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Posted Date: October 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11250952/v1

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Formaldehyde in locally landed and imported frozen fish from a Ghanaian fishing harbor: occurrence, compliance with regulatory limit, and dietary risk

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Highlights

- Formaldehyde was detected in all tuna, salmon and herring samples (0.77–11.01 mg/kg)
- Two of three imported salmon samples exceeded the 5 mg/kg reference limit for fish
- Salmon levels exceed plausible TMAO-derived natural formation in low-TMAO species
- Hazard quotients (0.004–0.072) indicate low non-carcinogenic risk at mean intake
- Targeted surveillance of the imported frozen-fish cold chain is recommended

Abstract

Formaldehyde, an IARC Group 1 human carcinogen, forms naturally in fish muscle through post-mortem demethylation of trimethylamine *N*-oxide (TMAO) but is also applied illegally as formalin to extend the apparent freshness of fish. No quantitative data were available for fish traded on the Sekondi–Takoradi coast of Ghana. Three individual fish each of locally landed tuna and herring and of imported frozen salmon (market names; $n = 9$) were purchased from separate vendors at the principal fishing harbour, extracted with 6% (w/v) trichloroacetic acid and analysed by 3-methyl-2-benzothiazolinone hydrazone (MBTH) spectrophotometry at 628 nm. Calibration was linear over 0.5–2.5 mg L^{-1} ($R^2 = 0.9992$), with limits of detection and quantification of 0.17 and 0.50 mg kg^{-1} (tissue basis). Formaldehyde was quantified in every sample, at 0.77–11.01 mg kg^{-1} wet weight (overall mean 4.21 ± 2.93 mg kg^{-1}). Species means were 2.12 ± 1.18 (tuna), 7.12 ± 3.52 (salmon) and 3.39 ± 0.66 mg kg^{-1} (herring). Two salmon samples (6.18 and 11.01 mg kg^{-1}) exceeded the 5 mg kg^{-1} reference limit, whereas all tuna and herring complied under the conventional extraction-volume calculation. Because both salmon extracts exceeded the absorbance of the highest calibration standard, these exceedances are robust even though their magnitudes were extrapolated. Interspecific differences were not significant (one-way ANOVA, $F_{2,6} = 4.29$, $p = 0.070$) despite a large effect size ($\eta^2 = 0.59$), reflecting low statistical power. The magnitude of the salmon values, the low-to-moderate TMAO status of all three species and their identical post-purchase storage history are more consistent with external adulteration than with natural formation, most plausibly within the imported frozen supply chain, although confirmation by a formaldehyde-selective method is required. Estimated daily intakes (0.76–10.86 × 10^{-3} mg kg^{-1} bw d^{-1}) yielded hazard quotients of 0.004–0.072, indicating negligible non-carcinogenic risk at average Ghanaian consumption (74 g d^{-1}). The findings support targeted surveillance of imported frozen salmon and the establishment of a national maximum limit for formaldehyde in fish.

Keywords: Formaldehyde; Food adulteration; MBTH spectrophotometry; Trimethylamine *N*-oxide; Hazard quotient; Ghana

1. Introduction

Fish is among the most perishable of foods. High water content, little connective tissue, and near-neutral post-mortem pH promote microbial growth and autolysis, especially in warm tropical climates (Olafsdottir *et al.*, 1997). Marine fish also accumulate trimethylamine *N*-oxide (TMAO), an osmolyte that stabilises proteins against osmotic and hydrostatic stress. After death, bacteria reduce TMAO to trimethylamine, the main cause of spoiled-fish odour, and TMAO is demethylated mainly by TMAO demethylase and partly non-enzymatically into equimolar dimethylamine (DMA) and formaldehyde. (Eq. (1)) (Benjakul *et al.*, 2004; Sotelo *et al.*, 1995).

$$(CH_3)_3NO \rightarrow (CH_3)_2NH + HCHO \quad (1)$$

Formaldehyde produced via this route accumulates in muscle during storage, especially frozen storage, and indicates post-mortem deterioration (Sotelo et al., 1995; Jinadasa et al., 2022). Levels vary with species, storage temperature and time, and tissue TMAO content and demethylase activity (Benjakul et al., 2004; Sotelo et al., 1995). Gadoids (e.g., cod, haddock, hake) with high TMAO and demethylase activity can accumulate substantial formaldehyde without external addition, whereas pelagic species (e.g., tunas, clupeids, salmonids) produce relatively little (Sotelo *et al.*, 1995; Jinadasa *et al.*, 2022).

Formaldehyde in fish may also be of deliberate anthropogenic origin (Jinadasa *et al.*, 2022; Mehta *et al.*, 2023). Formalin, an aqueous solution containing 37–40% formaldehyde, is a potent antimicrobial agent that cross-links muscle proteins, producing a firmer texture and fresher appearance that cannot readily be detected by visual or tactile inspection (Mehta *et al.*, 2023; Mohanty *et al.*, 2018). These properties have led to its illegal use by some fishers and traders to suppress bacterial growth and extend shelf life during handling, transport and marketing (Bhowmik *et al.*, 2017; Hoque *et al.*, 2018). Formaldehyde is not an approved food preservative in Ghana or in most other jurisdictions.

The International Agency for Research on Cancer classifies formaldehyde as carcinogenic to humans (Group 1), based on sufficient evidence for nasopharyngeal cancer and leukaemia (IARC, 2012). Ingestion of formaldehyde causes gastrointestinal irritation and, with prolonged exposure, may contribute to cumulative systemic toxicity (Tang *et al.*, 2009). The United States Environmental Protection Agency (USEPA) has established an oral reference dose (RfD) of 0.2 mg kg^{-1} body weight (bw) d^{-1} (USEPA, 1990), and the World Health Organization has established a tolerable daily intake (TDI) of 0.15 mg kg^{-1} bw d^{-1} (WHO, 2005).

Formaldehyde in market fish has been reported widely in South and South-East Asia, where surveys of retail and wet markets have documented concentrations ranging from trace levels to values well above regulatory reference limits (Bhowmik *et al.*, 2017, 2020; Devaraj *et al.*, 2021; Hoque *et al.*, 2018; Jinadasa *et al.*, 2022). Data for Ghana are sparse. Saba *et al.* (2015) screened 32 imported (mackerel, herring, horse mackerel, salmon and redfish) and locally farmed (tilapia) fish in the Tamale Metropolis with a qualitative test kit and detected no formaldehyde. Asare-Donkor *et al.* (2018) subsequently quantified formaldehyde in all sixty fish samples analysed in the Kumasi Metropolis, at 0.174–3.710 mg kg^{-1}, all below the 5 mg kg^{-1} maximum limit prescribed under the Malaysian Food Act and Regulations. More recently, rapid test-kit screening by the multi-institutional Fish4Food project detected formalin at landing sites, markets and processing centres in the Greater Accra, Western, Volta and Central regions, although not in Kumasi (Acquah, 2020).

Several analytical approaches are available for the determination of formaldehyde in fish (Supplementary Table S1). High-performance liquid chromatography after 2,4-dinitrophenylhydrazine derivatisation (HPLC–DNPH) and gas chromatography–mass spectrometry after derivatisation provide the lowest detection limits and the greatest selectivity (Bianchi *et al.*, 2007; Jinadasa *et al.*, 2022), but their cost restricts routine surveillance in many low- and middle-income settings. Among spectrophotometric methods, the Hantzsch (Nash)

acetylacetone reaction, measured at 412–415 nm (Nash, 1953), is susceptible to interference from yellow-brown co-extractives of fish tissue, which raises tissue-basis detection limits and can bias results relative to HPLC (Valadares *et al.*, 2021). The 3-methyl-2-benzothiazolinone hydrazone (MBTH) method (Sawicki *et al.*, 1961) yields a chromophore of higher molar absorptivity measured at approximately 628 nm, where matrix absorption is weak, and has previously been applied to Ghanaian market fish (Asare-Donkor *et al.*, 2018).

Despite this body of evidence, routine quantitative monitoring of formaldehyde in fish has not been established along the Ghanaian coast. Market-level screening, where performed, relies largely on qualitative test kits that do not support exposure assessment or enforcement (Mohanty *et al.*, 2018; Saba *et al.*, 2015). Few studies have explicitly examined whether measured concentrations are attributable to natural formation or to adulteration (Valadares *et al.*, 2021), and fewer still have combined occurrence data with quantitative risk characterisation (Bhowmik *et al.*, 2020). No quantitative data exist for the Sekondi–Takoradi metropolis, a major fish-landing centre in the Western Region in which formalin use has been reported (Acquah, 2020).

The present study therefore aimed to: (i) quantify formaldehyde in three commercially important fish types (tuna, salmon and herring) traded on the Sekondi–Takoradi coast using an MBTH spectrophotometric method; (ii) evaluate compliance with the 5 mg kg^{-1} reference limit; (iii) assess interspecific variation and evaluate whether measured concentrations are consistent with natural formation or external adulteration; and (iv) characterise the associated non-carcinogenic dietary health risk. Two null hypotheses were tested at $\alpha = 0.05$: that mean formaldehyde concentration does not differ among species ($H_{0,1}$), and that mean concentration does not exceed the 5 mg kg^{-1} reference limit ($H_{0,2}$).

2. Materials and methods

2.1. Study area

The Sekondi–Takoradi metropolis lies on the Gulf of Guinea in the Western Region of Ghana (Fig. 1). Its shoreline extends for approximately 25 km and comprises sandy beaches, rocky headlands, small lagoons and tidal creeks that receive urban runoff and discharges from small coastal streams; the Pra River enters the sea at Shama, immediately east of the metropolis. The climate is tropical maritime, with mean monthly temperatures of approximately 24 – 28°C, persistently high humidity, and a bimodal rainfall regime with a major season from April to July (peaking at approximately 280 mm in June) and a minor season from September to November (Supplementary Fig. S1). Such conditions accelerate post-harvest spoilage and may encourage reliance on chemical preservation where cold-chain capacity is limited (Saba *et al.*, 2015).

Fishing and fish trading underpin the coastal economy, and fish is the principal source of animal protein in local diets (Asare-Donkor *et al.*, 2018; Saba *et al.*, 2015). Tuna and herring are landed locally, whereas salmon is imported, predominantly frozen, and distributed through cold-store wholesalers and retailers.

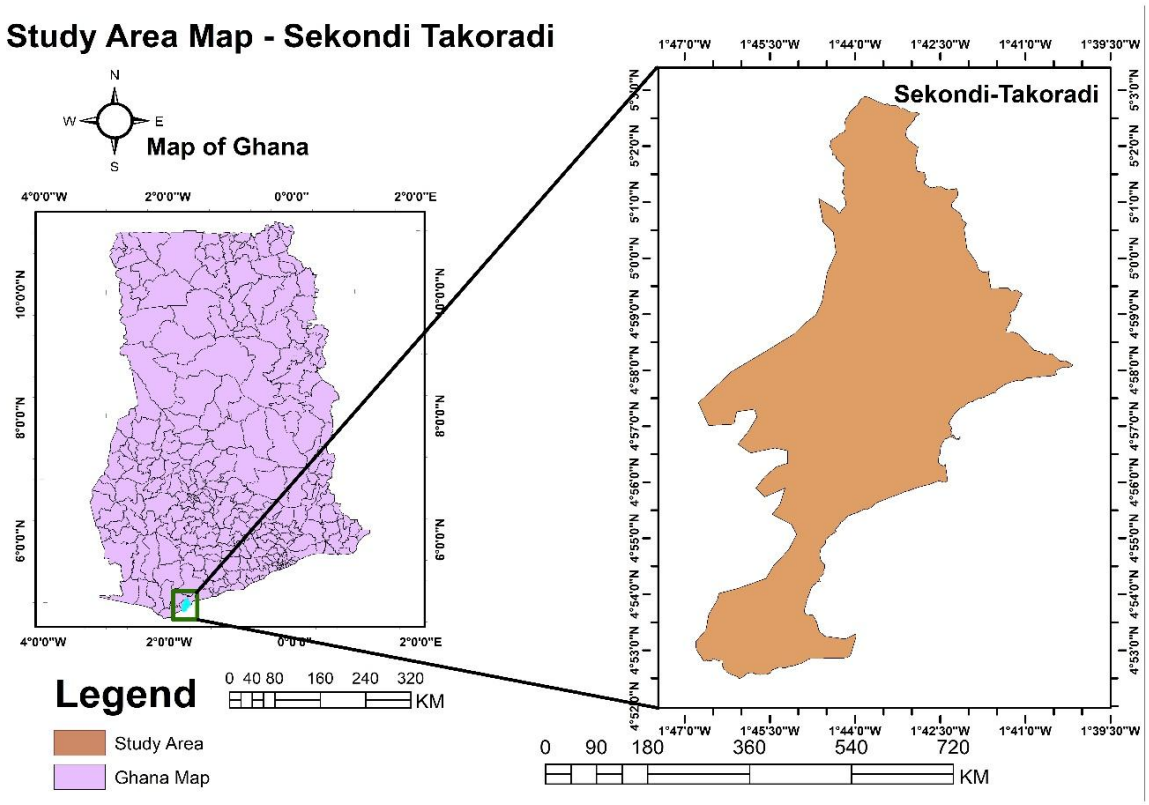


Fig. 1. Location of the study area: (a) map of Ghana showing the position of the Sekondi–Takoradi metropolis; (b) the Sekondi–Takoradi metropolitan area on the Gulf of Guinea coast.

2.2. Species selection and sample collection

Three fish types were selected by their local market names: tuna, salmon and herring. The Ghanaian tuna fishery lands skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*), and the fresh tuna purchased here belonged to one or more of these species; “herring” is the market name used in Ghana for fresh, locally landed sardinella (*Sardinella* spp.) and for imported frozen Atlantic herring (*Clupea harengus*); the herring purchased here was fresh and locally landed; and “salmon” denotes imported frozen salmonid sold under that name (most probably *Salmo salar* or *Oncorhynchus* spp.). Species were identified by market name without morphological or genetic confirmation (Section 4.4), and the three groups are referred to by market name throughout. They were selected on two grounds. First, they are traded in substantial volumes in the study area and are widely consumed, so that their contaminant burden is representative of local dietary exposure. Second,

tunas, clupeids and salmonids are low-to-moderate TMAO species in which natural formaldehyde formation is limited (Sotelo *et al.*, 1995; Jinadasa *et al.*, 2022). This choice provides an interpretive framework for objective (iii): elevated concentrations in these species are less readily explained by endogenous formation than equivalent concentrations in high-TMAO gadoids.

Samples were collected on a single occasion may 2026 at the Albert Bosomtwi-Sam Fishing Harbour, Sekondi, the principal landing and first-hand retail point on the Sekondi–Takoradi coast. Three individual fish of each type were purchased, each from a different vendor, giving nine biologically independent samples (Tuna 1–3, Salmon 1–3, Herring 1–3). Tuna and herring were purchased fresh from landing-site vendors; salmon was purchased frozen from cold-store retailers at the harbour. Restricting collection to one site and one occasion held the point of sale constant, so that observed variation reflects fish type and individual fish rather than market or date. Fish type, replicate number, vendor and date were recorded at purchase.

Each fish was wrapped individually in aluminium foil, labelled, and transported in an insulated cooler with ice to the laboratory of the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, where samples were stored frozen at 4 °C until analysis. All nine samples were subjected to the same freezing and thawing regime, so that any storage-related formaldehyde formation after purchase was common to all samples and did not confound interspecific comparison. The complete analytical workflow is summarised in Fig. 2.

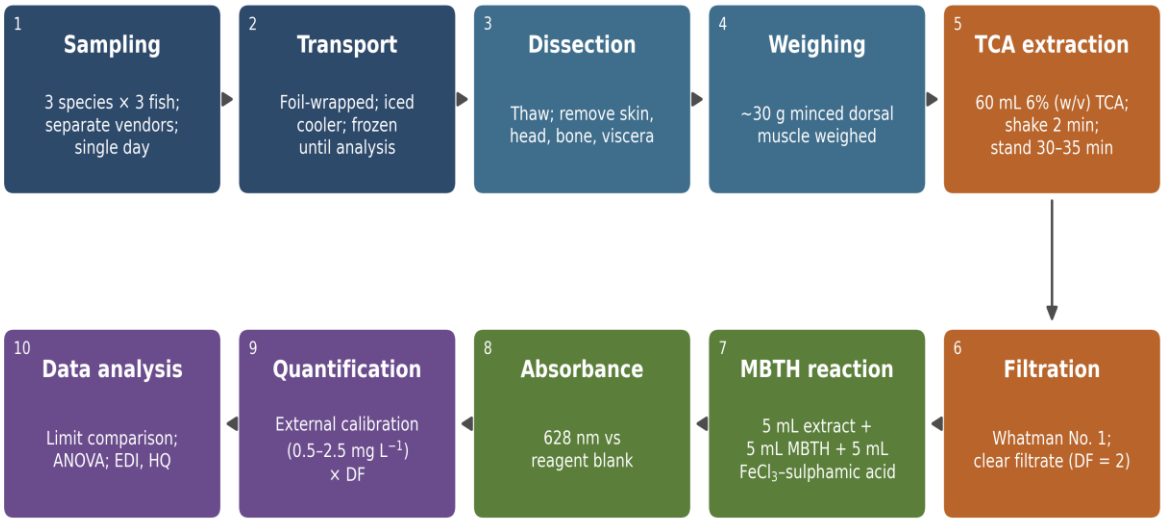


Fig. 2. Schematic of the analytical workflow for the determination of formaldehyde in fish muscle by the MBTH spectrophotometric method. DF, dilution factor; TCA, trichloroacetic acid; EDI, estimated daily intake; HQ, hazard quotient.

2.3. Reagents and instrumentation

All reagents were of analytical grade (Sigma-Aldrich, St. Louis, MO, USA), and all solutions were prepared in distilled water. Reagents comprised 6% (w/v) trichloroacetic acid (TCA) as extractant and protein precipitant; 0.05% (w/v) MBTH hydrochloride, prepared fresh daily; a ferric chloride–sulphamic acid oxidising reagent; and a formaldehyde stock solution prepared by dilution of 37% formaldehyde. Preparation details are given in Supplementary Text S1. Absorbance was measured on a Mettler Toledo UV5 UV–Vis spectrophotometer, and tissue was weighed on a Mettler Toledo electronic analytical balance. Extracts were filtered through Whatman No. 1 filter paper. The reagent system follows Asare-Donkor *et al.* (2018), adapted from Sawicki *et al.* (1961).

2.4. Sample preparation and extraction

Thawed fish were skinned, and the head, viscera and bones removed. A representative portion of dorsal muscle was excised and finely minced until visually homogeneous. Approximately 30 g of homogenised tissue was weighed accurately into a conical flask, and

60 mL of 6% (w/v) TCA was added to precipitate muscle proteins and release bound formaldehyde. The flask was stoppered, shaken vigorously for 2 min and left to stand at room temperature for 30–35 min to complete extraction and precipitation. The mixture was then filtered, and the clear filtrate was retained for analysis. Because formaldehyde from 30 g of tissue is distributed through 60 mL of extractant, the dilution factor (DF) relating extract concentration (mg L^{-1}) to tissue concentration (mg kg^{-1}) is 2. This convention, which assumes that the final extract volume equals the volume of extractant added, follows Asare-Donkor et al. (2018) and preserves direct comparability with the only previous Ghanaian dataset and with the 5 mg kg^{-1} reference limit as applied in that study. Because fish muscle contains approximately 65–80% water, which equilibrates with the TCA extractant, the true aqueous volume is approximately 80–84 mL, giving an effective dilution factor DFeff = (VTCA + m·w)/m ≈ 2.65–2.80, where m is the tissue mass and w its moisture fraction. Concentrations calculated with DF = 2 may therefore underestimate tissue concentrations by approximately 25–29%; tissue moisture was not determined, and the effect of this correction on compliance is evaluated in Section 3.3.

2.5. Spectrophotometric determination

In acidic solution, formaldehyde condenses with MBTH to form an azine (Eq. (2)). In the presence of Fe^{3+}, a second MBTH molecule is oxidised to a reactive cation that couples with the azine to yield a blue tetraazapentamethine cyanine dye with maximum absorption near 628 nm (Eq. (3)) (Sawicki *et al.*, 1961). Sulphamic acid is included in the oxidant to eliminate nitrite interference.

$$HCHO + MBTH \rightarrow azine + H_2O \quad (2)$$

$$azine + MBTH + 2Fe^{3+} \rightarrow blue\ cationic\ dye\ (\lambda_{max} \approx 628\,nm) + 2Fe^{2+} \quad (3)$$

To 5 mL of filtered extract were added 5 mL of 0.05% MBTH solution and, after mixing, 5 mL of oxidising reagent. Colour was allowed to develop for approximately 15 min at room

temperature. A reagent blank, in which 5 mL of distilled water replaced the extract, was prepared with every batch and used to zero the instrument at 628 nm. Each coloured solution was measured in duplicate, and the mean absorbance was used for quantification (Supplementary Fig. S2; raw readings in Supplementary Table S3).

2.6. Calibration and analytical quality assurance

Working standards of 0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1} were prepared by serial dilution of the stock solution and processed identically to the samples. Each standard was read in duplicate (Supplementary Table S2), and least-squares regression of mean absorbance (A) on concentration (C, mg L^{-1}) gave Eq. (4):

$$A = 0.5595C + 0.5577 \quad R^2 = 0.9992 \tag{4}$$

The formaldehyde concentration in fish tissue (C_{fish}, mg kg^{-1} wet weight) was calculated as:

$$C_{fish} = \frac{A - 0.5577}{0.5595} \times DF \tag{5}$$

where A is the mean sample absorbance and DF = 2 (Section 2.4). A worked example is provided in Supplementary Text S2. The limits of detection (LOD) and quantification (LOQ) were calculated following ICH (2005):

$$LOD = \frac{3.3 s_{y/x}}{b} \qquad LOQ = \frac{10 s_{y/x}}{b} \tag{6}$$

where $s_{y/x}$ is the residual standard deviation of the regression ($n - 2$ degrees of freedom) and b is the slope. Quality control comprised a reagent blank in every batch, duplicate absorbance readings, daily preparation of MBTH solution, and cleaning of all glassware with detergent followed by thorough rinsing with distilled water to prevent carry-over. No spike-recovery or certified reference material analysis was performed; method accuracy is therefore inferred from the previous application of the same procedure to Ghanaian fish (Asare-Donkor *et al.*, 2018) and from the original validation of the MBTH reaction (Sawicki *et al.*, 1961), and this is acknowledged as a limitation (Section 4.4).

2.7. Health risk assessment

Non-carcinogenic dietary risk was characterised deterministically following USEPA (1989) and the approach of Asare-Donkor *et al.* (2018). The estimated daily intake (EDI, mg kg^{-1} bw d^{-1}) was calculated as:

$$EDI = \frac{C \times IR}{BW} \quad (7)$$

where C is the formaldehyde concentration in fish (mg kg^{-1}), IR is the daily fish ingestion rate (kg d^{-1}) and BW is the mean adult body weight (kg). The hazard quotient (HQ) was calculated as:

$$HQ = \frac{EDI}{RfD} \quad (8)$$

HQ < 1 indicates that adverse non-carcinogenic effects are unlikely. HQ was computed against both the WHO TDI and the USEPA RfD to evaluate sensitivity to the toxicological benchmark. To quantify the margin of safety, the critical ingestion rate (IR_{crit}, kg d^{-1}) at which HQ reaches unity was derived by rearranging Eqs. (7) and (8):

$$IR_{crit} = \frac{RfD \times BW}{C} \quad (9)$$

Exposure parameters (Table 1) were adopted from Asare-Donkor *et al.* (2018) to ensure direct comparability with the only previous Ghanaian risk assessment.

Table 1. Exposure parameters and toxicological reference values used in the health risk assessment.

Parameter	Symbol	Value	Unit	Source
Fish ingestion rate	IR	0.074	kg d^{-1}	Asare-Donkor *et al.* (2018)
Per-capita fish consumption	–	27	kg yr^{-1}	Asare-Donkor *et al.* (2018)
Mean adult body weight	BW	75	kg	Asare-Donkor *et al.* (2018)
Tolerable daily intake (WHO)	RfD_{WHO}	0.15	mg kg^{-1} bw d^{-1}	WHO (2005)
Oral reference dose (USEPA)	RfD_{EPA}	0.20	mg kg^{-1} bw d^{-1}	USEPA (1990)

Parameter	Symbol	Value	Unit	Source
Maximum limit in fish[a]	–	5	mg kg^{-1}	Malaysian Food Regulations, cited in Asare-Donkor *et al.* (2018)

[a] Used for compliance assessment only (Section 3.3); not used in the HQ calculation.

2.8. Statistical analysis

Descriptive statistics (mean, standard deviation, range and coefficient of variation) were computed for each fish type and for the full dataset. $H_{0,1}$ was tested by one-way analysis of variance (ANOVA) with fish type as a fixed factor (n = 3 per group), and effect size was expressed as η^2 (between-group sum of squares/total sum of squares). Homogeneity of variance was assessed using the Brown–Forsythe (median-centred Levene) test and normality of residuals using the Shapiro–Wilk test. The Kruskal–Wallis test was applied as a distribution-free check, and Tukey's honestly significant difference (HSD) test was used for pairwise comparisons. $H_{0,2}$ was evaluated using two-tailed one-sample t-tests against 5 mg kg^{-1}. Analyses were performed in Microsoft Excel and Python 3 (SciPy), with significance set at $\alpha = 0.05$.

3. Results

3.1. Analytical performance

The calibration was linear over 0.5–2.5 mg L^{-1} (R^2 = 0.9992, $p < 0.001$; Fig. 3a), with no residual exceeding 0.02 absorbance units and no systematic curvature (Fig. 3b). Duplicate readings agreed to within 0.014 absorbance units at 0.5, 1.0 and 2.0 mg L^{-1}, with larger differences at 2.5 mg L^{-1} (0.046) and 1.5 mg L^{-1} (0.098). The LOD and LOQ were 0.083 and 0.251 mg L^{-1} in solution, corresponding to 0.17 and 0.50 mg kg^{-1} in tissue (Table 2). All sample concentrations exceeded the LOQ and are therefore reported as quantitative values. The intercept (0.5577 absorbance units) was large relative to the working range, indicating a

residual reagent or matrix contribution not removed by blank subtraction. Because standards and samples were processed identically and read against the same blank, interpolation remains internally consistent, but this offset is considered further in Section 4.4.

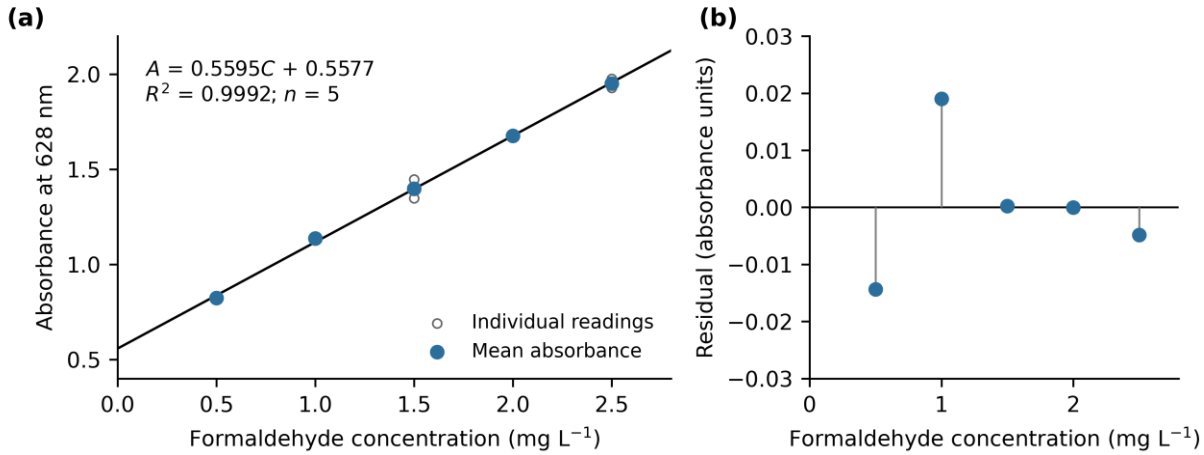


Fig. 3. Calibration of the MBTH method at 628 nm: (a) absorbance versus formaldehyde concentration, showing individual duplicate readings (open circles), mean absorbance (filled circles) and the least-squares regression line; (b) residuals of the mean absorbance from the regression line.

Table 2. Analytical performance characteristics of the MBTH spectrophotometric method.

Parameter	Value
Wavelength (nm)	628
Linear range (mg L^{-1})	0.5–2.5
Number of calibration levels	5 (duplicate readings)
Slope, b (AU L mg^{-1})	0.5595
Intercept (AU)	0.5577
Coefficient of determination, R^2	0.9992
Residual standard deviation, $s_{y/x}$ (AU)	0.0140
LOD, solution / tissue	0.083 mg L^{-1} / 0.17 mg kg^{-1}
LOQ, solution / tissue	0.251 mg L^{-1} / 0.50 mg kg^{-1}
Recovery	Not determined
Range of sample concentrations (tissue)	0.77–11.01 mg kg^{-1}

AU, absorbance units. Tissue-basis limits calculated using DF = 2 (Section 2.4).

3.2. Formaldehyde concentrations in fish

Formaldehyde was quantified in all nine samples (Table 3; Fig. 4a). Individual concentrations ranged from 0.77 mg kg^{-1} (Tuna 3) to 11.01 mg kg^{-1} (Salmon 2), a 14-fold difference, with an overall mean of 4.21 ± 2.93 mg kg^{-1}. Salmon had the highest mean concentration (7.12 ± 3.52 mg kg^{-1}), followed by herring (3.39 ± 0.66 mg kg^{-1}) and tuna (2.12 ± 1.18 mg kg^{-1}) (Table 4; Fig. 4b). Salmon also showed the largest absolute spread: the range among its three replicates (6.84 mg kg^{-1}) exceeded the combined range of all six tuna and herring samples. Herring was the least variable species (CV = 19.5%), compared with 49.4% for salmon and 55.5% for tuna. Three sample extracts fell outside the calibrated range: Tuna 3 lay slightly below the lowest standard, and Salmon 1 and Salmon 2 exceeded the highest standard (Table 3). Tuna 3 (0.385 mg L^{-1} in extract) lay below the lowest standard but above the LOQ (0.251 mg L^{-1}) and is therefore reported quantitatively, with greater relative uncertainty. Salmon 1 (A = 2.287) and Salmon 2 (A = 3.639) exceeded the absorbance of the highest standard (A = 1.956 by Eq. (4)), and their concentrations were obtained by linear extrapolation. Their magnitudes are therefore estimates. However, because the MBTH response increases monotonically with formaldehyde concentration, an extract absorbance greater than that of the 2.5 mg L^{-1} standard establishes an extract concentration greater than 2.5 mg L^{-1}, and hence a tissue concentration greater than 5.0 mg kg^{-1} (DF = 2), independently of the shape of the response above the calibrated range. Departures from linearity at high absorbance caused by stray light or the photometric limit of the instrument produce negative deviations from the Beer–Lambert law, so linear extrapolation is more likely to underestimate than to overestimate the true concentration. Chemical saturation of the reaction is also unlikely: the 5 mL of 0.05% MBTH added (≈ 11.6 μmol) exceeds by approximately six-fold the ≈ 1.8 μmol required to react stoichiometrically (2 mol MBTH per mol HCHO) with the formaldehyde present in 5 mL of the most concentrated extract (≈ 0.92 μmol). The exceedance

of the reference limit by Salmon 1 and Salmon 2 is therefore robust to extrapolation, whereas their exact concentrations (6.18 and 11.01 mg kg^{-1}) should be regarded as provisional estimates.

Table 3. Formaldehyde concentrations, estimated daily intake (EDI) and hazard quotients (HQ) for individual fish samples.

Sample	Mean absorbance	$C_{extract}$ (mg L^{-1})	C_{fish} (mg kg^{-1})	EDI ($\times 10^{-3}$ mg kg^{-1} bw d^{-1})	HQ_{WHO}	HQ_{EPA}
Tuna 1	1.302	1.331	2.66	2.625	0.0175	0.0131
Tuna 2	1.377	1.464	2.93	2.891	0.0193	0.0145
Tuna 3	0.773	0.385[a]	0.77	0.760	0.0051	0.0038
Salmon 1	2.287	3.091[b]	6.18[c]	6.098	0.0407	0.0305
Salmon 2	3.639	5.507[b]	11.01[c]	10.863	0.0724	0.0543
Salmon 3	1.724	2.084	4.17	4.114	0.0274	0.0206
Herring 1	1.602	1.866	3.73	3.680	0.0245	0.0184
Herring 2	1.623	1.904	3.81	3.759	0.0251	0.0188
Herring 3	1.293	1.315	2.63	2.595	0.0173	0.0130
Overall mean	–	–	4.21	4.154	0.0277	0.0208

Absorbance measured at 628 nm (mean of duplicate readings; raw data in Supplementary Table S3). C_{fish} calculated using Eq. (5); EDI and HQ calculated using Eqs. (7) and (8) with the parameters in Table 1.

[a] Below the lowest calibration standard (0.5 mg L^{-1}). [b] Above the highest calibration standard (2.5 mg L^{-1}); value obtained by linear extrapolation and regarded as a provisional estimate. Because the absorbance exceeds that of the highest standard, the tissue concentration is > 5.0 mg kg^{-1} irrespective of extrapolation (Section 3.2). [c] Exceeds the 5 mg kg^{-1} reference limit.

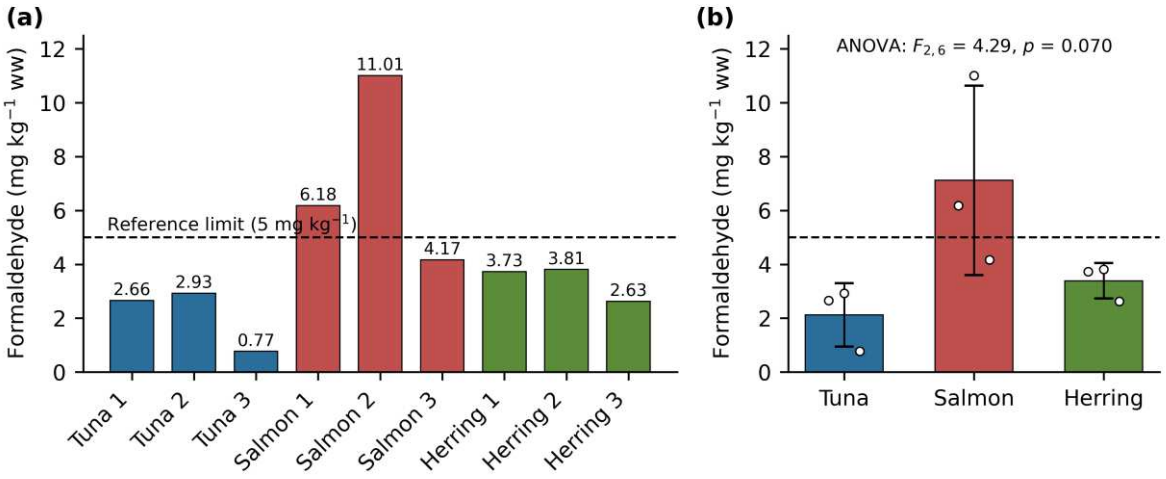


Fig. 4. Formaldehyde concentrations in fish muscle (wet weight): (a) individual samples; (b) species means ± standard deviation (n = 3), with individual values overlaid as open circles. The dashed line indicates the 5 mg kg^{-1} reference limit.

Table 4. Descriptive statistics of formaldehyde concentrations (mg kg^{-1} wet weight) by species and compliance with the 5 mg kg^{-1} reference limit.

Species	*n*	Mean ± SD	Range	CV (%)	Samples > 5 mg kg^{-1}	*t* vs 5 mg kg^{-1} (*p*)
Tuna	3	2.12 ± 1.18	0.77–2.93	55.5	0	−4.24 (0.051)
Salmon	3	7.12 ± 3.52	4.17–11.01	49.4	2	1.04 (0.41)
Herring	3	3.39 ± 0.66	2.63–3.81	19.5	0	−4.23 (0.052)
All samples	9	4.21 ± 2.93	0.77–11.01	69.7	2 (22%)	−0.81 (0.44)

SD, standard deviation (n − 1); CV, coefficient of variation. One-sample two-tailed t-tests against 5 mg kg^{-1} (df = 2 for species; df = 8 for all samples). One-way ANOVA among species: $F_{2,6}$ = 4.29, p = 0.070, η^2 = 0.59 (full output in Supplementary Tables S4–S6).

3.3. Compliance with the reference limit

Seven of the nine samples (78%) complied with the 5 mg kg^{-1} reference limit. All tuna samples (maximum 2.93 mg kg^{-1}, 59% of the limit) and all herring samples (maximum 3.81 mg kg^{-1}, 76% of the limit) were compliant. Within the salmon group, Salmon 3 (4.17 mg kg^{-1}) was compliant but approached the limit (83%), whereas Salmon 1 (6.18 mg kg^{-1}) and Salmon 2 (11.01 mg kg^{-1}) exceeded it by 24% and 120%, respectively. The salmon mean (7.12 mg kg^{-1}) exceeded the limit, but not significantly (t = 1.04, df = 2, p = 0.41), owing to the small sample size and large within-species variance. $H_{0,2}$ was therefore retained for the full dataset

(mean 4.21 mg kg^{-1}; $p = 0.44$) and for each species individually; the exceedance is evident at the level of individual samples but is not statistically established at the level of the species mean.

Compliance of samples close to the limit depends on the extraction-volume convention (Section 2.4). If the contribution of tissue water (65–80%) to the extract volume is taken into account, all concentrations increase by a factor of 1.33–1.40. Under this correction, Salmon 1 (8.2–8.7 mg kg^{-1}) and Salmon 2 (14.6–15.4 mg kg^{-1}) remain in exceedance, Salmon 3 (5.5–5.8 mg kg^{-1}) and Herring 2 (5.0–5.3 mg kg^{-1}) would also exceed the limit, and Herring 1 (4.9–5.2 mg kg^{-1}) would lie at the limit, whereas all tuna samples (≤ 4.1 mg kg^{-1}) and Herring 3 (≤ 3.7 mg kg^{-1}) would remain compliant. The exceedance by Salmon 1 and Salmon 2 is therefore robust to both the calibration extrapolation (Section 3.2) and the extraction-volume convention, whereas the compliance status of samples within approximately 30% of the limit (≥ 3.6 mg kg^{-1}) depends on the convention adopted. Concentrations calculated with DF = 2 are retained as the primary results for comparability with Asare-Donkor et al. (2018), but they should be regarded as conservative (lower-bound) estimates.

3.4. Interspecific variation

Model assumptions were satisfied (Brown–Forsythe, $p = 0.351$; Shapiro–Wilk on residuals, $W = 0.929$, $p = 0.471$). One-way ANOVA indicated no significant difference among species at $\alpha = 0.05$ ($F_{2,6} = 4.29$, $p = 0.070$; Supplementary Table S4), and the Kruskal–Wallis test gave a consistent result ($H = 5.96$, $p = 0.051$). $H_{0,1}$ was therefore retained. None of the Tukey HSD pairwise comparisons was significant; the largest contrast was salmon versus tuna (mean difference 5.00 mg kg^{-1}, 95% CI −0.45 to 10.45; adjusted $p = 0.068$) (Supplementary Table S5). Nevertheless, species accounted for 58.8% of total variance ($\eta^2 = 0.588$), a large effect by conventional criteria. Because the Kruskal–Wallis test is based on ranks, and Salmon 1 and Salmon 2 remain the two highest values whatever their exact magnitude above 5.0 mg kg^{-1}, its

outcome is unaffected by the extrapolation of these two values; the parametric statistics involving salmon (ANOVA, Tukey HSD, one-sample t-tests and descriptive statistics) should be regarded as provisional.

3.5. Dietary exposure and health risk

EDIs ranged from 0.760 × 10^{-3} mg kg^{-1} bw d^{-1} (Tuna 3) to 10.863 × 10^{-3} mg kg^{-1} bw d^{-1} (Salmon 2), with an overall mean of 4.154 × 10^{-3} mg kg^{-1} bw d^{-1} (Table 3). Corresponding HQs were 0.0051–0.0724 (mean 0.0277) against the WHO TDI and 0.0038–0.0543 (mean 0.0208) against the USEPA RfD (Fig. 5a). All HQs were at least an order of magnitude below unity. The highest value (Salmon 2, WHO TDI) represents approximately 7% of the tolerable intake. The critical ingestion rate required to reach HQ = 1 ranged from 1.02 kg d^{-1} (Salmon 2) to 14.61 kg d^{-1} (Tuna 3), and was 2.67 kg d^{-1} at the overall mean concentration, between 14 and 197 times the mean national intake of 0.074 kg d^{-1} (Fig. 5b).

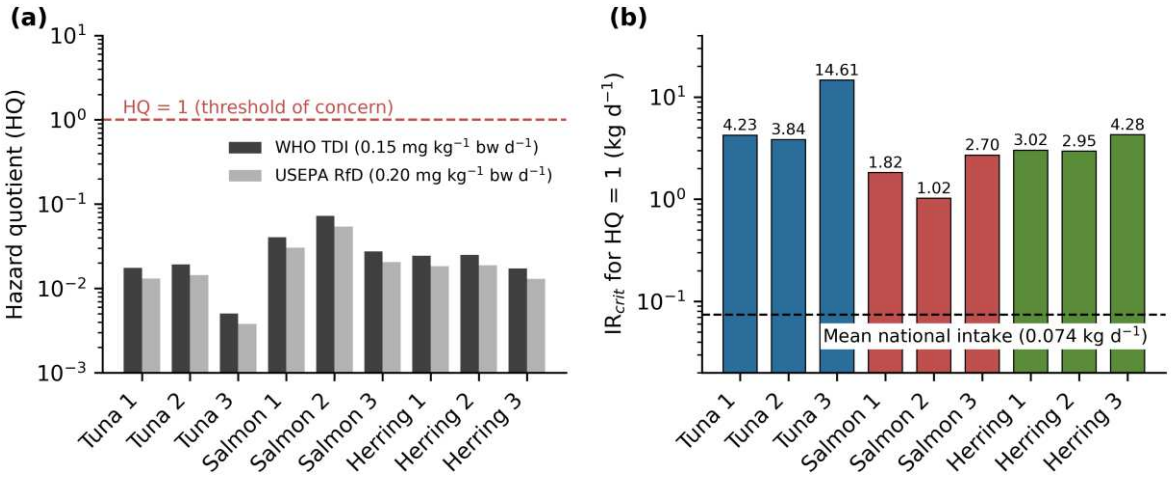


Fig. 5. Non-carcinogenic dietary risk of formaldehyde for adult consumers: (a) hazard quotients calculated against the WHO tolerable daily intake and the USEPA oral reference dose (logarithmic scale; dashed line, HQ = 1); (b) critical daily ingestion rate required to reach HQ = 1 (WHO TDI; logarithmic scale), compared with the mean Ghanaian fish intake (dashed line).

4. Discussion

4.1. Natural formation versus external adulteration

Because total formaldehyde was measured, the source of the formaldehyde in each sample cannot be determined directly. Three independent lines of evidence nevertheless bear on whether the measured concentrations are consistent with endogenous formation or indicate external addition.

First, the magnitude of the concentrations. Endogenous formaldehyde is stoichiometrically limited by the TMAO content of the tissue (Eq. (1)), and in species of low to moderate TMAO content, concentrations arising during normal commercial storage are typically no more than a few milligrams per kilogram (Benjakul *et al.*, 2004; Sotelo *et al.*, 1995). Concentrations in tuna (0.77–2.93 mg kg^{-1}) and herring (2.63–3.81 mg kg^{-1}) fall within this expectation, whereas those in Salmon 1 (6.18 mg kg^{-1}) and Salmon 2 (11.01 mg kg^{-1}) do not. The latter value is approximately three times the maximum reported among sixty samples in Kumasi (Asare-Donkor *et al.*, 2018).

Second, the species examined. Substantial endogenous formation is well documented in gadoids, in which TMAO demethylase activity is high (Sotelo *et al.*, 1995). In salmon, a concentration of 11 mg kg^{-1} cannot be attributed to this pathway with confidence. Third, the handling history. Frozen storage promotes TMAO demethylation and can raise formaldehyde over time (Benjakul *et al.*, 2004; Olafsdottir *et al.*, 1997), but all nine samples experienced an identical freeze–thaw regime after purchase, so post-purchase storage cannot explain the selective elevation of two salmon samples relative to six tuna and herring samples processed alongside them.

Taken together, these observations indicate that the tuna and herring concentrations are consistent with natural post-mortem formation, whereas the two salmon exceedances are more parsimoniously explained by external addition. This inference remains circumstantial: free and

412 protein-bound formaldehyde were not speciated, DMA was not co-determined, untreated
413 reference fish of known provenance were not analysed, and pre-purchase storage history of the
414 imported salmon was unknown. Prolonged frozen storage before import could have increased
415 endogenous formation, although the low TMAO demethylase activity of salmonids makes this
416 an unlikely sole explanation. A further possibility is analytical: the MBTH reaction is not
417 specific to formaldehyde and also responds to other aliphatic aldehydes (Sawicki et al., 1961).
418 Salmon is an oily species in which lipid oxidation during prolonged frozen storage generates
419 volatile aldehydes, which could contribute a positive bias; herring, also an oily species, showed
420 no comparable elevation, but this does not exclude the possibility. Confirmation of
421 formaldehyde identity by a selective method (e.g., HPLC–DNPH) is therefore required before
422 adulteration can be inferred with confidence. Regardless of mechanism, two of nine samples
423 exceeded the food-safety reference limit, which justifies regulatory attention.

424 *4.2. Comparison with previous studies and supply-chain considerations*

425 Table 5 compares the present findings with previous investigations. The closest comparator
426 is Asare-Donkor *et al.* (2018), who used the same analytical method and exposure parameters
427 in Kumasi. Agreement for locally landed fish is close: the Kumasi range (0.174–3.710 mg kg^{-1})
428 encompasses all tuna and two of the three herring values, and the third herring value (3.81 mg
429 kg^{-1}) exceeds it only marginally. HQs in both studies were well below unity. The divergence
430 is confined to salmon. Because the discrepancy is specific to one fish type rather than general
431 to the location, a systematic analytical or environmental explanation is unlikely; it points
432 instead to factors particular to the salmon supply chain.

Table 5. Comparison of formaldehyde concentrations in fish from the present study with previous reports.

Study	Location	Method	Concentration range	Exceedance of 5 mg kg^{-1}
Present study	Sekondi–Takoradi, Ghana	MBTH spectrophotometry (628 nm)	0.77–11.01 mg kg^{-1}	2 of 9 (22%); salmon only
Asare-Donkor *et al.* (2018)	Kumasi, Ghana	MBTH spectrophotometry	0.174–3.710 mg kg^{-1}	None (n = 60)
Saba *et al.* (2015)	Tamale, Ghana	Qualitative test kit	Not detected (n = 32; imported and local fish)	None
Fish4Food project (Acquah, 2020)	Greater Accra, Western, Volta and Central regions, Ghana	Rapid test kits (qualitative)	Formalin detected at landing sites, markets and processing centres; not quantified	Not applicable
Bhowmik *et al.* (2017)	Dhaka, Bangladesh	HPLC	5.1–39.7 mg kg^{-1}	All samples; attributed to natural origin
Bhowmik *et al.* (2020)	Bangladesh	HPLC	Risk-assessed market fish	Reported
Devaraj *et al.* (2021)	Chennai, India	Qualitative screening	Variable among species	Not reported

For fish tissue, 1 µg g^{-1} is equivalent to 1 mg kg^{-1}. Concentrations are reported as given in the original sources. Bhowmik *et al.* (2017) attributed the concentrations they measured in Dhaka markets to natural post-mortem formation rather than adulteration.

Unlike tuna and herring, which are landed locally and typically reach the market within hours, salmon is imported frozen and passes through international transport, cold storage, wholesale distribution and potentially repeated freeze–thaw episodes. Each additional stage extends the period over which spoilage must be controlled and increases the opportunity for chemical intervention. The concentration of all exceedances in the only imported fish type in the dataset is consistent with this interpretation, although three salmon samples cannot establish it. The finding is also consistent with the detection of formalin in the Western Region by the

Fish4Food project's rapid screening (Acquah, 2020), to which the present data add quantitative, species-specific evidence at a defined point in the value chain.

Concentrations reported from South Asian wet markets span a wide range. Bhowmik *et al.* (2017) measured 5.1–39.7 mg kg^{-1} in marketed fish in Dhaka, with the highest values in marine species, and attributed them to natural formation, whereas Hoque *et al.* (2018) found generally low concentrations in freshwater fish in southern Bangladesh, and concentrations an order of magnitude higher than those observed here have been reported in confirmed adulteration cases (reviewed by Jinadasa *et al.*, 2022). Against this background, the situation on the Sekondi–Takoradi coast appears comparatively moderate. It is, however, distinct from the uniformly compliant pattern reported for Kumasi, and is best characterised as a localised problem associated with a particular fish type and supply route rather than generalised contamination of fish traded on this coast.

4.3. Health risk and regulatory implications

The coexistence of limit exceedances and very low HQs is not contradictory. The 5 mg kg^{-1} limit functions as a compliance and adulteration indicator rather than a toxicological threshold, so a sample may exceed it while delivering an intake far below the tolerable dose. The IR_{crit} analysis shows that even for the most contaminated sample, a 75 kg adult would need to consume approximately 1.02 kg of fish daily to reach HQ = 1, indicating that the conclusion of negligible non-carcinogenic risk is robust to considerable underestimation of ingestion rates in high-consumption coastal households.

This reassurance is subject to three qualifications. First, HQ addresses non-carcinogenic effects only. Formaldehyde is a Group 1 carcinogen (IARC, 2012), but this classification rests on evidence from inhalation exposure (nasopharyngeal cancer and leukaemia). No oral cancer slope factor has been established (USEPA, 1990), and WHO (2005) concluded that there is no definitive evidence of carcinogenicity following ingestion. A quantitative lifetime incremental

cancer risk therefore could not be calculated without adopting an unvalidated slope factor. As an alternative point of reference, the highest EDI in this study (1.09×10^{-2} mg kg^{-1} bw d^{-1}) is approximately five orders of magnitude below the estimated endogenous turnover of formaldehyde in humans (878–1310 mg kg^{-1} bw d^{-1}), on the basis of which EFSA (2014) concluded that the contribution of dietary formaldehyde to systemic exposure is negligible. This comparison does not, however, exclude local effects on the gastrointestinal mucosa from repeated consumption of heavily adulterated fish. Second, the assessment considered average adult consumers only and did not address children, whose lower body weight increases intake per unit body weight. Third, aggregate exposure from other dietary and environmental sources was not considered. The results should therefore be regarded as a screening-level characterisation.

From a regulatory perspective, the species-specific pattern supports a risk-based allocation of surveillance resources. Targeted testing of imported frozen salmon by the Food and Drugs Authority and the Fisheries Commission, at the points of importation, cold storage and retail, would help identify the stage at which contamination is introduced, whereas routine periodic surveillance appears proportionate for locally landed species. Because Ghana currently has no statutory maximum limit for formaldehyde in fish, necessitating reliance on the Malaysian threshold, the establishment of a national limit and a prescribed analytical method by the Food and Drugs Authority and the Ghana Standards Authority would provide a domestic legal basis for enforcement. Investment in ice production and cold-storage capacity at landing sites, together with trader and consumer awareness programmes, would reduce the incentive for chemical preservation.

4.4. Limitations and future research

Several limitations should be considered when interpreting these results. (i) Sampling was confined to one site on one occasion with three replicates per fish type; the results characterise

that market at that time, and the low statistical power (six error degrees of freedom) prevented resolution of a large species effect ($\eta^2 = 0.59$). A follow-up study with at least ten replicates per fish type, multiple landing sites and sampling in both rainy seasons is required. (ii) The two exceedance values lay above the calibrated range, and the absorbance of Salmon 2 (3.64) is beyond the range in which photometric linearity is normally assured. Although exceedance of the reference limit is established by bracketing against the highest standard (Section 3.2), the magnitudes of these values are extrapolated estimates; future analyses should dilute high extracts into the calibrated range or extend the calibration to at least 6 mg L^{-1}. Tissue moisture was not determined, so concentrations were calculated with the conventional DF = 2, which may underestimate true concentrations by 25–29% (Section 3.3); moisture should be measured and the effective extract volume used in future work. The MBTH reaction is also not specific to formaldehyde, and confirmation by a selective method such as HPLC–DNPH is required, particularly for oily species subject to lipid oxidation. (iii) No spike-recovery or reference material analysis was conducted, and the large calibration intercept indicates a residual reagent or matrix contribution that a zero-concentration standard and matrix-matched calibration would characterise more fully. (iv) Fish were identified by market name only; “tuna” and “herring” in Ghana each cover more than one species, “salmon” may denote more than one imported salmonid, and mislabelling cannot be excluded. Taxonomic confirmation, ideally by DNA barcoding, should accompany future sampling. (v) Source attribution was circumstantial; future work should include speciation of free and bound formaldehyde, co-determination of DMA, whose equimolar formation with endogenous formaldehyde (Eq. (1)) offers a chemical basis for distinguishing natural from added formaldehyde, and analysis of reference fish of known provenance. (vi) Risk characterisation was limited to non-carcinogenic effects in average adults; carcinogenic risk could be contextualised only qualitatively in the absence of

an oral cancer slope factor (Section 4.3), and high-consumer and child scenarios should be evaluated, ideally using probabilistic methods (Putri *et al.*, 2018).

5. Conclusions

This study provides the first quantitative baseline for formaldehyde in fish traded on the Sekondi–Takoradi coast of Ghana. Formaldehyde was quantified in all nine samples of tuna, salmon and herring (0.77–11.01 mg kg^{-1}). Two of three imported salmon samples exceeded the 5 mg kg^{-1} reference limit, whereas all locally landed tuna and herring complied under the conventional extraction-volume calculation. Although interspecific differences were not statistically significant owing to low power, the magnitude of the salmon concentrations, the low-to-moderate TMAO status of the fish types examined and their common post-purchase storage history are more consistent with external adulteration than with endogenous formation, most plausibly within the imported frozen supply chain. Hazard quotients (0.004–0.072) indicate negligible non-carcinogenic risk at average national consumption, with a margin of safety of at least 14-fold in ingestion rate. The salmon exceedances are robust to calibration extrapolation and to the extraction-volume convention, although their exact magnitudes are provisional. These preliminary findings, which require confirmation by a larger, spatially and temporally replicated survey with taxonomic identification of samples, an extended calibration range, moisture-corrected quantification, recovery validation and confirmation by a formaldehyde-selective method, support targeted surveillance of imported frozen fish, the establishment of a national maximum limit for formaldehyde in fish, and improved cold-chain infrastructure at landing sites.

CRediT authorship contribution statement

Ebenezer Ashun: Conceptualization, Methodology, Supervision, Writing – review & editing. **Kwaku Abu Bonsrah:** Investigation, Formal analysis, Writing – original draft. **Louisa**

Kyennyine Domah: Investigation, Data curation, Writing – original draft. **Stanley Boavo Johnston:** Investigation, Visualization, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated in this study are included in the article and its Supplementary Material.

Acknowledgements

The authors thank the technical staff of the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, for assistance during the analytical work, and the fish traders at the Albert Bosomtwi-Sam Fishing Harbour, Sekondi, who permitted sample collection.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article (Supplementary Tables S1–S6, Supplementary Figs. S1–S2 and Supplementary Texts S1–S2) are provided in a separate file.

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