Evaluation of artificially contaminated fish with formaldehyde under laboratory conditions and exposure assessment in freshwater fish in Southern Bangladesh

Submitted to the Journal of Chemosphere

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Abstract

Formalin can be added as preservative to fresh foods to prevent spoilage and extend shelf life. Formalin contains 37-40% formaldehyde, which is classified as carcinogenic to humans. To assess the public health risk associated with formaldehyde exposure in freshwater fish in Southern Bangladesh, formaldehyde concentrations (mg/kg) were determined in tilapia, Indian major carp rui, Chinese carp and a minor carp from local market and in laboratory simulations (0.5, 1.0, 2.0 and 4.0% formaldehyde solution for 5, 15, 30 and 60 min) with spectrophotometric and high performance liquid chromatography (HPLC) methods. A food frequency questionnaire was used to collect fish consumption (kg/kg BW.d) data from 400 respondents. A probabilistic exposure assessment was conducted using @Risk® 7.0 software. Fish treated with formalin at increasing concentrations and exposure time showed increased trends of formaldehyde acquisition irrespective of fish species and analytical methods used (p<0.05). Compared to spectrophotometry, the HPLC method was shown to be more sensitive and is therefore the preferred method for formalin quantification. Maximum exposure to formaldehyde (0.28 mg/kg BW.d) was calculated for tilapia using HPLC analysis. Margin of exposure (MoE) provides high priority (<10,000) for tilapia and Indian major carp rui at P99 under spectrophotometric analysis whereas as determined using HPLC, tilapia had MoE values much lower than 10,000 at P99, P95 and P90 (both total population and consumers). Exposure to formaldehyde associated with freshwater fish consumption is a public health concern in Southern Bangladesh and needs further assessment and risk management strategies.

Key words: Fish, formalin, formaldehyde, exposure assessment, risk characterization.
1. **Introduction**

In Bangladesh, fish and fishery products are key for food security as they supply 60% of animal protein, provide employment opportunities (approx. 16 million people) and contribute 3.69% of the gross domestic product (DoF, 2015). Available reports suggest that formaldehyde is added in the form of formalin (by dipping or spraying) to marketed fish in Bangladesh to prevent spoilage and increase shelf life (Hoque et al. 2016; Jaman et al. 2015; Yeasmin et al. 2010). Formalin typically contains a 37-40% concentration of formaldehyde. Volatile toxic aldehydes like formaldehyde are considered food contaminants and a safety and public health concern (Bianchi et al. 2007; Claeys et al. 2009). A higher concentration of formaldehyde was reported for imported fish due to the additional time it takes through various steps along the supply chain before the fish reaches domestic retail markets in Bangladesh (Rahman et al. 2016). Consumer concerns regarding the formaldehyde content in fish and other food are growing and determination of the presence of formaldehyde is therefore needed.

Rapid, accurate, easy-to-use and affordable methods are available to screen for food contaminants and toxicants (Chiou et al. 2015). The Bangladesh Council of Scientific and Industrial Research (BCSIR) has developed a simple kit to detect presence of formaldehyde in fish (Rahman et al. 2016; Yeasmin et al. 2010). However, this kit can only be used for qualitative testing of formaldehyde, i.e. whether formaldehyde is present or absent. Qualitative tests and detection kits have been used previously in Bangladesh to determine formaldehyde in freshwater and marine water fish i.e. by Islam et al. (2015) for Indian major carp (rui and catla) fish in Dhaka region; Indian major carp (rui, catla), hilsa shad, minor carp fish in Jessore district (Paul et al. 2014) and Sylhet city (Rahman et al. 2012). Spectrophotometry and high performance liquid chromatography (HPLC) are quantitative methods for formaldehyde determination. The spectrophotometric
method using Nash’s reagent and trichloroacetic acid (TCA) based extraction is considered a
dependable, convenient, fast and safe procedure for quantitative estimation of formaldehyde in fish
(Benjakul *et al.* 2006; Zhang *et al.* 2015). Jaman *et al.* (2015) conducted quantitative tests of
formaldehyde presence in Indian major carp rui, tilapia, Thai climbing perch, Ganges river sprat,
bombay duck and ribbon fish in Mymensingh using spectrophotometry. Compared to
spectrophotometry, the HPLC method is more selective and more sensitive. HPLC requires
however more expensive equipment and more know-how to operate, which is not widely available
in a developing country such as Bangladesh. Wahed *et al.* (2016) used HPLC method for
determination of formaldehyde in marketed fish and other food in Bangladesh and concluded that
the method has good analytical performance in terms of specificity, linearity, precision, recovery
and robustness, i.e. potential as a reference standard method. The different formaldehyde content
found in the different studies was therefore due to differences in performance between methods
used for determination of formaldehyde content.

Fish and fishery products can contain high levels of formaldehyde from artificially added and
endogenous sources where the foremost source is endogenous (Zhang *et al.* 2015). Formaldehyde
can be produced naturally in fish by the degradation of trimethylamine oxide (TMAO) in presence
of enzyme trimethylamine oxide demethylase (TMAOase), which catalyzes the conversion of
TMAO into dimethylamine and methanal (also known as formalin) (Bianchi *et al.* 2007; Stanley
and Hultin, 1984; Yeh *et al.* 2013; Zhang *et al.* 2015). The naturally high levels of formaldehyde
in fish complicate the accurate detection of illegally added formaldehyde (Wahed *et al.* 2015). It
should be noted that in view of the high reactivity of formaldehyde towards proteins, a substantial
part of the formaldehyde will be bound to proteins (Vandemoortele and Meulenaer, 2015).
Naturally available formaldehyde in the fish muscle is covalently bound to functional groups of
proteins and forms a cross linkage among the peptide chains (Sikorski et al. 1982). Formaldehyde binds with lysine and arginine residues in peptides. The reaction of formaldehyde with a peptide or protein starts with N-formylated products (Liu et al. 2016) and formation of unstable methylol adducts on amino and thiol groups, and also Schiff base on a lysine residue can form stable cross-links with several amino acid residues (Metz et al. 2006). It is very likely that the formation of such formaldehyde-protein adducts would be stimulated by a cooking treatment, thus restricting further its evaporation, while increasing the amount of formaldehyde potentially liberated from these complexes during the digestion process, which is relevant from a food safety perspective (Vandemoortele and Meulenaer, 2015; Vandemoortele et al. 2017).

Formaldehyde has acute or chronic toxicity with carcinogenic and mutagenic properties. The International Agency for Research on Cancer hence categorized formaldehyde in the group 1, ‘as carcinogenic to humans’ (IARC, 2004). Formaldehyde has potential carcinogenic modes of action due to its mutagenicity (formaldehyde induced DNA–protein and protein–protein cross-links) (Claeys et al. 2009; Monakhova et al. 2012). Humans could be exposed to this hazardous chemical from eating of formaldehyde contaminated fish, or via inhalation, skin or eye contact (Claeys et al. 2009). Epidemiological studies reported that oral exposure to formaldehyde at 0.17 ppm and greater can induce ulceration of the gastrointestinal tract. Those working with chemicals, furniture and in the funeral service industry have greater prevalence of irritation of the eyes or respiratory tract including loss of cilia, goblet cell hyperplasia, and cuboidal and squamous cell metaplasia in the nasal cavity (Naya and Nakanishi, 2005).

Apart from cultivated mushrooms in Belgium (Claeys et al. 2009), marketed fish in Malaysia (Aminah et al. 2013) and different food products in China (Tang et al. 2009), research regarding the exposure associated with formaldehyde is scarce. To date, no assessment of exposure and risks
of formaldehyde through fish consumption in Bangladesh has been conducted. The aim of the present study is to (i) estimate intake data and to determine formaldehyde concentration in commonly consumed fish from different steps along the fish supply chain in Patuakhali district, Southern Bangladesh, and, (ii) to assess the effect of treatment conditions using two different analytical methods under laboratory conditions. Using these data, a further probabilistic exposure assessment and risk characterization is carried out. This study assesses the risks of formaldehyde exposure in fish and provides guidance for risk managers and the national food safety authorities in Bangladesh as well as in South East Asia.

2. Materials and methods

2.1 Study area and fish sample collection

Fresh fish samples were collected from different sites covering major steps along the fish distribution channel, viz. harvest site (fish farm in Patuakhali Science and Technology University; PSTU), landing center/auction center, wholesale market and retail market in Patuakhali district of Bangladesh. Four of the most commonly available freshwater fish i.e., tilapia (Oreochromis niloticus), Indian major carp (Labeo rohita), Chinese carp (Hypophthalmichthys molitrix) and a minor carp (Amblyparyngodon mola) were selected. Collected fish samples were covered in polythene pouch (zip-lock), preserved in an ice box and brought to the laboratory. In addition, tilapia from the PSTU fish farm were treated with formaldehyde in the laboratory at different concentrations and contact times (dipping into 0.5, 1.0, 2.0 and 4.0% formaldehyde solution for 5, 15, 30 and 60 min). Formaldehyde concentration of all fish samples from different steps along the fish supply chain in Patuakhali district of Bangladesh and laboratory treated fish were determined
using the spectrophotometric method. The difference in performance between the spectrophotometric and HPLC method was also compared using tilapia as representative sample.

2.2 Determination of formaldehyde concentration

2.2.1 Chemicals

Ammonium acetate, acetyl acetone, acetic acid, trichloroacetic acid (TCA), potassium hydroxide (KOH) and hydrochloric acid (HCl) were purchased from Merck, India. Formaldehyde (37% formaldehyde) in water certified reference material (CRM) (4815 mg/L) and solvents were of analytical grade (SIGMA–Aldrich, Buchs SG, Switzerland). 2,4 dinitrophenylhydrazine (2,4 DNPH) was purchased from Merck (Darmstadt, Germany).

2.2.2 Determination of formaldehyde concentration using spectrophotometry

The spectrophotometric method with some modification (Benjakul et al. 2003) using Nash’s reagent was applied to determine the formaldehyde content (mg/kg) in fish. Nash’s Reagent was used as an indicator which helps to detect the absorbance of formaldehyde (Nash, 1953). To prepare Nash’s reagent, 15 g ammonium acetate was diluted in a 100 ml Erlenmeyer flask with an addition of 0.3 ml of acetyl acetone and 0.2 ml of acetic acid. About 30 g fish flesh was blended for 10 minutes, using a Philips HR-2106 blender. Sixty (60) mL of 6% w/w TCA was added for extraction of formaldehyde from the fish flesh. The extracted solution was then filtered by a Whatman No.1 filter paper. The pH of the solution was determined by a pH meter (pH 211, Hanna Instruments, Italy). The addition of TCA resulted in a reduced pH value of the sample which was then adjusted between 6.00-6.50 by using potassium hydroxide (KOH) and hydrochloric acid (HCl). Five (5) mL of sample solution was taken in a 50 mL volumetric/conical flask and kept in a freezer (Walton W2D-1H5, Bangladesh) at -20 °C for 1 h. The sample was taken out of the
freezer and 2 mL of previously prepared Nash’s reagent was added as indicator. The fish sample was then heated in the water bath (Fisherbrand FB60301, China) at 60 °C for 30 minutes. The absorbance of the sample in a cuvette was measured at 415 nm immediately by UV/Vis spectrophotometer (T60 UV/Visible Spectrophotometer, PG Instruments, U.K). The sample reading (triplicate) was placed in the standard curve for the calculation of formaldehyde concentration in the fish sample.

2.2.3 Determination of formaldehyde concentration using HPLC

The modified method of Wahed et al. (2015) for the determination of formaldehyde concentration (mg/kg) using HPLC was applied. A 2,4 dinitrophenylhydrazine (2,4 DNPH) working solution was recrystallized prior to use by dissolving 10 g of 2,4 DNPH in 100 mL in hot analytical grade acetonitrile to form a saturated solution. After complete dissolution, the solution was cooled to room temperature, capped in a brown bottle and stored overnight at 4 °C for crystallization. The crystals were collected by vacuum filtration. A 150 mg of 2,4 DNPH crystals were accurately weighed, dissolved in 49.5 mL of acetonitrile and mixed with 0.5 mL of phosphoric acid (85%). Derivatization kinetics followed the procedure described by Claeys et al. (2009) with slight modification. Muscle parts of the fish were used for the analysis. To sample aliquots of 5 g, 5 mL of acetonitrile were added, the sample was vortexed and then sonicated for 30 min. The samples were then centrifuged at 5000 rpm for 5 min and the supernatant was passed through a 90 mm diameter Whatman®541 (Hardened Ashless) filter paper (SIGMA–Aldrich, Buchs SG, Switzerland). Two and half milliliter of 2,4 DNPH was added to the extract and mixed well. Samples were incubated at 40 °C for 60 min in a shaking water bath (model BS-11, Oxon, UK). Formaldehyde was quantitatively converted to its Schiff base in 60 min. During analysis,
derivatization time was set to 60 min. After incubation, the acetonitrile layer was collected, membrane filtered (0.45 µm) and injected into the HPLC. Analyses were performed on a C<sub>18</sub> Luna column (25 cm × 4.6 mm id., 5 µm particle size), (Phenomenex, Utrecht, The Netherlands) using a HPLC (model SPD-M20A) coupled to a photodiode array detector (both manufactured by Shimadzu, Kyoto, Japan). The wavelength was set to 355 nm and the oven temperature at 30 °C. Separation was achieved using isocratic elution with a mixture of water/methanol (35:65, v/v). The flow rate was 1.0 mL/min and the injection volume 20 µL. The total run time was 12 min. The regression square coefficient (R<sup>2</sup>), LODs and LOQs were 0.99, 0.39 (mg/L), 1.30 (mg/L) for matrix free and 0.99, 1.75 (mg/L), 5.83 (mg/L) for matrix-matched calibrations, respectively.

2.3 Collection of fish consumption data

Consumption data were collected via convenience sampling i.e. 400 adult respondents in Patuakhali district of Bangladesh from June 2015 to February 2016 were invited to participate in the survey. Socio-demographic (age, sex, weight, family member, income source etc.) data of the respondents were also collected. Fish consumption data were collected by Master students from PSTU who received training as interviewers beforehand, using face-to-face interviews. The interviewers explained the procedures and objective of the survey to the respondents, after which they administered a structured food frequency questionnaire. The respondents were asked to estimate the portion size of each fish consumed by the family. This estimate was divided by the total household size to obtain the quantity of fish consumed by the respondents, under the assumption that all consumers in the respondent’s family consume a similar amount of fish. Next, the respondents were asked ‘how often do you eat a particular fish, categorized as daily or 7 times a week, 5 times a week, 3 times a week, once a week, once per two weeks, once per four weeks or
never. The responses were first converted to a daily consumption using a conversion factor (i.e. 7
times a week corresponds to 1/day; 5 times a week corresponds to 5/7, 3 times a week corresponds
to 3/7, 1 time a week corresponds to 1/7, 1 time per two weeks corresponds to 1/15, 1 time per
four weeks corresponds to 1/30 and never corresponds to non-consumer who do not like or eat that
specific fish), followed by a multiplication of the amount of fish consumed by the respondent. The
estimate was divided by the body weight (kg) of the respective respondent as representing the body
weight of the whole family (adult members). The body weight of the respondent was measured
using a digital weighing scale (Sagas weighing scale, India). Using these estimates, the fish
consumption dataset kg/kg BW.d was obtained for both consumer and non-consumer in respect to
each fish species.

2.4 Probabilistic exposure assessment

To evaluate the population risk associated with consumption of formaldehyde contaminated fish,
a probabilistic exposure assessment was conducted. It was assumed that the food processing factor
(washing, freezing or cooking) of fish as a traditional consumer practice of Bangladesh does not
affect the formaldehyde concentration in fish (worst case scenario). As noted earlier in this paper,
in view of the high reactivity of formaldehyde towards proteins, part of the formaldehyde is likely
to be bound to proteins, and formation of such formaldehyde-protein adducts could be stimulated
by cooking, restricting its evaporation. Calculations were done for the actual fish consumer
(consumer of specific fish) and for the total population (consumers and non-consumers). The total
population (consumer and non-consumer) refers to the total number of respondents (e.g. 400)
whereas consumer refers to number of respondents who actually consume specific fish (e.g. out of
400, 350 respondents consume rui fish); in this case for rui, 350 respondents are consumers and
the remaining 50 respondents are non-consumers. The inclusion of non-consumers was used to assess the chronic exposure.

@Risk®7.0 for Microsoft Excel 2010 (Palisade Corporation, USA), was used with different fish consumption data (kg/kg BW.d) and formaldehyde concentration (mg/kg) distributions from both spectrophotometric and HPLC method were combined into an exposure distribution (mg/kg BW.d). Best fit distributions were determined for consumption and formaldehyde concentration (both spectrophotometric and HPLC method) using the Chi-square statistics, probability/probability (P/P) and quantile/quantile (Q/Q) plot. For the exposure calculations of the whole population (including consumers and non-consumers), a logical “if” function was applied combining the zero consumption of the fraction of non-consumers and the distribution of the consumption for the fraction of the consumers. First-order Monte-Carlo simulations were undertaken considering 50,000 iterations. The simulations were repeated three times to ensure that stable estimates. Formaldehyde intake (mean, standard deviation, maximum, minimum and percentiles) was determined from the output of the simulation model.

2.5 Risk characterization

Risk characterization of the carcinogenic and genotoxic formaldehyde was carried out compared with the results from the probabilistic exposure assessments with the corresponding margin of exposure (MoE) approach using the benchmark dose lower confidence level (BMDL_{10}) of 23 mg kg^{-1} day^{-1} for formaldehyde (Monakhova et al. 2012). To calculate the MoE, formaldehyde exposure estimated for both total population and consumers were used from both spectrophotometric (all fish species) and HPLC (Tilapia only) methods. MoE were calculated from a chosen point of departure (PoD) on the dose–response curve (lower limit of the benchmark dose
estimate at 95% confidence where 10% of responses achieved BMDL_{10} divided by the human dietary exposure estimated, using following formula:

\[ \text{MoE} = \frac{\text{BMDL}_{10}}{\text{Human exposure}} \]

2.6 Statistical analysis

For the both spectrophotometric and HPLC method, each sample was analyzed in triplicate (n=3). Data were subjected to analysis of variance (ANOVA) and comparison of means was carried out by Duncan’s multiple range test (Steel and Torrie, 1980). Statistical significance was accepted at a P value of <0.05. The statistical analysis was performed using the SPSS package (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Prevalence and concentration of formaldehyde in different fish from different steps along the supply chain in Southern Bangladesh

The formaldehyde concentration of four different fish collected in different steps along the supply chain is shown in Table 1. In spectrophotometric analysis, a significant variation in formaldehyde concentration was observed when the same species of fish was collected from different steps along the supply chain. Indian major carp rui had the highest (1.68 mg/kg) formaldehyde concentration when collected from the retail market while lowest concentrations were found (0.77 mg/kg) in samples collected from the fish farm (p<0.05). In case of Chinese carp, the highest and lowest formaldehyde concentrations were 0.82 and 1.50 mg/kg for samples collected from fish landing center and fish farm, respectively. Among the four different fish, the highest (2.08 mg/kg) formaldehyde concentration was found in tilapia samples from the landing site (p<0.05). Minor
carp contained a very low amount of formaldehyde compared to other fish and along any steps of the supply chain considered (range 0.43 to 0.93 mg/kg). Using the same analytical method, variations in formaldehyde concentration in tilapia from different locations were reported previously. Jaman et al. (2015) found formaldehyde concentrations of 1.85, 2.53 and 2.50 mg/kg in tilapia fish from different fish markets in Mymensingh district in Bangladesh, which were higher than the present findings. With the same spectrophotometric method, Jaman et al. (2015) reported formaldehyde concentrations of 1.45 mg/kg for rui and 7.00 to 7.35 mg/kg for small indigenous species kachki. Aminah et al. (2013), Bianchi et al. (2007) and Noordiana et al. (2011) also observed differences in formaldehyde content in different fish species.

A significant higher formaldehyde concentration was observed in HPLC analyzed fish irrespective of sample source/origin (p<0.05) (Table 1). Differences in formaldehyde concentration determined with the two different methods might be due to differences in sample preparation, extractions, recovery and detection limit of the respective methods. Chiou et al. (2015) reported that the accuracy of conventional testing methods was generally not as good as that of sophisticated instrumental methods such as HPLC, high performance liquid chromatography-mass spectrometry (HPLC-MS), gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) of which the limit of detection (LOD) is much lower and could be competent for quantitative analysis. Chiou et al. (2015) also reported that chromatography could easily separate and detect corresponding hydrazine derivates from reaction between formaldehyde and 2,4-dinitrophenylydrazine (2,4-DNPH) chemical derivatives. In the present study 2,4-DNPH was also used to determine the formaldehyde concentration in tilapia fish by using HPLC, this was not used in Nash’s reagent basis spectrophotometric method, and might explain the different results obtained from these two studies.
Furthermore, a non-significant difference in formaldehyde concentration between tilapia fish samples from the university fish farm and other steps along the supply chain was found (p<0.05, Table 1), indicating a natural occurrence of formaldehyde in fish. Several studies reported a natural occurrence of formaldehyde in fish, aquatic products and seafood (Bianchi et al. 2007; Chiou et al. 2015; Claeys et al. 2009). During post-mortem changes, TMAO is formed in fish from the post-mortem enzymatic reduction of TMAO to equi-molar amounts of formaldehyde and dimethylamine. The differences in formaldehyde content in different fish could be due to differences in the amount of TMAO formed upon death and during storage of fish (Bianchi et al. 2007; Nielsen and Jørgensen, 2004). Wahed et al. (2016) also reported that formaldehyde occurs naturally in free and bound forms. Therefore, the findings from present and previous studies indicate that formaldehyde content in fish could vary with species, habitat of fish, compositional differences, processing method used, storage time, storage temperature and differences in response to reaction between fish protein and formaldehyde.

3.2 Formaldehyde concentration in laboratory treated fish measured by spectrophotometric method

Figure 1 indicates that at the same concentration of formaldehyde dipping solution, formaldehyde concentration in fish muscle gradually increases with increasing dipping time regardless of fish species. On the other hand, at the same dipping time with increasing concentration of formaldehyde dipping solution, increased trends in formaldehyde concentration were found for each fish. At 0.5% concentration formaldehyde dipping solution for 5 min, Indian major carp fish muscle had a formaldehyde concentration of 5.65 mg/kg. For the same species, at the same concentration with the increasing dipping time at 15, 30 and 60 min, the formaldehyde concentration significantly (p<0.05) increased to 6.60, 7.77 and 8.32 mg/kg, respectively. The result also shows that for the same species and same dipping time (5 min), the formaldehyde
concentration gradually increased from 5.65 to 95.15 mg/kg when fish was treated with 0.5% to 4% formaldehyde solution (Figure 1). Similar trends in the increase of formaldehyde concentrations were observed for higher dipping time (15, 30 and 60 min) when other higher concentration (1, 2 and 4%) formaldehyde treatments were used. A significant increase (p<0.05) in formaldehyde concentration with increased concentrations of formaldehyde dipping solution and dipping time was also noticed, regardless of the fish. For all fish species treated under laboratory conditions, the concentration of formaldehyde dipping solution more markedly influenced the formaldehyde concentrations in the samples than dipping time. Yeasmin et al. (2013) also studied Indian major carp rui fish dipped in different concentrations (5 – 35%) of formalin solution for 5 min each and reported that at increased concentrations of dipping solution a correspondingly longer duration was required for removal of formalin in water. Removal of formalin from the surface of fish body also depends on the concentration of formalin with the time needed for removal being directly proportionate to the concentration of formalin used to treat the fish.

Figure 1 compares the formaldehyde concentration in different fish with the same concentration of formaldehyde dipping solution and dipping time used. There were differences (p<0.05) in formaldehyde concentration between the fish given a particular exposure concentration and dipping time. At 0.5% formaldehyde concentration dipping solution and 5 min dipping time, Indian major carp rui contained higher formaldehyde concentration (5.65 mg/kg) than any other species where Chinese carp contained the lowest value (2.92 mg/kg) (p<0.05). However, of the four species, the Chinese carp had a higher formaldehyde concentration when 1, 2 and 4% solutions were used for 5 and 15 min respectively (p<0.05). At the highest concentration dipping solution (4%) and dipping time (60 min) treatment, minor carp showed the highest formaldehyde
concentration value (186.82 mg/kg) (p<0.05). Under the same laboratory treatment condition, the variations in formaldehyde concentration between different fish species might be due to differences in biochemical composition of fish muscle. Analysis of proximate biochemical compositions of the studied fish (data not shown) confirm differences in major protein composition between the studied fish species. Chemical reactions between fish muscle of different biochemical composition and formaldehyde might have resulted in differences in formaldehyde concentrations between the species. Naturally produced or available formaldehyde in the fish muscle is covalently bound to functional groups of proteins and forms a cross linkage among the peptide chains (Sikorski et al. 1982). Yeh et al. (2013) distinguished formaldehyde bonding types in fishery products as free, bound and total formaldehyde. In another study Metz et al. (2006) and Yeh et al. (2013) reported several unstable and stable reactions of formaldehyde with several amino acid residues.

### 3.3 Formaldehyde concentration in laboratory treated tilapia fish measured by spectrophotometric and HPLC method

Formaldehyde concentrations in tilapia collected from different steps along the supply chain were determined using both spectrophotometric and HPLC methods. A considerable increase in detection of formaldehyde concentration by HPLC over spectrophotometric method (p<0.05) was noted (Table 1). Therefore, the HPLC method was also applied to measure formaldehyde concentrations in tilapia under laboratory conditions. Figure 2 compares formaldehyde concentrations of tilapia fish treated with different concentrations of formaldehyde dipping solution and dipping times under laboratory conditions determined with spectrophotometric and HPLC methods. At any concentration of formaldehyde dipping solution and dipping time used, significantly higher formaldehyde concentration readings were obtained by HPLC method in
comparison to the spectrophotometric method (p<0.05). At a condition of dipping into 0.5% for
5 min, the formaldehyde concentration determined by HPLC was approximately three times (from
3.43 to 10.87 mg/kg) higher than that by the spectrophotometric method. Similar to the
spectrophotometric results for different laboratory treated fish (Figure 1), the HPLC method also
showed increasing formaldehyde concentrations with formaldehyde dipping solutions at
increased concentrations and dipping time (Figure 2). At 0.5% dipping solution, formaldehyde
concentration detection at increasing dipping time from 5 to 60 min was about doubled for both
spectrophotometric (3.43 to 6.08 mg/kg) and HPLC (10.87 to 21.05 mg/kg) method. On the other
hand, with increasing concentrations of dipping solution from 0.5 to 4%, formaldehyde
concentration for the same time period (5 min), detection was around thirty times higher for both
spectrophotometric and HPLC methods. Nevertheless, the formaldehyde concentrations detected
by HPLC were much higher than those detected by the spectrophotometric method, which
indicates that the HPLC method is more sensitive and accurate than the spectrophotometric
method to determine formaldehyde concentrations in fish samples. In this last method,
colorimetric reaction in acid extracted sample distillates produces a purple color in presence of
formaldehyde. The intensity of the color is proportional to the formaldehyde concentration and
can be measured by UV spectrophotometer. Drawbacks of colorimetric methods are their poor
specificity, selectivity, prolonged analysis times and highly acidic conditions, which together lead
to over-reporting and/or false positives. Several studies reported that formaldehyde occurs
naturally in free and bound forms and produces different amino acid residues of protein with
different covalent cross-links, reversible, acid liable, acid resistant and schiff base reactions (Liu
spontaneous reaction of formaldehyde with protein could make formaldehyde unavailable for
colorimetric reaction. This is the likely reason that in this study spectrophotometric analysis showed lower formaldehyde concentrations than HPLC, under the same treatment. Formaldehyde reported by spectrophotometric procedure should hence be considered free formaldehyde as opposed to total formaldehyde. Yeh et al. (2013) found that the sum of the concentrations of free and reversibly bound formaldehyde was higher by 19.3 mg/kg than the free formaldehyde concentration in the HPLC method. Free formaldehyde constituted an average of 39% of total free and reversibly bound formaldehyde in the HPLC method. Mason et al. (2004) stated that under harsh conditions of steam distillation with acid, formaldehyde released was the sum of free and reversibly bound formaldehyde. Therefore, the measured formaldehyde concentrations were relatively high. From this study, it can be concluded that performance of the widely used spectrophotometric method is low compared to formaldehyde detection by HPLC.

3.4 Formaldehyde exposure assessment

Using the probabilistic exposure assessment in this study, one data set for formaldehyde concentration of all samples collected from different steps along the supply chain was constructed for each fish. No difference in formaldehyde concentration was observed in tilapia fish collected along different steps of supply chain using the HPLC method (Table 1).

3.4.1 Fish consumption data

Cumulative consumption (kg/kg BW.d) of different fish by all respondents (non-consumer and consumer) are presented in Figure 3. Fish consumption survey data were collected from 400 adults in the Patuakhali district of Bangladesh. Amongst the total sample (n=400), non-consumers were identified for the respective fish. Among the fish, the highest percentage (54.5%) of non-consumers was identified for Chinese carp, followed by minor carp (38.0%). Highest fish consumption was observed for Indian major carp rui and tilapia, where only 11.3 and 26.8% were
non-consumers, respectively. Mean fish consumption for Indian major carp rui, minor carp, Chinese carp and tilapia was 0.00042, 0.00015, 0.00027 and 0.00044 kg/kg BW.d, for consumers; and 0.00037, 0.00011, 0.00012 and 0.00031 kg/kg BW.d, for total population, respectively (Table 2).

Wahed et al. (2016) reported average consumption of fish at 50.3 g/person/d in Bangladesh. National data estimate average fish consumption in Bangladesh at 56 g/person/d (DoF, 2015). However, in our study, the estimated fish consumption was lower compared to the national average fish consumption. Only four species were considered in the present study whereas in the national data all fish consumption is considered. Differences in the consumption between species can be clarified due to differences in characteristics and sensory properties. Chinese carp is a bony fish with huge intramuscular bone and minor carp is a small indigenous species having less flesh, which is consumed as whole fish with its skeletal bones. As an Indian major carp rui is preferred by consumers for its high meat flesh, less bone and unique taste. However, non-consumption of Indian major carp rui fish might be a result of low purchasing power of consumers as Indian major carp rui is comparatively more expensive than the other three fish sampled. In Bangladesh, tilapia is cheaper and known to be fish for the poor people. Consumers from higher socio-economic classes generally do not consume tilapia due to its muddy flavor (Mikael et al. (2014). Belton et al., (2011) reported a preference for Indian major carp rui, tilapia and Chinese carp by 26, 23 and 8% respectively, of the consumers surveyed.

3.4.2. Probabilistic exposure assessment

To identify the best fit distribution in the probabilistic exposure analysis, fish consumption and natural occurrence of formaldehyde concentration in different fish measured with spectrophotometric and HPLC methods were fitted in the @Risk software. From the result, the P–
P plot provided roughly a straight line joining the diagonals for both consumption (consumers) of different fish and formaldehyde concentration in the respective fish. When fitting the distribution for consumption of different fish, the consumer and total population (including consumers and non-consumers) were separately considered due to the presence of zero consumption patterns of the respective fish. The best fit distributions for both the formaldehyde concentration and consumption (consumers and total population, including consumers and non-consumers) of fish are shown in Table 2. The calculated dietary exposures due to formaldehyde concentration in fish measured with two different methods are shown in Table 3. Based on spectrophotometric concentration data, the mean formaldehyde intake due to Indian major carp fish consumption was 2.41E-04 and 4.87E-04 mg/kg BW.d for total population and consumers, respectively. Mean formaldehyde intake was much lower for minor carp (4.61E-05 and 9.24E-05 mg/kg BW.d) and Chinese carp (1.64E-04 and 3.25E-04 mg/kg BW.d) than Indian major carp and tilapia, in case of both total population and consumers. Formaldehyde intake for tilapia had the highest mean value (3.64E-04 and 7.30E-04 mg/kg BW.d). Moreover, a further increase in mean value of formaldehyde exposure 1.45E-03 and 2.91E-03 mg/kg BW.d was observed for tilapia when exposure was calculated based on formaldehyde concentration data from HPLC method.

Based on HPLC determined formaldehyde concentration data, tilapia consumers were exposed to a maximum of 0.28 mg/kg BW.d. Exposure above zero level is considered harmful for formaldehyde, as genotoxic carcinogen (JECFA, 1998) and should be maintained as low as reasonable amount (ALARA). Limitations in the detection method (poor sensitivity, specificity explained in section 3.3) might result in an underestimate of concentrations of formaldehyde ingested (Wahed et al. 2016). The real exposure of formaldehyde could hence be higher than that suggested by the method of analysis. Based on a proper formaldehyde detection method,
formaldehyde exposure due to consumption of tilapia fish is a health concern for the population studied in Southern Bangladesh. However, from deterministic exposure analysis Wahed et al. (2016) reported low or no risk of human exposure to formaldehyde in fish in Bangladesh. Average human exposure to formaldehyde from alcoholic beverages was estimated at $8.0 \times 10^{-5}$ mg/kg/d and the resulting MoE was above 20,000 which may be considered a negligible risk of cancer from formaldehyde with alcohol consumption, but a priority for risk management (Monakhova et al. 2012). The formaldehyde exposure through the consumption of cultivated mushrooms was 0.19 µg/kg·day for consumers only and 99 µg/kg·day for total population and it was concluded that the dietary exposure to formaldehyde was not a cause for concern (Claeys et al. 2009). The present study indicates that, estimation of human exposure to formaldehyde in fish consumption is dependent on the method, with the HPLC method providing a more accurate way to determine free formaldehyde. Yeh et al. (2013) report a detailed analytical method applied for formaldehyde determination in fish and also suggested to measure the free formaldehyde as it is of toxicological interest.

### 3.5. Risk characterization due to formaldehyde exposure in fish based on toxicological value

To characterize the formaldehyde exposure risk, the margin of exposure (MoE) was calculated using the BMDL$_{10}$ of rodent data (Monakhova et. al. 2012) for the different fish under two different analytical methods (Table 4). According to EFSA (2005), a MoE greater than 10,000 could be considered as low health concern. Based on spectrophotometric data, the MoE for minor carp and Chinese carp calculated based on the exposures to formaldehyde concentrations at different percentiles (P50, P75, P95, P99) for both total population and consumers only were considerably higher than 10,000. However, calculated MoE at P99 was lower than 10,000 both for total population and consumers only, at 8,240 and 6,270 respectively for Indian major carp rui, and
5,560 and 3,940 respectively for tilapia. In addition, at P95 tilapia also showed MoE exposure (9,030) below 10,000 for consumers only.

Based on HPLC determined formaldehyde concentration data, MoE for tilapia was lower than 10,000 for formaldehyde exposure at P90, P95 and P99 in case of both total population and consumers only. For the same fish, MoE at the same percentile (P99) was much lower for the HPLC determined sample than the spectrophotometric one for both total population and consumer only. On the other hand, for the same analytical method (HPLC) and at higher percentile, MoE values were lower than that of lower percentile (MoE, P99<P95<P90) for both total population and consumer only, indicating potential health risk from consuming the respective fish in the studied area of Bangladesh.

3.6. Uncertainty evaluation of the exposure assessment

Uncertainties associated with exposure assessments need to be considered when interpreting the results. Factors intrinsic to fish consumption surveys such as under/over reporting of consumption data, misreporting of consumed fish and the erroneous estimation of consumed quantities (based on respondent’s perception) could contribute to both under and over estimation of fish consumption as well as concentration of contaminants which affects the exposure assessment. Moreover, freezing, proper washing and cooking of fish can alter the concentration of formaldehyde compared to that in fresh marketed fish. The analytically determined formaldehyde concentration in the food can underestimate the actual amount of formaldehyde liberated during the digestion process, which is relevant from a food safety perspective. Further research could elaborate the various aspects that affect the evaporation of formaldehyde. Variations in LOD, LOQ and recovery values for different formaldehyde detection methods might lead to over/under estimation of formaldehyde concentrations which is also supported by the present study results in
Table 1 and Figure 2. Due to the natural occurrence of formaldehyde in fish and high reactivity of added chemicals, reaction with food protein and formation of new adducts could contribute to underestimation of formaldehyde in fish.

4. Conclusion

Under both marketed and laboratory conditions, the HPLC method was more effective in determining formaldehyde concentration than the spectrophotometric method. Differences in formaldehyde concentration between different steps along the fish supply chain were observed by the spectrophotometric method. However the HPLC method had higher detection levels and yielded similar formaldehyde concentration estimates in tilapia from different steps along the fish supply chain. Increased trends in formaldehyde concentration were observed in fish treated under laboratory conditions with increasing concentrations of dipping solution and dipping time, irrespective of species. Natural occurrence of formaldehyde in fish and different reactions between highly reactive formaldehyde and fish protein amino acid residues might result in different magnitudes in determined formaldehyde concentration in fish varying by species, site, concentration of dipping solution and dipping time.

Formaldehyde exposure from the consumption of Indian major carp rui, minor carp, Chinese carp and tilapia fish was assessed by probabilistic exposure assessment using fish consumption and formaldehyde concentrations respectively analyzed under two different methods. Food frequency questionnaire results indicate higher consumption of Indian major carp rui and tilapia than Chinese carp and minor carp. Estimates of exposure to formaldehyde from consumption of four different fish were lower with spectrophotometric analysis than with HPLC analysis for both total population and consumer only. Maximum exposure of formaldehyde (0.28 mg/kg BW.d) was estimated for tilapia (consumers only) under HPLC method which might cause health concerns.
MoE provides high priority (<10,000) of risk from exposure for tilapia and Indian major carp rui at P99 under spectrophotometric analysis for both total population and consumer only. Under HPLC analysis, tilapia had much lower MoE values for both total population and consumers only at higher to lower percentiles (P99, P95 and P90) indicating risk priorities. Exposure to formaldehyde with tilapia fish consumption is a possible health concern for the population in the Southern district of Bangladesh. Therefore, priority should be given to formulating a proper risk management strategy on the basis of knowledge of endogenous formaldehyde present in fish. The MoE results from this study could be used to compare the risk of formaldehyde intake from consumption of different species of fish, between the methods used for formaldehyde determination, and to prioritize risk management strategies for the fish consumer in Bangladesh.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**Acknowledgments**

The authors are very grateful to the International Foundation for Science (IFS), Sweden for the research grant (research grant agreement number E/5670-1). The authors also would like to acknowledge ITP Food Safety program, Department of Food Safety and Food Quality, Faculty of Bioscience, Ghent University, Belgium and the Flemish Interuniversity Council (VLIR-UOS), Belgium for scientific and research stay support. The laboratory analytical support from National Food Safety Laboratory (NFSL), Dhaka, Bangladesh is also highly acknowledged. We acknowledge Dr. Martin L. van Brakel, Scientist, ECOFISH-Bangladesh project, WorldFish South Asia, Dhaka, Bangladesh for his additional comments on an earlier version of our manuscript.
References


EFSA, (2005). European Food Safety Authority opinion from the scientific committee on request from EFSA related to a harmonized approach of risk assessment of a substances which are both genotoxic and carcinogenic. EFSA J. 282, pp 1-31.


Table 1: Formaldehyde concentration of different fish obtained from different steps of fish supply chain measured by using spectrophotometric and HPLC method

<table>
<thead>
<tr>
<th>Method</th>
<th>Fish species</th>
<th>Formaldehyde concentration (mg/kg)</th>
<th>Fish farm</th>
<th>Fish landing center</th>
<th>Wholesale market</th>
<th>Retail Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometric</td>
<td>Indian major carp rui</td>
<td>0.77±0.03&lt;sup&gt;c&lt;/sup&gt;C</td>
<td>1.03±0.03&lt;sup&gt;b&lt;/sup&gt;B</td>
<td>1.03±0.03&lt;sup&gt;b&lt;/sup&gt;C</td>
<td>1.68±0.06&lt;sup&gt;a&lt;/sup&gt;A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minor carp</td>
<td>0.43±0.03&lt;sup&gt;d&lt;/sup&gt;D</td>
<td>0.55±0.00&lt;sup&gt;c&lt;/sup&gt;D</td>
<td>0.67±0.03&lt;sup&gt;b&lt;/sup&gt;D</td>
<td>0.93±0.03&lt;sup&gt;a&lt;/sup&gt;D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chinese carp</td>
<td>1.50±0.00&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>0.82±0.03&lt;sup&gt;d&lt;/sup&gt;C</td>
<td>1.35±0.00&lt;sup&gt;b&lt;/sup&gt;B</td>
<td>1.20±0.05&lt;sup&gt;c&lt;/sup&gt;C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Tilapia</td>
<td>B&lt;sup&gt;1.08±0.03&lt;/sup&gt;DB</td>
<td>B&lt;sup&gt;2.08±0.05&lt;/sup&gt;DB</td>
<td>B&lt;sup&gt;1.83±0.03&lt;/sup&gt;BA</td>
<td>B&lt;sup&gt;1.43±0.06&lt;/sup&gt;cB</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>*Tilapia</td>
<td>A&lt;sup&gt;6.62±0.84&lt;/sup&gt;aA</td>
<td>A&lt;sup&gt;6.92±0.66&lt;/sup&gt;aA</td>
<td>A&lt;sup&gt;6.44±0.65&lt;/sup&gt;aA</td>
<td>A&lt;sup&gt;5.60±0.53&lt;/sup&gt;a</td>
<td></td>
</tr>
</tbody>
</table>

Means ± standard deviation (n=3); Different small alphabet in the same row represent significant difference (p<0.05) in formaldehyde contents in same species from different steps of supply chain; and different capital alphabet in the same column represent significant difference (p<0.05) in formaldehyde contents in different fish species from same steps of supply chain.

(*) indicates the comparison between the methods and different capital alphabet in the right side of value indicates significant difference (p<0.05).
Table 2: Best fit distributions and descriptive statistics (min, mean, median and max) for different fish of formaldehyde concentrations (mg/kg) and consumption of fish (kg/kg BW.d) under both analytical methods applied for the probabilistic exposure assessment.

<table>
<thead>
<tr>
<th>Inputs of exposure assessment</th>
<th>Best fit distribution function</th>
<th>Min.</th>
<th>Mean</th>
<th>Median</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of formaldehyde (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indian major carp rui</td>
<td>RiskBetaGeneral(0.18516, 0.26237, 0.75000, 1.75000, RiskName(&quot;FA Concentration in Indian major carp rui fish (mg/kg)&quot;))</td>
<td>0.75</td>
<td>1.16</td>
<td>1.01</td>
<td>1.75</td>
</tr>
<tr>
<td>Minor carp</td>
<td>RiskLogistic(0.61200, 0.20802, RiskName(&quot;FA Concentration in Minor carp (mg/kg)&quot;))</td>
<td>-∞</td>
<td>0.61</td>
<td>0.62</td>
<td>+∞</td>
</tr>
<tr>
<td>Chinese carp</td>
<td>RiskExtValueMin(1.3351, 0.19389, RiskName(&quot;FA Concentration in Chinese carp fish (mg/kg)&quot;))</td>
<td>-∞</td>
<td>1.22</td>
<td>1.26</td>
<td>+∞</td>
</tr>
<tr>
<td>Tilapia</td>
<td>RiskExtValueMin(1.7954, 0.32482, RiskName(&quot;FA Concentration in Tilapia fish (mg/kg)_Spectro&quot;))</td>
<td>-∞</td>
<td>1.61</td>
<td>1.68</td>
<td>+∞</td>
</tr>
<tr>
<td>Tilapia_HPLC</td>
<td>RiskLogistic(6.38689, 0.44836, RiskName(&quot;FA concentration in Tilapia fish (mg/kg)_HPLC&quot;))</td>
<td>-∞</td>
<td>6.39</td>
<td>6.39</td>
<td>+∞</td>
</tr>
</tbody>
</table>

| Consumption of different fish species (kg/kg BW.d) by consumer | | | | | |
| Indian major carp rui | RiskInvGauss(0.00043263, 0.00023271, RiskShift(-0.000015392), RiskName("Consumption of Indian major carp rui (kg/kg BW.d)_Consumer")) | -1.54 | 0.00042 | 0.00022 | +∞ |
| Minor carp | RiskLognorm(0.00015215, 0.00019558, RiskShift(0.00000115741), RiskName("Consumption of Minor carp (kg/kg BW.d)_Consumer")) | 1.64 | 0.00015 | 0.00010 | +∞ |
| Chinese carp | RiskGamma(0.95289, 0.00026658, RiskShift(1.15741e-005), RiskName("Consumption of Chinese carp (kg/kg BW.d)_Consumer")) | 1.16 | 0.00027 | 0.00018 | +∞ |
| Tilapia | RiskLognorm(0.0004963, 0.000077965, RiskShift(5.26553e-006), RiskName("Consumption of Tilapia (kg/kg BW.d)_Consumer")) | 5.27 | 0.00044 | 0.00023 | +∞ |

| Consumption of different fish species (kg/kg BW.d) by total population | | | | | |
| Indian major carp rui | IF(RAND()>fraction of non-consumer,RiskInvgauss(0.00043263, 0.00023271, RiskShift(-0.000015392), RiskName("Consumption of Indian major carp rui (kg/kg BW.d)_Consumer")),0) | 0.00 | 0.00037 | 0.00017 | +∞ |
| Minor carp | IF(RAND()>fraction of non-consumer,RiskInvgauss(0.00043263, 0.00023271, RiskShift(-0.000015392), RiskName("Consumption of Minor carp (kg/kg BW.d)_Consumer")),0) | 0.00 | 0.00011 | 0.00005 | -∞ |
| Chinese carp | IF(RAND()>fraction of non-consumer,RiskGamma(0.95289, 0.00026658, RiskShift(1.15741e-005), RiskName("Consumption of Chinese carp (kg/kg BW.d)_Consumer")),0) | 0.00 | 0.00012 | 0.00 | +∞ |
| Tilapia | IF(RAND()>fraction of non-consumer,RiskLognorm(0.0004963, 0.000077965, RiskShift(5.26553e-006), RiskName("Consumption of Tilapia (kg/kg BW.day)_Consumer")),0) | 0.00 | 0.00031 | 0.00012 | +∞ |
Table 3: Probabilistic dietary exposures (mg/kg BW.d) associated with consumption of different fish contaminated with formaldehyde determined by using spectrophotometric and HPLC methods.

<table>
<thead>
<tr>
<th>Descriptive Level</th>
<th>Exposure due to formaldehyde contaminated fish consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spectrophotometric</td>
</tr>
<tr>
<td></td>
<td>Indian major carp</td>
</tr>
<tr>
<td></td>
<td>TP</td>
</tr>
<tr>
<td>Min.</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>2.41E-04</td>
</tr>
<tr>
<td>SD</td>
<td>5.79E-04</td>
</tr>
<tr>
<td>P50</td>
<td>5.77E-06</td>
</tr>
<tr>
<td>P75</td>
<td>2.34E-04</td>
</tr>
<tr>
<td>P90</td>
<td>6.65E-04</td>
</tr>
<tr>
<td>P95</td>
<td>1.15E-03</td>
</tr>
<tr>
<td>P99</td>
<td>2.79E-03</td>
</tr>
<tr>
<td>Max.</td>
<td>1.73E-02</td>
</tr>
</tbody>
</table>

'TP' and 'Cons.' Refer to Total Population and Consumers, respectively.
Table 4: Margin of exposures associated with consumption of different fish contaminated with formaldehyde determined by using spectrophotometric and HPLC methods.

<table>
<thead>
<tr>
<th>Different Percentile</th>
<th>Margin of Exposure</th>
<th>Spectrophotometric</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TP</td>
<td>Cons.</td>
</tr>
<tr>
<td>P50</td>
<td></td>
<td>3.99E+06</td>
<td>9.76E+04</td>
</tr>
<tr>
<td>P75</td>
<td></td>
<td>9.85E+04</td>
<td>4.20E+04</td>
</tr>
<tr>
<td>P90</td>
<td></td>
<td>3.46E+04</td>
<td>1.97E+04</td>
</tr>
<tr>
<td>P95</td>
<td></td>
<td>2.00E+04</td>
<td>1.28E+04</td>
</tr>
<tr>
<td>P99</td>
<td></td>
<td><strong>8.24E+03</strong></td>
<td><strong>6.27E+03</strong></td>
</tr>
</tbody>
</table>

Values exceeding the MoE with high priority (MoE<10,000) are shown in bold.

‘TP’ and ‘Cons.’ Refer to Total Population and Consumers only, respectively.

‘N/A’ referred to Not Available.
Figure 1:
Figure 2:
Figure 1: Formaldehyde concentration (mg/kg) in different fish treated with different concentration of formaldehyde and dipping time at laboratory condition measured by using spectrophotometric method. Means ± standard deviation (n=3).

Figure 2: Formaldehyde concentration (mg/kg) in tilapia fish treated with different concentration of formaldehyde at different dipping time at laboratory condition measured by using spectrophotometric and HPLC method. Means ± standard deviation (n=3).

Figure 3: Cumulative consumption (kg/kg BW.d) of different fish by total respondents (non-consumer and consumer).
Highlights

- Natural occurrence of formaldehyde in fish is species and source dependent
- Increased concentration and exposure time increase formaldehyde acquisition
- HPLC provides better quantification of formaldehyde in fish than spectrophotometry
- HPLC confirms risk associated with consumption of formaldehyde treated fish
- Formaldehyde exposure through tilapia consumption is a public health concern