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Omega-3 Fatty Acids versus Heavy Metals: A Quantitative Estimation of the Benefit-Risk Ratio for the Consumption of Commonly-Consumed Fish and Products in Metro Manila

Dave P. Briones[1], and Nancy Lazaro-Llanos[2]

[1] *Food and Nutrition Research Institute, Department of Science and Technology, Bicutan, Taguig City*
[2] *Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila*

Fish and other marine products consumption is the main route of heavy metal exposure to human which poses health risks if taken in high dosage. On the other hand, the nutritional benefits of fish are mainly due to the content of high-quality protein and high content of the two kinds of omega-3 polyunsaturated fatty acids (PUFA): eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The study aims to assess and compare the benefit-risk ratio for the consumption of commonly-consumed fish and products in Metro Manila. Fish and product samples were analyzed for moisture, crude fat, heavy metals (As, Cd, Pb) and fatty acid profile (saturated, mono- and polyunsaturated) including omega-3 fatty acids EPA and DHA. These findings generally indicate that the consumption of fish and products studied posed no risk to human health. Essential fatty acids EPA and DHA were highest for dried anchovy, *Stolephorus indicus* (1.5 and 7.3mg/g sample); mussel, *Mytilus smaragdinus* (1.5 and 1.0mg/g sample); round scad, *Decapterus macrosoma* (0.9 and 3.4mg/g sample); and skipjack tuna, *Auxis thazard* (1.1 and 3.9mg/g sample). The hazard quotients of essential fatty acids versus heavy metals were also less than 1 except for Cd in squid (1.8), In the case of dried sardine (19.9), squid (1.8), shrimp (3.4), and skipjack tuna (1.7), these foods should be monitored regularly. In conclusion, the target hazard quotients for most of the fish and products showed values less than 1, which suggest that health risks were insignificant.

Keywords: *ω-3 polyunsaturated fatty acids n-3 (PUFA); EPA; DHA; heavy metals; benefit-risk ratio*

INTRODUCTION

Fish is a healthy food because of its nutritional benefits related to its proteins of high biological quality, desirable lipid composition, valuable mineral compounds, and vitamins. The particular composition of its lipid fraction, rich in essential ω-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and poor in cholesterol makes it as a primer food (Castro-Gonzales 2002; Clarkson, 2002; Carvalho, *et al.,* 2005; Domingo, *et al.*, 2007; Castro-

Email: briones.dave@gmail.com[1], nancy.lazaro-llanos@dlsu.edu.ph[2]

Gonzales and Mendez-Armenta, 2008; Groth, 2010).

Currently there is major emphasis on the beneficial effects of omega-3 fatty acids in the diet (Yashodhara, *et al.*, 2009). There have been studies showing omega-3 fatty acid involvement in lowering cholesterol levels and blood pressure, which is essential for cardiovascular health (Bonaa, *et al.*, 1990; Horrocks, 1999; Connor, 1994; Stone, 1996; Connor and Connor, 1997; Schmidt, 1997). It has been estimated that consumption of one fatty fish meal per day could result in an omega-3 fatty acid intake of approximately 900 mg/day, an amount shown to lower coronary heart disease mortality rates (Kris-Etherton, *et al.*, 2002). Omega-3 fatty acids (EPA) have been proven to have protective effects in reducing arrhythmias and thrombosis (Kinsella, *et al.*, 1990; Oomen, *et al.*, 2000; Kris-Etherton, *et al.*, 2002), lowering plasma triglycerides levels (Harris, 1997; Ismail, 2005) and reducing blood clotting tendency (Agree, *et al.*, 1997; Din, *et al.*, 2004; Ismail, 2005). Studies have shown that omega-3 PUFAs appear to improve many metabolic consequences of insulin resistance in humans by lowering hypertension and plasma triglycerides (Berry, 1997). Moreover, fish oil intake has also been associated with a low incidence of diabetes mellitus (Malasanos and Stacopoole, 1991; Rustan, *et al.*, 1997; Toft, 1995). In addition, DHA was also found to be essential for infant brain development and eye function (Birch *et al.,* 1998). There is also a proposed mechanism that involves immune system modulation by DHA to reduce the action of inflammatory compounds (Darlington and Stone, 2001).

Humans cannot synthesize omega-3 fatty acids, instead they have to obtain omega-3 fatty acids from those that pass through the food chain and become incorporated in fish and other marine products. The types and levels of fatty acids found in fish vary with species, age, size, reproduction stage, season, geographical location and diet. Fatty acids from fish are considerably higher in proportions of 20 and 22 carbon chains in comparison with terrestrial animals (Nettleton and Exler, 1992). Saturated fatty acids such as hexadecaenoic acid (palmitic acid 16:0) and octadeconic acid (oleic acid 18:0) are also found in fish in substantial levels. Other fatty acids found to be present are 18:3 ω-3, 18:5 ω-3, 20:4 ω-3, and 22:5 ω-3 (Napolitano, 1998).

The American Heart Association (AHA), as well as various international organizations, has affirmed that omega-3 fatty acids benefit the heart of healthy people, and those at high risk or suffering cardiovascular disease. In 1996, the AHA released its Science Advisory, "Fish Consumption, Fish Oil, Lipids and Coronary Heart Disease" (Stone 1996). The current AHA's dietary guidelines recommend that healthy adults eat at least two servings of fish per week (Kris-Etherton, *et al.*, 2003; Kris-Etherton, *et al.,* 2002). However, the Scientific Statement of the AHA also indicates that fish and other seafood are potentially a major source of human exposure to various environmental contaminants such as heavy metals and metalloids. These compounds are of concern due to their toxicity, persistence, bioaccumulation, and biomagnifications into the food chain (Beijir and Jernelov, 1986; Falco *et al.,* 2006; Svenson, *et al.*, 1996 Takhata, *et al.*, 1998; Goyer, 1997; Papanikolau, *et al.*, 2005; Castro-Gonzales, and Mendez-Armeta, 2008; Groth, 2010). The content of toxic heavy metals in fish can counteract the positive effects of the omega-3 fatty acids present (Kris-Etherton, *et al*, 2003; Chan, *et al.*, 2004).

A variety of contaminants including toxic heavy metals are reported to be ubiquitously present in rivers and reservoirs and are disadvantageous for aquatic organisms (WHO, 1990; Olson, 1998; Carvalho, *et al.,* 2005; Alina *et al.*, 2012). Heavy metals in some commercial fish species from Manila Bay were analyzed by Prudente *et al.* (2007). In this study, the order of concentration of highly toxic metals in the fish examined were Hg > Pb > Cd. Heavy metal analysis in selected fish species collected from Laguna Bay was conducted by Madamba and Pamulaklakin (1994). Results have shown that the highest metal concentrations were observed in the inedible tissues, followed by whole tissues, and edible tissues. Santiago et. al. (2009) investigated the mercury levels of widely consumed fish species sold in public market in the Greater Manila Area. Total mercury concentrations were found to be highest in *Decarpterus macrosoma* (round scad) and with no significant difference in both *Chanos chanos* (milkfish) and *Oreochromis niloticus* (tilapia).

In the Philippines, according to the recent National Nutrition Survey, children 6 months to 5 years old consume 7.3% of fish and products from the total food intake in a day. Children 6 to 12 years old consume 12.7% of fish and products. Adolescents 13 to 19 years old, adults 20 to 59 years old and elderly 60 years old and older consume 13.4 %, 14.7% and 15.6% of fish and products, respectively (FNRI-DOST, 2010).

A number of researchers have started a quantitative estimation of risk versus benefit of fish intake for human health. Gladyshev, et. al. (2008) derived a formula to quantify the benefit-risk ratio of consuming food containing PUFAs versus that of heavy metals for Siberian grayling (*Thymallus arcticus*) commonly found in Yenisei River. Foran *et al.* (2005) estimated benefit-risk ratios for essential fatty acids (EFAs) versus many organic contaminants and methylmercury in wild and farmed salmon. In their calculation, Foran et. al. (2005) used the sum of concentrations for all of the measured contaminants. Besides the sum data, benefit-risk ratios for EFA versus individual organic toxic species, as well as versus heavy metal contents in consumed fish are very useful. Budtz-Jorgensen, et al. (2007) developed a complicated regression model, based on dietary questionnaire responses, clinical tests and neurobehavioral tests for a separation of risks and benefits of fish intake. This model seems to have a further development if supplemented with data on concentrations of given beneficial and risky components, e.g. EFA vs. metals in consumed fish of different species.

The study aims to compare and assess the quantitative estimation of the benefit-risk ratio of some of the commonly-consumed fish and products collected from four wet markets and a supermarket in Metro Manila. Proximate (moisture and crude fat) analyses, fatty acid profile and heavy metals composition of fish and products can serve as qualitative data that can be used for the food and nutrition researches. The study will also give quantitative estimation of risks versus benefits of fish intake for human health.

MATERIALS AND METHODS

Reagents and Materials Preparation. Fish and other marine products were collected and prepared from 4 wet markets and a supermarket in Metro Manila. Samples include: *Stolephorus indicus* (anchovy), *Sardinella longiceps* (sardine), *Mytilus smaragdinus* (mussel), *Loligo pealei* (squid), *Metapeneaus emsis* (shrimp), *Decapterus macrosoma* (round scad), *Chanos chanos (milkfish), Auxis thazard* (skipjack tuna), and *Oreochromis niloticus* (tilapia). The samples were washed, cleaned and scales were removed. Weight, dimension, and percent edible portion of samples were measured. Samples were pooled, ground and mixed thoroughly before taking sub-samples for analyses. Only edible portions were used in all the analyses. Portions were taken for moisture determination using AOAC International Official Methods for Analysis (Horwitz, 2000).

Acid Hydrolysis and Fat Extraction. Fish samples were weighed into a 250-mL digestion tube containing approximately 1 gram Celite. Acid hydrolysis was done using the Soxtec System 1047 Hydrolyzing Unit. Fat extraction cup was heated at 105^{0}C for 2 hours in the drying oven and was cooled in a desiccator for 30 minutes and weighed. The fat from the residue was extracted using the Soxtec System HT6 1043. Fat extraction was done with thimbles immersed in 50 mL ether and boiled for about 15 minutes. Fat extraction of sample with thimble was continued in the rinsing position for another 30 minutes. After extraction, the extraction cup with fat was removed from the Soxtec

System. The extraction cup was placed inside a fume hood and was allowed to dry, then dried in an oven at 105^{0}C for 2 hours, cooled for 30 minutes in a desiccator and will be weighed (Tecator Manual 1043; Tecator Manual 1047).

Heavy Metals (As, Cd, Pb,) Analyses by Graphite Furnace Atomizer-AAS. Approximately 2 grams of sample was weighed in a digestion vessel. A volume of 5 mL concentrated nitric acid and 2 mL 30% hydrogen peroxide were added to the sample. Digestion vessels were arranged in a digestion rack and placed inside the microwave digestor (AOAC Official Method 999.10). Digests were diluted to volume. A blank was included in every set. Intermediate standards and working standards used were 10 ppm and 200 ppb for As and Pb; 10 ppm and 100 ppm for Cd. Accurately 20μL of the standard and sample were injected until finished.

Method Suitability Test for Omega-3 PUFAs. Method suitability test of omega-3 PUFAs (EPA and DHA) were done using 4 saponification and transesterification method. FAPAS T1496 fish oil with consensus value for EPA and DHA was used for the method suitability test for quality assurance. The best method was tested to compare with the current method being used for fatty acid profile analysis. FAPAS T1464 vegetable oil with consensus value for total saturated, total mono- and polyunsaturated fatty acids was used for quality assurance.

Fatty Acid Profile Analysis. Saponification and transesterification were conducted based on AOAC method (AOAC Official Method 991.39; AOAC Official Method 996.06) as follows: 1 g of dried food sample or 25 mg of fish oil were placed into a 20 mL screw top glass tube to which 0.7 mL of 10 M KOH, and 5.3 mL of methanol was added. The tubes were incubated in a 55^{0}C water bath for 1.5 hour with vigorous hand-shaking for 5 seconds every 20 minutes to properly permeate, dissolve, and hydrolyze the sample. After cooling to below room temperature in a water bath containing tap water, 0.58 mL of 12 M H_2SO_4 was added. The tubes were mixed by inversion and when the precipitate appeared, the tubes were incubated again in a 55^{o}C water bath for 1.5 hours with hand-shaking for 5 seconds every 20 minutes. The tubes were cooled in a water bath containing tap water. To extract the fatty acid methyl esters (FAMEs) 3 mL of hexane was added to the tubes which were vortex-mixed for 5 min. The hexane layer containing the FAMEs was dried with anhydrous sodium sulfate. The upper layer of hexane was transferred into a GC vial.

Working standard was prepared by accurately weighing 0.01 to 0.1 g each of the methyl ester standard into 25 mL volumetric flask and diluted to volume with hexane. The standards used were caproic acid methyl ester (C6), caprylic acid methyl ester (C8), capric acid methyl ester (C10), lauric acid methyl ester (C12), myristic acid methyl ester (C14), palmitic acid methyl ester (C16), stearic acid methyl ester (C18), oleic acid methyl ester (C18:1), linoleic acid methyl ester (C18:2), linolenic acid methyl ester (C18:3), arachidic acid methyl ester (C20), behenic acid methyl ester (C22), docosahexaenoic acid methyl ester (C22:6) and lignoceric acid methyl ester (C24). Calibration standards for eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6) were prepared by quantitatively transferring 20, 40, 60, 80 and 100 μL of the working standard in 1 mL volumetric flask and were diluted with heptane to volume.

Gas chromatograph used was equipped with abonded phase capillary column, oxygen scrubber in carrier gas line, flame ionization detector and temperature programmable oven from 50^{0}C to 250^{0}C. Hydrogen gas was used as carrier gas with 1.2 to 1.5 mL per minute flow rate. CP-Sil88 capillary column was used with a 100 m length, 0.25 mm internal diameter and 0.25 μm film thickness. The column was coated with 100% cyanopropyl-polysiloxane and was purchased from Chrompack. Injector was set at 280^{0}C, detector at 300^{0}C and the oven temperature will be gradient. Initial temperature was 60^{0}C with holding time of 5 minutes; it was then be heated to 165^{0}C at a rate of 15^{0}C per minute and with holding time of 1 minute and the final temperature was increased to 225^{0}C at a rate of 2^{0}C per minute and with holding time of 17 minutes. The total run time was 61 minutes. From the above analysis, the concentration of each fatty acid methyl ester was automatically computed by the instrument using corrected normalization based on the area of the standard and area of the sample. Individual fatty acid concentration was compared by multiplying the fatty acid methyl ester concentration with the stoichiometric factor, which is the ratio of the molecular weights of fatty acid and fatty acid methyl ester.

Target Hazard Quotient (THQ). The formula derived by Gladyshev, et al., (2008) was used to estimate the benefit-risk ratio for the consumption of fish and products (THQ) as follows:

$$FP = \frac{R_{EFA}}{C} \quad \text{(Eq 1)}$$

$$DM = FP \times c \quad \text{(Eq 2)}$$

$$HQ = \frac{DM}{RfD \times AQ} \quad \text{(Eq 3)}$$

$$HQ_{EFA} = \frac{R_{EFA} \times c}{C \times RfD \times AW} \quad \text{(Eq 4)}$$

where FP = fish portion, g/day; R_{EFA} = recommended daily consumption of EPA + DHA, 1000 mg; C = content of EPA + DHA in a given fish, mg/g; DM = dose of metal, µg/day; c = content of a given metal in a given fish, µg/g; RfD = reference dose (maximum tolerable daily intake of a specific metal that does not result in any deleterious health effects, µg/kg/day; AW = average adult weight; HQ_{EFA} represents a hazard quotient for fish consumption when a human person aims to obtain from the fish the recommended dose of EFA or what is called risk-benefit ration for fish consumption due to metal and EFA intake, respectively. Evidently, $HQ_{EFA}<1$ means the health benefit from fish consumption, and $HQ_{EFA}>1$ means the risk.

RESULTS

Fish Samples and Proximate Analysis. The properties of the fish and product samples, percent moisture and percent crude fat are shown in Table 1. Percentage moisture was determined using AOAC 952.08A (Horwitz, 2005). The method is applicable to all marine products except for oyster. Milkfish, dried sardine, dried anchovy, skipjack tuna and round scad have the highest crude fat content among the fish and products.

Heavy Metal Levels in Fish and Products. The method used for heavy metals analysis was previously validated following the requirements of AOAC, NATA and Eurachem. FAPAS T0774 canned fish was used as a quality control test material to ensure the quality of data generated and was used every batch analysis.

The concentrations of different metals (As, Cd, Pb) (µg/g wet weight) in the edible portions of fish and products are given in Table 2. Evidently, dried anchovy has the highest Cd and Pb content since the fish is in the dried form. However, the heavy metal contents of dried anchovy are still below the maximum residue limit for fish set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2005) and the European Community (EEC, 2001). Next to dried anchovy, squid, dried Indian sardine and mussel have the highest content of Cd. Cadmium was detected in the lowest amount in the edible tissues of the studied fish and products. The lowest Cd level was found in shrimp with 0.001 µg/g wet weight. It has been established that Cd occurs in the aquatic organisms and marine environment in trace concentrations (Burger, *et al.,* 2008). A study of Bustamante et al. (2002) found out that the major part of cadmium is associated with lysosomes and cystolic proteins which play a key function in the storage and detoxification of this element.

High levels of As contents were observed for all fish and products. According to literature, 80-90% of arsenic in seafood is in organic form which is less toxic compared to the inorganic form (Abernathy *et. al.,* 2003; Al Rmalli *et al.,* 2005). Dried Indian sardine was observed to have the highest level of As. The influence of species and feeding habits on As accumulation is not clear in marine mammals. But a study of Storelli and Marcotrigiano (2004) revealed that organisms feeding on crustaceans and algae (such as Indian sardine) appear to retain arsenic concentrations than piscivorous species and the highest levels are found in muscle tissues.

Fresh water habitant milkfish and tilapia showed lower levels of heavy metals (As, Cd and Pb) as compared to other seafood. This may be due to the reality that seas and oceans are the larger bodies of water and a wider basin of more contaminants compared to smaller lakes and estuaries. The relatively lower levels of Cd and Pb in bangus may be due to the fact that milkfish stays near the surface of the water where they feed on phytoplanktons. Milkfish is a pelagic fish while tilapia is said to be benthic fish. Benthic fish dwells on the bottom where contaminants are found more concentrated (Storelli, 2008).

Table 1. General information of fish and products, percentage moisture and percentage crude fat.

Common Name and Local Name	Scientific Name	Length (cm)	Width (cm)	Weight (g)	Percentage Edible Portion (%)	Percentage Moisture (%)	Percentage Crude Fat (%)
anchovy (dried dilis)	*Stolephorus indicus*	48-70	8-10	0.34-1.03	100	17.90	3.99
dried sardine (dried tamban, Indian sardine)	*Sardinella longiceps*	38-60	25-30	8.04-12.85	46-52	40.22	5.93
Mussel (tahong)	*Mytilus smaragdinus*	55-65	28-32	7.09-11.84	-	85.18	1.72
Squid (pusit)	*Loligo pealei*	95-180	20-35	29.23-33.45	95-97	87.39	1.05
Shrimp, greasy back (hipong swahe) (shrimp, greasy back)	*Metapeneaus emsis*	-	-	11.36-16.86	49-63	85.07	0.48
Galunggong (round scad)	*Decapterus macrosoma*	70-210	35-40	100.93-128.13	59-65	72.13	3.02
Bangus (milkfish)	*Chanos chanos*	370-380	85	458.68-588.51	54-59	68.36	10.02
Skipjack tuna, frigate/bullet, (tambakol tulingan)	*Auxis thazard*	240-260	45-55	218.72-269.18	57-61	69.83	3.48
Tilapia	*Oreochromis niloticus*	210-215	80	281.46-304.33	36-38	77.40	2.06

Table 2. Range of concentrations (µg/g raw weight) of heavy metals (n=3).

Fish and Products	Heavy metals content (µg/g)		
	As	**Cd**	**Pb**
Stolephorus indicus (anchovy)	0.910 ± 0.091	0.273 ± 0.002	0.154 ± 0.023
Sardinella longiceps (sardine, Indian sardine)	9.282 ± 0.462	0.054 ± 0.052	0.056 ± 0.009
Mytilus smaragdinus (mussel)	0.572 ± 0.046	0.034 ± 0.002	0.030 ± 0.004
Loligo pealei (squid)	0.464 ± 0.042	0.064 ± 0.002	0.021 ± 0.002
Metapeneaus emsis (shrimp)	0.720 ± 0.066	0.0003 ± 0.0003	0.099 ± 0.019
Decapterus macrosoma (round scad*)*	0.414 ± 0.054	0.008 ± 0.0006	0.065 ± 0.018
Chanos chanos (bangus)	0.065 ± 0.013	0.005 ± 0.005	0.029 ± 0.011
Auxis thazard (skipjack tuna)	0.604 ± 0.059	0.026 ± 0.002	0.036 ± 0.002
Oreochromis niloticus (tilapia)	0.044 ± 0.010	0.011 ± 0.0003	0.055 ± 0.010
Quality control (reference material or spiking)	414-473	1.73-3.27	87-97%
Consensus value (µg/g), % recovery range	214-474	1.45-3.72	

*Mean ± SEM

Fresh water habitant milkfish and tilapia showed lower levels of heavy metals (As, Cd and Pb) as compared to other seafood. This may be due to the reality that seas and oceans are the larger bodies of water and a wider basin of more contaminants compared to smaller lakes and estuaries. The relatively lower levels of Cd and Pb in bangus may be due to the fact that milkfish stays near the surface of the water where they feed on phytoplanktons. Milkfish is a pelagic fish while tilapia is said to be benthic fish. Benthic fish dwells on the bottom where contaminants are found more concentrated (Storelli, 2008).

Fatty Acid Profile. Fatty acid compositions of fish and products are presented in Table 3. Among saturated fatty acids, the largest concentration was for palmitic acid (C16:0), varying from 0.455 to 6.780 mg/g sample. Dried anchovy, round scad, and skipjack tuna have the highest palmitic acid among the salt water seafood with 6.528, 5.861, and 6.358 mg/g wet weight, respectively. Fresh water milkfish and tilapia are also high in palmitic acid with 6.780 and 4.629 mg/g sample, respectively. Concerning the monounsaturated fatty acids, oleic acid (C18:1ω-9), fresh water fishes milkfish and tilapia have the highest content compared to the salt water seafood. Milkfish and tilapia gave 5.873 and 5.092 mg/g sample, respectively. Among the polyunsaturated fatty acids (PUFA), the outstanding results were linoleic acid (C18:2ω-

6), 13.144 mg/g and docosahexenoic acid (C22:6ω-3), 7.360 mg/g for dried anchovy. Fresh water milkfish posed the highest level of linoleic acid (C18:2ω-6) with 90.656 mg/g. This high concentration of linolenic acid of milkfish may be due to fish feeds that are given to them. Docosahexaenoic acid (C22:6ω-3), were also high for round scad and skipjack tuna which are deep sea habitants. Essential fatty acid eicosapentaenoic acid (C20:5ω-3) was highest for dried anchovy, mussel and skipjack tuna with 1.517, 1.543, and 1.085 mg/g sample, respectively.

Hazard Quotient of Essential Fatty Acids Versus Heavy Metals. Table 4 shows the hazard quotient of essential fatty acids versus heavy metals from the consumption of fish and products. Values of HQ_{EFA} for all the metals were less than 1 except for Cd in squid, and As in dried sardine, squid, shrimp and skipjack tuna, which means that the consumption of fish and products given has no deleterious health effects if to obtain the recommended portion of essential fatty acids. If hazard quotients of essential fatty acids versus Cd and As are to be considered, consumption of squid, dried sardine, shrimp and skipjack tuna for that particular sampling time and sampling site posed a risk to human health. This may be due to its high heavy metals content relative to its omega-3 fatty acids content.

Table 3. Fatty acid profile of the fish and products (n=3)

Fatty acids (mg/g sample)	*Stolephorus indicus (anchovy)*	*Sardinella longiceps (sardine, Indian sardine)*	*Mytilus smaragdinu (mussel)*	*Loligo pealli* (squid)	*Metapeneaus emsis (shrimp)*	*Decapterus macrosoma* (round scad)	*Chanos chanos (milkfish)*	*Auxis thazard (skipjack tuna)*	*Oreochromis niloticus (tilapia)*
C6:0	-	0.097 ± 0.001	-	0.025 ± 0.000	0.013 ± 0.003	0.050 ± 0.000	0.222 ± 0.002	0.059 ± 0.002	0.040 ± 0.001
C8:0	0.029 ± 0.000	0.022 ± 0.000	0.011 ± 0.000	-	0.006 ± 0.000	0.009 ± 0.000	-	0.036 ± 0.000	0.018 ± 0.004
C10:0	0.016 ± 0.000	0.139 ± 0.002	0.017 ± 0.000	-	0.009 ± 0.000	0.014 ± 0.001	0.023 ± 0.001	0.011 ± 0.001	0.039 ± 0.001
C12:0	0.036 ± 0.000	0.087 ± 0.000	0.099 ± 0.001	0.012 ± 0.000	0.065 ± 0.001	0.133 ± 0.003	0.861 ± 0.003	0.014 ± 0.000	0.910 ± 0.001
C14:0	1.197 ± 0.046	1.326 ± 0.007	0.900 ± 0.023	0.156 ± 0.014	0.074 ± 0.001	0.836 ± 0.004	1.236 ± 0.001	1.309 ± 0.010	1.103 ± 0.001
C16:0	6.528 ± 0.054	2.854 ± 0.010	2.440 ± 0.045	1.741 ± 0.028	0.455 ± 0.001	5.861 ± 0.026	6.780 ± 0.006	6.358 ± 0.102	4.629 ± 0.027
C18:0	2.417 ± 0.233	0.673 ± 0.004	0.745 ± 0.046	0.581 ± 0.024	0.427 ± 0.001	2.071 ± 0.087	1.192 ± 0.048	2.627 ± 0.1	1.155 ± 0.076
C18:1	1.332 ± 0.004	0.390 ± 0.007	0.218 ± 0.005	0.138 ± 0.007	0.164 ± 0.001	2.484 ± 0.053	5.873 ± 0.01	2.619 ± 0.034	5.093 ± 0.031
C18:2	13.144 ± 0.054	5.493 ± 0.293	3.315 ± 0.125	0.395 ± 0.001	0.742 ± 0.021	8.083 ± 0.125	90.656 ± 1.217	1.208 ± 0.010	4.961 ± 0.078
C18:3	0.550 ± 0.001	0.283 ± 0.000	0.144 ± 0.003	0.066 ± 0.000	0.059 ± 0.001	0.183 ± 0.001	0.330 ± 0.001	0.715 ± 0.02	0.651 ± 0.001
C20:0	-	0.048 ± 0.000	-	-	0.009 ± 0.000	-	-	-	-
C20:5	1.517 ± 0.040	0.379 ± 0.001	1.543 ± 0.05	0.180 ± 0.04	0.187 ± 0.002	0.882 ± 0.002	0.106 ± 0.003	1.085 ± 0.002	0.059 ± 0.002
C22:0	-	-	-	-	-	-	-	-	-
C24:0	0.233 ± 0.001	0.014 ± 0.000	0.064 ± 0.003	0.019 ± 0.000	0.022 ± 0.000	0.034 ± 0.000	0.098 ± 0.001	-	-
C22:6	7.360 ± 0.233	0.707 ± 0.009	1.022 ± 0.039	0.417 ± 0.036	0.313 ± 0.005	3.407 ± 0.063	0.413 ± 0.005	3.860 ± 0.027	0.534 ± 0.001

Fatty Acid	Consensus Value (g/100 g oil)	Range of Results using Method 4 (g/100g oil)
EPA	12.1 – 14.7	12.3 – 13.1
DHA	10.2 – 12.5	11.7 – 12.2

*Mean ± SEM

Table 4. Hazard quotient of essential fatty acids versus heavy metals

Fish and Products	Hazard Quotient		
	As	Cd	Pb
Stolephorus indicus (anchovy)	0.239	0.512	0.081
Sardinella longiceps (sardine, Indian sardine)	19.924	0.829	0.240
Mytilus smaragdinus (mussel)	0.520	0.223	0.055
Loligo pealei (squid)	1.814	1.775	0.168
Metapeneaus emsis (shrimp)	3.360	0.011	0.921
Decapterus macrosoma (round scad)	0.225	0.032	0.071
Chanos chanos (bangus)	0.365	0.199	0.322
Auxis thazard (skipjack tuna)	1.688	0.526	0.203
Oreochromis niloticus (tilapia)	0.173	0.320	0.436

$HQ_{EFA}<1$ means health benefit from fish consumption
$HQ_{EFA}>1$ means risk from fish consumption

CONCLUSIONS

The target hazard quotients for most of the fish and products showed values less than 1, which suggests that health risks were insignificant. All fish and products gave values less than 1 except for Cd of squid (1.8), As of dried Indian sardine (19.9), squid (1.8), shrimp (3.4), and skipjack tuna (1.7). The accumulation of heavy metals varies with the fish species, and the risk information given to the public allows people to choose from a diversity of fish for a healthy diet. On the basis of omega-3 fatty acids EPA and DHA and heavy metal content, the levels of Cd in squid and As in dried Indian sardine, squid, shrimp and skipjack tuna, may suggest risk.

It is concluded that to protect people from the deleterious effects of fish consumption and to ensure healthy fish diets, regular monitoring of hazard quotients for fish and products in wild conditions is recommended.

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