Toxicological Effects Of Thiobencarb Herbicide On *Clarias Lazera* – Some Biochemical Changes And Residue Analysis

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ABSTRACT

The use of insecticides and herbicides is increasing very rapidly in agriculture in order to control unwanted insects and weeds. The unlimited use of these chemicals is affecting the aquatic biota including fish. LC_{50} of thiobencarb in *Clarias lazera* was determined. The effect of $^{1}/_{2}$ LC_{50} for one week and 1/20 LC_{50} for 12 week as well as the field dose (2 liter per acre) on ALT, AST, ALP and ChE activities were carried out. Glucose, total protein, triglycerides, cholesterol, total bilirubin, reduced glutathione, lipid peroxidation and Ca, P levels were estimated. Results indicated an increase in enzymes activities while ChE was lowered. Hyper glycemia, an increase in Ca, P, total bilirubin, lipid peroxidation were recorded while reduced glutathione, cholesterol and triglycerides were decreased. Clinical signs, post mortem examination as well as residues were also investigated.

INTRODUCTION

Carbamates are probably the most familiar chemicals used as herbicide in agriculture for control of broad leaf weeds, they are cholinesterase inhibitors, exert their herbicidal action on the weeds by protein synthesis inhibition. The diagnosis of carbamate poisoning in fish requires detection of carbamates in the fish tissue and in the water *(1)*. Thiobencarb is a carbamate compound used as a broad spectrum herbicide affecting gramineous, cyperaceous weeds and some broad leaf weeds *(2)*. Several studies *(3-5)* reported that thiobencarb (s-4-chlorobenzyldithiocarbamate) act by inhibiting the acetyl choline esterase enzyme.

It is moderately toxic to fish *(6,7)*. It accelerates the swimming activity, increases the frequency of opercular beats and decreases growth rate *(5,9-11)* . Dithiocarbamate, cause wavy distortion of the notochord in Zebra fish and other fish embryos *(12,13)* .

Acid and alkaline phosphatase levels in fish exposed to carbamate pesticides were elevated *(14,15)*. Increased serum ALT, AST *(15-18)* and decreased serum ChE activity *(4,19-21)*

Benthiocarb treatment in rat was associated with significant increase in specific activity levels of proteases in hepatic and neuronal systems, and glycogenic aminotransferases showed a significant elevation in both tissues, while glutamate dehydrogenase GDH exhibited reduced activity *(22)*. Benthiocarb decreased activities of Mg^{2+} and Ca^{2+}-ATPases on developing rat brain *(23,24)*, inhibited succinate dehydrogenase (SDH) *(25)* benthiocarb and decreased activities of ATPases of European eels (*Anguilla anguilla*)

Repeated administration of benthiocarb was associated with significant decrease in proteins with a concomitant increase in free amino acids *(15,17,22)*. Treated fish showed hyperglycemia, while liver and muscle glycogen levels decreased markedly. *(5,26,27)*.

Anguilla anguilla exposed to thiobencarb showed a significant decrease in reduced glutathione and increased oxidized form *(28,29)*, increased activity of catalase and glutathione peroxidase *(20)*, increased lipid peroxidation *(30,31)*. Induced activity of glutathione S-transferase, superoxide dismutase *(29)*.

The ratio of calcium/phosphorus level in serum of air-breathing catfish, (*Clarias batrachus*) showed significant diminution in experimental groups compared to controls. *(32)*, hyperphosphatemia, hypocalcemia *(33)*,

increase in the levels of inorganic phosphate *(15)* was reported

Dithiocarbamates are selectively localized in various tissues, Pigmented tissues reported to be the target tissue for their toxic action. *(34). (35)* recorded that bioconcentration of thiobencarb in carp (*Cyprinus carpio*) were 25.5 in muscle. It has been stated that the bioconcentration factor for different herbicides in fish were thiobencarb > chlomethoxyfen > butachlor *(36).*

MATERIALS AND METHODS

Herbicide

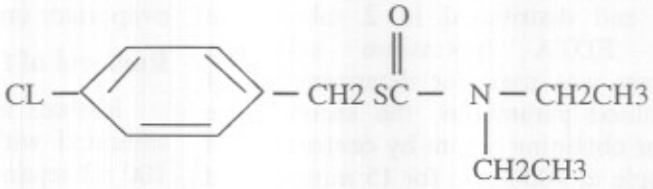
kafrosaturn was developed by Kumiai Chem. Ind. Co. Ltd, Japan and manfuctured by Kafr El-Zyat Chem. CO. Egypt. It contains 50 % active principle.

Thiobencarb is a member of the thiocarbamate group. It consists of a diethylthiocarbamate attached to a chlorobenzyl moiety. The chemical formula, structure, synonyms are listed as follows:

Common names: Thiobencarb; benthiocarb

Trade names: Bolero, Bolero 8EC, Bencarb, Saturn, Saturno, B 3015, IMC 3950, Siacarb, Tamariz

Structural formula



Aquaria

Six full glass aquaria (90 × 45 ×45 cm) were used for the experimental study. These aquaria were supplied with tap water. Continuous aeration and filtration were maintained using an air pump (Sicce–alls pietes, Italy) and filter (Sunny inc.,Japan), the water temperature was thermostatically adjusted at 20 ± 1°C using electrical heaters (type C M I, Germany). The pH was adjusted at 7.5 ± 0.4 throughout the experiment

Fish

Catfish (*Clarias lazera*) was the experimental fish. It was selected as it inhabits the lakes and drainage surrounding the cultivated area and in canals in which herbicides come with discharged water from applied area.

A total number of 230 catfish collected alive from Kafr El-sheikh market. All fish were apparently normal with average body weight 180gm and total length of 28 cm. The fish were transferred to the laboratory in a tank filled with water. All fish were kept in full glass aquaria supplied with tap water two weeks before experimentation to be adapted to the lab. environment.

METHODS

Determination of median lethal concentration (LC_{50}) of thiobencarb in cat fish (*Clarias lazera*)

The LC_{50} test was performed according to *(37).* A total number of 60 catfish were divided into 6 groups each of 10 fish. The fish groups were exposed to different concentration of technical product of thiobencarb (0, 2.3, 4.6, 9.2, 18.4, 36.8 ppm). The number of dead fish was recorded at 96 hours post exposure. The LC_{50} value was calculated according to the following formu¹⁻

$$M = X_k + 1/2\ d - \frac{dr}{N}$$

M = Log. LC_{50}
X_k = log. dose causing 100% mortality.
d = logarithmic interval of doses.
r = sum. of dead animals.
N = No. of animals per each group

Table 1. Design and grouping

	Groups.	No. of fish	Time (days)	Sampling time
Laboratory study	1- Control group	50	90	5 samples at the end of 1st, 2nd, 4th, 6th, 8th, 10th and 12th week
	2- Acute exposure toxicity group ($^1/_2$ LC_{50})	20	7	5 samples at the end of the week
	3- Chronic exposure toxicity group ($^1/_{20}$ LC_{50})	50	90	5 samples at the end of 2nd, 4th, 6th, 8th, 10th and 12th week
Field study	Exposed to the field recommended dose	50	90	5 samples at the end of , 2nd, 4th, 6th, 8th, 10th and 12th week

Blood samples were taken from caudal vein *(38)* and distributed in 2 tubes, one containing EDTA (potassium salt) as anticoagulant was used for determination of different blood parameters, the second tube was left for obtaining serum by centrifugation of the sample at 3000 rpm for 15 minutes and used for biochemical analysis.

Kits

Diamond diagnostics Kits were used for haemoglobin *(39)*, serum aspartate amino transferase (AST) *(40)*, serum cholinesterase *(41)*, cholesterol *(42)* and total protein *(43)*.

Biodiagnostic Kits were used for determination of serum alanine amino transferase (ALT) *(40)*, serum lipid peroxides (Malondialdehyde) *(44)*, blood reduced glutathione *(45)*, serum calcium *(46)*, serum phosphorus *(47)*, serum alkaline phosphatase *(48)*, serum triglycerides *(49)*, serum total bilirubin *(50)* and serum glucose*(51)*.

Determination of thiobencarb residues in catfish muscles

Extraction

Ten gms of muscles were homogenized with 20gms of anhydrous sodium sulfate with tissue homogenizer till having a fine homogenate. The homogenate was extracted with 50 ml of n-hexane: acetone (2:1) HPLC grade. Extraction was carried out using orbital shaker for 2 hours, and then extract was filtered through anhydrous sodium sulphate and evaporated till dryness using rotary evaporator under vacuum at 40°C *(52)*.

Removal of fat from extracts

Resides were dissolved in 2 ml n-hexane saturated with acetonitrile and transferred to 100 ml separating funnel then partitioned 3x20 ml with acetonitrile saturated with n-hexane. Acetonitrile layers were combined and evaporated just to dryness using rotary evaporator at 40°C *(53)*.

Cleaning up

Cleaning up was performed by a modification of the method of *(54)*. Six g activated florisil (60-100 mesh) were placed in a chromatographic column and topped with 1 g anhydrous Na_2SO_4. Column pre-wetted with 25ml n-hexane, samples were dissolved and loaded to column with n-heaxne (3x2ml nhexane), then eluted with 200 ml eluent of dichloromethane : n-hexane : acetonitrile at ratio of 50:48.5:1.5% at flow rate of 5ml/min, then elutes were evaporated till dryness at 40°C.

Condition of the chromatograph

The gas chromatograph used was Hewlett Packed GC Model 6890 equipped with Electron Capture Detector (ECD). A capillary HP –5 column (30m length x 0.25mm internal diameter x 0.25 µm film thickness), was used for the separation GC operating conditions were as the following: Injector and detector temperatures were 240°C and 250°C; initial

oven temperature, 170°C for 2 min, raised at 20 C˚ /min. then held at 280°C for 5 min.

Statistical analysis

Haematological and biochemical parameters were statistically analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System *(55)*. Means were compared using the Least Squares Means (LSM) of the same program.

RESULTS

Determination of LC_{50} of thiobencarb in catfish *Clarias lazera*.

The LC_{50} of thiobencarb was determined from Table 2 as follows:

$$M = 1.56585 + 1/2 \text{ X } 0.30103 - \frac{0.30103 \text{ X } 23}{10}$$

$M = 1.716365 - 0.692369$
$M = 1.023996$
96 hr LC_{50} = 10.57 mg /l (ppm).at confidence limit 95 %.

$$\text{Variance} = \frac{d^2}{N^2(N-1)} \text{ X } [r_1 (N_1-r_1)] [r_2 (N_2-r_2)] [\ldots\ldots]$$

$$= \frac{(0.30103)^2}{100(10-1)} \text{ X } [2(10-2)] [2(10-2)] [9(10-9)]$$

$$= 0.23198$$

$$\text{S.E} = \sqrt{\text{Variance}} = \sqrt{0.23198} = 0.481$$

Confidence limits = m ± t. SE =10.57 ± 0.47 X 0.48164
96 hr LC_{50} = 10.57 ± 0.23 ppm.
1/2 LC_{50} = 5.285 ppm.
1/20 LC_{50} = 0.5285 ppm.

Table 2. Determination of LC_{50} of thiobencarb in catfish *Clarias lazera* .

Group.	No. of animals per each group	Conc. (ppm)	Log. Conc.	Log. Conc. interval	Dead animals	Mortality %
1	10	0	—	—	0	0%
2	10	2.3*	0.36173	0.30103	0	0%
3	10	4.6	0.66276	0.30103	2	20%
4	10	9.2	0.96379	0.30103	2	20%
5	10	18.4	1.26482	0.30103	9	90%
6	10	36.8**	1.56585	0.30103	10	100%
Total.	60	—	—	—	23	—

Clinical signs and P.M finding of acute and chronic exposure of thiobencarb

The principal clinical signs observed in fish exposed to different concentrations of thiobencarb were signs of nervous manifestation, manifested by tremors, lethargy, circling movement and an increase in abnormal fish behaviour as swimming on its back, accelerated swimming activity. And respiratory signs manifested by an increase in the respiratory rhythm with larger opercular amplitude, increased the frequency of opercular beats, and increased surfacing. Other symptoms were observed such as decreased motility with loss of equilibrium, uncoordinated movements, decreased food intake and loss of body weight. The principal postmortem findings were congestion in liver, kidney, heart, spleen and gills.

Effect on enzymes and other biochemical parameters

The results revealed a significant increase in serum ALT, AST, ALP activities and serum total bilirubin level, decreased serum cholinesterase activity in catfish exposed to 1/2 LC_{50}, 1/20 LC_{50} and recommended field dose of thiobencarb compared with the control values (Table 3).

Table 3. Determination of ALT, AST, ALP, ChE activities and total bilirubin in catfish exposed to different concentrations of thiobencarb

		Time						
Parameter	group	1st w	2nd w	4th w	6th w	8th w	10th w	12th w
ALT u/L	Control	17.71±0.15	17.87±0.10 a C	17.82±0.15 a B	17.85±0.13 a B	17.93±0.09 a B	17.89±0.17 a B	17.99±0.20 a B
	Acute	30.44±0.27*	—	—	—	—	—	—
	Chronic	—	20.60±0.28 a A	20.93±0.37 a A	20.11±0.35 a A	20.35±0.49 a A	20.81±0.49 a A	21.08±0.43 a A
	Field	—	19.44±0.20 a B	20.05±0.39 a A	19.41±0.34 a A	20.17±0.15 a A	20.28±0.27 a A	20.32±0.59 a A
AST u/L	Control	140.42±0.65	140.32±0.90 a C	139.87±0.60 a C	139.66±0.57 a B	140.76±0.62 a C	140.75±0.80 a B	140.85±0.92 a B
	Acute	178.37±0.97*	—	—	—	—	—	—
	Chronic	—	149.77±0.54 c B	150.71±0.73 c B	153.32±0.93 b A	153.43±1.05 b B	157.06±1.01 a A	157.59±0.39 a A
	Field	—	152.59±0.59 d A	154.17±0.64 cd A	154.86±0.75 bc A	156.68±0.47 ab A	157.60±0.61 a A	157.20±0.63 a A
ALP u/l	Control	31.13±0.92	30.84±0.56 a B	31.34±0.71 a B	31.03±0.59 a C	31.85±0.51 a C	31.23±0.82 a C	31.52±0.81 a C
	Acute	49.06±1.70*	—	—	—	—	—	—
	Chronic	—	36.09±1.26 d A	36.78±1.00 c A	40.81±0.44 b A	41.28±0.50 b A	44.16±1.03 a A	45.67±0.66 a A
	Field	—	35.92±0.95 b A	37.48±0.69 ab A	37.79±0.64 ab B	37.92±0.73 ab B	39.20±0.76 a B	39.38±0.65 a B
Bilirubin Mg/dl	Control	1.20±0.12	1.25±0.06 a A	1.13±0.03 a B	1.23±0.10 a B	1.12±0.06 a B	1.26±0.09 a B	1.25±0.07 a B
	Acute	1.77±0.02*	—	—	—	—	—	—
	Chronic	—	1.29±0.04 c A	1.40±0.06 bc A	1.46±0.05 ab A	1.50±0.05 ab A	1.59±0.04 a A	1.62±0.04 a A
	Field	—	1.22±0.03 c A	1.29±0.04 bc A	1.34±0.03 bc AB	1.40±0.03 ab A	1.41±0.04 ab AB	1.51±0.04 a A
ChE u/ml	Control	14.30±0.10	14.32±0.07 a A	14.37±0.11 a A	14.35±0.06 a A	14.28±0.10 a A	14.33±0.10 a A	14.43±0.03 a A
	Acute	11.61±0.61*	—	—	—	—	—	—
	Chronic	—	10.77±0.34 b C	10.91±0.34 b C	10.75±0.30 b C	11.29±0.28 ab C	11.56±0.43 ab C	11.96±0.44 a C
	Field	—	12.50±0.26 ab B	12.16±0.40 b B	12.22±0.52 b B	12.92±0.17 ab B	12.75±0.56 ab B	13.27±0.32 a B

Values are means ± standard errors.
* Acute and control means of the first week differ significantly (P<0.05).
Means in the same column without a common capital letter differ significantly (P<0.05).
Means in the same row without a common small letter differ significantly (P<0.05).

The results revealed that there was a significant increase in blood reduced glutathione, increased serum lipid peroxides (malondialdhyde) in fish exposed to thiobencarb compared with the control values (Table 4).

Catfish exposed to thiobencarb showed a significant hyperglycemia, decreased serum total protein, decreased serum triglycerides, decreased serum total cholesterol, increased serum calcium and non significant increase in serum phosphorus level compared with the control values (Table 5).

Table 4. Determination of GSH, lipid peroxidation in catfish exposed to different concentrations of thiobencarb

		Time						
Parameter	group	1st w	2nd w	4th w	6th w	8th w	10th w	12th w
GSH Mg/dl	Control	39.80±0.69	39.59±0.43 a A	39.69±0.79 a A	40.07±0.48 a A	39.60±0.55 a A	39.43±0.57 a A	39.32±0.30 a A
	Acute	46.50±1.00*	—	—	—	—	—	—
	Chronic	—	31.60±1.58 a B	27.50±0.29 c C	28.34±0.54 c C	29.25±1.24 bc C	30.72±0.77 ab C	31.75±0.81 a C
	Field	—	31.58±0.68 c B	32.59±0.27 bc B	33.05±0.60 abc B	34.00±0.66 ab B	34.13±0.36 ab B	35.05±0.51 a B
Lipid peroxides nmol/ml	Control	2.58±0.30	2.49±0.18 a C	2.50±0.17 a C	2.76±0.13 a C	2.42±0.19 a C	2.45±0.24 a C	2.59±0.20 a C
	Acute	10.50±0.23*	—	—	—	—	—	—
	Chronic	—	13.53±0.91 a A	13.96±0.53 a A	14.79±0.49 a A	13.97±0.44 a A	13.33±0.74 a A	13.45±0.23 a A
	Field	—	10.81±0.59 a B	11.04±0.68 a B	10.47±0.34 a B	11.43±0.41 a B	11.17±0.50 a B	11.17±0.43 a B

Values are means ± standard errors.
* Acute and control means of the first week differ significantly (P<0.05).
Means in the same column without a common capital letter differ significantly (P<0.05).
Means in the same row without a common small letter differ significantly (P<0.05).

Table 5. Determination of glucose, total protein , triglycerides, cholesterol Ca and P in catfish exposed to different concentrations of thiobencarb

		Time						
Parameter	group	1st w	2nd w	4th w	6th w	8th w	10th w	12th w
Glucose Mg/dl	Control	62.16±3.25	61.89±1.43 a B	61.05±0.69 a B	61.36±0.77 a C	61.39±1.34 a C	61.95±0.79 a C	61.60±2.08 a C
	Acute	71.56±0.98*	—	—	—	—	—	—
	Chronic	—	72.28±3.08 c A	65.40±1.27 d A	72.16±0.72 c A	76.41±0.82 b A	78.83±0.73 b A	83.47±0.55 a A
	Field	—	65.10±0.45 a B	66.67±0.96 a A	65.65±0.31 a B	65.08±1.12 a B	68.57±0.99 a B	68.66±0.92 a B
Total protein g/dl	Control	3.70±0.01	3.72±0.02 a A	3.75±0.02 a A	3.71±0.02 a A	3.76±0.02 a A	3.79±0.02 a A	3.75±0.04 a A
	Acute	3.50±0.03*	—	—	—	—	—	—
	Chronic	—	3.50±0.01 ab B	3.52±0.02 a B	3.43±0.01 bc B	3.39±0.01 c B	3.31±0.04 d C	3.23±0.02 e C
	Field	—	3.41±0.02 c C	3.33±0.05 d C	3.29±0.03 d C	3.45±0.02 c B	3.55±0.03 b B	3.64±0.03 a B
Triglycerides Mg/dl	Control	136.79±5.34	139.19±4.01 a A	141.29±2.47 a A	139.66±3.65 a A	137.36±3.55 a A	137.28±1.82 a A	137.37±3.06 a A
	Acute	80.76±0.51*	—	—	—	—	—	—
	Chronic	—	62.64±0.71 c B	75.94±2.28 b B	74.04±1.38 b B	73.36±1.87 b B	78.54±1.10 b B	88.64±1.10 a B
	Field	—	64.17±0.91 a B	61.86±0.77 a C	61.92±0.78 a C	60.33±0.81 a C	59.76±0.60 a C	59.63±4.28 a C
Cholesterol Mg/dl	Control	175.78±0.14	175.88±0.38 a B	175.72±0.63 a B	175.59±0.37 a B	175.09±1.47 a B	176.42±0.47 a B	175.40±0.51 a C
	Acute	143.65±0.79*	—	—	—	—	—	—
	Chronic	—	162.26±0.86 e C	165.65±0.35 de C	166.93±0.42 d C	170.91±0.53 c C	176.25±0.69 b B	186.44±1.66 a B
	Field	—	184.54±1.63 d A	187.72±0.45 d A	191.49±0.76 c A	195.77±0.37 b A	198.52±0.80 b A	206.56±4.28 a A
Calcium Mg/dl	Control	12.73±0.48	12.29±0.28 a B	12.34±0.39 a B	12.65±0.47 a B	12.58±0.26 a B	12.47±0.30 a B	12.78±0.43 a B
	Acute	19.77±0.24*	—	—	—	—	—	—
	Chronic	—	19.21±0.25 a A	18.47±0.20 a A	16.60±0.24 b A	15.63±0.40 c A	15.04±0.09 c A	13.96±0.35 d A
	Field	—	12.92±0.24 a B	12.29±0.23 ab B	11.96±0.16 b B	12.13±0.16 ab B	11.69±0.16 b B	11.94±0.09 b C
Phosphorus Mg/dl	Control	8.35±0.26	8.23±0.25 *a A	8.07±0.09 a A	8.27±0.25 a A	8.15±0.12 a A	8.30±0.18 a A	8.46±0.33 a A
	Acute	9.42±0.71	—	—	—	—	—	—
	chronic	—	8.66±0.36 a A	7.26±0.30 b B	7.41±0.21 b B	7.59±0.22 b AB	7.05±0.44 b B	7.64±0.28 b B
	Field	—	7.09±0.36 a B	6.61±0.25 a B	6.94±0.23 a B	7.14±0.15 a B	7.10±0.26 a B	7.06±0.30 a B

Values are means ± standard errors.
Acute and control means of the first week do not differ significantly (P>0.05).
Means in the same column without a common capital letter differ significantly (P<0.05).
Means in the same row without a common small letter differ significantly (P<0.05).

Thiobencarb residues in fish muscles

The muscles samples in catfish exposed to different Conc. of thiobencarb showed presence of high amount of residues as shown in Table 6 and fig. (1,2,3,4) which showed the chromatogram produced by gas chromatograph.

Table 6. Determination of thiobencarb residues in catfish exposed to different concentrations of thiobencarb

Sample	Residues (mg/kg)	Mean
Fish exposed to ½ LC_{50} for one week (A1)	81.15	81.06
Fish exposed to ½ LC_{50} for one week (A2)	80.97	
Fish exposed to 1/20 LC_{50} for 12 week (C1)	0.996	1.05
Fish exposed to 1/20 LC_{50} for 12 week (C2)	1.115	
Fish exposed to field recommended dose for 12 week (F1)	0.525	0.53
Fish exposed to field recommended dose for 12 week (F2)	0.553	

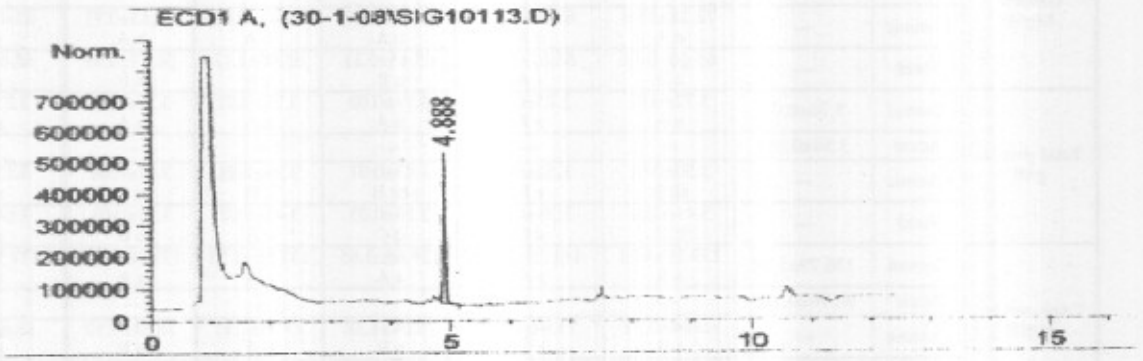


Fig. 1 Thiobencarb standard curve showing retention time 4.888 min.

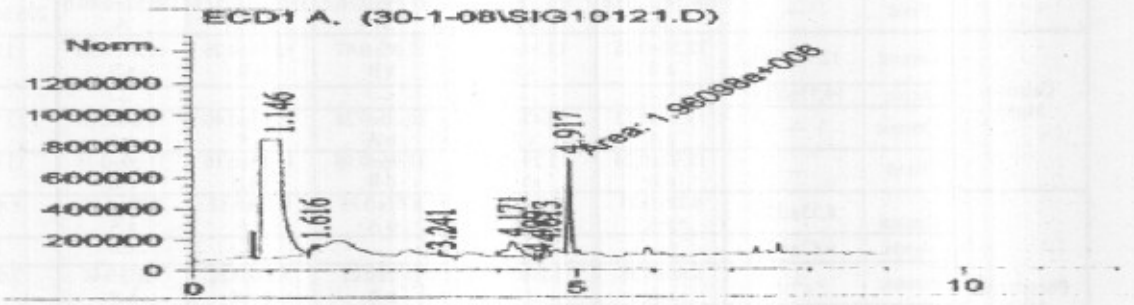


Fig. 2 Result of gas chromatographic analysis of thiobencarb in *Clarias lazera* fish muscles after dosing ½ LC_{50} for 1 week.

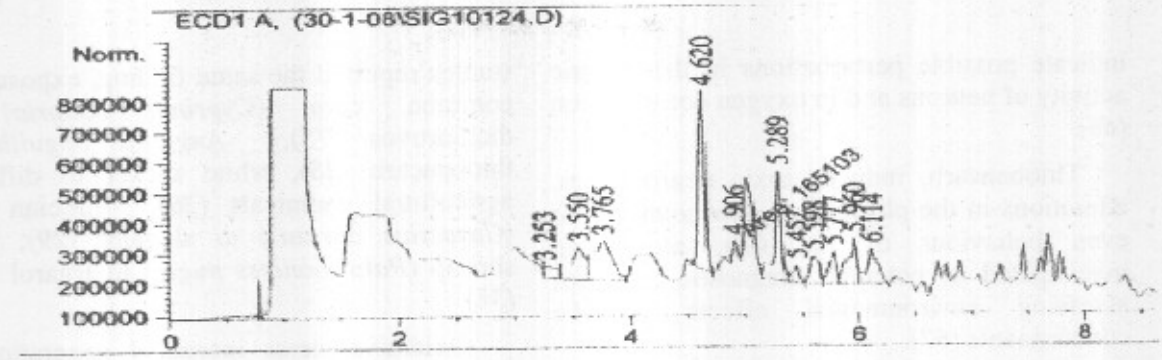


Fig. 3. Result of gas chromatographic analysis of thiobencarb in *Clarias lazera* fish muscles after dosing 1/20 LC_{50} for 12 week.

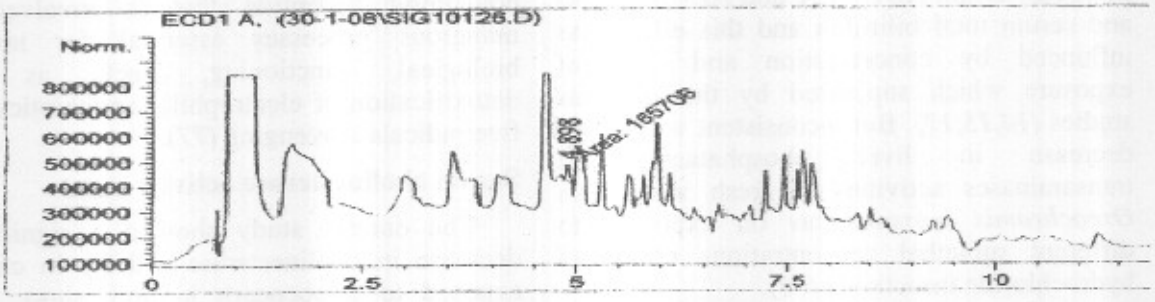


Fig. 4. Result of gas chromatographic analysis of thiobencarb in *Clarias lazera* fish muscles after exposure to the field recommended dose for 12 week.

DISCUSSION

Contamination of surface waters by pesticides from agricultural use is a problem of world wide importance *(56)*. The use of insecticides and herbicides is increasing very rapidly in agriculture in order to control unwanted insects and weeds. The unlimited use of these chemicals is affecting the aquatic biota including fish and other useful plants *(57)*. The traces of the pollutant found in the environmental water ways have been recognized as potentially toxic to the fish *(58)*.

Determination of LC_{50} of thiobencarb

The results showed that the estimated 96 hr LC50 of thiobencarb in catfish (*Clarias lazera*) was 10.57 ppm (mg/L). LC_{50} of thiobencarb in European eel *Anguilla anguilla* was 13.2 mg/L **(59)**, 96 hr LC50 in was 1.7 mg/l in blue gill, 6.5 ppm in craw fish and 1.2 rainbow trout **(60)**, 48hrs LC50 of thiobencarb in Mozambique tilapia was 1.99 ppm *(6)*. thiobencarb 96hrs LC50 in Channel catfish was 2.3 ppm *(61)*.

The previous results indicate that thiobencarb has lethal and toxic effects to aquatic life but the median lethal concentration differs according to temperature, PH, hardness, alkalinity of water, species and the size of life stage of fish *(62)*. Differs also according to water quality and fish species *(63,64)*

Clinical signs

The exposed catfish developed nervous, respiratory manifestations and reduction in growth rate. The observed signs were similar to that previously recorded by *(5,8-10,32,59,65)*. The signs may be attributed to stress condition of the toxicant *(8,9)*.

Inhibition of AChE resulted in excessive stimulation of cholinergic nerves, resulting in tremors and convulsions *(5)*. In addition the ionic profiles (Na(+)-K(+)-ATPase) altered by benthiocarb toxicity

indicate possible perturbations in the electric activity of neurons and in oxygen consumption *(66)*.

Thiobencarb, induced toxic neuropathies, alterations in the physiology, biochemistry, or even behaviour of the fish, are being investigated as potential diagnostic tools for assessing environmental effects of the contaminants *(67)*.

Serum liver enzymes and serum bilirubin

The results revealed a significant increase in catfish serum AST, ALT and ALP activities and serum total bilirubin and this effect was influnced by concentration and time of exposure which supported by the previous studies *(14,15,17)*. But inconsistent with that a decrease in liver phosphatases and transaminases activities in fresh water fish *Oreochromis mossambicus* on exposure to different sublethal concentrations of cartap hydrochloride insecticide *(68)*.

Liver of vertebrate generally and fish particularly is the principal organ of detoxification and the organ of bilirubin *(69)*. So exposure of fish to any toxic substance gives its indicator on liver. The recorded increase in ALT and AST may be attributed to hepatitis occurred due to thiobencarb *(70)*. Serum ALT increased essentially with liver diseases *(71)*. Elevation of these enzymes has been used as an indicator of cellular damage *(72)*. The increase of serum bilirubin was often related to the liver and bilary tract diseases *(73)*.

Oxidative stress biomarkers (Reduced glutathione and Lipid peroxides)

Antioxidants and lipid peroxidation could be used as markers of contaminant exposure in fish. *(20)*. It is possible to use non-enzymatic antioxidants as biomarkers of exposure to environmental contamination and subsequent validation as a sensitive system for biomonitoring and ecotoxicological risk assessment. *(74)*

The decreased level of GSH and increased level of lipid peroxidation is indicative of a process of oxidative stress. Several previous studies reported the same finding, exposure of common carp (*Cyprinus Caprio)* to dichlorovos *(75)*, *Anguilla anguilla* to thiobencarb *(28)*, white sucker to different agricultural chemicals *(20)*, Crucian carp (*Carassius auratus) to* alachlor *(29)*, grass shrimp (*Palaemonetes pugio*) to Irgarol 1051 *(31)*.

Oxidative stress increased generation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) *(76)*. Glutathione is an aubiqitous thiol – containing tripeptide that is involved in numerous processes essential for normal biologieal functioning, such as the detoxification of electrophilic xenobiotics and free radicals scavenging *(77)*.

Serum cholinesterase activity

The current study showed a significant decrease in cholinesterase activity in catfish exposed to thiobencarb in both short, long term exposure and even in catfish exposed to the field dose. There was a significant increase in cholinesterase activity in last weeks of the study compared with the 1st weeks.

Many previous studies reported the same finding, exposure of catfish *(Clarias batrachus*) to carbofuran *(32)*, eel to molinate *(4)*. Carbamates are inhibitors of AChE activity, but generally this inhibition is reversible with spontaneous reactivation (decarbamylation) *(78)*. Thiobencarb act by inhibiting the acetyl choline esterase enzyme *(59)*. Cholinesterase enzyme is a good biomarker of acute and subacute exposure to OP and carbamates *(3,21)*. The obtained results reveal that thiobencarb is a potent ChE inhibitor.

Serum glucose

From the previous investigation, the study showed that, the catfish exposed to various concentrations of thiobencarb for short and long period induced significant increase in serum glucose level. Hyperglycemia was developed in Indian catfish exposed to propoxur *(33)*, in eel exposed to lindane *(26)*, in goldfish (*Carassius auratus*) exposed to

carbofuran *(79)*, European eel during exposure to thiobencarb *(5)*, silver catfish exposed to clomazone *(18)*. On contrary it has been recorded decreased Glucose level in liver of Silver catfish (*Rhamdia quelen; Teleostei*) exposed to commercial formulation Roundup, a glyphosate herbicide *(80)*.

It is known that different toxicants promote a stress condition to the aquatic life. Stressed fish produce increased amount of cortisol, which may induce glycogenolysis in liver and muscles, so increasing blood glucose level *(81)*.

Serum cholesterol and triglycerides level

The obtained data showed a significant decrease in serum cholesterol value in catfish exposed to 1/2 LC50 of thiobencarb, 1/20 LC50 and even in the field concentration. There was a significant decrease in serum triglycerides level. Decreased total lipid in eel exposed to a sublethal concentration of lindane *(26)*, grass shrimp (*Palaemonetes pugio*) exposed to the anti-fouling herbicide Irgarol 1051 *(31)*. These results may be attributed to the pesticide toxic stress which induced glyconeogenesis from sources other than carbohydrate *(82)*.

Serum Calcium and Phosphorus

A significant increase in calcium level was recorded in catfish exposed to thiobencarb. 1/2 LC50 for one week showed non significant increase in phosphorus level, but Catfish exposed to 1/20 LC50 and field recommended dose of thiobencarb showed a significant decrease in serum phosphorus level specially at the end of the study. These data were recorded in catfish exposed to propoxur *(32,33)*.

The current results may be attributed to renal disturbance due to renal-toxic effect of thiobencarb *(32)*. These results were confirmed by histopathological study in our previous study.

Serum total protein

The results showed a significant decrease in serum total protein in catfish exposed to 1/2 LC50 of thiobencarb for one week, 1/20 LC50 of thiobencarb for 12 week and in group treated with the field dose. Repeated administration of benthiocarb to rat, propoxur to catfish, Carbaryl to *Clarias batrachus* resulted in hypoproteinemia *(22,33,15)*.

The decreased serum protein level observed during the experiment could be due to generalized stress response. It is known that different toxicants promote a reduction in protein content of the exposed fish. This reduction induced by the toxicants indicates the physiological adaptability of fish to compensate for the stressful situation. Stressed fish produce increased amounts of cortisol, which may induce synthesis of glucose from sources other than carbohydrate precursors *(83)*.

Residues of thiobencarb

The obtained results revealed that there was high concentration of thiobencarb in fish muscles exposed to different concentration. Similar findings were recorded in several previous studies *(34,35,84)*

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الملخص العربي

التأثير السمى للمبيد العشبى الثيوبنكارب على أسماك القرموط

بعض التغيرات البيوكيميائية وتحديد بقايا المبيد

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في الآونة الأخيرة, تزايد استخدام مضادات الحشرات و الأعشاب بصورة غير محدودة مما يؤثر على الحياة المائية. من هذه المواد الثيوبينكارب. تم تحديد الجرعة المميتة لنصف العدد من الأسماك . و قد استخدم نصف هذا التركيز لمدة أسبوع و واحد على عشرون منه لمدة ١٢ أسبوع بالإضافة إلى التركيز المستخدم في الحقل و هو ٢ لتر للفدان و ذلك لمعرفة تأثيره على إنزيمات الكبد و نشاط إنزيم الكولين استريز. و ذلك بالإضافة لمعرفة تأثيره على الجلوكوز و البروتين الكلي,التراى جلسرايد,الكوليسترول,البيليروبين الكلي, الجلوتاثيون المختزل, و مستوى كلا من الكالسيوم و الفسفور. و قد أسفرت النتائج عن ما يلي: زيادة في إنزيمات الكبد, و نقص في نشاط إنزيم الكولين استريز بالإضافة إلى زيادة الجلوكوز و الكالسيوم و الفسفور و البيليروبين الكلي و نقص في الجلوتاثيون و الكوليسترول و التراى جلسرايد. هذا بالإضافة إلى ملاحظة الأعراض الإكلينيكية و فحص ما بعد الوفاة و قياس بقاياه في العضلات.