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MULTIGENERATIONAL STUDY TO ACCESS THE MATERNOTOXICITY OF SLUDGE LEACHATE FROM TEXTILE INDUSTRIES AT PALI (INDIA) IN SWISS ALBINO MICE

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ABSTRACT

Water and soil pollution is inherited through the uncontrolled and indiscriminate discharge of pollutants from domestic, commercial and industrial sources into water bodies. This is visible everywhere and the water pollution problem is progressively mounting in our country. An attempt has been made to compare the maternotoxic effect of sludge leachate administered during two generations. Here the leachate obtained is from CETP, Pali which receives effluent exclusively from textile and dyeing industries located at Pali. It is diluted with water according to low level exposure dose concentration of 1/1000 and then administered to Swiss albino mice along with control group receiving simple tap water for consecutive three generations in order to access the long term effect of leachate. Research finding revealed that the treated sludge leachate from CETP, Pali at the tested dose level produced maternal toxicity to certain extent at both the generations. Maternal toxicity due to sludge leachate was evident by significant reduction in body weight gain during both prepartum and postpartum period, substantial loss of body fur and restlessness in F_1 generation. No significant adverse effect was seen in first generation while no second generation was observed indicating the potential toxicity of leachate and ability to accumulate and persist inside the body. The toxic symptoms in mothers were muscular tremors, ataxia, convulsions, hyper salivation, lacrimation and restlessness.

INTRODUCTION

Industrialization is believed to cause inevitable problems, such as pollution of air, water and soil. Water pollution due to industrial processes has attained serious dimensions in India [1]. Both, the quality and quantity of ground water is severely threatened by industrial sewage. Among the industries, textile industry plays a major role in modern civilization. It is an important industry in Rajasthan, accounting for nearly 20 percent of the investment made in the state, contributing over 7.5 percent of India's production of cotton and blended yarn (235,000 tonnes in 2002-03) and over5 percent of fabrics (60 million sq meters). Rajasthan has a leading position in spinning of polyester viscose yarn & synthetic suiting and processing. State is also famous for printing & dyeing of low cost, low weight fabric. Jodhpur, Pali, Balotra, Jasol and Bituja are the major clusters of small scale industries engaged in printing and dyeing of low cost fabric. Around 1640 industries are presently operating in these clusters [2].

Water pollution due to textile industry is the topic of major concern as they discharge large quantity of effluent into nearby water bodies. [3]. Central Pollution Control Board has listed the dye industry as one of the heavily polluted industries [4].

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Textile mill operations consist of weaving, dyeing, printing and finishing [5]. Many processes involve several steps, each contributing a particular type of waste, which may invite many diseases: both occupational and general [6] and consequently escalating the economic cost. Textile industry has long been known to pose health risks causing respiratory diseases like byssinosis and asthma from cotton dust exposure [7,8] and noise induced hearing loss [9]. The above situation can be well depicted in Western Rajasthan, in India, on both the sides of river Bandi (located in Pali) that is considered as the lifeline of people living there. It houses a number of industries (textile and dyeing) that have seen a phenomenal growth during the last two decades.

Studies conducted by Mohnot and Dugar [10] and Mohnot and Durve [11] have reported that various industrial units located in the three towns of Jodhpur, Pali and Balotra use about 77000 – 80000 tonnes of chemicals annually. However, potentially hazardous agents and situations are encountered in this industry, some of which might even influence the reproductive health [12]. These include solvents, dyes, noise, heat, vibrations, and prolonged standing, heavy metals etc. Humans may also be affected from exposure to agents that interfere with ovulation or spermatogenesis [13]. Exposure to such chemical compounds can produce a spectrum of adverse reproductive effects reproductive effects including chromosomal changes, mutations, sperm abnormalities, early or late foetal loss, still births, decreased birth weights, altered sex ratio, birth defects and childhood malignancies.

In the present study, an attempt has been made to study the undesired reproductive effects of chronic, low-level exposure to sludge obtained from CETP which receives effluent exclusively from textile and dyeing industries located at Pali, on reproductive parameters in Swiss Albino mice *as* some compounds may persistently accumulate in the body for longer period. The major part of the study involves finding out its long term teratogenic effect for continuous two generations.

MATERIALS AND METHODS

Study area

The industrial effluent affected area is located along the river Bandi. In Pali town there are about 1640 industries comprising of dyeing and printing units. Approximately 36 Million Litre Per Day (mlpd) industrial effluents containing high pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Dissolved Solid (TDS), Total Suspended Solid (TSS), sulphates and sodium are generated and discharged every day in the Bandi river [14,5,3,12,6,15,16].

Test substance

Test substance used is sludge, collected from drying beds of Combined Effluent Treatment Plant (CETP), Pali. The biologically and chemically treated sludge was collected, dried in oven, powdered and mixed in the ratio of 1:1. It was diluted 10 times with water and then homogenized and filtered. This filtrate served as 100% leachate, which was then diluted with water according to low-level exposure to sludge i.e. dose concentration of 1/1000.

Test animal

Five to six weeks old Swiss albino mice, weighing about 20 g, were paired in the ratio of 3 females: 1 male. The females were checked for the presence of vaginal plug every morning to determine the pregnancy in the females. The day a vaginal plug was seen was taken as day 0 of gestation and the female was presumed to be pregnant. Such females were caged singly and were given low-level exposure to sludge daily throughout in drinking water for three generations. The control animals received tap water *ad libitum* for the same period.

Experimental protocol

Weaning animals were randomly assigned to control or test groups. The males and females in each group (F_0 generation) were separately caged. The control animals received tap water and the test animals were given dose concentration of leachate 1/1000 in drinking water for 60 days. The animals in respective groups were then kept together for mating to obtain the F_1 pups. The F_1 pups underwent examination for any external malformations and their survival and weights were recorded till weaning. When the F_1 pups attained sexual maturity, they were caged to obtain the F_2 generation. The F_2 pups also underwent through similar observations as the F_1pups.The data was statistically evaluated using Student's t- test [12] and one tailed Mann - Whitney U - test [19]. Females showing the presence of the vaginal plug were separated and weighed on alternate days throughout the experimental period. Maternal weight gain during the gestation period i.e. prepartum weight of the pregnant females was noted along with the post partum weight till the pups weaned. Weights of neonatal male and female pups, their survival and sex ratios were recorded at birth, and then on days 4, 7, 14, & 21 after birth [10]. The food and water consumption by the test animals was also recorded on weekly basis.

RESULTS AND DISCUSSION

It was observed that for the first few weeks the amount of water consumed by the animals was more than their control counterparts during F_0 generation but after that it reduced significantly during F_1 generation. (Table 1). The experimental females of F_0 parental generations did not show any changes in maternal weight gain (Table 2). Similar relationship between maternal and fetal weight loss was observed by Hovland after treating mice with Cadmium [17]. The average prepartum weight gained by these dams during all the phases of gestation was less (12.05±2.32g) as compared to the weight gained by the control females (15.28±2.60) but no maternal mortality occurred.The postpartum weight gained by lactating females also seemed to be quite less (1.67±1.03) than that of the control females (3.43±1.34) but the difference was not found to be statistically significant (Table 3), when student t-test was applied to the available data. The probable cause for such an association, studied by Himani [15] may be that as a result of the administration of leachate containing various heavy metals, organochlorines, dyes, inorganic compounds, sulphates, acids and alkalies, the maternal body are put under stress and this in turn might affect the growing fetus leading to its growth retardation and hence reduced fetal weight.

The neonates of F_0 generation formed the F_1 generation. The litter size also didn't alter much (3.66±1.73) from the control (3.77±1.63)(Table 4). The surviving ability of the F_1 pups was assessed from their viability index and weaning index. Weight of F_1 pups was recorded on postnatal days 1, 4, 7,14and 21 to study their growth pattern. Neonates of dosed group revealed low viability index on postnatal days 4,7,14 and by the day 21 which was 79.62% as compared to 85.17% of the controls (Table 5). The observation of Growth of pups indicated that it was not affected much although reduced to 8.41±1.96 as compared to 10.93±2.05 of controls. (Table 6).The F_1 pups were observed for gross external malformations and their weights were taken. No stunted, immature or dead fetuses were born to any of the female of this group.

When the F_1 pups attained sexual maturity, they were housed in breeding cages in the ratio of three females to one male, in order to obtain the F_2 generation. Further when the F_1 pups reached sexual maturity after weaning, the treated animals showed significant increase in mortality rate. The control group produced F_2 generation successfully while no treated F_1 animals survived to produce F_2 generation. This could be due to the toxic effect of the leachate administered, on the reproductive performance of the mice. The observations of neonates revealed low viability index, reduced growth and weaning index. It may be attributed to the reason that harmful compounds present in the dose leachage were excreted in the milk without being detoxified and thus exerted their influence and proved lethal to the pups. Such toxic effect on suckling infants feeding only on mother's milk was studied by Kavlock et al, [18].

Large number of neonatal deaths may also be attributed to diminished secretory function of mammary gland [16]. Bagnell and Ellenberger [19] reported that tetrachloroethylene, a solvent present in dry-cleaning fluid, has been reported to cause cholestatic jaundice in a breast fed infant .The mother was exposed to vapours chronically, which accumulated in her breast milk. This chlorinated hydrocarbon is therefore fetotoxic. An extensive list of solvents shown to be fetotoxic in various animal systems was tabulated by Wilson [20].

Figure 1. F_0PARENTAL ANIMALS

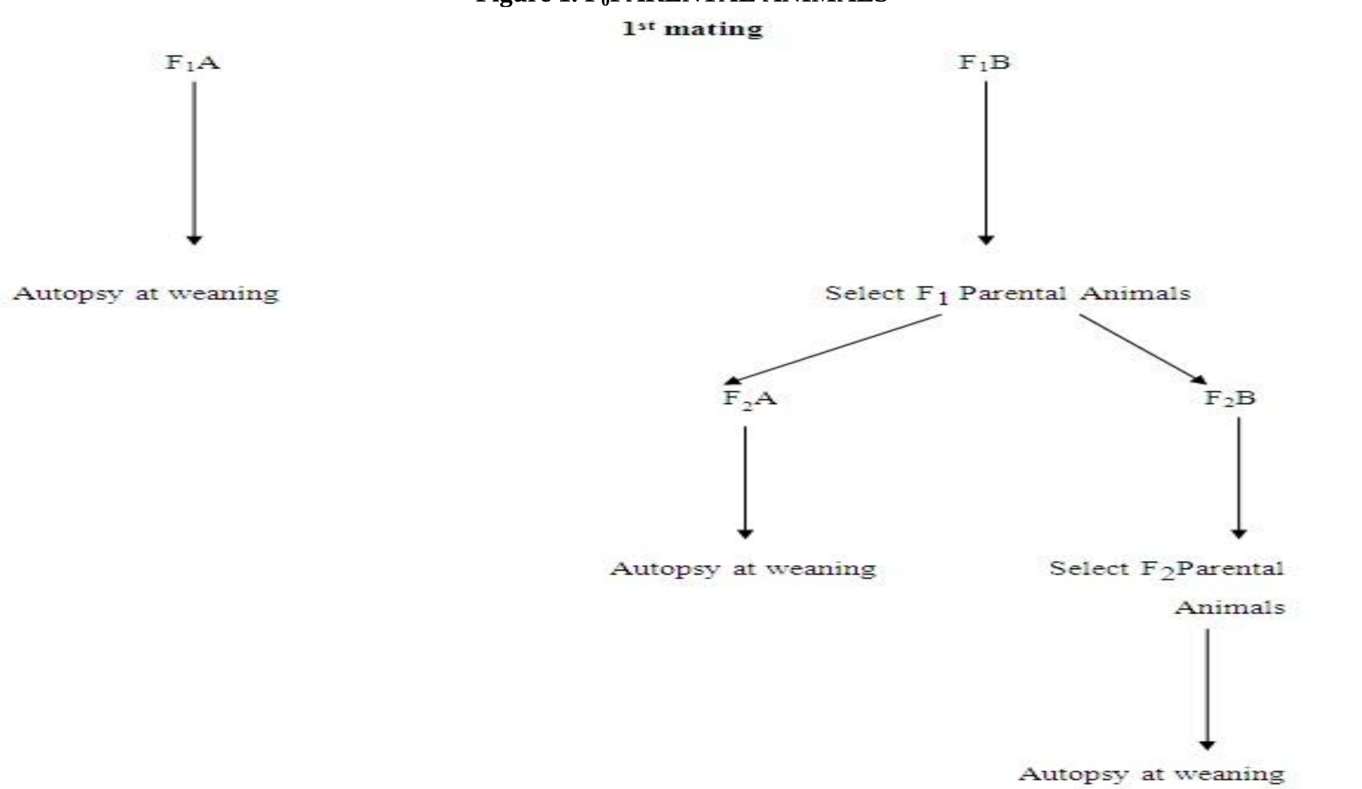


Table 1. Data on water consumption from F_0 to F_2 generations of mice

No. of weeks	Average water consumption for two months prior to mating F_0 parental generation		No. of weeks	Average water consumption by F_1 generation		No. of Weeks	Average water consumption by F_2 generation	
	Control	Experimental		Control	Experimental		Control	Experimental
1	4.03±0.27	10.34±0.26**	1	5.27±1.17	4.70±0.31	1	5.10±0.20	6.23±0.64
2	6.41±0.41	9.49± 0.78*	2	9.25±3.15	9.10±1.90	2	6.40±0.51	6.93±0.12
3	5.20±0.93	8.45±0.90	3	7.87±0.32	8.83±0.17	3	6.05±0.15	5.85±0.05
4	6.54±0.09	8.45±0.91	4	7.80±0.21	8.70±0.49	4	6.37±0.52	4.90±0.90*
5	6.33±0.26	7.79±0.50	5	7.75±0.15	9.40±0.40	5	4.53±0.17	2.60±0.30**
6	6.25±0.44	6.98±0.24	6	8.43±0.554	7.80±0.82	6	7.75±0.35	0
7	5.97±0.19	6.07±0.23	7	8.60±0.21	7.93±0.20	7	6.90±0.20	0
8	5.43±0.13	7.33±0.22**	8	8.50±0.25	7.50±0.27*	8	6.93±0.13	0
			9	7.67±0.81	7.23±0.83	9	4.53±0.37	0
			10	7.57±0.18	6.65±0.35	10	5.59±0.43	0
			11	8.33±0.22	7.10±0.10	11	6.78±0.21	0
			12	8.00±0.20	7.58±0.24	12	7.86±0.14	0
			13	7.57±0.18	6.65±0.35			
			14	7.87±0.67	6.60±0.15*			
			15	8.00±0.10	7.00±0.27			
			16	7.67±0.37	6.76±0.56			

1-8 Weeks Fo Parental gen. Given 2 months, prior mating treatment of 1/1000 leachate8-24 Weeks Fo Parental gen. Kept together to produce F1 pups (Treatment continued) 24-35 Weeks F1 Parental gen. kept together to produce F2 pups (treatment continued) Student's t test: *Significant difference (p<0.05) ** highly significant difference (p<0.01)

Table 2. Pre Partum weight of females

	Weight Gained in (g) byControl Females During					Weight Gained in (g) By Experimental females during				
	Pre-implantation period Day 1-6	Organogenetic period Day 6-14	Growth period Day 14-22	Complete gestation period	Maternal mortality	Pre Implantation Period Day 1-6	Oregano-genetic Period Day 6-14	Growth Period Day 14-22	Complete Gestation Period	Maternal Mortality
F0	2.72±1.08	6.40±1.60	6.16±1.98	15.28±2.60	0	1.8±1.20	5.40±1.12	4.84±1.88	12.05±2.32	0
F1	1.88±0.61	2.26±0.61	2.1±0.66	7.32±1.32	0	0.26±0.64	0.12±0.36	0.14±0.40	0.52±0.72	0

Student's t test: *Significant difference (p<0.05) ** highly significant difference (p<0.01)

Table 3.Post-partum weight of females

	Weight Gained in (g) byControl Females During				Weight Gained in (g) By Experimental females during			
	1st post-Partum Week	2nd post-Partum Week	3rd post-Partum Week	3 post-Partum Weeks	1st post-Partum Week	2nd post-Partum Week	3rd post-Partum Week	3 post-Partum Weeks
F0	1.05±0.99	1.73±1.03	0.85±0.86	3.43±1.34	0.72±0.67	0.73±0.68	0.6±0.73	1.67±1.03
F1	1.05±0.99	1.74±1.03	0.89±0.86	3.68±2.88	No female survived in F1 generation			

Student's t test: *Significant difference (p<0.05) ** highly significant difference (p<0.01)

Table 4. Effect of leachate on neonates of F_0 and F_1 generation

Control group					Experimental group			
Neonates of gen.	litter size	sex ratio M:F	average pup weight at birth (gm)	immature or stunted	litter size	sex ratio m: f	Average pup weight at birth(gm)	Immature or stunted
F_0	3.77±1.63	2:0	1.73±0.44	0	3.66±1.73	1:1	1.48±0.58	0
F_1	3.8 ±0.33	2:1	1.49±0.60	0	1 immature, 2 resorbed. All females died during gestation period			

Table 5.Effect of leachate on viability and weaning index of neonates

Viability Index (%) of control pups on postnatal days						Viability Index (%)of Experimental pups on postnatal days				
Gen.	**4th**	**7th**	**14th**	**21st**	**Weaning Index (%)**	**4th**	**7th**	**14th**	**21st**	**Weaning Index (%)**
F_1	96.9	95.04	85.17	85.17	85.17	93.33	72.95	72.95	72.95	79.62
F_2	94.42	91.08	73.32	73.32	73.32	No pups obtained in this gen.				

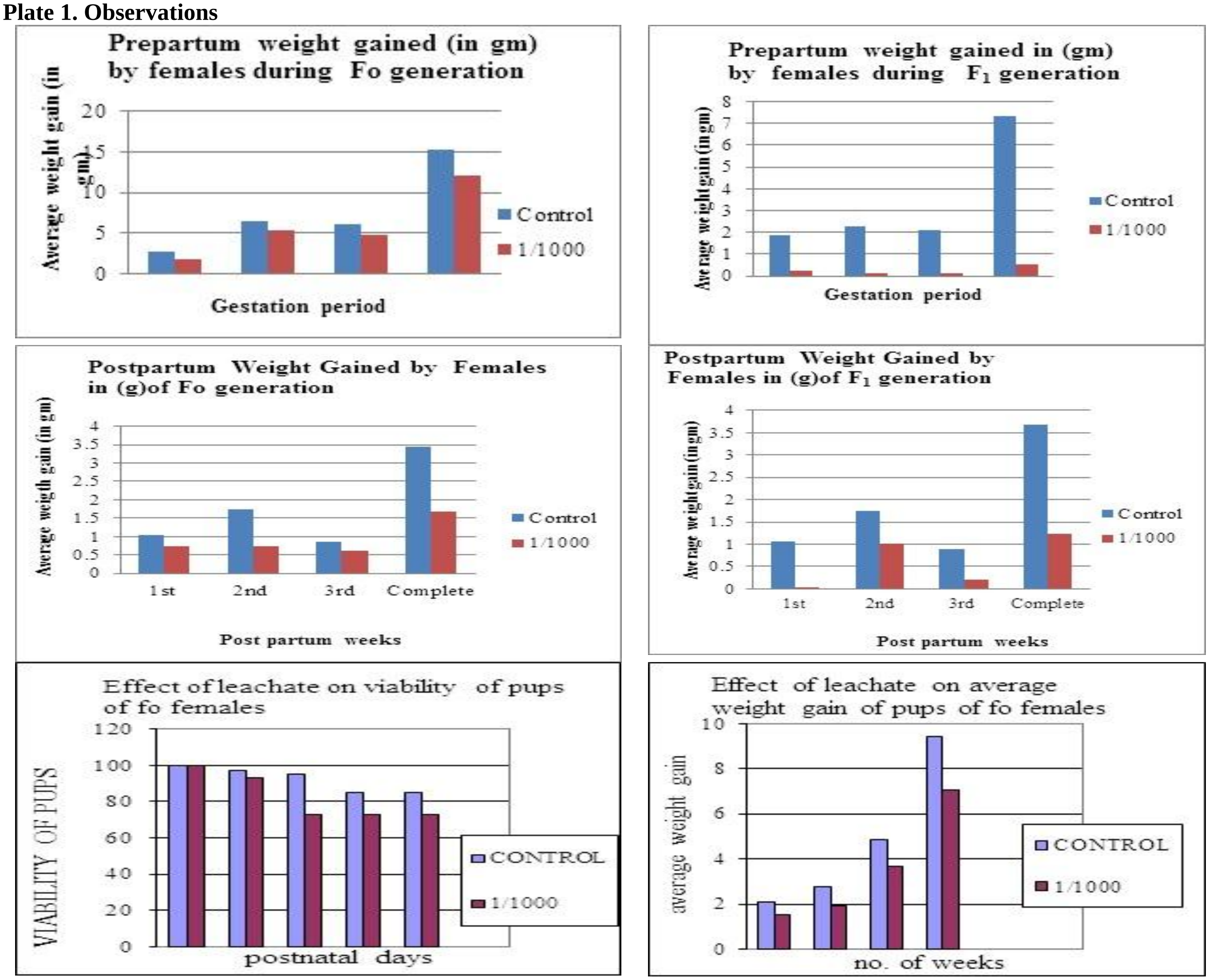
Student's t test: *Significant difference ($p<0.05$) ** highly significant difference ($p<0.01$)

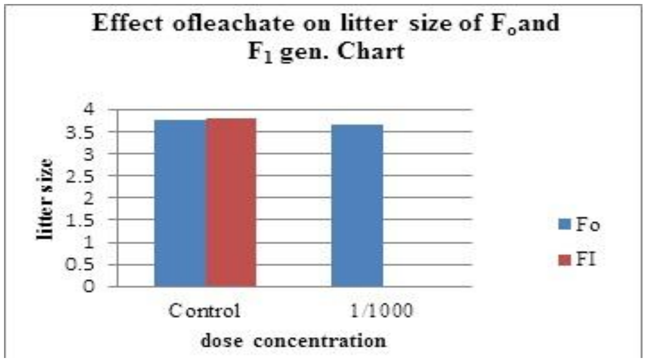
Table 6. Effect of leachate on growth index of pups

Effect of leachate on growth index of pups										
Growth Index (g) of Control pups On following postnatal days						Growth Index (g) of Experimental pups On following postnatal days				
Gen.	**1st**	**4th**	**7th**	**14th**	**21st**	**1st**	**4th**	**7th**	**14th**	**21st**
F_1	1.71±0.46	2.43±0.2	3.80±0.56	6.13±1.3	10.93±2.05	1.60±0.49	2.12±0.70	3.16±1.00	4.72±1.65	8.41±1.96
F_2	1.66±0.10	2.43±0.9	3.82±0.13	5.48±1.42	9.21±2.35	No pups obtained in this gen.				

Student's t test: *Significant difference ($p<0.05$) ** highly significant difference ($p<0.01$).

Plate 1. Observations





SUMMARY AND CONCLUSION

It can be concluded that no significant adverse effect was seen in first generation while no second generation was observed indicating the potential toxicity of leachate and ability to accumulate and persist inside the body. The present study has clearly brought out that the sludge from textile industry, which is dumped in open landfills, and with the potential of mixing with ground and surface water, may pose serious threat to human babies and fetuses and other mammalian fauna of the area. Thus our investigation is a step towards evaluating such adverse effects on the reproductive teratological and embryological aspects.

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