

## Toxicity Evaluation of Fluoride in Silkworm *Bombyx mori* L.

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**Abstract:** Toxicity evaluation of a pollutant will be highly useful in the final evaluation of designing 'safe level' or 'tolerable level' of pollution to the terrestrial biosphere and thus will have the way in establishing limits and levels of acceptability by biotic components. Keeping in view of this a static test was conducted to evaluate the toxicity of fluoride in silkworm *Bombyx mori*, L. As the 96 hr acute toxicity or short term test is the most commonly employed toxicity test, silkworms were treated orally with various doses of fluoride for 96 h and the percent mortality was recorded. The LD<sub>50</sub> value was found to be 27.5 mg/kg b.w.

**Key words:** Fluoride • Toxicity • Silkworm

### INTRODUCTION

Fluorides make their presence in the environment via soil, air, water. Ultimately their presence leads to the contamination of terrestrial ecosystems [1]. The fluorides from soil, air, water and fertilizers are well known to interfere with the efficient functioning of plants and animals. Many reports exhibited that the serious effects on the mulberry and silkworm cocoon rop production. Metabolic and physiological derangements are well documented in silkworm on exposure to fluoride toxicity. Physiological status leading to what is known as metabolic suppression which result in affected cocoon production [2-10].

Since the terrestrial habitats are more prone to hazardous pollutant like fluoride, toxicity evaluation of a pollutant will be highly useful in the final evaluation of designing safe level (or) tolerable level of pollution to the terrestrial biosphere and thus will have the way in establishing limits and level of acceptability by the biotic components. It also helps in programming preferential sericulture programmes depending upon the quality of environment and quality of pollutant.

LD<sub>50</sub>, a common measure of toxicity is the lethal concentration that causes death in 50 per cent of the treated animals. LD<sub>50</sub> is generally expressed as the dose in milligram (mg) of chemical per kilogram (kg) of body weight, LD<sub>50</sub> is often expressed as mg of chemical per volume of medium. The organism is exposed to chemicals are considered highly toxic when the LD<sub>50</sub> is small and practically non-toxic when the value is large. However the

LD<sub>50</sub> does not reflect any effects from long term exposure (i.e., cancer birth defects or reproductive toxicity).

For evaluating, toxicity of a substance there are different parameters such as lethal, sub-lethal median lethal dose, safe dose etc. The toxicity can be evaluated by exposing the animals to different doses to successive batches of the animals for fixed time and after suitable intervals by accounting the number of animals dead (or) alive [11]. However, the toxicity depends on factors like animal weight, Pickering *et al.*, [12] its development stage [13], the time of exposed, sex, age, nutritional status and environmental parameters like temperature, hydrogen ion concentration (pH) humidity light etc. In evaluating toxicity in silkworm, the young age silkworm (I instar to III instar) larva are sensitive to chemical substances even a lot lower dose. Hence, the toxicity evaluation has to be done in either IV or V instar. Several reports are available on toxicity evaluation of chemical substances on various insects including *B. Mori* [14-20]. LD<sub>50</sub> Values are important to evaluating the toxicity level and also to determine the sub-lethal doses, the present studies begin with 96 hrs LD<sub>50</sub> PM X CSR<sub>2</sub>. The present study is useful to observe the effect of fluoride and behavioral changes in silkworm on exposure to acute and sub-acute doses of fluoride.

### MATERIALS AND METHODS

Silkworm *Bombyx mori*, L (PM X CSR<sub>2</sub>) of V instar with an average weight of 1.5gm. were procured from RSRS, Anantapur, Andhra Pradesh and acclimatized to

laboratory and maintained the rearing conditions according to Krishnaswamy [21]. The temperature  $24.0 \pm 1$  °C and humidity  $75\% \pm 1$  % were maintained throughout the rearing. During acclimatization fresh mulberry leaves V1 variety fed adlibitum, sodium fluoride (Merk, Anlar ) was used. A stock solution of 100 mg/ml was prepared in distilled water. An appropriate amount of fluoride was drawn from this stock solution for the further experiment. Fluoride was given to silkworm through oral dosage by spraying on mulberry leaf of different doses. Preliminary tests were conducted to find out the median tolerance limit ( $LD_{50}$ ) of the silkworm for 96h by Probit analysis method [11]. The dose of fluoride at which 50% mortality occurred was taken as the median lethal dose ( $LD_{50}$ ) for 96 h, which was found to be 27.5 mg/kg b.w.

The mean values were derived following the method of Finney Probit Kill Theory, [11]. The data was subjected to following statistical equations for arriving at  $LD_{50}$  values.

A graph was drawn between percent mortality and log doses of fluoride and a sigmoid curve was obtained. A graph was drawn between probit mortality and log concentration of the pesticide and a linear curve was obtained.

To know the validity of data it was subjected to Dragstedt and Beheren's equation (Carpenter, 1975) as given below,

$$\text{Log } LD_{50} = \log A + \frac{50 - a}{a - b} \times \log 2$$

Where,

- A = concentration of fluoride below 50% mortality.
- a = percent kill just below 50% mortality.
- b = percent kill just above 50% mortality.

The mean  $LD_{50}$  value was calculated from the values obtained from the above three methods namely percent, Probit mortality and Dragstedt and Beheren's method.

## RESULTS AND DISCUSSION

The minimum dose at which zero percent mortality and maximum dose at 100% mortality was exhibited at 0.950 mg/kg b.w and 40.0 mg/kg b.w respectively in silkworm, *Bombyx mori.*, L.  $LD_{50}$  obtained through sigmoid curve is 27.5 mg/kg b.w.(Fig. 1) and linear curve is 27.5 mg/kg b.w (Fig.2). The  $LD_{50}$  values were further verified by Dragstedt and Behren's method and the  $LD_{50}$  value obtained was 27.58 mg/kg b.w. Hence, the average  $LD_{50}$  for 96 h is estimated to be 27.52 mg/kg b.w.

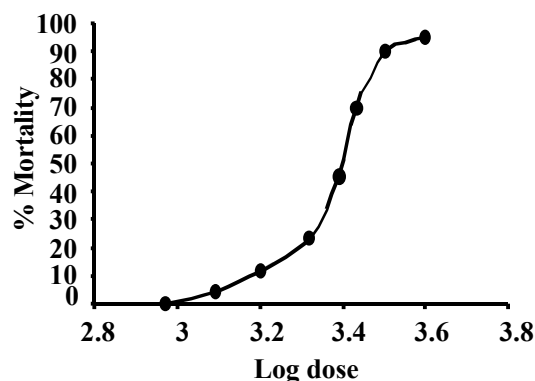


Fig. 1: The graph showing sigmoid curve between percent mortality (kill) of silkworm against log dose

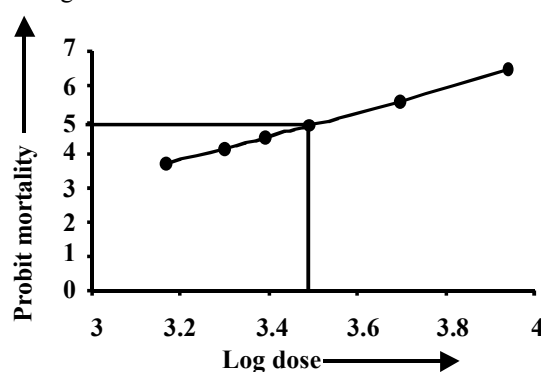


Fig. 2: The graph showing linear curve between probit mortality of silkworm against log dose

The toxicity studies of fluoride were also made by some workers on different animals. Ramakrishna *et al.*, [5] obtained V instar silkworm larvae  $LD_{50}$  82.37 ppm of fluoride at 216 hrs (in case of Pure Mysore) and  $LD_{50}$  52.00 ppm of fluoride at 168 hrs (in case of NB<sub>4</sub>D<sub>2</sub> race). According to Delopez *et al.*, [22] the  $LD_{50}$  for female rats weighing 80,150 or 200 g when given sodium fluoride by stomach intubation, the  $LD_{50}$  for 80 gm rats and 150 g rats was approximately the same (54 and 52) mg/kg b.w. Respectively, but these values were higher than that for 200 g rats (31 mg/kg b.w). Vijayabhaskara Rao, A. [23] showed a 24 hr  $LD_{50}$  or 50.56 mgF/kg b.w. in species mice, *Mus norvegicus albinus*, Pillai *et al.* [24, 25] demonstrated a 24 hr  $LD_{50}$  of 54.4 mg /kg bw, for male mice and slightly less for female mice (51.6 mg F/kg b.w).

Fujii and Honda [26] reported that mulberry leaves containing 10 15 ppm fluoride were lethal to silkworm larvae. Farhana Begum, A [27] reported a 24 hr  $LD_{50}$  as 23.9 mg/kg b.w. V instar silkworm *Bombyx mori.*, L. (PM X NB4D2) with fluoride. In the present study the 24 hr  $LD_{50}$  obtained for V instar silkworm PM X CSR2 silkworm

Table 1: Mortality of silkworm larvae in different doses of fluoride at 96 h exposure periods

S.No.	Dose of fluoride (mg/kg b.w.)	Log dose	No. of larvae exposed	No. of larvae dead	% Mortality	Probit mortality
1.	0.950	2.97	50	0	0	--
2.	1.000	3.00	50	5	10	3.72
3.	1.500	3.17	50	10	20	4.16
4.	2.000	3.30	50	15	30	4.48
5.	2.500	3.39	50	20	40	4.75
6.	3.000	3.47	50	35	70	5.52
7.	3.500	3.54	50	45	90	6.28
8.	4.000	3.60	50	50	100	--

Table 2: LD<sub>50</sub> values of silkworm larvae after 96 h treatment

S. No.	Name of the Method	LD <sub>50</sub> value (in mg/kg b.w.)
1.	Percent mortality (Sigmoid cure)	27.500
2.	Probit mortality (linear curve)	27.500
3.	Dragsted and Beheren's method	27.580
	Mean	27.520
	Standard deviation	2.065

*Bombyx mori.*, L. The results of the present study also support the statement in which it is noted that silkworm is highly sensitive to fluoride perhaps the higher metabolic rate of silkworm could be one of the reason for their susceptibility to fluoride due to higher metabolic rate rapid incorporation of heavy metals into the tissues would occur [28]. LD<sub>50</sub> values are useful in determining the sub lethal and sub-sub lethal doses of fluoride.

Typical symptoms of exposure to fluoride intoxication involving the determination of LD<sub>50</sub> include were observed, the controlled silkworm exhibited in their usual manner i.e., the silkworm were very actively feeding and movements were well co-coordinated, silkworm were alert and at any slight disturbance, moved fast. The silkworm exposures to lethal doses of fluoride became irritable and hyper excited movements abnormal crawling movements were observed, other symptoms that have been observed are slowly becoming restlessness slowly becoming sluggish with sharp jerky movements on exposure to lethal dose. Finally the silkworm settles down at some place with the loss of equilibrium and caused to death. Following sub lethal dose exposure, symptoms of fluoride poisoning are not severe, include appearance of some hyper excitable movements and slight vomiting at the initial hours and decreases food consumption. Fluoride, as a complex mode of action and the knowledge of toxicity studies and behavioral observations are certainly useful to establish limits and levels of susceptibility of the silkworm *Bombyx mori.*, to fluoride.

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