

An Enzymatic Methodolgy For Determining PAH Effects On The Cholinesterase Activity of Fish Brains

Hauser-Davis, R.A¹; Vianna, M.S¹; Ziolli, R.L¹.

¹ Departamento de Química, PUC-Rio, RJ, Brasil.

Acetylcholinesterase is the chemical mediator responsible for transmitting nervous impulses. Measuring its activity in fish is a diagnostic biomarker, in which reduced activities indicate site contamination by anti-cholinergic substances, resulting in acetylthiocholine accumulation in the synapses, causing muscle and neural failure. Studies indicate that oil derived products, such as Polycyclic Aromatic Hydrocarbons, may inhibit AChE in marine organisms. Analytical techniques that measure the catalytic activity of cholinesterases are inexpensive and adequate for many environmental analyses, allowing quantification of variations in enzymatic activities and observations of possible inhibition signs. These methods have already been used for determining pesticides in soils (Vianna, 2008) and AChE inhibition by PAH using eel extracts (Kang & Fang, 1997). This study aimed to evaluate the viability of applying enzymatic methodologies in identifying PAH presence in environmental monitoring programs, using fish enzymatic biomarkers. AChE extracts from *Mugil liza* brains were prepared according to Moura (1998), and AChE activity was quantified according to Ellman (1961), modified by Vianna (2008). Enzymatic tests were conducted in triplicate, using PAH standards (purity =99%) of Phenantrene, Naphthalene, Pyrene (Acros Organics), 1-Hidroxy-pyrene, Chrisene (AccuStandard) and 2-Naphtol (Chem Service) in Ethanol 100%, A.G. (Merck). AChE extracts from rat brains were used as the control group, since they possess known enzymatic activity. After incubation the samples were loaded onto 96-well microplates and read by an ELISA spectrophotometer (Vitor R³ – PERKIN ELMER), at 412 nm for 3 min, to determine enzymatic activity. Results demonstrated that the AChE verified the presence of PAH, and that this methodology can, therefore, be applied in the verification of enzyme inhibition due to AChE inhibitors.

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Key-Words: AChE, Enzymatic Biomarker, *Mugil liza*, PAH.