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Mercury and selenium concentrations in the internal organs of toothed whales and dolphins marketed for human consumption in Japan

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Abstract

Small cetaceans (toothed whales *odontoceti* and dolphins *delphinidae*) have been traditionally hunted along the coast of Japan and fresh red meat and blubber, as well as boiled internal organs such as liver, kidney, lung and small intestine, are still being sold for human consumption. We surveyed mercury contamination in boiled liver, kidney and lung products marketed in Japan between 1999–2001. The average ±S.D. of total mercury (T–Hg) was 370±525 (range: 7.60∼1980, $n=26$) μg/g in liver, 40.5±48.5 (7.30∼95.1, $n=15$) μg/g in kidney and 42.8±43.8 (2.10∼79.6, $n=23$) μg/g in lung. A high correlation was observed between T–Hg and selenium (Se) concentrations in these organs, supporting the formation of a Hg–Se complex. The formation of a Hg–Se complex probably contribute to the detoxification of Hg for cetaceans and allows a very large accumulation of Hg in livers. The provisional permitted level of T–Hg in marine foods set by the Japanese Ministry of Health and Welfare is 0.4 μg/g, and the provisional permitted weekly intake (PTWI) set by WHO is 5 μg/kg bw/week. The maximal T–Hg detected in boiled liver (1980 μg/g) exceeds the permitted level by approximately 5000 times and the consumption of only 0.15 g of liver exceeds the PTWI of 60 kg of body weight of the consumer, suggesting the possibility of an acute intoxication by T–Hg even after a single consumption of the product.

Keywords: Mercury; Liver; Toothed whale; Dolphin; Selenium; Human consumption; Metallothionein

1. Introduction

Environmental pollutants such as mercury (Hg), PCB and DDT are known to concentrate in biota via biomagnification as a result of their persistent and lipophilic nature. Apex predators, odontocete cetaceans (toothed whales and dolphins) are exposed to high levels of these pollutants (Haraguchi et al., 2000; Simmonds et al., in press). Mercury is known to accumulate in their internal organs, especially in the liver (Honda et al., 1983; Itano et al., 1984; Andre et al., 1991; Leonzio et al., 1992; Holsbeek et al., 1998; Meador et al.,

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1999; Zhou et al., 2001). To our knowledge, a maximal Hg concentration of 1500 μg/g on wet weight basis has been reported in a striped dolphin's liver (Andre et al., 1991).

In the marine environment, almost all of the Hg present in fish and squid is methylated (Caurant et al., 1996; Das et al., 2000). However, the major part of Hg accumulated in marine mammal internal organs, especially in the liver, exists as inorganic mercury (I–Hg), suggesting the demethylation of methyl mercury (M–Hg) occurs in their livers (Caurant et al., 1996; Holsbeek et al., 1998; Wagemann et al., 1998; Meador et al., 1999). The high correlation between Hg and selenium (Se) in the organs of marine mammals is well known (Itano et al., 1984; Wagemann et al., 1998; Meador et al., 1999; Das et al., 2000). The formation of the Hg–Se complex appears to be the last step of the detoxification process through the demethylation of M–Hg, leading to the fossilization of Hg and Se in the form of non-biodegradable compounds (Caurant et al., 1996). Metallothioneins (MTs) are well-known proteins, which are important in the detoxification of non-essential elements, such as cadmium (Cd), and I–Hg. In spite of a strong affinity for MTs, only a small part of I–Hg in the liver and kidney of mammals is bound to MTs (Das et al., 2000). According to literature (Caurant et al., 1996; Das et al., 2000), Se plays a key role in M–Hg detoxification processes in marine-mammals, and hence MTs would appear to play only a minor role, probably limited to the detoxification of I–Hg.

Small cetaceans (toothed whales and dolphins) have traditionally been hunted in the coastal water of Japan. Some people have consumed red meat and blubber, but also in Wakayama Prefecture they have consumed the internal organs such as the liver, kidney, lung, stomach and the small intestine of odontocete. In spite of a very large Hg contamination, the mixed package of boiled internal organs is sold for human consumption in Japanese retail outlets. Previously we reported on Hg contamination (Haraguchi et al., 2000), but no specific inspection and attention on the actual Hg contamination in the boiled internal organ products had been paid for human health. In response to the Minamata disease tragedy, the Japanese Ministry of Health and Welfare has set the provisional permitted levels of T–Hg and M–Hg in marine foods at 0.4 and 0.3 μg/g, respectively (JMHW, 1973). However, it is not clear whether whale products are covered by it or not.

The aim of the present study was to survey the level of T–Hg contaminated in the boiled internal organ products (liver, kidney and lung) of cetaceans marketed for human consumption in Japan. We also analyzed essential heavy metals such as zinc (Zn), copper (Cu), iron (Fe) and Se to estimate the existing forms of Hg in the internal organs.

2. Materials and methods

2.1. Sampling

Samples of a mixture of boiled internal organs marketed for human consumption were purchased between 1999 and 2001 from retail outlets at Taiji, Katsuura and Kushimoto in the Wakayama Prefecture, as well as one sample from the Tokyo Metropolitan area in Japan. The mixtures had been vacuum packed and sold as frozen foods. To minimize duplicate sampling of the same animal, we purchased only one package from each vender or a few packages labeled at different dates of manufacture. The boiled liver, kidney and lung were chosen for the determination of total mercury and essential heavy metals.

2.2. Chemical analyses

Total mercury (T–Hg) in these internal organs were analyzed using a flameless atomic absorption spectrophotometer (Hiranuma, HG–1) after digestion of samples by a mixture of HNO_3, $HClO_4$ and H_2SO_4 (Simmonds et al., in press). A cross-check of the T–Hg determination in the products was undertaken by the National Institute for Minamata Disease and by the Hokkaido Pharmaceutical Association Public Health Examination Center. We confirmed the T–Hg values we analyzed were almost the same as those by these laboratories. Selenium (Se) was determined by the method of Watkinson (1966) using an Hitachi Fluorescence Spectrophotometer F-450. Zinc (Zn), copper (Cu)

Table 1
Total mercury and essential heavy metals levels in whale liver products (μg/wet g)

	T–Hg	Zn	Cu	Se	Fe
1	414	49.0	13.4	155	147
2	353	52.5	10.7	115	169
3	286	61.6	15.0	144	327
4	645	56.6	12.8	191	398
5	30.2	50.4	6.70	11.2	143
6	36.8	53.8	10.7	12.6	106
7	204	59.0	6.60	86.3	156
8	504	83.9	8.74	166	816
9	1980	87.2	13.6	656	392
10	914	85.3	11.4	346	197
11	422	88.8	12.0	174	190
12	390	91.9	12.5	145	282
13	39.0	59.4	4.74	13.6	178
14	22.7	N.A	N.A	48.5	N.A
15	41.2	N.A	N.A	12.7	N.A
16	55.9	N.A	N.A	27.7	N.A
17	41.1	N.A	N.A	14.8	N.A
18	72.6	58.9	8.68	24.1	199
19	58.5	59.9	9.08	22.7	231
20	47.4	58.4	9.23	18.1	226
21	174	62.7	6.34	71.1	41.8
22	7.60	56.0	6.71	3.64	90.5
23	393	50.6	10.3	160	215
24	1973	57.5	9.14	694	883
25	134	62.2	11.9	47.4	191
26	368	66.7	13.4	157	137
Mean ± S.D.	370 ± 525 (n=26)	64.2 ± 13.61 (n=22)	10.2 ± 2.8 (n=22)	155 ± 188 (n=26)	254 ± 215 (n=22)

N.A. not determination

and iron (Fe) were determined using an Hitachi Polarized Zeeman flame atomic absorption spectrophotometer Z-8100 after digestion by a HNO_3–$HClO_4$ mixture. We used DOLT-2 (National Research Council of Canada) as an analytical quality control (data not shown). Metal concentrations in the boiled organs were expressed as wet weight. To measure the dry weight of the products, some boiled organs were heated overnight at 80 °C.

3. Results and discussion

T–Hg concentrations in the boiled livers of cetaceans, purchased mainly from Wakayama Prefecture, were listed in Table 1. The mean value ±S.D. of T–Hg as expressed on wet weight was 370±525 μg/g (range: 7.60∼1980 μg/g, n=26). The values were similar to the results by Honda et al. (1983) who reported that the hepatic concentrations of T–Hg in striped dolphins (*Stenella coeruleoalba*) caught off Taiji, Wakayama Prefecture, were 205±139 μg/g (1.7∼485 μg/g, n=59). The highest concentration of T–Hg has been so far reported to be 1500 μg/g (wet weight) in a liver of striped dolphin (Andre et al., 1991). The highest concentrations of T–Hg in liver on dry weight were 13156 μg/g in a bottle-nosed dolphin (*Tursiops truncatus*) and 4400 μg/g in a striped dolphin (Leonzio et al., 1992), which correspond to approximately 3300 and 1100 μg/g on wet weight, respectively, assuming a ratio (0.26) of dry to wet weights (Meador et al., 1999). T–Hg concentrations in the boiled kidney and lung were an order of magnitude lower than those in liver (Tables 1–3). Similar distributions of T–

Table 2
Total mercury and essential heavy metals levels in whale kidney products (μg/wet g)

	T–Hg	Zn	Cu	Se	Fe
1	24.0	32.4	3.13	14.1	118
2	23.9	28.9	2.92	11.6	166
3	13.7	31.9	2.16	6.39	74.4
4	28.8	38.5	2.29	11.9	161
5	95.1	24.6	1.83	37.8	105
6	24.1	24.7	2.02	11.2	165
7	142	47.9	1.89	52.6	177
8	7.30	50.0	2.79	5.68	97.4
9	8.67	39.0	3.20	3.23	62.7
10	20.8	47.6	2.00	11.0	124
11	2.28	45.3	2.59	3.75	55.5
12	27.5	48.0	1.98	9.80	113
13	153	36.5	2.32	56.6	159
14	7.85	42.6	2.30	4.50	297
15	29.2	42.0	2.97	8.25	125
Mean ± S.D.	40.5 ± 48.5 (n = 15)	38.7 ± 8.6 (n = 15)	2.43 ± 0.47 (n = 15)	16.6 ± 17.5 (n = 15)	133 ± 60 (n = 15)

Table 3
Total mercury and essential heavy metals levels in whale lung products (μg/wet g)

	T–Hg	Zn	Cu	Se	Fe
1	20.5	16.0	0.50	7.63	103
2	11.5	20.9	0.24	6.33	125
3	145	22.0	0.76	26.8	160
4	2.38	15.8	0.48	1.66	66.0
5	23.6	22.9	0.66	4.46	122
6	76.6	22.9	0.71	13.3	196
7	63.0	48.9	1.14	25.3	94.9
8	12.2	47.5	1.23	6.64	102
9	2.60	40.6	1.12	1.54	105
10	2.10	37.6	2.16	1.44	112
11	19.1	18.1	0.93	3.09	209
12	21.9	17.6	0.67	8.14	82.7
13	63.3	15.8	0.87	19.5	93.3
14	52.9	21.3	1.41	14.7	76.6
15	1.54	22.2	0.71	1.12	143
16	14.3	30.0	0.75	4.19	175
17	39.4	32.0	0.69	11.7	131
18	79.6	34.3	0.82	21.3	85.8
19	70.9	26.7	1.08	29.9	91.9
20	21.2	31.1	0.77	6.16	110
21	15.2	36.4	0.84	3.55	176
22	164	25.5	0.81	58.6	168
23	62.1	22.3	0.831	21.8	134
Mean ± S.D.	42.8 ± 43.8 (n = 23)	27.3 ± 9.8 (n = 23)	0.88 ± 0.38 (n = 23)	13.0 ± 13.4 (n = 23)	124 ± 40 (n = 23)

Hg in small cetaceans have been reported by many researchers (Honda et al., 1983; Itano et al., 1984; Andre et al., 1991; Leonzio et al., 1992; Holsbeek et al., 1998; Meador et al., 1999; Zhou et al., 2001).

In the present study, we found that the ratio of dry to wet weights of the boiled liver, kidney and lung (Tables 1–3) were 0.39 ± 0.02, 0.35 ± 0.03, and 0.23 ± 0.03 (mean $\pm$ S.D., $n=5$), respectively. Meador et al. (1999) reported that the ratios of fresh liver and kidney from bottlenose dolphins stranded on the coast of Texas were 0.26 ± 0.04 and 0.22 ± 0.03, respectively. It appears that the moisture content of cetacean organs are reduced by boiling and the concentrations of T–Hg as well as essential metals in boiled organs are somewhat higher than those in fresh organs.

It is well known that a one-to-one molar association of Hg and Se, tiemannite HgSe, forms in the liver of many mammals (Itano et al., 1984; Wagemann et al., 1998; Meador et al., 1999; Das et al., 2000). HgSe granules are reported to be located mainly in the liver macrophages, Kupffer cells, the proximal tubles of the kidney, lungs and hilar lymh nodes of cetaceans (Das et al., 2000). We also observed a high correlation between T–Hg and Se concentrations in the boiled liver, kidney and lung (Fig. 1). In most cases, the molar ratios of T–Hg to Se in these organs were >1.0 (Tables 1–3). According to the slopes of regression lines, the molar ratios of T–Hg to Se in the boiled liver, kidney and lung were 1.18, 1.11 and 1.43, respectively. The higher ratios suggest a contribution of the binding of I–Hg to MTs and the existence of M–Hg (Wagemann et al., 2000).

To confirm the formation of HgSe complex and the binding of I–Hg to MTs, we homogenized the boiled liver contaminated with 1980 ppm T–Hg. The homogenate was centrifuged at $104\,000\times g$ for 1 h, and the supernatant fraction was subsequently chromatographed using a Sephadex G-75 column, according to the conventional method to isolate MTs (Vasak, 1991). Only a small percentage of T–Hg was retained in the supernatant fraction, and almost all of the T–Hg was detected in the sediment fraction which was in agreement with the subcellular distribution of HgSe (Wagemann et al., 1984; Caurant et al., 1996). Overlapping peaks of Hg, Zn and Cu, binding to MTs (approximately Ve/Vo $=2$), was not observed in the supernatant fraction. Although MTs are relatively heat-stable proteins (Caurant et al., 1996), the boiling could denature the intracellular MTs and centrifugation may cause the denatured MTs to settle. Positive correlations were observed between Zn and Cu concentrations in the boiled liver ($P>0.05$) and lung ($P<0.05$) (Table 4). These correlations may reflect the induction of MTs by I–Hg and/or Cd in these organs (Holsbeek et al., 1998; Zhou et al., 2001).

Significant correlations were observed between Fe and T–Hg or Se concentrations in the liver (Table 4). It has been reported that the hepatic concentrations of Hg, Cd and Fe in minke whales (*Balaenoptera acutorostrata*) increase with age (Honda et al., 1987), and the hepatic concentrations of Se in striped and bottlenose dolphins also increase with age (Itano et al., 1984; Meador et al., 1999). Thus, the significant correlations between Fe and T–Hg or Se concentrations in the boiled liver may be related to age.

Weak positive correlations were observed between T–Hg and Zn or Cu concentrations in the boiled liver (Table 4). This is similar to the results reported in the liver of minke whales (Honda et al., 1987). The positive correlation may reflect the induction of MTs by the toxic metals. In contrast, a negative correlation was observed between T–Hg and Cu concentrations in the kidney (Table 4). Negative correlations between T–Hg and Cu or Zn concentrations have previously been reported in the internal organs of striped dolphins (Honda et al., 1983). T–Hg concentrations tend to increase with age (Honda et al., 1983; Itano et al., 1984; Holsbeek et al., 1998; Meador et al., 1999) and that Zn and Cu concentrations are higher in adult than in juvenile or fetus (Honda et al., 1982, 1983; Beck et al., 1997; Zhou et al., 2001). These facts may explain the negative nature of these correlations, although further work is required to elucidate the reason for the opposite correlations between T–Hg and Zn or Cu concentrations.

T–Hg concentrations in cetaceans are markedly different among species (Honda, 1990) and apparently increase with age (Honda et al., 1983; Itano et al., 1984; Holsbeek et al., 1998; Meador et al.,

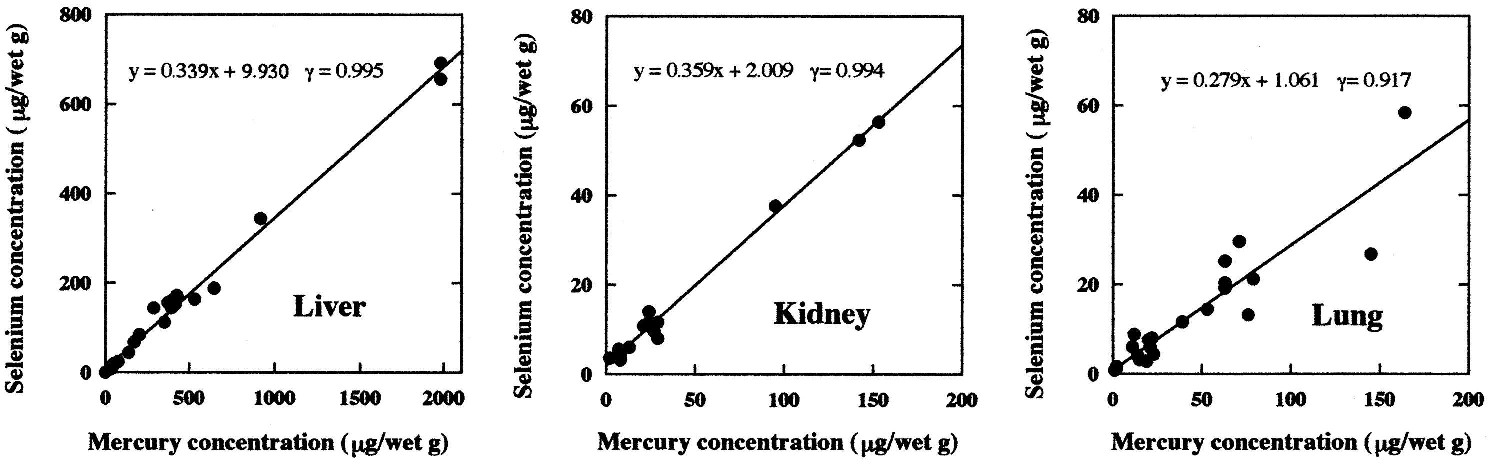

Fig. 1. Correlation between total mercury and selenium in boiled whale liver, kidney and lung. See Tables 1–3.

Table 4
Correlation coefficient (γ) between heavy metal concentrations in whale organs

	Zn	Cu	Se	Fe
Liver				
Hg	0.372	0.340	0.995[a]	0.634[a]
Zn		0.308	0.384	0.253
Cu			0.360	0.073
Se				0.620[a]
Kidney				
Hg	0.086	−0.420	0.994[a]	0.194
Zn		0.013	−0.117	−0.018
Cu			−0.424	−0.249
Se				0.191
Lung				
Hg	0.105	−0.052	0.917[a]	0.205
Zn		0.510[a]	−0.015	−0.079
Cu			−0.004	−0.157
Se				0.103

Negative values indicate a negative correlation.
[a] Significant at 5% level.

1999). In addition, contamination may vary according to living area, gender (Andre et al., 1990; Beck et al., 1997; Meador et al., 1999; Zhou et al., 2001) and stage of lactation (Caurant et al., 1996). Further surveys of T–Hg that allow for these variations are necessary. The concentration ratio of M–Hg to T–Hg in the internal organs is reported to decrease with an increase of T–Hg (Holsbeek et al., 1998; Storelli et al., 1998; Meador et al., 1999). Determination of M–Hg is also necessary to fully investigate the human health problems related to the consumption of whale's internal organs.

The provisional permitted level of T–Hg in marine foods set by JMHW is 0.4 μg/g (JMHW, 1973), and the provisional tolerable weekly intake (PTWI) set by WHO is 5 μg/kg bw/week (WHO, 1972). It is noticeable that the highest value of T–Hg detected in the boiled liver (1980 μg/g) exceeded the permitted level by approximately 5000 times, and the consumption of only 0.15 g this liver exceeds the PTWI of 60 kg body weight consumer. Webb (1966) reported that the lethal dose for $HgCl_2$ in human (p.o.) is approximately 2.5 mgHg/kg, and the LD_{50} of $HgCl_2$ (p.o.) for experimental animals is roughly 10 mgHg/kg. Not only chronic intoxication but also acute intoxication of T–Hg could result from a single ingestion of whale internal organs (Endo et al., 2001), although most of the Hg appears to exist as the less toxic compound HgSe.

The government of the Faroes Island Health Authorities advising, Denmark, issued the following recommendations to the public for the consumption of toothed whale products (Anonymous, 1998).

- Adults should only eat blubber and meat once or twice a month;
- Girls and women should not eat blubber until they have given birth to all theirchildren;
- Meat should not be eaten within three months of planned pregnancy and not eatenat all by pregnant and nursing women; and
- Organs (e.g. liver and kidney) should not be eaten at all.

More attention must be paid to these recommendations, and the Japanese government should issue some regulations concerning the human consumption of whale internal organs.

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