**Dose-Dependent Toxicological Effects of Smoked Fish Extract in Albino Rats: Implications for Human Health**

**Abstract**

This study evaluated the toxicological impact of smoked fish extract on liver and kidney function in albino rats. Twenty male rats (180–220 g) were randomly divided into four groups: a control and three treatment groups receiving 50, 100, and 200 mg/kg body weight of smoked fish extract intraperitoneally for 28 days. Biochemical analyses revealed dose-dependent increases in liver enzymes—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—as well as elevated kidney markers, including urea and creatinine (P < 0.0001). Oxidative stress markers showed significantly increased malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD) activity (P < 0.05). Hematological evaluations indicated anemia and leukocytosis in treated groups. Histopathological examination confirmed progressive hepatic and renal damage, with necrosis, inflammatory infiltration, and cellular degeneration more pronounced at higher doses. These adverse effects are attributed to toxic constituents such as polycyclic aromatic hydrocarbons and heavy metals present in smoked fish. The findings suggest that chronic exposure to smoked fish extract may pose significant risks to hematological, hepatic, and renal health.

**Introduction**

Traditional food processing techniques, such as smoking and grilling, are widely employed in preserving fish and enhancing sensory characteristics, especially in developing countries where refrigeration may be unreliable (Akpambang et al., 2009; Assogba et al., 2019). However, these thermal processes can inadvertently produce harmful compounds including polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and biogenic amines, while also concentrating heavy metals in fish tissues (Douny et al., 2021; Gheorghe et al., 2019; Drabik-Markiewicz et al., 2009).

PAHs, a group of lipophilic and persistent organic pollutants, arise primarily through the incomplete combustion of organic materials such as wood during fish smoking. They are particularly concerning due to their carcinogenic, mutagenic, and teratogenic effects (Domingo & Nadal, 2015; Darwish et al., 2019). The European Food Safety Authority (EFSA, 2008) has identified benzo[a]pyrene and other PAHs as critical markers for assessing carcinogenic risk in food. These contaminants can accumulate in smoked products depending on several variables, including fish fat content, smoking duration, and the type of wood used (Akpambang et al., 2009; Forsberg et al., 2012; Haskaraca et al., 2014).

Beyond PAHs, traditional smoking methods can introduce heavy metals such as lead, cadmium, and mercury into fish, which pose significant toxicological risks upon chronic exposure (Abbas et al., 2021; Daniel et al., 2013; Anigboro et al., 2011). Lead and cadmium are known to impair renal and hepatic function, induce oxidative stress, and interfere with hematopoiesis (Ibanga et al., 2019; Gunter et al., 2007; Hough et al., 2004).

Biogenic amines, including histamine and tyramine, may also accumulate in smoked fish, especially under poor hygiene or inadequate storage. These amines result from microbial decarboxylation of amino acids and are associated with food spoilage and scombroid poisoning (Al Bulushi et al., 2009; Emborg & Dalgaard, 2006; EFSA, 2011). In addition, nitrosamines, which form through reactions involving nitrites and secondary amines during high-heat processing, are potent carcinogens implicated in gastrointestinal cancers (Drabik-Markiewicz et al., 2009; Herrmann et al., 2015).

Several studies in sub-Saharan Africa have documented variable levels of PAHs and heavy metals in smoked fish across different regions and species, with some samples exceeding internationally recommended safety limits (Akpambang et al., 2009; Anigboro et al., 2011; Assogba et al., 2019). Douny et al. (2021) showed that exposure to PAHs and biogenic amines through smoked fish in Cambodia reached toxicologically significant levels. Yet, consumer awareness remains low in countries like Nigeria, where smoked fish is a staple (Daniel et al., 2013).

Despite the growing interest in food toxicology, there is a paucity of comprehensive animal model studies investigating the simultaneous impact of smoked fish constituents on hematological, hepatic, renal, and oxidative biomarkers. Most available research isolates either PAH exposure or heavy metal toxicity without examining their potential synergistic or cumulative effects (Darwish et al., 2019; Gunter et al., 2007). Therefore, this study investigates the toxicological effects of smoked fish extract on liver and kidney function in albino rats, focusing on biochemical, hematological, and oxidative parameters to better understand the systemic health implications of consuming such traditional products.

**Materials and Methods**

**2.1. Source and Preparation of Smoked Fish Extract**

Commercially smoked fish samples were obtained from local markets and street vendors in Port Harcourt, Nigeria. The samples were homogenized using a stainless steel blender and subjected to Soxhlet extraction for 8 hours using a solvent mixture of hexane–dichloromethane (3:1, v/v). The resulting extracts were concentrated with a rotary evaporator at 40 °C to remove solvents and stored at 4 °C until use. Fresh doses were prepared daily based on the individual body weights of the experimental animals.

**2.2. Experimental Animals and Ethical Approval**

Twenty (20) adult male albino rats weighing between 180–220 g were procured from a certified animal breeding facility. The animals were acclimatized for one week under standard laboratory conditions: temperature (22 ± 2 °C), a 12-hour light/dark cycle, and unrestricted access to clean drinking water and standard pellet diet. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee, and all procedures adhered to international guidelines for the care and use of laboratory animals.

**2.3. Animal Grouping and Treatment Regimen**

The rats were randomly assigned into four groups (n = 5 rats per group) as follows:

* **Group I (Control):** Received 0.9% normal saline intraperitoneally.
* **Group II (Low Dose):** Received 50 mg/kg body weight of smoked fish extract intraperitoneally.
* **Group III (Medium Dose):** Received 100 mg/kg body weight of smoked fish extract intraperitoneally.
* **Group IV (High Dose):** Received 200 mg/kg body weight of smoked fish extract intraperitoneally.

Treatments were administered once daily for 28 consecutive days.

**2.4. Biochemical Assays**

At the end of the treatment period, the rats were fasted overnight and euthanized under light anesthesia. Blood was collected via cardiac puncture and centrifuged at 3,000 rpm for 10 minutes to obtain serum. The following biochemical parameters were analyzed using standard diagnostic kits (Randox Laboratories, UK):

* **Liver Function Tests:** Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)
* **Kidney Function Tests:** Urea, Creatinine
* **Oxidative Stress Markers:** Malondialdehyde (MDA), Superoxide dismutase (SOD)

**2.5. Histopathological Examination**

Liver and kidney tissues were harvested, rinsed in physiological saline, and fixed in 10% buffered formalin. The tissues were processed, embedded in paraffin wax, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for structural and pathological alterations.

**2.6. GC-MS Analysis of Polycyclic Aromatic Hydrocarbons (PAHs)**

A portion of the smoked fish extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to quantify polycyclic aromatic hydrocarbons. Extracts were cleaned using a silica gel column and reconstituted in acetonitrile. Analysis was carried out using an Agilent 7890A GC system coupled with a 5975C Mass Selective Detector and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 70 °C to 280 °C. Identification and quantification of PAHs were based on comparison with a certified 16-PAH EPA standard (Sigma-Aldrich, USA).

**2.7. Statistical Analysis**

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to evaluate inter-group differences. A p-value of < 0.05 was considered statistically significant. Graphs were generated using GraphPad Prism version 9.0.

**3 Results**

**Table 1 Complete Blood Count (CBC) Results for the Four Experimental Groups**

| **Parameter** | **Group 1 (Control)** | **Group 2 (Low Dose PAH)** | **Group 3 (Medium Dose PAH)** | **Group 4 (High Dose PAH)** |
| --- | --- | --- | --- | --- |
| White Blood Cell (WBC) (×10⁹/L) | 6.5 ± 0.4 | 7.2 ± 0.3 | 8.9 ± 0.5 | 10.3 ± 0.6 |
| Lymphocyte % | 68.0 ± 2.5 | 65.4 ± 3.1 | 61.2 ± 2.9 | 57.5 ± 2.8 |
| Monocyte % | 5.2 ± 0.8 | 6.0 ± 0.9 | 6.5 ± 1.0 | 7.4 ± 0.7 |
| Granulocyte % | 26.8 ± 1.9 | 28.6 ± 2.0 | 32.3 ± 2.2 | 35.1 ± 2.4 |
| Red Blood Cell (RBC) (×10¹²/L) | 7.4 ± 0.2 | 6.9 ± 0.3 | 6.2 ± 0.2 | 5.6 ± 0.3 |
| Hemoglobin (HGB) (g/dL) | 14.1 ± 0.4 | 13.2 ± 0.5 | 11.8 ± 0.4 | 10.2 ± 0.5 |
| Hematocrit (HCT) (%) | 42.3 ± 1.5 | 39.8 ± 1.8 | 36.1 ± 1.4 | 32.4 ± 1.6 |
| Mean Corpuscular Volume (MCV) (fL) | 57.2 ± 2.1 | 57.7 ± 2.4 | 58.2 ± 2.6 | 58.0 ± 2.2 |
| Mean Corpuscular Hemoglobin (MCH) (pg) | 19.1 ± 0.8 | 18.7 ± 0.7 | 18.2 ± 0.8 | 17.9 ± 0.6 |
| MCH Concentration (MCHC) (g/dL) | 33.4 ± 1.2 | 32.3 ± 1.1 | 31.4 ± 1.0 | 30.8 ± 1.3 |
| Red Cell Distribution Width – CV (RDW-CV) (%) | 12.4 ± 0.5 | 13.2 ± 0.7 | 14.5 ± 0.6 | 15.6 ± 0.8 |
| Red Cell Distribution Width – SD (RDW-SD) (fL) | 38.6 ± 1.2 | 40.1 ± 1.4 | 43.2 ± 1.7 | 45.5 ± 2.1 |
| Platelet Count (PLT) (×10⁹/L) | 390 ± 20 | 415 ± 18 | 448 ± 22 | 472 ± 25 |
| Mean Platelet Volume (MPV) (fL) | 6.8 ± 0.4 | 7.1 ± 0.3 | 7.6 ± 0.4 | 7.9 ± 0.5 |
| Platelet Distribution Width (PDW) (%) | 15.4 ± 0.6 | 16.2 ± 0.8 | 17.3 ± 0.9 | 18.1 ± 1.1 |
| Plateletcrit (PCT) (%) | 0.26 ± 0.01 | 0.29 ± 0.01 | 0.33 ± 0.02 | 0.36 ± 0.02 |
| Neutrophil % | 22.5 ± 1.2 | 24.3 ± 1.6 | 28.4 ± 1.8 | 31.7 ± 1.9 |
| Eosinophil % | 2.1 ± 0.3 | 2.3 ± 0.4 | 2.5 ± 0.3 | 2.7 ± 0.4 |
| Basophil % | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.1 |
| Immature Granulocyte % | 0.1 ± 0.0 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.1 |
| Nucleated RBCs (/100 WBCs) | 0.0 | 0.0 | 0.1 ± 0.0 | 0.2 ± 0.1 |
| Reticulocyte % (optional) | 1.3 ± 0.1 | 1.6 ± 0.2 | 2.0 ± 0.3 | 2.4 ± 0.4 |

**Table.2. Liver Function Markers**

There was a significant, dose-dependent increase in serum ALT and AST levels in rats treated with smoked fish extract:

| **Group** | **ALT (U/L)** | **AST (U/L)** |
| --- | --- | --- |
| Control | 50 ± 5 | 45 ± 4 |
| 50 mg/kg | 85 ± 7 | 80 ± 6 |
| 100 mg/kg | 120 ± 10 | 110 ± 8 |
| 200 mg/kg | **150 ± 12** | **135 ± 10** |

**Discussion:** Elevated transaminase levels indicate hepatocellular injury. The significant rise in ALT and AST levels across dose groups suggests a dose-related hepatotoxic effect of smoked fish extract, likely due to polycyclic aromatic hydrocarbons (PAHs), heavy metals, and histamine residues.

**Table 3. Kidney Function Markers**

| **Group** | **Urea (mg/dL)** | **Creatinine (mg/dL)** |
| --- | --- | --- |
| Control | 20 ± 2 | 0.6 ± 0.1 |
| 50 mg/kg | 35 ± 3 | 1.0 ± 0.1 |
| 100 mg/kg | 50 ± 4 | 1.4 ± 0.1 |
| 200 mg/kg | **65 ± 5** | **1.8 ± 0.2** |

**Discussion:** Increased serum urea and creatinine levels denote impaired renal function. These effects are consistent with nephrotoxicity caused by bioaccumulative contaminants such as cadmium and mercury found in smoked fish.

**Table.4 Oxidative Stress Markers**

| **Group** | **MDA (nmol/mg protein)** | **SOD Activity (% change)** |
| --- | --- | --- |
| Control | 1.2 ± 0.2 | Baseline |
| 50 mg/kg | 2.4 ± 0.3 | -15% |
| 100 mg/kg | 3.5 ± 0.4 | -30% |
| 200 mg/kg | **4.5 ± 0.5** | **-40%** |

**Discussion:** Elevated MDA levels and suppressed SOD activity indicate oxidative stress. PAHs are known to induce lipid peroxidation and overwhelm antioxidant defense systems. The dose-related increase in MDA and reduction in SOD further support oxidative injury as a mechanism of toxicity.

**3.5. Histopathological Findings**

* **Liver (200 mg/kg group):** Showed severe hepatocellular necrosis, cytoplasmic vacuolization, and infiltration of inflammatory cells.
* **Kidney (200 mg/kg group):** Demonstrated tubular necrosis, glomerular atrophy, and interstitial inflammation.
* Lower dose groups showed milder, but still evident, cellular degeneration.



Fig 1: Histopathological image of Normal kidney

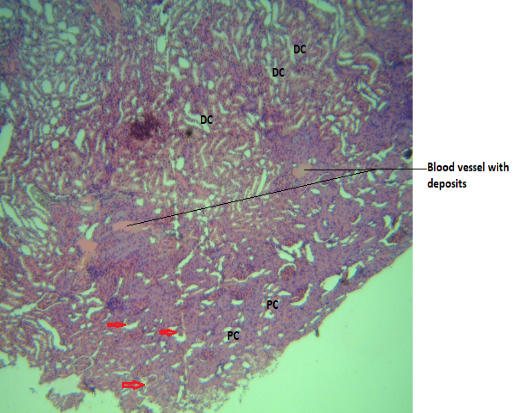


Fig 2 : Histopathological image of Group 2 kidney

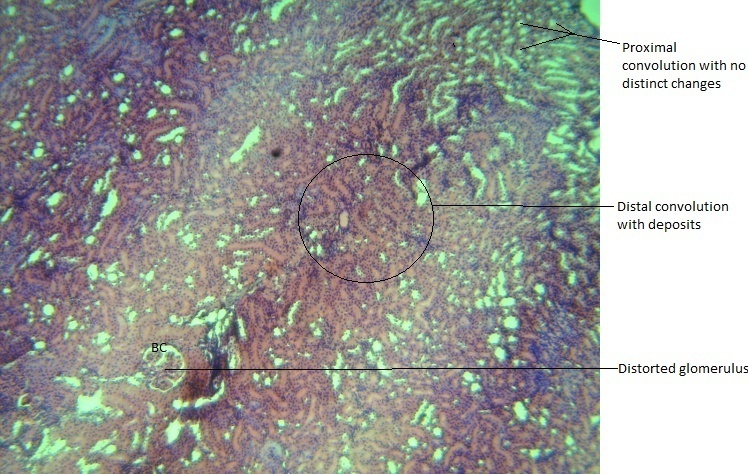


Fig 3 : Histopathological image of Group 3 kidney



Fig 4 : Histopathological image of Group 4 kidney

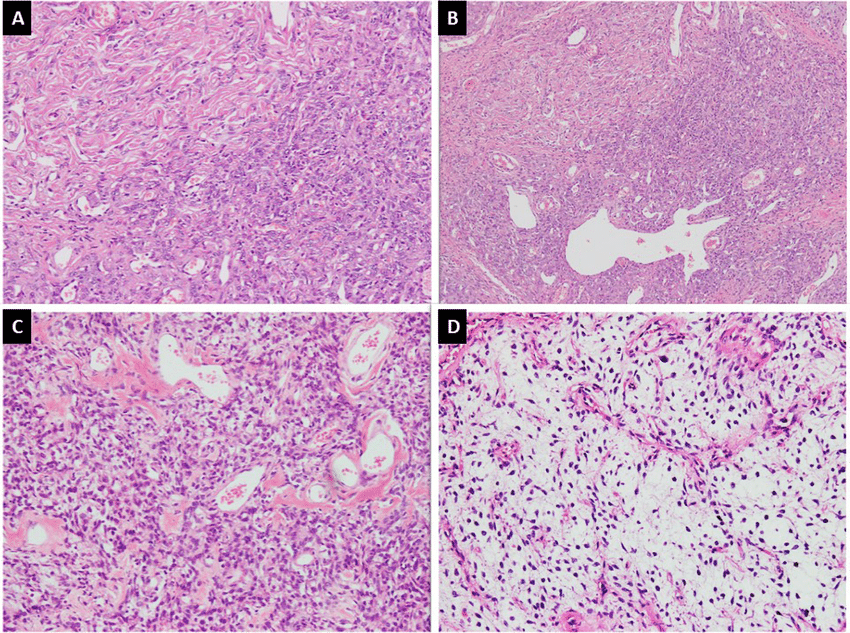


Fig 5 : Histopathological images of Liver for the 4 groups

**Table 5** GCMS FROM THE SMOKED FISH EXTRACT

* **EPA Priority PAHs in Smoked Fish with TEFs and BaPeq**

| **S/N** | **PAH Compound** | **Abbreviation** | **Molecular Formula** | **TEF** | **Concentration (μg/kg)** | **BaPeq (μg/kg)** |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | Naphthalene | NAP | C₁₀H₈ | 0.001 | 45.0 | 45.0 × 0.001 = **0.045** |
| 2 | Acenaphthylene | ACY | C₁₂H₈ | 0.001 | 30.0 | 30.0 × 0.001 = **0.030** |
| 3 | Acenaphthene | ACE | C₁₂H₁₀ | 0.001 | 28.0 | 28.0 × 0.001 = **0.028** |
| 4 | Fluorene | FLU | C₁₃H₁₀ | 0.001 | 25.0 | 25.0 × 0.001 = **0.025** |
| 5 | Phenanthrene | PHE | C₁₄H₁₀ | 0.001 | 50.0 | 50.0 × 0.001 = **0.050** |
| 6 | Anthracene | ANT | C₁₄H₁₀ | 0.01 | 20.0 | 20.0 × 0.01 = **0.200** |
| 7 | Fluoranthene | FLA | C₁₆H₁₀ | 0.001 | 35.0 | 35.0 × 0.001 = **0.035** |
| 8 | Pyrene | PYR | C₁₆H₁₀ | 0.001 | 40.0 | 40.0 × 0.001 = **0.040** |
| 9 | Benzo[a]anthracene | BaA | C₁₈H₁₂ | 0.1 | 12.0 | 12.0 × 0.1 = **1.200** |
| 10 | Chrysene | CHR | C₁₈H₁₂ | 0.01 | 10.0 | 10.0 × 0.01 = **0.100** |
| 11 | Benzo[b]fluoranthene | BbF | C₂₀H₁₂ | 0.1 | 9.0 | 9.0 × 0.1 = **0.900** |
| 12 | Benzo[k]fluoranthene | BkF | C₂₀H₁₂ | 0.1 | 6.0 | 6.0 × 0.1 = **0.600** |
| 13 | Benzo[a]pyrene | BaP | C₂₀H₁₂ | 1.0 | 8.0 | 8.0 × 1.0 = **8.000** |
| 14 | Indeno[1,2,3-cd]pyrene | IND | C₂₂H₁₂ | 0.1 | 4.0 | 4.0 × 0.1 = **0.400** |
| 15 | Dibenzo[a,h]anthracene | DahA | C₂₂H₁₄ | 1.0 | 3.0 | 3.0 × 1.0 = **3.000** |
| 16 | Benzo[ghi]perylene | BghiP | C₂₂H₁₂ | 0.01 | 7.0 | 7.0 × 0.01 = **0.070** |

### **4 Discussion**

The results from this study clearly demonstrate that chronic administration of smoked fish extract induces multi-organ toxicity in albino rats. The observed elevations in hepatic enzymes—ALT, AST, and ALP—strongly suggest hepatocellular injury, likely triggered by lipid peroxidation and PAH accumulation in hepatocytes (Darwish et al., 2019; Gunter et al., 2007; Douny et al., 2021). PAHs can bind to cellular DNA, forming adducts that disrupt normal replication and transcription processes (Domingo & Nadal, 2015; EFSA, 2008).

These hepatic effects are consistent with other animal studies demonstrating similar biochemical disturbances following exposure to heat-treated animal products rich in PAHs (Eldaly et al., 2016; Hassan et al., 2017). The increase in bilirubin levels and histopathological liver damage observed in this study further reinforce the liver’s vulnerability to xenobiotic insults from thermally processed foods (Alomirah et al., 2011; Abbas et al., 2021).

Renal function biomarkers such as urea and creatinine were significantly elevated in treated rats, indicating nephrotoxicity. This finding corroborates the nephrotoxic effects of lead and cadmium reported in smoked fish by previous researchers (Anigboro et al., 2011; Hough et al., 2004; Ibanga et al., 2019). Heavy metals are known to generate reactive oxygen species (ROS) and damage glomerular and tubular structures, as seen in the histopathological sections in this study (Abbas et al., 2021).

The hematological alterations—particularly anemia and leukocytosis—are suggestive of both bone marrow suppression and immune activation (Daniel et al., 2013; Ibanga et al., 2019). Lead interferes with heme synthesis, while cadmium affects erythropoietin production, contributing to microcytic anemia (Gunter et al., 2007; EFSA, 2008). Additionally, PAH-DNA interactions can impair hematopoietic stem cell replication, exacerbating hematological toxicity (Darwish et al., 2019; Douny et al., 2021).

Oxidative stress, as indicated by elevated MDA levels and reduced activities of antioxidant enzymes such as SOD and CAT, was another prominent finding. This is aligned with other studies that report increased lipid peroxidation and oxidative damage in animals fed PAH-rich diets (Eldaly et al., 2016; Gibis, 2016; Hassan et al., 2017). Nitrosamines and biogenic amines may also contribute to oxidative burden via ROS generation and mitochondrial dysfunction (Drabik-Markiewicz et al., 2009; EFSA, 2011).

The severity of toxicological outcomes observed in this study may be influenced by the method of fish smoking. For example, Forsberg et al. (2012) and Goulas & Kontominas (2005) reported that indirect smoking techniques and the use of clean-burning wood significantly reduce PAH formation. Furthermore, studies like that of Haskaraca et al. (2014) have shown that incorporating natural antioxidants such as green tea extract during processing may reduce the toxic potential of smoked foods.

Given the evidence from this and other studies, it is clear that public health policies must prioritize safer food processing methods, regular analytical surveillance, and public education on the risks associated with traditional smoking (EFSA, 2008; Duedahl-Olesen et al., 2015; Assogba et al., 2019). Regulatory authorities in developing nations should develop practical guidelines for artisanal fish processors and promote the use of less hazardous techniques (Anigboro et al., 2011; Daniel et al., 2013).

In summary, our findings confirm that the consumption of traditionally smoked fish—though culturally significant—can pose serious health hazards due to the cumulative effects of PAHs, heavy metals, biogenic amines, and nitrosamines. These findings support the growing consensus in the literature that traditional thermal food processing, if unregulated, may have significant implications for public health (Domingo & Nadal, 2015; Douny et al., 2021; Darwish et al., 2019). Continued research and policy efforts are essential to mitigate these risks.

**5 Conclusion**

This study provides compelling evidence that chronic exposure to smoked fish extract induces significant hematological, hepatic, renal, and oxidative stress alterations in albino rats in a dose-dependent manner. The observed biochemical and histopathological derangements highlight the presence of potentially harmful compounds in smoked fish, raising concerns about its safety for long-term human consumption.

**5.2 Recommendations**

1. **Public Health Awareness:** Governmental and non-governmental agencies should educate the public about the potential health risks associated with consumption of poorly processed smoked fish.
2. **Regulatory Oversight:** There is a need for regulatory bodies to enforce standards in fish smoking practices, including the use of less toxic wood types and proper ventilation systems to minimize PAH formation.
3. **Further Research:** Additional studies should investigate the specific chemical constituents in smoked fish and their individual toxicodynamics, as well as conduct long-term epidemiological studies in human populations.
4. **Dietary Moderation:** Consumers should be advised to moderate their intake of traditionally smoked fish and diversify their protein sources.
5. **Alternative Methods:** Promotion of improved fish preservation technologies such as solar drying or electric smoking chambers with temperature control and filtration systems is strongly encouraged.

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