Mercury concentrations in the tissues of bottlenose dolphins (*Tursiops truncatus*) and striped dolphins (*Stenella coeruloalba*) stranded on the Croatian Adriatic coast

J. Pompe-Gotal, E. Srebocan, H. Gomercic, A. Prevendar Crnic

Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

ABSTRACT: The concentrations of total mercury in muscle (17 samples), liver (16 samples) and kidney tissues (16 samples) of both bottlenose *(Tursiops truncatus)* and striped dolphins *(Stenella coeruloalba)* were analyzed by cold vapour atomic absorption (AA) spectroscopy. The dolphins were found dead at different locations along the east Adriatic over a 10-year period starting in 1990. In this study, the magnitude of mercury contamination of dolphins which are indicators of contamination of the Adriatic as well as the Mediterranean Sea was determined. Our results represent the first investigation of heavy metal concentrations in dolphins sampled along the Croatian coast of the Adriatic Sea. Total mercury levels were high, ranging from 1.51 to 136.7 mg/kg in muscle, from 2.04 to 143.1 in kidney and from 10.35 to 1 833 mg/kg in liver tissues (expressed as wet weight). These results were examined for differences based upon age and tissue type. A high correlation was observed between total mercury concentration and age, and adult dolphins (10 samples) contained statistically significant higher concentrations of mercury compared to juvenile (seven samples) dolphins. Mercury concentrations were generally the highest in liver tissue. The presence of high mercury levels in dolphins is attributed to natural as well as anthropogenic sources.

Keywords: mercury; contamination; bottlenose dolphins; striped dolphins; Adriatic Sea

Over the last two decades, a number of studies have been carried out on heavy metals in marine organisms and, more particularly, on the levels of mercury found in cetaceans. All of these studies point to the variability of mercury levels. Due to its lenghty persistence and high mobility in the marine ecosystem, mercury shows a high level of biomagnification in the upper levels of the food web (Leonzio et al., 1992; Augier et al., 1993; Storelli et al., 1998; Wagemann et al., 1998; Cardellicchio et al., 2002). Mercury can be accumulated by marine organisms through a variety of pathways, including respiration, adsorption and ingestion (Law, 1996). As marine mammals do not breathe with gills, their uptake of metals directly from water is assumed to be negligible, and the three main routes of uptake are across the placenta before birth, in milk during the suckling period and from ingested food in adults (Leonzio et al., 1992; Beck et al., 1997; Siebert et al., 1999; Storelli and Marcotrigiano, 2000; Zhou et al., 2001; Lahaye et al., 2007).

Bottlenose dolphins (*Tursiops truncatus*) can be found in all cold to tropical seas worldwide. Along with striped dolphins (*Stenella coeruleoalba*), they appear to be common in the Mediterranean Sea. These marine mammals, as inshore organisms, have been considered to be directly subjected to pollution because coastal areas are also the repositories for toxic agents and hazardous materials such as heavy metals and organochlorine compounds

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from industrial, agricultural and urban sources (Storelli and Marcotrigiano, 2000). These assumptions, together with the consideration that dolphins are typical end-points in the biomagnification of persistent pollutants in the pelagic food chain, has led to many studies on the levels and effects of contaminants in marine mammals.

The objective of the present study was survey the level of total mercury concentration in muscle, liver and kidney tissues of both bottlenose and striped dolphins sampled along the Croatian coast of Adriatic Sea in order to assess and compare them with results of extensive investigations of mercury levels in Mediterranean Sea areas.

MATERIAL AND METHODS

Animals

Seventeen stranded dolphins aged between less than one year and 23 years were collected between 1990 and 1999 at different locations from the Istria peninsula to Lastovo island and the city of Dubrovnik (Table 1). The coastal area of the Adriatic Sea where dolphins were found is depicted in Figure 1.

Analysis

Liver, kidney and muscle samples for mercury analysis were collected during necropsies and stored at –20°C until processing. Total mercury was determined by cold vapor AAS and concentrations were expressed as μg Hg/g of wet weight of tissue, according to the method of Hatch and Ott (1968) using a Coleman Mercury Analyser MAS 50, Perkin Elmer. The accuracy of the method was checked using standard reference material (oyster tissue SRM – 1566a).

Statistics

All data were analyzed by Statgraphics 4.0 software. The significance of mean differences was based on a P value of < 0.05.

RESULTS

The results are presented in two tables. In Table 1. the species of dolphins, location and year of stranding, sex and age in years, individual mercury concentrations (μg/g w.w.) in liver, kidney and

Figure 1. Locations along the Croatian Adriatic Coast where dolphins were found

Table 1. Mercury concentrations (μg/g w.w.) in liver (L), kidney (K) and muscle (M) tissues of bottlenose (Tt) and striped (Sc) dolphins sampled between 1990 and 1999 along the Eastern Adriatic Sea and descriptive statistics of mercury concentrations in tissues of dolphins

Species	Location and year of stranding	Sex	Age (years)	Hg		
				L	K	M
Tt	W of penninsula Istra, 1990	F	4	–	5.78	3.63
Tt	Rovinj,1990	F	< 1	29.31	3.45	3.05
Tt	Rovinj, 1992	M	7	58.50	9.01	5.73
Tt	island Lošinj, 1993	M	3–4	33.05	8.21	3.53
Tt	island Lastovo, 1994	M	< 1	10.35	2.04	1.69
Tt	Rovinj, 1994	F	6	23.48	6.52	1.51
Tt	Medulin, 1994	F	6	330.38	143.13	8.06
Tt	Šibenik, 1995	M	9	198.06	17.11	19.14
Tt	Banjole, 1996	F	5	29.71	8.44	3.54
Tt	Rovinj, 1996	M	12	116.97	6.75	9.29
Tt	Pirovac, 1997	F	21	469.40	12.98	92.54
Tt	Rogoznica, 1998	M	3	53.89	13.20	3.41
Tt	Dubrovnik, 1999	M	11	908.40	60.53	90.61
Tt	Nin, 1999	F	23	916.70	98.57	136.70
Sc	island Krk, 1999	F	5	37.05	–	2.38
Sc	Skradin (river Krka), 1999	M	11	332.90	27.67	32.26
Tt	Split (bay Žnjan), 1999	M	16	1 833.80	35.09	57.90
N				16	16	17
Median				87.73	10.99	5.73
Mean ± SD				336.40 ± 498.16	28.65 ± 39.76	27.94 ± 41.23
Min–Max				10.35–1833.8	2.04–143.1	1.51–136.7

muscle tissues of bottlenose and striped dolphins sampled between 1990 and 1999 along the Eastern Adriatic Sea are shown. Descriptive statistics of mercury concentrations (μg/g w.w.) in liver, kidney and muscle tissues are also presented. The large standard deviation of the means reflects the wide range of mercury contamination. The highest total Hg concentrations were found in liver with values ranging between 10.35 and 1 833.8 (median 87.73) μg/g. In muscle tissue total Hg concentrations were much lower with values ranging from 1.51 to 136.7 (median 5.73) μg/g. Hg values in kidney ranged from 2.04 to 143.1 (median 10.99) μg/g wet weight tissues (Table 1).

Table 2 displays the median values of total mercury concentrations in all tissues of adult (n = 10; age > 6 years) and juvenile (n = 7; age < 6 years) dolphins.

Statistically significant differences ($P < 0.05$) are observed only between muscle tissues of adult and juvenile dolphins (Figure 2)

Table 2. Median values of total mercury concentrations in tissues of adult (n = 10; > 6 years) and juvenile (n = 7; < 6 years) dolphins

	Hg (μg/g w.w.)		
	Muscle	Liver	Kidney
Adult	25.7*	331.6	22.39
Juvenile	3.42	31.38	6.99

*$P < 0.05$

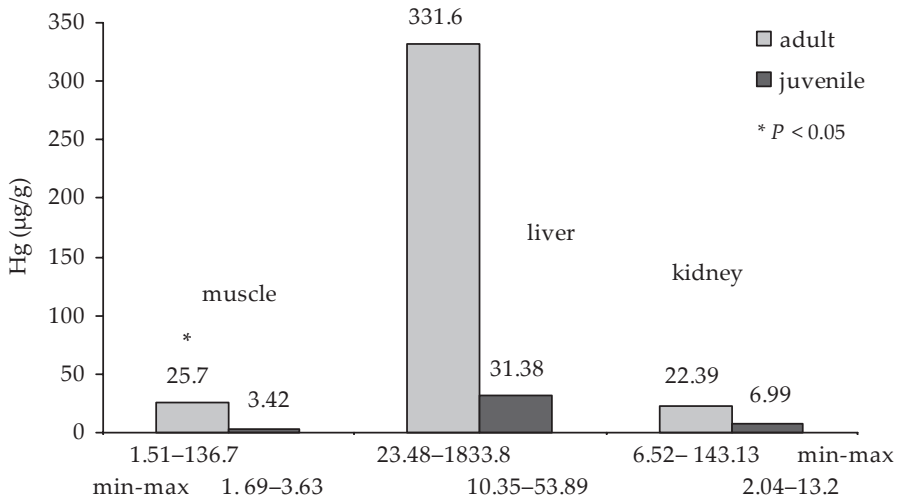

Figure 2. Median values of total mercury concentrations in all tissues of adult (n = 10; > 6 years) and juvenile (n = 7; < 6 years) dolphins

DISCUSSION

Cetaceans from the Mediterranean Sea have very high mercury burdens (Andre et al., 1991; Augier et al., 1993; Cardellicchio et al., 2000; Lahaye et al., 2006) and a maximal mercury concentration of about 13 000 µg/g dry weight (about 4 250 µg/g wet weight) has been reported in the liver of one bottlenose dolphin captured in the Thyrrhenian Sea (Leonzio et al., 1992) probably due to the natural mercury deposits in the Mediterranean basin (Andre et al., 1991).

Both biotic (species, sex, age, diet, metabolism) and abiotic factors (contamination gradients and physico-chemical parameters of the aquatic environment) affect metal accumulation in marine organisms (Storelli et al., 2005). In order to fully understand the impact of contamination of marine organisms, many factors have to be studied in detail. With regard to the abiotic factors, the contamination gradient for the Mediterranean Sea is the most important, as it is generally believed to harbour higher mercury concentrations than other seas or oceans due to numerous deposits of mercury ores and metallic mercury in surrounding countries. The most important biotic parameter to be considered is the age of the animals, since mercury potentially accumulates throughout life. Other factors such as growth features, gender and reproductive status and/or health aspects may also be important. Feeding preference is probably the key factor controlling Hg levels because upper-level predators are mainly exposed to metals through their food (Aguilar et al., 1999). Considering the high mobility of cetaceans, the levels of mercury concentrations in their tissues reflect the general contamination of the broad and poorly defined area in which the cetaceans live.

A wide range of mercury concentrations was observed in our analyzed samples: from 1.51 (muscle sample) to 1 833.8 (liver sample) µg Hg/g wet weight. The highest concentration of mercury was found in the liver of an adult male dolphin (16 years) from the Znjan bay near the city of Split, and the lowest concentration of mercury in liver was seen in a young male dolphin (< 1 year) stranded on Lastovo island. These findings confirm the well-known relationship between mercury accumulation and age, and such an increase of Hg concentrations with age has previously been demonstrated in several cetacean species (Honda et al., 1983; Paludan-Muller et al., 1993; Caurant et al., 1994; Monaci et al., 1998; Meador et al., 1999; Agusa et al., 2008; Capelli et al., 2008). Moreover, age has been described to be the major factor affecting hepatic Hg concentrations in bottlenose and striped dolphins from Atlantic and Mediterranean waters (Lahaye et al., 2006).

The high mercury levels found in liver, kidney and muscle tissues of the animals studied in this work are in general agreement with the results of other studies on the contamination of these top Mediterranean predators. The study of Andre et al. (1991) on the total mercury concentrations of dolphins from the French Mediterranean coast reported a highest concentration in liver of about 1 500 mg Hg/kg wet weight and is of the same order of magnitude as our assessment of a maximal value of 1 833.8 mg/kg wet weight in dolphins from the Eastern Adriatic coast. The highest concentration of total mercury in the muscles of dolphins from the French coast was 80 mg/kg wet weight, while a concentration of 136.7 mg Hg/kg wet weight was

found in the muscle of a 23 year old dolphin in our study. Hg levels in dolphins stranded in Apulia (Cardellicchio et al., 2000, 2002), on the Corsician coast (Frodello et al., 2000), on the Mediterranean coast of Israel (Roditi-Elasar et al., 2003), on Spanish and Italian Mediterranean coasts (Monaci et al., 1998) are lower than those found in our study and dolphins from the French Mediterranean, Ligurian Sea and Northern Tyrrhenian coasts (Leonzio et al., 1992; Augier et al., 1993; Capelli et al., 2000, 2008). The mean values of total mercury concentrations in liver samples (336.4 μg/g) in this study are in accordance with the observations of Storelli et al. (1998) and Storelli and Marcotrigiano (2000) who examined striped and bottlenose dolphins from Apulian coasts (South Adriatic Sea) and found 277.4 and 393.4 μgHg/g w.w. of liver.

With regard to the tissue type examined in our work, median mercury values increased in the order: muscle < kidney < liver. This is in agreement with all published reports dealing with mercury accumulation in marine mammals. Wagemann and Muir (1984) have considered the limit of Hg tolerance for the mammal's liver to be in the range of 100–400 μg/g wet weight. The concentrations presented in Table 1 fall within this range. Such high levels of mercury without overt evidence of deleterious effects can occur due to efficient Hg detoxification. The dolphin accumulates Hg mainly as methyl mercury (HgMe) through the diet; a methyl mercury biological half-life of about 1 000 days has been estimated for striped dolphins by Itano and Kawai (1981). A proportion of less than 10% of HgMe in the liver of adult specimens confirms a demethylation process in the liver. Demethylation is probably activated when HgMe concentrations exceed a threshold value (Palmisano et al., 1995). It has been proposed that at high HgMe concentrations the liver accumulates Se, which is involved in the detoxification process. Koeman et al. (1973) found the molar ratio of Hg'/Se (Hg' = Hg_{tot} – HgMe) in the liver of dolphins to be approximately 1. Such observations led to the hypothesis that the final product of HgMe demethylation could be HgSe (tiemannite), a form in which Hg is immobilized and prevented from exerting cytotoxic effects (Nigro and Leonzio, 1996; Lahaye et al., 2007). Concerning mercury concentrations in the muscle, due to its proportion in the total mass of the individual, it is not surprising that this tissue also generally contains moderate concentrations of mercury.

In conclusion, our results represent the first investigation of total mercury concentrations in dolphins sampled along the Croatian coast of the Adriatic Sea, and are mostly comparable with the results of extensive investigations of mercury levels in the Mediterranean Sea area: on the French coast, Corsician coast, Spanish and Italian Mediterranean coasts, Ligurian Sea and Northern Tyrrhenian coasts, Mediterranean coast of Israel, and especially the Italian coast of the Adriatic Sea (Apulia). We hope that our results contribute to the long-term database of contaminants in marine mammals and assist the further ecotoxicological investigation of Adriatic Sea.

REFERENCES

Andre J.M., Boudou A., Ribeyre F., Bernhard M. (1991): Comparative study of mercury accumulation in dolphins (*Stenella coeruleoalba*) from French Atlantic and Mediterranean coasts. The Science of the Total Environment, 104, 191–209.

Aguilar A., Borrel A., Pastor T. (1999): Biological factors affecting variability of persistent pollutant levels in cetaceans, 83–116. In: Reijnders, P., Aguilar, A., Donovan, G. (eds.): Chemical Pollutants and Cetaceans. Journal of Cetacean Research and Management, Special Issue 1.

Agusa T., Nomura K, Kunito T., Anan Y., Iwata H., Miyazaki N., Tatsukava R., Tanabe S. (2008): Interelement relationships and age-related variation of trace element concentrations in liver of striped dolphins (*Stenella coeruleoalba*) from Japanese coastal waters. Marine Pollution Bulletin, 57, 807–815.

Augier H., Prak W.K., Ronneau C. (1993): Mercury contamination of the striped dolphin *Stenella coeruleoalba* Meyen from the French Mediterranean coasts. Marine Pollution Bulletin, 26, 306–311.

Beck K.M., Fair P., McFee W., Wolf D. (1997): Heavy metal in livers of bottlenose dolphins stranded along the South Carolina coast. Marine Pollution Bulletin, 34, 734–739.

Capelli R., Drava G., De Pellegrini R., Minganti V., Poggi R. (2000): Study of trace elements in organs and tissues of striped dolphins (*Stenella coeruleoalba*) found dead along the Ligurian coasts (Italy). Advances in Environmental Research, 4, 31–43.

Capelli R., Das K., De Pellegrini R., Drava G., Lepoint G., Miglio C., Minganti V., Poggi R. (2008): Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. The Science of the Total Environment, 390, 569–578.

Cardellicchio N., Giandomenico S., Ragone P., Di Leo A. (2000): Tissue distribution of metals in striped dolphins (*Stenella coeruleoalba*) from the Apulian coasts, Southern Italy. Marine Environmental Research, 49, 55–66.

Cardellicchio N., Decataldo A., Di Leo A., Misino A. (2002): Accumulation and tissue distribution of mercury and selenium in striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea (Southern Italy). Environmental Pollution, 116, 265–271.

Caurant F., Amiard J.C., Amiard-Triquet C., Sauriau P.G. (1994): Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean. Marine Ecology Progress Series, 103, 207–219.

Frodello J.P., Romeo M., Viale D. (2000): Distribution of mercury in the organs and tissues of five toothed-whale species of the Mediterranean. Environmental Pollution, 108, 447–452.

Hatch W.R., Ott W.L. (1968): Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Analytical Chemistry, 40, 2085–2087.

Honda K., Tatsukawa R., Itano K., Miyasaki N., Fujiyama T. (1983): Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. Agricultural and Biological Chemistry, 47, 1219–1228.

Itano K., Kawai S. (1981): Changes of mercury contents and biological half-life of mercury in the striped dolphins, 49–73. In Fujiyama N. (ed.): Studies on the levels and organochorine compounds and heavy metals in the marine organisms. University of the Ryukyus, Nishihara, Okinawa. 132 pp.

Koeman J.H., Peters W.H.M., Koudstaal-Hol C.H.M., Tjioe P.S., De Goeij J.J.M. (1973): Mercury-selenium correlations in marine mammals. Nature, 245, 385–386.

Lahaye V., Bustamante P., Dabin W., Van Canneyt O., Dhermain F., Cesarini C., Pierce G.J., Caurant F. (2006): New insights from age determination on toxic element accumulation in striped and bottlenose dolphins from Atlantic and Mediterranean waters. Marine Pollution Bulletin, 52, 1219–1230.

Lahaye V., Bustamante P., Dabin W., Churlaud C., Caurant F. (2007): Trace element levels in foetus-mother pairs of short-beaked common dolphins (*Delphinus delphis*) stranded along the French coasts. Environment International, 33, 1021–1028.

Law R.J. (1996): Metals in Marine Mammals, 357–365. In: Beyer W.N., Heinz G.H., Redmon-Norwood A.W. (eds.): Environmental Contaminants in Wildlife. Interpreting Tissue Concentrations. SETAC Special Publication Series. Lewis Publishers Inc/CRC, Boca Raton. 494 pp.

Leonzio C., Focardi S., Fossi C. (1992): Heavy metals and selenium in stranded dolphins of the Northern Tyrrhenian. The Science of the Total Environment, 119, 77–84.

Meador J.P., Ernest D., Hohn A.A., Tilbury K., Gorzelany J., Worthy G., Stein J.E. (1999): Comparison of elements in bottlenose dolphins stranded on the beaches of Texas and Florida in the Gulf of Mexico over a one-year period. Archives of Environmental Contamination & Toxicology, 36, 87–98.

Monaci F., Borrel A., Leonzio C., Marsili L., Calzada N. (1998): Trace elements in striped dolphins (*Stenella coeruleoalba*) from the western Mediterranean, Environmental Pollution, 99, 61–68.

Nigro M., Leonzio C. (1996): Intracellular storage of mercury and selenium in different marine vertebrates. Marine Ecology Progress Series, 135, 137–143.

Palmisano F., Cardellicchio N., Zambonin P.G. (1995): Speciation of mercury in dolphin liver. A two-stage mechanism for the demethylation accumulation process and role of selenium. Marine Environmental Research, 40, 109–121.

Paludan-Muller P., Agger C.T., Dietz R., Kinze C.C. (1993): Mercury, cadmium, zinc, copper and selenium in harbour porpoise (*Phocoena phocoena*) from West Greenland. Polar Biology, 13, 311–320.

Roditi-Elasar M., Kerem D., Hornung H., Kress N., Shoham-Frider E., Goffman O., Spanier E. (2003): Heavy metal levels in bottlenose and striped dolphins off the Mediterranean coast of Israel. Marine Polution Bulletin, 46, 503–512.

Siebert U., Joiris C.R., Holsbeek L., Benke H., Failing K., Frese K., Petzinger E. (1999): Potential relation between mercury concentration and necropsy findings in cetaceans from German waters and Baltic Sea. Marine Pollution Bulletin, 38, 285–295.

Storelli M.M., Marcotrigiano G.O. (2000): Environmental contamination in bottlenose dolphin (*Tursiops truncatus*): Relationship between levels of metals, methylmercury and organochlorine compoun ds in an adult female, her neonate and a calf. Bulletin of Environmental Contamination and Toxicology, 64, 333–340.

Storelli M.M., Ceci E., Marcotrigiano G.O. (1998): Comparison of total mercury, methylmercury and selenium in muscle tissues and in the liver of *Stenella coeruleoalba* (Meyen) and *Caretta caretta* (Linnaeus). Bulletin of Environmental Contamination and Toxicology, 61, 541–547.

Storelli M.M., Giacominelli-Stuffler R., Storelli A., Marcotrigiano G.O. (2005): Accumulation of mercury, cadmium, lead and arsenic in swordfish and bluefin tuna from the Mediterranean Sea: A comparative study. Baseline/Marine Pollution Bulletin, 50, 993–1018.

Wagemann R., Muir D.C.G. (1984): Concentrations of heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation. Canadian Technical Report of Fisheries and Aquatic Sciences, 1279, 1–97.

Wagemann R., Trebacz B., Boila G., Lockhart W.L. (1998): Methylmerury and total mercury in tissues of arctic marine mammals. The Science of the Total Environment, 218, 19–31.

Zhou J.I., Salvador S.M, Liu Y.P., Sequeira M. (2001): Heavy metals in the tissues of common dolphins (*Delphinus delphis*) stranded on the Portuguese coast. The Science of the Total Environment, 273, 61–76.

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Corresponding Author:

Doc. Andreja Prevendar Crnic, University of Zagreb, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Heinzelova 55, 10000 Croatia
Tel. +385 1 2390 164, Fax +385 1 244 1390 e-mail: apcrnic@vef.hr