VOLUME 8 ISSUE 1 2022

ISSN 2454-3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 8, No. 1, 01-17 (2022)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Evaluation of Oxidative Stress and Genotoxicity Induced by Triclosan in the Freshwater Fish, *Anabas testudineus* (Bloch, 1792)

Chokki Veettil Priyatha and Kumari Chidambaran Chitra*

Endocrinology and Toxicology Laboratory, Department of Zoology, University of Calicut, Malappuram District, Kerala 673635, India

**Corresponding Author*

Received: 4th December, 2021; **Accepted:** 30th December, 2021; **Published online:** 5th January, 2022

https://doi.org/10.33745/ijzi.2022.v08i01.001

Abstract: The antimicrobial property of triclosan benefits human in many consumer products, while its adverse effects on non-target organisms raise environmental concern. In this study we attempted to investigate possible role of triclosan in the antioxidant defense system and genotoxicity in the fish, *Anabas testudineus*. Fish were exposed to the sublethal (176.7 µg L^{-1}) and environment concentrations (0.009 and 9 µg L^{-1}) of triclosan for 4, 7, 30, and 60 days along with the control groups. Oxidative stress of triclosan was evaluated using biomarkers such as glutathione reductase, levels of reduced glutathione, hydrogen peroxide generation, lipid peroxidation, and protein carbonyl content in gonads. Besides, the frequencies of micronucleus, nucleo-cytoplasmic abnormalities, and comet assay were performed to estimate the genetic damage in the peripheral erythrocytes. Triclosan caused a significant ($P<0.05$) decrease in the activity of the glutathione reductase, and the level of reduced glutathione. However, the levels of hydrogen peroxide generation, lipid peroxidation, and protein carbonyls increased significantly ($P<0.05$) in the gonads. Micronucleus test showed a significant increase in the formation of micronucleus and other nucleo-cytoplasmic abnormalities in the peripheral erythrocytes. Furthermore, a significant ($P<0.05$) increase in the comet tail DNA per cent and tail length in the fish erythrocytes revealed the genotoxicity of triclosan. Thus, the present results suggest that triclosan exposure could induce oxidative stress, and potentially lead to DNA damage in the fish, *Anabas testudineus.*

Keywords: Triclosan, Oxidative stress, Comet assay, Micronucleus, *Anabas testudineus*

Citation: Chokki Veettil Priyatha and Kumari Chidambaran Chitra: Evaluation of Oxidative Stress And Genotoxicity Induced By Triclosan In The Freshwater Fish, *Anabas testudineus* (Bloch, 1792). Intern. J. Zool. Invest. 8(1): 01-17, 2022.
https://doi.org/10.33745/ijzi.2022.v08i01.001

Introduction

Pharmaceuticals and personal care products (PPCPs), which include diverse organic compounds have confirmed their presence in various environmental compartments, and exert the biological responses in several non-target organisms. Triclosan, one of the PPCPs, widely used as an antimicrobial agent in several consumer products is continuously discharged into the aquatic ecosystems through various point and non-point sources (Li *et al.*, 2014). The United States Food and Drug Administration and the European Union have banned the use of triclosan

in few developed countries from some consumer products and reformulated with healthier alternatives (US-FDA, 2016; EU, 2016). However, the regulation of triclosan on other personal care products like liquid soaps, toothpaste, and medical devices is not enforced throughout the world (Dodson *et al.,* 2020). The bactericidal property of triclosan prevents it from several degradation steps in the wastewater sewage treatment plants and potentially escapes to reach the aquatic environment (Ricart *et al.,* 2010). The acute toxicity of triclosan in aquatic invertebrates such as zooplankton (Gonzalez-Perez *et al.,* 2018), larval midges (Martinez-Paz *et al.,* 2017), and crustaceans (Perron *et al.,* 2012) has been well established. In aquatic vertebrates, particularly in fish species, the endocrine-disrupting effects of triclosan have been evidenced by developmental and reproductive defects (Carmosini *et al.,* 2016; Song *et al.,* 2020). Triclosan exerts its toxicity by the direct action on mitochondria by disrupting mitochondrial membrane fluidity and impairment of mitochondrial uncoupling in embryos of zebrafish *Danio rerio* (Shim *et al.,* 2005). Mitochondrial dysfunction is indirectly associated with the generation of reactive oxygen species (ROS) that contribute to several pathologies in the organism (Murphy, 2013).

Environmental stressors released into the aquatic environment are known to activate the endogenous production of ROS leading to an imbalance in the pro-oxidant and antioxidant defense system in fish (Livingstone, 2001; Lushchak, 2011). The disturbances in cellular oxidative homeostasis cause oxidative stress that eventually damages the biological systems often prelude to the onset of lipid peroxidation, protein and DNA damage by inhibiting the activities of antioxidant enzymes (Lushchak, 2016). The alteration in the antioxidant defenses can be used as a diagnostic tool of oxidative stress, and also play a significant role in predicting the toxic effects of the stressors. The recommended biomarkers of oxidative stress in fish include the activities of antioxidant enzymes (Doyotte *et al.,* 1997; Pandey *et al.,* 2003), non-enzymatic antioxidants (Parvez *et al.,* 2006), generation of hydrogen peroxide (Tkachenko *et al.,* 2013), lipid peroxidation (Yoshida *et al.,* 2013), and protein oxidation (Parvez and Raisuddin, 2005). The free radicals formed as a result of ROS production could also lead to DNA damage that is usually detected using micronucleus formation and comet tail (Beedanagari *et al.,* 2014; Hussain *et al.,* 2018).

The measurement of changes in the activities of certain antioxidant enzymes is largely used as an early warning system to elucidate the effects of toxicants. The glutathione-related enzyme activity appeared to be a relevant biomarker that attempts to find the relation between the effects of toxicants in the tissues and the response of the antioxidant defense system (Habashy *et al.,* 2019). The non-enzymatic antioxidant glutathione is a cysteine-containing peptide that prevents free radical-induced cellular or tissue damages, and is widely used as an indicator of the 'redox status' of the cell (van der Oost *et al.,* 2003). The failure in the activation of the key antioxidant enzymes namely superoxide dismutase, catalase, and glutathione peroxidase prevents the conversion of hydrogen peroxide and hydroperoxides to harmless water and oxygen molecules (Chelikani *et al.,* 2004). The low level of hydrogen peroxide generated regulates several physiological processes including signaling in cell proliferation, apoptosis, normal mitochondrial function, carbohydrate metabolism, platelet activation, and balancing the thiol-redox status in cells (Droge, 2002). However, high concentrations of hydrogen peroxide are deleterious to the cells that could lead to free radical-induced tissue damages (Clement and Pervaiz, 2001).

Free radicals primarily target lipids, the essential components of the cell membrane, and generate lipid peroxidation that leads to a profound impact on the biological systems by causing oxidative damage (Yin *et al.,* 2011). The levels of malondialdehyde, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides, and 4-hydroxyalkenals are frequently measured endpoints to detect the rate

of lipid peroxidation or oxidation of polyunsaturated fatty acids in the cells (Livingstone, 2001; Ayala *et al.,* 2014). Besides, proteins are considered as an important target of free radical attack in cells (Du and Gebicki, 2004), and the formation of protein carbonyl by oxidative modifications contributes to protein degradation and associated cellular pathologies (Dalle-Donne *et al.,* 2003). The free radical formation also leads to modifications of DNA bases, formation of abasic sites, and strand breaks that potentially damage both mitochondrial and nuclear genomes (Aitken and Krausz, 2001; Barzilai and Yamamoto, 2004).

Few researchers have identified that triclosan toxicity has been mediated through the induction of oxidative stress and DNA methylation in eggs of zebrafish (Falisse *et al.,* 2017). Besides, alteration of the antioxidant defense system and genetic damage in the liver of goldfish, *Carassius auratus* (Wang *et al.,* 2018a), and in liver and brain tissues of adult zebrafish (Gyimah *et al.,* 2020) also contribute to free radical formation. Triclosan exposure activates cellular defense mechanism for the induction of cytotoxicity in zebrafish embryos (Parenti *et al.,* 2019) while the binary mixture of triclosan and triclocarban has known to alter antioxidant enzyme activities in the embryos of silver catfish (Gomes *et al.,* 2021). Although the adverse effects of triclosan are evident in other fish species, the present study was aimed to investigate the gap of knowledge on the oxidative stress response in gonadal tissues of the native adult climbing perch, *Anabas testudineus*. The most reliable oxidative stress biomarkers were considered for the appropriate evaluation of triclosan toxicity, and genotoxic effects was also assessed using micronucleus test and comet assay.

Materials and Methods

Chemicals:

Triclosan (5-chloro-2-(2, 4-dichlorophenoxy) phenol; TCS of 97% purity), dimethyl sulfoxide (DMSO; 99% purity), thiobarbituric acid, fetal bovine serum, Giemsa stain, Triton X-100, ethidium bromide, and agarose were obtained from HiMedia Research Laboratories Pvt. Ltd, Mumbai, India. All other chemicals of analytical grade were purchased from the local commercial sources, India.

Test animal:

Healthy adult freshwater fish, *Anabas testudineus* (8 ± 1 g; 8.5 ± 0.75 cm) were randomly selected from the stock maintained in the Endocrinology and Toxicology Laboratory, University of Calicut, India. The physico-chemical features of water such as temperature 28 ± 2 C, pH 7.4 to 7.6, dissolved oxygen 8.64 ± 0.6 mg L^{-1}, and salinity < 100 ppm, were maintained in the range throughout the study (APHA, 1998). Each group consisting of a total 10 fish per sex, in replicates were introduced into well-aerated and dechlorinated water in glass tanks (40 L; 30 cm width × 60 cm length × 30 cm depth) under a natural photoperiod (12: 12 h; light: dark). Fish were fed daily with standard fish pellets and water was renewed on every 96 h by maintaining the appropriate test concentrations. The care and use of the fish complied with the Animal Welfare Board of India and Committee for Control and Supervision of Experiments on Animals (CPCSEA) under the Ministry of Environment, Forest and Climate change, Government of India.

Experimental setup:

Control (n=10 per group, in replicates)	**Experimental** (n=10 per group, in replicates)		
	Sublethal concentration (One-tenth of LC_{50} 96 h) (Priyatha and Chitra, 2018)	**Environmentally relevant concentration** (Ramaswamy *et al.,* 2011; Nag *et al.*, 2018)	
No Toxicant/Vehicle	176.7 µg L^{-1}	0.009 µg L^{-1}	9 µg L^{-1}
Exposure period: 4, 7, 30 and 60 days			

Preparation and analysis of samples:

After every exposure period, fish were caught using a small dip net with the least disturbance, and blood from both sexes was collected from the caudal vein using a heparinized microsyringe. The collected fresh blood was transferred into microcentrifuge tubes for the analysis of

cytonuclear abnormalities and DNA damage. The blood smear for the micronucleus test was prepared according to the standard method of Heddle (1973) and Schmid (1975). Frequencies of cellular anomalies were classified into cytoplasmic and nuclear abnormalities (Carrasco *et al.,* 1990) and identified as described by Sumi and Chitra (2019a). The comet assay was performed according to Singh *et al.* (1988). The images were captured using a C-mount camera (Optika pro5 CCD camera) and analyzed using Open Comet-Image J, version 1.3.1 software. DNA damage was estimated based on the percentage of DNA in the comet tail and categorized into five grades ranging from 0 to 4. The tail DNA damages less than 5%, 5–20%, 20–40%, 40–95%, and more than 95% were considered as grade zero, one, two, three, and four, respectively (Collins, 2004). The total score was calculated as follows:

Total score = (% of cells in grade 0 × 0) + (% of cells in grade 1 × 1) +(% of cells in grade 2 × 2) + (% of cells in grade 3 × 3) + (% of cells in grade 4 × 4)

The ovary and testis were extirpated from 10 fish per sex from both control and treatment groups by sacrificing the animal. Gonadal homogenates (1% w/ v) were prepared in 0.9% ice-cold normal saline using a motor-driven glass Teflon homogenizer on crushed ice for 1 min, centrifuged at 800 g for 15 min at 4 C. The gonadal supernatants were collected and used for the analysis of glutathione reductase (Carlberg and Mannervik, 1985), reduced glutathione (Moron *et al.* 1979), levels of hydrogen peroxide generation (Pick and Keisari, 1981), lipid peroxidation (Ohkawa *et al.,* 1979), and protein carbonyl content (Levine, 1990; Reznick and Packer, 1994). The total protein was estimated using bovine serum albumin as the standard (Lowry *et al.,* 1951).

Statistical analyses:

All data represented in Figures and Tabels were expressed as Mean ± Standard Deviation (SD) for ten animals per sex in each group, in replicates. The mean differences between the groups were considered to be significant at $P<0.05$ against the control groups, which were shown as asterisks (*). Comparison of mean among the groups was performed using one-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range as a post-hoc test using SPSS 21.0 statistical software. All biochemical analyses were carried out in triplicate to minimize the statistical errors.

Results

Antioxidant status in gonads:

The activity of glutathione reductase (Fig. 1) and the level of reduced glutathione (Fig. 2) decreased significantly ($P<0.05$) after sublethal and environmental concentrations of triclosan exposure in both ovary and testis in duration and concentration-dependent manner when compared with the respective control groups. The levels of hydrogen peroxide generation (Fig. 3), lipid peroxidation (Fig. 4), and protein carbonyl content (Fig. 5) were significantly ($P<0.05$) increased in both ovary and testis of triclosan-exposed groups than the corresponding control groups.

Micronucleus formation:

Micronucleus test performed in the peripheral blood of control groups showed no nucleo-cytoplasmic abnormalities, and it showed clear cytoplasm and distinct compact nucleus (Fig. 6A, B). Triclosan exposure caused cytoplasmic and nuclear abnormalities in the peripheral blood of the fish, *Anabas testudineus*. Cytoplasmic abnormalities include vacuolated and degenerated cytoplasm (Fig. 6C), formation of sticky cells (Fig. 6D), cytoplasmic bridge (Fig. 6E), cells without cytoplasm (Fig. 6F), and granular cytoplasm (Fig. 6H). The nuclear abnormalities observed were irregular and blebbed nucleus (Fig. 6), binucleated and serrated nucleus (Fig. 6), micronucleus (Fig. 6), and notched nucleus (Fig. 6). The percentage of nuclear and cytoplasmic abnormalities increased with an increase in time and concentrations (Tables 1, 2).

Comet tail:

The control and vehicle-control groups showed 96% of Grade A comet score, which was 0.75 and

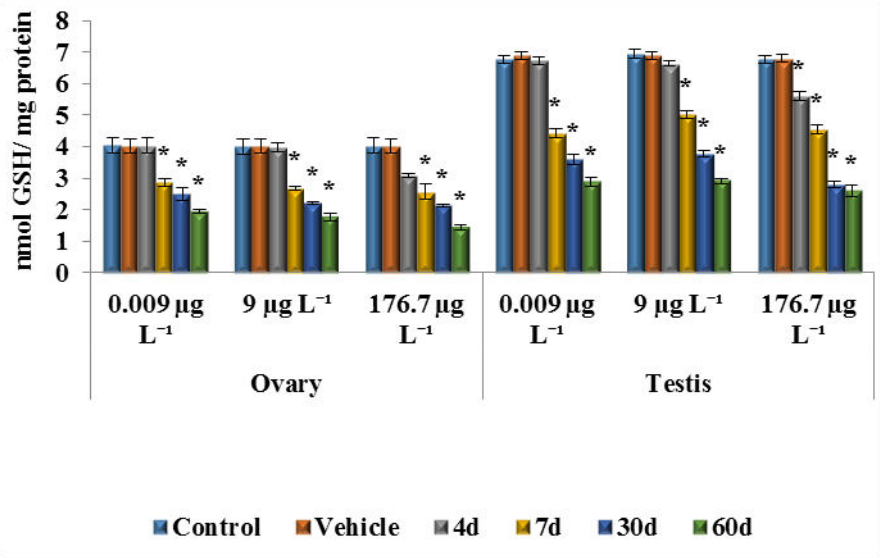

Fig. 1: Effect of triclosan on the activity of glutathione reductase in the gonads of the fish, *Anabas testudineus* (Mean ± SD; n = 10/ group, in replicates; Asterisks (*) denotes P < 0.05 against the control groups).

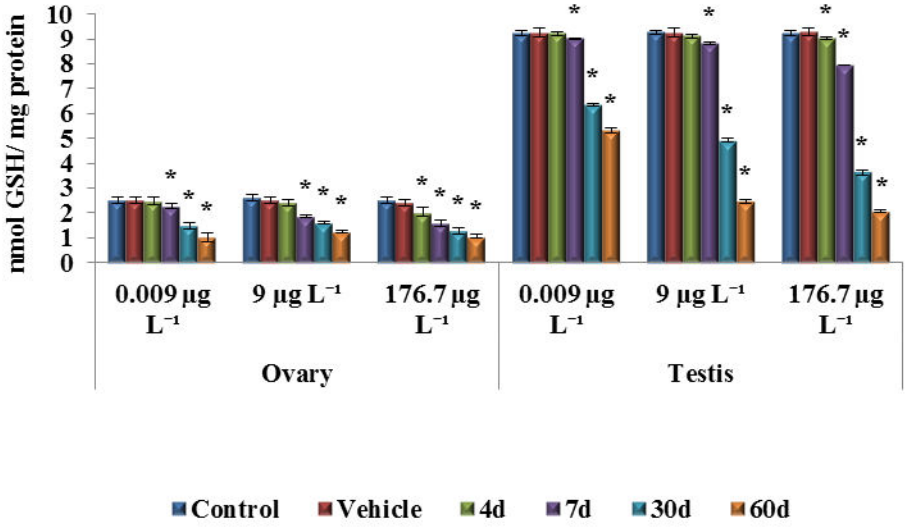

Fig.2: Effect of triclosan on the level of reduced glutathione in the gonads of the fish, *Anabas testudineus* (Mean ± SD; n = 10/ group, in replicates; Asterisks (*) denotes P < 0.05 against the control groups).

0.88 of the total scores, respectively (Table 3). In the triclosan-exposed group at 0.009 µg L^{-1} concentration, there was a significant (P<0.05) increase in the grades of DNA damage with an increase in the duration of exposure showing 2.21 to 14.77 of the total scores (Table 3). The grades of DNA damage were observed to increase at 9 µg L^{-1} concentration of triclosan ranging from Grade 0 to Grade 3 with an increase in total score from 2.51 to 17.79 based on the duration of exposure (Table 3). In the sublethal triclosan exposure group, there was a significant (P<0.05) increase in the grades of DNA damage than the environmentally relevant concentration groups (0.009 and 9 µg L^{-1} concentrations) with the total score ranging between 2.82 and 18.72 based on an increase in exposure time (Table 3).

The comet parameters such as per cent tail DNA and tail length were also scored after

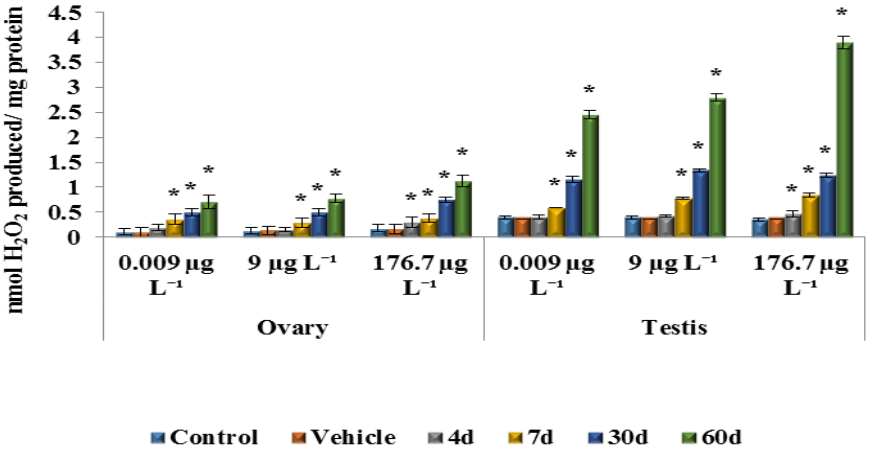

Fig. 3: Effect of triclosan on the level of hydrogen peroxide generation in the gonads of the fish, *Anabas testudineus* (Mean ± SD; n = 10/ group, in replicates; Asterisks (*) denotes P < 0.05 against the control groups).

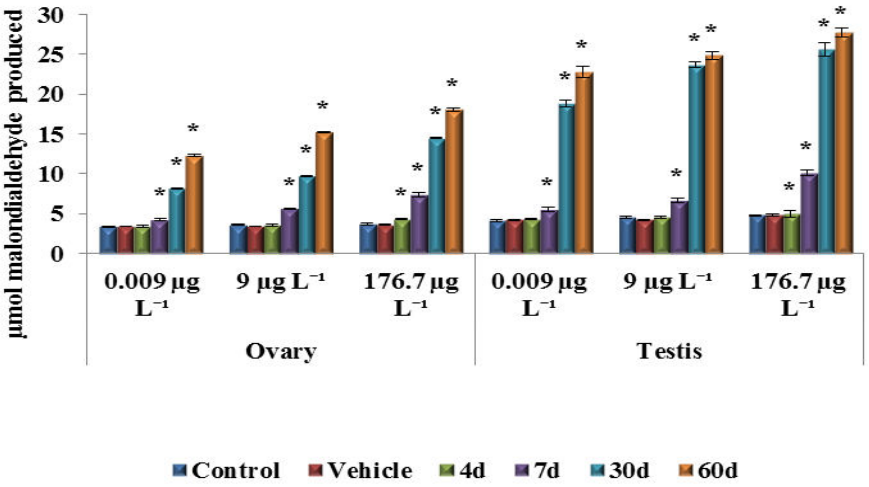

Fig. 4: Effect of triclosan on level of lipid peroxidation in the gonads of the fish, *Anabas testudineus* (Mean ± SD; n = 10/ group, in replicates; Asterisks (*) denotes P<0.05 against the control groups).

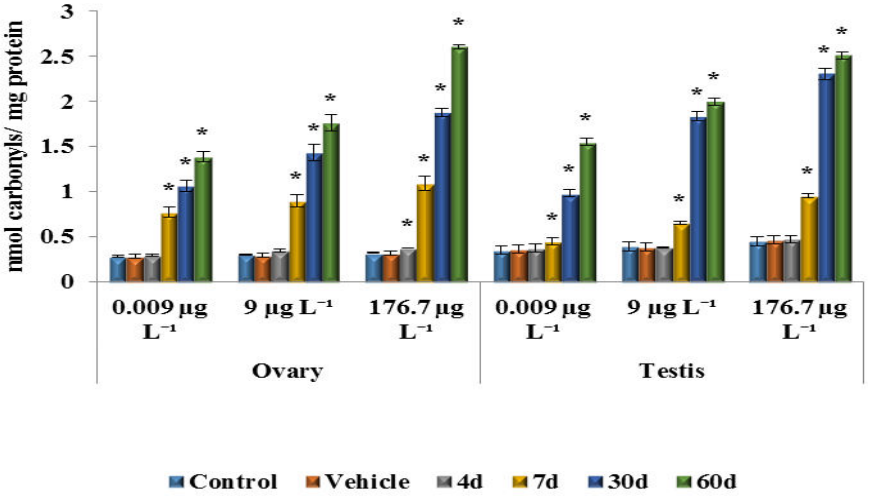

Fig. 5: Effect of triclosan on the level of protein carbonyl content in the gonads of the fish, *Anabas testudineus* (Mean ± SD; n = 10/ group, in replicates; Asterisks (*) denotes P < 0.05 against the control groups).

Table 1: Effect of triclosan on nuclear abnormalities in the erythrocytes of the fish, *Anabas testudineus* (Mean ± SD; asterisks (*) indicated significance at P < 0.05 against the control groups; n = 10/ group, in replicates; Total cells scored-1000 Nos.)

Treatment groups		Blebbed Nucleus	Notched Nucleus	Micronucleus	Irregular nucleus
Control		1.36±0.26	0	0	1.47±0.15
Vehicle		1.29±0.10	0	0	1.45±0.03
0.009 µg L^{-1}	**4 d**	3.07±0.064*	1.41±0.13	1.15±0.02	6.54±2.07*
	7 d	4.11±0.364*	3.03±0.33*	1.32±0.21	11.69±2.15*
	30 d	10.20±1.014*	3.49±1.32*	2.18±0.53*	12.18±1.64*
	60 d	12.02±2.34*	5.37±1.73*	3.76±1.26*	21.64±0.12*
9 µg L^{-1}	**4 d**	3.35±0.06*	1.47±0.06	1.66±0.01*	7.29±0.80*
	7 d	4.74±1.18*	3.08±0.84*	3.92±1.05*	12.41±1.27*
	30 d	11.51±1.95*	4.76±1.51*	10.08±0.90*	15.47±1.53*
	60 d	13.37±0.11*	5.43±1.98*	15.91±1.20*	24.99±0.39*
176.7 µg L^{-1}	**4 d**	4.511±0.14*	1.68±0.02	1.76±0.01*	12.52±0.21*
	7 d	5.66±0.67*	3.67±1.32*	6.09±1.58*	13.75±1.88*
	30 d	12.34±1.26*	4.63±1.17*	13.06±0.62*	15.72±1.60*
	60 d	14.26±0.85*	5.58±2.42*	18.24±1.85*	27.30±2.61*

Table 2: Effect of triclosan on cytoplasmic abnormalities in the erythrocytes of the fish, *Anabas testudineus* (Mean ± SD; asterisks (*) indicated significance at P < 0.05 against the control groups; n = 10/ group, in replicates; Total cells scored-1000 Nos.)

Treatment groups		Sticky cells	Cytoplasmic degeneration	Cytoplasmic vacuoles	Cytoplasmic bridge formation
Control		0.077±0.24	0	0.16±0.36	0
Vehicle		0.064±0.20	0	0.16±0.52	0
0.009 µg L^{-1}	**4 d**	0.97±2.19	0	0	0.15±0.47*
	7 d	13.23±1.11*	0	9.65±1.68*	3.76±0.90*
	30 d	14.46±2.74*	3.76±0.68*	18.26±3.66*	5.04±1.40*
	60 d	17.26±3.65*	9.43±1.27*	25.50±1.54*	11.61±2.01*
9 µg L^{-1}	**4 d**	6.63±1.76*	0	1.44±0.26*	3.83±0.80*
	7 d	10.92±1.94*	5.17±0.59*	6.78±2.75*	4.47±1.00*
	30 d	8.16±2.04*	14.64±1.91*	21.87±2.02*	10.14±1.65*
	60 d	11.57±1.74*	25.12±2.30*	15.86±2.36*	4.35±0.95*
176.7 µg L^{-1}	**4 d**	14.67±1.42*	0	20.74±2.84*	3.74±0.66*
	7 d	32.02±3.07*	13.21±2.01*	25.45±2.37*	6.44±1.27*
	30 d	19.13±1.88*	32.56±1.88*	33.58±0.92*	11.49±0.89*
	60 d	12.86±1.09*	35.27±0.92*	20.46±0.97*	7.79±0.54*

Table 3: Grades of DNA damages after triclosan exposure in erythrocytes of the fish, *Anabas testudineus* (Mean ± SD; asterisks (*) indicated significance at P<0.05 against the control groups; n = 10/ group, in replicates; Total cells scored-100 Nos.)

Treatment groups		Grades of DNA damage					Total score (%)
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	
Control		96.88±0.66	3.13±0.66	0	0	0	0.75
Vehicle		96.33±0.23	3.67±0.23	0	0	0	0.88
0.009 µg L^{-1}	**4 d**	90.75±0.61*	9.25±0.61*	0	0	0	2.21
	7 d	88.42±0.65*	11.58±0.65*	0	0	0	2.77
	30 d	78.36±0.99*	19.83±0.49*	1.82±0.49*	0	0	5.62
	60 d	60.94±0.36*	24.25±0.20*	7±0.82*	7.81±0.26*	0	14.77
9 µg L^{-1}	**4 d**	89.5±0.41*	10.5±0.41*	0	0	0	2.51
	7 d	86.25±0.61*	13.75±0.61*	0	0	0	3.29
	30 d	76.52±0.72*	14.59±0.79*	6.45±0.22*	2.44±0.15*	0	8.34
	60 d	57±0.82*	19.59±0.48*	15.47±0.38*	7.94±0.05*	0	17.79
176.7 µg L^{-1}	**4 d**	88.22±0.48*	11.78±0.48*	0	0	0	2.82
	7 d	83±0.82*	14±1.63*	3±2.45*	0	0	4.79
	30 d	60.65±0.89*	20.75±0.61*	14.94±0.36*	3.66±0.08*	0	14.75
	60 d	54.28±0.69*	23.25±0.20*	12.47±0.48*	10±0.00*	0	18.72

Table 4: Effect of triclosan on the percent tail DNA, and tail length in erythrocytes of the fish, *Anabas testudineus* (Mean ± SD; asterisks (*) denotes significance at P<0.05 against the control groups; n = 10/ group, in replicates)

Treatment groups		Tail DNA (%)	Tail length
Control		2.73±0.60	0.43±0.30
Vehicle		2.74±0.77	0.43±0.34
0.009 µg L^{-1}	**4 d**	3.02±0.84	0.69±0.35
	7 d	4.08±1.73	3.57±1.36*
	30 d	7.39±1.64*	11.96±2.21*
	60 d	18.84±3.59*	29.14±3.58*
9 µg L^{-1}	**4 d**	3.51±0.61	0.93±0.39
	7 d	4.27±1.35	3.93±1.83*
	30 d	8.58±2.49*	13.87±1.50*
	60 d	24.43±2.84*	30.18±2.67*
176.7 µg L^{-1}	**4 d**	3.84±0.75	1.00±0.34
	7 d	7.00±1.94*	4.75±2.19*
	30 d	16.25±2.27*	14.69±0.58*
	60 d	28.39±3.38*	39.67±3.68*

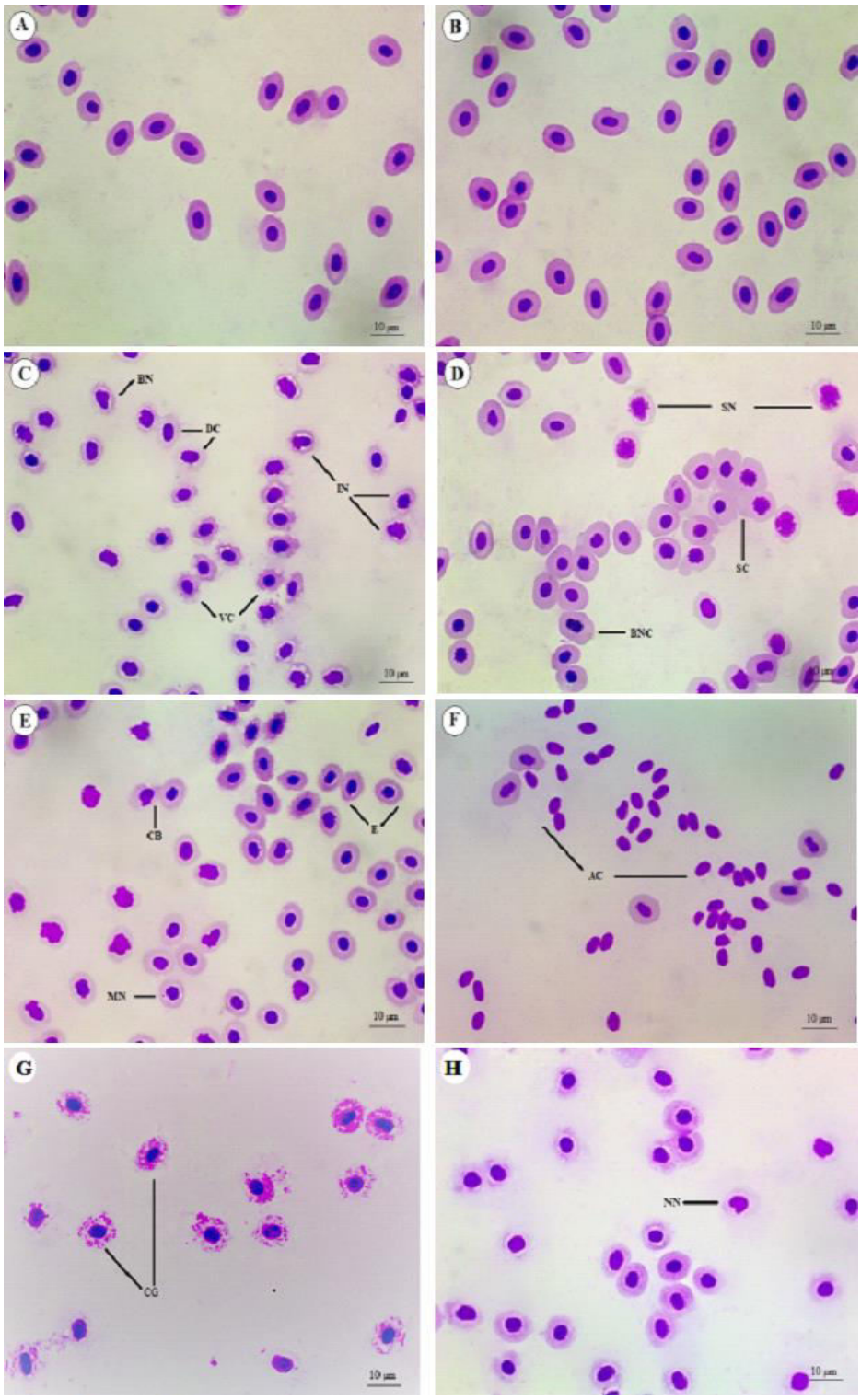

Fig. 6: Effect of triclosan showing cyto-nuclear abnormalities in the peripheral blood of the fish, *Anabas testudineus*. A-Control; B-Vehicle; C-Vacuolated cytoplasm (VC), degenerated cytoplasm (DC), irregular nucleus (IN), blebbed nucleus (BN); D-Sticky cells (SC), binucleated cell (BNC), serrated nucleus (SN); E-Cytoplasmic bridge (CB), echinocytes (E), micronucleus (MN); F-Cells without cytoplasm (AC); G-Cytoplasmic granules (CG); H-Notched nucleus (NN); Scale:10 µm.

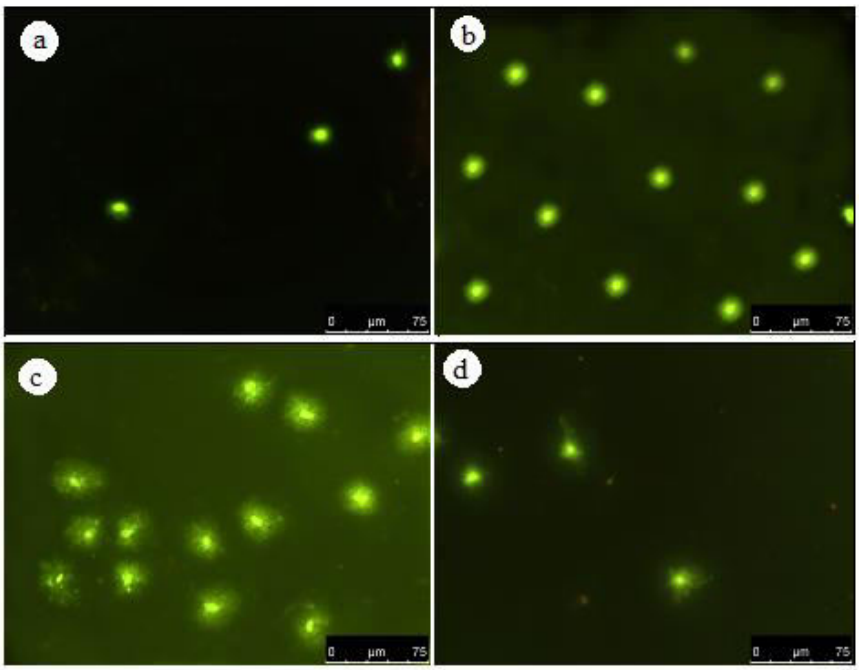

Fig. 7: Representative images of grades of DNA damage in *Anabas testudineus* (a) Grade 0-control and vehicle-control cells; (b) Grade 1; (c) Grade 2; (d) Grade 3.

triclosan exposure, which showed a significant ($P<0.05$) increase in both the parameters and the severity of DNA damages were time- and concentration-dependent (Table 4; Fig. 7). However, control groups showed no significant differences in the per cent tail DNA and tail length.

Discussion

The ubiquity of triclosan in the aquatic environment, and its crucial role in the antioxidant defense system in various animal models gained attention to explore its toxicity in the fish model, *Anabas testudineus*. There is overwhelming evidence suggesting an association between oxidative stress and DNA damage in both mitochondrial and nuclear genomes. In this study, we have investigated that triclosan toxicity was mediated through the induction of oxidative stress in the gonadal tissues, and associated DNA damage in the peripheral erythrocytes. The inactivation of antioxidant enzymes results in the overproduction of reactive oxygen species (ROS), which are highly toxic, and cause damage to carbohydrates, proteins, lipids, and DNA thereby leading to oxidative stress (Patlevic *et al.,* 2016). The interaction of enzymatic and non-enzymatic antioxidant defense systems protects the biological systems from the production of free radicals and their metabolites (Deponte, 2013).

In most aerobic organisms, glutathione (GSH) functions as the central redox agent by neutralizing ROS and 2-oxoaldehyde formations probably functioning together with the thioredoxin system (Kanzok *et al.,* 2001). GSH gets oxidized to its disulfide dimer, GSSG by the reaction with the free radicals or by the catalytic action of glutathione peroxidase (GPx) or glutathione S-transferase (GST) enzymes while glutathione reductase (GR) enzyme regenerates GSH through NADPH-dependent reaction (Grant, 2001). The glutathione redox system consisting of GSH, GSSG, GPx, GST, and GR is considered as the second-line of the defensive system against the oxidative stress induced by free radicals and lipid peroxides (Szalai *et al.,* 2009; Wu *et al.,* 2011). In the present study, the level of glutathione, as well as the activity of glutathione reductase, decreased in both ovary and testis of the fish in time- and concentration-dependent manner illustrating an altered redox state in the gonads. These observations were in accordance with previous findings where triclosan exposure at 50, 100, and

150 µg L^{-1} concentrations to male zebrafish lowered the level of GSH along with a reduction in the activity of glutathione reductase thus GSH could not be regenerated to restore its normal level in the liver tissues (Gyimah *et al.,* 2020). Furthermore, another study has revealed that sublethal exposure of triclosan to the early-life stage of zebrafish altered the redox state of glutathione (Falisse *et al.,* 2017).

Hydrogen peroxide is one of the low molecular weight, non-radical two-electron reduction products of oxygen that emerge as a central hub in redox signaling, sensing, and regulation related to oxidative stress (Sies, 2014). Hydrogen peroxide also functions as a second messenger molecule and regulates many cellular functions including initiation of cell proliferation, differentiation and migration, maintenance of cell shape and structure, and the recruitment of immune cells (Stone and Yang 2006). However, high concentrations of hydrogen peroxide evoke growth arrest causing cell death and other inflammatory responses (Alvarez *et al.,* 2016). Superoxide dismutases are the first-line antioxidant defense enzymes that catalyze superoxide radicals into hydrogen peroxide, which is consequently converted to water and molecular oxygen by catalase or glutathione peroxidase enzymes (Wang *et al.,* 2018b). In the current study, triclosan exposure elevated the level of hydrogen peroxide generation in gonads of the fish, which indicated the failure of the antioxidant enzyme system to eliminate the oxygen-derived free radical into non-toxic compounds. It is noteworthy that phenol, the environmental stressor, similarly provoked the generation of hydrogen peroxide in serum, liver, and ovary of the fish, *Cyprinus carpio* thereby causing oxidative stress (Das *et al.,* 2016). In our laboratory, it has been proved that exposure to fullerene C_{60} nanomaterial has been shown to increase the level of hydrogen peroxide generation leading to an oxidative imbalance in the gonads of the fish, *Anabas testudineus* (Sumi and Chitra, 2019b).

Cell membranes formed of the lipid bilayer are more susceptible to oxidative stress as the polyunsaturated fatty acids (PUFA) in the membrane degrade to small, more reactive particles such as conjugated dienes, lipid hydroperoxides, and thiobarbituric acid-reactive substances (TBARS) (Janero, 1990; Halliwell and Gutteridge, 1999). The products of lipid peroxidation such as acrolein, 4-oxo-2-nonenal, 4-hydroxynonenal, and malondialdehyde exhibit free radical activity, and enhance membrane damage associated with several pathologies (Zieba *et al.,* 2000). It is well-known that about 40% of gonadal membrane lipids of fish are rich in PUFA, which forms a critical risk factor for peroxidative attack (Ebeid *et al.,* 2008). In the present study, malondialdehyde, the advanced oxidation product of lipid peroxidation, was measured as an oxidative stress biomarker in the gonadal tissues to evaluate the triclosan-induced stress. Triclosan exposure at all concentrations increased the level of lipid peroxidation in both ovary and testis of the fish suggesting that the lipophilic nature of the toxicant probably evoked free radical attack on the membrane lipids of gonadal tissue. The present results are in agreement with the findings of Gallego *et al.* (2021) who have reported an increase in the level of lipid peroxidation after ibuprofen and triclosan exposure at 25 and 50 µg L^{-1} concentrations in striped catfish *Pseudoplatystoma magdaleniatum*. Dar *et al.* (2020) has reported a similar increase in the level of lipid peroxidation in embryos of four food fishes namely *Cyprinus carpio*, *Ctenopharyngodon idella*, *Labeo rohita,* and *Cirrhinus mrigala* after exposure to different sublethal concentrations of triclosan. In another study, exposure to triclosan alone or in combination with different polymers of microplastics has been shown to elevate the level of lipid peroxidation in the liver of zebrafish (Sheng *et al.,* 2021).

ROS generation also induces irreversible protein modification and degradation formed by the two most reactive products of lipid peroxidation namely 4-oxo-2-nonenal and 4-hydroxynonenal, which diffuses from the cell membrane into the cytoplasm and nucleus, and

react with a variety of proteins and nucleic acids (Dean *et al.,* 1997; Dalle-Donne *et al.,* 2003). Protein carbonyls were measured from the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone product derived from 2,4-dinitrophenylhydrazine (DNPH) and used as a biomarker for protein oxidation elicited by ROS production (Kolgiri and Patil 2017). In the present study, triclosan exposure caused an increase in the level of protein carbonyl content in the gonads of the fish *Anabas testudineus* thus indicating oxidative modifications of protein. In another study, sublethal concentrations of triclosan increased the levels of carbonyl protein in the brain of adult zebrafish (Gyimah *et al.,* 2020). On the contrary, environmental concentrations of triclosan did not cause any alteration in the protein carbonyl content in the zebrafish embryos (Parenti *et al.,* 2019).

One of the possible consequences of free radical formation is genetic damage, in which blood was found more sensitive to genotoxicity (Garcia-Medina *et al.,* 2017). Thus, the current study monitored the genotoxicity of triclosan in the peripheral erythrocytes using micronucleus test and comet assay as biomarkers of DNA damage. The peripheral blood of the fish is widely used to evaluate the micronucleus test and comet assay since it avoids the complex cell preparation and also able to obtain repeated samples from the same animal. Micronucleus are small, round dark structures of the whole or fragments of chromosomes that were failed to incorporate into the nucleus of daughter cells during cell division (Bolognesi and Hayashi, 2011). The test for micronucleus formation detects clastogens or aneugens, which are involved in the chromosomal breakage and rearrangement or induce aneuploidy or an abnormal number of chromosomes, respectively (Al-Sabti and Metcalfe, 1995; Oliveira *et al.,* 2009; Obiakor *et al.,* 2014). The results obtained showed several nucleo-cytoplasmic abnormalities in the peripheral blood of the fish after triclosan exposure, which includes the formation of micronucleus, and other nuclear aberrations like irregular, binucleated, serrated and notched nucleus. Besides, the cytoplasmic abnormalities such as vacuolated and degenerated cytoplasm, formation of sticky cells, cytoplasmic bridges, presence of cytoplasmic granules, and cells without cytoplasm were more prominent features found among the treatment groups. Echinocytes, a spiculated erythrocytes with small and numerous projections were also noted after triclosan exposure as a result of the altered membrane permeability or impaired metabolic functions, which could be caused due to the overproduction of ROS (Hale *et al.,* 2011). Micronucleus formation and other nuclear abnormalities have been reported in the liver cells of goldfish, *Carassius auratus* (Wang *et al.,* 2018a), in gill and ovary of zebrafish (Wang *et al.,* 2019), in the peripheral erythrocytes of *Oreochromis niloticus* (Vijitha *et al.,* 2017), *Pangasianodon hypothalamus* (Paul *et al.,* 2019), and *Clarias gariepinus* (Jimoh and Sogbanmu, 2021) after triclosan exposure. The cytoplasmic anomalies observed in the current study coincided with other findings on fullerene C_{60} nanomaterial exposure in the erythrocytes of the fish, *Anabas testudineus* (Sumi and Chitra, 2019a), and in *Oreochromis mossambicus* exposed to silicon dioxide, titanium dioxide, aluminum oxide, and iron oxide nanoparticles (Vidya and Chitra, 2018).

The primary genetic damage induced by triclosan was also evaluated by comet assay or single cell gel electrophoresis assay using specific parameters such as percent tail DNA and tail length in erythrocytes of the fish. Comet assay is widely used to detect single-strand DNA breaks, alkali labile sites, and crosslinking caused by environmental stressors in the aquatic environment (Tice *et al.,* 2000; Bolognesi and Cirillo, 2014). The amount of DNA that leaves the nucleus was known by scoring the percent tail DNA, which denotes the actual percentage of DNA damage in a particular cell, while the tail length or DNA migration represents the extent of genetic damage (Kumaravel *et al.,* 2009; Cabello *et al.,* 2018). Exposure of triclosan at all concentrations for long-term duration showed grade 3 DNA damage, and it was noted that the intensity of the

damage increased with time and concentration. A study conducted on zebrafish embryos revealed that triclosan exposure caused a slight primary genotoxic effects only at high concentrations (Parenti *et al.,* 2019). Genetic damage scored using per cent tail DNA has been found associated with the generation of ROS in erythrocytes of the Indian major carp *Labeo rohita* (Hemalatha *et al.,* 2019), and in *Oncorhynchus mykiss* (Capkin *et al.,* 2017). The tail moment and tail length scored in the hepatocytes of the goldfish, *Carassius auratus*, and adult zebrafish showed significant DNA damage only after high concentration of triclosan exposure (Wang *et al.,* 2018a; Gyimah *et al.,* 2020). The overall results of antioxidant and genotoxic parameters suggest that triclosan-induced oxidative stress in gonads thereby contributed to DNA damage in erythocytes of the fish *Anabas testudineus*.

Conclusion

To our knowledge, this is the first study to investigate the sublethal and environmental concentrations of triclosan-induced gonadal toxicity mediated through oxidative stress, which could also impart genetic damages in erythrocytes of the fish, *Anabas testudineus*. Taken together, the evaluation of oxidative stress and cytogenetic damages provides a diagnostic and predictive tool for the environmental quality assessment.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Kerala State Council for Science Technology and Environment (KSCSTE), Government of Kerala, India.

References

Aitken RJ and Krausz C. (2001) Oxidative stress, DNA damage and the Y chromosome. Reproduction 122(4):497-506.

Al-Sabti K and Metcalfe CD. (1995) Fish micronuclei for assessing genotoxicity in water. Mutat Res. 343(2-3): 121-135.

Alvarez LA, Kovacic L, Rodriguez J, Gosemann JH and Kubica M. (2016) NADPH oxidase-derived H_2O_2 subverts pathogen signaling by oxidative phosphotyrosine conversion to PB-DOPA. Proc Nat Acad Sci USA. 113:10406-10411.

APHA (1998) Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association, American water works association and water environmental federation, Washington DC.

Ayala A, Munoz MF and Arguelles S. (2014) Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longv. 2014: 360438.

Barzilai A and Yamamoto K. (2004) DNA damage responses to oxidative stress. DNA Rep. 3(8-9): 1109-1115.

Beedanagari S, Vulimiri SV, Bhatia S and Mahadevan B. (2014) Genotoxicity biomarkers: Molecular basis of genetic variability and susceptibility. In: Biomarkers Toxicology, (ed.) Gupta R.C., Academic Press, Oxford, pp. 729-742.

Bolognesi C and Cirillo S. (2014) Genotoxicity biomarkers in aquatic bioindicators. Curr Zool. 60(2): 273-284.

Bolognesi C and Hayashi M. (2011) Micronucleus assay in aquatic animals. Mutagenesis 26(1): 205-213.

Capkin E, Ozcelep T, Kayis S and Altinok I. (2017) Antimicrobial agents, triclosan, chloroxylenol, methylisothiazolinone and borax, used in cleaning had genotoxic and histopathologic effects on rainbow trout. Chemosphere 182: 720-729.

Carlberg I and Mannervik B. (1975) Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem. 250(14): 5475-5480.

Carmosini N, Grandstrand S and King-Heiden TC. (2016) Developmental toxicity of triclosan in the presence of dissolved organic carbon: Moving beyond standard acute toxicity assays to understand ecotoxicological risk. Zebrafish 13(5):424-431.

Carrasco KR, Tilbury K and Myers M. (1990) Assessment of the piscine micronucleus test as an *in situ* biological indicator of chemical contaminant effects. Canad J Fish Aquat Sci. 47: 2123-2136.

Chelikani P, Fita I and Loewen PC. (2004) Diversity of structures and properties among catalases. Cell Mol Life Sci. 61: 192-208.

Clement MV and Pervaiz S. (2001) Intracellular superoxide and hydrogen peroxide concentrations: A critical balance that determines survival or death. Commun Free Rad Res. 6(4): 211-214.

Collins AR. (2004) The comet assay for DNA damage

and repair: Principles, applications, and limitations. Mol Biotechnol. 26(3): 249-261.

Dalle-Donne I, Rossi R, Giustarini D, Milzani A and Colombo R. (2003) Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta. 329(1-2): 23-38.

Dar OI, Sharma S, Singh K, Sharma A, Bhardwaj R and Kaur A. (2020) Biochemical markers for prolongation of the acute stress of triclosan in the early life stages of four food fishes. Chemosphere 247: 125914.

Das S, Majumder S, Gupta S, Dutta S and Mukherjee D. (2016) Effects of phenol on ovarian P450arom gene expression and aromatase activity *in vivo* and antioxidant metabolism in common carp *Cyprinus carpio*. Fish Physiol Biochem. 42(1): 275-286.

Dean RT, Fu S, Stocker R and Davies MJ. (1997) Biochemistry and pathology of radical-mediated protein oxidation. Biochem J. 324: 1-18.

Deponte M. (2013) Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochim Biophys Acta. 1830(5): 3217-3266.

Dodson RE, Boronow KE, Susmann H, Udesky JO, Rodgers KM, Weller D, Woudneh M, Brody JG and Rudel RA. (2020) Consumer behavior and exposure to parabens, bisphenols, triclosan, dichlorophenols, and benzophenone-3: Results from a crowd sourced biomonitoring study. Int J Hyg Environ Hlth. 230: 113624.

Doyotte A, Cossu C, Jacquin MC, Babut M and Vasseur P. (1997) Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*. Aquat Toxicol. 39: 93-110.

Droge W. (2002) Free radicals in the physiological control of cell function. Physiol Rev. 82: 47-95.

Du J and Gebicki JM. (2004) Proteins are major initial cell targets of hydroxyl free radicals. Int J Biochem Cell Biol. 36(11): 2334-2343.

Ebeid T, Eid Y, Saleh A and Abd El-Hamid H. (2008) Ovarian follicular development, lipid peroxidation, antioxidative status and immune response in laying hens fed fish oil-supplemented diets to produce n-3-enriched eggs. Animal. 2(1): 84-91.

European Union. (2016) Commission implementing decision (EU) 2016/110 of 27 January 2016 not approving triclosan as an existing active substance for use in biocidal products for product-type 1. OJ L 21, 28.1.2016, pp. 86-87.

Falisse E, Voisin AS and Silvestre F. (2017) Impacts of triclosan exposure on zebrafish early-life stage: Toxicity and acclimation mechanisms. Aquat Toxicol. 189: 97-107.

Gallego RSE, Penuela GA and Martinez-Lopez E. (2021) Enzymatic activity changes in striped catfish *Pseudoplatystoma magdaleniatum*, induced by exposure to different concentrations of ibuprofen and triclosan. Chemosphere 271: 129399.

Garcia-Medina S, Galar-Martinez M, Gomez-Olivan LM, Ruiz-Lara K, Islas-Flores H and Gasca-Perez E. (2017) Relationship between genotoxicity and oxidative stress induced by mercury on common carp (*Cyprinus carpio*) tissues. Aquat Toxicol. 192: 207-215.

Gomes MF, de Carvalho Soares de Paula V, Rocha Martins LR, Esquivel Garcia JR, Yamamoto FY and Martins de Freitas A. (2021) Sublethal effects of triclosan and triclocarban at environmental concentrations in silver catfish (*Rhamdia quelen*) embryos. Chemosphere 263: 127985.

Gonzalez-Perez BK, Sarma SSS, Castellanos-Paez ME and Nandini S. (2018) Multigenerational effects of triclosan on the demography of *Plationus patulus* and *Brachionus havanaensis* (Rotifera). Ecotoxicol Environ Saf. 147: 275-282.

Grant CM. (2001) Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions. Mol Microbiol. 39(3): 533-541.

Gyimah E, Dong X, Qiu W, Zhang Z and Xu H. (2020) Sublethal concentrations of triclosan elicited oxidative stress, DNA damage, and histological alterations in the liver and brain of adult zebrafish. Environ Sci Pollut Res Int. 27(14): 17329-17338.

Habashy WS, Milfort MC, Rekaya R and Aggrey SE. (2019) Cellular antioxidant enzyme activity and biomarkers for oxidative stress are affected by heat stress. Int J Biometeorol. 63(12): 1569-1584.

Hale JP, Winlove CP and Petrov PG. (2011) Effect of hydroperoxides on red blood cell membrane mechanical properties. Biophys J. 101: 1921-1929.

Halliwell B and Gutteridge JMC. (1999) Free Radicals in Biology and Medicine. Oxford University Press, Oxford, pp. 1-25.

Heddle JA. (1973) A rapid *in vivo* test for chromosomal damage. Mutat Res. 18(2): 187-190.

Hemalatha D, Rangasamy B, Nataraj B and Ramesh M. (2019) Assessment of triclosan impact on enzymatic biomarkers in an Indian major carp, *Catla catla*. J Basic Appl Zool. 80(23): 1-8.

Hussain B, Sultana T, Sultana S, Masoud MS, Ahmed Z and Mahboob S. (2018) Fish eco-genotoxicology: Comet and micronucleus assay in fish erythrocytes as *in situ* biomarker of freshwater pollution. Saudi J Biol Sci. 25(2): 393-398.

Janero DR. (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 9: 515-554.

Jimoh RO and Sogbanmu TO. (2021) Sublethal and environmentally relevant concentrations of triclosan and triclocarban induce histological, genotoxic, and embryotoxic effects in *Clarias gariepinus* (Burchell, 1822). Environ Sci Pollut Res Int. 28(24): 31071-31083.

Kanzok SM, Fechner A, Bauer H, Ulschmid JK, Muller HM, Botella-Munoz J, Schneuwly S, Schirmer R and Becker K. (2001) Substitution of the thioredoxin system for glutathione reductase in *Drosophila melanogaster.* Science 291: 643-646.

Kolgiri V and Patil VW. (2017) Protein carbonyl content: A novel biomarker for aging in HIV/AIDS patients. Braz J Infect Dis. 21(1): 35-41.

Kumaravel TS, Vilhar B, Faux SP and Jha AN. (2009) Comet assay measurements: A perspective. Cell Biol Toxicol. 25: 53-64.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S and Stadtman ER. (1990) Determination of carbonyl content in oxidatively modified proteins. Meth Enzymol. 186: 464-478.

Li WC. (2014) ccurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. Environ Pollut. 187: 193-201.

Livingstone DR. (2001) Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar Pollut Bull. 42: 656-666.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem. 193(1): 265-275.

Lushchak VI. (2011) Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. Comp Biochem Physiol-Toxicol Pharmacol. 153(2): 175-190.

Lushchak VI. (2016) Contaminant-induced oxidative stress in fish: A mechanistic approach. Fish Physiol Biochem. 42: 711-747.

Martinez-Paz P, Morales M, Urien J, Morcillo G and Martinez-Guitarte JL. (2017) Endocrine-related genes are altered by antibacterial agent triclosan in *Chironomus riparius* aquatic larvae. Ecotoxicol Environ Saf. 140: 185-190.

Moron MS, Depierre JW and Mannervik B. (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta. 582(1): 67-78.

Murphy MP. (2013) Mitochondrial dysfunction indirectly elevates ROS production by the endoplasmic reticulum. Cell Metabol. 18(2): 145-146.

Nag SK, Das Sarkar S and Manna SK. (2018) Triclosan-an antibacterial compound in water, sediment and fish of River Gomti, India. Int J Environ Health Res. 28(5): 461-470.

Obiakor MO, Okonkwo JC and Ezeonyejiaku CD. (2014) Genotoxicity of freshwater ecosystem shows DNA damage in preponderant fish as validated by *in vivo* micronucleus induction in gill and kidney erythrocytes. Mutat Res-Genet Toxicol Environ Mutagen. 775-776: 20-30.

Ohkawa H, Ohishi N and Yagi K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95(2): 351-358.

Oliveira R, Domingues I, Koppe Grisolia C and Soares AMVM. (2009) Effects of triclosan on zebrafish early-life stages and adults. Environ Sci Pollut Res. 16(6): 679-688.

Pandey S, Parvez S, Sayeed I, Haque R, Binhafeez B and Raisuddin S. (2003) Biomarkers of oxidative stress: A comparative study of river Yamuna fish *Wallago attu* (Bl. and Schn.). Sci Total Environ. 309(1-3): 105-115.

Parenti CC, Ghilardi A, Della Torre C, Mandelli M, Magni S, Del Giacco L and Binelli A. (2019) Environmental concentrations of triclosan activate cellular defence mechanism and generate cytotoxicity on zebrafish (*Danio rerio*) embryos. Sci Total Environ. 650:1752-1758.

Parvez S, Pandey S, Ali M and Raisuddin S. (2006) Biomarkers of oxidative stress in *Wallago attu* (Bl. and Sch.) during and after a fish-kill episode at Panipat, India. Sci Total Environ. 368(2-3): 627-636.

Parvez S and Raisuddin S. (2005) Protein carbonyls: Novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). Environ Toxicol Pharmacol. 20(1): 112-117.

Patlevic P, Vaskova J, Svorc P, Vasko L and Svorc, P. (2016) Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integr Med Res. 5(4): 250-258.

Paul T, Kumar S, Shukla SP, Pal P, Kumar K, Poojary N, Biswal A and Mishra A. (2020) A multi-biomarker approach using integrated biomarker response to assess the effect of pH on triclosan toxicity in *Pangasianodon hypophthalmus* (Sauvage, 1878). Environ Pollut. 260: 114001.

Perron M, Ho KT, Cantwell MG, Burgess RM and Pelletier MC. (2012) Effects of triclosan on marine benthic and epibenthic organisms. Environ Toxicol Chem. 31(8): 1861-1866.

Pick E and Keisari Y. (1981) Superoxide anion and hydrogen peroxide production by chemically elicited peritoneal macrophages-induction by multiple nonphagocytic stimuli. Cell Immunol. 59(2):301-318.

Priyatha CV and Chitra KC. (2018) Acute toxicity of triclosan on the native freshwater fish, *Anabas testudineus* (Bloch, 1792): Behavioral alterations and histopathological lesions. Int J Life Sci. 6(1): 166-172.

Ramaswamy BR, Shanmugam G, Velu G, Rengarajan B and Larsson DG. (2011) GC-MS analysis and ecotoxicological risk assessment of triclosan, carbamazepine and parabens in Indian rivers. J Hazard Mater. 186(2-3): 1586-1593.

Reznick AZ and Packer L. (1994) Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Meth Enzymol. 233: 357-363.

Ricart M, Guasch H, Alberch M, Barcelo D, Bonnineau C, Geiszinger A, La Farre M, Ferrer J, Ricciardi F, Romani AM, Morine S, Proia L, Salaf L, Sureda D and Sabater S. (2010) Triclosan persistence through wastewater treatment plants and its potential toxic effects on river biofilms. Aquat Toxicol. 100(4): 346-353.

Schmid W. (1975) The micronucleus test. Mutat Res. 31(1): 9-15.

Sheng C, Zhang S and Zhang Y. (2021) The influence of different polymer types of microplastics on adsorption, accumulation, and toxicity of triclosan in zebrafish. J Hazard Mater. 402: 123733.

Shim J, Weatherly LM, Luc RH, Dorman MT, Neilson A, Ng R, Kim CH, Millard PJ and Gosse JA. (2016) Triclosan is a mitochondrial uncoupler in live zebrafish. J Appl Toxicol. 36(12): 1662-1667.

Sies H. (2014) Role of metabolic H_2O_2 generation. J Biol Chem. 289(13): 8735-8741.

Singh NP, McCoy MT, Tice RR and Schneider EL. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 175(1): 184-191.

Song X, Wang X and Bhandari RK. (2020) Developmental abnormalities and epigenetic alterations in medaka (*Oryzias latipes*) embryos induced by triclosan exposure. Chemosphere 261: 127613..

Stone JR and Yang S. (2006) Hydrogen peroxide: A signaling messenger. Antioxid Redox Signal. 8: 243-270.

Sumi N and Chitra KC. (2019a) Cytogenotoxic effects of fullerene C_{60} in the freshwater teleostean fish, *Anabas testudineus* (Bloch, 1792). Mutat Res-Genet Toxicol Environ Mutagen. 847: 503104..

Sumi N and Chitra KC. (2019b) Fullerene C_{60} nanomaterial induced oxidative imbalance in gonads of the freshwater fish, *Anabas testudineus* (Bloch, 1792). Aquat Toxicol. 210: 196-206.

Szalai G, Kellos T, Galiba G and Kocsy G. (2009) Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. J Plant Growth Regul. 28(1): 66-80.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC and Sasaki YF. (2000) Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen. 35(3): 206-221.

Tkachenko H, Kurhaluk N and Grudniewska J. (2013) Biomarkers of oxidative stress and antioxidant defences as indicators of different disinfectants exposure in the heart of rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquac Res. 46(3): 679-689.

United States - Food and Drug Administration (US-EPA) (2016). Safety and effectiveness of consumer antiseptics; Topical antimicrobial drug products for over-the-counter human use. Final rule, Federal register, 81(172): 61106-61130.

van der Oost R, Beyer J and Vermeulen NPE. (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ Toxicol Pharmacol. 13(2): 57-149.

Vidya PV and Chitra KC. (2018) Evaluation of genetic damage in *Oreochromis mossambicus* exposed to selected nanoparticles by using micronucleus and comet bioassays, Croat J Fish. 76(3): 115-124.

Vijitha C, Asifa KP and Chitra KC. (2017) Assessment of genotoxic and haematological consequence of triclosan in the fish, *Oreochromis niloticus* (Linnaeus, 1758). Int J Appl Res. 3: 101-109.

Wang Y, Branicky R, Noe A and Hekimi S. (2018b) Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol. 217(6): 1915-1928.

Wang F, Xu R, Zheng F and Liu H. (2018a) Effects of triclosan on acute toxicity, genetic toxicity and oxidative stress in goldfish (*Carassius auratus*). Exp Anim. 67(2): 219-227.

Wang F, Zheng F and Liu F. (2019) Effects of triclosan on antioxidant- and apoptosis-related genes expression in the gill and ovary of zebrafish. Exp Anim. 69(2): 199-206.

Wu M, Xu H, Shen Y, Qiu W and Yang M. (2011) Oxidative stress in zebrafish embryos induced by short-term

exposure to bisphenol A, nonylphenol, and their mixture. Environ Toxicol Chem. 30(10):2335-2341.

Yin H, Xu L and Porter NA. (2011) Free radical lipid peroxidation: Mechanisms and analysis. Chem Rev. 111(10): 5944-5972.

Yoshida Y, Umeno A and Shichiri M. (2013) Lipid peroxidation biomarkers for evaluating oxidative stress and assessing antioxidant capacity *in vivo*. J Clin Biochem Nutr. 52(1):9-16.

Zieba M, Suwalski M, Kwiatkowska S, Piasecka G, Grzelewska-Rzymowska I, Stolarek R and Nowak D. (2000) Comparison of hydrogen peroxide generation and the content of lipid peroxidation products in lung cancer tissue and pulmonary parenchyma. Respir Med. 94(8): 800-805.