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Bioaccumulation of Commercial Grade Thiodicarb 75% WP Pesticide in Different organs of the Freshwater Major Carp, *Labeo rohita* (Hamilton)

KEYWORDS: High pressure liquid chromatography, thiodicarb, Residue analysis, Labeo rohita

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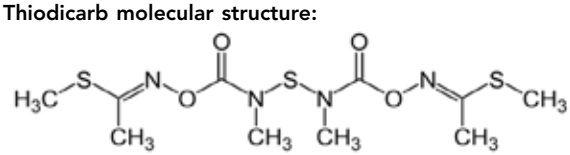
ABSTRACT *Bioaccumulation studies of thiodicarb 75% WP were conducted on Indian major carp fish, Labeo rohita (Hamilton). The LC50 values were calculated for thiodicarb for 24, 48, 72 and 96 h, in static system 14.2, 13.0, 12.0 and 7.0 ppm respectively. The fish were exposed to Sub-lethal (Static 1/10th of 96h) and lethal concentrations of commercial grade at 96 hours. The vital tissues viz., gill, muscle, brain, liver and kidney were analyzed for residue estimations. In all the tissues, analyzed maximum residue was recovered in the time period 96h. After exposed to sub-lethal and lethal concentrations the highest residue concentration of thiodicarb were observed in kidney (0.082µg and 0.246µg) followed by, muscle (0.064µg and 0.192µg), Liver (0.057µg and 0.171µg), brain (0.046µg and 0.138µg) and Gill (0.031µg and 0.093µg). For the calculation of thiodicarb concentration in different organs, peak heights were taken. The LOD and LOQ for thiodicarb were 0.04 (µg/g) and 0.05 (µg/g) respectively. The decline in residue levels along with the period of exposure indicates the fast acting nature of thiodicarb and metabolites.*

Introduction

Pesticides were used at all stages of food production, storage and transport to protect from the pest infestation. Pesticides are inherently toxic in nature and can cause harmful health effect on human beings and animals if not used properly (Sharma, 2005). Thiodicarb, a carbamate insecticide has been used for the last several years in agriculture. It was moderately toxic after oral administration and was classified as moderately hazardous by WHO.

Thiodicarb molecular structure:



The pesticide residues are present in very small quantities in the aquatic medium in which the fish exists, the residues in the fish tissue are in higher orders of magnitude because of bioaccumulation, and are at detectable quantities (Edwards, 1973). Unfortunately, pesticides do not stay at the site of application but move to different compartments of the globe. During their sojourn the pesticides pose harmful effects to the non-target organisms. The undue persistence, high mammalian toxicity and developing resistance of the organochlorine, organophosphate and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries (Veeraiah,2002; Praasada Rao,2012). The studies on the residue levels in the environment of both aquatic and terrestrial are more (Pandurangadu, 1988; Flora 16, 2008; Kirmani *et al,* 2010; Gurava Reddy, 2011 and Jonnalagadda, 2011). Residue studies on the fish were also reported from Guntur region (Veeraiah and Durga Prasad, 1996; Tilak *et al,* 2003 & 2007; Butchi Ram and Tilak, 2009; Praasada Rao, 2012). The residues of such widely used hydrocarbon insecticides have been reviewed by Murty (1986). Unfortunately, not all farmers follow the legal practice and due to the tremendous number of pesticides and crops in production, a number of analytical methods was designed to determine multiple pesticide residues (Food and Drug Administration, 1999; Luke *et al.,* 1975). Thus, analysis of pesticide residue in foods, especially fishes, becomes an essential requirement for consumers, producers, and food quality control authorities (Ashutos*h et al.,* 2011).

In the present study an attempt has been made to observe residue levels in certain vital tissues like gill, muscle, brain, liver and kidney of *Labeo rohita* qualitatively and Quantitatively after exposure to Sub-lethal (1/10th of 96 h LC_{50}) and lethal concentrations of thiodicarb 75% WP.

MATERIALS AND METHODS

Freshwater fish, Indian major carp *Labeo rohita* (size 6-7 $\pm$ ½ cm and weight 10-12 $\pm$ ½ gm) were collected from local fish market and brought to the laboratory and stored in large plastic tubs containing tap water. The fish were acclimated to laboratory conditions for one week. The A.P.H.A *et al.,* (1998) recommendations of committee on toxicity tests of Aquatic organisms were followed. The fish were fed with rice bran. The water in the fish storage tanks was changed daily. The feeding was stopped one day prior to experiments. Such acclimatized fish only were, used for toxicity evaluation. If mortality exceeded 5% in any batch of fish during acclimatization, the total batch of that fish were discarded.

In the present study fish were exposed to sub-lethal (1/10th of 96 hrs LC_{50}) and lethal concentrations of commercial grade thiodicarb 75 % WP. The fish were exposed to 96 h after that the fish were sacrificed and analyzed for residues. The control fish were treated with pure acetone. The

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

residues from the fish tissue of gill, muscle, brain, liver and kidney were extracted by the modified method of Mills and Olney (1977). Extracts were cleaned up on silica gel columns covered with a layer of anhydrous sodium sulfate and packed with hexane (Goughan *et al.,* 1978). The pesticide residues were analyzed by qualitatively by using TLC (method of Moats, 1966), cleaned up by using Column Chromatography. The quantitative estimation was carried out by High performance liquid chromatography (HPLC).

The quantitative residue analysis was carried out by using the Reverse Phase High Performance Liquid Chromatography (RP-HPLC). The analytical conditions of HPLC were as follows: Model, LC-UV100 plus (Ver.1.0) with UV-Detector fitted with Reverse-phase, Varian Pursuit XRs 5 performance column C_{18} 250 X 4.6 mm at ambient temperature, mobile phase was Acetone : water (90:10, v/v), with a flow rate of 1ml/min^{-1}, 12 minutes run time for single sample. UV-Detector wavelength was 232 nm and injection volume was 20 µl. The quantitative analysis was carried out with the external standard method. The linear correlation coefficient was 0.9688.

RESULTS AND DISCUSSION

The carbamate pesticide, thiodicarb residues analysis found in vital organs of freshwater Indian major carp, *Labeo rohita* were shown in Fig.5. The highest total thiodicarb pesticide residue found in kidney followed by muscle, liver, brain, gill. The appearance of brown spot observed on the TLC plates in different ratios of developing solvents in different solvent systems in the test tissues of brain, liver, muscle, gill and kidney. The R_f values varied with the change in ratios as well as change in solvents. The suitable solvents were selected after several attempts. The results of the qualitative resiude analysis in thin layer chromatographic method were presented in Fig.I. The quantitative estimations of thiodicarb residues by HPLC method were presented in Table.1. and Fig.5.

After 96 h exposure period of sub-lethal and lethal concentrations of thiodicarb the residue spots appeared prominently in almost all the tissues, the residues appeared prominently except in brain where in the residue spot is not very prominent. Fig.1. In the present study, it was observed that the prominence in residue spots increased steadily throughout the investigation period. This may be due to continuous bathing of the fish in the toxicant medium. Though the excretion and metabolism processes are continuous, the uptake will lead to the maintenance of residue load.

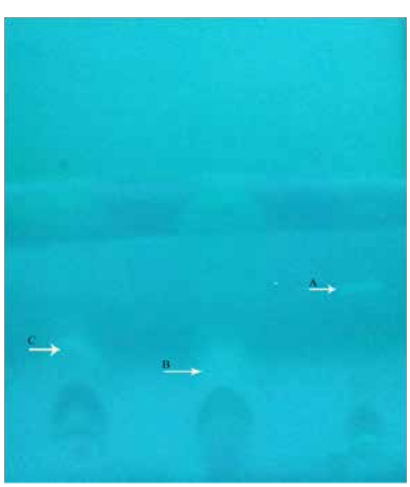
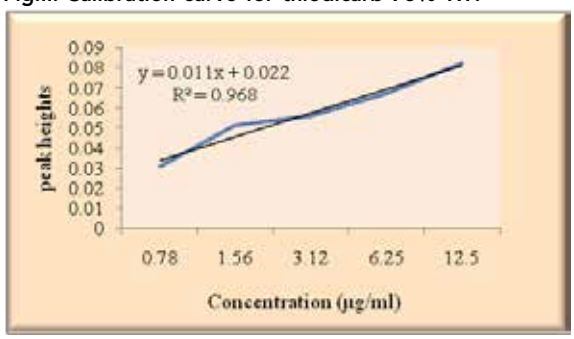


Fig. I. Chromatogram showed comparison of Rf values of thiodicarb with standard (A), Sub-lethal (B) and lethal (C) concentrations of 96h.

Fig.II. Calibration curve for thiodicarb 75% WP.



Thiodicarb residues were quantified from the respective peak heights and the concentrations in tissue homogenates were determined by means of calibration curves obtained on analysis of blank tissue homogenates spiked with thiodicarb. The different increased thiodicarb concentrations were spiked 0.78, 1.56, 3.12, 6.25 and 12.5 µg/ml. The Chromatograms of spiked concentrations (Fig.III), Retention time and Calibration data (Table.1.), Retention time Chromatogram (Table. IV) of commercial grade thiodicarb 75% WP were described.

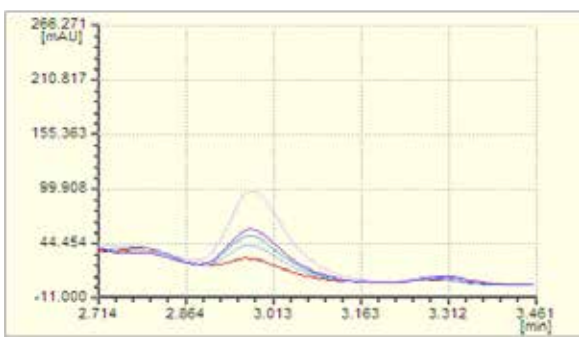


Fig.III. Chromatograms of spiked concentrations of thiodicarb in freshwater fish *Labeo rohita***.**

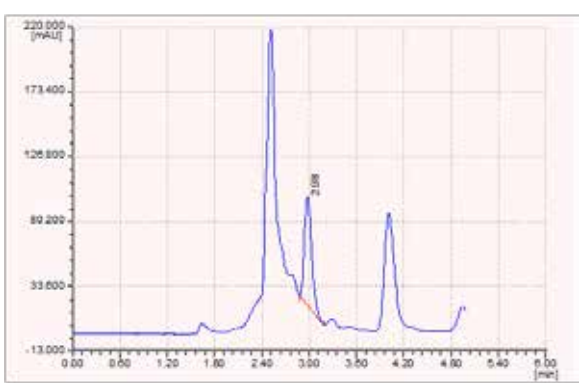


Fig.IV. Chromatogram showed Retention time for commercial grade thiodicarb pesticide.

Table.I. Retention time, Calibration data (n=6) and Sensitivity for commercial grade thiodicarb pesticide.

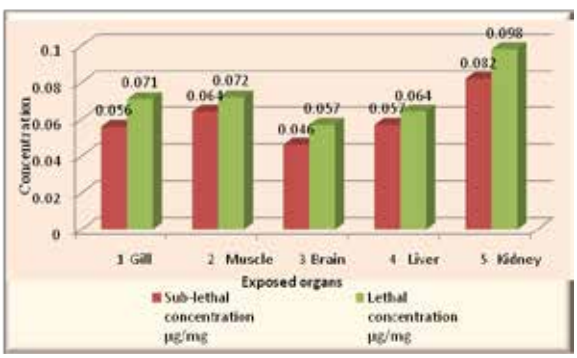
Pesticide	t_R (min)	Equation	t	Sensitivity	
				LOD (µg/g)	LOQ(µg/g)
Thiodicarb	2.98	y = 0.0118x + 0.022	0.9688	0.04	0.05

The linear correlation coefficient (t) was 0.9688. Limit of detection (LOD) of thiodicarb was calculated at a single-to-single ra-

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

tio of 3, while the limit of quantification (LOQ) was obtained at a single-to-single ratio of 10. The LOD and LOQ for thiodicarb were 0.04 (µg/g) and 0.05 (µg/g) respectively. The mobile phase, flow rate and wavelengths were suitable for estimation of sub-lethal and lethal residue concentrations of commercial grade thiodicarb (Larvin 75% WP) in freshwater fish Labeo rohita. The highest residue concentration of thiodicarb were found in kidney (0.082µg and 0.246µg) followed by, muscle (0.064µg and 0.192µg), Liver (0.057µg and 0.171µg), brain (0.046µg and 0.138µg) and Gill (0.031µg and 0.093µg). for the calculation of thiodicarb concentration in different organs, peak heights were taken. The variations in the residue analysis are attributed to factors like difference in uptake rate and lipid content of respective animal tissue and the duration of exposure. The chemical structure, solubility, fish interaction and metabolic pattern are responsible for pesticide uptake.

Fig.V. The residual concentrations over the control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of thiodicarb (Larvin 75% WP) for 96h.



The results of present study revealed that increase in the time of exposure to sub-lethal and lethal concentrations of thiodicarb led to increase in the accumulation of residues. The present results agreement with the earlier reports (Tilak *et al.*, 2003 & 2004; Tripathi, 1992; Bradbury *et al.*, 1987). The accumulation is a factor responsible for changes in biochemical actions or pathological changes and also disturbance of overall biochemical cyclic reactions which are cumulative causing lethal actions even when the concentrations are sub-lethal.

Two carbamate pesticides, kresoxim and thiodicarb were found in alzahraa market fish, *Labeo rohita* and the values were 0.18 and 0.038mg/kg respectively, which is higher than the MRL (Mohamed Ahmed Ibrahim Ahmed *et al.,* 2014). Veeraiah, (2001) reported qualitatively the presence of cypermethrin in brain, liver, gill and kidney of the fish *Labeo rohita.* A chromatographic analysis of cypermethrin in the animal material was studied by Jebakumar *et al.,* (1992).

Bradbury and Coats (1986) reported high levels of fenvalerate residues in bile, followed by gill and kidney tissues after a 48 hours exposure in rainbow trout.

Conclusions:

The results of the present study revealed that prolonged exposure to Sub-lethal and lethal concentrations of thiodicarb in freshwater fish, *Labeo rohita* leads to increased accumulation of residues. Thus the uptake and persistence of thiodicarb depends not only on a number of physical and chemical conditions, but also varies according to the biological conditions. Furthermore, a periodical monitoring of carbamate pesticides residue in vegetables and other foods are the recent need for the consumers.

Tough scientifically the risk analysis and assessment of pesticides look to be very sound but the human being is exposed to different chemicals through food, drinking water, soil etc. There is a need to make cumulative assessment of risk posed by exposure to multiple chemicals by multiple pathways.

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REFERENCE

APHA (1998). Standard methods for examination of water and waste water, 16th Edn. American public health association, Washington D.C. | | Ashutosh, KS., Purushottam, T., Srivastava, MK., Lohani M (2011). Monitoring of pesticide residues in market basket samples of vegetable from Lucknow city, Inida: QuEChERS method. Environ.Monit.Assess. 176:465-472. | | Bradbury, S.P., Coats, J.R, and Mckim, J.M (1986). Toxicokinetics of fenvalerate in rainbow trout, Environ. Toxicol. Chem., 5: 567-576. | | Bradbury, S.P., Coats, J.R and McKim, J.M (1987).Different toxicity and uptake of two fenvalerate formuations in the fat head minnows (Pimephales promelas), Environmental Toxicology and Chemistry, 4: 533-541. | | Butchiram, M. S., Tilak, K. S and Raju, P W (2009). Studies on histopathological changes in the gill, liver and kidney of Channa punctatus (Bloch) exposed to Alachlor. J. Environ. Biol, 30(2), 303-306. | | Edwards, C.A (1973). Environmental Pollution by Pesticides 542 pp, Plenum Press. London and New York. | | Flora, J., S Swaran (2008). Status of Toxicological Research in India. Chem. Res. Toxicol, 21 (7), 1317–1319. | | Food and Drug Administration (1999). Pesticide Analytical Manual Volume I: Multi residue methods, 3rd Edition, U.S. Department of health and Human Services, Washington,DC. | Goughan, L,C., R.H. Robinson and J.E. Casida (1978).Distribution and metabolic fate of trans and cis permethrin in laying hens. J. Agric. Food Chem., 26, 1374-1380. | | Gurava Reddy, K., Subbarami Reddy, A., Satish babu, J and Chandra Sekhara Reddy, M (2011). Adoption of Integrated Pest Management (IPM) in chilli (capsicum annuum) a case study in Guntur district of Andhra Pradesh. International Journal of Applied Biology and Pharmaceutical Technology, 2, 2. | | Jeba Kumar, S.R.D., Flora, S. D. J., Ganesan, R. M., Jagadeesan, G and Jayaraman, J (1992). Effect of short-term sublethal exposure of cypermethrin on the organic constituents of the freshwater fish Lepidocephalicthys thermalis, J. Environ. Biol, 11(2): 203-209. | | Jonnalagadda, P.R., Jahan, P., Venkatasubramanian, S., Khan, I. A., Prasad, A.Y. E., Reddy, K.A., Rao, M.V., Venkaiah, K and Q, Hasan (2011). Genotoxicity in agricultural farmers from Guntur district of South India—A case study, Hum Exp Toxicol, 31, 7741-747. | | Kirmani, N., Jamil, K., Naidu, U.R.M (2010). Occupational and environmental carcinogens in epidemiology of lung cancer in South Indian population. Biology and Medicine, 2 (4), 1-11. | | Luke, MA., Froberg, J.E., Masumoto, H.T (1975). Extraction and clean-up of organochlorine, organophosphate, organonitrogen, and hydrocarbon pesticides in produce for determination by gas-liquid chromatography. J. Assoc. Off. Anal. Chem. 58:1020-1026. | | Mills and Olney (1977). Pesticidal Analytical Manual, Food and Drug Administration, Washington D.C., 1-5. | | Moats, W. A (1966). Analysis of dairy products for chlorinate insecticide residues by thin layer chromatography, J. Assoc. Off. Anal. Chem, 49-795. | | Mohamed Ahmed Ibrahim Ahmed, Nasr Sobhy Khalil, Tarek Abd Elaliem Abd El Rahman (2014). Carbamate pesticide residues analysis of potao tuber samples using high-performance liquid chromatography (HPLC). Vol. 6 (1), pp. 1-5, March,2014. | | Murty, A.S (1986). Toxicity of Pesticides to fish Vol.I and Vol.II, C.R.C. Press Inc, Bocaraton, 483 pp and 355 pp. | | Pandurangadu, K (1988). Economics of pesticide use in major commercial crops of Guntur district of Andhra Pradesh. M. Sc. (Agri) Thesis, angaru. Hyderab. | | Prasada Rao, G.D.V (2012). Toxicity and effect of cyhalothrin on freshwater fish Catla catla (Hamilton), Labeo rohita, Cirrhinus mrigala (Hamilton), Ciprinus carpio (Linnaeus), and Ctenopharingodon idellus (Valanciennes)". Ph.D. Thesis, Acharya Nagarjuna University, Nagarjuna Nagar, India, Effect of sublethalconcentrations of aldrin on the testis of frog Rana hexadactyla. 20-22 pyrethroid ester, J. Agric. Bio. Chem., 37: 2681-2682. | | Sharma, (2005).Proceedings of the National Seminar on pesticide Residues and their Risk Assement, January 20-21, 2005. | | Tilak, K.S., Veeraiah.K and Koteswara Rao, D (2004). Toxicity and bioaccumulation of chloropyrifos in Indian carp Catla catla (Hamilton), Labeo rohita (Hamilton) and Cirrhinus mrigala (Hamilton), 3(5): 933-941. | | Tilak, K. S., Veeraiah, K and Milton, P.R .J (2007). Effects of ammonia, nitrite and nitrate on hemoglobin content and oxygen composition of fresh water fish, Cyprinus carpio (Linnaeus). J. Environ. Biol, 28: 45-47. | | Tilak, K.S., Veeraiah, K., Bhaskara Thathaji, P and Butchiram, M.S (2007). Toxicity studies of butachlor to the freshwater fish Channa punctata (Bloch). Journal of Environmental Biology, 28(2), 485-487. | | Tilak,K.S., Veeraiah, K., Vardhan, K.S (2003): Toxicity and Residue Studies of fenvalerate, a synthetic pyrethroid to freshwater fish Channa punctatus (Bloch). Bulletin of Environmental Contamination and Toxicology, Florida, USA, 71(6), 1207-1212. | | Tripathi, P.K., Srivastava, V.K and Singh,A (1992). Toxic effects of dimethoate (Organophosphate) on metabolism and enzyme system of freshwater teleost fish Channa punctatu. asian fisheries society, manila, Philippines, Asian fisheries science, 16 : 349-359. | | Veeraiah, K and Durga Prasad, M. K (2001): Studies on ventilatory patterns of fish under normal and stressed conditions using indigenously designed electronic recording instrument, proceeding of the International Conference of ICIPACT. | | Veeraiah, K (2002). 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. | | Veeraiah, K and Durga Prasad, M.K (1996). A study on the organochlorine pesticide residue input into lake Kolleru through Tammileru river, Ecol. Env. and Cons, 2: 182. |