African Journal of Biotechnology Vol. 8 (11), pp. 2640-2644, 3 June, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

The effects of sodium perchlorate on the liver of Molly Fish (*Poecilia sphenops*, Cyprinidae, Teleostei)

Kolbaşı Tekkan Burcu*, İşisağ Üçüncü Sema and Önen Özlem

Ege University, Faculty of Science, Department of Biology, 35100 Bornova, Izmir, Turkey.

Accepted 13 March, 2009

Adult male molly fishes were reared up to ten days in control water or in water containing sodium perchlorate at concentrations of 1, 5, 25 and 125 ppm. Remarkable steatosis, fibrosis, hyperemia and necrosis were distinguished in parallel with increasing sodium perchlorate concentrations. The striking cellular damages were observed especially in 25 and 125 ppm sodium perchlorate exposed animals. The exposition to 25 ppm sodium perchlorate induced hepatocellular breakdowns and nuclear polymorphism. In 125 ppm sodium perchlorate exposed fish, hyperemia and widened necrotic areas were discovered. The results were evaluated as being the first documentation of histopathological effects of sodium perchlorate on teleost liver.

Key words: Perchlorate, molly fish, liver, histopathology.

INTRODUCTION

Perchlorate is a persistent environmental contaminant and largely due to widespread military use as a propellant (Urbansky, 1998). The main target organ of perchlorate is thyroid. Undoubtedly, it inhibits iodide uptake by the thyroid follicles, thus impairing the production of thyroid hormones (Crane et al., 2005; Liu et al., 2006), which regulate the basal metabolic rate of all cells. Although it was noted that exposure to perchlorate causes kidney lesions and developmental abnormalities in zebrafish (Capps et al., 2004), its effects on other physiological systems and organs of the teleosts are not investigated in detail.

Because of the liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health (Myers et al., 1998), and exhibit the effects of a variety of environmental pollutants (Hinton et al., 1992). Moreover, the liver has play a major role in complex enzymatic processes of tetraiodothyronine (thyroxine)-tri-iodothyronine (T_4 - T_3) conversion. The metabolic rate of hepatocytes is certainly modulated by thyroid hormones. Thyroid dysfunction may perturb liver function, and liver disease effects thyroid hormone metabolism (Malik and Hodgson, 2002). Therefore, clearly more attention has to be paid on the functions of liver when affected by perchlorate. In the present study, we aimed at elucidating the acute toxic effects of sodium perchlorate (SP) on liver from a freshwater fish, molly fish *Poecilia sphenops.*

MATERIALS AND METHODS

Twenty-five adult male molly fish (average weight 0.45 ± 0.05 g and length 32.70 ± 0.9 mm) were purchased from commercial dealers, and allowed to acclimate in 20 L aquaria for 30 days. The same conditions for temperature (24 ± 2ºC); salinity (% 0.1), pH (7.5 ± 0.4) and hardness degree (31.0 ± 1 French degree) were maintained, and the photoperiod was set at 15L/9D during the acclimation and experimental periods.

Fish were divided randomly into one control and four test groups, each containing five specimen. Temperature and pH were measured daily. The water quality parameters, which were also measured at the beginning and the end of exposure duration, had been noted as 18 ± 1 mg/L for sodium, 2.9 ± 0.1 mg/L for potassium, 0.030 ± 0.008 mg/L for aluminium, 0.08 ± 0.055 mg/L for iron, and 6 ± 1 mg/L for nitrate.

Sodium perchlorate 1-hydrate (Panreac Quimica SA-PA 134387, CAS Number: 7791-07-3; Eksper Ltd. İzmir, Turkey) was used as the source of perchlorate and was added directly to system water.

*Corresponding author. E-mail: sema.isisag@ege.edu.tr or semaisisag@gmail.com. Tel.: +90 0232 388 40 00 /2403. Fax: +90 0232 388 10 36-388 18 90.

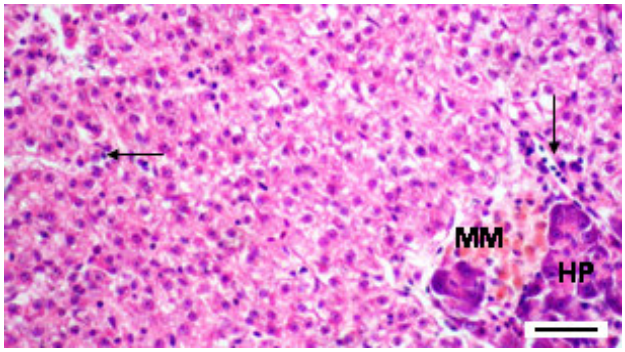


Figure 1. The liver of the molly fish reared in control water, showing hepatocytes with their uniform nuclei, and sinusuids (arrows). Note also the slight vacuolation of the hepatocytes close to hepatopancreas (HP) and melanomacrophage centers (MM); H&E, bar = 20 µm

The nominal perchlorate concentrations were 0 (control), 1, 5, 25 and 125 ppm, and exposure duration was 10 days. Fish were fed once daily with commercial fish food (Sera-San), leftover materials were removed by siphoning every evening, and one-third of the water volume was replaced every morning. At the end of the exposure duration, the entire specimen was sacrificed with MS222 (ethyl-ester.3.aminobenzoic acid, Sigma) and immediately dissected. The liver tissues were fixed in Bouin's solution at room temperature for 24 h. Tissue samples were embedded into paraffin, and at least 15 serial sections (4 to 6 µm) were obtained from each of the fishes. Sections were stained with hematoxylin-eosin (H&E), mounted and examined by light microscope.

RESULTS

The liver of *Poecilia sphenops* reared in control water (Figure 1), was exhibited the typical teleost liver that not organized in lobuli or acini. It was parenchymatous in appearance and mainly consisted of polygonally shaped, eosinophilic stained hepatocytes with their central, round or ovoid nuclei. Sinusoids, which are irregularly distributed between the polygonal hepatocytes, were fewer in number. Hepatopancreatic alveoli of the exocrine pancreas were seen to be placed in the parenchyma, and the melanomacrophage centers stained light-brown in color, were located close to hepatopancreas. Some of the hepatocytes nearby the hepatopancreas were slightly vacuolated, most likely due to storage of the glycogen and lipid.

Fish exposed to all sodium perchlorate (SP) concentrations exhibited fibrosis, presumptive steatosis, hyperemia and necrosis. In general, the degree of the histopathological findings was seemed to be related to the increasing concentrations. The fibrosis was prominently seen in 1 ppm (Figure 2) and 5 ppm (Figure 3) SP exposed fishes, and also distinguished in other experimental groups. The steatosis characterized with intensive hepatocellular vacuolization, was arised in 1ppm exposed fish (Figure 2); pronounced in 5 ppm SP exposed animals (Figure 3) and noted as the most distinctive feature for 25 ppm SP exposed ones (Figure 4). Although a relative decrease in steatosis was distinguished in the 125 ppm exposed fish, the most intensive hyperemia and the most widened necrotic areas were observed in this group (Figure 5).

The striking cellular deformities were seen in both of 25 and 125 ppm SP exposed experimental animals. Individuals exposed to 25 ppm SP were showed a range of state of hepatocellular and nuclear polymorphism (Figure 6).

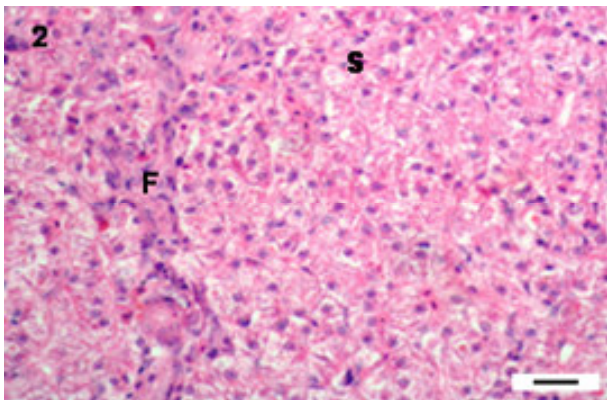
Cellular breakdowns and cytoplasmic vacuolation were distinguished in combination with pyknosis and karyorrhexis. The enlarged, hyperchromatic nuclei was indicated to megalocytic hepatosis. Some eosinophilic clusters were also seen. In 125 ppm SP exposed fish, the noticeable necrotic areas located around the hyperemic hepatopancreas, were characterized with cellular shrinkage (Figure 7).

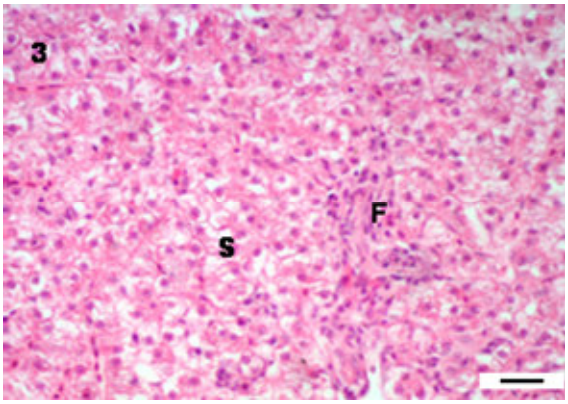
DISCUSSION

Like the mammals, perchlorate causes hypothyroidism in fish, which is arised in many histopathological determinants (Liu et al., 2006). Although the kinetics of uptake and elimination rates of the sodium perchlorate in fillet, gills, gastrointestinal tract, liver, and head of channel catfish, *Ictalurus punctatus* (Park, 2003) and whole body of mosquitofish, *Gambusia holbrook* (Bradford, 2004) were investigated in detail, there is no report about the histopathological effects of perchlorate in the liver of molly fish.

As mentioned above, thyroid hormones regulate the basal metabolic rate of all of the cells including hepatocytes, and the liver in turn metabolizes the thyroid hormones and regulates their endocrine effects (Malik and Hodgson, 2002). By other means, thyroxine and triiodothyronine are essential for normal liver function, and a healty liver is essential for thyroidal metabolism. Although it is not conformable to make a final decision on the mode of action of perchlorate on thyroid-liver axis, the results presented in our preliminary investigation suggest that, at least for *Poecilia sphenops*, acute exposure to sodium perchlorate is likely to affect not only the thyroid (not presented), but also the liver. While our observation remains unexplained at the moment, we could note that this is the first documentation demonstrated the histopathological effects of perchlorate on hepatic tissue of teleosts.

The fibrosis, steatosis, hyperemia and necrosis; which were formed a rectangle of hepatic tissue changes, are similar to those reported for fish caught in contaminated water or exposed ones to various chemicals in laboratory conditions (Brand et al., 2001; Koehler, 2004; Olojo et al.,





Figures 2 and 3. Fibrosis (F) and steatosis (S) in 1 and 5 ppm SP exposed molly fish, respectively. H&E, bar = 20 µm.

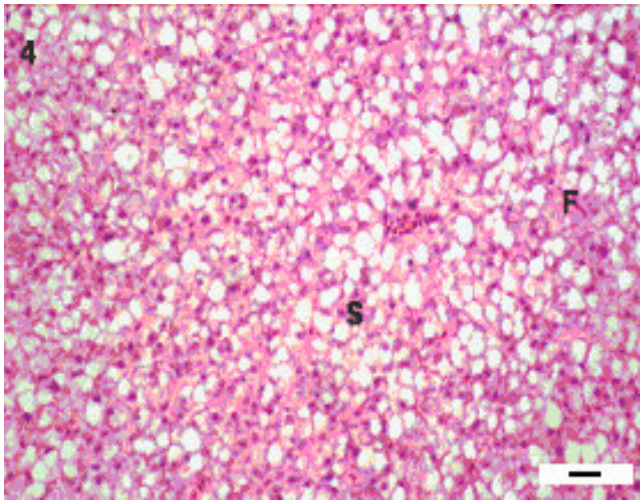


Figure 4. Fibrosis (F) and intensive steatosis (S) in 25 ppm SP exposed fish, bar = 20 µm.

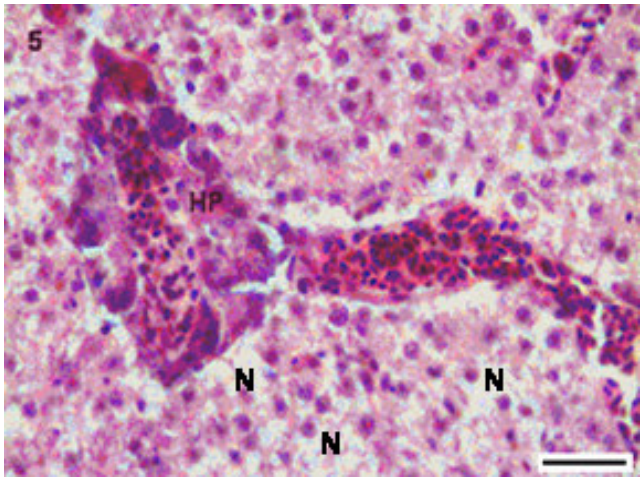


Figure 5. Relatively increased steatosis and necrotic areas (N) in 125 ppm SP exposed fish. Note the hyperemic hepatopancreas (HP) also bar = 20 µm.

2005; Camargo and Martinez, 2007; Wahbi and El-Greisy, 2007; Aniladevi et al., 2008). Fibrosis and local blood congestions in the liver sinusoids of the flounder, *Platichthys flesus*, the ruffe, *Gyrnnocephalus cernua*, and the smelt, *Osmerus eperlanus*, were reported as a consequence of pollution by Peters et al. (1987). Radhaiah and Jayantha (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels, and pyknotic nuclei in the liver of *Tilapia mossambica* exposed to fenvalerate. Tilak et al. (2005) observed the same changes in liver of *Catla catla* exposed to chlorpyrifos; and, Weisman and Miller (2006) noted those kinds of morphopathology as being the symptoms of lipoid liver disease in the sapphire damsel, *Pomacentrus pavo*.

Our observations on the pathological patterns were also in consistent with the report on elimination rate of perchlorate. As noted by Park (2003), perchlorate was not totally eliminated after 10 days, and its slowest rate of elimination was demonstrated in the liver. This is surely due to that the liver is the principal organ of detoxification and excretion.

Spisni et al. (1998) noted that, when compared with naturally fed, artificially fed marine fish showed a more extensive steatosis and a higher mortality rate. In our investigation, no mortality was recorded in control and experimental fish, however, the control ones were expressed just a slight steatosis. An increase in steatosis was distinguished in 1 and 5 ppm SP exposed ones, respectively, and the most distinctive feature of 25 ppm SP exposed fish was intensive steatosis. In the light of these findings, we state that the slight steatosis observed in control fish was due to artificial feeding. By the way, we

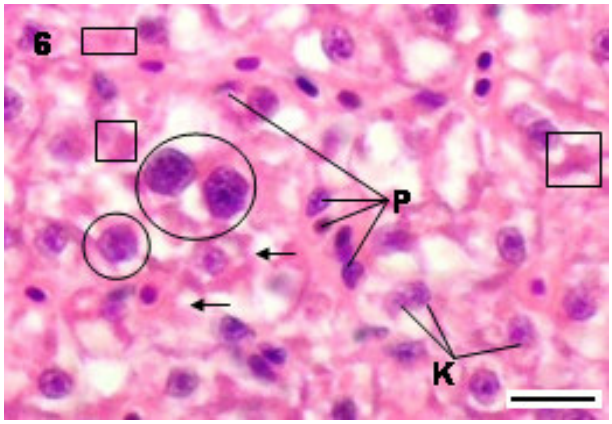


Figure 6. Pyknotic (p), karyorrhexic (k), and enlarged (encircled) nuclei of widely vacuolated hepatocytes in 25 ppm SP exposed fish. Note also cellular breakdowns of the hepatocytes (arrows) and eosinophilic clusters (rectangulated), bar = 20 µm.

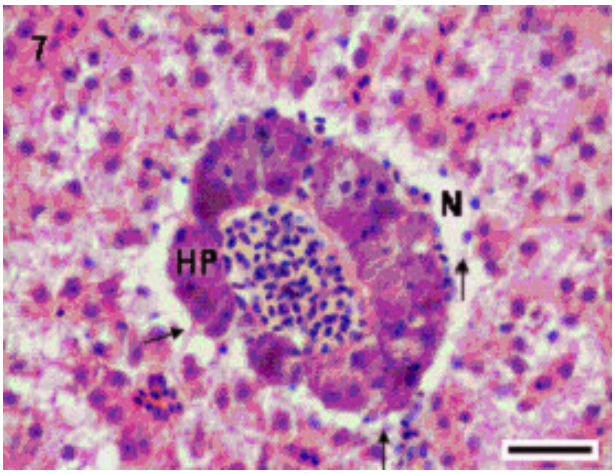


Figure 7. Hepatopancreas (hp) surrounded by necrotic areas (n) including clusters of shrinkage (arrows) in 125 ppm SP exposed fish. H&E, bar = 20 µm.

have to note the unforeseen finding about the relative decrease of steatosis observed in 125 ppm expose animals. When taken together the most widened necrotic areas and the most intensive hemorragie, this finding is not so unexpected in fact. It is strongly possible that, the decreased steatosis is in relation with the pronounced parenchymatous deformation. However, the homeostatic mechanisms, which were not investigated in the present paper, may also have been triggered. One can expect that these mechanisms will be satisfactory for the whole repair of the liver, but we cannot judge that the defectiveness observed in this study were either reversible or not upon removal of the SP from the water.

Nuclear polymorphism that is known to be a precursor of megalocytic hepatosis, could be induced by a wide spectrum of hepatotoxicants and hepatocarcinogens in the sole (*Parophrys vetulus*; Myers et al., 1987), the dab (*Limanda limanda*; Koehler et al., 1992) and the flounder (*Platichthys flesus*; Koehler, 2004). Koehler (2004) has reported that early non-neoplastic lesions were characterised by hepatocellular and nuclear polymorphism in flounder from the highly polluted Elbe estuary. According to the author, cell types including hepatocytes, bile ducts epithelial cells and sinusoidal endothelial cells may give rise to neoplasms in fish liver. Nuclear vacuolization and pyknotic nuclei were also found in *Prochilodus lineatus* (Langiano and Martinez, 2008) affected by a glyphosate-based herbicide. At this preliminary step, we certainly cannot make a conclusion about neoplasmic formation; however, the role of steatosis in the progression of neoplastic lesions had certainly been overemphasized.

Conclusion

Although it is too early to conclude all of the unknown effects of perchlorate, our findings strongly suggest that perchlorate is at least hepatotoxic for *Poecilia sphenops*. Surely more information is needed to decide either sodium perchlorate affects the liver directly or indirectly. By this context, the perchlorate has to be taken into more consideration as an environmental contaminant. Further details should be obtained from advanced investigations.

REFERENCES

Aniladevi Kunjamma KP, Philip B, Smitha V, Bhanu SV, Jose J (2008). Histopathological effects on *Oreochromis mossambicus* (Tilapia) exposed to chlorpyrifos. J. Environ. Res. Develop. 2(4): 553-559.

Bradford CM (2004). Perchlorate uptake and effects on thyroid function in fish. etd.lib.ttu.edu/theses/available/etd-07312008-31295017082008/unrestricted/31295017082008.pdf

Brand DG, Fink R, Bengeyfield W, Birtwell IK, McAllister CD (2001). Salt water-acclimated pink salmon fry (*Oncorhynchus gorbuscha*) develop stress-related visceral lesions after 10-day exposure to sublethal concentrations of the water-soluble fraction of North Slope crude oil. Toxicol. Pathol. 29(5): 574-584

Camargo MMP, Martinez CBR (2007). Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. Neotropical Ichtyol. 5(3): 327-336.

Capps T, Patino R, Blazer V (2004). Kidney lesions induced by ammonium perchlorate in adult zebrafish. http://abstracts.co.allenpress.com/pweb/setac2003/document/29356.

Crane HM, Pickford DB, Hutchinson TH, Brown JA (2005). Effects of ammonium perchlorate on thyroid function in developing fathead minnows, *Pimephales promelas*. Environ. Health Perspect. 113: 396-401

Hinton DE, Baumann PC, Gardner GR, Hawkins WE, Hendricks JD, Murchelano RA, Okihiro MS (1992). Histopathologic biomarkers. In: Huggett RJ, Kimerle RA, Mehrle JR, Bergman HL, Editors, Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress, Lewis Publishers, Boca Raton (1992), pp. 155-209.

Koehler A, Deisemann H, Lauritzen B (1992). Histological and cytochemical indices of toxic injury in the liver of dab *Limanda limanda*. Mar. Ecol. Prog. Ser. 91: 141-153.

Koehler A (2004). The gender-specific risk to liver toxicity and cancer of flounder [(*Platichthys flesus* (L.)] at the German Wadden Sea coast. Aquat. Toxicol. 70(4): 257-276

Langiano VDC, Martinez CBR (2008). Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. Comp. Biochem. Physiol. Cancer Toxicol. Pharmacol. 147(2):222-231.

Liu FJ, Wang JS, Theodorakis CW (2006). Thyrotoxicity of sodium arsenate, sodium perchlorate, and their mixture in zebrafish *Danio rerio*. Environ. Sci. Technol. 40(10): 3429-3436.

Malik R, Hodgson H (2002). The relationship between the thyroid gland and the liver. Quart. J. Med. 95:559-569.

Myers MS, Rhodes LD, McCain BB (1987). Pathologic anatomy and pattern of occurrence of hepatic neoplasms, putative preneoplastic lesions and other idiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. J. Natl. Cancer Inst. 78: 333-363.

Myers MS, Johnson LL, Olson OP, Sther CM, Horness BH, Collier TK, McCain BB (1998). Toxicopathic hepatic lesions as biomarkers of chemical contaminant exposure and effects in marine bottomfish species from the northeastand Pacific Coast. USA. Mar. Pollut. Bull. 37: 92-113.

Olojo EAA, Olurin KB, Mbaka G, Oluwemimo AD (2005). Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to lead. Afr. J. Biotechnol. 4(1): 117-122.

Park JW (2003). Toxicological effects and kinetics of perchlorate on fishes. http://etd.lib.ttu.edu/theses/available/etd-06272008-31295017115311/unrestricted/31295017115311.pdf

Peters N, Köhler A, Kranz H (1987). Liver pathology in fishes from the lower Elbe as a consequence of pollution. Dis. Aquat. Organ, 2: 87-97.

Radhaiah V, Jayantha Rao K (1992). Fenvalerate toxicity to the liver in a freshwater teleost, *Tilapia mossambica* (Peters). Comp. Physiol. Ecol. 17(2): 48-53.

Spisni E, Tugnoli M, Ponticelli A, Mordenti T, Tomasi V (1998). Hepatic steatosis in artificially fed marine teleosts. J. Fish Dis. 21(3): 177-184.

Tilak KS, Koteswara R, Veeraiah K (2005). Effects of chlorpyrifos on histopathology of the fish *Catla catla*. J. Ecotoxicol. Environ. Monit., 15(2): 127-140.

Urbansky ET (1998). Perchlorate chemistry: implications for analysis and remediation. Bioremed. J. 2: 81-95.

Wahbi OM, El-Greisy ZA (2007). Comperative impact of different waste sources on the reproductive parameters and histology of gonads, liver and pituitary gland of *Siganus rivaltus*. J. App. Sci. Res., 3(3): 236-244.

Weisman JL, Miller DL (2006). Lipoid liver disease and steatitis in captive sapphire damsel *Pomacentrus pavo*, Acta Ichtiyol. et Piscatoria., 36(2): 99-104.