Total and Organic Mercury in Marine Fish

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Introduction

The past year has seen an increasing awareness of the problems associated with the redistribution of mercury in the environment. The problem, however, is not entirely a new one. In 1966 mercury-containing pesticides were implicated in a series of bird kills in Sweden (BORG, et al. 1966). The Minamata poisonings in Japan between 1953 and 1960 were responsible for neurological damage and death to people consuming fish and shellfish contaminated with organic mercury being discharged from an industrial plant (KURLAND, et al. 1960). Recently, high levels of mercury were found in fish from Swedish lakes and streams. The principal mercury contaminant of these fish was reported to be an organic form of methyl mercury (JOHNELS, et al. 1967; WESTÖÖ 1966). The more toxic organic mercurials, particularly the short chain alkyl salts (e.g. methyl mercury), may produce irreversible damage to the central nervous system with acute exposure (KURLAND, et al. 1960; CURLEY, et al. 1971) or chronic exposure (BERGLAND and BERLIN 1969).

The ability of some micro-organisms to methylate inorganic mercury (JENSEN and JERNELOV 1969) to the more biologically stable alkyl forms further increases the dangers of contamination. The alkyl mercury thus enters the food chain and because of low excretion rates, tends to accumulate there. In the past year in Hawaii, the Pacific Blue Marlin was found to have total mercury levels well above the Food and Drug Administration tolerance limit of 0.5 ppm. This fish has been voluntarily removed from the market and a moratorium declared on its use for human consumption (MINETTE 1971).

This study was undertaken to determine the total and organic levels in the muscle tissue of certain pelagic and inshore fish of Hawaii which are used for human consumption.

Methods and Materials

Total mercury levels in the fish tested were determined by variation of the flameless Atomic Absorption technique (KIMURA and MILLER 1962; HATCH and OTT 1968; JEFFUS, et al. 1970). All muscle tissue tested came from skinless fillets of fish. Fifty grams of muscle tissue was homogenized in 50 ml of distilled water with an Omnimixer (Ivan Sorval Co., Norwalk, Connecticut). Ten grams of homogenate were digested in concentrated nitric acid and further oxidized with potassium permanganate solution.

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Excess oxidizing agents were reduced with hydroxylamine solution. Mercury ions were reduced to elemental mercury with stannous sulfate solution, and the mercury was vaporized in the flameless atomic absorption apparatus (MANNING 1970).

Organic mercury in the fish samples was determined by a modification of methods developed by GAGE (1961) and WESTÖÖ (1966). Five to ten grams of the homogenate (2.5 to 5 grams of fish) were weighed and transferred with a total of 70 ml of distilled water to a 250-ml separatory funnel containing 15 ml of conc. HCl and 10 grams of NaCl (sample fortifications were made at this point). The contents of the separatory funnel were mixed, 65 ml of benzene were added and the mixture was shaken vigorously for 5 minutes. The entire sample was then transferred to a 250-ml centrifuge bottle and spun at 2500 rpm until the benzene layer (top) was clear. Fifty ml of the benzene layer were transferred with a volumetric pipet to a 60-ml separatory funnel. Seven ml of a 1% cysteine solution (1.000 gm of L-cysteine HCl, 0.775 gm of sodium acetate, and 12 gm of sodium sulfate in 100 ml of distilled water) were added and the mixture was shaken vigorously for 2 minutes. After phase seperation, the aqueous (bottom) layer was drained into a glass testtube. Two ml of this extract were transferred to a 125-ml erlenmeyer flask containing 1 ml of conc. HCl and 5 ml of 5% potassium permanganate solution. The flask contents were mixed and allowed to stand for 15 minutes. One ml of 5% sodium persulfate solution was then mixed into the flask contents, and the mixture was allowed to stand for an additional 30 minutes. The sample was then diluted with 30 ml of acid solution (85 ml H_2SO_4 and 70 ml HNO_3 diluted to 1 liter with distilled water). One ml of hydroxylamine solution (15 gm each of hydroxylamine sulfate and sodium chloride in 100 ml of distilled water) was added and the flask contents were swirled until the solution became clear. Five ml of 10% stannous sulfate solution (in 0.5 N H_2SO_4) were added to the sample which was immediately aerated to volatilize the reduced mercury into the flameless Atomic Absorption instrument. Absorption due to mercury was measured at 2573 Å with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., Norwalk, Connecticut). Standard operating conditions were used, and mercury content was calculated by comparison of sample responses to a standard curve derived from responses for oxidized methyl mercury chloride standard (10 ug Hg/ml).

Results

Recovery data are presented in Table 1. Ten grams of fish muscle tissue (mullet) were fortified with 2, 4, 6, 8, and 10 ug/g organic mercury (methyl mercury chloride). Recovery percentages for the organic mercury method decreased with higher concentrations of mercury in the sample. This difficulty was avoided in the routine analytical procedure by utilizing smaller sample sizes for tissues with high levels of organic mercury.

Table 2 reports the mean and range of total and organic concentrations in all species examined. In all species, except the Pacific Blue Marlin, there was close correlation between the organic and total

TABLE 1

Recovery of organic mercury from fish muscle tissue utilizing total and modified organic methods

Sample wt.[a] gm.	CH_3HgCl Added ug.	CH_3HgCl Rec. ug.	% Recovery
Total Hg method			
10.00	0.00	<0.05[b]	--
10.00	2.00	1.94	97
10.00	4.00	4.08	102
10.00	6.00	6.00	100
10.00	8.00	8.16	102
10.00	10.00	10.60	106
Organic Hg method			
10.00	0.00	<0.05[b]	--
10.00	2.00	2.08	104
10.00	4.00	4.00	100
10.00	6.00	5.70	95
10.00	8.00	7.36	92
10.00	10.00	8.60	86

[a]Mullet (Mugil cephalus)
[b]Limit of Detectability

TABLE 2

Average and range of organic and total mercury in muscle tissue of all fish analyzed

Common (local) Scientific name	No. of fish Analyzed	Organic Hg ppm*	Total Hg ppm*
Pelagic species:			
Pacific Blue Marlin (A'u)	29	X 0.93	4.78
Makaira ampla		Range 0.23-1.79	0.35-14.0
Yellow Fin Tuna (Ahi)	22	X 0.48	0.54
Neothunnus macropterus		Range 0.25-1.00	0.24-1.32
Skip Jack Tuna (Aku)	20	X 0.41	0.38
Katsuwonus pelamis		Range 0.20-0.57	0.27-0.52
Dolphin (Mahimahi)	10	X 0.25	0.25
Coryphaena hippurus		Range 0.15-0.30	0.17-0.31
Inshore species:			
Squirrel fish (Uu, Menpachi)	14	X 0.21	0.21
Myripristis arayomus		Range 0.10-0.40	0.10-0.43
Bigeyed Scad (Akule)	10	X 0.10	0.09
Trachurops crumenophthalmus		Range 0.07-0.11	0.07-0.11
Red Goat Fish (Weke-ula)	10	X <0.05	<0.05
Mulloidichthys auriflamma		Range --	--
Mullet (Amaama)	10	X <0.05	<0.05
Mugil cephalus		Range --	--
Parrot Fish (Panahunuhu)	10	X 0.05	0.05
Scaridae		Range <0.05-0.10	<0.05-0.08

*Mercury calculated as inorganic mercury

mercury content. All pelagic species had rather high levels of both organic and total mercury. The two species of tuna examined had levels approaching the 0.5 ppm level. The inshore fish all had very low mercury levels, except for the squirrel fish which had a higher average and a greater range of values than the other species. High mercury levels in the squirrel fish have not been explained. Inshore fish examined ranged in weight from approximately 250 grams to 1300 grams. There was no obvious relationship between weight and mercury levels.

The Pacific Blue Marlin had higher levels of organic and inorganic mercury than all other species examined, but the total mercury in this fish was not comparable to the organic mercury levels. Evidently, the total mercury contamination of the marlin is not necessarily of the organic type. Figure 1 shows the regression of total and organic mercury on fish weight, using data summarized in Table 3. Correlation coefficients for these relationships are shown in Table 4.

The correlation of organic and total mercury with size of fish in the Yellowfin tuna (Tables 5,6) indicates that factors different from those affecting the marlin may be at work. There was close correlation between the organic and total mercury levels in this fish.

Liver samples were available from twenty-two of the Pacific Blue Marlin. These were analyzed for both total and organic mercury to see if any relationship existed between the mercury content of this tissue and the weight of the individual fish and proportions of organic and inorganic mercury (Tables 7,8). No correlation between any of the three parameters considered was statistically significant. Additional statistical analyses were performed on the mercury content of muscle tissue, liver tissue, fish size, and proportions of organic and inorganic mercury (Tables 9, 10). There is a relationship between organic mercury in liver and total mercury in muscle and also between organic mercury in liver and organic mercury in muscle. The ratio between total and organic mercury (average of 17 samples) in liver was approximately 13:1 as compared to the muscle ratio of approximately 6:1.

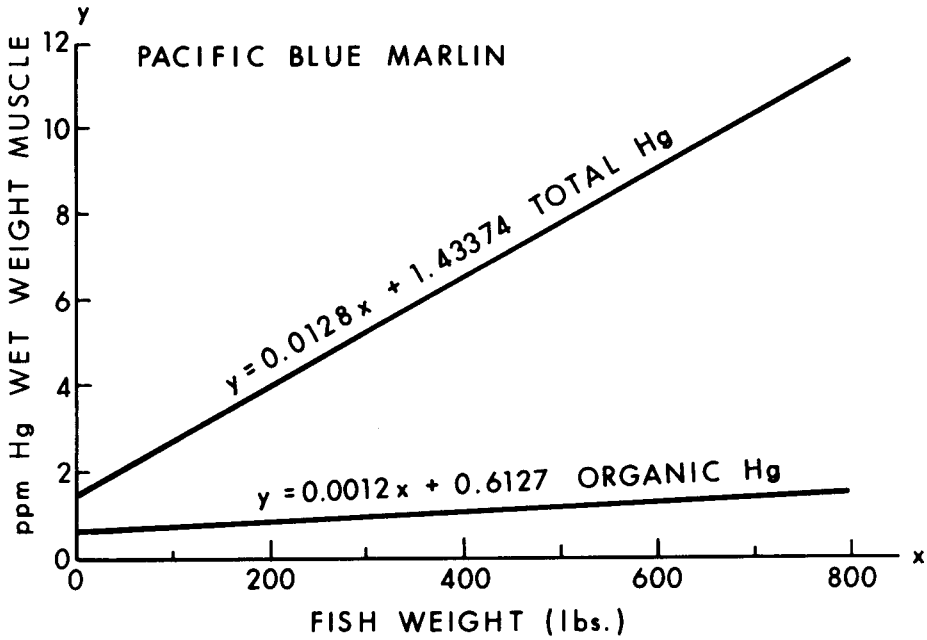
Discussion

A modified method for determining organic mercury concentrations was employed for the comparison with total mercury by an established method (see Methods section). This comparison appears valid (see Table 1). Recoveries of methyl mercury chloride by the modified method, when compared to the total mercury method, were in the same percentile range, satisfactory for the purposes of the study. It was not possible with this method to seperate alkyl and aryl organic compounds. No confirmatory tests were performed, but the literature suggests that methyl mercury is the principal organic contaminant in fish tissue (WESTÖÖ 1966, 1967).

Of the nine species examined (see Table 2) only one, Pacific Blue Marlin, showed a large disparity between the total and organic mercury levels in the muscle tissue. Levels of 3.11 ppm methyl

FIGURE 1

Regression curves describing the relationship of total and organic mercury levels in muscle tissue of the Pacific Blue Marlin to fish weight



mercury as compared to 3.35 ppm total mercury have been reported in Pike (Esox lucius L.) by WESTÖÖ (1967). Organic mercury in Pacific Blue Marlin was found to be somewhat lower than those levels, averaging 0.93 ppm (29 fish) with a range of 0.23 to 1.79 ppm (see Table 2). The total mercury, however, in these fish averaged 4.78 ppm with a range of 0.35 to 14.0 ppm. The organic mercury levels were closely comparable to the total mercury levels in the other eight species examined.

Pacific Blue Marlin is the only species in which organic mercury levels averaged over 0.5 ppm. However, the Pacific Blue Marlin used in this study were all caught in the same area and within the same week. The area of capture, the Kona Coast of the island of Hawaii, is near Hawaii's active volcano. Since Pacific Blue Marlin are not believed to migrate great distances it may be postulated that these fish are living in an area of high natural mercury contamination (ESHLEMAN, et al. 1971). BERGLUND and BERLIN (1969, p. 93) have reported that in a situation of organisms living in a contaminated area the mercury levels in the liver are greater than those in other tissues. The data on muscle and liver in the marlin support this hypothesis.

The disparity between total and organic mercury levels in the

Pacific Blue Marlin further suggests a biotransformation in which the animal, at a certain mercury concentration in the tissue, muscle or liver, begins to biotransfer the toxic organic mercury to the less toxic and more easily excreted inorganic form (NORSETH and CLARKSON 1970a, 1970b). This type of data suggests the existence of some substrate optimum above which demethylation begins.

TABLE 3

Total and organic mercury of muscle tissue in Pacific Blue Marlin

Sample No.	Weight, lb.	Total Hg ppm*	Organic Hg ppm*
1	96	0.35	0.23
2	100	1.4	0.83
3	115	1.3	0.92
4	118	1.3	0.29
5	142	2.2	0.73
6	143	3.3	0.36
7	146	2.2	1.54
8	150	3.5	0.54
9	150	4.5	1.34
10	155	2.4	0.54
11	162	1.2	0.67
12	176	3.0	0.86
13	185	4.0	0.83
14	198	11.4	1.79
15	211	3.6	0.65
16	223	4.8	0.67
17	253	5.5	0.47
18	282	11.2	0.89
19	296	2.4	1.08
20	309	3.9	1.09
21	368	2.7	1.19
22	409	4.8	1.63
23	438	12.6	1.53
24	443	8.6	1.22
25	497	7.0	1.09
26	502	14.0	0.70
27	782	5.8	1.42

*Mercury calculated as inorganic mercury

TABLE 4

Correlation coefficients(SNEDECOR 1956) of relationships between total mercury, organic mercury, and fish weight for Pacific Blue Marlin

Organic Hg/Weight	Total Hg/Weight	Organic Hg/Total
0.47*	0.56**	0.41*

*p 0.05
**p 0.01

TABLE 5

Total and organic mercury of muscle tissue in Yellow Fin Tuna

Sample No.	Weight lb.	Total Hg ppm*	Organic Hg ppm*
1	68	0.24	0.25
2	89	0.32	0.29
3	104	0.31	0.29
4	104	0.55	0.46
5	115	0.40	0.32
6	117	0.34	0.33
7	133	0.64	0.62
8	139	0.50	0.48
9	140	0.32	0.34
10	143	0.47	0.48
11	151	0.44	0.41
12	155	0.32	0.32
13	160	0.41	0.32
14	160	1.32	0.81
15	161	0.54	0.44
16	165	0.60	0.56
17	170	1.10	1.00
18	180	0.55	0.54
19	182	0.48	0.48
20	201	0.66	0.60
21	210	0.61	0.54
22	215	0.64	0.64

*Mercury calculated as inorganic mercury

TABLE 6

Correlation coefficients of relationships between total mercury, organic mercury, and fish weight for Yellow Fin Tuna

Organic Hg/Weight	Total Hg/Weight	Organic Hg/ Total
0.60**	0.47*	0.93**

*p 0.05
**p 0.01

This study represents only a beginning in the complicated problem of defining background mercury levels and identifying sources of mercury contamination in the world's food supply. Researchers have examined many situations where industry was the source of pollution, but with high mercury levels being found in pelagic fish in the middle of the Pacific Ocean, the possibility of natural contamination must also be considered.

TABLE 7

Total and organic mercury of liver tissue in Pacific Blue Marlin

Sample No.	Weight lb	Total Hg ppm*	Organic Hg ppm*
1	96	0.39	0.16
2	100	2.8	0.54
3	115	2.1	0.39
4	118	1.2	0.22
5	142	7.4	0.54
6	142	4.7	0.44
7	143	7.4	0.38
8	150	3.3	1.00
9	155	10.4	0.35
10	155	4.2	0.38
11	162	1.4	0.61
12	176	9.0	0.41
13	185	3.4	0.64
14	198	5.0	1.34
15	211	16.3	0.49
16	223	5.0	0.65
17	253	14.8	0.32
18	282	13.7	0.58
19	309	4.2	0.39
20	443	7.1	1.16
21	497	36.0	0.91
22	782	6.8	0.77
	average	7.57	0.58

*Mercury calculated as inorganic mercury

TABLE 8

Correlation coefficients of relationships between total mercury and organic mercury in liver and fish weight for Pacific Blue Marlin

Organic Hg/Weight	Total Hg/Weight	Organic Hg/Total
0.43	0.44	0.19

*p 0.05
**p 0.01

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TABLE 9

Comparison of total and organic mercury in muscle and liver of the Pacific Blue Marlin

Number of fish analysed		Muscle		Liver	
		Organic Hg ppm*	Total Hg ppm*	Organic Hg ppm*	Total Hg ppm*
17	X	0.80	4.57	0.59	8.03
	Range	0.23-1.79	0.35-11.4	0.16-1.34	0.39-36.0

*Mercury calculated as inorganic mercury

TABLE 10

Correlation coefficients of relationships between fish weight, total and organic mercury in muscle and liver tissue for the Pacific Blue Marlin

	Weight	Total Hg muscle	Organic Hg muscle	Total Hg liver
Weight				
Total Hg muscle	0.47			
Organic Hg muscle	0.56*	0.67**		
Total Hg liver	0.43	0.42	0.15	
Organic Hg liver	0.47	0.74**	0.83**	0.24

*p 0.05
**p 0.01

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