Review



# Fishery Anesthetics in Aquaculture Products: Safety Concerns and Analytical Methods

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Posted Date: 29 September 2025

doi: 10.20944/preprints202509.2352.v1

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# Fishery Anesthetics in Aquaculture Products: Safety Concerns and Analytical Methods

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**Abstract**

Fishery anesthetics are extensively employed in aquaculture to mitigate stress and reduce mortality during handling, transportation, and processing of fish. While they enhance operational efficiency and reduce economic losses for fish merchants, the potential residual presence of these anesthetics raises concerns regarding human health risks and environmental impact. This review examines six commonly used anesthetics in aquaculture—eugenol, MS-222, benzocaine, 2-phenoxyethanol, diazepam, and quinaldine—focusing on their mechanisms of action, application risks, ecotoxicological effects, and methods for residue analysis. The objective is to promote the safe and informed application of these anesthetics, mitigate their biological toxicity, and minimize their ecological impact. Furthermore, the review provides technical insights into monitoring and managing anesthetic residues in aquaculture to ensure the safety of aquatic products and safeguard environmental health, while also supporting the development of sustainable aquaculture practices.

**Keywords:** aquatic products; fishery anesthetics; analytical methods; food safety

## 1. Introduction

Fresh aquatic products are an important source of high-quality protein and essential nutrients,playing a valuable role in a balanced human diet [1]. Owing to their desirable flavor, high nutritional value, and low cholesterol content, these products are widely consumed worldwide. With the expansion of the seafood market, however, ensuring the safety of aquatic products has become a major concern. Consumers are increasingly attentive not only to nutritional benefits but also to potential hazards such as chemical residues, contaminants, and improper handling practices.

The application of fishery anesthetics is a critical practice to mitigate stress during aquaculture operations [2]. Procedures such as transport and handling can induce intense stress, leading to injury, mortality, and compromised product quality [3]. The judicious use of anesthetics helps minimize these adverse effects, supports animal welfare, and can enhance antioxidant capacity, thereby extending shelf life [4,5]. However, the safety and efficacy of these compounds are dose-dependent. It is therefore essential to establish optimal dosage regimes and understand their physiological impacts to safeguard both fish health and consumer safety.

In many regions, the application of anesthetics is not sufficiently regulated. Dosages and withdrawal periods are often determined empirically, increasing the risk of unsafe residue

 doi:10.20944/preprints202509.2352.v1



levels in edible tissues. Moreover, regulatory standards for maximum residue limits (MRLs) and withdrawal periods vary significantly among countries, and some anesthetics still lack established guidelines (Table 1). These regulatory gaps underline the need for more research on rapid detection methods, residue elimination kinetics, and anesthetic efficacy.

**Table 1.** Withdraw period and maximum residue limit standards for different anesthetics in some countries/organization.

| Anesthetic | MRL | | Withdrawal period | Reference |
|---|---|---|---|---|
| Eugenol | New Zealand | 100ng $mL^{-1}$ | — | [123] |
| | Japan | 50ng $mL^{-1}$ | 7 d | [28] |
| Isoeugenol | European Conformity (CE) | 6mg $kg^{-1}$ | — | [124] |
| AQUI-S | Australia and Chile | — | 0d | [125] |
| MS-222 | FDA | 1μg $mL^{-1}$ | 21 d | [126] |
| | Canada | — | 7 d | [39] |

This review provides a comprehensive analysis of commonly used fishery anesthetics, with a focus on their safety evaluation, residue detection techniques, and implications for food safety. Recent advances in residue depletion studies and analytical methodologies are highlighted, with the aim of supporting the rational and safe use of anesthetics in aquaculture. The paper also identifies current research gaps and suggests future directions to strengthen regulatory frameworks and ensure the protection of public health.

## 2. Overview of Fishery Anesthetics

Fishery anesthetics are chemical agents employed in aquaculture to induce sedation or temporary immobility in fish,thereby reducing stress and physical injury during operations such as transport, handling, sampling, and artificial reproduction [6,7]. By suppressing stress responses, these compounds help maintain fish welfare, lower mortality, decrease disease susceptibility, and preserve flesh quality [8]. Stress-related metabolic changes—such as lactic acid accumulation—can impair meat texture and overall product quality; anesthetic use helps mitigate these effects.

Common fishery anesthetics include tricaine methanesulfonate (MS-222), isoeugenol, eugenol, benzocaine, 2-phenoxyethanol, quinaldine, tetracaine, and bupivacaine. These substances differ in chemical properties, efficacy, and safety profiles. Depending on their solubility (water-soluble or fat-soluble), they act on the central nervous system to produce reversible sedation or anesthesia. Figure 1 illustrates their chemical structures, aiding in understanding their molecular characteristics and potential residue behavior.

 doi:10.20944/preprints202509.2352.v1



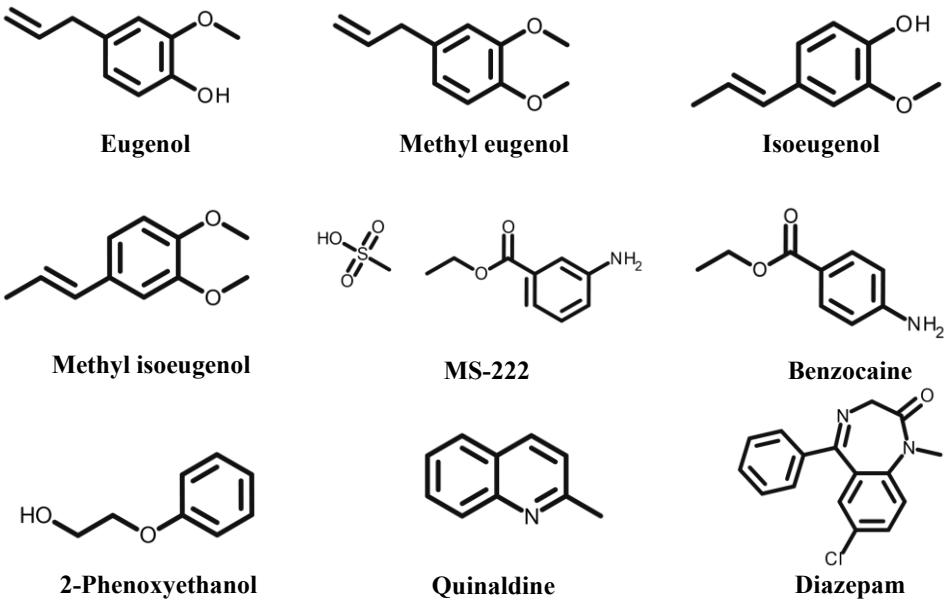


**Figure 1**

Appropriate selection and dosing of anesthetics are critical not only for fish welfare but also for compliance with humane treatment standards and food safety regulations. Moving forward, a deeper exploration of the pharmacological principles of commonly used anesthetics, will help further clarify their mechanisms of action and safety profiles in aquaculture.

## 3. Pharmacological Actions and Safety Assessment

### *3.1. Eugenol*

Eugenol, derived primarily from clove oil, contains 85-95% eugenol as the main active ingredient, with minor components such as methyl eugenol and isoeugenol [9,10]. It acts as an effective anesthetic by inducing temporary immobility in fish, reducing stress responses during procedures like transportation, handling, and artificial reproduction. This leads to minimized physical injuries, such as mechanical damage and scale loss, and lowers the risk of subsequent infections [7]. Additionally, eugenol decreases ammonia excretion in fish by reducing metabolic rates, which enhances water quality and lowers ammonia toxicity [11]. Eugenol also possesses antioxidant and anti-inflammatory properties that help mitigate stress responses during anesthesia [12].

Fishery anesthetics are primarily absorbed through the gills, accumulating in tissues and being eliminated via the same route [13,14]. Studies on eugenol in Pacific white shrimp revealed that, regardless of treatment duration (e.g., 300 mg $L^{-1}$ for 5 minutes or 10 mg $L^{-1}$ for 24 hours), residual eugenol concentrations fell below 2.5 mg $kg^{-1}$ within 24.5 hours. This indicates a rapid depletion of eugenol residues, suggesting a low risk of exceeding the FAO/WHO acceptable daily intake (ADI) when shrimp are consumed [15]. Moreover, the half-life of eugenol in seabass varies with water temperature, demonstrating faster metabolism at higher temperatures (e.g., 2 hours at 20°C vs. over 4 hours at 13°C) [16]. These findings suggest that temperature control can enhance eugenol metabolism, accelerating residue elimination in fish tissues.

Although eugenol is effective as an anesthetic, it poses cytotoxic risks at high concentrations. In vitro studies have shown that eugenol can cause developmental

 doi:10.20944/preprints202509.2352.v1



abnormalities in zebrafish embryos, including skeletal deformities and pericardial edema, when exposed to concentrations between 0.5 and 2 mg $L^{-1}$ [17]. Tao et al. also found that eugenol exposure delayed the hatching of zebrafish embryos, reduced their body length, and decreased the inflation rate of their swim bladders [18].Additionally, eugenol has been linked to cytotoxicity in various cell lines [19], including mouse fibroblasts [20], rat hepatocytes [21], and human dental pulp fibroblasts [22,23]. In vivo studies have also demonstrated its potential for oral mucosal damage in rats [24,25]. Despite these risks, studies on rats and other animals have shown no toxicity at low concentrations, further supporting its safety when used according to guidelines [26].

Eugenol has been approved as a fish anesthetic in several countries, including New Zealand, Japan, Australia, Chile, and Finland [27]. In Japan, the MRL is 50 ng $mL^{-1}$, with a withdrawal period of 7 days for fish and 10 days for crustaceans [28]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set an acceptable daily intake (ADI) of 2.5 mg $kg^{-1}$ for eugenol [29]. However, there are no established MRLs or withdrawal periods for eugenol in the United States and China. The U.S. FDA permits eugenol as a food additive at concentrations up to 1.5 g $kg^{-1}$ but does not approve it for use as a fish anesthetic [30]. Given the variability in regulation across regions, it is essential to adhere to local guidelines to ensure the safety of seafood for human consumption.d mesenchymal stem cells (HASCs), indicating cytotoxic effects [31].

### *3.2. MS-222*

MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate), commonly known as tricaine, is widely used as an anesthetic for aquatic animals. It is absorbed into the bloodstream through the gills and skin and distributed throughout the body [32]. MS-222 primarily induces anesthesia by blocking sodium ion channels in muscle cells and, to a lesser extent, potassium ion channels in nerve membranes. This mechanism leads to a loss of consciousness and reduced metabolic activity [33]. The anesthetic efficacy of MS-222 depends on biological factors such as fish species, size, and environmental factors like water temperature, pH, and salinity [34]. MS-222 is preferred for its low effective dosage, rapid onset, quick recovery, and minimal side effects, making it a widely used anesthetic in fisheries worldwide [35]. Importantly, MS-222 does not accumulate in fish tissues, leaving minimal residues in muscle, ensuring its safety and effectiveness.

MS-222, initially developed as a substitute for cocaine, is the only anesthetic approved by the U.S. FDA for aquaculture, although its use is limited to specific fish families, such as *Ictaluridae*, *Salmonidae*, *Esocidae*, and *Percidae* [36]. It is absorbed and eliminated through diffusion across the gill membranes, with no involvement of unmetabolized parent compounds [37]. In fish, MS-222 is mainly metabolized via two pathways: (i) hydrolysis of the ethyl ester to yield m-aminobenzoic acid, which is subsequently N-acetylated, and (ii) N-acetylation of the parent compound followed by hydrolysis [38]. These processes predominantly occur in the liver and gills. In adult rainbow trout, MS-222 and its metabolite, ethyl-meta-acetylaminobenzoate, are excreted through the kidneys. Fish treated with MS-222 in Canada must be held in clean water for 21 days before marketing, with a mandatory 7-day waiting period [39].

Prolonged exposure to MS-222 can cause skin and respiratory irritation and, in rare cases, retinal damage [40]. While MS-222 has been shown to be toxic to certain fish species at high concentrations, its high water solubility and absence of documented human health risks support its use as a safe anesthetic when the recommended withdrawal period is followed. Studies have shown that MS-222 significantly reduces stress in fish during anesthesia,

 doi:10.20944/preprints202509.2352.v1



improving animal welfare during aquaculture operations [41]. However, it is essential to monitor exposure to avoid potential toxicity, especially in species with higher sensitivity .

MS-222 is approved as a fish anesthetic in several countries, including the United States, Norway, and New Zealand, with withdrawal periods of 21, 21, and 10 days, respectively, before consumption [38]. However, it is not approved in China, Spain, Greece, or France, and no official national standard methods for residue detection have been established in these countries. Despite this, MS-222 remains one of the most widely used anesthetics in global aquaculture, with established withdrawal periods and guidelines in regions where it is permitted [38]. The U.S. FDA allows its use in aquaculture with specific restrictions on fish families and requires adherence to withdrawal periods to ensure food safety.

### *3.3. Benzocaine*

Benzocaine (ethyl p-aminobenzoate) is a widely used local anesthetic known for its rapid onset, low toxicity, stable efficacy, and prolonged effect. It works by binding to sodium ion channels, blocking nerve impulse transmission. These properties make benzocaine an ideal anesthetic for various aquatic species, including juvenile *Colossoma macropomum* and *Aulonocara nyassae*, offering a cost-effective alternative to other anesthetics [42,43].

Benzocaine is absorbed into fish and metabolized into N-acetylbenzocaine, which is then excreted through the gills, kidneys, and bile [44]. A study by Meinertz et al. on rainbow trout demonstrated that 59.2% of benzocaine residues were excreted through the gills within 3 hours, while renal elimination was slower, with only 2.7% excreted through the kidneys after three hours, and 9.0% after 24 hours. Bile also contributed to the elimination of 2.0% of the administered dose after 24 hours [45]. These findings highlight that the gills are the primary route of elimination, with the kidneys and biliary system serving secondary roles.

While benzocaine is considered safe for use in aquaculture, excessive exposure can lead to toxicity, including arrhythmias, coma, and pulmonary complications [46]. It may also cause allergic reactions, such as contact dermatitis and hypersensitivity [47]. Despite its generally low toxicity, the use of benzocaine as an aquatic anesthetic has been prohibited in several countries, mainly due to concerns about residue control and food safety. Nevertheless, genotoxicity studies have demonstrated that benzocaine does not possess genotoxic properties. [48].

Benzocaine is subject to regulatory oversight in several countries. In the United States, the FDA has set an import tolerance of 50 μg $kg^{-1}$ for benzocaine residues in fish muscle, with the expectation that residue levels will diminish to minimal concentrations within 24 hours after treatment. Regulatory authorities in Australia and New Zealand have set a maximum residue limit (MRL) of 0.05 mg $kg^{-1}$ in finfish, ensuring detectable residues are minimal or below the detection limit [49]. However, China has yet to establish specific regulations for the use of benzocaine in aquaculture.

### *3.4. 2-Phenoxyethanol*

2-Phenoxyethanol is a colorless, oily liquid, soluble in water, and commonly used as a local anesthetic in aquaculture [50]. In fish, it is absorbed across the gills and skin and transported by arterial blood to the central nervous system, while excretion takes place mainly through branchial respiration. In rainbow trout, its biological half-life is approximately 30 minutes [51]. Although the exact mechanism of action remains unclear, studies suggest that it may act by expanding neuronal cell membranes [52].

While it offers low cost and strong bactericidal properties, 2-phenoxyethanol poses residue concerns, has a prolonged duration of effect, and may be harmful to fish. Compared to anesthetics like MS-222 and eugenol, its use is more restricted, and its efficacy in preserving fish during transport is lower [27]. In the U.S. and European Union, 2-phenoxyethanol is mainly

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used for non-food fish, aquaculture research, and sedation of ornamental fish during transport [53,54]. The compound is not approved for use in food fish, and experts including Priborsky and Velisek recommend avoiding its application in aquaculture species intended for human consumption because of legal, safety, and environmental considerations [55].

The true anesthetic mechanism of 2-phenoxyethanol in fish is still under investigation. Based on studies in other vertebrates, it is believed to inhibit neural activity in higher regions of the nervous system [56]. Side effects observed include reduced ventilation rate, heart rate, and blood oxygen partial pressure. Exposure in rainbow trout and brown trout has been linked to reductions in red blood cells and platelet counts [57].

Toxicological studies have demonstrated that 2-phenoxyethanol is neither clastogenic nor mutagenic, as evidenced by negative outcomes in the in vivo micronucleus and Ames tests [58]. However, it has been reported to cause toxicity in skin and upper airway tissues [59]. Chronic exposure across multiple species suggests that it may induce hepatotoxicity, renal toxicity, and hemolysis [60]. Notably, a study by Velisek and Svobodova found elevated alanine aminotransferase (ALT) levels in juvenile carp, indicating potential hepatotoxic effects [61].

Currently, no countries explicitly authorize the use of 2-phenoxyethanol for fish intended for human consumption. Its use in food fish is prohibited in the U.S. and the European Union, where it is restricted to non-food fish, aquaculture research, and ornamental fish sedation. Concerns over food safety, legality, and environmental impact limit its broader application in aquaculture.

### *3.5. Diazepam*

Diazepam is a long-acting benzodiazepine sedative that acts as a positive allosteric modulator of type-A γ-aminobutyric acid receptors (GABAARs), enhancing the effect of γ-aminobutyric acid (GABA) and reducing neuronal activity [62]. It is commonly used to treat neurological disorders such as epilepsy, anxiety, and sleep disturbances. In aquaculture, diazepam reduces the metabolic rate in fish, promoting growth, alleviating stress, and improving survival rates. It also induces a schooling effect in fish when added to their feed. However, the use of diazepam at any stage can lead to its residues in fish, which are persistent and can transfer through the food chain to humans. Studies have shown that diazepam residues in freshwater fish such as Parabramis pekinensis and Carassius auratus can reach concentrations of 0.5-118.6 μg $kg^{-1}$, with a detection rate as high as 26.8% [63].

The pharmacokinetics of diazepam in aquatic species has not been extensively studied. However, in mammals, diazepam is well absorbed after oral administration, reaching peak plasma concentrations within 30 to 90 minutes [64]. Its metabolism in humans is influenced by factors such as age, gender, liver disease, and genetic variations, particularly those affecting cytochrome P450 enzymes [65]. In pregnant women, diazepam rapidly crosses the placenta, and its accumulation in the fetus can lead to prolonged sedation in newborns.

The use of diazepam in aquaculture and during fish transportation is prohibited; however, residues have still been detected in animal products. A survey conducted by the Hunan Provincial Department of Agriculture identified residues above permissible limits in carp, with 4.9% of 286 samples from various species testing non-compliant [66]. These findings highlight ongoing concerns regarding human exposure through the food chain.

Diazepam residues in fish can be harmful if consumed by humans. Ingesting contaminated fish may cause symptoms such as fatigue, drowsiness, ataxia, and mental confusion. In severe cases, it may lead to coma, arrhythmias, or carcinogenic effects [67,68]. The drug's persistence in fish and its transfer through the food chain highlight the potential health risks associated with illegal use in aquaculture.

 doi:10.20944/preprints202509.2352.v1



Many countries prohibit the use of diazepam in food animals. In China, this restriction was formalized by the Ministry of Agriculture through Announcement No. 193 (2002), which listed sedative-hypnotic drugs, including diazepam, as banned substances in food-producing animals. The current national standard, Maximum Residue Limits of Veterinary Drugs in Food (GB 31650-2019), further stipulates that while diazepam may be administered to non-food animals, residues are not permitted in edible animal products. Similarly, the European Union's Food and Feed Safety Law and Residue Regulation (Regulation (EC) No 470/2009) prohibit diazepam in food products, especially in raw materials like meat, dairy, and poultry [69]. The Codex Alimentarius Commission (CAC) has not set specific standards for diazepam but requires all drug residues in food to be below safe levels and proven to be safe for human consumption. Despite its prohibition, illegal use of diazepam in aquaculture may occur, leading to potential contamination of fish and seafood products. The detection of diazepam residues in such products is considered illegal, and regulatory actions are typically taken to prevent human exposure to contaminated aquatic products.

### *3.6. Quinaldine*

Quinaldine (2-methylquinoline) has antibacterial and antipyretic properties and is widely used as a precursor for pharmaceuticals, including biocides and bactericides [70]. Since the 1950s, it has been studied as a fish anesthetic and has since been applied in fisheries for capture, transportation, sampling, and measurement [70]. At low doses, quinaldine can effectively anesthetize species such as Rohu (Labeo rohita) and Silver Carp (Hypophthalmichthys molitrix), prolonging anesthesia for up to 6 hours, reducing stress, and preventing bacterial growth, which improves survival during transport [71]. Combination strategies have also shown potential; for example, co-administration of quinaldine with diazepam significantly shortened induction time and reduced excitability in seabream compared with quinaldine alone [72].

Systematic pharmacokinetic studies of quinaldine in aquatic species are limited. Current knowledge is largely based on observations of anesthetic onset and duration, with low doses producing several hours of sedation [71]. Data on absorption, distribution, metabolism, and excretion in fish remain scarce, leaving the residue profile of quinaldine insufficiently characterized.

Research on the toxicological effects of quinaldine is also limited. Existing studies suggest that low concentrations are generally safe for fish anesthesia, but its environmental impact raises concern. Quinaldine-containing wastewater is resistant to degradation and may pose long-term ecological risks [73]. The potential for bioaccumulation in fish and subsequent risks to human consumers has not been fully evaluated, highlighting the need for further investigation.

At present, no international or national authority explicitly authorizes quinaldine for use in food fish. Its application is generally restricted to research and fish transport. Due to the lack of clear residue data, toxicological evidence, and food safety assessments, regulators remain cautious, and quinaldine is not approved for aquaculture use in species intended for human consumption.

## 4. Analytical Methods for Residue Detection

### *4.1. Instrument detection*

Monitoring residual anesthetic levels in aquatic products is essential for ensuring food safety and regulatory compliance. However, detection is challenging due to the complex composition of aquatic products, which contain proteins, fats, and other components that can

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interfere with accurate quantification. Moreover, anesthetic residues are usually present at low concentrations, and their metabolites may exhibit toxic or pharmacological effects, necessitating highly sensitive and reliable detection methods.

The detection is further complicated by their diverse chemical properties, including volatility, solubility, and polarity. Each anesthetic requires a specific detection protocol tailored to its characteristics, necessitating flexible methodologies to account for variations in chemical behavior. Pre-treatment steps, such as extraction and purification, are crucial for minimizing interference and ensuring accurate quantification. Common pre-treatment methods include Soxhlet extraction (SE) [74], liquid-liquid extraction (LLE) [75], solid-phase extraction（SPE）[76], headspace solid-phase microextraction (HS-SPME) [77], Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) [78], and molecular imprinting techniques (MIT) [79]. In summary, detecting anesthetics in aquatic products requires advanced analytical instruments, coupled with complex sample preparation workflows, to ensure accuracy and sensitivity. Each method must be optimized based on the chemical properties of the anesthetic, making the process particularly challenging. Table 2 summarizes key analytical parameters for the instrumental analysis of anesthetics in aquatic products.

**Table 2.** Representative analytical parameters for the analysis of anesthetics using instrumental methods in aquatic products.

| Methods | Anesthetic | Sample | Pretreatment | Linearity | Repeatability / Reproducibility* | Detection limits | Reference |
|---|---|---|---|---|---|---|---|
| GC-MS | eugenol | fish back, fish belly, and fish tail carp muscle tissues | VALLME/ HS-SPME | 15.0-750.0 μg $kg^{-1}$ | RSD<20%, n=3 | 0.5 μg $kg^{-1}$ | [81] |
| | eugenol | | SPE | 5.0-500.0 μg $L^{-1}$ | RSD<12%, n=6 | 2.5 μg $kg^{-1}$ | [28] |
| | Isoeugenol | shrimp, tilapia, and salmon | Headspace solid-phase microextraction | 0-160 ng $g^{-1}$ | RSD 5-13%, n=9 | below 15ng $g^{-1}$ | [120] |
| GC-MS/MS | diazepam | water samples | dispersive solid-phase microextraction | 10-1000 ng $mL^{-1}$ | RSD=6%, n=5 | 3 ng $mL^{-1}$ | [121] |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | eugenol, isoeugenol, and methyleugenol | groupers | SPE | 5–500μg $L^{-1}$ | RSD 2.18%-15.5%, n=4 | eugenol 0.4μg $kg^{-1}$, isoeugenol 1.2μg $kg^{-1}$, and methyleugenol 0.2μg $kg^{-1}$ | [27] |
| | 2-Phenoxyethanol | rainbow trout | SPME | 0.1-250 mg $kg^{-1}$ | RSD 3%-11%, n=5 | 0.03mg $kg^{-1}$ | [122] |
| GC-IT-MS/MS | eugenol | mandarin | QuEChERS | 5-1000μg $L^{-1}$ | RSD 1.82%-9.74%, n=6 | 5.0 μg /kg | [123] |
| Orbitrap GC-MS | eugenols | prawns | *m*-PFC column | 0.001–0.1 μg $mL^{-1}$ | RSD 1.2%–7.5%, n=6 | 2-10 μg $kg^{-1}$ | [82] |
| | | carp and eel | QuEChERS | 2–1000 μg $L^{-1}$ | RSD<6%, n=3 | 2.5μg $kg^{-1}$ | [124] |
| | | / | SPE | 0.05-10μg $L^{-1}$ | RSD<9.36%, n=5 | 0.01μg/ L | [83] |
| HPLC-MS/MS | MS-222 | finfish | extracted with acetone using a tissue homogenizer, followed by derivatization with dansyl chloride | 2.5-40.0 ng $g^{-1}$ | RSD 2.6%–8.0%, n=6 | 0.2-1μg $kg^{-1}$ | [75] |
| | | marine fish and freshw | isotope dilution assay | 2.0-200.0 μg $L^{-1}$ | inter- and intra-assay relative standard | 1μg $kg^{-1}$ | [125] |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | ater fish | | | deviations (RSD values) were 0.39-3.01 and 0.85-2.77% | | |
| LC-MS/MS | diazepam | fish and shrimp tissue | $C_{18}$ cartridge solid-phase extraction | 0.05-5ng $mL^{-1}$ | RSD<4.9%, n=3 | 0.01μg $kg^{-1}$ | [126] |
| LC | AQUI-S® 20E (eugenol) | standard water containing fish feed | SPE | 5-500mg $L^{-1}$ | RSD<0.7%, n=3 | 0.0011 mg $L^{-1}$ | [127] |
| HPLC-QTRAP-MS/MS | tricaine, tetracaine, and bupivacaine | fish samples | QuEChERS | 1.0–50.0 μg $L^{-1}$ | RSD<15%, n=3 | 2.0 μg $kg^{-1}$ | [128] |
| PGD-IMS）/ LC-MS/MS | MS-222 | fish-raising water samples | *m*-PFC | 0.005-0.2 mg $L^{-1}$ | PGD-IMS RSD 6.9%–10.3%, n=5 LC-MS/MS RSD 1.3%–3.4%, n=5 | 6μg $kg^{-1}$/0.6μg $kg^{-1}$ | [39] |
| LC-QLIT-MS/MS | eugenol | fish samples | dispersive solid-phase extraction (DSPE) | 1–100 μg $kg^{-1}$ | RSD 1.9%–8.9%, n=6 | 0.03-0.4 μg $kg^{-1}$ | [129] |
| HPLC-UV | Diazepam | water samples | Dispersive micro solid phase extraction | 0.3-450 ng $mL^{-1}$ | RSD 3.42-3.75%, n=3 | 0.09ng $mL^{-1}$ | [130] |

5.1.1. GAS chromatography (GC, GC-MS)

 doi:10.20944/preprints202509.2352.v1



GC and GC-MS are widely preferred for anesthetic residue analysis due to their high sensitivity, effective separation capabilities, and precise quantification of trace anesthetics. Li *et al*. developed a new pretreatment method for eugenol in fish samples using the Stable Isotope Dilution Assay (SIDA) and SPE, effectively minimizing matrix effects during GC-MS analysis. The combined SIDA-SPE-GC-MS/MS approach demonstrated accuracy and precision that meet bioanalytical assay requirements. Among the evaluated techniques, Vortex-Assisted Liquid-Liquid Microextraction (VALLME) combined with HS-SPME pretreatment achieved the lowest detection limits, making it highly effective for eugenol detection [80]. Liang *et al*. established an HS-SPME method for eugenol extraction from fish, ensuring GC-MS stability while incorporating VALLME to reduce matrix effects and enhance sensitivity. The optimized pretreatment demonstrated good repeatability, linearity, and sensitivity, making it suitable for long-term GC-MS analysis [81]. Huang *et al.* were the first to employ a Multiplug Filtration Cleanup (m-PFC) method (Figure 2A), derived from QuEChERS, in combination with Gas Chromatography-Orbitrap Mass Spectrometry (Orbitrap GC-MS) for the determination of six eugenol anesthetics in aquatic products [82]. This rapid pretreatment method exhibited strong resistance to matrix interference, enabling accurate detection in large sample quantities.

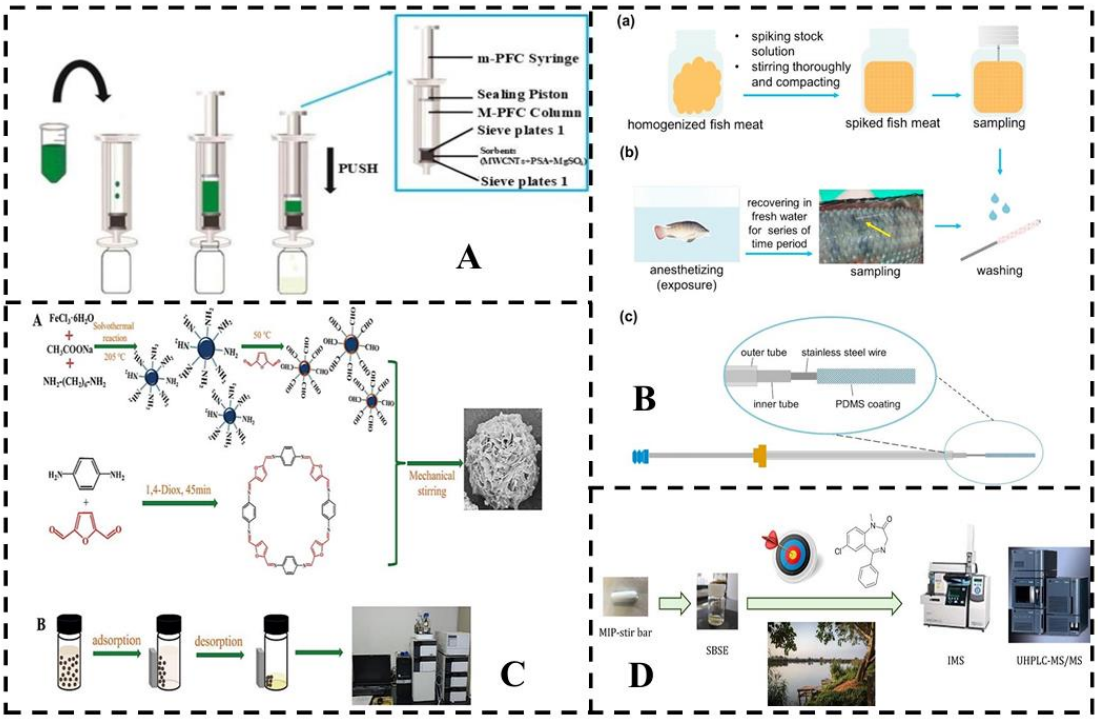


**Figure 2.**

For MS-222, a water-soluble anesthetic, GC can be employed for detection [83]. As shown in Figure 2B, a biocompatible PDMS fiber head extraction technique demonstrates balanced in *vitro* and in *vivo* extraction of five anesthetics from tilapia, highlighting its efficiency in complex biological matrices. This approach offers a short analysis time and prevents analyte loss due to degradation or sample storage [84].

Abreu *et al.* developed and compared SPME and single-drop microextraction (SDME) techniques for detecting 2-phenoxyethanol residues in fish fillets, using a central composite design (CCD) to enable accurate assessments with minimal sample volume. Both methods demonstrated good precision, with SDME achieving detection and quantification limits of 0.2 μg $mL^{-1}$ and 0.62 μg $mL^{-1}$, respectively, while SPME achieved limits of 0.18 and 0.56 μg $mL^{-1}$. At anesthetic concentrations of 450-1050 μg $mL^{-1}$, the elimination times for 2-phenoxyethanol

 doi:10.20944/preprints202509.2352.v1



were 12 hours for SDME and 24 hours for SPME, indicating that both techniques are feasible for residue analysis [77].

#### 4.1.2. Liquid Chromatograph (LC or HPLC)

Unlike GC, liquid chromatography (LC) and high-performance liquid chromatography (HPLC) do not require analytes to be volatilized, making them particularly suitable for detecting non-volatile or thermally unstable anesthetics. In complex matrices such as aquatic products, HPLC offers superior precision and repeatability in quantification, establishing it as a more reliable method for quantitative analysis [85].

Scherpenisse and Bergwerff investigated various extraction columns for detecting MS-222 residues in the tissues of three fish species. Their findings revealed that the $C^{-1}8$ column achieved a higher fortified recovery rate, complying with FDA and Canadian standards for MS-222 [86]. For the rapid determination of diazepam and its main metabolites in fish samples, Li *et al.* employed primary secondary amine (PSA) and multi-walled carbon nanotubes (MWCNT) as QuEChERS sorbents, coupled with high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). The study showed that PSA exhibited superior extraction efficiency than MWCNT, effectively removing interfering substances. This approach yielded excellent recovery and an acceptable relative standard deviation (RSD), with a limit of quantification (LOQ) of 2.5 μg $kg^{-1}$ [67]. Xie *et al.* developed a stable isotope dilution assay coupled with HPLC-tandem mass spectrometry to quantify MS-222 levels in grass carp, utilizing a synthesized stable isotope-labeled Tricaine-$D_5$ to enhance accuracy and precision. This method demonstrated high adsorption capacity for MS-222, providing a rapid and accurate approach for detecting trace levels in aquatic products [87]. In another study, Xia *et al.* synthesized uniform magnetic covalent organic framework (MCOF) microflowers by embedding aldehyde-functionalized $Fe_3O_4$ nanoparticles into a covalent organic framework (COF) under mechanical stirring at room temperature. Under optimal conditions, these MCOF microflowers exhibited rapid adsorption (10 minutes) and high extraction efficiency (over 84.02%) for eugenol anesthetic (Figure 2C). The developed MSPE-HPLC-UV method demonstrated high precision and accuracy, enabling effective quantification of three eugenol anesthetics in tilapia, shrimp, and crab samples [88].

To achieve selective recognition of diazepam, Aitor *et al.* used magnetic stir bars with an activated polytetrafluoroethylene (PTFE) surface, onto which a molecularly imprinted polymer (MIP) monolith was covalently bound. This MIP system enhanced extraction performance (Figure 2D). In addition, two analytical techniques have been proposed for determining diazepam in water extracts: ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS/MS) and ion mobility spectrometry (IMS). Among these, UHPLC-MS/MS demonstrated greater sensitivity, with an limit of detection (LOD) of 1.2 ng $L^{-1}$, compared to IMS (LOD of 1.2 μg $L^{-1}$) [89].

### *4.2. Rapid detection*

Although the instrument exhibits high analytical accuracy, its applicability for the rapid detection of large quantities of on-site samples is limited due to labor-intensive pre-processing and substantial resource consumption, requiring specialized and costly technical personnel [90]. In contrast, rapid analytical methods for detecting fishery anesthetics offer significant advantages, including efficiency, sensitivity, and simplified pre-processing. These methods enable the rapid detection of on-site samples, addressing the limitations associated with conventional instrumental detection [91]. Immunoassay and electrochemical detection methods are currently utilized to rapidly determine anesthetic residues in fish.

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4.2.1. Immunoassays

Immunoassay is a rapid analytical technique that relies on the specific binding reaction between antigens and antibodies. Immunoassay-based detection of fishery anesthetics provides rapid and sensitive screening alternatives to instrument-based methods. Common immunoassay methods include enzyme-linked immunosorbent assay (ELISA), colloidal gold immunochromatographic assay (GICA), and fluorescent immunoassay (FIA) [92].

ELISA is a versatile analytical technique capable of both qualitative and quantitative detection, relying on the immobilization of antigens or antibodies on a solid carrier, followed by colorimetric detection and analysis [93]. This technique is highly valued for its sensitivity, simplicity, minimal sample pretreatment requirements, and capacity to efficiently process large numbers of small-volume samples [94]. GICA is an innovative immunolabeling technique that utilizes colloidal gold as a tracer for labelling monoclonal antibodies [95]. In this method, a competitive reaction occurs between the target analyte in the sample and the antigen on the test line, producing visible chromogenic results that can be observed with the naked eye [96]. FIA integrates the specificity of immune responses with the sensitivity of fluorescence techniques, offering a highly effective tool for detection and analysis [97].

Lateral flow immunochromatography is a cost-effective, user-friendly, and time-efficient method that offers sufficient sensitivity, accuracy, and specificity [98,99]. For instance, Shen *et al*. developed a colloidal gold-based immunoassay for detecting four eugenol compounds (EUGs) in water, with a detection range of 5–100 μg $mL^{-1}$ [95]. As shown in Figure 3A, the lateral flow immunochromatographic strip (LF-ICS) enables portable and rapid MS-222 detection, with visual detection and cut-off limits of 0.1 μg $mL^{-1}$ and 1 μg $mL^{-1}$, respectively [100]. Subsequent research by the group led to the development of a four-layer immunochromatographic assay (Qua-ICS) based on colloidal gold, which incorporated four highly sensitive monoclonal antibodies (mAbs) to simultaneously detect 11 anesthetic residues in fish within 10 minutes. The method exhibited detection ranges of 3.3–10, 11–222, 100–2000, 0.37 and 3.3, and 111–10000 μg $kg^{-1}$ (Figure 3B). Quantitative analysis was performed using a portable strip reader, with detection ranges of 0.15–2.6, 6.3–677, 0.13–2.8, and 83–1245 μg $kg^{-1}$ for procaine, eugenol, bupivacaine, and tetracaine, respectively. Compared to traditional single ICS methods, this multiplex ICS enables the simultaneous detection of 11 anesthetic residues within 10 minutes, providing a more comprehensive screening approach for anesthetic residues in fish [101].

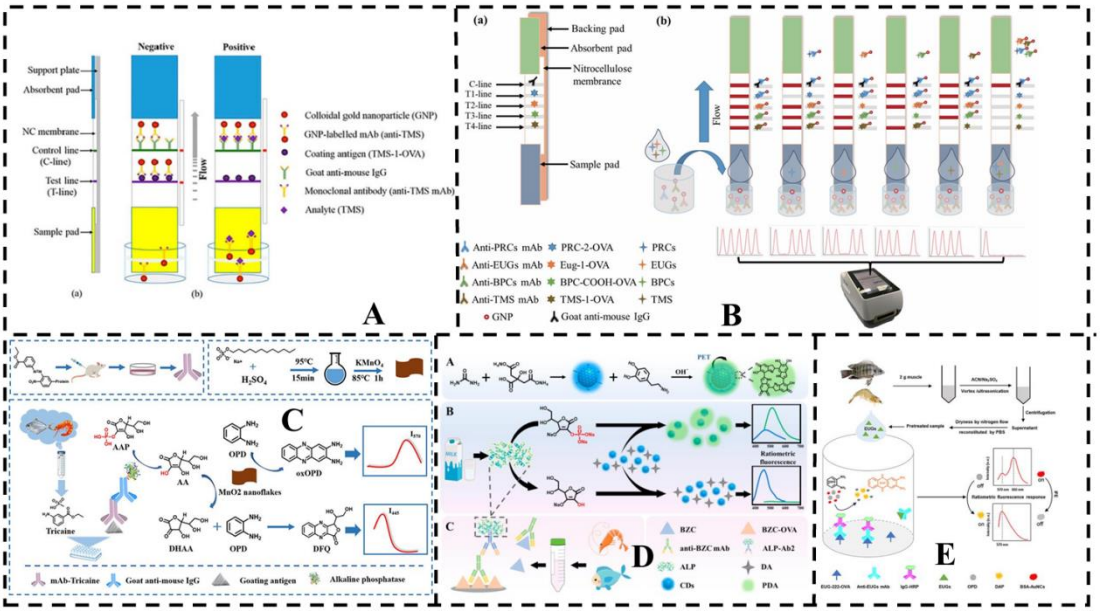


**Figure 3.**

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GICA is primarily suitable for qualitative or semi-quantitative detection. However, for precise quantification, fluorescent immunoassays have been developed [102,103]. Integrating nanomaterials in ELISA enhances the fluorescence signals, improving detection efficiency. Ratiometric fluorescence detection further advances this technique by quantifying analytes based on the intensity ratio of emissions at two different wavelengths [104,105]. This method not only preserves the high sensitivity of fluorescence detection but also incorporates an internal calibration mechanism, effectively mitigating erroneous signals caused by external environmental factors.

For example, our group developed a monoclonal antibody specific to MS-222 and utilized manganese dioxide nanosheets to mediate fluorescence reactions, generating two distinct fluorescence signals for detecting MS-222 residues in aquatic products (Figure 3C). The resulting ratiometric fluorescence ELISA (RF-ELISA) achieved a limit of detection of 0.28 ng $mL^{-1}$ in buffer, which is 376 times lower than that of conventional colorimetric ELISA. In shrimp and tilapia samples, the LODs were 2.8 ng $g^{-1}$ and 5.6 ng $g^{-1}$, respectively [90].

Additionally, our group developed an expandable ratiometric fluorescence sensing platform (Figure 3D). The core mechanism of this platform is based on the alkaline phosphatase (ALP)-catalyzed hydrolysis of ascorbic acid 2-phosphate (AAP), releasing ascorbic acid (AA), which inhibits the dopamine (DA)-mediated synthesis of luminescent polydopamine (PDA). Simultaneously, PDA effectively quenches the fluorescence of carbon dots (CDs), generating a distinct ratiometric fluorescence signal. This multifaceted system enables the RF sensor to achieve ultra-sensitive detection of ALP activity, with a detection limit as low as 0.01 mU $L^{-1}$ for benzocaine [106]. Similarly, our group employed red-emitting gold nanoclusters (Au NCs) as fluorescence probes to quench the fluorescence of 2,3-diaminophenazine (DAP), the oxidation product of o-phenylenediamine (OPD) catalyzed by horseradish peroxidase (HRP) (Figure 3E). Utilizing broad-spectrum monoclonal antibodies against EUGs and enzyme-labeled secondary antibodies, we recently developed a ratiometric fluorescence immunoassay with sensitivity at the pg $mL^{-1}$ level [107]. This approach offers a rapid and efficient screening method for eugenol-based anesthetics in aquatic products while also serving as a reference for developing immunoassays targeting other small molecule contaminants, contributing to food safety and public health.

#### 4.2.2. Electrochemical Sensor

Electrochemical sensor leverages the electrochemical properties of target analytes to convert their chemical quantities into electrical signals [108]. As a rapid detection technology, it offers key advantages such as ease of operation, high sensitivity, and strong potential for miniaturization. Furthermore, the superior electrochemical performance of novel nanomaterials in electrode applications has accelerated advancements in electrochemical detection [109]. These attributes have contributed to its widespread application in diverse fields, including food safety and environmental monitoring.

Rafael *et al.* developed a batch injection analysis (BIA) system utilizing screen-printed carbon electrodes for the rapid and precise quantification of the anesthetics benzocaine and MS-222 in fresh fish fillets [110]. This method exhibits high sensitivity, enabling over 300 injections per hour, with low detection limits and excellent reproducibility. In addition, it effectively minimizes matrix interference in the samples, enhancing analytical accuracy. Regarding MS-222, Cai *et al*. developed a nanoporous gold (NPG) electrochemical sensor using a simple and efficient one-step corrosion method with concentrated nitric acid (Figure 4A). Their study demonstrated that the NPG sensor exhibits high sensitivity, a broad linear range, and reasonable recovery rates, along with user-friendly operation, making it well-suited for rapid on-site sample testing [111]. Moreover, as shown in Figure 4B, Shi *et al.* developed a

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platinum nanoparticle/raspberry-like $SiO_2$-modified glassy carbon electrode (Pt NPs@RL-$SiO_2$/GCE), which demonstrated excellent electrocatalytic activity for the simultaneous detection of eugenol and methyleugenol [112]. Similarly, Chen *et al.* constructed a chitosan-reduced graphene oxide/multimetal oxide/poly-L-lysine (CS-rGO/$P_2Mo_{17}Cu$/PLL) modified electrode via electrodeposition, achieving a detection limit of 0.4490 μM. This electrode demonstrated effective applicability for detecting EU residues in the kidneys, liver, and muscle tissues of freshwater bass [113] (Figure 4C).

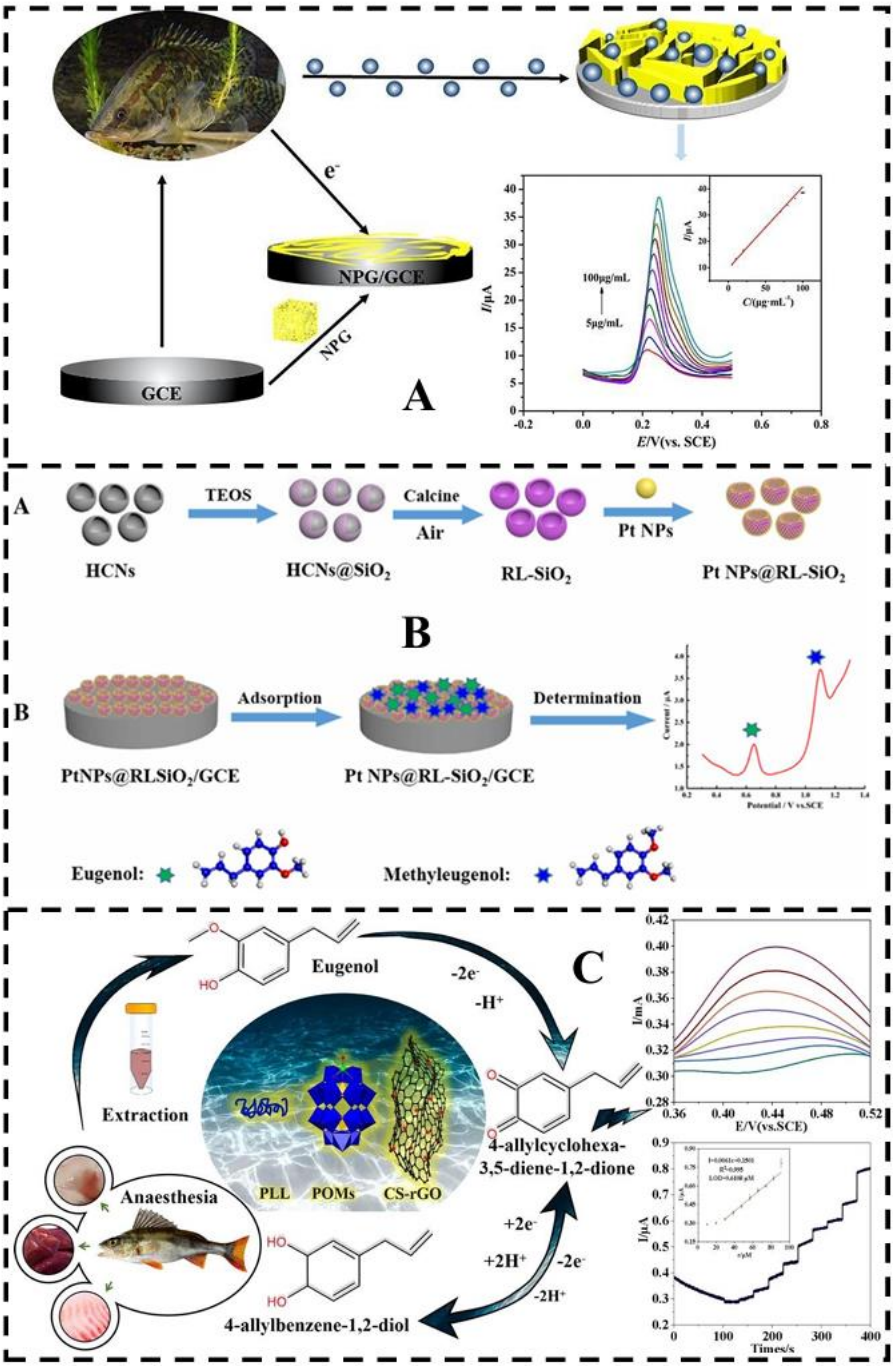


**Figure 4.**

Finally, several innovative electrochemical sensor electrodes have recently been developed for the sensitive voltammetric quantification of benzocaine. For example, Pysarevska *et al.* were the first to utilize a miniaturized boron-doped diamond thick film electrode as an advanced electrochemical sensor [114]. Meanwhile, Wang *et al.* leveraged the

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remarkable properties of β-cyclodextrin to fabricate a carbon black/β-CD nanocomposite electrode [115]. However, current research on the detection of fishery anesthetics remains limited, underscoring the need for further exploration in this field. Advancing the application of electrochemical sensors for the rapid detection of fishery anesthetics represents a promising direction for future research.

## 5. Conclusion

Fishery anesthetics such as eugenol, MS-222, and benzocaine are indispensable for reducing stress and mortality during aquaculture operations, thereby improving product quality and animal welfare. However, the lack of harmonized regulations and the potential accumulation of residues in edible tissues raise concerns for food safety, environmental health, and consumer confidence. In particular, the illegal use of sedatives such as diazepam in aquaculture has been reported, posing serious food safety risks due to their persistence, bioaccumulation, and potential transfer through the food chain. These cases highlight the urgent need for stricter monitoring and enforcement. Current detection approaches—primarily instrumental analysis and rapid screening—each have strengths and limitations, and their combined application represents an effective strategy for ensuring both accuracy and practicality in residue monitoring. Looking ahead, research should prioritize the development of eco-friendly anesthetics with low toxicity, rapid degradability, and minimal residue risks. Advancements in multi-residue detection technologies, including biosensors and point-of-care assays, will enable faster and more accessible monitoring. A One Health perspective is needed to integrate human, animal, and environmental safety considerations, while harmonized global regulatory frameworks will help reduce trade barriers and protect public health. Finally, sustainable aquaculture practices and targeted training programs for producers should be promoted to minimize anesthetic use and foster a culture of responsibility in the industry.

**CRediT authorship contribution statement**

Bao-Zhu Jia: Investigation, Methodology, Writing – original draft. Xue-Ying Rui: Data curation, Investigation, Methodology, Editing the draft. Yu Wang: Writing – review & editing, Resources. Xi Zeng: Writing – review & editing. Shu-Jing Sheng: Supervision, Writing – review & editing. Bi-Jian Zeng: Conceptualization, Funding acquisition. Zhen-Lin Xu: Resources, Funding acquisition. Lin Luo: Investigation, Resources, Funding acquisition.

**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:** Data will be made available on request.

**Acknowledgments:** This work was supported by the Guangdong Basic and Applied Basic Research Foundation (2023A1515030011, 2021A1515012422), Guangzhou Planned Program in Science and Technology (202102021002).

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