ウナギ(Anguilla anguilla)におけるチオベンカルブによるストレスの生理的影響

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Original Article

Physiological Effects of Thiobencarb Stress in the Eel (*Anguilla anguilla*)

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The effects of thiobencarb (0.22 mg/l) on the intermediary metabolism of the European eel, *Anguilla anguilla*, and its recovery from intoxication were evaluated. Physiological measurements were made in plasma, muscle and liver of the eels during exposure and after an 8-day recovery period in non-contaminated water. Hyperglycemia was detected during exposure, while liver and muscle glycogen levels decreased markedly. Blood lipid values increased, however, a decrease of the lipidic reserves was determined in liver tissue. Mobilization of proteins was detected specially in muscle tissue. Most of the metabolic disorders persisted after eight days in clean water. The observed effects of thiobencarb on intermediate metabolism are discussed in relation to a stress syndrome, as an indicative status of the physiological adaptability of the fish to compensate pesticide stress.

Key words: liver, plasma, muscle, eel, metabolism, thiobencarb, stress, recovery.

INTRODUCTION

Stress is a mechanism of physiological compensation that an organism adopts in response to a stressing physical or chemical factor. Collectively, those compensations are named the General Adaptation Syndrome (GAS), because they form a group of stereotyped responses which do not differ with the original cause.[1] From a purely physiological standpoint, pesticides can be considered as another extremely interesting environmental factor to which a fish will physiologically respond.[1] When stress agent exceeds the tolerance limit to compensate the stress situation, the death of the individual results. However, lethal effects are rare in nature because the organisms are usually exposed to low concentrations, which are normally sublethal.[2] As a result of the presence of sublethal pollutant concentrations in the aquatic medium, some biochemical parameters in animals can be altered. Within the sublethal range a wide variety of reversible and irreversible processes take place in order to maintain homeostasis. The dynamics of intermediary metabolism are greatly influenced by any sort of stressors (any sort of change altering the homeostasis of the animal). Biochemical parameters are very sensitive to sublethal concentrations of many stress agents. Their main disadvantage is that they are often specific to special responses. It is therefore possible to observe no change in experimental results if the appropriate biochemical system is not chosen. For this reason, it is preferable to choose general parameters (e.g. glucose, glycogen, lactate) to determine a stress situation in the organisms under study.[3]

It is known that fish utilize protein and lipids for energy to a greater extension than mammals. However, under stress, fish are somewhat similar to other vertebrates in that they mobilize and use carbohydrates. Stress exhibits a generalized group of physiological responses (GAS). There is a rapid elevation in adrenalin and noradrenalin which mobilizes muscle glycogen into blood sugar, causes blood pressure to rise, and in general causes the body to undergo the "fight or flight" response. If the stressful condition continues, the adrenal cortex is stimulated to release increased amounts of cortisol which sustain the changes caused by the adrenalin and also cause a mobilization of some of the body protein into plasma amino acids and an assortment of other physiological changes. The hormonal changes are referred to as primary effects of the stress and the other physiological alterations produced by them are called secondary effects.[1]

Herbicides are commonly used to control nuisance aquatic vegetation when alternative controls are not feasible. Little is known about the sublethal effects of herbicides on fish. Animals are often exposed to levels

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of herbicides below those that cause acute effects. The harmful effects (specially the sublethal ones) retard the development of the surviving individuals and/or exert a harmful influence on their normal metabolic processes.[4]

Thiobencarb is a powerful carbamate herbicide widely used in Spanish rice fields protection. Although changes in rice management practices have greatly reduced the concentration of the pesticides in the runoff waters, the concentrations of thiobencarb, while not acutely toxic, deserve possible concern for their sublethal effects.

The european eel (*Anguilla anguilla*) differs from most freshwater fish species in a number of ways, the life cycle of the eel is unique among European fishes; eels contain large amounts of fat, so pesticides will accumulate in eel tissues more than in other fish species and combining this fact with the long migration, using the fat deposits as energy reserves, eels, specially during its freshwater stage (yellow eel) seems to be a species highly exposed to pollutants.[5]

In the present study, we investigated the impact of a sublethal concentration of the herbicide thiobencarb during 4 days-exposure on eel metabolism and its recovery from intoxication when the exposed fish were transfer to clean water for 8 more days.

MATERIAL AND METHODS

1. Test Chemical

Technical grade thiobencarb (98.1% purity) was obtained from ARGOS S.A. (Spain). Sublethal test concentration (0.22 mg/l) corresponding to 1/60 LC_{50}-96 hr, used for this study, was based on its 96-hr LC_{50} value for *A. anguilla*.[6]

2. Test Animals

Individuals of *A. anguilla* (weight, 20–30 g; length, 16–20 cm) were collected from Albufera Lake (Valencia, Spain). They were acclimatized to the laboratory conditions for one week in 300-l glass tanks before the start of the experiments.[7] The tanks were supplied with a continuous flow of tap water (temperature: 20±2°C; total hardness: 240±10 ppm as $CaCO_3$; pH: 7.9±0.2 and alkalinity: 4.1±0.5 mmol/l). A 12-hr photoperiod (light on from 8.0 to 20.0 hr) was maintained. Eels did not respond to feeding attempts in our laboratory, but all animals were healthy.[8] No mortality was observed during the acclimation period.

3. Test System

Thiobencarb experiments were carried out in a continuous flow-through system based on OECD Guidelines.[7] Eels were exposed to a constant concentration of thiobencarb for 96 hr and then an elimination period of 192 hr in clean water was allowed. For the exposure period, the herbicide was dissolved in acetone and the solution was supplied to a glass mixing chamber with tap water and connected to a perfusor pump (Gilson, Minipulse 3) that generated a constant solution flow. The outlet was connected to a 300-l glass test aquarium. This diluted the pesticide to the desired concentration (0.22 mg/l) by a constant water flow. In this way, the aqueous test solution was renewed 3 times a day. This system was connected 48 hr before the start of the experiments to reach a balance of thiobencarb contaminated water in the test aquaria. Every 24 hr the mixture stock was renewed. Final concentration of solvent (acetone) in the test aquarium was 17 μl/l.

After 0, 2, 12, 24, 48, 72 and 96 hr six eels were removed, rinsed, anaesthetized with 100 mg/l of MS222[9] and weighted. Blood was removed from the heart with a heparinized syringe (1 ml), centrifuged (15 min, 5000 rpm, 4°C) and the plasma frozen for metabolite determination until analyzed. Muscle and liver tissues were removed quickly, weighted and stored frozen (−24°C). Gas chromatography analysis confirmed the presence of thiobencarb in the water at the desired concentration over the entire exposure period.[10] Recovery from water samples was not less than 90%.

In a second part of the experiment, eels previously exposed to thiobencarb during 96-hr were transferred to clean water in a 300-l glass aquarium with the same flow-through system under the above described conditions but without toxicant (recovery period). Six eels were removed after 8, 24, 72, 96, 144 and 192 hr. The same tissues were taken out and stored at −24°C until analyses for its biochemical parameters determination.

Finally, another experiment was performed to test if the solvent used (acetone, 17 μl/l) had any effect on eel metabolism in the same test conditions and at the same exposure times. Therefore, acetone and blank controls were used for each exposure and recovery period and eel tissues dissected for metabolic determination.

4. Analytical Procedures

Plasma glucose levels were determined spectrophotometrically (610 nm) according to Boehringer-Mannheim kits.

Muscle and liver glycogen was determined by the anthrone reagent method.[11] The samples were homogenized by adding 0.5 ml of KOH (60%) and 1 ml of KOH (30%), both in water. The mixture was incubated in a water bath (100°C) for 30 min and 4 ml of ethanol was added to the homogenates before they were placed in the refrigerator overnight. Twenty-four hours later, samples were centrifuged at 3000 rpm for 20 min. The pellet was resuspended in 1 ml of destilled water and 0.25 ml was mixed with 1.75 ml of anthrone reagent for 15 min, in boiling water (100°C). Glycogen was determined spectrophotometrically at 620 nm.

Total lipids were analyzed spectrophotometrically

(530 nm) with a Merckotest kit after extraction according to Bligh and Dyer.[12] Tissue homogenates were prepared in a mixture of chloroform: methanol: water and the resulting organic phase was analyzed.

Total proteins in plasma and tissues were estimated spectrometrically (750 nm) by the method of Lowry *et al.*[13] using a Protein assay kit from Sigma Diagnostics®. Tissue homogenates from every fish were prepared individually in 0.1 M phosphate buffer, pH 7.2 at 0°C, and centrifuged at 3500 rpm for 7 min, and the kit was applied individually to the resulting supernatant. Bovine serum albumin was used to construct the standard curve.

5. *Statistical Analysis*

Mean values and standard deviations were calculated for each test group based on the values obtained for each individual tissue from four fishes. These values were compared by analysis of variance (ANOVA) and Duncan's multiple range test. All statistical analyses were performed on an IBM computer using SPSS+ Programme. The significance level was set at 0.05.

RESULTS AND DISCUSSION

Tables 1 and 2 indicate the results obtained on eel metabolism in those animals exposed only to acetone (solvent) compared with those called blank controls (without acetone) during all the selected time periods. As can be seen in these Tables, no significant differences ($p>0.05$) were found between them, so the small amount of acetone used in the experiment as well as exposure time did not produce any disturbance in eel metabolism. Therefore, we can compare our control values (0 hr) with those obtained after herbicide treatment at different exposure times, for all the selected physiological parameters.

Similar findings were reported by Sancho *et al.*[16] or Ferrando and Andreu[8] when studied the effect of different pesticides on *A. anguilla* metabolism, the authors concluded that the acetone used as a pesticide solvent during the exposure period did not alter the physiological studied parameters.

However, a constant sublethal concentration of thiobencarb in the surrounding water during 96 hr appears to be physiologically stressful to the european eel (Table 3, Figs. 1-3). The most obvious sign of thiobencarb intoxication was started with tremors and convulsions, followed by lethargy and an hipoactive state (from 32 to 72 hr) accompained by a reduced opercular movements activity, but all of the animals survived the estipulated exposure period. Lethargy is a consequence observed in other fish species exposed to herbicides.[14]

As we can see in Table 3, a significant elevation in plasma glucose was observed in treated fish (Fig. 1) from the first hours of contact to the toxicant (12 hr). Thus, thiobencarb exposure caused hyperglycemia in *A. anguil-*

Table 1 Changes in glucose (mg/100 ml), lipid (g/100 ml), protein (g/ml) plasma levels and glycogen (mg/g wet wt), total lipids (mg/g wet wt) and proteins (mg/g) in liver and muscle of european eel (*A. anguilla*) in acetone and blank controls, during the exposure period.

Parameter	Exposure Period						
	0 hr	2 hr	12 hr	24 hr	48 hr	72 hr	96 hr
Plasma							
Glucose	645.5±131.84	803.82±341.44	1411.19±236.70*	1200.29±188.94*	723.03±256.48	1019.57±188.65*	1038.19±125.18*
Proteins	2.99±0.49	3.37±1.21	3.08±0.79	2.51±0.63	2.75±0.22	2.61±0.31	3.26±0.81
Lipids	1236.01±171.38	1508.20±240.14	1543.17±297.89	1869.20±316.78*	1567.25±344.02*	1780.70±294.39*	1528.07±179.56
Liver							
Glycogen	227.13±21.55	207.84±61.82	61.66±4.24*	61.27±0.39*	58.70±32.95*	53.89±32.79*	17.35±7.23*
Proteins	181.03±35.69	106.88±27.32*	154.37±42.06	187.00±47.5	130.97±75.48*	217.75±34.57	192.57±20.01
Lipids	78.60±16.84	38.21±9.14*	19.86±6.52*	12.03±3.19*	10.51±3.48*	12.85±2.87*	12.78±2.69*
Muscle							
Glycogen	7.83±1.75	4.27±0.97*	3.56±1.03*	2.71±0.90*	2.57±1.25*	4.29±1.78*	4.03±1.87*
Proteins	160.36±31.56	160.57±15.64	134.58±22.02*	105.58±17.34*	104.89±25.71*	108.85±18.43*	126.64±17.58*
Lipids	10.47±2.06	7.71±4.68	11.00±3.97	5.67±3.04	7.78±2.80	12.64±5.89	14.56±3.82

*=significant differences ($p<0.05$).

Table 2 Changes in glucose (mg/100 ml), lipid (g/100 ml), protein (g/ml) plasma levels and glycogen (mg/g wet wt), total lipids (mg/g wet wt) and proteins (mg/g) in liver and muscle of european eel (*A. anguilla*) in acetone and blank controls, during the recovery period.

	Recovery Period					
	104 hr	120 hr	168 hr	192 hr	240 hr	288 hr
Plasma						
Glucose	1093.64±312.19*	1150.44±265.30*	1413.95±288.18*	1461.11±253.87*	1395.03±136.62*	1346.03±74.49*
Proteins	2.98±0.34	3.03±0.17	2.93±0.1	2.83±0.13	3.07±0.27	2.92±0.04
Lipids	937.85±125.20	966.10±139.87	1180.11±256.79	1217.06±304.87	1787.80±165.35*	1421.71±316.58
Liver						
Glycogen	67.95±19.62*	74.73±13.04*	115.67±58.15*	126.81±20.69*	—	213.83±33.90
Proteins	193.70±31.92	191.99±11.7	212.18±21.56	189.02±26.39	144.86±25.29	179.28±48.01
Lipids	17.39±4.19*	14.60±2.61*	16.84±1.96*	17.99±3.04*	17.57±8.68*	11.68±2.91*
Muscle						
Glycogen	3.11±1.02*	2.37±0.47*	3.64±1.93*	4.03±2.83*	5.41±2.68*	3.42±1.18*
Proteins	121.88±55.61*	108.55±30.52*	122.83±17.09*	130.94±18.84*	95.83±15.72*	87.07±12.20*
Lipids	11.55±4.50	8.99±2.88	11.07±2.21	12.52±4.25	17.43±5.01*	11.71±3.62

*=significant differences ($p<0.05$).

Table 3 Changes in glucose (mg/100 ml), lipid (g/100 ml), protein (g/ml) plasma levels and glycogen (mg/g wet wt), total lipids (mg/g wet wt) and proteins (mg/g) in liver and muscle of european eel (*A. anguilla*) during exposure to 0.22 mg/l thiobencarb.

Parameter	Exposure Period						
	0 hr	2 hr	12 hr	24 hr	48 hr	72 hr	96 hr
Plasma							
Glucose	687.58±18.36	682.02±15.46	686.71±15.44	688.82±13.5	680.55±26.52	679.81±18.05	688.25±18.21
Proteins	4.40±0.15	4.45±0.28	4.24±0.16	4.31±0.26	4.40±0.28	4.71±0.51	4.37±0.27
Lipids	1236.01±171.38	1208.20±140.14	1243.17±197.89	1169.20±216.78	1267.25±144.02	1280.70±194.39	1328.07±179.56
Liver							
Glycogen	166.05±36.37	195.80±21.82	140.28±29.51	147.66±13.02	165.95±17.67	158.13±26.52	160.26±27.06
Proteins	76.82±18.21	64.86±19.45	70.68±13.42	66.86±13.63	63.62±20.38	61.30±7.20	79.38±20.40
Lipids	144.68±44.35	133.82±40.47	168.43±41.39	178.20±33.19	187.41±40.81	177.12±32.37	172.78±32.69
Muscle							
Glycogen	5.34±1.75	5.65±1.97	5.47±1.03	4.35±1.90	5.11±1.25	5.53±1.78	6.66±1.87
Proteins	120.36±30.56	120.57±15.34	124.58±21.02	115.58±18.34	114.89±25.21	128.85±17.43	126.84±17.48
Lipids	18.47±2.16	17.71±3.68	19.00±3.12	15.87±3.24	17.88±2.40	18.60±5.19	16.56±3.42

*=significant differences ($p<0.05$).

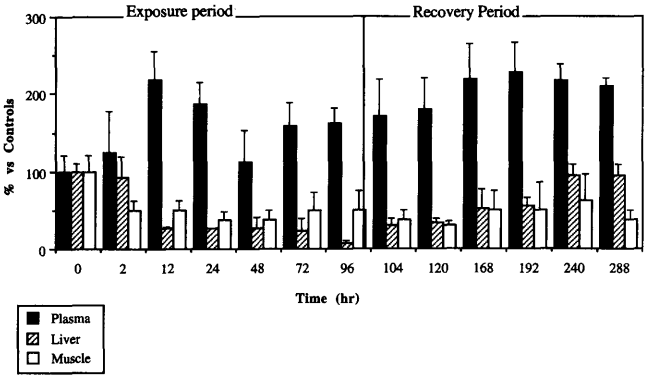

Fig. 1 Changes in plasma glucose and muscle-liver glycogen content versus blank controls after 96 hr treatment with 0.22 mg/l of thiobencarb (exposure period) and after 192 hr in clean water (recovery period).

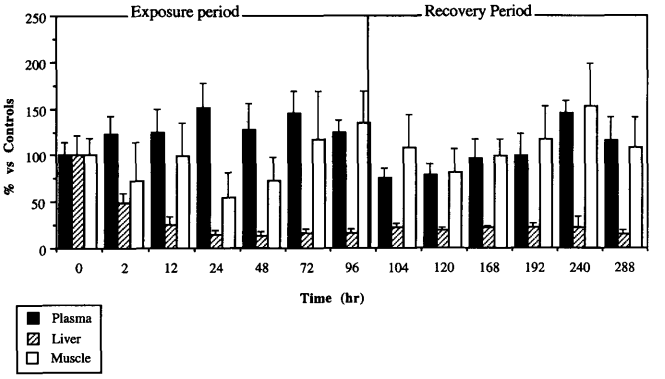

Fig. 2 Changes in plasma lipid levels and muscle-liver total lipidic content versus blank controls after 96 hr treatment with 0.22 mg/l of thiobencarb (exposure period) and after 192 hr in clean water (recovery period).

la. The increase in glucose level after toxicant exposure was 118% at 12 hr as compared with control values and it was maintained from this time onward. The hyperglycemic status was supported by a rapid glycogenolysis in both reserve tissues, liver and muscle. This mobilization was already observable after 12 hr in contaminated water. Higher depletion in muscle glycogen was specially observed between 24–48 hr (67%), while in liver the maximum depletion was determined at the end of the exposure period (90%).

Hyperglycemia is frequently reported in *A. anguilla* exposed to sublethal concentrations of different pesticides.[8,9,15,16] Juvenils of the european eel exposed to a sublethal concentration of thiobencarb manifested a consistent hyperglycemia correlated with a spectacular depletion on glycogen stores in liver and muscle. This initial hyperglycemia in plasma could due, effectively, to hepatic and muscle glycogenolysis releasing glucose to the blood. To meet the increased energy demands of such stressed animals, glycogen, because of its easy availability for energy production, is rapidly catabolized, resulting in huge losses of this energy reserve. The high reduction in glycogen content observed in the present study supports this view.

The dynamics of stress response in mammals (and to a certain extent in fish) is generally characterized by an alarm phase during which the levels of adrenaline rise rapidly causing a mobilization of the liver glycogen into blood glucose. This is followed by a resistance phase in which the glucose levels remain elevated and liver glycogen may or may not be low, depending on the extent of hyperglycemia, diet, etc. If the stressor continue, then either adaptation or exhaustion occurs.[1] Adaptation implies changes in several related physiological processes which permit the return of homeostasis. When blood glucose is involved, this means a return to near normal levels. Exhaustation may occur if the extent and duration of stress is sufficient and characterized by a depletion of liver glycogen, decreasing levels of cortisol, depressed imune response, and a host of other changes that make the organism less able to survive, specially if other types of stressors are present.[1,17]

The rapid activation of carbohydrate catabolism observed during the exposure, was accompanied by an increased metabolism of lipidic reserves in liver as it is showed in Fig. 2. A gradual and consistent lipolysis was observed during the first 24 hr of exposure, after that time a maximum decline was observed from 48 to 96 hr, when stored lipids were diminished until a 85% below to the control level (227.13 mg/g wet wt.). This reduction on lipidic content in liver was correlated with an increased hyperlipidemia from 24 to 72 hr in plasma (Fig. 2). Similar results were found in *A. anguilla* exposed to PCB,[18] or to different organochlorinated[8,15] or organophosphate insecticides.[9,16,19] When stress persist, other mechanisms may also be involved in mobilization of energy reserves. Classically, cortisol from the adrenal cortex (in mammals) acts to reduce glucose utilization by the muscle and other tissues and glycogen is stored in the liver. The net effect is to maintain an elevated blood glucose level. In fish it is not clearly established.[20] Prolonged blood glucose elevation in fish, as seen with the chronic stress, must be maintained by mechanisms other than cortisol and it may also be large species differences. In teleosta, as stress response operating via hypothalamus, the first effect is to activate the cromaffin cells which are located in the walls of the cardinal veins and in some cases the head kidney.[21] These cells release adrenaline and a small amount of noradrenaline which stimulate the conversion of liver glycogen into blood glucose and the utilization of glucose by the muscle. These adrenergic effects may result in increases of blood glucose within minutes of the onset of the stress, elevations in the concentration of blood lactic acid frequently occur coincident with the stress, but is not clear whether this is due to adrenergic stimulation of glycolysis, or a

direct effect of the stress (e.g. hypoxia) on the muscle tissues. It could, of course, involve both mechanisms.

Liver plays an important role in both metabolism equilibrium between the rates of synthesis and degradation and as the main detoxifying tissue.[19] Moderate hepatomegaly has been reported in other cases of exposure to chemical stressors.[22] Liver weight relative to body weight (Hepatosomatic Index) was moderately increased during exposure to thiobencarb, being especially significant during the first 2 hr of treatment. However, after this time onward the fish did not show any abnormality for this index. Prolonged exposure to endosulfan resulted in significant elevation of the hepatosomatic index (HSI) in the freshwater fish *Brachionus conchonious*.[23] Increase in the HSI in the european eel, *A. anguilla*, as consequence of exposure to PCP and fenitrothion, had been also reported.[16,18] This increase was explained by an enlargement of the liver as a result of the pesticide action. Pathological lesions, particularly in the liver, related to the pesticide exposure have also been described in fish.[23]

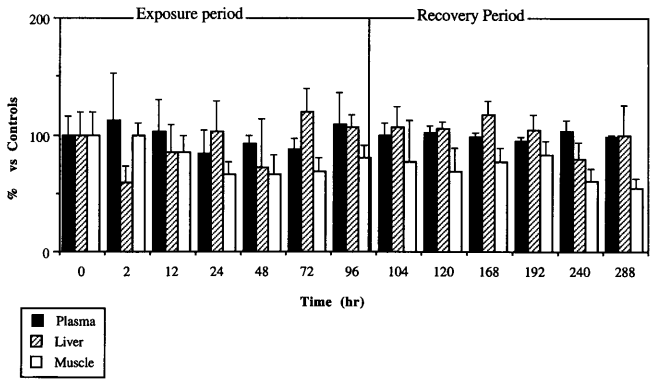

Fig. 3 Changes in plasma protein levels and muscle-liver total proteins content versus blank controls after 96 hr treatment with 0.22 mg/l of thiobencarb (exposure period) and after 192 hr in clean water (recovery period)

A. anguilla exposed to sublethal levels of the organophosphate insecticide fenitrothion showed a decrease in lipid content as well as in glycogen levels after 2 hr of pesticide treatment.[16] It is showed in the present study how lipid content in eel liver tissue decreased during the exposure period, due to its use as energy reserve parallel to glycogen and the observed decrease in protein content in thiobencarb intoxicated fish as indicative of the fish adaptability to compensate for pesticide stress. Muscle lipidic content did not change after exposure to 0.22 mg/l of thiobencarb. In contrast, a rapid and significant mobilization on proteinic reserves began after 24 hr and was maintained over the entire experiment (Fig. 3). Significant proteolysis could be ocurring in liver during the first part of the exposure period (until 48 hr exposure), but from this time onward no significant differences were detected from the controls. In spite of these disorders, no alterations was reflected in plasma levels which were maintained constant during the exposure. Decrease in tissue lipid and proteins under pesticide stress could be due to several mechanisms. These include formation of lipoproteins which are utilized for repair of damage cell and tissue organelles, direct utilization by cells for energy requirements and increased lypolyses.[16,27] A reduction in total protein content indicates that tissue protein may undergo proteolysis which results in the production of free amino acids and then used in the TCA cycle for energy production during stress conditions.[24,26]

The greatest amount of carbohydrates are stored in liver as glycogen. Although skeletal muscle glycogen is also an important store, concentrations found in fish are generally one order of magnitude less than those in liver.[28] Stressed fish secrete increased amount of cor-

Table 4 Changes in glucose (mg/100 ml), lipid (g/100 ml), protein (g/ml) plasma levels and glycogen (mg/g wet wt), total lipids (mg/g wet wt) and proteins (mg/g) in liver and muscle of european eel (*A. anguilla*) during the recovery period, after exposure to 0.22 mg/l thiobencarb.

	Recovery Period					
	104 hr	120 hr	168 hr	192 hr	240 hr	288 hr
Plasma						
Glucose	689.38±12.19	680.35±15.30	684.14±18.18	675.88±13.87	678.34±16.62	681.85±16.49
Proteins	4.40±0.14	4.23±0.27	4.41±0.24	4.35±0.30	4.39±0.17	4.52±0.14
Lipids	1167.85±115.20	1266.10±129.77	1280.11±156.49	1237.16±104.80	1287.60±135.35	1321.21±116.08
Liver						
Glycogen	195.86±50.73	161.27±45.37	129.89±42.73	141.38±24.24	134.50±12.63	161.51±48.25
Proteins	79.38±30.40	79.54±22.80	72.56±13.80	76.40±18.60	81.16±25.20	66.30±16.80
Lipids	159.85±35.80	161.96±33.87	145.87±23.89	161.18±34.88	179.24±28.68	154.61±22.91
Muscle						
Glycogen	6.42±1.49	7.97±2.12	6.28±2.90	7.01±2.33	5.81±2.68	6.65±1.01
Proteins	116.87±35.61	118.55±20.51	122.05±17.08	120.64±18.54	121.23±15.01	124.76±22.20
Lipids	18.65±3.50	18.99±3.88	19.07±2.81	15.52±4.35	16.43±4.01	17.73±3.82

*=significant differences ($p<0.05$).

tisol which may induce synthesis of glycogen from sources other than carbohydrate precursors. The ocurrence of liver glycogenesis joint to the maintenance of the elevated hyperglycemia would show that the pesticide exposure induced an alteration on eel intermediate metabolism derived in either inhibited glycogenolysis or promoted gluconeogenesis.[25] Hyperglycemia continued during the entire recovery period (with a mean increased level of 103% respect to the control) (Table 4). In fact, during this part of the experiment, glucose levels showed a tendency to increase from 70% after 8 hr in clean water to 109% after 8 days in these conditions. That is correlated with the significant depletion which was still maintained in energetic reserves in muscle and liver (Table 4). These findings indicate the activation of both anabolic and catabolic metabolism's pathways in order to generate a new homeostatic steadiness. Liver glycogen reserves were replenished after 96 hr in clean water, as long as total lipids stores in this organ were reduced to a less than a 50% at the end of the entire experiment. Parallely, muscle reserves were also maintained in a 50%. That would reflect a general metabolism disorder in the full organism induced by this concentration of toxicant. Similarly, fenitrothion induced a decrease of tissue energy reserves as glycogen, protein and lipids, and this status was maintained after some days in fenitrothion-free water.[29]

It is known the capacity of the european eel to mobilizate and metabolizate proteins for glucose resource under starvation situations before using glycogen stores.[30] There is also accumulated evidence that chronic high levels of cortisol in fish cause utilization of protein for energy, so that growth would be inhibited.[20] Lipids form the richest energy reserves whose caloric value was reported to be twice that of an equivalent weight of carbohydrate or proteins. Therefore, mobilization of lipidic reserves during the recovery period would also be indicative of high energy demand. Moreover, the use of lipidic reserves from a determined tissue would contribute to the toxicity of the compound (as a lipophylic dependent compound) but, at the same time, to its elimination when mobilization is started.[31]

Commonly, the european eel seems to respond to pesticides, by exhibiting a degradation in total protein and lipid reserves,[9,24] and maintain even after exposure.[16,19,31] Exposure to pesticides is known to alter intermediate metabolism of fish, and this disturbance has been already reported in *A. anguilla*.[8,9] But there are a few data about how metabolic effects of the stressor are minimized after stress agent cessation.

As response to the presence of thiobencarb, additional requirements of energy became evident for juvenil eels, not only during the exposure time, but also during the recovery period, that could affect their own normal growth if the stress situation persists.

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REFERENCES

1) A. G. Heath: "Water Pollution and Fish Physiology," CRC Press, Boca Raton, pp. 20–28, 1995
2) P. F. Dehn & V. R. Schirff: *Comp. Biochem. Physiol.* **84**, 523 (1986)
3) J. P. Giesy, S. R. Denzer, G. W. Duke & G. W. Dickson: *Verh. Int. Verein. Limnol.* **21**, 205 (1981)
4) T. Bâlint, T. J. Ferenzy, F. Katai, I. Kiss, L. Kraczer, O. Kufcak, G. Lang, C. Polyhos, I. Szabo, T. Szegletes & J. Nemcsok: *Ecotox. Environ. Saf.* **37**, 17 (1997)
5) P. Larsson, S. Hamrin & L. Okla: *Environ. Pollut.* **69**, 39 (1991)
6) C. Fernández-Vega, E. Sancho, M. D. Ferrando & E. Andreu-Moliner: *J. Environ. Sci. Health* **B34(1)**, 61 (1999)
7) OECD (Organization for Economic Cooperation): "Guidelines for Testing Chemicals," p. 305, Paris, 1981
8) M. D. Ferrando & E. Andreu-Moliner: *Comp. Biochem. Physiol.* **101C**, 337 (1992)
9) J. Cerón, E. Sancho, M. D. Ferrando, C. Gutierrez & E. Andreu: *Toxicol. Environ. Chem.* **60**, 201 (1997)
10) Y. H. Moon & S. Kubatsuka: *J. Pesticide Sci.* **9**, 745 (1984)
11) S. Seifter, S. Dayton, B. Novic & I. Montwyler: *Arch. Biochem.* **25**, 191 (1950)
12) E. G. Bligh & W. J. Dyer: *Can. J. Biochem. Physiol.* **37**, 911 (1959)
13) O. H. Lowry, A. L. Rosebrough, A. L. Farr & R. J. Randall: *J. Biol. Chem.* **193**, 265 (1951)
14) J. A. Johansen & G. H. Green: *Arch. Environ. Contam. Toxicol.* **19**, 610 (1990)
15) L. Gimeno, M. D. Ferrando, S. Sanchez & E. Andreu: *Ecotox. Environ. Saf.* **31**, 153 (1995)
16) E. Sancho, M. D. Ferrando, C. Fernández & E. Andreu: *Ecotox. Environ. Saf.* **41**, 168 (1998)
17) A. D. Pickering: "Stress and Fish," ed. by A. D. Pickering, Academic Press, New York, pp. 42–53, 1981
18) B. Holmerg, S. Jensen, A. Larsson, K. Lewander & M. Olsson: *Comp. Biochem. Physiol.* **43B**, 171 (1972)
19) E. Sancho, M. D. Ferrando & E. Andreu-Moliner: *Ecotox. Environ. Saf.* **36**, 57 (1997)
20) J. Van der Boon, G. Van den Thillart & A. Addink: *Comp. Biochem. Physiol.* **100A**, 47 (1991)
21) M. M. Mazeaud & F. Mazeaud: "Stress and Fish," ed. by A. D. Pickering, Academic Press, New York, pp. 12–27, 1981
22) J. A. Couch: "The Pathology of Fishes," ed. by W. E. Ribelin & G. Magaki, Univ. Wisconsin Press, Madison, pp. 585–612, 1975
23) T.S. Gill, J. Pande & H. Tewari: *J. Environ. Sci. Health* **25**, 653 (1990)
24) P. Baskaram, G. Gapalakrishnasamy & P. G. Sathiyabama: *J. Ecotoxicol. Environ. Monit.* **1**, 31 (1991)
25) N. N. Singh, V. K. Das & S. Singh: *Bull. Environ. Contam. Toxicol.* **57**, 204 (1996)

26) G. Begum & S. Vijayaraghavan: *Bull. Environ. Contam. Toxicol.* **57**, 223 (1996)
27) R. J. Padmaja & B. M. Rao: *Bull. Environ. Contam. Toxicol.* **53**, 142 (1994)
28) E. C. Black, A. R. Connor, K. Lam & W. Chiu: *J. Fish. Res. Bd. Can.* **19**, 409 (1962)
29) E. Sancho, M. D. Ferrando & E. Andreu-Moliner: *J. Environ. Sci. Health.* **B31**, 87 (1996)
30) S. Hayashi & Z. Ooshiro: *Nippon Suisan Gakkaishi* **41**, 791 (1975)
31) E. Sancho, M. D. Ferrando & E. Andreu: *Fresenius Envir. Bull.* **8**, 416 (1999)

要　　約

ウナギ(*Anguilla anguilla*)におけるチオベンカルブによるストレスの生理的影響

Cristina FERNANDEZ-VEGA, Encarna SANCHO, Dolores FERRANDO, Enrique ANDREU

ヨーロッパウナギ *Anguilla anguilla* の代謝系へのチオベンカルブの影響と，中毒からの回復とを評価した．暴露期間ならびにそれに続く非汚染水中での8日間のウナギの血漿，筋肉，肝臓についてグリコーゲン，脂質およびタンパクを測定した．暴露により血糖値の上昇が認められたが，肝臓と筋肉におけるグリコーゲンレベルは著しく低下した．血中の脂質は増加したが，肝臓での脂質は顕著に減少した．タンパク質の易動化は，とくに筋肉組織で認められた．ほとんどの代謝障害は，非汚染水へ移した8日後まで継続した．以上のチオベンカルブの代謝系への影響はある種のストレス症状であり，魚類の農薬ストレスを代償しようとする生理的適応の具体例と考えられた．