



Determination of arsenic and mercury in longtail tuna (*Thunnus tonggol*) collected from Terengganu waters: risk assessment of dietary exposure

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Abstract

Despite the beneficial aspect of fish consumption, bioaccumulation of toxic metals such as arsenic (As) and mercury (Hg) can enhance the health risk for the consumers. Arsenic and Hg concentrations were measured in edible tissue and two targeted organs, namely gill and liver of longtail tuna species (*Thunnus tonggol*) from Terengganu waters, including Kuala Besut, Kuala Terengganu, Dungun and Kemaman. The concentration of As and Hg were analysed by using inductively coupled plasma-mass spectrometry. The mean concentrations of both elements were significantly different ($p < 0.05$) among the locations and targeted organs. The hierarchy of As and Hg mean concentrations in muscle samples were Dungun > Kuala Besut > Kemaman > Kuala Terengganu. The mean concentration of As in all samples, including muscle, exceeded the permitted level set by Malaysia Food Act. Estimate Weekly Intake (EWI) was conducted to assess the health risk effect, and 63 kg was used as the average body weight of Malaysian adults. However, the EWI values show that the weekly intake of As and Hg does not exceed the provisional tolerable weekly intake limit suggested by Food and Agricultural Organization for the United Nations and is considered safe to be consumed.

Keywords: *Thunnus tonggol*, Seafood safety, Health risk, Heavy metals, Commercial fishes

Introduction

Terengganu is located at the east coast of Peninsular Malaysia and has the longest coastline with 244 kilometers in the East

Coast of Peninsular Malaysia. The discovery of offshore oil and gas in 1974 has granted Terengganu a significant change to its economic, technology and social structure. Fish are the main aquatic product of this state, and longtail tuna (*Thunnus*

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tonggol) is one of the most preferred species consumed by local people. *Thunnus tonggol* is found in water temperatures between 16°C and 31°C and is considered a highly migratory species (Hedayatifard, 2007). It is a small to medium-sized tuna species and feeds on marine crustaceans, fish and cephalopods. It avoids surrounding with low salinity and high turbidity such as in estuaries. It lives in shallow waters less than 200 m depth and is most common at less than 50 m. About 97% of landings for this species were in the coastal waters of seven developing countries namely, Iran, Indonesia, Pakistan, India, Oman, Malaysia and Thailand (Asiapacific-fishwatch, 2018). However, the landings for this species in Terengganu, Malaysia had decreased by 17.2% for the past five years from 2013 to 2018 (Lembaga Kemajuan Ikan Malaysia, 2018).

In Malaysia, fish has always been an important protein source for most of the population compared to other sources such as chicken and beef (Anual et al., 2018). The high demand for fish is not only for their taste but also for nutritional values. Beneficial polyunsaturated fatty acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported to be present in sufficient quantity in fish. These compounds can both prevent and cure some critical illnesses, including cancers, heart disease, rheumatoid arthritis, and inflammation (Njinkoué et al., 2002; Raatz et al., 2013). Furthermore, people tend to consume fish as their principal source of protein, nutrients, vitamins and energy and in addition, fish have low level of fat and calories (Ahmed et al., 2019). However, the presence of heavy metals in high amounts is detrimental to fish and, by extension, humans and other organisms who consume them. According to Rahmani et al. (2018), the most significant foodborne diseases for humans were derived from microbiological and chemical hazards sources. Principally, a human can be exposed to heavy metals through the ingestion of fish or seafood (Al-Busaidi et al., 2011).

Heavy metals are a significant component of chemical pollution in the marine ecosystem. This is due to its toxicity, persistence, accumulation, and ability to bio-magnify along the food chain (Ayanda et al., 2019). The anthropogenic activities and industries have made the significant upsurging of heavy metals level in the marine ecosystem via the anonymous usage and discharge of the metal effluents that turned into chemical hazards (Li et al., 2015). Nowadays, human health risks through chemical pollution in fish or aquatic products are recognized as one of the most compelling issues globally (Ahmed et al., 2019; Gan et al., 2017). Past studies suggested that food intake is one of the major pathway humans may have acquired heavy

metals from the environment, alongside other routes such as inhalation and dermal contact (Zhang et al., 2015). Arsenic distribution in the environment which basically derived from the geological process and human activities such as smelting, mining and industrial production (Du et al., 2021). Arsenic from different sources that entered the marine ecosystem caused a serious environmental problem in coastal areas. Marine organisms including fish generally contain high As concentration as they have high capacities for As accumulation and metabolic transformation (Smedley & Kinniburgh, 2002). On the other hand, Hg exposure to humans primarily through the intake of fish and seafood has accumulated a considerable amount of Hg via the biomagnification process (Visha et al., 2015).

Ahmad et al. (2015) proclaimed that 2% of samples from marine fish sampled in Peninsular Malaysia exceeded the permitted level of mercury which is 0.5 µg/g wet weight (Ibrahim et al., 1985). A study by Tengku Nur Alia et al (2020) reported that the concentration of Hg in the raw muscle of seabass (*Lates calcarifer*) from Setiu Lagoon, Terengganu was 0.032 mg/kg wet weight while As concentration was 3.90 mg/kg wet weight. Similarly, Hg concentration in fish from West Peninsular Malaysia is relatively lower and safe for human consumption (Anual et al., 2018). The contaminants in the marine environment have become a concern, as they can accumulate in the organism. Fish may accumulate a huge amount of toxic metals through the bioaccumulation process from the contaminated water (Chakraborty et al., 2019). Hence, we determined the concentration of mercury and arsenic in muscle and two targeted organs, namely gill and liver of *Thunnus tonggol* from four different locations in Terengganu during the dry season and analysed the risk factor of consuming this fish species.

Materials and Methods

Sampling location

The northern district of Terengganu bordering the state of Kelantan is Besut, followed by Kuala Terengganu (capital), Dungun and Kemaman district in the southward direction (Fig. 1). The samples of *Thunnus tonggol* were purchased from these four different fish landing ports LKIM Complex. A total of 100 samples from all sampling locations, with 25 individuals for each sampling location. All the purchased fish were caught along Terengganu waters as a part of the South China Sea. All the samples were sent back to the laboratory and frozen for further analysis (Ong & Gan, 2017).

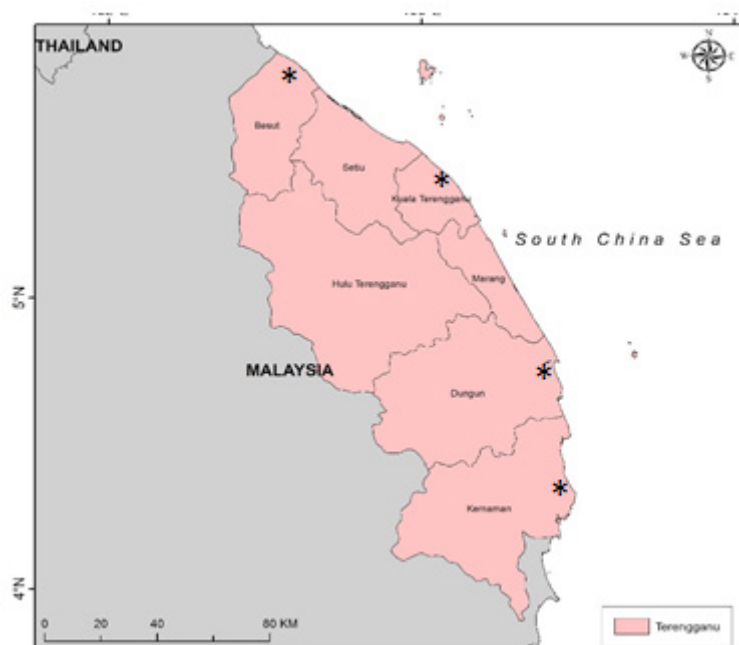


Fig. 1. The four different (*) sampling locations namely Besut, Kuala Terengganu, Dungun and Kemaman.

Sample treatment

All the samples were thawed at room temperature prior to the dissection process. The morphometric information such as total length and wet body weight were measured and recorded. The dissection of muscle and the removal of targeted organs (gills and liver) of the samples were performed using ceramic knives. Through the whole dissection process, the possibilities of samples losing blood were minimized to prevent contamination. The dissected muscles and organs were rinsed with distilled water to remove impurities before putting in the clean polyethene container for drying purposes. All the samples were dried in the oven at 60 °C for three days or until they reached a constant weight (Ong et al., 2014).

Analytical method

Arsenic (As) and mercury (Hg) analyses were based on the modified procedures described in Ong et al. (2014). A total of 0.05 g of grinded powder samples was mixed with 1.5 mL of suprapur nitric acid (HNO₃) for the digestion process. Both blank reagent and certified reference materials (Dolt-4 fish liver) were carried out simultaneously to control the exactitude of the procedure used (Poong et al., 2020). For acid digestion, all the samples were kept in a Teflon bomb and heated at 100 °C

for 8 hours in the oven. The digested samples were transferred into a centrifuge tube and diluted with deionized water up to 10 mL. The concentration of total As and Hg were measured using inductively coupled plasma-mass spectrometry (ICP-MS) to obtain quick and precise data (Chuan et al. 2018; Ong et al., 2014).

Tuning procedure of inductively coupled plasma-mass spectrometry (ICP-MS)

The application of ICP-MS with the model of Perkin Elmer Elan 9000 combined high-temperature Inductively Coupled Plasma sources with a mass-spectrometer. Argon gas was used to form argon discharge or plasma. Generally, the atoms of the elements in the samples were converted into ions that separated and brought into the mass spectrometer via the interface cones. The ICP-MS has transmitted the ions in the argon sample streams at atmospheric pressure ($< 1 \times 10^{-5}$ torr). The ions that enter the mass spectrometer are then separated according to their mass-to-charge ratio and detected by the detector. The number of ions striking the detector into an electrical sign that can be measured and related to the number of atoms of the particular elements in the samples through the calibration methods.

Human risk assessment

Based on Norhazirah et al. (2020a), Terengganu people consume 22.88 kg of *Thunnus tonggol* per person per year. This value showed that the estimated *Thunnus tonggol* consumption per week was 0.44 kg/person/week. The average body weight for Malaysian adults used as an indicator for this study was 63 kg. This value was obtained from survey conducted by Malaysian Adult Nutritional Survey (MANS) from October 2002 to July 2003 (Norimah et al., 2008). The value of provisional tolerable weekly intake (PTWI) for As and Hg suggested by Food and Agricultural Organization (1983) were 15.0 and 1.60 µg/kg body weight, respectively. The estimate of weekly intake (EWI) for this study were calculated based on the following equation to estimate the amount of As and Hg intake through consumption of muscle of *Thunnus tonggol* over a week. (Suhaimi et al., 2005).

$$EWI \left(\frac{\text{mg}}{\text{kg}} \text{ wet wt.} \right) = \frac{\text{Mean heavy metal concentration in fish muscle} \left(\frac{\text{mg}}{\text{kg}} \text{ wet weight} \right) \times \text{Weekly fish consumption (kg)}}{\text{Bodyweight (kg)}}$$

The data calculated were compared with the permitted values provided by Food and Agricultural Organization (1983).

Statistical analysis

Results of As and Hg concentrations were normally distributed ($p > 0.05$) based on normality test analysed using SPSS software. Two-way ANOVA and Tukey HSD (pairwise comparison) were used to examine differences among locations and relationships between targeted organs. A significant level of 5% was used.

Results

A total of 100 individual samples were collected from Terengganu waters at four different fishing ports, namely Kuala Besut, Kuala Terengganu, Dungun and Kemaman. All the details are illustrated in Table 1. The total lengths of all samples were ranged from 38.1 to 51.5 cm whereas the wet body weight ranged from 891.0 to 1,782.9 g.

The concentration of As and Hg in muscle and targeted organs of *Thunnus tonggol* were expressed in µg/g wet weight and presented in Table 2. There were significant differences ($p < 0.05$)

Table 1. Statistical information on total length and wet weight of longtail tuna collected from four sampling locations in Terengganu, Malaysia

Location	n	Total length (cm)	Total weight (g)
Kuala Besut	25	51.5 ± 1.86 (48.4–54.0)	1,690.2 ± 196.1 (1,406.1–1,787.6)
Kuala Terengganu	25	44.3 ± 3.3 (38.0–49.5)	1,159.6 ± 230.4 (799.3–1,537.2)
Dungun	25	49.7 ± 3.8 (46.0–57.4)	1,782.9 ± 426.8 (1,346.0–2,622.1)
Kemaman	25	38.1 ± 5.1 (30.2–46.7)	891.9 ± 355.6 (891.9–355.6)

Table 2. The mean concentrations of total arsenic (As) and mercury (Hg) in muscle and two targeted organs (gills and liver) of *Thunnus tonggol*

Location	Organ	Total arsenic (As) (µg/g) w.w	Total mercury (Hg) (µg/g) w.w
Kuala Besut	Muscle	17.8 ± 4.1	0.460 ± 0.09
	Gill	17.11 ± 4.9	0.107 ± 0.06
	Liver	30.24 ± 5.28	0.23 ± 0.03
Kuala Terengganu	Muscle	8.74 ± 0.19	0.19 ± 0.03
	Gill	7.50 ± 2.9	0.038 ± 0.02
	Liver	24.3 ± 11.9	0.16 ± 0.03
Dungun	Muscle	30.5 ± 6.8	1.57 ± 0.33
	Gill	8.02 ± 2.9	0.142 ± 0.03
Kemaman	Muscle	16.7 ± 5.1	0.467 ± 0.1
	Gill	4.56 ± 1.6	0.02 ± 0.01

All values expressed in mg/kg wet weight ± SD.

between the concentration of As and Hg to the location and targeted tissues in this study. The muscle of fish collected from Dungun had the highest concentrations of As and Hg compared to other locations with the mean concentrations of 30.5 ± 6.8 and 1.57 ± 0.33 mg/kg wet wt., respectively. Other than that, the range of Hg concentration in muscle samples from another three locations was 0.24 to 0.78 µg/g wet weight. The divergence of Hg concentration between the location and targeted tissues might be due to few factors that played a significant role in the bioaccumulation process such as characteristics of the contaminants, habitat and trophic level (Jayapal et al., 2017). The geographic differences in *Thunnus tonggol* metal concentration are therefore most likely due to the various types of prey and food web exploited (Norhazirah et al., 2020b). Additionally, fish may accumulate Hg from the surrounding waters and the accumu-

lation rate into the fish may differ with both on the uptake and elimination rate (Mansour & Sidky, 2002). Furthermore, fish can assimilate the metals to their body in varied ways including the ingestion of other marine organisms, ion-exchange transversely into lipophilic tissues such as gills, along with adsorption on tissue and skin surface (Ahmed et al., 2016). Overall, the concentration of Hg in gills and liver samples were detected in a small range and none of the samples contained Hg above the permitted level set by Ibrahim et al. (1985).

Generally, As can exist in different concentrations of both abiotic and biotic components of the marine environment (Perera et al., 2016). These non-essential elements uptake by fish may differ fundamentally from the land animal as they are continually immersed into the contaminated aquatic environment. Accurate measurement of As was a very difficult task, however the total estimation of As consist of at least 10% of inorganic As that can expose human to carcinogenic if they were consumed for a long time (Ahmed et al., 2019). In this study, the concentration of As in the analysed fish muscle and organs varied between the location. The results showed that all the As levels were very high and exceeded the permitted level. Among all locations, muscle samples were recorded from 11.19 up to 55.6 µg/g wet weight with the highest level was in samples from Dungun followed by Kuala Besut, Kemaman and Kuala Terengganu. Muscle of *Thunnus tonggol* from Dungun was recorded as 55.6 ± 32.6 µg/g wet weight. From the details recorded, the *Thunnus tonggol* sampled from Dungun has the biggest sizes and weights. The trophic position of fish species can have a significant effect on the bioaccumulation of As element in fish (Perera et al., 2015). Notably, the differences in As concentration might be due to the geographical location, the sizes and the propensity of the metal to be subjected to biomagnifications in the food chain (Al-Busaidi et al., 2011). On the other hand, Mohamed (2008) suggested that the distribution of As in fish is determined mainly by its content in water and food.

Discussion

Metals can be absorbed into aquatic organism such as fish through digestive tract related to dietary exposure and through the gills by the waterborne exposure (Ptashynski et al., 2002). By comparing the As level in gills samples, the concentration was in the following decreasing order of Besut > Kuala Terengganu > Dungun > Kemaman. The highest concentration of As in gills of *Thunnus tonggol* from Besut was probably caused by the size

of gills. According to Anandkumar et al. (2018), the size of gills plays a role in the accumulation of metal and the metal concentration was higher in a larger gill fish. However, the level of As in gills showed slightly different in samples from Kuala Terengganu and Dungun which is 27.0 ± 10.4 µg/g and 25.1 ± 17.0 µg/g wet weight, respectively while Kemaman was 15.4 ± 6.17 µg/g wet weight. The level of As concentration in the enclosing waters is emulated by the gills because of the direct contact with the water and suspended material during the osmoregulation process (Anandkumar et al., 2018). During the gas exchange process, the gills serve as a barrier to the metal ion-exchanged from the water (Ahmed et al., 2016). Furthermore, the surface of gills was very large, thus facilitating rapid diffusion of toxic metals such as As and Hg (Dhaneesh et al., 2012).

On the other hand, liver samples were only obtained from Kuala Besut and Kuala Terengganu area. The As level obtained in this samples revealed that this element was very high in both locations which is 44.3 ± 24.7 µg/g wet weight in Besut and 36.7 ± 20.5 µg/g wet weight in Kuala Terengganu. It might be caused by metallothioneins protein that is bound naturally to the hepatic tissues like a liver (Görür et al., 2012). Metallothioneins are the natural protein binding presented in the fish liver that plays a role in the bioaccumulation of toxic metals and detoxification (Samuel et al., 2021). These proteins act as a defence mechanism to adapt to metal toxicity including As and Hg. Furthermore, the liver is also recognised as primary metal storage and plays a role in the particular function of amassing and transporting metals in the organism (Anandkumar et al., 2018).

The result apparently recognized that As and Hg accumulated higher in liver than other analysed parts such as gills and muscle. Thus, it can be concluded that the metal accumulation rate varied among the tissues and organs of the fish and it was determined by the process of bioaccumulation and the intrinsic factors of the fish such as the sizes, habitat and feeding habit (Anandkumar et al., 2018). Among the organs analysed, the liver functions as the elementary organ for metal depository and they played a big role in the metabolic and detoxification process in fish (Staniskiene et al., 2006).

Literally, it is crucial to assess the toxicity of metal contaminated in *Thunnus tonggol* that can be transferred to humans through frequent consumption. Consequently, PTWI proposed by Joint Food and Agricultural Organization for the United Nations (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA) is required. This calculation evaluates the amount of metal pollutants by humans over

a lifetime without pose any risk. Table 3 presented the estimated weekly dietary intake of As and Hg from the consumption of *Thunnus tonggol* edible tissue, muscle.

The PTWI value for As and Hg calculated in muscle samples from all sampling stations was very high and exceeded the PTWI standard provided by FAO. The highest PTWI value for As was in muscle samples from Dungun (386.0) followed by Kemaman, Kuala Terengganu and Kuala Besut. On the other hand, the range of PTWI value for Hg calculated in muscle samples was from 1.68 to 19.9. It showed that Kuala Besut has the lowest value while Dungun was recorded the highest value with 19.9. Notably, all samples were exceeded the PTWI value set by FAO.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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Table 3. The estimation weekly intake (EWI) of *Thunnus tonggol* from Terengganu waters

Location	EWI (mg/kg body weight) As	EWI (mg/kg body weight) Hg
Kuala Besut	0.124 ± 0.04	0.003 ± 0.002
Kuala Terengganu	0.06 ± 0.03	0.0013 ± 0.001
Dungun	0.199 ± 0.10	0.011 ± 0.008
Kemaman	0.115 ± 0.04	0.003 ± 0.002
PTWI mg/kg body weight/ week)	0.015	1.6
PTWI (mg/kg body weight/ week) for a 63 kg adult	0.945	100.8

All values were expressed in mean ± SD mg/kg body weight. PTWI, provisional tolerable weekly intake.

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