National Research Council Canada
Conseil national de recherches Canada

Certified Reference Material

CARP-2

Ground Whole Carp Reference Material for Organochlorine Compounds

This reference material replaces CARP-1, supplies of which have been exhausted. Each unit of CARP-2 contains six individually sealed ampoules.

This reference material is primarily intended for use in the calibration of procedures and the development of methods used for the determination of PCB's, PCDD's, PCDF's and pesticides in biological materials.

Preparation of CARP-2

The material was prepared from ground whole carp (*Cyprinus carpio*), harvested in March 1994 near the warm water discharge of the Consumer's Power Plant, Saginaw Bay, Lake Huron. It was frozen to -30°C and shipped to the Canadian Institute for Fisheries Technology for further processing. It was comminuted four times, an antioxidant (ethoxyquin powder) was added followed by distilled water to increase the moisture content to 85%. The slurry was passed four times through a high pressure homogenizer and aliquoted in 10 g quantities into nitrogen flushed glass ampoules and sealed. The vials were thermally sterilized in a steam retort at 118°C for 11 minutes.

Except for the addition of some water and an antioxidant, CARP-2 is a natural biological material.

A lipid content of approximately 7% was determined by pressurized fluid extraction (PFE).

Storage

It is recommended that the reference material be stored in a cool, clean location. The ampoules should only be opened immediately prior to use. This material has been stored at 22°C since packaging.

Handling of the Material Prior to Extraction

The following procedure is recommended for weighing and transferring CARP-2 quantitatively for extraction. Weigh the ampoule. Sonicate it in an ultrasonic bath for fifteen minutes. Break the seal at the neck and transfer as much material as possible out of the ampoule. Wash the inside of the ampoule and the top with a small amount of the extraction solvent and transfer the wash quantitatively. Weigh the empty ampoule, the top and any glass slivers. Calculate the CARP-2 slurry weight by difference. Use the whole ampoule contents for analysis.

NRC · CNRC

Certified Concentrations for Selected Polychlorinated biphenyl (PCB) Congeners in CARP-2.

Congener (IUPAC No.)	µg/kg, (wet weight basis)		
18	27.3	±	4.0
28	34.0	±	7.2
44	86.6	±	25.9
52	138	±	43
118	148	±	33
128	20.4	±	4.4
153	105	±	22
180	53.3	±	13.0
194	10.9	±	3.1
206	4.4	±	1.1

The certified results are expressed as the mean value ± the expanded uncertainty. These results were derived from four contributions: (1) the NRC analysis, (2) the mean of three methods performed at NIST, (3) the recommended value arising from the NIST/NOAA intercomparison [3], (4) the mean from three results performed by an external laboratory.

Reference Concentrations for Selected Polychlorinated biphenyl (PCB) Congeners and Pesticides in CARP-2.

These concentrations are provided as reference values because (1) the variance from multiple methods was greater than desired for certified values or (2) in the case of PCBs, limited data were available concerning the relative amounts of co-eluting congeners.

Congener (IUPAC No.)	µg/kg, (wet weight basis)		
8	4.8	±	1.8
66/95	174	±	52
101/90	145	±	48
105	53.2	±	15.6
138/163/164	103	±	30
170/190	20.6	±	2.9
187/182	37.1	±	6.3
209	4.6	±	2.0

Pesticides	µg/kg (wet weight basis)		
gamma-chlordane	4.5	±	0.7
2,4'-DDE	2.9	±	0.5
trans-nonachlor	11.0	±	0.9
dieldrin	8.3	±	0.8
4,4'-DDE	158	±	14
2,4'-DDD	21.8	±	0.7
4,4'-DDD	90.9	±	8.5

The results are expressed as the mean value ± the expanded uncertainty. The PCB reference results were derived from four contributions: (1) the NRC analysis, (2) the mean of three methods performed at NIST, (3) the recommended value arising from the NIST/NOAA intercomparison[3] and (4) the mean of three results performed by an external laboratory. The pesticide reference results were derived from process 1,2 and 3 only.

Reference Concentrations for Selected PCDF's and PCDD's in CARP-2.

The following table lists the polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners for which reference values have been established for CARP-2. These concentrations are provided as reference values because limited data were available to permit certification.

Congener	ng/kg, (wet weight basis)
Polychlorinated dibenzofuran (PCDF)	
2,3,7,8-Tetrachlorodibenzofuran	18.2 ± 1.6
1,2,3,7,8-Pentachlorodibenzofuran	5.6 ± 0.3
Polychlorinated dibenzo-*p*-dioxin (PCDD)	
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	7.4 ± 0.7
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	5.3 ± 1.3
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	1.6 ± 0.3
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	5.8 ± 0.8
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	0.78 ± 0.12
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	6.4 ± 0.9
Octachlorodibenzo-*p*-dioxin	9.4 ± 1.7

The results are expressed as the mean value ± the expanded uncertainty. The reference result is the mean of five independent analyses performed at NRC.

The results used to calculate both certified and reference values in CARP-2 were judged to be independent measurements. The uncertainty in these values is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the ISO Guide [2] and k is the coverage factor. The value of u_c is intended to represent at the level of one standard deviation the combined effect of all the uncertainties in the certified or reference value. Here u_c is given by the standard error of the mean of the analyses. The coverage factor, k , is the Student's t-value for a 95% confidence interval with the appropriate degrees of freedom.

Analytical Methods used at NRC

PCBs: (Method1a, b) Soxhlet extraction using 1:1 acetone/hexane followed by Florisil and alumina column cleanup. Measurement by both GC-ECD and GC/MS using a DB5 fused silica column (30m x 0.25mm id x 0.25µm film).

PCBs and Chlorinated Pesticides: (Method 2) Pressurized fluid extraction using dichloromethane solvent with alumina in the extraction cell. Glass Silica SPE tubes were used for the final cleanup step. Samples were spiked with selected Carbon-13 labelled PCBs and selected Carbon-13 or Deuterium labelled pesticides prior to extraction. Final analysis was by GC/HRMS using an HT8 fused silica column (50m x 0.22mm id x 0.25µm film).

PCDDs and PCDFs: Carp-2 was analysed using five combinations of different extraction/cleanup techniques followed by GC/HRMS. Carp-1 samples were analysed concurrently using the same techniques to validate the methods. The extraction steps consisted of either Soxhlet (1:1 acetone/hexane) [4], shaking with acetonitrile or sonication with HCl. A sulphuric acid shake step was used for bulk lipid removal when required. The final cleanup steps were completed using either in house prepared glass columns (acid-base silica, Florisil, carbon) or commercially available glass SPE tubes (C-18, SCX, SAX, SiOH, Florisil, alumina). In all of these methods, the samples were spiked with a mixture of Carbon-13 labelled PCDDs and PCDFs prior to the extraction step. GC/HRMS was performed at a resolution of 5000 or 10000. A DB5 fused silica column (50m x 0.25mm id x 0.1µm film) was used for the determination of these compounds.

Further details concerning any of these methods are available upon request.

Homogeneity and Stability

CARP-1 was monitored at NRC for a period of twelve years. No signs of inhomogeneity or instability were evident and CARP-2 is expected to be similarly stable with respect to its organochlorine contents.

Certification

The bulk of the analytical and certification work was performed at the Institute for National Measurement Standards, National Research Council of Canada. Results for selected analytes were also used from twenty-one laboratories that participated in an intercomparison exercise coordinated by R. Parris and S. Wise [3] of the National Institute of Standards and Technology , Gaithersburg, MD.

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[2] Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9, 1st ed. ISO, Geneva, Switzerland (1993).

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[4] J.J. Ryan, P.Y. Lau, J.C. Pilon, D. Lewis, H.A. McLeod, and A. Gervais; Incidence and Levels of 2,3,7,8-TCDD in Lake Ontario Commercial Fish., Environ. Sci. Technol., 18, 719-721, 1984.

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It is expected that as more data becomes available, the reference values may be updated and/or certified, and values assigned to additional compounds. These updates will be forwarded to all users of this reference material.

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