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Article *in* Journal of Environmental Biology · May 2007

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April 2007, 28(2) 485-487 (2007)
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Toxicity studies of butachlor to the freshwater fish *Channa punctata* (Bloch)

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(Received: May 17, 2005 ; Revised received: December 18, 2005 ; Accepted: January 14, 2006)

Abstract: *The toxicity studies were conducted on the fish Channa punctata (Bloch) by employing static and continuous flow through systems, for the toxicant butachlor (technical grade*) and its commercial formulation* (machete 50% EC). The LC_{50} values are 297.89 ppb and 247.46 ppb for 24 hr and 48 hr in static for technical and 636.45 and 546.09 for machete. In continuous flow through the values are 270.05, 233.52 to the technical and 567.85 and 481.49 respectively for machete. The tissues show qualitative accumulation and were quantitatively analysed by gas liquid chromatography (GLC).*

Key words: *Toxicity, Channa punctata, Butachlor, Machete, Gas liquid chromatography*

Introduction

Butachlor was the first rice herbicide to be introduced in India. It is chemically 2-chloro 2, 6 diethyl N, butoxymethyl acetanilide (Fig. 1).

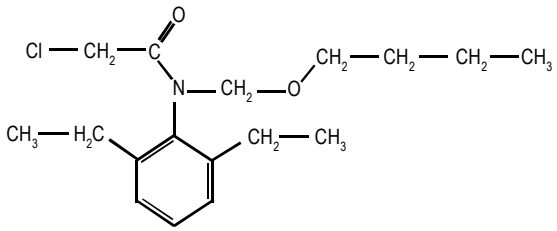

Fig. 1: Chemical formula of machete

For many years, bioassays have remained simple tools to assess acute and chronic toxicity. Even in sublethal concentrations butachlor effects are multifold ranging from simple respiratory distress, accumulation, effecting the biochemical pathway at cellular neurological levels and finally culminating in inhibition/decrease of the neuromotor enzyme acetyl cholinesterase. In the present study, an attempt has, therefore, been made to assess the toxicity of acute nature and the residue study of qualitative and quantitative nature of the toxicant butachlor after exposure of the fish *Channa punctata* (Bloch) to sublethal doses of the herbicide.

Materials and Methods

The fish *Channa punctata* (Bloch) of 8 to 10 cm length and 6 to 9 g of weight were brought from the river Krishna. Toxicity studies were conducted using the machete (50% EC) by employing static and continuous flow through systems as per the recommendations of APHA (1998). For flowthrough system, test solutions of desired concentrations were prepared once every five hours in glass reservoirs and delivered into the test containers through thin walled polyethelene tubes. The flow rate was adjusted with regulators such that 4 liter of water passed through containers in one hour. The conditions of the test medium were: temperature 28°C ± 2 °C, oxygen 6-8 mg l^{-1}, hardness 80 mg l^{-1}, alkalinity 425 mg l^{-1} and pH 8.3. Pilot experiments were conducted to determine the concentrations causing 10-90% mortality of test fish. For each concentration, 10 fish were tested and the experiment was repeated thrice. Probit analysis (Finney, 1971) as recommended by Roberts and Boyce (1972) was followed to calculate the LC_{50} values.

For the residue analysis, the fish were acclimatized at room temperature (28°C ± 2) in the laboratory conditions for 7 days in well aerated water and these acclimatized fish were exposed for 10 days to sublethal concentration ($^1/_5^{th}$ of static LC_{50}) of butachlor. After the exposure, the residues were extracted from the tissues by the method of Seleh *et al.* (1986), but the brain tissue was extracted in acetonitrile, in the presence of anhydrous sodium sulphate, the residue was finally washed in 4 ml of hexane.

Clean up: The extracts of the tissues *viz.*, gill, liver, kidney, muscle and brain were cleaned up by column chromatography using silica gel as adsorbent covered with a layer of anhydrous sodium sulphate, packed with hexane. The butachlor was eluted in a mixture of hexane and acetone (9 : 1). A 100 ml portion of the elutant was collected and evaporated to a final volume of 1 ml according to Goughan *et al.* (1978).

Gas liquid chromatographic (GLC) analysis: The quantitative analysis by gas liquid chromatography was performed according to Bradbury and Coats method (1982). Residues of butachlor were determined by using GC-17A. GC 17A is an autocontrol system of Shimadzu, Japan equipped with electron capture detector and coiled glass column (S.E-30) of 25 metres and 0.31 mm inner diameter with 0.17 micro meters film thickness. Helium was used as carrier gas through 1.5 ml/min. The standard curves

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based on the 8 cm of peak heights were used for quantification. The amount of butachlor residue was calculated against known standard values by employing the following formula.

$$\text{Concentration} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{Amount of standard}$$

The residue analysis by GLC is being done by Indian Institute of Chemical Technology (IICT), a CSIR Laboratory, Hyderabad-500 007, India.

Results and Discussion

The LC_{50} values of machete for 24 hr and 48 hr to the fish, *Channa punctata* (Bloch) are given in Table 1. Farah *et al.* (2004) reported the LC_{50} values and stress behaviour in the fish and mosquito larvae under exposure to three widely used xenobiotic compounds like pentachlorophenol (PCP), 2,4-D and butachlor. Mosquito larvae were generally more resistant than the fish.

Junghans *et al*. (2003) studied the effects of chloroacetanilides (acetachlor, alachlor, butachlor, dimethachlor, metazachlor, pretilachlor and propachlor) individually and in combination on the reproduction of the green algae, *Scenedesmus vaculatus*. Individually, chloroacetanilides impaired algal reproduction with EC_{50} values ranging from 3 to 232 mg l^{-1}. The differences in EC_{50} values were strongly correlated with the lipophilicites of the compound synergistically. The acute toxicity of butachlor on earthworm *Drawide willsi* was determined by Smeeth and Sanjat (2002). The reported 96 hr LC_{50} values were 7.72 to 10.22 mg/kg for juveniles as well as for adults respectively. Tantawy (2002) studied the effect of two herbicides on biological and biochemical parameters of *Biomphalaria alexandrina* and reported that after 6 hr of exposure to 6.5 mg l^{-1} concentration of butachlor, the mortality rate was increased. Suseela (2001) reported that the herbicide, as toxicant, did not have any adverse effects but it accelerated the nitrogen fixation. Hashimoto and Nishituchi (1983) studied the toxic effects of butachlor not only in fish but also in *Daphnia* and other aquatic organisms and concluded that it is toxic. Wang *et al.* (1991) reported that the residues of butachlor in paddy field, even though lower than the safe concentration, caused toxic effect to *Cyprinus carpio*.

Alachlor, closely related to butchlor is also toxic to fish. The LC_{50} value for rainbow trout is 2.4 mg l^{-1}, bluegill fish is 4.3 mg l^{-1}, catfish is 6.5 mg l^{-1} and carp is 4.6 mg l^{-1} after exposure of 96 hr (Kidd and James, 1991; Usnlm, 1995). Hill *et al.* (1997) studied the most widely used herbicides of chloroacetalnilides, alachlor and butachlor and made the hypothesis that they exert genotoxic effect in cultured lymphocytes.

Hard *et al.* (1995) studied the stomach tumours that were induced in Sprague Dawley rats during two chronic bioassays with acetanilide herbicide, butachlor at a dietery concentration of 3000 mg l^{-1}, the results in the experimental animals indicated that butachlor has low mammalian toxicity following acute oral, dermal and inhalation exposure. Some earlier reports revealed that commercial formulations are more toxic than technical grade pesticides, but in the present study the technical grade herbicide *i.e.*, butachlor was found more toxic than commercial formulations *i.e.*, machete 50% E.C. The reason is that the ingredients mixed in the formulations are not causing toxicity, an observation which is contrary to the earlier reports of Bradbury *et al.* (1987) and Tilak *et al.* (2003).

Generally aquatic organisms are chemically affected by three different classes of pesticides *viz.*, organochlorine, organophosphates and carbamates (Edwards, 1973; Brown, 1978; Rand and Sam, 1985). Among them, organochlorines act as contact poison which effect the central nervous system of the organisms, whereas organophosphates and carbamates inhibit the enzyme acetylcholinesterase. The acetanilide group of herbicides, as per the earlier reports, proved to have different toxic values for different aquatic organisms and the mechanism of action is of cumulative nature.

The results of tissue analysis of *Channa punctata* (Bloch) exposed to sublethal concentration are given in Table 2 and the residues are in the following order:

Liver > Kidney > Muscle > Gill > Brain

The variations in the residue analysis are due to factors like difference in uptake rate, lipid content of respective animal tissue, chemical structure, solubility, and metabolic pattern. (Zitko

Table - 1: The LC_{50} values and their confidence limits of butachlor technical grade and machete (50% EC) formulation to fish, *Channa punctata* (Bloch) for 24 and 48 hr static and continuous flow (CF) through system

Toxicant	Fish		24 hr		48 hr	
	Length cm	Weight g	Static ppb	C.F. ppb	Static ppb	C.F. ppb
Technical grade of butachlor	8 - 10	6 - 9	297.89 (4.56-4.38)	270.05 (4.51-4.35)	247.46 (4.49-4.28)	233.52 (4.49-4.24)
Machete 50% E C	8 – 10	6 - 9	636.45 (4.86-4.74)	567.85 (4.91-4.60)	546.09 (4.78-4.68)	481.49 (4.73-4.63)

CF = Continuous flow through, ppb = parts per billion

Table - 2: Residues of butachlor analysed by gas liquid chromatography (GLC)

Fish	Type of tissue	Amount of residue mg/g of tissue weight
Channa punctata (Bloch)	Gill	0.1255
	Liver	0.3515
	Kidney	0.3145
	Brain	Traces
	Muscle	0.2350

et al., 1977; Mulla *et al.,* 1978; Coats and O' Donnel Jaffery, 1979; Zitko *et al.,* 1979; Bradbury *et al.,* 1987; Haya, 1989; Tripathi, 1992; Tilak 1982; Tilak, *et al.,* 1980, 2003 and 2004). The accumulation of residue is a factor responsible for changes either in biochemical or pathological disturbance of overall biochemical cyclic reactions which are cumulative, causing lethality even at the sublethal concentrations.

The results of present study revealed that prolonged exposure to sublethal concentrations led to increase in the accumulation of residue. Several chemicals are absorbed and metabolised. Radio labelled butachlor absorption was measured in human skin and the unchanged compound and metabolites were quantified by high pressure liquid chromatography (HPLC) and qualified by thin layer chromatography (TLC) (Ademola *et al*. 1993). The final action of toxicity is due to metabolism and accumulation of the residues in fish tissues as reported by Susan *et al*. (1999a and b). The residues are accumulated in different tissues, causing toxicity to the fish which ultimately results in bio-magnification through the food chain.

References

Ademola, J.I., R.C. Wester and H.I. Maibach: Absorption and metabolism of 2-chloro-2,6-di ethyl-N-(butoxymethyl) acetonilide (butachlor) in human skin *in vitro*, *Toxicol. Appl. Pharmacol.,* **121(1)**, 78-86 (1993).

APHA.: Standard methods for examination of water and waste water, 16th *Edn.* American public health association, Washington D.C. (1998).

Bradbury, S.P. and J.R. Coats: Toxicity of fenvalerate to bobwhite quail including brain and liver residues associated with mortality. *J. Toxicol. Environ. Hlth.,* **10**, 307-319 (1982).

Bradbury, S.P., D.M. Symonik, J.R. Coats and G.J. Atchison: Toxicity of fenvalerate and its constituent isomers to the fat head minnow *(Pimephales promelas*), bluegill (*Lepomis macrochirus*). *Bull. Environ. Contam. Toxicol.,* **38**, 727-35 (1987).

Brown, A.W.A.: Insecticides and fish. *In:* Ecology of pesticides, John Wiley and Sons, Inc., New York, USA. p. 525 (1978).

Coats, J.R. and N. O'Donnell Jaffery: Toxicity of four synthetic pyrethroid insecticides to rainbow trout. *Bull. Environ. Contam. Toxicol.,* **23**, 250-255 (1979).

Edwards, C.A.: Environmental pollution by pesticides. Plenum Press, London, New York (1973).

Farah, M.A., B. Ateeq, M.N. Ali, R. Sabir and W. Ahmad: Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. *Chemosphere,* **55(2)**, 257-65 (2004).

Finney, D.J.: Probit Analysis, 3rd Edn. Cambridge University Press (1971).

Goughan, L,C., R.H. Robinson and J.E. Casida: Distribution and metabolic fate of trans and cis permethrin in laying hens. *J. Agric. Food Chem.,* **26**, 1374-1380 (1978).

Hard, G.C., M.J. Iatropoulos, D.C. Thake, D. Wheeler, M. Tatematsu, A. Magiwara, G.M. Williams and A.G. Wilson: Identity and pathogenesis of stomach tumors in sprague - Dawley rats associated with the dietary administration of butachlor. *Exp. Toxicol. Pathol.,* **47(2-3)**, 95-105 (1995).

Hashimoto, Y and Y. Nischituchi: IUPAC Pesticides chemistry, human welfare and the environment. (*Eds*.: J Miyamoto and PC Kearney), Pergamon Press, New York, Vol. 2, pp. 355-358, (1983).

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

Hill, A.B., P.R. Jefferies, G.B. Quistad and J.E. Casida: Dialkylquinonelmine metabolites of chloroacetanilide herbicide induce sister chromatid exchanges in cultured human lymphocytes. *Mutat. Res.,* **395(2-3)**, 159-71 (1997).

Junghans, M., T. Backhaust, M. Faust, M. Scholze and L.H. Grimme: Predictability of combined effects of eight chloroacetanilide herbicides on algal reproduction. *Pest Manage. Sci.*, **59(10)**, 1101-10 (2003).

Kidd, H. and D.R. James: The agrochemicals handbook. 3rd Edn., Royal Society of Chemistry Information Services, Cambridge, UK (as updated). pp. 10-2 (1991).

Mulla, M.S., H.A. Navvab Gojrati and H.A. Darwazeh: Toxicity of mosquito larvicidal pyrethroids to four species of freshwater fishes. *Environ. Entomol.,* **7**, 428-430 (1978).

Rand, G.M. and R.P. Sam: Fundamentals of aquatic methods and application, Hemisphere Publishing Corp. Washington (1985).

Roberts, M. and C.B.C. Boyce: *In*: Methods in Microbiology. 7-A Edn. (*Eds*: J.R. Norris and D.W. Ribbows), Academic Press, New York. p. 479 (1972).

Seleh, M.A., N.A. Ibrahim, N. Solina and M.K.E. Sheimy: Persistence and distribution of cypermethrin, deltamethrin and fenvlerate in laying chickens. *J. Agric. Food Chem.,* **34(5)**, 895-899 (1986).

Smeeth, P. and K.S. Sanjat: Acute toxicity assessment of three pesticides to the earthworm *Drawida wellsi*. *J. Ecotoxicol. Environ. Monit.,* **12(3)**, 215-223 (2002).

Susan, A.T., K. Veeraiah and K.S. Tilak: Biochemical changes in the tissues of the fish *Catla catla* exposed to fenvalerate a synthetic pyrethroid. *J. Ecobiol.,* **11(2)**, 109-116 (1999a).

Susan, A.T., K. Veeraiah and K.S. Tilak: A study on the bioaccumulation of fenvalerate, a synthetic pyrethroid in the whole body tissue of *Labeo rohita, Catla catla, Cirrhinus mrigala* by GLC. *Enviromedia,* **18(1)**, 57-59 (1999b).

Suseela, M.R.: Effect of butachlor on growth and nitrogen fixation by *Anabaena sphaerica*. *J. Environ. Biol.,* **22**, 201-203 (2001).

Tantawy, A.A.: Effect of two herbicides in some biological and biochemical parameters of *Biomphalaria alexandrina*. *J. Egypt Soc. Paragitol.,* **32(3)**, 837-47 (2002).

Tilak, K.S., D.M.R. Rao, A.P. Devi and A.S. Murthy: Toxicity of carbaryl and 1-naphthol to the fresh water fish *Labeo rohita*. *J. Expt. Biol.,* **18(1)**, 75-76 (1980).

Tilak, K.S.: Relative toxicity of carbaryl 1-naphthol and three formulations of carbaryl to *Channa punctatus* (Bloch). *Matsya*, **8**, 47-48 (1982).

Tilak, K.S., K. Satyavardhan and P.B. Thathaji: Biochemical changes induced by fenvalerate in the freshwater fish *Channa punctata*. *J. Ecotoxicol. Environ. Monit.,* **13(4)**, 261-270 (2003).

Tilak, K.S., K. Veeraiah and D. Koteswara Rao: Toxicity and bioaccumulation of chlorpyrifos in Indian carp *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.). *Bull. Environ. Contam. Toxicol.,* **3(5)**, 933-941 (2004).

Tripathi, G.: Relative toxicity of aldrin, fenvalerate, captan and diazinon to the fresh water food fish *Claries batrachus***.** *Biomed. Environ. Sci.*, **5**, 33-38 (1992).

USNLM (US National Library of Medicine): Hazardous substances data bank, Bethesda, MD. 10-9 (1995).

Wang, S., C.C. Cheng, H. Liu and C. Chiangh: Residue of three herbicides in paddy water and their danger level to carp. *J. Chin. Agric. Chem. Soc.,* **29(2)**, 195-202 (1991).

Zitko, V., W.G. Carson and C.D. Metcalfe: Toxicity of pyrethroids to juvenile atlantic salmon. *Bull. Environ. Contam. Toxicol.,* **18**, 35-41 (1977).

Zitko, V., D.W. McLeese, C.D. Metcalfe and W.G. Carlson: Toxicity of permethrin, decamethrin and related pyrethroids to Salmon (*Salmo saler*) and lobster. *Bull. Environ. Contam. Toxicol.,* **21**, 338-343 (1979).