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Developmental and Neurobehavioral Effects of Mycotoxin Fumonisin B1 in Rats

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ABSTRACT *: The objective of this experiment is to investigate neurobehavioral and developmental effects of fumonisin B1 (FB1) after prenatal FB1 administration in rats. FB1 (0.8 or 1.6 mg/kg) was orally exposed to pregnant rats during gestational days 13 to 20, whereas the vehicle alone was administered to control group. Maternal and offspring body weights, physical landmarks of incisor eruption, eye opening, testes descending and vaginal opening, open field activity, running wheel activity, and complex maze performance were included as endpoints for developmental and neurobehavioral measurement. Maternal body weights were not significantly altered after FB1 exposure. Percentage of maternal weight gain difference between control and 1.6 mg/kg FB1 groups was about 4%. Pre- and post-weanling weight of offsprings after prenatal exposure to FB1 was not significantly changed, suggesting that FB1 at 0.8 or 1.6 mg/kg doses may not cross the placenta. Significant gender difference in running wheel activity on postnatal days 57 to 63 and complex maze performance on postnatal days 75 to 78 was observed.*

Key Words *: Fumonisin B1, Mycotoxin, Developmental toxicity, Neurobehavioral toxicity, Pregnant rats*

I. INTRODUCTION

Fumonisins are produced predominantly by the fungus *Fusarium moniliforme* throughout the world and contaminate dietary staples such as corn (Sydenham *et al.*, 1990; Nelson *et al.*, 1991; Visconti and Doko, 1994). Fumonisin B1 (FB1), the major toxic metabolite, has been shown to have cancer-promoting effects in rats (Gelderblom *et al.*, 1988; Marasas *et al.*, 1984), to cause swine pulmonary edema (Harrison *et al.*, 1990), and to be associated with human esophageal cancer (Marasas *et al.*, 1988a).

Equine leukoencephalomalacia, the most common toxicity caused by *Fusarium moniliforme* in horses and donkeys, is a neurotoxic disease that is characterized by multifocal liquefactive necrosis of predominantly the white matter of the brain (Wilson *et al.*, 1973; Wilson and Maronport, 1971; Marasas *et al.*, 1988b). Horses intravenously administered FB1 showed clinical signs of neurotoxicity including severe edema and focal necrosis in the brain, providing evidence that FB1 causes equine leukoencephalomalacia (Marasas *et al.*, 1988b).

Pregnant rats were dosed from days 8 to 12 of gestation with 30 or 60 mg FB1/kg body weight (Lebepe-Mazur *et al.*, 1995). At the 60 mg/kg dose, FB1 decreased the relative litter weight. In addition, fetal bone development was significantly impaired by FB1 treatment. In another study, pregnant CD1 mice exposed to *Fusarium moniliforme* culture material containing 0, 12.5, 50 or 100 mg FB1/kg (po, day 7-day 15 of gestation) were sacrificed on day 18 of gestation and the litters were examined (Gross *et al.*, 1994). Dose-dependent decreases in both the number of live offsprings per litter and the body weights of the offspring occurred at 25 mg FB1/kg or higher doses. The percentage of embryonic implants resorbed was dose-dependently increased. In hammsters, a significant increase in the number of litters with fetal deaths, was observed at 18 mg FB1/kg or culture-extracted fumonisins (18 mg FB1 and 4.5 mg FB2) after gavage on days 8 and 9 of gestation. Frequency of dead fetuses after administration of FB1 culture material by gavage was higher than that after treat-

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ment with pure FB1 at 12 or 18 mg/kg doses (Floss *et al.*, 1994). However, the mean fetal weights and crown-rump lengths of living term fetuses per litter were not affected in hamsters given 6 mg FB1/kg from day 8 to 10 or 12 of gestation. At the 12 mg FB1/kg dose, placentas had extensive necrosis and 100% of the fetuses were dead or resorbed (Casteel *et al.*, 1994). In New Zealand white rabbits gavaged daily on gestation day 3~19 with FB1 at 0.1, 0.5 or 1 mg/kg, fetal weight was decreased at 0.5 and 1 mg/kg doses where maternal toxicity was also occurred (LaBorde *et al.*, 1997).

In low dosage of FB1 that maternal toxicity was not observed, the developmental toxicity and fetotoxicity of FB1 in rats remain unclear and whether FB1 might result in neurotoxicity and/or functional effects in the offspring needs to be investigated. This experiment was conducted to investigate some of the neurobehavioral and developmental effects after relatively low concentration ranges of FB1 administration in prenatal rats.

II. MATERIALS AND METHODS

1. Fumonisin compounds

Fusarium moniliforme M-1325 culture material containing known concentrations of FB1 and FB2 was provided by Rottinghaus Laboratory of University of Missouri (Columbia, MI, USA). The method of preparation was described in the literature (Weibking *et al.*, 1993). Water soluble FB1 was isolated from the cultured powder as an aqueous extract, and water soluble material was analyzed for FB1 and FB2 by high performance liquid chromatography. It was free of aflatoxin, ochratoxin, T-2 toxin, vomitoxin, and fusarin C. The filtrate contained FB1 and FB2 in a ratio of 19 : 1.

2. Animals and Treatment

Plug-positive Sprague-Dawley rats (NCTR breeding colony, Jefferson, Arkansas, USA) were used and housed individually in polycarbonate cages with woodchip bedding (plug date=gestational day 0). Food (NIH-31) and water were provided *ad lib*. The housing room was maintained on a 12 : 12 hour light-dark cycle, and temperature and humidity were maintained at 23±3°C and 50±20%, respectively.

Each rat was gavaged on gestational days 13~20 with an aqueous solution of control (n=7), 0.8 mg FB1/kg (n=7), or 1.6 mg FB1/kg (n=7). The day of birth was designated postnatal day (PND) 1. Litters were weighed on PND 1 and culled to 10 pups, maintaining equal numbers of each sex where possible. All litters remained with their biological dam; no cross-fostering was done. Each pup was tattooed on the dorsal surface of the paw for identification purposes. Weanling was made on PND 22.

3. Maternal measurement

Body weight was measured for each dam on gestation days 13 to 21 and approximately weekly through post-parturition day 40. Gestation length, number of dams non-pregnant, number of dams giving birth, and number of dams dead during treatment were measured.

4. Offspring measurement

1) Body weight

Pre- and post-weaning body weight of offspring were measured on PND 1, 7, 14, 21, 30 and 60 in designated male and female of litters.

2) Physical land mark

Endpoints used for physical development of offspring were included measurement of the day of incisor eruption, eye opening, testes descending and vaginal opening. The criteria of recording these endpoints were clear appearance of bilateral incisor, full opening of bilateral eyes, full testes descent and full vaginal patency using methods previously described (Mohammad and St. Omer, 1986).

3) Open field activity

Open field activity was assessed in pup #3 of each sex on PND 19~22 and 33~36 as previously described (Ferguson *et al.*, 1993). The apparatus was a Plexiglas cube (45×45×45 cm) bisected by photobeams. Duration of session was 9 min, and each subject was tested for 4 consecutive daily sessions in lighted conditions.

4) Running wheel activity

Residental running wheel activity was observed in

pup #3 of each sex on PND 57~64 as previously described (Ferguson *et al.*, 1993). Each apparatus was a standard Plexiglas housing cage equipped with a running wheel (34 cm diameter). Number of wheel revolutions was recorded.

5) Complex maze performance

Complex maze test was assessed on PND 75~78 in pup #2 of each sex in lighted conditions. The apparatus was an array of 24 acrylic arms, two of which were consistently designated as goal arms (1~2 drops tap water/reinforcer). The rats had been water-deprived for 24 hours, to locomote between the two goal arms to obtain reinforcers. There was no limit on the number of reinforcers a subject could obtain in any given session which lasted 15 min on each of 4 consecutive days.

6) Statistical treatment

Data are presented as mean±SE. The general linear model procedure of SAS (SAS Institute Inc., Cary, NC) was used. Repeated measures analyses over day or session were evaluated with multivariate techniques. Duncan's multiple range test and probability difference were used for one- and two-way analysis when F-values showed significance. Values were considered statistically significant when $p<0.05$.

III. RESULTS

1. Development of physical landmarks in offspring

Results of physical land marks are shown in Table 1. No statistical difference was observed between FB1-treated and vehicle-treated control groups.

2. Developmental endpoints in offspring

As shown in Table 2, the offspring weight at PND 1 did not show dose-dependency and statistical significance. There was no significant difference in numbers of male or female offsprings, and gender ratios, although the number of total pups shows a trend of decreases. Number of total dead pups are higher in the FB1 treated group, and the number of dams related to dead pups is 1 dam in control and 2 dams in 0.8 and 1.6 mg/kg groups.

Table 1. Development of physical landmarks in offspring

Endpoints	Dose of fumonisin B1		
	Control	0.8 mg/kg	1.6 mg/kg
Incisor eruption			
Male (days)	13.1±0.5	12.6±0.2	12.9±0.4
Female (days)	13.0±0.4	12.3±0.2	12.6±0.2
Eye opening			
Male (days)	16.0±0.2	15.3±0.3	15.6±0.2
Female (days)	15.9±0.3	15.0±0.3	15.9±0.3
Reproductive organs			
Testes descent (days)	20.7±0.2	21.3±0.3	21.7±0.4
Vaginal opening (days)	41.3±1.3	43.0±1.0	42.1±1.9

Each value presents mean±SE of n=7 per group.

Table 2. Summary of developmental endpoints in offspring

Endpoints	Dose of fumonisin B1		
	Control	0.8 mg/kg	1.6 mg/kg
Offspring weight/litter (g) (PND 1)	6.17±0.16	6.45±0.21	5.88±0.25
No. of sex and ratios			
No. total	14.6±0.7	13.6±0.7	13.0±1.3
No. male (M)	7.4±0.6	5.6±1.3	5.6±1.0
No. female (F)	7.0±0.4	7.0±0.5	7.0±1.4
Ratio (M/F) total	1.1±0.1	0.9±0.3	1.1±0.3
No. total dead pups	1	7	3
Male	0	3	2
Female	1	4	1
No. dams related to dead pups	1	2	2

Each value presents mean±SE (n=7).

3. Maternal weight gain during gestation

Weight gain of dam during about the third term of gestation showed insignificant but dose-dependent tendency from gestational days 13. Direct exposure of fumonisin B1 to pregnant dams shows dose-dependent tendency in change of body weight gain although not statistically significant (Fig. 1).

4. Offspring weights

Body weight of pre- and post-weaning offspring in both sex are not significantly different as shown in Fig. 2, suggesting that prenatal exposure of these doses on gestational days 13~20 may not be potentially toxic. Significant gender difference in body weight was observed in postnatal days 1, 7, 14, and 60 (PND 1, F(1,

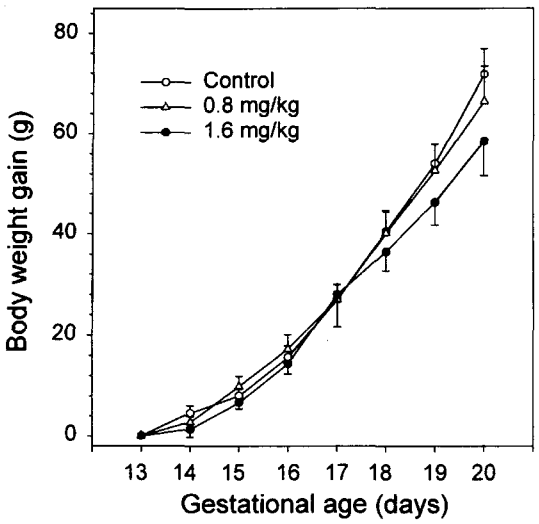

Fig. 1. Maternal body weight gains during gestation. Fumonisin B1 was administered to rats by gavage on gestational days 13 to 20. Body weight gains were calculated based on the weight of gestational days 13 of each group.

16)=19.48, Pr=0.0004; PND 7, (1, 15)=8.02, Pr=0.0126; PND 14, F(1, 16)=8.58, Pr=0.0098; PND 60, F(1, 16)=18.25, Pr=0.0006).

5. Open field activities for pre- and post-weaning periods

1) Open field activities on days 19-22

Open field activity observed during preweaning periods is presented by numbers of photobeam break for

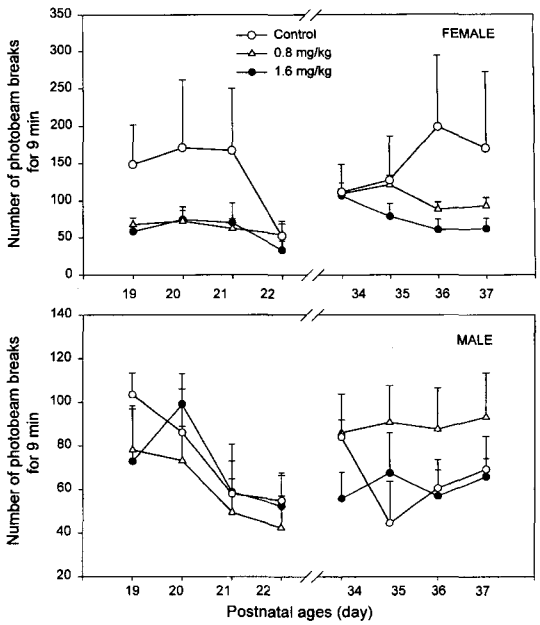

Fig. 3. Open field activity observed by number of photobeam breaks for 9 min period. The photobeam breaks were measured on postnatal days on 19 to 22, and 34 to 37 in female and male.

9 min period and resulted in no significant treatment effects in female and male offspring. In female, FB1 treatment showed a tendency of insignificant decreases of the open field activity (Fig. 3).

2) Open field activities on days 34-37

Postweaning open field activity showed a similar

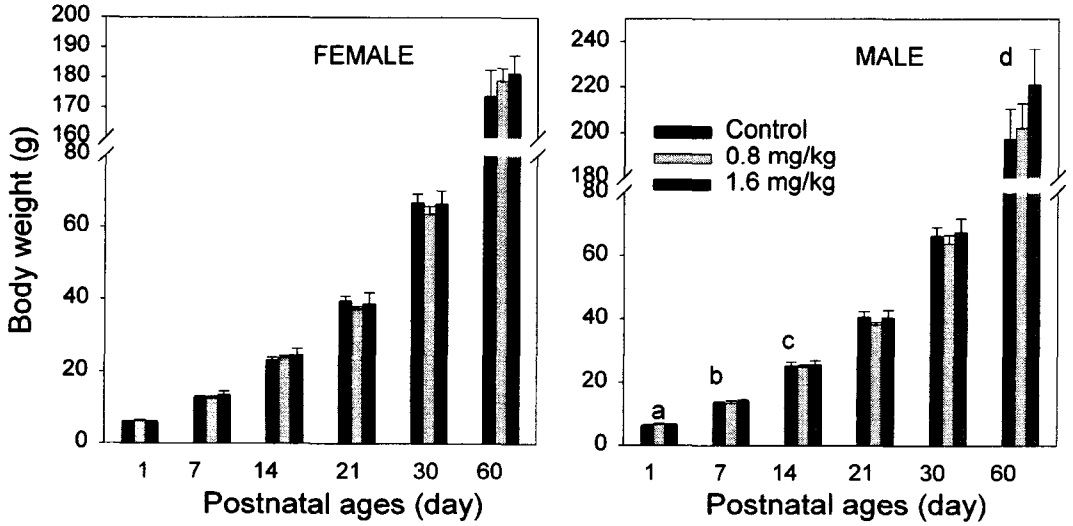

Fig. 2. Offspring body weight gains after prenatal treatment of fumonisin B1 from PND 1 to PND 60 in female and male. PND 1, 7, 14 and 60 showed significant gender difference.

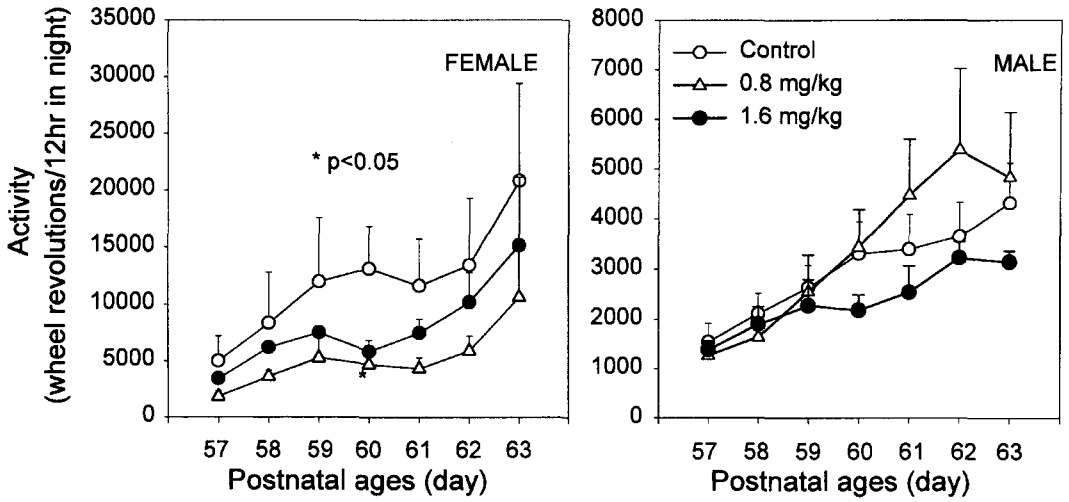

Fig. 4. Running wheel revolution activity for 12 hour in night during postnatal days 57 to 63. Running wheel activity of fumonisin B1 treated rats (0.8 mg/kg) was significantly ($p < 0.05$) decreased at postnatal days 60, compared to the control.

pattern to the results obtained from preweaning duration. At postnatal days 35, 36, and 37, activity of FB1 treated female rats was insignificantly decreased in comparison with control and showed a dose-dependent tendency. Interaction of ages by treatment effect for female was significantly different (Age * Treatment, F(6, 45)=2.50, Pr=0.0355). This indicates that FB1 may cause sex-specific changes in open field activity (Fig. 3).

6. Running wheel activity

Running wheel activity is shown in Fig. 4 for night 12 hr. FB1-treated groups in female rats showed low levels of running wheel activity compared to that of control. Significant sex difference was shown in this test. Activity at PND 60 showed a significant treatment effects by Duncann's multiple range test. This results confirmed the result of pre- and post-weaning open field activity, suggesting that FB1 may decrease activity in female after prenatal exposure (Sex, F(1, 9)=10.18, Pr=0.0110).

7. Complex maze performance

No significant effects in the number of total arm entry and number of water rewards were observed in female. In male offsprings, the number of water rewards was increased in 0.8 mg/kg FB1 treated group (Treatment F(2, 18)=5.83, Pr=0.0111; Fig. 5). Significant gender-related effect was also observed (Sex, F(1, 18)=7.01, Pr=0.0163).

IV. DISCUSSION

With our assumption that FB1 is developmentally toxic in rodents, prenatal exposure study was conducted to investigate developmental and neurobehavioral effects of FB1. The assumption is based on the following several reports: FB1 is reportedly known to be associated with equine leukoencephalomalacia which is a neurotoxic disease. The disease was experimentally produced in equine species by either cultured or purified material of FB1 (Wilson *et al.*, 1971; Marasas *et al.*, 1988a). This neurotoxic disease is characterized by an acute and severe neurologic disorder, and its clinical symptoms include nervousness, apathy, trembling, ataxia, paresis of the lower lip and tongue, and anorexia. The toxic effects of the fumonisins in target organs may be dose-dependent and species specific. Even though most of neurotoxic phenomena of fumonisins were manifested in equine species, the effects of fumonisins on neurotransmitters and its metabolites were investigated in rats (Porter *et al.*, 1990; 1993). Increases in both 5-hydroxy indol acetic acid (5-HIAA), major metabolite of neurotransmitter serotonin (5-HT), and 5-HIAA/5-HT ratios were manifested in rats fed the cultured corn of *Fusarium moniliforme*. 5-HT metabolism, however, was not altered in rats fed pure FB1 added to rat

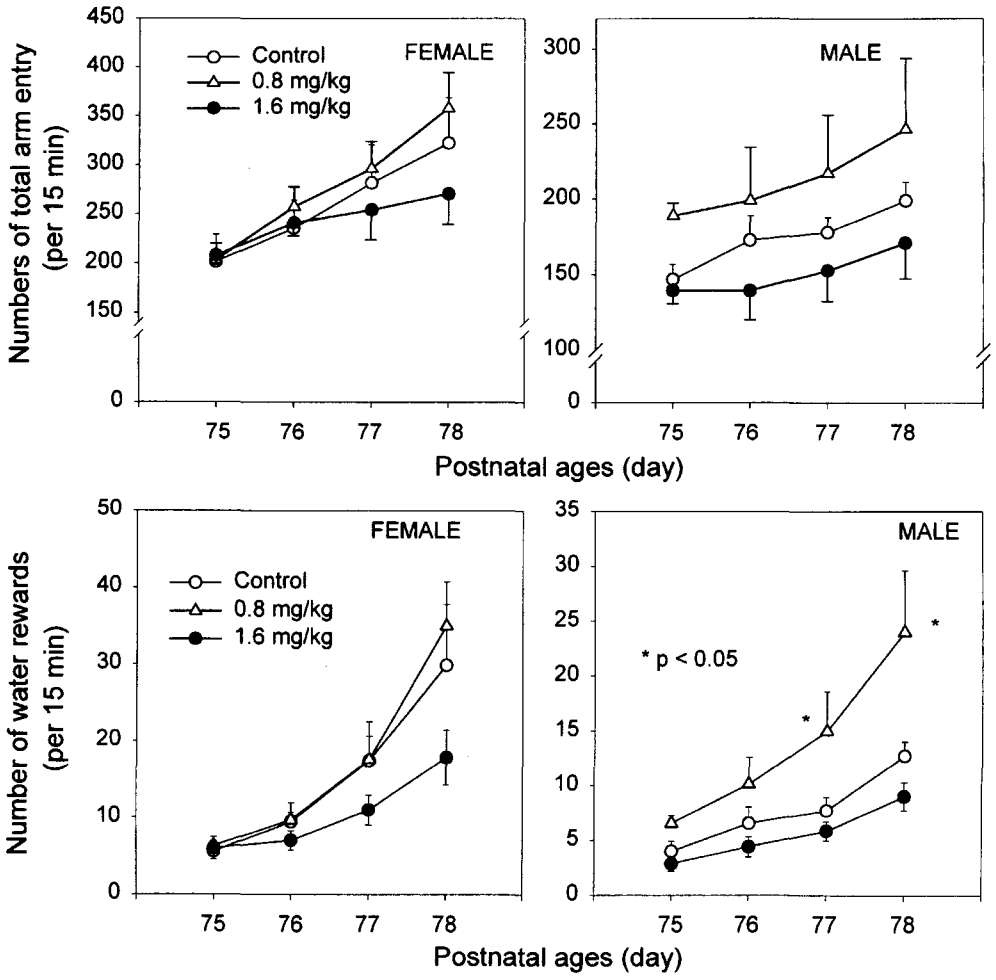

Fig. 5. Complex maze performance. Number of total arm entry and number of water rewards were measured on postnatal days 75 to 78. Number of water rewards was significantly ($p<0.05$) increased in 0.8 mg/kg FB1 group.

chow.

FB1 levels in corn and corn screenings from 1988-1991 crop years were reported to be 2.3~2.9 μg/g of corn as mean concentrations in Iowa, Wisconsin and Illinois (Murphy *et al.*, 1993). FB1 concentrations measured in feeds from equine leukoencephalomalacia cases were higher than 10 μg FB1/g, whereas horse feed samples not associated with the disease contained less than 8 μg FB1/g (Ross *et al.*, 1991). The no observed adverse effect level in the experimemt for inducing liver damages was less than 12 μg FB1/g (Motelin *et al.*, 1994). Because the developing nervous tissues may be more sensitive to many foreign chemicals and toxic effects may be manifested as subtle disturbance of behavior long before any apparent symptoms, low concentrations of FB1 that hepatotoxicity are not caused and are reported as the concentration ranges similar to the concentration of FB1 measured in corn were applied to the pregnant rats.

Exposure of rats to the low concentrations of FB1 prenatally on gestational days 13~20 did not induce significant changes in several endpoints used. Minor significant changes in running wheel activity at PND 60 in female was observed, and significant gender differences in body weight, running wheel activity, and complex maze performance were showed. Collins *et al.* (1998) showed that pregnant rats dosed orally with 50 mg/kg FB1 on gestation days 3~16 resulted in maternal and fetal toxicities. Due to hydrophilic properties of FB1, FB1 may not cross easily the placenta at low dosage of FB1.

In summary, weight gain of dams during gestation (13~20 days) showed an insignificant dose-dependent

tendency. Percentage of weight gain difference between control and 1.6 mg/kg of FB1 was about 4%. Significant sex differences in running wheel activity (PND 57-63) and complex maze performance (PND 75-78) were observed. Pre- and post-weaning weight of offspring after prenatal exposure to FB1 was not significantly altered, suggesting that FB1 of 0.8 or 1.6 mg/kg doses may not cross the placenta.

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