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Research Article

CHANGES IN BIOCHEMICAL PARAMETERS OF FRESHWATER FISH LABEO ROHITA EXPOSED TO LETHAL AND SUB-LETHAL CONCENTRATIONS OF INDOXACARB

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Abstract: The Indian major carp *Labeo rohita* (Hamilton) was exposed to the new generation pesticide Indoxacarb for 24, 48, 72 and 96 h and the acute static LC_{50} values were determined as 0.0589, 0.05669, 0.05522 and 0.0531 respectively. These LC50 values indicate that the indoxacarb is highly toxic to fish. The fish were exposed to sub-lethal concentration ($1/10^{th}$ 96 h LC_{50}) for 8 days and the changes in the biochemical constituents of the vital organs viz, Gill, Liver, Brain, Muscle, and Kidney were studied. Significant changes in these organs of fish were observed. The changes were noted as tissue specific and time dependent. Several behavioral changes during the period of exposure were also observed and noted. The results obtained were discussed at length with the available literature.

Keywords: Indoxacarb, *Labeo rohita,* LC50 nucleic acids, and proteins.

INTRODUCTION

Pesticides are widely used in modern agriculture to aid in the production of high quality food. However, some pesticides have the potential to cause serious health and/or environmental damage. Repeated exposure to sub-lethal doses of some pesticides can cause physiological and behavioral changes in fish that reduce populations, such as abandonment of nests and broods, decreased immunity to disease, and increased failure to avoid predators. (Helfrich, *et al.,* 1996)

Pesticides can contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants. Exposure to higher concentrations of persistent, bioaccumulative, and toxic contaminants such as DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane) and PCBs has been shown to elicit adverse effects on reproductive and immunological functions in captive or wild aquatic mammals (Helle *et al*., 1976; Reijnders, 1986; Martineau *et al*., 1987; Kannan *et al*., 1997 ; Ross *et al*., 1995; Ross *et al*., 1995 Colborn and Smolen, 1996). The undue persistence, high mammalian toxicity and developing resistance of the organo chlorine, organophosphate and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries. Thus, attention was focused on the synthesis of less persistent, low mammalian toxicity new generation compounds like indoxacarb.

New generation pesticides such as nicotinodis and indoxacarb are relatively non-persistent and do not accumulate in the environment. However, some are bioaccumulated by various organisms during exposure to sub-lethal concentrations but levels rapidly return to normal after exposure ceases. Indoxacarb also has very good toxicological and ecotoxicological profile. This low use rate material will reduce environmental loading, particularly when compared to OP's and carbamates. It provides a much safer alternative to pyrethroids from the standpoint of aquatic safety. The relatively low mammalian toxicity with this product provides improved safety to workers, as well as terrestrial mammals and birds when compared to competitive OP's and carbamates. Moreover, the novel mode of action together with the lack of cross resistance to existing insect control products, environmental compatibility and its safety to non-target organisms (Wing *et al*., 2000) makes indoxacarb an excellent candidate for integrated pest management programs.

Hence in the present study an attempt has been made to study the impact of indoxacarb, a new generation compound, which is extensively used on the commercial crops for the control of insect pests, on the Indian Major carp *Labeo rohita* (Hamilton).

MATERIALS AND METHODS

Fish *Labeo rohita* of size 6-7 $\pm^{1}/_{2}$ cm and 6-8$\pm^{1}/_{2}$ g weight were brought from a local fish farm and acclimated at 28 ± 2°C in the laboratory for 96 h. Such acclimated fish were exposed to sub-lethal concentration of indoxacarb 14.5% SC formulation for 8 days (0.00567 mg/L).

Indoxacarb (14.5% SC) was supplied by Rallies India Ltd. Hyderabad. Stock solutions are prepared in acetone and the working solutions are prepared in

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distilled water. The water used for acclimatization and conducting experiments was clear unchlorinated ground water. Experiments were conducted to determine the toxicity of indoxacarb in various concentrations in static system by employing the method of APHA *et al.*, (1998). The data on the mortality range from 10% to 90% for 24, 48, 72 and 96 h in static and continuous flow-through systems were recorded. Finney's probit analysis (Finney, 1971) as reported by Roberts and Boyce (1972) was followed to calculate the LC_{50} values.

The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of total proteins, glycogen, and nucleic acids. Total protein content was estimated by the modified method of Lowry *et al.,* (1951). The glycogen was estimated by the method of Kemp *et al.,* (1954). The nucleic acids, deoxyribo nucleic acid (DNA) and ribo nucleic acid (RNA) were estimated by the method of Searchy and Maclinnis 1970 (a &b).

RESULTS AND DISCUSSION

The LC_{50} values of indoxacarb to the fish *Labeo rohita* indicate that the pesticide indoxacarb is extremely toxic to the fish Kannan, (1997). Indoxacarb, the DPX-KN127 isomer, and associated degradates are moderately to very highly toxic to freshwater fish and invertebrates on an acute basis with LC_{50}s ranging from 0.024 to 2.94 mg/L. They are also moderately toxic to very highly toxic to estuarine/marine fish and invertebrates on an acute basis with EC_{50}s (50 percent effective concentrations) ranging from 0.0542 to > 0.37 mg/L. Chronic toxicities range from 0.0036 to 0.25 mg/L for freshwater fish and invertebrates and from 0.017 to 0.042 mg/L for estuarine fish and invertebrates (U.S. EPA, 2000; Hetrick *et al*., 2005). Acute restricted use and endangered species levels of concern (RQ = 0.1) are exceeded by estuarine/marine invertebrate acute risk quotients calculated for indoxacarb (RQ = 0.1) and the depredates IN-JT333 (RQ = 0.2) for the two highest exposure concentrations (peanuts and alfalfa). Indoxacarb and its degradate IN-JT333 are not expected to reach surface water concentrations high enough to trigger acute risk concerns (RQ = 0.5) or chronic concerns (RQ =1) in fish or invertebrates in either freshwater or estuarine/marine systems (Hetrick *et al*., 2005). However, due to the very high toxicity of indoxacarb to some estuarine/marine fish and invertebrates, direct exposure or runoff into surface waters could be of concern.

The calculated values for total proteins were graphically represented in fig. 1. The variation in distribution suggests differences in metabolic calibers of various tissues. The present trend in the tissues is justifiable in the wake of mechanical tissue of muscle intended for mobility and does not participate in metabolism. The liver is also much in proteins because of metabolic potential being oriented towards it and is the seat for the synthesis of various proteins besides being the regulating center of metabolism.

Under sub-lethal exposure, the total protein was found to decrease in all the tissues. Gill tissues of *Labeo rohita* evidenced a highly significant decrease in the protein content under sub-lethal concentrations of indoxacarb followed by kidney. Biochemical changes in the muscle and liver of the fish suggests that they are relatively less affected than other tissues under indoxacarb toxicity. The decreased trend of the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; or due to the directing of free amino acids for the synthesis of necessary proteins, or, for the maintenance of osmotic and ionic regulation (Schmidt Nielson, 1975).

The investigations of Koundinya and Ramamurthy (1980) revealed a decrease in protein content in *Sarotherodon mossambica* exposed to different pesticides. Sastry and Siddiqui (1984) reported that the protein content was decreased in liver, muscle, kidney, intestine, brain and gill of *Channa punctatus* treated with quinolphos. The levels of protein decreased significantly in liver, kidney and muscle of *Catla catla* treated with endosulfan (Rao, 1989). Yaragi *et al.,* (2000) observed decreased levels of proteins in gills, testis, ovaries and muscle of marine crab, *Uca merionis* exposed to acute and chronic levels of malathion. Aruna Khare *et al.,* (2000) observed that the sublethal concentrations of malathion showed a significant increase in total protein content in kidney of exposed fish, *Clarias batrachus* during the first week and there after a gradual decrease in protein content was observed in the later periods of exposure.

Several other investigations also revealed a decrease in protein profiles with organophosphate compounds. All these investigations support the present study of decreasing trend of proteins in the tissues of the fish *Channa punctatus* exposed to sublethal concentration of indoxacarb.

The calculated values for glycogen were graphically represented in fig. 1. Among the test tissues higher glycogen content was observed in liver. Highest glycogen content of liver is acceptable due to its involvement in glycogen synthesis and utilization. Glycogen is the major storage form of carbohydrate in animals which occurs mainly in liver and muscle. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within

the muscle itself (Harper, 2003). Though brain tissue is metabolically active, lower glycogen content was observed, since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities (Lehninger, 2004).

The results indicate depletion of glycogen in various tissues. A fall in glycogen levels indicates its rapid utilization to meet the enhanced energy demands in pesticide treated animals through glycolysis or hexose monophosphate pathway (Cappon and Nicholas, 1975). Pesticides are known to act on endocrine system (Edwards, 1973). Hence, it contributes to the decreased glycogen synthesis. Decreased glycogen synthesis is also attributed to the inhibition of the enzyme glycogen synthatase which mediates glycogen synthesis.

The earlier observation on the effect of pesticides on carbohydrate metabolism in various species indicates an attenuation of the energy reserve under pesticide stress (Holden, 1973; Radhaiah, 1988; Rama Murthy, 1988). It appears that exposure to indoxacarb leads to enhancement of energy requirement. Since the glycogen is considered to be the first among the organic nutrients, it initially gets affected and decrease under any physiological stress conditions imposed on the animal. A drop in tissue glycogen content may also be either due to decreased synthesis as a consequence of toxic stress or breakdown (Dezwaan and Zandee, 1972).

Long-term exposure to sub-lethal concentrations of quinolphos decreased the glucose level in the fish, *Channa punctatus* (Sastry and Siddiqui, 1984). Methylparathion sublethal exposure on freshwater mussel, *Lamellidens marginalis* decreased the glycogen content (Moorthy *et al.,* 1985). Monocrotophos exposure to *Channa punctatus* reduced the glycogen levels (Miny Samuel and Sastry, 1989). Exposure of *Rana tigerina* to aldrin reduced glycogen level in the tissues of liver, kidney, muscle, testis and brain (Vijaya Joseph, 1989). Muscle glycogen decreased in cadmium toxicity on *Channa punctatus* (Sastry and Shukhla, 1990) and in crab *Scylla serrata* also, same trend was observed in the selected tissues tested (Srinivasula Reddy and Bhagyalakshmi, 1994). Endosulfan 96 h exposure decreased the glycogen level in the fish, *Clarias batrachus* (Asfia Parveen and Vasantha, 1994). Decrease of glycogen content in liver and muscle tissue in Atlantic salmon was observed under sub-lethal exposure of fenvalerate (Haya, 1989); hexachlorocyclohexane exposure on *Channa punctatus* (Ganathy *et al.,* 1994); monocrotophos exposure on freshwater crab *Barytelphusa guerini* (Venkateswarlu and Sunita, 1995); heptachlor on Swiss albino mice (Nagabhushanam *et al.,* 1994) and phosphamidon on *Gambusia affinis* (Govindan et *al.*, 1994). Sublethal concentrations of cypermethrin induced depletion of glycogen in *Tilapa mossambica* (Reddy and Yellamma, 1991) in *Labeo rohita* (Veeraiah and Durga Prasad, 1998; Veeraiah, 2002) and in *Cyprinus carpio* (Ravisankar *et al.,* 1992). Sobha Rani *et al.,* (2000) observed a significant depletion in glucose and glycogen levels in various tissues of freshwater teleost, *T. mossambica* under sublethal concentration of sodium arsenate and stated that these changes were tissue specific and time dependent. Bhamre *et al.,* (2001) observed decreased levels of glycogen under acute and chronic exposure of mercuric chloride in the tissues of whole body, foot, digestive gland and mantle of freshwater mussel, *Parreysia favidens.*

The decreased glycogen level is also attributed to the conversion of carbohydrates into aminoacids (Gaiton *et al.,* 1965). Koundinya and Ramamurthy, (1980) reported that stepped up glycogenolysis leads to a decrease in glycogen content. Similar changes were observed in *Sarotherodon mossambicus* exposed to endosulfan (Vasanthi and Ramaswamy, 1987) and in *Channa striatus* to metasystox exposure (Natarajan, 1981).

In the present study, it was observed that exposure to sub-lethal concentrations of indoxacarb in the fish *Labeo rohita caused* changes in the total glycogen level which may be attributed to toxic stress, resulting in the disruption of enzymes associated with carbohydrate metabolism.

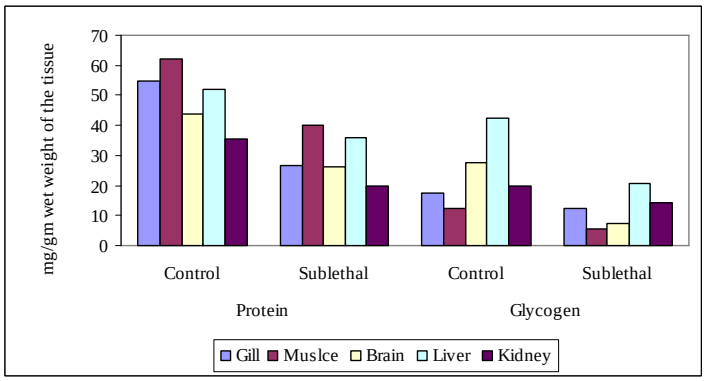

Fig.1. Changes in the protein (mg/gm wet weight of the tissue) and glycogen (mg/gm wet weight of the tissue) of the fish *Labeo rohita* exposed to sub-lethal concentration of indoxacarb

Nucleic Acids (DNA and RNA):

The calculated values of nucleic acids were graphically presented in fig. 2. Under exposure to sub-lethal concentration, the amount of RNA decreased in all of the tissues of *Labeo rohita.* The results indicate heterogeneous levels of DNA and RNA in the tissues of brain, liver, muscle, gill and kidney. The level of DNA in different tissues indicates the cell number and is constant for a species. Increasing levels of DNA in liver

supports the earlier findings of Holbrook (1980). According to Holbrook (1980), thymidine incorporation into hepatic DNA is markedly increased after 1-3 days of administration of various toxicants. The increase of DNA in gill region may be due to hypertrophic nature of chloride cells. These results are in agreement with the works of Natarajan (1981); Durai Raj and Selvarajan (1992), which reveal the enlargement of nuclei in the chloride secreting cells in *Channa striatus* exposed to metasystox and in *Oreochromis mossambicus* exposed to quinolphos respectively. In other tissues, no significant change was observed in DNA levels. The RNA level reflects the intensity of protein synthesis (Bracht, 1955) and metabolic activity of the tissue (Bulow, 1970). The decrease of RNA supports the view of Holbrook (1980). The work of Durai Raj and Selvarajan (1988) also supports the present study.

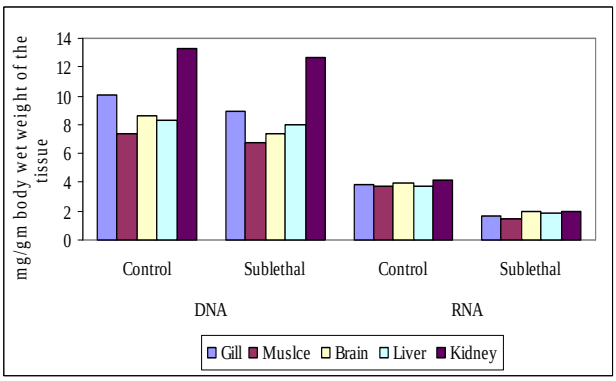

Fig. 2: Changes in the DNA (mg/gm body wet weight of the tissue) and RNA (mg/gm body wet weight of the tissue) of the fish *Labeo rohita* exposed to sub-lethal concentration of indoxacarb.

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REFERENCES

1. APHA, AWWA and WEF. Standard methods for the examination of water and wastewater, 20th ed. Washington, D.C., American Public Health Association, American Water Works Association and Water Environment Federation.,1998.

2. Aruna Khare, Sudha Sing and Keerthy Shrivastava. Malathion induced biochemical changes in the kidney of freshwater fish Clarias batrachus. J. Ecotoxicol. Environ. Monit.2000, 10(1): 11-14.

3. Asfia Parveen and Vasantha, N., Evaluation of pesticide endosulfan impact on respiratory metabolism of fish Clarias batrachus. Ind. J. Comp. Ani. Physiol., 1994, 12(1): 83-89.

4. Bhamre, P.R., Desai, A.E. and Deoray, B.M., Effect of mercuric chloride on glycogen content of the freshwater mussel Parreysia favidens. Poll. Res., 2001, 20(1): 13-15.

5. Bulow, F.J., RNA-DNA ratios as indicators of recent growth rates of a fish. J. Fish. Res. Bd. Can., 1970, 27: 2343-2349.

6. Cappon, I.D. and Nicholas, D.M., Factors involved in increased protein synthesis in liver microsomes after administration of DDT. Pestic. Biochem. Physiol., 1975, 5: 109-118.

7. Colborn T, Smolen MJ., Epidemiological analysis of persistent organochlorine contaminants in cetaceans. Rev Environ Contam Toxicol., 1996, 146:91–172.

8. Dezwaan, A. and Zandee, D.T., The utilization of glycogen accumulation of some intermediates during anaerobiosis in Mytilus edulis L. Comp. Biochem. Physiol., B, 1972, 43: 47-54.

9. Durai Raj, S. and Selvarajan, V.R., Influence of quinolphos an organo phosphorous pesticide, on the biochemical constituents of the tissues of fish Oreochromis mossambicus. J. Environ. Biol.1992, 13(3): 181-185.

10. Edwards, C.A., Environmental pollution by pesticides, Plenum Press, London, New York., 1973.

11. Finney, D.J., Probit Analysis 3rd edition, Cambridge University Press, London/New York., 1971.

12. Harper, H.A., In: Harper's Review of Biochemistry (eds.) D.W. Martin, P.A. Mayes and V.W. Rodwell. Lange Medical Publications, Marusen, Asia (Singapore)., 2003.

13. Hetrick, J., Evans, W. and Abel, S., Environmental Fate and Effects Division risk assessment for proposed new uses of indoxacarb on grapes, fire ants, mole crickets, alfalfa, peanut, soybeans, Brassica leafy vegetables (Group 5), and turnip greens. PC Code 067710. U.S. EPA, Washington, D.C., 2005.

14. Holbrook (Jr.) D.J., Effect of toxicants on nucleic acid and protein metabolism In: Introduction to biochemical toxicology (Eds: E. Hodgson and F.E. Guthrie), Blackwell Scientific Publications, Oxford, 1980, 261-284.

15. Gaiton, M.K., Dahl, D.R. and Elliott, K.A.L., Entry of glucose carbon in to amino acids of rat brain and liver in vivo after injection of uniformly 14C labelled glucose. J. Biochem., 1965, 94: 345-352.

16. Ganathy, V., Srinivasa Reddy, D., Reddy, S.L.N. and Shankaraiah, K., Effect of hexachlorocyclohexane on biochemical composition of the fish Channa punctatus. J. Ecotoxicol., Environ. Monit., 1994, 4(1): 015-020.

17. Govindan, V.S., Jacob, L. and Devika, R., Toxicity and metabolic changes in Gambusia affinis exposed to phosphamidon. J. Ecotoxicol. Environ. Monit., 1994, 4(1): 1-6.

18. Haya, K., Toxicity of pyrethroid insecticides to fish. Environ. Toxicol. Chemistry, 1989, (8): 381-391.

19. Helfrich, LA, Weigmann, DL, Hipkins, P, and Stinson, ER, Pesticides and aquatic animals: A guide to reducing impacts on aquatic systems. Virginia Cooperative Extension. Retrieved on, 1986, 2007-10-14.

20. Helle E, Olsson M, Jensen S., DDT and PCB levels and reproduction in ringed seal from
21. the Bothnian Bay. Ambio.1976, 5:188–189.

22. Holden, A.V., Chapter 6 in environmental pollution by pesticides (ed. C.A. Edwards), Plenum Press, New York.1973.

23. Kannan K, Tanabe S, Borrell A, Aguilar A, Focardi S, Tatsukawa R., Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in striped dolphins affected by an epizootic in the western Mediterranean Sea. Arch Environ Contam Toxicol., 1993, 25: 227–233.

24. Kannan, K., Fundamentals of Environmental Pollution. S. Chand & Company Ltd., New Delhi, 1997.

25. Kemp, A., Adrinene, J.M., Kits and Van Hejnenga, A colorimetric method for the determination of glycogen in tissues. J. Biol. Chem., 1954, 56: 646-648.

26. Koundinya, P.R. and Ramamurthy, R., Toxicity of sumithion and sevin to the freshwater fish Sarotherodon mossambicus. Curr. Sci., 1980, 49: 875.

27. Koundinya, P.R. and Ramamurthy, R., Effect of pesticide sumithion (fenetriothion) on some aspects of carbohydrate metabolism in freshwater fish Tilapia mossambica (Peters). Experientia, 1979, 35: 1632-1633.

28. Lehninger, A.L., In: Biochemistry, Kalyani Publications Ludhiana, New Delhi, 2004.

29. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. J., Protein measurement with folin phenol reagent, J. Biol. Chem.,1951, 193: 265-275.

30. Martineau D, Be'land P, Desjardins C, Lagace' A., Levels of organochlorine chemicals in tissues of beluga whales (Delphinapterusleucas) from the St. Lawrence Estuary, Que'bec, Canada. Arch Environ Contam Toxicol., 1987, 16:137–147.

31. Miny Samuel and Sastry, K.V., In vivo effect of monocrotophos on the carbohydrate metabolism of the freshwater snakehead fish Channa punctatus. Pestic. Biochem. Physiol., 1989, 34: 1-8.

32. Moorthy, K.S., Kasi Reddy, B., Swami, K.S., and Chetty, C.S., Glucose metabolism in hepatopancreas and gill of Lamellidens marginalis during methyl parathion toxicity. Pestic. Biochem. Physiol., 1985, 24: 40-44.

33. Nagabhushanam, M., Suhasini, P.B., Rao, M.R. and Jayantha Rao, K., Effect of heptachlor on certain aspects of carbohydrate metabolism in Swiss albino mice. Bull. Environ. Contam. Toxicol., 1994, 52: 905-911.

34. Natarajan, G.M., Effect of lethal concentration of metasystox on selected oxidative enzymes, tissue respiration and haematology of the freshwater air breathing fish, Channa striatus (Bleeker). Pest. Biochem. Physiol., 1981.

35. Radhaiah, V., Studies on the toxic impact of a pyrethroid insecticide, fenvalerate on some metabolic aspects and histopathology of a freshwater teleost, Tilapia mossambica (Peters). Ph.D. Thesis. S.V. University, Tirupathi, India, 1988.

36. Rama Murthy, K., Impact of heptachlor on haematological, histological and selected biochemical parameters on freshwater edible fish, Channa punctata (Bloch). Ph.D. Thesis, S.V. University, Tirupathi, India, 1988.

37. Rao, D.M.R., Studies on the relative toxicity and metabolism of endosulfan to the Indian major carp Catla catla with special reference to some biochemical changes induced by the pesticide. Pestic. Biochem. Physiol., 1989, 33: 220-229.

38. Ravisankar, P., Sarala Waghray, S. and Indira Devi, The effect of sublethal concentration of synthetic pyrethroid, cypermethrin to the common carp, Cyprinus carpio. J. Environ. Biol., 1992, 13(2): 89-94.

39. Reddy, A.T.V. and Yellamma, K., Perturbations in carbohydrate metabolism during cypermethrin toxicity in fish, Tilapia mossambica (Peters). J. Biochem., 1991, 23(4): 633-638.

40. Reijnders PJH., Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature.; 1986, 324:456–457.

41. Ross PS, de Swart RL, Reijnders PJH, Loveren HV, Vos JG, Osterhaus ADME. Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. Environ Health Perspect., 1995,103:162–167.

42. Roberts, M. and Boyce, C.B.C., Methods in Microbiology (7-Q ed). Norris, J.R. and Ribbows, 479 pp. D.W. Academic Press, New York.1972.

43. Sastry, K.V. and Shukla, V., Acute and chronic toxic effects of cadmium on some biochemical and enzymatic parameters in fish. Paper submitted to the second Indian fisheries forum held on 27th May, 1990 at Mangalore, Karnataka, India, 1990.

44. Sastry, K.V. and Siddiqui, Some haematological, biochemical and enzymological parameters of a freshwater teleost fish, Channa punctata exposed to sublethal concentrations of quinolphos. Pestic. Biochem. and Physiol.,1984, 22: 8-13.

45. Schmidt Nielson, B., Osmoregulation: Effect of salinity and heavy metal. Fed. Proc., 1975, 33: 2137-2146.

46. Searchy, D.G. and MacLenis, A.J., Determination of DNA by the Burton Diphenylamine technique. In: experiments and techniques in parasitology. (Eds: Mac Lenis, A. J. and Voge, M). W.H. Freeman and Co., San-Francisco, 1970a, 190-191.

47. Sobha Rani, A., Sudharsan, R., Reddy, T.N., Reddy, P.V.M. and Raju, T.N., Effect of sodium arsenate on glucose and glycogen levels in freshwater teleost fish Tilapia mossambica. Poll. Res., 2000, 19(1): 129-131.

48. Srinivasula Reddy, M. and Bhagyalakshmi, A., Changes in the oxidative metabolism in the selected tissue of the crab Scylla serrata in response to cadmium toxicity. Ecotoxicol and Environ. Saf., 1994, 29: 255-264.

49. U. S. EPA. Pesticide Fact Sheet: Indoxacarb. U.S. EPA, Washington, DC, 2000.

50. Vasanthi, M. and Ramaswamy, M., A shift in metabolic pathway of Sarotherodon mossambicus (Peters) exposed to thiodon (endosulfan) Proc. Indian. Acad. Sci., (Anim. Sci) 1987, 96: 56-69.

51. Vijaya Joseph, K., Effect of aldrin an organo- chlorine compound on frog, Rana hexadactyla, Ph.D. Thesis submitted to S.V.University, Tirupathi, India., 1989.

52. Venkateswarlu, M. and Sunita, A., Impact of monocrotophos on tissue carbohydrates of the freshwater crab Barytelphusa guerini (Milne Edwards) Ind. J. Comp. Ani. Physiol., 1995, 13(1): 35-36.

53. Veeraiah, K., Cypermethrin toxicity and its impact on histochemical and histological changes in the Indian major carp Labeo rohita (Hamilton). Ph.D. Thesis submitted to Nagarjuna University, Nagarjuna Nagar, Guntur, A.P. India., 2002.

54. Veeraiah, K. and Durga Prasad, M.K., Study on the toxic effects of cypermethrin (technical) on organic constituents of freshwater fish, Labeo rohita (Hamilton). Proc. Acad. Environ. Biol., 1998, 7(2): 143-148.

55. Wing, K.D., Sacher, M., Kagaya, Y., Tsurubuchi, Y., Mulberig, L., Connair, M. and Schnee, M., Bioactivation and mode of action of the oxadiazine indoxacarb in insects. Crop. Prot. 2000, 19: 537-545.

56. Yaragi, S.G., Koli, V.A. and Yaragi, S., Effect of pesticide malathion on protein metabolism of the marine crab Uca merionis. J. Ecotoxicol. Environ. Monit., 2000, 10 (1): 59-62.

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