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Environmental Effects of Dredging Technical Notes

A Comparison of Three Lipid Extraction Methods

Purpose

This technical note summarizes results from studies that compare three commonly used lipid extraction methods: Bligh-Dyer, hexane:acetone, and dichloromethane.

Background

Organism lipid content is a critical component of both theoretical bioaccumulation potential, currently recommended in the "Green Book" (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers 1991), and the sediment quality criteria (SQC) proposed by the USEPA. Since no standard extraction method exists for quantitating lipids, many different methods are used, leading to questions concerning the comparability of lipid data obtained using different extraction methods. Research examining the relationship between various extraction methodologies can help reduce uncertainty in interpreting and utilizing data obtained from different studies, leading to better environmental assessments concerning the long-term effects of dredging.

Additional Information

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Introduction

The role of lipids as the major compartment for neutral organic chemical partitioning in organism tissues has been well documented (Connell 1988; Esser 1986; Roberts, de Frietas, and Gidney 1977; Schneider 1982). Lipid normalization is used in the calculation of accumulation factors, which express the equilibrium distribution of neutral chemicals between sediments and aquatic biota (Ankley and others 1992; Ferraro and others 1990, 1991; Lake and others 1990; McFarland and others 1994; Young, Mearns, and Gossett 1991). A screening test used to estimate the bioaccumulation potential of neutral chemicals associated with dredged sediments relies on equilibrium partitioning to organism lipids (USEPA/USACE 1991). Additionally, the USEPA seeks to promulgate SQC that will require lipid normalization of data (USEPA 1993).

No standardized method exists for lipid determinations in environmental samples. Typically, analysts will either reserve an aliquot of a residue-analysis tissue extract for lipid analysis or run a separate tissue sample for lipid analysis concurrently. In the former case, hexane:acetone or dichloromethane is commonly used as the solvent (Ryan and others 1985, Schwartz and others 1993). In the latter case, the chloroform:methanol (Bligh-Dyer) method is commonly used, as it is specifically intended for lipid analysis and is routinely used to measure the lipid content of foods (Bligh and Dyer 1959). Often, the amount of tissue used in either of the above cases varies due to differing amounts of tissue required (or available) for chemical analysis, or to the amount of sample remaining for lipid analysis after that required for chemical analysis has been taken. Knowledge of the variability that may be introduced due to sample size or solvent used is required in order to compare lipid-normalized data obtained from different studies.

The purpose of the study described in this technical note was to assess these sources of variability by comparing percent lipid determinations made on different sample sizes of the same homogenized fish tissue. Three commonly used lipid extraction methods (Bligh-Dyer, hexane:acetone, and dichloromethane) and six sample sizes representing a 200-fold range of tissue weights were compared.

Materials and Methods

Tissue

Nine kilograms of frozen commercial whiting fish was purchased from a local supermarket, skinned, and filleted. The fillets were homogenized with a Waring blender, divided into 100-g fresh weight aliquots, and stored at -80 °C until use. For each lipid extraction method, four replicate tissue samples were used for each tissue sample size. All procedures were performed at room temperature.

Bligh-Dyer

Tissue samples of 0.5, 1, 5, 10, 50, and 100 g were analyzed for lipid content using the Bligh-Dyer method (Bligh and Dyer 1959). The samples were homogenized for 2 min in a Waring blender with chloroform and methanol in the proportion of 1 g tissue:1 ml chloroform:2 ml methanol. Solvent volumes were adjusted for each sample size to maintain the same proportions. For the 0.5-, 1-, 5-, and 10-g tissue samples, a Polytron homogenizer was used rather than a Waring blender. An additional equivalent amount of chloroform was added, and the mixture was homogenized for another 30 sec. Deionized water (1 ml water/1 g tissue) was then added, and the mixture was homogenized again for 30 sec. The final mixture proportion was 1 g tissue:2 ml chloroform:2 ml methanol:1 ml deionized water.

The mixture was filtered through Whatman No. 1 filter paper, and the remaining tissue was homogenized for 2 min with another 1 ml chloroform/1 g tissue. After filtering the mixture again, the combined filtrate was transferred to a graduated cylinder and allowed to separate. Lipid content was determined gravimetrically by measuring triplicate aliquots of the chloroform layer into tared containers, air-drying the solvent, and weighing. Percent lipid determinations were then calculated.

Hexane:Acetone Extraction

Tissue samples of 0.5, 1, 5, 10, and 50 g were analyzed for lipid content by homogenizing each sample three times with 20 ml hexane:acetone (1:1, v/v) for 2 min using a Polytron homogenizer. The three extracts were filtered and pooled. Percent lipids were calculated on triplicate extract aliquots as with the Bligh-Dyer method.

Dichloromethane Extraction

Tissue samples of 0.5, 1, 5, and 10 g were placed into 25- or 150-ml screw-capped centrifuge tubes according to sample size along with one to two times the tissue weight of anhydrous sodium sulfate. Dichloromethane in a proportion of 5 ml to 1 g tissue was added to the samples, which were rolled for 18 to 24 hr. The mixture was filtered through Whatman No. 1 filter paper, and percent lipid determinations were made for triplicate aliquots of the dichloromethane extracts as with the Bligh-Dyer method.

Statistical Analysis

All data were analyzed using PC SAS (SAS Institute 1988). Two-way analysis of variance was performed using PROC GLM, and mean comparisons were made using Fisher's Protected Least Significant Difference. The normality assumption was tested using the Shapiro-Wilk's test, and homogeneity of variances was assessed using Levene's test (Snedecor and Cochran 1989).

Results and Discussion

Percent lipid data for the three methods used are listed in Table 1. The hexane: acetone method was impractical for use with the 100-g sample size. Similarly, the dichloromethane method could not be performed using sample sizes of 50 and 100 g.

Table 1.Mean Percent Lipids ± Standard Error on a Fresh-Weight Basis of Commercial Whiting Fish Fillets

Sample Size (g)	Method		
	Bligh-Dyer	Hexane:Acetone	Dichloromethane
0.5	2.07 ± 0.20 A[1] b[2]	1.29 ± 0.12 A b	5.77 ± 0.37 A a
1	1.47 ± 0.29 B a	0.63 ± 0.05 C b	0.28 ± 0.05 B b
5	1.12 ± 0.26 B a	0.93 ± 0.14 B a	0.71 ± 0.11 BC a
10	1.06 ± 0.02 B a	0.92 ± 0.06 BC a	1.14 ± 0.37 C a
50	1.25 ± 0.12 B a	1.00 ± 0.08 AB a	—
100	1.39 ± 0.19 B	—	—

[1]For a given method, sample size means followed by the same uppercase letter are not significantly different from each other ($p < 0.05$).
[2]For a given sample size, method means followed by the same lowercase letter are not significantly different from each other ($p < 0.05$).

The data indicate that sample size has a significant effect on lipid analysis results regardless of method. The whiting tissue apparently had a percent lipid value of approximately 1 percent, since all three methods yielded results encompassing this value at one or more sample weights. Lipid determinations made using the 0.5- and 1-g sample weights were the most variable and yielded significant differences among the methods, while the 5- and 10-g sample sizes were not significantly different. The Bligh-Dyer method generally gave higher percent lipid values, yielding significantly higher results for the 1-g sample size.

Randall and others (1991) found a 3.5-fold variation among several extraction methods which included acetonitrile extraction with pentane partitioning, acetone extraction with hexane partitioning, Bligh-Dyer, and acetonitrile extraction using sample sizes of 1 to 5 g. Results from this study suggest that larger sample sizes (5 to 10 g) may yield less variable results and would be comparable using the three methods investigated.

The 0.5-g sample size for all three extraction methods yielded an aberrantly high percent lipid, indicating that this sample size is below the lower limit of practical application of the three methods. Samples of less than 5 g should probably be analyzed using a micromethod such as the method described by Gardner and others (1985).

Conclusions

Sample sizes of 5 to 10 g were optimal for the three lipid extraction methods studied, and produced similar results for all three methods. The 100-g sample size called for in the original Bligh-Dyer method (Bligh and Dyer 1959) is usually impractical for environmental studies involving small organisms, and is not necessary. However, if less than 5 g of tissue is available, a micromethod should be used.

References

Ankley, G. T., Cook, P. M., Carlson, A. R., Call, D. J., Swenson, J. A., Corcoran, H. F., and Hoke, R. A. H. 1992. "Bioaccumulation of PCBs from Sediments by Oligochaetes and Fishes: Comparison of Laboratory and Field Studies," *Canadian Journal of Fisheries and Aquatic Science*, Vol 49, pp 2080-2085.

Bligh, E. G., and Dyer, W. J. 1959. "A Rapid Method of Total Lipid Extraction and Purification," *Canadian Journal of Biochemistry and Physiology*, Vol 37, pp 911-917.

Connell, D. W. 1988. "Bioaccumulation Behavior of Persistent Organic Chemicals with Aquatic Organisms," *Reviews in Environmental Contamination and Toxicology*, Vol 101, pp 121-154.

Esser, H. O. 1986. "A Review of the Correlation Between Physicochemical Properties and Bioaccumulation," *Pesticide Science*, Vol 17, pp 265-276.

Ferraro, S. P., Lee, H., II, Ozretich, R. J., and Specht, D. T. 1990. "Predicting Bioaccumulation Potential: A Test of a Fugacity-Based Model," *Archives of Environmental Contamination and Toxicology*, Vol 19, pp 386-394.

Ferraro, S. P., Lee, H., II, Smith, L. M., Ozretich, R. J., and Specht, D. T. 1991. "Accumulation Factors for Eleven Polychlorinated Biphenyl Congeners," *Bulletin of Environmental Contamination and Toxicology*, Vol 46, pp 276-283.

Gardner, W. S., Frez, W. A., Chichocki, E. A., and Parrish, C. C. 1985. "Micromethod for Lipid Analysis in Aquatic Invertebrates," *Limnology and Oceanography*, Vol 30, pp 1099-1105.

Lake, J. L., Rubinstein, N. I., Lee, H., II, Lake, C. A., Heltsche, J., and Pavignano, S. 1990. "Equilibrium Partitioning and Bioaccumulation of Sediment-Associated Contaminants by Infaunal Organisms," *Environmental Toxicology and Chemistry*, Vol 9, pp 1095-1106.

McFarland, V. A., Feldhaus, J., Ace, L. N., and Brannon, J. M. 1994. "Measuring the Sediment/Organism Accumulation Factor of PCB-52 Using a Kinetic Model," *Bulletin of Environmental Contamination and Toxicology*, Vol 52, pp 699-705.

Randall, R. C., Lee, H., II, Ozretich, R. J., Lake, J. L., and Pruell, R. J. 1991. "Evaluation of Selected Lipid Methods for Normalizing Pollutant Bioaccumulation," *Environmental Toxicology and Chemistry*, Vol 10, pp 1431-1436.

Roberts, J. R., de Frietas, A. S. W., and Gidney, M. A. J. 1977. "Influence of Lipid Pool Size on Bioaccumulation of the Insecticide Chlordane by Northern Redhorse Suckers (*Moxostoma macrolepidotum*)," *Journal of the Fisheries Research Board of Canada*, Vol 34, pp 89-97.

Ryan, J. J., Schecter, A., Lizotte, R., Sun, W., and Miller, L. 1985. "Tissue Distribution of Dioxins and Furans in Humans from the General Population," *Chemosphere*, Vol 14, pp 929-932.

SAS Institute, Inc. 1988. "SAS/STAT Procedures Guide, Release 6.03 Edition," Cary, NC.

Schneider, R. 1982. "Polychlorinated Biphenyls (PCBs) in Cod Tissues from the Western Baltic: Significance of Equilibrium Partitioning and Lipid Composition in the Bioaccumulation of Lipophilic Pollutants in Gill-Breathing Animals," *Meeresforsch.*, Vol 29, pp 69-79.

Schwartz, T. R., Tillitt, D. E., Feltz, K. P., and Peterman, P. H. 1993. "Determination of Mono- and Non-o,o'-Chlorine Substituted Polychlorinated Biphenyls in Aroclors and Environmental Samples," *Chemosphere*, Vol 26, pp 1443-1460.

Snedecor, G. W., and Cochran, W. G. 1989. *Statistical Methods*, 8th ed., Iowa State University Press, Ames, IA.

U.S. Environmental Protection Agency. 1993. "Technical Basis for Deriving Sediment Quality Criteria for Nonionic Organic Contaminants for the Protection of Benthic Organisms by Using Equilibrium Partitioning," EPA-822-R-93-011, Office of Water, Washington, DC.

U.S. Environmental Protection Agency/U.S. Army Corps of Engineers. 1991. "Evaluation of Dredged Material Proposed for Ocean Disposal (Testing Manual)," EPA-503/8-91/001, Office of Marine and Estuarine Protection, Washington, DC.

Young, D. A., Mearns, A. J., and Gossett, R. W. 1991. "Bioaccumulation of *p,p'*-DDE and PCB 1254 by a Flatfish Bioindicator from Highly Contaminated Marine Sediments of Southern California," *Organic Substances and Sediments in Water: Biological Processes*, R. A. Baker, ed., American Chemical Society, Washington, DC, pp 159-169.

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