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Age and Sex Related Behavioral Changes Induced by Dibutyltin-Dilaurate in Rats

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Dibutyltin-dilaurate (DBTL) is extensively used as a stabilizer in polyvinyl chloride (PVC) formulations, as a catalyst in industry and as a biocide in agriculture (Piver, 1983 and Shoeij et al., 1987). Industrial workers are exposed to varying amounts of organotin compounds during processing, and the general population including children and pregnant women are also exposed through the food chain, environmental contamination and due to migration from finished plastics to food stuffs and biological fluids (Woggen and Uhde, 1967 and Koch and Figge, 1971). Lower homologues such as triethyltin,trimethyltin and tributyltin oxide are known to produce convulsions, behavioral abnormalities, decrease in the level of brain gamma aminobutyric acid (GABA), biogenic amines and cause cerebral oedema and diarhoea in experimental animals (Kimbrough, 1976, 1982; Mailman et al., 1983; Dwivedi et al., 1985 and Stanton, 1991). Previous observations from our laboratory have shown a decrease in the level of brain biogenic amines, impairment in motor activity and learning ability in DBTL exposed rats (Alam et al., 1988).

In view of the possibility of exposure of growing children, pregnant women and industrial workers to varying amounts of organotin compounds, information on its age and sex related effects on neurobehavioral function is needed. Therefore, in the present investigation the implications of DBTL on behavioral indices (locomotor activity and learning ability), using albino rats of different ages (weanling, juvenile and adults) and sexes (male and female) as experimental model were studied.

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MATERIALS AND METHODS

Dibutyltin dilaurate $(C_4 H_9)_2 Sn (OO C_{11} H_{23})_2$ of the highest purity was procured from Fluka A.G., Switzerland. The amphetamine was obtained from Sigma Chemical Company, USA.

Weanling, juvenile and adult rats of both sexes were obtained from ITRC animal breeding colony and maintained on standard pellet diet (Hindustan Lever Laboratory Animal Feed, India) and water ad libitum. The age and body weight ranges of the rats were: weanling (4 weeks old,35-40 gm), (8 weeks old, 115-125 gm) and adults (24 weeks old, 340-355 gm). The rats of either sex were randomly assigned into three groups of 20 animals each. The animals of group I and II of each sex were given DBTL in ground nut oil by oral gavage at dose levels of 20 and 40 mg/kg body weight/day respectively for three consecutive days. The animals of group III of both sexes, were given an equivalent amount of ground nut oil to serve as control. Seven animals from each group were used for the locomotor activity and another set of seven animals for the learning ability. In view of the possibility of mortality, each group had some extra rats.

All behavioral assessment were performed between 10.00 and 18.00 hours under standard laboratory conditions. Twenty four hours after the last exposure to DBTL, spontaneous and drug induced motor activity was monitored using a digital photoactometer (Techno, India) by the method of Kuhn and Van Maaner (1961).

Another set of 7 rats from each group were removed from the home cage and placed in the chamber of the Cook's Pole Climbing Apparatus (Techno, India). Conditioned avoidance response (CAR) was measured according to the procedure of Cook and Weidley (1956). Data were evaluated by the Student's 't' test for a comparison of means (Fisher, 1960). A value of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The animals exposed to DBTL (20 and 40 mg/kg), were found to be lethargic, dull and weak throughout the experimental period as compared to the controls. Swelling around the mouth area was accompanied with brown pigmentation on the central body surface and the animals exposed to 40 mg/kg body weight also showed hind limb weakness. A gradual loss in the body weight gain of DBTL treated rats was observed in weanling, juvenile and adult rts in comparison to the age matched controls in a dose dependent manner, throughout the

experimental period. The decrease in body weight was significant in the animals receiving the higher dose of DBTL, more prominent in juvenile rats of either sex (data not shown).

The percent mortality in animals of different age and sex, 24 hours after the last exposure to DBTL is shown in Table 1. No mortality was recorded in the controls

Table 1. Effect of various concentrations of DBTL* on the mortality** in rats of different age and sex

Kind of animals	CONTROL		DBTL EXPOSED RATS 20 mg/kg		40 mg/kg	
	Male	Female	Male	Female	Male	Female
Weanling	0%	0%	0%	5%	10%	20%
Juvenile	0.1%	0%	10%	15%	20%	25%
Adult	0%	0%	10%	20%	25%	30%

*DBTL was administered orally for 3 consecutive days.
**Each group was having 20 animals.

except in the juvenile males. Exposure of 20 mg/kg DBTL to weanling female rats showed 5% mortality, while weanling males exhibited no mortality. The juvenile male and female rats showed 10% and 15% mortality respectively, while adult male and female showed 10% and 20% mortality respectively. When the exposure of DBTL was increased to 40 mg/kg, the mortality rate was 10% and 20% in weanling males and females respectively, and 20% and 25% in juvenile males and females respectively. The mortality rate in adult males and females of this group was 25% and 30% respectively.

Effect of various doses of DBTL on the spontaneous and drug induced motor activity in different groups of rats is shown in Figure 1. The weanling male rats showed 54% and 65% decrease in the spontaneous motor activity (SMA) while in females it was 72% and 80% at 20 and 40 mg DBTL/kg body weight respectively. Amphetamine challanege resulted in an increase in SMA in both sexes. The percent increase was 66 and 67 in males and 65 and 67 in females respectively at the two doses. In Juvenile group the percent decrease in SMA was 70 and 77 in males and 82 and 86 in females at lower and higher dose respectively. Percent increase in the drug induced motor activity was 72 and 74 in males and 80 and 82 in females while in adult rats the percent decrease in the SMA was 60 and 65 in males and 59 and

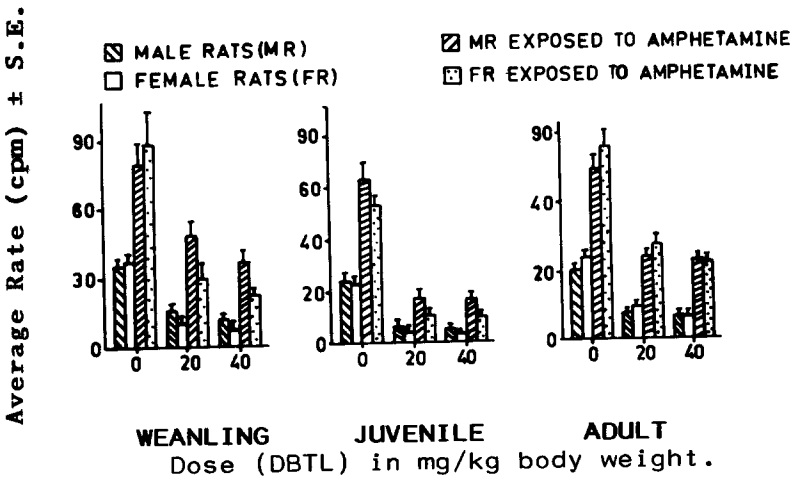

Figure 1. Effect of various concentrations of DBTL on spontaneous and drug induced motor activity in rats of different age and sex.

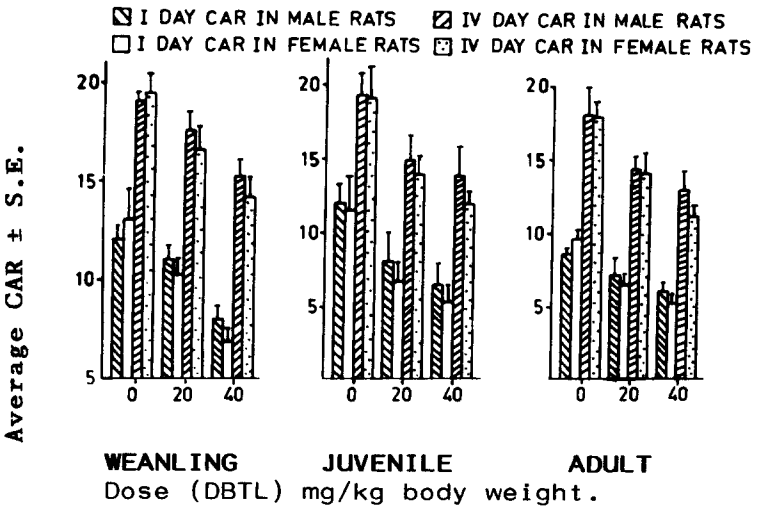

Figure 2. Effect of various concentrations of DBTL on learning ability in rats of different age and sex.

71 in females. The drug induced motor activity showed percent increase to 67 and 70 in males and 65 and 67 in females in the two treated groups.

Effect of variou doses of DBTL on CAR in different groups of rats is shown in Figure 2. In weanling rats the decrease in the 1st day CAR was 9% and 33% in males and 21% and 45% in females, whereas on the 4th day it was 7% and 19% in males and 15% and 28% in female at 20 and 40 mg doses of DBTL respectively. In juvenile rats

the decrease in the 1st day CAR was 33% and 45% in males and 42% and 53% in females whereas on 4th day it was 22% and 28% in males and 26% and 37% in females at the two doses respectively. In adults the decrease in the 1st day CAR was 16% and 28% in males and 32% and 45% in females and on 4th day it was 20% and 27% in males and 23% and 39% in females in the two treated groups respectively.

Certain organotin compounds have been reported to effect the growth, food intake and cause anemia in experimental animals (Kimbrough, 1976). Unpalatability of diet, due to mixing of organotin compounds in food grain has been suggested to be one of the factors for such effects. A reduced body weight gain, lethargic conditions, hind limb weakness and swelling around the mouth area in the DBTL treated rats were observed in the present study. However, these changes can not be related to unpalatability since DBTL was given by gavage. The reduced food intake may be due to hyperactivity of the rats or slow absorption of nutrients from gastrointestinal tract. Generalized illness, muscular weakness and paralysis has been reported both in animals and humans exposed to organotin compounds (Barnes and Stonner,1959; Bierkamper and Basset, 1984).

Maximum decreases in the CAR and SMA were noted in the juvenile rats and minimum in the weanlings. Rapid metabolism of DBTL and formation of toxic metabolites in the liver of juvenile rats may partly account for these effects. On the contrary the immature brain of weanling rats may exhibit slow turnover of catecholamines and serotonin, which may probably explain the small decrease in % CAR and SMA. Such a diversity in the rates of metabolism of organotins has already been reported in the literature which further supports our findings (Atchison et al., 1982). Sex related differences in the metabolism of drugs and toxicants are also documented in the literature (Hurst, E.W., 1985; Rumke, 1965). The rapid metabolism of amphetamine by male rats is also well established (Groppetti and Costa, 1969). Besides, considerable variation in male and female CNS (structurally and functionally) may further contribute to the greater behavioral responses of females to amphetamine. In the present study the incidence of behavioral alterations were more pronounced in females at all ages in comparison with males of the same age. Taken together these observation indicate a sex related effect of DBTL, which could be attributed to hormonal influences. Variations in hormone levels may also effect the metabolism of DBTL, leading to higher concentration of toxic metabolites in females. In our earlier studies we have observed an impairment in the levels of biogenic amines in rats

following exposure to DBTL (Alam et al., 1988), since catecholamines and serotonin are involved in regulating a variety of behavioral functions chiefly, motor coordination,learning and memory. The significant reduction in CAR and SMA may presumably be due to alterations in the levels of these amines. Our data indicate that DBTL exposure in rats could lead to neurobehavioral dysfunctions, which may be more pronounced in juvenile rats specially in the females.

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