Discussion
CHAPTER - V  
DISCUSSION

The studies on effects of metal and metal oxide nanoparticles of personal care products discharged through domestic wastewater on freshwater fish *Labeo rohita* analyzed for short-term (96 h) and long-term (30 days) periods and the damage caused in the Hematological, Histopathological, Enzymological and Bio accumulation of nanoparticles in the tissues of gill, liver and kidney were studied by TEM/EDX. Moreover the concentration of elements present in the wastewater is analyzed by ICP-AES. The present study show that toxicity data of PCPs contains domestic wastewater is less toxic to *Labeo rohita* when compared to other toxicants like pharmaceuticals, pesticides, insecticides and heavy metals. However the bio accumulation study shows significant changes in the tissues of gill, liver and kidney.

Preliminary toxicological data suggested that metal oxide NPs were being called for a warning to possible sub-lethal or long-term toxic effects (Handy *et al.*, 2008a; Hao *et al.*, 2009; Zhao *et al.*, 2011). Hence, the 30-days exposure duration was chosen not only to enable some obvious physiological or biochemical responses, but also to consider the ethical constraint of using the minimum experimental period likely to achieve the most scientific objectives.

5.1. Hematology

To the best of our knowledge, no data was available on the effects of hematological parameters by nanomaterials. Few studies were carried out on the toxicity of different types of nanoparticles but not much data available on nanoparticles of personal care products. We have described the first detailed report on the toxicity of metal and metal oxide nanoparticles of personal care products to the freshwater fish *Labeo rohita*.

Blood parameters can be useful for the measurement of physiological disturbances in stressed fish and thus provide information about the level of damage in the fish. (Tavares-Dias and Moraes 2006). As hematology has been used to assess the health status of most animals, several studies have shown hematological changes in fish exposed to
numerous stress agents, including those relating to current aquaculture practices. (Mattsson et al., 2001; Affonso et al., 2002; Cerquire and Fernandes 2002) reported that hematological parameters allow the most rapid detection of changes in fish. Disrupted hematological patterns appear very quickly and precede changes in fish behavior and visible lesions. Evaluation of toxic effects of the metals is facilitated by results of the basic hematological assays (RBC, WBC, Hb, Hct, leucocyte and erythrocyte appearance).

In the present study, the results of hemoglobin level (gm/dl), for the short-term and long-term studies showed there were no considerable changes in control and treated. All the values are highly significant at P<0.001 level except 24 h. During long-term exposure the hemoglobin value increased in 20th day. On the whole study we found a fluctuation in hemoglobin level. Several authors have observed an increase in the hemoglobin content in fish when exposed to heavy metals. The hyper activity may be due to hypoxia faced by the fish due to gill damage by the irritants and increased hemoglobin content may be a response to compensate impaired respiratory efficiency.

Similar study conducted in the same fish Labeo rohita exposed to silver nanoparticle by Vignesh et al. (2013), they observed a fluctuation in hemoglobin content. The fluctuation in hemoglobin content is may be due to in blood orally absorbed silver from nanoparticles can enter the blood circulation and be distributed to other organs. Since there is no much report for soluble silver, it is to be assumed that these effects are due to particles as opposed to ionized silver. The effects from AgNPs may begin from undeviating effects of nanoparticles (Choi et al., 2010).

Smith et al. (2007), investigated the effects of single walled carbon nanotubes (SWCNT) to rainbow trout (Oncorhynchus mykiss), who reported that SWCNT did not cause any major disturbance to hematology, although a few individual data points were statistically different from the freshwater control. Notably, exposure to the highest SWCNT concentration caused a statistically significant decrease in haematocrit and whole blood hemoglobin content by day 10 compared to the freshwater control. Mean erythrocyte hemoglobin content (MEH) was not affected, except for a statistically significant decrease in MEH at the highest SWCNT concentration compared to the solvent control at day 10. The WBC counts tended to decrease with time, but there was no
treatment effect compared to controls. There were no effects of the solvent on hematology, apart from a small transient decrease in haematocrit and red cell counts on day 4.

Boyle et al. (2014) reported that exposure to SWCNT did not affect hematology in rainbow trout. Neither the haematocrit nor the concentration of hemoglobin in blood was significantly different between controls and fish exposed to SWCNT.

Increases in hematocrit values were associated with osmotic shifts, as the pH of the blood decreased, erythrocytes swelled and plasma volume decreased. Folmar (1993). In our present investigation hematocrit values were more or less similar in short-term and long-term exposure when compared with control groups. In short-term exposure the hematocrit values increased up to 48 h and decreased in 72 and 96 h gradually. In long-term exposure hematocrit values increased gradually up to 20th day and decreased in 30th day.

In the present study erythrocyte counts were gradually increased in short-term exposure, in contrast the erythrocyte counts were gradually decreased in long-term exposure.

Erythrocyte level was found to be depressed in fishes subjected to stressful conditions (Natarajan, 1981; Ahmad and Munshi 1989; kumar and Barathwal 1991; Hota, 1995). Toxicant caused hemolysis in fish has been demonstrated by Nwude and Pearson (1978). The reduction in RBC may be caused either by the inhibition of erythropoiesis or by the destruction of red cells (Sakthivel, 1988).

The reduction in number, increase in surface area and distorted shape of RBCs in ammonia exposed fish Cirrhinus mirgala may have resulted from the hypoxic condition (Jeney et al., 1992; Das et al., 2004). Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage and nature of the changes shows a release of erythrocytes from the blood depots (Drastichova et al., 2004).

Leucocytes are involved in the regulation of immunological function and their numbers increase as protective response in fish to stress (Witeska, 2001, 2004; Joshi et al., 2002; Nussy et al., 2002). Nelson and Rani (1992) reported that the strength of toxicant and the count of leucocytes are directly proportional.

In our present investigation the WBC count gradually increases both short and long-term exposure. Increase in WBC count in fishes exposed to chronic and lethal doses.
indicates leucocytosis (Garg et al., 1989). McLeay and Brown (1974) found a significant increase in WBC count in *Onchorhynchus kisutch* exposed to bleached kraft pulpmill effluent and suggested that leucocytosis probably due to the tissue damage and subsequent removal of cell debris. Sen. (1992) reported significant increase in WBC count in *Channa punctatus* exposed to sublethal does of zinc. They reported that the increase in WBC count may be due to leucocytosis, which is an adaptation to meet stressful condition by animal.

Fink and Salibian (2005) reported that WBC increase could be due to an induced proliferation as a result of the metal’s toxicity of pluripotential hematopoietic cells that in turn may be consequences of a depletion circulating differentiated cells.

In the present investigation the hematological indices MCV, MCH and MCHC also studied. During short-term exposure the MCV and MCH values were decreased. In long-term exposure they were increased till 20th day and decreased in 30th day. But the MCHC value was increased in short-term exposure. In long-term exposure the MCHC value was increased till 20th day and decreased in 30th day.

Reddy and Bashamohideen (1989) reported that the increase in the MCV and MCH in carp may be due to symptoms of hypochromic microcytic anaemia, accompanying iron deficiency in the body.

Nikinmaa, (1982) reported that lowered MCHC, elevated hematocrit denotes the swelling of erythrocytes a mechanism by which blood O2 transport capacity is increased when fishes were subjected to less effective gas exchange. Besides, lowered MCHC might result from the release of young erythrocytes containing less hemoglobin than the older ones into the circulation.

5.2. Enzyme studies

When foreign chemicals and changes in physical environmental factors such as temperature, dissolved oxygen activity, and TDS occur, there may be inhibition or acceleration of the catalyzed reaction rates controlled by enzyme activity. The mechanisms are described as due to changes in the enzyme quantity or to a direct effect on the enzyme molecule (Heath, 1995). This means that the enzyme chosen as a biomarker of water
pollution should be sensitive to toxicants present in small concentrations and should respond faster than the organism as a whole (Giesy et al., 1988).

The gills are the initial target sites for many toxicants due to their close association with the external environment. Pollutants such as metals are known to disturb gill function at low exposure concentrations and to cause respiratory distress at high concentrations (Playle and Wood 1989). The use of gill-related enzymes as biomarkers of pollutants seemed suitable for the identification of pollution of the water body before irreversible damage occurs. Again the enzymes are not pollutant specific, making fluctuations in the enzyme activities impossible to link to a specific kind of pollution.

In the present study, the significant increase and decreases values of GOT and GPT activity in gill and liver was observed when the experimental fish *Labeo rohita* exposed to nanoparticles of personal care products discharged with domestic wastewater. During short-term exposure we found significant decrease in GOT activity in gill but the GPT activity was increased. In long-term exposure there was a fluctuation of GOT and GPT activity in gills. In the case of liver, GOT and GPT activities were increased in short-term exposure. During long-term exposure GOT activity in liver was increased up to end of 20 days and suddenly decreased at end of 30th day. But the GPT activity was gradually decreased.

Vignesh et al. (2013) reported that the activity of GOT, GPT was measured in *Labeo rohita* tissue damaging enzymes of GOT and GPT were significantly lower in the administration of leaf extract, leaf extract with silver nanoparticles, and silver nanoparticles, when comparing with the administration of silver nitrate solution. The activity of GOT and GPT was noticed much higher in the vital tissue of liver than gill and kidney.

In the present investigation, the significant increase and decreases values of GOT and GPT activity in gill and liver was found. The increasing level of these enzymes in blood is an indication of liver damage (Bouk et al., 1978). In hepato toxicity studies, GOT and GPT are known to be a predominant biomarker (Karthikeyeni et al., 2013).

Elevation in GOT activity may indicate muscle damage especially cardiac muscle, where as higher GPT activity indicates damage in liver cells. Damage in liver, kidney and gills is evident from elevated transaminase activities (Bernet et al., 2001).
Manavalaramanujam and Ramesh (1996) reported that the elevation of GOT and GPT activity in fish indicate cumulative toxicity of the toxicant or possibly to meet the increased energy demand under sustained and prolonged toxic stress. Sivakumari et al. (1997) reported that the inhibition of GOT and GPT activity in fish indicate enzyme loss from soluble region of the hepatocytes and not from the mitochondrial fraction.

Adenosine triphosphate (ATP) is the primary energy source for nearly every energy-requiring process in the body. (Barnhoorn and Vuren 2004).

\( \text{Na}^+/\text{K}^+ - \text{ATPase} \) is a very important enzyme to sustain the inner equilibrium of cell and responsible for regulating the \( \text{Na}^+ \) and \( \text{K}^+ \) level in the body (Federici et al., 2007).\( \text{Na}^+/\text{K}^+ - \text{ATPase} \) molecules which are located at the basolateral membrane of the gill epithelium of freshwater fish plays a direct role in active transport of \( \text{Na}^+ / \text{Cl}^- \) from the water into the extra cellular fluid. Aquatic pollutants could alter the gill \( \text{Na}^+/\text{K}^+ - \text{ATPase} \) activity on direct interaction with the enzyme or through disruption of energy producing metallic pathways (Watson and Beamish 1980). Aquatic pollutants may separate the enzyme complex, which may induce allosteric change resulting inhibition of ATPase activity. The mechanism used to adapt to hyper osmotic environment and primary lethal lesions in gills could be the reason of decrease in \( \text{Na}^+/\text{K}^+ - \text{ATPase} \) activity. Furthermore, aquatic pollutants may affect the ATPase system by partitioning in the enzyme complex resulting inhibition of ATPase activity (Reddy et al., 1992). Toxic substances in the aquatic environment may alter the structure or permeability of gill membrane of fish resulting alterations in the branchial ionoregulation or osmoregulatory dysfunction (McCarty and Houstan et al., 1976).

In the present investigation, there was a gradual declination of \( \text{Na}^+/\text{K}^+ - \text{ATPase} \) activity with increasing exposure duration, these significant decreases were observed throughout the study of both short and long-term exposure in gill \( \text{Na}^+/\text{K}^+ - \text{ATPase} \) activity of the freshwater fish \textit{Labeo rohita} when exposed to nanoparticles of personal care products discharged with domestic wastewater.

Similar result reported by Hao et al. (2013), when ZnO NPs and bulk-ZnO caused significant decreases in \( \text{Na}^+/\text{K}^+ - \text{ATPase} \) activity in gill and liver tissues of \textit{Cyprinus carpio}.

The inhibition of \( \text{Na}^+/\text{K}^+ - \text{ATPase} \) in gills probably disturbs \( \text{Na}^+ \), \( \text{K}^+ \) pump, resulting in an erratic entry of \( \text{Na}^+ \) into the cell along the concentration gradient and the
water molecule follows along the osmotic gradient. This process may cause swelling of the cell and finally membrane ruptures (Oruc et al., 2002).

Similar result reported by Griffitt et al. (2007) exposure to copper nanoparticles caused inhibition of Na+/K+ ATPase activity in the gill cells of Zebrafish (Danio rerio).

Chen et al. (2011) reported that TiO2 NPs caused statistically significant decrease in Na+K+-ATPase activity in the gills and intestine as well as a trend of decreasing enzyme activity in the brain.

Wang et al. (2014) The Na+/K+ -ATPase activity in the liver and gills of on juvenile Epinephelus coioides exposed to copper NPs and copper sulphate was decreased with an increase in CuSO4 or Cu-NPs dose, and was significantly inhibited at 100 µg Cu L−1 as CuSO4 or Cu-NPs compared with the control.

In contrast Smith et al. (2007) reported that the SWCNT exposure caused statistically significant increases in Na+/K+ -ATPase activity in the gills and intestine of rainbow trout (Oncorhynchus mykiss).

The inhibition in the Na+/K+ -ATPase activity in gill may also be due to primary lethal lesions in gills. Inhibition or stimulation of ATPase activity could be expected to have metabolic or ionic effect in fishes in relation to osmoregulation (Gopal et al., 1993; Parvez et al., 2006).

5.3. Histopathology and Uptake of NPs

Histological analyzes have been extensively used tool in monitoring of fish exposed to contaminants and showing the initial signs of lesions or alterations not easily identifiable during the morphological or macroscopic examination. And, in this study, the changes of structure and physiology of gill, liver and tissues could be regarded as the effective indicators of water contamination and the degrees of toxicity to fish induced by NPs of personal care products.

In the present investigation transition electron microscopy also used to identify the uptake of NPs into the cells. In addition EDX spectrum showed the elemental percentage in the tissues of gill, liver and kidney of the freshwater fish Labeo rohita exposed NPs of PCPs contains wastewater.
Histopathology of gill

It is well known that gills are important respiratory organs and participate in many physiological activities, including metabolites excretion, body fluid permeability balance and acid–base regulation balance, which were vulnerable to water pollution. Hence gill is the major target organ for chemical pollutants to elicit toxic effects. Previously, some researchers defined two types of gill injuries: the first type of injury results from defense response, including hyperplasia of the gill filaments epithelium, oedema of gill lamellae; the second type is the direct injury, including necrosis and shedding of gill epithelium (Richmonds and Dutta 1989; Fanta et al., 2003; Cengiz and Unlu 2006).

Gill plays a primer role in respiration, and its direct contact with aquatic environment makes it an important tissue for histopathology study. The histological examinations have demonstrated that exposure to NPs resulted in obvious hyperplasia of the gill filaments epithelium and oedema of gill lamellae of carp (Hao et al., 2009), zebrafish (Griffitt et al., 2007; Chen et al., 2011) and rainbow trout (Federici et al., 2007).

In the present investigation during 96 h period the damage was high as compared to other exposures. In this period the overlapping of secondary lamella, detachment of secondary lamella, degeneration of primary lamella and haemorrhage were observed in the treated fish. In addition fusion of adjacent secondary lamellae and necrosis in the primary lamellae were also observed. At the end of 10th day haemorrhage, degeneration of primary lamella, overlapping of secondary lamellae and detachment of secondary lamella were observed. During day 20 and 30 the damage was decreased compared to 96 h duration. During day 20 and 30, necrosis in the primary lamellae, epithelial lifting, necrosis and curling of secondary lamellae were identified.

Smith et al. (2007) reported that the histological examination of the gills at the end of the experiment showed normal anatomy in the freshwater controls, with a normal background incidence of injuries on <4% of the secondary lamellae. Exposure to SWCNT resulted in some increases in the incidence of oedema in the secondary lamellae, changes in mucocyte morphology, and hyperplasia in the primary lamellae of rainbow trout.
The swollen and disrupted gill cells exposed to ZnO NPs might reduce the contact surface and affect the exchanges of air and ion. And, some black blocks accumulated on the mucus of chloride cell, which was suspected as ZnO NPs aggregates, showing that ZnO NPs might directly enter into the fish body through the injured epithelial cell membrane and induce the undesirable toxic effects. (Hao et al., 2013).

Farmen et al. (2012) reported that the commercial Ag NP suspension caused acute gill lamellae necrosis at high concentrations (100 µg/L) to juvenile Atlantic salmon exposed to Ag nanoparticles.

The histopathology was examined in the target tissues of carp after 30 days of exposure. Under TEM examination, the control fish possessed normal cytological structures of gill tissue including epithelial cell and chloride cell with intact cell membranes, nuclei and organelles and normal appearance of the cytoplasm. The bulk-ZnO exposure caused some cytological changes including irregular cell outlines, abnormal pyknotic nuclei, shrinkage or loss of cell cytoplasm. However, more remarkable pathologies with exposure to ZnO NPs such as atrophic and damaged cell membrane, swollen and distorted organelles and a trend of total disruption of gill cells could be observed in this study. Additionally, some epithelioid granulomas were found to appear on the epithelial cell exposed to ZnO NPs (Hao et al., 2013).

Griffitt et al. (2007) reported that exposure to copper sulfate and nanocopper suspensions caused damage to gill lamellae characterized by proliferation of epithelial cells as well as edema of primary and secondary gill filaments. Effects of nanocopper suspensions were dose dependent, with significantly greater damage observed at higher concentrations. No significant histological evidence of injury was observed in major internal organs, though all fish had some degree of vacuolation of the liver. There was no hepatocellular necrosis and eosinophilic vacuolation was minimal and no significant difference between livers in the control and treated fish were detected.

Chen et al. (2011) reported that the prominent accumulation of TiO₂ NPs was observed in the gills of zebrafish. A hyperplasia like thickening of the primary lamellae was found in the gill filaments after the treatment of TiO₂ NPs. Hyperplasia of the gill epithelium was commonly observed in foci of oedema. The gill injuries aggravated with
the increasing TiO$_2$ concentration in aqueous solution. These symptoms were not observed in the control fishes. External histological examination indicated that no significant abnormal histology alteration from gross anatomy was found in the livers, hearts and brains. Furthermore, there was no evidence showed gross inflammation, vacuolation, oedema, cellular atrophy, or necrosis in these tissues.

Boyle et al. (2014) reported that waterborne exposure to SWCNT did not cause acute gill pathology in rainbow trout. At the end of the exposure (day10), fish examined from each of the treatment groups showed normal gill morphology with a few incidences of gill pathologies, including club tips, epithelial lifting and fusion of the secondary lamellae.

Chen et al. (2011) reported, the histological examination demonstrated that exposure to TiO$_2$ NPs resulted in the obvious hyperplasia and gill lamellae, which belonged to the defense response injury. With the increasing concentration (5.0, 7.0mg L$^{-1}$) of TiO$_2$ NPs, some areas of lamellae were fused and disrupted in filaments because of serious hyperplasia that led to significant direct injury.

In previous studies, the short-term exposure to TiO$_2$ NPs displayed the lower toxicity in zebrafish than other NPs, such as nanocopper or nanosilver (Grosell et al., 2007).

Due to the accumulation of harmful substance in gills, the efficiency of gills in gas exchange decreases, which result in subsequent reduction in respiratory efficiency and deleterious effects on circulatory system. These toxic responses affect the normal physiological function and endanger the health of fish, even lead to the death of fish (Cengiz and Unlu 2006; Liao et al., 2006).

Previous studies investigating the effects of TiO$_2$ nanoparticles on rainbow trout have done so by exposure via the water column and via the diet (Federici et al., 2007; Handy et al., 2008c; Johnston et al., 2010; Moger et al., 2008). In some of this work, uptake of nanoparticles from the water into gill was shown to occur at very low levels using the imaging technique of Coherent Anti- Stokes Raman microscopy (Moger et al., 2008). In another study moderately toxic effects were reported, which included decreases in Na$^+$/K$^+$ATPase activity in the gills and intestine, increases in analysis of thiobarbituric reactive substances (TBARS) in the gill, intestine and brain and increases in the total
glutathione levels in the gills, (Federici et al., 2007). The present study also represents significant changes in the tissues of histological organs.

**Histology of the liver**

The liver tissue of fish is an important organ of active metabolism and detoxification and extremely sensitive to pollutants. Extraneous xenobiotic compounds biotransformations occur in liver (Brusle and Anadon 1996).

Linhua et al. (2009) reported that the exposure to TiO$_2$ NPs caused cellular pathologies to liver with the more severe changes observed at higher TiO$_2$ NPs concentrations. The liver of the control group showed normal histology such as the regular hepatocytes and integrated nucleus and clear boundaries between cells, and the livers of the fish exposed to TiO$_2$ NPs showed characteristic cytoplasm vacuolation and apoptosome including necrotic cell bodies and nuclear fragments which appeared to be apoptotic bodies, and a few foci of lipidosis with minor fatty change, mainly at high TiO$_2$ NPs concentrations.

In this present study at the end of 10$^{th}$ day we found several changes in the liver tissues of *Labeo rohita*, a marked change in fat infiltration, though the size of the hepatocytes was decreased when compared to control. Kupffer cell prominence was also noted due to inflammation. Sinusoidal hypertrophy persisted; the number of binucleated cells became reduced when compared to control, showing regeneration of cells. Central vein was reduced considerably in diameter when compared to control.

At the end of 20$^{th}$ day, hepatocytes had regained their structure partially as still individual cellular demarcation was lost compared to the control. Sinusoidal spaces were still filled with fat at many places; however, hepatic cord arrangements became normal. The number of Kupffer cells was reduced and the fat distribution within the hepatocytes was reduced. Central vein diameter reversed to normal size. The number of Kupffer cells was reduced and the diffused fat distribution along hepatocytes was lost when compared to control. In 30$^{th}$ day, hepatocytes damage such as necrosis was still persistent in few areas. Kupffer cell prominence was also noted. Negligible lipid deposition was still persistent in sinusoids. The number of binucleated cells was considerably reduced.
Absence of cellular demarcation was still persistent in some places. The fatty infiltration was reduced in the sinusoids spaces and was mobilized in the large vessels only.

Hao et al. (2013) reported that the liver section of the control fish showed normal hepatocyte structures such as intact cytoplasm and well-developed organelles as well as normal appearance of the cytoplasm. The bulk-ZnO group caused some pathologies including cell edema, nuclei deformation, hyperplasia swelling of rough endoplasmic reticulum and abnormal mitochondrial structure, but cell membrane was still intact. TEM observation revealed that injured hepatocytes of the ZnO NPs exposure group exhibited more distinct pathological changes such as vacuolization, apoptosis, cell membrane rupture, desquamation of hepatocytes, even severe cellular necrosis. Congestive enlargement of lysosomes led to vacuolar degenerations in liver sinusoids which hindered the rapid exchange of molecules between blood vessels and surrounding tissues through their small pores.

The liver of fish is an important tissue of active metabolism and detoxification and extremely sensitive to pollutants. Federici et al., 2007; Hao et al., 2009; Smith et al., 2007) reported that the livers of some fish exposed to TiO₂ NPs and single walled carbon nanotubes (SWCNTs) showed condensed nuclear bodies and minor fatty change. Hao et al., (2013) reported that exposure to ZnO NPs caused the cellular pathological changes in liver tissue.

The liver of fish from the freshwater control and solvent controls showed normal histology, and apart from some foci of lipidosis during SWCNT exposure there were no signs of fatty change that could be associated with oxidative stress. However, all fish from the 0.25 mg l⁻¹ SWCNT treatment showed changes to nuclear morphology with condensed nuclear bodies that have the appearance of apoptotic bodies, and cells also show nuclear division with condensed nuclear material. This was also evident to a lesser extent at the highest SWCNT concentration.

**Histology of Kidney**

In the present investigation at the end of 10th day, there was marked shrinkage and degeneration in the tubular elements namely, proximal tubules, distal tubules as well as Henle’s loop and collecting tubules. Fat deposition was seen invariably in all tubules so that the entire region showed opacity. degeneration appears in the basal parietal cells
around Bowman’s capsule. There was hyperplasia of mesangial matrix with tufts of glomerulus leading to decrease Bowman’s space and the glomerular apparatus was filled with fat and showed opacity. Larger blood vessels in the kidney showed lipid deposition. Lipid droplets appeared as round the goblet cells for forming foam cells. Macrophage infiltration was more in the areas where fat had accumulated. During 20th day, the mesangial matrix showed shrinkage. All the blood vessels were still clogged with fat as plaques in many regions and prominent so that the opaqueness persisted. Blood vessel congestion was noted. Still opaqueness persisted. In 30th day, there was hyperplasia of tubules; mesangial matrix became hyperplastic with reduction in Bowman’s space. Regeneration of proximal convoluted tubules (PCT) in many places was noted. All the blood vessels were dilated and filled with fat.

Similarly Al-Bairuty et al. (2013) reported that some damage to the epithelial cells of the renal tubules, changes in the Bowman’s space, and an increase in the foci of melanomacrophage deposits when Rainbow Trout exposed copper sulphate and copper NPs.

Boyle et al. (2014) reported that exposure of waterborne SWCNT to rainbow trout, the injuries sustained included the mild necrotic degeneration of epithelium cells in some of the renal tubules, which was observed in half (n = 4/8) of the fish examined. There was also evidence of mild glomerular necrosis, some glomeruli surrounded by an increased Bowman’s space and evidence of increased extracellular space between the renal tubules in the cortex. Blood cells were also present in this space around the tubules of half (n = 4/8) of the fish examined, indicating some local blood loss to the extracellular space. However, these minor injuries did not cause any overall change in the mean diameters of the renal tubules or glomerulus in the kidneys of any treatment.

Scown et al. (2009) reported that, In the first injection study, the kidneys were found to be the main target organ for accumulation of titanium, with a lower level of accumulation in the liver. Six hours after injection, 10–19% of the total burden of injected titanium was found in the kidneys and this level persisted up to three weeks after injection. TEM imaging of the kidneys of trout sampled at 21 days revealed clusters of nanoparticles in the hematopoietic cells surrounding the kidney tubules and in some cases these clusters were compartmentalized in lysosomes. After 90 days, however, the level of
titanium in the kidney was significantly lower (49–57%) than the concentrations seen at 6 and 12 h and at 504 h, suggesting that the titanium was beginning to be eliminated from the kidney. The level of titanium did not significantly change in the liver throughout the study period (6 h to 90 days). These data suggest that approximately 20% of nanoparticles persist in the hematopoietic tissue of the kidney for periods of weeks and do not reach the kidney tubules to be excreted in the urine. After a prolonged period, however (90 days), there was evidence of some elimination of the TiO$_2$. It is not clear whether this elimination was related to an active excretion of the TiO$_2$ or simply due elimination of the TiO$_2$ along with dead cells during normal tissue cell turnover. Little is known, however, about the turnover time for kidney cells in fish.

The kidney of rainbow trout also has a role in maintaining blood volume by producing dilute urine to compensate for the general osmotic influx of body water. Some minor pathologies were occasionally observed in kidney of fish exposed to SWCNT compared to control groups Boyle et al. (2014).