Pertanika J. Sci. & Technol. **13(2)**: 249 - 256 (2005)
ISSN: 0128-7680

Bioaccumulation of Carbofuran and Endosulfan in the African Catfish *Clarias gariepinus*

†Isam Eldin Mohamed Abu Zeid, Mohd Arif Syed, Johari Ramli, Juzu Hayati Arshad, Ismail Omar & *Nor Aripin Shamaan

†College of Applied and Industrial Sciences
University of Juba, P.O. Box 321/1
Khartoum, Sudan
Department of Biochemistry
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Received: 13 October 2003

ABSTRAK

Ikan keli Afrika *Clarias gariepinus*, telah didedahkan kepada dos submaut ^{14}C-carbofuran dan ^{14}C-endosulfan. Selepas tempoh pendedahan 1, 6, 12, 24, 72 dan 144 jam, ikan dibunuh dan sisa racun tersebut ditentukan dalam hepatopankreas, usus, insang, otak dan otot skelet. Paras tertinggi sisa racun serangga tersebut (per g berat kering tisu) didapati dalam hepatopankreas ((354 ± 6 μg carbofuran dan 1409 ± 43 ng endosulfan selepas 24 jam), diikuti usus (239 ± 1 μg carbofuran dan 1147 ± 21 ng endosulfan selepas 72 jam), insang (108 ± 2 μg carbofuran dan 817 ± 19 ng endosulfan selepas 24 jam), otak (57 ± 2 μg carbofuran selepas 24 jam, 555 ± 19 ng endosulfan selepas 72 jam) dan otot skelet (25 ± 1 μg carbofuran selepas 144 jam, 364 ± 13 ng endosulfan selepas 6 jam). Bermula pada pendedahan selepas 1 jam, paras sisa carbofuran dan endosulfan meningkat dengan pantas ke paras tertinggi pada masa yang berbeza dalam tisu berkenaan selepas 6 jam pendedahan. Selepas sisa carbofuran dan endosulfan meningkat ke paras maksimum dalam semua tisu tersebut, ia menurun mengikut masa.

ABSTRACT

The African catfish *Clarias gariepinus* was exposed to a sublethal dose of ^{14}C-carbofuran and ^{14}C-endosulfan. After 1, 6, 12, 24, 72 and 144 h, the fish were removed and insecticide residues determined in the liver, intestines, gills, brain and skeletal muscles. Highest levels of residues, per g dry weight of tissues, was found in the liver (354 ± 6 μg carbofuran and 1409 ± 43 ng endosulfan after 24 h) followed by the intestines (239 ± 1 μg carbofuran and 1147 ± 21 ng endosulfan after 72 h), the gills (108 ± 2 μg carbofuran and 817 ± 19 ng endosulfan after 24 h), brain (57 ± 2 μg carbofuran after 24 h, 555 ± 19 ng endosulfan after 72 h) and skeletal muscle (25 ± 1 μg carbofuran after 144 h and 364 ± 13 ng endosulfan after 6 h). The residue levels were rapidly accumulated in the tissues. Based on the levels of pesticide residues after 1 h, all the tissues recorded very high levels of residues after 6 h. After reaching

* Corresponding Author: Nor Aripin Shamaan
E-mail: *naripin@biotech.upm.edu.my*

maximum levels in the tissues at various times of exposure, the residue levels in all the tissues began to decrease over time.

Keywords: Carbofuran, endosulfan, African catfish, bioaccumulation

INTRODUCTION

The applications of the insecticides carbofuran and endosulfan in rice fields as pest control had resulted in increased fatalities of freshwater fishes which is an important source of protein for rice growers and also as their economic subsistence (Liong *et al.* 1988). The rice field freshwater fishes had been reported to be sensitive to the insecticides as shown by the low LC_{50} values of the insecticides.

Currently, most of the data on bioaccumulative studies of pesticides in the African catfish, *Clarias gariepinus,* have been obtained from field studies while in the present study, the work was done under controlled laboratory conditions and juvenile fish of the same age and body weight was selected.

It was reported that following endosulfan application for testse fly control in Botswana, an increased fatality in freshwater fish was observed and endosulfan residues were detected in the tissues (Mathiessen *et al.* 1982). This is an example of non-target organism being affected by applications of insecticides in vector control. High concentration of pesticide residues was detected in the liver of the indigenous fish sampled for the study followed by the intestines, gills, brain and skeletal muscles (Heath 1992). The pesticide residues were rapidly absorbed into the body through the gills of freshwater fish species (Lloyd 1992) and accumulated in the various organs, sometimes bioconcentrated by as much as 2000-fold over the concentration of the residue in the water (Devi *et al.* 1981). The liver has been shown to be the major organ for the metabolism of the residues in fish (Peterson and Bately 1993).

Due to the high toxicity of carbofuran and endosulfan to freshwater fish, a study was carried out to investigate the cause of toxicity in the African catfish, *Clarias gariepinus,* with particular emphasis on bioaccumulation of the residues in the tissues of the fish. We report on work done on the bioaccumulation of two insecticides, carbofuran and endosulfan, in the African catfish, *Clarias gariepinus.*

MATERIALS AND METHODS

Technical grade carbofuran (99.1% purity) and endosulfan (99.3% purity) were purchased from Ehrenstorfer GmbH Co., Germany. Labelled ^{14}C-carbofuran and ^{14}C- endosulfan were supplied by the International Atomic Energy Agency, Vienna, Austria. The insecticides were initially dissolved in acetone and then serially diluted in water prior to application. All other chemicals and reagents used were of the highest purity commercially available.

Juvenile African catfish *Clarias gariepinus,* (14 ± 2 cm length, 30 ± 2 g body weight) were reared in the university hatchery. The fishes were initially acclimatised for 14 days in glass aquaria containing 25 L dechlorinated water at ambient temperature of 29°C, pH 7.5-7.9, dissolved oxygen 5.6-7.8 mg/L and ammonia 0.4-1.4 mg/L and fed commercial fish pellet *ad libitum* before testing. These conditions were maintained throughout testing. The fish were starved for 24 h prior to exposure to the insecticides and throughout the test period to avoid prandial effects.

To the water in the test aquaria, 0.1 mL (10 μCi) ring-labelled ^{14}C-carbofuran and 0.18 mL (10 μCi) ring-labelled ^{14}C-endosulfan were added and shaken to uniformly distribute the pesticides. The carbofuran and endosulfan concentrations were maintained at 5.2 mg/L and 10.8 μg/L, respectively. These values were 50% of the LC_{50} (96h) for carbofuran and endosulfan estimated previously in our laboratory (unpublished data). The fishes were then placed in the aquaria and three fishes were subsequently removed after 1, 6, 12, 24, 72 and 144 h.

After removal, the fishes were immediately rinsed in distilled water to remove surface insecticide residue. The fishes were then sacrificed with a single blow on the head and the liver, gills, brain, skeletal muscle and intestines immediately excised, weighed and analyzed. The excised organs were oven dried at 45°C over 24 h and the dry weight determined. Subsequently, 0.1 g dried organ was then combusted in a biological oxidiser at 900°C and the labelled CO_2 released trapped in 10 mL Harvey cocktail. Radioactive carbon was determined by liquid scintillation.

RESULTS

Fig. 1 shows the distribution of the insecticide residues in fish organs after the $^{14}CO_2$ counts were converted into actual amount of insecticide per dry weight of the organ.

Juvenile African catfish generally seemed to accumulate carbofuran and endosulfan very rapidly. After 1 h exposure, there were considerable amounts of insecticide residues in the selected tissues. The residue concentrations were markedly elevated after 6 h exposure and these levels were maintained for the test period.

Bioaccumulation of carbofuran in the tissues reached a maximum in the liver, gills and brain after 24 h, intestines after 72 h and skeletal muscle after 144 h. Carbofuran residues were found to accumulate in the liver, intestines, gills, brain and skeletal muscle, in descending order, at any given time.

Endosulfan accumulation in the tissues of the African catfish reached a maximum in the liver and gills after 24 h, intestines and brain after 72 h, and skeletal muscle after 6 h. Surprisingly, accumulation of endosulfan was more rapid in the skeletal muscle than carbofuran.

An interesting observation were the high levels of carbofuran and endosulfan residues located in the organs after 6 h, which showed the insecticides were rapidly absorbed and distributed to the organs.

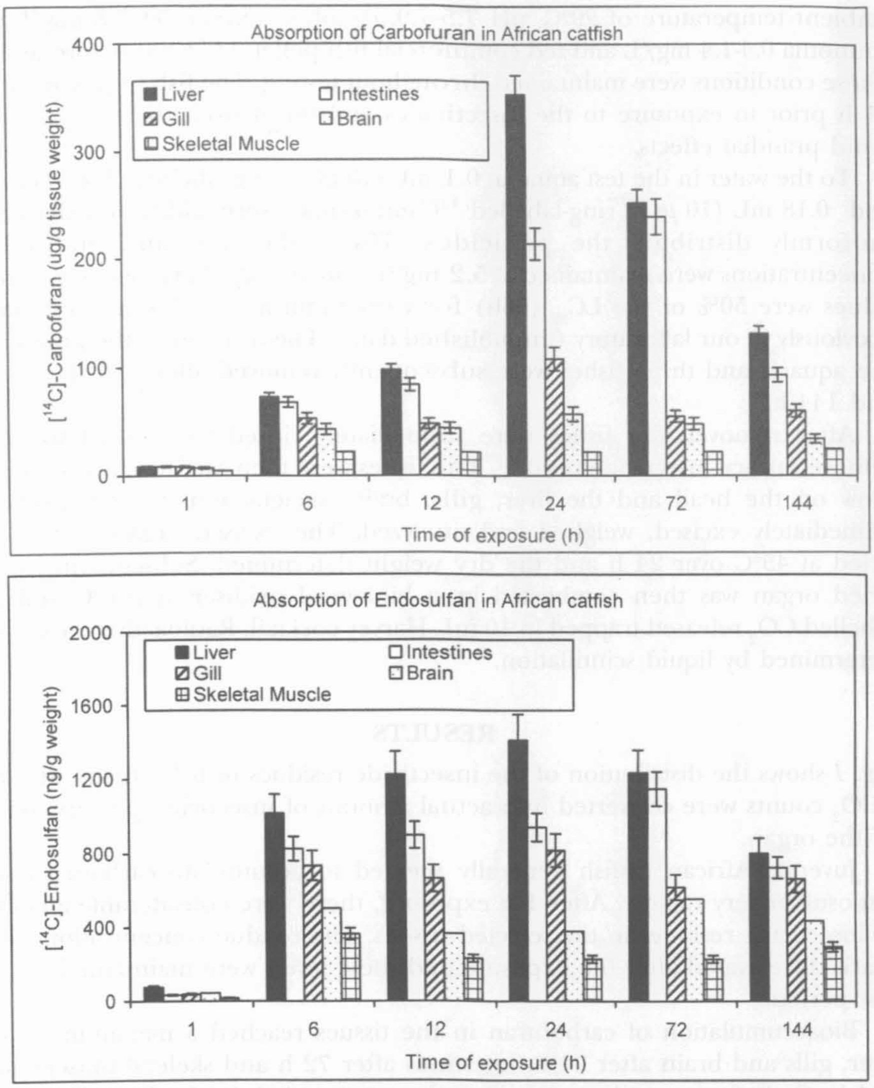

Fig. 1: Levels of carbofuran and endosulfan residues in selected tissues of the African catfish over time. Values shown are mean ± standard deviation. Values for carbofuran are μg carbofuran per g dry weight of tissue; endosulfan, μg endosulfan per g dry weight tissue

DISCUSSION

It is evident from the present study that *Clarias gariepinus* can bioaccumulate carbofuran and endosulfan to elevated levels in the tissues and that the distribution of these pesticides is different from one tissue to the other. Generally, the liver contained the highest levels of pesticide residues while the skeletal muscle contained the lowest amount of pesticide residues.

Heath (1992) reported that pesticide residues were determined in tissues of several freshwater fish species caught in two rivers in South Africa. Of the species caught, the African catfish was suggested as an indicator species for pollution due to the high concentrations of residues found in the fish compared with the other species. Grobler (1994) and Siwela *et al.* (1996) detected and measured pesticide residues in the catfish caught in the Olifants river, South Africa and dams in Zimbabwe, respectively. They found that the catfish contained elevated levels of pesticide residues compared with other freshwater fish species caught in the study.

Bioaccumulation of carbofuran and endosulfan in tissues had been demonstrated in several other species of fish (Berbert *et al.* 1989; Ferrando *et al.* 1992; Kale *et al.* 1996; Singh and Garg 1992). Of the tissues analysed for residues, the liver contained the highest level of carbofuran and endosulfan residues followed by the intestines, gills, brain and skeletal muscle. The liver and gills of *Clarias gariepinus* caught in the Olifants river, South Africa, were also found to accumulate high levels of heavy metals (Du Preez *et al.* 1997). The freshwater fish *Oreochromis mossambicus,* showed tissue selective bioaccumulation of cadmium with the liver and kidney being high accumulators while the gills, intestines and scales contained low levels of the metal (Rani 2000). The findings obtained in the present study are consistent with the liver and gills as the major organs for accumulation and metabolism of pesticides. The tissue distribution of carbofuran and endosulfan in *Clarias gariepinus* could be attributed to differential mechanism of binding and regulation, as shown for cadmium and zinc, in the green-lipped mussel, *Perna viridis* (Yap *et al.* 2003).

Endosulfan had been found to be metabolised in fish liver (Devi *et al.* 1981; Rao and Murty 1980), by oxidation reactions and conjugation with sulphate, glucuronate and bile salts (Mathiessen *et al.* 1982; Peterson and Bately 1993). In the present work, after reaching the maximum level of accumulation, the level of both carbofuran and endosulfan in the liver was observed to decrease, indicating possible metabolism, excretion and elimination of the insecticides. This is supported by Toledo and Jonsson (1992) who worked on the bioaccumulation and elimination of endosulfan in the Zebra fish. In the other tissues, the residue levels were also slightly decreased.

The gills had been shown to be the major organ for the uptake of pollutants in freshwater fishes (Lloyd 1992). Since the fish were not given any food 24 h before and throughout the test period, uptake and accumulation of residues by feeding could be ruled out. Therefore, the major uptake of carbofuran and endosulfan into tissues was through the gills, consistent with the work of Takimoto and Miyamoto (1976). It had also been reported that freshwater fish can bioconcentrate endosulfan over 2000-fold the concentration of the insecticide in the water (Devi *et al.* 1981).

The rapid bioaccumulation of carbofuran and endosulfan residues in the brain and skeletal muscles of the African catfish gives rise to concern. Within 6 h of exposure, both the brain and skeletal muscle of the catfish contained at least 4-fold the initial level of insecticide residues. Since both carbofuran and endosulfan had been shown to be highly toxic to freshwater fishes, the rapid absorption of the insecticides in these tissues could possibly provide an explanation as to why freshwater fishes are highly susceptible to the toxic effect of carbofuran and endosulfan.

Both carbofuran and endosulfan had been shown to induce biochemical changes in fish. Induction of cytochrome P450 oxidases, glutathione S-transferase and amino acid transaminases had been reported (Arnold *et al.* 1995; Rodriguez-Ariza *et al.* 1993; Trotter 1991; Verma *et al.* 1981). Since both insecticides are anti-cholinesterases, inhibition of the enzyme had also been documented (Gupta and Gupta 1971; Gupta 1994). Thus, the freshwater fish is sensitive to the insecticides used in the present study.

In conclusion, the data obtained in the study reveals that the African catfish is able to accumulate carbofuran and endosulfan rapidly with the liver and intestines being the major tissues for bioaccumulation. The gills provided the major uptake tissues for the African catfish. The distribution of the residues in the selected tissues reveal tissue selective bioaccumulation and could contribute to the possible cause of susceptibility of the fish to the toxic effect of the insecticides. Finally, the potential use of *Clarias gariepinus* for biomonitoring of carbofuran and endosulfan is suggested although its use should be verified by field and depuration studies done under laboratory conditions.

ACKNOWLEDGEMENTS

This work was funded by a research grant no. IRPA 01-02-04-078, from the Ministry of Science, Technology and Innovation, Malaysia

REFERENCES

ARNOLD, H., H.J. PLULA and T. BRAUNBECK. 1995. Simultaneous exposure of fish to endosulfan and disulfoton *in vivo*: Ultrastructural, stereological and biochemical reaction in hepatocytes of male rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* **33:** 17-43.

BERBERT, P.R.T., J.M. ABREU, M.P. GRADVOHL and J.M. DE-ABRE. 1989. Toxicity of endosulfan to native and exotic fishes and crustaceans from the south of Bahia. *Agrotropica* **1**:144-152.

DEVI, A.P., D.M.R. RATO, K.S. TILAK and A.S. MURTY. 1981. Relative toxicities of technical materials, isomers, and formulations of endosulfan to *Channa punctatus*. *Bulletin of Environmental Contamination and Toxicology* **27:** 239-243.

DU PREEZ, H.H., M. VAN DER MERWE and J.H.J. VAN VUREN. 1997. Bioaccumulation of selected metals in African sharptooth catfish, *Clarias gariepinus* from the lower Olifants river, Mpumalanga, South Africa. *Koedoe* **40:** 77-90.

FERRANDO, M.D., M. GAMON and E. ANDREU. 1992. Accumulation and distribution of pesticides in *Anguilla anguilla* from Albiferra lake (Spain). *Journal of Environment and Biology* **13:** 75-82.

GROBLER, D.F. 1994. A note on PCBs and chlorinated hydrocarbon pesticide residues in water, fish and sediment from Olifants river, eastern Transvaal, South Africa. *Water SA* **20:** 187-194.

GUPTA, P.K. and R.C. GUPTA. 1971. Pharmacology, toxicology and degradation of endosulfan. A review. *Toxicology* **13:** 115-130.

GUPTA, R.C. 1994. Carbofuran toxicity. *Journal of Toxicology and Environmental Health* **43:** 383-418.

HEATH, R.G.M. 1992. The levels of organochlorine pesticides in indigenous fish from two rivers that flow through the Kruger National Park, South Africa. *Water Supply* **10:** 177-185.

KALE, S.P., G. SARMA, U.C. GOSWAMI and K. RAGH. 1996. Uptake and distribution of ^{14}C-carbofuran and ^{14}C-HCH in catfish. *Chemosphere* **33:** 449-451.

LIONG, P.C., W.P. HAMZAH and V. MURUGAN. 1988. Toxicity of some pesticides towards freshwater fishes. *Malaysian Agricultural Journal* **54:** 147-156.

LLOYD, R. 1992. What is pollution? Definition and effects. In *Pollution and Freshwater Fish*, pp. 12-23. Bodmin, Cornwall: Hartnolls.

MATHIESSEN, P., P.J. FOX, R.J. DOUTHWAITE and A.B. WOO. 1982. Accumulation of endosulfan residues in fish and their predators after aerial spraying for the control of tsetse fly in Botswana. *Pesticide Science* **13:** 39-48.

PETERSON, S.M. and G.E. BATELY. 1993. The fate of endosulfan in aquatic ecosystem. *Environment and Pollution* **82:** 143-152.

RANI, A.U. 2000. Cadmium induced bioaccumulation in the selected tissues of a freshwater teleost, *Oreochromis mossambicus*. *Annals of New York Academy of Sciences* **919:** 318-320.

RAO, D.M.R. and A.S. MURTY. 1980. Toxicity, biotransformation and elimination of endosulfan in *Anabas testineus* (Bloch). *Indian Journal of Experimental Biology* **18:** 664-666.

RODRIGUEZ-ARIZA, A., J. PEINADO, C. PUEYO and J. LOPEZ-BAREA. 1993. Biochemical indicators of oxidative stress in fish from polluted littoral areas. *Canadian Journal of Fisheries and Aquatic Science* **50:** 2568-2573.

SINGH, B. and A.K. GARG. 1992. Observation on the accumulation of ^{14}C-labelled carbofuran in different organs of fish. *National Academy of Sciences Letters* **15:** 343-344.

SIWELA, A.H., G. MARUFU and A.T. MHLANGA, 1996. A comparison of organochlorine pesticide residues in upper Ncema and lower Umguza dams, Zimbabwe. *Journal of Applied Sciences South Africa* **2:** 23-30.

TAKIMOTO, Y. and J. MIYAMOTO. 1976. Studies on accumulation and metabolism of sumithion in fish. *Journal of Pesticide Science* **1:** 261-271.

TOLEDO, M.C.F. and C.M. JONSSON.1992. Bioaccumulation and elimination of endosulfan in Zebra fish (*Brachydanio rerio*). *Pesticide Science* **36:** 207-211.

TROTTER, D.M. 1991. Aquatic fate effect of carbofuran. *Critical Review in Environmental Control* **21:** 137-176.

VERMA, S.R., S. RANI and R.C. DALELA. 1981. Isolated and combined effects of pesticides on serum transaminases in *Mystus vittatus* (African catfish). *Toxicological Letters* **8:** 67-71

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2003. Accumulation, depuration and distribution of Cadmium and zinc in the green-lipped mussel *Perna viridis* (Linnaeus) under laboratory conditions. *Hydrobiologia* **498:** 151-160.