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Recovery of ^{14}C-Labelled Polychlorinated Biphenyls (PCB) in Fish Tissue Using a Combustion and a Solubilization Method of Sample Preparation for Scintillation Analysis

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The use of ^{14}C-labelled industrial chemicals in pollutant dynamics studies has provided a complementary analytical method to gas-liquid chromatography which allows a rapid quantitative estimate at a low level of detection. Its application has been used to establish the fate and effects of environmental contaminants within an organism (ROUBAL et al. 1977, CRAWFORD and GUARINO 1976), and its bioaccumulative properties in an ecosystem (SANDBORN and YU 1973).

Liquid scintillation analysis for ^{14}C in most biological materials require some preparation to render the sample suitable for counting. Use of a tissue solubilizer is by far the most common practice (e.g. SANDBORN et al. 1975), although an oxidative combustion method has been used (METCALF et al. 1975). This study evaluated the two methods to determine if one was more desirable than the other.

MATERIALS AND METHODS

^{14}C-labelled PCB (Aroclor 1242, specific activity 31.1 mCi/mM, New England Nuclear, Boston, Massachusetts) was incorporated into the tissue of rainbow trout (Salmo gairdneri) through the food-chain according to the following procedure. Chorella were cultured in 50% Bristol's solution at 20 C, until a dense, log-phased growth was attained. An aliquot of the culture was innoculated with PCB dissolved in methanol at concentrations of 10-100 ug/L, and incubated for 4 h. Adult Daphnia magna were then introduced and allowed to graze on the algae for 12-60 h before being fed to the trout.

Eleven groups of trout containing 5 fish each, with each fish averaging 4 g, were held at 18 C in 17 L flow-through aquaria. Approximately 2 g of Daphnia was introduced daily into each tank. One group was fed exclusively on ^{14}C-labelled Daphnia, while other groups were fed a mixture of labelled and unlabelled Daphnia. To attain a range of PCB tissue concentrations, fish were fed from 2-15 days, with all groups fed unlabelled Daphnia on the final day to ensure no residual labelled material would be present in their digestive system. Fish were then starved 24 h before being frozen for analysis.

Each group of five fish was ground to a uniform consistency using a homogenizer. The homogenate was divided into 0.2 g sub-

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samples, and prepared for ^{14}C liquid scintillation count following one of two procedures. One group was subjected to an oxidation combustion process while the second group was treated with a tissue solubilizer. There were 15 replicates for each treatment.

Samples prepared by combustion utilized a Biological Material Oxidizer (R.J. Harvey Instrument Corp., Hillsdale, New Jersey). Tissue samples were weighed on 2 cm^2 ashless filter paper, then combused at 900 C in the presence of oxygen and a platinum catalyst. This procedure oxidizes the sample to carbon dioxide and water, and the $^{14}CO_2$ emitted is trapped in a scintillation vial containing a 2:1 cocktail of PCS and CO_2mMET (Amersham/Searle Corp., Arlington Heights, Illinois). The operating conditions are sufficient to combust PCB, and recovery rates of 98% can be expected (NIIMI 1979).

The tissue solubilizer NCS (Amersham/Searle Corp.) was used to prepare the second group of samples. Following the manufacturer's recommendations, tissue samples were weighed in scintillation vials, 3.6 ml NCS added, and incubated at 50 C for 3 h. A 1.1 ml solution of 20% benzoyl peroxide dissolved in toluene was added as a decolorizing agent and the samples were incubated again for 30 minutes. After cooling, 15 ml of PCS was added before counting.

RESULTS

For most replicates, no significant differences in the mean recovery level was demonstrated between the two treatments at PCB tissue concentrations of 2-7600 ng/g (TABLE 1). This was due in part to the large variations of the NCS treated samples at PCB concentrations below 220 ng/g. The oxidative combustion method was able to measure the labelled compound in the pg/g range, while the NCS treatment was not able to consistently provide measurements above background levels.

DISCUSSION

The oxidative combustion technique would be the preferred method for preparing tissue samples for ^{14}C liquid scintillation analysis. Mean recovery levels were only slightly higher than the solubilization method, but variations among the replicates were less, and more importantly, the detection limit was well into the pg/g range. A need for the latter would be particularly relevant in pollutant bioaccumulation studies where body burdens in the picogram range may be found in organisms occupying the lower trophic levels.

Detection limit of the combustion method is dependent on the specific activity of the chemical under investigation, and its relative concentration in the material to be tested. This study monitored the ^{14}C-labelled PCB that was biologically incorporated into tissue where concentrations in the 0.3 ng/g range were consistently detectable. Detection limit could be lowered by

TABLE 1

Recovery of tissue incorporated ^{14}C-labelled PCB in rainbow trout using an oxidation and a solubilization method of sample preparation. Each estimate represents a replicate of 15 samples.

Biological Material Oxidizer				NCS Tissue Solubilizer			
^{14}PCB, ng/g				^{14}PCB, ng/g			
Min.	Mean	Max.	SD	Min.	Mean	Max.	SD
7260	7680	7890	190	7380	7600	7840	120
1860	1940	2010	40	1850	1920	1980	40
207	215	221	4	197	207	229	7
109	128	136	6	119	123	137	5
47	49	51	1	43	45	49	2
10	11	12	1	5	8	11	2
8	9	10	0.5	4	7	11	3
4	5	5	0	2	5	9	2
2	2	2	0	0.3	2	3	0.8
0.2	0.3	0.5	0.1	Background		0.3	-
Background		0.3	-		Background		

working with dried material, where feasible, which would allow a larger sample weight to be combusted. This method would be applicable to all ^{14}C-labelled compounds that would combust to carbon dioxide and water. The operating conditions of the instrument used was sufficient to combust thermally stable compounds such as PCB and hexachlorobenzene (NIIMI 1979). Earlier applications of this procedure used combustion temperatures in the 600 C range which may not have been entirely suitable for complete combustion of compounds of this nature (PETERSON et al. 1969). A preparation time of 5 minutes per sample would also enhance the use of this method.

The NCS treated samples also showed high quench although the degree of quench was within the range of the external standards used. This appears to be a factor common to solubilization methods that have used agents such as nitric acid, formamide, Hyamine hydroxide, ans potassium hydroxide as the active ingredient (PFEFFER et al. 1970, POLLAY and STEVENS 1970, HANSEN and BUSH 1967). Attempts to reduce quench following these applications have been met with limited success.

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