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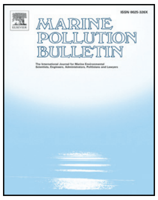

# Tissue concentrations of four Taiwanese toothed cetaceans indicating the silver and cadmium pollution in the western Pacific Ocean

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ABSTRACT

Muscle, lung, kidney and liver tissues of 45 bycatch and stranded cetaceans, including 14 *Grampus griseus* (*Gg*), 7 *Kogia simus* (*Ks*), 10 *Lagenodelphis hosei* (*Lh*), and 14 *Stenella attenuata* (*Sa*), were collected in the waters off Taiwan from 1994 to 1995, and from 2001 to 2012. Baseline concentrations (in $\mu g\ g^{-1}$ dry weight) of the cetaceans were lung (<0.05) = muscle (<0.05) < kidney (0.08 ± 0.04) < liver (0.43 ± 0.28) for Ag, and muscle (0.03 ± 0.03) = lung (0.22 ± 0.19) < liver (3.82 ± 3.50) < kidney (16.22 ± 18.81) for Cd. Unhealthy and critically dangerous Ag and Cd tissue concentrations in the toothed cetaceans are suggested. Marked high concentrations of Ag and Cd found in *Gg* and *Lh* are highly related to their squid-eating and deep diving habits. The highest ever recorded concentrations of liver-Ag and kidney-Cd were found in two *Lh*. These Taiwanese cetaceans indicate marked Ag and Cd pollution in the recent two decades in the western Pacific Ocean.

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## 1. Introduction

Silver (Ag) and Cadmium (Cd) are non-essential elements for animals, including cetaceans (Eisler, 1985; ATSDR, 1990). They are not easy for any animal to eliminate (Eisler, 1985), and certain concentrations of these elements in the body are toxic and may even be lethal (Eisler, 1985).

Silver (Ag) has a long history of use by humans in the production of jewelry and silverware. More recently, it has been widely used in electronic equipment and dental fillings, and in various other human activities such as mining, the photographic industry, combustion of wastes (fossil fuel, municipal and industrial), electronic applications, cloud seeding and medicines (ATSDR, 1990). This widespread use of Ag has led to its entry into the marine environment. Moreover, since the 1980s, when their excellent antibacterial ability was discovered, silver nanoparticles (AgNPs) have been even more widely used in many commercial products (Yu et al., 2013). It was estimated that by 2008, 500 tons of Ag had been used for AgNP production worldwide (Mueller and Nowack, 2008). This may have resulted in Ag becoming more bioavailable to marine organisms.

In comparison to Ag, the history of the use of Cd is not as long, but it has also been widely used in industrial processes such as plastic production, electroplating, the manufacturing of alloys, batteries and fertilizers, in addition to being present in the combustion products of fossil fuels, and emissions from smelting and refining plants throughout the past 200 years (Tataruch and Kierdorf, 2003).

These two metals have been widely used since the Industrial Revolution, and have become widespread environmental pollutants, with a remarkable increase in Cd levels in the ecosystem recorded over the past 100 years (Korte, 1982). The effects of Cd on the marine environment have long been monitored through marine mammals, with reports of high levels of tissue bioaccumulation in toothed whales worldwide (Fujise et al., 1988; Morris et al., 1989; Marcovecchio et al., 1990; Law et al., 1991; Noda et al., 1995; Wagemann et al., 1996; Wood and Van Vleet, 1996; Holsbeek et al., 1998; Parsons, 1999; Siebert et al., 1999; Shoham-Frider et al., 2002, 2014, 2016). However, the marine environmental impact of Ag has seldom been reported. In one of the few studies reported in the literature, Savery et al. (2013) used skin biopsy samples to establish a global baseline (16.9 ± 14.1 $\mu g\ g^{-1}$ wet weight) for Ag in sperm whales (*Physeter macrocephalus*).

Due to their widespread use in various industrial, medical, agricultural and household products, Cd and Ag are creating an environmental

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loading which is highly related to the density of the human population (Bossart, 2006). For example, one-third of the world's population borders the western Pacific Ocean and Indian Ocean, a region which has seen the highest population growth and industrial development in the last two decades. It is therefore hypothesized that the resulting significant anthropogenic environmental loading may be harming the marine mammals in these oceans.

Taiwan is situated in the western Pacific Ocean tropical volcanic chain. To the east of the island, the Kuroshio Current brings heat from the tropics (Su and Pu, 1986; Mensah et al., 2014), triggering many upwellings along its way (Udarbe-Walker and Villanoy, 2001). This results in high primary production which creates an abundance of food resources for the top marine predators, especially toothed cetaceans (Ku et al., 2014). Therefore, in our study, we took advantage of the location of Taiwan as a biodiversity hot spot, where at least 31 species of cetaceans have been recorded (Chou, 2008), and obtained as samples four of the five most dominant dolphin species which appear in eastern Taiwan, namely Risso's dolphin (*Grampus griseus*), pantropical spotted dolphin (*Stenella attenuata*), Fraser's dolphin (*Lagenodelphis hosei*), and dwarf sperm whale (*Kogia sima*) (Chou, 2008).

Toothed cetaceans are ideal sentinels for marine environmental health due to their longevity and situation at the highest trophic level of the ocean (Bossart, 2006). Once pollutants enter the environment, they will gradually accumulate in the bodies of these cetaceans, thus affecting their health while also reflecting the marine pollution status (Reif, 2011). However, such information from the highly populated and industrialized western Pacific Ocean region is scarce. From the study of the Ag and Cd concentrations in the tissues of the four toothed cetaceans, we have established baselines for these two metals for small cetaceans, as well as unhealthy and critically dangerous thresholds, in order to gain insights into their health, and to establish an early warning system in the ocean which is vital to ensure our environmental safety and public health.

## 2. Materials and methods

A total of 45 stranded or bycatch individuals of 4 cetacean species, including 14 *Grampus griseus* (*Gg*), 7 *Kogia simus* (*Ks*), 10 *Lagenodelphis hosei* (*Lh*), and 14 *Stenella attenuata* (*Sa*), were collected in the waters off Taiwan from 1994 to 1995, and from 2001 to 2012. Their muscle, lung, kidney and liver tissues were collected by the Taiwanese Cetacean Stranding Network, the Taiwan Cetacean Society, and by many volunteers from the Cetacean Laboratory (Prof. Lien-Siang Chou), the Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, and the National Museum of Marine Biology and Aquarium (Dr. Chiou-Ju Yao), Taichung.

The samples we used were mostly fresh/edible at Code 2, with three samples were died from live animals (Code 1) and two samples in fair condition (Code 3) (Decomposed, but organs basically intact) (Geraci and Lounsbury, 1993; Dr. Chiou-Ju Yao personal communication) (also see Appendix table). The tissue samples collected for Ag and Cd analyses were firstly trimmed of their outer layer by stainless steel scalpel. Only the inner part of the metal-free tissue samples were then put into zip lock plastic bags and stored at −20 °C as analytical samples. Before analysis, about 10 g of each tissue sample were homogenized and freeze-dried for at least 72 h.

The Ag and Cd analyses were digested following the method established in M.-H. Chen's lab (Chen, 2002). Approximately 0.3 g of homogenized freeze-dried non-lipid extracted sample was used for the analysis. At the same time, the standard reference materials, DOLT-2 (dogfish liver, NRCC) and DORM-2 (dogfish muscle, NRCC) from the National Research Council of Canada (NRCC) were used to verify the analytical quality.

Cd was measured by graphite furnace atomic absorption spectrometry (Hitachi Z-5000, tube type: 7JO-8885), using the standard addition method to avoid unknown interference. In this method, each unmeasured sample is mixed with 0, 2, 4 $\mu g\ L^{-1}$ of 1 $\mu g\ mL^{-1}$ cadmium standard solution. Ag concentrations were measured by ICP-MS (Inductively coupled plasma-mass spectrometer, Perkin-Elmer Elan). The recovery of the standard materials of DORM-2 and DOLT-2 with six replicates (vs. certified value) were 0.037 ± 0.004 (vs. 0.041 ± 0.013) and 0.601 ± 0.09 (vs. 0.608 ± 0.03) $\mu g\ g^{-1}$ dry weight for Ag, and 0.041 ± 0.008 (vs. 0.043 ± 0.008) and 18.5 ± 2.46 (vs. 20.8 ± 0.50) $\mu g\ g^{-1}$ dry weight for Cd. For those data presenting as wet weight in the literature, we used conversion factors of 4.2, 4.6, 3.5 and 4.5, respectively, for muscle, lung, liver, and kidney to dry weight, based on our calculations from fresh samples, which were 4.16 ± 0.41 ($n = 12$) for muscle, 4.56 ± 0.27 ($n = 8$) for lung, 3.46 ± 0.14 ($n = 7$) for liver, and 4.51 ± 0.10 ($n = 6$) for kidney.

To establish the baseline data, we first excluded one sample each of *Gg*, *Ks* and *Lh*, namely Gg(TP20080430), Ks(TP20060813), and Lh(HU20091210), which had at least one instance of extraordinarily high Ag tissue concentration, due to being diagnosed with symptoms of emphysema, serious malnutrition, dehydration, fatty liver, pulmonary fibrosis, darkening of the kidney, sclerosis of the liver, bronchitis pneumonia, periplentis, and suprarenal-capsule hematoma. We then calculated the Ag and Cd means of the total remaining sample for each kind of tissue, identifying 20 individual dolphins including 9 *Gg*, 5 *Lh*, and 6 *Sa*, with Ag and Cd concentrations in their muscle, lung, kidney and liver which did not exceed any of the total tissue means. These 20 individuals were considered in this study to be healthy specimens, and their Ag and Cd tissue concentrations were taken as the baseline (see Appendix table).

All other individuals with any Ag or Cd tissue concentrations higher than the total sample means were then pooled together to recalculate the mean and standard deviation for the unhealthy standard. Then the unhealthy mean plus one standard deviation was taken in this study to signify the critically dangerous threshold. Any samples with at least one data exceeding the critically dangerous threshold were assumed to be seriously ill. These samples were then pooled together to yield the dangerous thresholds as shown in Table 1.

Due to the limitation of our data, they did not fit the normality. Therefore, Non-parametric ANOVA (Kruskal-Wallis) using the Dunn Test as a post-hoc test was used to test the species-specific differences in the Ag and Cd concentrations ($p < 0.05$). All of the statistical analyses were performed using SAS® Version 9.3 (SAS Institute Inc., Cary, NC, USA, 1988).

## 3. Results

The baselines for the Ag and Cd concentrations (in $\mu g\ g^{-1}$ dry weight) in the four tissues of the four dolphins were established as muscle (<0.05) = lung (<0.05) < kidney (0.08 ± 0.03) < liver (0.43 ± 0.28) for Ag, and muscle (0.03 ± 0.03) < lung (0.22 ± 0.19) < liver (3.82 ± 3.50) < kidney (16.22 ± 18.81) for Cd. Significant tissue differences were found as follows: muscle = lung ≤ kidney < liver for Ag, and muscle ≤ lung ≤ liver ≤ kidney for Cd ($p < 0.05$, Table 1, Figs. 1 and 2). The Ag and Cd concentrations are of the same magnitude in the muscle tissues, but the Ag concentrations are one magnitude lower than those of Cd in the lung and liver tissues, and three magnitudes lower than those of Cd in the kidney.

The unhealthy Ag and Cd concentrations of the tissues in the small cetaceans are one magnitude higher than their baseline counterparts, except for the Ag concentrations in the muscle and lung tissues and the Cd concentration in the kidney, which are of the same magnitude as the baseline. The unhealthy concentrations (in $\mu g\ g^{-1}$ dry weight) are lung (0.06 ± 0.02) = muscle (0.09 ± 0.11) < kidney (0.33 ± 0.30) < liver (4.45 ± 5.36) for Ag, and muscle (0.42 ± 0.49) = lung (1.55 ± 0.97) < liver (32.19 ± 36.91) = kidney (99.34 ± 100.23) for Cd (Table 1, Figs. 1 and 2).

The critically dangerous thresholds of Ag and Cd concentrations of the tissues in the small cetaceans are mostly within the same magnitude

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