Trihalomethanes in marine mammal aquaria: Occurrences, sources, and health risks

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1. Introduction

Around the world, there are at least 240 large marine aquaria/ocean life centers in ~40 countries displaying marine mammals or serving as rehabilitation facilities listed by MarineBio.org (2012). Chemical disinfectants are widely used in such facilities to maintain water quality and minimize transmission of pathogens among animals and to personnel...
(Huguenin et al., 2003; Spotte, 1991). However, these disinfectants can react with water constituents, such as dissolved organic matter, ammonia, and bromide (Br\(^-\)), to form a variety of potentially hazardous disinfection byproducts (DBPs) (Croué et al., 2000; Xie, 2004). Among more than 600 DBPs identified in disinfected water (Krasner et al., 2009), the trihalomethanes (THMs), typically comprised of chloroform (CHCl\(_3\)), bromodichloromethane (CHClBr\(_2\)), dibromochloromethane (CHCl\(_2\)Br), and bromoform (CHBr\(_3\)), make up the largest class of DBPs. The THMs have been documented to be potentially genotoxic and mutagenic, and may result in adverse health effects with long-term exposure, such as bladder cancer and spontaneous abortions (Bull et al., 2001; Plewa et al., 2002; Villanueva et al., 2004). Health risk assessments of THMs in tap water and recreational swimming pools have been increasingly reported (Dyck et al., 2011; Glauner et al., 2005; Kim et al., 2002; Lee et al., 2009; Zwiener et al., 2007), but little information is available for marine mammal aquaria (Shi and Adams, 2012).

Dissolved organic carbon (DOC) has been well recognized as a DBP precursor (Xie, 2004), suggesting that DOC from marine mammal foods and wastes left in pools can produce DBPs during water disinfection (likely at levels exceeding well-studied municipal water supplies and recreational swimming pool waters; Spotte, 1991) may produce DBPs during water disinfection. In marine mammal aquaria for porpoises and pinnipeds (Huguenin et al., 2003), a disinfecting chlorine residual level of 0.4–0.7 mg/L (sometimes >1.0 mg/L) is usually maintained. Also, seawater containing approximately 65 ppm bromide could contribute to brominated DBP formation if applied (e.g., oceanaria) (Chow et al., 2005; Hua et al., 2006). With the required precursors in place, DBP formation will occur and may pose health risks to marine mammals and their trainers in captive facilities. However, distinct from municipal water supplies, in which DOC commonly origins from humic substances from soil or litter leachates (Chow et al., 2007; Zhang et al., 2009), or recreational swimming pools, where DOC are mainly from swimmers’ hair, sun block lotion, saliva, and urine (Kim et al., 2002; Zwiener et al., 2007), the main sources of DOC in marine mammal pools are probably the uneaten food (e.g., fish), urine, and scat. As the reactivity of these readily biodegradable organic matters for forming THMs is unclear, understanding their THM formation potential (THMFP) (Chow et al., 2005; Xie, 2004) is important to quantify and control the contribution of different DOC sources, facilitating better engineering and treatment processes.

Typical marine mammals workers, including animal trainers, veterinarians, biologists, and volunteers in the marine mammal rehabilitation centers, as well as field researchers and workers at aquaria and oceanaria that exhibit marine mammals, are at risk of health effects from THMs exposure. The public may also be at risk when attending recreational activities like ‘swim-with-the-dolphin’ programs by contact with the water directly in marine mammal aquaria (Hunt et al., 2008). Typically, people are in close contact with the water, and at risk of health effects. Regulations are currently lacking for THMs in swimming or working pool environments, even though the concentration of THMs has been regulated in drinking water to a maximum contaminant level (MCL) of 80 μg/L by the US Environmental Protection Agency (US EPA, 2004). Trihalomethanes can be taken up by the swimmers via the skin and inhalation, along with the unintentional swallowing of water, due to the high dermal permeability and volatility of THMs (Erdinger et al., 2004).

In the present study, we monitored the DOC and THMs generated in different compartments of two large tank systems housing California sea lions (Zalophus californianus) for seven months to evaluate the exposure and potential health risk for marine mammal workers. To identify the precursors of THMs, we set up an isolated tank system to house each of 9 sea lions and recorded the material flows (food input and waste production) in detail and determined the THM formation potential from DOC of different sources. Our objective was to systematically understand THM formation in marine mammal aquaria and its potential impact on health risk for marine mammal workers.

2. Materials and methods

2.1. Monitoring water quality in large captive facilities

2.1.1. Study site and water sample collection

To investigate the occurrence of DOC and THM in facilities housing marine mammals, we monitored the water quality at The Marine Mammal Center (TMMC) located in the Golden Gate National Recreation Area, Sausalito, California, USA. One of the largest marine mammal rescue and rehabilitation facilities in the world, TMMC had the capacity to care for 200 sick and injured pinnipeds, including California sea lion (Z. californianus), elephant seal (Mirounga angustirostris), and harbour seal (Phoca vitulina), and had two independently closed freshwater systems maintaining 45,000 gallons of freshwater with settling tanks, sand filters, and protein skimmers. Water was primarily disinfected by ozone with supplemental chlorine and bromine. A flow chart of the water system is depicted in Fig. A.1.

The pool water samples were collected three to five times per month from February to August for DOC and THM determinations. Water samples from four other sampling locations through the treatment systems were also collected once monthly from April to June, when the number of housed animal was greater than 100. These other four sampling locations were: the drain line (from the animal pools to the water treatment system), the settling tank, water storage tank (after the sand filter treatment), and the return line (from the treatment system to the animal pools). Water samples were always collected without headspace in two clean amber glass bottles (one for ultra-violet absorbance and DOC and one for THM quantification), stored at 4 °C, and analyzed within 2 weeks.

2.1.2. DOC and THM determination

Water samples for ultra-violet absorbance and DOC were filtered through a 0.45 μm polyethersulfone membrane filter.
et al., 2007). The GC oven was programmed to hold an initial temperature of 35 \(^{\circ}\)C for 10 min, increased to 140 \(^{\circ}\)C at a rate of 3.5 \(^{\circ}\)C/min and to 220 \(^{\circ}\)C at a rate of 5 \(^{\circ}\)C/min, and finally held at 220 \(^{\circ}\)C for 3 min. Precision, accuracy and detection limits of all chemical analyses (e.g., DOC and THM quantification) were tested for QA/QC using repeating tests on blank and standard solutions and spike-and-recovery tests. The values of percent relative standard deviation (%RSD) were all within 5%, and the recovery values were within 100\% \pm 5\% for DOC (method detection limit 0.1 mg/L) and 100\% \pm 10\% for four THMs (method detection limit \(< 0.055 \mu g/L\)).

2.2. Exploring THM sources in an isolated tank

2.2.1. Animals and husbandry
To quantify the THM precursors and their THM formation potential in pinniped husbandry, isolated tank with freshwater was used to house each of nine California sea lions, with feeding and waste production recorded in detail. All sea lions were in the final rehabilitation stages, being held only for final observation prior to release after being determined to be clinically recovered by veterinarians. Observers recorded sex, age class, and weights of all sea lions. Each meal being fed to sea lion was detailedly weighed and recorded. The market squid (Loligo opalescens), Pacific sardine (Sardinops sagax), and Atlantic herring (Clupea harengus) were among the common prey items in the present controlled study (Sweeney and Harvey, 2011). Meals were fed once or twice per day depending on meal size, and the total amount (kg) per day for individual sea lion remained constant, varying from 2.8\% to 6.5\% of body weight depending upon different individual needs and optimal target weights for release (Table A.1).

The freshwater tank housing individual sea lions was 6.1 m in diameter and 1.1 m in water depth with a 2.1 m width haul-out site (Fig. A.2). The tank floor was slanted to facilitate the collection of all fecal remains when the pool was drained. Scat and spew deposited (noted as pool scat) on the haul-out site and in the water during the monitoring periods were collected immediately with pertinent data recorded. The tank was drained every 24 h to collect drainpipe scat in a screened drain-basket, located externally to the tank (noted as drain scat). Volume and weight were recorded for each collected scat. A small portion (<30\% in mass) of solid wastes were collected from the drain screen, but the majority of solid wastes were collected directly from the pools by husbandry personnel. Husbandry staff (or researchers) closely monitored each sea lion for 12 h per day for 2–5 weeks. Because sea lions commonly micturated in the pool, making it difficult to collect urine from the pool, comparative urine (n = 18) samples were collected from recently deceased sea lions at necropsy (collected within 2 days of death). All scat and urine samples were immediately frozen at –20 \(^{\circ}\)C until analysis.

2.2.2. Organic carbon extraction and trihalomethane formation potential
We examined three commonly frozen foods used in feeding pinnipeds (Atlantic herring, market squid, and Pacific sardine; n = 9 each), as well as pool scat (n = 18), urine (n = 18), and drain scat (n = 10), to estimate their potential water extractable organic carbon (WEOC) yield in the marine mammal pool. Five grams of each solid sample (on a wet-weight basis) were mixed vigorously with 45 mL deionized water in a 50 mL centrifuge tube for 1 min to prepare a 1:10 (w:v) extraction (Seguin et al., 2004; Wu and Ma, 2002). Then, the mixture was centrifuged for 20 min at 250 g relative centrifugal force. The supernatant was withdrawn and filtered through a 0.45 \(\mu\)m polyethersulfone filter, and stored at 4 \(^{\circ}\)C within one week before analyses.

All water extracts and urine samples were analyzed for DOC and ultra-violet absorbance at 254 nm (UVA\(_{254}\)), whereas water extracts/samples of each potential source (herring, sardine, squid, pool scat, drain scat, and urine; n = 5 each) were chosen for THM formation potential (THMFP) measurement. We employed the dose-based THMFP method developed by Btrye Laboratory of the California Department Water Resources (California Department of Water Resources, 1994). Briefly, samples were chlorinated with freshly prepared NaOCl/H\(_3\)BO\(_3\) buffered at pH 8.3 \pm 0.1, representing the pH of natural seawater. Each sample was diluted to a DOC value of less than 10 mg/L and treated with a constant chlorine dosage (120 mg/L). The high chlorine dosage was used to ensure that there was enough reactive chlorine for complete chlorination because high ammonia levels. Samples were stored in a 40 mL borosilicate amber vial without headspace. The vials were incubated for 7 days (20 \pm 1 \(^{\circ}\)C) and then 0.15 mL of 10\% sodium sulfite solution was added to quench residual chlorine. In parallel to the direct THMFP measurement, an aliquot of 1 ppm bromide was added to the 30 chosen samples prior to chlorination in order to evaluate the effects of bromide in the THM formation. All samples were refrigerated at 4 \(^{\circ}\)C and analyzed for THM concentrations using GC-ECD within two weeks. The reactivity of DOC in forming THM, as determined by specific THMFP (STHMFP, in \(\mu g/-THM/mg-C\)), was also calculated as the amount of THM formation divided by DOC concentration of the tested water.

2.3. Health risk assessment
Lifetime cancer risk commonly refers to the chance a person has, over the course of his or her lifetime (from birth to death), of being diagnosed with or dying from cancer. Based on the US Environmental Protection Agency (EPA) guidelines on carcinogen risk assessment and the Swimmer Exposure Assessment Model (SWIMODEL 3.0 (US EPA, 2006)), we estimated the lifetime cancer risk for marine mammal workers exposed to THMs from marine mammal pool water through dermal absorption, inhalation, and incidental oral ingestion. Using measured aqueous concentrations of THMs and ambient vapor concentrations, the lifetime risk of THMs can be estimated according to SWIMODEL using Henry’s law. Briefly, the cancer risks through dermal absorption, inhalation, and oral ingestion were calculated as:
RiskDermal = LADD_{Dermal} \times SF_{Dermal}
= \frac{C_W \times K_F \times SA \times ET_{Dermal} \times EF \times ED \times 10^{-3}}{BW \times AT \times 365 \text{ d/yr}} \times SF_{Dermal}

RiskInhalation = LADD_{Inhalation} \times SF_{Inhalation}
= \frac{C_W \times IR_{Inhalation} \times ET_{Inhalation} \times EF \times ED \times 10^{-3}}{BW \times AT \times 365 \text{ d/yr}} \times SF_{Inhalation}

RiskOral = LADD_{Oral} \times SF_{Oral}
= \frac{C_W \times IR_{Oral} \times ET_{Oral} \times EF \times ED \times 10^{-3}}{BW \times AT \times 365 \text{ d/yr}} \times SF_{Oral}

where LADD is lifetime average daily dose (mg kg\(^{-1}\) day\(^{-1}\)) and SF is the slope factor (an upper bound of the approximated 95% confidence limit on the increased cancer risk from a lifetime exposure to an agent by route, in kg mg\(^{-1}\) day). The slope factors for different THM species were obtained from US EPA (2005). The components of the LADD are: \(C_W\) for chemical concentration in water (µg L\(^{-1}\)) or \(C_V\) for chemical concentration in vapor (µg m\(^{-3}\)); \(K_F\) for the chemical-specific dermal permeability constant (m h\(^{-1}\)); \(SA\) for skin surface area available for contact (m\(^2\)); \(IR\) for intake rate (L h\(^{-1}\) for ingestion and m\(^3\) h\(^{-1}\) for inhalation); \(ET\) for exposure time (h event\(^{-1}\)); \(EF\) for exposure frequency (event year\(^{-1}\)); \(ED\) for exposure duration (years); \(BW\) for body weight (kg); and \(AT\) for average lifetime of marine mammal worker (yr). Based on the recommendation of SWIMODEL (US EPA, 2006) and exposure risks for marine mammal workers who work in the water with the animals, such as cetacean rehabilitation staff, marine mammal trainers, or swim-with-the-dolphin program workers (hereafter “trainers”) (Hunt et al., 2008), we assumed the parameters for maximal risk exposure as follows: body surface areas (SA): male = 1.94 m\(^2\), female = 1.69 m\(^2\), inhalation rate (IR\(_{Inhalation}\) = 1.00 m\(^3\)/h); oral ingestion rate (IR\(_{Oral}\) = 0.010 L h\(^{-1}\)), swimming exposure frequency (EF = 250 events year\(^{-1}\)), career exposure duration (ED = 20 years), body weight (BW: male = 78.1 kg, female = 65.4 kg), swimming exposure time (ET = 2 h event\(^{-1}\) for dermal and oral working in pool; 6 h event\(^{-1}\) for inhalation considering the addition of a 4 h event\(^{-1}\) working around the pool), and average life span (AT = 70 years). Note that when swimming in a marine mammal pool or ocean, people are much more conservative and careful about letting water get in their mouth than when playing in a swimming pool, and thus we adopted a lower IR\(_{Oral}\) of 0.010 L h\(^{-1}\) compared to that of 0.025 L h\(^{-1}\) recommended in SWIMODEL.

Noncarcinogenic risks of THMs were also evaluated using the hazard index (HI) for different exposure routes (Lee et al., 2009). This index is calculated as the ratio of the average daily doses to the reference doses (RFDs). As US EPA recommended, the RFD values are 1.00 \times 10^{-2} mg kg\(^{-1}\) day\(^{-1}\) for chloroform, and 2.00 \times 10^{-2} mg kg\(^{-1}\) day\(^{-1}\) for bromodichloromethane, dibromochloromethane, and bromoform.

3. Results

3.1. Occurrences of DOC and THMs in the rehabilitation facility

As presented in Fig. 1, the monthly THM concentrations of pool water in the large freshwater systems of TMMC ranged from 1.9 \pm 0.6 µg/L in August to 105.3 \pm 23.8 µg/L in May. Across different months, mean THM concentrations generally increased with both DOC concentrations and the number of patients admitted in TMMC (\(P < 0.05\)). For speciation, the bromoform dominated all THM species (>90%). Concentrations decreased along the line in the water treatment systems (Fig. 2) from pool water, drain line, and settling tank (averages of 25–31 mg/L) to storage tank and return line (averages of 9–11 mg/L; \(P < 0.001\)). The most significant drop (58% on average) occurred from settling tank to post-sand filter. Dissimilarly, the THM concentrations in the storage tank and return line after disinfection (averages of 120–130 µg/L) were higher than those in the diluted in pool water, drain line, and settling tank (averages of 40–55 µg/L; \(P < 0.001\)).

3.2. Information on California sea lions in the isolated tank study

The sub-adult female weights (\(n = 2\)) were 45–50 kg (kg); the sub-adult male weights (\(n = 3\)) were 77–119 kg; the adult female weights (\(n = 2\)) were 53–80 kg; and the adult male weights (\(n = 3\)) were 190–215 kg (Table A.1). The amount of solid wastes (including pool and drain scat) generated by a California sea lion in captivity generally increased with the body weight (\(R^2 = 0.81, P = 0.001\)), as daily meals were fed with a fixed ratio to body weight. When the ratios of meal weight to body weight stood between 2.8% and 6.5%, California sea lions converted a range of 3.2–7.7% (5.4% on average) of their meal weights to solid wastes (Table A.1).

3.3. Waste and DOC production per animal housed

The amounts of DOC produced from food items (herring, sardine, and squid) and wastes (pool scat, urine, and drain scat) were determined by water extraction methods (Chow et al., 2005). Foods yielded higher DOC concentrations in pool water with averages of 22–34 mg-C/g-wet sample, whereas wastes showed averages of 2–16 mg-C/g-wet sample (Fig. 3). The levels of water extractable organic carbon among the 5 groups of solid samples were all significantly different (\(P < 0.01\)) with squid > herring > sardine > pool scat > drain scat. The concentration of DOC in urine was 12 \pm 1 g/L.

3.4. Reactivity of DOC in THM formation

The reactivity of DOC in forming THMs for different wastes is summarized in Fig. 3. The WEOC from solid wastes (pool and drain scat) had higher reactivity in forming THMs compared to other groups.

2.4. Statistical analyses

All statistical analyses were conducted using the software SPSS 13 for windows. One way analysis of variance (ANOVA) and subsequent multiple comparisons (Tukey test) were used to determine the differences between means of different groups (significance level of \(P < 0.05\)). Pearson correlations were performed when appropriate (Shapiro–Wilk’s test, \(P > 0.05\)).
food items \((P < 0.05)\). The drain scat samples showed the highest formation potential \((58 \pm 8 \mu g\cdot THM/mg-C)\), followed by pool scat \((48 \pm 10 \mu g\cdot THM/mg-C)\). Among food sources, herring and sardine had comparable STHMFP \((P > 0.05)\). Although squid had the highest WEOC potential, its STHMFP \((31 \pm 4 \mu g\cdot THM/mg-C)\) was significantly lower than that of herring \((41 \pm 5 \mu g\cdot THM/mg-C)\) or sardine \((38 \pm 6 \mu g\cdot THM/mg-C; P < 0.05)\). The urine samples had the lowest STHMFP \((14 \pm 6 \mu g\cdot THM/mg-C)\) compared to solid wastes and food items.

The presence of bromide enhanced the formation of THMs by 25–124%, on a mass basis in all samples (Fig. 3). In addition to the increase in THM production, bromide changed the speciation of THMs. Before bromide addition, chloroform was the only THM formed in all samples. With 1 ppm of bromide, other forms of THMs, including bromodichloromethane, dibromochloromethane, and bromoform, were produced in all samples. Utilizing ‘drain scat’ as an example, the primary species was bromodichloromethane, accounting for 42%, followed by chloroform (31%), dibromochloromethane (23%), and bromoform (4%).

### 3.5. Correlations among DOC, THMs, and UVA254

Positive linear correlations \((R^2 = 0.64–0.74; both P < 0.001)\) between WEOC concentration and UVA254 in the food items or waste extracts (Fig. 4a) and between DOC concentrations and UVA254 in pool water (Fig. 4c) were observed without statistically different slopes or intercepts \((P > 0.05)\). Significant but weak correlations (Figs. 4b and d) were found between THMFP and UVA254 of all extracted samples \((R^2 = 0.40)\), as well as between THM concentration and UVA254 in pool water at TMMC \((R^2 = 0.44)\).

### 3.6. Health risk assessment of THMs in large rehabilitation facility

The cancer risks from exposure to THMs in marine mammal pool water through oral ingestion, dermal absorption, and inhalation for both male and female trainers are shown in Table 1. Among the three exposure routes, inhalation of THMs poses the highest cancer risk \((5.16 \times 10^{-4} to 1.30 \times 10^{-3})\) for marine mammal trainers who regularly swim in aquaria, followed by dermal absorption \((4.63 \times 10^{-8} to 2.86 \times 10^{-7})\) and oral ingestion \((5.40 \times 10^{-9} to 8.64 \times 10^{-8})\). This risk from inhalation is much higher than the negligible cancer risk level \((10^{-6})\) defined by the US EPA, while those from dermal absorption and oral ingestion were within the negligible range. Comparing different genders, female trainers have slightly higher cancer risks than male trainers through all different exposure routes. For noncarcinogenic risks, the hazard index of THMs for the oral ingestion (maximum of 4.52 \times 10^{-4}) and dermal absorption (maximum of 1.20 \times 10^{-3}) were below the EPA acceptable level of 1 by a factor of about 1000 (Table 1).

### 4. Discussion

#### 4.1. DOC and THM loads in marine mammal facilities

The variation of DOC and THM concentrations along different compartments of the marine mammal water treatment
the concentration of THMs in pool water was less than half of drinking water industry (commonly 10 mg/L) (Lee et al., 2009; Zwiener et al., 2007). However, our documentation of weak correlations with monitored THM levels in marine mammal aquaria (Spotte and Adams, 1979), but much higher than those commonly found in municipal water (commonly <5 mg/L) or swimming pool water (commonly <10 mg/L) (Lee et al., 2009; Zwiener et al., 2007). However, evaluating the THMs, the concentration in pool water at TMMC (1.1–144.2 μg/L with average of 44.1 μg/L) was comparable to that observed in seawater/saltwater aquaria (up to 140 μg/L) by Shi and Adams (2012), and those reported in swimming pools (average of 17.5–132.4 μg/L for swimming pools in 5 different countries reviewed by Lee et al. (2009)). Of 70 pool water samples taken at TMMC, 11 had a higher THM concentration than 80 μg/L, the MCL of THM in drinking water implemented by USEPA.

The positive correlations between monthly THM concentration and both DOC concentration and the number of marine mammals admitted to TMMC (Fig. 1) indicate that the increase in food inputs and waste generation yielded higher risk of exposure to unsafe levels when high numbers of marine mammals were present.

4.2. UV\textsubscript{254} reflected DOC sources but failed to predict THM formation

Ultraviolet absorption spectrophotometry provides an inexpensive and convenient tool to characterize the aromaticity of DOC. The regression between DOC and UV\textsubscript{254} (Fig. 4c) in pool water at TMMC is consistent with the regression between WEOC and UV\textsubscript{254} of food and wastes (Fig. 4a), with slopes greater than those for soil extract (DOC = 17.85 × UV\textsubscript{254} - 0.071; \(R^2 = 0.98\)) or natural water (DOC = 30.3 × UV\textsubscript{254} - 0.212; \(R^2 = 0.93\)) (Chow et al., 2008), suggesting that the food input and waste production are the major DOC sources in marine mammal aquaria. Humic substances from plant or soil leachates or natural water that commonly have high aromaticity have been well identified as THM precursor in tap water (Croue et al., 2000). In contrast to the humic substance, DOC from food and waste primarily contained readily biodegradable nitrogen-enriched organic matter, such as fatty acids and protein, which are relatively smaller molecules with less aromatic characteristics (Her et al., 2002; Leenheer and Croue, 2003; Stevenson, 1994), contributing to the STHMFP of WEOC of marine mammal pool water (36 μg-THM/mg-C on average) being significantly lower than those of natural waters (typically 60–100 μg-THM/mg-C) (Chow et al., 2005).

Because of its low cost and convenience, UV\textsubscript{254} has been widely applied to predict DOC levels and THM formation in the drinking water industry (commonly \(R^2 > 0.8\) (Diaz et al., 2009; Dobbs et al., 1972; Edzwald et al., 1985)). However, our documentation of weak correlations with monitored THM levels in marine mammal aquaria (\(R^2 = 0.44\), Fig. 4d) and THMFP from food and waste materials (\(R^2 = 0.40\), Fig. 4b) suggests it can hardly be a good surrogate to predict THM levels in marine mammal aquaria.

4.3. Contribution of different DOC sources to THM formation

Combining the WEOC and STHMFP results, we can calculate the mass-normalized THMFP from different sources. The average THM formation potential per mass sample followed the order of: herring (1066 μg-THM/g-wet-sample) > squid (1054 μg-THM/g-wet-sample) > sardine (836 μg-THM/g-wet-sample) > pool scat (768 μg-THM/g-wet-sample) > urine (168 μg-THM/mL/g-wet-sample) > drain scat (116 μg-THM/g-wet-sample).

Consider the example of a fully grown male sea lion with a body weight of 200 kg in a pool being fed 6 kg (3% of its body weight) of squid daily with all food consumed: 3.0–7.4 g DOC and 0.15–0.35 g THM would likely be dissolved in the water.
from his fecal production, according to the relatively stable metabolic capability (range of the mass ratio of feces to food in Table A.1: $3.2 \text{e}^{7.7\%}$). Although few data on urine volume of the adult sea lion are available, based on a urine volume of $800\text{e}^{950}$ mL per day for a 50 kg sub-adult male California sea lion (Pilson, 1970), the DOC and THM generated per day by urine would equal $9.6\text{e}^{11.4}$ g and $0.13\text{e}^{0.16}$ g, respectively.

However, all offered food is not always completely consumed, and thus the percentage of uneaten food should influence the total DOC and THM productions. Taking the same example above, if we assumed 0%, 1%, 5%, and 10% of the 6 kg of squid input went uneaten, then the total DOC yield from food and waste would be 15.7 g, 17.6 g, 25.1 g, and 34.5 g, respectively, while the corresponding THM potential production would amount to $0.39\text{e}^{0.10}$ g, $0.45\text{e}^{0.10}$ g, $0.69\text{e}^{0.10}$ g, and $0.99\text{e}^{0.09}$ g, respectively (Fig. 5). The food-derived THM contribution would increase rapidly with the percentage of food remaining, contributing almost half (46%) of the THM when even just 5% of food remained. In an extreme case, if all 6 kg of squid were left uneaten and not removed, the potential generation would be 204 g of DOC and 6.32 g THM. Under this condition, a minimum of 79,000 L of water would be required to house only one adult sea lion in captivity to guarantee a safe level of THMs below the $80\mu\text{g}/\text{L}$ threshold.

### 4.4. Risk assessment

We calculated the cancer and noncarcinogenic health risks of THMs to marine mammal trainers to help workers understand their risks and add to the body of literature for different exposure pathways. The average risk from inhalation exposure to THMs in marine mammal pool water ranged from

| Table 1 – Lifetime cancer risks and hazard indices (for noncarcinogenic risks) from THM exposure for male and female trainers working in marine mammal aquaria. |
|----------------------------------|------------------|------------------|
|                                  | Male Trainer     | Female trainer   |
|                                  | Arithmetic mean  | Minimum          | Maximum          |
| Cancer risks:                    |                  |                  |
| Oral                            | 2.80E-08         | 5.40E-09         | 7.20E-08         |
| Dermal                          | 1.51E-07         | 4.63E-08         | 2.75E-07         |
| Inhalation                      | 5.88E-04         | 5.16E-04         | 1.09E-03         |
| Noncarcinogenic hazard indices: |                  |                  |
| Oral                            | 1.16E-04         | 1.80E-05         | 3.76E-04         |
| Dermal                          | 4.57E-04         | 2.49E-04         | 1.16E-03         |
5.16 \times 10^{-4} \text{ to } 1.30 \times 10^{-3}, two orders of magnitude higher than the negligible risk level (10^{-9}) defined by the US EPA. This estimate highlights the significant risk from the inhalation of THMs around marine mammal pools. Inhalation exposure was the primary risk for THM exposure, similar to what has been found for recreational swimming pools in other studies (Erdinger et al., 2004; Lee et al., 2009). The calculated hazard index for noncarcinogenic risk ranging from 10^{-5} to 10^{-3} was much lower than 1, which is the EPA-defined acceptable level. Notably, the health risk we calculated here is only from occupational exposure to the THMs in marine mammal pools. The marine mammal trainers are also exposed to THMs when they drink tap water daily and swim in recreational swimming pools; therefore, their total THM exposure and the cancer or noncarcinogenic risks should be higher depending on other activities.

Compared to humans, marine mammals have a greater fat and lipid content, predisposing marine mammals to accumulate THMs. By implication, the marine mammals in aquaria may also face potential health risks from THMs. To our knowledge, the only available document discussing the possible impact of THM on marine mammals is Spotte’s risk assessment (1991). His assessment considered THM intake by ingestion only and concluded that the risk THM posed to marine mammals is negligible. However, dermal contact and inhalation are also important routes for THM exposure (Erdinger et al., 2004; Miles et al., 2002). Considering an adult California sea lion (200 kg) with a lung volume of 55 mL/kg and an average resting respiration rate of 438 breaths/h (Wise et al., 2009), its inhalation rate would be 4.82 m³/h. Also, because of the increased sea lion exposure time to water in aquaria (assuming 14 h/day), we calculated the potential dose rate via inhalation exposure to be at least one order of magnitude higher than that of a trainer. However, the lifetime cancer health risks are difficult to estimate because of likely varying exposure frequency and duration (how much time in water and duration of captivity), as well as lack of information on cancer development in sea lions.

Notably, our study has not accounted for the health risk from other, more toxic, DBPs such as haloacetic acids, haloacetonitriles and halonitromethanes (Shi et al., 2013). The water in marine mammal aquaria have low organic C/N ratio (here \(<3.5\) and large amounts of protein, which suggests the haloacetonitriles yield could account for a large fraction of DBPs (Wang et al., 2013, 2012). Much is still unknown about the risks of DBPs to marine mammals in captivity and marine mammal workers, which should not be simply considered as “negligible” but should be further explored.

4.5. Implications for marine mammal aquaria management

We have shown that food and waste materials are important DOC sources and THM precursors in marine mammal aquaria. If not collected by the pool-operator, the uneaten foods and feces (\(\geq16 \pm 1\) mg-WEOC/g-wet sample) compose the drain scat (\(2 \pm 0\) mg-WEOC/g-wet sample) with the water flow, therefore most of the DOC (\(\geq14\) mg-WEOC/g-wet sample) is released into pool water. It is noteworthy that drain scat still releases DOC with the highest STHMFP, even after long retention time in water. Thus, it is necessary to inspect the pool regularly and remove all solid waste materials from the water systems as soon as possible to minimize the THM generation.

According to the monitoring results along the water treatment system (Fig. 2), approximately 30% of the DOC on average was retained and re-entered the pool water after treatment through the settling tank, protein skimmer, sand filter, and chlorination. This finding is consistent with a previous study, which showed carbon fed to the animals could remain in the water systems after water treatment, along with the organic carbon which accumulated linearly with time in the closed water system (Spotte and Adams, 1979). By implication, the THM generation likely increases with the accumulating DOC level due to water recirculation. The pool operator should therefore add and exchange water on a regular basis to avoid deteriorating water quality. Also, as bromide has long been identified as one of the major precursors in forming more toxic brominated THM (Richardson et al., 2007) during water sanitization (Bull et al., 2001; Croue et al., 2000; Hua et al., 2006; Xie, 2004), marine mammal holding facilities should avoid using Br-containing disinfectants to minimize the production of more toxic THMs.

5. Conclusions

(1) Trihalomethanes were detected in a model marine mammal rehabilitation facility at levels ranging from 1.1 to 144.2 µg/L with an average of 44.1 µg/L. Monthly average THM levels increased with the number of animals admitted.

(2) Uneaten food in the tank and animal wastes were identified as major sources of DOC and THM precursors. Each gram of food or waste material can potentially generate 22–34 mg or 2–16 g of DOC in water, respectively. The THMFP of food and waste ranged from 14 to
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Appendix A. Supplementary data

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