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UNIVERSITY OF CALIFORNIA SANTA CRUZ

**Tuna Consumption and Tuna Mercury Concentration - Implications for Human
Health**

A thesis submitted in partial satisfaction
of the requirements for the degree of

MASTER OF SCIENCE

in

Microbiology and Environmental Toxicology

by

Yasuhiko Murata

September 2018

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ABSTRACT

Title: Tuna Consumption and Tuna Mercury Concentration - Implications for Human Health

Author: Yasuhiko Murata

To our knowledge, no studies have analyzed tuna consumption levels, hair mercury, and knowledge in a population, let alone a high risk population such as college students that are provided tuna daily at dining facilities. We examined the relationship between tuna consumption, hair mercury levels, and knowledge of methylmercury exposure risk from tuna consumption in university students that were served tuna daily at university-run dining halls. Total mercury concentrations in tuna and hair samples were measured using atomic absorption spectrophotometry while tuna consumption levels and knowledge was assessed through surveys. Average hair total mercury levels in tuna consumers was higher than non-tuna consumers ($0.443 \mu\text{g/g} \pm 0.333 \text{ SD}$, $n = 22$ versus $0.113 \mu\text{g/g} \pm 0.100 \text{ SD}$, $n = 32$, respectively) ($p < 0.0001$, Mann-Whitney U), with tuna eaters exhibiting a positive relationship between self-reported tuna consumption and their hair total mercury levels ($r = 0.852$, $p < 0.0001$, $n = 22$, linear regression). For tuna eaters, about half (46%) of the students self-reported eating > 3 tuna meals per week, potentially exceeding the U.S. Environmental Protection Agency's reference dose for methylmercury of $0.1 \mu\text{g/kgbw/day}$. Eight percent of study participants ate > 20 tuna meals per week, corresponding to hair total mercury levels $> 1 \mu\text{g/g}$ - a level of concern. We also found that for tuna eating students, self-reported tuna consumption was negatively

related to knowledge about risk of methylmercury exposure from tuna and confidence in their knowledge. Only one out of the 107 students surveyed illustrated knowledge about the United States Environmental Protection Agency's recommended limit on tuna consumption, high confidence in their knowledge, and high awareness of the risk of mercury exposure from tuna consumption. Our study highlights the importance of education about the risks of tuna consumption, particularly in institutional settings where individuals have access to tuna products on a regular basis.

Introduction

Fish, in particular tuna, is a common food source across the world but also known to contain high concentrations of mercury (FDA, 1997), with the majority (~90%) of total mercury in tuna consisting of methylmercury (MeHg) (Storelli et al., 2002). Methylmercury is a potent neurotoxin (NRC, 2000) that is readily (~95%) absorbed across the GI tract (Silbernagel et al., 2011). Methylmercury exposure can cause delayed neural development, and lead to symptoms such as reduced brain size (Bose-O'Reilly, 2010) and poor cognitive skills (Grandjean et al., 1997). Methylmercury has also been shown to be a fetotoxic compound (Rice et al., 2014). Due to the toxicity of methylmercury, governmental and world health organizations have assigned a reference dose for methylmercury exposure, which is an “estimate of a daily exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime.” (EPA, 1997). The United States Environmental Protection Agency’s (USEPA) reference dose for methylmercury is 0.1 µg/kg bw/day (EPA, 1997).

Unlike other methylmercury exposure sources, such as industrial waste (Harada, 1995), tuna has also been proven to provide nutritional benefits. Tuna has high levels of DHA (Docosahexaenoic acid), an omega 3 fatty acid (Hosomi et al., 2012; Horrocks and Keo, 1999) which has been found to be neuro-developmentally beneficial (Hong et al., 2015) and supports brain development (Lauritzen et al., 2016), which is associated with higher IQ in children (Kuratko et al., 2013). Indeed,

some studies have suggested that the DHA levels in tuna may mask the adverse effects of methylmercury (Strain et al., 2015).

Although human studies have shown a positive relationship between tuna consumption and tuna hair mercury levels (Strain et al., 2015; Hightower, 2003), and populations that consume large quantities of tuna are exposed to potentially harmful levels of methylmercury (Bradley et al., 2017), little is understood about the risk of mercury exposure to people who eat at dining halls and have access to tuna on a daily basis. This study's full population of students typically spend their first few years living on campus and eat the majority of their meals at university-run dining halls (University of California, Santa Cruz, 2018). These university students may be a higher risk population from mercury exposure because a majority are of reproductive and neurodevelopmental age (Deliens et al, 2012; Ruthig et al., 2011; Cavazos-Rehg et al., 2015; Bellieni, 2016; Silveri 2012).

In order to better understand methylmercury exposure risk from tuna consumption in people, in particular young adults and adolescents, that frequent dining halls with unlimited access to tuna, we 1) assessed exposure to methylmercury in university students who ate tuna served daily at the university-run dining halls through self-report questionnaires and 2) examined how knowledge about methylmercury exposure risk from tuna consumption was related to tuna consumption levels. The results of our study can help inform institutions, especially university or boarding schools, about the risks of methylmercury exposure and about possible recommendations to moderate consumption.

Materials and Methods

Sample Collection and Processing. Tuna samples. Tuna samples were collected from buffet stations at the Rachel Carson Dining Hall on the University of California Santa Cruz campus (Santa Cruz, CA USA) between February 2016 and June 2017 over 15 different days ($n = 15$), and from Crown/Merrill Dining Hall, Cowell/Stevenson Dining Hall, Porter/Kresge Dining Hall, and Nine/Ten Dining Hall between May 2017 and June 2017 over 2 different days ($n = 10$). Tuna samples were obtained using tweezers, placed in plastic containers, and frozen at -20°C until analysis.

Survey 1. Individuals were randomly selected by counting persons who exited the dining hall by increments dictated through a sequence of randomly generated integers. Randomly selected individuals ($n = 168$) exiting the Rachel Carson Dining Hall on the University of California Santa Cruz campus between May 2017 and June 2017 were approached and asked to consent to complete a survey regarding their tuna consumption behavior (SI Figure 1). Out of the 168 individuals approached, 62% agreed to take the survey ($n = 105$). Individuals who completed a survey were given a further option to consent to giving a hair sample for total mercury analysis. A total of 54 individuals (51%) that took the survey provided a hair sample.

Survey 2. Individuals were randomly selected through the same criteria as previously mentioned for survey 1. Randomly selected individuals ($n = 238$) exiting

the Rachel Carson Dining Hall on the University of California Santa Cruz campus between March 2018 and June 2018 were approached and asked to consent to complete a survey regarding their tuna consumption behavior as well as associated risk of mercury exposure from eating tuna (SI Figure 2). Almost half of the individuals approached consented to completing the survey (n = 107 out of 238).

Survey quality control. Initial cohort size of survey 1 was 105. Quality control eliminated responses considered to be not possible or inappropriate for the question. After quality control, 2 responses were removed. Thus, 103 responses were used for calculations for survey 1. Initial cohort size for survey 2 was 107. All responses from survey 2 were kept after quality control. All 107 survey responses from survey 2 were used for calculations. Survey responses were also assessed for consistency across survey answers. For example, for question 5 in survey 2 (SI Figure 2) asking a person to respond how many meals per week of tuna was related to the United States Environmental Protection Agency's (USEPA) recommended limit, all participants' survey responses for the number below the recommended limit were below their responses for the number around the recommended limit, and all survey responses for the number above the recommended limit were above their responses for the number around the recommended limit, illustrating consistency across their answers for this question. In addition, overall demographic information gathered across both surveys were similar to each other (SI Figure 7).

Human hair sample collection. Human hair samples were collected between May 2017 and June 2017 from 54 consenting individuals who completed survey 1

(see above). Samples were collected anonymously as individuals who gave a hair sample were assigned a unique identification number to match their survey responses with their hair sample. About 0.2 g of hair was trimmed from the occipital lobe proximal to the scalp using scissors, placed in paper envelopes, and stored at room temperature. Between 0.00362 g and 0.0689 g of hair ~1 cm proximal to the scalp was used in analysis.

Hair washing assessment. Hair samples were obtained from a separate set of four individuals and washed following the methods of Eastman et al. (2013). Briefly, hair samples were sonicated for 20 min in 0.5% Triton X-100, rinsed five times with Milli-Q water, sonicated for 10 min in 1 N trace metal grade nitric acid, rinsed once with 1 N trace metal grade nitric acid, and then rinsed five-times with MQ water. A subset of samples was washed with the above procedure three times while control samples had no washing or rinsing treatment.

We found that, similar to prior studies (Morton et al., 2002; Li et al., 2016; Schwedler et al., 2017), hair washing did not affect hair total Hg measured (SI Figure 3) and thus we did not wash our hair samples from study participants who took the survey.

Tuna and hair analysis for total mercury

Total mercury concentrations in tuna and hair samples were measured using a Direct Mercury Analyzer-80 (DMA-80). Between 24 and 99 mg of each tuna sample was weighed, placed into individual quartz sample boats for analysis, and each sample was analyzed in triplicates. At least 5 mg of hair was weighed and placed into

individual quartz sample boats for analysis. Triplicate blank sample boats, triplicate fish protein reference material and hair reference material were analyzed during each analytical run. The sample detection limit for total mercury was 0.001 ng. Standard reference material for fish protein (DORM-4) average total mercury recovery was 85.5% recovery (range 80 - 91%, n = 5) and the % RSD (residual standard deviation) for the triplicate samples was 3% to 21%. Standard reference material for hair (IAEA-86) average total mercury recovery was 94% (range 90 - 103%, n = 16) and the % RSD for the triplicate samples was 1.11% to 2.85%.

Statistical Analysis

All statistical tests were performed using JMP Pro 13 (SAS Institute Inc., Cary, North Carolina, USA). Mercury measurements and survey data were analyzed with parametric and non-parametric statistics as appropriate with significance reported if $P < 0.05$. Linear regression was used for determining the relationship between reported tuna consumption and hair mercury concentrations. Spearman's test for correlation was used for the survey responses for knowledge assessment and tuna consumption levels. Mann-Whitney U was used to determine whether there was significantly different levels of hair mercury between groups.

Setting mercury exposure limits from tuna consumption.

To help assess knowledge related to mercury exposure from tuna consumption, an accurate response of 2-3 tuna meals per week was assigned using the United States Environmental Protection Agency's (EPA) recommended fish consumption limit of 2~3 times a week (EPA, 2017).

Results

Tuna mercury concentrations.

The average total mercury concentration in tuna collected during the same timeframe of hair sample collection (between May 2017 and June 2017) was $0.075\mu\text{g/g} \pm 0.007$ SD (range = 0.067 to 0.088 $\mu\text{g/g}$, $n = 10$). Tuna samples collected approximately a year prior (between February 2016 to June 2016) had about 3-fold higher total mercury concentrations (average = 0.251 ± 0.171 $\mu\text{g/g}$, range = 0.060 to 0.548 $\mu\text{g/g}$, $n = 15$) (SI Table 1). Due to the difference in appearance of the tuna collected during these two collection intervals, and discussions with dining facility managers, we believe a switch to a different brand of tuna between the two collection times (2016 and 2017) is responsible for the different mercury levels, highlighting the large variation of mercury levels in tuna products sold.

We calculated the number of times a person could consume either the low mercury tuna (0.075 $\mu\text{g/g}$) or high mercury tuna (0.251 $\mu\text{g/g}$) from the dining hall per week and stay below the United States Environmental Protection Agency's (USEPA) reference dose for mercury exposure of 0.1 μg mercury/ 1 kg body weight per day (EPA, 1997). We used an average body weight of 63.5 kg (Walpole et al., 2012), and a serving size of 212 grams (~ equivalent to approximately three scoops using the dining hall serving utensils¹) and found that a person could consume up to three low mercury tuna meals per week but less than one high mercury tuna meals per week to

¹ Students who served themselves tuna took on average 3 scoops (± 3 SD, range = 2-13, $n= 19$ observational days) (Y. Murata, pers. obs.)

stay below the United States Environmental Protection Agency's (USEPA) reference dose.

Survey 1 study participants.

105 individuals agreed to take survey 1 (SI Figure 1) which included asking people how often they consumed tuna at the university-run dining hall (measured by their input of an integer value for their weekly frequency of tuna consumption) and if they would consent to donate a hair sample for mercury analysis. Everyone surveyed was of reproductive age (between 18 and 34 years), with more male responders than females (males = 61%, females = 37%, non-binary/other = 2%). The proportion of individuals who reported tuna consumption at the dining halls was 25 out of 103 (24%) and the average weekly tuna consumption of the students that ate tuna was 6 ± 6 SD (range = 1 to 25) meals per week. Using the United States Environmental Protection Agency's (USEPA) recommended limit of 2-3 times fish consumption per week (EPA, 2017), 12 out of 25 (48%) of tuna consuming study participants reported weekly tuna consumption greater than 3 meals per week.

Survey 2 study participants.

107 individuals agreed to take survey 2 (SI Figure 2) which included asking people how often they consumed tuna at the dining hall (measured by their input of an integer value for their weekly frequency of tuna consumption) and about their knowledge of mercury exposure risk from tuna consumption. Everyone surveyed was of reproductive age (between 18 and 34 years), with close to an equal number of males and females (males = 53% females = 47%). The proportion of individuals who

reported tuna consumption (28 out of 107, 26%) and average weekly consumption (6 meals per week \pm 6 SD, range = 1 to 25) was similar to survey 1. Using the United States Environmental Protection Agency's (USEPA) recommended limit of 2-3 times fish consumption per week (EPA, 2017), 12 out of 28 (48%) of tuna consuming study participants reported weekly tuna consumption greater than 3 meals per week.

Hair mercury and tuna consumption.

Average total mercury concentration in hair for all study participants was $0.247 \mu\text{g/g} \pm 0.275 \text{ SD}$ (range = 0.009 to $1.22 \mu\text{g/g}$, $n = 54$) (SI Table 2) and there was no difference between males and females ($p > 0.8$, Mann-Whitney U) (males = $0.241 \mu\text{g/g} \pm 0.249 \text{ SD}$, $n = 35$, females = $0.287 \mu\text{g/g} \pm 0.333 \text{ SD}$, $n = 17$) (SI Table 2). The average concentration of hair mercury in people that self-reported they did not consume tuna at the dining halls was $0.113 \mu\text{g/g} \pm 0.100 \text{ SD}$ (range = 0.009 to 0.40, $n = 32$) and lower than the average concentration of hair mercury in self-reported tuna consumers ($0.443 \mu\text{g/g} \pm 0.333 \text{ SD}$, range = 0.021 to 1.22, $n = 22$) ($p < 0.0001$, Mann-Whitney U).

Self-reported tuna eating study participants had hair total mercury levels that were positively related to their reported number of tuna meals eaten per week at university dining halls ($R^2 = 0.852$, $p < 0.0001$, $n = 22$, linear regression) (Figure 1). Two study participants reported eating > 20 tuna meals per week and had total mercury concentrations in hair that were above $1 \mu\text{g/g}$. Interestingly, we found no relationship between the number of times a person self-reported they consumed tuna

weekly outside of the dining halls and their hair total mercury levels ($R^2 = 0.0210$, $p > 0.3$, $n = 22$, linear regression).

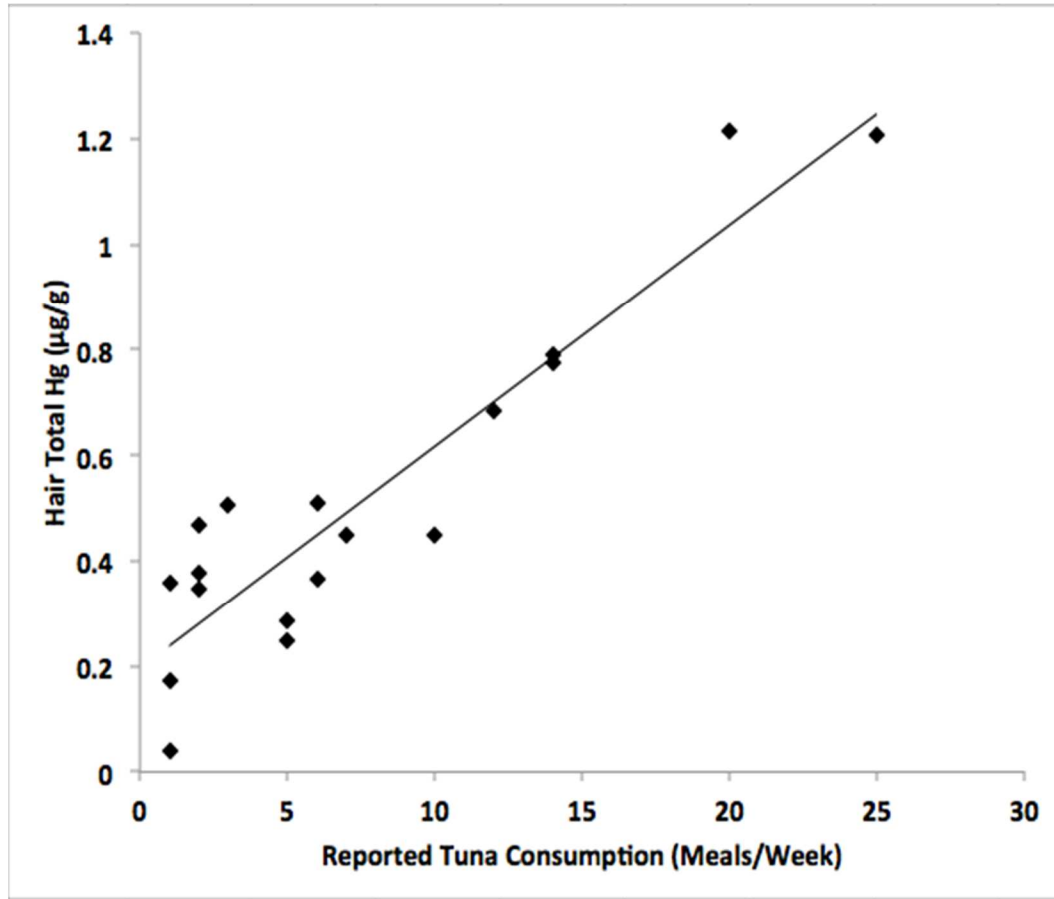


Figure 1.

The reported frequency that a person consumed meals with tuna at one of the dining facilities was significantly related to their hair mercury levels ($R^2 = 0.852$, $p < 0.0001$, $n = 22$, linear regression) for students that responded they ate one meal or greater of tuna per week. Two of the study participants (9%) reported that they ate greater than 20 tuna meals per week and had hair mercury levels above the United

States Environmental Protection Agency's (USEPA) recommended threshold of $1\mu\text{g/g}$ (EPA, 1997), suggesting that some students are ingesting potentially harmful levels of mercury from tuna consumption at dining halls.

Survey responses related to knowledge about tuna mercury levels and consumption rates with respect to the United States Environmental Protection Agency's (USEPA) recommended limit (SI Figure 2 Question 5).

Using the United States Environmental Protection Agency's (EPA) recommended number of times a week a person should limit their fish consumption with respect to methylmercury exposure ((2-3 times/week, EPA, 2017), the number of tuna meals per week would be <2 to be under, 2 to 3 to be around, and >3 to be over.

Number below the recommended limit (accurate response assigned to be <2 meals). The study participants answered what they thought was the number of tuna meals that could be consumed per week and remain below the United States Environmental Protection Agency's (USEPA) recommended limit for mercury exposure. For self-reported tuna consumers, the average response was 5 ± 5 SD (median = 4, range = 1 to 20, $n = 28$) tuna meals per week. For self-reported non-tuna consumers, the average response was 5 ± 3 SD (median = 4, range = 1 to 14, $n = 79$) tuna meals per week. Thus, on average, students responded that the amount of tuna meals per week was ~ 2 times higher than what is recommended to stay below the United States Environmental Protection Agency's (EPA) limit. About 17% (18 of 107) of all study participants marked the accurate response of <2 meals per week.

Number around the recommended limit (accurate response assigned to be 2-3 meals). The study participant answered what they thought was the number of tuna meals that could be consumed per week and remain around the United States Environmental Protection Agency's (USEPA) recommended limit for mercury exposure. For self-reported tuna consumers, the average response was 19 tuna meals per week \pm 6 SD (median = 6.5, range = 1 to 20, n = 28). For self-reported non-tuna consumers, the average response was 8 tuna meals per week \pm 4 SD (median = 7, range = 1 to 20, n = 79). Thus, on average, self-reported tuna consumers reported a number ~6-fold higher and self-reported non-tuna consumers a number ~3-fold higher of weekly tuna meals than what would be around the United States Environmental Protection Agency's (USEPA) recommended limit for mercury exposure. About 8% (9 of 107) of all study participants marked the accurate response. For tuna eating study participants, there was a significant positive correlation between the number of tuna meals a study participant reported they ate per week and the number of tuna meals that they reported were around the United States Environmental Protection Agency's (USEPA) recommended limit ($\rho = 0.732$, $p < 0.0001$, Spearman's Correlation) (Fig. 2A). Interestingly, 25% of the self-reported tuna consumers (7 out of 28) reported they ate more tuna per week than they report a person should consume to remain around the United States Environmental Protection Agency's (USEPA) recommended limit for mercury exposure.

Number above the recommended limit (accurate response assigned to be >3 meals). The study participants answered what they thought was the number of tuna

meals consumed per week to be above the United States Environmental Protection Agency's (EPA) recommended limit for mercury exposure. For self-reported tuna consumers, the average was 9 tuna meals per week \pm 7 SD (median = 9, range = 1 to 21, n = 28). For self-reported non-tuna consumers, the average response was 11 tuna meals per week \pm 6 SD (median = 10, range = 2 to 21, n = 79). Thus, study participants answered, on average, a 3-4-fold higher number of tuna meals per week than the lowest amount that would be above the recommended limit, consistent with the above findings that study participants are over reporting the amount of tuna that can be consumed per week to stay below the United States Environmental Protection Agency's (EPA) recommended limit of mercury exposure. Noteworthy is that only 0.9% of students surveyed had a high degree of knowledge about tuna methylmercury and safe consumption levels in terms of answering accurately questions related to all three categories of safe consumption levels (i.e., the number of tuna meals per week that would be under, around, and over the recommended daily limit of methylmercury exposure).

Confidence in Survey Response (SI Figure 2 Question 6).

This question evaluated the study participant's confidence in their answers for how much tuna can be consumed per week to be around to the United States Environmental Protection Agency's (USEPA) limit (EPA, 1997) (SI Figure 2, question 5) on a scale of 1 to 5 with one being 'just guessing' and 5 being 'very confident'. For self-reported tuna consumers, the average response was 3 ± 1 SD (range = 1 to 5, n = 28). For self-reported non-tuna consumers, the average response

was 3 ± 2 SD (range = 1 to 5, n = 79). Using a score of 4 or 5 for 'confidence', 32% of self-reported tuna consumers reported confidence in their answers while 29% of self-reported non-tuna consumers reported confidence. For self-reported tuna consumers, there was a negative correlation between the number of tuna meals they reported they ate each week and their confidence in their knowledge about the amount of tuna consumption related to the United States Environmental Protection Agency's (USEPA) recommended limit ($\rho = -0.898$, $p < 0.0001$, Spearman's Correlation, n=28) (Figure 2B).

Awareness of risks of methylmercury exposure from tuna consumption
(SI Figure 2, Question 7)

This question evaluated whether the study participant was aware of the risks of methylmercury exposure from tuna consumption prior to taking the survey on a scale of 1 to 5 with one being 'not aware' and 5 being 'very aware'. For self-reported tuna consumers, the average response was 3 ± 1 SD (range = 1 to 5, n = 28). For self-reported non-tuna consumers, the average response was 2 ± 1 (range = 1 to 5, n = 79). Using a score of 4 or 5 for 'aware', 32% of self-reported tuna consumers marked that they were aware of the risks of methylmercury exposure from tuna consumption while 29% of self-reported non-tuna consumers marked that they were aware of the risks of methylmercury exposure from tuna consumption.

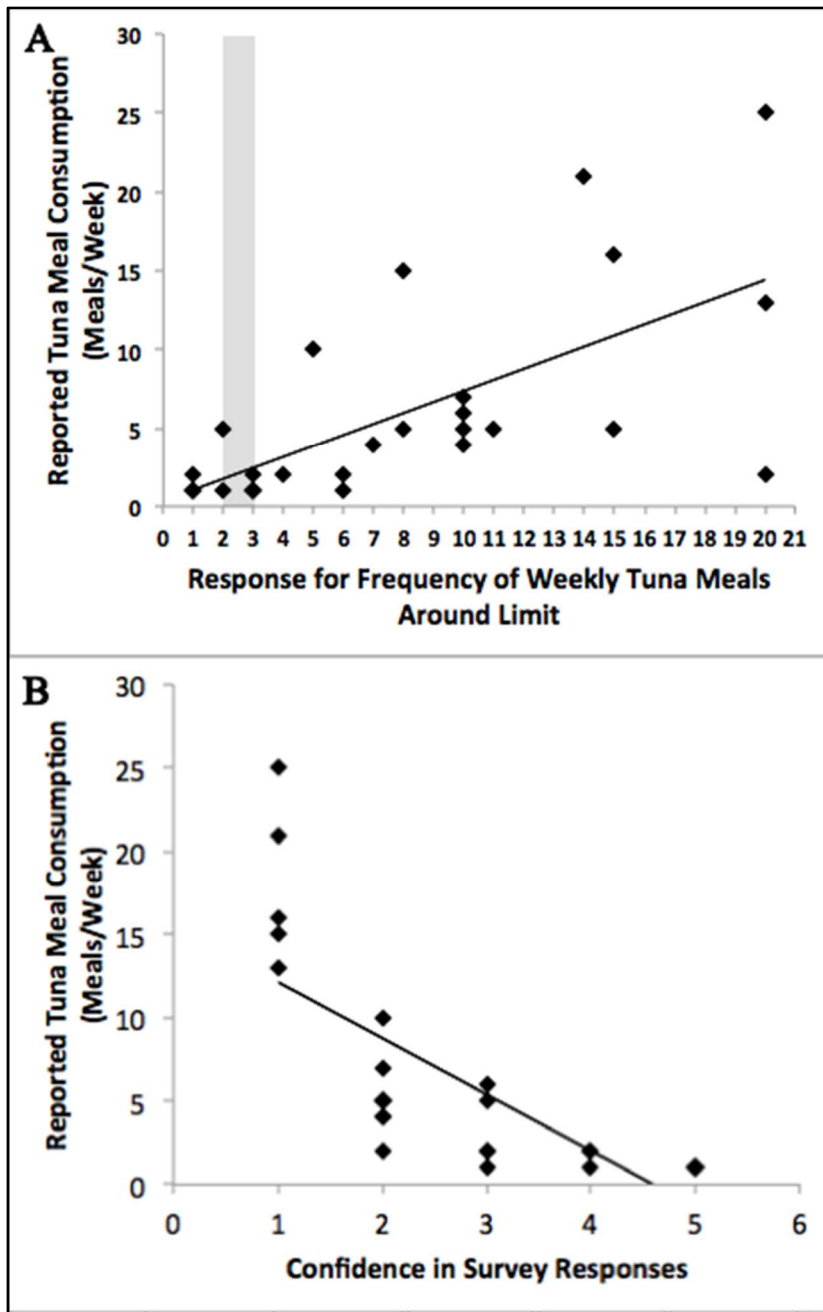


Figure 2.

Relationship between tuna consumption and other survey responses for study participants that reported they ate tuna A. Reported weekly tuna consumption (SI Figure 2, Question 4a) and response for frequency of weekly tuna meals around

United States Environmental Protection Agency's (USEPA) reference dose of mercury exposure (SI Figure 2, Question 5b) were positively correlated with each other ($\rho = 0.732$, $p < 0.0001$, Spearman's Correlation, $n=28$). Thus, the more meals a person believes is the limit for safe tuna consumption, the more likely they are to consume a greater quantity of tuna, leading to higher mercury exposure (Figure 1). Shaded area corresponds to 2-3 meals/week - the number of weekly fish consumption the United States Environmental Protection Agency's (USEPA) recommends to remain at or below with respect to methylmercury exposure (EPA, 2017). **B.** Reported weekly tuna consumption and confidence in responses were negatively correlated with each other ($\rho = -0.898$, $p < 0.0001$, Spearman's Correlation, $n=28$). Confidence was represented on a scale of 1 to 5 with one being 'just guessing' and 5 being 'very confident'. Study participants that had higher confidence in their responses about how much tuna would be associated with the United States Environmental Protection Agency's (EPA) limit for mercury exposure ate less tuna per week.

Discussion

Tuna mercury.

Total mercury concentrations in tuna samples collected between May and June 2017 and concurrent with hair collections were about 4-fold lower compared to other studies on mercury concentrations in canned tuna ($0.075\mu\text{g/g}$ this study versus $0.298\mu\text{g/g}$ (González-Estecha et al., 2013), $0.325\mu\text{g/g}$ (Dabeka et al., 2014), and

0.361 $\mu\text{g/g}$ (Burger and Gochfeld 2006)). However, even given the lower tuna mercury levels, we still saw a significant increase in mercury exposure with tuna consumption at the dining halls (Figure 1) with two study participants having hair mercury levels over $1\mu\text{g/g}$, which is considered an exposure threshold (EPA, 1997). Thus, our results demonstrate that even tuna containing relatively ‘low’ levels of mercury, if consumed in large frequencies such as observed here (e.g., >20 times/week), can result in potentially harmful levels of mercury exposure.

Mercury exposure in study participants.

Hair mercury levels measured were comparable to the NHANES study of 1999 to 2000 ($0.2\mu\text{g/g}$ versus $0.25\mu\text{g/g}$ for this study) (McDowell et al., 2004), but over an order of magnitude lower than hair mercury concentrations reported in populations that consumed high levels of fish as well as whales (e.g, from the Faroe Islands, geometric mean hair mercury = $3\mu\text{g/g}$, Grandjean et al., 1999). Even though the participants had lower hair mercury levels than those observed in populations that consume large quantities of fish and other marine species (e.g., whales) (Blanco et al., 2008; Grandjean et al., 1999), the mercury concentrations found in the study participants for this study are of concern, as almost 50% that ate tuna were potentially exceeding the United States Environmental Protection Agency’s (USEPA) reference dose of $0.1\mu\text{g/g mercury/kg body weight/day}$ (EPA, 1997). Further, about 8% of self-reported tuna eaters (2 out of 22) reporting they eat > 20 tuna meals per week, which was related to hair mercury levels greater than $1\mu\text{g/g}$ (Figure 1), above a level ($1.0\mu\text{g Hg/g hair}$) considered to be potentially harmful (Nuttal, 2006; NRC, 2000).

Overall, ~13% of all study participants (self-report tuna and non-tuna eaters combined, n = 212) reported weekly tuna consumption that could exceed the United States Environmental Protection Agency's (USEPA) daily reference dose (i.e., >3 times/week), which is comparable to the proportion of a Spanish population exceeding safe limits for methylmercury exposure based on their reported fish consumption (12% (Moreno-Ortega et al., 2017)). The mercury exposure in our study participants is of additional concern as college students are typically 18-24 years old (Carter et al., 2010), an age range that overlaps with reproductive (Pal and Santoro, 2005) and neurological (Griffin, 2017) development. Underscoring this concern is mercury's high degree of reproductive toxicity (NRC, 2000).

Tuna consumption and knowledge of risks from mercury exposure.

The positive correlation between a study participant's reported weekly tuna consumption and the number of weekly tuna meals they reported could be consumed without exceeding the United States Environmental Protection Agency's (USEPA) recommended limit (Figure 2A) can be interpreted as the less a person knows about the risks of tuna consumption, the more likely they are to eat more tuna than is recommended. A study which focused on pregnant women showed that while 82% of the surveyed women consumed canned tuna (Gliori et al., 2006), 65% reported knowledge about the risks of methylmercury exposure from fish consumption. This was more than twice the 29~32% of participants in our study who indicated prior awareness of methylmercury exposure from tuna consumption. However, the pregnant women in the aforementioned study (Gliori et al., 2006) obtained

knowledge about the risks of methylmercury exposure from fish consumption from their physician.

Eating tuna could be interpreted as risky in terms of mercury exposure but also tuna is known to be a healthy food choice with high levels of omega 3 fatty acids such as DHA (Maqbool et al., 2011). Thus, tuna could be considered to have a ‘health halo effect’, where people have incomplete perceptions about the health benefits of a product (Roe et al., 1999). Other fish, such as anchovies, are low in mercury but high in DHA (Mahaffey et al., 2011) while some plant-based products (e.g., walnuts, flax seeds) also contain high levels of omega 3 fatty acids (Burns-Whitmoe et al., 2014). Knowledge about the risks and benefits of tuna consumption is essential, as studies show that a significant base knowledge is important for making informed risk assessments for fish consumption with respect to health advisory notices (Burger et a., 2009; Burger et al., 1999).

Conclusion and Recommendations.

Students that eat regularly at dining halls and have unlimited access to tuna may be at a higher risk of MeHg exposure effects on their reproductive and neurological development, as they are of reproductive (Pal and Santoro, 2005) and neurological (Griffin, 2017) development. In terms of MeHg exposure recommendations, our finding that half of the tuna consuming students surveyed reported eating more tuna per week than is recommended (> 3 times/week) and 8% eating tuna over six times the recommend limit (> 20 times/week) is of concern. We

also found that the vast majority of the study participants scored low knowledge (>90%) and reported low awareness (~70%) about tuna MeHg and safe consumption levels. The overall lack of knowledge reported by the study participants for their survey answers and the negative association between knowledge and reported tuna consumption indicates that efforts to increase knowledge about the risks of tuna consumption are important. However, there was insufficient evidence to indicate whether there should be a priority on knowledge or awareness with respect to developing educational materials (SI Figure 6). Overall, universities or other institutions, such as boarding schools or summer camps, with dining facilities where tuna is served regularly, should be aware of the risks of mercury exposure from frequent tuna consumption and take action to either limit the amount of tuna consumed (for younger age children) or provide information about safe levels of tuna consumption (for teens and adults).

Appendix

Supplement 1

Figure 1:

*****SURVEY BEGINS HERE*****

Survey on Dining Hall Fish Consumption

This survey is part of a study being conducted by faculty at UC Santa Cruz. Your participation in this survey is anonymous and completely voluntary. If there's a question you don't wish to answer, you may leave it blank. You may decide to discontinue your participation in this survey at any time.

Please answer the following questions as honestly as you can. We appreciate your help — thank you!

1. How long have you attended UCSC?

This is my first year This is my second year This is my third year

This is my fourth year Longer than four years

2. What is your age? 18-34 35-50 51 or older

3a. What is your gender? Male Female Non-binary / other

3b. Are you biologically capable of becoming pregnant? _____ Yes _____ No

4. Around how many meals do you currently eat at a UCSC dining hall during a typical week?

of breakfasts: _____ # of lunches: _____ # of dinners: _____

5. If you eat fish, do you normally eat tuna, or another type of fish? Please indicate how often you eat tuna (for example, canned tuna, tuna steak, tuna melts, tuna salad, tuna sushi, albacore, yellowfin, ahi) vs. another type of fish (for example, salmon, lox, mackerel, sardines, swordfish, halibut, tilapia). Please also indicate how often you eat the fish at a UCSC dining hall vs. elsewhere.

5a. Meals per week that include tuna:

eaten at a UCSC dining hall: _____ # eaten elsewhere: _____

5b. Meals per week that include a type of fish other than tuna:

eaten at a UCSC dining hall: _____ # eaten elsewhere: _____

5c. or: _____ I don't eat fish.

6. If you eat fish other than tuna, what two main types of fish do you tend to eat?

1st fish type: _____ 2nd fish type:

or: _____ I don't eat fish other than tuna / I don't eat fish.

7. If you eat tuna, how concerned are you about possible harm from mercury exposure due to how often you eat tuna?

1	2	3	4	5
not at all		moderately		very
concerned		concerned		concerned

or: _____ I don't eat tuna.

8. How often do you think people need to eat tuna before they are likely to experience harm from mercury exposure due to the amount of tuna they eat?

number of meals per week with tuna: _____

or: _____ It doesn't matter, because mercury toxicity from too much tuna consumption is unlikely.

9. How confident are you in your answer to #7 above?

1	2	3	4	5
just		moderately		very
guessing		confident		confident

10. Are you willing to anonymously give a small hair sample to find out the mercury concentration in your hair, and support our study? If you are, or you might be, please check the space below and speak to us when you return this survey.

_____ Yes, I am willing to anonymously give a small hair sample to find out the mercury concentration in my hair.

*****SURVEY ENDS HERE*****

Supplement 2

Figure 2:

*****SURVEY BEGINS HERE*****

Survey on Dining Hall Fish Consumption

This survey is part of a study being conducted by faculty at UC Santa Cruz. Your participation in this survey is anonymous and completely voluntary. If there's a question you don't wish to answer, you may leave it blank. You may decide to discontinue your participation in this survey at any time.

Please answer the following questions as honestly as you can. We appreciate your help — thank you!

1. What is your age? _____ 18-34 _____ 35-50 _____ 51 or older

2. What is your gender? _____ Male _____ Female _____ Non-binary / other

3. Around how many meals do you currently eat at a UCSC dining hall during a typical week?

of breakfasts: _____ # of lunches: _____ # of dinners: _____

4 Meals per week that include tuna:

eaten at a UCSC dining hall: _____ # eaten elsewhere: _____.

5 How many tuna meals per week does a person need to eat before exceeding the Environmental Protection Agency's recommended limit for mercury exposure? In the table below, please indicate whether a person is **unlikely** to exceed the recommended limit, will be exposed around the recommended limit, or is **likely** to exceed the recommended limit, based on the number of meals per week they eat that include tuna.

# of meals per week that include	Exposure is <u>unlikely</u> to exceed the recommended	Exposure will be around the recommended limit	Exposure is <u>likely</u> to exceed the recommended limit
----------------------------------	--	---	--

tuna	limit		
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			

16			
17			
18			
19			
20			
21			

6 How confident are you in your ratings in the above question?

1	2	3	4	5
just		moderately		very
guessing		confident		confident

7 Prior to this survey, were you aware that there is a risk of mercury exposure from tuna consumption?

1	2	3	4	5
not		somewhat		very
aware		aware		aware

SURVEY ENDS HERE

Supplement 3

Hair Washing Study:

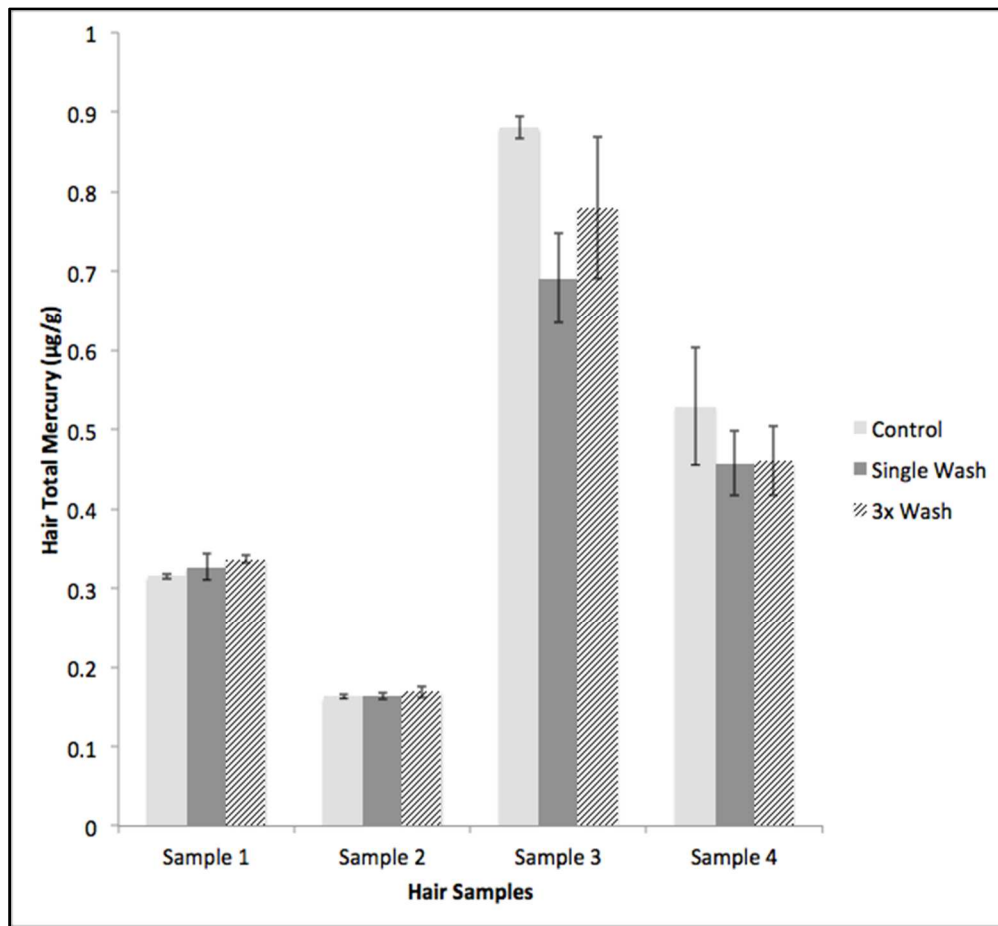


Figure 3. Total hair mercury concentrations were not substantially affected by washing procedure. Due to negligible difference in hair total mercury concentration after application of washing procedures, it was concluded that hair washing was not

necessary for total mercury analysis, similar to other studies (Morton et al., 2002; Li et al., 2016; Schwedler et al., 2017; Gerstenberger et al., 2006).

Supplement 4

Table 1:

Date (m/dd/yy)	Mean Total Hg ($\mu\text{g/g}$)	SD	Simultaneous with Hair Sampling
2/17/16	0.441	0.012	No
2/22/16	0.314	0.010	No
2/22/16	0.339	0.014	No
2/29/16	0.247	0.009	No
3/9/16	0.425	0.013	No
1/14/16	0.486	0.015	No
4/12/16	0.249	0.054	No
4/13/16	0.226	0.018	No
4/20/16	0.089	0.009	No
4/21/16	0.063	0.003	No
4/26/16	0.075	0.001	No
4/26/16	0.064	0.004	No
5/3/16	0.549	0.033	No
5/4/16	0.098	0.002	No
5/9/16	0.060	0.003	No
5/19/17	0.088	0.002	Yes
5/19/17	0.071	0.009	Yes
5/19/17	0.082	0.017	Yes
5/19/17	0.076	0.009	Yes
5/19/17	0.075	0.010	Yes
6/3/17	0.085	0.009	Yes
6/3/17	0.070	0.007	Yes
6/3/17	0.067	0.007	Yes
6/3/17	0.068	0.007	Yes
6/3/17	0.071	0.008	Yes

Table 1. Distribution of measured total Hg measured from tuna samples, with standard deviation and measured in three replicates, represented by individual collection days that the tuna samples were collected and the dates that the collection periods occurred. Mean total mercury measured from all samples was $0.179 \mu\text{g/g} \pm 0.157 \text{ SD}$ (range = 0.06 to $0.589 \mu\text{g/g}$, $n = 25$). Mean total mercury measured from 2016 samples was $0.248 \mu\text{g/g} \pm 0.171 \text{ SD}$ (range = 0.06 to $0.589 \mu\text{g/g}$, $n = 15$). Mean

total mercury measured from 2017 samples was $0.075 \mu\text{g/g} \pm 0.007 \text{ SD}$ (range = 0.07 to $0.088 \mu\text{g/g}$, $n = 10$). This distinct decrease in total mercury levels was attributable to a possible change in the tuna that was available for consumption based also on tuna color and conversations with dining facility staff.

Supplement 5:

Table 2 Hair Data:

Subset of responses from the first survey (SI Figure 1) and measured total hair mercury concentrations. All study participants reported between ages 18-34 years.

Years attending UCSC	Gender	Reported meals per week eaten at dining halls that include tuna	Total Hair Mercury ($\mu\text{g/g}$)
4th year	Male	20	1.22
1st year	Female	25	1.21
1st year	Female	14	0.790
1st year	Female	14	0.776
1st year	Male	12	0.684
2nd year	Male	6	0.511

1st year	Male	3	0.504
3rd year	Male	2	0.469
1st year	Male	10	0.449
1st year	Male	7	0.447
1st year	Male	1	0.405
1st year	Male	2	0.377
1st year	Male	6	0.365
1st year	Male	0	0.364
2nd year	Male	1	0.357
1st year	Female	2	0.348
2nd year	Female	0	0.314
1st year	Female	5	0.288
2nd year	Male	0	0.276
1st year	Male	0	0.276
1st year	Female	5	0.248
1st year	Female	0	0.206

4nd year	Female	0	0.205
1st year	Male	0	0.198
1st year	Male	0	0.176
2nd year	Male	1	0.172
1st year	Female	0	0.130
1st year	Male	0	0.130
1st year	Male	0	0.125
3rd year	Male	0	0.118
2nd year	Male	0	0.0892
2nd year	Female	0	0.0889
1st year	Female	0	0.0874
1st year	Male	0	0.0847
1st year	Male	0	0.0746
1st year	Female	0	0.0731
1st year	Male	0	0.0728
1st year	Male	0	0.0682

1st year	Male	0	0.0677
2nd year	Male	0	0.0643
2nd year	Male	0	0.0582
2nd year	Male	0	0.0566
4th year	Female	1	0.0401
1st year	Male	1	0.0388
1st year	Male	0	0.0387
1st year	Male	0	0.0348
2nd year	Female	0	0.0322
2nd year	Other	0	0.0320
1st year	Female	1	0.0231
2nd year	Male	0	0.0227
2nd year	Other	1	0.0208
2nd year	Male	0	0.0201
2nd year	Female	0	0.0159
2nd year	Male	0	0.00945

Supplement 6:

Correlations between Knowledge, Awareness, and Confidence

Variable	by Variable	Spearman ρ	Prob> ρ
Confidence	Response for Frequency of Tuna Meals around Limit (Meals/Week)	-0.8043	<.0001*
Awareness	Response for Frequency of Tuna Meals around Limit (Meals/Week)	-0.6798	<.0001*
Awareness	Confidence	0.6608	0.0001*

Figure 4: There were significant correlations between knowledge responses, awareness responses, and confidence responses.

Supplement 7:

Table 3 Survey Results:

	Survey 1	Survey 2
People surveyed	105	107
Males/Females	61/37	53/46
Reported Tuna Consumers	25	28
Average reported tuna consumption by tuna consumers	6 meals weekly	6 meals weekly

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