



Toxicity Assessment of Novaluron on Zebrafish (*Danio rerio*) : A Model for Aquatic Ecosystem Risk Evaluation

C Komaladevi

**Research Scholar in Zoology Bharatiya Engineering Science & Technology innovation
University (BESTIU), Sri Sathya Sai, Andhra Pradesh-515231**

Dr. M. Rajasekhar

Assistant professor in Zoology Palamuru University, Mahbubnagar, Telangana-509002

ABSTRACT

Biocide use in a broad variety of goods has grown crucial and pervasive in recent years. Some of the most common ones in industrial settings are triclosan, parabens, polyhexamethylene, and quaternary ammonium compounds. Nevertheless, there are valid concerns about the toxicological profile of these biocides when they are included. The biosphere is seriously threatened by the environmental discharge of these chemicals, and they also pose concerns to people who use items that include biocide. Approximately 150 papers were reviewed in our comprehensive study; at least 88 of these articles were sourced from reputable sources such as PubMed, MDPI, and Google Scholar. The scope of our research included investigations into a wide range of toxicological effects, including general toxicity, behavioral toxicity, cardiovascular toxicity, and neurotoxicity. The zebrafish, or *Danio rerio*, is a well-known model organism in toxicological studies, and we investigate it in depth using this method. The purpose of this review is to compile studies that have used zebrafish to assess the toxicity of biocide and to determine whether or not this model is appropriate for studying biocidal agents and their byproducts in depth.

Keywords: Zebrafish, Quaternary ammonium compound, Polyhexamethylene, Paraben, Triclosan, Biocides

INTRODUCTION

We humans have been fighting off monsters that prey on our food, our livestock, and even ourselves since the dawn of time. In the past, they relied on drying and smoke coating in addition to natural substances like salt, honey, and vinegar as biocides for pathogen elimination. These methods gave rise to the chemical biocides used today. Medical professionals had a key role in the early acceptance of chemical biocides as disinfectants, which has a rich historical backdrop.

In order to stop the spread of contagious illnesses, doctors realized in the early 1800s that microbial contamination management was crucial. Their ground-breaking work paved the way for chemical biocides to be widely used in healthcare and other fields. Modern human health is profoundly affected by them. Their poisonous properties and ability to eradicate many diseases make them

indispensable. Water treatment, food processing, health goods, healthcare facilities, etc. are just a few of the many human activities that make use of biocides. The four most common types of biocides are guanidine, triclosan, parabens, and quaternary ammonium compounds (QACs). Now we will explore these important classes of biocide in further detail. Biocides have become ubiquitous in human life, used for everything from personal hygiene to industrial processes, and this has raised concerns about their impact on the environment and human health. There has been some research on biocides' harmful effects, but no systematic study that specifically looks at zebrafish as a model organism is currently available. This study fills that need by compiling studies that have looked at the toxicological effects of zebrafish exposed to triclosan, parabens, polyhexamethylene, and quaternary ammonium compounds (QACs). It also takes into account the potential use of new technology, such as artificial intelligence, for the analysis and tracking of morphological images. The purpose of this review is to determine if zebrafish are a good model to study biocidal chemicals and their byproducts in depth.

QAC

Quaternary ammonium compounds (QACs) disinfectants have a nitrogen atom and a large hydrophobic group. Scientists discovered these compounds' bactericidal properties in the 19th century. QACs are widely used as household disinfectants after a century of development and research. QACs have been used as disinfectants in medical, industrial, and residential settings for decades. Personal care products often contain cetylpyridinium chloride (CPC) as an active agent or detergent. Recently, CPC has become popular for preventing microbial contamination in processed foods. Due to their cleaning, antibacterial, and surfactant qualities, benzoalkonium chloride (BAC) and berythonium chloride (BEC) are used in numerous consumer and commercial products. BAC is a popular QAC used to clean hospitals and stabilize pharmaceuticals. Many research have examined QACs' toxicological effects on *Danio rerio*, or zebrafish. This review aims to clarify key study results and effects.

Beatriz Sousa and colleagues investigated if short-term exposure to different benzalkonium chloride concentrations negatively impacted zebrafish. The Fish Embryo Toxicity (FET) test indicated that BAC did not substantially cause embryonic death in zebrafish at 2.5 mg/l. Researchers noticed increased swimming, thigmotaxis, and erratic movement. Though CYP1A1 and catalase were increased, CYP1A2, GSTs, and GPx were reduced. The research found that CYP1A1 metabolizes BAC, activating CAT and increasing H₂O₂ production. Results showed an increase in AChE activity. The study emphasizes the environmental ramifications of BAC's deleterious developmental, behavioral, and metabolic effects, given the predicted rise in BAC consumption and discharge.

Another 2018 study studied the toxicological effects of benzalkonium chloride (BAC) and benzethonium chloride (BEC) on zebrafish. It was compared to banned antimicrobials triclosan (TCS) and triclocarban (TCC). These alternative substances are as harmful as illicit antibiotics, the data show. Zebrafish were harmed by 0.05–5 mg/L pesticides. Neurotoxicity, morphological abnormalities, embryonic mortality, and delayed hatching were negative effects. BAC was the most lethal compound at ecologically relevant quantities (hundreds of mg/L), with acute death toxicity equal to the banned TCC. Curiously, BAC and TCC had distinct toxic effects at various periods,

suggesting separate toxic mechanisms. Changing secondary motoneuron axonal projections demonstrated that all compounds except TCS induced neurotoxicity in fish larvae. Previous studies on these antimicrobials have mostly disregarded this neurotoxicity component, stressing the need for greater research to understand its ecological importance and mechanisms.

Xuchun Qiu and colleagues also evaluated zebrafish's early-life cetylpyridinium chloride (CPC) exposure. To maintain zebrafish survival after 120 hours post-fertilization, various CPC dosages (0, 4, 40, 400, and 1200 µg/L) were administered. CPC exposure at 400 and 1200 µg/L resulted in significant mortality, with a 120h-EC50 value of 175.9 µg/L. The study found that CPC exposure significantly reduced heart rates in 48-hpf embryos (4-400 µg/L) and 72-hpf larvae (40 and 400 µg/L). CPC altered zebrafish motility in two ways 120 hours after fertilization. At 400 µg/L, it decreased, but at 4 and 40 µg/L, it increased. At 400 µg/L, zebrafish larvae exhibited elevated reactive oxygen species, glutathione, and superoxide dismutase levels. The study's association analysis revealed that CPC-induced oxidative stress may cause heart and behavior damage in zebrafish larvae. CPC may affect zebrafish oxidative equilibrium, development, and behavior at ambient doses, according to studies. Erena Christen et al. examined three Quaternary Ammonium Compounds (QACs): ADBAC, Barquat, and Benzalkonium Chloride. All of these compounds were assessed for cytotoxicity in ZFL using the MTT assay.

We then tested these compounds in binary and ternary combinations. QACs exhibited significant cytotoxicity in both cell lines, with EC50 values of low µg/ml. Most binary and ternary combinations showed synergistic action at any equi-effective concentration. The study examined gene transcription changes connected to endoplasmic reticulum (ER) stress, inflammation, apoptosis, and general stress. ER stress genes were upregulated in ZFL cells treated with non-cytotoxic Barquat and BAC. Both compounds also induced *tnf-α* expression. Live zebrafish embryos exposed to particular environmental conditions over 120 hours showed similar gene expression changes. Both BAC and Benzalkonium Chloride boosted cell death gene expression at high concentrations. The study discovered that binary, ternary, and quintuple QACs are particularly toxic to cells and have diverse impacts on the body. Barquat and BAC reduced ER stress and inflammation in vitro. Expression of *tnf-α* was primarily increased by Benzalkonium Chloride in vivo. These findings suggest more research on QAC health impacts.

Polyhexamethylene

Polymeric guanidine family member polyhexamethylene guanidine hydrochloride (PHMG) has high in vitro virucidal and bactericidal properties. High water solubility, non-corrosive, and odorless. To reiterate, in vitro cytotoxicity tests at 0.04% and 0.005% w/v showed no harm. PHMG hydrochloride disinfects spores and has various applications. An in vitro investigation showed that at concentrations of 0.36% (w/v) and 0.52% (w/v), respectively, it eliminated all spores after 90 seconds and 3 minutes of contact. It is recommended for use in households, labs, hospitals, and the food sector. The chemical is being researched for its numerous applications. Polyhexamethylene biguanide (PHMB), also known as polyhexanide, polyaminopropyl biguanide, polymeric biguanide hydrochloride, and polyhexanide biguanide, is antiviral and antibacterial. This chemical is used in

wound dressings, contact lens cleaners, perioperative cleaning solutions, and pool sanitizers. Many cosmetics and personal care products employ it as a preservative. PHMB is widely used, although environmental amounts are unknown. Next, we shall discuss some key Zebrafish studies on Polyhexamethylenes' toxicological effects.

PHMG-P and PGH were tested on embryonic and adult zebrafish by Jae-Yong Kim and colleagues. After injecting the sterilizing agent into zebrafish eggs at 4 hours post-fertilization, mortality increased significantly after 48 hours. In contrast to water-injected embryos, PHMG-injected embryos survived 31%. The study found that sterilized embryos perished too quickly, displayed acute inflammation, and matured too slowly. After 70 minutes of exposure to PHMG (0.3%) and PGH (10 mM), zebrafish perished. This was associated to fatty liver and a sudden rise in blood triacylglycerol. After killing the zebrafish, its heart showed fibrous collagen and reactive oxygen species.

A 2020 study used a zebrafish embryo/larvae model to assess cardiotoxic and developmental effects and transcriptome alterations by RNA-sequencing. PHMG-P was administered to zebrafish embryos at 0.1 to 2 mg/L from 3 to 96 hours after conception. After 96 hours of 2 mg/L PHMG-P exposure, the LC50 was 1.18 mg/L, causing death. Despite no substantial development changes, zebrafish larvae's heart rate altered dramatically. Transcriptome analysis demonstrated significant effects on inflammatory and immunological responses, supporting epidemiological findings. After 96 hours of exposure to 0.4 mg/L PHMG-P, our qPCR analysis (Itgb1b, TNC, Arg1, Arg2, IL-1 β , Serpine-1, and Ptgs2b) supported this. Ha-Na Oh et al. (2024) studied PHMB's effects on zebrafish DNT. The researchers observed that PHMB dramatically influenced zebrafish embryo viability and heart rate, which altered hatching time. In transgenic zebrafish, PHMB dosages from 1 to 4 μ M caused brain and spinal cord constriction and myelination loss. PHMB also altered neurodevelopmental gene expression and increased ROS in zebrafish. These data show that PHMB's ROS-dependent DNT actions may constitute a concern.

PHMB and other biocidal disinfectants live in water. However, their effects on fish, especially in combinations, remain unknown. PHMB's MTT cytotoxicity in zebrafish liver cells (ZFL) was measured by Verena Christen and colleagues to determine its synergistic effects. To study molecular effects, they performed quantitative PCR in vitro and in zebrafish eleuthero-embryos using a targeted gene expression method. PHMB has lower cytotoxicity than other compounds. Combining all five medications at their no-effect levels produced strong cytotoxicity, indicating a synergistic interaction. Additionally, they found transcription changes in target genes connected to endoplasmic reticulum (ER) stress, basic stress, inflammation, and cell death. Zebrafish eleuthero-embryos revealed comparable transcriptional changes after 120 hours of exposure. These data reveal that PHMB is cytotoxic and causes inflammation and endoplasmic reticulum stress in lab and live zebrafish..

Parabens

Esterification of p-hydroxybenzoic acid produces parabens, synthetic preservatives used in personal care, food, and pharmaceuticals. Physicochemical properties depend on whether they have aromatic rings or alkyl chains as substituents. The most frequent linear alkyl chain substituent parabens are MeP, EtP, PP, BuP, and PeP. The extensive use of MeP and PP in aquatic settings has led to their presence. Parabens are utilized globally at 8,000 tons per year. In sewage sludge, landfill effluent, and wastewater treatment plant effluent, these chemicals can leak into the environment during manufacturing and disposal. Parabens and their metabolites can be consumed, breathed, or absorbed via skin contact. Some have been found in air, dust, wastewater, surface water, human urine, and cancers. Learning where parabens move in the environment and how much are in ecosystems is crucial to assessing their risks and toxicological effects. A full review of paraben toxicity research in zebrafish followed. Carmine Merola and colleagues studied the neurological consequences of butylparaben (BuP), ethylparaben (EtP), and methylparaben (MeP) exposure in early development using zebrafish larvae behavioral models. Zebrafish were given three doses of each paraben up to four days following fertilization. These amounts were based on the benchmark-dose lower limit for zebrafish larvae and ecologically appropriate human exposure. At 4, 5, and 6 days post fertilization, behavioral circadian rhythms were studied in connection to thigmotaxis, visual startle response, photic synchronization, and familiar and unfamiliar activity. Zebrafish larvae exposed to BuP 500 µg/L and EtP 5000 µg/L demonstrated increased discomfort in new settings. Larvae treated with 5000 µg/L EtP showed hyperactivity in familiar environments, but those treated with 500 µg/L BuP showed reduced activity in both familiar and unfamiliar situations. Parabens did not affect visual reflex reactions or light-synchronized circadian cycles in zebrafish larvae.

Ceyhun Bereketoglu and colleagues conducted a comprehensive study on the harmful effects of propylparaben (PP) and methylparaben (MeP) on early zebrafish development. The study examined how both substances affected fetal development, hatching, gene expression abnormalities, and mortality.

In a concentration-dependent manner, embryos are toxic to MeP (≥ 100 µM) and PP (≥ 10 µM) during semi-static exposure, leading to developmental abnormalities. After MeP and PP therapy, spinal abnormalities, pericardial fluid accumulation, and pigmentation abnormalities occurred. Late hatching, mortality, and aberrant development suggest PP is more detrimental than MeP. MeP and PP were tested at 1 and 10 µM doses for gene expression. MeP and PP influenced inflammation, stress response, fatty acid metabolism, endocrine activity, cell cycle, and DNA damage. Gene expression patterns suggest parabens disrupt fatty acid metabolism, causing oxidative stress, DNA double-strand breaks, and apoptosis. Parabens may be antiandrogenic and estrogenic in zebrafish due to *ar* and *esr2a* gene expression alterations. This study illuminates MeP and PP's deleterious effects on physiological endpoints and calls for greater research into paraben toxicity's chemical foundation.

According to a study by Vrinda Yatin Dambal and colleagues, methylparaben (MeP) was tested on embryo-larval zebrafish for 96 hours after fertilization (hpf) at levels from 100 µM to 1000 µM. MeP-exposed embryos were evaluated for survival, hatching, heart rate, and developmental abnormalities. MeP-exposed embryos showed reduced heart and hatching rates. Abnormalities such

pericardial edema, blood cell accumulation, and bent spine increased with concentration, observed in all treatment dosages except 100 μ M. The MeP 96 hpf LC50 was 0.065 mg/L. Real-time polymerase chain reaction (RT-PCR) showed that larval zebrafish exposed to 100 μ M (0.015 mg/L) methane had significantly higher expression of vitellogenin I (Vtg-I) compared to the control group until 96 hpf. These results imply that dysregulated expression of the estrogenic biomarker gene Vtg-I occurs at lower doses of MeP, even if these amounts do not generate phenotypic abnormalities. Additionally, C. Merola and colleagues examined the embryotoxicity of methylparaben (MeP) in early zebrafish. OECD guideline 236's Fish Embryo Acute Toxicity (FET) testing and benchmark-dose (BMD) technique were employed. To assess injury, lethal endpoints, hatching rate, and sublethal alterations were monitored daily. Five dosages of MeP were given to zebrafish eggs from 96 hours after fertilization (hpf): 1, 10, 30, 60, and 80 mg/L. Fatal concentration 50 was 72.67 mg/L. The BMD confidence interval for fatal endpoints was 40.8-57.4 mg/L, and for the toxicity index (sublethal and lethal alterations), it was 16-26.5 mg/L. Sublethal alterations in MeP-treated zebrafish embryos included notochord curvature, decreased heartbeat, blood circulation, pericardial and yolk edema. The greatest MeP exposure increased the incidence of embryos with sublethal alterations between 24 and 96 hours after fertilization. The only zebrafish larvae showing behavioral problems were treated with 30 mg/L MeP.

Zebrafish eggs were examined for Butylparaben (BuP)-induced developmental damage in 2023. Growth of Neural Crest Cells (NCCs), which are crucial to gastrulation in zebrafish embryos, was studied. The study employed BuP solutions at 0.5, 0.75, and 1 mg/L, per international safety regulations.

Five days after exposure, Zebrafish larvae had heart dysplasia, periocular edema, delayed otolith development, and significant craniofacial cartilage abnormalities. Oxidative stress reactivity increased. Biochemical assays showed that MDA concentrations rose while CAT and SOD activities decreased. Alkaline phosphatase (ALP) activity, another osteoblast biomarker, dropped. The chondrocyte marker genes *col2a1a*, *sox9b*, and *sox9a* were down-regulated by RT-qPCR. Zebrafish larvae also have altered maxillofacial chondrocyte morphology and decreased cranial NCC proliferation.

Due to BuP-induced extreme oxidative stress, larval Zebrafish develop craniofacial deformities and NCC development is prevented. Larissa Cristine de Carvalho Penha et al. studied the potential harm of methylparaben (MeP) on adult and larval zebrafish using toxicity tests and physiological and biochemical markers. To test biomarkers, fish were exposed to 30 μ g/L MeP in the environment. NOEC levels were 60 mg/L for larvae and 50 mg/L for adults. Adults were much more sensitive to MeP than larvae, with an LC50 of 105.09 mg/L versus 211.12 mg/L. When exposed to 50 mg/L MeP, an adult fish's phase 1 biotransformation activity (ethoxyresorufin O-deethylase) decreased, gill lipoperoxidation (LPO) increased, and erythrocyte micronuclei increased. MeP-exposed fish tissues had lower integrated biomarker response (IBR) scores, indicating biological processes are dampened. For fish gills exposed to 50 mg/L methylparaben (MeP), LPO was the predominant IBR score factor. Sublethal exposure to MeP at 30 μ g/L and 60 mg/L did not affect larvae, regardless of LPO amount. The researchers wondered if MeP's antibacterial effect on adults' gut flora explains why larvae are less susceptible. Although it may be a response to environmental stress, eating more

carbon sources did not affect diversity or abundance. However, MeP is genotoxic and produces LPO in adult zebrafish, making it crucial to manage its ambient presence and clean wastewater treatment facilities.

Triclosan

Due of its antibacterial qualities, shampoo, skin cream, soap, and toothpaste include triclosan (TCS), a chemical known as 5-chloro-2-(2,4-dichlorophenoxy) phenol. It has been found in wastewater, surface water, sediments, and even breast milk and urine due to its ubiquitous use. The content of TCS in surface water can vary by 10,000 times, from 0.0014 to 40 µg/L. TCS is stable and lipophilic, however it can undergo transformation processes in the aquatic environment, forming hazardous and bioaccumulative chemicals or derivatives. These modified substances can harm algae, invertebrates, fish, and amphibians. TCS forms methyltriclosan (MTCS), which biological wastewater treatment systems cannot entirely remove. MTCS bioaccumulates in aquatic species more than TCS due to its higher environmental persistence and lipophilicity. We examined zebrafish studies of triclosan toxicity in the following areas.

Rhauil Oliveira and colleagues evaluated the harmful effects of Triclosan (TCS) on embryos and adults of zebrafish (*Danio rerio*). Death, embryo development and behavior, hatching, micronuclei, and biochemical markers like GST, ChE, and LDH were examined in TCS-exposed organisms. The embryo/larvae testing model followed the OECD Fish Embryo Toxicity Test standard. Embryos were exposed to TCS concentrations from 0.1 to 0.9 mg/l during 6 days. Mortality, development, and hatching were monitored daily with a stereomicroscope. In a parallel test, larvae were collected for ChE, GST, and LDH analysis. Semi-static conditions were used for the adult test per OECD Guideline TG 203. Adult zebrafish of equal age and length were exposed to TCS. TCS was given at 0.1-0.5 mg/l doses, with a 96-hour exposure time. Daily inspections monitored mortality and behavior. A later test obtained organs for biomarker analysis. Results showed TCS induced acute toxicity to embryo/larvae (96 h LC₅₀ = 0.42 mg/l) and delayed hatching. In addition, embryotoxicity caused delays in otolith development, body and eye coloration, and deformities. Exposure to 0.25 mg/l resulted in increased ChE and LDH activity in larvae, and increased GST activity in larvae subjected to 0.25 and 0.35 mg/l. TCS caused acute toxicity in adult zebrafish, with a 96-hour LC₅₀ of 0.34 mg/l.

However, TCS did not generate micronucleus or affect biomarker levels in adulthood. Although *D. rerio* embryos and adults had similar 96-h LC₅₀ values (0.42 and 0.34 mg/l, respectively), the embryo assay revealed more profound effects, including teratogenic response, hatching delay, and biomarker changes. TCS does not appear to be genotoxic to adult fish or affect biomarker levels at studied amounts.

Falisse Elodie and colleagues studied zebrafish early-life stress response and Triclosan (TCS) acclimatization. After fertilization, zebrafish eggs were exposed to four sublethal TCS concentrations (2, 20, 50, and 100 µg/L) for seven days before being subjected to a lethal TCS dose (1000 µg/L). To predict resistance, mortality was monitored during the time-to-death (TTD) exposure. Interestingly, larvae exposed to 50 µg/L TCS showed greater sensitivity, resulting in

delayed hatching, increased mortality after sub-lethal exposure, and considerably lower mean TTD values compared to other groups. Fish exposed to 100 µg/L TCS had identical mean TTD values as controls and much greater survival than embryos exposed to 50 µg/L, indicating an adaptation process. Proteomic and enzymatic studies of larvae exposed to 50 µg/L and 100 µg/L TCS at 7 days post-fertilization (dpf) revealed functional alterations. TCS appears to alter cytoskeleton, stress response, ocular, and neural development proteins. This was supported by enzymatic studies showing glutathione metabolism impairment and acute neurotoxicity. After TCS exposure, AChE activity increased 2.5-fold and 3-fold. While GPx activity was greatly boosted, GR activity was significantly inhibited, suggesting that de novo synthesis of reduced GSH may occur to preserve the reduced/oxidized GSH ratio. Potential candidate proteins for acclimation in larvae exposed to 100 µg/L TCS were identified using proteomic analysis. Integrative study showed complicated effects of non-monotonic concentrations on early-life zebrafish, with resistance increasing between 50 and 100 µg/L exposures.

Jing Fu and colleagues used gas chromatography–mass spectrometry to assess Triclosan (TCS) toxicity in developing zebrafish (*Danio rerio*) embryos. The embryos were exposed to TCS concentrations from 10 ng/L to 500 mg/L. Endogenous metabolites were extracted using 3:3:2 acetonitrile, isopropanol, and water. Metabolites were derivatized before GC–MS identification and quantification. The embryos tested positive for 29 metabolites. Univariate (one-way analysis of variance) and multivariate (principal components analysis and projection to latent structure-discriminant analysis) studies were used to assess TCS-exposed embryo metabolic profile alterations. In embryos exposed to TCS, eight metabolites (urea, citric acid, D-(p)-galactose, D-glucose, stearic acid, L-proline, phenylalanine, and L-glutamic acid) showed significant changes ($p < 0.05$). The findings imply that TCS may disrupt energy, amino acid, nitrogen, and gill metabolism in developing zebrafish embryos. These findings should aid TCS and other developing pollutant risk evaluations.

Another research by Jing Fu and colleagues examined the harmful effects of Triclosan (TCS) and its derivative, Methyl-Triclosan (MTCS), on zebrafish embryos. The study employed TCS and MTCS at ecologically relevant (ng/L) and high-dose sublethal doses. Metabolomics and reverse transcription qPCR were used to study these impacts. Zebrafish embryos showed alterations in metabolite and transcript expression following 96 hours of exposure to TCS (30 µg/L, 300 µg/L, 400 µg/L), MTCS, and a combo of TCS/MTCS (30 µg/L TCS + 3 µg/L MTCS and 300 µg/L TCS + 30 µg/L MTCS). The expression of various enzymes and transporters, including UT, G6PD, ALT, GDH, PGM, and FASN, was significantly altered. TCS, MTCS, and TCS/MTCS treatments also altered alanine, urea, glucose, 6-phosphogluconalactone, and palmitic acid. Fold alterations in mRNA expression linked to nitrogen metabolism, energy metabolism, and fatty acid synthesis were identified in the MTCS treatment group, indicating biological pathway disruption in zebrafish embryos. TCS, MTCS, and the TCS/MTCS mixture treatment alter metabolites and gene expressions in starch and sucrose metabolism, nitrogen metabolism, fatty acid synthesis, and phenylalanine, tyrosine, and tryptophan biosynthesis. Thus, this work illuminates the hazards of the parent substance (TCS) and its by-product (MTCS) and their biological pathway disruptions in aquatic habitats.

In 2016, zebrafish were utilized to research Triclosan (TCS)-induced developmental and metabolic problems. In situ hybridization staining was conducted at four embryonic stages (70-85% epiboly, 10-12 somites, prim-5, and 5 days after fertilization) to study the impact of TCS on dorsal ventral patterning, segmentation, brain development, and organ creation. After 5 days of exposure to 250 µg/L TCS, no phenotypic or molecular alterations were found in terms of developmental toxicity. However, such a dose of TCS caused yolk sac lipid droplets, perhaps due to beta-oxidation transcript mRNA deregulation. This study found that 250 µg/L TCS exposure does not impact normal embryogenesis or organogenesis. Lipid metabolism impairment is a problem. TCS may not directly affect development, but it may disturb metabolic pathways, warranting additional study.

Alisha Saley and colleagues examined the effects of Triclosan (TCS) on zebrafish cardiac function to determine its potential dangers to aquatic life. From 8 to 120 hours post-fertilization, zebrafish were exposed to different TCS concentrations (0, 0.4, 40, and 400 µg/L) utilizing a static waterborne exposure approach with daily modifications. The study examined pericardial edema and its effects on heart anatomy and function. Exposure to TCS concentrations of 40 µg/L or above caused pericardial edema and cardiac structural changes. A reduction in cardiac output was only observed at the highest exposure level of 400 µg/L. Even at a modest exposure level of 0.4 µg/L, a small but substantial percentage of embryos showed increased regurgitation. These data suggest that growing fish may experience modest cardiac damage from short-term TCS exposure. Further assessments are needed to properly comprehend TCS's dangers to aquatic life and human health.

Zebrafish

As a result, it is of the utmost importance to continue monitoring their utilization and disposal, as well as to seek out safer alternatives wherever they are feasible. When it comes to the field of biological research, there is a wide variety of approaches that may be utilized to evaluate the toxicity of different substances. The cost-effectiveness of in vitro procedures, such as cell cultures, has contributed to the fact that they have become increasingly popular among these. On the other hand, the difficulty of generalizing the results of these tests to complete organisms is a substantial obstacle that needed to be overcome. On the other hand, in vivo approaches provide testing that is more complete, but they also come with their own unique assortment of difficulties. The zebrafish, also known as *Danio rerio*, is an organism that has been shown to be an extremely useful asset in this particular subject.

In addition to being simple to care for and preserve, this species is also characterized by a quick growth rate and a very short lifetime. In a remarkable way, the zebrafish and humans have a substantial amount of genetic similarities. As a consequence of this, it has managed to become one of the most effective and helpful animal models in the field of toxicology. The Zebrafish Embryo Toxicity Test, often known as the ZET, is a procedure that is frequently used to determine the level of toxicity that any given chemical agent possesses. The evaluation of a broad variety of potentially harmful chemical compounds may be accomplished with the help of this test, which is not only quick and uncomplicated but also adaptable. This article discusses the many harmful consequences that are linked with these four regularly used chemical biocides when they are given to zebrafish eggs. These toxic effects include genotoxicity, developmental toxicity, and behavioral toxicity.

CONCLUSION

When it comes to the manufacture and development of different biocides, it is necessary to conduct stringent toxicity tests. There have been a great number of papers that have brought attention to the widespread prevalence of these biocides in the environment, particularly highlighting the significance of their non-toxicity from an environmental point of view. The results of this research highlight the potential of zebrafish as a good model for regulatory testing of biocides. This is due to the fact that zebrafish are genetically similar to humans, they develop quickly, and they are very inexpensive. The zebrafish model offers a method that is both efficient and cost-effective for evaluating the toxicological profiles of biocides. This gives valuable information that can be used to guide regulatory choices and the development of safer alternatives. It is also possible that these findings can help influence the development of safer biocides that can be used in industrial, cosmetic, and domestic goods. This will help reduce the negative impacts that these biocides have on human health and the environment. This study emphasizes the significance of the zebrafish model for conducting comprehensive assessments of the toxicity of biocides.

REFERENCES

1. Russell A. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J. Appl. Microbiol.* 2002;92(s1):121S–135S.
2. Jones I.A., Joshi L.T. Biocide use in the antimicrobial era: a review. *Molecules.* 2021;26(8):2276. doi: 10.3390/molecules26082276.
3. Maillard J.-Y. Antimicrobial biocides in the healthcare environment: efficacy, usage, policies, and perceived problems. *Ther. Clin. Risk Manag.* 2005;1(4):307–320.
4. Maillard J.-Y. Resistance of bacteria to biocides. *Microbiol. Spectr.* 2018;6(2) doi: 10.1128/microbiolspec.arba-0006-2017
5. Sreenivasan P., Gaffar A. Antiplatelet biocides and bacterial resistance: a review. *J. Clin. Periodontol.* 2002;29(11):965–974. doi: 10.1034/j.1600-051x.2002.291101.x.
6. Hernández-Moreno D., et al. Acute hazard of biocides for the aquatic environmental compartment from a life-cycle perspective. *Sci. Total Environ.* 2019;658:416–423. doi: 10.1016/j.scitotenv.2018.12.186.
7. Gerba C.P. Quaternary ammonium biocides: efficacy in application. *Appl. Environ. Microbiol.* 2015;81(2):464–469. doi: 10.1128/AEM.02633-14.
8. Arnold W.A., et al. Quaternary ammonium compounds: a chemical class of emerging concern. *Environ. Sci. Technol.* 2023;57(20):7645–7665. doi: 10.1021/acs.est.2c08244.
9. Tezel U., Pavlostathis S.G. Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. *Curr. Opin. Biotechnol.* 2015;33:296–304. doi: 10.1016/j.copbio.2015.03.018.

10. Bragg R., et al. Infectious Diseases and Nanomedicine II: First International Conference (ICIDN–2012), Dec. 15-18, 2012, Kathmandu, Nepal. Springer; 2014. Bacterial resistance to quaternary ammonium compounds (QAC) disinfectants
11. Sączewski F., Balewski L. Biological activities of guanidine compounds, 2008–2012 update. *Expert Opin. Ther. Pat.* 2013;23(8):965–995. doi: 10.1517/13543776.2013.788645.
12. Park Y.J., et al. Guanidine-based disinfectants, polyhexamethylene guanidine-phosphate (PHMG-P), polyhexamethylene biguanide (PHMB), and oligo (2-(2-ethoxy) ethoxyethyl guanidinium chloride (PGH) induced epithelial-mesenchymal transition in A549 alveolar epithelial cells. *Inhal. Toxicol.* 2019;31(4):161–166. doi: 10.1080/08958378.2019.1624896.
13. Oule M.K., et al. Polyhexamethylene guanidine hydrochloride-based disinfectant: a novel tool to fight meticillin-resistant *Staphylococcus aureus* and nosocomial infections. *J. Med. Microbiol.* 2008;57(12):1523–1528. doi: 10.1099/jmm.0.2008/003350-0.
14. Qian L., et al. Modified guanidine polymers: synthesis and antimicrobial mechanism revealed by AFM. *Polymer.* 2008;49(10):2471–2475.
15. Lee J.H., Yu I.J. Human exposure to polyhexamethylene guanidine phosphate from humidifiers in residential settings: cause of serious lung disease. *Toxicol. Ind. Health.* 2017;33(11):835–842. doi: 10.1177/0748233717724983
16. Fransway A.F., et al. Parabens. *Dermatitis.* 2019;30(1):3–31. doi: 10.1097/DER.0000000000000429.
17. Wei F., et al. Parabens as chemicals of emerging concern in the environment and humans: a review. *Sci. Total Environ.* 2021;778 doi: 10.1016/j.scitotenv.2021.146150.
18. Torfs E., Brackman G. A perspective on the safety of parabens as preservatives in wound care products. *Int. Wound J.* 2021;18(2):221–232. doi: 10.1111/iwj.13521.
19. Błędzka D., Gromadzińska J., Wąsowicz W. Parabens. From environmental studies to human health. *Environ. Int.* 2014;67:27–42. doi: 10.1016/j.envint.2014.02.007. [
20. Soni M., Carabin I., Burdock G. Safety assessment of esters of p-hydroxybenzoic acid (parabens) *Food Chem. Toxicol.* 2005;43(7):985–1015. doi: 10.1016/j.fct.2005.01.020.
21. Crovetto S.I., et al. Bacterial toxicity testing and antibacterial activity of parabens. *Toxicol. Environ. Chem.* 2017;99(5-6):858–868
22. Giuliano C.A., Rybak M.J. Efficacy of triclosan as an antimicrobial hand soap and its potential impact on antimicrobial resistance: a focused review. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* 2015;35(3):328–336. doi: 10.1002/phar.1553.
23. Jones R.D., et al. Triclosan: a review of effectiveness and safety in health care settings. *Am. J. Infect. Control.* 2000;28(2):184–196.

24. Bhargava H., Leonard P.A. Triclosan: applications and safety. Am. J. Infect. Control. 1996;24(3):209–218. doi: 10.1016/s0196-6553(96)90017-6.

25. Dann A.B., Hontela A. Triclosan: environmental exposure, toxicity and mechanisms of action. J. Appl. Toxicol. 2011;31(4):285–311. doi: 10.1002/jat.1660.