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FETOTOXICITY OF TREATED SLUDGE FROM CETP, PALI (INDIA) IN SWISS ALBINO MICE: A MULTIGENERATIONAL STUDY

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ABSTRACT

Water is a precious commodity and very essential part of all lives on earth. Unfortunately, it is polluted through the uncontrolled and indiscriminate discharge of pollutants from domestic, commercial and industrial sources. This is visible everywhere and the water pollution problem is progressively mounting in our country. An attempt has been made to compare the fetotoxic 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, 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 fetotoxicity to certain extent at both the generations. It was evident by significant reduction in body weight gain, substantial loss of body fur and restlessness. Visceral malformation included abnormalities in eye (like anophthalmia, microphthalmia), pulmonary edema, reduced gonadal size in dose related manner. Skeletal malformation included poor ossification of skull bones, Sternal defects, rib anomalies in all the fetuses of dose group followed by reduction in body weight gain during both prepartum and postpartum period, substantial loss of body fur and restlessness in F_1 generation. No second generation was observed indicating the potential toxicity of leachate and ability to accumulate and persist inside the body.

INTRODUCTION

Water is not only essential for well-being of human, animals and plants but is also important for industrial development. Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water. The quality of water is of vital concern for mankind since it is directly linked with human welfare.

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Water plays an important role in the economy, as it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation. The major sources of water pollution are domestic waste from urban and rural areas, and industrial wastes which are discharged in to natural water bodies. Both, the quality and quantity of ground water is severely threatened by industrial sewage. More than 80% of sewage in developing countries is discharged untreated, polluting rivers, lakes and coastal areas. Water pollution due to industrial processes has attained serious dimensions in India [1]. During the last decades, environmental issues associated with dyestuff production and application have grown significantly and are indisputably among the major driving forces affecting the textile dye industry today. Considerable amounts of dyes have been noticed in these textile wastewaters, due to their incomplete use and washing operations. The dyes disposed off, can be found in dissolved state or in suspension in the wastewater. These dyestuffs are highly structured polymers and are very difficult to decompose biologically [2]. The most obvious impact of the discharge of dye colored effluent is the persisting nature of the color. It is stable and fast, difficult to degrade, toxic, rendering the water unfit for its intended use [3]. Such dyestuffs can reach the aquatic environment, primarily dissolved or suspended in water, since the conventional treatment of wastewaters from textile mills and dyestuff factories are unable to remove most of the azo and other dyes effectively.

Textile industry 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 over 5 percent of fabrics (60 million sq meters). 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 [4]. Water pollution due to textile industry is the topic of major concern as they discharge large quantity of effluent into nearby water bodies [5].Central Pollution Control Board has listed the dye industry as one of the heavily polluted industries [6].Textile mill operations consist of weaving, dyeing, printing and finishing [7,8]. Many processes involve several steps, each contributing a particular type of waste, which may invite many diseases: both occupational and general [9] 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 [10,9] and noise induced hearing loss [11]. 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 (1987) [12] and Mohnot and Durve (1989)[13] 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 [14]. 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 [15]. 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 [16].

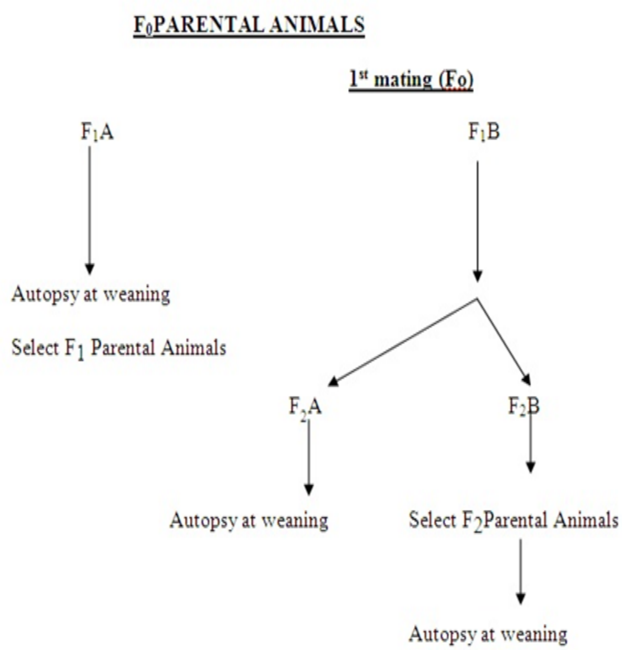
In this paper, multigenerational study was conducted to evaluate the toxicity of compound or agent, which is persistent and tends to accumulate in the body. Here, the animals were continuously exposed to the test agent in drinking water for three generations. The control animals received tap water *ad libitum* for the same period. 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 .

MATERIALS AND METHODS

Study area: The industrial effluent affected area is located along the river Bandi, a tributary of the Luni River in Pali district. 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 [,5,7,8,15,18,26]. **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_1 pups. The data was statistically evaluated using Student's t- test [19] and one tailed Mann - Whitney U - test [20]. 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 [17]. 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 average prepartum weight gained by these dams during all the phases of gestation was less (12.05$\pm$2.32g) as compared to the weight gained by the control females (15.28$\pm$2.60) (table 2) which may be attributed to the effect of TCA *(trichloroacetic acid*) used as an auxiliary in textile dying processes [9] but no maternal mortality occurred. The pregnant animals showed some signs indicative of leachate poisoning i.e. pus formation in eyes followed by lacrimation, deformities in limbs and facial tumors (Plate 1) which may be due to presence of 1-(4-Chlorophenyl)-ethanone, also known as *p*-chloroacetophenone, used as an intermediate for manufacture of heteroaryl thiazole dyes [21,22]. The ethylene glycol ethers widely as solvents in textile industry to prevent spotting in printing or dyeing is moderately irritating to the eye, producing acute pain, inflammation of the membranes, and corneal clouding which persists for several hours [23]. NP Nonylphenol cause severe irritation of the eye of rabbits. Studies have reported conjunctive redness and lesion of the iris [24].

The postpartum weight gained by lactating females seemed to be quite less than that of the control females but the difference was not found to be statistically significant (Table 3), when student t-test was applied to the available data [25]. One fourth of the females of F_0 were sacrificed on day 18^{th} of gestation their uterine contents were examined for teratological observations. External examination of the fetuses did not reveal any other gross anomaly except for open eyelid and deformed limbs in some along with megacephaly of head (Plate 2). However, two fetuses exhibited enlarged cerebral ventricles, micropthalmia (reduced eye lens) when razor sections through the head were observed. (Plate3). Pulmonary edema was seen as spaces or gaps in lungs, and enlarged renal pelvis was observed in few fetuses. The alizarin preparations of the specimen revealed poor ossification of skull bones specially the frontal. Sternal defects (dumbbell shaped sternebrae, Plate 4) and rib anomalies (wavy ribs, gap between the ribs) (Plate 5) were also seen but not significant. The remaining F_0 females of both the groups were allowed to deliver the pups to get F_1 generation .The reproductive performance of F_1 The litter size of F_1 generation was comparable with that of control group i.e. 3.66$\pm$1.73(control=3.77$\pm$1.63).The average fetal body weight was slightly lower i.e. 1.48$\pm$0.58g (control=1.73$\pm$0.44g) (Table 4). No immature or stunted fetuses were seen in this generation. Few fetuses showed external abnormalities like open eyes and enlarged head. 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. It was found that the male and female mice gradually started dying and there was no vaginal plug seen in the rest of the females. The control group generated F_2 generation while the treated group didn't produce even a single fetus rather 1 immature and 2 resorbed were found (Plate 6). All females died during gestation period. This could be due to the toxic effect of the leachate administered, on the reproductive performance of the mice. It may be attributed to the reason that harmful compounds present in the dose leachate 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, 1987[26]. Large number of neonatal deaths may also be attributed to diminished secretary function of mammary gland [26]. Bagnell and Ellenberger [27] 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 (1977) [28].

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

	Average water consumption for two months prior to mating F_0 parental generation			Average water consumption by F_1 generation			Average water consumption by F_2 generation	
No of weeks	**Control**	**Experimental**	**No of weeks**	**Control**	**Experimental**	**No of Weeks**	**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 leachate
8-24 Weeks Fo Parental gen. Kept together to produce F1 pups (Treatment continued)
24-35 Weeks F_1 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) by Control 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) by Control Females during F0 and F1generation					Weight Gained in (g) By Experimental females during F0 and F1 generation			
	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 to get F2 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; no F_2 generation			

Figure 1. Observations

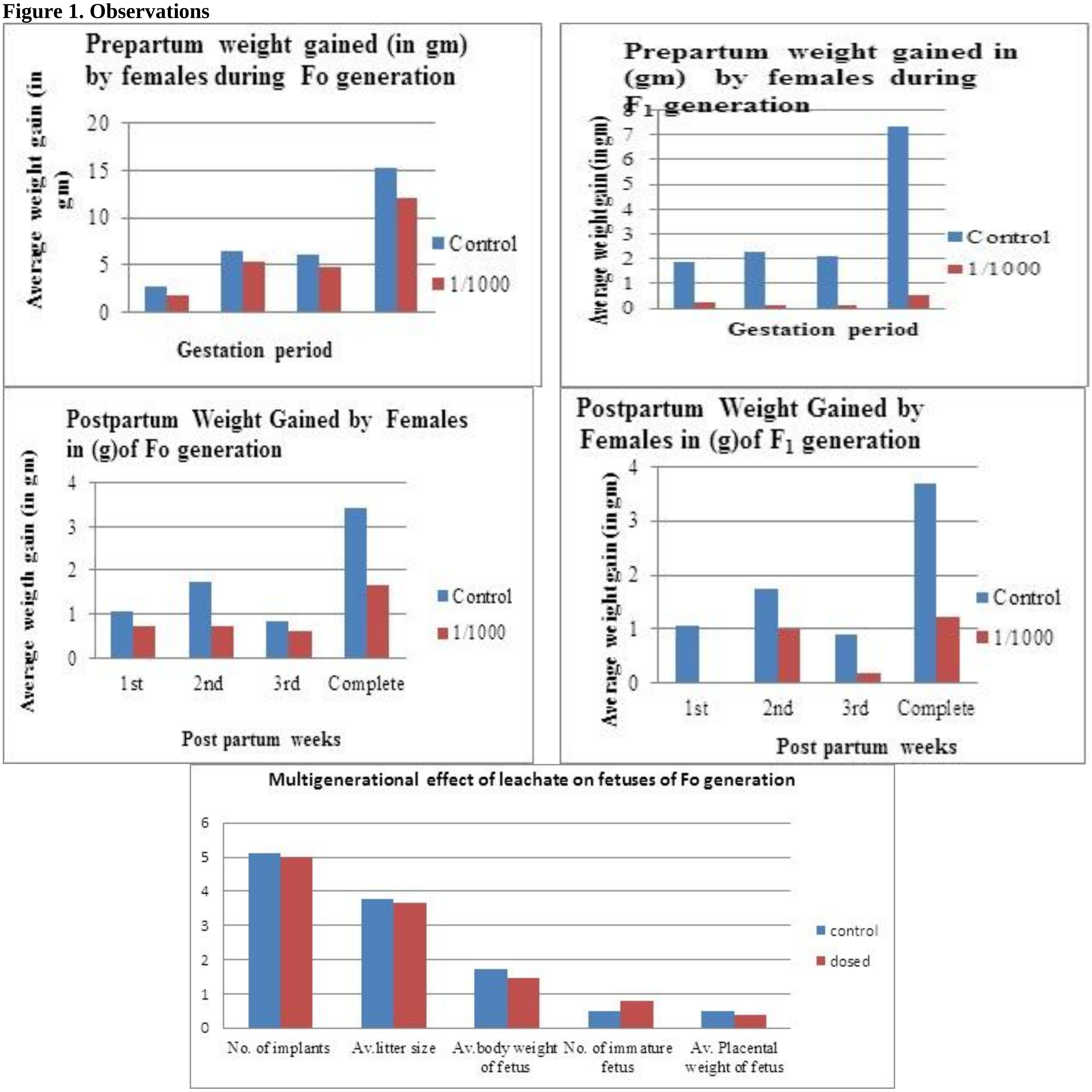


Plate 1. Facial Tumor

Plate 2. Megacephaly

Plate 3. Micropthalmia

Plate 4. Dumbell shaped ribs

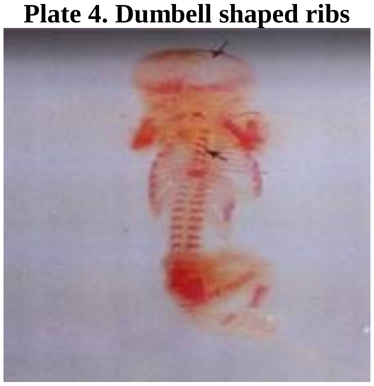


Plate 5. Wavy ribs

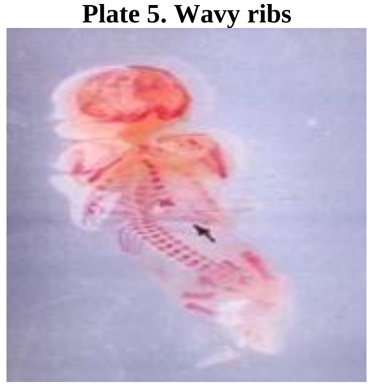
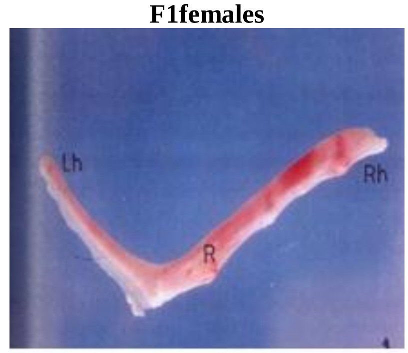


Plate 6. Resorbed fetuses of F1females



SUMMARY AND CONCLUSIONS

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. In the multigenerational experiment with leachate, 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 occurrence of both visceral and skeletal variations indicates towards the potential of leachate to cause dysmorphogenic effects.

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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