IRMM HCB and HCBD in Blankvoorn (testmaterial for reference material)

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Summary

Blankvoorn Nieuwe Merwede (Roach, *Rutilus rultilus*) has been tested as a reference material for the components HCBD and HCB for biota monitoring according to the Water Framework Directive.
Both components in the blankvoorn are present at moderate levels (overall average levels of respectively HCBD 1.6 µg/kg and HCB 6.2 µg/kg). These concentrations are far above reporting limits (respectively 0.1 µg/kg for HCBD and 0.2 µg/kg for HCB) and should therefore be suitable for use in a reference material.

1 Introduction

The Institute for Reference Materials and Measurements (IRMM) started a new project in order to produce reference material for biota monitoring according to the Water Framework Directive.
The matrix of the reference material will be fish tissue including the whole edible part. The analytes of interest are hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD).
Based on the measured concentration of HCB and HCBD in eel from De Nieuwe Merwede (respectively 42 and 9.2 µg/kg), blankvoorn from the same location could probably be a good candidate for preparing the desired reference material. IRMM asked IMARES to analyze HCB, HCBD and fat in blankvoorn from De Nieuwe Merwede to see if the levels of both components are appropriate for using blankvoorn from this location as a reference material.

2 Materials and Methods

On 21/07/2010 a professional fisherman catched a few blankvoorns from the Nieuwe Merwede. IMARES took two of the blankvoorns, fish A and fish B for analyses in the edible part of the fish tissue. Each fish was analyzed three times as an individual sample for the contaminants HCB and HCBD and fat content was only measured once in the edible part of the two fishes.

The method of analysis used by IMARES for HCB and HCBD:
Sample mixed with sodium sulphate (for removing moisture) is Soxhlet extracted for 6.5 hours with pentane/dichloromethane (1:1). After addition of 5 ml of iso-octane as a keeper, the extract is concentrated to about 5 ml in a rotary evaporator. Further clean-up and fractionation is then performed by putting the extract on top of a 15 g alumina · 9% H_2O column and elution with 170 ml n-pentane. After the addition of 2 ml of iso-octane as a keeper, the eluate is concentrated to about 2 ml in a rotary evaporator. The concentrate is transferred to a silica column (1.6 g silica · 2% H_2O) and eluted with 11 ml of iso-octane. Test determination by GC-ECD is then performed to estimate concentration of the analytes in the eluate. If values are found to be out of the calibration curve, dilution or concentration of the eluate is performed. Final determination is then performed by gas chromatography (GC) coupled to electron capture detector (ECD) using two column system: Cp-Sil 8 (50 m long with internal diameter of 0.15 mm and film thickness of 0.20 µm) and Cp-Sil 19 (50 m long with internal diameter of 0.15 mm and film thickness of 0.20 µm).

The analyte HCB in fish tissue is accredited by the Council for Accreditation.
IMARES is registered as a Reference Laboratory at The Europese Commission for Reference Materials and Measurements (IRMM) for the determination on PCB's.

The method of analysis used by IMARES for fat:
The analysis of free extractable fat is performed within the analysis of HCB and HCBD. After Soxhlet extraction a part of the extract is evaporated until dry and the residue is weighted.
The analysis of total fat is performed according to an adapted version of the Bligh and Dyer method, based on a cold chloroform-methanol extraction.
The method of Bligh en Dyer is accredited by the Council for Accreditation.

3 Results and discussion

The results of the analyses are given in appendix 1.
In appendix 1 A the results of the requested analyses in the blankvoorns, fish A and B, are given both on product base and on fat base.
In appendix 1 B results of eel from the same location measured in 2005 are shown also on product base and on fat base. The results for the components HCBD (9.2 µg/kg on product base) and HCB (42 µg/kg on product

base) in eel Nieuwe Merwede were the highest levels we could find in our historical (2003-2008) results of all kinds of fishes we analyzed for both components. Based on those concentrations in eel it was assumed that blankvoorn of the same location could probably be an appropriate fish to use as a reference material.

It appears that the results on fat base for HCBD and HCB in blankvoorn Nieuwe Merwede (2010) have the same level as the results on fat base of eel from the Nieuwe Merwede (2005) for both components. Thus, the level of contamination for both kinds of fishes (blankvoorn and eel) is comparable for HCBD and HCB accumulated in the fat of both fishes.

On a product base however we find average levels of HCBD of 1.8 µg/kg in fish A (rsd=8.6 %) and 1.4 µg/kg in fish B (rsd=4.2 %), with an overall average of 1.6 µg/kg HCBD. For the component HCB the level on a product base is 7.6 µg/kg in fish A (rsd=0.8 %) and 4.8 µg/kg in fish B (rsd=8.5 %), with an overall average of 6.2 µg/kg HCB. Thus, on a product base the overall levels of HCBD and HCB are about a factor 6 lower in the blankvoorn than in the eel. Still the concentrations in blankvoorn Nieuwe Merwede are at such levels that are well measurable with GC-ECD, the reporting limits of both components are respectively 0.1 µg/kg for HCBD and 0.2 µg/kg for HCB. We therefore consider the levels of both components in blankvoorn Nieuwe Merwede appropriate for use as a reference material.

Next to that it will be very difficult to obtain another fish species naturally contaminated with higher concentrations for both components on a product base. Eel Nieuwe Merwede could be a good alternative, but it wouldn't be a proper decision to use eel for that purpose now, because it is an endangered animal species at the moment.

4 Conclusion

Blankvoorn Nieuwe Merwede could be used as a reference material for the components HCBD and HCB for biota monitoring according to the Water Framework Directive, although the levels of contamination are moderate for both components.

5 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 December 2012. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V.
Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.
IMARES has a focus at constant improvement in quality; a substantial amount of analyses are accredited by the Council for Accreditation. The accuracy of our methods is tested by attendance to Performance Laboratory Studies (Quasimeme).
For every sequence our results are tested on internal or certified reference materials (IRM/CRM).
IMARES has a prominent role in Quasimeme preparing reference materials for certifying purposes and organizing international interlaboratory tests for contaminants in environmental samples.

Justification

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Project Number: 4305106501

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved:	Dr. Ir. M.J.J. Kotterman Projectleader
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Date:	13 september 2010

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