LEVELS OF SELECTED ORGANIC POLLUTANTS IN HADDOCK FROM GEORGES BANK - ABSOLUTE CONCENTRATIONS AND VARIABILITY BETWEEN AGE CLASSES, INDIVIDUALS AND SAMPLE POOLINGS*

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1. Administrative Information

1.1 Principal Investigator

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1.2 Organization

ERCO (Energy Resources Co. Inc.)
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1.3 Present Level of Funding

$20,010 (this segment)

1.4 Title of Investigation

Levels of Selected Organic Pollutants in Haddock from Georges Bank - Absolute Concentrations and Variability between Age Classes, Individuals and Sample Poolings

1.5 NEMP Cruises

None

1.6 Reports or Publications Produced

(see references)

1.7 NEMP Work Unit Monitor

Mr. Frank Steimle

1.8 Duration of Work Unit

23 September 1981 - 1 June 1982

2. Objectives

There were several objectives sighted as part of this program. Firstly the analytical work focused on the determination of levels of petroleum hydrocarbons (PHC) including normal alkanes, specific polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and DDT family compounds in two sample sets of haddock (Melanogrammus aeglefinus) from Georges Bank, to provide additional benchmark information on this species. Secondly by analyzing both individual fish and poolings of different numbers of indivduals from 25-cm juveniles and adult sample sets, we aimed to provide information on: (1) the differences between constituent levels in juveniles and adults; (2) the variability in organic constituent levels between individual fish, (3) the efficacy of sample pooling as producing a sample representative of a population of fish, and (4) the variability between sample pooling sizes.

Although marine organisms including fish have been analyzed in the past for a variety of inorganic and organic components there is little data on the variability of the concentrations of these chemicals between fish within a given population. Although the procedure of pooling organism tissue has been used to create a sufficient amount of sample for analysis and to derive "a number" supposedly representative of a given population, there is little data on the efficacy of this pooling procedure (i.e., homogenization of tissue subsamples from several different animals).

3. Summary of Activities and Rationale

3.1 Sampling Activities

Two sample sets of fish, one an adult Haddock sample set (30 individuals) and the other a juvenile (25 cm) Haddock sample set (30 individuals) were obtained from the Georges Bank area by NMFS personnel.

3.2 Analytical Methodology

Individual fish from each sample set were randomly selected for either individual analysis, tissue pooling or both. Six adult individuals were selected and sufficient tissue (muscle) (100 g wet) obtained for individual analyses. To obtain the triplicate pooling of six, three tissue subsamples were obtained from each fish and combined with one of three subsamples from the other five. To obtain the triplicate pooling of 12, an additional 6 fish were randomly selected to comprise the set of 12 from which samples were obtained. The pooling of 30 fish included these 12 plus 18 others.

The juveniles were treated differently owing to the smaller amount of tissue available from each fish. There was enough tissue from the 6 individuals selected to provide enough sample for the individual sample only. An additional 6 fish had to be selected to provide the sample poolings of 6 individuals. Thereafter these 6 were used to provide the pooling of 12 and 30 with, of course, an additional 6 and 12 fish respectively.

All samples were processed according to procedures previously used on NEMP programs (Boehm, 1980; Boehm and

Hirtzer, 1981). Samples were digested in 5 N aqueous KOH at room temperature overnight and then extracted five times with hexane. The hexane was dried over sodium sulfate and subjected to an alumina column cleanup to remove polar lipids. The sample extract was then charged to an alumina/silica gel column on which the extract was fractionated to yield a saturated fraction, and an aromatic fraction (PAH and PCB). The saturated fraction was analyzed by capillary gas chromatography (GC) and the PCB/PAH fraction by electron capture packed column gas chromatography (PCB) and by GC/MS with selected ion monitoring for 10 key PAH compounds. PCB and ΣDDT (as DDE) were quantified using external Aroclor and DDE calibration curves while saturates and PAH were quantified using spiked internal standards carried through the entire procedure.

4. Summary of Findings

This section presents the absolute levels of the organic parameters in the 30 samples analyzed and treats the data for each parameter statistically.

4.1 Polychlorinated Biphenyls and ΣDDT

PCB and ΣDDT (as DDE) concentrations are presented in Table 1. It is evident from the data that the adult fish population contains roughly 2 to 2.5 times more PCB (a statistically significant $\alpha = .05$ difference) and DDE than do the juveniles (approximately 30 ppb versus 12 ppb for PCB). There is moderate variation in the analytical results from the individual fish, with adult PCB levels ranging from 13.2 to 31.7 ppb ($\bar{x} = 19.7 \pm 6.3$; coefficient of variation; i.e., $S/\bar{x} = 32\%$). Considerably less variation is noted with poolings of 6 ($\bar{x} = 32.3 \pm 2.9$; coefficient of variation = 9%) and 30 ($\bar{x} = 29.1 \pm 4.8$; coefficient of variation = 16%). Variability in the pooling of 12 samples is somewhat greater ($\bar{x} = 40.4 \pm 14.2$; coefficient of variation = 35%). Juvenile haddock concentration variations are less, with individual levels ($\bar{x} = 8.2 \pm 1.3$; 16%) somewhat more variable than the poolings (coefficients of variation = 8-10%). Levels of PCB are somewhat higher than previously determined (Boehm 1980) (1-26 ppb; $\bar{x} = 6$ ppb) on adult haddock from Georges Bank.

The discrepancy in the mean of the PCB and ΣDDT measurement of the 6 adult individuals (19.7 ± 6.3 for PCB) and that for the pooling of 6 (32.3 ± 2.9; see Table 1) is surprising in view of the fact that the same fish were involved in these two sets of analysis. Analytical variability would not alone account for this difference. It is

possible that within-fish variations, i.e., PCB variations within the muscle tissue of a given fish, might be important, thus contributing to this discrepancy. However, this hypothesis was not explored in the present study.

Statistical analysis of the data using a two-tailed t test indicated that the results for the poolings of 6, 12 and 30 individuals are different from those for the individuals for both the adults and juveniles (Table 2). However, the poolings were not statistically different from one another with probabilities of similarity (i.e., interpolation of t statistic) between .06 and 0.73. Thus for PCB measurements analyses of a pooling of 6 individuals appears sufficient to characterize a population of both juvenile and adult haddock.

A similar statistical result is observed for ΣDDT concentrations. Analysis of a pooling of 6 individuals sufficiently describes the population as all pooled results are similar. Again the results for the mean of 6 individual fish are not the same as the pooled results probably owing to within-fish variations.

4.2 Saturated Hydrocarbon Levels

Although not of great toxicological interest, distributions of saturated hydrocarbons can reveal both the presence of a petroleum insult as well as dietary influences. Two sets of measurements were compiled based on GC data. The sum of the n-alkanes from n-C_{14} to n-C_{24} (see Figure 1 for a typical saturated hydrocarbon GC trace) and the concentration of the biogenic branched alkane, pristane. The former set of compounds can be acquired from petroleum

Table 1. Summary of Analytical Data for Chlorinated Organics.

ADULT HADDOCK SAMPLE	PCB (ng/g)	ΣDDT (ng/g)	JUVENILE HADDOCK SAMPLE	PCB (ng/g)	ΣDDT (ng/g)
A. Individuals			A. Individuals		
1	16.6	0.10	1	9.9	.13
2	18.9	.07	2	8.9	.10
3	13.2	.08	3	7.1	.06
4	31.7	.57	4	8.2	.08
5	18.4	.25	5	8.6	.08
6	19.7	.18	6	6.2	.04
$\bar{x}$ individuals $\pm$S	19.7$\pm$6.3	.21$\pm$.19	$\bar{x}$ individuals $\pm$S	8.2$\pm$1.3	.08$\pm$.03
B. Pooling of 6			B. Pooling of 6		
1	35.6	.40	1	10.5	.12
2	30.1	.38	2	12.5	.15
3	31.1	.33	3	11.9	.14
$\bar{x}$ pooling of 6$\pm$S	32.3$\pm$2.9	.37$\pm$.04	$\bar{x}$ pooling of 6$\pm$S	11.6$\pm$1.0	.14$\pm$.02
C. Pooling of 12			C. Pooling of 12		
1	55.5	.42	1	10.8	.18
2	38.3	.28	2	13.6	<.01
3	27.3	.35	3	11.5	.14
$\bar{x}$ pooling of 12$\pm$S	40.4$\pm$14.2	.35$\pm$.07	$\bar{x}$ pooling of 12$\pm$S	12.0$\pm$1.5	.11$\pm$.09
D. Pooling of 30			D. Pooling of 30		
1	28.5	.33	1	14.7	.19
2	34.1	.41	2	12.3	.14
3	24.6	.28	3	15.0	.18
$\bar{x}$ pooling of 30$\pm$S	29.1$\pm$4.8	.34$\pm$.07	$\bar{x}$ pooling of 30$\pm$S	14.0$\pm$1.5	.17$\pm$.03

Table 2. Comparisons of means of organic parameter results using two tailed t-test

Parameter	Comparison	PROBABILITY ALIKE[a] Adult	Juvenile	Adults vs. Juveniles
PCB				
	1 fish/6[b] fish	.01	.005	
	1 fish/12[b] fish	.02	.005	
	1 fish/30[b] fish	.06	.0005	
	6 fish/12 fish	.34	.73	
	6 fish/30 fish	.33	.06	
	12 fish/30 fish	.22	.13	
	6 fish adult/6 fish juvenile	--	--	.000
DDE				
	1 fish/6 fish	.20	.02	
	1 fish/12 fish	.001	.46	
	1 fish/30 fish	.002	.004	
	6 fish/12 fish	.66	.56	
	6 fish/30 fish	.51	.18	
	12 fish/30 fish	.59	.29	
	6 fish adult/6 fish juvenile	--	--	.000
ALKANES				
	1 fish/6 fish	.000	.009	
	1 fish/12 fish	.000	.000	
	1 fish/30 fish	.008	.03	
	6 fish/12 fish	.006	.51	
	6 fish/30 fish	.98	.62	
	12 fish/30 fish	.09	.86	
	6 fish adult/6 fish juvenile	--	--	.93
PRISTANE				
	1 fish/6 fish	.61	.000	
	1 fish/12 fish	.67	.000	
	1 fish/30 fish	.39	.002	
	6 fish/12 fish	.000	.13	
	6 fish/30 fish	.37	.55	
	12 fish/30 fish	.19	.33	
	6 fish adult vs. 6 fish juvenile	--	--	.55

[a](>.05 = same at the 95% level)

[b]6, 12, 30 denotes poolings of these numbers of fish

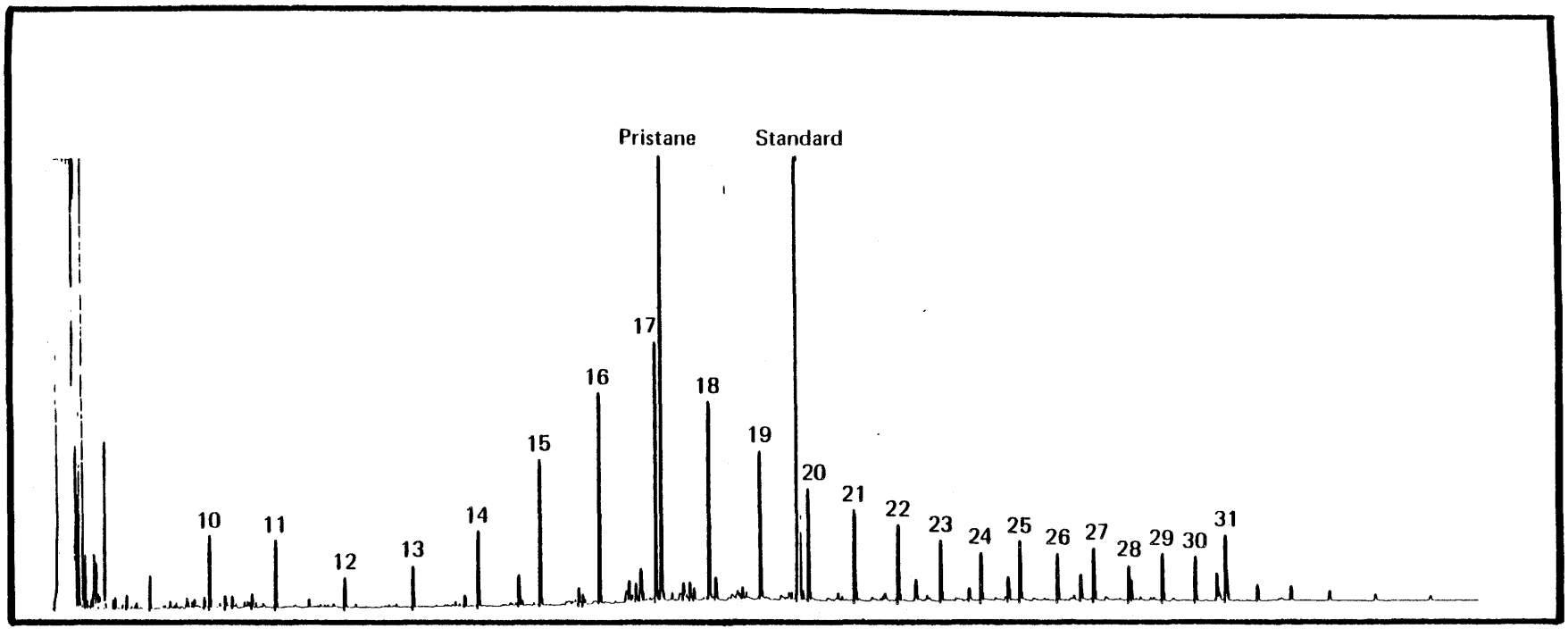

Figure 1. Typcial saturated hydrocarbon capillary GC trace of juvenile and adult haddock.

(although the n-alkanes n-C_{15} and n-C_{17} also have contributing biogenic sources) and the latter through normal feeding routes.

Results for the n-alkanes and pristane are presented in Table 3. There appears to be no direct relationship between the variability in the measurement (coefficient of variation $\bar{x}/s$) and the number of individuals pooled. For example the variability in the n-alkane numbers, $\pm$11%, for the adult pooling of 6, decreases to 5% for 12 individuals, but increases to $\pm$43% for 30 individuals. In addition the pooling of 12 result, 610 $\pm$ 28 ppm is significantly (α = .05) greater than either of the other two poolings. The results for the juveniles indicate higher n-alkane and pristane levels in all of the poolings than in the mean of the 6 individuals.

Results for the saturated hydrocarbons indicate the unlike the PCB results which indicate that the adults have higher levels than the juveniles, the n-alkanes and pristane are statistically similar in the two sample sets (Table 2).

As Table 2 indicates, the individual fish analyses yield results significantly different than the poolings. For both the adult and juveniles, results from a pooling of 6 individuals adequately describe the n-alkane concentrations. However, note that pooling of 12 adults yielded a significantly different result from the pooling of 6 and only having a marginal probability of similarity with the pooling of 30 (p = .09).

Due to the large coefficient of variation ($\bar{x}/s$ = .83) in the adult individual pristane numbers, the mean of the adult individuals (204 ppb) is similar to all of the pooling

Table 3. Summary of Analytical Data for Saturates.

ADULT HADDOCK SAMPLE	N-Alkanes (ng/g)	Pristane (ng/g)	JUVENILE HADDOCK SAMPLE	N-Alkanes (ng/g)	Pristane (ng/g)
A. Individuals			A. Individuals		
1	190	360	1	140	200
2	160	460	2	160	170
3	110	110	3	190	160
4	63	30	4	200	80
5	110	180	5	170	210
6	120	80	6	140	80
$\bar{x}$ individuals $\pm$S	123$\pm$43	204$\pm$170	$\bar{x}$ individuals $\pm$S	167$\pm$24	148$\pm$58
B. Pooling of 6			B. Pooling of 6		
1	390	140	1	410	290
2	330	160	2	210	270
3	410	150	3	520	370
$\bar{x}$ pooling of 6$\pm$S	370$\pm$42	150$\pm$8.9	$\bar{x}$ pooling of 6$\pm$S	380$\pm$155	310$\pm$54
C. Pooling of 12			C. Pooling of 12		
1	590	260	1	470	370
2	630	270	2	410	360
3	-	-	3	520	360
$\bar{x}$ pooling of 12$\pm$S	610$\pm$28	262$\pm$5.7	$\bar{x}$ pooling of 12$\pm$S	460$\pm$57	364$\pm$5.5
D. Pooling of 30			D. Pooling of 30		
1	360	200	1	790	390
2	540	240	2	240	290
3	210	110	3	390	330
$\bar{x}$ pooling of 30$\pm$S	370$\pm$160	180$\pm$63	$\bar{x}$ pooling of 30$\pm$S	470$\pm$290	340$\pm$70

results. The pooling of 12 fish appears to yield a high result as it did for the n-alkane mean as well. Juvenile haddock pristane determinations require a minimum pooling of 6 individuals to yield an adequate result.

4.3 Aromatic Hydrocarbons

GC/MS analyses of the juvenile and adult poolings indicate very low levels (Table 4) of three-ringed (phenanthrene) and four-ringed (fluoranthene, pyrene) aromatics and no detectable levels of the toxic alkylated naphthalene compounds. In general as aromatic compounds get larger by either increased ring size or methyl substitution up through the alkyl-phenanthrenes and fluoranthene, they become more toxic. Beyond this molecular size the molecules become less soluble in water and hence less readily available in the sense of resulting in acute toxicity. However many of the five-ringed compounds are carcinogenic (see Tables 5 and 6) once introduced to the animal via uptake routes other than through the water soluble fraction (i.e., sediment uptake, or uptake of contaminated food). Thus the PAH compounds are both acute toxicants and carcinogens depending on molecular size.

Levels of phenanthrene, for example (Table 4), are the same in all pooling sizes for the adults (approximately 3-4 ppb) as well as for the poolings of 6 and 30 organisms for the juveniles. The juvenile pooling of 12 is higher in concentration than either the pooling of 6 or 30. Furthermore, note that pyrene is detected in this pooling yet was less than 1 ppb (=nd) in all other juvenile samples examined. Thus due to the low PAH levels encountered, heterogeneity perhaps within the muscle tissue of a given fish resulted in this slight variation.

Table 4. Summary of Analyses for Haddocks.

ADULT HADDOCK SAMPLE	PHEN.[a] (ng/g)	FLUOR.[a] (ng/g)	PYR[a] (ng/g)	JUVENILE HADDOCK SAMPLE	PHEN.[a] (ng/g)	FLUOR.[a] (ng/g)	PYR[a] (ng/g)
A. Individuals				A. Individuals			
1	NA	NA	NA	1	NA	NA	NA
2	NA	NA	NA	2	NA	NA	NA
3	NA	NA	NA	3	NA	NA	NA
4	NA	NA	NA	4	NA	NA	NA
5	NA	NA	NA	5	NA	NA	NA
6	NA	NA	NA	6	NA	NA	NA
$\bar{x}$ individuals $\pm$S	NA	NA	NA	$\bar{x}$ individuals $\pm$S	NA	NA	NA
B. Pooling of 6				B. Pooling of 6			
1	3.0	ND	1.0	1	2.4	ND	ND
2	5.3	ND	7.5	2	1.0	ND	ND
3	1.3	ND	1.0	3	5.5	ND	ND
$\bar{x}$ pooling of 6$\pm$S	3.2$\pm$2.0	ND	3.2$\pm$3.8	$\bar{x}$ pooling of 6$\pm$S	3.0$\pm$2.3	ND	ND
C. Pooling of 12				C. Pooling of 12			
1	4.0	ND	1.0	1	6.3	ND	1.5
2	6.4	ND	6.1	2	5.8	ND	1.0
3	1.0	ND	1.0	3	5.1	ND	1.1
$\bar{x}$ pooling of 12$\pm$S	3.8$\pm$2.7	ND	2.7$\pm$2.9	$\bar{x}$ pooling of 12$\pm$S	5.4$\pm$1.1	ND	1.2$\pm$2.6
D. Pooling of 30				D. Pooling of 30			
1	5.2	ND	4.3	1	3.7	ND	ND
2	2.1	ND	1.0	2	3.1	ND	ND
3	0.6	ND	0.5	3	1.9	ND	ND
$\bar{x}$ pooling of 30$\pm$S	2.6$\pm$2.3	ND	2.2$\pm$1.9	$\bar{x}$ pooling of 30$\pm$S	2.9$\pm$.9	ND	ND

NA = Not analyzed
ND = < 1 ng/g
a = Only aromatics detected out of target list of twenty compounds

Table 5. Acute toxicity, measured as LC50 (Concentration causing 50% mortality in the time indicated), to selected species of marine animals (from Neff, 1982)

Compound	Species	Concentration (ppm)	Effect[a]
Naphthalene	Neanthes arenaceodentata (marine polychaete)	3.8	96h LC50
	Cancer magister (Stage I Zoeae, Dungeness crab)	2.0	96h LC50
	Elasmopus pectenicrus (marine amphipod)	2.7	96h LC50
	Eurytemora affinis (marine copepod)	3.8	24h LC50
	Palaemonetes pugio (grass shrimp)	2.4	96h LC50
	Cyprinodon variegatus (sheepshead minnow)	2.4	24h LC50
	Oncorhynchus kisutch (coho salmon fry)	3.2	96h LC50
1-Methylnaphthalene	Cancer magister	1.9	96h LC50
	Cyprinodon variegatus		
2-Methylnaphthalene	Cancer magister	1.3	96h LC50
	Palaemonetes pugio	1.1	96h LC50
	Eurytemora affinis	1.5	24h LC50
	Cyprinodon variegatus	2.0	24h LC50
2,6-Dimethylnaphthalene	Neanthes arenaceodentata	2.6	96h LC50
	Palaemonetes pugio	0.7	96h LC50
	Eurytemora affinis	0.9	96h LC50
2,3,6-Trimethyl-naphthalene	Neanthes arenaceodentata	2.0	96h LC50
2,3,5-Trimethyl-naphthalene	Eurytemora affinis	0.3	24h LC50
Fluorene	Neanthes arenaceodentata	1.0	96h LC50
	Palaemonetes pugio	0.3	96h LC50
	Cyprinodon variegatus	1.7	96h LC50
Phenanthrene	Neanthes arenaceodentata	0.6	96h LC50
	Palaemonetes pugio	0.3	96h LC50
1-Methylphenanthrene	Neanthes arenaceodentata	0.3	96h LC50
Fluoranthene	Neanthes arenaceodentata	0.5	96h LC50
Chrysene	Neanthes arenaceodentata	1.0	NAT[b]
Benzo(a)pyrene	Neanthes arenaceodentata	1.0	NAT
Dibenz(ah)anthracene	Neanthes arenaceodentata	1.0	NAT

[a]LC50 = median lethal concentration in the time specified.

[b]NAT = not acutely toxic in 96 hours.

Table 6. Relative carcinogenicity of PAH to laboratory mammals (from NAS, 1972).

Compound	Carcino-genicity[a]	Compound	Carcino-genicity[a]
Anthracene	-	Aceanthrylene	-
Phenanthrene	-	Benz(j)aceanthrylene = cholanthrene	++
Benz(a)anthracene	+	3-Methycholanthrene	++++
7,12-Dimethylbenz(a)-anthracene	++++	Naphthacene	-
Dibenz(aj)anthracene	+	Pyrene	-
Dibenz(ah)anthracene	+++	Benzo(a)pyrene	+++
Dibenz(ac)anthracene	+	Benzo(e)pyrene	-
Benzo(a)phenanthrene	+++	Dibenzo(al)pyrene	±
Fluorene	-	Dibenzo(ah)pyrene	+++
Benzo(a)fluorene	-	Dibenzo(ah)pyrene	+++
Benzo(b)fluorene	-	Dibenzo(cd,jk)pyrene	-
Benzo(c)fluorene	-	Indeno(1,2,3-cd)pyrene	+
Dibenzo(ag)fluorene	+	Chrysene	±
Dibenzo(ah)fluorene	±	Dibenzo(b,def)chrysene	++
Dibenzo(ac)fluorene	±	Dibenzo(def,p)chrysene	+
Fluoranthene	-	Dibenzo(def,mno)chrysene = anthanthrene	-
Benzo(b)fluoranthene	++	Perylene	-
Benzo(j)fluoranthene	++	Benzo(ghi)perylene	-
Benzo(k)fluoranthene	-	Coronene	-
Benzo(mno)fluoranthene	-		

[a] - not carcinogenic
± uncertain or weakly carcinogenic
+ carcinogenic
++, +++, ++++ strongly carcinogenic.

In any event, levels of PAH in these haddock are very low. Due to metabolic transformations of aromatic hydrocarbons to polar metabolites one would not expect to find that elevated concentrations would be long-lived in these fish unless continuous (chronic) uptake was occurring or unless the fish had been exposed to a recent dosage of petroleum hydrocarbons. Neither appears to have been the case here.

There is no statistical reason for going beyond a pooling of 6 individuals to yield a valid PAH result.

5. Interpretation of Findings

The data on PCB, ΣDDT, saturated and aromatic hydrocarbons illustrate that almost without exception one can have confidence that the mean of triplicate analyses of poolings of tissue from 6 individuals is sufficient to yield a valid baseline data point for chemical body burdens of fish. Whether this conclusion is valid for more highly polluted scenarios (e.g., spills, chronic discharges, etc.) wherein greater patchiness might be expected, is not known. Similarly it is not clear whether one can extrapolate these results to benthic species. However, in view of the fact that one would expect sessile bivalves to integrate exposure over time at a specific location, it can be assumed for the time being that statistical results vis-a-vis pooling results would be similar to non-sessile fish.

The lack of statistical agreement between juvenile individuals and the poolings can be explained by the fact that different individuals were being pooled. However, the parallel result for the adults is puzzling and may be due to the within-fish variability, a factor not examined in this study.

As predicted, the coefficient of variation of many of the parameter measurements is higher in the analysis of individuals than in the poolings. For example the variability of the PCB ΣDDT measurements is higher in the adult individuals than in the pooling of 6. The coefficient of variation then increases again in the pooling of 12 before decreasing again to approximately 15-20% in the pooling of 30 adults. Hydrocarbon analyses (alkanes and pristane) are subject to somewhat greater variability.

Thus it can be concluded that:

1. PCB and ΣDDT pollutant levels in adult haddock are greater than for juveniles by a factor of 2-3.

2. Levels of saturated hydrocarbons in these baseline samples are the same in juveniles and adults.

3. The steady-state aromatic hydrocarbon levels in adults and juveniles are similar.

4. A pooling of 6 individuals yields an analytical result adequate to describe pollutant levels in a given population of fish in baseline samplings.

6. References

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