Original Research
ISSN (Print): 2455-1244
ISSN (Online): 0971-913X
J Endocrinol Reprod. *Vol 26(3), 171-178, September 2022, DOI: 10.18311/jer/2022/29363*

Effects of Sub-lethal Concentrations of Plasticizer - Diethyl Phthalate on a few Reproductive Indices of Female Freshwater Murrel, *Channa striatus* (Bloch)

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Abstract

Diethyl phthalate (DEP) is used as a plasticizer and arrives at the aquatic environment from different industries and can cause deleterious effects to fish. Great concern regarding the effects of these compounds in nature has evolved as a consequence to their endocrine-disrupting properties. Most of the studies on the endocrine-disrupting effects of chemicals in nature are based on their effects on the reproduction of fish and on changes in their genital structures. In the present study, the effect of exposure of female *Channa striatus* to DEP has been investigated. The treatment caused reduction in gonadosomatic index, ova diameter and fecundity. This may be due to the endocrine disrupting activity of this chemical. Histopathological examination of the ovary of DEP-exposed fish showed dose-dependent regressive changes as a result of estrogenic endocrine disruption. The present study strongly suggests that DEP induces endocrine disruption adversely affecting the reproductive potential of female *Channa striatus.*

Keywords: *Channa striatus*, Diethyl Phthalate, Endocrine Disruption, Plasticizer

1. Introduction

Endocrine disruptors are substances or compounds that interfere with the normal functioning of the endocrine system which otherwise helps organisms to live in harmony with their nature. Phthalate, a known Endocrine Disrupting Chemical (EDC), used as a matrix for plastics and cosmetics, is an alkyl di-ester of phthalic acid based on the length of chains[1]. Phthalate is widely used in day to day life. It is also used as a plasticizer, solvent and additive in many day-to-day consumption products, especially in food packaging. Phthalates increase the flexibility and durability of plastics. These are used in the manufacture of Polyvinyl Chloride (PVC), medical supplies, and packaging of food and personal care items[2]. Due to the lipophilic nature, phthalates penetrate into the high fat diets such as milk and milk products, meat and meat products, fish, vegetable oils and fats. The European Zone produces and imports more than 103 tonnes of DEP and DMP per year and more than 105 tonnes of DPHP and DEHP per year[3]. Continuous emission from industrial areas and the resulting pollution adversely affects fish reproduction. Previous findings show that diisononyl phthalate and di (2-ethyl hexyl) phthalate did not produce any change in the body weight at 300 ppm and 60 ppm concentrations after 60 days of exposure to the fish *Oreochromis mossambicus*[4,5]. On the other hand, high levels of mucus production have been observed in freshwater fish *Cyprinus carpio* subjected to Di-Butyl Phthalate (DBP) and Di-Ethyl Phthalate (DEP) exposure[6].

In jawed vertebrates, the gonadal growth and development is controlled by the follicle stimulating hormone (FSH) and Leuteinizing Hormone (LH). The synthesis and release of these hormones is regulated by the Hypothalamo-Pituitary-Gonadal (HPG) axis[7].

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Article Received: 25.03.2022 Revised on: 06.10.2022 Accepted on: 10.10.2022

The synchronization of the HPG axis is contributed by the endocrine feedback of gonad-derived activin and inhibin on[8]. The fish populations which are exposed to chemicals possessing endocrine disrupting properties show reproductive disorders in both male and female fishes. Moreover, studies in our laboratory revealed that exposure to various chemicals disturbed the physiological and metabolic activities in different fresh water species[9, 10]. Therefore, the side effects of chemicals that disrupt the functioning of the endocrine system in fish are reproductive behavior, delay in sexual maturity, alteration of gonado-somatic indices, level of vitellogenin, sperm parameters, activities of steroidogenic enzymes and changes in hormonal parameters are identified by different assessment methods[11].

Early research suggests that phthalate exposure may be associated with diabetes, insulin resistance, obesity, breast cancer, immunity, disruption in endocrine system and other metabolic disorders[12]. Adverse neurodevelopment and autistic behaviors in children may be associated with impaired cognitive and motor development caused due to phthalate contamination[13,14]. Phthalates have also been found in packaging materials and in non-packaged foods. Product pollution is caused by water, air and soil contamination, overuse and discharge into landfills. Phthalates can bind to estrogen receptors and alter the production of vitellogenin (VTG) in aquatic species thereby causing an endocrine disrupting activity. In the present study, we investigated the effects of different sub-lethal concentrations of Di-Ethyl Phthalate (DEP) on the reproductive functions of freshwater murrel, *Channa striatus*.

2. Materials and Methods

Healthy female *C. striatus* (80 ± 4 g body weight, 20 ± 2 cm length) were collected from local paddy fields, and carefully brought to the laboratory. The fish were acclimatized to laboratory conditions for a couple of weeks in a large tank (1000 L capacity) when they were fed with natural food *viz*., tilapia fingerlings, insects, earthworm, *etc*. Water was renewed daily to avoid accumulation and contamination of excretory materials. Feeding was withheld for 24 hrs before to the beginning of the experiment. Fish showing any abnormal behavior was removed from the tank as soon as possible. The physicochemical characteristics of the tap water used in this study were as follows: temperature -22 ± 2 ^{0}C; pH-7, dissolved O_2 - 4.48 ± 1.6 ml l-1; alkalinity - 35 ± 2ppm and CO_2 - 8 ± 2ppm.

Diethyl phathalate (99.99%) was procured from the Central Drug House, Mumbai. Stock solution was prepared by dissolving 1 mL of DEP in 9 mL distilled water (10 times dilution). Test concentrations were prepared by diluting appropriate aliquots of the stock solution. LC_{50} of DEP was statistically determined using probit analysis based on least squares (normal distribution) method generated by Statplus 2009 version 5.8.4.0 software (Figure 1). For this, a preliminary range finding assay was conducted in which eight fishes were randomly selected from the stock and exposed to increasing test concentrations of DEP (10 to 80 ppm at 10 ppm interval) for 96 hrs. Water was changed every day with fresh DEP-mixed water to sustain stable level of DEP during the exposure period. The LC_{50} value for DEP was found to be 70 ppm. For the experiment, three sub-lethal concentrations (i.e. 0.4 ppm, 4 ppm and 40 ppm) were selected, along with a negative control. Observations were made on 7th, 14th & 21st day of the experiment when the fishes were sacrificed by decapitation; gonads were dissected out, and the gonadosomatic index, diameter of ova and fecundity were determined. Gonads were fixed in Bouin's fixative and processed for histological examination adopting the standard methods such as dehydration, embedding, sectioning and staining in haematoxylin and eosin. The stained sections were examined in a research microscope. Photomicrographs were obtained using Lumenera- Infinity 1 (Model N9032789) camera fitted to Olympus S761 (Model SZ2-ILST) research microscope.

3. Results

In the present study, sub-lethal effects of diethyl phthalate on the reproductive indices of *Channa striatus* were evaluated.

The Gonadosomatic Index (GSI) of control and DEP-exposed fish is show in Table 1. The GSI decreased at all exposure levels of DEP in a dose-dependent manner. This decrease in GSI was significant ($p<0.05$) in 4 ppm (for day 14 and day 21) and 40 ppm (for all exposure periods) concentrations of DEP.

The diameter of ova was found to be decreased in 0.4 ppm, 4 ppm and 40 ppm concentrations of DEP in all exposure periods, compared to control. Reduction in

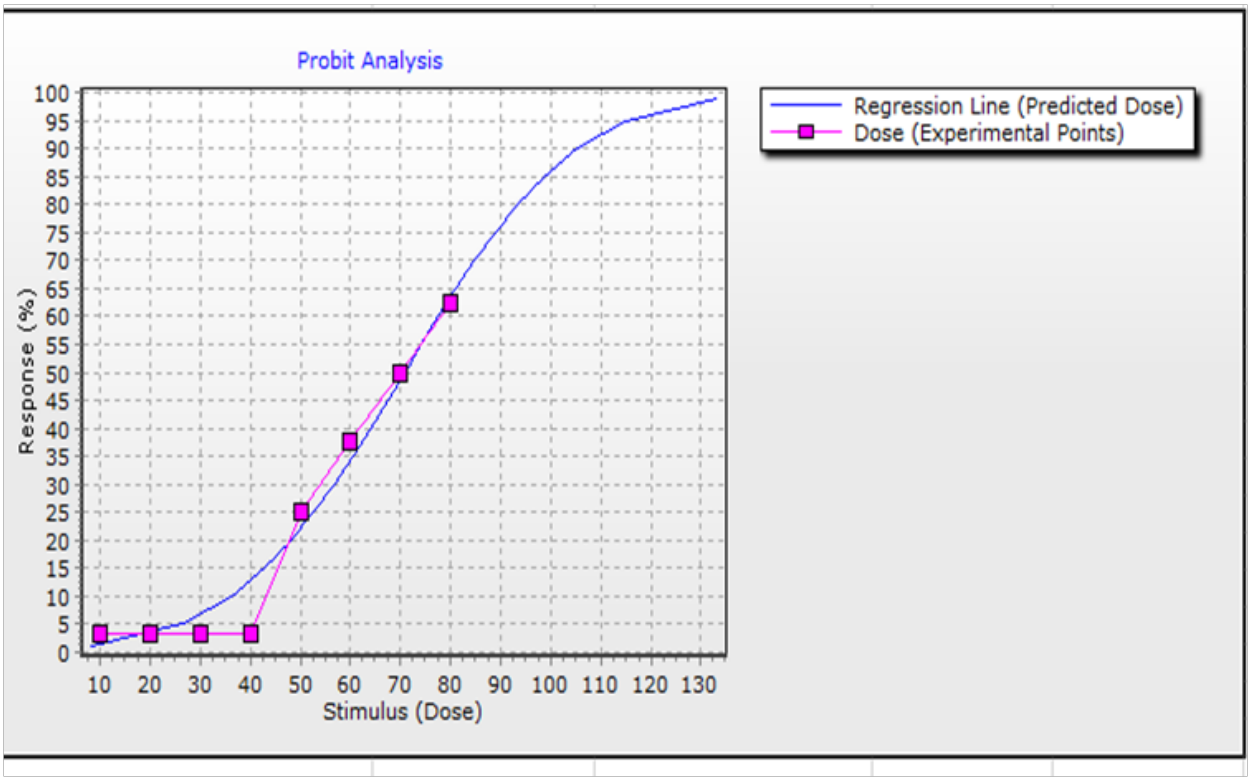

Figure 1. Screenshot of probit analysis based on least squares [Normal Distribution] method generated by Statplus 2009 version 5.8.4.0 software.

Table 1. Gonadosomatic index (Mean ± SE) in control and DEP-exposed fish

Gonadosomatic Index (%)	Control	0.4 ppm	4 ppm	40 ppm
Day 7	37.4±0.3	35.24±0.4	32.53±0.4	*30.15±0.2
Day 14	37.4±0.3	34.72±0.3	*31.2±0.08	*28.62±0.6
Day 21	37.4±0.3	33.31±0.6	*30.43±0.4	*25.17±0.3

*Significant ($p<0.05$)

Table 2. Diameter of ova (Mean ± SE) in control and DEP-exposed fish

Ova diameter (µm)	Control	0.4 ppm	4 ppm	40 ppm
Day 7	1317.64 ±0.6	1315.5 ± 0.9	1313.6 ±0.4	*1283.5±0.2
Day 14	1317.64 ±0.6	1312.7 ±0.4	1311.9 ±0.3	*1280.4 ±0.5
Day 21	1317.64 ±0.6	1310.8 ± 0.5	*1285.3 ±0.2	*1278.4±0.9

*Significant ($p<0.05$)

Table 3. Fecundity (Mean ± SE) in control and DEP-exposed fish

Fecundity	Control	0.4 ppm	4 ppm	40 ppm
Day 7	965.23 ±0.3	963.7±0.5	960.7±0.2	*943.5±0.4
Day 14	965.23 ±0.3	958.4±0.4	956.6±0.9	*935.6±0.2
Day 21	965.23 ±0.3	954.5±0.3	*945.7±0.2	*930.5±0.4

*Significant ($p<0.05$)

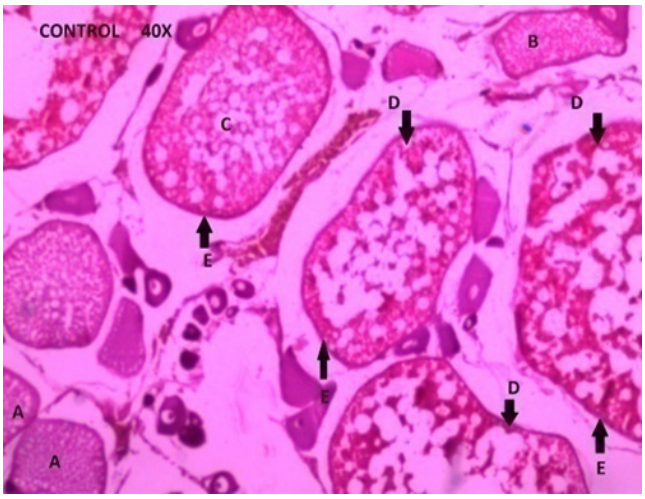

Figure 1. Histology of ovary of control fish. **(A)** Vitellogenicstage **(B)** Previtellogenic stage **(C)** Cytoplasm **(D)** Oocytes **(E)** Zona pellucida.

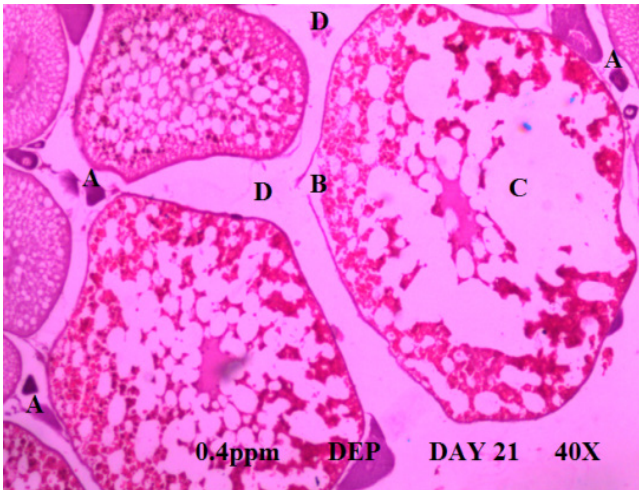

Figure 2. Histology of ovary of DEP (0.4 ppm) exposed (Day 21)Fish. **(A)** Atresia of oocyte **(B)** Disrupted ovarian wall **(C)** Vacuolation.

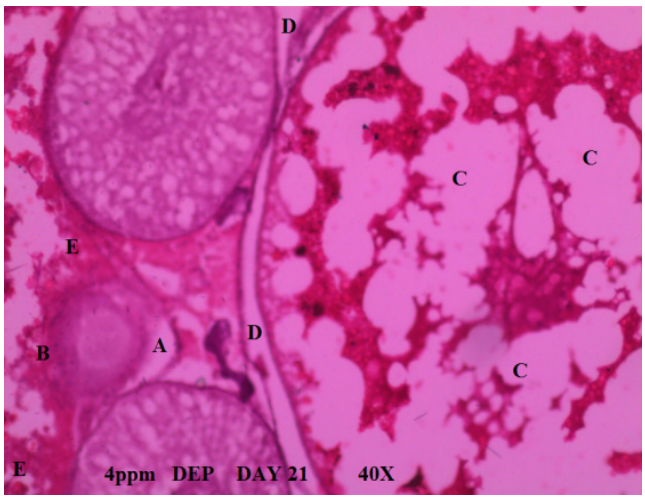

Figure 3. Histology of ovary of DEP (4 ppm) exposed (Day 21) fish. **(A)** Retraction of cytoplasm **(B)** Disrupted ovarian wall **(C)** Vacuolation **(D)** Interfollicular space **(E)** Necrosis.

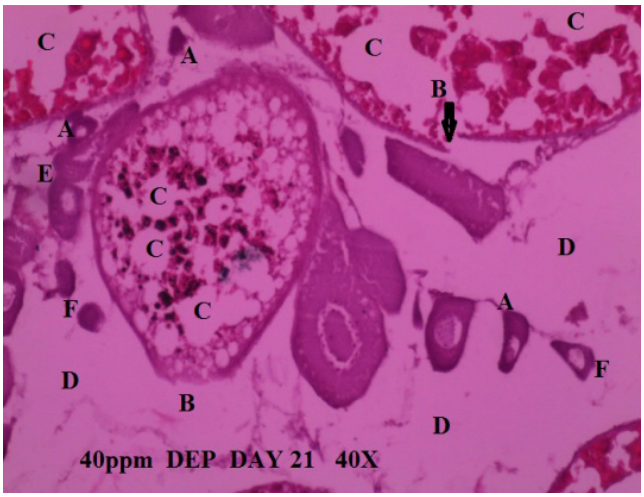

Figure 4. Histology of ovary of DEP (40 ppm) exposed (Day 21) fish. **(A)** Atresia of oocyte **(B)** Disrupted ovarian wall **(C)** Vacuolation **(D)** Inter follicular space **(E)** Adhesion of oocytes **(F)** Deformed oocyte.

diameter of ova was found significant ($p<0.05$) in 4 ppm (i.e., on day 21) and 40 ppm (for all exposure periods) concentrations of DEP in comparison to control (Table 2).

A decrease in fecundity was observed in all exposure periods of DEP in a dose-dependent manner. This decrease was found to be significant ($p<0.05$) in the fish exposed to 4 ppm (i.e., on day 21) and 40 ppm (for all exposure periods) concentrations, when compared to control (Table 3).

The histo-architecture of ovary of DEP- exposed (0.4 ppm, 4 ppm & 40 ppm) fish on day 21 showed atresia of oocyte, disrupted ovarian wall, retraction of cytoplasm, vacuolation, necrosis, inter follicular space, adhesion of oocytes and deformed oocyte.

4. Discussion

Given the over-production, overuse and the consequent presence of phthalates in the environment, phthalates have the potential to target the ovaries at all stages of development, including in the adult. The harmful nature of phthalates can lead to premature ovarian failure, decreased ovulation, infertility and impaired steroidogenesis[15,16]. Therefore, exposure to phthalate interferes with different ovarian functions in different systems, leading to abnormal reproductive and post-reproductive functions. Earlier studies in our laboratory revealed that as an endocrine disruptor, plasticizers like Bisphenol A and DEP disrupted the haematopoietic system, metabolic machinery and histoarchitecture of organs of freshwater fish species[17,18,19]. Reduction in gonadosomatic index, ova diameter and fecundity of DEP-exposed fish can be attributed to the endocrine disrupting activity of this chemical. In aquatic species, phthalates influence female reproduction, although mainly interfering with egg production and biasing sex ratio. Changes observed in the histo-architecture of ovary of DEP-exposed fish may be due to disruption of oogenesis. DEP is an Estrogenic Endocrine Disrupting Chemical (EEDC). The outcomes of EEDCs on aquatic organisms are properly established as this has a tendency to be the most comprehensively investigated facet of endocrine disruption in fishes. These changes were noticeable at conflicting levels of biological importance depending on several facets comprising the length and rate of recurrence of exposure, the specific exposure chemical(s), age, developmental stage, season, and sex.

Decreased gonadosomatic index in *Heteropneustes fossilis* fish after long-term exposure to DEHP and acetyl tri-butyl has been reported[20]. Another study found that exposure to high concentration of DBP in adult male Murray rainbow fish caused testicular degeneration, including vacuolated cells, apoptotic bodies, interstitial fibrosis and overgrowth[21]. Phthalate exposure affects oocyte replication in peri-ovulatory phase and MEHP exposure at 5 to 100 μM for 22 to 24 hrs causes denuded oocytes, the cumulus-oocyte complex, and decrease the number of cells that have progressed from meiosis II to advanced metaphase II[22]. Contact with di-isononyl phthalate (DINP) and di-(2-ethylhexyl) phthalate (DEHP) has been shown to reduce the activity of some of the steroidogenic ezymes present in the ovaries and testicles of *Oreochromis mossambicus* during proximal activity[23,24]. Studies have shown that DBP causes oxidative stress through extracellularly controlled pathways as well as through calcium ion signals. In addition, it causes testicular damage in mice[25].

Studies have shown that phthalates at 10-100 μm concentration treated to mice for 72 hrs caused collapse of the spermatogenic cell store and affect the primordial follicle assembly[26]. Exposure to DEHP causes heretable changes in the DNA affecting methylation process in rat oocytes, which in turn would affect oocyte growth[27]. A single intraperitoneal injection of DIBP to pregnant mice changed the structure of the foetus' follicles[28]. DEHP at 20-750 μg/kg/day for 10 to 30 days in mature mice accelerates the recruitment of the primordial follicles, resulting in decrease of the primordial follicles and increase of the primary follicles[29]. MEHP exposure of mouse uterus enhances follicular formation, and 100 to 1000 mg/kg/day MEHP exposure from 17 to 19 days of gestation increases preantral and antral follicles of F1 mice[30].

DEHP exposure of mice at 0.5 to 5 mg/kg/day dose during pregnancy and lactation reduces the number of oocytes that reach the stage of ovulation and meiosis II in adults[31]. Exposure of 25 to 50 mg/kg/day DEHP to adult sheep has been shown to reduce the copora-lutea and the luteal phase of the estrous cycle[32]. Experiments with phthalate levels that are within human exposure range are useful for deciphering monotonic dose responses[33,34]. It has been shown that DEHP exposure of mice at 1000 to 3000 mg/kg/day dose through oral gavage reduces serum estradiol levels[35]. Similarly, DEHP exposure results in decreased levels of serum testosterone, progesterone, LH and FSH[36]. DEHP exposure of adult mice at 500 to 2000 mg/kg/day dose through oral gavage for 16 weeks caused increase in the length of estrous cycle, apoptosis of granulosa cells and cell cycle arrest, as well as decrease in serum progesterone levels[37]. In human luteal cells extracted from corpora-lutea, the production of progesterone, which stimulates basal and human chorionic gonadotropin, is reduced by exposure to DEHP, DBP and BBP at levels of 10^{-6} to 10^{-9} M for 24 hrs[38]. A survey of women between the ages 6-20 and 40-60 by the National Health and Nutritional Examination Survey, found that the amount of phthalate metabolites present in the urine of women was associated with a decrease in the total testosterone level[39].

A 2012 study in Swedish children found that phthalates from PVC flooring enter children's bodies not only through food but also through the skin and breath[40]. In adult Murray rainbow fish, exposure to high levels of Di-Butyl Phthalate (DBP) caused the testicles to degenerate, so as to result in vacuolated cells, apoptotic bodies, interstitial fibrosis, asynchronous development and a significant reduction in sperm count[21]. In the human body, phthalates are hydrolyzed into monoesters, which are then converted to more hydrophilic metabolites by enzymatic oxidation of the alkyl network, and later oxidized and converted as mono-2-ethyl-5-hydroxyhexyl phthalate and mono-2-ethyl-5-oxohexyl phthalate[41]. An epidemiological study in adult men has shown that phthalate exposure is associated with testicular function, especially low sperm quality relationships[42]. In addition to the direct effects of phthalates, viz. interaction with steroid hormone receptors, phthalates cause proliferation of cancer cells that are susceptible to hormone deficiency, as well as the malignant invasive formation of breast cancer. In addition, they show inverse estrogenic or androgenic effects[42].

5. Conclusion

The present study leads to the conclusion that di-ethyl phthalate (DEP), as an EEDC, adversely affects the functioning of the female reproductive system of the freshwater murrel *Channa striatus*.

6. References

1. Bukola D, Zaid A, Olalekan EI, Falilu A. Consequences of anthropogenic activities on fish and the aquatic environment. Poultry Fisheries & Wildlife Sciences. 2015; 3:2. https://doi.org/10.4172/2375-446X.1000138
2. Wang Y, Zhu H, Kannan K. A review of biomonitoring of phthalate exposures. Toxicol. 2019; 7(2):21. https://doi.org/10.3390/toxics7020021 PMid:30959800 PMCid:PMC6630674
3. European Chemicals Agency (ECHA). Information on Chemicals. Helsinki Finland European Chemicals Agency; 2018
4. Revathy V, Chithra KC. Effects of diisononyl phthalate on the antioxidant status in gill, liver and muscle tissues of the fish, *Oreochromis mossambicus.* Asian J Adv Basic Sci. 2018a; 6(1):37-48.
5. Revathy V, Chithra K. Di (2- ethyl hexyl) phthalate induced lipid peroxidation and associated oxidative stress in gill, liver and muscle tissues of fish, *Oreochromis mossambicus* (Peters, 1852). Int J Theor Appl Sci. 2018b; 10(2):1-9.
6. Poopal RK, Ramesh M, Maruthappan V, Rajendran RB. Potential effects of low molecular weight phthalate esters ($C_6H_{22}O_4$ and $C_{12}H_{14}O_4$) on the freshwater fish *Cyprinus carpio.* Toxicol Res. 2017; 6(4):505-20. https://doi.org/10.1039/C7TX00084G PMid:30090519 PMCid:PMC6062309
7. Sower SA, Freamat M, Kavanaugh SI. Erratum to "The origins of the vertebrate hypothalamic- pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) endocrine systems: New insights from lampreys. Gen Comp Endocrinol. 2009; 161(1):20-9. https://doi.org/10.1016/j.ygcen.2008.11.023 PMid:19084529
8. de Kretser DM, Hedger MP, Loveland KL, Philips DJ. Inhibins, activins and follistatin in reproduction. Hum Reprod Update 2002; 8(6): 529-41. https://doi.org/10.1093/humupd/8.6.529 PMid:12498423
9. Roy GK, Malini NA, Archana R, Deepa R. Enzymatic changes in the kidney and brain of freshwater murrel, *Channa striatus* (Bloch) on short term exposure to sub-lethal concentration of lead nitrate. Indian J Fish. 2011; 58: 91–94.
10. Roy GK, Malini, NA, Sandhya RGO. Biochemical changes in liver and muscle of the cichlid, *Oreochromis mossambicus* (Peters, 1852) exposed to sub-lethal concentration of mercuric chloride. Indian J Fish. 2012; 59:147–152.
11. Scholz S, Kluver N. Effects of endocrine disrupters on sexual, gonadal development in fish. Sex Dev. 2009; 3(2-3):136-51. https://doi.org/10.1159/000223078 PMid:19684458
12. Giulivo M, Lopez de AM, Capri E, Barceló D. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environ Res. 2016; 151:251-264. https://doi.org/10.1016/j.envres.2016.07.011 PMid:27504873
13. Factor LP, Insel B, Calafat AM, *et al.* Persistent Associations between Maternal Prenatal Exposure to Phthalates on Child IQ at Age 7 Years. PLoS ONE. 2014; 9(12): e114003. https://doi.org/10.1371/journal.pone.0114003 PMid:25493564 PMCid:PMC4262205
14. Balalian AA, Whyatt RM, Liu X, *et al.* Factor LP. Prenatal and childhood exposure to phthalates and motor skills at age 11 years. Environ Res. 2019; 171:416-27. https://doi.org/10.1016/j.envres.2019.01.046 PMid:30731329 PMCid:PMC6814270
15. Bhattacharya P, Keating AF. Impact of environmental exposures on ovarian function and role of xenobiotic metabolism during ovotoxicity. Toxicol Appl Pharmacol. 2012; 261(3):227-35. https://doi.org/10.1016/j.taap.2012.04.009 PMid:22531813 PMCid:PMC3359424

16. Craig ZR, Wang W, Flaws JA. Endocrine-disrupting chemicals in ovarian function: effects on steroidogenesis, metabolism and nuclear receptor signaling. Reproduction 2011; 142(5):633-46. https://doi.org/10.1530/REP-11-0136 PMid:21862696
17. Roy GK, Malini NA, Praveena GS, Rejani MK.Haematological and biochemical alterations in short term exposure to sub lethal concentration of Bisphenol A in *Oreochromis mossambicus*(Peters, 1852). Poll Res. 2017; 36(1): 57-62.
18. Roy GK, Gokul, GN, Malini NA . Effects of different sub-lethal concentrations of plasticizer-diethyl phthalate on Fresh water murrel, *Channa striatus* (Bloch). J Appl Nat Sci. 2017; 9(1): 476–481.
19. Elvin T, Malini NA, Roy GK. Deleterious Effect of Short Term Exposure to Xenoestrogen- Bisphenol A on Certain Haematological and Physiological Profile of Freshwater Murrel, *Channa Striata* (Bloch, 1793). Poll Res. 2020; 39: 126-133
20. Muhammad S, Zhang Z, Pavase TR, Guo H. Long-term exposure of two plasticizers di (2-ethylhexyl) phthalate (DEHP) and acetyl tributyl citrate (ATBC): Toxic effects on gonadal development and reproduction of zebra fish (Danio rerio). Indian J Marine Sci. 2018; 47(4):789-97.
21. Bhatia H, Kumar A, Ogino Y, *et al*. Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbow fish *(Melanotaenia fluviatilis)*. Aquatic Toxicol. 2014; 149:103-115. https://doi.org/10.1016/j.aquatox.2014.01.025 PMid:24576492
22. Grossman D, Kalo D, Gendelman M, Roth Z. Effect of di-(2-ethylhexyl) phthalate and Mono-(2-ethylhexyl) phthalate on in vitro developmental competence of bovine oocytes. Cell Biol Toxicol. 2012; 28(6):383-96. https://doi.org/10.1007/s10565-012-9230-1 PMid:22956148
23. Revathy V, KC Chitra. Di-isononyl phthalate (DINP) impairs reproduction in the freshwater fish, *Oreochromis mossambicus* (Peters, 1852). Asian Fish Sci. 2018c; 31(4):284-96. https://doi.org/10.33997/j.afs.2018.31.04.004
24. Revathy V, KC Chitra. Role of di-(2-ethylhexyl) phthalate on the antioxidant status in testes and ovary of the fish, *Oreochromis mossambicus* (Peters, 1852). Int J Pharm Biol Sci. 2019; 9(1):1186-94.
25. Li J, Wu Z, Cheng J. Testicular injury induced by DBP involved in activation of ERK pathway in KM mice. Wei Sheng Yan Jiu. 2018; 47(6):956-62.
26. Zhang T, Li L, Qin XS, *et al*. Di-(2-ethylhexyl) phthalate and bisphenol A exposure impairs mouse primordial follicle assembly in vitro. Environ Mol Mutagen. 2014; 55(4):343-53. https://doi.org/10.1002/em.21847 PMid:24458533
27. Li L, Zhang T, Qin XS, *et al*. Exposure to di (2-ethylhexyl) phthalate (DEHP) results in a heritable modification of imprint genes DNA methylation in mouse oocytes. Mol Biol Rep. 2014; 41(3):1227-35. https://doi.org/10.1007/s11033-013-2967-7 PMid:24390239
28. Ray B, D'Souza AS, Kumar V, *et al*. Ovarian development in Wistar rat treated prenatally with single dose diisobutyl phthalate. Bratisl Lek Listy. 2012; 113(10):577-82. https://doi.org/10.4149/BLL_2012_129 PMid:23094893
29. Hannon PR, Peretz J, Flaws JA. Daily exposure to Di (2-ethylhexyl) phthalate alters estrous cyclicity and accelerates primordial follicle recruitment potentially via dysregulation of the phosphatidylinositol 3-kinase signalling pathway in adult mice. Biol Reprod. 2014; 90(6):136. https://doi.org/10.1095/biolreprod.114.119032 PMid:24804967 PMCid:PMC4435463
30. Moyer B, Hixon ML. Reproductive effects in F1 adult females exposed in utero to moderate to high doses of mono-2-ethylhexylphthalate (MEHP). Reprod Toxicol. 2012; 34(1):43-50. https://doi.org/10.1016/j.reprotox.2012.02.006 PMid:22401849 PMCid:PMC3367132
31. Pocar P, Fiandanese N, Secchi C, *et al*. Exposure to di (2-ethylhexyl) phthalate (DEHP) in utero and during lactation causes long-term pituitary-gonadal axis disruption in male and female mouse offspring. Endocrinology 2012; 153(2):937-48. https://doi.org/10.1210/en.2011-1450 PMid:22147016
32. Herreros MA, Gonzalez-Bulnes A, Inigo-Nunez S, *et al*. Toxicokinetics of di(2-ethylhexyl) phthalate (DEHP) and its effects on luteal function in sheep. Reprod Biol. 2013; 13(1):66-74. https://doi.org/10.1016/j.repbio.2013.01.177 PMid:23522073
33. Beausoleil C, Ormsby JN, Gies A, *et al*. Low dose effects and non-monotonic dose responses for endocrine active chemicals: science to practice workshop: workshop summary. Chemosphere. 2013; 93(6):847-56. https://doi.org/10.1016/j.chemosphere.2013.06.043 PMid:23932820
34. Vandenberg LN. Non-monotonic dose responses in studies of endocrine disrupting chemicals: Bisphenol a as a case study. Dose Response 2014; 12(2):259-76. https://doi.org/10.2203/dose-response.13-020. PMid:24910584 PMCid:PMC4036398
35. Liu T, Li N, Zhu J, *et al*. Effects of di-(2-ethylhexyl) phthalate on the hypothalamus-pituitary-ovarian axis in adult female rats. Reprod Toxicol. 2014; 46:141-47. https://doi.org/10.1016/j.reprotox.2014.03.006 PMid:24675100
36. Li N, Liu T, Zhou L, *et al*. Di-(2-ethylhcxyl) phthalate reduces progesterone levels and induces apoptosis of ovarian granulosa cell in adult female ICR mice. Environ Toxicol Pharmacol. 2012; 34(3):869-75. https://doi.org/10.1016/j.etap.2012.08.013 PMid:22986106

37. Romani F, Tropea A, Scarinci E, *et al*. Endocrine disruptors and human reproductive failure: The in vitro effect of phthalates on human luteal cells. Fertil Steril. 2014; 102(3):831-37. https://doi.org/10.1016/j.fertnstert.2014.05.041 PMid:25016925
38. Meeker JD, Ferguson KK. Urinary phthalate metabolites are associated with decreased serum testosterone in men, women, and children from NHANES 2011-2012. J Clin Endocrinol Metab. 2014; 99(11):4346-52. https://doi.org/10.1210/jc.2014-2555 PMid:25121464 PMCid:PMC4223430
39. Carlstedt F, Jonsson BAG, Bornehag CG. PVC flooring is related to human uptake of phthalates in infants. Indoor Air. 2013; 23(1): 32-9. https://doi.org/10.1111/j.1600-0668.2012.00788.x PMid:22563949
40. Gomez C, Gallart-Ayala H. Metabolomics: A tool to characterize the effect of phthalates and bisphenol A, Environ Rev. 2018; 26(4): https://doi.org/10.1139/er-2018-0010 Joensen UN, Fredericksen H, Jensen MB, *et al*. Phthalate excretion pattern and testicular function: A study of 881 healthy Danish men. Environ Health Perspect. 2012; 120:1397-403. https://doi.org/10.1289/ehp.1205113 PMid:22832070 PMCid:PMC3491947
41. Hsieh TH, Tsai CF, Hsu CY, *et al*. Phthalates induce proliferation and invasiveness of estrogen receptor-negative breast cancer through the AhR/HDAC6/c-Myc signaling pathway. FASEB J. 2012; 26(2):778-87. https://doi.org/10.1096/fj.11-191742 PMid:22049059
42. Joensen UN, Fredericksen H, Jensen MB, et al. Phthalate excretion pattern and testicular function: a study of 881 healthy Danish men. Environ Health Perspect. 2012; 120: 1397-403