IAEA-SM-354/33

FRESH BIOLOGICAL REFERENCE MATERIALS — USE IN INTER LABORATORY STUDIES AND AS CRMs

DE BOER, J.,
Dlo-Netherlands Institute For Fisheries Research
P.O. Box 68
197 Ab Ijmuiden
The Netherlands

Abstract

Biological reference materials were prepared and packed in tins and glass jars to be used in inter laboratory studies on chlorobiphenyls and organochlorine pesticides, and trace metals, respectively. The materials were homogenised, sterilised and packed as wet tissue, which is unique for the purpose of inter laboratory studies and offers the advantage of studying the extraction and destruction steps of the analytical methods. In addition to their use in inter laboratory studies, some materials have been prepared or are being prepared as certified reference material for chlorobiphenyl analysis.

1. INTRODUCTION

During the European project QUASIMEME (Quality Assurance of Information on Marine Environmental Monitoring in Europe) (Standards, Measurements and Testing Programme, SMT) from 1993-1996 five inter laboratory studies were organised both for trace organics (chlorobiphenyls (CBs) and organochlorine pesticides (OCPs)) and trace metals [1-3]. During the 3-years programme eleven materials were prepared, six for use in trace metal inter laboratory studies - three cod (*Gadus morhua*) livers, cod (*Gadus morhua*) muscle tissue, plaice (*Pleuronectes platessa*) muscle tissue and mussels (*Mytilus edulis*), and five materials for use in CB/OCP inter laboratory studies - two cod (*Gadus morhua*) livers, mackerel (*Scomber scombrus*) muscle tissue, plaice (*Pleuronectes platessa*) muscle tissue and mussels (*Mytilus edulis*). After 1996, when the QUASIMEME programme continues on a subscription basis, more materials were made.

The use of wet fish or shellfish tissue for inter laboratory studies on trace metal analysis was not known until now. The use of wet cod muscle tissue in an inter laboratory study on CBs was only reported once [4]. The obvious advantage of the use of wet fish or shellfish tissues in inter laboratory studies is that the participating laboratories are given the opportunity to demonstrate their ability of analysing CBs, OCPs and trace metals in a matrix which very closely resembles the matrices which have to be analysed in their routine monitoring work.

Until now for CBs and OCPs fish oils or fish fat were commonly used in inter laboratory studies [5-8]. Possible errors made in the extraction could obviously not be studies then. For trace metals freeze-dried or spray-dried materials have been used before [9-10]. These materials could show a different behaviour during destruction and their stability before or longer periods was not always guaranteed.

Because the stability of the wet materials made over a period of more than three years was good, a shellfish materials (mussels - *Mytilus edulis*) was prepared as a candidate certified reference material (CRM) for CB analysis.

2. MATERIALS AND METHODS

2.1 Preparation and packing of CB/OCP reference materials

All CB/OCP reference materials were thoroughly homogenised in a Stephan cutter. Attention was paid to avoid contact with any plastic materials to prevent contamination with plasticizers. The reference materials for CBs and OCPs were packed in tins. Two types of tins have been used. The mussels were packed in tins of ca. 80 g, height 55 mm and diameter 52 mm (Thomassen and Drijver, Deventer, The Netherlands). These tins, used for food preservation, are coated inside to prevent formation of rust. With regard to this rust formation the use of uncoated tins was considered too risky for the high-salinity mussel sample. Because the basic idea of using tins for the reference materials for CB and OCP determination was based upon avoiding all synthetic materials because of possible interferences during the analysis, these coated tins were subject to a blank test to check if any interferences could be transferred from the coating to the matrix. Two tests were carried out: 1) Extraction of the tin with 80 ml n-pentane, concentration of the n-pentane extract (20 to 1) and comparison with a blank of concentrated n-pentane that had not been in contact with a coated tin. This experiment was carried out to mimic the storage of a fatty tissue in a coated tin. 2) Extraction of the tin with 80 ml distilled water containing 30 g/L NaCl; extraction of the water with n-pentane and concentration of the pentane (20 to 1); comparison of this concentrated pentane extract with a blank concentrated pentane extract of distilled water. This experiment was carried out to mimic the storage of a watery matrix in a coated tin.

Fig. 1 shows that the first experiment caused a number of negative peaks in the GC/ECD chromatogram. This means that fatty tissue, particularly cod liver, will possibly extract interferences from the coating of the tin that may considerably disturb the GC/ECD chromatogram.

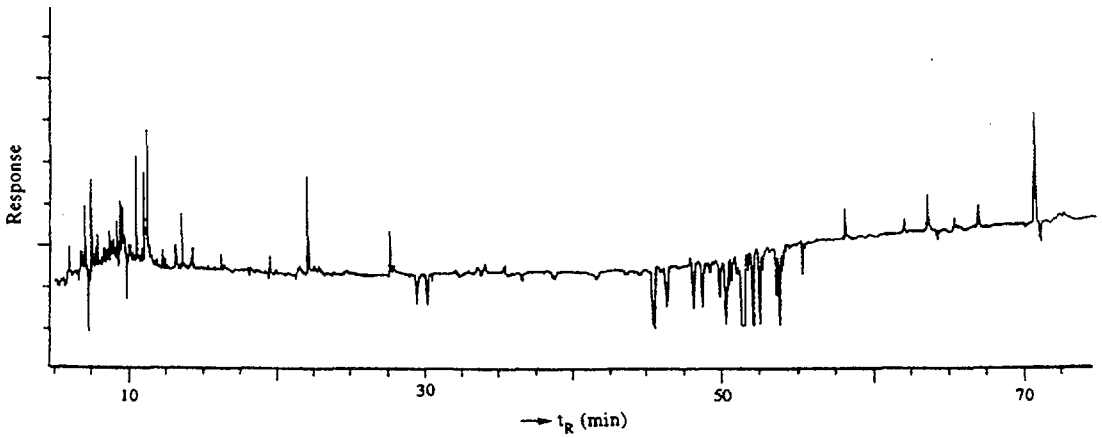

FIG. 1. Chromatogram of blank analysis of coated tin on a CP Sil 19CB column (exp.1) (Temperature programme: 3 min 90°C, 40°C/min to 215°C, 5°C/min to 270°C).

No significant differences between the test chromatogram and that of the blank were found in experiment 2. Considering the risk of rust formation when storing mussels in uncoated tins, these coated tins were selected for the storage of the mussel sample.

Uncoated tins are not used in the food industry. Therefore, they are not regularly available. A batch of uncoated tins was especially produced for this project by Carnaud Metal Box in France and delivered through Eurocan Food, Mechelen, Belgium. These tins were extracted with 80 ml n-pentane which was concentrated (20 to 1) and compared with a blank of concentrated pentane. This experiment was carried out in triplicate. No extra positive or negative peaks were present in the test extract in comparison with the blank pentane. These tins were consequently used for cod liver, mackerel and plaice.

In later experiments it appeared that the interferences shown in Fig. 1 do not pass conventional clean-up methods for CBs and OCPs such as alumina and silica columns. The materials packed in the uncoated tins showed a blackish layer inside after opening the after a few months or more. Although an effect of this blackish layer on the results of the inter laboratory studies was not seen, given the trapping of the interferences from the coatings during clean-up, it was considered to be safe to use coated tins also for more fatty samples.

The tins were filled to the brim (ca. 70 g) with the cold materials, directly from the Stephan cutter. Only cod liver was firstly transferred from the Stephan cutter to a round bottomed flask provided with a tap at the bottom and a stirring device. The cod liver was tapped from the flask during stirring, this to prevent separation of liquid and solid particles of the cod liver.

All tins were sealed by a Lanico TVM335 sealing machine (Thomassen and Drijver, Deventer, The Netherlands).

2.2 Separation and packing of trace metal reference materials

All trace metal reference materials were homogenised in a Stephan cutter. Initially some materials were packed in a plastic foil, a sterilisable laminate Steriflex PA, PE 30/100 (Sengewald, Germany). However, in spite of guarantees to be waterproof and fat resistant, a small sweating effect was found for lean materials stored at 37°C (ca. 1% decrease of moisture content after six months). In addition the lipid-rich (50%) cod liver caused leakages of the foils. The use of small glass jars, used in the food industry for packing caviar, appeared to be an acceptable alternative. These jars were equipped with twist-off lids. The inner part of these lids, which could come in contact with the reference materials, was provided with an epoxyphenol/eopxyphenyl lacquer, with a zinc carbonate content of less than 0.5 mg/kg. Although the producer guaranteed that the percentage of trace metals other than zinc in this lacquer was negligible, the jars were filled with distilled water, closed with the lid, shaken for 2 h and equilibrated for one day to test the possible presence of some trace metals. The contents of Hg, Cu and Zn were determined in the water. The contribution of the trace metal contamination to the concentration of the metals in the biological samples was less than 0.2% (Zn) which was considered acceptable.

The cod livers were, similarly to the CB/OCP cod liver materials, tapped from a round bottomed flask during stirring, while the other materials were taken directly from the cutter. Butylhydroxytoluene (0.02% w/w) was added to the cod liver as an antioxidant.

2.3 Sterilisation

The materials were sterilised in a 80 L Muvero-mat steriliser. The temperature was kept at 120°C for 30 minutes. The pressure was 2 bar. The total cooling time was 30 minutes. Because of the large quantity of samples, the temperature between the tins or plastic packages decreased somewhat slower than the water temperature. The effect of the slightly larger cooling time must, however, be regarded negligible. No irregularities were observed during the sterilisation process. A minimum stability of three years under moderate conditions is guaranteed for each of the materials.

2.4 Homogeneity tests

The homogeneity of the samples was initially tested by sodium and potassium determinations together with total lipid determinations according to Bligh and Dyer [11]. Later these determinations were replaced by determinations of some of the target compounds: CBs 118 and 153 for organic materials and zinc for trace metal materials. Each time 15 between-batch tests were carried out and 5 within-batch tests. The total number of lots in the batches was 200 for the tins and 300 for the glass jars.

F teste were carried out to identify a possible difference between the between- and within-batch test results. The contribution of inhomogeneity of the samples to the final result of inter laboratory studies was negligible. However, because during sterilisation some water or liquid lipids

can separate from the bulk of the sample during sterilisation, the users of the materials are advised to rehomogenise the samples after opening and before taking the sub-sample for analysis.

2.5 Stability

An example of the stability of a CB material is given in Table 1. Given the persistent nature of CBs instability is unexpected and was not found indeed. Also for trace metals a good stability was found over a period of more than three years.

TABLE I. STABILITY: CBS IN MUSSELS 3 YEARS AFTER PREPARATION (CONCENTRATIONS IN µg/kg WET WEIGHT)

CB	t=0	Assigned value[1]	t=3 yr.
28	0.18	0.22	0.18
52	0.46	0.49	0.43
101	1.2	1.5	1.5
118	0.92	1.1	1.1
153	3.2	3.5	3.4
§38	1.8	2.2	2.2
180	0.38	0.39	0.41

[1] Assigned value from inter laboratory study in which the material was used.

3. RESULTS AND DISCUSSION

The fresh biological materials produced are suitable for use in inter laboratory studies on CBs and trace metals. The materials cover a wide range of concentrations (CBs: 0.1-400 µg/kg wet weight, metals: 0.02-40 mg/kg wet weight). The tins used are a suitable packing material for the CB/OCP materials. They offer a quantity of 70 g material to the participant of inter laboratory studies, which enables a sufficiently high sample intake in the case of lean samples. No disadvantages such as chromatographic interferences from the coating of the tins were observed during use. Fresh fish and shellfish samples to be used for trace metal analysis were successfully packed in glass jars.
It was possible to produce these samples with a very good degree of homogeneity. The contribution to the variance of the final results of an inter comparison exercise due to the inhomogeneity of most samples is less than 1%.

Because of the good homogeneity and stability of the materials made for inter laboratory studies, and because of the need for good quality CRMs, another European project was started under the SMT programme in which a candidate CRM, consisting of several thousands of tins with fresh, homogenised and sterilised mussels (*Mytilus edulis*), was prepared to be used for CB analysis. This material is currently being certified by fourteen expert laboratories.

Two other candidate CRMs will be prepared under the SMT programme in 1999: herring (*Clupea harengus*) for CBs and chub (*Squalius cephalus*) for non-ortho CBs.

References

[1] WELLS, D.E., AMINOT, A., DE BOER, J., COFINO, W, KIRKWOOD, D., PEDERSEN, B., A review of the achievements of the EU Project 'QUASIMEME' 1993-1996. Mar. Pollut. Bull. **35** (1997) 3-17.

[2] DE BOER, J., WELLS, D.E., Chlorobiphenyls and organochlorine pesticides in fish and sediments - three years of QUASIMEME laboratory performance studies. Mar. Pollut. Bull. **35** (1997) 52-63.

[3] PEDERSEN, B, COFINO, W., DAVIES, I. The 1993-1995 QUASIMEME laboratory performance study: trace metals in sediments and biota. Mar. Pollut. Bull. **35** (1997) 42-51.

[4] DE BOER, J., VAN DER MEER, J., BRINKMAN, U.A.Th., Determination of chlorobiphenyls in seal blubber, marine sediment, and fish: inter laboratory study. J. Assoc. Off. Anal. Chem. **79** (1996) 83-96.
[5] MUSIAL, C.J., UTHE, J.F., Interlaboratory calibration results of polychlorinated biphenyl analyses in herring. J. Assoc. Off. Anal. Chem. **66** (1983) 22-31.
[6] TUINSTRA, L.G.M.Th., ROOS, A.H., WERDMULLER, G.A. Capillary gas chromatograpic determination of some chlorobiphenyls in eel fat: inter laboratory study. J. Assoc. Off. Anal. Chem. **68** (1985) 756-759.
[7] UTHE, J.F., MUSIAL, C.J., MISRA, R.K. Multi-laboratory study of measurement of chlorobiphenyls and other organochlorines in fish oil. J. Assoc. Off. Anal. Chem. **71** (1988) 369-372.
[8] WELLS, D.E. DE BOER, J. TUINSTRA, L.G.M.Th., REUTERGÅRDH, L. GRIEPINK, B. Improvement in the analysis of chlorobiphenyls prior to the certification of seven CBs in two fish oils. Fresenius Z. Anal. Chem. **332** (1988) 591-597.
[9] TOPPING, G. Report on the sixth ICES trace metal intercomparison exercise for cadmium and lead in biological tissue. ICES Cooperative Research Report No. 111, Intern. Council for the Exploration of the Sea, Copenhagen, Denmark (1982).
[10] BERMAN, S., BOYKO, V.J. ICES seventh round intercalibration for trace metals in biological tissue - ICES 7/TM/BT/BT (Part 2). ICES Cooperative Research Report No. 289, Intern. Council for the Exploration of the Sea, Copenhagen, Denmark (1992).
[11] BLIGH, E.G., DYER, W.J. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37** (1959) 911-917.