

## Toxicity of Swascol® 1P (SLS) to *Channa punctatus* and *Cirrhina mrigala*: Biochemical Alterations

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In recent years there has been a rapid increase in the production of synthetic detergents (syndets), used in industries and house hold purposes. As the syndets are not fully consumed during use, fairly good amount find their way into the fresh water resources, harbouring diverse fauna and flora. Most of these syndets are non-biodegradable and so remained as such for a considerable period in aquatic environment, adversely affecting the life living therein. It is evident from the literature available (HENDERSON et al. 1959, CAIRNS and SCHEIER 1964, DOOLEY and CAVIL 1964, FOSTER et al. 1966, THATCHER and SANTNER 1966, PICKERING and THATCHER 1970, LOCKHART et al. 1975, VERMA and MOHAN 1976) that these are the great contributory toxic substances for life, creating water pollution problems. Very little is known about the toxic effects of syndets on the fish enzyme system. BONDY et al. (1960 and 1973) studied the acute toxicity of tri-aryl-phosphates and their effects upon cholinesterase. In vivo effect of syndets, Idet 5L and Swanic 6L, has been studied on ATPase system by VERMA et al. (1978) in fish, Channa punctatus.

The present study was undertaken to determine the effect of various sublethal concentrations of a syndet on the activity of enzymes, acid and alkaline phosphatases, and succinic dehydrogenase in the liver and kidney of few fresh water teleosts, exposed for 15 and 30 days time intervals.

### MATERIALS AND METHODS

Healthy fish specimens, collected from the neighbouring fresh water resources, were kept in clean rectangular glass aquaria in the laboratory at  $24 \pm 4^\circ\text{C}$ . The size of the Channa punctatus varies from 118-170 mm and weight from 72-108 g while in Cirrhina mrigala, the size varies from 147-194 mm and weight from 90-111 g. The syndet, Swascol® 1P with active matter sodium lauryl sulphate (SLS), an anionic detergent, manufactured by Swastik oil mills, Bombay and marketed by Industrial

Products Sales Division, Bombay was used.

The fishes were transferred to different experimental aquaria, after the normal process of acclimatization and washing with 1%  $\text{KMnO}_4$  solution to avoid any possibility of infection. LC(50) values of both the fishes for the syndet were determined by adopting dilution techniques (STANDARD METHODS, 13th ed. 1971). The sublethal concentrations, 1/2, 1/3 and 1/6th of the LC(50) values were calculated and then the fishes were exposed for 15 and 30 days. During exposure, the fishes were provided with an artificial diet, so as to avoid any possible alteration in enzymes activities due to starvation. Experimental solutions were regularly changed after 48 h interval. Controls were set side by side for comparison.

Control as well as treated fishes were sacrificed and the liver and kidney were removed, washed, weighed, and homogenized in 0.25 M sucrose solution by Potter-Elvehjem homogenizer, and centrifuged. The homogenization and centrifugation were carried under cold conditions.

In the supernatant, acid and alkaline phosphatases were estimated by the methods described by SHINOWARA et al. (1942) and BODANSKY (1932). The inorganic phosphate liberated in both the cases was determined by the method of FISKE and SUBBAROW (1925). Protein was determined by the procedure developed by LOWRY et al. (1951). The succinic dehydrogenase was measured by the method given by KUM and ABOOD (1949), where the tissue homogenate in the presence of succinate reduced the tetrazolium salt to red formazan.

The mean  $\pm$  standard error (S.E.) of three observations and statistical significance between control and experimental values were calculated by student's 't' test (FISHER 1950).

## RESULTS AND DISCUSSION

TOVELL et al. (1975) studied the route of absorption and tissues distribution of anionic detergent, sodium lauryl sulphate (SLS) in gold fish (Carassius auratus), using ( $^{35}\text{S}$ ) or ( $1\text{-}^{14}\text{C}$ ) SLS and found the principal route of entry through the oral or respiratory membrane. Absorbed SLS entered the circulation and was distributed throughout the body tissues. We also assume the possibility of the same route for the absorption and distribution of the syndet used, in different tissues.

This study indicates the sensitivity of enzymes, assayed from liver and kidney of C. punctatus and C. mrigala, chronically exposed to various sublethal concentrations of syndet. The results obtained are compiled and tabulated (Table 1 to 3). The inhibition pattern of acid and alkaline phosphatases activities was found quite similar and was concentration dependant. Differences in the response of alkaline phosphatase towards various levels of inhibitors were also noted by FISHMAN et al. (1962). GUPTA et al. (1975), DIKSHITH et al. (1975) and KSHIRSAGAR (1975) observed the diverse effects of various toxicants, viz. heavy metals, pesticides etc. on the activity of acid and alkaline phosphatases.

Alterations noted in the enzymes activities in general were more pronounced in liver than in kidney. The highest fall (41.71%) has been observed in the activity of alkaline phosphatase in liver of C. mrigala, exposed to highest concentration for 30 days. KSHIRSAGAR (1975) while studying the effect of  $Sr^{++}$  on rat, reported the significant ( $P < 0.001$ ) loss in the alkaline phosphatase activity.

As regards the sensitivity of acid phosphatase activity, it altered moderately and lies between alkaline phosphatase and succinic dehydrogenase. The observed depression of phosphatases may actually represent an inhibition of the synthesis of a greatly increased turnover as a result of the action of toxicant. It is clear that the effect of this syndet is not specific to only single enzyme, it may disturb the activities of other enzymes as well. The capability of syndets to interact with proteins (SWISHER 1970) and alter membrane permeability (GOLDACRE 1968) also suggests that they act as general tissue poisons rather than a highly specific inhibitors.

It is further noted that at lowest level the SLS stimulates the activity of phosphatases in the kidney. However, certain concentrations stimulate the SDH activity in liver of C. punctatus and C. mrigala, even significantly ( $P < 0.05$ ), but the highest syndet concentration with increase in exposure time inhibits the SDH activity. This fall in the activity of SDH suggests the impairment of the energy metabolism after the exposure to syndet. The transient depression of SDH, following the injection of fluorescent carcinogen-4-dimethylaminostibene (BITENSKY et al. 1960) and sodium malonate (ZIMMERMANN 1961) has been observed.

It is admitted that there is no clear cut explanation for the alterations in the enzyme activities since many factors may be involved in it. The possible cause

TABLE 1

Alkaline phosphatase activity in the liver and kidney of C. punctatus and C. mrigala exposed to Swascol 1P (SLS).

Fish	Conc. in mg/L	Enzyme activity: mg inorganic phosphate liberated/mg of tissue protein/hr			
		15 days exposure		30 days exposure	
		Liver	Kidney	Liver	Kidney
<u>C. punctatus</u>	Control	0.0488±0.0028 (--)	0.1284±0.0076 (--)	0.0483±0.0031 (--)	0.1261±0.0075 (--)
	9.75	0.0383±0.0016 (21.50)*	0.1118±0.0058 (13.41)	0.0296±0.0010 (38.42)**	0.0957±0.0041 (24.13)*
	6.50	0.0409±0.0020 (16.28)	0.1142±0.0062 (11.04)	0.0346±0.0013 (28.45)**	0.1012±0.0049 (19.75)*
	3.25	0.0449±0.0026 (7.88)	0.1368±0.0077 +(6.54)	0.0426±0.0026 (11.75)	0.1139±0.0065 (9.56)
	Control	0.0475±0.0028 (--)	0.1107±0.0072 (--)	0.0469±0.0026 (--)	0.1108±0.0071 (--)
<u>C. mrigala</u>	8.40	0.0343±0.0014 (27.87)*	0.0957±0.0053 (13.57)	0.0273±0.0010 (41.71)***	0.0836±0.0041 (24.52)*
	5.60	0.0388±0.0018 (18.42)*	0.0998±0.0057 (9.85)	0.0318±0.0012 (32.11)**	0.0899±0.0052 (18.87)
	2.80	0.0416±0.0023 (12.35)	0.1197±0.0081 +(8.10)	0.0407±0.0021 (13.22)	0.0971±0.0054 (12.41)

Values are the mean ± Standard error of 3 observations.

Values in parenthesis indicate the percent inhibition and + indicates percent activation.

Values are statistically significant \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

TABLE 2

Acid phosphatase activity in the liver and kidney of C. punctatus and C. mrigala exposed to Swascol 1P (SLS).

Fish	Conc. in mg/L	Enzyme activity: mg inorganic phosphate liberated/mg of tissue protein/hr			
		15 days exposure		30 days exposure	
		Liver	Kidney	Liver	Kidney
<u>C. punctatus</u>	Control	0.0573±0.0031 (--)	0.0997±0.0016 (--)	0.0564±0.0030 (--)	0.0985±0.0017 (--)
	9.75	0.0468±0.0026 (18.37)*	0.0909±0.0016 (8.85)*	0.0375±0.0017 (33.51)**	0.0795±0.0010 (19.24)**
	6.50	0.0490±0.0026 (14.50)*	0.0933±0.0015 (6.45)*	0.0411±0.0016 (27.06)**	0.0834±0.0011 (15.38)**
	3.25	0.0549±0.0029 (4.23)	0.1034±0.0017 (8.72)	0.0463±0.0022 (15.93)*	0.0959±0.0013 (2.55)
	Control	0.0511±0.0029 (--)	0.0984±0.016 (--)	0.0512±0.0029 (--)	0.0987±0.0016 (--)
<u>C. mrigala</u>	8.60	0.0407±0.0021 (20.25)*	0.0906±0.0014 (7.93)*	0.0320±0.0018 (37.42)**	0.0785±0.0089 (20.47)**
	5.60	0.0433±0.0023 (15.12)	0.0924±0.0014 (6.11)*	0.0412±0.0019 (29.51)*	0.0830±0.0009 (15.89)**
	2.80	0.0488±0.0026 (4.35)	0.1026±0.0016 (4.28)	0.0435±0.0023 (15.04)	0.0997±0.0015 (1.03)

Values are the mean ± Standard error of 3 observations.

Values in parenthesis indicate the percent inhibition and + indicates percent activation.

Values are statistically significant \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

TABLE 3

Succinic dehydrogenase activity in liver and kidney of C. punctatus and C. mrigala exposed to Swascol 1P (SLS).

Fish	Conc. in mg/L	Enzyme activity: $\mu$ g tetrazolium reduced/ $\mu$ g/10 min			
		15 days exposure		30 days exposure	
		Liver	Kidney	Liver	Kidney
<u>C. punctatus</u>	Control	1.1724 $\pm$ 0.073 (--)	1.5872 $\pm$ 0.091 (--)	1.1747 $\pm$ 0.077 (--)	1.6430 $\pm$ 0.111 (--)
	9.75	1.0535 $\pm$ 0.047 (10.14)	1.4702 $\pm$ 0.052 (7.37)	0.9314 $\pm$ 0.031 (20.71)*	1.4348 $\pm$ 0.039 (12.67)
	6.50	1.2747 $\pm$ 0.078 +(8.47)	1.5102 $\pm$ 0.082 (4.85)	1.0286 $\pm$ 0.040 (12.43)	1.5222 $\pm$ 0.056 (7.35)
	3.25	1.4251 $\pm$ 0.091 +(21.55)*	1.6096 $\pm$ 0.091 +(1.41)	1.2528 $\pm$ 0.092 +(6.65)	1.5759 $\pm$ 0.082 (4.08)
<u>C. mrigala</u>	Control	1.0143 $\pm$ 0.071 (--)	1.6741 $\pm$ 0.093 (--)	1.0892 $\pm$ 0.074 (--)	1.7341 $\pm$ 0.092 (--)
	8.60	0.8600 $\pm$ 0.045 (15.21)*	1.5179 $\pm$ 0.066 (9.33)	0.8367 $\pm$ 0.031 (23.18)*	1.4626 $\pm$ 0.040 (15.65)*
	5.60	1.1121 $\pm$ 0.071 +(9.64)	1.5862 $\pm$ 0.073 (5.25)	0.9260 $\pm$ 0.039 (14.98)	1.5714 $\pm$ 0.059 (9.38)
	2.80	1.2382 $\pm$ 0.081 +(22.07)*	1.6369 $\pm$ 0.084 (2.22)	0.9939 $\pm$ 0.075 (8.75)	1.6402 $\pm$ 0.071 (5.41)

Values are the mean  $\pm$  Standard error of 3 observations.

Values in parenthesis indicate the percent inhibition and + indicates percent activation.

Values are statistically significant \* $P < 0.05$ .

of depletion of phosphatases may be due to the uncoupling of oxidative phosphorylation followed by intoxication. The uncoupling of phosphorylation was also reported by GALLAGHER and REES (1960) and DIANZANI and MARINARI (1961) after carbon tetrachloride administration.

It is in vivo study, therefore, if it has a bearing on the environmental conditions, it would serve to illustrate the possible damage due to exposure with this syndet. As such, if the uptake of syndet by fishes reaches tissue concentration equal to those used in this study, the resulting alterations in enzyme activities may be sufficient to impair normal organ function.

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