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ABSTRACT

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This report describes research performed in 1984 by the Halifax and St. Andrews components of the Fisheries Contaminants and Toxicology Section, Fisheries and Environmental Sciences Division. The research dealt with trace metals in shellfish, interlaboratory calibrative studies, polycyclic aromatic hydrocarbons in lobsters, steroid hormone isolation and identification in lobsters, the effects of acid precipitation on sexual maturation and reproduction in caged Atlantic salmon, pesticide hazards, the VINLAND wellsite blowout, and a hatchery fish kill due to the fungicide OBPA.

RÉSUMÉ

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On décrit dans le présent rapport les travaux de recherche effectués en 1984 par les composantes Halifax et St. Andrews de la Section des polluants et de la toxicologie des pêches, Division des sciences halieutiques et environnementales. Les travaux ont porté sur les métaux présents à l'état de traces chez les crustacés et les mollusques, l'étalonnage entre laboratoires, les hydrocarbures aromatiques polycycliques du homard, l'isolement et l'identification des hormones stéroïdiennes chez ce dernier, l'effet des précipitations acides sur la maturation sexuelle et la reproduction de saumons atlantiques maintenus en cages, les dangers que présentent les pesticides, l'éruption au site pétrolifère VINLAND et, enfin, les mortalités massives de poissons d'élevage causées par le fongicide OBPA.

MARINE ENVIRONMENTAL CONTAMINATION

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Marine environmental contamination studies are designed to determine the levels of a variety of chemical contaminants in commercially important and other marine species, and spatial and temporal changes in such levels. Levels of contaminants in selected fishery products are also investigated to ensure the continued marketability of such products originating from areas of potential or actual contamination. Research during the past year has concentrated on trace metals in lobsters from Belledune Harbour [cadmium (Cd), copper (Cu), arsenic (As), silver (Ag), and lead (Pb)], Cd in scallops captured in clean and contaminated areas, polycyclic aromatic hydrocarbons (PAH) in lobsters from Sydney Harbour and on analytical methods for these contaminants.

TRACE METALS IN LOBSTER CAPTURED IN THE AREA OF BELLEDUNE, NEW BRUNSWICK

Cd levels in lobsters (*Homarus americanus*) captured at specified sites within and around Belledune Harbour, New Brunswick, have been monitored since 1980 (Uthe 1984). During 1984, in addition to determining Cd concentrations in digestive glands and cooked meat samples, Cu, As, Ag, and Pb concentrations were measured in selected samples of cooked lobster meat.

Mean Cd concentrations changed little between 1983 and 1984 compared to changes observed earlier (Table 1; Uthe et al. 1983, 1984). Changes in mean Cd levels are obscured by differences in the weights of captured animals in 1983 and 1984. Overall, there appears to be a tendency for the levels to have decreased slightly.

Cd concentrations in cooked meat have decreased since monitoring was initiated in 1981 (Table 2) to the point where it is possible to consider reopening of Belledune Harbour to lobster fishing. Cd concentrations in digestive glands from harbour lobsters are still too high ($\bar{X}_g > 70$ mg Cd/kg wet wt) to allow lobsters from the harbour to be sold on the open market. Harbour lobsters could be processed in the same manner as lobsters from the controlled fishery zone surrounding the harbour by removing and cooking the tails and claws while disposing of the remainder, including the digestive gland. It would be cumbersome to keep the lobsters captured in the harbour separate from those captured in the controlled fishery zone. One possibility is the combination of the present harbour and controlled fishery zone as a new controlled fishery zone. All of the lobsters captured within this new zone would be processed as described above. The catches within the harbour and the present controlled fishery zone are similar. It is therefore possible to calculate what the Cd concentration would have been had a combined controlled fishery zone been in effect and to compare these levels with those actually found in cooked meat from the present controlled fishery zone (Table 2). Had a product been prepared from a single controlled fishery zone (including the harbour catch) in 1984, the mean Cd level would have been 0.20 mg/kg, a level equal to that in product prepared annually from the controlled fishery zone since 1980 where a concentration of 0.20 mg Cd/kg wet wt was judged safe for sale (Uthe et al. 1982).

There does not seem to be any compelling reason based on Cd levels to continue a complete ban on lobster fishing within Belledune Harbour.

Following a recommendation from National Health and Welfare officials, we have determined Pb, Cu, As, and Ag concentrations in cooked lobster meat (Table 3). Only Pb concentrations were elevated in harbour cooked meat samples compared to concentrations in samples from outside Belledune Harbour. The mean Pb concentration (1.19 mg/kg wet wt) in cooked meat from Harbour West lobsters, while elevated compared to the Control Site mean of 0.052 mg Pb/kg wet wt, was within the range of Pb levels reported in a study of commercial lobster meat (0.01-2.3 mg Pb/kg wet wt; mean value = 0.65; N = 115) carried out by the Inspection Service of the Department of Fisheries and Oceans in the early 1970's (A. Gervais, personal communication). This same group reported that Pb levels in cooked meat commercial packs prepared from lobsters captured within the controlled fishery zone around Belledune Harbour in 1981 ranged from 0.05 to 1.56 mg Pb/kg wet wt. Thus Pb levels in cooked lobster meat from Belledune Harbour lobster do not appear to pose any threat to human health.

Concentrations of As, Cu, and Ag in Belledune Harbour meat were not significantly higher than those in meat from lobsters sampled outside the harbour, i.e. Heron Island. Chou and Uthe (1978) reported a geometric mean Cu concentration of 26.3 mg/kg in uncooked lobster claw muscle from animals captured at Petit Rocher, New Brunswick, a site approximately 17 km downstream of Belledune Harbour in the Bay of Chaleur. Reid et al. (1982) reported that Cu concentrations in lobster muscle ranged from 2.27 to 15.48 mg/kg wet wt. No information was given on which muscles were included in the sample. The sample was collected in the area of the Long Island Sound-New York Bight. Levaque Charron (1981) reported that Cu concentrations in pooled, uncooked lobster claw and tail meat from Belledune Harbour ranged between 10 and 27 mg/kg wet wt while two animals captured at a 'distant' site contained 19 and 35 mg/kg. We do not know if cooking elevates Cu levels in the same manner as it does for Cd where cooking resulted in higher Cd levels in the cooked meat (Uthe et al. 1982). The foregoing suggests that little, if any, Cu contamination is present in cooked meat from lobsters captured in Belledune Harbour. This is not surprising since studies on Cu levels in juvenile lobsters suggest that muscle Cu levels are under biological control and that the major Cu storage organ is the digestive gland.

The mean concentration of As in cooked meat from harbour lobsters (3.92 mg/kg) was lower than levels reported by Uthe et al. (1974) for uncooked lobster tail muscle. The Department of Fisheries and Oceans found a mean of 7.6 mg As/kg cooked lobster meat in commercial samples (Gervais, personal communication). These data suggest that Belledune Harbour lobsters contain lower average levels of As than are generally present. Silver concentrations are within the range reported by Reid et al. (1982) in lobster muscle tissue (0.01-0.73 mg/kg).

Overall, there is no demonstrable elevation of concentrations of Cu, As, and Ag in cooked meat prepared from lobsters captured within Belledune Harbour nor any compelling reason based on the concentrations of these metals to continue the ban

Table 1. Geometric mean Cd levels (mg/kg wet wt) in cooked meat and uncooked digestive gland from lobsters captured in the area of Belledune Harbour, New Brunswick, in 1983 and 1984. (Sample sites are described in Uthe et al. 1982).

Sample Site	Year	Mean animal weight (g) \pm s.d.	N	Cooked meat \bar{X}_g	Range	Digestive gland \bar{X}_g	Range
Within Belledune Harbour							
Harbour West	1983	465 \pm 252	25	0.480	0.110-1.59	74.2	11.3 -348
	1984	539 \pm 243	30	0.427	0.054-2.69	75.2	11.8 -262
Harbour East	1983	467 \pm 177	11	0.240	0.070-1.53	44.5	12.0 -254
	1984	632 \pm 293	15	0.203	0.069-1.08	77.0	8.24-537
Within Controlled Fishery Zone							
L1E	1983	297 \pm 117	11	0.110	0.020-0.660	29.4	8.39-107
	1984	491 \pm 195	15	0.167	0.010-1.80	33.1	1.81-204
L4E	1983	436 \pm 235	14	0.140	0.030-1.32	20.7	9.75-157
	1984	480 \pm 190	15	0.070	0.019-0.214	17.3	8.14- 82.1
Outside Controlled Fishery Zone							
L1W	1983	373 \pm 136	12	0.022	0.052-1.06	17.4	5.98-193
	1984	446 \pm 196	15	0.079	0.010-0.450	19.9	4.52-170
Petit Rocher	1983	310 \pm 56	12	0.044	0.020-0.069	9.02	2.63- 20.5
	1984	310 \pm 103	15	0.033	0.014-0.052	7.90	4.10- 25.8
Heron Island	1983	410 \pm 211	13	0.020	0.010-0.028	4.50	1.88- 9.91
	1984	387 \pm 181	15	0.011	0.010-0.028	3.30	1.60- 13.8

Table 2. Estimated mean Cd levels (mg/kg wet wt) in cooked lobster meat commercial packs prepared from various combinations of lobsters captured in the area of Belledune Harbour.

Observed Cd levels					Calculated Cd levels in commercial packs		
Year	Harbour West	Harbour East	L1E	L4E	B Hb ^a	CFZ ^b	B Hb+ CFZ ^c
1981	0.89	0.35	0.21	0.19	0.62	0.20	0.41
1982	0.74	0.23	0.18	0.11	0.49	0.15	0.32
1983	0.480	0.240	0.110	0.140	0.36	0.13	0.24
1984	0.427	0.203	0.167	0.072	0.32	0.12	0.20

^aHarbour estimate (Harbour West/2 + Harbour East/2).

^bControlled Fishery Zone estimate (L1E/2 + L4E/2).

^cHarbour estimate/2 + Controlled Fishery Zone estimate/2.

Table 3. Mean levels (mg/kg wet wt) \pm s.d. of trace metals in cooked meat from lobsters captured in the area of Belledune Harbour, New Brunswick. (Capture year is in parentheses; number of animals analyzed is in parentheses following mean metal concentration).

Sample site	Pb (1984)	Cu (1983)	As (1983)	Ag (1983)
Heron Island (Control)	0.052 \pm 0.014(16)	19.60 \pm 3.16(10)	2.30 \pm 0.41(10)	0.407 \pm 0.862(10)
Harbour West	1.199 \pm 0.690(30) ^a	22.77 \pm 3.62(10)	3.92 \pm 2.75(10)	0.493 \pm 0.127(10)
Harbour East	1.137 \pm 0.658(15) ^a	-	-	-
L1W	- ^b	19.74 \pm 6.65(10)	1.93 \pm 0.68(10)	0.432 \pm 0.215(10)

^aSignificantly different from Heron Island ($p \leq 0.05$).

^bNot determined.

on lobster fishing within the harbour. Reopening the harbour as part of a controlled fishery zone would have an additional consumer benefit. Tagging-recapture studies, carried out in 1980-1981 on lobsters captured and released within Belledune Harbour, showed that 20.8% of the lobsters tagged in 1980 were recaptured outside the harbour during the 1981 commercial fishery. Of the total number of tagged lobsters recaptured, 46% were recaptured outside the current controlled fishery zone (Uthe et al. 1982). This reflects the usual wandering rate of lobsters in the area (Levaque Charron and Eljarbo 1981). Lobsters wandering out of the harbour into the open fishery are sold within the normal commercial operation. Fishing the harbour in the manner suggested above would prevent a significant number of lobsters from wandering out of the harbour into the commercial fishery. Overall the effect would be a reduction in consumer exposure to Cd, especially for those in the vicinity of Belledune Harbour who consume relatively large numbers of lobsters compared to the population at large.

Cd IN SCALLOPS

In continuing our investigation (Uthe 1984) of Cd in sea scallops (*Placopecten magellanicus*) we have expanded the study zone from Georges Bank to other areas of the fishery, namely, Browns Bank and two commercial areas within the Bay of Chaleur, off Belledune Harbour, and Petit Rocher, New Brunswick, both near a major source of Cd contamination (Uthe and Zitko 1980). A sample was also obtained from the Mascarene area of Passamaquoddy Bay. All scallops were transported to the Halifax Fisheries Research Laboratory and held live in the seawater aquarium until sampled, generally less than 1 wk. The effect of holding on Cd levels and tissue burdens was assessed in scallops from Georges Bank which were held without feeding for 14 mo. Cd was determined in acidic tissue digests, using either flame or graphite furnace atomic absorption spectrophotometry. In general, Cd was measured only in the digestive gland and the adductor muscle. Last year Uthe (1984) reported that over 90% of the

total amount of Cd in the soft tissue of scallop was present in the digestive gland and that this tissue may be appropriate for investigating temporal and spatial trends. The amount of Cd in the adductor muscle, although only a small percentage of the total Cd present in the animal, is also important because of human consumption of this tissue and the concern that it can contain excessive amounts of Cd in certain instances. Sometimes the soft tissues remaining after removal of the digestive gland and the adductor muscle were pooled, homogenized and analyzed as a 'tissue residue'. Samples were removed from the living animal as rapidly as possible. Fluid was lost from the soft tissues during dissection but no Cd could be detected in this fluid. Fluid loss from starved scallops was particularly great with the recovered tissue (digestive gland plus adductor muscle plus residue) weight being only 35% of the calculated (total weight of animal minus shell weight) amount (Table 4).

To investigate the utility of scallops as monitors of Cd contamination in their environment, we measured Cd concentrations and tissue burdens in similarly sized scallops (approximately 100 mm shell height) from each site (Table 4, 5). It was not possible to properly rank the four areas by simply comparing either concentrations or burdens by using either the adductor muscle or the digestive gland values. For example, consideration of digestive gland burdens would suggest that Browns Bank is more contaminated than the Bay of Chaleur (Belledune and Petit Rocher sites), whereas the adductor muscle burdens indicate the opposite! These results suggest that tissue Cd values are of limited use in ranking areas of chemical contamination in any straightforward, meaningful manner. It is likely that tissue levels/burdens are a complex response controlled not only by the input of Cd to the animals' immediate environment, but also by factors such as age and nutritional status of the animal.

It is interesting to compare Cd ratios of digestive gland to adductor muscle for both

Table 4. Mean total animal and tissue weights in 100-mm scallops from various locations within the Maritimes area.

Location	Weight (g)				
	Total (Weighed)	Adductor muscle	Digestive gland	Shell	Tissue residue
Petit Rocher	101.0 +10.8	8.18(8.1) ^a +1.01	2.15(2.13) +0.17	55.88(55.3) + 6.17	16.17 +2.51
Belledune Harbour	128.4 +10.8	12.71(9.90) +0.90	2.74(2.13) +0.67	71.07(55.4) + 9.06	-
Browns Bank	153.3 + 8.6	7.33(4.78) +0.79	2.72(1.78) +0.30	107.30(78.0) + 8.47	20.58 +3.15
Passamaquoddy Bay (Mascarene)	179.1 +34.1	14.23(7.9) +3.81	2.83(1.58) +0.53	124.09(69.3) +35.13	18.92 +3.37
Georges Bank	141.8 +10.4	16.52(11.65) +1.44	3.33(2.35) +0.40	81.53(57.5) + 4.97	23.57 +3.10
Georges Bank ^b (held live for 14 mo)	146.0 ^{ns} +22.0	4.60 ^s (3.15) +1.33	1.45 ^s (0.99) +0.27	91.04 ^{ns} (62.4) +17.44	13.37 ^s +1.19

^aFigure in parentheses is % of total weight.

^bns and s indicate not significant and significant ($p < 0.05$) differences compared to Georges Bank values.

Table 5. Mean (\pm s.d.) Cd values in scallop (100 mm) tissues from various locations within the Maritimes area.

Location	mg Cd/kg wet wt			Tissue burden (μ g Cd)			
	Adductor muscle	Digestive gland	Tissue residue	Adductor muscle	Digestive gland	Tissue residue	Total burden
Petit Rocher	0.348 +0.119	78.38(225) ^a +15.27	1.51 +0.33	2.82 +0.89	166.3(60) ^a + 21.4	24.28 + 4.80	191.4 +24.8
Belledune Harbour	0.257 +0.066	61.15(237) +11.72	-	3.30 +1.05	164.5(50) + 38.9	-	-
Browns Bank	0.338 +0.125	291.3(861) +44.7	-	2.46 +0.90	789.7(321) +119.3	-	-
Passamaquoddy Bay (Mascarene)	0.121 +0.224	69.41(574) +12.41	-	1.70 +0.36	183.8(103) + 40.7	-	-
Georges Bank	0.121 +0.033	98.25(812) + 9.32	1.08 +0.44	1.93 +0.54	313.8(163) + 35.3	24.81 + 7.97	340.4 +33.2
Georges Bank ^b (held live for 14 months)	0.756 ^s +0.257	217.7 ^s (287) +43.0	1.90 ^{ns} +0.79	3.25 ^s +0.93	307.3 ^{ns} (95) + 26.5	25.95 ^{ns} +10.07	336.5 ^{ns} +18.7

^aRatio of mean digestive gland Cd to mean adductor muscle Cd for concentration and tissue burden.

^bns and s indicate not significant or significant ($p < 0.05$).

concentrations and tissue burdens (Table 5). The ratios for Petit Rocher and Belledune Harbour scallops are much lower than those for scallops from other areas. This may reflect increased exposure of the Petit Rocher and Belledune Harbour scallops to higher environmental levels of Cd which would result in relatively higher muscle levels if the deposition rate of Cd in the digestive gland is somewhat limited compared to the uptake of Cd by the animal. Some evidence for this was suggested by the results from starving Georges Bank scallops. Georges Bank scallops held for 14 mo in our aquarium without feeding showed that little, if any, Cd is lost from the animal over time since the total mean burden of Cd in starved animals was $336 \pm 18.7 \mu\text{g}$ compared to $340.4 \pm 33.2 \mu\text{g}$ prior to starvation (Table 5). Cd concentrations in both digestive gland and adductor muscle rose dramatically over the period of starvation, not surprisingly since both tissues lost significant mass (Table 4). Cd burden in the digestive gland was essentially unchanged by starvation while the adductor muscle burden was raised significantly. The cause of this elevation is unknown. It is possible that it reflects an internal redistribution of Cd caused by starvation or external Cd input which ends up in muscle due to the inability of the digestive gland to take it up during catabolism. The small absolute amount of Cd being considered here is lost in the variation in tissue burdens. If this hypothesis is correct, scallops may prove to be a useful monitoring animal through consideration of tissue ratios of metal levels.

The observed effects of starvation on tissue mass, Cd concentration, and Cd burden offer an explanation of the Browns Bank observations. Browns Bank scallops had low adductor muscle to total animal weight ratios compared to scallops from other areas. The digestive gland-total animal weight ratios were also lower than most, Passamaquoddy Bay also being low. This suggests that Browns Bank scallops are in a state of marginal nutritional adequacy. One can thus postulate a mechanism where animals receiving either minimal amounts of their usual foodstuffs or nutritionally less desirable foodstuffs would have relatively high tissue levels and burdens of Cd even in an area of minimal anthropogenic input of Cd.

The use of similarly sized animals in ranking contaminated areas is also subject to the criticism that each area would yield animals of different ages. The lack of appreciable Cd elimination in scallops makes this a particularly valid criticism. We have investigated the effect of age on Cd values in adductor muscle and digestive gland (Table 6). In this table, age is relative, based simply on the number of growth rings observed on the shell of each animal. As expected, tissue burdens increased with relative age, as did concentrations. The animals were also aged (years) by two professionals within the Department. The ages reported were numerically lower than the relative ages reported here but there were differences in the data reported by the two individuals. In the case of Georges Bank scallops the results reported by one individual differed from the other by an average of -0.25 ± 0.69 yr. While not a significant mean difference, the results showed that this individual's results were consistently lower than the other's when aging scallops of less than 90-mm shell height and higher with scallops of greater than 90-mm shell height. In the case of Passamaquoddy Bay scallops the error averaged -1.62 ± 1.01 yr, with the magnitude of the

error tending to increase with shell height. Because of these findings we did not use age in Table 6. Table 6 also compares the Georges Bank-Passamaquoddy Bay ratio for mean Cd values for each relative year-class. Based on concentrations, the relative ratios of Cd in adductor muscles or digestive glands for Georges Bank to Passamaquoddy Bay were quite constant (Table 6). The concentrations of Cd in digestive gland increased with increasing relative age for both sites. Cd concentrations in adductor muscle increased only slightly, if at all, with increasing relative age. Tissue burdens, reflecting both the increasing concentrations and sizes of the two tissues with age, also increased with relative age. In the case of the youngest scallops (relative age - 6 and 7), the digestive Cd burden ratios for the two sampling sites were greater than those for older animals (8-13). Since the same was not observed with concentration ratios, this likely reflects changes in digestive gland masses with age between the two sites. The increase in Cd values with increasing age in both digestive gland and adductor muscle can be described mathematically (Table 7). The tissue burden in the adductor muscle gave a much higher coefficient of determination than did concentration (0.865 vs 0.122). Of the relationships found between Cd values and age or shell height, the best relationship was that between the tissue burden of Cd in the digestive gland and shell height ($r = 0.978$). The age relationships were less highly correlated, probably reflecting the difficulty encountered in aging the animals and the large contribution of an error of one unit of age.

Studies are continuing; however the results obtained to date show that:

- 1) No significant human health concerns are present with regard to Cd levels in commercial scallop product (the adductor muscle) even in areas of known anthropogenic Cd input.
- 2) Extremely high Cd levels are present in scallop digestive gland.
- 3) Scallops did not lose Cd from their soft tissues (tissue burden) although tissue concentrations were markedly increased by starvation. Starvation resulted in a significant increase in Cd in the adductor muscle (both concentration and tissue burden).
- 4) Cd values in both adductor muscle and digestive gland increased with increasing shell height. There was a relatively consistent ratio in Cd values between Georges Bank and Passamaquoddy Bay for both adductor muscle and digestive gland for animals of the same relative age, with the exception of digestive gland burdens in young scallops.
- 5) Significant relationships were found between Cd values and age/shell height with the best relationship between shell height and Cd burden in the digestive gland. The less significant relationship with age was probably due to difficulties encountered in aging the animals.

Scallop Cd levels may reflect environmental levels of Cd in a rational manner; however, the overriding influence of the scallop's internal metabolism suggests that Cd values in soft tissues are not related to environmental Cd levels in a

Table 6. Cd mean values (+s.d.) vs relative age for scallops from Passamaquoddy Bay and Georges Bank. [Cd] - concentration in mg/kg wet wt; Cd_T-μg Cd in tissue; subscripts A and DG are adductor muscle and digestive gland.

Relative age ^a	N	Passamaquoddy Bay				N	Georges Bank			
		[Cd] _A	[Cd] _{DG}	Cd _{TA}	Cd _{TDG}		[Cd] _A	[Cd] _{DG}	Cd _{TA}	Cd _{TDG}
6	3	0.120 +0.056	53.57 + 6.31	0.286 +0.061	29.17 + 15.88	5	0.094(0.78) ^b +0.105	87.32(1.63) +21.41	0.560(1.96) +0.376	106.0(3.67) + 79.4
7	9	0.099 +0.035	55.64 + 8.41	0.429 +0.193	48.97 + 18.23	8	0.083(0.84) +0.023	92.63(1.66) +11.93	0.670(.136) +0.161	149.3(2.96) + 31.1
8	8	0.130 +0.030	64.28 +16.31	1.794 +1.182	176.7 +128.9	13	0.086(0.66) +0.033	113.71(1.78) +15.70	1.032(0.58) +0.455	24.62(1.39) + 71.23
9	4	0.118 +0.022	70.82 + 4.54	2.229 +1.149	218.9 + 47.5	10	0.130 (1.0) +0.068	133.50(1.89) +26.67	2.168(0.97) +1.444	379.5(1.73) +114.2
10	5	0.107 +0.034	90.44 + 7.56	2.678 +0.546	355.4 + 61.5	6	0.144(1.35) +0.101	167.70(1.85) +22.86	2.425(0.91) +1.410	478.7(1.35) + 40.21
11	2	0.169 +0.057	91.89 + 2.38	5.399 +3.570	425.4 +113.0	6	0.086(0.51) +0.020	149.30(1.62) +31.3	2.292(0.42) +1.015	685.6(1.61) +227.3
12	4	0.149 +0.025	98.51 +11.34	6.479 +1.179	617.2 + 54.4	3	0.118(0.79) +0.016	157.71(1.60) +16.9	3.240(0.50) +0.243	845.1(1.38) +161.7
13	2	0.208 +0.156	104.4 + 8.65	9.052 +6.574	653.9 + 37.97	2	0.106(0.51) +0.018	169.24(1.60) +18.42	3.060(0.34) +0.523	925.3(1.42) +109.1

^aNumber of growth rings counted on shell outer surface.

^bRatio of Georges Bank mean value to Passamaquoddy Bay mean value given in parentheses.

Table 7. Regression relationship between Cd in tissue and age or shell height in scallops from Georges Bank. Linear, semi-log and power relationships were tested in each case. Only best relationship is given out of each. Cd concentrations and tissue burdens are defined in Table 6.

Relationship	Line equation	Coefficient of determination
[Cd] _A vs [Cd] _{DG}	$\log [Cd]_A = -1.4339 + 0.390 \log [Cd]_{DG}$	0.122
Cd _{TA} vs Cd _{TDG}	$\log Cd_{TA} = -2.046 + 0.874 \log Cd_{TDG}$	0.865
Cd _{TA} vs Age ^a	$\log Cd_{TA} = -3.000 + 3.247 \log Age$	0.770
Cd _{TDG} vs Age ^a	$\log Cd_{TDG} = -1.009 + 3.699 \log Age$	0.882
Cd _{TDG} vs Age ^b	$\log Cd_{TDG} = -0.330 + 3.998 \log Age_{(1)}$	0.895
Cd _{TDG} vs Age ^b	$\log Cd_{TDG} = -1.820 + 6.611 \log Age_{(2)}$	0.934
[Cd] _A vs S.H. (shell height)	$\log [Cd]_A = -1.699 + 0.337 \log S.H.$	0.060
Cd _{TA} vs S.H.	$\log Cd_{TA} = -6.429 + 3.306 \log S.H.$	0.850
[Cd] _{DG} vs S.H.	$\log [Cd]_{DG} = -0.015 + 1.049 \log S.H.$	0.726
Cd _{TDG} vs S.H.	$\log Cd_{TDG} = -4.984 + 3.776 \log S.H.$	0.978

^aRelative age based on number of growth rings.

^bAge in years using two age-reading specialists (1 & 2). Average difference in ages = 0.25+0.69 yr with negative differences in scallops < 90-mm shell height and positive differences in scallops > 90-mm shell height.

straightforward enough manner to enable scallops to be used as pseudo-integrating water monitors for Cd. Rather, they appear to function only as indicators of the interaction of scallops with environmental Cd. Ratios of tissue Cd levels may be of value in detecting polluted areas.

INTERCOMPARATIVE STUDY ON PAH DETERMINATION IN LOBSTER TISSUE PREPARATIONS

Under the auspices of the International Council for the Exploration of the Sea, an intercomparative exercise was carried out during 1983-84 to determine the degree of comparability in the determination of PAH in biological tissue.

Vials containing the acetone powder and oil prepared from lobster (*Homarus americanus*) digestive gland were sent to 25 laboratories for an inter-laboratory study of the determination of trace levels of common PAH compounds. Participants had been requested to determine as many different PAH as they could with a minimum of 12 common non-alkylated PAH generally present in combustion products (Uthe and Sirota 1982). In spite of a commitment on the part of the 25 participants to carry out the study, only 11 sets of results were received.

The results were compiled for each of two analytical methodologies used, i.e. capillary gas chromatography-mass spectroscopy (CGC-MS) or high pressure liquid chromatography-ultraviolet absorption and fluorescence detection (HPLC-UVAF). PAH compounds on which three or more laboratories submitted data were tabulated (Table 8). It is immediately apparent from Table 8 that both methodologies resulted in a wide range of results for every PAH tabulated. Less apparent is the

observation that, generally, the use of CGC-MS gave lower results than did the HPLC. This is not surprising when one considers the superior resolving power of CGC columns currently in use compared to HPLC. Mass spectroscopy is also a superior detection system. Benzo[a]pyrene measurements were exceptional in that both methodologies gave approximately the same range in results. This probably reflects specific tuning of the ultraviolet detection systems for benzo[a]pyrene or the lack of co-eluting, interfering, materials in the benzo[a]pyrene region of the HPLC.

The small number of returns and the variety of PAH compounds reported make statistical assessment of the data difficult. In all instances the range and standard deviation among laboratories greatly exceeded the range and standard deviation observed within our own laboratory. This suggests that a large amount of systematic error is present in the various participating laboratories. Interlaboratory relative standard deviations ranged from 39 to 96% for seven PAH compounds in lobster oil determined by HPLC. Some of this large variance may have resulted from whether or not the participants saponified the two materials prior to extracting PAH. The data set was not large enough to identify differences between approaches utilizing saponification and those which did not. No participant compared saponification with simple extraction. The greatest difference should have been observed in the results for the powder but nothing could be observed in the results since most analysts had difficulty measuring the low levels of PAH present in the powder. The fact that the preparation of the acetone powder (three serial extractions with cold acetone) resulted in an oil which contained the bulk of the PAH suggests that, at least for lobster, saponification does not result

Table 8. Ranges in levels of PAH reported by participants using either CGC-MS or HPLC-UVAF. Figures are given only when the number of reporting laboratories was three or greater.

Methodology PAH	Oil		Powder	
	CGC-MS	HPLC-UVAF	CGC-MS	HPLC-UVAF
Naphthalene	5.2- 118.4	-	12 - 60.6	-
Phenanthrene	672 -1650.4	-	24.6-783.3	-
Anthracene	197 -1012.1	-	6.0-131.0	-
Fluoranthene	1071.6-6340	5650 -15400	13 - 90.9	-
Pyrene	383 -3555	2453.8- 8300	10.1-150.7	85 -163
Benz[a]anthracene	348.7-1365.4	523.9- 3680	0.8- 7.3	0.8- 71.78
Chrysene	-	638 - 2800	-	8 -216.76
Benzo[e]pyrene	478.1- 650.1	928.7- 1773.43	-	6.3-131.32
Benzo[k]fluoranthene	-	980 - 3410	-	10 - 74.79
Benzo[a]pyrene	521.6-1100	220 - 980	-	0.6- 49.94
Benzo[ghi]perylene	-	140.3- 325	-	3.6- 60.34
Indeno[1,2,3-cd]pyrene	-	68 - 1682	-	3 - 50.54
Benzo[k]fluoranthene	-	565.2- 1834	-	1.1- 29.99

in markedly higher levels of PAH being found. Analysts also employed a variety of clean-up methods for separating PAH from other constituents in their extracts. This may have also contributed to the overall error. Other sources of error in addition to those associated with the manipulative part of the determination are associated with the supply of PAH used to prepare the standard (qualitative) and the preparation of the standard solution itself (quantitative).

There is no reason to believe that a satisfactory degree of interlaboratory comparability cannot be achieved. A recommended method for determining traces of PAH in foodstuffs has been published (Grimmer and Bohnke 1976). The method was recommended by the Commission on Food Additives, IUPAC, in 1976. The tests of interlaboratory error indicated that the error was small if all analysts employed the method under rigid control (reviewed by Howard and Fazio 1980).

PAH IN LOBSTER FROM SYDNEY HARBOUR

In 1982 the South Arm of Sydney Harbour, Nova Scotia, was closed to the commercial lobster fishery due to the presence of high PAH levels in digestive gland and muscle tissues of lobsters captured in the area (Sirota et al. 1984). The Sydney Harbour area was resampled in May 1984 during the early part of the commercial lobster fishery. Lobsters from the South Arm of Sydney Harbour were captured under special permit. Although a number of sites within the harbour were sampled in both 1982 and 1984, only the results for the South Arm and the control site (Port Morien) will be considered in this report. PAH were measured by HPLC with ultraviolet fluorescence detection (UFD) following clean-up of saponified tissue extracts by size-separation chromatography on Bio-Beads SX-3. With the exception of fluoranthene and pyrene, the tissue levels of PAH in South Arm lobsters have gone down slightly, if at all, over the 2 yr (Table 9). Levels of benz[a]anthracene appear to have dropped precipitously between 1982 and 1984; however, since the same precipitous drop

was observed in the control site data where little change occurred in levels of other PAH, it is likely that the change in benz[a]anthracene levels is due to an analytical problem. Comparisons of standard chromatograms from the 2 yr suggest that the actual amount of benz[a]anthracene in the 1982 standard was only one-tenth of the amount believed to be present. It is likely that the real drop in benz[a]anthracene levels over the 2 yr is less than the drop observed for pyrene. The magnitude of the difference in levels of PAH in the two tissue pools makes it difficult to accurately estimate the change in average tissue levels over the 2-yr interval. Fluoranthene and pyrene levels have decreased, possibly along with benz[a]anthracene levels but the levels of other PAH have not changed enough to say with certainty that levels have decreased.

There are two coking ovens located near the harbour, one of which was shut down in November 1981. After 1981, the second oven ran at half-capacity until shut down in November 1983. When one considers the greater water solubility and vapor pressure of pyrene and fluoranthene compared to the other PAH studied here (NRCC 1984a), it is easy to postulate a mechanism based on these chemical properties by which an aging PAH source would result in the observed results. Unfortunately, the relatively small change observed in levels of higher molecular weight PAH between 1982 and 1984 predicts a long-term lobster contamination problem with relatively slow changes in tissue concentrations of the higher molecular weight PAH over time. Since many of these are carcinogenic (NAS 1972), it is likely that the harbour should remain closed to the commercial lobster fishery for many years unless action is taken to remove or isolate the source of PAH. The decision to sample only every 2 yr was justified in light of the above results. In the absence of ameliorating action, biennial assessments should continue.

Table 9. PAH levels (ng/g wet wt) in lobster captured in May in the South Harbour compared to Port Morien lobsters (control). Values in parentheses are digestive gland/tail muscle PAH ratios.

	Sample Site									
	South Arm					Port Morien				
	Digestive gland		Tail muscle			Digestive gland				
	1982	1984	1982	1984		1982	1984			
PAH										
Fluoranthene	15200	12400 ^a	4216	5237	420	442 (32)	68	68(70)	156	162
Pyrene	13100	9150	3176	2912	333	70 (53)	59	63(49)	42	46
Benz[a]anthracene	32700	18000	762	1154	678	900 (43)	17	19(53)	79	74
Chrysene	1030	252	770	1243	20	15 (37)	24	24(42)	7	2
Benzo[e]pyrene	3600	1990	1552	2867	35	35 (80)	36	36(61)	17	15
Benzo[b]fluoranthene	3820	2460	1022	1554	835	72	29	35(40)	10	7
Benzo[k]fluoranthene	955	640	502	813	26	19 (35)	15	19(39)	2.8	1.9
Benzo[a]pyrene	1430	930	711	1262	43	33 (21)	27	37(32)	2.5	1.6
Benzo[ghi]perylene	769	479	232	459	20	10 (41)	10	18(25)	2.4	3.8
Indeno[1,2,3-cd]pyrene	739	525	486	931	40	30 (18)	12	21(42)	2.1	2.5
									5	trace

^aThe two values are results for two tissue pools (equal weights of tissue), generally from 10 animals, with 5 in each pool.

THE DETERMINATION OF PAH IN SHELLFISH

A novel method has been developed for the rapid screening of shellfish for PAH contamination. The method can also be used to give detailed quantitative information on individual non-alkylated PAH compounds present in the samples.

The method begins with the saponification of small quantities (1-8 g) of tissue in Folin-Wu tubes with 25 mL 1N ethanolic potassium hydroxide. The contents are gently refluxed on a micro-Kjeldahl heating rack using BDH boiling chips. The top portion of the tube acts as an air condenser and little ethanol is lost over the 1½- to 2-h reflux period. The hot saponification mixture is added to an equal volume of water (25 mL) in a stoppered mixing cylinder and PAH extracted into three consecutive 10-mL portions of isooctane. After mixing and separating, each isooctane phase is transferred to a 100-mL round-bottom flask using a glass syringe-operated Pasteur pipet. The isooctane is removed by rotary evaporation and the PAH are separated from coextractives by gel permeation chromatography (GPC) using Bio-Beads SX-3. The PAH fraction can be collected and individual PAH compounds determined using published methods. We employed reversed phase HPLC (C-18 column) with UVAF detection.

Screening for PAH is carried out by monitoring the GPC effluent with ultraviolet absorption and refractive index detectors. In a recent study of contaminated lobster from Sydney Harbour, Nova Scotia, the method was used to study "total PAH" (equated to the sum of ten non-alkylated PAH compounds measured in the extract by HPLC in lobster tissues at concentrations ranging from approximately 25 to 20,000 ng/g wet wt). The ultraviolet absorption response at 254 nm was linear (peak height, peak area paper weight) over the range with a coefficient of determination of 0.9959.

Recoveries of added amounts of individual PAH were essentially quantitative for the complete procedure (Table 10).

The advantages of this method over existing methods are: the use of small quantities of tissue, resulting in lesser amounts of interfering materials; reduction in complexity and costs of glassware; easily cleaned glassware; fewer transfer steps; reductions in solvent quantities; and reduction in the operational time requirements per analysis. It is possible to carry out 23 analyses in 3-4 technician days, depending on whether screening or full quantitative analysis is required, using the automated gel permeation and HPLC. We estimate that the method is two to four times faster than other current methods. With minor maintenance the gel permeation column packing can be used for many hundred clean-ups without requiring replacement or column repacking.

In the course of this work it was discovered that Bio-Beads SX-3 did not separate PAH from interferences strictly on the basis of molecular size. In fact, the order of elution of individual non-alkylated PAH compounds was opposite to that predicted on the basis of molecular size, indicating that absorption chromatographic effects were major. Based on this observation, research is in progress to determine if a rapid method for measuring various size classes (number of rings) of PAH can be developed.

POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS IN LOBSTER

Further to the 1983 work on polychlorinated dibenzodioxins in lobsters from the Miramichi Estuary and the Bay of Chaleur, we arranged to have samples of lobster digestive glands from Sydney Harbour, Petrie Point and Port Morien (control), Nova Scotia, screened for these contaminants by the

Table 10. Recoveries of PAH added to lobster digestive gland homogenate.

PAH	Total amount added (µg)	Recovery (%)	Relative standard deviation (%)
Fluoranthene	3.4	105.4	8.3
Pyrene	1.7	123.6	5.7
Benz[a]anthracene	1.7	112.4	9.4
Chrysene	1.7	136.8	10.0
Benzo[b]fluoranthene	3.4	108.9	3.8
Benzo[k]fluoranthene	1.7	108.5	0.0
Benzo[a]pyrene	1.7	99.1	6.8
Dibenz[a,h]anthracene	3.4	104.6	3.6
Benzo[ghi]perylene	3.4	88.8	6.9
Indeno[1,2,3-cd]pyrene	1.7	104.6	6.0

Ontario Ministry of the Environment. Sample sites are described in Sirota et al. (1984).

The analytical procedure used by the Ontario Ministry of the Environment is complicated, involving high resolution mass spectroscopy (MS) following an elaborate clean-up procedure. The data have yet to be fully evaluated. Therefore, some of the following results may be revised in their final report.

Generally the results from high resolution MS agreed well with those from the Miramichi reported last year (Uthe 1984) and the current results from low resolution MS for the Sydney Harbour lobsters. Also, the polychlorinated dibenzodioxin/polychlorinated dibenzofuran relative abundances in Sydney Harbour lobster paralleled the Miramichi findings, i.e. very small amounts of hepta- and octachlorodibenzodioxins and relatively larger quantities of the polychlorinated dibenzofurans. The concentration of tetrachlorodibenzofurans (sum of all isomers) in Sydney Harbour lobster was of the order of 200 pg/g wet wt while the Port Morien animals ranged from 20-40 pg/g and Petrie Point lobsters had 10-15 pg/g. One Petrie Point sample gave an atypical pattern, therefore requiring further study.

No tolerance levels for polychlorinated dibenzofurans have been set in Canada. It is relevant to note that the no observable effects level (NOEL) for 2,3,7,8-tetrachlorodibenzo-p-dioxin for reproductive and carcinogenic effects in rats and mice is reported to be 1 ng/kg body wt/day (NRCC 1984b), and it was further suggested that, in the absence of more appropriate data, this NOEL be used as a first approximation of the toxicity of 2,3,7,8-tetrachlorodibenzofuran. The NRCC panel also concluded that a dangerous concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin in fish would be of the order of 3 to 30 pg/kg if fish were the sole source of the dioxin eaten once a week and only this isomer was of concern (NRCC 1981). Based on this information, further research into the presence and congener composition of these highly toxic contaminants may be warranted.

CONTAMINANT TRENDS IN MARINE SPECIES R.K. Misra

Since the late 1970's the International Council for the Exploration of the Sea has been interested in scientific approaches to the accurate determination of spatial and temporal trends in contaminant levels in marine stocks. In 1977, we began a study of common trace metal and organochlorine contaminants in Atlantic cod (*Gadus morhua*) captured in the southern part of the Gulf of St. Lawrence. Contaminant levels were measured in length-stratified samples captured in September of 1977, 1978, and 1979. Both liver and muscle were analyzed. The stock was resampled in 1982 but all trace contaminant levels were not determined due to lack of resources and other commitments. The sampling strategy was designed by Dr. D.P. Scott of the Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba. Statistical analysis of the data was carried out by Dr. Scott using a standard multiple regression analysis program (Scott et al. 1983). Both concentration and tissue burden (concentration times the total tissue weight) were investigated first in a non-interactive model, then in an interactive one. The approach has been the subject of discussion within the Council community (e.g. Lassen 1983; Hansen 1982; Hansen et al. 1982; ICES 1981).

In early 1984, a workshop was held in Halifax to review the approach (Uthe et al. 1984). As a result of the discussion and recommendations from this meeting a full statistical analysis of the 1977-1979 data is being carried out by Dr. R.K. Misra of the Halifax Fisheries Research Laboratory. The workshop expressed a feeling that, even after this analysis, sampling over a period longer than 3 yr would be required to detect small changes in contaminant levels and to examine the stability of the model.

Full statistical analysis of the data requires a number of steps: 1) development of a statistical program suited to the data set; 2) design of an appropriate model for testing; 3) identification of influential observations (outliers) with respect to their X and Y values and then to ascertain whether

or not they significantly (statistically) influence the fit of the regression function; and 4) statistical analysis for time trends. To date, the work is about half completed. Due to problems identified in applying commercially available statistical packages to the data set, Dr. Misra has written a Fortran program for the analysis, supported under IBM.

Interactive and non-interactive models are being studied. Dr. Scott's model may have to be replaced by a model which allows for examination of time trend in conjunction with that of deviations from it. A series of univariate statistical analyses carried out separately for each of the contaminants will be insufficient as it ignores the interactions among contaminants. Multivariate analyses that consider all contaminants simultaneously will also be done.

The following analyses are now completed: analysis for outliers when all biological parameters were included in the analysis (one individual fish was identified in each year as influential enough to lead to serious distortion with that of deviations from it. A series of univariate statistical analyses carried out separately for each of the contaminants will be insufficient as it ignores the interactions among contaminants. Multivariate analyses that consider all contaminants simultaneously will also be done.

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CONTAMINANTS AND HORMONE METABOLISM

H.C. Freeman
G.B. Sangalang
G.R. Sirota

The objective is to develop and apply methods that can detect the sublethal chronic effects of trace quantities of chemical contaminants on fish and shellfish. Fish are sensitive to low levels of some chemicals and can be used as early warning indicators of environmental effects so that adjustments can be made before the problem becomes severe and irreversible.

STEROID HORMONES IN THE LOBSTER

Previously our laboratory has demonstrated that changes in steroid hormone metabolism in fish and

marine mammals induced by chemical contaminants can be used as a sensitive early warning test for the presence of pollution effects (Freeman et al. 1984a,b) on animal reproduction, physiology and biochemistry.

In the past few years we have extended our studies to include the American lobster (*Homarus americanus*). This has mainly involved isolation and identification of the major steroidal compounds in lobster hemolymph. The effort has been complicated by the observation that steroid levels in lobster hemolymph are much lower than levels characteristic of finfish blood, requiring the processing of large quantities of hemolymph to obtain sufficient quantities of steroids for further study. To date, testosterone has been confirmed as a major steroid in lobster hemolymph (Burns et al. 1984a). Twenty alpha-dihydroprogesterone as well as several, as yet unidentified, steroids have been isolated from lobster testicular incubations (Burns et al. 1984b).

RADIOIMMUNOASSAY (RIA) PROCEDURES

During 1984 our laboratory has devised two RIA procedures for determining levels of 17-alpha-hydroxy-20-beta-dihydroprogesterone and 20-alpha-dihydroprogesterone in tissue extracts. The former method will be valuable in determining changes in concentrations of 17-alpha-hydroxy-20-beta-dihydroprogesterone concentrations in female Atlantic salmon during sexual maturation under normal and pollution conditions. This will complement our studies on androgen levels in male salmon during ripening under normal and adverse environmental conditions.

The RIA method for the determination of 2-alpha-dihydroprogesterone will be useful in determining hemolymph levels of this steroid in lobster. Following confirmation of this steroid as a major hormone in the lobster, its usefulness as an early warning effects detection system will be assessed.

ACID RAIN STUDIES

Last year it was reported that Atlantic salmon (*Salmo salar*) held in cages in the more acidic Westfield River during the period of sexual maturation gained less weight, produced smaller eggs, had higher egg mortality, and had abnormal sex hormone levels compared to equivalent salmon held for the same period of time in the less acidic Medway River (Freeman et al. 1984a). During the fall of 1984 the holding part of the experiment was repeated. Atlantic salmon were purchased from the Cape Breton Marine Farms and were River Phillip stock. They were acclimatized to freshwater and then transported to the cages on September 28, 1984. They ripened and were manually spawned over the period December 4-12, 1984. During holding the fish were fed daily with dry (6.3 mm) trout grower pellets. Every 2-3 wk, the fish were anesthetized, weighed and bled for steroid hormone (androgen and estrogen) determinations. When ripe, the fish were spawned and eggs fertilized with milt from fish from the same river. The eggs are being incubated at the Mersey Hatchery to determine viability. Fecundity of female salmon from the Westfield cage was 31% lower than for the Medway salmon, even though the pH of the Westfield River remained above 5.0 during most of the holding period (Fig. 1) due to the low rainfall during the period.

Westfield and Medway Rivers

River Water pH and Water Temperatures (Oct 10 to Dec 24.84)

Change in Water Level (Dec 5 to Dec 24.84)

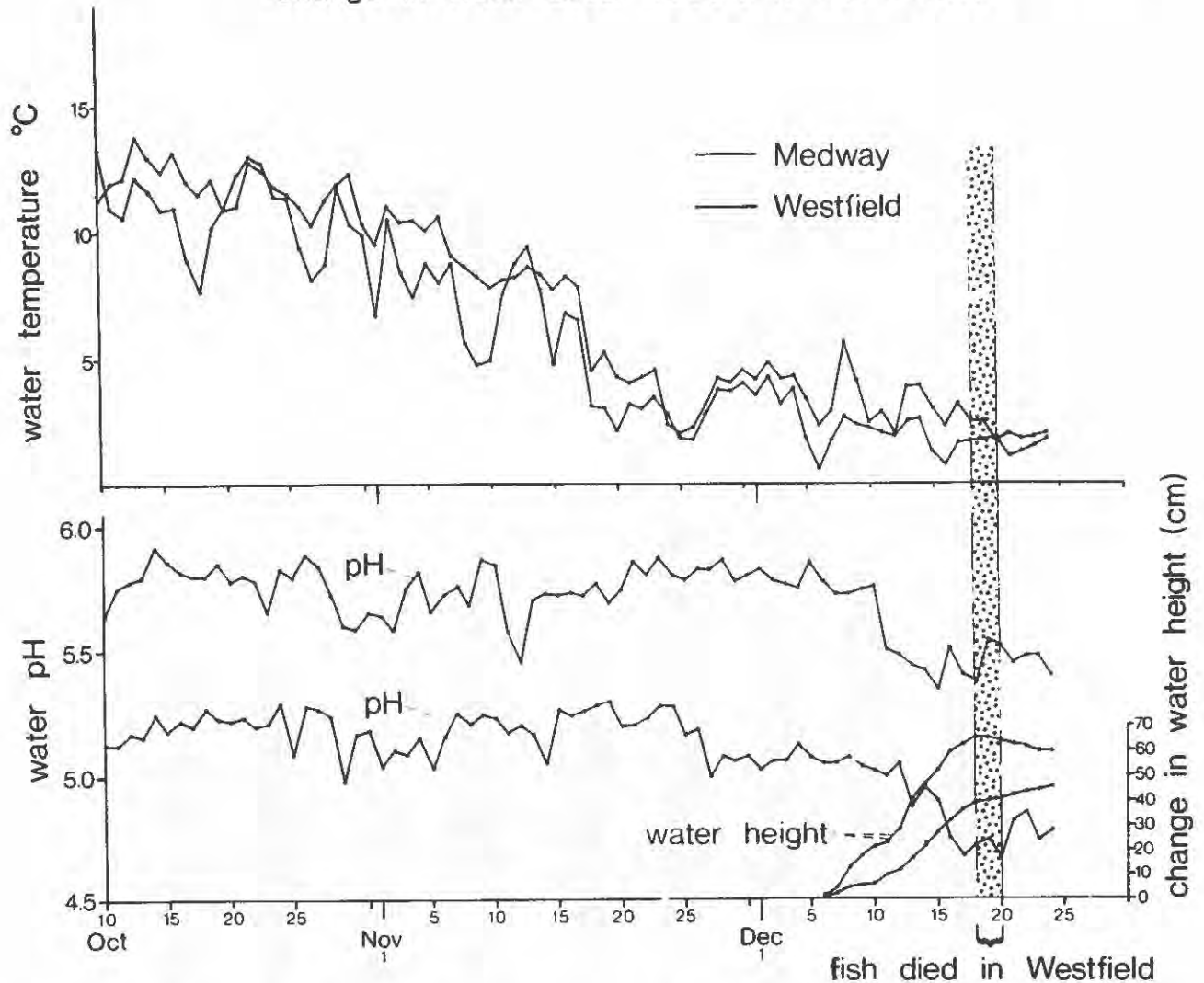


Fig. 1. The pH of both rivers declined during heavy rains in November and December 1984. The greatest pH drop was in mid-December during several days of the heaviest rains. At this time, on the Westfield River, the water height peaked, the pH lowered to 4.7, the water temperature dropped to 1.8°C, and 23% of the post-spawned salmon died on December 18, 19, and 20. The remaining lethargic moribund fish survived when transferred immediately to the higher pH Medway River (pH 5.5) which was approximately at the same temperature. In 1983, 75% of similar Atlantic salmon died over a period of a few days when the same conditions occurred in the Westfield River and the fish were not removed immediately to a higher pH river.

During the period December 18-19, nine salmon in the Westfield cage died. The remaining 30 fish appeared lethargic and moribund. This occurred when the temperature of the Westfield River was approximately 2°C and the pH had dropped to 4.7 due to heavy rains (Fig. 1). No such problem was observed in the Medway River cage. The remaining Westfield fish were transferred to the Medway River on December 20 where the pH was 5.52 and the water temperature 1.9°C. The fish recovered and no further mortalities were recorded. A similar post-spawning mortality (75%) was recorded in the Westfield cage in 1983 where transfer of the remaining fish also resulted in complete survival and apparent recovery. It appears that post-spawning Atlantic salmon cannot tolerate simultaneous low pH and low water temperature.

The *in vitro* steroidogenesis tests (Freeman et al. 1980) showed that the Westfield fish had abnormal steroid hormone metabolism. This is obvious from the autoradiograms of extracts of head kidney incubations for the two rivers (Fig. 2). The proportion of polar metabolic products produced by the head kidneys of the Westfield River salmon was greater than that produced in the Medway incubations since the concentrations of less polar compounds (the metabolites near the "P" area, Fig. 2) were lower compared to those of the Medway fish. A compound isopolar with cortisol (F) was the principal steroid produced by the head kidneys of

both Westfield and Medway fish (Fig. 2). In the Medway River fish, 11-desoxycortisol (S) was also a major product as normally occurs in salmonids, but in the Westfield fish the steroid hormone corticosterone (B) replaced (S) as a major metabolite (Fig. 2). This altered metabolism is an indicator of stress in the Westfield fish (Freeman et al. 1981; 1984b). This could be related to the sluggishness and deaths observed later in the course of the experiment.

Autoradiograms (Fig. 3) demonstrated impaired biosynthesis of both androgens, testosterone and 11-ketotestosterone in Westfield River fish compared with that observed in the Medway fish. This impaired synthesis explains the abnormally low blood levels of these two hormones reported in 1983 (Freeman et al. 1983).

Transfer of fish from the Westfield to the Medway River after spawning and during a period of observed lethality indicated that the observed stress effects in the metabolism of steroid hormones by the head kidneys may be reversible since there were no further mortalities observed. It is not known if the same can be said of the effects observed in the testes. Whether or not the fish native to the Westfield River can survive after spawning by escaping to an area of higher pH or return to the ocean is unknown.

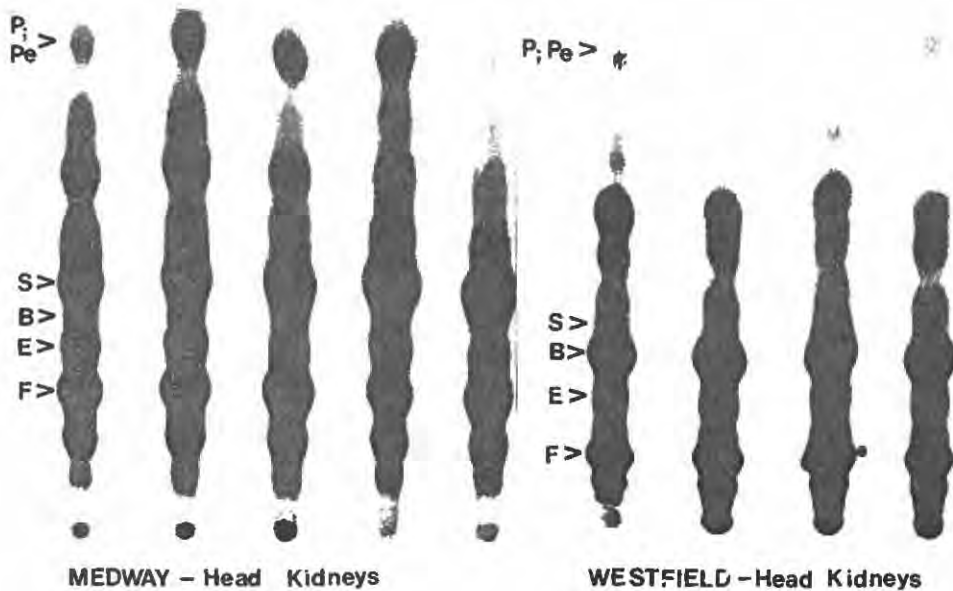


Fig. 2. Biosynthesis of steroids (*in vitro*) from precursors [^{14}C]-progesterone plus [^3H]-pregnenolone by head kidneys (adrenal homologues) of sexually mature Atlantic salmon held in the acidic Westfield River and the normal pH Medway River.

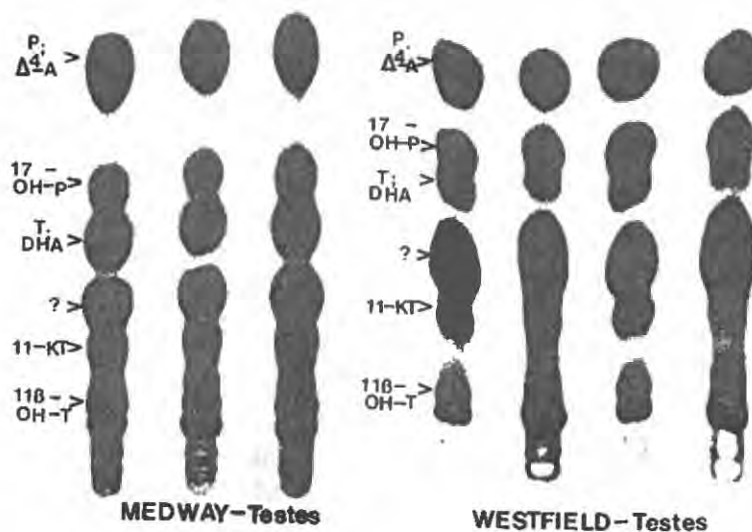


Fig. 3. Biosynthesis of steroids (in vitro) from precursors [^{14}C]-progesterone plus [^3H]-pregnenolone by testes from sexually mature Atlantic salmon held in the acidic Westfield River and the normal pH Medway River.

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Figure 1. A grid of small, dark, rectangular objects arranged in rows and columns, possibly a microarray or a set of small components.

The first part of the document discusses the importance of maintaining accurate records of all transactions and the role of the accounting department in ensuring the integrity of the financial data. It also highlights the need for regular audits and the importance of transparency in financial reporting.

The second part of the document focuses on the challenges faced by the organization in managing its resources and the strategies implemented to overcome these challenges. It discusses the importance of efficient resource allocation and the role of management in ensuring the organization's long-term sustainability.

The third part of the document provides a detailed analysis of the organization's financial performance over the past year, including a breakdown of revenue, expenses, and profit. It also includes a comparison of the organization's performance with industry benchmarks and a discussion of the factors that have influenced its financial results.

The final part of the document offers conclusions and recommendations for the future, based on the findings of the analysis. It emphasizes the need for continued improvement in financial management and the importance of staying abreast of industry trends and developments.

ENVIRONMENTAL CHEMISTRY

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S. Ray
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M. Woodside (contract)

The objective of this project is to identify hazards and to estimate risks of chemicals to fisheries. The work includes specific chemicals, development of techniques, and response to events and emergencies. The main clients are other government agencies, industry, the scientific community and the general public.

In our context, the hazards are almost always acute or chronic toxicity. A man-made chemical detected in fish, shellfish, water, or sediment is considered a hazard. Risk is the probability that a chemical may cause harm. Main factors of risk are exposure level and duration. In other words, toxicity of a chemical and often just its presence are the hazard. The chance that harmful exposure may be reached is the risk.

For example, the concentrations of the pesticide permethrin causing 50% mortality of salmon and lobster in 96 h (96h-LC50) are 10 and 0.7 µg/L, respectively. Chronic toxicity is not known. This is the available information on the hazard of permethrin. Permethrin is proposed for forest spraying against spruce budworm at an application rate of 1900 g/km², applied once, in early May. The solubility of permethrin in water is 0.28 mg/L, log(octanol-water partition coefficient)=5.1, and permethrin is probably reasonably biodegradable or at least is hydrolyzed to relatively nontoxic components. Does permethrin pose a risk to the aquatic environment under these conditions?

The identification of hazards includes the detection of chemicals (contaminants) in fish, shellfish, water or sediment. The chemicals may be either known contaminants such as the polychlorinated biphenyls (PCB) or cadmium (Cd), or new and not-anticipated pollutants. For the known pollutants the emphasis is on the determination of levels and trends. The knowledge of levels of pollutants is the first step in risk estimation. (Are the levels observed associated with toxic effects under experimental conditions or elsewhere in natural situations?) The trends of the levels show whether the situation is improving or getting worse, how efficient are the regulations limiting discharges of a pollutant, etc. The search for new and not-anticipated pollutants is performed routinely during some analyses. It may also be initiated on the basis of reports in scientific or trade journals or other information.

Exposure is affected by discharge and dissipation rates of the chemical in the environment. Solubility, adsorption, decomposition, and evaporation are the main factors determining the dissipation. The estimation of these parameters is also a part of risk estimation.

Physico-chemical parameters such as those above, parameters needed for the detection and identification of chemicals (spectra, response factors, etc.), and toxicity may be estimated usually from values determined for related chemicals. This is important in view of the large

number of chemicals encountered in the environment. The estimates are based on Quantitative Structure Activity Relationships (QSAR). Their development depends among other things on the accuracy with which one can describe quantitatively the various parameters (structure, toxicity, bioaccumulation, etc.). The development and improvement of QSAR is a part of the project.

The determination and identification of chemicals at trace levels in environmental samples require complex chemical analytical instrumentation. Maintenance, modification, and development of this instrumentation also require considerable effort and are very time-consuming and costly.

MASS SPECTROMETRY

In a mass spectrometer, chemicals are fragmented into ions and the mass of the ions is determined; actually it is the mass/charge ratio, m/e or m/z, but the charge is almost always unity. The original chemical is identified from the fragments. Electrons are used to fragment chemicals in the Electron Impact (EI) mode. Ions formed from other chemicals (most frequently methane) are used in the Chemical Ionization (CI) mode. The investigated chemicals are usually introduced into the mass spectrometer from a gas chromatograph. This separates mixtures of chemicals into components and further aids the identification.

The capacity of our Finnigan Model 4500 mass spectrometer was increased by the addition of a 16-megabyte disk drive with 52,000 sectors (unit of space on the disk). Of these, about one-third is occupied by software. The results of one analysis occupy about 1000 sectors. The system has also two 5-megabyte drives with a total of about 40,000 sectors but, until recently, all three drives could not be operated simultaneously. The gas chromatograph was improved by the addition of an on-column injector. This injector ensures quantitative recovery of chemicals with higher boiling points.

The mass spectrometer was used to identify or confirm organic chemicals in fish, shellfish and various other substrates. The emphasis of our own projects was on PCB and organochlorine pesticides such as chlordane, with the objective of reaching sensitivity sufficient to measure chlorinated dibenzodioxins (CDD) and dibenzofurans (CDF). In addition, two time-consuming nondiscretionary projects were completed. One consisted of analyses of fish after the VINLAND wellsite blowout in February 1984; the other dealt with the identification of organic compounds in fish and a fish tank liner, following a major fish kill in a commercial salmon hatchery.

VINLAND WELLSITE BLOWOUT

The blowout occurred on February 22, 1984, at a wellsite located about 16 km north of the eastern tip of Sable Island. Gas and gas condensate were escaping from the wellhead until March 3, 1984. A sheen was observed extending from the rig for approximately 16 km in a southeasterly direction. The discharge of the gas condensate was about 300 barrels/d.

Because of the expected relatively high evaporation rate of the gas condensate, wide-ranging contamination of fish was not anticipated. To

confirm this, samples of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) were analyzed by gas chromatography-mass spectrometry and by spectrofluorometry (Zitko et al. 1984). Spectrofluorometry measured fluorescence of the extract. This is a technique extremely sensitive to hydrocarbons containing benzene rings, but only in exceptional cases can it be used to identify individual chemicals.

Samples of fillet and liver were extracted by steam distillation. The gas condensate contained hydrocarbons with somewhat higher boiling points than expected. On the scale of straight chain paraffins they ranged from C₉H₂₀ to C₂₆H₅₄, with those from C₁₀H₂₂ to C₁₆H₃₄ present in about equal proportions. Straight chain paraffins are widely distributed in nature and are not good indicators of the contamination by gas condensate. Xylenes (dimethyl benzenes), tri- and tetramethyl benzenes, naphthalene, methyl- and dimethyl naphthalene were used as indicators by mass spectrometry. Fluorescence emission at 339 nm (excitation at 290 nm) was used in the spectrofluorometric characterization of the extracts.

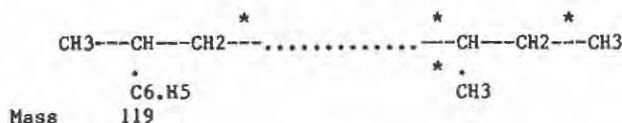
Only xylenes were detectable in livers of fish from the blowout areas and the extracts also had somewhat elevated fluorescence in comparison with fish from control areas. It appears that the fish were exposed to very low concentrations of the gas condensate and the likelihood of adverse effects was minimal. On the other hand, the potential of the gas condensate to contaminate fish is higher than anticipated. Large-scale or prolonged blowouts could lead to considerable contamination of fish, probably lasting for several months after the event. One obvious result of the contamination would be tainting which could make the fish unsuitable for human consumption. High-level exposure (in the 200 µg/L range for about a day) may cause mortality. Blowouts of this magnitude appear unlikely.

The analyses by gas chromatography-mass spectrometry detected several interesting compounds. One of them is a phenyltridecane, C₁₉H₃₂. Tridecylbenzene (1-phenyltridecane) is a commercially available detergent intermediate. Mass spectrum is usually presented as a bar graph with the m/e values on the X-axis and the abundance of ions on the Y-axis. The latter is scaled to the abundance of the most abundant "base" ion set at 100. The mass spectrum of the detected phenyltridecane is presented in Fig. 1. The absolute intensity of the base ion is 1106 units (scale on the right). The spectrum is dominated by a series of ions spaced 14 mass units apart: 91, 105, 119, 133, ..., 203. These are ions of alkylbenzenes containing an alkyl chain consisting of 1, 2, 3, 4, ..., 9 CH₂ groups (atomic weight of carbon (C) is 12, that of hydrogen (H) is 1; CH₂ = 12+2=14):



	* Alkyl chain				
Benzene ring					
Mass					
77	14	14	14		15
Cumulative mass					
77	91	105	119		260

The most favorable fragmentation point of alkylbenzenes under electron impact is at the second bond from the benzene ring (*). Since the base ion of the detected phenyltridecane is at m/z=119, it appears that the benzene ring is located on the second alkyl group:



In addition, the alkyl chain is probably branched at the "aliphatic" end, since ions containing 10 and 11 CH₂ groups have not been detected. (The likely fragmentation pattern is indicated by *.)

The gas chromatogram of cod liver sample is presented in Fig. 2. The chromatogram is "constructed" by the data system by adding the intensities of all ions in the mass spectra. The numbers of the mass spectra ("scans") are on the X-axis. The times from the beginning of the run are also given since scan numbers may change depending on the scanning rate. The total intensity or "Reconstructed Ion Current" (RIC) is on the Y-axis.

In the analysis of environmental samples, the largest peaks are usually not the most interesting ones. In this example the peak in scan 821 is hexadecanoic acid, a frequently encountered fatty acid. More specific information can be obtained by plotting intensities of selected ions rather than the RIC. Such plots are "Mass chromatograms". A mass plot for masses 100, 256, 284, 312, and 340 is given in Fig. 3. The mass spectra were obtained under CI conditions.

These compounds were conspicuous by EI mass spectra containing only the ion at m/e=100. The most likely elemental composition of this ion is C₆H₁₄N or C₅H₁₀NO. The lack of other fragment ions is typical for secondary and tertiary alkyl amines. There are usually additional fragments in the spectra of alkyl amides (the latter formula). CI spectra contain the "molecular weight +1" [M+1] ions and also the much less intense [M-1] ions. This identifies the compounds as dodecyl, tetradecyl, hexadecyl, and octadecyl N-alkyl amine. The N-alkyl may be pentyl, methyl and butyl, or propyl and ethyl. If the compounds were alkanoyl amides, the N-substituents may be butyl, propyl and methyl, or diethyl, with the amides ranging from dodecanoyl to octadecanoyl. Fish containing these compounds were from control and from the blowout areas.

Fatty amines, fatty acid amides, fatty acid polyamine condensates, imidazolines, and cyclic ammonium quats are used as corrosion inhibitors and drilling mud additives in the oil industry and could be the source of the contamination, but the compounds could have other sources as well.

These examples illustrate the detection of unanticipated chemicals, leading to followup toxicological studies and source identification, and possibly regulation. The search for not-anticipated compounds is very time-consuming and may at times depend on serendipity. On the other hand, the search for specific compounds is much easier and can be carried out by the data system, after a considerable initial programming effort.

MASS SPECTRUM

29/03/84 8:46:00 + 24:22

SAMPLE: VINLAND COD 192 LIVER(5ML EVAP TO .5ML)(.5UL @ 10-8)

ENHANCED (S 158 2N 0T)

DATA: MI192LIVER2 #731

CALI: FC290384P #1

BASE M/E: 119

RIC: 6624.

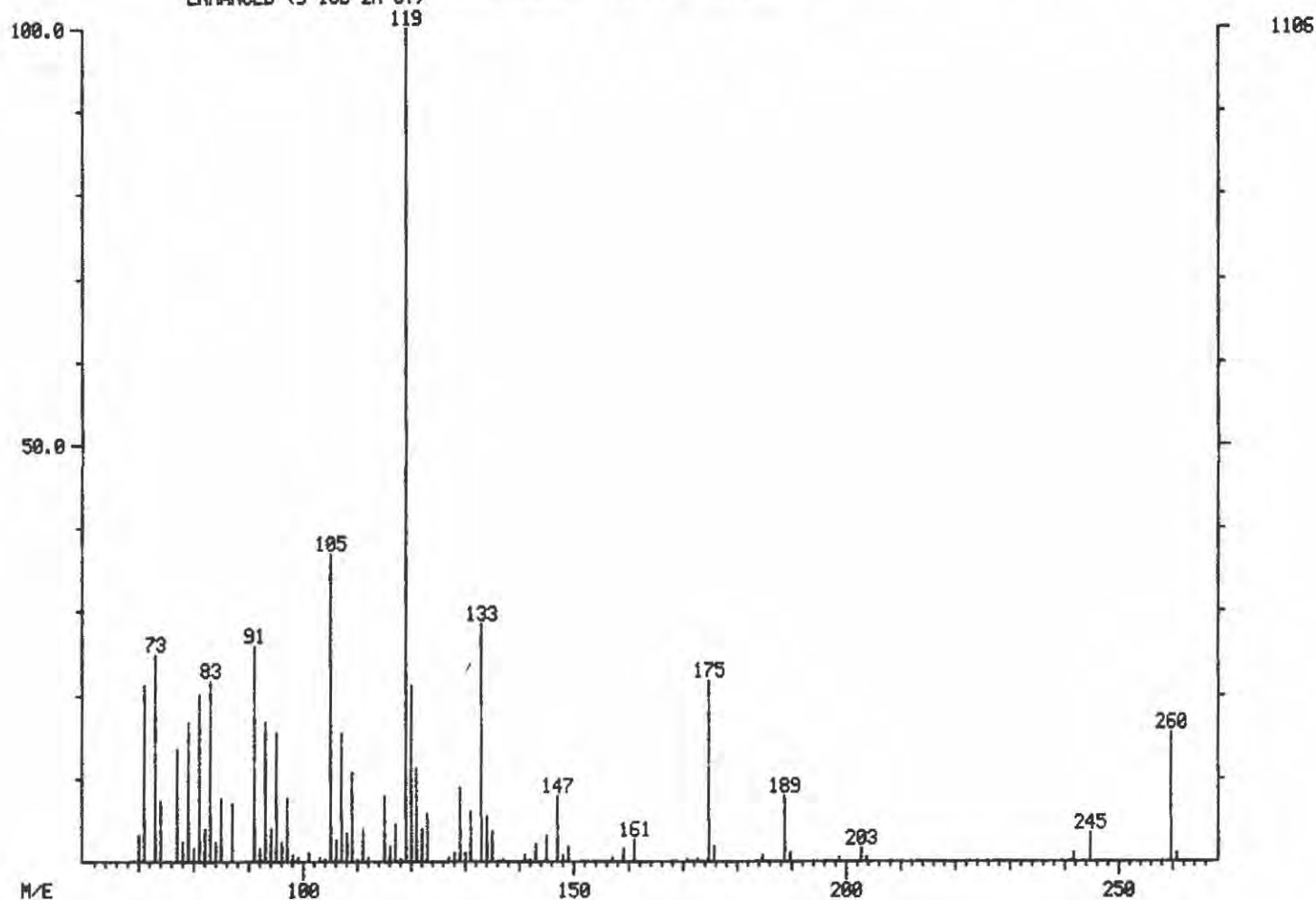


Fig. 1. Mass spectrum (EI) of phenyltridecane (C₁₉H₃₂) detected in a cod liver.

X-axis: Mass/charge (m/e) of ions

Y-axis: Intensity of ions (relative to m/e 119 on the left; absolute on the right)

Molecular ion at m/e=260.

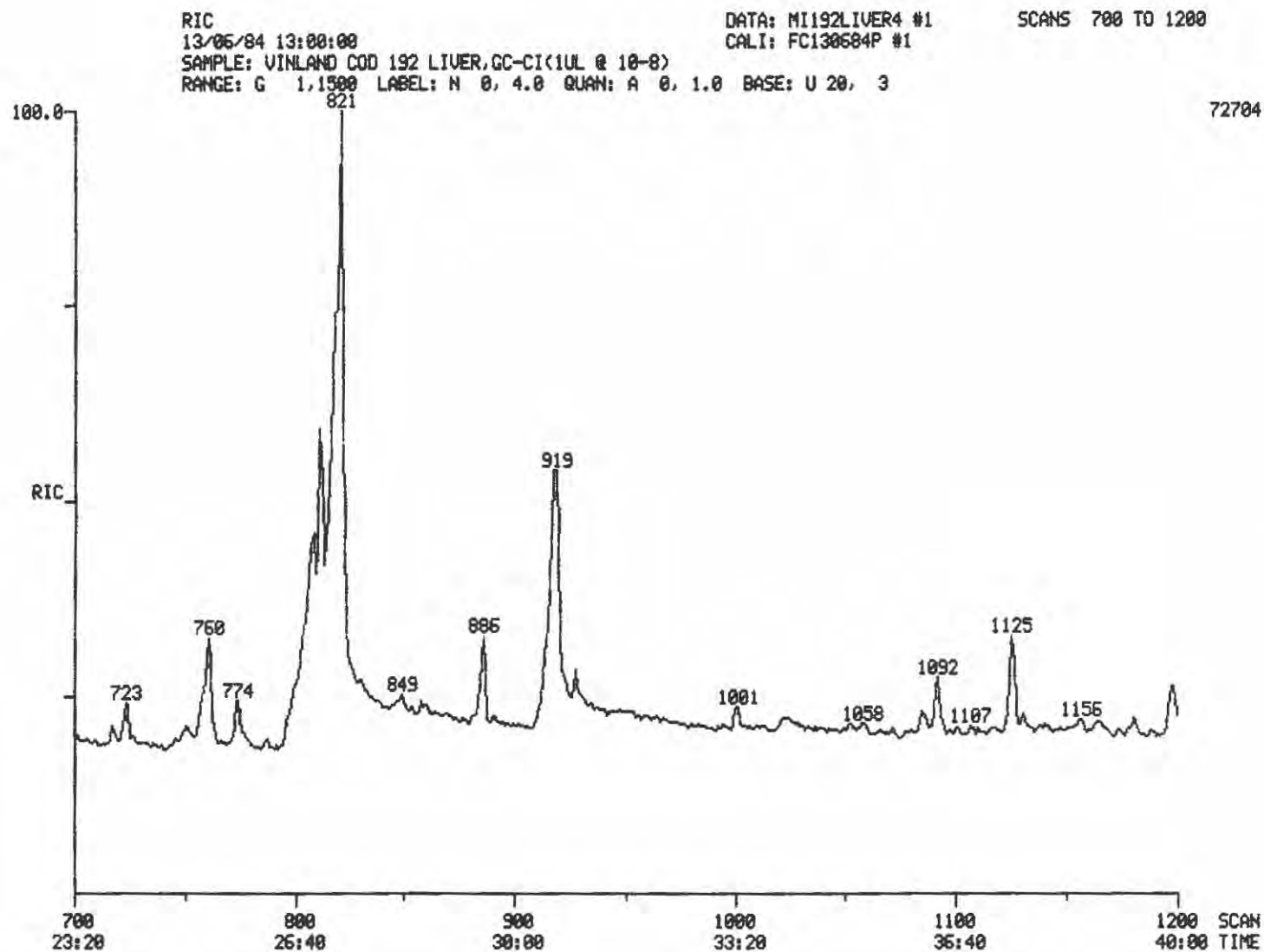


Fig. 2. Reconstructed gas chromatogram of a cod liver extract.

X-axis: Scan number and time from sample injection
 Y-axis: Reconstructed Ion Current (RIC, summed intensities of all ions).

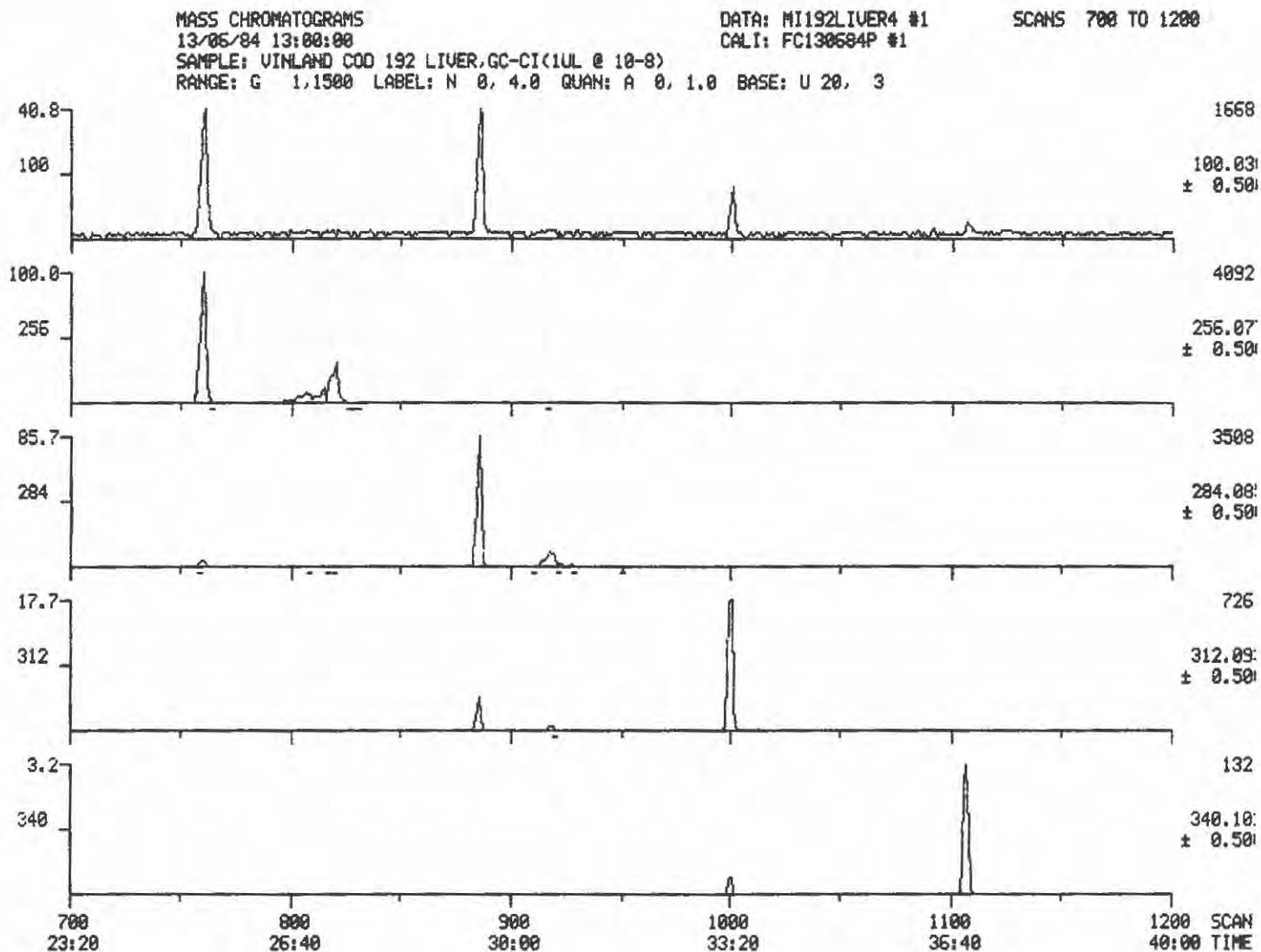


Fig. 3. Mass chromatograms of alkylamines tentatively identified in cod liver extracts.

X-axis: Scan number and time from sample injection
 Y-axis: Intensity of ions with m/e specified on the left
 Ions with m/e=100 are probably C₆H₁₄N
 Ions with m/e=256, 284, 312, and 340 are molecular ions
 of a polymer-homologous series dodecyl - octadecyl.

HATCHERY FISH KILL

In September 1984 a commercial hatchery in New Brunswick suffered extensive mortalities of juvenile Atlantic salmon. The mortalities occurred a few days after the fish were moved into tanks equipped with a new plastic liner. Analyses of water, and fish disease investigation by other agencies, did not provide a clue. However, the latter noted that the surface of the fish was unusually free of bacteria. We determined that the plastic tank liner was a plasticized polyvinyl chloride (PVC). This material is always protected by fungicides against degradation and a few inquiries established that it contains the fungicide OBPA (10,10'-oxybis-10H-phenoxarsine) which is extremely toxic to fish (Zitko et al. 1985).

The liner contains a variety of organic compounds. Major components are phthalate plasticizers ranging from dihexyl to diundecyl. This is somewhat surprising since in the past such liners were plasticized mostly by di(2-ethylhexyl) phthalate (DEHP). Toxicological implications of this change are uncertain. Phthalates with longer alkyl chains are less toxic and probably also less bioaccumulative, but may be biodegraded more slowly.

Mass spectra obtained in the CI mode were invaluable in the identification of the phthalates. The liner also contained nonylphenol which is very toxic to fish, but was probably only a minor contributor to the toxicity of the liner caused by OBPA. Additional organic compounds leachable from the liner by water included phenol, 2-ethylhexanoic acid, benzoic acid, fatty acids ranging from C9 to C16 and, probably, bisphenol A. These are the compounds that were detected and identified. There are others that were detected but remain unidentified and, without doubt, other compounds are present below the detection limit of the technique.

Of the trace metals, the liner contained Cd and lead (Pb) at 400 and 1500 µg/g, respectively. The content of arsenic (As, from BPA) was 80 µg/g. These values were obtained by atomic absorption spectrophotometry.

The variety of compounds present in the liner may have chronic effects on the fish during long-term exposure and the problem of chemicals in aquacultural operations deserves further attention.

STRUCTURE-ACTIVITY STUDIES

PCB Resolution, Nomenclature and Data Management

There are 209 chlorobiphenyls. The majority of these are not present in environmental samples but the high resolution of capillary columns in gas chromatography indicates the complexity of mixtures present in environmental samples (Fig. 4).

The conventional designation of chlorobiphenyls becomes very cumbersome at higher degrees of chlorination. This is shown in the list of

pentachlorobiphenyls given below. The list contains the conventional, IUPAC, and "shorthand" (Zitko 1983) designation. The IUPAC numbering requires a list for decoding; the shorthand can be decoded directly.

IUPAC	Standard name	Shorthand
82	22'33'4'-pentachlorobiphenyl	73
83	22'33'5'-pentachlorobiphenyl	B3
84	22'33'6'-pentachlorobiphenyl	331
85	22'344'-pentachlorobiphenyl	75
86	22'345'-pentachlorobiphenyl	F1
87	22'345'-pentachlorobiphenyl	79
88	22'346'-pentachlorobiphenyl	711
89	22'346'-pentachlorobiphenyl	712
90	22'34'5'-pentachlorobiphenyl	B5
91	22'34'6'-pentachlorobiphenyl	351
92	22'355'-pentachlorobiphenyl	B9
93	22'356'-pentachlorobiphenyl	B11
94	22'356'-pentachlorobiphenyl	B12
95	22'35'6'-pentachlorobiphenyl	391
96	22'366'-pentachlorobiphenyl	313
97	22'3'45'-pentachlorobiphenyl	D3
98	22'3'46'-pentachlorobiphenyl	531
99	22'44'5'-pentachlorobiphenyl	D5
100	22'44'6'-pentachlorobiphenyl	551
101	22'455'-pentachlorobiphenyl	D9
102	22'456'-pentachlorobiphenyl	D12
103	22'45'6'-pentachlorobiphenyl	591
104	22'466'-pentachlorobiphenyl	513
105	233'44'-pentachlorobiphenyl	76
106	233'45'-pentachlorobiphenyl	F2
107	233'4'5'-pentachlorobiphenyl	B6
108	233'45'-pentachlorobiphenyl	7A
109	233'46'-pentachlorobiphenyl	721
110	233'4'6'-pentachlorobiphenyl	361
111	233'55'-pentachlorobiphenyl	BA
112	233'56'-pentachlorobiphenyl	B21
113	233'5'6'-pentachlorobiphenyl	3A1
114	2344'5'-pentachlorobiphenyl	F4
115	2344'6'-pentachlorobiphenyl	741
116	23456'-pentachlorobiphenyl	F1
117	234'56'-pentachlorobiphenyl	B41
118	23'44'5'-pentachlorobiphenyl	D6
119	23'44'6'-pentachlorobiphenyl	561
120	23'455'-pentachlorobiphenyl	DA
121	23'45'6'-pentachlorobiphenyl	5A1
122	2'33'45'-pentachlorobiphenyl	E3
123	2'344'5'-pentachlorobiphenyl	E5
124	2'3455'-pentachlorobiphenyl	E9
125	2'3456'-pentachlorobiphenyl	E12
126	33'44'5'-pentachlorobiphenyl	E6
127	33'455'-pentachlorobiphenyl	EA

The shorthand designation uses numbers in base 16 (Hex). Values assigned to substituents in positions 2,3,4, and 5 are 1, 2, 4, and 8, respectively. These are added and expressed in Hex (values 10 - 15 are A - F). The designation contains up to 3 digits; the digits from left to right refer to the first ring, the primed ring, and to substituents in the "6" positions, respectively:

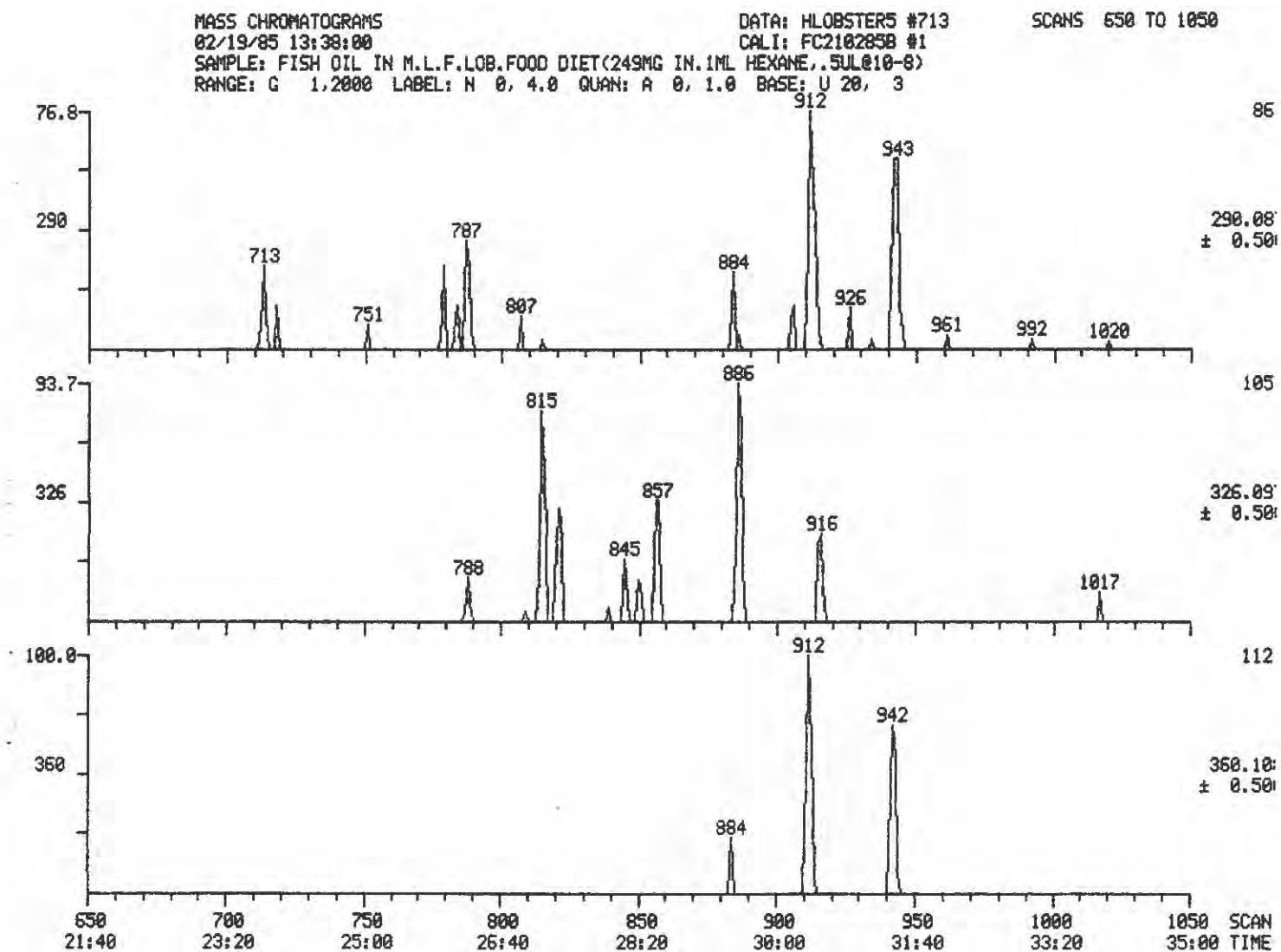
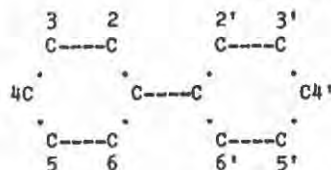


Fig. 4. Mass chromatograms of PCB in a sample of fish oil.

X-axis: Scan number and time from sample injection
 Y-axis: Intensity of ions with m/e specified on the left
 Ions with m/e=290, 326, and 360 are tetra-, penta-, and hexachlorobiphenyls, respectively.



Position	2	3	4	5	2'	3'	4'	5'	6	6'
Value	1	2	4	8	1	2	4	8	1	2
Example substitution: 2,2',3,4',5,5',6										
	X	X		X	X		X	X	X	
	1+2+8=11=B				1+4+8=13=D				1	
Shorthand	BD1									

A similar approach can be used for "shorthand" designation of substitution patterns in other compounds when groups of positions are involved, for example CDD and CDF (Zitko 1985).

Full resolution of individual chlorobiphenyls is a difficult task even for a capillary column operating under optimal conditions, and it is useful to have a "flexible" resolution reference in a program simulating the resolution under different resolving power, to know what to expect. An example of the output is given below for two resolutions. The numbers are retention times relative to octachloronaphthalene. For unresolved mixtures of chlorobiphenyls their retention times are averaged.

Resolution (High 1, Low 100) 50

3924	92 +52
4023.25	511+94 +54
4173.25	7 +61 +32 +912
4254.5	512+34
4354.5	311+A2
4489.5	312+A4 +521
4670.41	1A1+99 +B1 +E +59 +55 +541+D1 +B01+701
4747.5	62 +513
4861.25	39 +64 +321+35
5036.91	9A +161+71 +341+5A +313
5138.5	33 +591+B2
5213	551+D2
5278.5	3A +B4
5336.	B12+F +D4
5494.88	96 +E1 +531+D12+B11+56 +391+AA +711+5A1
5555.5	351+72
5673.5	553+36 +74
5761.	B9 +331+712
5876.63	B5 +D9 +3A1+D5 +6A
6040.83	561+353+B21+721+E2 +B3 +B13
6292.7	
D3 +F1 +F01+E12+713+E4 +B41+741+79 +BA +75 +B52+DA +	
333+66 +361	
6349	D52
6476	73 +B91
6700.32	B32+791+E9 +B51+7A +B6 +E5 +3D1+F2 +D6 +
	752+751
6863.52	F12+B31+F4 +F11+731+E3 +BB
6952.75	B53+BA1+BD +7A1
7065.97	753+732+DD +76 +5E1+EA

Resolution (High 1, Low 100) 25

3937	52
4023.25	511+94 +54
4135	7

4176.75	61 +32 +912
4254.5	512+34
4334	311
4375	A2
4450	312
4499	A4 +521
4555.5	1A1+99
4600	B1 +E +59
4658.5	55 +541+D1 +B01
4685	701
4747.5	62 +513
4832	39
4864.5	64 +321+35
4993.62	9A +161+71 +341
5048.5	5A +313
5102	33
5148.5	591+B2
5213	551+D2
5278.5	3A +B4
5336.	B12+F +D4
5438.03	96 +E1 +531+D12+B11+56
5475	391+AA +711
5518	5A1
5555.5	351+72
5673.5	553+36 +74
5743	B9 +331
5779.	712
5815	B5 +D9
5882.5	3A1+D5 +6A
5977.25	561+353+821
6024.5	721+E2 +B3
6062	B13
6102.5	D3 +F1
6175.12	F01+E12+713+E4 +B41+741+79 +BA
6250.87	75 +B52+DA +333
6304.5	66 +361
6349	D52
6453	73
6499	B91
6573.5	B32+791+E9
6622.5	B51+7A +B6
6700.94	E5 +3D1+F2 +D6 +752+751
6792.5	F12+B31
6845.5	F4 +F11+731
6871.	E3 +BB
6920	B53+BA1
6961.5	BD +7A1
7039.88	753+732+DD +76
7073	5E1+EA
7204	F9 +B33

Data Management and Information Extraction

The volume of data produced by mass spectrometers, gas chromatographs with a capillary column, or spectrophotometers is very large. Efficient ways are needed to extract information from the raw data and the data must be reduced to manageable size or presented in a comprehensible manner. Data must also be communicated between systems since not all systems perform different tasks equally well. As much evaluation as possible should be delegated to computers while the operator retains control over the crucial steps and is able to detect unusual occurrences. Existing programs or described algorithms and numerical techniques have to be implemented on the existing equipment. This takes considerable effort.

A technique to plot data received from a host computer on an intelligent graphics terminal was developed (Zitko 1984a).

An optimization program for general laboratory applications ranging from curve fitting to tuning of chemical analytical equipment was written, based on the simplex algorithm (Zitko 1984b).

Simplex is an n -dimensional object with $(n+1)$ vertices; a triangle in two-, a tetrahedron in three-, dimensions. The vertices are sets of values of parameters (n parameters per vertex) at which the response is determined. The best (B), worst (W) and next-to-worst (N) vertices are located. The mean of all vertices except W is the centroid (C, Fig. 5). Points NC, C, PC, R, and E are negative contraction, centroid, positive contraction, reflection, and expansion, respectively. The mean of all vertices (not shown) is the center of gravity.

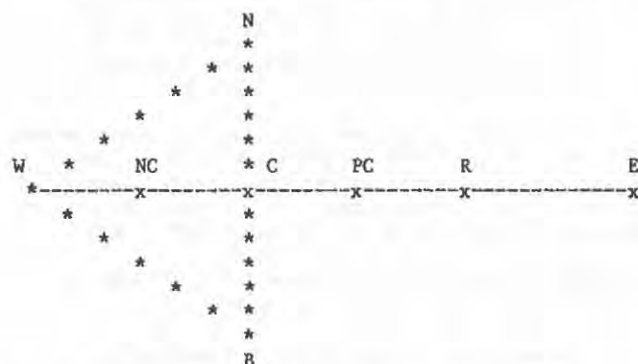


Fig. 5. A two-dimensional simplex. Responses in vertices are: worst(W), next-to-worst(N), and best(B). NC, C, PC, R, and E indicate negative contraction, centroid, positive contraction, reflection, and expansion, respectively.

As can be seen from Fig. 5, all the points of potential simplex movement (NC, PC, R, and E) lie on the straight line through W and C, given by:

$$\text{Point} = C + F * (C - W).$$

where Point = NC, PC, R, or E and F=-0.5, 0.5, 1 or 2, respectively.

Consider for example a two-dimensional simplex of the function $z=2-x^2-y^2$:

x	1	3	2
y	1	1	3
<hr/>			
z	0	-8	-11

The best response (0) is at $x=y=1$; the worst (-11) is at $x=2, y=3$. The coordinates of C are $x=2$ and $y=1$ $[(1+3)/2; (1+1)/2]$ and those of R are $x=2$ and $y=-1$ $[2+(2-2); 1+(1-3)]$. The vertex with the worst response is discarded and a new vertex is chosen at R or E when the response is improving in this direction. If this is not the case, the new vertex is selected at PC or NC. The process is repeated until the size of the simplex decreases below a preset level.

Measurement of several parameters ("variates") per sample gives an opportunity to extract information by factor analysis. Examples of multiparameter measurements are mass spectra where the intensities of ions are measured routinely at 400-500 m/e values. The simultaneous measurements of Cu, Zn, Pb, and Cd or of a number of organochlorine compounds or polynuclear aromatic hydrocarbons are other examples.

Factor analysis is able to determine the number of components in mixtures and, at times, even their composition. This aids the presentation of the data and recognition of patterns in the data.

Factor analysis projects points on axes. The axes are at right angles (orthogonal) and are selected to maximize the sum of the squared projections. For the points A, B, and C these axes are f_1 and f_2 (Fig. 6). The projection of the points

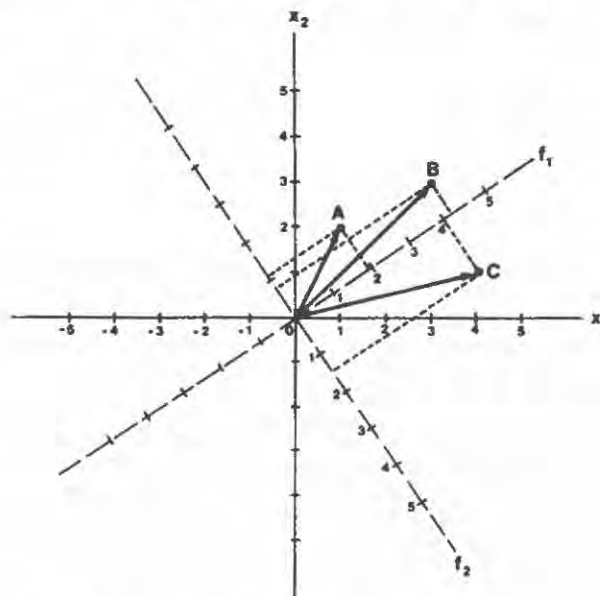


Fig. 6. Principle of factor analysis. Points A(1,2), B(3,3), and C(4,1) are projected on an axis located to maximize the sum of the squared projections (f1). Second axis (f2) is selected perpendicular to the first. Imagine yourself in the center of a swarm of mosquitos. Draw the first axis through the largest width of the swarm. Then search the perpendicular plane for the second largest width to place the second axis, and so on. Try a mental experiment in 4 dimensions.

on the fl axis retains most of their arrangement in the plane. The projection reduces the dimensions of the data from 2 to 1 while retaining most of their "shape". This is important for gaining comprehension of multidimensional data. The following example illustrates the principle of factor analysis. Consider chemicals A and B with the mass spectra:

m/e	57	71	85	99	119
Compound A	100	50	30	0	0
Compound B	100	2	0	22	5

The compounds are eluted from a gas chromatograph into a mass spectrometer and their concentrations in 5 consecutive scans are:

Scan	1	2	3	4	5
Compound A	0.3	0.5	0.4	0.2	0.1
Compound B	0.1	0.2	0.4	0.3	0.2

The peaks of A and B do not coincide. The maximum of A is in scan 2, that of B in scan 3, but the mixture will appear as one broad peak. The resulting mass spectra will be weighted sums of the mass spectra of A and B. For example, mass spectrum in scan 1 will be $0.3 \times (\text{mass spectrum of A}) + 0.1 \times (\text{mass spectrum of B})$. In general, the weighting factors are amounts or concentrations of components; the components are described by their "composition" (in this case mass spectra).

The mass spectra of the 5 scans are:

m/e	57	71	85	99	119
Scan					
1	40	15.2	9	2.2	.5
2	70	25.4	15	4.4	1.0
3	80	20.8	12	8.8	2.0
4	50	10.6	6	6.6	1.5
5	30	5.4	3	4.4	1.0

Factor analysis is this process "in reverse". Mass spectra of A and B are not known. Only the mass spectra of scans 1-5 are available. It is not even immediately obvious that the spectra are caused by a mixture of compounds. Factor analysis first attempts to establish the number of components. This is done by forming the covariance matrix and extracting its eigenvalues. The data matrix is usually preprocessed by subtracting its mean row. The number of "large" eigenvalues is the number of components. Each eigenvalue has an associated direction (eigenvector). The eigenvectors are the constituting components (factor scores, abstract column matrix). A mixture is reconstituted by adding the eigenvectors in amounts given by the "weights" (factor loadings, abstract row matrix).

For the given example:

Abstract components (eigenvectors, factor scores)

.927342	.308919
.317	-.724683
.186533	-.456788
6.72244E-02	.40294
1.52783E-02	9.15774E-02

Abstract amounts (concentrations, row matrix, factor loadings)

-13.2893	-5.42712
19.0391	-5.35979
26.6058	4.29783
-5.72261	4.2305
-26.633	2.25858

The components or factor scores are the mass spectra of A and B. Their amounts are the factor loadings. The scan 1 then is Mean spectrum (row) - $13.2893(\text{eigenvector 1}) - 5.427(\text{eigenvector 2})$.

The components and their weights are different from the "real" ones. This is because factor analysis tries to preserve the "span" of the data. It draws the first coordinate axis in the direction of the largest span, the second axis in the direction of the second largest span perpendicular to the first, etc. Techniques are available to find the "real" components or at least components that could be added in positive amounts to reproduce the data.

Plotting even the abstract row matrix facilitates visual inspection of the data and helps to detect pattern. An example is given in connection with heavy metals.

QSAR

Properties, environmental distribution, and effects of chemicals are functions of its structure. Structure may be considered the "independent" variable; properties, distribution, and effects are the "dependent" variables. The task of QSAR is to relate quantitatively structure to properties, distribution, and effects. To be able to do this, one must express quantitatively all four variables by various "descriptors". Structural descriptors include topology (which atom is bound to which), geometry (shape of the molecule), and the presence of functional groups (chlorine, hydroxyl).

Effects are described by biological descriptors such as LC50 or a dose-response curve; environmental distribution is described by compartmental models (Zitko 1984c). Bioaccumulation of chemicals by fish is usually depicted by a "one compartment" model. In many cases this is a considerable oversimplification and techniques for fitting more complex models to data are being developed.

Compartment is a kinetically homogeneous, distinct amount of a chemical. It may be associated with a tissue or an organ (i.e. gills may be considered a compartment), but an organ may contain several compartments ('bound' and 'free' zinc in the gills). A model consists of one or several compartments. The chemical is transported between compartments at rates characterized by rate coefficient k_{ij} , where k is the value whose dimension is $1/\text{time}$ and i =originating, j =destination compartment. The model is 'open' when the chemical is also exchanged with the environment (compartment 0, Fig. 7). In nonlinear models, at least one of the rate coefficients is a function of concentration and has a dimension of $1/(\text{time} \times \text{concentration})$. Differential equations describing the model are derived from material balances of the chemical in individual compartments (change = input-output):

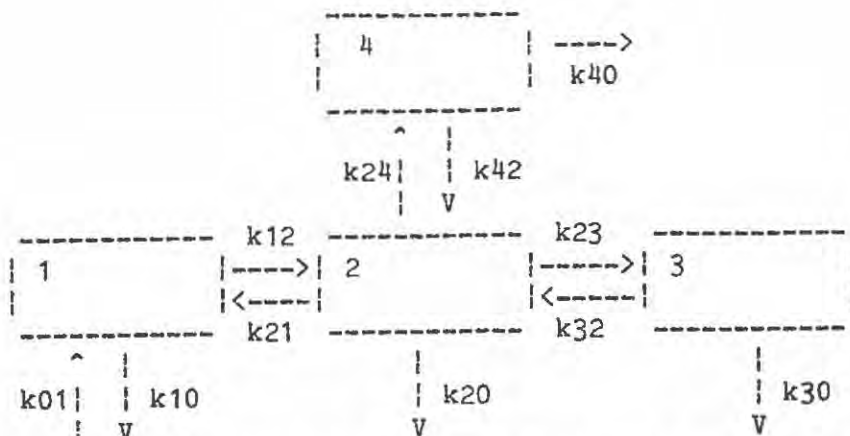


Fig. 7. A four compartment mammillary (peripheral compartments 1,3,4 connected to the central compartment 2) open (a chemical enters from, and may be excreted to the environment (compartment 0)) model. The compartments 1-4 may be, for example, gills, blood, liver, and kidney, respectively.

A mass balance for compartment 1 (Fig. 7) is:

Input = $k_{01} \cdot c_w + k_{21} \cdot c_2 \cdot V_2$ (unit: mass/time)
Output = $k_{10} \cdot c_1 \cdot V_1 + k_{12} \cdot c_1 \cdot V_1$ (unit: mass/time)

Change = $V_1 \cdot dc_1/dt = k_{01} \cdot c_w + k_{21} \cdot c_2 \cdot V_2 - k_{10} \cdot c_1 \cdot V_1 - k_{12} \cdot c_1 \cdot V_1$

or, $dc_1/dt = -(k_{10} + k_{12}) \cdot c_1 + k_{21} \cdot V_2/V_1 \cdot c_2 + k_{01}/V_1 \cdot c_w$,

where k_{ij} = transfer rate coefficients (unit 1/time, except for k_{01} which is volume/time),
 c_w = concentration in water,
 c_1 = concentration in compartment 1 (unit mass/volume),
 V_1 = volume of compartment 1 (unit volume).

Differential equations for the other compartments may be derived in the same way. The model is then described by a system of differential equations.

The task is to obtain values of the transfer rates k_{ij} from the compartment sizes V_i and measured concentrations of the chemical in the compartments. Techniques are being adapted for this purpose. Two environmental compartmental models are operational. One is based on the fugacity approach of Dr. Mackay (University of Toronto), the other is the NRCC Environmental Secretariat model, adapted to our computer. A wider use of these models in verification and risk estimation is desirable. The U.S. EPA model "EXAMS" is too large and complex for our computer.

Biodegradation is an important factor in environmental distribution of chemicals. Biodegradation intermediates are often quite difficult to identify and measure. The state of the art in chemical analytical techniques characterizing biodegradation was reviewed (Zitko 1984d).

HEAVY METALS IN SEDIMENTS

Knowledge of the effects of heavy metals in sediments is needed mainly for the estimation of risk to fisheries from dredging and dredge spoil

dumping. The metals of prime concern are Cd and lead (Pb). Copper (Cu) and zinc (Zn), although very toxic to fish in water, are of lesser concern in sediments.

In sediments the metals are present as insoluble salts, usually sulfides or carbonates or, for Pb as lead sulfate, and also bound by organic matter. Additionally, a portion of the metals is present in crystal lattices of minerals of the earth crust. The portion present as salts or bound by organic matter is to some extent leached by water and may be toxicologically important. The ease of leaching affects the "bioavailability" of the metals. Bioavailability is, as a rule, not related to the "total" concentration of the metals in the sediments (Ray and McLeese 1983).

Metal content of suspended particulate matter (SPM) was studied in the Miramichi estuary (Ray and Macknight 1983). The average concentrations of Cu, Zn, Cd, and Pb were 1.2, 2.6, 0.03, and 0.6 mg/g dry SPM, respectively. SPM contained about 4% of organic carbon. In most cases the concentration of heavy metals in SPM was much higher than that in the sediment. The variations were large and effects of dredging in the estuary were not detectable.

Zinc ore concentrates shipped from the port of Dalhousie, N.B., contaminate sediments in the harbour. Seawater leaches Cu, Zn, Cd, and Pb from the concentrates and from contaminated sediments. Organic carbon of the sediments decreases somewhat their bioavailability. Only Pb and Zn were accumulated by *Crangon septemspinosus* and by *Nereis virens* exposed to contaminated sediments in the laboratory (Ray and Peterson 1983).

The concentrations of Cu, Zn, Cd, Pb, molybdenum (Mo), nickel (Ni), manganese (Mn), and mercury (Hg) were low in sediments in Saint John Harbour, but anthropogenic input in one area was detectable (Ray and Macknight 1984).

Metal	Cu	Zn	Pb	Cd	Mo	Ni	Mn	Hg
Site	$\mu\text{g/g}^{-1}$ dry wt							
1 Inner harbour	15.9	53.3	24.3	0.16	3.1	16.3	296	0.05
2 Grand Bay	11.1	41.2	24.3	0.18	3.3	17.1	328	0.04
3 Narrows	14.5	41.9	20.5	0.14	2.9	15.8	248	0.02
4 Rodney Terminal	12.6	46.7	20.9	0.10	3.5	16.3	307	0.02
5 Courtenay Bay	20.6	65.9	30.8	0.22	2.6	16.7	285	0.03
6 Dumpsite	11.5	44.2	18.5	0.06	4.2	15.1	283	0.03
7 Outer harbour	15.1	69.2	21.9	0.08				0.03

As an example of the application of factor analysis, the data for Cu - Cd could be reasonably well reproduced by using two factors (Fig. 8). This may indicate two sources. The figure presents well the similarity relationships of the samples.

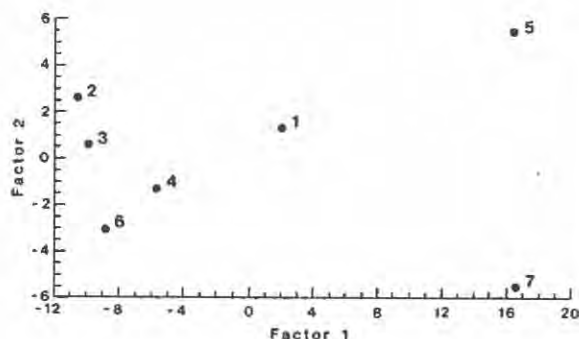


Fig. 8. Heavy metals (Cu, Zn, Pb, and Cd) in sediments in Saint John Harbour, a two-factor projection.

X-axis: First abstract factor
Y-axis: Second abstract factor
1=Inner harbour; 2=Grand Bay; 3=Narrows;
4=Rodney Terminal; 5=Courtenay Bay;
6=Dumpsite (in outer harbour); 7=Outer harbour.

HEAVY METALS IN SCALLOPS

Cu, Zn, Cd, and Pb were measured in scallops from Chaleur Bay, N.B. (Ray et al. 1984a) and from Georges and Browns Banks (Ray et al. 1984b). The levels of Cd and Pb in scallops from all three sites were higher than those from a control site in Passamaquoddy Bay, N.B. With the exception of a few sites in Chaleur Bay with known anthropogenic inputs, the elevated levels reflect local geological conditions.

RISK ASSESSMENT

The bioaccumulation of Cd in marine organisms was reviewed (Ray 1984). In spite of its extremely low concentration in seawater (about 40 ng/L), molluscs and crustaceans accumulate Cd to a considerable extent and do not appear able to excrete it. The literature on the accumulation of Cd and on conditions affecting it is very extensive and significant progress has been made in the

studies of the metal-binding protein, metallothionein, and on storage mechanisms of Cd as well as of other metals. At the same time much remains to be learned.

With a few exceptions it appears that levels of Cd as well as those of other metals have not been elevated by anthropogenic activities in marine fauna of the western North Atlantic (Ray and Bowers 1984).

In freshwater, Cd at 2 $\mu\text{g/L}$ slowed the growth of Atlantic salmon *Salmo salar* alevins and caused 80-90% mortality of feeding fry. The mortality of fry reared previously in the presence of Cd at 2 $\mu\text{g/L}$ was lower (Peterson et al. 1983). The accumulation of Cd by salmon eggs is rapid and decreases with decreasing pH (Peterson et al. 1985).

FIELD STUDIES

It is always difficult to determine causes of changes (usually declines in populations of fish and shellfish) and only a few cases exist where a causal relationship has been established, even tentatively. One more case can now be added to the record. In recent years, striped bass (*Morone saxatilis*) have not reproduced successfully in the Annapolis River, N.S., and a high embryonic mortality has been reported for striped bass eggs from the St. John River, N.B. Levels of PCB were higher (1.4 $\mu\text{g/g}$) in the gonads of the fish from the Annapolis River than from the Shubenacadie River, N.S. (0.04 $\mu\text{g/g}$) (Ray et al. 1984c). The effect of PCB on fish reproduction has been documented in several species in the laboratory.

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BIOCHEMICAL TOXICOLOGY

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Exposure of animals to sublethal levels of xenobiotics may cause stress on mechanisms required for maintaining a healthy physiological state. This stress may result in changes in biochemical, physiological or behavioral processes in aquatic organisms. Quantitative measurements of these sublethal effects on biochemical parameters will increase our ability to predict acceptable levels of pollutants in the environment. Such indicators could also be developed as early warning signals of potential pollution problems or for determining the health of an aquatic population. The objective of the Biochemical Toxicology Program is to develop biochemical parameters as indicators of sublethal effects caused by xenobiotics. We have focused our attention on energy metabolism.

Adenosine triphosphatases (ATPases) are a group of enzymes which hydrolyze adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (Pi). In the process, the energy released becomes available for cation transport. These enzymes, especially sodium, potassium-activated ATPase (Na,K-ATPase), play a central role in whole body osmoregulation (Towle 1981). Na,K-ATPase activity is inhibited by ouabain and those ATPases not inhibited by ouabain are referred to collectively as residual ATPase. Exposure of aquatic animals to various chemicals alters the activity of ATPases and Na,K-ATPase activity is a potentially useful indicator of sublethal toxicity (Haya and Waiwood 1983).

Adenylate energy charge (AEC) is another potential indicator of sublethal effects in multicellular aquatic organisms (Haya and Waiwood 1983; Ivanovici 1980). $AEC = \frac{(ATP + 0.5ADP)}{(ATP + ADP + AMP)}$ is a measure of the amount of energy available from the adenylate pool and is a prime factor in the regulation of the flux of energy in catabolic and anabolic processes of cells (Atkinson 1977). Changes in AEC of aquatic animals during environmental (salinity, temperature, pH) or xenobiotic stress have been reviewed (Haya and Waiwood 1983).

AEC may not be applicable in systems where phosphagens dominate as energy carriers (e.g. muscle) or where adenylate kinase is not sufficiently active (e.g. mitochondrial matrix) (Reich and Sel'kov 1981). Creatine phosphate (CP) in vertebrates and arginine phosphate in invertebrates are the main phosphagens that accept or return energy equivalents from the adenylate pool. Cells use this process to maintain a constant AEC until the phosphagen pool is exhausted, provided that inorganic phosphate concentration is well buffered. In those systems where inorganic phosphate levels are limited or weakly buffered, its concentration is expected to be a factor in mechanisms controlling the energy state of cells (Erecinska et al. 1977). In this situation the

phosphorylation potential ($PP = ATP/(ADP + P_i)$) may be a better measure of the metabolic energy state (Erecinska et al. 1977; Erecinska and Wilson 1982; Reich and Sel'kov 1981). Therefore phosphagen levels or PP may be altered on, or may be responsive to, exposure to sublethal levels of chemicals in cases where AEC remains constant.

Glucose and glycogen are principal substrates in central pathways of energy-producing carbohydrate metabolism in the liver, and their concentrations were also determined. Alterations in levels of these substrates can be caused by starvation, hypoxia or sublethal exposure to chemicals. Recent projects of this program are:

1. Sublethal effects of xenobiotics on juvenile Atlantic salmon;
2. Hypoxia and organochlorine toxicity to Nereis virens;
3. Low pH and smoltification of Atlantic salmon parr.

1. SUBLETHAL EFFECTS OF XENOBIOTICS ON JUVENILE ATLANTIC SALMON

The purpose of this project was to evaluate ATPase activity of gills and intermediary energy metabolism of liver in juvenile Atlantic salmon, Salmo salar, as indicators of sublethal effects of chemicals. Gills are the main osmoregulatory surface organ in aquatic animals and are the primary site of uptake of waterborne pollutants. Therefore, sublethal effects of chemicals on Na,K-ATPase activity would be expected in gills. The liver is metabolically a very active tissue, the site of biotransformation of most xenobiotics, an organ in which bioconcentration of chemicals can be large and the target organ of many toxic compounds. Thus the liver is a likely organ in which sublethal effects on energy metabolism will occur. The project includes results from three separate experiments.

The Na,K- and residual ATPase activity in gill and AEC, PP, CP, glucose and glycogen in liver after exposure of juvenile Atlantic salmon to sublethal levels of 18 chemicals were determined. The chemicals included eight chlorinated or alkylated phenols, five organochlorine pesticides and five ester type pesticides (Table 1). Exposures were for 96 h at nominal concentrations that were 50-90% of nominal LC50 values for the phenols, and nominal concentrations that were equal to or twice LC50 values based on average measured concentrations for all pesticides (for details of methodology see Haya et al. 1985).

A UV-Vis spectrophotometric kinetic method of analysis for ATPase activity was developed for salmon parr gills. Previously we used a technique where accumulation of one of the end products (inorganic phosphate) after 20 min of incubation was measured. This method was cumbersome and had several potential sources of error that had to be carefully monitored. The new method was adapted from a technique used to measure ATPase activity of mammalian renal tubules (Schwartz et al. 1983) and is a more sensitive and "direct" method of determining ATPase activity. It also has the advantage that both Na,K- and residual ATPase activity can be determined on the same sample.

In all three experiments, Na,K- and residual ATPase activities in gill of 0-h control fish were not significantly different from that of 96-h controls within each experiment. The significant differences in activities of gill ATPases from control fish among the three experiments may be related to factors such as life stage, size or season. Gills of fish exposed to eight of the 18 chemicals had significant differences in activity of ATPases from those of their respective 96-h controls. Na,K-ATPase activity of fish exposed to endosulfan, kepone, toxaphene and aminocarb increased while those exposed to 4-methylphenol decreased compared to those of their respective 96-h controls. Residual ATPase activity increased on exposure to 2-chlorophenol, p-nonylphenol, toxaphene, kepone, azinphosmethyl and malathion but decreased on exposure to aminocarb. Variability in the response of ATPases to xenobiotics has been noted previously (see Haya and Waiwood 1983, for review). There is no obvious relationship between structure and effect. The lack of response on exposure to most of the chemicals may be due to dose; that is the exposure concentration may have been below the threshold concentration required to produce an effect on the ATPases. Our limited data suggest that with kepone the threshold for increase in activity of ATPases is between 0.08 and 0.12 mg/L.

Some of the parameters of energy metabolism measured in liver of control salmon were different between experiments and with time within experiments (Table 2). There was a decrease in glycogen levels from 0-h controls to 96-h controls. This was probably an effect of starvation as the fish were not fed during the exposure period. The cause of the differences in glycogen levels or PP between experiments is not known but may be related to season, size, nutritional status and sexual maturity.

After 96 h of exposure to the chemicals, differences in parameters of energy metabolism between the treated and controls were not consistent. However, glycogen levels were consistently higher in the 0-h controls than in those of the 96-h controls which in turn were higher than in those of fish exposed to chemicals. AEC also showed a similar trend and values were lower in salmon exposed to organochlorines or ester-type pesticides, but not in those exposed to phenols. The results suggest that the increased energy requirements caused by acute exposure to chemicals are met by increased glycolysis resulting in a depletion of glycogen stores.

2. HYPOXIA AND ORGANOCHLORINE PESTICIDE TOXICITY TO NEREIS VIRENS

Relatively high levels of organochlorine pesticides are not as toxic to the polychaete worm, Nereis virens, as they are to other invertebrates (McLeese et al. 1982). Some marine invertebrates, including N. virens, survive extended periods of anoxia by switching to anaerobic pathways of energy metabolism. The present study investigated the possibility that the tolerance of N. virens to relatively high levels of organochlorines was related to its ability to switch to anaerobic metabolism. Specifically, would organochlorines stimulate a response similar to that induced by anoxia and thus decrease uptake of or alter

Table 1. Nominal exposure concentration, means of weight, Na,K- and residual ATPase activity in gill of juvenile Atlantic salmon exposed to phenols and pesticides for 96 h.

Treatment	n	Exposure conc. (mg/L)	Mean wt (g)	Mean ATPase activity (μ mole Pi/mg protein x h)		
				Na,K	Residual	Total
Phenols						
0-h control	27		4.6	0.4	2.0	2.4
96-h control	23		3.8	0.5	2.3	2.8
Pentachlorophenol	18	0.04	4.4	0.4	2.0	2.4
p-Nonylphenol	15	0.5	4.2	0.7	3.1 ^a	3.8 ^a
2-Chlorophenol	15	0.6	4.0	0.5	3.4 ^a	3.9 ^a
4-Methylphenol	15	1.5	4.4	0.3 ^a	1.9	2.2
2,4,5-Trichlorophenol	14	1.6	3.7	0.4	2.3	2.7
Phenol	21	2.7	3.9	0.4	2.3	2.7
2,6-Dimethylphenol	23	10.5	4.9	0.4	2.7	3.1
3,5-Dimethylphenol	15	12.0	5.0	0.4	2.3	2.7
Organochlorine pesticides						
0-h control	20		108	1.8	6.4	8.2
96-h control	25		81	1.9	6.2	8.1
DDT	14	0.13	93	1.7	7.1	8.8
Endosulfan	15	0.02	100	2.7 ^b	7.2	9.9 ^b
Dieldrin	14	0.10	90	2.3	6.9	9.2
Toxaphene	13	0.14	96	2.5 ^a	6.4 ^b	8.9
Kepone	4	0.20	95	1.8	8.5 ^b	10.3 ^a
	8	0.12	82	2.9 ^b	7.5 ^a	10.4 ^b
	10	0.08	82	2.0	6.1	8.1
Ester-type pesticides						
0-h control	15		74	2.8	7.4	10.2
96-h control	15		72	3.4	6.5 ^b	9.9 ^b
Malathion	14	0.2	76	3.1	9.1 ^b	12.2 ^b
Fenitrothion	15	1.0	66	3.3	6.8	10.1
Azinphosmethyl	15	2.0	71	2.8	8.1 ^a	10.9
Carbaryl	2	4.6	82	3.8 ^b	6.6	10.4
Aminocarb	15	8.3	67	4.3 ^b	5.7 ^a	10.0

^aSignificantly different ($p < 0.05$) from 96-h controls in t-test.

^bSignificantly different ($p < 0.01$) from 96-h controls in t-test.

Table 2. Biochemical parameters of energy metabolism ($\mu\text{mol/g}$ wet weight) in liver of control juvenile Atlantic salmon.

Experiment	Phenols control		Organochlorines control		Ester-type pesticides control	
	0-h	96-h	0-h	96-h	0-h	96-h
n	5	5	15	15	15	15
ATP	0.99	1.19	1.58	1.25	1.31	1.31
ADP	0.81	0.85	0.94	0.85	0.74	0.95
AMP	0.05	0.06	0.21	0.19	0.05	0.08
TOTAL ADEN	1.85	2.10	2.72	2.29	2.10	2.34
AEC ^a	0.751	0.767	0.752	0.723	0.798	0.762
CP	0.53	0.19	0.26	0.20	0.08	0.14
Pi	6.8	8.4	5.5	4.9	4.2	4.5
PP ^b	0.23	0.19	0.33	0.32	0.46	0.33
GLUCOSE	6.2	4.6	3.1	4.7	3.0	4.6
GLYCOGEN	402	250	539	394	214	176

^aunitless

^bper mM

intermediary energy metabolism to overcome the toxic effects of organochlorines?

In a series of uptake and excretion studies, *N. virens* were acclimated to hypoxic or normoxic conditions for 24 h, exposed to several organochlorines for 96 h (Table 3) and then placed in unspiked normoxic or hypoxic seawater for 336 h. Worms were sampled periodically and analyzed for organochlorine content or, in the case of non-radioactive experiments, analyzed for levels of energy metabolism substrates. Dissolved oxygen levels were 94% saturation for normoxic treatment and 6% saturation for hypoxic treatment.

Worms exposed to hypoxia at 7°C in this experiment survived longer than 400 h. However, worms sampled late in the excretion phase of the experiment appeared to be near death (flaccid, inactive and showing very little reaction to stimuli).

There were no differences in rate of excretion of pesticide for worms held in hypoxic or normoxic conditions. Generally little if any excretion was indicated. Uptake was greater during hypoxic exposure than that observed during normoxic exposure for both DDT and endosulfan. There appears to be no difference in uptake by worms exposed to dieldrin in hypoxic or normoxic seawater. The pesticides continue to be taken up by the worms regardless of oxygen concentration in the surrounding water. Therefore tolerance of *N. virens* to DDT, dieldrin, and endosulfan cannot be explained in terms of uptake and excretion kinetics under normoxic or hypoxic conditions.

The levels of ATP, ADP, AMP, Pi, glucose and glycogen of 0-h normoxic control worms were 2.03, 1.11, 0.52, 10.3, 0.51, and 99 $\mu\text{mol/g}$ wet weight, respectively. The levels of these substrates were not significantly different from those of the 96-h normoxic controls, or the 0- and 96-h hypoxic controls. Thus the values of AEC and PP (0.764 and 0.38/mM, respectively, for 0-h normoxic worms) which were derived from the concentrations of these substrates were not significantly different among the four control groups. Unlike most invertebrates,

Table 3. Average measured concentrations of organochlorine pesticides in water during 96 h that *Nereis virens* were exposed.

Organochlorine	$\mu\text{g/L}$	
	Hypoxia	Normoxia
pp-DDT	3.5	4.6
14C-DDT	3.3	4.3
Dieldrin	7.1	9.0
14-Dieldrin	2.2	1.9
Endosulfan	55.4	64.3

N. virens have CP rather than arginine phosphate. CP levels in 0- and 96-h hypoxic control worms (1.89 and 1.94 $\mu\text{mol/g}$ wet weight, respectively) were significantly lower than those of 0- and 96-h normoxic controls (2.24 and 2.49 $\mu\text{mol/g}$ wet weight, respectively) (Fig. 1). CP levels were not different between 0- and 96-h controls within each oxygen treatment.

There were no differences in the biochemical parameters between controls and worms exposed to organochlorines under normoxic conditions. Exposure to both hypoxia and organochlorines caused significant differences (ANOVA; $p < 0.01$). The levels of Pi and glucose increased and glycogen decreased in worms exposed to anoxia and organochlorines for 96 h compared to all other treatments. CP concentration was lower in worms exposed to hypoxia and organochlorines than in those exposed to hypoxia, which in turn was lower than normoxic controls or normoxic organochlorine-exposed worms (Fig. 1). These results indicate that exposure to hypoxia and organochlorines has a synergistic effect and that the resulting increase in energy requirements is met by an increase in glycolysis and utilization of CP to maintain ATP levels and AEC.

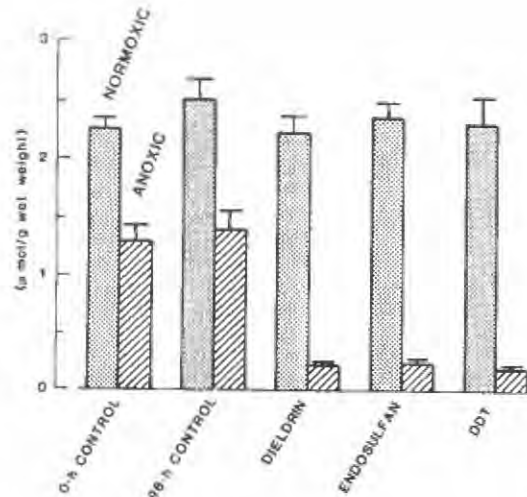


Fig. 1. Levels of CP in *Nereis virens* exposed to dieldrin, DDT, and endosulfan under normoxic and hypoxic conditions for 96 h. Each bar represents the mean of six worms (+ 1 S.E.).

3. LOW PH AND SMOLTIFICATION OF ATLANTIC SALMON PARR

Smoltification does not proceed normally when parr are exposed to sublethal levels of low pH. Exposure of Atlantic salmon parr to low pH prevents the increase in Na,K-ATPase activity and plasma Na⁺ and Cl⁻ concentrations which occurs during smoltification (Saunders et al. 1983). Acute exposure to low pH causes alterations in carbohydrate metabolism in both red and white muscle tissue of *Tilapia mossambica* (Murthy et al. 1981), an increase in blood glucose and lactate concentrations of *Coregonus peled* and *Salmo trutta* (Nieminen et al. 1982), and a decrease in adenylate energy charge (AEC) of four organs of *Fundulus grandis* (MacFarlane 1981). The purpose of this study was to determine the effects on intermediary energy metabolism when Atlantic salmon were exposed to low pH during the period when smoltification normally occurs.

LABORATORY EXPERIMENT

Atlantic salmon parr were held for 76 d at pH 4.7 during the period when the final stages of smoltification normally occur. Control salmon (pH 6.5) had a significant increase in weight, length, and liver somatic index which was not observed in those held at low pH. Condition factor decreased in both groups but to a significantly greater extent in the low pH group. Decreased food intake probably contributed to the lack of growth by salmon parr exposed to low pH.

At the start of the experiment the mean levels in muscle of adenine nucleotides were 6.93, 0.86, 0.09 µmol/g wet weight for ATP, ADP and AMP, respectively, and the AEC was 0.910. The concentrations of CP, glucose and glycogen (glucose equivalents) were 5.8, 1.1 and 18 µmol/g wet weight,

respectively. In the liver the initial control concentrations were 1.04, 0.8 and 0.2 µmol/g wet weight for ATP, ADP and AMP, respectively, and AEC was 0.689. CP, glucose and glycogen were 0.27, 8.6 and 163 µmol/g wet weight, respectively.

The concentrations of energy metabolism substrates which we determined in liver of control salmon varied significantly ($p < 0.05$) with time but in a random fashion. An exception was the level of CP, which decreased steadily after the 20th day. These random variations were probably of no physiological significance. Similar random effects with time were found in livers of fish exposed to low pH except that AMP, CP and glycogen levels did not vary significantly and the glucose level had a definite pattern (see below). In muscle of control salmon ADP and glucose levels decreased ($p < 0.1$ and $p < 0.5$, respectively), and CP and glycogen levels increased ($p < 0.1$) with time. AEC values fluctuated ($p < 0.5$) with time randomly but all values were greater than 0.9 and the changes were probably of no physiological significance. Glucose levels in muscles of fish held at low pH increased with time and were particularly elevated from day 13 to 28. A similar pattern to that of muscle glucose was observed for liver glucose levels. The rise in concentration for muscle glucose lagged behind that for liver glucose by at least several days. This supports the hypothesis that the source of muscle glucose is from liver via blood.

After 15 d the levels of substrates in muscle, except for AMP, differed in a consistent manner between the two treatments. ADP and glucose levels were higher in acid-exposed than in control fish. However, the difference in levels of ADP was due to a decrease in ADP concentration in control muscle rather than an increase in concentration of salmon exposed to low pH. AEC, CP and glycogen levels were lower in muscles of the acid-exposed group compared to those of controls.

A sustained decrease in ATP and total adenylate levels occurred after 62 d of exposure to low pH. AEC remained only slightly lower in muscles of acid-exposed salmon compared to those of controls after 15 d of exposure to low pH. AEC values in the acid-exposed group remained above 0.9 which is in the range expected for fish muscles in a healthy metabolic energy state. However, the lower levels of CP, ATP, total adenylates, and glycogen would suggest that muscle energy metabolism is stressed and energy reserves are depleted under low pH conditions.

The concentrations of ATP, total adenylates, AEC and glucose were consistently higher (ANOVA; $p < 0.01$) in the livers of acid-exposed salmon than those of controls. There were no differences between the liver concentrations of AMP, CP and glycogen in the two treatments. However, if amount of substrates was considered on the basis of total amount in the liver rather than on concentration the results were different. There was an increase in the amount of all substrates in liver of controls but no change with time in livers of acid-exposed salmon. This is related to the increase in liver weight of controls. The small decrease in liver weight of acid-exposed fish did not increase the concentration of substrates significantly. An exception is the high concentration of glucose in liver of acid-exposed salmon which probably resulted from an increase in gluconeogenesis using dietary protein rather than glycogen consuming pathways. The increased glucose concentrations may also indicate a decrease in glycolysis.

MERSEY FISH HATCHERY EXPERIMENT

This study was carried out at the Mersey Fish Hatchery, Nova Scotia, and included three exposure conditions: normal hatchery water at pH 4.9 (control), acid-treated water at pH 4.5 (low pH) and lime-treated water at pH 6.5 (limed). Fish were divided into the three groups on December 18, 1982, and sampled on March 15, April 19, and May 4, 1983 (56, 109 and 124 d of experiment, respectively). This experiment was conducted as part of a larger study (Saunders, Fish Physiology Section, Fisheries and Environmental Sciences) and sampling procedures were such that only liver could be sampled without introducing changes in biochemical parameters of energy metabolism due to sampling stress.

Only fish of the limed group had a significant increase in length and weight during the experiment. The CF progressively decreased in fish held at low pH. Similarly to the laboratory experiment, liver weight and CF were always lower in the fish exposed to low pH than in the limed or control groups.

The levels of substrates for liver of control fish on March 15 were 1.07, .074, .09, .051, 15.35, 342, 4.97 $\mu\text{mol/g}$ wet weight for ATP, ADP, AMP, CP, glucose, glycogen and Pi, respectively. AEC and PP values were 0.756 and 0.33/mM, respectively. There were no significant differences in any of the biochemical parameters we measured between fish held in limed water and those of controls throughout the experiment.

As in the laboratory experiment, exposure of salmon parr to low pH caused an increase in energy requirements which were partially met by an increase in gluconeogenesis. The effects were not as definite as in the laboratory study as the fish were sampled less frequently and muscles were not sampled. Liver glycogen levels were lower in fish

held at low pH fish than that in controls on April 19 and May 4. Glucose levels were higher in fish held at low pH compared to controls on all three sampling days. There was a trend towards higher levels of total adenylates and values of AEC for the low pH group.

Some effects observed in the hatchery study were not significant in the laboratory study. These were a higher Pi concentration in liver of low pH group compared to those of controls on April 19 and May 4. PP was also significantly lower in the low pH group on May 4. These results are also consistent with an increased energy metabolism in liver of fish exposed to low pH.

The results of both the laboratory and hatchery experiments indicate that exposing Atlantic salmon parr to low pH causes an increase in energy requirements which is met by an increase in gluconeogenesis. The result is consumption of protein which probably occurs after or along with a depletion of lipid reserves. Gluconeogenesis with amino acids rather than carbohydrates as the initial substrate is the preferred route of maintaining glucose and glycogen levels in fish. Thus dietary and endogenous protein is the primary source of energy in fish, and in conjunction with decreased food intake and increased energy consumption there is no protein available for growth. This may be a contributing factor in impairment of smoltification of Atlantic salmon parr during exposure to low pH.

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INVERTEBRATES AND CONTAMINANTS

D.W. McLeese

LETHAL BODY BURDENS OF CONTAMINANTS

Contaminant levels within aquatic organisms that were sampled from nature may indicate whether the contaminant in question has accumulated above background levels or not. However, because of lack of information, it is more difficult, usually impossible, to assess whether the level within the organisms is approaching sublethal or lethal levels.

To investigate lethal body burden, shrimp (*Crangon*) were exposed to Cd in seawater to determine concentration of Cd that kills 50% within various specified times (LC50's). Shrimp were exposed to Cd at 0.06 to 2.7 mg/L. No deaths occurred at 0.06 mg/L in 18 d. The estimated LC50's were 2, 1.7, 1.2, 1.0, and 0.47 mg/L for 4, 6, 7, 8, and 11 d, respectively. Based on these lethality data, more shrimp were exposed to Cd at 0.4 to 2.0 mg/L for 13-33 d. Shrimp were sampled from each test concentration when close to 50% mortality had occurred and were analyzed to determine Cd concentration within the tissues (M.R. Peterson, unpubl. data).

The lethal threshold concentrations for Cd in tissues (LTCT) were estimated as the geometric mean of the concentration in the tissue associated with no mortality and the lowest concentration that caused 50% mortality. These estimates are shown

(Table 1). Because of the wide spread between the exposure concentrations of 0.06 and 0.4 mg Cd/L, the LTCT values may be overestimated.

The Cd concentration in tissues increased with increased Cd concentration in the water even though exposure time decreased. In other words, the Cd concentration in tissues of shrimp exposed to lethal levels of Cd for tissues approximating LT50's exceeded the LTCT. An overshoot of the LTCT is to be expected in parallel with the well documented inverse relationship between concentration during exposure and time to 50% mortality seen in lethality studies (examples, McLeese and Metcalfe 1980; McLeese et al. 1982). The fact that Cd is not metabolized or excreted probably contributes to the magnitude of the overshoot.

The LTCT is the main information required for the assessment of the significance of contaminant level within an organism sampled from nature. Such information can be generated for various contaminants from information such as that in Table 1. A simpler approach would be to measure contaminant levels within organisms that had been exposed to the contaminants in water at concentrations just below lethal threshold levels, if known from previous lethality tests. Exposure times should approximate the maximum time utilized in the lethality tests. Such information should provide reasonably reliable estimates of the LTCT's.

FILTRATION RATE OF MUSSELS

Study of the effects of contaminants on filtration rate of mussels was initiated. The overall objective was to examine the comparative uptake of contaminants from water and from food. Reasonably consistent control filtration rates were obtained by using mussels of uniform size and by maintaining constant temperature and reasonably constant algal cell (*Dunaliella*) concentration in the water. The filtration rate varied from about 800 to 2100 mL/h/mussel (estimated feeding rate 30 to 90 million cells/h/mussel) when algal cell concentration in the solution around the mussels was 20×10^3 to 50×10^3 cells/mL. A trend for filtration rate to vary with cell density over this range could not be recognized.

Considering that certain contaminants might produce irreversible changes in filtration rate (sublethal or lethal effects), background information on control filtration rates was required to reduce time required to "stabilize" filtration rates when different groups of mussels were substituted in the test chamber.

The effects of cadmium, copper, endosulfan, dieldrin, and DDT present in the algal cell stock solution and in the seawater around the mussels on filtration rate are summarized (Table 2). Also the effects of endosulfan and dieldrin present in the algal cell stock solution are summarized.

Examination of the partitioning of endosulfan and DDT between algal cells and the seawater medium indicated that practically all of these compounds would be associated with the cells (in or on the cells).

Increasing levels of cadmium, copper, and endosulfan in cells and water reduced the filtration rate progressively. Dieldrin and DDT did not reduce filtration rate, at least up to the highest levels tested.

Table 1. Cd concentration in tissues of shrimp exposed to Cd in seawater at concentrations that caused close to 50% mortality at specified times.

Cd (mg/L)	Exposure Times (d)	Cd concentration in tissues (mg/kg tissues, dry wt)			
		Whole shrimp	Hepatopancreas	Tail muscle	Remainder
Control ^a	28	0.8	17	0.2	0.3
0.06 ^a	18	10.2	74	0.4	8.4
0.4	11	18.7	284	1.3	19.6
0.64	7	19.2	-	-	-
1.3	6	26.6	-	-	-
2.0	3.3	37.2	627	5.9	60.2
Estimated threshold concentration in tissues		15	225	1	15

^aNo deaths.

Table 2. Effect of various chemicals on filtration rates of mussels.

Chemical	Concentration of chemical (mg/L)		
	No reduction in rate	50% reduction in rate	Filtration not initiated
Cadmium (cells and water)	2	3	8
Copper (cells and water)	0.075	0.09	0.15
Endosulfan (cells and water)	0.5	1.0	1.25
Dieldrin (cells and water)	3.0 (highest level tested)		
DDT (cells and water)	8.0 (highest level tested)		
Endosulfan (cells)	2.5 (highest level tested)		
DDT (cells)	8.0 (highest level tested)		

Endosulfan in cells and water at 1.0 mg/L reduced the filtration rate by 50%. However, endosulfan in cells at 2.5 mg/L (the highest level tested) did not alter the filtration rate. From this limited evidence with endosulfan, it appears that the stimulus for reduction in filtration rate may be dependent on the concentration of the contaminant in the water around the mussels rather than directly associated with the algal cells. Depending on the validity of this relationship, there may not be a sensory mechanism to lower the filtration (and feeding) rate unless, at the same time, the contaminant level in water is relatively high. In other words, maximum accumulation of contaminants by mussels might occur when food cells have concentrated the contaminant and the resultant contaminant level in the water is low.

RELATIVE HAZARD OF PYRETHROIDS TO THE AQUATIC ENVIRONMENT

Four pyrethroid insecticides were evaluated in relation to their potential hazard to the aquatic environment in case they are recommended sometime for use in forest spraying. The evaluation was based on a six-compartment model (air, soil, water, suspended solids, aquatic biota, and sediment) developed previously (Zitko and McLeese 1980).

The relative hazard indices (RHI) are 40 for permethrin, 35 for deltamethrin, 190 for fenvalerate, and 750 for cypermethrin compared with a RHI of 1 for fenitrothion. These were calculated on the basis of water solubility of 0.28 mg/L for each of the pyrethroids. The RHI's decrease if their actual solubilities are lower. For example, if water solubilities for permethrin, deltamethrin, fenvalerate, and cypermethrin are 0.02, 0.04, 0.04, and 0.009 mg/L, respectively, the RHI's are 5, 14, 38, and 100, respectively. The magnitude of the RHI's indicates that the pyrethroids are considerably more hazardous than fenitrothion for the aquatic environment.

TOXICITY FACTORS TO AQUATIC ORGANISMS FOR INSECTICIDES USED ON POTATOES

Seventeen insecticides (21 formulations) were registered for use on potatoes for 1983 (A.C.P.G. 1983). Toxicity factors (TF) for the insecticides to aquatic organisms, calculated as application rate (kg a.i./ha)/96-h LC50 (mg/L), ranged from 0.02 to 9250. This wide range in TF indicates a wide range in potential hazard to aquatic organisms from little or no hazard to extreme hazard.

The TF's for a particular insecticide are larger for crustaceans than for freshwater fish. Three of the insecticides were pyrethroids (fenvalerate, permethrin, and deltamethrin) and these had the lowest application rates (0.0125-0.14 kg a.i./ha). In that order, their TF's for freshwater fish were 120, 100, and 20, and they ranked 6, 7, and 11 out of 16 in toxicity hazard. For crustaceans, the TF's for the pyrethroids in the same order were 3500, 5000, and 8930, and they ranked 6, 5, and 1 out of 13.

In addition to the pyrethroids, TF's for phorate, endosulfan, azinphos-m, chlorpyrifos, and phosmet were 620, 470, 430, 250, and 180, respectively, for freshwater fish (ranks of 1, 2, 3, 4, and 5 out of 16). For crustaceans, the TF's for these insecticides in the same order were 5290, 1400, 5730, 5450, and 275 (rank of 4, 7, 2, 3, 10 out of 13).

The TF's indicate high potential hazard of pyrethroids and certain other of the insecticides to aquatic organisms, particularly to crustaceans. Whenever possible, insecticides with the lowest TF's should be chosen for use on potatoes to minimize the hazard to non-target aquatic organisms and the aquatic environment.

POLYNUCLEAR AROMATIC HYDROCARBONS

Lobsters (20) were exposed to 5 PAH for 80 h and delivered live to Dr. J. Uthe to test for possible changes in distribution and concentration of the PAH in lobster tissues following cooking of the lobsters. Concentrations of the 5 PAH in water during the exposure were determined by liquid chromatography (see J.F. Uthe section).

TBTO FLEXGARD PAINT

An antifouling paint, tributyltin oxide, or TBTO, was assessed for toxicity by exposing lobsters for 9 wk to netting that had been soaked in the paint. No mortality occurred that could be attributed to the effects of the paint.

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