

Health Risk Assessment of Adults Consuming Commercial Fish Contaminated with Formaldehyde

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Abstract The health benefits of consuming fish as a source of omega-3 fatty acids have been established. It can reduce cholesterol levels and the incidence of stroke and can protect against cardiovascular disease, improve cognitive development in children and slow cognitive decline in the elderly. Formaldehyde was used as antibacterial agent and preservative in food processing such as dried food, fish, certain oil and fats and disinfectants for container. Formaldehyde classified by the International Agency for Research on Cancer (IARC) in the Group 1 as carcinogenic to humans. If the amount of formaldehyde is small, it does not harm health. However, it can cause minor to serious problems such as pain, vomiting, coma and possible death when with large doses of formaldehyde is taken. Survey was conducted for adults, teenagers and children to identify the commercial fish consumption pattern in order to assess the risks of consuming different commercial fish contaminated with formaldehyde. Seven types of commercial fish species based on the survey were analysed. All the samples were purchased in different wet market and analysed under different circumstances; fresh, boiled and fried. Formaldehyde were determined in all fish circumstances analysed. Formaldehyde content was in range 2.38 to 2.95 µg/g for fresh, 2.08 to 2.35 µg/g for boiled and 2.28 to 2.49 µg/g for fried. This study showed that there is formaldehyde content in fish sample analysis. However, formaldehyde content among all fish species and fish circumstances were still lower than amount that set by Malaysian Food Act (1985) and Malaysian Food Regulation (1985) that the maximum limit value for formaldehyde in fish and fish products are 5 mg/kg. The effect of cooking shows a reduction of the formaldehyde content. There is no adverse health effects on human related to the fish consumption contaminated with formaldehyde from the risk assessment calculation. Thus, the fish from wet market can be considered safe for consumption because of low formaldehyde content. Furthermore, some methods have suggested cooking and washing can potentially reduce the formaldehyde content in fish.

Keywords Risk Assessment, Formaldehyde, Fish

1. Introduction

The health benefits of consuming fish are well documented such as a source of omega-3 fatty acids that can reduce cholesterol level, the incidence of stroke and protect against cardiovascular disease[1],[2], improve cognitive development in children[3] and slow cognitive decline in the elderly[4].

Fish also can cause harm to human health. The most significant sources of foodborne diseases are related to the micrological and chemical hazards. Contamination of chemical in food may include natural toxicants such as mycotoxins[5] and marine toxin[6], environmental contaminants such as mercury and lead[7] and naturally occurring substances. Among the contaminants, attention has been paid to volatile toxic aldehydes such as formaldehyde

classified by the International Agency for Research on Cancer (IARC) in the Group 1 as carcinogenic to humans[8].

Formaldehyde is metabolised naturally in our bodies by normal metabolism and can also be found in the air, natural food, some skin-care products as well as preservatives in processed food, especially in dried and frozen food. If the amount of formaldehyde is small, it does not harm health. However, it can cause minor to serious problems such as pain, vomiting, coma and possible death when with large doses of formaldehyde is taken. Formaldehyde an acceptable daily intake (ADI) of 0.2 mg/kg body weight has been set by the United States Environmental Protection Agency[9].

In the food industry, formaldehyde is used as anti bacterial agent and preservative in food processing. It widely used in food processing for its bleaching effect and also as a preservative in order to prevent spoilage by microbial contamination. Formaldehyde is also used as a preservative in dried foods, fish, certain oil and fats and disinfectants for containers[10]

In seafood and crustaceans, formaldehyde is known to form post-mortem from the enzymatic reduction of

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trimethylamine-N-oxide (TMAO) to formaldehyde and dimethylamine[11],[12]. This compound accumulates during frozen storage, reacts with protein and subsequently causes protein denaturizing and muscle toughness[11].

Protein sources in the Malaysian diet are mainly provided by fish and seafood[13]. Fish and seafood are an important part of a healthy diet and considered as the biggest protein sources[14]. Fish contain fat, free amino acids and water by composition which is susceptible to spoilage by microorganisms and biochemical reactions during post mortem process[15]. Unfortunately, commercial fish consumed by population maybe contaminated with formaldehyde in order to keep the freshness of the fish and seafood because they are very perishable and can be only kept fresh in ice for 8 to 14 days depending on the species[16].

Risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to food borne hazards. It is structured process for determining the risk associated with any type of hazard for biological, chemical or physical in food. It has as its objective a characterization of the nature and likelihood of harm resulting from human exposure to agents in food. The characterization of risk typically contains both qualitative and quantitative information and is associated with a certain degree of scientific uncertainty[17].

Formaldehyde is known to be carcinogenic to human. Therefore, it is important to investigate the formaldehyde content in the fish since it is claimed to be the major contaminant in seafood in order to understand better the risks of fish consumption, to manage the risk of consumption and to provide additional information in food safety.

2. Materials and Methods

The survey area was around 1 kilometre radius from the wholesale wet market. There were 3 wholesale markets selected, namely Pasar Borong Selayang, Pasar Harian Selayang and Pasar Borong Klang. The calculation of the required sample size for adults will be calculated based on the formula[18] as follow:

$$N = \frac{Z^2 P (1-P)}{d^2} \quad (1)$$

Where;

N = Sample size needed

Z = Z statistic for a level of confidence (level of confidence of 95%, Z value is 1.96)

P = Prevalence of the marine fish consumed in the central zone (28.9%) - According to Malaysian Adult Nutrition Survey (2003).

d = precision (In proportion of one; if 5%, d = 0.05).

From the calculation, minimum sample size needed for adults are 316 respondents. However, the sampling for ethnicity was stratified according to quota Malaysian population which is Malay 59% (186), Chinese 32% (101) and Indian 9% (29).

2.1. Survey Form

The form used The form used in the survey consisted of 16 questions and was administered by face to face interview, where respondents was asked on the frequency commercial fish intake either per day, per week, per month. Respondents were also requested to respond on the number of servings consumed each time they ate the fish and duration of fish storage before cooking. Questionnaires also consisted on socio-demographic variables which included gender, body weight, ethnicity, age group, family members, how much they buy fish per week from market, the type of fish they consume, how often they consume the fish.

2.2. Sample Preparation

Four types of commercial fish were selected as target sample based on the frequency of consumption in the short survey. Each of the fish sample weighed 500 g and purchased from the different wet markets. The sample was collected into sterile plastic bag and stored in ice box. Each of the fish species was analysed under different circumstances; fresh, fried and boiled. The flesh was separated from skin and bones without damaging the gut by using sterile scalpel. For the boiled and fried fish, each sample was boiled in hot water or cooked at 180 °C for 20 min. Thirty g of each sample was then minced and homogenized with 60 ml of 6% w/w trichloroacetic acid (TCA). A calibration curve in the range of 0–5 mg/L was used for the analysis of the samples. Six concentration levels were analysed using 3 measurements at each concentration level.

2.3. Chemicals

Nash's Reagent[19] was used as an indicator by diluting 15 g ammonium acetate with an addition of 0.3 ml of acetyl acetone and 0.2 ml of acetic acid. Nash's Reagent was kept in amber glass reagent bottle at all times because the reagent is light sensitive. TCA was used to adjust the pH of fish flesh appropriately. 0.1 N potassium hydroxide (KOH) and 0.1 N hydrochloric acid (HCl) were used to adjust the pH of the distillate in order to be in the range from 6.0 to 6.5. The working standard solution was ranged in 0 – 5 mg/l and it was prepared from the intermediate standard solution (10 µg/g) for the graph calibration.

2.4. Determination of Formaldehyde

The fish samples were cut into small pieces and 30 g of the sample was homogenized with 60 ml of 6% w/w TCA. The mixture was filtered and the pH of the filtrate was adjusted to 7.0 with 30% w/w KOH and stored in ice for 1 h. The test was performed by mixing 5 ml of the standard solution, TCA, fish extract, 2 ml of Nash's Reagent and then heated in the water bath at 60°C for 30 min. The absorbance at 415 nm was measured immediately by UV/vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

2.5. Statistical Analysis

All experiments were done in triplicate. The data were recorded as mean (standard deviations) and were analysed using the statistical computer software 'Statistical Package for Social Science' (SPSS Windows Version 17.0). Based on the collected data, a descriptive statistical analysis was carried out to study the average content of formaldehyde in fish. One-way ANOVA was used to compare mean formaldehyde content in fish based on fish species, fresh, fried and boiled fish. Multiple regressions were used to determine the exposure assessment of consuming formaldehyde contaminated commercial fish.

3. Results and Discussions

There were a total of 319 respondents who agreed to participate in this study, yielding a high response rate which is more than 0.95% of sample size. The exposure was obtained by calculating the formaldehyde concentration in fish in order to identify the hazard quotient (HQ)

3.1. Socio-demographic Characteristics of Respondents

The mean age of the respondents was 34 years old while the mean weight was 60.27 kg with standard deviation (13.10). The majority of the respondents are female (90.6%). The respondents comprised of 58.6% Malay, 32% Chinese, 9.1% Indian and the other 0.3%. For family's average monthly income, the majority of them had monthly income below RM3000 (43.9%). Most of the respondents have less than four children (90%) that eat fish in their family. For the favourite part of consumption, majority of the respondents like to eat the meat part (49.5%) more than tail (7.2%) and head (1.6%) part. About 41.6 % of the consumer likes to eat all part or some of the part of the fish. From the survey, 23.3 % of the respondent chose to fry the fish, while 11.3% of them boiled the fish and 7.0% of them roasted the fish. More than half of the respondents (63.3%) have selected to cook the fish by using all of the style or some of the style above. Overall, 23.2 % to 60.6 % of the respondents consumed all types of commercial fish once to twice a week.

3.2. The Consumption Pattern of Commercial Fish

The four types of fish frequently consumed by the respondents were Kembong, Selar, Bawal and Kerisi. The Kembong fish had the highest consumption rate (76.5 %), followed by Selar (46.7 %), Bawal (40.1 %) and Kerisi (37.9 %). According to ethnicity, Kembong fish had the highest rating among Malay (82.7 %) followed by Bawal (48.9 %), Siakap (44.3 %) and Merah (42.0 %). Among the Chinese, the highest fish species consumed was Kembong (60.7 %) followed by Bawal (60.3 %), Merah (46.2 %) and Selayang (45.3 %). Among the Indian, the highest fish species consumed was Kembong 64.2 %, followed by Merah (58.0 %), Bawal (46.9 %) and Selayang (40.7 %). The consumptions pattern of commercial fish contaminated with

formaldehyde (Kembong, Merah, Bawal and Siakap) were significantly different ($p < 0.05$) between the ethnics. Thus, the study showed that the most frequent fish consumed by the respondents was Kembong fish. According to Fisheries Development Authority of Malaysia (2005)[20], Kembong fish have high consumption rate because they are inexpensive.

Fish are the main supply of healthy and cheap protein to a large percentage of the world's population. In most of the Asian countries, fish is a main protein of the diet especially in Southeast Asia. It is particularly valuable for providing proteins of high quality comparable with those meat, milk or eggs. Fish also a good source of *n*-3 fatty acids, calcium, phosphorus, iron, trace elements like copper and a fair proportion of the B-vitamins[21]. Consumption of fish oils contain in fish is beneficial to the development of fetal and the brain[22].

3.3. Formaldehyde Content in Fish

Since fish products are generally consumed after cooking, the samples were analysed immediately after boiling and frying in order to determine the formaldehyde content. As reported in Table 1, a decrease in a formaldehyde concentration was generally observed in all fish species.

Comparisons of the mean formaldehyde concentration between fish sample is presented in Table 1. The results showed that there were significant differences ($p < 0.05$) in formaldehyde concentration between fish samples for Kembong, Kerisi, Selar, Selayang, Merah and Bawal fish. Post Hoc comparisons using Tukeys test indicated that the mean formaldehyde concentration were significantly different ($p < 0.05$) between fresh and boiled fish samples of Kembong, Kerisi, Selar, Merah, Bawal and in Selayang there was significantly different between fresh, boiled and fried. However there was no statistically significant difference ($F = 1.736, p = 0.198$) in the mean formaldehyde concentration of Siakap fish samples.

The comparisons of the amount formaldehyde content in the whole fish of commercial fish species are shown in Table 1. Generally, results showed that all the fresh, boiled and fried samples had low formaldehyde concentration. Data obtained found that the amount of formaldehyde in all fish species and fish circumstances were still lower than the permissible level set by the Malaysian Food Act (1983) and Malaysian Food Regulation (1985) which stated that the maximum limit for formaldehyde in fish and fish products are 5 mg/kg[23].

According to Swiss[24], Danish[25] and Swedish Products Registers[26], formaldehyde is found in a large number of products and available for consumer. In addition, formaldehyde is used in fish farming as preservative agents. Formaldehyde formed in fish reacts with protein and subsequently causes muscle toughness[27].

Based on the result, there were significant differences in the amount of formaldehyde content between fish samples. The formaldehyde content generally decreased in all of the

fish species analysed after cooking. The decrease in levels occurred when the samples were cooked in open pots, thus allowing the evaporation of formaldehyde during the cooking process since the formaldehyde is soluble in water at 20 °C. In addition, the boiling point for formaldehyde is 101 °C, so the evaporation of formaldehyde maybe occurred due to its low volatility[28]. Thus, the results showed that exposure to formaldehyde will decrease if the fish was cooked.

Table 1. Amount of Formaldehyde in Commercial Fish Species Consumed by respondents

Fish Type	Mean (SD)			<i>F</i>	<i>p</i>
	Fresh	Boiled	Fried		
Kembong (<i>Mackerel</i>)	2.557 (0.219)	2.233 (0.341)	2.407 (0.114)	3.968	0.032*
Kerisi (<i>Red sea beam</i>)	2.381 (0.162)	2.082 (0.235)	2.283 (0.190)	5.272	0.013*
Selar (<i>Scad</i>)	2.447 (0.204)	2.118 (0.357)	2.327 (0.227)	3.995	0.032*
Selayang (<i>Sardine</i>)	2.946 (0.431)	2.357 (0.178)	2.495 (0.156)	10.558	0.001***
Merah (<i>Red snapper</i>)	2.498 (0.158)	2.292 (0.210)	2.403 (0.124)	3.403	0.050*
Siakap (<i>Golden snapper</i>)	2.409 (0.157)	2.237 (0.247)	2.327 (0.172)	1.736	0.198
Bawal (<i>Black pomfret</i>)	2.615 (0.316)	2.108 (0.386)	2.427 (0.226)	5.887	0.008**

*Significant at $p < 0.005$

** Significant at $p < 0.01$

***Significant at $p < 0.0001$

The differences in the formaldehyde levels among fish could be explained on the basis of the different trimethylamine-oxide (TMAO) levels usually found in these products. In seafood, formaldehyde is present naturally. It is formed from the post-mortem of enzymatic reduction of TMAO to equimolar amounts of formaldehyde and dimethylamine (DMA)[29]. The amount of formaldehyde formed depends mainly on the time and temperature of the storage which causes muscle toughening and water loss in fish, leading to lower acceptability as well as functionality [11]. Different levels of formaldehyde in fish species depend on the level of TMAO and reaction to reduce TMAO to formaldehyde and DMA obtained from the frozen seafood [29]. The reduction of TMAO process also caused the bacteria activity to increase[11].

3.4. Risk Assessment

The average daily dose (ADD) was used for carcinogenic risk assessment. The estimation of ADD expressed as mg per kg of body weight (BW) per day[30] is shown in Table 2.

The choice of fish consumed may vary considerably from one individual to another. The daily intake of formaldehyde concentration from food consumption is dependent on the fish and amount of fish consumed. Statistic showed that the average age of Malaysian consumed 169 g/day of fish

serving and the demand for fish consumption are increasing over years[31].

The survey made by Malaysian Adults Nutrition Survey [13] through their survey on Habitual Food Intake of Adults Aged 18 to 59 years showed that one medium size fish particularly marine fish are eaten daily. Protein sources in the Malaysian diet are mainly obtained from fish and seafood.

Since seafood is one of the most important food sources in Southeast Asia, intake of formaldehyde from fish is of great concern for human health risk. Assessment of human health risk from ingestion of fish contaminated with formaldehyde required information on the quantities of fish consumed. The ADD values in adults were calculated by assuming that 70 kg individual[30] will consume 169 g/day fish serving[31].

Table 2. The Value of Average Daily Dose (ADD)

Fish	C	IR	E	CF	BW	D
Selayan	2.946	16900	1	10^{-6}	70**	0.07112
Selayan	2.357	16900	1	10^{-6}	70**	0.05691
Selayan	2.495	16900	1	10^{-6}	70**	0.06024

Note : *Intake rate according to Ministry of Health 2006

**Body weight according to US EPA 1997

Table 3. Hazard Quotient for Formaldehyde

Fish types	D (mg/kg / day)	Oral RfD (mg/kg/day)	HQ (≤ 1)	Conclusion
Selayang fresh	0.07112	0.2	0.3556	No adverse effect
Selayang boiled	0.05691	0.2	0.2845	No adverse effect
Selayang fried	0.06024	0.2	0.3012	No adverse effect

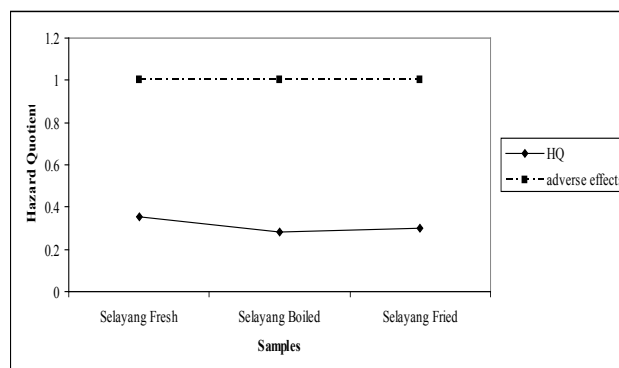


Figure 1. The Range of HQs for a Fish Sample Contaminated with Formaldehyde

The estimation of risk assessment is shown in Table 2 and Table 3. Generally, the findings showed that the risks are computed as a hazard quotient (HQ, the ratio of ingested formaldehyde to the reference dose, or RfD). Since the value of HQ is less than 1, the concentration of formaldehyde in fish is unlikely to cause adverse effects on human when the fish are consumed. In fact, the ADI value (0.2mg/kg-day) is sufficient to guarantee the consumer safety. Additionally, fish is usually consumed after cooking. The boiling point of formaldehyde is 101 °C and the formaldehyde content in fish will be decreased during the cooking process at high temperature. This is due to the fish is cooked in the open pot

that may cause formaldehyde to evaporate easily[32]. However, the study showed that the consumption of commercial fish could influence the risk of formaldehyde.

Studies on human acute toxicity showed that serious ulceration and damage of the gastrointestinal tract have been observed after ingestion of formaldehyde[33]. Formaldehyde can also cause allergic reaction in human. Systemic or localized allergic reactions have been associated with formaldehyde exposure[34]. For patch testing of product containing formaldehyde, the threshold for elicitation of allergic contact dermatitis in sensitized respondents range from 30 ppm to 60 ppm aqueous solution. A threshold for induction is estimated to be less than 5 % of aqueous solution[35].

Formaldehyde is weakly genotoxic and was able to induce gene mutations and chromosomal aberrations in mammalian cells. DNA-protein cross-links are a sensitive measure of DNA modification by formaldehyde. However, the genotoxic effects were limited to the cells, which are in direct contact with formaldehyde, and no effects could be observed in distant-site tissues. In conclusion, formaldehyde is a direct acting locally effective mutagen[36].

Epidemiological studies of potential carcinogenic hazards associated with the ingestion of formaldehyde were not identified. However, consistent with the known reactivity of this substance with biological macromolecules in the tissue or organ of first contact, histopathological and cytogenetic changes within the aerodigestive tract, including oral and gastrointestinal mucosa, have been observed in rats administered formaldehyde orally. These observations and additional consideration of the mode of induction of tumour by formaldehyde lead to the conclusion that under certain conditions of exposure, potential carcinogenic hazard associated with the ingestion of formaldehyde cannot be eliminated[37].

There are no indications of a specific toxicity of formaldehyde to fetal development and no effects on reproductive organs were observed after chronic oral administration of formaldehyde to male and female rats. Amounts of formaldehyde which produce marked toxic effects at the portal of entry do not lead to an appreciable systemic dose and thus do not produce systemic toxicity. This is consistent with formaldehyde's high reactivity with many cellular nucleophiles and its rapid metabolic degradation[37].

3.4. Methods to Control Formaldehyde in Fish

There are a few suggestions that can be considered to overcome the formaldehyde content in fish. Several methods have been taken and proposed in order to control or reduces formaldehyde content in fish. In Hong Kong, the government advised to the public to choose the fish that are fresh and avoid those with unusual smell and also avoid buying noodle fish that are stiff (formaldehyde could stiffen flesh of fish). Freshness is a property of fish that has a considerable influence on its quality[38].

Besides, public also advised to wash and cook the fish thoroughly as formaldehyde is water soluble and it could be dissipated upon heating. When formaldehyde is released into water, it does not move into other media but it is broken down because formaldehyde is readily soluble in water, alcohols and other polar solvent[37]. The USEPA's Exposure Factors Handbook[30] has reported cooking the fish will result in weight (moisture and fat) loss which subsequently decreases the formaldehyde concentration in cooked fish. The formaldehyde concentration was decrease after roasting and boiling. The decrease of formaldehyde content was due to the evaporation of the sample during the cooking process[39].

4. Conclusions

This study showed that there was formaldehyde content in fish sample analysis. However, formaldehyde content among all fish species and fish circumstances were still lower than the amount set by Malaysian Food Act 1985 [1 October 1985, P.U. (B) 446/1985] and Malaysian Food Regulation 1985 (in exercise of the powers conferred by section 34 of the Food Act 1985), where the maximum limit for formaldehyde in fish and fish products are 5 mg/kg. The effect of cooking showed the reduction of the formaldehyde content in fish.

However, there were some limitations in this study such as the temperature change, time of storage and handling could possibly influenced the concentrations of formaldehyde since it is a volatile compound. Additionally, only edible parts of fish were analysed and no results were shown in the bones and fins. The fish consumption was an approximate since no diet recall interview was carried out.

There was no adverse health effects on human due to the formaldehyde contaminated fish consumption based on the risk assessment calculation. Thus, the fish from wet market can be considered safe for consumption because of low formaldehyde content. Furthermore, frying and boiling the fish can potentially reduce the formaldehyde content .

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