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Hazard Assessment of a Mixture of Pollutants from a Sugar Industry to Three Fish Species of Western Mexico by the Responses of Enzymes and Lipid Peroxidation

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The discharge of pollutants by several industries represents a serious water pollution problem due to the toxic properties of these contaminants and their adverse effects on the biota (Pflugmacher et al. 2000). The Ameca River, located in the Pacific slope of western México of the Ameca basin is characterized by the presence of springs and a reservoir (De La Vega Reservoir). Behind the reservoir is a sugar industry that discharges its wastewater directly to the main tributary of De la Vega Reservoir. Among the pollutants of the sugar industry are pressed pulp, lignin and phenol, molasses, vinasses, sulfur, organochlorines, organophosphates, herbicides and fungicides that may produce multiple damages in fish. The Ameca river is considered a center of fish endemism but has undergone severe loss of fish biodiversity in last decade (López-López and Paulo-Maya 2001), the changes in fish fauna has been related to the pollution and water extraction in the basin.

Very little is known about the health of the fish populations inhabiting Mexican water bodies despite the significant contamination of water, sediments and biota by organophosphate and organochlorine insecticides in them (Albert 1996, De la Vega Salazar et al. 1997). Since, toxic pollutants often affect the activity of enzymes at least to some degree, changes in enzyme levels such as alkaline phosphatase, gamma-glutamyl transpeptidase and acetylcholinesterase are logical candidates to use as biomarkers (Mayer et al. 1992). Also, lipid peroxidation appears to have considerable potential as a biomarker (Mayer et al. 1992). In Mexico there is no information about the biochemical responses of native fish to the exposure to a mixture of xenobiotics in aquatic ecosystems, in order to establish their degree of tolerance for conservancy policies. In the present study we have investigated the biomarker data of three fish species inhabiting at a spring in the head waters of Ameca basin, aimed at comparing biomarker responses of fish exposed to effluents of a sugar industry.

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MATERIALS AND METHODS

Live fish of the three local species of the Ameca basin *Ameca splendens* (Butterfly Goodeid), *Goodea atripinnis* (Blackfin Goodea) and *Xiphophorus helleri* (Swordtail) were collected from a spring located 0.5 km north of de la Vega Reservoir (20° 41'25"N, 103° 50'28"0) and used as bioindicator organisms. These species were chosen because of the first of them is an endemic and endangered species of the basin (NOM-059-ECOL-2000), the second species is a widespride native fish of the Central Plateau (Soto-Galera et al. 1998) and the last one is an introduced aquarium fish. Gamma-glutamyl transpeptidase (GGTP), alkaline phosphatase (ALP) and acetylcholinesterase activities (AchE) and the level of lipoperoxidation (LP) were the biomarkers used.

Prior to exposure fish were preadapted for three days in dechlorinated water with the following characteristics: hardness 150 mgl^{-1}, alkalinity 31 mgl^{-1}, dissolved oxygen 12 mgl^{-1} and pH= 8.2. Six adult healthy specimens of both sexes of fish species, were taken directly from dechlorinated water tanks and placed in three reservoir water aquaria containing 15 liters of water. The water was collected at De La Vega Reservoir that contains a mixture of pollutants of sugar industry and lixiviates of the adjacent agriculture areas. Characteristics of the reservoir water were as follows: temperature 22.0 °C; pH 8.0; conductivity 0.6 $\mu s\ cm^{-1}$ dissolved oxygen 10 mg L^{-1}; nitrate 0.50 mg L^{-1}, nitrite 0.2 mg L^{-1}, ammonium 1.0 mg L^{-1} and phosphate 1.5 mg L^{-1} and water concentration of contaminants are: malathion 4.25 ± 0.17 $\mu g\ L^{-1}$, methyl parathion 2.76 ± 0.30 μg^{-1} and chloropyriphox 6.83 ± 0.78 $\mu g\ L^{-1}$. Also, six fish of the each species were stocked in three separated aquaria containing dechlorinated water which was circulated through a filter and served as controls (C).

The period of exposure was 4 hr. All experimental animals survived this exposure time. In each aquarium the fish were kept at constant aeration at a temperature of 22 ± 1°C during the exposure period. After exposure fish liver, muscle and gill were extracted and homogenized with Tris buffer at pH=7 in a Potter-Elvehjem glass homogenizer using a teflon pestle. The homogenized material was used to determinate 1) ALP activity by spectrophotometric method (Berger and Rudolph 1963), 2) GGTP activity assayed by the colorimetric method using p-nitroaniline as substrate (Glossman and Neville 1972), 3) AchE activity by the Hestrin method (1949) using acetylcholine as substrate and 4) LP levels by the quantification of malondialdehide (MDA) (Buege and Aust 1978). Protein concentration was measured using the method of Bradford (1976). All these assays were done in triplicate.

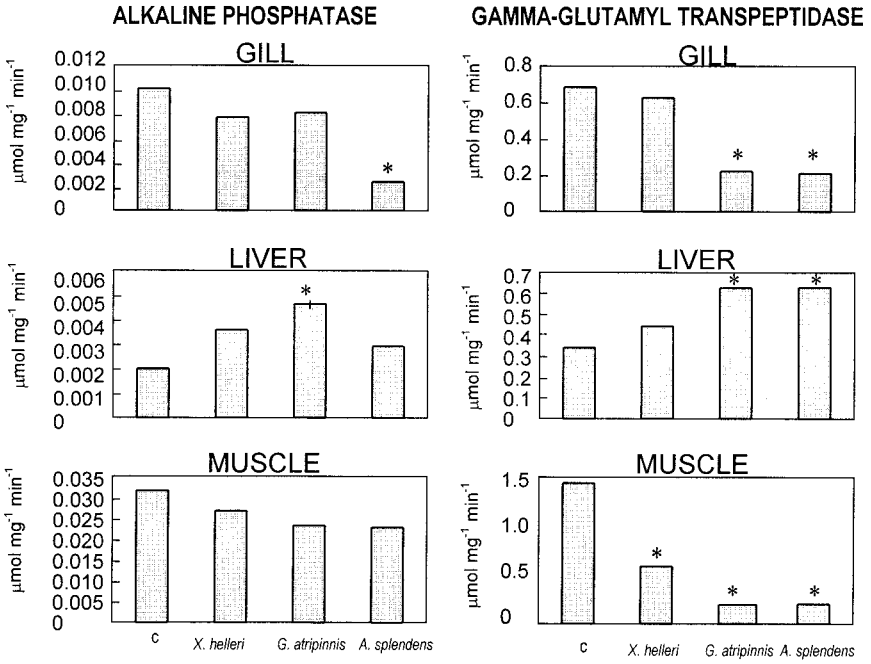

Figure 1. ALP and GGTP activities determined in gill, liver and muscle of fishes exposed to De la Vega water. Each bar represents the mean value of 6 fishes ± SD. Significant differences ($p < 0.05$) are marked *.

Data were statistically analyzed using one way ANOVA and mean differences of each group were compared using the Tukey test (Zar 1984). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The contaminated water caused statistically significant increase of the ALP activity in the liver only in *G. atripinnis*. However, the gill ALP activity was lowered in *A. splendens* and in muscle a slight decrement in ALP was observed in the three species but this was statistically no significant (Fig. 1). Elevated hepatic ALP activity could be attributed to alterations in their active sites induced by pesticides and /or disruption of membranes due to toxic action of toxicants (Nicholls *et al.* 1989).

The decreased activity may be due to the structural damage to the cellular machinery of enzyme production leading to the enzyme inhibition or decreased synthesis of the enzyme (Gill et al. 1990). Also, a decreased ALP activity in muscle and gills of *Heteropneustes fossilis* was reported following dimethoate exposure (Borah and Yadav 1996).

In the liver, the GGTP activity showed an increase over the control in *A. splendens* and *G. atripinnis* but the activity was decreased in the gill and

muscle of that species (Fig. 1). In the gill, *X. helleri* showed insignificant decrease compared to control but in muscle the decreases were significant in the three species exposed; the highest decrease were noted in *G. atripinnis* and *A. splendens.* GGTP is a plasma membrane bound enzyme and has been associated with amino acid transport, it catalyses transfer of the gamma-glutamyl moiety of glutathione to a number of acceptors as well as hydrolysis of gamma-glutamyl compounds (Meister 1981). García and Mourelle (1984) propose that the increase in liver GGTP provides a detoxification mechanism from chronic subletal exposure through the glutathione transferases which can dealkylate the insecticide in mammals. Since certain membrane enzymes may be affected in fish as well as in mammals (Nicholls *et al.* 1981), it is possible that occurs the same in fish. Inhibitory effects on gill and muscle GGTP activities could indicate a decrease in protein synthesis induced by water contaminants.

The inhibitory action of pesticides on the AchE activity was clearly evident in the gill ($p<0.05$). In the three species the reservoir water provoked 87, 89 and 93% inhibition for *X. helleri, Goodea atripinnis and Ameca splendens,* respectively, suggesting greater susceptibility of the last species attributed to the stress exerted by the mixture of contaminants on the fish (Fig. 2). Straus and Chambers (1995) found that AchE activity was inhibited 84% in *Ictalurus punctatus* after 8 hrs of exposure to chlorpyrifos.

The organophosphate insecticides induce toxic effects by inhibition of acetylcholinesterase and as a consequence blockade of synaptic transmission in the cholinergic parts of the nerve system (Escartín and Porte 1996). This could interfere with vital functions such as respiration, resulting in asphyxia and death (Gill et al. 1990).

Lipid peroxidation levels were affected by exposure to reservoir water (Fig. 2). The gill MDA levels were increased in all fish after exposure. There were the same elevation of LP in *Xiphophorus helleri* and *G. atripinnis* but *Ameca splendens* showed the highest increase in LP. MDA is one of the main products of degradation of polyunsaturated fatty acids that are essential to the entire cell supporting system. Disruption of their structural properties can have serious consequences for cellular function.

Lipid peroxidation provides a link between free radical initiated processes and associated cellular membrane dysfunction from many diverse xenobiotics (Muriel 1997).

The alteration of the enzymes activities by pollutants in tissues show the potential of these enzymes for use as biomarkers of sublethal stress. The results clearly indicate that the high level of pollution of De la Vega reservoir might impose a considerable stress on the fish species assayed due to increased level of LP and changes of enzymatic activities. The

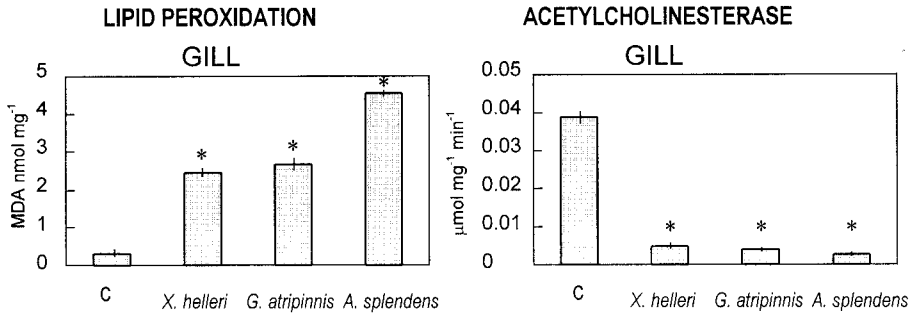

Figure 2. Malondialdehide formation and AchE activities in gill homogenates from 6 fishes exposed to De la Vega water. Each bar represents the mean value ± S.D. * means statistically different from control, $p < 0.05$.

differences in enzyme responses suggested that *Ameca splendens* followed by *G. atripinnis* are the more sensitive species, the exotic species *X. helleri* is the least sensitive.

It is recognized that some pollutants may be more or less toxic to native species compared to test species because of interspecific differences in sensitivity (USEPA 1983). Information on the toxic effect of the mixture of pollutants on enzyme activities in Mexican fishes is not enough. The establishment of realistic water quality criteria for pollutants entering critical habitats requires information on the response of native endangered species to these pollutants. The present study is a contribution toward the assessment of the toxic impact of wastewater inputs into waterbodies on the fish fauna. The use of the tissue enzyme activities measurements let us detect exposure to dangerous levels of xenobiotics which may lead to the impairment of the reproductive success, to changes in either the population size and structure of the most sensitive species and to endangering some species. Adverse effects of the mixture of pollutants in De La Vega Reservoir may be a contributing factor in the decline of the endangered fish in Ameca River.

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REFERENCES

Albert LA (1996) Persistent insecticides in México. Rev Environ Contam Toxicol 147: 1-44

Berger L, Rudolph GN (1963) Alkaline and acid phosphatase In: Meites E (ed) Standard Methods of Clinical Chemistry, vol 5. Academic Press,

New York, p 56
Borah S, Yaday RNS (1996) Effect of Rogor (30% w/w Dimethoate) on the activity of lactate dehydrogenase, acid and alkaline phosphatase in muscle and gill of a fresh water fish *Heteropneustes fossilis*. J Environ Biol 17:279-283
Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of dye-binding. Anal Biochem 72: 248-254
Buege A, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 53: 302-310
De la Vega-Salazar MY, Martínez-Tabche L, Macías-García C (1997) Bioaccumulation of methyl parathion and its toxicology in several species of the freshwater community in Ignacio Ramírez dam in México. Ecotoxicol Environ Saf, 38:53-62
Escartin E, Porte C (1996) Acetylcholinesterase inhibition in the crayfish *Procambarus clarkii* exposed to fenitrothion. Ecotoxicol Environ Saf 34:160-164
García M, Mourelle M (1984) Gamma-glutamyl transpeptidase: a sensitive marker in DDT and toxaphene exposure. J Appl Toxicol 4: 246-248
Gill TS, Pande J, Tewari H (1990) Enzyme modulation by sublethal concentrations of aldicarb, phosphamidon and endosulfan in fish tissues. Pest Biochem Physiol 38:231-244
Glossmann H, Neville DM jr (1972) γ-Glutamyl transferase in kidney brush border membranes. FEBS Letters 19: 340-344
Hestrin S (1949) Reaction of acetylcholine and other carboxylic acids derivates with hydroxylamine and its analytical application. J Biol Chem 180: 249-261
López-López E, Paulo-Maya J (2001) Changes in the fish assemblages in the upper Rio Ameca, México. J Freshwater Ecol 16: 179-187
Mayer FL, Versteeg DJ, McKee MJ, Folmar LC, Graney RL, Mc Cume DC, Rattner B A (1992) Physiological and nonspecific biomarkers. In:
Huggett RA, Kimerle RA, Mehrle PM Jr, Bergman HL (eds) Biomarkers, biochemical physiological and histological markers of anthropogenic stress, vol 1. Lewis Publishers, Boca Raton, p 5-85
Meister A (1981) On the cycles of glutathione metabolism and transport. In: Eastbrook RW, Srere P (eds) Current Topics in Cellular Regulation, vol 18. Academic Press, New York, p 21-58
Muriel P (1997). Peroxidation of lipids and liver damage. In: Baskin SI, Salem H (eds) Oxidants, antioxidants and free radicals, vol.14. Taylor and Francis, Washington, p 238-257
Nicholls DM, Teicher-Kuliszewska K, Kuliszewski MJ (1981) The activity of membrane enzymes in homogenate fractions of rat kidney following the administration of cadmium. Toxicol Appl Pharmacol 67:193-199
Nicholls DM, Teichert-Kuliszewska K, Girgis GR (1989) Effect of chronic mercuric chloride exposure on liver and muscle enzymes in fish. Comp Biochem Physiol 94:265-270

Norma Oficial Mexicana (1994) NOM-059-Ecol-2000. Diario Oficial de la Federación (primera sección) 16 de mayo de 1994 México. p 54
Pflugmacher S,Schwarz S, Pachur HJ,Steinberg CEW (2000) Effects of tributyltin chloride (TBTCL) on detoxication enzymes in aquatic plants. Environ Toxicol 15:225-233
Soto-Galera E, Díaz-Pardo E, López-López E, Lyons J (1998) Fish as indicators of environmental quality in the Rio Lerma Basin México. Aquat Ecosys Health Manag 1:267-276
Straus DL, Chambers JE (1995) Inhibition of acetylcholinesterase and aliesterases of fingerling channel catfish by chlorpyrifos, parathion and S, S, S-tributyl phosphoro
U.S. Environmental Protection Agency (1983) Water quality standars.
Handbook Office of Water Regulations and Standars. US. Environmental Protection Agency, Washington, D.C
Zar JH (1984) Biostatistical analysis.Prentice Hall, New Jersey