# The Application of A Biological CRM ( *CARP-1* ) in the Development of an Extraction and Cleanup Procedure based upon Commercially Available Solid Phase Extraction Cartridges for the Determination of Chlorinated Dioxins and Furans in Biological Tissues

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## 1. Introduction

A recent article [1] mentioned that until very recently, no real-matrix certified reference materials existed. This is especially true for biological tissue reference materials certified for the concentrations of polychlorinated dioxins, furans and PCB's. The National Research Council of Canada has recently introduced for sale ***CARP-1,*** a ground whole carp reference material for organochlorine compounds. The concentration of selected priority PCDD's, PCDF's and PCB's has been determined and certified. A brief history of the preparation of this material will be given in the first portion of this paper.

The determination of nanograms-per-kilogram and picograms-per-kilogram levels of polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) congeners in environmental samples has traditionally been time consuming and labour intensive. Even in the hands of skilled operators, the slow extraction steps and the multi column cleanup steps required to prepare a purified extract suitable for high resolution gas chromatography (HRGC) / high resolution mass spectrometry (HRMS) often means that only a few samples can be processed per week. The actual quantification of these analytes is relatively straight forward especially when auto samplers coupled with automated gas chromatographs and mass spectrometers are used. The rate determining steps in the analysis are the extraction and cleanup stages. The second portion of this paper will illustrate the use of ***CARP-1*** as a sample used in the development of an extraction and clean up procedure for PCDD/F's in biological samples that is less time consuming and tedious than the current conventional method [2]. This procedure utilizes commercially available solid phase extraction cartridges to replace a number of in-house-prepared cleanup columns.

## 2. Preparation of *CARP-1*

The material was prepared from ground whole carp (Cyprinus carpio) harvested near the warm water discharge of the Consumer's Power Plant, Saginaw Bay , Lake Huron on December 2, 1988. The sample was ground up and stored at -20°C. About 30 kg of the sample was made available to us courtesy of the U.S. Fish and Wildlife Service in East Lansing, Michigan. The frozen sample was shipped to the Canadian Institute of Fisheries Technology, Technical University of Nova Scotia where further processing took place.

The sample was first comminuted a total of four times in a food cutter. After the first pass, a finer cutter was installed on the machine. Approximately 16 kg remained after the first pass through the cutter. An antioxidant, 9.5 g of ethoxyquin powder, was added to the sample and then it was processed three more times in the food cutter. Distilled water was added to raise the moisture content from 62 to 85%. The resultant slurry then underwent high pressure emulsification and homogenization. This process was carried out four times and a relatively free flowing material was created. An ampouling machine was used to divide and bottle the material. Each ampoule was filled with approximately 10 ml (about 9 g) of the slurry and then sealed under a nitrogen atmosphere. To stabilize the homogenate, the ampoules were heated in a steam retort at 118°C for 11 minutes which achieved lethalities, $F_0$, between 6.0 and 6.6 minutes. The ampoules were individually heat sealed in trilaminate pouches and packed six to a box. The material is available as ***CARP-1*** from the Institute for Environmental Research and Technology, National Research Council of Canada. Each unit of ***CARP-1*** contains six ampoules. More details concerning the production of ***CARP-1*** can be found in references 3 and 4.

The following table lists the PCDD, PCDF and PCB congeners for which certified values have been established for ***CARP-1***. Certified concentrations are listed on a wet weight basis, the uncertainties are 95% confidence limits for an individual sample (ampoule).

| **Congener** | **Concentration (nanograms/kilogram)** |
|---|---|
| **Polychlorinated dibenzofuran (PCDF)** | |
| 2,3,7,8-Tetrachlorodibenzofuran | 11.9 ± 2.7 |
| 1,2,3,7,8-Pentachlorodibenzofuran | 5.0 ± 2.0 |
| **Polychlorinated dibenzo-p-dioxin (PCDD)** | |
| 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 6.6 ± 0.6 |
| 1,2,3,7,8-Pentachlorodibenzo-p-dioxin | 4.4 ± 1.1 |
| 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin | 1.9 ± 0.7 |
| 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin | 5.6 ± 1.3 |
| 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin | 0.7 ± 0.4 |
| 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin | 6.5 ± 1.8 |
| Octachlorodibenzo-p-dioxin | 6.3 ± 1.9 |

| **Polychlorinated biphenyl (PCB)** | | **Concentration (micrograms/kilogram)** |
|---|---|---|
| **IUPAC No.** | 52 | 124 ± 32 |
| | 101/90 | 124 ± 37 |
| | 105 | 54 ± 24 |
| | 118 | 132 ± 60 |
| | 138/163/164 | 102 ± 23 |
| | 153 | 83 ± 39 |
| | 170/190 | 22 ± 8 |
| | 180 | 46 ± 14 |
| | 187/182 | 36 ± 16 |

Note : These values were determined by statistical analysis of data submitted by several expert laboratories using their own methods of extraction and quantification.

3) Method Development Studies using ***CARP-1***

The data obtained by this laboratory for the certification values for ***CARP-1*** were all obtained using the conventional method[2] developed by Ryan and co-workers at Health and Welfare Canada. Figure 1 is a schematic representation of this method. It can briefly be described as a 7.5 hour Soxhlet extraction using acetone/hexane followed by a sulphuric acid defatting step and several column cleanup steps. While this method has performed very well for us, it suffers from three drawbacks : 1) the long extraction time required (7.5 hours), 2) the requirement to handle concentrated sulphuric acid and 3) the stability of the in-house fabricated sulphuric acid-impregnated silica columns. The method produces reasonably clean solutions for HRGC-HRMS analysis that are free from other chlorinated interferences and yields Carbon-13 recoveries in the 70% or better range. This laboratory still uses this method in an on-going storage/stability test being performed on ***CARP-1*** and it will be used as one of the techniques used in the certification of ***CARP-2*** which is now taking place.

Recent publications[5,6] by Chang and coworkers describe the extraction of PCDD/F congeners from tissue samples using acetonitrile followed by extract cleanup using commercially available, Sep-Pak type cartridges including C-18, benzenesulphonic acid, acidic silica and Florisil. Figure 2 is a schematic diagram of this method. Acetonitrile acts as a selective extractant by removing the analytes of interest while dissolving a much smaller portion of the lipids (about 10%)[5,6] than hexane/acetone (1:2) mixture. The small amount of lipids extracted obviates a time-consuming bulk lipid removal step. Advantages of this type of approach include : 1) greater sample throughput due to decreased sample extraction and cleanup time, 2) minimized use of undesirable solvents or chemicals (no sulphuric acid, no strong base, no ether and only tens of millilitres of chlorinated solvents) and 3) the elimination of in-house column prepartion. It was the goal of our work to use ***CARP-1*** to assess and/or verify the Chang method and to develop a modified version in which the separation chemistry more closely resembles that of the conventional cleanup. The modified version adds an SAX-type cartridge and incorporates an additional Alumina fractionation after the Florisil step. Details of this cleanup method are still under development.

Experimental

All solvents used were distilled-in-glass grade; the commercial cartridges were purchased from Varian Analytical and Waters. PCDD/F congeners (native and C-13-labelled) were purchased from Cambridge Isotopes Laboratories. The homogenizing device used was a Brinkman Polytron and the Visiprep apparatus from Supelco was used for cartridge handling (solvent flows and drying). A VG AutoSpecQ ( EBEqQ geometry) mass spectrometer equipped with an autosampler (CTC A200S) and HP 5890 GC was used in the SIR Voltage mode for all PCDD/F congener measurements. The instrument was normally operated at a resolution of 10,000. A DB5-MS column (60 m X 0.25mm X 0.1 um film) programmed as follows was used for all runs. The initial column temperature of 120°C was held for 2 min., programmed to 180°C at 20°C per min. and then 2.5°C per min. to 282.5°C with a 14 min. hold at the final temperature. The total run time was 60 minutes. 1 uL injections were made in the splitless mode with a valve closed time of 2 minutes. 1 uL of $^{13}C_{12}$-1,2,3,4-TCDD was added to all solutions to correct for instrument variability.

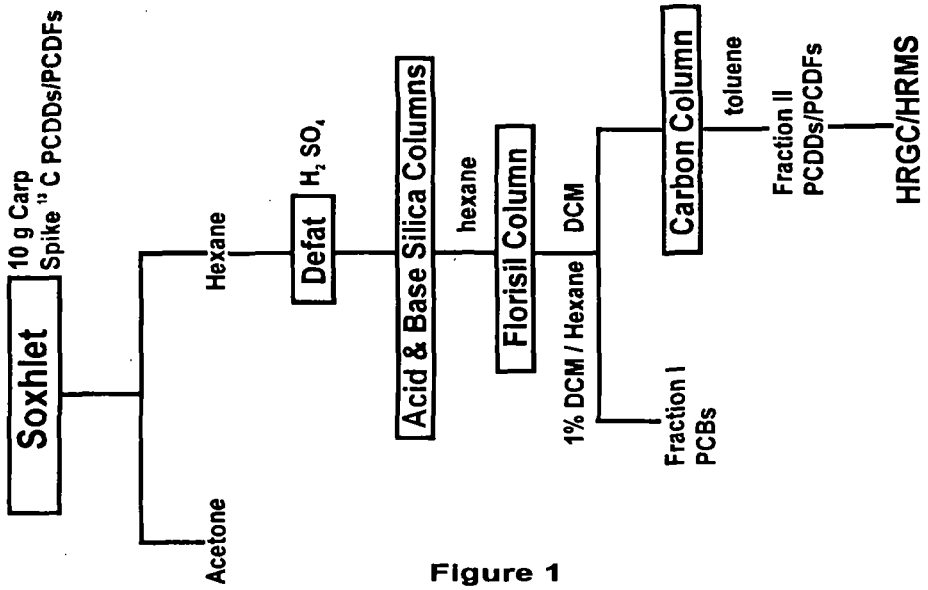


Figure 1

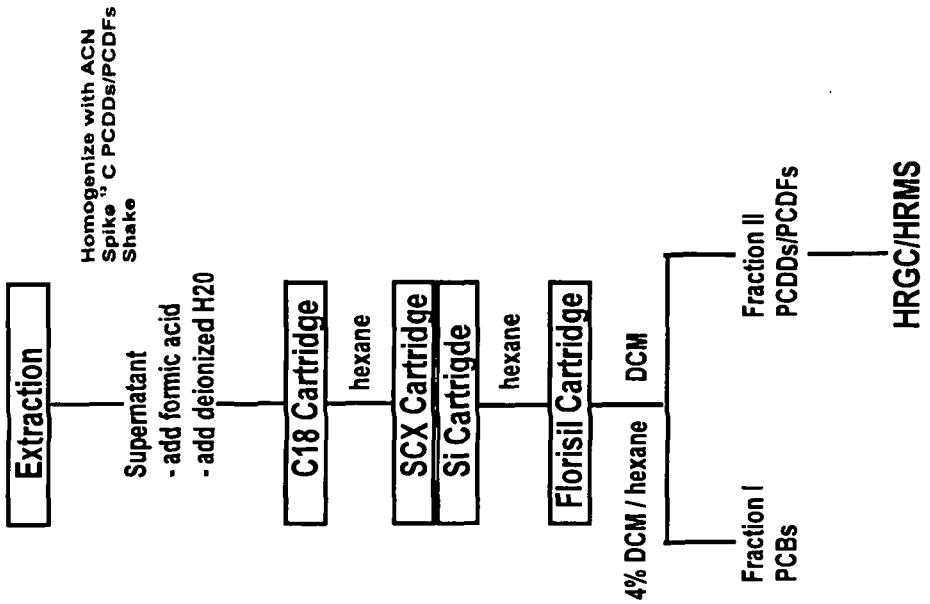


Figure 2

Results and Discussion

Our preliminary results indicate that the Chang method, as published, does not appear to work successfully with the carp material unless a sulphuric acid defatting step is inserted prior to the column cleanup steps. In all experiments where this step was omitted, there was evidence of lipid materials present in the solutions submitted for HRGC-HRMS analysis. This resulted in very poor chromatograpy --misshapen peaks, delayed elution times and large unknown peaks. Visual inspection of the injection port liner and the first few centimetres usually revealed the presence of a viscous oily material. When the sulphuric acid defatting step was employed, the results were in good agreement with the certified values. The Carbon-13 recoveries were usually 70% or better except for OCDD (50%) and 2,3,7,8-TCDF (low). In addition, the concentration values obtained for 2,3,7,8-TCDF were quite variable (± 3X the certified value). The failure of the Chang method to handle the carp material is not completely understood at this time and experiments are continuing to develop a modified version which will overcome these difficulties and eliminate the necessity for the sulphuric acid defatting step.

4) References

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