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Zebrafish as a model for assessing biocide toxicity: A comprehensive review

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ABSTRACT

The utilization of biocides in a myriad of products has become a widespread and critical practice in recent years. Among these, quaternary ammonium compounds, polyhexamethylene, parabens, and triclosan are notably prevalent across various industrial applications. However, the incorporation of these biocides raises significant concerns regarding their toxicological profile. Not only do these chemicals pose potential risks to consumers using biocide-containing products, but their environmental discharge also represents a substantial threat to the biosphere. In our meticulous review, we examined approximately 150 articles from esteemed databases including PubMed, MDPI, and Google Scholar, ultimately utilizing at least 88 of these articles to inform our analysis. Our investigation encompassed studies that probe general toxicity, behavioral toxicity, cardiovascular toxicity, and genotoxicity, among other toxicological impacts. With this comprehensive approach, we explore the zebrafish (Danio rerio) as a prominent model organism in toxicology research. This review article aims to synthesize research employing zebrafish to evaluate biocide toxicity and ascertain the suitability of this model for comprehensive analysis of biocidal agents and their associated products.

1. Introduction

Since ancient times, people have striven to combat harmful beings affecting their food, animals, and themselves. They use biocides to eliminate pathogens, initially relying on natural ingredients like salt, honey, vinegar, and methods such as drying and smoke coating. Over time, these practices have evolved into today's chemical biocides [1]. The utilization of chemical biocides as disinfectants has a rich historical context, with physicians playing a pivotal role in their early adoption. During the early 19th century, physicians recognized the importance of controlling microbial contamination to prevent the spread of infectious diseases. Their pioneering efforts laid the foundation for the widespread use of chemical biocides in healthcare settings and beyond [2,3]. They have significant implications for human health in today's world. They serve essential functions by exerting toxic effects and effectively eliminating various pathogens. Biocides are utilized across multiple facets of human life, including water treatment, food industries, health products, healthcare centers etc [4–6]. Among all available biocides, four distinct categories stand out as the most widely used: quaternary ammonium compounds (QACs), parabens, guanidine, and triclosan. Let's delve into each of these essential biocide categories. The use of biocides has been an integral part of human activities across various domains, from household disinfection to industrial applications, leading to increased environmental presence and potential health risks. While several studies have examined the toxic effects of biocides, a comprehensive review focusing on the use of zebrafish as a model organism for this purpose is lacking. This review addresses this gap by synthesizing research on the toxicological effects of quaternary ammonium compounds (QACs), polyhexamethylene, parabens, and triclosan in zebrafish, while also considering the integration of emerging technologies like AI for morphological image analysis and tracking. By doing so, this review aims to ascertain the suitability of zebrafish for comprehensive analysis of biocidal agents and their associated products.

2. QAC

Quaternary Ammonium Compounds (QAC) play a crucial role as beneficial biocides across diverse applications. These compounds have gained recognition as one of the most effective disinfection materials over the past century [7]. They have remarkably low toxicity levels, making them suitable for a wide range of industrial activities, including cosmetic and healthcare products [8,9]. The mechanism of action for this all-purpose biocide involves exposing the cytoplasm by disrupting the cell wall [10].

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3. Polyhexamethylene

Polyhexamethylene play a pivotal role as disinfection agents, falling into two distinct categories: natural and synthetic [11]. These versatile compounds find applications across various domains, including natural and cosmetic products, swimming pools, medicine, and even the food industry, thanks to their remarkable broad-spectrum efficiency [12,13]. The primary mechanism of action for Polyhexamethylene involves the disruption of cell membranes [14]. However, recent research has raised concerns about the use of humidifier disinfections containing Polyhexamethylene. These disinfectants have been associated with severe effects, particularly lung injuries in humans [15].

4. Paraben

Parabens are a group of synthetic chemicals widely used as artificial preservatives in cosmetic and healthcare products. These chemicals are added to prevent and reduce the growth of microbial agents, thereby increasing the shelf life of the product [16]. They are used not only for their broad-spectrum antimicrobial activity, but also because they are inexpensive, easy to handle, and have low toxicity [17,18]. These qualities place parabens at the center of attention. On the other hand, they exhibit some adverse effects on the human body, notably endocrine disruption [19]. In addition, parabens have adverse effects on the environment, leading to soil and water pollution [20]. The main mechanism of action of parabens involves inhibiting membrane transport and mitochondrial activity [21].

5. Triclosan

Triclosan, a substance with broad-spectrum effects, has been utilized as a pesticide for a period [22]. Widely incorporated into lotions, including oral and skin care solutions, triclosan plays a role in human health products [23]. However, its impact varies from acute to chronic, depending on usage duration, affecting various organs [24]. Notably, triclosan is detected in water sources due to its release into wastewater [25]. It is worth noting that the mechanism of action of triclosan involves inhibiting fatty acid synthesis in pathogenic agents [26].

6. Zebrafish

As such, it's crucial to continue monitoring their use and disposal, and to develop safer alternatives where possible. In the realm of biological research, a multitude of methods exist for assessing the toxicity of various materials. Among these, in vitro techniques, such as cell cultures, have gained popularity due to their cost-effectiveness [27]. However, a significant challenge lies in the difficulty of generalizing the results of these tests to entire organisms. In contrast, in vivo techniques offer more comprehensive testing but come with their own set of challenges. One organism that has proven to be a valuable asset in this field is the zebrafish (Danio rerio). This species is not only easy to nourish and conserve, but it also exhibits rapid growth and a short lifespan [28]. Remarkably, the zebrafish shares a significant degree of genetic similarity with humans. This has led to its emergence as one of the most successful and useful animal models in toxicology. A commonly employed method for measuring the toxicity of chemical substances is the Zebrafish Embryo Toxicity Test (ZET). This test is not only quick and simple but also versatile, as it can be used to assess a wide range of hazardous chemical substances [29,30]. This article reviews various aspects of toxic effects, including genotoxicity, developmental toxicity, and behavioral toxicity, associated with these four commonly used chemical biocides when applied to zebrafish embryos (Figs. 1–5).

7. Methodology

We conducted an extensive literature search across reputable databases, including ScienceDirect, Google Scholar, and the Science Citation Index (SCI-Expanded) of Web of Science. Our search criteria involved keywords related to biocides (such as Quaternary ammonium compounds, polyhexamethylene, parabens, and triclosan) in conjunction with zebrafish (Danio rerio). From the retrieved pool of 200 research papers, we meticulously analyzed at least 101 articles that employed zebrafish as an experimental organism to investigate the effects of biocides. Our focus centered on assessing the toxicities associated with four commonly used disinfectants found in household and cosmetic products. By leveraging the zebrafish model, we aimed to elucidate the impact of these biocides on various physiological and developmental endpoints. Studies were excluded if they did not use zebrafish, focused on other types of biocides, or were not peer-reviewed journal articles.

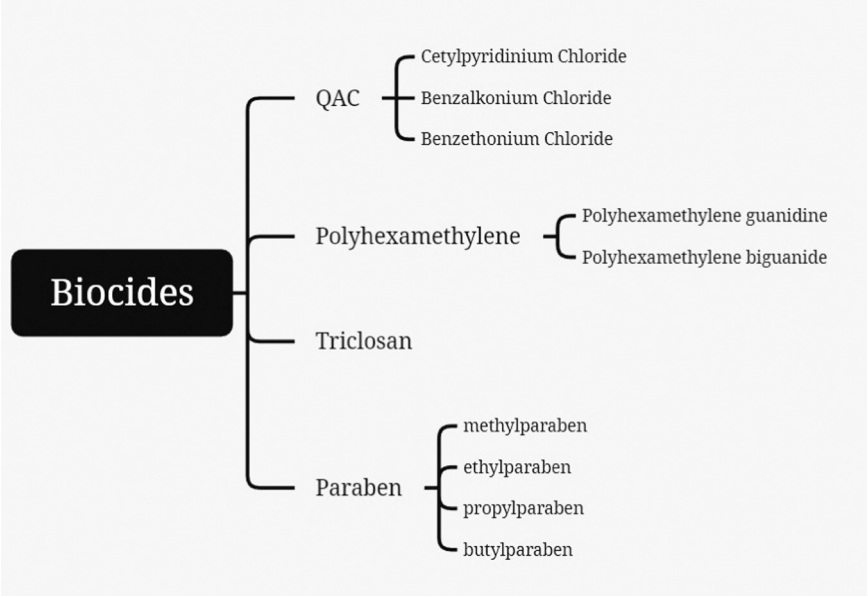


Fig. 1. Classification of biocides.

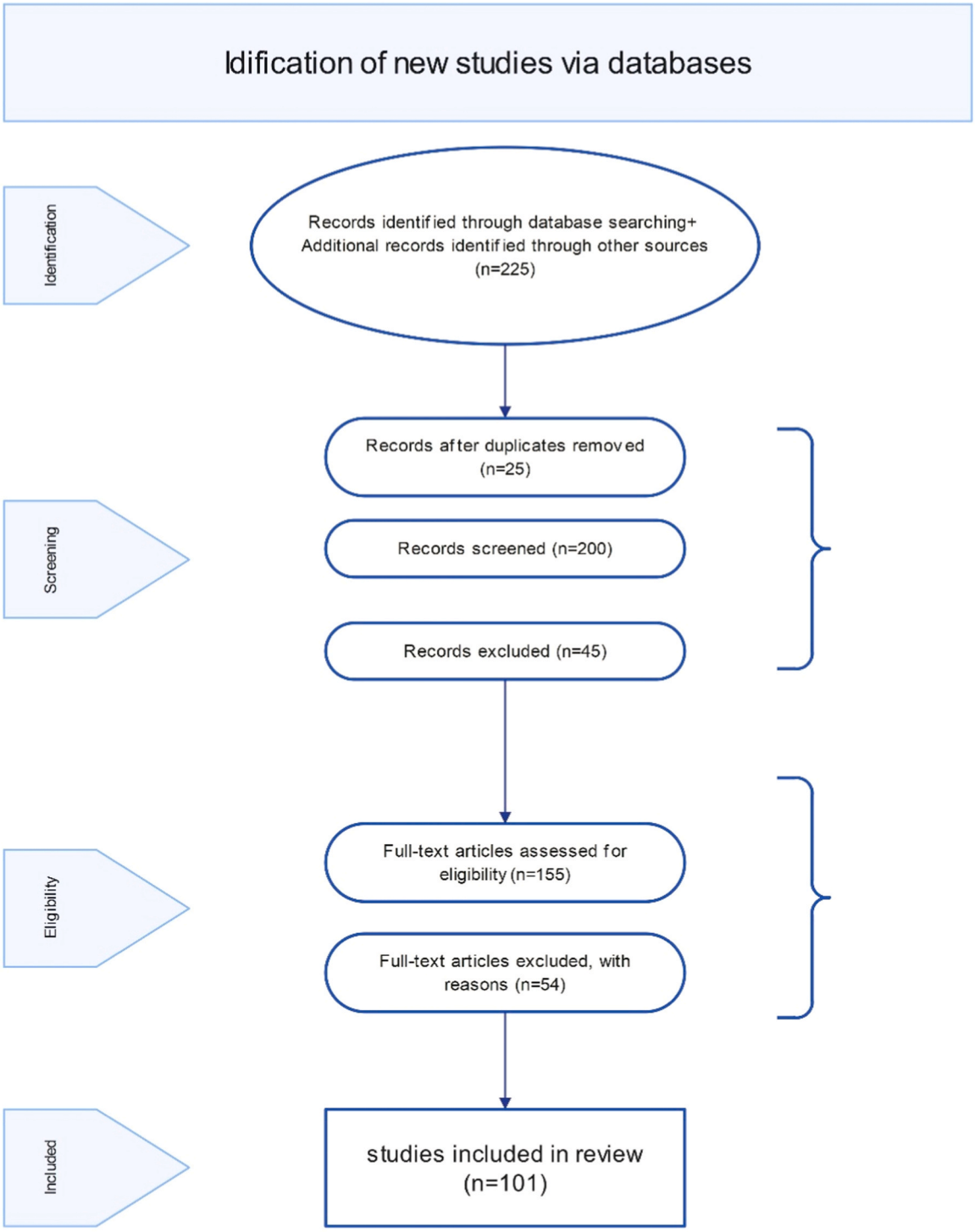


Fig. 2. Identification of new studies via databases.

8. Biocides and their regulatory implications

The toxicity of biocides, particularly Parabens, Quaternary Ammonium Compounds (QACs), Triclosan, and Polyhexamethylene, has significant policy and regulatory implications concerning environmental discharge and public health. These compounds are widely used for their antimicrobial properties in various applications. However, their persistent nature and potential adverse effects on human health and ecosystems necessitate careful regulatory oversight.

Studies have shown that QACs can act as irritants and sensitizers, leading to skin and respiratory issues in exposed populations. The increased use of QACs during the COVID-19 pandemic has raised alarms about chronic exposure and its implications for public health [31]. Furthermore, the environmental persistence of QACs poses a risk to aquatic ecosystems, where they can accumulate and exert toxic effects on aquatic organisms [32]

Triclosan, another widely used biocide, has been scrutinized for its potential endocrine-disrupting effects and contribution to antibiotic resistance [33]. The long-term environmental impact of Triclosan, particularly its ability to persist in aquatic environments, raises

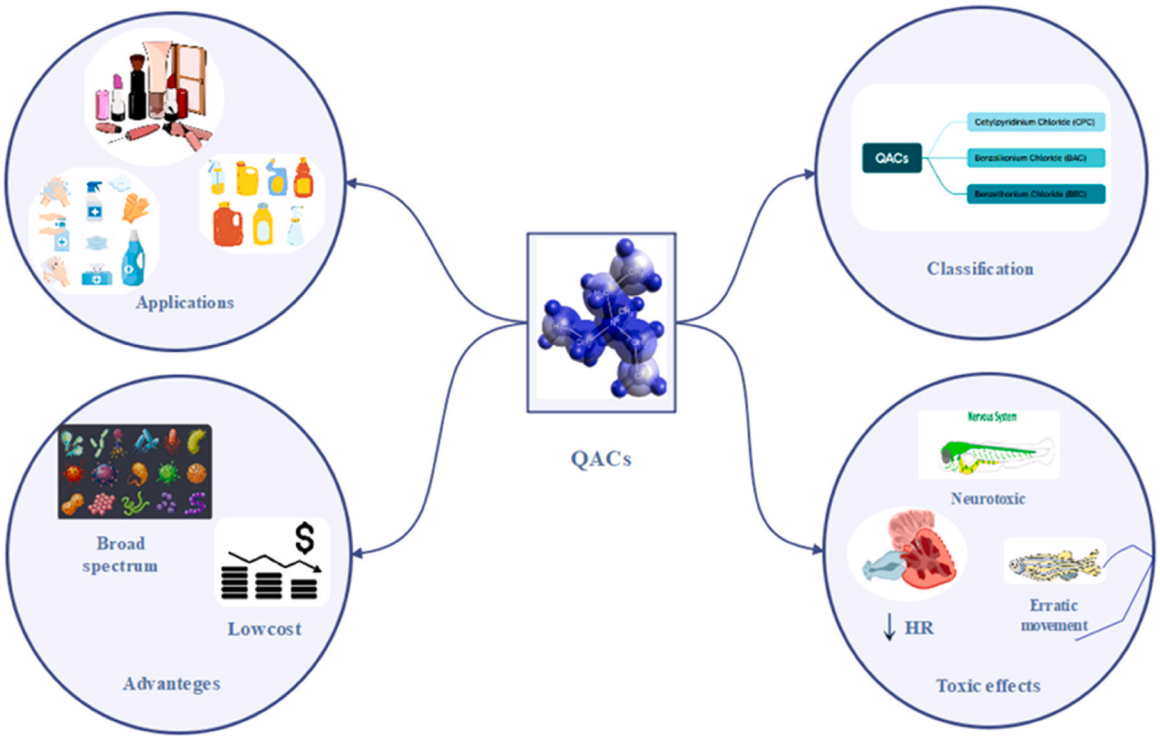


Fig. 3. QACs classification, applications, advantages and toxic effects.

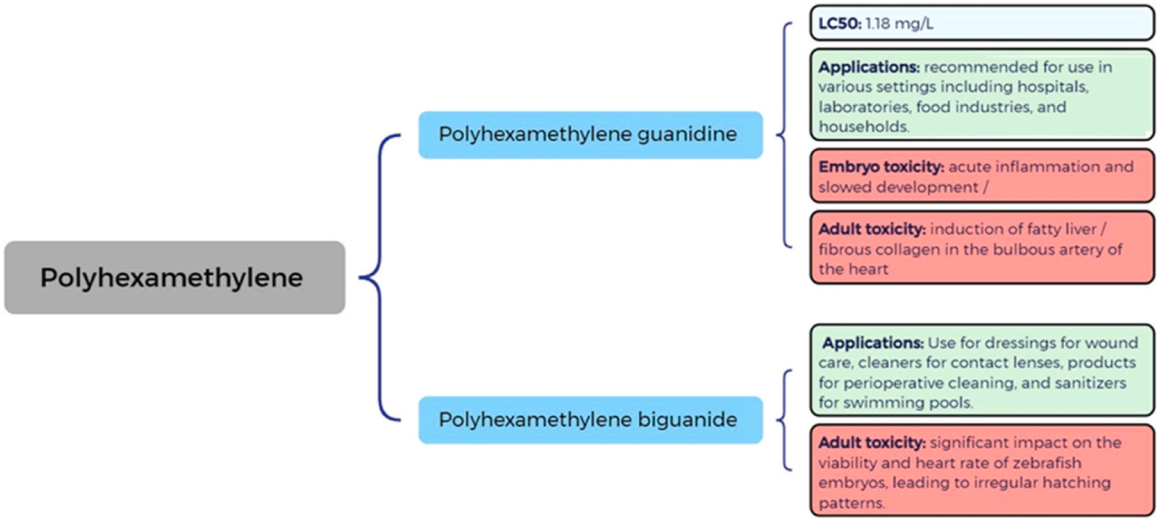


Fig. 4. classification of Polyexamethylene, applications and toxic effects.

significant concerns about its regulation. The need for policies that limit the use of Triclosan in consumer products and its discharge into wastewater systems is critical to protect both public health and the environment [34].

The presence of biocides like Polyhexamethylene in fecal sludge can disrupt the biological processes in wastewater treatment plants, leading to operational failures and environmental contamination [35].

The presence of parabens in human breast tumors has heightened public concern and prompted calls for stricter regulations on their use in consumer products [36].

From an environmental perspective, parabens are classified as emerging contaminants by the U.S. Environmental Protection Agency (EPA) due to their widespread detection in various water sources Their persistence in aquatic environments poses risks to wildlife, as parabens can disrupt endocrine functions in aquatic organisms, leading to population declines and ecosystem imbalances [37].

the toxicity of biocides such as Parabens, QACs, Triclosan, and Polyhexamethylene has profound implications for public health and environmental policy. Regulatory frameworks must evolve to address the risks associated with these compounds, focusing on limiting their use, ensuring safe disposal practices, and promoting the development of safer alternatives. Enhanced monitoring and stricter regulations will be essential to safeguard public health and protect environmental integrity.

8.1. *Quaternary ammonium*

Quaternary Ammonium Compounds (QACs) represent a category of disinfectants, characterized by the presence of a quaternary nitrogen

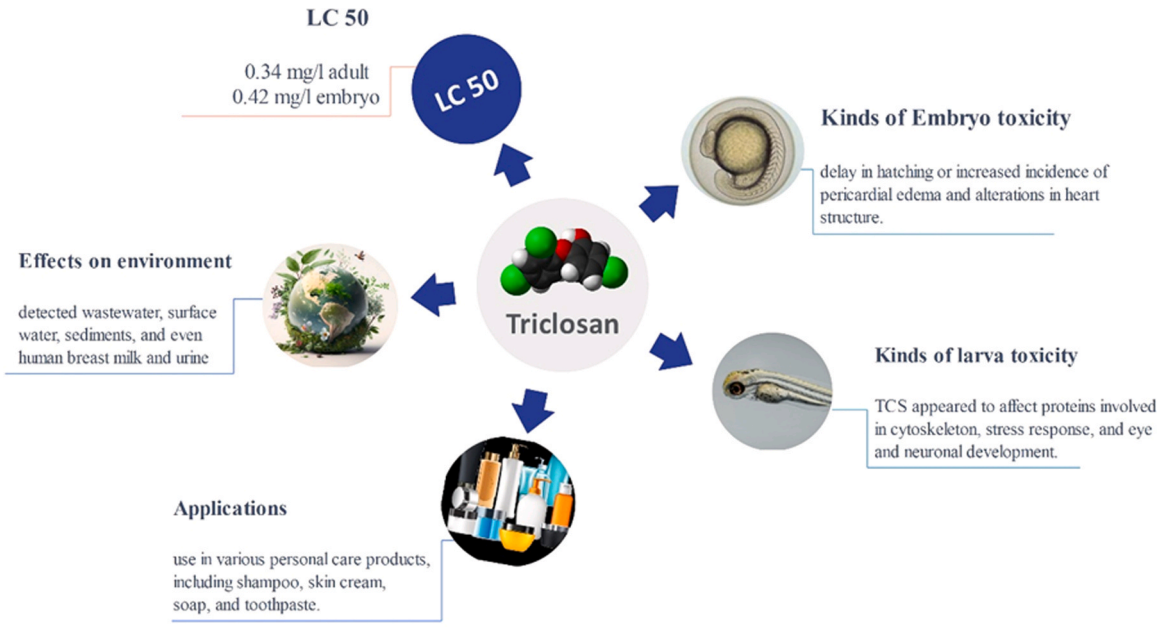


Fig. 5. Triclosan as biocide and its adverse effects.

atom linked to a significant hydrophobic substituent [38,39]. In the 19th century, researchers discovered that these compounds exhibited specific bactericidal properties. Following a century of evolution and extensive research, QACs have become commonplace as household disinfectants. QACs, have been extensively utilized as disinfectants across various sectors such as industrial, medical, and domestic for several decades [40–42]. Cetylpyridinium Chloride (CPC) is commonly employed as an active ingredient or detergent additive in personal care products [43, 44]. Recently, CPC has gained acceptance for use in food processing to combat microbial contamination. Benzalkonium Chloride (BAC) and Benzethonium Chloride (BEC), both members of the QACs group, are frequently used as disinfectants, antimicrobials, or surfactants in both industrial and household products. BAC, a prominent member of the QACs, is frequently utilized as a stabilizing agent in pharmaceutical compositions and as a sanitizing agent in medical facilities and health institutions [45–47]. Extensive research has been undertaken to assess the toxicological impact of QACs on Danio rerio (Zebrafish). This review aims to elucidate the pivotal findings and implications of these investigations.

Beatriz Sousa and colleagues aimed to assess the potential negative impacts of acute exposure to varying levels of benzalkonium chloride (BAC) on zebrafish. The Fish Embryo Toxicity (FET) test revealed that BAC did not cause significant mortality in zebrafish embryos, with the highest mortality rate (under the maximum tested concentration of 2.5 mg/l) consistently remaining under 20 %. The study observed an escalation in overall swimming activity, thigmotaxis behavior, and erratic movements. There was a noted increase in CYP1A1 and catalase activities, contrasted by a decrease in CY1A2, GSTs, and GPx activities. The study found that BAC is metabolized by CYP1A1, which leads to an increase in H2O2 production, subsequently activating the antioxidant enzyme CAT. The data also indicated an increase in AChE activity. The study underscores the adverse embryonic, behavioral, and metabolic effects of BAC, which hold significant environmental implications, particularly given the anticipated rise in the use and discharge of BAC in the foreseeable future [48].

Another research in 2018 delved into the toxicological impacts of two frequently used substitute antimicrobials, benzalkonium chloride (BAC) and benzethonium chloride (BEC), on zebrafish (Danio rerio). These were compared to the prohibited antimicrobials triclosan (TCS) and triclocarban (TCC). The findings revealed that these substitute compounds are not safer than the banned antimicrobials. In the zebrafish, the compounds, within the tested concentration ranges of 0.05–5 mg/L, exhibited toxic effects. These effects were manifested as delayed or inhibited hatching, embryonic mortality, morphological abnormalities, and neurotoxicity. BAC emerged as the most toxic compound, with acute lethal toxicity observed at environmentally relevant concentrations (hundreds of mg/L), a level comparable to the banned TCC. Interestingly, the toxic effects of BAC and TCC manifested within different time frames, potentially indicating distinct toxicity mechanisms. Moreover, all the compounds except TCS induced neurotoxicity in fish larvae, as evidenced by changes in secondary motoneuron axonal projections. This aspect of neurotoxicity has been largely overlooked in previous studies on these antimicrobials, underscoring the need for further research to understand its underlying mechanisms and ecological relevance [49].

Furthermore, in study conducted by Xuchun Qiu and colleagues, the impact of Cetylpyridinium Chloride (CPC) exposure on zebrafish during their early life stages was investigated. The zebrafish were exposed to varying concentrations of CPC (0, 4, 40, 400, and 1200 μg/L) up to 120 hours post-fertilization (hpf). The findings revealed that exposure to 400 and 1200 μg/L of CPC resulted in significant mortality, with a 120h-EC50 value of 175.9 μg/L. The study also found that CPC notably reduced the heart rate of embryos at 48 hpf (4–400 μg/L) and larvae at 72 hpf (40 and 400 μg/L). At 120 hours post-fertilization, CPC exhibited a two-fold impact on the zebrafish's movement activity. It led to a reduction at a concentration of 400 μg/L, while it caused an enhancement at concentrations of 4 and 40 μg/L. Furthermore, the levels of reactive oxygen species, superoxide dismutase, and glutathione in zebrafish larvae were elevated at 400 μg/L. The study's correlation analysis indicated that the oxidative stress triggered by CPC might play a pivotal role in the observed cardiac and behavioral toxicity in zebrafish larvae. The research findings suggest that at concentrations relevant to the environment, CPC possesses the capability to interfere with the growth, behavior, and oxidative equilibrium of Zebrafish [50].

erena Christen et al. reported the combined effects of three Quaternary Ammonium Compounds (QACs) - Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC), Barquat, and Benzalkonium Chloride. The cytotoxicity of these compounds was individually assessed using the MTT assay in zebrafish liver cells (ZFL). This was followed by an evaluation of binary and ternary mixtures of these compounds. The QACs

exhibited significant cytotoxicity in both cell lines, with EC50 values in the low μg/ml range. The majority of the binary mixtures and all ternary mixtures demonstrated synergistic activity at all equi-effective concentrations. Additionally, the study investigated transcriptional changes in target genes associated with endoplasmic reticulum (ER) stress, general stress, inflammation, and apoptosis. Non-cytotoxic concentrations of Barquat and BAC in ZFL cells led to the induction of (ER) stress genes. Both these compounds also triggered the expression of tumor necrosis factor alpha (tnf-α). Upon exposing zebrafish eleuthero-embryos to certain conditions for 120 hours, analogous transcriptional changes were noted in vivo. BAC was found to stimulate the expression of genes linked to apoptosis, and this was also observed with Benzalkonium Chloride at the highest concentration. The research concluded that QACs exhibit potent cytotoxicity, and that binary, ternary, and quintuple combinations demonstrate synergistic activity. In vitro, Barquat and BAC were found to induce ER stress and inflammation. However, only Benzalkonium Chloride was found to stimulate the expression of tnf-α in vivo. These observations underscore the necessity for additional research into the potential health impacts of QACs [51]

9. Polyhexamethylene

Polyhexamethylene guanidine (PHMG) hydrochloride, a member of the polymeric guanidine family, is a chemical disinfectant known for its potent virucidal and bactericidal properties in vitro. It is characterized by its odorless nature, non-corrosive properties, and high solubility in water. Notably, it has demonstrated non-toxicity in in vitro cytotoxicity studies at low concentrations (0.04 % and 0.005 %, w/v). PHMG hydrochloride also serves as an effective sporicidal disinfectant, as evidenced by an in vitro study where it eradicated all spores at a concentration of 0.52 % (w/v) within 90 seconds of contact and 0.36 % (w/v) within 3 minutes [52–54]. Consequently, it is recommended for use in various settings including hospitals, laboratories, food industries, and households. The compound has garnered significant interest and acceptance, and is being explored for potential multipurpose applications [55,56]. Polyhexamethylene biguanide (PHMB), also known as polyhexanide, polyaminopropyl biguanide, polymeric biguanide hydrochloride, and polyhexanide biguanide, is an antiseptic recognized for its antiviral and antibacterial properties [57]. This substance finds its application in a variety of uses, including dressings for wound care, cleaners for contact lenses, products for perioperative cleaning, and sanitizers for swimming pools. It is also frequently employed as a preservative in cosmetics and personal care items. Despite its extensive use, there is a scarcity of data concerning the environmental concentrations of PHMB [58–60]. Subsequently, we will present a selection of pivotal studies that have utilized Zebrafish as a model organism to investigate the toxicological effects of Polyhexamethylenes.

In a study led by Jae-Yong Kim and colleagues, zebrafish embryos and adult zebrafish were subjected to exposure to polyhexamethylene guanidine phosphate (PHMG-P) and oligo-[2-(2-ethoxy)-ethoxyethyl)-guanidinium-chloride (PGH). The injection of the sterilizing agent into zebrafish embryos at 4 h post-fertilization (hpf) led to a significant increase in mortality by 48 hours post-injection. The survival rate for PHMG-injected embryos was found to be 31 %, compared to 73 % for those injected with water. The researchers observed that the embryos exposed to the sterilizer experienced premature death, accompanied by acute inflammation and slowed development. When zebrafish were exposed to the operational concentrations of PHMG (final 0.3 %) and PGH (final 10 mM), all of them perished within 70 minutes. This was associated with a sharp rise in serum triacylglycerol levels and the induction of fatty liver. Post-mortem examination of the zebrafish revealed a substantial buildup of fibrous collagen in the bulbous artery of the heart, along with an increase in reactive oxygen species [61].

According to a published study in2020, a model of zebrafish embryo/larvae was utilized to assess both developmental and cardiotoxic impacts, as well as alterations in the transcriptome via RNA-sequencing. The zebrafish embryos were subjected to varying concentrations of PHMG-P, ranging from 0.1 to 2 mg/L, from 3 h to 96 h post-fertilization. Exposure to 2 mg/L of PHMG-P led to complete mortality, with an LC50 value determined at 1.18 mg/L after 96 hours. While no significant developmental alterations were observed, there was a notable change in the heart rate of the zebrafish larvae. Transcriptome analysis revealed significant effects on immune and inflammatory responses, mirroring findings from epidemiological studies. This was further corroborated by our qPCR analysis (Itgb1b, TNC, Arg1, Arg2, IL-1β, Serpine-1, and Ptgs2b) following a 96-hour exposure to 0.4 mg/L of PHMG-P [62].

Recently, Ha-Na Oh et al. (2024) assessed the developmental neurotoxicity (DNT) effects of PHMB in zebrafish. The study revealed that PHMB had a significant impact on the viability and heart rate of zebrafish embryos, leading to irregular hatching patterns. When exposed to PHMB concentrations ranging from 1–4 μM, there was a noticeable reduction in the width of the brain and spinal cord in transgenic zebrafish, and a decrease in myelination processes. Additionally, PHMB was found to alter the expression of neurodevelopmental genes in zebrafish and induce the accumulation of reactive oxygen species (ROS). These findings suggest that PHMB may pose a risk due to its DNT effects, which appear to operate through a ROS-dependent mechanism [63].

Commonly used biocidal disinfectants, including PHMB, are found in aquatic environments. However, their potential impacts on fish, particularly when present as mixtures, are not well understood. In an effort to explore their combined effects, Verena Christen and colleagues evaluated the cytotoxicity of PHMB using the MTT assay in zebrafish liver cells (ZFL). They also examined molecular impacts by conducting quantitative PCR in vitro and in zebrafish eleuthero-embryos using a targeted gene expression approach. PHMB displayed less cytotoxicity compared to other compounds. When mixed at their no observed effect concentrations, a mixture containing all five compounds exhibited strong cytotoxicity, suggesting a synergistic interaction. Furthermore, they identified changes in the transcription of target genes associated with endoplasmic reticulum (ER) stress, general stress, inflammation, and apoptosis. Similar transcriptional changes were observed in vivo upon exposure of zebrafish eleuthero-embryos for 120 hours. These findings demonstrate the cytotoxicity of PHMB and its potential to induce ER stress and inflammation in zebrafish, both in vitro and in vivo [51].

10. Paraben

Parabens, synthetic preservatives extensively used in cosmetics, food products, and medicines, are esters of p-hydroxybenzoic acid (PHBA) [64–66]. Their physicochemical properties are influenced by the type of substituent, which can be either an alkyl chain or an aromatic ring [67]. The most frequently encountered parabens with a linear alkyl chain as a substituent include methylparaben (MeP), ethylparaben (EtP), propylparaben (PP), butylparaben (BuP), and pentylparaben (PeP) [68,69]. The pervasive use of MeP and PP has led to their detection in aquatic ecosystems [70]. Annually, around 8000 tons of parabens are consumed globally, and these compounds can seep into the environment during their manufacture and disposal, leading to their detection in landfills, effluent from wastewater treatment plants, and sewage sludge [71]. Parabens and their metabolites have been found in air, dust, wastewater, surface water, human urine, and tumors, and can be absorbed through ingestion, inhalation, and skin contact [72]. It is crucial to understand the environmental fate of parabens and determine their levels in ecosystems to assess their potential risks and toxicological effects on populations. In the subsequent sections, we meticulously examined studies that explored the toxicity of parabens utilizing the zebrafish as an animal model.

The study conducted by Carmine Merola and colleagues aimed to explore the impact of early exposure to butylparaben (BuP), ethylparaben (EtP), and methylparaben (MeP) on the nervous system, using behavioural models of zebrafish larvae. The zebrafish were exposed to

three concentrations of each paraben until 4 days post-fertilization (dpf), taking into account the environmentally realistic concentrations of human exposure and the benchmark-dose lower limit calculated for zebrafish larvae. The activity in new and familiar environments, thigmotaxis, visual startle response, and photic synchronization of behavioural circadian rhythms were analyzed at 4, 5, and 6 dpf. It was observed that zebrafish larvae exposed to BuP 500 μg/L and EtP 5000 μg/L exhibited increased anxiety-like behaviour in a new environment. Larvae treated with 500 μg/L of BuP showed reduced activity in both familiar and slightly unfamiliar environments, while larvae exposed to 5000 μg/L of EtP displayed hyperactivity in a familiar environment. The contact with parabens did not influence the visual reflex response or the light-induced synchronization of circadian rhythms in zebrafish larvae [68].

In a comprehensive study conducted by Ceyhun Bereketoglu and the team, the adverse effects of propylparaben (PP) and methylparaben (MeP) on the early developmental stages of zebrafish were examined. The investigation encompassed mortality, hatching, developmental abnormalities, and gene expression profiles in embryos exposed to both compounds. The semi-static exposure conditions revealed that both MeP (≥100 μM) and PP (≥10 μM) are toxic to the embryos in a concentration-dependent manner, leading to developmental abnormalities. Abnormalities such as defects in the spine, pericardial fluid accumulation, and pigmentation irregularities were noted following the administration of both MeP and PP. Observations on delayed hatching, mortality, and developmental anomalies suggest that PP exhibits greater toxicity than MeP. For the analysis of gene expression, doses of 1 and 10 μM of MeP and PP were examined. Genes involved in physiological pathways such as stress response, cell cycle and DNA damage, inflammation, fatty acid metabolism, and endocrine functions were impacted by MeP and PP. The gene expression patterns imply that parabens induce toxicity by initiating oxidative stress, causing DNA double-strand breaks, apoptosis, and by modifying fatty acid metabolism. The altered expression of the androgen receptor (ar) and estrogen receptor 2 alpha (esr2a) indicates an antiandrogenic and estrogenic activity of parabens in zebrafish. In conclusion, this study provides substantial information on the adverse effects of MeP and PP using physiological endpoints and encourages further studies to delve into the molecular mechanism of the toxicity associated with parabens [73].

According to the study conducted by Vrinda Yatin Dambal and colleagues, the developmental impacts of methylparaben (MeP) were assessed in embryo-larval zebrafish at concentrations ranging from 100 μM to 1000 μM for 96 hours post-fertilization (hpf). Observations were made on survival, hatching, heart rate, and developmental abnormalities in the embryos exposed to MeP. Exposure to MeP resulted in a decrease in heart rate and hatching rate. Abnormalities such as pericardial edema, blood cell accumulation, and bent spine were observed in all treated concentrations, except at 100 μM, with the frequency of these defects increasing with concentration. The 96 hpf LC50 of MeP was calculated to be 0.065 mg/L. Furthermore, RT-PCR results showed that in larval zebrafish exposed to 100 μM (0.015 mg/L) of MeP until 96 hpf, the expression of vitellogenin I (Vtg -I) was significantly upregulated compared to the control group. This data suggests that even though lower concentrations of MeP do not cause phenotypic malformations, they lead to the dysregulated expression of the estrogenic biomarker gene Vtg-I [74].

In another study conducted by C. Merola and colleagues, the embryotoxicity of methylparaben (MeP) was evaluated in the early life stages of zebrafish using the benchmark-dose (BMD) methodology in accordance with the Fish Embryo Acute Toxicity (FET) tests - OECD guideline 236. The toxic effects were assessed daily by examining lethal endpoints, hatching rate, and sublethal alterations. Zebrafish fertilized eggs were exposed to five concentrations of MeP (1 mg/L, 10 mg/L, 30 mg/L, 60 mg/L, and 80 mg/L) until 96 hours post-fertilization (hpf). The lethal concentration 50 was determined to be 72.67 mg/L. The BMD confidence interval was 40.8–57.4 mg/L for lethal endpoints and 16–26.5 mg/L for the toxicity index, which includes both lethal and sublethal alterations. Zebrafish embryos exposed to MeP exhibited sublethal alterations such as pericardial edema, yolk edema, blood stasis, reduced blood circulation, decreased heartbeat, and notochord curvature. The number of embryos exposed to the highest concentrations of MeP exhibiting sublethal alterations increased between 24hpf and 96hpf. Behavioural changes were observed only in zebrafish larvae treated with 30 mg/L of MeP [75].

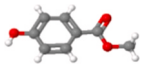
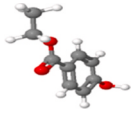
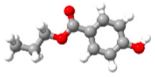
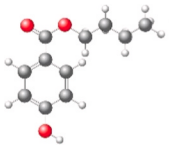
In new research published in 2023, the potential developmental toxicity caused by exposure to Butylparaben (BuP) was investigated using Zebrafish embryos. The development of Neural Crest Cells (NCCs), which is highly active during gastrulation in Zebrafish embryos, was a key focus. The study utilized BuP solutions of 0.5 mg/L, 0.75 mg/L, and 1 mg/L, in line with international safety standard dosages. Observations made 5 days post-exposure revealed severe craniofacial cartilage deformities, periocular edema, cardiac dysplasia, and delayed otolith development in the Zebrafish larvae. An enhanced oxidative stress response was also noted. Biochemical analysis showed significant reductions in the activities of catalase (CAT) and superoxide dismutase (SOD), and a significant increase in the concentration of malondialdehyde (MDA). Alkaline phosphatase (ALP) activity, an osteoblast activity marker, was also reduced. RT-qPCR results indicated down-regulation of chondrocyte marker genes sox9a, sox9b, and col2a1a. Changes were also observed in the morphology of maxillofacial chondrocytes in Zebrafish larvae, and inhibition of cranial NCC proliferation. The findings suggest that the strong oxidative stress induced by BuP inhibits NCC proliferation in larval Zebrafish, leading to craniofacial deformities [76] (Table 1).

In the comprehensive study led by Larissa Cristine de Carvalho Penha et al. the potential harmful effects of methylparaben (MeP) on both larvae and adult zebrafish were evaluated through toxicity tests and physiological and biochemical biomarkers. For the assessment of biomarkers, the fish were subjected to an environmental exposure level of 30 μg/L of MeP and the non-effect concentration (NOEC) was determined to be 60 mg/L for larvae and 50 mg/L for adults. The median lethal concentration (LC50) of MeP was found to be 105.09 mg/L for adults and 211.12 mg/L for larvae, surprisingly indicating a higher sensitivity in adults compared to larvae. Exposure to 50 mg/L of MeP in adult fish led to a significant decrease in phase 1 biotransformation (ethoxyresorufin O-deethylase activity) and an increase in lipoperoxidation (LPO) in gills, as well as an increased frequency of micronuclei in erythrocytes. The results from the biomarkers were integrated (integrated biomarker response [IBR] index), revealing lower IBR scores in tissues of fish exposed to MeP, suggesting a suppression of biological responses.The primary contributor to the IBR score, as determined for the gills of fish subjected to a concentration of 50 mg/L Methylparaben (MeP), was LPO. When considering the amount of LPO, it was observed that sublethal exposure to MeP at concentrations of 30 μg/L and 60 mg/L did not induce toxicity in the larvae. The team investigated whether the difference in sensitivity between adults and larvae could be associated with the antimicrobial action of MeP that could affect the intestinal microbiota of adults. An increase in the number of carbon sources consumed by them was found without effects on diversity and abundance, which can be considered an adaptation to environmental stress, but not a negative effect. However, the LPO and genotoxicity caused by MeP to zebrafish adults underscore the importance of regulating the presence of this compound in the environment and improving cleaning processes adopted by wastewater treatment plants [77].

11. Triclosan

Triclosan (TCS), a compound known as 5-chloro-2-(2,4-dichlorophenoxy) phenol, is a common ingredient in various personal care products, including shampoo, skin cream, soap, and toothpaste, due to its antimicrobial properties [78,79]. Its widespread use has led to its detection

Table 1
Paraben classification.

Paraben	Substance	Chemical structure	Zebra or embryo	Kinds of toxicity	Reference
	MeP		embryo	Deformities such as spinal defects, pericardial edema, and pigmentation defects / decrease in heart rate and hatching rate	[73,74]
	EtP		Larvae	displayed hyperactivity in a familiar environment.	[68]
	PP		embryo	Deformities such as spinal defects, pericardial edema, and pigmentation defects	[73]
	BuP		embryo	severe craniofacial cartilage deformities, periocular edema, cardiac dysplasia, and delayed otolith development	[76]

in numerous environmental matrices, such as wastewater, surface water, sediments, and even human breast milk and urine [80–82]. The concentration of TCS in surface water can vary significantly, ranging from 0.0014 to 40 μg/L, a factor of 10,000 [83]. Despite its stability and lipophilic nature, TCS can undergo various transformation reactions in the aquatic environment, leading to the formation of more toxic and bioaccumulative compounds or derivatives. These transformed compounds can have detrimental effects on aquatic life, including algae, invertebrates, fish, and amphibians [84]. One such derivative, methyltriclosan (MTCS), is formed from TCS and is not fully removed by biological wastewater treatment plants (WWTP). MTCS is reported to have a higher environmental persistence and increased lipophilicity compared to TCS, leading to greater bioaccumulation in aquatic organisms [78,79]. In the ensuing sections, we scrutinized investigations that explored triclosan toxicity using zebrafish as an animal model.

In the research conducted by Rhaul Oliveira and colleagues, the toxic effects of Triclosan (TCS) on zebrafish (Danio rerio), both embryos and adults, were examined. The study encompassed an analysis of several lethal and sub-lethal endpoints in organisms exposed to TCS, including mortality, embryo development and behaviour, hatching, micronuclei, and biochemical markers such as glutathione S-transferase (GST), cholinesterase (ChE), and lactate dehydrogenase (LDH). The experimental design adhered to the OECD guideline on Fish Embryo Toxicity Test for the embryo/larvae assay. Embryos were subjected to varying concentrations of TCS, ranging from 0.1 to 0.9 mg/l, over a period of 6 days. Daily observations were made using a stereomicroscope to monitor mortality, developmental parameters, and hatching. A parallel test was conducted to obtain larvae for ChE, GST, and LDH analysis. The adult test was performed in semi-static conditions following the OECD Guideline TG 203. The adult zebrafish, exhibiting similar age and length, were subjected to TCS exposure. The TCS was administered at nominal concentrations that varied between 0.1 and 0.5 mg/l, and the exposure duration was maintained at 96 h. Daily inspections were carried out to monitor mortality and behavioural changes. A subsequent test was conducted to obtain organs for biomarker analysis. The results indicated that TCS exhibited acute toxicity for embryo/larvae (96 h LC50 =0.42 mg/l) and caused a delay in hatching. Furthermore, embryotoxicity was apparent with delays in otolith formation, body and eye pigmentation, and malformations were also observed. Biomarker levels were affected, with increased ChE and LDH activity in larvae exposed to 0.25 mg/l, and increased GST activity in larvae exposed to 0.25 and 0.35 mg/l. Acute toxicity was also exhibited by TCS towards adult zebrafish, with a 96-hour LC50 value of 0.34 mg/l. However, TCS did not alter biomarker levels or induce micronucleus in adults. Even though similar 96 h LC50 values were found for D. rerio embryos and adults (0.42 and 0.34 mg/l, respectively), the embryo assay provided more comprehensive information, revealing significant effects at several levels, including teratogenic response, hatching delay, and alteration of biomarker levels. TCS does not appear to be genotoxic for adult fish or to interfere with biomarker levels at the tested concentrations [80].

According to the study conducted by Falisse Elodie and colleagues, the occurrence of acclimation to Triclosan (TCS) and the biological mechanisms that underpin the stress response in the early-life stage of zebrafish were examined. Initially, zebrafish eggs were exposed to four distinct sublethal concentrations of TCS (2, 20, 50, and 100 μg/L) for seven days post-fertilization, followed by exposure to a lethal concentration of TCS (1000 μg/L). Mortality was continuously recorded during the time-to-death (TTD) exposure to assess the development of increased resistance. Interestingly, larvae exposed to 50 μg/L of TCS exhibited higher sensitivity, as evidenced by delayed hatching, increased mortality during the sub-lethal exposure, and a significantly lower mean TTD value compared to other groups. In contrast, fish exposed to the highest concentration of TCS (100 μg/L) showed a similar mean TTD value as controls and significantly better survival compared to embryos exposed to 50 μg/L, suggesting the initiation of an acclimation process at this concentration. Proteomic and enzymatic analyses were performed on larvae at 7 days post-fertilization (dpf) exposed to 50 μg/L and 100 μg/L of TCS, providing insights into the functional changes induced at these specific concentrations. TCS appeared to affect proteins involved in cytoskeleton, stress response, and eye and neuronal development. This was corroborated by the enzymatic results, which indicated impairment in glutathione metabolism and acute neurotoxicity. A significant 2.5-fold and 3-fold increase in AChE activity was observed following TCS exposure. Furthermore, GPx activity was significantly increased, whereas a significant inhibition of GR activity was observed, suggesting that de novo synthesis of reduced GSH might occur to maintain the ratio between reduced and oxidized GSH. The proteomic results revealed potential candidate proteins involved in the acclimation process of larvae exposed to 100 μg/L of TCS. The integrative analysis revealed complex non-monotonic concentration-related effects on zebrafish early-life stages, with increased resistance observed between 50 and 100 μg/L exposures [85].

The analytical results from the research conducted by Jing Fu and colleagues evaluated the toxicity of Triclosan (TCS) in developing zebrafish (Danio rerio) embryos using a metabolomics approach based on gas chromatography–mass spectrometry (GC–MS). The embryos

were subjected to a broad spectrum of TCS concentrations, ranging from 10 ng/L to 500 mg/L. Endogenous metabolites were extracted using a mixture of acetonitrile, isopropanol, and water in a 3:3:2 ratio. Prior to their identification and quantification via GC–MS analysis, the metabolites underwent a process of derivatization. A total of 29 metabolites were positively identified in the embryos. To determine the changes in the metabolic profile of TCS-exposed embryos, both univariate (one-way analysis of variance) and multivariate (principal components analysis and projection to latent structure-discriminant analysis) analyses were utilized. Eight metabolites, namely urea, citric acid, D-(þ)-galactose, D-glucose, stearic acid, L-proline, phenylalanine, and L-glutamic acid, were significantly altered ($p < 0.05$) in embryos exposed to TCS. The results suggest that exposure to TCS can lead to the impairment of several pathways in developing zebrafish embryos, with potential implications for energy metabolism, amino acid metabolism, nitrogen metabolism, and gill function. These findings are expected to contribute to future risk assessments of TCS and other emerging contaminants of concern [84].

In another study led by Jing Fu and team, the adverse effects of Triclosan (TCS) and its derivative, Methyl-Triclosan (MTCS), on zebrafish (Danio rerio) embryos were explored. The concentrations of TCS and MTCS used in the study ranged from environmentally relevant levels (ng/L) to high-dose sublethal concentrations. The team employed metabolomics and reverse transcription qPCR to investigate these effects. The analysis of metabolism and transcriptome revealed changes in the expression of metabolites and transcripts in zebrafish embryos after 96 hours of exposure to 30 μg/L and 300 μg/L of TCS, 400 μg/L of MTCS, and a mixture of TCS/MTCS (30 μg/L TCS + 3 μg/L MTCS and 300 μg/L TCS + 30 μg/L MTCS). Significant dysregulations were observed in the expression of several enzymes and transporters, including the urea transporter (UT), glucose-6-phosphate dehydrogenase (G6PD), alanine transaminase (ALT), glutamate dehydrogenase (GDH), phosphoglucomutase (PGM), and fatty acid synthase (FASN). Additionally, changes were noted in the levels of alanine, urea, glucose, 6-phosphogluconalactone, and palmitic acid in the TCS, MTCS, and TCS/MTCS treatments. Particularly in the MTCS treatment group, fold changes were observed in the mRNA expression related to nitrogen metabolism, energy metabolism, and fatty acid synthesis, indicating a disruption of the biological pathways in zebrafish embryos. The changes in metabolites and gene expressions induced by the TCS, MTCS, and the TCS/MTCS mixture treatment demonstrate alterations in several metabolic pathways, including starch and sucrose metabolism, nitrogen metabolism, fatty acid synthesis, and the biosynthesis of phenylalanine, tyrosine, and tryptophan. Therefore, this study provides deeper insights into the risks posed by the parent compound (TCS) and its by-product (MTCS), as well as the perturbations in biological pathways induced by these two compounds in aquatic environments [79].

According to published study in 2016, the potential developmental and metabolic abnormalities caused by Triclosan (TCS) exposure were investigated using zebrafish as the experimental model. Four developmental stages (70–85 % epiboly, 10–12 somite, prim-5, and 5 days post fertilization) were chosen to perform in situ hybridization staining to examine the effects of TCS on dorsal ventral patterning, segmentation, brain development, and organ formation. The results, in terms of developmental toxicology, showed that neither phenotypic nor molecular changes were observed after 5 days of exposure to 250 μg/L TCS. However, such a dosage of TCS exposure resulted in the accumulation of lipid droplets in the yolk sac, which might be due to the deregulated mRNA expression level of beta-oxidation transcripts. This study demonstrated that 250 μg/L TCS exposure does not affect normal embryogenesis or organogenesis. However, there are concerns regarding the possible impairment of lipid metabolism. This suggests that while TCS may not directly impact the developmental stages, it could potentially disrupt metabolic processes, warranting further investigation [86].

In their study, Alisha Saley and colleagues investigated the effects of Triclosan (TCS) on the cardiac function of zebrafish, with the aim of understanding the potential threats TCS could pose to aquatic life. The zebrafish were subjected to varying concentrations of TCS (0, 0.4, 40, and 400 μg/L) from 8 to 120 h post-fertilization, using a static waterborne exposure method with daily changes. The research focused on the occurrence of pericardial edema and its impact on the heart's structure and functionality. It was observed that exposure to TCS concentrations of 40 μg/L or higher led to an increased incidence of pericardial edema and alterations in heart structure. However, a decrease in cardiac output was only noted at the highest exposure level of 400 μg/L. Interestingly, even at the lowest exposure level of 0.4 μg/L, a small yet significant number of embryos exhibited an increased incidence of regurgitation. These findings indicate that even short-term exposure to TCS can potentially induce mild cardiac toxicity in developing fish. Therefore, further assessments are necessary to fully understand the risks TCS poses to both aquatic life and human health [87].

12. Discussion

Animal models play a crucial role in toxicology research, providing valuable insights into the effects of various substances on biological systems. Commonly used animal models include rats, mice a and zebrafish. These models help researchers understand the mechanisms of toxicity, assess the safety of new compounds, and develop therapeutic interventions. For example:

In a study conducted by Daniel Arismendi and colleagues, the effects of triclosan on the growth hormones of 40 female rats were examined. The findings revealed that rats exposed to triclosan exhibited significantly reduced growth compared to the control group [88].

In another study conducted by Fátima C. Martins and her team, the effects of methyl and butyl parabens on genotoxicity in 6-week-old male Wistar rats were investigated. The results of the DNA damage assessment showed that the treatment group exhibited higher GDI values compared to the control group [89].

In a 2024 study, researchers investigated the toxicity of polyhexamethyleneguanidine phosphate in young and adult mice. Histopathological examination revealed dose-dependent, sex-independent inflammatory and fibrotic changes in the lungs of both juvenile and adult mice, with higher severity observed in adults [90].

But The zebrafish stands out as the only widely studied vertebrate with the capacity for high-throughput systems-level screens of extensive small molecule libraries or numerous genes. This scale of screening enables the identification of suppressors for complex disease phenotypes, as well as the determination of on-target and off-target toxicities [91].

In addition, zebrafish embryos are considered suitable for Replacement or Refinement methods because they do not experience pain, distress, or suffering during these developmental stages [92].

The zebrafish possesses unique advantages, including high fecundity, rapid development, and optical transparency. These attributes make it an ideal model for developmental, reproductive, and transgenerational toxicity studies [93].

13. Future directions and considerations

In the contemporary era, marked by the rapid production and development of personal health products, the necessity for a comprehensive animal model to investigate the toxicity of these products is increasingly recognized. This review article underscores the suitability of the zebrafish animal model for such comprehensive toxicity investigations, encompassing developmental toxicity, cardiotoxicity, behavioral toxicity, genotoxicity, and more. The unique characteristics of the zebrafish model, including its rapid development, genetic manipulability, and physiological similarity to mammals, make it an ideal candidate for these studies. Furthermore, the transparency of zebrafish embryos and larvae allows for real-time, in vivo observation of

developmental processes and toxic responses. With the advent of new technologies such as artificial intelligence, the field of zebrafish testing is poised for significant advancements [94,95]. These technologies facilitate tasks such as the analysis of morphological images [96,97], tracking [98,99], and sorting of eggs [100,101], thereby enabling rapid and high-quality zebrafish tests for a large number of chemicals. This technological progress, coupled with the inherent advantages of the zebrafish model outlined in this review, positions zebrafish as a highly suitable animal model for toxicity studies of biocides. As such, the zebrafish model stands at the forefront of efforts to ensure the safety and efficacy of personal health products.

14. Conclusion

The production and development of various biocides necessitate rigorous toxicity checks. Numerous reports have highlighted the pervasive presence of these biocides in the environment, emphasizing the importance of their non-toxicity from an environmental perspective. The findings from this review underscore the potential of zebrafish as a valuable model for regulatory testing of biocides, due to its genetic similarity to humans, rapid growth, and cost-effectiveness. The zebrafish model provides a cost-effective and efficient means to assess the toxicological profiles of biocides, which can inform regulatory decisions and the development of safer alternatives. These findings can also guide the development of safer biocides for use in household, cosmetic, and industrial products, reducing their adverse effects on human health and the environment. This review highlights the importance of the zebrafish model for comprehensive biocide toxicity assessment.

CRediT authorship contribution statement

Hassan Jalal: Writing – review & editing, Validation, Supervision. **Rezazadeh Ali:** Writing – review & editing, Writing – original draft, Software, Investigation, Data curation. **Pourshabn-Shahrestani Ali:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization.

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. (All authors have no competing interests to declare.

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Data availability

No data was used for the research described in the article.

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