repeated, the solids separated by centrifugation for 5 min, and the pentane layer transferred to a separate vial. The pentane extract was dried over anhydrous magnesium sulfate (ca. 0.30 g) and cleaned by column chromatography using Florisil that was eluted with petroleum ether and methyl tertiary butyl ether (99:1).

The eluate was reduced in volume to 0.5 mL under a stream of nitrogen and transferred to a crimp-cap vial for GC-MS analysis.

NILU (Appendix 3): Liver homogenates (ca. 0.3 g) were extracted in duplicate using a 3:1 solvent-to-sample mass ratio of hexane. Isotopically-labeled internal standard were not available. Samples were placed on a vortex mixer for 30 min, the solids separated by centrifugation for 10 min, and the hexane layer transferred to a separate vial for GC-MS analysis.

### ***Sample Analysis (Table 4)***

DCC (Appendix 1): DCC used gas chromatography with low-resolution mass selective detection (GC-MS) to measure cVMS materials in tetrahydrofuran (THF) extracts of the homogenized liver samples. Analyses were conducted in a general laboratory with no process in place to minimize potential contamination from air. Except for D<sub>3</sub>, isotopically-labeled internal standards were used to correct for analytical bias that may have resulted from matrix effects and potential loss of analyte from the samples.

Evonik (Appendix 2): Evonik used gas chromatography with low-resolution mass selective detection (GC-MS) to measure cVMS materials in pentane extracts of the homogenized liver samples. Analyses were conducted in a general laboratory with no process in place to minimize potential contamination from air. Except for D<sub>3</sub>, isotopically-labeled internal standards were used to correct for analytical bias that may have resulted from matrix effects and potential loss of analyte from the samples.

NILU (Appendix 3): NILU used gas chromatography with high-resolution mass selective detection (GC-HRMS) to measure cVMS materials in hexane extracts of the homogenized liver samples. Liver collection and sample preparation was conducted in a clean room with processes in place to minimize potential contamination from air. Analyses were conducted in a general laboratory. Internal standards were not used to correct for analytical bias that may have resulted from matrix effects and potential loss of analyte from the samples. However, the results were recovery corrected based on spiked samples of cod liver.

### ***Detection Limits***

No attempt was made to standardize analytical methods and procedures across the three laboratories, including methods for determination of levels of detection and quantification. As a result, levels of detection and quantification reported by the three laboratories were not defined on a uniform basis and were not always directly comparable. Of the different detection levels typically reported for environmental monitoring studies, the lower level of detection (LOD), the method detection level (MDL),

and the level of quantification (LOQ) are the most useful. These detection levels were defined and reported by the three laboratories as:

- DCC: The LOD was defined as 3 times the standard deviation ( $n=4$ ) of the response from analysis of matrix blanks (non-spiked cod liver). If the matrix blank response values were negative the LOD was estimated as 1/3 of LOQ. The LOQ was defined as ten times the standard deviation of the matrix blank response. The MDL was not specifically defined or calculated in the report.
- Evonik: The LOD was defined as 3 times the standard deviation ( $n=5$ ) of the response from analysis of matrix blanks (non-spiked wolffish liver). The LOQ was defined as 3 times of the LOD. The MDL was not specifically defined or calculated in the report.
- NILU: The LOD was defined as 3 times the hexane blank response ( $n=3$ ). In the absence of a blank response for a particular siloxane, the compound LOD was calculated as the peak-to-peak signal-to-noise ratio of 3:1. The MDL and LOQ were not specifically defined or calculated in the report.

For comparison across the three laboratories, detection levels were identified as defined by APHA (1999) and recalculated using data provided in the laboratory-specific reports (Appendices 1-3). Although the terminology used by APHA (1999) may differ from that provided in more definitive sources (ASTM 2003; IUPAC 1995, 2002) the definitions are analogous. Methods for determining detection levels are available in various publications (Analytical Methods Committee 1987; Taylor 1987; Kateman and Buydens 1993; IUPAC 1995; Berger et al. 1996; Smith 1997; Eurachem 1998; APHA 1999; IUPAC 2002). The LOD, MDL, and LOQ are similar in concept to the signal-to-noise ratio, and were defined as:

- LOD: The minimum level of target analyte that can be measured and reported with 99% confidence that the level of analyte is greater than zero. The LOD is based on the ability to distinguish between signal and noise of the instrument and is determined by repetitive analysis of matrix-free blanks.
- MDL: The minimum level of target analyte in a specified matrix that can be measured and reported with 99% confidence that the level of analyte in the matrix is greater than zero. The MDL is a measure of a methods ability to quantify an analyte in a sample matrix and is determined by repetitive analysis of a sample matrix having a small, but measurable amount of analyte (2-5 times the MDL). The MDL will be greater than the LOD because of additional background noise resulting from the sample matrix and variability introduced through processing the sample for analysis.
- LOQ: The minimum level of target analyte in a sample that can be reported with 99% confidence of having an estimation error no greater than 30% when based on a single measurement. The LOQ is the minimum level of target analyte in a

sample that can be detected and accurately quantified on a routine basis, and is typically defined as 3.3 times the LOD or, preferably, as 2.5 times the MDL.

Detection levels (DL) are determined as a function of the variance associated with replicate analyses of matrix-free blanks (LOD) and replicate analyses of samples containing a small, but measurable amount of target analyte (MDL, LOQ). The DL (LOD and MDL) at 99% confidence are then defined as:

$$DL = t_{0.99, n-1} \times s_0$$

where  $t_{0.99, n-1}$  is the one-tailed t-statistic at 99% confidence for the performed number of sample analyses (degrees of freedom = n-1) and  $s_0$  is the standard deviation for the replicate analyses. In order to avoid skewing the resulting detection levels, the recommended minimum number of replicate analyses is n = 7 or 8 ( $t_{0.99, n-1} = 3.143$  or  $2.998$ , respectively), which yields the desired signal to noise ratio of about 3:1.

For comparison across the three laboratories, LOD values were calculated using the amounts of the cVMS materials measured in reagent blanks that were carried through the entire analytical procedure. The MDL values were calculated using the amounts of the cVMS materials measured in samples, as discussed below:

- DCC Data: Results for non-spiked cod liver were used to calculate MDLs as shown below. Except for D<sub>3</sub>, mean measured concentrations in the samples were between 9.4 and 19 times the calculated MDLs, indicating that the MDLs may be high (especially for D<sub>5</sub>). Mean measured concentrations of D<sub>3</sub> in the samples were less than the calculated MDL, indicating that the MDL for D<sub>3</sub> may be low.

Parameter	Concentration (ng/g ww) in Cod Liver			
	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>	D <sub>6</sub>
mean:	2.97	93.9	638	119
sd:	0.725	1.437	4.726	1.561
n:	3	3	3	3
$t_{0.99, n-1}$ :	6.965	6.965	6.965	6.965
MDL:	5.05	10.0	32.9	10.9

- Evonik Data: Results for non-spiked wolffish liver were used to calculate MDLs as shown below. Mean measured concentrations in the samples were all less than the calculated MDLs, indicating that the MDLs may be low.

Parameter	Concentration (ng/g ww) in Wolffish Liver			
	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>	D <sub>6</sub>
mean:		-0.68	4.01	5.97

sd:	2.252	4.514	1.923
n:	6	6	6
$t_{0.99,n-1}$ :	3.365	3.365	3.365
MDL:	7.58	15.2	6.47

- **NILU Data:** Results for spiked cod liver were used to calculate MDLs as shown below. Mean measured concentrations in the samples were between 2.8 and 7.8 times the calculated MDLs, indicating that the MDLs may be high (especially for D<sub>3</sub> and D<sub>4</sub>) but acceptable.

Concentration (ng/g ww) in Spiked Cod Liver				
Parameter	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>	D <sub>6</sub>
mean:	24.6	26.7	24.9	27.7
sd:	0.828	1.286	2.277	2.628
n:	5	5	5	5
$t_{0.99,n-1}$ :	3.747	3.747	3.747	3.747
MDL:	3.14	4.73	8.45	9.78

### **Left Censored Data**

For environmental monitoring, a measurement that is less than a specified detection level (DL) may be: 1) reported as "below detection", 2) reported as zero, 3) reported as less than (<) the value of the DL, 4) reported as some value between zero and the DL, for example one-half the DL, or 5) reported as the actual value (positive or negative), whether or not it is below the DL. The last option, the reporting of the actual value, is generally considered the recommended procedure (Clarke 1998; Frome and Wambach 2005; Antweiler and Taylor 2008); otherwise the data is considered to be "left censored". For this project, each laboratory was requested to report actual measured concentrations even if they were negative, less than the limit of detection (LOD), or less than the method detection limit (MDL).

### **Statistical Analyses**

Statistical analyses were performed using SAS Version 9.1.3 (SAS 2001). Actual measured values were used for all calculations even if results were negative or less than the limits of detection. Censored data was not used. A Type I error ( $\alpha$ ) of 0.05 was used to judge the significance of all statistical tests. The Pearson, Spearman, Kendall and Hoeffding correlation coefficients were used to evaluate correlations between the continuous variables (total length of fish, fresh weight of fish, condition factor of fish, liver condition index, and liver mass of fish) and between the continuous variables and concentrations of the cVMS materials measured in the liver by each laboratory.

Linear regression was used to determine the effects of length, weight and the interaction of length and weight on liver condition index and liver mass. Regression of cVMS concentrations on liver mass by analysis laboratory was done so that there was an independent regression line for each laboratory. Transformed values (discussed below) were used for the regression analyses so that residuals were normally distributed. Significant differences in slopes across the three laboratories were indicative of a matrix bias during the analyses. Significant differences in the intercept across the three laboratories were indicative of contamination or loss of analyte from the matrix.

Analysis of variance (ANOVA) for unbalanced designs (General Linear Model) was used to test for differences between processor for total length, weight, condition factor, liver condition index, and liver mass. Homogeneity of variance was determined by Levene's test (Levene 1960) and normality of the residuals was determined by the Shapiro-Wilk test (Shapiro and Wilk 1965). Because both random data (length, weight, condition factor, liver condition index, and liver mass) and fixed data (processor and analysis laboratory) were present, mixed modeling was used to test for differences in the cVMS concentrations in the liver determined by the three laboratories and if the processor affected those values. Because length was highly correlated with weight, only weight, liver mass, condition factor, and liver condition index were included as random effects in the model in order to effectively nest the fish data. The analysis laboratory, processor, and the interaction of the two were included as fixed effects in the model and least squares means were provided to compare between processor and analysis laboratory. A compound symmetry covariance structure was used for the random effects. Normally distributed data is a requirement of the mixed modeling regression analyses. The following transformations of the cVMS concentrations in the liver were done to achieve normality:

- For  $D_3$ , a log transformation with a translation of axis  $\ln(D_3 + 1)$  was done because negative concentration values were present in the data set.
- For  $D_4$ , a square root transformation with a translation of axis  $\sqrt{D_4 + 1}$  was done because negative concentration values were present in the data. A square root-transformation with axis translation was used because a log-transformation with axis translation did not result in normally distributed data.
- For  $D_5$ , a square root transformation was done since there were no negative concentration values present in the data. A square root-transformation with axis translation was used because a log-transformation did not result in normally distributed data.
- For  $D_6$ , a square root transformation with a translation of axis  $\sqrt{D_6 + 10}$  was done because negative concentration values were present in the data. A square root-transformation with axis translation was used because a log-transformation with axis translation did not result in normally distributed data.

## Results and Discussion

Of the different quality control parameters and detection levels typically reported for environmental monitoring studies, background levels, the lower level of detection (LOD),

the method detection level (MDL), and the level of quantification (LOQ) are the most useful. Generally, background levels of the cVMS materials were lower by a factor of 10x or more at NILU compared to DCC and Evonik (Table 5). Similarly, the LODs for NILU (range 0.25 to 1.55 ng) were considerably lower than the LODs for DCC (range 4.72 to 11.3 ng) or for Evonik (range 1.59 to 6.64 ng). Presumably, these differences were the result of NILU conducting the analyses in a clean room facility whereas DCC and Evonik conducted the analyses in standard laboratories. Except for D<sub>3</sub>, concentrations of the cVMS materials in the cod liver samples were generally greater than the MDLs and LOQs generated for each laboratory (Figs. 3-4). This suggests that use of a clean room did not appear to be required for samples from a highly contaminated system such as the Inner Oslofjord. Nevertheless, the lower backgrounds and LODs for the cVMS materials reported by NILU suggests that use of clean room technology may be required for analysis of samples collected from remote areas where trace contamination may have a significant impact. Enhanced monitoring of background levels in the field and laboratory, as well as a more robust quality control program may also be required for samples collected from remote areas. This would include collection, processing, and analysis of additional solvent blanks, appropriate matrix blanks, and reference samples. Without appropriate quality control in the field, use of clean room technology for analysis of the samples would be ineffective and may result in erroneous conclusions.

Because analytical methods and procedures were not standardized, levels of detection and quantification reported by the three laboratories were not defined on a uniform basis and were not always directly comparable. In order to make direct comparison across the three laboratories, detection levels were identified, redefined (APHA 1999), and recalculated using data provided in the laboratory-specific reports (Appendices 1-3). However, comparison of detection levels was still somewhat confounded because the number of replicate blanks (used to estimate LOD) and samples (used to estimate MDL) that were analyzed was not consistent across the three laboratories (Table 5). In addition, the MDL calculations were based on replicate analyses of livers from different species of fish having different concentrations of cVMS.

Because MDLs are concentration dependant, they should be based on repetitive analyses ( $n \geq 7$ ;  $t_{0.99, n-1} = 3.14$ ) of a sample matrix having a small, but measurable amount of analyte that is in the range of about 2 to 5 times the MDL. Only the MDLs generated for NILU appeared to meet these criteria. The MDLs for NILU were based on  $n=5$  replicate analyses ( $t_{0.99, n-1} = 3.75$ ) of spiked cod liver (commercially obtained; origin from northwest coast of Norway) having mean cVMS concentrations that were 2.9-7.7 times the MDL. The MDLs for DCC were based on  $n=3$  replicate analyses ( $t_{0.99, n-1} = 6.97$ ) of cod liver (from Oslofjord fish) having mean cVMS concentrations that were 0.6 times the MDL for D<sub>3</sub> and 9.4-19 times the MDL for the other materials. The MDLs for Evonik were based on  $n=6$  replicate analyses ( $t_{0.99, n-1}=3.37$ ) of wolffish liver (commercially obtained; origin not identified) having mean cVMS concentrations that were 0.1-0.9 times the MDL. Under these conditions, the MDLs calculated for NILU ranged from 3.14 to 9.78 ng/g ww (Table 5) and were the lowest across the three laboratories. The MDLs for the other laboratories ranged from 5.05 to 32.9 ng/g ww for

DCC, and from 6.47 to 15.2 ng/g ww for Evonik. Considering the differences between laboratories on how the MDLs were obtained, the level of agreement of the MDLs was reasonable and, except for D3, appeared to be adequate for cVMS materials in livers of cod collected from Inner Oslofjord. However, for future studies consideration should be given to establishing a standard definition and approach for calculating and reporting MDLs, as used in this report. This would be especially important for samples collected from remote areas where concentrations at or below the levels of detection may be encountered.

Generally, condition indices for cod from the Inner Oslofjord were comparable to indices for cod from polluted environments (Schnell et al. 2008; Hylland et al. 2009), and appeared to be indicative of fish in poor condition compared to free-ranging cod from non-polluted areas (Lambert and Dutil 1997; Yaragina and Marshall 2000; Mello and Rose 2005; Hylland et al. 2009). Fish characteristics of total length, fresh weight, condition factor ( $K$ ), and liver condition index ( $LCI$ ) were similar within the population of cod sampled and across the three processing laboratories that the fish were distributed (Table 1). In contrast, liver mass of the cod was highly variable, ranging from 4.5 to 35.9 g ww.

Atlantic cod may contain high concentrations of contaminants, particularly in the liver, which because of its very high lipid content, tends to accumulate persistent lipophilic compounds (Schneider et al. 2000; Falandysz 2003; Schnell et al. 2008). The cytochrome P450 system plays an important role in metabolism of contaminants in fish and is known to be induced by exposure to certain materials, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dioxins, and furans (Stagg and McIntosh 1998). Exposure to these materials results in increased liver size as the fish maximize detoxification of these compounds in the liver. Livers in cod from contaminated areas, such as the Inner Oslofjord, may be larger in size because of exposure to these contaminants. However, condition indices such as liver size do not provide a simple separation between polluted and pristine locations (Hylland et al., 2009).

Comparison of fish characteristics (Table 1) between the two size classes of cod (i.e., fresh weight < 1000 g and fresh weight > 1000 g) showed highly significant differences for length and weight ( $p < 0.001$ ) but no differences for liver mass, condition factor ( $K$ ), or liver condition index ( $LCI$ ). No significant differences were observed between fish characteristics across the three processors for the small size-class fish. However, liver mass of the large size-class fish processed by Evonik were significantly greater ( $p=0.04$ ) compared to the liver mass of fish processed by DCC and NILU.

The correlation analyses between fish characteristics using the four different correlation measurements (Pearson, Spearman, Kendall, and Hoeffding correlation coefficients) indicated that strong correlations existed only between length and weight of the fish ( $p < 0.01$ ) and between liver condition index and liver mass ( $p < 0.01$ ). Significant correlations did not exist ( $p > 0.09$ ) between condition factor and liver mass, length, or weight. Liver mass was correlated with weight only for Kendall's test ( $p=0.04$ ) but was

not correlated ( $p > 0.05$ ) with length or condition factor. Regression analysis showed a significant effect of weight on the liver mass ( $p=0.03$ ), but not with length ( $p=0.07$ ) or the interaction of length and weight ( $p=0.05$ ).

The correlation analyses between fish characteristics and concentrations of the cVMS materials in liver were statistically significant ( $p < 0.05$ ) for liver mass and liver condition index (Table 6; Figs. 3-4) but were mixed (i.e., the correlations may or may not be significant, depending on correlation coefficient used) or not significant for condition factor, weight, or length. For  $D_3$  concentrations, correlation coefficients were statistically significant for liver mass and liver condition index but not for length, weight, or condition factor. For  $D_4$  concentrations, correlation coefficients were statistically significant for liver mass and liver condition index, were mixed for condition factor and weight, but were not significant for length. For  $D_5$  concentrations, correlation coefficients were statistically significant for liver mass, liver condition index, condition factor, and weight, but were not significant for length. For  $D_6$  concentrations, correlation coefficients were statistically significant for liver mass and liver condition index, were mixed for weight and length, but were not significant for condition factor. These results demonstrated that the *a priori* decision to use a randomized design blocked on size of fish was not effective at controlling variability in cVMS concentrations. Consequently, the fish characteristics of weight, liver mass, liver condition index, and condition factor were included as random effects in the mixed ANOVA model used to test for differences between the three laboratories. However, it should be recognized that concentrations of the lipophilic cVMS materials in the cod livers were likely related to lipid content of the liver rather than to mass of the liver. One laboratory, DCC, determined lipid content of the livers that they harvested ( $n=6$ ). Correlation analyses (Pearson product moment correlation coefficient only) based on this limited data set (degrees of freedom = 4) indicated that cVMS concentrations were not correlated ( $p>0.05$ ) with liver mass, that concentrations of  $D_4$  and  $D_5$  (but not  $D_3$  and  $D_6$ ) were correlated ( $p<0.05$ ) with lipid content of the liver, and that liver mass was marginally correlated ( $p=0.06$ ) with lipid content of the liver. Although liver mass and liver condition index are reasonable surrogates for lipid content of the liver (Lambert and Dutil 1997), incorporation of measured lipid content as a random effect in the mixed ANOVA model would have been much more effective at controlling variability in cVMS concentrations.

Generally, comparison of cVMS concentrations shows good agreement in performance across the three laboratories (Table 7). Except for  $D_3$ , comparison of analytical results without including fish characteristics as random effects indicated no significant differences between the three laboratories that could be attributed to processor (Figs. 5-6), analysis (Figs. 7-8), or to the interaction of processor and analysis (Figs. 9-10). A highly significant difference ( $p < 0.01$ ) was observed for  $D_3$  concentrations that could be attributed to analysis, but not to processor or the interaction of processor and analysis. The linear regression of liver mass on  $D_3$  concentrations showed significant differences in intercepts of the regression lines but not the slopes, suggesting that samples analyzed by NILU may have been contaminated with  $D_3$  or that  $D_3$  was lost from samples analyzed by Dow Corning.

When fish characteristics were taken into account, no significant differences in cVMS concentrations were observed across the three processors. However, the mixed modeling comparisons of cVMS concentrations indicated that significant differences existed between the three analysis laboratories that were not related to fish characteristics or to processing of the fish. Mixed modeling comparisons of cVMS concentrations across the three analysis laboratories indicated:

- For D<sub>3</sub> (Fig. 11): Concentrations of D<sub>3</sub> measured by NILU were significantly ( $p < 0.01$ ) greater than concentrations measured by DCC (Evonik did not report concentrations for D<sub>3</sub>). Ranking of mean measured concentrations of D<sub>3</sub> in Oslofjord cod livers followed the order: NILU > DCC (Table 7).
- For D<sub>4</sub> (Fig. 11): Concentrations of D<sub>4</sub> measured by DCC were significantly ( $p=0.01$ ) greater than concentrations measured by NILU. There were no statistically significant differences between concentrations of D<sub>4</sub> measured by DCC and Evonik or between concentrations of D<sub>4</sub> measured by Evonik and NILU. Rank of mean measured concentrations of D<sub>4</sub> in Oslofjord cod livers followed the order: DCC ≥ Evonik ≥ NILU (Table 7).
- For D<sub>5</sub> (Fig. 12): Concentrations of D<sub>5</sub> measured by DCC and Evonik were significantly ( $p < 0.01$ ) greater than concentrations measured by NILU. There were no statistically significant differences between concentrations of D<sub>5</sub> measured by DCC and Evonik ( $p=0.53$ ). Rank of mean measured concentrations of D<sub>5</sub> in Oslofjord cod livers followed the order: DCC ≥ Evonik > NILU (Table 7).
- For D<sub>6</sub> (Fig. 12): Concentrations of D<sub>6</sub> measured by DCC and Evonik were significantly ( $p < 0.01$ ) greater than concentrations measured by NILU. There were no statistically significant differences between concentrations of D<sub>6</sub> measured by DCC and Evonik ( $p=0.98$ ). Rank of mean measured concentrations of D<sub>5</sub> in Oslofjord cod livers followed the order: DCC ≥ Evonik > NILU (Table 7).

Concentrations of D<sub>3</sub> in cod liver measured by DCC were consistently less than concentrations measured by NILU (Fig. 11). Concentrations of D<sub>3</sub> measured by DCC were greater than the DCC MDL of 5.05 ng/g ww (Table 5) in only 1 of 15 samples. In contrast, concentrations of D<sub>3</sub> measured by NILU were greater than the NILU MDL of 3.14 ng/g ww (Table 5) in 15 of 18 samples, and were greater than the DCC MDL in 8 of 18 samples. These results can not be attributed to problems associated with analyzing samples having concentrations that are less than or equal to the level of detection. If detection levels were an issue, individual concentrations of D<sub>3</sub> measured by NILU and DCC would have been randomly distributed about the mean concentrations across the two laboratories. However, the distribution of D<sub>3</sub> concentrations was not random and concentrations measured by NILU were always greater than concentrations measured by DCC (Fig. 11). While the differences between the two laboratories may be related to possible contamination issues, the fact that background levels and LODs for D<sub>3</sub> were

lowest for NILU (Table 5) makes the possibility of contamination appear to be small. Rather, differences between the two laboratories for D<sub>3</sub> are more likely related to NILU not using an internal standard for the analyses and DCC using an internal standard (M<sub>4</sub>Q) that may not have been appropriate for D<sub>3</sub>.

Except for D<sub>3</sub>, concentrations of the cVMS (D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>) materials in cod liver measured by NILU were consistently lower than concentrations measured by DCC and Evonik (Table 7; Figs. 11-12). The lower concentrations measured by NILU could not be attributed to fish characteristics or processing of the fish. Consequently, the lower concentrations measured by NILU appear to be related to the analytical process itself. In contrast to DCC and Evonik, isotopically-labeled internal standards were not available to NILU for the analyses. Use of an appropriate internal standard corrects analytical bias that may result from matrix effects and any loss of analyte from the sample. Recovery of the cVMS materials from spiked samples analyzed by NILU ranged from 64% to 72% (Table 5), suggesting that significant loss of analyte and possible matrix effects may have occurred. However, this possible bias would not have been apparent from the results generated by DCC and Evonik because isotopically-labeled internal standards were used for the analyses. The octanol-to-air partition coefficients (Log K<sub>OA</sub>) for the cVMS materials range from 4.2 for D<sub>4</sub> to 5.8 for D<sub>6</sub>. These high values of Log K<sub>OA</sub> suggest that volatile loss from the sample extracts to the headspace of the sample container may account for the low recoveries observed for the NILU analyses. If this is correct, then appropriate internal standards (e.g., isotopically-labeled structural analogs) should be included in the analytical method. Moreover, the internal standard should be added to the sample and incorporated into the matrix as soon as possible (preferably in the field at the time of collection, if practical) to control for loss from the matrix during storage, processing, and analysis.

## Conclusions

Methods of processing, extraction, and analysis were variable across the three laboratories, which was attributed to the lack of standard procedures. Although concentrations of the cVMS materials measured in the cod livers were similar, there were statistically significant differences that were not related to fish characteristics or to processing of the fish. Based on these differences the following recommendations should be taken into consideration:

- International shipment of samples (organisms, tissues, etc.) may present a risk from the perspective of sample loss and sample integrity. Reliability of shipper, shipping requirements, and customs requirements need to be carefully considered very early in the process.
- Clean room technology may be required for samples collected from remote areas where trace contamination may have a significant impact.
- Enhanced monitoring of background levels in both the field and laboratory may be required for samples collected from remote areas. This would include

collection, processing, and analysis of additional solvent blanks, appropriate matrix blanks, and reference samples.

- Isotopically-labeled internal standards should be used as a requirement of the analytical method. Addition of the internal standard should be implemented as soon as possible (preferably in the field at the time of collection, if practical) to control for loss from the matrix during storage, processing, and analysis.
- Calculation and reporting of detection levels (LOD, MDL, and LOQ) should be based on a standard definition and approach, which must be clearly identified. This is especially important for samples collected from remote areas where concentrations would be expected to be at or below the levels of detection.
- It is proposed that detection levels (DLs) be determined as a function of the variance associated with replicate analyses of matrix-free blanks (LOD) and replicate analyses of specific matrices containing small, but measurable amounts of target analyte (MDL, LOQ). The DLs at 99% confidence are then defined as:

$$DL = t_{0.99, n-1} \times s_0$$

where:  $t_{0.99, n-1}$  is the one-tailed t-statistic at 99% confidence for the performed number of sample analyses (degrees of freedom = n-1) and  $s_0$  is the standard deviation for the replicate analyses. In order to avoid skewing the resulting detection levels, the recommended number of replicate analyses is n = 7 or 8 ( $t_{0.99, n-1} = 3.143$  or  $2.998$ , respectively), which yields the desired signal to noise ratio of about 3:1.

- It is recommended that reference materials of various matrices and concentrations of cVMS materials be developed and made available so that analytical performance between laboratories can be qualified. If reference materials are not available, consideration should be given to having a separate and independent laboratory analyze a subset of samples.
- Reporting of only left censored data should be avoided. Rather actual measured values (positive or negative) should be reported with the estimated detection level provided for comparison. If left censored data is reported then it must be very clear how the data was censored.
- Lipid content should be determined for each sample in order to control for variability in cVMS concentrations that are related to variability in the lipid content of the matrix. In the absence of measured lipid content, liver mass or liver condition factor should be used for controlling variability in cVMS concentrations.

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## Tables

Table 1. Characteristics of Atlantic cod (*Gadus morhua*) collected by trawl from Inner Oslofjord on 10 December 2007. Cod were collected from aboard the *F/F Trygve Braarud* by the Norwegian Institute of Water Research (NIVA), and measured for total length (cm) and fresh weight (g) in the field. Liver mass was determined by the laboratory responsible for processing the fish. Condition factor ( $K$ ) and the liver condition index ( $LCI$ ) were calculated from the fresh weight, total length, and liver mass of each fish.

Fish IDNO	Processor	Length (cm)	Weight (g)	Liver (g)	$K^1$ (g/cm <sup>3</sup> )	$LCI^2$ (% fw)
OCF-07	DCC	40.0	615	6.9	0.961	1.12
OCF-08	DCC	40.0	632	12.8	0.988	2.02
OCF-10	DCC	45.5	870	35.9	0.924	4.13
OCF-02	DCC	46.0	972	31.7	0.999	3.26
OCF-15	DCC	49.7	1120	12.6	0.912	1.13
OCF-13	DCC	52.5	1265	14.0	0.874	1.11
	Mean:	45.6	912	19.0	0.943	2.13
	SD:	5.0	261	11.8	0.048	1.29
OCF-09	Evonik	40.3	650	6.1	0.993	0.94
OCF-06	Evonik	45.5	862	18.8	0.915	2.18
OCF-12	Evonik	44.8	887	36.2	0.986	4.08
OCF-14	Evonik	48.3	1240	31.7	1.100	2.56
OCF-11	Evonik	49.5	1272	34.8	1.049	2.74
	Mean:	45.7	982	25.5	1.009	2.50
	SD:	3.6	267	12.8	0.070	1.13
OCF-01	NILU	44.0	605	4.5	0.710	0.74
OCF-03	NILU	43.0	781	21.4	0.982	2.74
OCF-05	NILU	43.0	810	15.3	1.019	1.89
OCF-04	NILU	42.0	829	10.1	1.119	1.22
OCF-17	NILU	50.5	1032	6.1	0.801	0.59
OCF-16	NILU	48.0	1120	18.0	1.013	1.61
	Mean:	45.1	863	12.6	0.941	1.46
	SD:	3.4	185	6.8	0.153	0.80
	Grand Mean:	45.4	915	18.6	0.961	2.00
	Grand SD:	3.8	228	11.3	0.101	1.11

<sup>1</sup>  $K$  is the Fulton condition factor of the fish

<sup>2</sup>  $LCI$  is the liver condition index of the fish

Table 2. Summary of chain-of-custody for Atlantic cod (*Gadus morhua*) collected from Inner Oslofjord on 10 December 2007. Livers from individual fish were analyzed for cVMS concentrations.

	Dow Corning	Evonik	NILU
<b>Distribution of Fish</b>			
• Number of fish received	6	5	6
• Date sent from NIVA	11-Feb-08	11-Feb-08	25-Jan-08
• Date received	14-Feb-08	13-Feb-08	25-Jan-08
• Condition received	frozen	frozen	frozen
• Storage temperature	-80°C	-20°C	-18°C
<b>Sample Processing</b>			
• Date livers processed	23-Apr-08	24-Apr-08	12-May-08
• Collection method	Dissection (thawed)	Dissection (thawed)	Dissection (thawed)
• Homogenization method	scissors	Ultra-Turrax	Ultra-Turrax
• Storage temperature	-80°C	-20°C	-18°C
<b>Distribution of Liver Samples by Dow Corning</b>			
• Date samples sent			
• Date samples received		06-May-08	06-May-08
• Condition received		frozen	frozen
• Storage temperature		-20°C	-18°C
<b>Distribution of Liver Samples by Evonik</b>			
• Date samples sent	28-Apr-08		05-May-08
• Date samples received	30-Apr-08		07-May-08
• Condition received	frozen		frozen
• Storage temperature	-80°C		-18°C
<b>Distribution of Liver Samples by NILU<sup>†</sup></b>			
• Date samples sent	03-Jun-08	14-May-08	
• Date samples received	05-Jun-08	16-May-08	
• Condition received	frozen	not frozen	
• Storage temperature	-80°C	-20°	

<sup>†</sup> Frozen homogenized liver samples from the 6 cod were initially shipped from NILU to Dow Corning Corporation on 13 May 2008, but the samples were never received. A second set of samples from 4 of the 6 cod were shipped to Dow Corning Corporation on 03 June 2008 and received on 05 June 2008 in frozen condition.

Table 3. Summary of procedures used to extract cVMS materials (D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>) from livers of Atlantic cod collected from Inner Oslofjord on 10 December 2007.

Parameter	DCC	Evonik	NILU
Sample mass extracted	1.5 g	0.4 g	0.3 g
Solvent	THF	pentane	hexane
Solvent-to-sample ratio	2:1	10:1	3:1
Equipment	vortex mixer	Ultra-Turrax	vortex mixer
Number of extractions	2	1	1
Internal standards			
• D <sub>3</sub>	M <sub>4</sub> Q <sup>1</sup>		
• D <sub>4</sub>	<sup>13</sup> C-D <sub>4</sub>	<sup>13</sup> C-D <sub>4</sub>	
• D <sub>5</sub>	<sup>13</sup> C-D <sub>5</sub>	<sup>13</sup> C-D <sub>5</sub>	
• D <sub>6</sub>	<sup>13</sup> C-D <sub>6</sub>	<sup>13</sup> C-D <sub>6</sub>	
Clean-up		Florisil	
Drying agent	MgSO <sub>4</sub>		
Blow-down		N <sub>2</sub>	

<sup>1</sup> M<sub>4</sub>Q (tetrakis(trimethylsiloxy)silane; CAS No. 3555-47-3) was used as the internal standard for D<sub>3</sub> because a <sup>13</sup>C isotopically-labeled standard was not available.

Table 4. Summary of analytical methods used to measure concentrations of cVMS materials (D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>) in extracts of livers from Atlantic cod collected from Inner Oslofjord on 10 December 2007.

Parameter	DCC	Evonik	NILU
Instrument			
• Gas chromatograph	Agilent 6890 GC	Agilent 6890 GC	Agilent 5890N GC
• Mass spectrometer	Agilent 5973 MSD	Agilent 5973 MSD	Waters Autospec-V Ultima HRMS (R>10,000)
Inlet conditions			
• Injection	Split-less	Cool on-column	Split-less
• Volume	1 µL	1 µL	1 µL
• temperature	150°C		200°C
Column			
	Zebron ZB-5 (30m x 0.25mm x 0.25µm)	Agilent DB5-HT (30m x 0.25mm x 0.10µm)	Agilent J&W Ultra-2 (25m x 0.20mm x 0.11µm)
Oven start temperature	50°C (hold 3 min)	40°C	35°C (hold 3 min)
Transfer line temperature	280°C	300°C	270°C
Analyte m/z			
• D <sub>3</sub>	207	207	207.0329
• D <sub>4</sub>	281	281	281.0517
• D <sub>5</sub>	355	355	355.0705
• D <sub>6</sub>	429	341	429.0893
Internal standard m/z			
• M <sub>4</sub> Q	281		
• <sup>13</sup> C-D <sub>4</sub>	285	285	
• <sup>13</sup> C-D <sub>5</sub>	360	360	
• <sup>13</sup> C-D <sub>6</sub>	435	345	

Table 5: Summary of quality control parameters of measure used for the determination of cVMS materials (D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>) in livers of Atlantic cod collected from Inner Oslofjord on 10 December 2007.

Parameter of Measure	D <sub>3</sub>			D <sub>4</sub>			D <sub>5</sub>			D <sub>6</sub>		
	DCC	NILU	n	DCC	Evonik	NILU	DCC	Evonik	NILU	DCC	Evonik	NILU
<u>Background</u>												
• Mean (ng)	5.88	0.39		10.6	16.4	1.16	16.2	24.0	0.59	-19.3	7.14	0.29
• n	4	8		4	7	8	4	7	8	4	7	8
<u>Lower Level of Detection</u>												
• LOD (ng)	8.60	0.25		4.72	4.53	0.63	8.75	6.64	0.42	11.3	1.59	1.55
• n	4	3		4	5	3	4	5	3	4	5	3
• $t_{0.99,n-1}$	4.54	6.97		4.54	3.75	6.97	4.54	3.75	6.97	4.54	3.75	6.97
<u>Lower Level of Detection</u>												
• LOD (ng/g ww)	5.73	0.84		3.15	11.3	2.09	5.83	16.6	1.41	7.52	3.98	5.17
• Average mass (g)	1.50	0.30		1.50	0.40	0.30	1.50	0.40	0.30	1.50	0.40	0.30
<u>Method Detection Level</u>												
• MDL (ng/g ww)	5.05	3.14		10.0	7.58	4.73	32.9	15.2	8.45	10.9	6.47	9.78
• n	3	5		3	6	5	3	6	5	3	6	5
• $t_{0.99,n-1}$	6.97	3.75		6.97	3.37	3.75	6.97	3.37	3.75	6.97	3.37	3.75
<u>Level of Quantitation</u> <sup>2</sup>												
• LOQ <sub>LOD</sub> (ng/g ww)	18.9	2.77		10.4	37.3	6.90	19.2	54.8	4.65	24.8	13.1	17.1
• LOQ <sub>MDL</sub> (ng/g ww)	12.6	7.85		24.9	18.9	11.8	82.3	38.0	21.1	27.3	16.2	24.5
<u>Recovery</u>												
• Mean (%)	98.0	65.2		103	97.6	67.4	103	101	63.9	97.6	93.0	71.8
• Spike (ng/g)	160	37.1		171	547	39.1	179	545	39.0	166	587	37.2
• n	3	5		3	5	5	3	5	5	3	5	5
• RSD (%)	3.5	0.9		1.4	5.9	2.4	1.7	3.6	7.1	1.9	2.5	8.6

<sup>1</sup> MDLs were based on replicate analyses of cod liver (DCC; origin Oslofjord), wolffish liver (Evonik; origin not identified), and spiked cod liver (NILU; origin northwest coast of Norway).

<sup>2</sup> LOQ<sub>LOD</sub> was determined as 3.3 times the LOD. LOQ<sub>MDL</sub> was determined as 2.5 times the MDL.

Table 6. Correlation coefficients used to evaluate relationships between fish characteristics (length, weight, liver mass, condition factor, liver condition index) and concentrations of the cVMS materials measured in the liver by each laboratory.

Correlations	Pearson Coefficient <sup>1</sup>			Spearman Coefficient <sup>1</sup>			Kendall Coefficient <sup>1</sup>			Hoeffding Coefficient <sup>1</sup>		
	DCC (n=15)	Evonik (n=17)	NILU (n=17)	DCC (n=15)	Evonik (n=17)	NILU (n=17)	DCC (n=15)	Evonik (n=17)	NILU (n=17)	DCC (n=15)	Evonik (n=17)	NILU (n=17)
<b>D<sub>3</sub> Concentration</b>												
• Fresh weight	0.11	0.13	0.36	0.33	0.27	0.39	0.22	0.22	0.26	-0.02	0.01	0.01
• Total length	0.03	-0.04	0.32	0.12	0.04	0.33	0.09	0.09	0.26	-0.05	0.04	0.04
• Liver mass	0.54*	0.61*	0.48*	0.58*	0.61*	0.43	0.53*	0.53*	0.33	0.23*	0.08*	0.08*
• K <sup>2</sup>	0.37	0.44	0.11	0.52*	0.54*	0.20	0.36	0.36	0.12	0.04	-0.04	-0.04
• LCI <sup>3</sup>	0.55*	0.64*	0.36*	0.56*	0.63*	0.40	0.46*	0.46*	0.31	0.14*	0.08*	0.08*
<b>D<sub>4</sub> Concentration</b>												
• Fresh weight	0.09	0.13	0.36	0.30	0.27	0.51*	0.21	0.22	0.47*	-0.02	0.01	0.23*
• Total length	0.03	-0.04	0.18	0.21	0.04	0.26	0.16	0.02	0.20	-0.04	-0.03	0.06*
• Liver mass	0.61*	0.61*	0.70*	0.52*	0.61*	0.65*	0.40*	0.48*	0.47*	0.09*	0.11*	0.10*
• K <sup>2</sup>	0.33	0.44	0.50*	0.44	0.54*	0.58*	0.31	0.36*	0.40*	0.02	0.02	0.05
• LCI <sup>3</sup>	0.63*	0.64*	0.63*	0.54*	0.63*	0.63*	0.39*	0.44*	0.44*	0.05	0.10*	0.09*
<b>D<sub>5</sub> Concentration</b>												
• Fresh weight	0.48	0.53*	0.61*	0.48	0.49*	0.60*	0.40*	0.39*	0.54*	0.08*	0.08*	0.23*
• Total length	0.32	0.30	0.37	0.34	0.26	0.34	0.27	0.14	0.26	0.02	0.00	0.05
• Liver mass	0.71*	0.75*	0.73*	0.65*	0.68*	0.62*	0.44*	0.49*	0.42*	0.10*	0.15*	0.06*
• K <sup>2</sup>	0.53*	0.54*	0.56*	0.61*	0.62*	0.63*	0.45*	0.46*	0.46*	0.12*	0.11*	0.08*
• LCI <sup>3</sup>	0.52*	0.58*	0.52*	0.62*	0.66*	0.56*	0.45*	0.47*	0.35	0.10*	0.17*	0.07*
<b>Two-tail p &lt; 0.01<sup>a</sup></b>												
	0.64	0.61	0.61	0.65	0.62	0.62	0.51	0.47	0.47	0.13	0.11	0.11
<b>Two-tail p &lt; 0.05<sup>b</sup></b>												
	0.51	0.48	0.48	0.52	0.49	0.49	0.39	0.36	0.36	0.07	0.06	0.06

<sup>1</sup> An \* indicates a statistically significant correlation (p < 0.05).

<sup>2</sup> K is the Fulton condition factor of the fish

<sup>3</sup> LCI is the liver condition index of the fish.

<sup>a</sup> Value of coefficient required for correlation to be considered significant at p < 0.01.

<sup>b</sup> Value of coefficient required for correlation to be considered significant at p < 0.05.

Table 6. Continued.

Correlations	Pearson Coefficient <sup>1</sup>			Spearman Coefficient <sup>1</sup>			Kendall Coefficient <sup>1</sup>			Hoeffding Coefficient <sup>1</sup>		
	DCC (n=15)	Evonik (n=17)	NILU (n=17)	DCC (n=15)	Evonik (n=17)	NILU (n=17)	DCC (n=15)	Evonik (n=17)	NILU (n=17)	DCC (n=15)	Evonik (n=17)	NILU (n=17)
D <sub>6</sub> Concentration												
• Fresh weight	0.26	0.30	0.43	0.48	0.41	0.49*	0.40*	0.35	0.45*	0.16*	0.11*	0.23*
• Total length	0.25	0.15	0.26	0.44	0.20	0.25	0.43*	0.22	0.22	0.19*	0.07*	0.07*
• Liver mass	0.58*	0.67*	0.71*	0.61*	0.69*	0.70*	0.42*	0.50*	0.53*	0.07*	0.13*	0.17*
• K <sup>2</sup>	0.11	0.39	0.43	0.18	0.45	0.53*	0.09	0.28	0.34	-0.04	0.02	0.03
• LC <sup>3</sup>	0.52*	0.63*	0.60*	0.57*	0.68*	0.68*	0.39*	0.47*	0.50*	0.05	0.14*	0.14*
Two-tail p < 0.01 <sup>a</sup>	0.64	0.61	0.61	0.65	0.62	0.62	0.51	0.47	0.47	0.13	0.11	0.11
Two-tail p < 0.05 <sup>b</sup>	0.51	0.48	0.48	0.52	0.49	0.49	0.39	0.36	0.36	0.07	0.06	0.06

<sup>1</sup> An \* indicates a statistically significant correlation (p < 0.05).

<sup>2</sup> K is the Fulton condition factor of the fish

<sup>3</sup> LC<sup>i</sup> is the liver condition index of the fish.

<sup>a</sup> Value of coefficient required for correlation to be considered significant at p < 0.01.

<sup>b</sup> Value of coefficient required for correlation to be considered significant at p < 0.05.

Table 7. Summary of cVMS concentrations (specifically D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>) measured in livers of Atlantic cod (*Gadus morhua*) collected from Inner Oslofjord on 10 December 2007. Livers were collected, processed, and analyzed by Dow Corning Corporation (DCC; Auburn, Michigan), Evonik Goldschmidt (Evonik; Essen, Germany), and the Norwegian Institute for Air Research (NILU; Kjeller, Norway).

Fish IDNO	Processor	D <sub>3</sub> (ng/g ww)			D <sub>4</sub> (ng/g ww)			D <sub>5</sub> (ng/g ww)			D <sub>6</sub> (ng/g ww)		
		DCC	NILU	Processor	DCC	Evonik	NILU	DCC	Evonik	NILU	DCC	Evonik	NILU
OCF-07	DCC	0.1	2.9		51.6	70.0	10.0	379	384	62	24.2	32.5	5.9
OCF-08	DCC	0.9	3.6		66.0	49.0	41.7	778	743	520	66.6	74.0	43.6
OCF-10	DCC	0.3	3.4		93.9	82.0	77.3	1477	1698	1221	119.2	120.0	81.4
OCF-02	DCC	2.8	5.6		127.5	130.0	113.9	3137	3141	2790	395.5	387.0	282.1
OCF-15	DCC	-0.1	4.3		120.2	114.0	103.8	1456	1489	1238	203.9	195.0	140.4
OCF-13	DCC	0.7	2.1		16.5	-0.2	5.4	111	116	36	-6.2	2.2	1.6
	Mean:	0.8	3.7		79.3	74.1	58.7	1223	1262	978	133.9	135.1	92.5
	SD:	1.1	1.2		42.7	46.8	46.8	1089	1107	1034	148.1	140.8	106.3
OCF-09	Evonik	-0.1	2.8		55.2	27.5	38.2	433	368	347	47.1	55.0	41.5
OCF-06	Evonik	1.9	5.5		95.4	77.0	70.6	638	591	495	149.4	132.0	99.3
OCF-12	Evonik	7.9	13.4		220.8	280.0	224.9	1785	1938	1865	222.7	242.0	196.5
OCF-14	Evonik	2.2	6.0		135.7	131.0	145.0	2770	3023	3007	121.4	122.0	115.8
OCF-11	Evonik	3.0	14.6		102.6	82.0	105.2	2788	2921	2999	174.0	177.0	184.2
	Mean:	3.0	8.5		121.9	119.5	116.8	1683	1768	1743	142.9	145.6	127.5
	SD:	3.0	5.2		62.2	96.9	72.3	1126	1253	1294	65.2	69.3	63.8
OCF-01	NILU		3.9			9.0	7.9		252	40		3.7	5.6
OCF-03	NILU	2.1	6.0		99.5	63.0	59.3	707	703	462	88.8	83.0	51.9
OCF-05	NILU	0.6	2.6		134.4	131.0	68.4	2009	2199	1078	138.4	136.0	61.8
OCF-04	NILU	4.0	5.5		102.8	100.0	110.3	1212	1185	1419	76.5	78.0	87.6
OCF-17	NILU		11.0			43.0	62.4		1081	1106		29.0	38.6
OCF-16	NILU	1.0	4.0		76.4	68.0	84.1	1542	1664	1697	171.4	170.0	154.3
	Mean:	1.9	5.5		103.3	69.0	65.4	1368	1181	967	118.8	83.3	66.6
	SD:	1.5	3.0		23.8	42.7	33.8	549	689	614	44.1	62.6	50.7
Grand Mean:		1.8	5.7		99.9	85.7	78.1	1415	1382	1199	132.8	119.9	93.7
Grand SD:		2.1	3.7		47.3	64.1	55.0	944	995	1001	97.8	97.1	77.4

## Figures

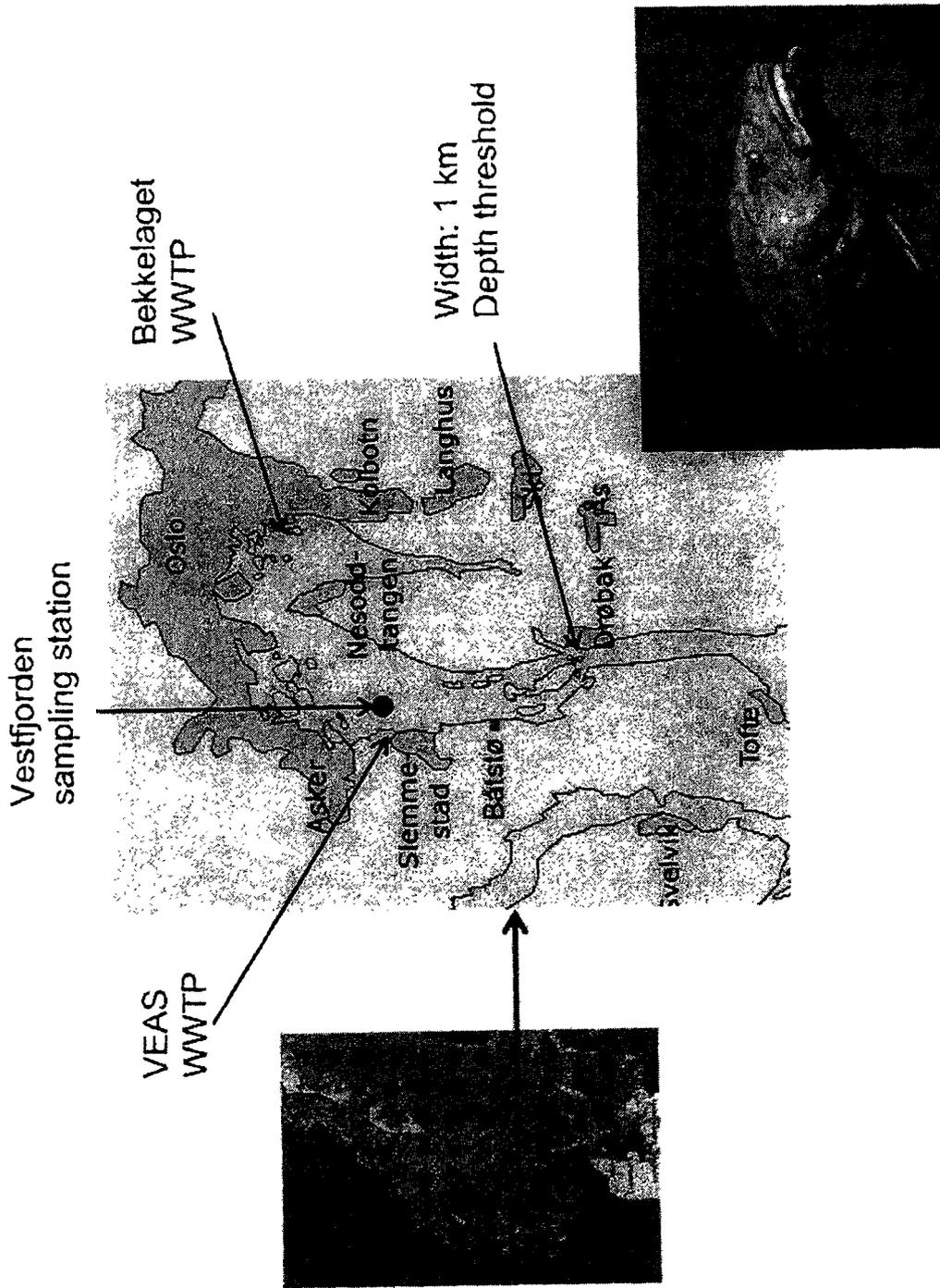


Figure 1. Location where Atlantic cod (*Gadus morhua*) were collected from Inner Oslofjord on 10 December 2007. Livers from individual fish were analyzed for cVMS concentrations (specifically D3, D4, D5, and D6).

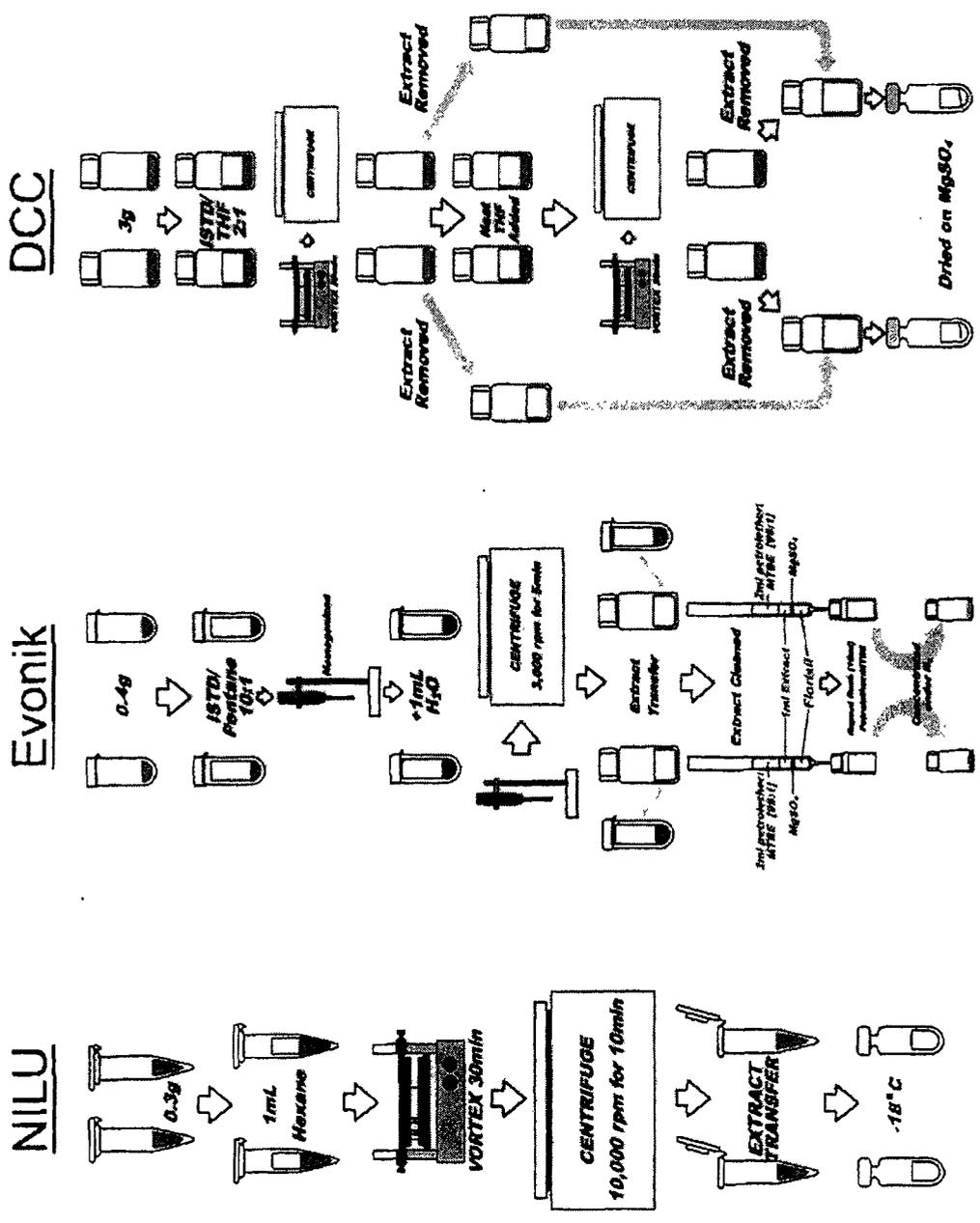


Figure 2. Schematic of procedures used to extract cVMS materials (D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>) from livers of Atlantic cod collected from Inner Oslofjord on 10 December 2007.

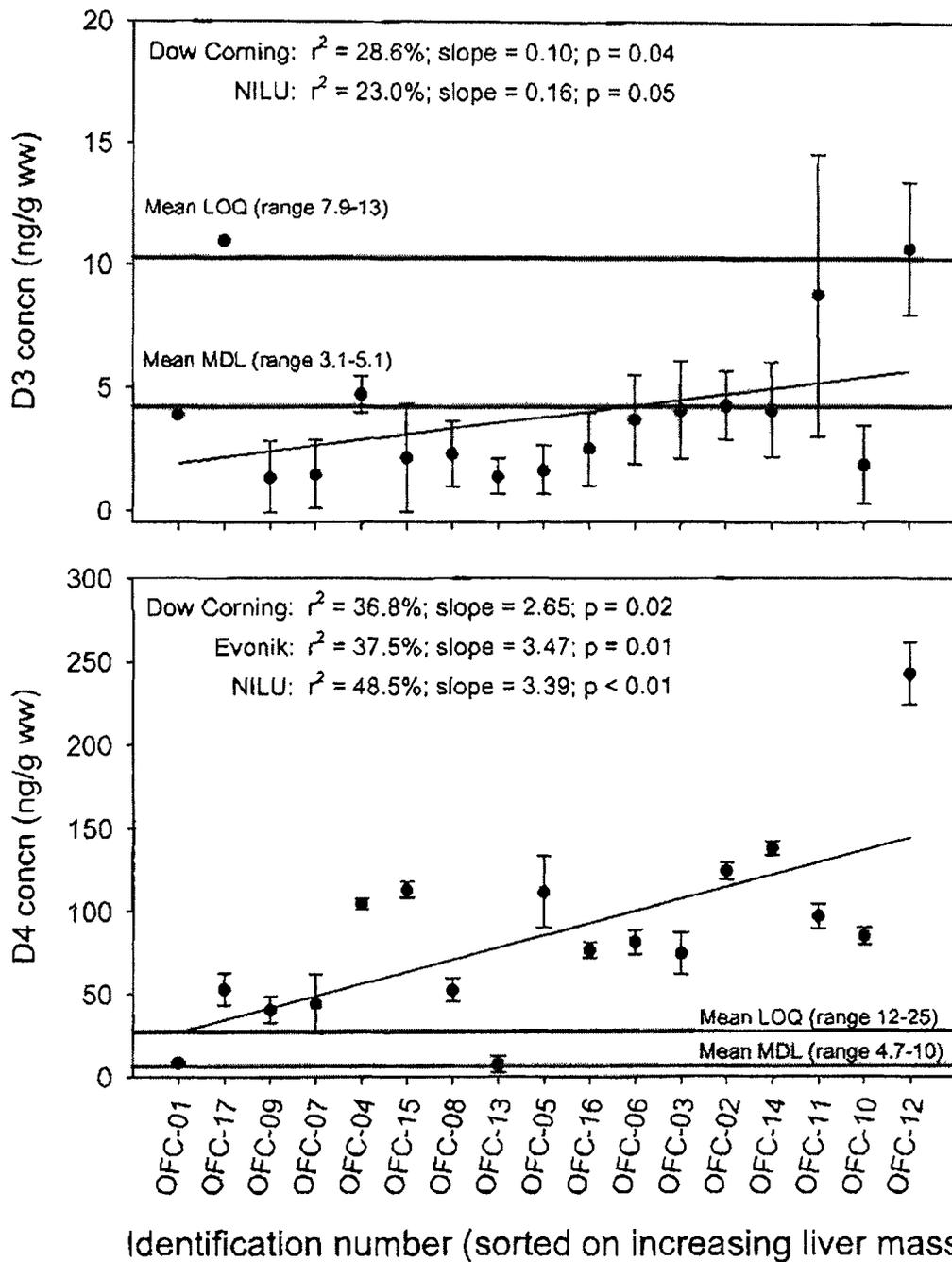


Figure 3. Measured concentrations (mean  $\pm$  sd) of D<sub>3</sub> and D<sub>4</sub> in Oslofjord cod (OFC) livers, sorted on increasing liver mass (lipid content was not determined for all fish). Concentrations are expressed as the mean across the three analysis laboratories for each individual fish. The mean method detection level (MDL) and mean level of quantification (LOQ) are represented by the red and blue lines, respectively. Regression statistics for cVMS concentration on liver mass are provided by laboratory.

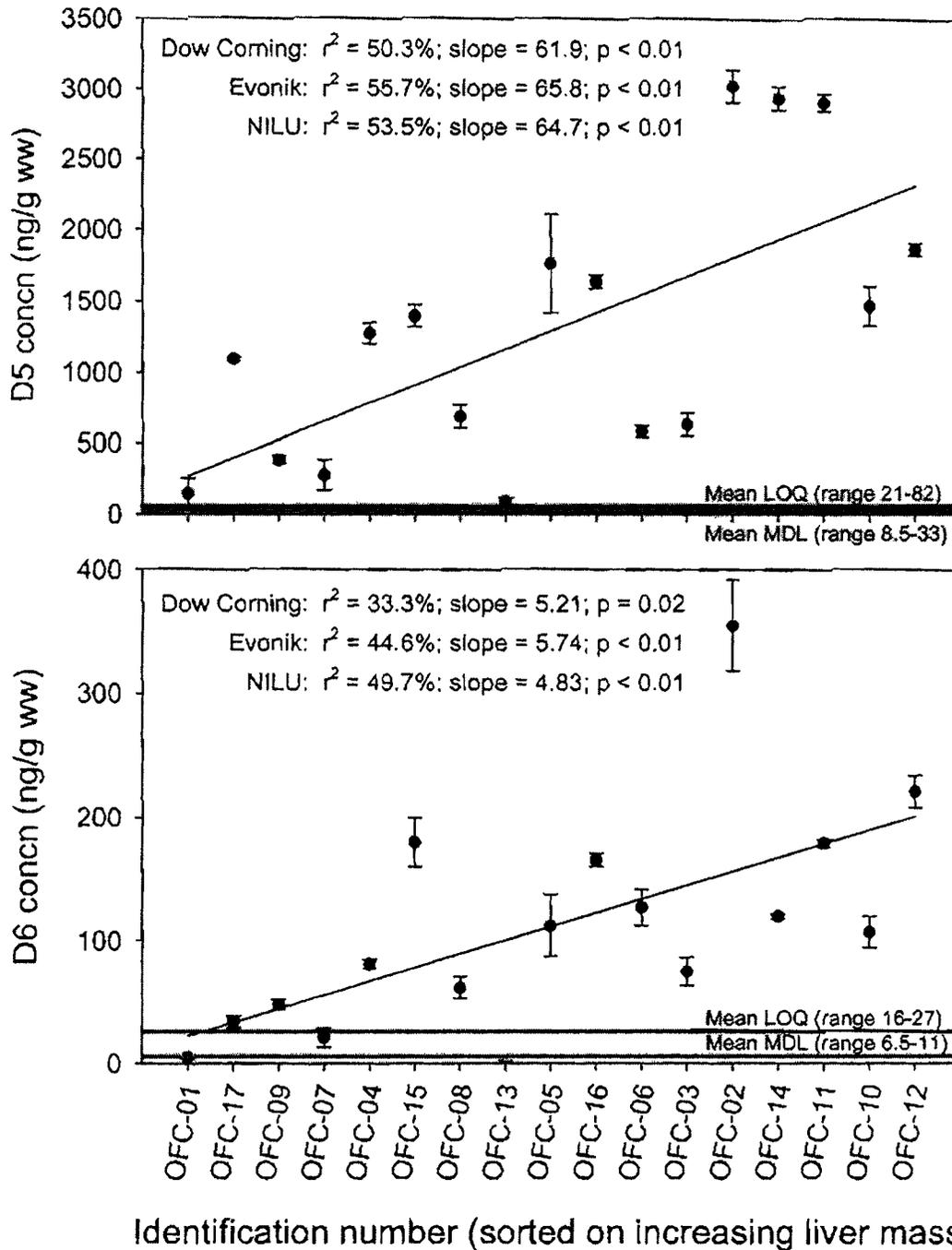


Figure 4. Measured concentrations (mean  $\pm$  sd) of D<sub>5</sub> and D<sub>6</sub> in Oslofjord cod (OFC) livers, sorted on increasing liver mass (lipid content was not determined for all fish). Concentrations are expressed as the mean across the three analysis laboratories for each individual fish. The mean method detection level (MDL) and mean level of quantification (LOQ) are represented by the red and blue lines, respectively. Regression statistics for cVMS concentration on liver mass are provided by laboratory.

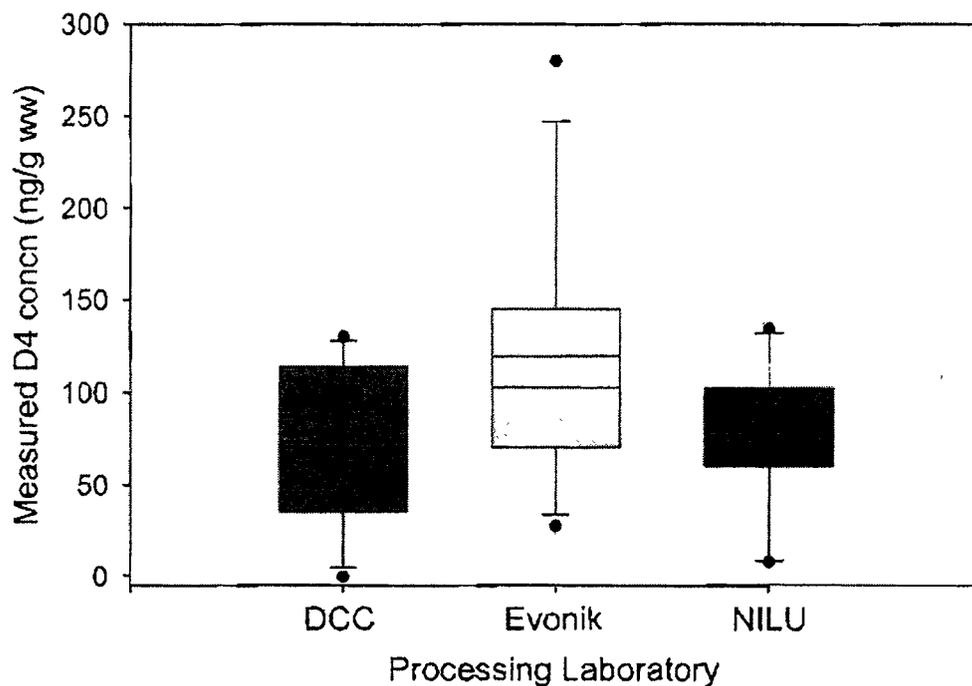
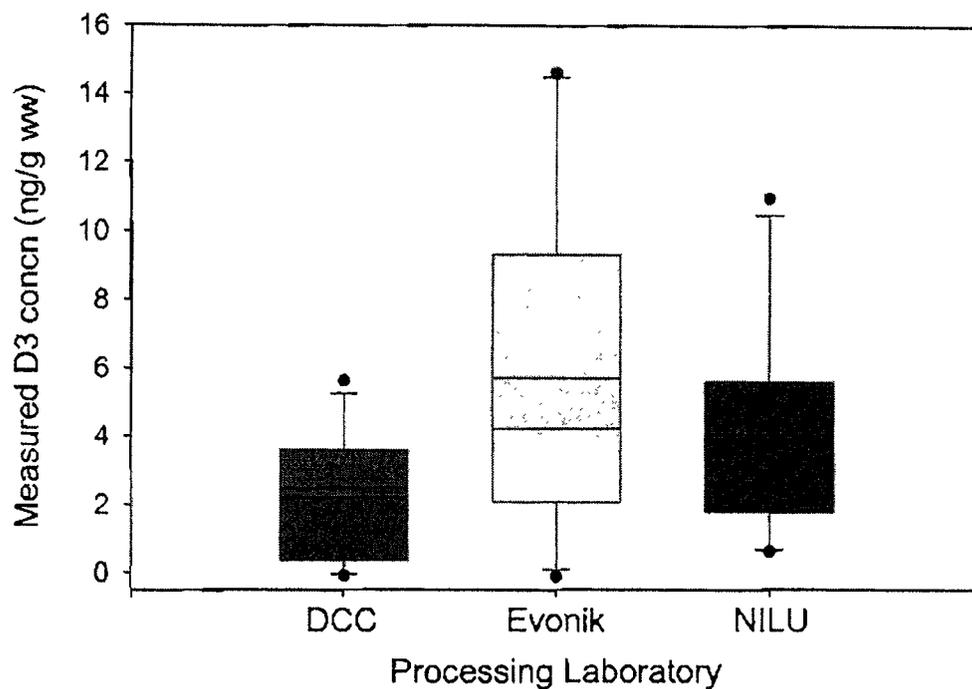


Figure 5. Box plots for measured concentrations of D<sub>3</sub> and D<sub>4</sub> in Oslofjord cod livers, grouped by processing laboratory. The upper and lower boundaries of each box represent the 75th and 25th percentiles, the lines within each box mark the median and mean, the whiskers above and below each box indicate the 90th and 10th percentiles, and the points represent outlying values.

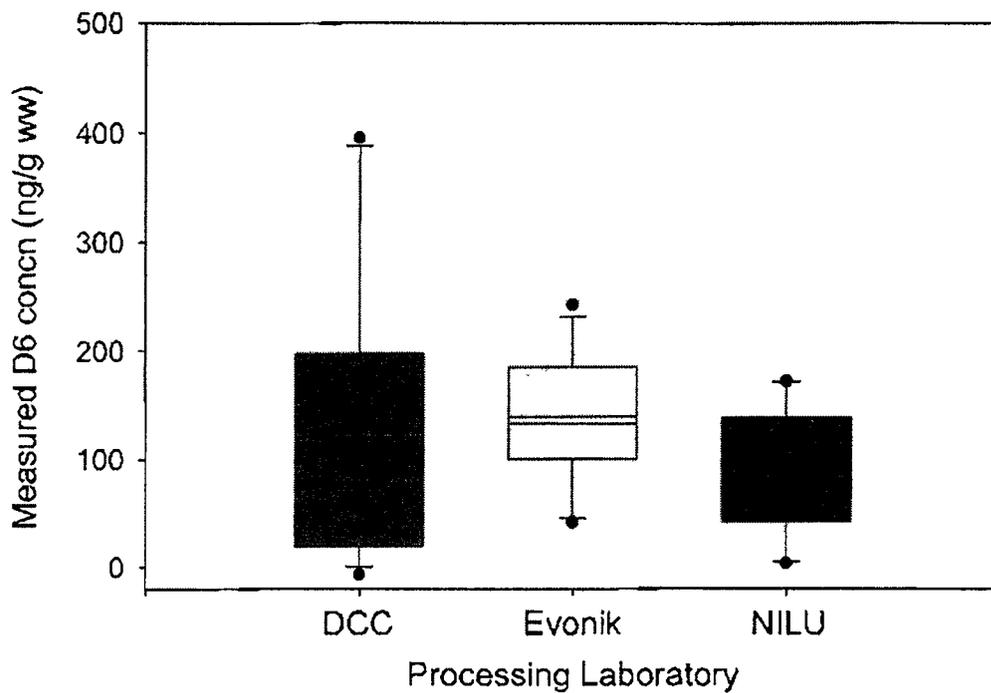
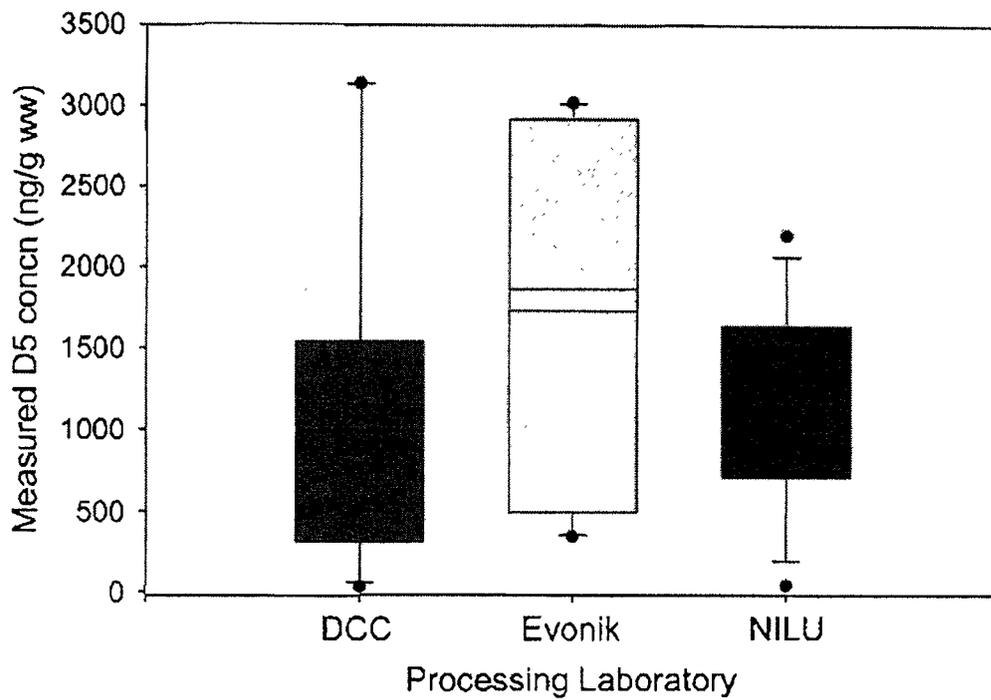


Figure 6. Box plots for measured concentrations of D<sub>5</sub> and D<sub>6</sub> in Oslofjord cod livers, grouped by processing laboratory. The upper and lower boundaries of each box represent the 75th and 25th percentiles, the lines within each box mark the median and mean, the whiskers above and below each box indicate the 90th and 10th percentiles, and the points represent outlying values.

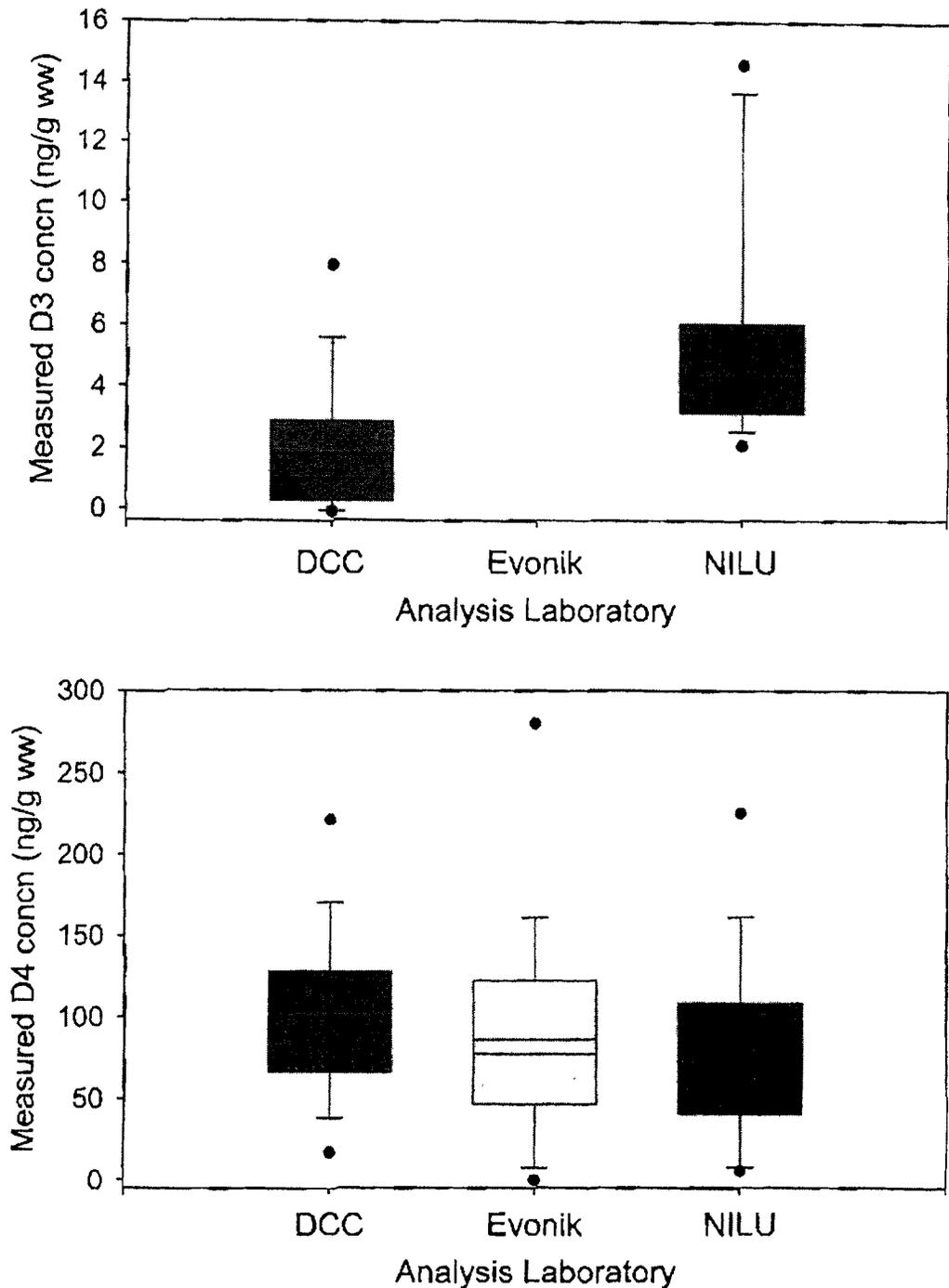


Figure 7. Box plots for measured concentrations of D<sub>3</sub> and D<sub>4</sub> in Oslofjord cod livers, grouped by analysis laboratory. The upper and lower boundaries of each box represent the 75th and 25th percentiles, the lines within each box mark the median and mean, the whiskers above and below each box indicate the 90th and 10th percentiles, and the points represent outlying values.

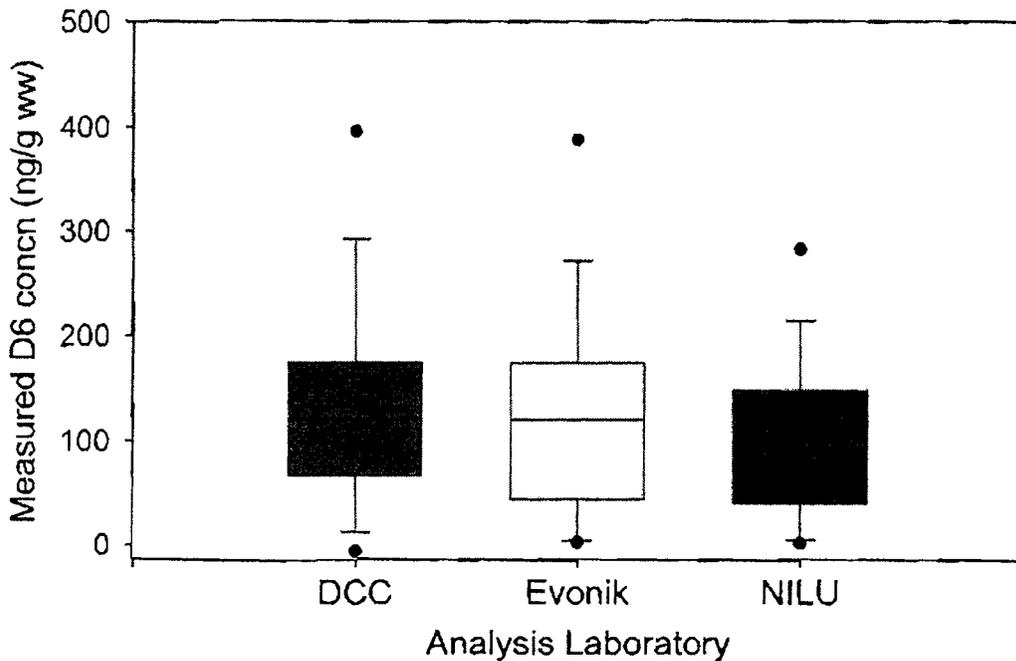
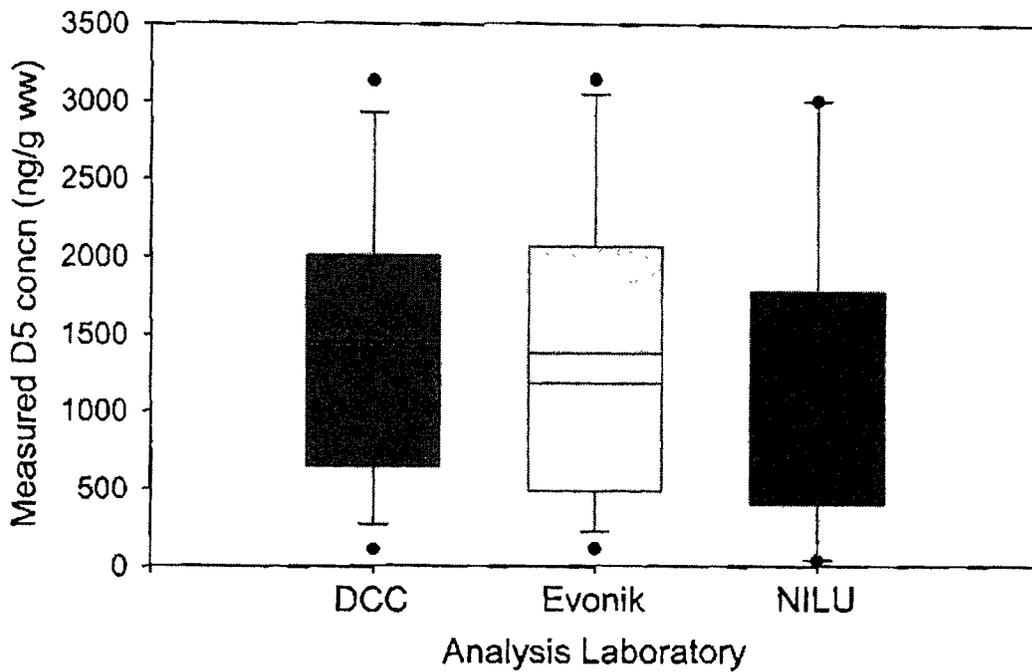


Figure 8. Box plots for measured concentrations of D<sub>5</sub> and D<sub>6</sub> in Oslofjord cod livers, grouped by analysis laboratory. The upper and lower boundaries of each box represent the 75th and 25th percentiles, the lines within each box mark the median and mean, the whiskers above and below each box indicate the 90th and 10th percentiles, and the points represent outlying values.

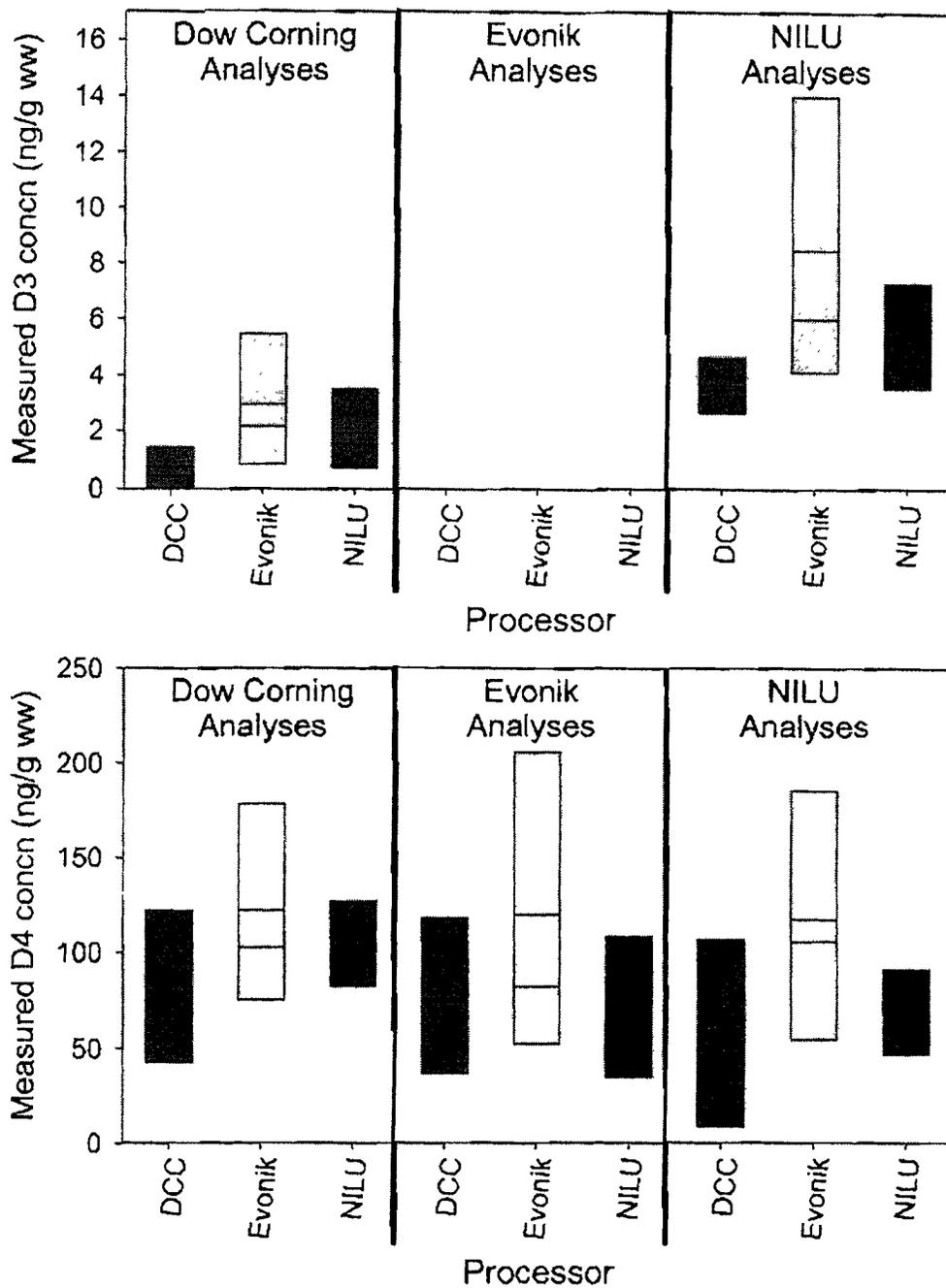


Figure 9. Box plots for measured concentrations of D<sub>3</sub> and D<sub>4</sub> in Oslofjord cod livers, grouped by processing laboratory within analysis laboratory. The upper and lower boundaries of each box represent the 75th and 25th percentiles, and the lines within each box mark the median and mean. There were not a sufficient number of fish analyzed to calculate the 90th and 10th percentiles.

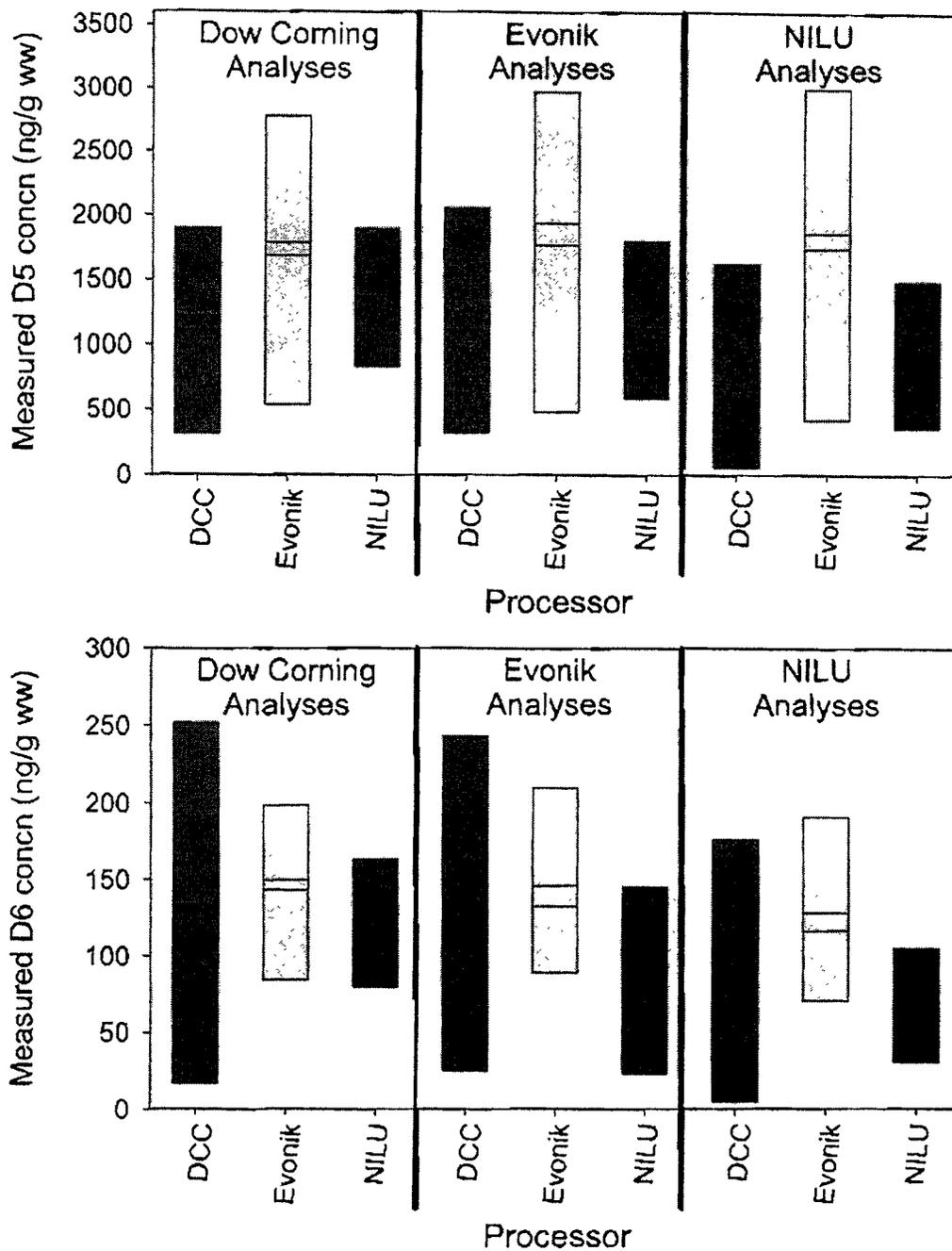


Figure 10. Box plots for measured concentrations of D<sub>5</sub> and D<sub>6</sub> in Oslofjord cod livers, grouped by processing laboratory within analysis laboratory. The upper and lower boundaries of each box represent the 75th and 25th percentiles, and the lines within each box mark the median and mean. There were not a sufficient number of fish analyzed to calculate the 90th and 10th percentiles.



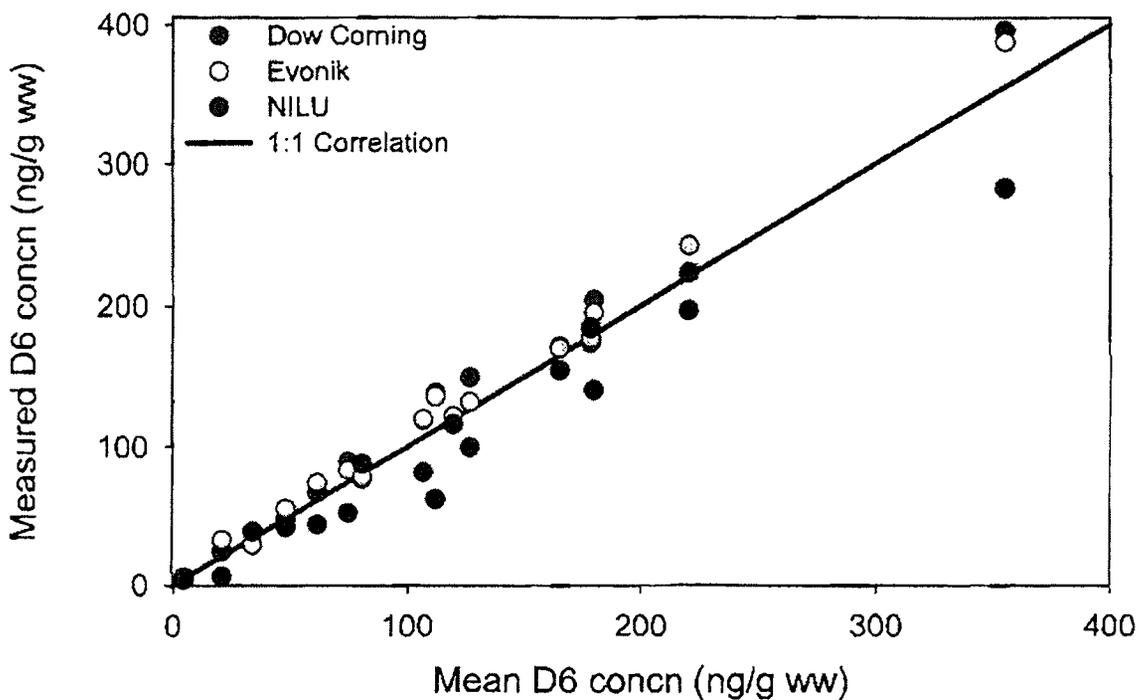
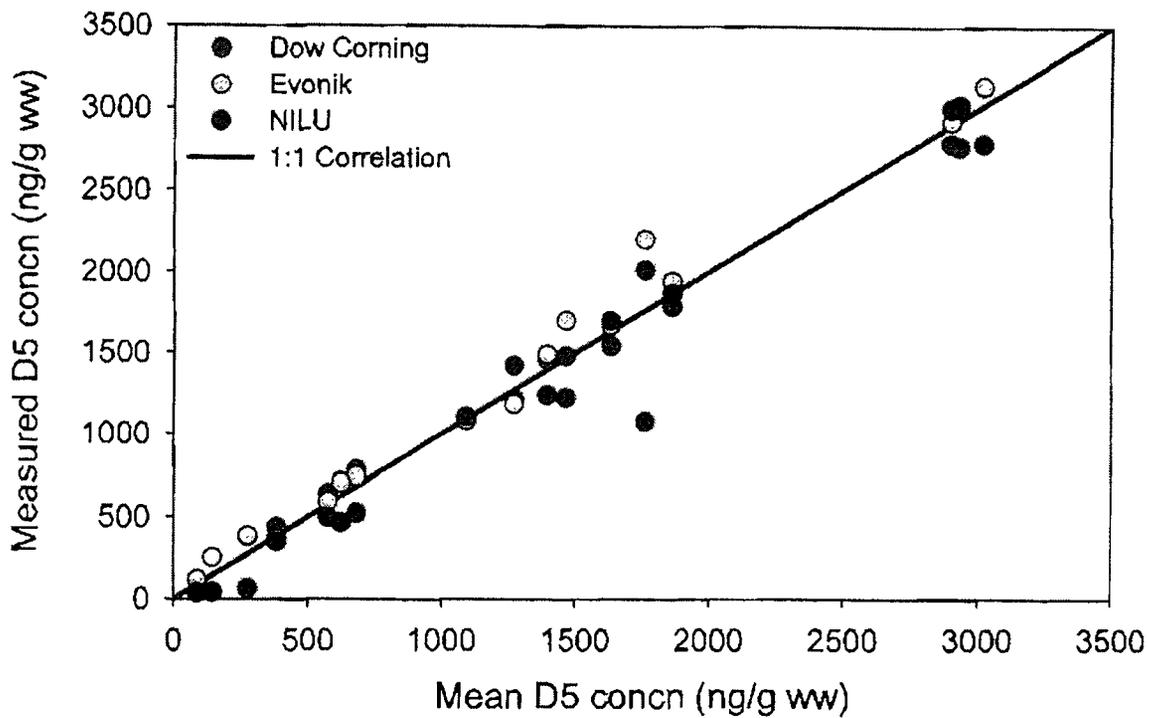


Figure 12. Scatter graph of measured concentrations of D<sub>5</sub> and D<sub>6</sub> in Oslofjord cod livers, relative to the mean concentrations across the three analysis laboratories.

## Appendices

# NON-REGULATED TECHNICAL REPORT

**Study Number:** 10922

**Study Leader:** Jeremy Durham

**Supervisor:** Debra McNett

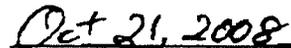
**Department:** Chemistry and Environment

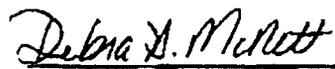
**Location:** Midland Corporate, Michigan USA, Americas

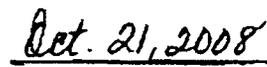
**Date:** October 21, 2008

**Title:** Non-Regulated Study: eVMS Analysis of Cod fish livers collected by Norway  
(Norwegian Institute for Air Research, NILU)

  
\_\_\_\_\_  
Jeremy Durham  
Study Leader

  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Debra McNett  
Supervisor Bioanalytical

  
\_\_\_\_\_  
Date

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## I. OBJECTIVE

Quantitation of cyclic volatile methyl siloxanes (cVMS) (D3, D4, D5 and D6) in cod livers from environmental monitoring samples collected by the Norwegian Institute for Air Research (NILU), as part of a 3 lab comparison between Dow Corning, NILU and Evonik.

## II. TEST SYSTEM

The test system for this study consisted of livers, removed from cod collected by NILU of Norway. Dow Corning received 6 of 17 fish, identification numbers; 2, 7, 8, 10, 13 and 15.

## III. REFERENCE ARTICLE INFORMATION

Reference article characterization was done in compliance with the EPA Toxic Substances Control Act (TSCA), Good Laboratory Practice Regulations (40CFR Part 792).

### A. Reference Article 1

- Identification: Hexamethylcyclotrisiloxane (D<sub>3</sub>)
- Lot Number: 1N44 (supplied as GE Silicones TSL8433 Hexamethylcyclotrisiloxane)
- Expiration Date: July 27, 2008
- Purity: 99.996 % by GC-FID

### B. Reference Article 2

- Identification: Octamethylcyclotetrasiloxane (D<sub>4</sub>)
- Lot Number: LL084732 (supplied as Dow Corning™ 244 Fluid)
- Expiration Date: January 19, 2009
- Purity: 99.75 % by GC-FID

### C. Reference Article 3

- Identification: Decamethylcyclopentasiloxane (D<sub>5</sub>)
- Lot Number: 0000341832 (supplied as Dow Corning™ 1693 Fluid)
- Expiration Date: September 7, 2008
- Purity: 99.19 % by GC-FID

### D. Reference Article 4

- Identification: Dodecamethylcyclohexasiloxane (D<sub>6</sub>)
- Lot Number: LL114030 (supplied as Dow Corning™ 246 Fluid)
- Expiration Date: June 8, 2009
- Purity: 99.54 % by GC-FID

#### IV. SAMPLE PROCESSING AND ANALYSIS

Liver samples were collected from 6 fish that were shipped to Dow Corning frozen on dry ice from NILU on February 11, 2008. The samples were received on February 14, 2008 and stored frozen upon receipt at  $-80 \pm 10^\circ\text{C}$ . The organization of the samples received from NILU is shown in Table 1. The cod fish were removed from  $-80^\circ\text{C}$  frozen storage and allowed to come to refrigerated temperature  $+5^\circ\text{C}$  prior to removal of livers. The liver was removed from each fish with a scalpel and trimmed of any fat. The liver was placed in pre-weighed 4 ounce jar and stored on ice until homogenization. A weight of the sample was obtained and then the liver was cut with scissors until homogenous. At this time an aliquot of the homogenate was removed and placed into pre-weighed vials supplied by each of the laboratories. A vial plus liver weight was recorded and the samples placed in  $-80^\circ\text{C}$  storage until processing or shipment.

The analysis was based upon the principle of calibrating a gas chromatograph with mass selective detection (GC-MS) using solvent standards of tetrahydrofuran (THF) spiked with various concentrations of  $\text{D}_3$ ,  $\text{D}_4$ ,  $\text{D}_5$ , and  $\text{D}_6$  and  $\text{M}_4\text{Q}$ ,  $^{13}\text{C}-\text{D}_4$ ,  $^{13}\text{C}-\text{D}_5$ , and  $^{13}\text{C}-\text{D}_6$  as internal standards, and then analyzing THF extracts of the liver against the calibration curves. Extraction of the liver was performed by using 2:1 solvent to sample ratio of THF containing the internal standards. After addition of a weighed amount of THF containing internal standard the samples were vortexed for 30 minutes using a bed vortexer set on high. The samples were then centrifuged for 5 minutes at a setting of 2000 revolutions per minute. The extractant from each sample was transferred to a separate vial. A second extraction was performed using THF without internal standard. The sample was vortexed for 30 minutes and centrifuged for 5 minutes. The extractant from each sample was transferred to the vial containing the first extract and dried using  $\text{MgSO}_4$  (~250 mg).

A linear regression analysis was performed relating the concentration of the calibration standards to the relative chromatographic response ratio (test article/internal standard). The standards were split into two ranges in order to accommodate the large range in concentration. The linearity parameters; y - intercepts, slopes, and correlation coefficients, were determined for each of the standard curves. The solvent standard concentrations ranges validated were approximately 6 to 2000 ng for  $\text{D}_3$ , 6 to 4000ng for  $\text{D}_4$ ,  $\text{D}_5$  and  $\text{D}_6$ . The minimum acceptance criterion for the linearity of a standard curve was a regression coefficient ( $R^2$ ) of 0.99. In the event that the linearity criteria was not met, concentrations could be excluded on the following basis: 1) over the concentration range four non zero concentrations remained with which to build a standard curve, and 2) a valid explanation could be offered, which would be documented in the study file as to why those concentrations were removed.

Peak responses from the GC-MS system were quantitated by automatic electronic integration either by using integration parameters in the software or by drawing a baseline manually in the software for the peak. If the baselines were drawn manually, this was indicated on the chromatogram printout in the study file. Detailed records of the GC-MS system and data system software used can be found in the study file. Calibration curves were determined from linear regression analysis of the data using Microsoft Excel Version 9.

#### V. QUALITY CONTROL

Control liver was obtained from Rainbow Ranch Trout Farm Tawas City, Michigan. Extraction of liver spikes and controls were done the same way as samples. In this manner, an assessment of accuracy, limit of detection (LOD), and limit of quantitation (LOQ) was derived. These aspects of the method that

are assessed can be collectively referred to as figures of merit. The target analytical concentration was ~200 ng of D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> per sample. This method was developed based on the methodology developed for the extraction and analysis of D<sub>4</sub> from biological samples (Varaprath 1998, *et. al.* and Varaprath 2000, *et. al.*). Additionally triplicate aliquots were taken from one of the samples and a spike into the solvent layer before extraction was completed at the same concentration as the control liver sample. This QC was run to check for cod matrix effect.

Specificity/selectivity was assessed by ensuring that the response in any of the spiked matrix blanks or solvent blanks did not significantly interfere with the analytes or internal standards of interest.

Peak responses from the GC-MS system were quantitated by automatic electronic integration either by using integration parameters in the software or by drawing a baseline manually in the software for the peak. If the baselines were drawn manually, this was indicated on the chromatogram printout in the study file. Detailed records of the GC-MS system and data system software used can be found in the study file. Calibration curves were determined from linear regression analysis of the data using Microsoft Excel Version 9.

## VI. DETERMINATION OF LIMITS OF DETECTION AND QUANTIFICATION

The limit of detection (LOD) was defined as three times the standard deviation (SD) of the average matrix blank response. During the calculation of the LOD if the values are negative due to the slope or intercept, the LOD will be estimated as 1/3 of the LOQ.

The limit of quantification (LOQ) was defined as ten times the standard deviation (SD) of the average matrix blank response.

## VII. RESULTS AND DISCUSSION

### A. Study Samples

The ug/g amounts of D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> found for the liver samples is presented in Table 2. Detailed results can be found in the study file.

### B. Quality control

A set of QC samples were prepared and analyzed in conjunction with the samples. The set included solvent blanks, control liver from rainbow trout, and spike addition to liver from control trout as well as cod liver from sample 10. The nominal spike level and the results for quantifying D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> are presented in Table 3. One spike level concentration was prepared and used to spike triplicate control trout liver samples as well as a spike addition to triplicate aliquots of Cod liver Identification number 10 samples. The method produced acceptable accuracies for all of the samples. The solvent standard accuracy and standard curves obtained from the analysis set are presented in Table 4 and Figure 1, respectively. The solvent standards were separated into two ranges in order to accommodate a wide range of concentrations. In all cases, the correlation coefficient ( $R^2$ ) value was greater than 0.99. The GC-MS conditions are listed in Table 5. The limit of quantification and detection was set at the level determined using the liver matrix, this data is summarized in Table 6. For this set, the limits of quantification were set at approximately 18.9, 1.7, 13.1 and 3.0 ng/g assuming 1.5 gram sample size for D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> respectively.

## TABLES

Table 1. Organization of Test Samples

Processing Laboratory	ID	Weight (g)	Length (cm)	Liver wt (g)
NILU	1	605	44	4.5
DCC	2	972	46	31.6567
NILU	3	781	43	21.4
NILU	4	829	42	10.1
NILU	5	810	43	15.3
Evonik	6	862	45.5	18.79
DCC	7	615	40	6.9154
DCC	8	632	40	12.7682
Evonik	9	650	40.3	6.129
DCC	10	870	45.5	35.9099
Evonik	11	1272	49.5	34.84
Evonik	12	887	44.8	36.163
DCC	13	1265	52.5	14.0174
Evonik	14	1240	48.3	31.71
DCC	15	1120	49.7	12.6133
NILU	16	1120	48	18.0
NILU	17	1032	50.5	6.1

Table 2. Summary of D3, D4, D5 and D6 Concentrations in Liver

Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
<b>NILU 1</b>				
<b>No Sample</b>				
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
DCC 2-1	B-LOQ	126.0	3113.9	393.98
DCC 2-2	B-LOQ	127.9	3169.1	392.55
DCC 2-3	B-LOQ	128.5	3127.7	399.90
Average =	NA	127.50	3136.9	395.48
Std. Dev. =	NA	1.3	28.72	3.896
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
NILU 3-4	B-LOQ	98.8	698.0	83.57
NILU 3-5	B-LOQ	100.3	716.3	92.06
Average =	NA	99.53	707.1	88.82
Std. Dev. =	NA	1.1	12.97	4.590
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
NILU 4-4	B-LOQ	100.3	806.9	76.31
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
NILU 5-4	B-LOQ	130.9	2012.9	135.38
NILU 5-5	B-LOQ	138.0	2004.6	141.40
Average =	NA	134.42	2008.8	138.39
Std. Dev. =	NA	5.0	5.86	4.260
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
Evonik 6-1	B-LOQ	95.0	642.1	143.54
Evonik 6-2	B-LOQ	93.7	633.1	156.11
Evonik 6-3	B-LOQ	97.4	640.0	148.48
Average =	NA	95.37	638.4	149.38
Std. Dev. =	NA	1.9	4.73	6.335
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
DCC 7-1	B-LOQ	49.9	377.4	26.18
DCC 7-2	B-LOQ	53.4	379.7	22.15
Average =	NA	51.60	378.6	24.16
Std. Dev. =	NA	2.5	1.66	2.848
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
DCC 8-1	B-LOQ	66.6	781.6	64.79
DCC 8-2	B-LOQ	65.3	774.0	68.47
Average =	NA	65.96	777.8	66.63
Std. Dev. =	NA	0.9	5.36	2.606

B-LOQ = Below Limit of Quantitation, NA = Not Applicable

Table 2. Continued: Summary of D3, D4, D5 and D6 Concentrations in Liver

Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
Evonik 9-1	B-LOQ	52.1	435.5	49.67
Evonik 9-2	B-LOQ	58.4	430.6	44.44
Average =	NA	55.24	433.0	47.06
Std. Dev. =	NA	4.5	3.50	3.699
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
DCC 10-1	B-LOQ	95.1	1482.4	120.99
DCC 10-2	B-LOQ	94.2	1458.2	118.53
DCC 10-3	B-LOQ	92.3	1491.2	118.09
Average =	NA	93.85	1477.3	119.20
Std. Dev. =	NA	1.4	17.08	1.561
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
Evonik 11-1	B-LOQ	101.1	2762.4	170.13
Evonik 11-2	B-LOQ	104.6	2805.8	167.33
Evonik 11-3	B-LOQ	102.0	2796.9	184.48
Average =	NA	102.56	2788.4	174.00
Std. Dev. =	NA	1.8	22.94	9.191
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
Evonik 12-1	B-LOQ	220.6	1779.4	222.43
Evonik 12-2	B-LOQ	221.1	1791.2	222.94
Average =	NA	220.83	1785.3	222.94
Std. Dev. =	NA	0.4	8.33	0.361
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
DCC 13-1	B-LOQ	14.8	111.7	B-LOQ
DCC 13-2	B-LOQ	18.2	110.9	B-LOQ
Average =	NA	16.50	111.3	NA
Std. Dev. =	NA	2.4	0.58	NA
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
Evonik 14-1	B-LOQ	129.1	2666.3	108.10
Evonik 14-2	B-LOQ	135.5	2821.8	138.25
Evonik 14-3	B-LOQ	142.4	2822.2	117.93
Average =	NA	135.67	2770.1	121.43
Std. Dev. =	NA	6.7	89.90	15.377
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
DCC 15-1	B-LOQ	119.7	1465.6	211.49
DCC 15-2	B-LOQ	120.6	1447.4	196.24
Average =	NA	120.19	1456.5	203.86
Std. Dev. =	NA	0.6	12.88	10.778
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
NILU 16-1	B-LOQ	76.4	1541.3	171.35
Replicate ID	Conc. (ng/g D3)	Conc. (ng/g D4)	Conc. (ng/g D5)	Conc. (ng/g D6)
NILU 17	No Sample			

B-LOQ = Below Limit of Quantitation, NA = Not Applicable

Table 3. Quality Control results for D3, D4, D5 and D6

QC Name	Expected [D3] ng	Recovered [D3] ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)	%RSD (Precision)
QC 0-1	0				
QC 0-2	0				
QC 0-3	0				
QC spike 1	211	215	2%	-2%	4%
QC spike 2	211	202	-4%		
QC spike 3	211	203	-4%		
10S-1	211	203	-3%	-4%	2%
10S-2	211	204	-3%		
10S-3	211	197	-6%		

QC Name	Expected [D4] ng	Recovered [D4] ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)	%RSD (Precision)
QC 0-1	0				
QC 0-2	0				
QC 0-3	0				
QC spike 1	226	235	4%	3%	1%
QC spike 2	226	230	1%		
QC spike 3	226	235	4%		
10S-1	226	231	2%	1%	1%
10S-2	226	226	0%		
10S-3	226	227	0%		

QC Name	Expected [D5] ng	Recovered [D5] ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)	%RSD (Precision)
QC 0-1	0				
QC 0-2	0				
QC 0-3	0				
QC spike 1	237	244	3%	3%	2%
QC spike 2	237	247	4%		
QC spike 3	237	239	1%		
10S-1	237	272.56	15%	5%	9%
10S-2	237	229.66	-3%		
10S-3	237	243.02	3%		

QC Name	Expected [D6] ng	Recovered [D6] ng	%Relative Error (Accuracy)	Average % Relative Error (Accuracy)	%RSD (Precision)
QC 0-1	0				
QC 0-2	0				
QC 0-3	0				
QC spike 1	220	218.62	-1%	-2%	2%
QC spike 2	220	210.45	-4%		
QC spike 3	220	215.18	-2%		
10S-1	220	232.83	6%	0%	5%
10S-2	220	218.35	-1%		
10S-3	220	209.02	-5%		

Table 4. Method Results for Solvent Standards

Standard ID	Expected [D <sub>s</sub> ] ng	[D <sub>s</sub> ] found ng	%Relative Error (Accuracy)
STD B	5.519	5.36	2.9
STD C	11.17	9.90	11.4
STD D	23.07	23.40	1.4
STD E	48.24	49.72	3.1
STD F	90.55	90.36	0.2
STD G	219.15	218.94	0.1
STD G	219.1	208.3	5.0
STD H	439.2	442.0	0.6
STD I	960	975.9	1.7
STD J	1807	1798.6	0.5
STD K	Standard Not used		

Standard ID	Expected [D <sub>s</sub> ] ng	[D <sub>s</sub> ] found ng	%Relative Error (Accuracy)
STD B	6.163	6.31	2.4
STD C	12.47	13.42	7.6
STD D	25.76	25.19	2.2
STD E	53.87	52.73	2.1
STD F	101.1	101.7	0.6
STD F	101.1	101.2	0.1
STD G	244.7	244.8	0.0
STD H	490.3	484.3	1.2
STD I	1072	1069	0.3
STD J	2018	2033	0.7
STD K	4203	4197	0.1

Standard ID	Expected [D <sub>s</sub> ] ng	[D <sub>s</sub> ] found ng	%Relative Error (Accuracy)
STD B	6.059	6.01	0.9
STD C	12.26	11.87	3.2
STD D	25.32	25.87	2.2
STD E	52.96	52.90	0.1
STD F	99.4	99.3	0.1
STD F	99.4	104.1	4.7
STD G	240.6	244.0	1.4
STD H	482.1	481.5	0.1
STD I	1054	1046	0.8
STD J	1984	1982	0.1
STD K	4132	4135	0.1

Standard ID	Expected [D <sub>s</sub> ] ng	[D <sub>s</sub> ] found ng	%Relative Error (Accuracy)
STD B	5.893	5.4521	7.5
STD C	standard error		
STD D	24.6	23.1637	5.9
STD E	51.5	54.6543	6.1
STD F	96.7	95.4176	1.3
STD F	96.7	91.79	5.1
STD G	234	232.60	0.6
STD H	469	467.23	0.3
STD I	1025	1024.18	0.1
STD J	1930	1945.00	0.8
STD K	4019	4012.15	0.2

Table 5. GC Conditions

Parameter	Setting
Instrument/Software	HP6890 Series Gas Chromatograph HP5973 Mass Selective Detector HP ChemStation version D.03.00
Inlet conditions	splitless, Purge Flow 10ml/min Purge Time 0.10 min 1 $\mu$ L injection
Carrier	Helium, 1 mL/min constant flow
Column	Zebron ZB-5, 30 m $\times$ 250 $\mu$ m ID $\times$ 0.25 $\mu$ m film thickness
Oven Program	50 $^{\circ}$ C (hold 3 min) to 190 $^{\circ}$ C at 25 $^{\circ}$ C/min, to 250 $^{\circ}$ C at 40 $^{\circ}$ C/ min
Transfer line	280 $^{\circ}$ C
MSD	Selective ion monitoring, m/z D3-207, D4-281, M4Q-281, 13C-D4-285, D5-355, 13C-D5-360, D6-429, 13C-D6-435; 100 ms dwell time

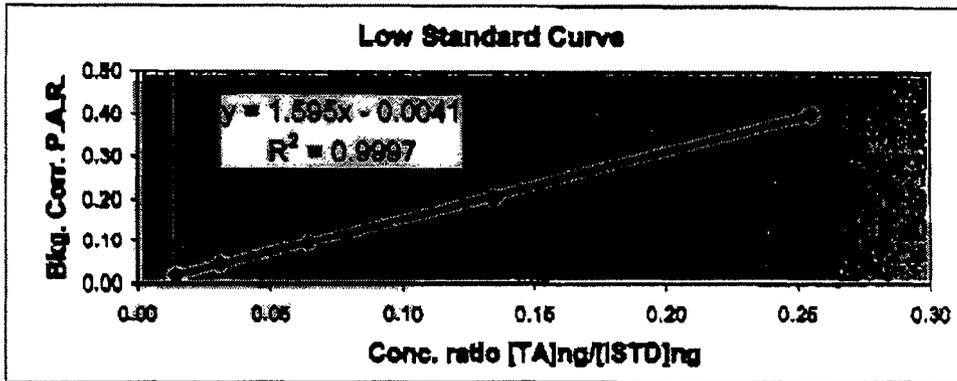
Table 6. Limit of Quantification and Detection

Control Trout Liver Matrix

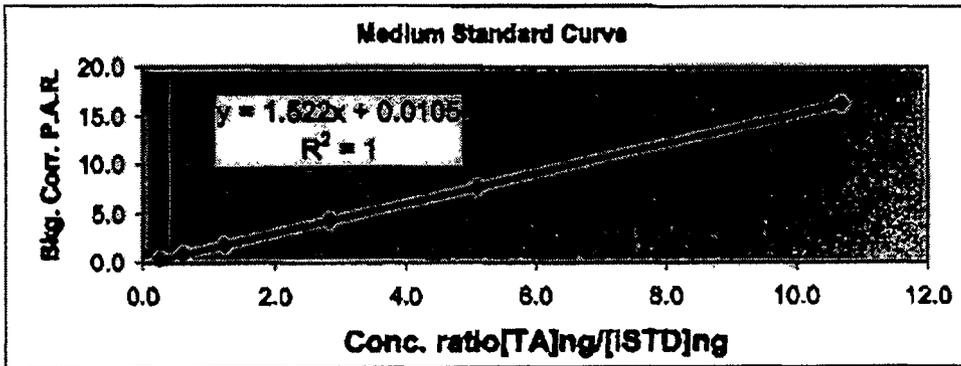
QC Name	Area Ratio D <sub>7</sub> /ISTD	Area Ratio D <sub>8</sub> /ISTD	Area Ratio D <sub>9</sub> /ISTD	Area Ratio D <sub>6</sub> /ISTD
QC 0-a	0.018	0.028	0.026	0.019
QC 0-b	0.018	0.026	0.036	0.011
QC 0-c	0.010	0.027	0.033	0.011
Std. Dev.	0.0045	0.0010	0.0052	0.0048
10 x Std. Dev =	0.045	0.010	0.052	0.048
LOQ ng/g	18.9	1.7	13.1	3.0
3x Std. Dev =	0.013	0.003	0.015	0.014
LOD ng/g	4.9	0.1	4.2	0.9

Figures

Figures 1. D5 Tetrahydrofuran Standard Curves from analysis for cVMS



slope = 1.5950      y-intercept -0.0041       $R^2 = 0.9997$



slope = 1.5220      y-intercept 0.0105       $R^2 = 1.0000$

Norsk institutt for luftforskning  
Norwegian Institute for Air Research



## Project Report

# Cyclic siloxanes in codfish from the Oslo Fjord

Henriette Leknes, Martin Schlabach

*Delbaker i CEENS og Miljøalliansen / Associated with CEENS and the Environmental Research Alliance of Norway  
ISO-sertifisert etter / ISO certified according to NS-EN ISO 9001*

NILU  
P.O. Box 100  
Instituttveien 18  
NO-2027 KJELLER, Norway  
Phone: +47 63 89 80 00/Fax: +47 63 89 80 50

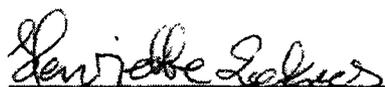
NILU Tromsø  
Polarmiljøsentret / The Polar Environmental Centre  
Hjalmar Johanssens gt. 14  
NO-9296 TROMSØ, Norway  
Phone: +47 77 75 03 75/Fax: +47 77 75 03 76

e-mail: [nifu@nifu.no](mailto:nifu@nifu.no)  
[nifu-tromso@nifu.no](mailto:nifu-tromso@nifu.no)  
Internet: [www.nifu.no](http://www.nifu.no)  
Bank: 5102.05.19030  
Fvretaksnr./Enterprise no. 941705561

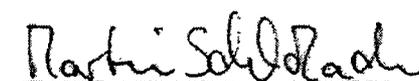
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**Cyclic siloxanes in codfish from the Oslo Fjord****Project Report**

**NILU Project Number:** O-108063  
**Project Leader:** Henriette Leknes  
**Supervisor:** Martin Schlabach  
**Institute:** Norwegian Institute for Air Research (NILU)  
**Department:** Environmental Chemistry Department (MILK)  
**Location:** Kjeller, Norway  
**Date:** October 7<sup>th</sup>, 2008

  
**Henriette Leknes**  
**Project Leader**

08.10.2008  
**Date**

  
**Martin Schlabach**  
**Supervisor**

08.10.2008  
**Date**

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## I. Objective

Quantitation of cyclic volatile methyl siloxanes (cVMS) (D3, D4, D5 and D6) in cod livers from environmental monitoring samples collected in Norway, as part of a 3 lab comparison between Dow Corning, Evonik and NILU.

## II. Test System

The test system for this study consisted of livers, removed from cod collected by the Norwegian Institute for Water Research (NIVA) of Oslo, Norway. NILU received 6 of 17 fish, identification numbers; 1, 3, 4, 5, 16 and 17 (Table 1).

## III. Reference Compounds

D3: Hexamethylcyclotrisiloxane, 98 %, Lot S42782-277, Aldrich

D4: Octamethylcyclotetrasiloxane,  $\geq 99.0$  %, Lot 1330251, Fluka

D5: Decamethylcyclopentasiloxane,  $\geq 97$  %, Lot 1281960, Fluka

D6: Dodecamethylcyclohexasiloxane,  $>95$  %, Lot 4A-4348, Gelest Inc., Morrisville, PA.

## IV. Sample Processing and analysis

All handling and sample preparation was carried out in a clean room laboratory. The clean room is built according to international standards (US Federal Standard 209e) for class 10000 and 100000 particles per ft<sup>3</sup>. However, the air quality inside the clean room has been measured to be better than the specifications for class 1000. All glassware was heated to 450°C overnight before use. Other equipment was rinsed thoroughly with hexane to minimize siloxane contamination.

Cod liver samples were collected from 6 fish caught by NIVA on December 11<sup>th</sup>, 2007. The fish were received in frozen condition on January 25<sup>th</sup>, 2008, and were stored at -18°C until dissection. The fish were allowed to thaw, and the livers removed with a scalpel. The samples were placed in glass bottles and homogenized using an Ultra Turrax. The steel homogenizer was rinsed several times in hexane between samples. Immediately after homogenization, aliquots of the samples were weighed into vials supplied from each laboratory. The samples were stored at -18°C until shipment or processing.

Two aliquots of 0.3 g of each sample were weighed into 2.0 mL Eppendorf Protein LoBind centrifuge tubes. 1.00 mL of hexane was added, the samples were shaken by hand and then vortex mixed for 30 min. After centrifuging at 10000 rpm for 10 min, the extracts were transferred to pre-weighed autosampler vials, weighed, and stored at -18°C until analysis.

The chromatographic analysis was performed on an Agilent 5890N gas chromatograph with Agilent 7683B autosampler. The isomer identification was performed by high-resolution mass

spectrometry on a Waters Autospec-V Ultima in positive electron ionisation mode (EI+, 35 eV). Two masses were monitored for each analyte, corresponding to the  $[M-CH_3]^+$  fragment. For parameters, see Table 4. Chromatograms are shown in Figure 1 to Figure 4.

Quantitative analysis was performed by external standard calibration. Multiple injections of a single-level calibration solution of cyclic siloxanes at 35-50 ng/mL in hexane were performed before, during, and after sample analysis. Additionally, injections of the hexane used for sample extraction was performed several times during each analytical run. The hexane injections were included in the calibration curve to correct blank values from the solvent and injector system. Integration and quantitative analysis was performed using MassLynx 4.1 software.

## V. Quality control samples

Quality control samples of cod liver were obtained from Hallvard Lerøy AS; two fresh, intact cod fish were ordered through a local supermarket. The fish were caught at a remote site outside Nordmøre at the northwestern coast of Norway, and stored on ice until frozen whole at NILU. The livers were collected and analysed as described above. The liver showing the lowest trace of siloxanes of the two was selected for quality control and blank samples.

Five aliquots of control liver was weighed into Eppendorf tubes and spiked with 15  $\mu$ L of a hexane solution of cyclic siloxanes giving concentrations of approximately 40 ng/g wet weight. The five QC samples and two blank samples of the control liver was prepared and analysed together with the Oslo Fjord cod liver samples. In addition to the cod liver blanks, three replicate system blank samples were prepared and analysed. The system blanks were treated exactly as the cod liver samples, but did not contain liver tissue.

## VI. Determination of Limits of detection

The limit of detection (LOD) was set at three times the siloxane response in the blank hexane injections. In the absence of a blank response for a particular siloxane, the compound LOD calculated from the MassLynx software was used. This LOD is calculated as the concentration giving a signal-to-noise ratio of 3:1. None of the system blanks gave concentrations above the LOD set in this manner.

## VII. Results and Discussion

### A. Study Samples

Results from the analysis of cyclic siloxanes in cod liver samples are presented in Table 2. All samples were analysed in duplicate. The results are corrected according to the average recoveries listed in Table 3.

**B. Quality control samples**

Results from the quality control samples are shown in Table 3. The QC sample set included five spiked samples of cod liver from a remote area, two non-spiked samples of the same cod liver, and three system blanks. A concentration of D5 above the LOD was detected in the cod liver blanks. To calculate D5 recovery in the spiked samples, the detected concentration was corrected by the average D5 level from the blanks.

Average recoveries obtained were between 64 and 74 %. Recoveries may have been affected by long storage time (two weeks) before analysis. This may have caused some loss of siloxanes due to adsorption or degradation. However, concentrations were recovery corrected based on results obtained from QC samples, thus the results for the Oslo Fjord cod liver should not be affected.

Precision, measured as relative standard deviation of the five spiked samples, was between 0.7 and 8.4 %.

## TABLES

Table 1. Organization of Test Samples

Processing Laboratory	ID	Weight (g)	Length (cm)	Liver wt (g)
NILU	1	605	44	4.5
DCC	2	972	46	31.6567
NILU	3	781	43	21.4
NILU	4	829	42	10.1
NILU	5	810	43	15.3
Evonik	6	862	45.5	18.79
DCC	7	615	40	6.9154
DCC	8	632	40	12.7682
Evonik	9	650	40.3	6.129
DCC	10	870	45.5	35.9099
Evonik	11	1272	49.5	34.84
Evonik	12	887	44.8	36.163
DCC	13	1265	52.5	14.0174
Evonik	14	1240	48.3	31.71
DCC	15	1120	49.7	12.6133
NILU	16	1120	48	18.0
NILU	17	1032	50.5	6.1

Table 2. Summary of D3, D4, D5 and D6 concentrations in cod liver.  
Results have been corrected for recovery (Table 3)

Sample ID*	Fish ID	Result, ng/g w.w.			
		D3	D4	D5	D6
2-B-1	Proc. blank NILU 1	<3.3	<2.4	2.4	<3.8
2-B-2	Proc. blank NILU 2	<3.3	<2.4	2.6	<2.7
	Average	<3.3	<2.4	2.5	<2.7
2-1-1	1	<2.2	5.5	52	<6.0
2-1-2	1	<2.2	5.5	52	<6.0
	Average**	<2.2	5.5	52	<6.0
2-3-1	3	6.5	55	441	52
2-3-2	3	<3.2	59	483	48
	Average	6.5	57	462	50
2-4-1	4	4.0	111	1569	88
2-4-2	4	3.9	102	1268	81
	Average	3.9	107	1419	84
2-5-1	5	<3.0	56	962	49
2-5-2	5	<2.3	75	1195	70
	Average	<2.3	66	1078	60
2-16-1	16	<2.6	88	1815	158
2-16-2	16	3.0	74	1579	140
	Average	3.0	81	1697	149
2-17-1	17	<2.4	39	847	29
2-17-2	17	17	80	1366	46
	Average	17	60	1106	37
3-B-1	Proc. blank Evonik 1	<3.3	2.5	2.5	<4.6
3-B-2	Proc. blank Evonik 2	<3.3	3.0	2.6	<3.7
3-B-3	Proc. blank Evonik 3	<2.6	5.6	3.5	<3.9
	Average	<2.6	3.7	2.9	<3.7
3-6-1	6	2.7	66	448	88
3-6-2	6	5.4	70	543	104
	Average	4.0	68	495	96
3-9-1	9	<1.9	33	300	34
3-9-2	9	2.4	40	393	46
	Average	2.4	37	347	40
3-11-1	11	17	109	3142	191
3-11-2	11	6.6	94	2883	164
	Average	12	101	3012	178
3-12-1	12	11	226	1935	199
3-12-2	12	10	210	1809	180
	Average	11	218	1872	190
3-14-1	14	<5.2	147	3179	124
3-14-2	14	<4.9	134	2861	100
	Average	<4.9	140	3020	112

Sample ID	Fish ID	Result, ng/g w.w.			
		D3	D4	D5	D6
1-B-1	Proc. Blank DCC 1	<4.6	7.9	1.7	<0.3
1-B-2	Proc. Blank DCC 2	<4.6	5.8	1.5	<0.2
1-B-3	Proc. Blank DCC 3	<4.6	12	3.6	1.3
	<b>Average</b>	<b>&lt;4.6</b>	<b>8.6</b>	<b>2.3</b>	<b>1.3</b>
1-2-1	1	<5.1	113	2889	283
1-2-2	1	<5.2	106	2715	262
	<b>Average</b>	<b>&lt;5.1</b>	<b>110</b>	<b>2802</b>	<b>272</b>
1-7-1	7	<4.7	8.2	62	5.9
1-7-2	7	<7.4	8.3	61	5.4
	<b>Average</b>	<b>&lt;4.7</b>	<b>8.3</b>	<b>61</b>	<b>5.7</b>
1-8-1	8	<5.0	43	562	44
1-8-2	8	<4.7	36	480	40
	<b>Average</b>	<b>&lt;4.7</b>	<b>40</b>	<b>521</b>	<b>42</b>
1-10-1	10	<5.2	73	1164	75
1-10-2	10	<4.8	76	1288	82
	<b>Average</b>	<b>&lt;4.8</b>	<b>74</b>	<b>1226</b>	<b>79</b>
1-13-1	13	<4.4	5.2	38	<2.8
1-13-2	13	<4.3	3.2	33	2.4
	<b>Average</b>	<b>&lt;4.3</b>	<b>4.2</b>	<b>35</b>	<b>2.4</b>
1-15-1	15	<4.9	96	1192	132
1-15-2	15	<5.0	104	1293	139
	<b>Average</b>	<b>&lt;4.9</b>	<b>100</b>	<b>1243</b>	<b>136</b>
	System blank 1	<0.7	<0.5	<0.5	<1.8
	System blank 2	<0.7	<0.5	<0.9	<3.6
	System blank 3	<0.7	<0.5	<0.3	<1.1
	<b>Average</b>	<b>&lt;0.7</b>	<b>&lt;0.5</b>	<b>&lt;0.3</b>	<b>&lt;1.1</b>

\*: The samples were labeled according to lab ID (Lab 1: Dow Corning, lab 2: NILU, lab 3: Evonik), fish number and replicate number. Sample 1-8-1: Lab 1, fish 8, replicate 1. Processing blanks are labeled "B" instead of fish number.

\*\*\*: The sample amount for sample 2-1-2 was very low. The used for confirmation only.

Table 3. Quality Control results for D3, D4, D5 and D6 in spiked samples of cod liver.  
D5 concentrations have been corrected according to blank levels.

Sample Text	D3			D4		
	Expected, ng/g	Conc., ng/g	Rec., %	Expected, ng/g	Conc., ng/g	Rec., %
System blank 1		<1.0			<0.6	
System blank 2		<0.9			<0.6	
System blank 3		<0.9			<0.6	
Cod liver blank 1		<2.0			<1.4	
Cod liver blank 2		<2.0			<1.4	
Spike 1	37.4	25.0	66.8	39.5	28.6	72.3
Spike 2	35.7	24.1	67.5	37.7	26.3	69.8
Spike 3	37.8	25.4	67.2	39.9	27.2	68.2
Spike 4	34.7	23.4	67.4	36.7	25.1	68.4
Spike 5	36.8	25.1	68.2	38.9	26.5	68.1
Average		24.6	67.4		26.7	69.4
RSD, %		3.4	0.7		4.8	2.6
Sample Text	D5			D6		
	Expected, ng/g	Conc., ng/g	Rec., %	Expected, ng/g	Conc., ng/g	Rec., %
System blank 1		<0.7			<2.3	
System blank 2		<1.1			<4.6	
System blank 3		<0.4			<1.4	
Cod liver blank 1		6.5			<4.9	
Cod liver blank 2		4.2			<4.8	
Average		5.3				
Spike 1	40.0	28.2	70.5	38.2	32.4	84.7
Spike 2	38.1	25.1	65.7	36.5	26.0	71.3
Spike 3	40.4	24.5	60.6	38.6	26.6	68.9
Spike 4	37.1	21.8	58.9	35.5	26.8	75.5
Spike 5	39.4	25.1	63.7	37.6	26.9	71.5
Average		24.9	63.9		27.7	74.4
RSD, %		9.1	7.1		9.5	8.4

Table 4. GC-HRMS conditions

Parameter	Setting
Instrument	Agilent GC 5890N Autosampler 7683B Waters Autospec-V Ultima
Software	Opus V3.6X MassLynx 4.1
Inlet	Splitless, 0.3 min, 200 °C, 1 µL
Column	J&W Ultra2, 25 m × 0.2 mm ID × 0.11 µm film thickness
Temperature program	35 °C (3 min), 7 °C/min to 130 °C (0 min), 30 °C/min to 325 °C (5 min)
Flow	He, 1.0 mL/min, constant flow
HRMS	EI+, 35 eV R = 10000 at 5 % valley.
Mass	Fragment [M-CH <sub>3</sub> ] <sup>+</sup> D3: m/z 207.0329 / 208.0336 D4: m/z 281.0517 / 282.0524 D5: m/z 355.0705 / 356.0712 D6: m/z 429.0893 / 430.0900

Figures

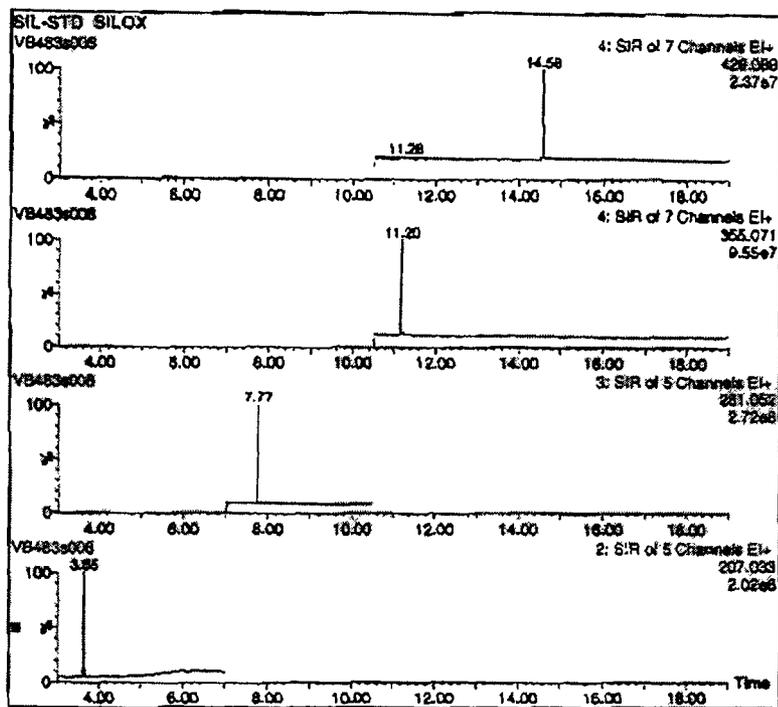


Figure 1. Selected ion chromatogram of a siloxane standard, 40 ng/mL.

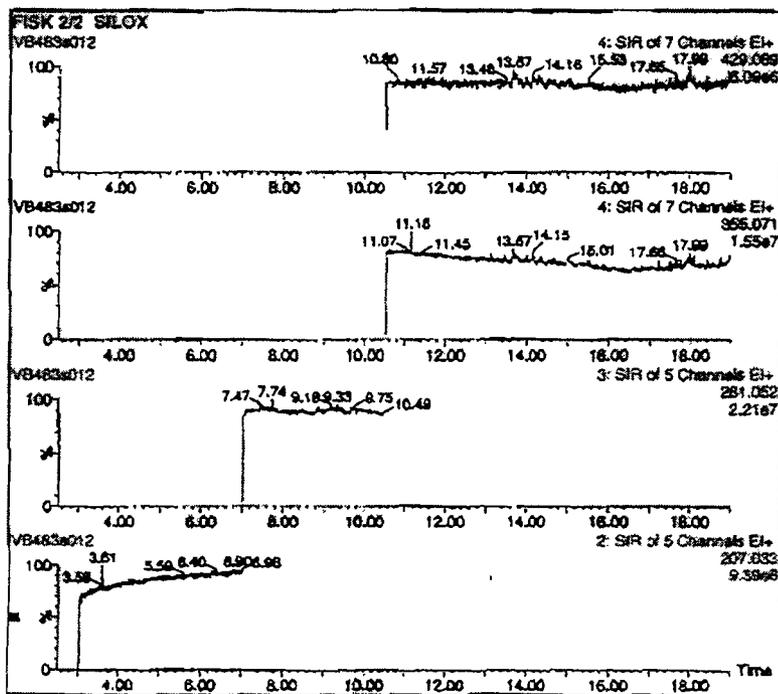


Figure 2. Non-spiked cod liver from Nordmøre

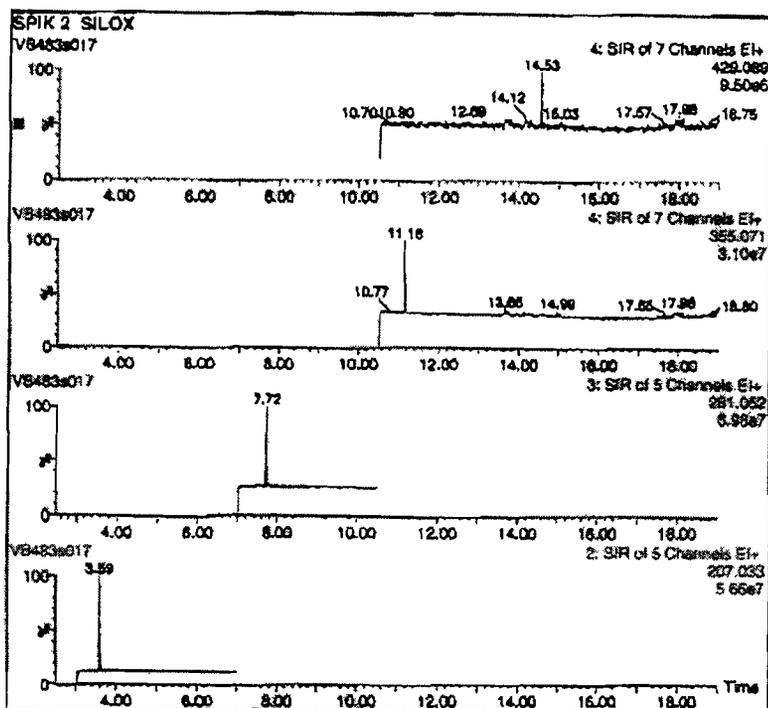


Figure 3. Quality control sample of cod liver spiked to 40 ng/g w.w.

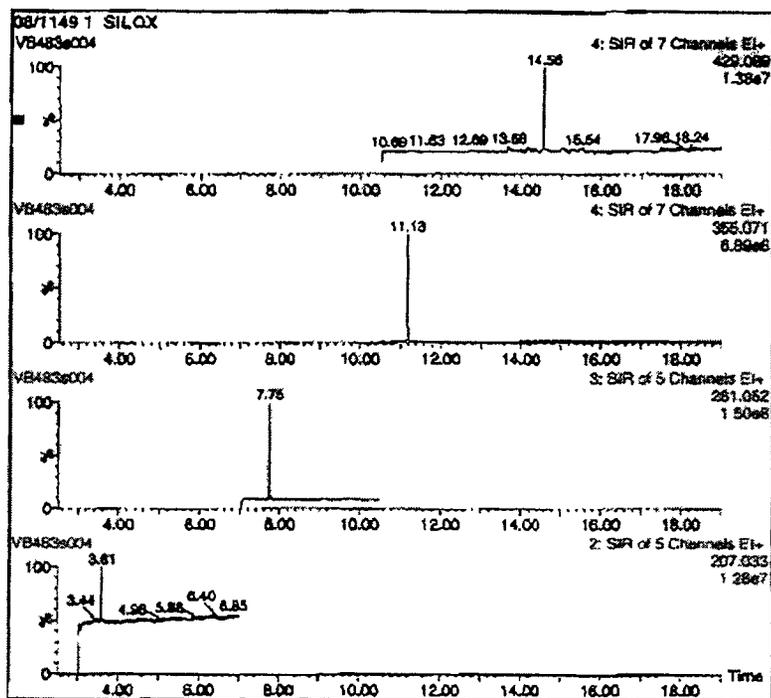
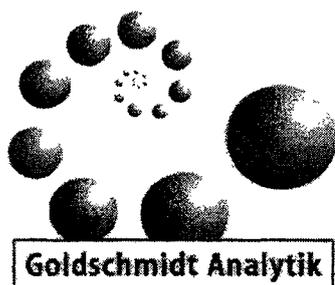


Figure 4. Oslo Fjord cod liver, fish no. 4.



**degussa.**

## **cVMS Analysis of Cod Fish Livers Collected by Norway (NILU)**

**A study carried out by:**

**Thomas Böhmer  
Reinhard Gerhards  
Martin Koerner  
Regina Unthan  
Evonik Goldschmidt GmbH  
EK WD AL  
Goldschmidtstrasse 100  
45127 Essen  
Germany**

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## 1. Objective

Quantitation of cyclic volatile methyl siloxanes (cVMS) (D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub>) in Cod livers from environmental monitoring samples collected by Norway, as part of a 3 lab comparison between Dow Corning, NILU and Evonik Goldschmidt.

## 2. Test System

The test system for this study consisted of livers, removed from Cod collected by NILU of Norway. Evonik Goldschmidt received 5 of 17 fish, identification numbers; 6, 9, 11, 12 and 14.

## 3. Reference Article Information

Reference article characterization was done in our own laboratory by GLC/FID analysis.

### 3.1. Reference Article 1

- Identification: Hexamethylcyclotetrasiloxane (D<sub>3</sub>)
- Lot Number: Hexamethylcyclotrisiloxane
- Purity: 99.7 % by GC-FID

### 3.2. Reference Article 2

- Identification: Octamethylcyclotetrasiloxane (D<sub>4</sub>)
- Lot Number: Wacker >99.5%
- Purity: 99.8 % by GC-FID

### 3.3. Reference Article 3

- Identification: Decamethylcyclopentasiloxane (D<sub>5</sub>)
- Lot Number: Aldrich, 97%
- Purity: 97.5 % by GC-FID

### 3.4. Reference Article 4

- Identification: Dodecamethylcyclohexasiloxane (D<sub>6</sub>)
- Lot Number: ABCR, > 95 %, checked by GC
- Purity: 99.5 % by GC-FID

As a considerable amount of the individual cVMS was found in blank solutions – currently unavoidable contaminations from solvents and analytical devices used- all peak area ratios in the calibration samples were corrected by the corresponding peak area ratios of a calibration blank. The resulting data pairs area ratio vs. concentration ratio were used for a linear regression calibration function ( $y = a * x$ ).

The linearity parameters; slopes, and correlation coefficients, were determined for each of the standard curves. The minimum acceptance criterion for the linearity of a standard curve was a regression coefficient (R2) of 0.99.

Calibration curves were determined from linear regression analysis of the data using Microsoft Excel Version 2003.

#### Analysis:

At first preliminary analyses were performed in accordance to the methodology applied for the determination of Cyclic Volatile Methyl Siloxanes in Mussels (see report "Cyclic Volatile Methyl Siloxanes In Mussels - Screening of Mussels from some Intertidal Areas of the Southern North Sea- A study carried out by Thomas Böhmer et al.; April 17<sup>th</sup> 2007)

However, due to the high content of triglycerides and other fatty constituents a direct GC/MS analysis of the raw extracts was not possible, because the matrix components irreversibly contaminated the GC column.

Hence the extraction procedure was modified and an additional liquid chromatography pre-cleaning procedure was applied.

#### Extraction:

Extraction of the liver was performed using a 10:1 solvent to sample ratio of pentane (4 ml pentane vs. 400 mg tissue sample) containing the internal standards. After addition of a measured volume of pentane containing internal standard the samples were homogenised with the help of an Ultra-Turrax device for 1 minute. Subsequently 1 ml of pure water was added and the mixture was mixed again by an Ultra-Turrax. The samples were then centrifuged for 5 minutes at a setting of 3000 RPM's. The extractant from each sample was transferred into a separate vial.

#### Pre-cleaning

The raw extract was cleaned by column chromatography. As sorbent 2 g of pre-cleaned Florisil (activated at 140°C) were filled into a 15 cm glass column and covered with 300 mg of MgSO<sub>4</sub> (pre-treated at 540 °C). 1 ml of the raw extract were transferred onto the MgSO<sub>4</sub> layer and flushed into the column bed with 2 ml of a mixture of petrolether (30-40°C) and methyl tertiary butyl ether (MTBE) [99:1]. Subsequently the cVMS were eluted from the column with additional 10 ml of of petrolether : MTBE [99:1]. The eluate was carefully reduced by volume to 0.5 ml by a gentle stream of nitrogen at 25 °C and analysed by GC/MS.

#### Evaluation:

Peak responses from the GC-MS system were quantitated by automatic electronic integration either by using integration parameters in the software or by drawing a baseline manually in the software for the peak. If the baselines were drawn manually, this was indicated on the chromatogram printout in the study file. All calculations were done using Microsoft Excel Version 2003.

#### 4. Sample Processing and Analysis

The cods used for this study were caught on December 10, 2007 in the Oslo Fjord and kept frozen till shipping to the laboratories. Details on the collection area are provided by NILU. Details on Fish weights and lengths are compiled in Table 1. The 17 cods collected were divided into three batches, with one batch being sent to each of the three laboratories involved. For Evonik Goldschmidt the batch consisted of 5 fish which were sent from Oslo on February 11, and arrived in the laboratory on February 13. These fish arrived frozen in an thermo-insulated parcel still containing lot of dry ice at arrival time. The fish were transferred into a refrigerator set to a temperature of -20 °C. There they were kept till April 24, 2008, when they were defrosted and their livers were removed. Each of the livers then was homogenized by applying an Ultra-Turrax device, which was carefully cleaned prior to the homogenisation of each fish liver. The homogenates then were divided into three sub-samples and filled into pre-cleaned containers that had been provided by DC, NILU and Evonik individually. All containers then were placed into the refrigerator at -20 °C. The sub-samples of each liver homogenates in the DC-containers were placed into a thermo-insulated parcel together with a sufficient amount of dry-ice to keep the frozen status during transport and sent to Dow Corning, Midland, on April 28, 2008, where they arrived in good condition on April 30. The NILU-containers with the homogenates were sent on May 5 and arrived in good conditions on May 7, 2008 in Oslo.

Dow Corning and NILU followed a similar procedure with the fish they had received in February 2007 and sent sub-samples of the liver homogenates generated to the Evonik laboratories. The Dow Corning samples were sent on May 6 and arrived on May 8, 2008 in frozen condition, while the NILU samples, sent on May 14 at arrival time on May 16 were no longer frozen. All these samples were placed into the refrigerator at -20 °C until analysis.

The set of samples to be analysed in the Evonik laboratories thus consisted of three sets of cod-liver homogenates samples, each of them prepared in one of the three laboratories involved

##### Calibration

The analysis was based upon the principle of calibrating a gas chromatograph with mass selective detection (GC-MS) using solvent standards pentane spiked with various concentrations of D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub> and <sup>13</sup>C-D<sub>4</sub>, <sup>13</sup>C-D<sub>5</sub> and <sup>13</sup>C-D<sub>6</sub> as internal standards, and then analyzing pentane extracts of the liver against the calibration curves.

The solvent standard concentrations ranges validated were adapted to the working range resulting from the appearance of the individual cVMS in the cod liver samples and ranged from approximately 4 to 80 ng/ml D4 and D6 and from approximately 4 to 850 ng/ml D5.

Peak responses from the GC-MS system were quantitated by automatic electronic integration either by using integration parameters in the software or by drawing a baseline manually in the software for the peak. If the baselines were drawn manually, this was indicated on the chromatogram printout in the study file. Detailed records of the GC-MS system and data system software used can be found in the study file.

A linear regression analysis was performed relating the concentration of the calibration standards to the relative chromatographic response ratio (test article/internal standard).

As a considerable amount of the individual cVMS was found in blank solutions – currently unavoidable contaminations from solvents and analytical devices used- all peak area ratios in the calibration samples were corrected by the corresponding peak area ratios of a method blank. The method blanks were repeatedly determined over the course of the sample preparation work.

All samples were worked up using the same batch of solvents.

The corrected peak area ratios were transformed into their corresponding concentrations and the cVMS were calculated as ng/g referred to the original sample weight.

## **5. Quality control**

A wolffish liver commercially obtained was used as a control.

A 50 g portion of homogenised wolffish liver spiked with a defined amount of cVMS was used as a spike.

Sample preparation of the liver spikes and controls were done the same way as samples. In this manner, an assessment of accuracy, linearity, limit of detection (LOD), and limit of quantitation (LOQ) was derived.

Specificity/selectivity was assessed by ensuring that the response in any of the spiked matrix blanks or solvent blanks did not significantly interfere with the analytes or internal standards of interest.

## **6. Determination of Limits of detection and quantification**

In accordance to IUPAC guideline (2002 IUPAC, Pure and Applied Chemistry 74,850) the limit of detection (LOD) is defined as 3 times of the standard deviation of the control sample (matrix blank, low level material resp.)

The limit of quantitation (LOQ) is defined as 3 times of the defined LOD.

## 7. Results and Discussion

### 7.1. Study Samples

The ug/g amounts of D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> found for the study sample liver are presented in **Table 2**.

### 7.2. Quality control

A set of QC samples were run along in conjunction with the samples. The set included solvent blanks, control liver from wolffish, and spike addition to liver from the control wolffish. The nominal spike levels and the results for quantifying D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> are presented in **Table 3**. The method produced acceptable accuracies for all of the samples. The solvent standard accuracy and standard diagrams obtained from the analysis set are presented in **Table 4** and **Figure 1**, respectively. The solvent standards were adapted for each cVMS to the required range of concentrations. In all cases, the correlation coefficient ( $R^2$ ) value was greater than 0.99. The GC-MS conditions are listed in **Table 5**. The limit of quantification was derived from the standard deviation of a replicate test of the control wolffish.

## 8. D<sub>3</sub> Analysis

Originally it was planned to also analyse the cod liver samples for their D<sub>3</sub> content. We performed the same procedures as described above for D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub>. As we did not have access to <sup>13</sup>C-labelled D<sub>3</sub> we choose to use the <sup>13</sup>C-labelled D<sub>4</sub> as internal standard for quantifying the D<sub>3</sub> content.

With the conditions used for the separation of D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> the retention time for D<sub>3</sub> was rather close to the solvent peaks and so we first interpreted the observed problems with quantification (scattering data) of D<sub>3</sub> as a result of the poor resolution.

In order to overcome this problem we switched the chromatographic system completely and used a polar column (Agilent HP-FFAP) for separation. In contrast to the usually used GC columns, this column does not contain a siloxane-based phase for separation, so contamination induced by GC-column phase degradation can be excluded definitely by using this column.

While the chromatographic separation conditions seemed to be perfectly suited for D<sub>3</sub>, the quantification of trace D<sub>3</sub> concentrations in the lower ppb range still failed. The repeatability of the peak area ratios D<sub>3</sub> / <sup>13</sup>D<sub>4</sub> determined for blanks and controls were significantly worse compared to the other cVMS. These results still would allow to determine limits of detection and limits of quantification as described for the other cVMS. Nevertheless we finally decided to refrain from reporting values, since we unpredictably observed an extremely increased number of outliers, without having any explanation. These outliers were observed as well for calibration solutions, as for extracts from the liver of the wolffish samples used as blank und spike, and for actual samples from the livers of the cods to be analysed.

Anyhow, we did not see any systematic difference in the concentrations of D<sub>3</sub> in all cod liver samples and the blanks. The quality of the data generated however, is too low to report quantitative results.

Further work is necessary to identify the reasons for the recurring observations of apparent high concentrations of D<sub>3</sub>.

**TABLES****Table 1. Organization of Test Samples**

Processing Laboratory	ID	Weight (g)	Length (cm)	Liver wt (g)
NILU	1	605	44	4.5
DCC	2	972	46	31.6567
NILU	3	781	43	21.4
NILU	4	829	42	10.1
NILU	5	810	43	15.3
Evonik	6	862	45.5	18.79
DCC	7	615	40	6.9154
DCC	8	632	40	12.7682
Evonik	9	650	40.3	6.129
DCC	10	870	45.5	35.9099
Evonik	11	1272	49.5	34.84
Evonik	12	887	44.8	36.163
DCC	13	1265	52.5	14.0174
Evonik	14	1240	48.3	31.71
DCC	15	1120	49.7	12.6133
NILU	16	1120	48	18.0
NILU	17	1032	50.5	6.1

**Table 2. Summary of D3, D4, D5 and D6 Concentrations in Liver**

Specimen:	D4	D5	D6
Evonik 12/1	257 ng/g	1919 ng/g	240 ng/g
Evonik 12/2	303 ng/g	1958 ng/g	244 ng/g
average	280 ng/g	1938 ng/g	242 ng/g
Evonik 8/1	86 ng/g	624 ng/g	139 ng/g
Evonik 8/2	68 ng/g	558 ng/g	125 ng/g
average	77 ng/g	591 ng/g	132 ng/g
Evonik 14/1	118 ng/g	2999 ng/g	127 ng/g
Evonik 14/2	144 ng/g	3048 ng/g	118 ng/g
average	131 ng/g	3023 ng/g	122 ng/g
Evonik 9/1	37 ng/g	347 ng/g	54 ng/g
Evonik 9/2	< 20 ng/g	389 ng/g	56 ng/g
average		368 ng/g	55 ng/g
Evonik 11/1	74 ng/g	2884 ng/g	177 ng/g
Evonik 11/2	90 ng/g	2958 ng/g	177 ng/g
average	82 ng/g	2921 ng/g	177 ng/g
DCC 2/1	125 ng/g	3092 ng/g	388 ng/g
DCC 2/2	135 ng/g	3189 ng/g	387 ng/g
average	130 ng/g	3141 ng/g	387 ng/g
DCC 7/1	74 ng/g	401 ng/g	35 ng/g
DCC 7/2	66 ng/g	367 ng/g	30 ng/g
average	70 ng/g	384 ng/g	
DCC 8/1	41 ng/g	718 ng/g	71 ng/g
DCC 8/2	57 ng/g	769 ng/g	77 ng/g
average	49 ng/g	743 ng/g	74 ng/g
DCC 10/1	84 ng/g	1717 ng/g	121 ng/g
DCC 10/2	79 ng/g	1679 ng/g	119 ng/g
average	82 ng/g	1698 ng/g	120 ng/g
DCC 13/1	< 22 ng/g	99 ng/g	< 19 ng/g
DCC 13/2	< 21 ng/g	133 ng/g	< 18 ng/g
average		116 ng/g	
DCC 15/1	131 ng/g	1564 ng/g	206 ng/g
DCC 15/2	97 ng/g	1414 ng/g	184 ng/g
average	114 ng/g	1489 ng/g	195 ng/g

**Table 2 (continued). Summary of D3, D4, D5 and D6 Concentrations In Liver**

	D4	D5	D6
NILU 1/1	< 21 ng/g	285 ng/g	< 18 ng/g
NILU 1/2	< 21 ng/g	238 ng/g	< 18 ng/g
average		252 ng/g	
NILU 3/1	61 ng/g	679 ng/g	79 ng/g
NILU 3/2	64 ng/g	728 ng/g	87 ng/g
average	63 ng/g	703 ng/g	83 ng/g
NILU 4/1	83 ng/g	1041 ng/g	89 ng/g
NILU 4/2	117 ng/g	1330 ng/g	86 ng/g
average	100 ng/g	1185 ng/g	78 ng/g
NILU 5/1	128 ng/g	2238 ng/g	143 ng/g
NILU 5/2	134 ng/g	2169 ng/g	129 ng/g
average	131 ng/g	2199 ng/g	136 ng/g
NILU 18/1	62 ng/g	1574 ng/g	159 ng/g
NILU 18/2	74 ng/g	1754 ng/g	180 ng/g
average	68 ng/g	1664 ng/g	170 ng/g
NILU 17/1	48 ng/g	1098 ng/g	30 ng/g
NILU 17/2	38 ng/g	1064 ng/g	27 ng/g
average	43 ng/g	1081 ng/g	29 ng/g

**Table 3. Quality Control results for D3, D4, D5 and D6**

Limit of Detection (LOD) – Limit of quantification (LOQ)

Control (wolffish)	nominal content:	D4	D5	D6
sample 1		1.60 ng/g	10.19 ng/g	8.88 ng/g
sample 2		1.23 ng/g	0.32 ng/g	5.31 ng/g
sample 3	average	-0.22 ng/g	6.88 ng/g	6.19 ng/g
sample 4		-1.32 ng/g	6.81 ng/g	6.32 ng/g
sample 5		-0.52 ng/g	3.01 ng/g	6.18 ng/g
sample 6		-4.72 ng/g	-2.13 ng/g	2.91 ng/g
average		-0.68 ng/g	4.01 ng/g	5.97 ng/g
standard deviation		2.25 ng/g	4.51 ng/g	1.92 ng/g
LOD (3* std. dev.)		6.76 ng/g	13.54 ng/g	5.77 ng/g
LOQ (3* std. dev.)		20.28 ng/g	40.62 ng/g	17.31 ng/g

## Recovery and Reproducibility

Spike solution	D4	D5	D6
methanol solution	0.275 ng/µl	0.274 ng/µl	0.274 ng/µl

Spiking: 100µl of the spiking solution mixed with 50.3 g homogenised wolffish liver homogenised by ultra turrax.

Spike	D4	D5	D6
nominal concentration	546.7 ng/g	544.7 ng/g	588.5 ng/g

## Analytical Results:

Spike	D4	D5	D6
spike sample 1	583.0 ng/g	573.8 ng/g	576.1 ng/g
spike sample 2	506.2 ng/g	529.8 ng/g	539.9 ng/g
spike sample 3	512.3 ng/g	538.3 ng/g	544.8 ng/g
spike sample 4	519.5 ng/g	555.1 ng/g	548.1 ng/g
spike sample 5	540.7 ng/g	568.6 ng/g	553.0 ng/g

**Table 3 (continued). Quality Control results for D4, D5 and D6**

Recovery (wolffish)	D4	D5	D6
spike sample 1	107%	105%	97%
spike sample 2	93%	96%	91%
spike sample 3	94%	98%	92%
spike sample 4	95%	101%	92%
spike sample 5	99%	103%	93%
average	97%	101%	93%
standard dev.	8%	3%	2%

**Table 4. Method Results for Solvent Standards**

Standard ID	[D <sub>6</sub> ] Expected ng/ml	[D <sub>6</sub> ] found ng/ml	%Relative Error (Accuracy)
STD 1	3.8	3.7	2.1%
STD 2	7.6	7.1	7.4%
STD 3	15.3	16.2	5.8%
STD 4	30.6	30.1	1.6%
STD 5	38.2	37.7	1.3%
STD 6	76.4	76.7	0.4%
STD 7	-*		
STD 8	-*		

Standard ID	[D <sub>6</sub> ] Expected ng/ml	[D <sub>6</sub> ] found ng/ml	%Relative Error (Accuracy)
STD 1	-*		
STD 2	-*		
STD 3	17.0	16.9	0.6%
STD 4	34.1	33.2	2.5%
STD 5	42.8	41.8	1.8%
STD 6	85.2	84.8	0.5%
STD 7	426.0	422.6	0.8%
STD 8	852.0	853.8	0.2%

Standard ID	[D <sub>6</sub> ] Expected ng/ml	[D <sub>6</sub> ] found ng/ml	%Relative Error (Accuracy)
STD 1	4.3	4.1	4.3%
STD 2	8.6	8.5	0.5%
STD 3	17.1	17.9	4.3%
STD 4	34.2	34.1	0.5%
STD 5	42.8	40.7	5.0%
STD 6	85.6	86.6	1.2%
STD 7	-*		
STD 8	-*		

-\* : not used

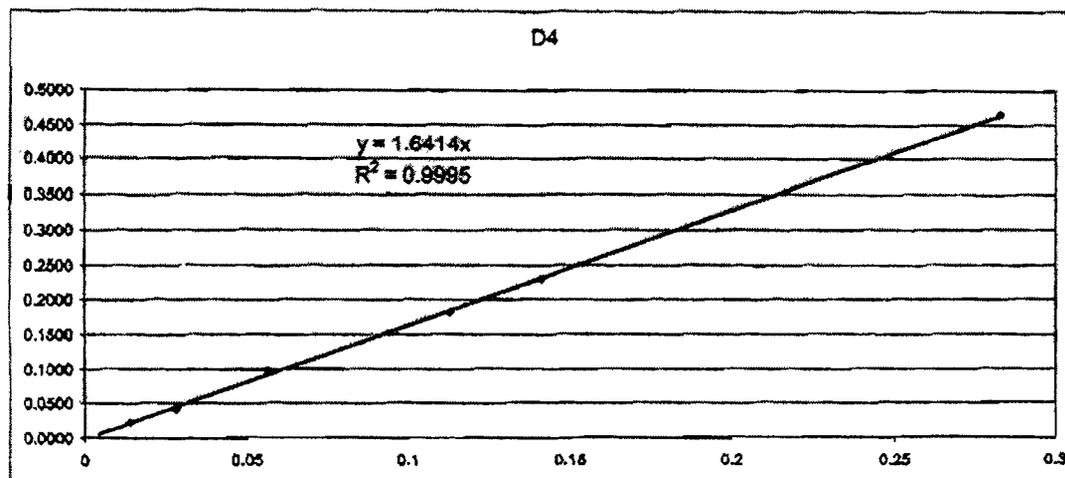
**Table 5. GC Conditions**

Parameter	Setting
Instrument/Software	HP6890 Series Gas Chromatograph HP5973 Mass Selective Detector HP ChemStation version E 01.01.
Inlet conditions	cool on column ; oven tray ; 1 µl
Carrier	Helium, 1 mL/min constant flow
Column	Agilent 122-5731 (DB-5HT) 30m * 0.25 mm * 0.10µm
Oven Program	40 °C to 65°C at 25°C/min; (hold 6 min) to 130°C at 6°C/min, post run 10 min at 310 °C
Transfer line	300 °C

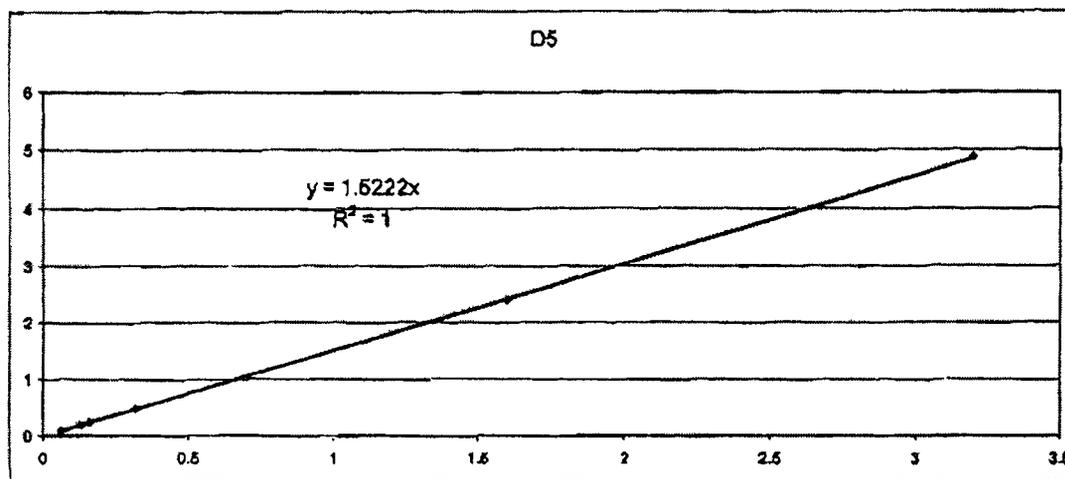
Acquisition Mode: Single Ion Detection			
	Retention time	Quantifier	Qualifier
D4	6.04 min.	281 amu	265, 249 amu
D5	11.94 min.	355 amu	268, 2523 amu
D6	16.75 min.	341 amu	429, 147 amu
<sup>13</sup> C-D4	6.04 min.	285 amu	268, 252 amu
<sup>13</sup> C-D5	11.94 min.	360 amu	270, 253 amu
<sup>13</sup> C-D6	16.75 min.	345 amu	435, 150 amu

## Figures

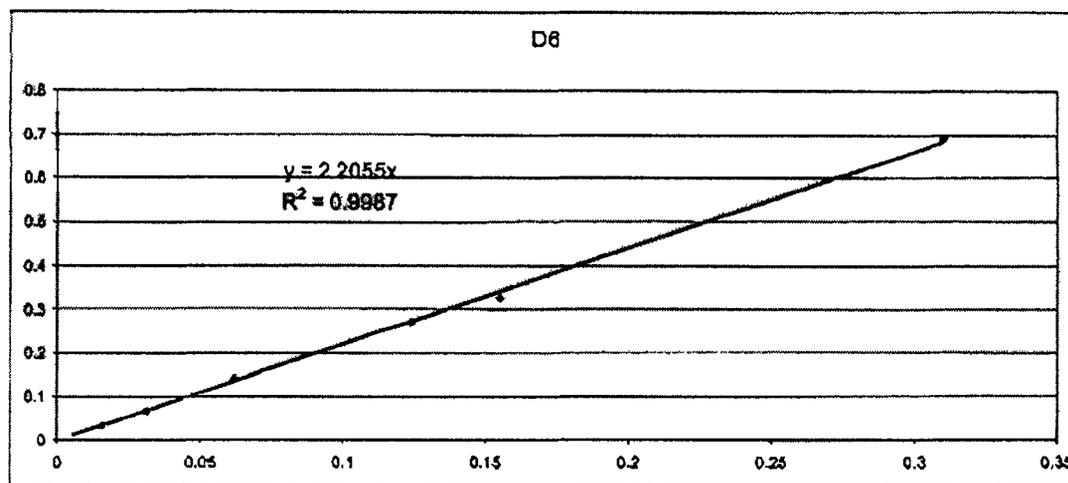
Figures 1. Pentane Standard Curves from analysis for cVMS



$$\text{Area Ratio } D_4 / {}^{13}D_4 = 1.6414 * \text{Amount Ratio } D_4 / {}^{13}D_4$$



$$\text{Area Ratio } D_5 / {}^{13}D_5 = 1.522 * \text{Amount Ratio } D_5 / {}^{13}D_5$$



$$\text{Area Ratio D}_8 / ^{13}\text{D}_8 = 2.2055 * \text{Amount Ratio D}_8 / ^{13}\text{D}_8$$

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Study Title: Cyclic volatile methylsiloxane materials (D3, D4, D5, and D6) in livers of Atlantic cod (*Gadus morhua*) from Oslofjord, Norway. Comparison and assessment of analytical methods utilized by Dow Corning Corporation, Evonik Goldschmidt, and the Norwegian Institute for Air Research.

HES Study No: 10922-108

Test Substances: Hexamethylcyclotrisiloxane (D<sub>3</sub>; CAS No. 541-05-9)  
Octamethylcyclotetrasiloxane (D<sub>4</sub>; CAS No. 556-67-2)  
Decamethylcyclopentasiloxane (D<sub>5</sub>; CAS No. 541-02-6)  
Dodecamethylcyclohexasiloxane (D<sub>6</sub>; CAS No. 540-97-6)

Author: David E. Powell, Dow Corning Corporation

Contributing Scientists: Jeremy Durham, Dow Corning Corporation  
Darren Huff, Dow Corning Corporation  
Thomas Böhmer, Evonik Goldschmidt GmbH  
Reinhard Gerhards, Evonik Goldschmidt GmbH  
Martin Koerner, Evonik Goldschmidt GmbH  
Regina Unthan, Evonik Goldschmidt GmbH  
Henriette Leknes, Norwegian Institute for Air Research  
Martin Schlabach, Norwegian Institute for Air Research  
Norman Green, Norwegian Institute for Water Research  
Merete Schøyen, Norwegian Institute for Water Research

Sponsor: Centre Européen des Silicones (CES)

Testing Facility: Health and Environmental Sciences  
Dow Corning Corporation  
2200 West Salzburg Road  
Auburn, MI 48611

HES Group Manager: Roy A. Campbell

Date: 13 July 2009

## Abstract

An inter-laboratory comparison of processing and analytical procedures for analysis of cyclic volatile methylsiloxane (cVMS) materials in fish liver was performed across three separate laboratories: Norwegian Institute for Air Research (NILU), Evonik Goldschmidt GmbH, and Dow Corning Corporation. Whole Atlantic cod (*Gadus morhua*) used for the inter-laboratory comparison were collected from Inner Oslofjord, Norway by the Norwegian Institute for Water Research (NIVA). Each Laboratory received five or six whole frozen fish, which were processed according to each laboratory's protocol. Each laboratory was responsible for harvesting livers that were free of adipose and mesenteric tissue from their assigned fish, homogenizing the liver samples, and for providing samples of the homogenized livers to the other two laboratories for analysis. Livers from individual fish were harvested and processed following laboratory-specific protocols. Similarly, each laboratory analyzed the homogenized liver samples following laboratory-specific protocols for hexamethylcyclotrisiloxane (D<sub>3</sub>), octamethylcyclotetrasiloxane (D<sub>4</sub>), decamethylcyclopentasiloxane (D<sub>5</sub>) and dodecamethylcyclohexasiloxane (D<sub>6</sub>) by GC-MS. No attempt was made to standardize sample collection, sample processing, or sample analysis across the three laboratories. Quality control procedures, sample processing, analytical methods, and measured concentrations of the cVMS materials were compared for consistency across the three laboratories. Methods of processing, extraction, and analysis were variable across the three laboratories, which was attributed to the lack of standard procedures. Although concentrations of the cVMS materials measured in the cod livers were similar, there were statistically significant differences that were not related to fish characteristics or to processing of the fish. Based on these differences recommendations are provided for collection, processing, and analysis of samples for cVMS materials.

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## Signatures and Approval

This report consists of 47 pages with 3 Appendices of 41 pages for a total of 88 pages. The undersigned have read and approved this report.

Author: David E. Powell  
David E. Powell, Ph.D.  
Chemistry and Environmental Sciences  
Dow Corning Corporation

13-Jul-2009  
Date

Management: Roy A. Campbell  
Roy A. Campbell, B.S.  
Chemistry and Environmental Sciences  
Dow Corning Corporation

13-July-2009  
Date

## Introduction

Cyclic volatile methylsiloxane (cVMS) materials, specifically octamethylcyclotetrasiloxane (D<sub>4</sub>; CAS No. 556-67-2), decamethylcyclopentasiloxane (D<sub>5</sub>; CAS No. 541-02-6), and dodecamethylcyclohexasiloxane (D<sub>6</sub>; CAS No. 540-97-6), are widely used in industrial, personal care, and household applications (Hori and Kannan 2008), and the wastewater stream represents a major post-use disposal route. Generally, cVMS materials have relatively low molecular weight (297 to 445 amu), are volatile (vapor pressure 4.7 to 132 Pa at 25°C), have very low water solubility (5 to 56 µg/L), and are very lipophilic (Log K<sub>ow</sub> 6.5 to 9.1). These properties are a consequence of the weak dispersion interactions in the neat liquid due to the low polarizability of the methyl siloxane materials, as reflected by their low molar refractivity, and their relatively large molecular size (Himer et al. 2003; Ahmed et al. 2007). Because of these properties, cVMS materials occupy a somewhat unique chemical space, which makes analysis of these materials in environmental matrices challenging. In addition, as a result of the widespread use of the cVMS materials it may be difficult to collect, process, and analyze environmental matrices that are free of contamination.

Relatively little data is currently available on the behavior of cVMS materials in the environment (reviewed by Brooke et al. 2009a;b;c). Cyclic volatile methylsiloxane materials have been measured in wastewater effluents (reviewed by Himer et al. 2003; Brooke et al. 2009a,b,c). A Nordic survey (Kaj et al. 2005) found concentrations of cVMS materials ranging from < 0.1-6 µg/L in wastewater effluents with D<sub>5</sub> predominating. However, the cVMS materials were not detected in urban or background surface waters. Urban sediments in Scandinavia had concentrations of cVMS materials ranging from <1 to 2200 ng/g dry weight (dw) while they were not detected at background sites. Schlabach et al. (2007) measured D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub> in wastewater effluent, surface water, sediment and biota from Inner Oslofjord. Concentrations of cVMS materials ranged from < 0.02-12 µg/L in wastewater and from < 4-920 ng/g dw in sediment, with D<sub>5</sub> predominating, but were not detected in surface waters. Concentrations of cVMS materials in biota were 1.3-8.7 ng/g wet weight (ww) in mussels, 0.9-27 ng/g ww in livers of flounder, 70-2200 ng/g ww in livers of cod. Kaj et al. (2004, 2005) analyzed fish, seabird eggs, and marine mammals from Norway and Iceland for cVMS materials, finding D<sub>4</sub>, D<sub>5</sub> and D<sub>6</sub> in livers of fish (flounder, sculpin, dab) from urban areas and from several "background" marine locations. The cVMS materials were also detected in freshwater fish (pike, vendace) from Finland but not in fish from background sites (arctic char and brown trout, Faroe Islands). Cyclic volatile methylsiloxane materials were not detected in seabird eggs (fulmar, herring gull). However, cVMS materials were reported at low ng/g concentrations in blubber of harbor seals from Denmark and pilot whale from the Faroe Islands. Knudsen et al. (2007) measured D<sub>5</sub> residues ranging from 32.2-68.8 ng/g (wet weight; ww) in livers of dead or dying glaucous gulls from Bear Island, located midway between the mainland of Norway and the southern tip of Svalbard Archipelago. Kierkegaard et al. (2008) measured D<sub>5</sub> residues averaging 10-12 ng/g ww in Arctic char taken from Lake Vattern, a large lake in Sweden receiving both industrial and wastewater discharge, while residues in a remote Swedish lake were detectable but below the limit of quantitation of 1 ng/g ww. A

Nordic survey (Evenset et al. 2009) on Svalbard Archipelago measured cVMS residues in livers of Arctic fish (polar cod and Atlantic cod) ranging from below detection (< 4.3 ng/g ww) to 10.4 ng/g ww for D3, from 2.6 to 9.2 ng/g ww for D4, from 2.7 to 19.1 ng/g ww for D5, and from below detection (< 9.7 ng/g ww) to 10.7 ng/g ww for D6. Concentrations in whole-body polar cod ranged from 3.6-9.9 ng/g ww for D3, from 3.6 to 7.8 ng/g ww for D4, from 2.2 to 5.1 ng/g ww for D5, and from 2.2 to 3.8 ng/g ww for D6. Low levels of the cVMS materials were measured in livers of sea birds (kittiwake and eider) that ranged from below detection (< 3.1 ng/g ww) to 3.8 ng/g ww, but were also detected in the field blanks suggesting that samples may have been contaminated during collection and processing. While these limited measurements appear to suggest that cVMS materials may accumulate in top predator fish and marine mammals and birds, it is unclear whether these results represent actual food web biomagnification or are simply a result of continuous exposure and rapid elimination or contamination of samples during collection, processing and analysis.

The objective of the project described in this report was to compare and contrast analytical results and laboratory quality control measures for analysis of hexamethylcyclotrisiloxane (D<sub>3</sub>; CAS No. 541-05-9), D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub> in livers of Atlantic cod (*Gadus morhua*) collected from Inner Oslofjord, Norway. This report does not evaluate field quality control measures or potential contamination of samples that may have occurred during collection. Field contamination of the samples was considered unlikely because only livers of the codfish were analyzed and the fish were kept intact until the livers were collected in the laboratory. The laboratories selected for this inter-laboratory comparison were actively involved with analysis of cVMS materials in environmental matrices and included Dow Corning Corporation (DCC, located in Auburn, Michigan, USA), Evonik Goldschmidt GmbH (Evonik, located in Essen, Germany) and the Norwegian Institute for Air Research (NILU, located in Kjeller, Norway).

## **Materials and Methods**

### **Sample Collection**

A total of 17 Atlantic cod (*Gadus morhua*) were used for this project (Table 1) and were collected by trawl from Inner Oslofjord on 10 December 2007 (Fig. 1). Cod were collected from aboard the *F/F Trygve Braarud* by the Norwegian Institute of Water Research (NIVA), measured for total length (cm) and fresh weight (g) in the field, and individually frozen in plastic bags for distribution to the three analytical laboratories for processing and analysis.

Before being distributed to the laboratories for processing the 17 cod were first separated into two size classes to control for possible confounding effects of size and age on concentrations of cVMS. The age-growth relationship for Atlantic cod is dependant upon location and water temperature. Age and growth relationships reported for Atlantic cod (Brander 1995) collected from the North Sea (T=8.6°C) and off the West Coast of Scotland (T=10°C) suggested that Oslofjord cod less than 1000 g

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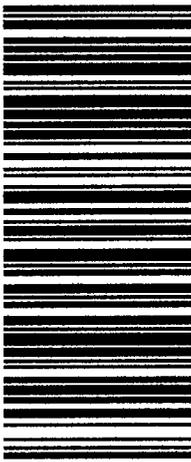
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