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Intercalibration of Gas Chromatographic Analyses for Hydrocarbons in Tissues and Extracts of Marine Organisms

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Gas chromatographic analyses of hydrocarbons separated from tuna meal samples and cod liver lipid extracts have been intercalibrated among three laboratories. Measurement of petroleum hydrocarbons spiked to samples of cod liver oil gave values as follows: $\hat{x}$, $(\hat{x} - \bar{x})/\hat{x}$, $s/\bar{x}$; distillate cut of South Louisiana crude oil—372 μg/g, 0.09, 0.06; No. 2 fuel oil—1163 μg/g, 0.50, 0.26; Wilmington crude oil—913 μg/g, 0.69, 0.34. The estimates of petroleum hydrocarbons in tuna meal subsamples gave $\bar{x} \pm s$ of 37.7 ± 4.6 μg/g dry weight. Measurements of pristane in cod liver lipid samples gave $\bar{x} \pm s$ of 35.7 ± 3.5 μg/g lipid and 271 ± 4.5 μg/g lipid. Measurements of pristane in tuna meal were less precise with $\bar{x} \pm s$ of 2.4 ± 1.5 μg/g dry weight. Some limitations to current methods of analysis as applied in this study and in several current oil pollution studies are demonstrated and discussed.

Petroleum and its refined products such as fuel oils and lubricating oils continue to be discharged to the world's oceans with little hope in the immediate future for substantial reduction of the quantities released (*1*). Studies of the inputs, effects, and fate of these discharges have recently been reviewed and recommendations for future studies set forth (*1*). These studies will require the efforts of many laboratories throughout the world because of the complexity and global nature of the oil pollution problem. This is already apparent to some degree in the oil pollution research literature.

An important factor in field or laboratory studies is the precision, accuracy, and intercomparability of the data gathered using the same or different methods of analyses in different laboratories (*1–3*). We initiated a program of intercalibration between our laboratories in 1971 as part of a study program to identify problems related to oceanic environmental quality under the auspices of the Office for the International Decade of Ocean Exploration, National Science Foundation. Three laboratories were involved in studying the distribution of hydrocarbons in the biota, water, air–sea interface and sediments of the North Atlantic Ocean. Unfortunately, suitable samples for intercalibration were not available, and we proceeded to develop our own. We choose to intercalibrate using a lipid material containing indigenous biogenic hydrocarbons and spiked petroleum hydrocarbons. Most current methods of extracting hydrocarbons from sediment, tissue, whole organism, or water involve an initial step of organic solvent extraction to obtain a lipid extract (*1–4*). Thus, it seemed that an initial intercalibration program with a lipid matrix sample would apply to a wide variety of marine samples. It also excluded the influence of initial lipid extraction which would have to be investigated at a later date if the first intercalibration program was successful.

We have reported the result of one phase of this intercalibration program (*5, 6*). Later we received a working intercalibration sample of tuna meal from the National Bureau of

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Standards. We emphasize that this was not a standard reference material for hydrocarbon analysis. It was used only because it was a relatively homogeneous sample of marine tissue for which we had no knowledge of the hydrocarbon composition and concentration. A fourth laboratory participated in this phase of the intercalibration and we have issued a brief report on the results of this study (*7*).

We report here the result of our entire intercalibration program to date. We hope that by reporting our experience and data in detail, we will stimulate others to develop similar and even more comprehensive intercalibration efforts.

EXPERIMENTAL

Preparation of Intercalibration Samples. a) Cod liver oil sample A and sample B were manufactured by Squibb Pharmaceutical Company and purchased at a local pharmacy.

b) IDOE-1: Cod liver oil sample B was spiked with 1163 μg of No. 2 fuel oil/g cod liver oil.

c) IDOE-3: Cod liver oil sample B was spiked with 913 μg of Wilmington crude oil/g cod liver oil.

d) IDOE-5: Cod liver lipid extract was prepared by Virtis homogenization extraction in hexane (*8*) of a liver excised from a cod caught in the East Greenland Current on Cruise B-9-71 of *R/V Bjarni Saemudson* of the Marine Research Institute, Reykjavik, Iceland. The concentrated extract was spiked with 372 μg of a distillate fraction of a South Louisiana crude oil/g cod liver lipids. The distillate cut was prepared by vacuum distillation and had a boiling range between *n*-hexadecane (*n*-C_{16}, 287 °C, 760 mm Hg) and *n*-octacosane (*n*-C_{28}, 432 °C, 760 mm Hg).

e) Tuna meal sample: this was provided as a working intercalibration sample by the National Bureau of Standards, courtesy of Philip A. LaFleur.

Procedures. *IDOE-1 and IDOE-3,* QUINN AND WADE. Each subsample was transferred to a 50-ml centrifuge tube and 20 ml of 0.5 N KOH in absolute methanol was used to rinse out the sample vial and then added to the centrifuge tube. Three ml of distilled H_2O and 1 ml of methanol containing 100 μg of *n*-eicosane (internal standard) were added to the tube. The tube was flushed with nitrogen, capped, and heated at 100 °C for 10 min to ensure complete saponification of the sample. After cooling, 5 ml of distilled H_2O were added to the tube followed by 10 ml of ethyl ether. The contents of the tube were shaken and the phases separated by centrifugation.

The ether phase was removed and evaporated to dryness under reduced pressure at room temperature on a rotary evaporator. The residue was dissolved in a small amount of chloroform and applied to predeveloped (in chloroform) 0.37- or 0.50-mm thick silica gel G plates (20 × 20 cm). The solvent system was petroleum ether/ethyl ether/acetic acid (95/5/1). After development, the plates were visualized by brief exposure to iodine vapors. The total hydrocarbon band (corresponding to cochromatographed *n*-hexadecane and phenanthrene standards) was scraped from the plate and extracted with chloroform/methanol (9/1). After evaporation of the solvent under vacuum, the hydrocarbons were dissolved in a small volume of carbon disulfide and analyzed using a Hewlett-Packard Model 700 gas chromatograph equipped with a flame ionization detector (FID).

The chromatographic columns employed were 2.2-mm i.d. stainless steel and ranged in length from 1.8 to 2.0 m. The nonpolar column contained 2% Apiezon L on Anakrom Q (90/100 mesh), and the polar column contained 12% FFAP on Chromosorb W (H.P., 80/100 mesh). Both columns were temperature-programmed from 80 to 280 °C at 5°/min with nitrogen carrier gas at a flow rate of 10 ml/min. Quantitative analyses of samples involved comparison of the area of the chromatogram attributed to petroleum hydrocarbons with the area of the internal standard (IDOE-1) or pristane (IDOE-3, the concentration of pristane is known from analyses of the cod liver oil as described below). Peak areas were calculated by multiplying peak height times peak width at half height. The area of the unresolved complex mixture signal was determined by planimetry.

FARRINGTON. Subsamples of IDOE-1 and IDOE-3 were column-chromatographed on 10 g of alumina over 20 g of silica (both 5% deactivated with water) in a 1- to 1.5-cm i.d. column. Hydrocarbons were eluted with three column volumes of 5% benzene in pentane. The eluate was concentrated under reduced pressure to a volume of 10–15 ml and transferred to a 50-ml pear-shape flask. The eluate was then evaporated until the manometer in the vacuum line of the rotary evaporation unit showed a rapid drop in pressure indicating the last of the solvent had been evacuated from the flask. The residue was transferred to a small vial with ~0.5–1.0 ml of CS_2 and evaporated to dryness in the vial on the rotary evaporator. A known volume of CS_2 was added, usually 50–100 μl, and an aliquot was weighed on the Cahn balance to determine total hydrocarbon concentration. The hydrocarbons were analyzed by gas chromatography using a 2.3-m 3% Apiezon L on Chromasorb W 80/100 mesh column, 2.2-mm i.d. stainless steel, programmed from 80 to 290 °C at 6°/min. The column had 2160 plates as determined using *n*-C_{15} at 160 °C. Nitrogen carrier gas flow rate was 10–15 ml/min at the start of the program. Varian Aerograph Model 1200 and Model 1700 gas chromatographs equipped with FID's were used for these analyses.

The hydrocarbons were checked for purity by thin layer chromatography on silica gel G plates developed with 3% benzene in isooctane. No spots attributable to other lipids such as mono-, di-, and triglycerides, wax esters, sterol esters, methyl ketones, sterols, or fatty acids were found when plates were visualized in an iodine chamber. Several of the hydrocarbon samples were checked by infrared spectroscopy and no functional group absorption bands, which could be assigned to these other lipid compounds in the cod liver oil, were noted.

The concentration of petroleum hydrocarbons in the samples was determined as previously described in Quinn's section on methods using *n*-eicosane or *n*-docosane as an internal standard.

TEAL AND BURNS. Subsamples of IDOE-1 and IDOE-3 were analyzed by column chromatography using 20 g of 1/1 (v/v) deactivated alumina packed over silica in a 1-cm i.d. column. The columns were routinely eluted with three column volumes of pentane. Two analyses were also conducted by eluting the column with three column volumes of 5% benzene in pentane. Column eluates were concentrated by evaporation under reduced pressure. Aliquots of the residue, dissolved in CS_2, were weighed on the Cahn balance to determine total hydrocarbon concentration. The hydrocarbons were then analyzed by gas chromatography on a 3.0-m 3% Apiezon L on Chromasorb W column, 2.2-mm i.d. stainless steel, programmed from 80 to 280 °C at 5°/minute. Nitrogen carrier gas flow rate was 10–15 ml/min at the start of the program. The column had 4281 theoretical plates at the start of the analysis of the samples but had deteriorated to 1835 plates at the end of this series of analyses based on *n*-C_{14} analysis at 125 °C. A Hewlett-Packard Model 700 gas chromatograph with FID was used for these analyses.

The peak areas in chromatograms of a known portion of sample were calculated and multiplied by the quantity of hydrocarbons/unit area calculated from chromatograms of a series of standard *n*-alkanes of known amounts, i.e., external standard technique.

Cod Liver Oil—Samples A and B (Quinn, Wade, and Farrington). Samples of cod liver oil were analyzed to determine the concentration of pristane. Methods of analysis by Quinn were the same as those described above using *n*-eicosane as an internal standard. Farrington analyzed both saponified and nonsaponified samples using the same column procedure as described above. A thin-layer chromatography procedure employing silica gel G plates developed with 5% benzene in pentane was also used to analyze a saponified sample of cod liver oil. Samples were saponified under reflux for 2 h with 0.5 N KOH in methanol/benzene (1/1).

Comparison of the peak area of an internal standard of *n*-eicosane, *n*-docosane, *n*-tetradecane, or *n*-octacosane added prior to analysis, with the peak areas of the pristane was used as a means of determining concentrations. Gas chromatographic procedures were the same as described above.

IDOE-5 and Tuna Meal. The methods of analysis used to measure hydrocarbons in these samples have been presented in detail elsewhere (*5–7, 9*) and were in principle the same as those described above.

Measurement of Petroleum Hydrocarbons. The problems associated with distinguishing between pollutant hydrocarbons and those biosynthesized by organisms have been discussed elsewhere (*1, 4*). In our measurements, we have integrated the unresolved complex mixture signal,resolved and partially resolved peaks due to the petroleum spike (*5*).

RESULTS AND DISCUSSION

Pristane Concentration Measurements. The concentrations of pristane in the various samples analyzed are given in Table I. The agreement and precision of the measurement is good for the cod liver lipid samples where the pristane concentration was between 30 and 40 μg/g lipid and 260 to 280 μg/g lipid. The precision for the pristane in the tuna meal

Table I. Reproducibility of Pristane Analyses

Analyst	Technique		Pristane concn, μg/g Lipid
Cod Liver Sample A			
Quinn, Wade	Saponification–TLC, GC[a]		30.1
Farrington	Saponification–TLC, GC, 3 analyses by GC		35.8 ± 1.6
	Saponification–CC, 2 analyses by GC		39.4 ± 2.3[b]
	No saponification–CC, GC, Subsample A-1		36.4 ± 1.6[b]
	No saponification–CC, GC, Subsample A-2		37.3 ± 1.0[b]
		Mean =	35.7 ± 3.5
Cod Liver Sample B			
Farrington	No saponification–CC-GC		
	Subsample 1		38.7 (n-C_{14})[c]
			40.3 (n-C_{28})
	Subsample 2		37.1 (n-C_{14})
			39.7 (n-C_{28})
		Mean =	39.0 ± 1.2
IDOE-5			
Quinn, Wade	Saponification–TLC, GC, 2 analyses 259, 268; mean =		264
Teal, Burns	Saponification–CC, GC, 1 analysis		276
Farrington	Saponification–CC, GC, 6 analyses, range 225 to 308; mean =		272
	Mean for 3 laboratories		271 ± 4.5
			μg/g dry wt.
Tuna meal			
Quinn, Wade	Saponification–TLC, GC, 2 analyses 3.0, 3.6; mean =		3.3
Teal	Saponification–CC, GC, 1 analysis		2.0
Farrington	Saponification–CC, GC, 2 analyses; 1.9, 2.0; mean =		2.0
	Mean for 3 laboratories		2.4 ± 1.5

[a] TLC = Thin layer chromatography; CC = Column chromatography; GC = Gas chromatography. [b] Mean ± 1 σ estimated from 2 or 3 analyses by GC of hydrocarbons isolated from same sample. [c] Internal standard used to calculate concentration. Both n-C_{14} and n-C_{28} added to subsamples as internal standard.

Table II. Results of Hydrocarbon Analyses of IDOE-1 and IDOE-3 Intercalibration Samples, (μg hydrocarbons/g cod liver lipid)

		Gas chromatography analysis		
Sample	Analyst	Petroleum hyd. peaks + unresolved complex mixture	Total hyd.	Total hydrocarbons by weighing
IDOE-1	Quinn, Wade[a]	498 μg/g	770 μg/g	N.A.[b]
	Teal, Burns	782	823	1200 μg/g ± 310(2)[c]
	Farrington	449 ± 85 (4)	628 ± 131 (4)	2948 ± 192 (4)
	Mean	576 ± 147	740 ± 82	2074 ± 874
IDOE-1: $\hat{x}$ = 1,163 μg No. 2 fuel oil/g cod liver lipid[d]				
IDOE-3	Quinn, Wade[a]	410 μg/g	523 μg/g	N.A.
	Teal, Burns	178 ± 47 (2)	242 ± 54 (2)	1178 μg/g ± 313(2)
	Farrington	262 ± 122 (3)	438 ± 92 (3)	1080 ± 260 (5)
	Mean	283 ± 96	401 ± 118	1129 ± 49
IDOE-3: $\hat{x}$ = 913 μg Wilmington crude oil/g cod liver lipid[d]				

[a] The average coefficient of variation for total hydrocarbons estimated to be 20%. [b] N.A. = not analyzed or calculated. [c] Standard deviations calculated or estimated from number of analyses shown in parentheses. [d] An unknown but minor portion of the fuel oil and crude oil are not hydrocarbons.

sample was not as good. This may have been the result of the inclusion of the additional extraction procedure in the analysis. The starting mass of the lipid extract is not the reason because 200 to 600 mg of lipid were used for saponification, separation, and gas chromatographic analysis for both cod liver oil, IDOE-5, and tuna meal analyses.

IDOE-1 and IDOE-3 Measurements. The results of the analyses of samples of cod liver oil spiked with No. 2 fuel oil or Wilmington crude oil are given in Table II. The discrepancy between the actual amount of No. 2 fuel oil added to the sample (IDOE-1, 1163 μg/g lipid) and that found (576 ± 147 μg/g lipid) is probably due to losses by volatilization of hydrocarbons boiling below the C_{14} n-alkane during the isolation and concentration procedures. Comparison of the gas chromatograms of hydrocarbons isolated from IDOE-1 with the gas chromatogram of No. 2 fuel oil confirmed that there were losses of hydrocarbons boiling below n-tetradecane in the analysis of IDOE-1. This is consistent with previously reported recovery values of 70% by weight for No. 2 fuel oil from similar column chromatography procedures (8).

The values of total hydrocarbons by weighing are reasonably precise. However, the values found by Farrington were much higher than the calculated amounts of hydrocarbons in the sample. This is attributed to elution of cod liver oil com-

Table III. Results of Hydrocarbon Analyses of IDOE-5 Intercalibration Sample IDOE-5: $\hat{x}$ = 372 μg petroleum/g cod liver lipid (μg hydrocarbons/g cod liver lipid)

Analyst	(Petroleum hydrocarbons) Peaks and unresolved complex mixture	Peaks[c]	Unresolved complex mixture	Pristane
Subsamples date, January 1972. Analyses, January to October, 1972				
Quinn, Wade[a]	373	85.5	288	264
Teal, Burns[a]	438	87.7	350	276
Farrington[a]	407	64.4	343	272
Mean std dev	406 ± 26	79.2 ± 10.5	327 ± 27.7	271 ± 4.5
Subsample date, August 1972. Analysis, February 1974				
Medeiros, Robinson[b]	455	71	384	267
Subsample date, October 1972. Analysis, February 1974				
Medeiros, Robinson[b]	426	59	367	270
Subsample date, June 1974 Analyses, November 1974				
Quinn, Wade	474	78	396	262
	493	108	385	256
	676	63	613	272

[a] Data taken from Ref. (5). [b] Data taken from Ref. (6). [c] Does not include pristane or squalene.

Table IV. Results of Hydrocarbon Analyses of Tuna Meal[a] (μg/g dry wt tuna meal)

Analyst	Unresolved complex mixture n-C_{14} to n-C_{30}	Resolved peaks[b]	Total
Quinn, Wade	32.4	9.4	41.8
Teal, Burns	39.7	19.3	59.0
Farrington	41.0	6.5	47.5
Mean ± std dev	37.7 ± 4.6	11.7 ± 6.7	49.4 ± 8.8

[a] Taken from Ref. (6). [b] This does not include pristane or squalene.

ponents from the alumina–silica column which were not measured by gas chromatography because of its high molecular and very late elution, if at all, from the GC column. These components do not contain carbonyl, carboxyl, or carboxylate functional groups based upon infrared spectroscopy of the concentrated column eluate. Further analyses are needed to determine the nature of this material.

The petroleum hydrocarbon concentration in IDOE-3 as determined by gas chromatography has a greater relative standard deviation (34%) among the three laboratories than the analysis of IDOE-1 (26%). In addition, the measured IDOE-3 concentration values (283 ± 96 μg/g lipid) are much lower than the true value (913 μg/g lipid) compared to results of the analyses of IDOE-1.

The reported values for petroleum hydrocarbons can be explained as follows for the IDOE-3 analyses. Column and thin-layer chromatography as employed would exclude the more polar fractions of the crude oil from the hydrocarbon isolates. Also, the fraction of the crude oil which was obtained for gas chromatographic analysis would not include volatile low-boiling hydrocarbons which would be lost in the same manner as the volatile components of the No. 2 fuel oil in IDOE-1. Furthermore, the fraction analyzed by gas chromatography may contain high-boiling hydrocarbons which do not elute from the gas chromatographic column or elute at the end of the temperature program and during the isothermal operation at the upper limit. Their signal may be difficult to distinguish from the column bleed signal which is present at these high temperatures. This problem did not occur during analysis of the No. 2 fuel oil in IDOE-1 because its boiling range is such that the hydrocarbons elute from the column prior to the appearance of the column bleed signal.

There is evidence available to support our explanation of the low results obtained for IDOE-3 petroleum hydrocarbons. A comparison of the gas chromatograms of hydrocarbons in IDOE-3 with the gas chromatograms of the crude oil showed that lower-boiling hydrocarbons have been lost during the analysis. The recovery of Wilmington crude oil from the column chromatography procedure was 30–48% by GC analysis.

IDOE-5 Measurements. The results of the first series of intercalibrations with IDOE-1 and IDOE-3 led to the preparation of sample IDOE-5 as described previously. We prepared this sample to test our interpretation of the problems associated with analyses of IDOE-1 and IDOE-3. We reasoned our methods should lead to more precise and accurate measurements for the spike of the distillate fraction of South Louisiana crude oil because the spike contained only compounds with a molecular weight range within the range which could be measured by our methods, i.e., molecular weight range n-tetradecane to n-triacontane. Also, a check of the distillate fraction by column chromatography showed that 95+% of the components should be recovered by column chromatography and thin-layer chromatography as analyzed by weighing and GC.

The results of intercalibration with IDOE-5 were more precise and accurate as expected. The data are presented in Table III, as taken from our earlier reports (5, 6). The measured concentration of petroleum hydrocarbons is in fair agreement with the actual concentration spiked to the sample—372 μg/g lipid—with the exception of one measurement of the June 1974 subsample. We cannot explain this discrepancy except that it may represent inhomogeneity in this

Table V. Comparison of Results of IDOE-5 Analyses by One Laboratory Using Internal and External Standards (μg hydrocarbons/g lipid)

	Peaks and unresolved complex mixture	Resolved Peaks[a]	Unresolved complex mixture	Pristane
External standard method–6 subsample analyses	$41 \times 10^1 \pm 7 \times 10^1$	58 ± 18	$35 \times 10^1 \pm 6 \times 10^1$	$27 \times 10^1 \pm 3 \times 10^1$
Internal standard method–4 subsamples	400 ± 83	69 ± 28	331 ± 69	261 ± 24

[a] Does not include pristane or squalene.

sample. There are no large changes in the concentrations of hydrocarbons as a result of two years' storage in the dark, under N_2, at 0 °C. However, there appears to be a trend of a small increase in the concentration of petroleum hydrocarbons with time. The pristane concentration has remained constant within the interlaboratory variability. One explanation is that the bulk IDOE-5 sample has suffered some inhomogeneity imposed during subsampling. We intend further analyses and testing to establish if this trend is real.

Tuna Meal Measurements. The results of our analyses of tuna meal samples as reported elsewhere (7) are presented in Table IV. These analyses required extraction in addition to the procedures of analysis previously described in this paper. Despite this additional step, there is good agreement for the measurement of the unresolved complex mixture hydrocarbons. The agreement for the analyses of resolved peaks is not as good and is probably a result of the small ratio of peak signal to unresolved complex mixture signal for many of the peaks.

Comparison of Measurements Using Internal and External Standards. During the program of analyses of IDOE-5, we tested measurements of hydrocarbons by gas chromatography using both internal and external standards. Internal standards used for these experiments were the *n*-alkanes, *n*-tetradecane, *n*-eicosane, *n*-docosane, and *n*-octacosane. The gas chromatogram FID peak area of the internal standard was compared with the areas of the sample hydrocarbon peaks and unresolved complex mixture to quantitate the sample hydrocarbons. The second method employed was to analyze an accurately measured volume of a mixture of known concentrations of even carbon number *n*-alkanes from *n*-tetradecane to *n*-octacosane using the same gas chromatographic conditions as those used for analysis of the sample. The FID response is calibrated with the *n*-alkane standard and used to quantitate the hydrocarbon peaks and unresolved complex mixture signal of the sample.

A comparison of results obtained by analyzing subsamples spiked with an internal standard and quantitating hydrocarbons using this standard with results obtained by quantitation of hydrocarbons using external standards is presented in Table V. The results compare quite favorably.

There are advantages gained in using an internal standard. If the standard is added with a volumetric pipet under carefully controlled conditions, an accurate measurement of hydrocarbons in the sample to three significant figures is possible. In addition, the exact amount of sample injected into the GC need not be measured. The external standard method requires a more accurate measurement of the volume of external standard injected into the GC. The commonly used 10-μl Hamilton syringe or other similar syringes offer only two-figure volume measurements, thus limiting the accuracy and precision to two significant figures.

An additional advantage of the internal standard method is that once the standard is well mixed into the sample, any subsequent accidental losses do not negate the analysis. The disadvantage of the internal standard method is that it requires the analysis of a separate subsample without internal standard or with a different internal standard to determine which hydrocarbons present in the sample interfere with the measurement of a given internal standard. An error of this type results in calculation of a lower than true μg/unit area of peak. This disadvantage can be overcome by using ^{14}C-labeled hydrocarbon which would act as an internal standard for manipulations prior to the GC analysis and not interfere with FID-GC analysis due to the trace amount of label required.

GENERAL DISCUSSION

We have demonstrated the feasibility of interlaboratory calibration for hydrocarbon analyses in marine tissues and extracts. We have also shown that an intercalibration sample of marine lipids spiked with petroleum hydrocarbons can be stored for at least two years with minimal sample alteration as far as concentrations of hydrocarbons in lipid are concerned. The data suggest that after two years the sample may begin to deteriorate. However, we think that storage of subsamples under N_2 in sealed ampules at 0 °C could overcome this problem. We have stored the bulk sample under N_2 in a brown bottle with Teflon-lined screw cap at 0 °C in the dark. Subsamples were withdrawn in batches and it may be that, during the last subsampling, the homogeneity of the sample was altered and resulted in the data for one of the June 1974 subsamples.

The results of the first intercalibration effort confirms that the present methodology we employ and which is employed by others (*1–4*) has certain limitations. Some of these limitations have been discussed in this paper.

The agreement among three laboratories for the IDOE-5 and tuna meal samples was generally good and provides guidelines for comparing data reported by any of the laboratories and allows a more comprehensive interpretation of hydrocarbon biogeochemistry in the marine environment.

Despite these advances, we have only focused on a few aspects of the problem. A more thorough and widespread intercalibration effort is needed to investigate precision and accuracy from extraction through the entire analysis up to and including gas chromatography–mass spectrometry and other instrumental methods employed in oil pollution studies (*1*). Other methods of analyses for different molecular weight ranges and types of molecules need to be intercalibrated. Analyses for specific petroleum components, such as known carcinogenic polynuclear aromatic hydrocarbons, also need to be intercalibrated. These efforts would best be served if working intercalibration materials could be provided by an organization such as the United States National Bureau of Standards.

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LITERATURE CITED

(1) National Academy of Sciences, "Inputs, Fate and Effects of Petroleum in the Marine Environment", A Report of the Ocean Affairs Board, National Academy of Sciences, Washington, D.C., 1975.
(2) "Baseline Studies of Pollutants in the Marine Environment and Research Recommendations", Office of the International Decade of Ocean Exploration, National Science Foundation, Washington, D.C., 1972.
(3) "Marine Pollution Monitoring (Petroleum)", *Nat. Bur. Stand. (U.S.), Spec. Publ.*, **409**, 29 (1974).
(4) J. W. Farrington, and P. A. Meyers, Chapter 5 in "Environmental Chemistry, Vol. I", Specialist Periodical Report No. 35, The Chemical Society, U.K., 1975.
(5) J. W. Farrington, J. M. Teal, J. G. Quinn, T. Wade, and K. A. Burns, *Bull. Environ. Contam. Toxicol.*, **10**, 129 (1973).
(6) G. C. Medeiros and J. W. Farrington, in Ref. 3, p 167.
(7) J. W. Farrington, J. M. Teal, J. G. Quinn, P. L. Parker, J. K. Winters, T. L. Wade, and K. Burns, in Ref. 3, p 163.
(8) J. W. Farrington and G. C. Medeiros, "Proceedings, 1975 Conference on Prevention and Control of Oil Pollution", American Petroleum Institute, Washington, D.C., 1975, p 115.
(9) J. G. Quinn and T. L. Wade, Marine Memorandum Series No. 33, Graduate School of Oceanography, University of Rhode Island, Kingston, R.I. 02881, 1974.

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