LEVELS OF LIPID METABOLITES IN *Cyprinus carpio* (L.) ON SUBLETHAL EXPOSURE TO SYNTHETIC DETERGENT LINEAR ALKYL BENZENE SULFONATE (LAS)

S. GOPAL, M. NAGABHUSHAN REDDY, M. VENKATA RAMUDU AND P. INDIRA

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SUMMARY

Synthetic detergents deposited in the aquatic environment may accumulate in the food chain and cause ecological damage and even threat to human health. Linear alkyl benzene sulfonate (LAS) is an anionic surfactant and placed head list of chemical pollutants posing a great potential risk than the organic wastes and eutrophicating nutrients. During this research sublethal effects of LAS on the levels of lipid metabolites in various tissues of freshwater fish *Cyprinus carpio* were studied. The levels of total lipids decreased initially at 24h in relation to control and up to day 7. After day 7, these levels increased gradually and maximum increase was observed at day 15 from where there was again decrease in those levels up to day 20 and reached nearer to control at day 30. The increase in the levels of total lipids was more in muscle than liver followed by gill. The levels of both free fatty acids and lipase activity followed a reverse trend to that of total lipids.

See end of the article for authors' affiliations

Correspondence to :
P. INDIRA
Department of Zoology, Sri Krishnadevaraya University, ANANTAPUR (A.P.) INDIA

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Lipids are heterogenous group of complex macromolecules such as fatty acids, acylglycerols, phosphoglycerides, steroids, terpenes and prostaglandins having high calorific value. Changes in lipid metabolism provide demanding energy to survive from toxicological effects of the fish under stressed conditions. They form the rich energy reserves whose calorific value was reported to be twice that of an equivalent weight of carbohydrates or proteins (Oser, 1979). Lipids are generally stored in the liver, adipose tissues and muscles and are one of the most important fish energy sources. They are mobilized, when food intake cannot supply the energy demands of growth and maintenance (Moreira *et al.*, 2002). The mobilization of lipid reserves in an organism testifies the imposition of high-energy demands (Srinivasulu Reddy and Raman Rao, 1989). Lassiter and Hallam (1990) proposed the survival of the fattest model which means the fish with higher body lipid content will survive longer since they are more resistant to toxic effects of chemicals than those with lower lipid content. Since lipids undergo rapid breakdown, resynthesis and interconversion in response to different stimuli, it is essential to consider simultaneously various lipid fractions in different tissues to provide a clear cut picture of lipid metabolism.

Synthetic detergents are one of the resultants of these modern technologies emerged as major contributors to the problem of pollution. Synthetic detergents are head list of chemical pollutants at this moment, posing a great potential risk than the organic wastes and eutrophicating nutrients. All surfactants are potentially harmful to most of the organisms, aquatic as well as terrestrial, at some level or other and are reported to produce toxic effects (Belanger *et al.,* 2002). Several reports have come to light in recent years explaining that synthetic detergents interfere with various metabolic aspects of organisms and cause death (Goodrich *et al.*, 1991; Toshima *et al.*, 1992).

Linear Alkyl benzene Sulfonate (LAS) is one the most widely used anionic surfactants in commercial use today. It has a variety of industrial uses and is a common ingredient in laundry detergents, household cleaning products and personal products (e.g. shampoos and cosmetics) with an annual use of approximately 1 million metric ton. LAS accounts for 28% of the surfactants produced in United States, Western Europe and Japan (Federle *et al.,* 1989). Although most LAS is discarded as sewage and effectively removed during sewage treatment, in some areas raw sewage containing LAS is discharged directly into the environment (Rapaport *et al.,* 1990). In India, per capita consumption of detergent in 1994 was 2kg per annum and was projected to rise to over 4kg per annum by 2005 (Indian Toxics Link, 2002). Most detergents are formulated products containing surfactants, which remove dirt, stain and soil from surfaces or textiles. Surfactants consist of a hydrophobic and hydrophilic component and have the ability to change the surface properties of water. In aqueous solutions, surfactants tend to accumulate at air/solution or solid/liquid interfaces, whereby the surface tension of water is reduced.

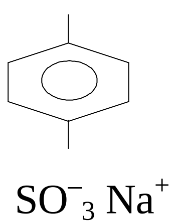
Several biochemical changes are involved during utilization of lipid reserves. Energy yielding processes of fatty acid oxidation is known to proceed by the release of fatty acids from triglycerides and then they are transported to the site of utilization. The physiological role of lipase in mobilization of lipids consists of essentially promoting the hydrolysis of fat deposits (Bilinski, 1969) and the synthesis of neutral fats by its reverse action (George and Talesara, 1962). The product of fat hydrolysis is fatty acids through β-oxidation from acetyl-CoA, which are partly channeled into TCA cycle for metabolic energy production and the rest to the production of acetoacetate. In order to mitigate any stress generally more energy is needed to the fish that may be obtained from carbohydrates, proteins and lipids.

Piska *et al.*, (1992) reported decline lipid content in liver, gill and brain of *Cyprinus carpio* on exposure to sublethal concentration of synthetic pyrethroid cypermethrin. Based on the above results, it is of present interest to know how the common carp could respond to the stress caused by the sublethal concentration of synthetic detergent by involving in lipid metabolism.

MATERIALS AND METHODS

Commercial grade of household green arial surf was selected as the representative toxicant for the study of physiological changes in freshwater fish *Cyprinus carpio*.

Chemical name	: Linear alkyl benzene sulfonate (LAS)
Structural formul	: $CH_3 – (CH_2)_m – CH – (CH_2)_n – CH_3$
Molecular weight	: 342.4 g/mol
CAS Registry No.	: 68411–30–3
Physical state	: Powder
Solubility in	: Water, oils & other aqueous solutions under certain conditions



Experimental design :

Cyprinus carpio were collected from the Department of Fisheries in big fish containers and brought to the laboratory. Then they were released in large tanks containing dechlorinated tap water. The fish were fed with groundnut pellets having around 40% protein content once in two days and allowed to acclimatize for 15 days. Then LC_{50} was determined using Dragstedt-Behren's method as given by Carpenter (1975). Later, the fish (10 ± 2g) were separated into the 6 batches, each consisting of 6 fishes and were renewed everyday to provide freshwater rich in oxygen. During experimentation, water was aerated once a day to prevent hypoxic conditions. As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of the animal, size, sex etc. precautions were taken throughout this investigation to control all these factors as far as possible. As a part of it, water from same source has been used for the maintenance of fish. The animals were starved 24hr prior to each experiment, to avoid any influence of differential feeding. The size of the animal selected also maintained strictly throughout the investigation.

Fixation of sublethal concentration :

Taking into consideration the fact that the effect of a synthetic detergent LAS on fish becomes consistent within 24h of exposure, sublethal concentration was taken to study the lipid metabolism in fish, *Cyprinus carpio*. However, knowledge on the concentration of toxicant that kills 50% of the test animals in fixed period of time could become insufficient to assess various responses of the animal to toxicant (Nobbs and Pearu, 1976). Further studies on acute toxicity have significant limitations such as the occurrence of adaptation of the test animals to the imposed toxicity (Stockner and Aueta, 1976). Perkin (1979) also viewed the need for sublethal studies because distinct changes involving a sequence of events in the responses of test animal could occur during sublethal concentration. Hence, about $1/10^{th}$ (3.111mg/l) of the LC_{50} (31.11mg/l) was taken as the sublethal concentration to evaluate further studies.

Fixation of exposure periods :

Since the duration of exposure is having a great influence on the toxicity of synthetic detergent in an organism, 24hr, day 7, day 15, day 20 and day 30 were chosen to observe the short-term and long-term effects of synthetic detergent LAS on freshwater fish *Cyprinus carpio*.

The experiments were carried out both in normal fish (control) and exposed fish (LAS treated). The fishes stunned to death, gill, liver and muscle were dissected out from each of them using sterilized instruments. These were weighed separately to nearest milligram on a sartorius electrical semi-microbalance and transferred into separate microbeakers containing fish ringer solution (Ekberg, 1958). An equilibration time of 15min was allowed to the above tissues to enable them to regain normally from a state of stroke, if any due to handling and dissecting. The entire procedure was carried out in a sterilized cold room (temperature at $15±1^0C$). Total lipids, free fatty acids and lipase activity were estimated in each of the dissected out by the methods of Folch *et al.* (1957), Natelson (1971) and Colowick and Kaplan (1955), respectively. The experimental data were subjected to statistical analysis by using Students t-test in which 'P' indicates the level of significance.

RESULTS AND DISCUSSION

The levels of total lipids were initially decreased relative to the control in all the tissues of fish from 24hr up to day 7. This decrease was highly significant (P<0.001) at day 7. Later from day 7 onwards, the levels of total lipids were increased up to day 15. After day 15, these levels were gradually decreased up to day 20 through day 30 relative to the control. Among these tissues, the per cent increase in the levels of total lipids followed the order: Gill < Liver < Muscle (Table 1 and Fig. 1).

The levels of free fatty acids and lipase activity reciprocal to total lipids, increased initially relative to control in all tissues of fish at 24hr. After 24hr, these levels were decreased through day 7 to day 15. This decrease was highly significant (P<0.001) at day 15 (Table 2 and Fig. 2). The levels of free fatty acids gradually increased

Table 1 : Levels of total lipids (mg/g wet wt. of tissue) in *Cyprinus carpio* at different exposure periods of sublethal concentration of synthetic detergent LAS besides control medium. % change was calculated in relation to control medium. % recovery at day 30 was calculated in relation to control medium (which was fixed at 100%). Each Mean represents value (n=6). 'P' denotes the level of significance

Name of the tissue	Control	Sublethal exposure period				
		24h	day 7	day 15	day 20	day 30
Gill						
Mean	62.0	30.8*	43.2*	65.3#	57.1¶	52.7#
S.D.±	2.48	1.23	1.72	2.61	2.28	6.32
% change	-	-50.0	-30.3	+5.3	-7.9	-15.0
% recovery	-	-	-	-	-	85.0
Liver						
Mean	74.3	59.5*	69.8#	79.9#	71.2#	67.9#
S.D.±	2.97	2.38	2.79	3.19	2.84	5.43
% change	-	-19.9	-6.0	+7.5	-4.1	-8.6
% recovery	-	-	-	-	-	91.4
Muscle						
Mean	24.1	17.6*	22.7#	29.6*	22.8#	19.4$
S.D.±	0.96	0.70	0.90	1.18	1.14	4.65
% change	-	-26.9	-5.8	+22.8	-5.3	-19.5
% recovery	-	-	-	-	-	80.5

* indicates P<0.001, ¶ indicates P<0.01,
# indicates P<0.05, $ indicates Non-Significant

Table 2 : Levels of free fatty acids (mg/g wet wt. of tissue) in *Cyprinus carpio* at different exposure periods of sublethal concentration of synthetic detergent LAS besides control medium. % change was calculated in relation to control medium. % recovery at day 30 was calculated in relation to control medium (which was fixed at 100%). Each Mean represent value (n=6). 'P' denotes the level of significance.

Name of the tissue	Control	Sublethal exposure period				
		24h	day 7	day 15	day 20	day 30
Gill						
Mean	2.50	2.96#	2.15*	1.73*	1.89¶	2.20$
S.D.±	0.10	0.35	0.08	0.06	0.30	0.30
% change	-	+ 18.4	-14.0	-30.8	-24.4	-12.0
% recovery	-	-	-	-	-	88.0
Liver						
Mean	6.54	7.01¶	6.31$	5.50*	5.81*	6.15#
S.D.±	0.26	0.28	0.25	0.22	0.23	0.24
% change	-	+ 7.18	-3.5	-15.9	-11.1	-5..1
% recovery	-	-	-	-	-	94.9
Muscle						
Mean	1.52	2.11¶	1.43#	1.01*	1.25*	1.43$
S.D.±	0.06	0.29	0.05	0.04	0.05	0.07
% change	-	+ 38.8	-5.7	-33.5	-17.5	-5.9
% recovery	-	-	-	-	-	94.1

* indicates P<0.001, ¶ indicates P<0.01,
# indicates P<0.05, $ indicates Non-Significant

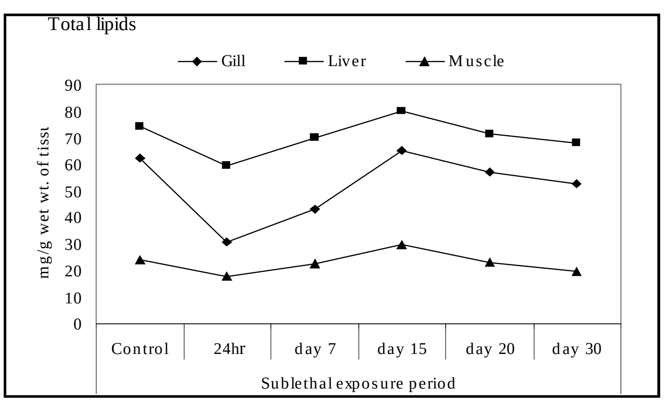


Fig. 1 : Levels of total lipids in *Cyprinus carpio* in control and at different sublethal exposure periods of synthetic detergent LAS

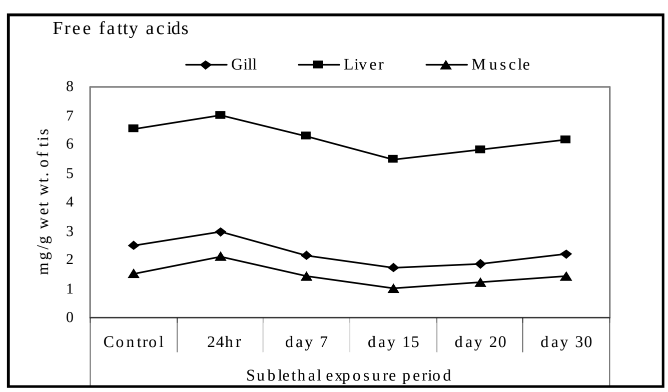


Fig. 2 : Levels of free fatty acids in *Cyprinus carpio* in control and at different sublethal exposure periods of synthetic detergent LAS

through day 20 to day 30. The percent decrease in the levels of free fatty acids and lipase activity in all the tissues followed the order: Gill > Muscle > Liver and Liver > Muscle > Gill.

Involvement of lipids in fishes exposed to toxicants can reasonably be expected on two grounds. One is to meet additional energy demands and the other is to provide an extra amount of water needed for osmoconcentration of body fluids. Studies on the involvement of lipids in freshwater fishes exposed to pesticides and metal were many and to synthetic detergents are very limited (Kaphalino *et al.*, 1981; Luvizotto *et al.*, 2003).

Lipase activity indicates the initial stage of lipid utilization besides bringing about the mobilization of lipids through hydrolysis leading to the release of fatty acids and glycerol and also synthesis of neutral fats by its reserve action (George and Talesara, 1962; Bilinski, 1969). In the present study, it was noticed the elevation in lipase activity and free fatty acids followed by significant decrease in total lipids. The elevation in lipase activity on exposure to sublethal concentration of synthetic detergent LAS indicates stepping up of lipid metabolism involving the mobilization and utilization of triglycerides (Table 3 and Fig. 3).

Table 3 : Lipase activity (µM/mg wet wt. of tissue/h) in *Cyprinus carpio* at different exposure periods of sublethal concentration of synthetic detergent LAS besides control medium. % change was calculated in relation to control medium. % recovery at day 30 was calculated in relation to control medium (which was fixed at 100%). Each Mean represent value (n=6). 'P' denotes the level of significance

Name of the tissue	Control	Sublethal exposure period				
		24h	day 7	day 15	day 20	day 30
Gill						
Mean	161.5	169.8$^{\$}$	153.4$^{\#}$	143.8*	150.1$^{\#}$	155.8$^{\$}$
S.D.±	6.46	6.79	3.32	4.83	6.00	6.23
% change	-	+ 5.1	-5.0	-10.9	-7.0	-3.5
% recovery	-	-	-	-	-	96.5
Liver						
Mean	381.3	420.1¶	370.6$^{\#}$	310.4*	330.4*	360.2$^{\#}$
S.D.±	15.25	16.80	14.82	12.41	13.21	14.40
% change	-	+ 10.1	-11.7	-18.5	-13.3	-5.5
% recovery	-	-	-	-	-	94.5
Muscle						
Mean	84.1	89.3$^{\#}$	82.7$^{\#}$	71.2*	75.6*	80.5$^{\$}$
S.D.±	3.36	3.57	3.30	2.84	3.02	3.22
% change	-	+ 6.1	-1.6	-15.3	-10.1	-4.2
% recovery	-	-	-	-	-	95.8

* indicates P<0.001, ¶ indicates P<0.01,
# indicates P<0.05, $ indicates Non-Significant

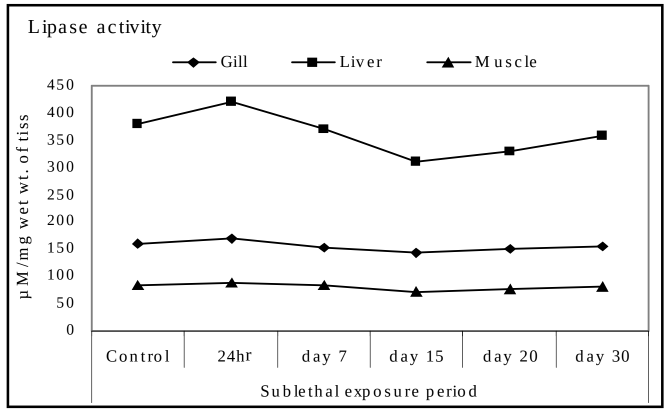


Fig. 3 : Lipase activity in *Cyprinus carpio* in control and at different sublethal exposure periods of synthetic detergent LAS

Kamble and Muley (2000) reported a significant decrease in total lipids in *Sarotherodon mossambicus* on exposure to endosulfan and chloropyrifos. Lemos *et al.*, (2001) have also demonstrated salinity change–induced alteration in lipid content in post larvae of *Farfantepenaeus paulensis*. Rao and Rao (1981) reported a decline in total lipids, phospholipids and an elevation in free fatty acids and total cholesterol in the red muscle, gill, liver and brain tissues of freshwater teleost, *Sarotherodon mossambicus* on exposure to sublethal concentrations of methyl parathion. All the above reports corroborated with the present decline in total lipids and elevation of free fatty acids. The length of the alkyl chain is an important factor determining the aquatic toxicity, shorter chain lengths being less toxic than the longer ones (Tolls *et al.*, 1997). The toxicity of LAS to fish generally increases with increasing alkyl chain length and approximately a 10-fold difference in toxicity between homologues separated by two carbon atoms has been observed (Painter, 1992).

The elevated free fatty acids may undergo -oxidation leading to the formation of acetyl–CoA in order to meet impending energy demand arising out of toxic stress. Synthetic detergent LAS induced mobilization of lipids by stimulating the lipase activity there by breaking down the lipids into free fatty acids. These free fatty acids enter the utilization cycle in the tissue of fish. Swamy *et al.* (1983) observed a metabolic shift from carbohydrate to lipid metabolism through acetyl-CoA barrier leading to an increment in lipids in the organs of freshwater mussel, *Lamellidens marginalis* under pesticide toxicity. This also supports the present trend to total lipids increment corresponding to increased lipogenesis in the tissues of fish under sublethal synthetic detergent stress. After day 15, lipase activity and free fatty acids were increased and came nearer to control at day 30 of exposure periods. Where as total lipids declined initially and came nearer to control at day 30. This indicates the ability of the animal to survive in less toxic environment by stabilization of the organ system to activate all the detoxifying mechanisms underlying in them. Metabolic compensation involves breakdown and synthesis of products necessary to cope up altered situation. In conclusion the shift in lipid metabolism is to compensate the situation shown by the fish for its survival.

Authors' affiliations

S. GOPAL, M. NAGABHUSHAN REDDY, M. VENKATA RAMUDU, Department of Zoology, Sri Krishandevaraya University, ANANTAPUR, (A.P.) INDIA

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