Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(4): 272-276
© 2021 JEZS
Received: 17-04-2021
Accepted: 30-05-2021

VR Bhatta
Assistant Professor, Department of Genetics, Jyoti Nivas College Autonomous Hosur Road, Bengaluru, Karnataka, India

PS Oza
Student, Department of Genetics, Jyoti Nivas College Autonomous, Hosur Road, Bengaluru, Karnataka, India

M Reddy
Student, Department of Genetics, Jyoti Nivas College Autonomous, Hosur Road, Bengaluru, Karnataka, India

M Vinitha
Student, Department of Genetics, Jyoti Nivas College Autonomous, Hosur Road, Bengaluru, Karnataka, India

Corresponding Author:
VR Bhatta
Assistant Professor, Department of Genetics, Jyoti Nivas College Autonomous Hosur Road, Bengaluru, Karnataka, India

Cytotoxic effect of paraphenylenediamine (PPD) on *Danio rerio* (Zebra Fish)

VR Bhatta, PS Oza, M Reddy and M Vinitha

DOI: https://doi.org/10.22271/j.ento.2021.v9.i4c.8770

Abstract
Paraphenylenediamine (PPD) is a synthetic chemical widely used as a substitute for henna as a permanent hair dye because it gives a natural look to the hair and the dyed hair can also be shampooed or permed without losing the colour. However, the intermediate and partially oxidized form of PPD is reported to cause adverse reactions in people. Effects of sublethal concentrations of PPD in the zebra fish were examined for 15 days following exposure to the chemical. On the 7th day and the 15th day following the exposure, blood smears were examined for presence of micronuclei using the Giemsa stain. The micronuclei percentage indicating the cytotoxic effect of PPD was significantly greater in the exposed fishes and the extent of toxicity was directly proportional to the concentration of PPD and to the length of exposure. Therefore, waste water reaching any aquatic ecosystem should be monitored for PPD in order to prevent any adverse impacts on the aquatic ecosystem and to minimize the threat to human beings due to biomagnification.

Keywords: Hair dyes, paraphenylenediamine, cytotoxic, micronucleus, aquatic animals

Introduction
A hair dye changes the colour or tone of hair. People have used hair dyes since ancient times, different types of plant extracts were used by Europeans and Asians although before the invention of modern dyes [1].

To colour the hair permanently, chemical hair dyes use a combination of ammonia and hydrogen peroxide: ammonia breaks down the outer cuticle around the hair shaft, allowing the chemicals to enter the hair to facilitate colour development, and hydrogen peroxide bleaches out the natural colour and releases oxygen to facilitate chemical reactions. These chemicals are quite harsh and may harden and thin the hair and also have adverse effects on eyes and the respiratory system [1].

The chemicals generally used in most of the dyes include the following: cetearyl alcohol, deceth-3, propylene glycol, laureth12, oleth-30, ammonium hydroxide, lauric acid, hexadimethrine chloride, glycol distearate, p-phenylenediamine, resorcinol, ethanolamine, polyquaternium-22, silica dimethyl silylate, 2,4 diamino phenoxyethanol, 2 m-Aminophenol, ascorbic acid, ammonium thioacetate, dimethicone, penta sodium pentaacetate, N, N-BIS (-hydroxyethyl)-p-phenylene diamine sulphate, and carbomer.

Paraphenylenediamine (PPD), a derivative of paranitoanaline, is a major and most commonly used chemical in several formulations of hair dyes including oxidizable hair dyes. Such formulations contain up to 9% of PPD [2]. Whereas fully oxidized PPD does not usually trigger a response, its intermediate and partially oxidized form can cause contact allergic dermatitis in sensitive individuals [3]. Semi-permanent and permanent hair dyes are considered to pose greater risk compared other alternate farms of hair colours [4]. Permanent hair dyes that contain PPD account for three-quarters of such dyes used globally, and their adverse effects including allergy, mutagenicity, and toxicity have been debated over a decade. Mutagenicity tests show the oxidized form PPD to be strongly mutagenic compared to its non-oxidized form [5].

In 2006, the American Contact Dermatitis Society declared PPD as a contact allergen. The chemical was tested on a few model animals. The tests not only confirmed that PPD was an allergen but also showed it to be carcinogenic [5]. A study based on immune response in mice exposed to PPD-containing hair dyes induced local inflammation in the form of swelling, additionally the chemical was also seen to have infiltrated into mice cells [6].

Risk assessments of chemicals used in hair dyes have indicated their carcinogenicity and the ability to cause chromosomal aberrations in animal models [7, 8]. Similar studies on zebra fish embryos at concentration levels 100, 200, and 275 µM have also reported morphological and physiological abnormalities such as increased mortality, delayed hatching, slow blood circulation, pericardial sac edema, yolk sac edema, abnormal body axes, twisted notochord, tail deformation, weak heartbeat, and growth [9].

Waste water containing such harmful dyes can be hazardous, especially for aquatic organisms exposed to industrial and domestic wastes, which are also of great concern in public health. Increasing quantities of PPD are entering water bodies. PPD being an aromatic amine can be easily dispersed, leading to possible adverse effects on aquatic organisms. It is therefore important to assess the effects of PPD on the ecology of aquatic environments to address the safety issues related to products containing PPD.

However, data on the environmental impacts of PPD are rare and there is limited knowledge on its impact on aquatic animals. Thus the present study sought to assess the effect of PPD on zebra fish, which is considered an ideal and cost-effective model for studying chemical toxicity in aquatic ecosystems [10].

Materials and Methods

Test animal

The present study was carried out on Zebra fish, *Danio rerio* having an average weight of 0.259g. They were purchased from the local aquarist. The fishes were then acclimatized to laboratory conditions for 7 days in a 500 ml glass aquarium containing water collected from the local groundwater source with no detectable presence of paraphenylenediamine. They were fed twice a day with packaged fish food (Red fin).

Test substance

The compound used in this study was Paraphenylenediamine (97% PPD) which is a major component of many powder hair dyes.

Experimental setup for toxicity test

After 7 days of acclimatization in laboratory, the fishes were divided into 15 groups, 12 groups having 4 test groups in triplicates and 3 set of control groups containing 4 fishes each in 500 ml glass bowls with water (Figure 1). The test groups were exposed to a sub-lethal dose of PPD dissolved in water (w/v) at concentrations ranging from 0.1 to 0.4% (Table 1). The fishes were kept in the water containing different concentrations of PPD for a period of 15 days. Similarly, the control groups were kept in 500 ml of water in glass bowls without PPD. During the study period both the test and control groups of fishes were fed with packaged fish food twice a day. The experimental water was renewed, and the glass bowls were also cleaned regularly on alternate days to avoid any contamination and to ensure constant test concentration throughout the exposure time. Experiments were conducted with a natural photoperiod and at ambient water temperature.

Collection of blood for micronucleus assay

The fishes were screened for the micronucleus by collecting the peripheral blood samples from the peripheral veins after 7th and 15th day of the exposure.

Preparation of slide

This smear of blood was made on a clean slide by slide drawn method, air-dried for 10 minutes. Slides were then stained using Giemsa staining technique and finally rinsed in running tap water to remove the excess stain particles.

Screening for micronucleus

Stained slides were observed for micronucleus in the stained red blood cells, under the light microscope under 45X magnification lens (Figure 2).

The micronucleus frequency (MN %), was calculated using the formula:

$$MN\% = \frac{\text{Number of MN cells}}{\text{Total number of cells screened.}} \times 100$$

Statistical analysis

All data in graphs were presented as mean and standard deviation. Data were analyzed using analysis of variance (ANOVA). Values of $p < 0.01$ were considered as level statistical significance.

Result and Discussion

Zebra fish exposed to different concentrations of PPD in water showed a significantly greater percentage of micronuclei on the 7th and the 15th day following exposure to PPD, and the extent of such increase was directly proportional to the concentration and to the duration of exposure.

After seven days of exposure, the mean percentage of micronucleus for different concentrations of PPD was as follows: 4.80% (±1.32%) when PPD concentration was 0.1%; 10.79% (±2.93%) when it was 0.2%; 11.24%±1.21% at 0.3%; and 15.85%±5.66% at 0.4% (Table 2). Significant increase in the micronucleus was observed with the increasing concentration of PPD. Significant increase ($p=0.02$) in the micronucleus was observed between 0.1% and 0.2% of Similarly the corresponding value of p for the difference between 0.1% and 0.3% was 0.01 and that for the difference between 0.1% and 0.4% was 0.0006 (Figure 3). This confirms the cytotoxic effect of PPD on the fishes even at the sub-lethal concentration and also its vulnerability for biomagnification.

The same pattern was seen after 15 days of exposure: the percentage increased significantly, the mean value being 22.51%±5.34% at 0.3% PPD and 27.20%±6.29% at 0.4% PPD. Between day 7 and day 15, the micronucleus percentage increased twofold at 0.3% ($p = 0.0005$). The increase was also significant ($p = 0.008$) at 0.4% (Table 3, Figure 4).

In biological systems, PPD is shown to cause mutations following its oxidation by the enzyme P450 oxidase. Ingestion of PPD is shown to cause oedema, skeletal muscle rhabdomyolysis, and renal failure in acute cases [11].

The toxic effects of contaminants such as PPD on aquatic animals were manifested in the form of inappropriate behavioural responses to environmental and physiological stimuli and lower survival [12].

According to the National Cancer Institute, regular use of hair dyes increases the risk of cancers of the blood and bone marrow, such as non-Hodgkin lymphoma and leukaemia. Studies on carcinogenicity associated with hair dye use have shown increased risk of breast and bladder cancer [13, 14].

Zebra fish (*Danio rerio*) embryos exposed for 96 h to sublethal concentrations (100 µM, 200 µM, and 275 µM) of henna 1 h after fertilization showed morphological and

physiological abnormalities, delayed hatching, slow blood circulation, pericardial sac edema, yolk sac edema, abnormal body axes, twisted notochord, tail deformation, weak heartbeat, retarded growth, and high mortality—findings that point to possible ill effects on development in human beings [15].

Fish exposed to PPD also showed behavioural abnormalities. The toxic effects of xenobiotics include disruption of sensory, hormonal, neurological, and metabolic systems, which have profound implications for the behaviours of fish. The more common behavioural disruptions include the inhibition of cholinesterase, altered levels of brain neurotransmitters, sensory deprivation, and impaired gonadal or hormone levels [16]. Lastly, PPD can cause significant morbidity and mortality in children, because clinical manifestations and outcomes in children are similar to those in adults.

Similar to hair dye studies on the toxicity of metal dyes have also shown the xenobiotic and recalcitrant nature of the dyes with adverse effects on aquatic biological systems due to changes in ecosystem functioning [17]. Textile dyes consisting of heavy metals such as nickel, copper, cobalt, and chromium also pose a threat to aquatic ecosystems given their resistance to degradation and prolonged half-lives of 2–13 years. The damage caused by the dye industry to the environment, however, is mainly from the discharge of untreated effluents into water bodies, which typically account for 80% of the total emissions from textile industry [18].

Table 1: Experimental set up showing the number of trials and test concentrations of PPD

	No. of experimental bowls with fish per concentration	No. of fishes per bowl each Concentration	Concentration of PPD (w/v) in water
Control	3	4	100 ml
Test concentration 1	3	4	0.1 mg / 100 ml
Test concentration 2	3	4	0.2 mg / 100 ml
Test concentration 3	3	4	0.3 mg / 100 ml
Test concentration 4	3	4	0.4 mg / 100 ml

Table 2: Micronucleus percentage at different concentration of PPD (w/v in water) in the red blood cells of the Zebra fish after seven days of exposure.

	Concentration of PPD (%)	% of MN (Mean±SD)	Maximum (in %)	Minimum (in %)
Control	0	0	0	0
Test 1	0.1	4.80±1.32	6.27	3.17
Test 2	0.2	10.79±2.93	14.81	6.71
Test 3	0.3	11.24±1.21	12.53	9.42
Test 4	0.4	15.85±5.66	25.14	9.83

Table 3: Comparison of Micronucleus percentage at different concentration of PPD (w/v) in water) in the red blood cells of the Zebra fish after seventh and fifteenth days of exposure.

	After 7 days of exposure			After 15 days of exposure			
Concentration of PPD (%)	% Of MN (Mean±SD)	Maximum (In %)	Minimum (In %)	% Of MN (Mean±SD)	Maximum (In %)	Minimum (In %)	P value
0.3	11.24±1.21	12.53	9.42	22.51±5.34	31.69	17.09	0.0005*
0.4	15.85±5.66	25.14	9.83	27.20±6.29	34.4	17.04	0.008*

*significance at $p<0.01$

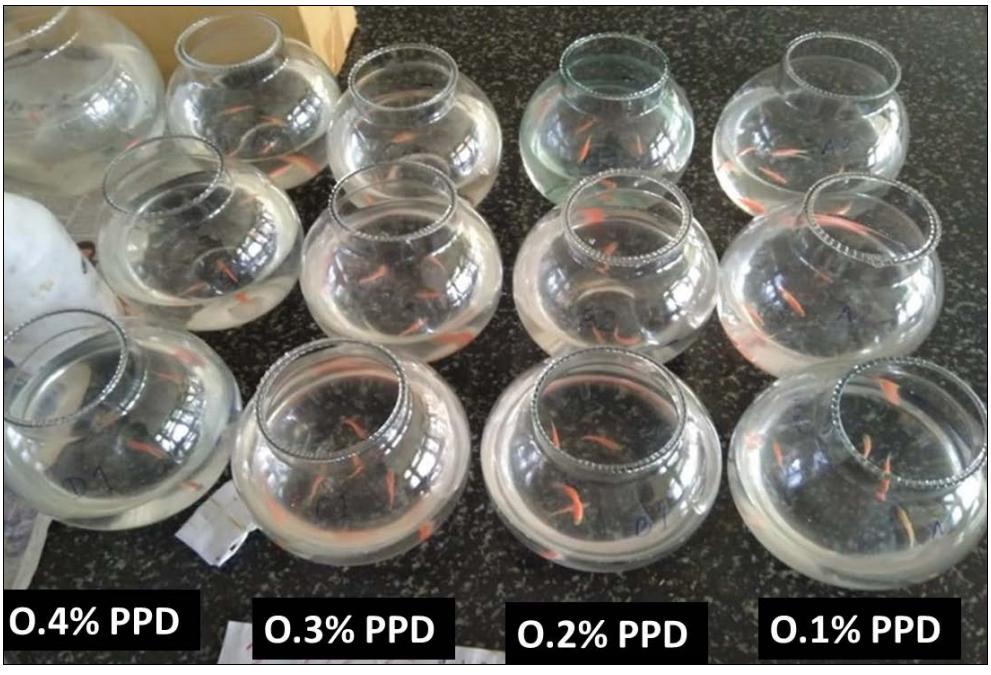

Fig 1: Experimental set up showing triplicates of different test concentration of PPD in 500 ml of water with 4 zebra fishes each.

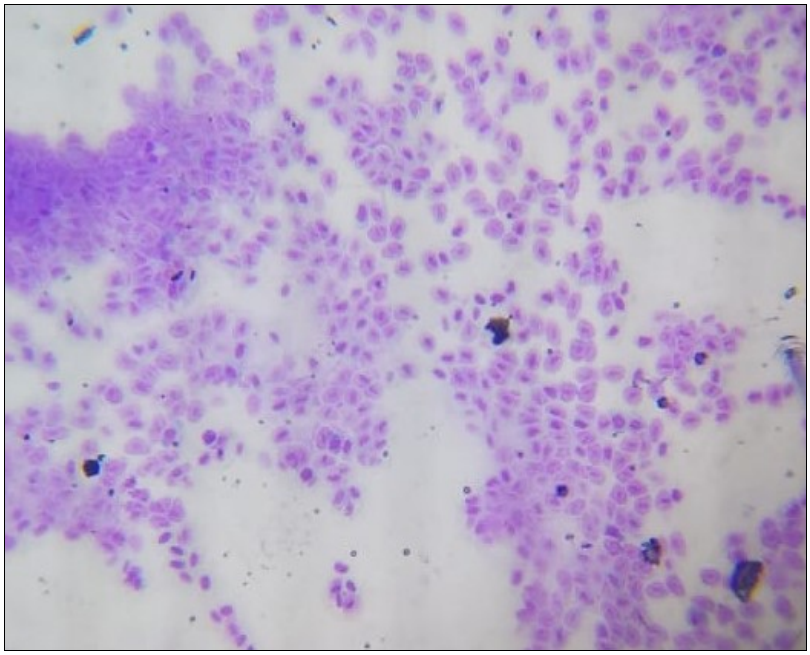

Fig 1: Giemsa-stained Peripheral blood smear showing the presence of micro nucleus.

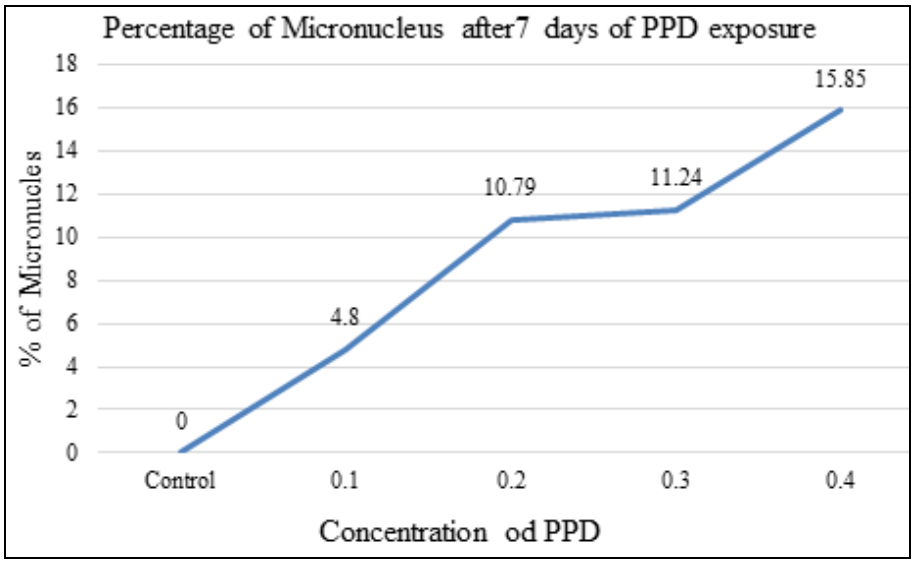

Fig 3: Percentage of micronucleus in the peripheral blood cells of Zebra fish after seven days of exposure under laboratory condition

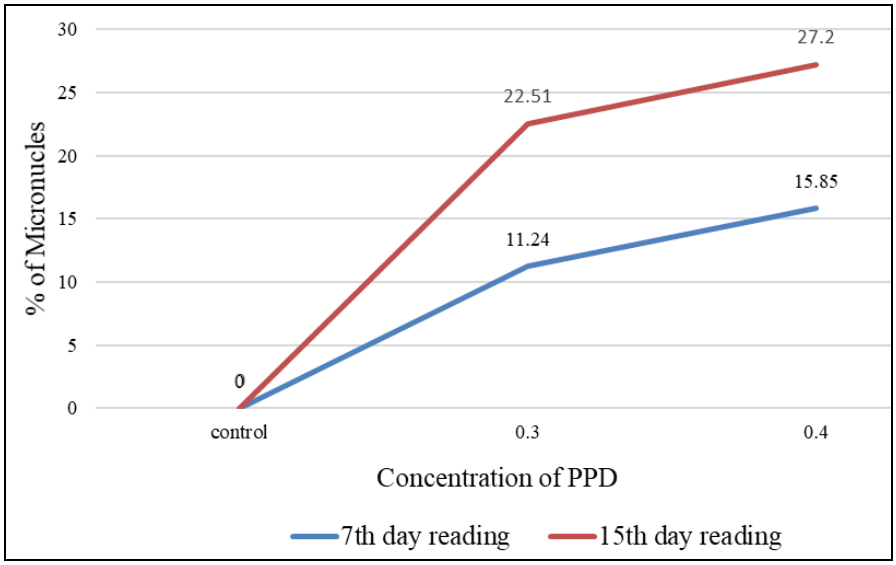

Fig 4: Comparison of percentage of micronucleus in the red blood cells of Zebra fish after seven and fifteen days of exposure at 0.3 and 0.4% of PPD.

Conclusion

The concentration of such harmful chemicals as PPD in industrial and domestic waste water requires strict monitoring before such water is discharged into aquatic ecosystems. In hair dyes, PPD should be strictly at sublethal concentrations and such dyes must be treated properly before they reach water bodies. Control and release of untreated henna or hair dyes and many other domestic wastes into the environment should be given special attention for a cleaner and safer environment and better quality of life for all including aquatic organisms. If such control is neglected, entire ecosystems can be affected and the process of biomagnification will ultimately pose serious threats to people.

References

1. Steilinga W, Kreutzb J, Hoferc H. Percutaneous penetration/dermal absorption of hair dyes *in vitro*. Toxicology *in Vitro* 2001;15:565-570.
2. Jain, PK, Agarwal N, Sharma AK, Akhtar A, Prospective study of ingestional hair dye poisoning in Northern India (Prohina). Journal of clinical medicine and research, 2011;3(1):9-19.
3. Meyer A, Fischer K. Oxidative transformation processes and products of para-phenylenediamine (PPD) and para-toluene diamine (PTD)-A Review. Environmental Sciences Europe 2015;27:11.
4. Ames BN, Kammen HO, Yamasaki E. Hair dyes are mutagenic: identification of a variety of mutagenic ingredients. Proceedings of National Academy of Sciences 1975, 2423-2427.
5. Chong HP, Reena K, Chye SM. Para-Phenylenediamine Containing Hair Dye: An Overview of Mutagenicity, Carcinogenicity and Toxicity. Journal of Environmental & Analytical Toxicology 2016;6(5).
6. Rubin IMC, Dabelsteen S, Nielsen M, White IR, Johansen JD, Geisler C *et al*. Repeated exposure to hair dye induces regulatory T cells in mice. British Journal of Dermatology 2010;163(5):992-8.
7. Burnett C, Loehr R, Corbett J. Heritable translocation study on two hair dye formulations. Fundamental Applied Toxicology 1981,325-328.
8. Sasaki YF, Fujikawa K, Ishida K, Kawamura N, Nishikawa Y, Ohra S. *et al*. The alkaline single cell gel electro-phoresis assay with mouse multiple organs: results with 30 aromatic amines evaluated by IARC and US NTP. Mutation Research 1999;440:1-18.
9. Fent K, Meier W. Effects of Triphenyltin on Fish Early Life Stages. Archives of Environmental Contamination and Toxicology 1994;27:224-231.
10. Volz DC, Belanger S, Embry M, Padilla S, Sanderson H, Schirmer K *et al*. Adverse outcome pathways during early fish development: a conceptual framework for identification of chemical screening and prioritization strategies. Toxicological Sciences 2011;123:349-358.
11. Ram R, Swarnalatha G, Prasad N, Dakshinamurty KV. Paraphenylenediamine ingestion. An uncommon cause of acute renal failure, Journal of Postgraduate Medicine, 2007;53:181-2.
12. Weber DN, Spieler RE. Behavioral Mechanisms of Metal Toxicity in Fishes. Molecular Biological Approaches to Aquatic Toxicology 1994.
13. Huncharek M, Kupelnick B. Personal use of hair dyes and the risk of bladder cancer: results of a meta-analysis, Public Health Reports 2005;120(1):31-8.
14. Stavraky KM, Clarke EA, Donner A. Case-control study of hair dye use by patients with breast cancer and endometrial cancer. Journal of the National Cancer Institute 63(4):941-945.
15. Manjunatha B, Wei-bing P, Ke-chun L, Marigoudar SR, Xi-qiang C, Xi-min W *et al*. The effects of henna (hair dye) on the embryonic development of zebrafish (*Danio rerio*), Environmental Science and Pollution Research, 2014;21(17):10361-10367.
16. Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behavior: integrating behavioral and physiological indicators of toxicity, Aquatic Toxicology 2004;68(4):369-392.
17. Copaciu F, Opris O, Coman V, Ristoiu D, Niinemets U, Copolovici L. Diffuse water pollution by anthraquinone and azo dyes in environment importantly alters foliage volatiles, carotenoids and physiology in wheat (Triticum aestivum), Water, Air, and Soil Pollution, 2013;224(3):1-11.
18. Orts F, del Río AI, Molina J, Bonastre J, Cases F, Electrochemical treatment of real textile wastewater: Trichromy Procion HEXL, Journal of Electroanalytical Chemistry 2018;808:387-394.