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## Toxicity of non-ionic surfactant 4-nonylphenol an endocrine disruptor: A review

**Madhu Sharma and Pooja Chadha**

### Abstract

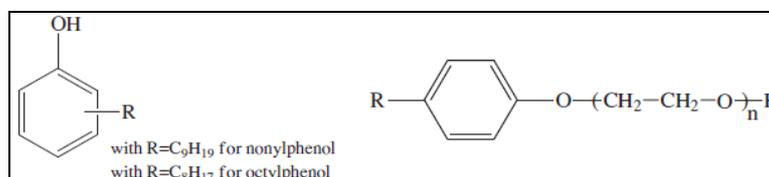
Nonylphenol is the ultimate breakdown product of alkyphenol ethoxylate which is widely used in household and industrial products. As a result of widespread use and lipophilic nature there pervasiveness in the environment have been found everywhere. After that nonylphenol ethoxylates and their metabolites became a major focus of environmental research. Various studies have been performed regarding its endocrine disrupting effects, adverse developmental, reproductive, neurological effects on different organism which have been reviewed by various scientists. But the primary concern of the present review is the measurement of toxic effect of 4-nonylphenol (4-NP) ultimate degradation product of nonylphenol ethoxylates in different organism. Review is limited to, the studies related to genotoxicity, haemotoxicity and histopathological alterations in effect to 4-NP. This review provides evidence for environmental endocrine disruption and sequelae on genotoxic, haemotoxic and histopathological effects, as well as its status in India regarding its concentration in river water and research status.

**Keywords:** Alkyphenol, 4-nonylphenol, genotoxicity, endocrine disruptors

### 1. Introduction

Surfactants are a group of chemicals consisting of two parts: a polar head group and a nonpolar hydrocarbon tail. Surfactant reduces the surface tension of water and form a bridge between two chemicals that don't promptly combine [1]. In 2006, worldwide production of surfactants was 12.5 million tons [2]. Over [3] million tons were produced in Western Europe alone in 2007 (CESIO, 2007).

Among the varied classes of surfactants, nonionic surfactants play a massive role within the World surfactant market. Alkyphenol (AP) is among the most commonly used nonionic surfactant that embody the two groups of compounds nonylphenol ethoxylates (NPEO) and ocyphenol ethoxylates (OPEO). 80% of alkyphenols are made from NPEO while the rest 20% is made from octylphenol (OPEO) [4].



**Fig 1:** General formula of alkyphenol (left) and alkyphenol ethoxylate (n=1-100)

Nonylphenol ethoxylates (NPEO) are conjointly utilized in many industrial applications, namely: textile, leather processing, latex paint, plastic, pulp and paper and pesticide production. Due to extensive use its major percentage is discharged into the sewage [5]. Major source of NPE in the environment is industrial, institutional and domestic. Industrial processes contribute 55% of the total use, while 30% and 15 % is from industrial and household cleaning products respectively. So both industrial and household wastewater contributes NPE to the environment [5]. Once place out into the environment, nonylphenol ethoxylates are transformed into metabolic intermediates that's nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate and different associated compounds by microbial transformation. These breakdown products are relentless in the environment so called as "biorefractory" [6].

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Microbial Transformation usually reduces the environmental toxicity of a compound; but in some cases, breakdown products are much more toxic than the parent compounds [7]. Therefore, to gauge the environmental toxicity of such compounds toxicity testing might be done on final degradation products. Ubiquitous, persistent and easily bioaccumulative nature of NP and NPEs has attracted researcher's attention from the past couple of years [8]. After their discharge into water bodies, 49-59% of them remain in water, 41-50% entered into soil and only 1% remains within the air. On the other hand, if the discharge takes directly on soil, 99% of them remain within the soil [9]. Once they enter into the environment, these will get into the food chain and ultimately to humans in multiplying concentrations as get bioaccumulated. Humans may be exposed to NP by taking contaminated food and water and also due to direct contact

with the products having NP. The presence of 4-nonylphenol, bisphenol and triclsan was reported by [10] in tap water, water bottles and baby bottles. NP was found in all water samples from the bottles. Five samples of tap water were found to have NP out of total six samples taken. It was estimated that every day with 2 liter of water 1410 ng/l of 4-NP is taken by every adult. Recently NP has been found in human milk, urine as well as in blood [11].

NP has attracted the attention due to its pervasiveness in the environment. Concerns have increased recently as it can mimic natural hormones and its copious level in environment can disrupt endocrine, immune, and reproductive systems [12, 13]. Most research till date has focused on occurrence and fate in the environment as well as its potential endocrine disrupting effects and neurotoxic effect and number of reviews has been compiled regarding these aspects (Table 2).

**Table 2:** Various reviews on Nonylphenol

| S. No | Review  | Reference                               |
|-------|---|---|
| 1.    | Endocrine-disrupting chemicals: review of toxicological mechanism using molecular pathway analysis.                               | Yang <i>et al.</i> , 2015               |
| 1.    | Alkyl phenol and alkylphenol polyethoxylates in water and wastewater: a review of option for their elimination                    | Priac <i>et al.</i> , 2014              |
| 2.    | Neurotoxic effects of nonylphenol: a review   | Jie <i>et al.</i> , 2013                |
| 3.    | Occurrence and degradation in the environment   | Mao <i>et al.</i> , 2012                |
| 4.    | Nonylphenol, octylphenol and bisphenol-A in the aquatic environment: A review on occurrence, fate and treatment                   | Sharma <i>et al.</i> , 2009             |
| 5.    | Toxicological profile for nonylphenol   | David <i>et al.</i> , 2009              |
| 6.    | Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters.                     | Soraes <i>et al.</i> , 2008             |
| 7.    | Endocrine disrupting potential of bisphenol A Dimethanolate 4-n-nonylphenol and 4-n-octylphenol: New data and brief review        | Bonefeld-Jargensen <i>et al.</i> , 2007 |
| 8.    | Fate, Behavior, and effects of surfactants and their degradation products in the environment                                      | Ying, 2006                              |
| 9.    | Biologically directed environmental monitoring fate and transport of estrogenic endocrine disrupting compounds in water: A review | Campbell <i>et al.</i> , 2006           |
| 10.   | Nonylphenol, an integrated vision of a pollutant. Scientific Review   | Vazquez-Dahalt <i>et al.</i> , 2005     |
| 11.   | Environmental fate of alkylphenols and alkylphenols ethoxylates-a review  | Ying and Kookana, 2002                  |
| 12.   | Review of aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates.             | Servos, 1999                            |
| 13.   | Chronic toxicity of surfactants and detergents builders to algae: A review and risk assessment                                    | Lewis, 1990                             |

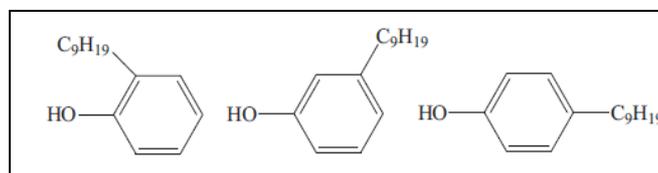
EPA has accepted the risk of nonylphenol so most countries have banned the usage of alkylphenol ethoxylates as surfactants and its production has decreased gradually in these countries [14]. However, in many developing countries, including India, China and South America, no action has been taken to decrease the usage of NP step by step. Rather the use and production of NPEs in these countries have increased annually [15].

The present review first focuses on physicochemical properties and pervasiveness of nonylphenol in the environment and few studies revealing its endocrine disrupting effect have been compiled. Then the emphasis is on revealing its genocytotoxicity, haemotoxicity and histopathological effects.

## 2. Physico-chemical properties

Fate of any compound in the environment depends upon the physico-chemical properties and these properties in turn depend upon the structure of the compounds. Toxicity of a compound is related to its hydrophobicity and electrophilicity. Tissue penetration, adsorption and excretion are related to hydrophobicity and acute toxicity is determined by electrophilicity. Nonylphenol ethoxylates (NPEOs) are aromatic compounds with polyethoxylate chain and a alkyl chain having nine carbon, which are attached to a benzene

ring. Structure of nonylphenol may vary, as carbon chain may attach at various positions on the phenol group, it may be ortho, para and meta in position and can be either linear or branched representing a different isomer. The para isomer is the typical member of family making up to 90% of the commercial form and is used in experimentation and environmental analysis.



**Fig 2:** Ortho, meta and para isomer of alkylphenol

NPEOs have different physical and chemical properties and as increase in the ethoxylate chain length occurs, decrease in hydrophobicity and solubility has been found. Lower ethoxylates NP compounds are found to be more persistent and dangerous for living organisms. The half-life of these compounds is above 60 years in sediments [16]. Nonylphenol ethoxylates are non ionic in water. Due to this property they are used in variety of applications like detergents, cleaners and emulsifiers etc. Being amphipathic in nature, it may

surround the substances like oil and grease and can isolate them from water.

**Table 1:** Physico-chemical properties of 4-Nonylphenol <sup>[14]</sup>

| Molecular formula  | C <sub>15</sub> H <sub>24</sub> O   |
|--------------------|---|
| Molecular weight   | 220.34g/mole  |
| Appearance         | Transparent or light straw-colored, high-viscosity liquid; slight phenolic odor |
| Specific gravity   | 0.95 (20°C)   |
| Melting point      | approx. -8°C  |
| Vapor pressure     | 0.3Pa or lower (25°C)   |
| Aquatic solubility | 6mg/L (20°C)  |
| Log Kow            | 4.48  |

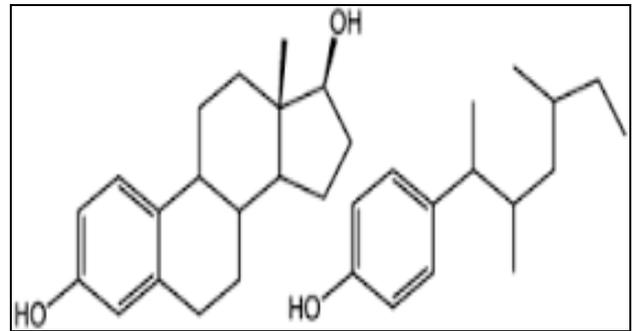
### 3. Pervasiveness in the environment

NP has been detected in freshwater, saltwater and estuaries. Different studies have supported the presence of NP in rivers, lakes, rainwater, ground water, sea, soil and air. <sup>[17]</sup> emphasized that NP level should not exceed 6.6 µg/l in fresh water and 1.7 µg/l in marine water. <sup>[18]</sup> reported the annual average concentration of NP to be 0.3µg/l in the surface water. While in the sea water, it was absolutely detected in the range of 0.000002-4.1 µg/l. Similarly <sup>[19]</sup> tested 122 samples from different rivers and found 90% of samples had 0.268 µg/l of NP. In keeping these <sup>[20]</sup> evaluated that rain water had 0.03-1.20 µg/l of NP. <sup>[21]</sup> tested water samples from San Francisco and detected 0.024-6.25 µg/l of NP. <sup>[4]</sup> discovered that the rivers flowing near the vicinity of commercial area have 644 µg/l of NP <sup>[22]</sup> evaluated the seasonal and spatial distribution of nonylphenol in the Yellow River in China. The highest concentration was observed in the month of November further NP was analyzed in 60 food stuffs commercially available in Germany and range varied between 0.1 and 10.4 µg/kg regardless of fat content of foodstuff. Daily intake for adult was calculated 7.5 µg/day NPs and for infants fed with infant formula daily 1.4 µg/day <sup>[23]</sup> analyzed water samples from three rivers of South India for the presence of octylphenol, nonylphenol and bisphenol. Concentration of NP was found to be highest (2200ng/l) from the water samples of Kavari River among all the three tested chemicals. NP was found to show highest hazard quotient (HQ) for fish in Kavari River. On the other hand, recently <sup>[24]</sup> observed the bioaccumulation of 4-NP in different tissues of fish *H. fossilis*. The concentration of 4-NP was found in the range of 12.40 ± 1.1 µg/L and 16.29 ± 1.18 µg/L from the samples collected from River Ganga and Varuna. Among the different tissues of fish brain was reported to accumulate the highest concentration of 4-NP and muscles showed least accumulation, while gill, liver, kidney and ovary showed intermediate accumulation. Further, it was also revealed that all the tissues were involved in the metabolism of 4-NP uptake except the muscle. So it is clear from the studies that in Indian rivers the concentration of 4-NP is above permissible levels. But very few studies are available revealing its the toxicity at national level.

### 4. Endocrine disrupting effect-

Further review expands arguments presented in favor of its endocrine disrupting effect. A number of studies have been conducted to show that NP acts as an endocrine disruptor as it has a benzene ring with hydrophobic moiety which mimics the B and C ring of 17β-estradiol<sup>[25]</sup>. Endocrine disruptors are the chemicals that target the endocrine system and alter its functions in a number of ways <sup>[26, 27]</sup> found that these mimic

the steroid hormones and get hooked up to natural receptors. These are reported to alter the synthesis and breakdown of the hormones. Nonylphenol is found to induce the incidences of hermaphrodites, delay in metamorphosis, developmental delay, and reduction in larval survival and increase in female sex ratio <sup>[28, 29]</sup>.



**Fig 3:** 17β-estradiol and 4-NP showing how 4-NP structure mimic the structure of estradiol hormone

NP has been found to show impact on freshwater and marine animals, humans as well as laboratory animals. These effects are mainly on reproductive and endocrine systems. It is proved to have several implications to human health as human sperm count have dropped significantly, mainly in the industrial area and testicular cancer cases have also been found to increase in number of times in the same region <sup>[30]</sup>. A decrease in sperm count, increase incidences of congenital malformation in case of male reproductive tract and in young men and cases of testicular cancer due to the exposure with 4-NP has been found <sup>[31]</sup>. Similarly, delay in spermatogenesis in fish exposed to xenoestrogen has been found by <sup>[32, 33]</sup> found increased vitellogenesis in *Zoarcetes viviparous* in effect to 4-NP. Along with this developmental toxicity have also been reported by a number of studies <sup>[34, 30]</sup>. Similarly, when endocrine disruptor was applied on the breast a significant increase in the number of cells has been observed <sup>[35, 36]</sup> reported that when these are applied to chicken embryo, trout and rat estrogen receptors responded to them. In human it may also cause destruction of respiratory system, eyes and skin <sup>[35]</sup>.

<sup>[33]</sup> Studied the impact of nonylphenol and 17β-estradiol on vitellogenesis, testicular structure and the cytology of *Zoarcetes viviparous*. They found that the effect increased with an increase in the concentration of the nonylphenol and 17β-estradiol and an increase in plasma vitellogenesis was also observed <sup>[37]</sup> had shown the effects of NP on gonadal differentiation as well as in the development of fish *Rivulus marmoratus* and found the significant inhibition of oogenesis. <sup>[38]</sup> tested the estrogenic and non-estrogenic effects of nonylphenol using mouse as model and observed that at high dose NP was very similar to estradiol in uterus tissue. However, in liver tissue no effects were seen. So it was suggested to consider the tissue specific effects <sup>[39]</sup> studied the effect of 4-NP and estrogen -17β on the expression of receptor of α gene involved in smolting. Nonylphenol isomer downregulate the steroid hormone receptor expression and activation by cell receptors and cell signaling transduction pathway <sup>[40, 41]</sup> studied the protective effect of vitamin E on sperm parameters and reproductive hormones in rats after treatment with 4-NP. NP treatment caused a greater reduction in testis weight, sperm motility and number. Treatment with vitamin E was found to cut back the effect considerably when

compared with the NP treated group. Endocrine disruptors have been shown to affect courtship and reproductive behavior [42, 34] investigated the developmental toxicity caused by 4-nonylphenol in Zebrafish (*Danio rerio*) embryo. NP was found to cause developmental abnormalities and morphological alterations as the dose increases [43] revealed that spermatozoa motility gets remittent altogether in the experimental groups in response to NP addition [44] investigated the toxic effects of 4-NP on embryonic development of African catfish *Clarias garipinus* and found that fertilization and hatching rate were significantly decreased while incubation period, the mortality rate and malformed embryos ratio were increased and number of morphological and histopathological alterations were observed. [45] investigated the profound effect induced by combined effect of nonylphenol and letrozole on male Gold fish *Carassius aumatus*. They found that coexposure results in liver mitochondria impairment uneven distribution of cytoplasm but no significant damage was observed in the testes [46] studied the comparative endocrine disrupting compounds exposed Atlantic Salmon with three doses of 17  $\alpha$ -ethinylestradiol, 17  $\beta$ -estradiol and nonylphenol on four early life stages embryo, yolk sac larvae, feeding fry and 1 year old smolts and suggest fry as most responsive life stage in early development.

Although a wealth of individual studies regarding its toxicity in relation to genocytotoxicity, haematotoxicity and histopathological alterations has not been compiled so we summarize the key findings from the recent scientific literature.

## 5. Toxicity of 4-NP

Chronic and acute toxicities of NP on aquatic organisms are reviewed by [47, 14, 48, 49] analyzed the induction of micronuclei (MN) and other nuclear abnormalities in erythrocytes of peripheral blood and cephalic kidney treated with crude oil and nonylphenol. Significant increase in MN was observed in turbot kidney and blood in *Scophthalmus maximus* and *Gadus morua* after exposure to 30ppb of nonylphenol, 0.5ppm of crude oil and after the exposure to 0.5ppm oil spiked with an additional mixture of alkylphenol and PAHs. A significant inter tissue difference was found only in the induction of fragmented apoptosis [50] revealed the toxicity of 4-nonylphenol in aquatic invertebrates in Taiwan. They found genotoxicity in six aquatic invertebrate species and urged to manage its ecological impact on aquatic ecosystems. [51] investigated the mutagenic effect of nonylphenol and octylphenol on *Salmonella thyphimirium* by using mutation assay. They used six concentrations from 0.93 to 4.68 $\mu$ g/l of NP in the experiment and observed non-mutagenic impact of NP. Lethal concentration of nonylphenol determined for fish *Oreochromis niloticus* was found to be 0.032 mg/l by [52] and have shown the genotoxicity and effects on reproduction of nonylphenol in fish *Oreochromis niloticus*. Genotoxicity was evaluated by micronucleus assay and comet assay in peripheral erythrocytes and found no genotoxic effect on fish. However 3 days exposure show increased frequency of reproductive stages in male and female. [53] showed the destructive effect of 4-nonylphenol on African catfish (*Claria gariepinus*). The sublethal concentrations of 4-nonylphenol (0.05, 0.08 and 0.1mg/l) were used for the study of apoptosis, erythrocytes alterations, micronucleus test and blood parameters count as biological indicators. 4-nonylphenol was found to cause genotoxicity in erythrocytes with several

malformations in shape and number besides with other blood parameters [54] tested the acute and subacute toxicity of nonylphenol on fish, Rosy barb (*Puntius conchonious*). Effects were seen for sub lethal concentrations after determining LC<sub>50</sub> on structural and biochemical parameters of gill, liver and kidney. After 14 days exposure NP was reported to alter structural and biochemical parameters. [55] found 1.5 mg/l LC<sub>50</sub> for NP at 96 hours in fish *Oreochromis mossambicus*. Significant induction of micronuclei along with other nuclear abnormalities was also reported in fish after treatment with 4-NP. [56] studied DNA damaging effects of 4-nonylphenol in fish *C. punctatus* on erythrocytes from gill and kidney and observed a significant increase in micronucleated cells and aberrant cells. Sharma *et al.* [57] revealed the cytotoxic effects of 4-nonylphenol after acute and sub chronic exposure to fish *C. punctatus* by determining polychromatic erythrocytes frequency (PCE frequency). PCE frequency was found to decrease after both acute and sub chronic exposure and revealed its cytotoxic potential. [58] observed significant induction of nuclear buds and fragmented apoptotic cells after exposure to 30 $\mu$ g/l of NP for the 2nd generation in the blood of exposed fish (Tilapia group). Exposing fish to 4-nonylphenol resulted in an increased amount of both damaged DNA and RNA showing its cytogenetic effect on *Oreochromis spilurus* fish. [59] studied the change in gene activity in response to 4-NP and 17 $\beta$ -estradiol and observed a variation in gene expression. Further, they found that 4-NP repress the glutathione peroxidase expression and inhibit the protective mechanism of the cell. Glutathione (GSH) and glutathione dependent enzymes are the two important enzymes involved in cascade which are involved in removal of intermediate toxins formed by xenobiotics during their bio activation. When there is a drop in GSH below a critical level, then it is enable to bind with xenobiotics and they get covalently combined with DNA, RNA and proteins and leads to cellular damage [60]. So DNA damaging potential may be due to drop in GSH level or also due to the formation of reactive oxygen species (ROS). Metabolism of surfactant by the organism may produce highly reactive oxygen species which may cause the damage to the organism [61]. DNA damaging effect of 4-NP must be due to its micro tubular disrupting activity [62] or due to its biotransformation into reactive intermediates which may cause changes at the DNA level. Nonylphenol is reported to induce DNA adduct formation and mutation or genomic rearrangements [62, 63] demonstrated that 100  $\mu$ g/ml concentration induce apoptosis by causing DNA breaks in bovine sperm cells as well as oocyte maturation showing adverse effects on integrity of sperm DNA and oocyte maturation. Increased DNA damage may also led to apoptosis and apoptosis was observed in fish sertoli cells when exposed to nonylphenol [64, 65, 66]. [67] revealed the toxicity of nonylphenol and octylphenol in fish *Claris garipinus* by using hematological, biochemical and hormonal effects. The exposure to NP and OP led to anemia, increased leukocyte count along with bilirubin followed by reduction in protein content. On the other hand the toxicity of para nonylphenol was revealed by [68] using male *Mus musculus* as animal model. The group treated with para-nonylphenol showed reduction in body weight; along with these RBCs, Hb, neutrophils and monocytes were found to decrease significantly, while WBCs and lymphocytes increased significantly after 30 and 60 days of exposure. [69] investigated the haematological profile of fish, *Channa punctatus* after exposure to 4-nonylphenol for 96 hours and

found that values RBC count, Hb, PCV, MCH, MCHC gets decreased after exposure while value of WBC count was found to increase. This is an indication of disruptive effects of 4-nonylphenol on erythropoietic tissues as well as cells viability <sup>[70]</sup>. It is also possible that 4-nonylphenol adversely suppressed fish osmoregulation. The disturbed osmoregulation may finally results in dilution of blood. Other reasons for anemia may be altered membrane permeability or increased mechanical fragility or may be defective iron metabolism or intestinal uptake of iron due to mucosal lesions. NP has higher affinity for membrane phospholipids, which accounts for its lytic activity <sup>[71]</sup>. Chronic effect of nonylphenol and ethinlestradiol for 70 days was evaluated for histopathological and hematological alterations in *Cyprinus carpio*. Severe anemia was observed after both the treatments. Histological alterations were found in kidney, liver and spleen after treatment with ethinlestradiol, while NP exposed fish did not show any tissue lesions <sup>[71, 72]</sup> tested the effect of 4-nonylphenol on hormonal balance and histopathology of endangered Caspian brown trout. They observed that no significant effect was seen on male plasma TSH levels where as in female NP reduces significantly TSH level. Various histopathological changes were observed in gill and intestine tissue.

<sup>[73]</sup> evaluated the pathological alterations in liver tissue of fish, Nile tilapia exposed to 4-NP for long duration starting from beginning of life and during the sexual maturity. Larvae and mother were exposed to different concentrations of 4-NP. Both liver and muscles showed increased bioaccumulation as compared to control group. Liver showed lysis, loss of nuclei, necrosis and fatty infiltration. The studies done by <sup>[74, 75]</sup> revealed the histological changes observed in different fish species after treatment with 4-NP. Higher nephrotoxicity as compared to hepatotoxicity were observed by <sup>[76]</sup> in Zebra fish after 4-NP exposure. The surfactants are known to exert antimicrobial activity by binding to various proteins and phospholipids of membranes. Binding leads to increase in the permeability of membranes and formation of vesicles, causing leakage of compounds with low molecular mass. This results in cell death due to the damage through loss of ions or amino acids <sup>[77]</sup>. Increased ion permeability and sodium efflux of gill has been reported for rainbow trout exposed to 4-nonylphenol <sup>78</sup>. NP found to adversely affect the active transport of calcium to sarcoplasmic reticulum and cause cell death <sup>[79, 80]</sup>. <sup>[81]</sup> studied the histopathological alterations in *Clarias garipinus* when exposed to 4-nonylphenol in gill, skin and kidney for 15 days. Gill was found to have epithelium lifting, edema, deformed secondary lamellae, while skin showed ruptured epithelial cells and enlarged mucus cells, necrotic cells, granulated cells and vacuoles. Kidney showed degeneration, rupture of Bowman's capsule, necrosis, pyknosis, proliferation of renal tubules and haemopoietic tissue.

<sup>[82]</sup> studied the combined effect of diazinon and 4-nonylphenol on *Daphnia pulex*. They observed a significant effect on the swimming behavior of *D. pulex* and the effect was concentration and time dependent. The 4-NP was found to enhance the toxic effect of insecticide diazinon. <sup>[83]</sup> observed number of behavioural changes in fish *C. punctatus* when exposed to different concentrations of 4-NP. <sup>[84]</sup> have reported the deleterious effects of 4-NP on central nervous system as well as neuroendocrine homeostasis and cognitive functions. Inhibition of AChE activity due to the exposure of 4-NP activity in *Mytilus galloprovincialis* has been reported by <sup>[85]</sup>.

So the abnormal behavior shown by fish may be due to abnormal level of neurotransmitters.

## 6. Conclusion

Conclusively our study indicates that 4-NP is genotoxic, haematotoxic and cause histopathological alterations to fish and other organisms. It is pervasive in the environment further its use is increasing day by day in developing countries like India, so there is a dire need of specialized research to rescue the polluted habitats and also to explore the alternatives for minimizing its ill effects. On the other hand future research is needed to know the mechanism of toxicity and also to explore the safer alternatives of these toxic surfactants to be used in various industries. A novel solution should be found for elimination of alkylphenols from water as well as from real waste, which should be technically feasible, efficient, economic and nature friendly. For this a multidisciplinary approach is required to adopt with microbiologist, biologist, chemist and engineers working together towards a common goal.

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