Teratological Evaluation of Zearalenone Administered Orally to the Rat

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Zearalenone (F-2 toxin) is an estrogenic mycotoxin produced by several species of Fusarium (STEELE et al., 1974, KALLELA and KORPINEN, 1973, and EUGENIO et al., 1970). Its estrogenic effects involving enlargement of the uteri and mammary glands, vulvular swelling, testicular atrophy and vaginal prolapse in rats, mice, swine and guinea pigs have been reported (MIROCHA et al., 1968 and MIROCHA et al., 1971). A fetopathic effect has been incriminated by observations of still birth and "splayleg" following injection of pregnant sows (MILLER et al., 1973). The mycotoxin occurs in food and feed (MIROCHA et al., 1974, EPPLEY et al., 1974) at levels of up to 2000 ppm (MIROCHA et al., 1971). In order to evaluate its embryopathic potential a study of zearalenone by oral dosing another species, the rat, was undertaken.

Materials and Methods

Female Wistar rats (175-200 g, Woodlyn Farms, Guelph, Ontario) were bred by pairing them overnight with proven sires. The morning that a sperm positive smear was observed was designated as the first day of gestation. Mated females were randomly assigned to 4 test groups (10/group) and were caged individually. They were provided food (Purina cubes) and water ad libitum.

The mated females were treated orally on days 6 through to 15 of gestation by stomach intubation with 0, 1, 5 and 10 mg of zearalenone per kg per day. Zearalenone (Commercial Solvents Corporation, Terre Haute, Indiana, U.S.A., purity 97.1% by weight, m.p. 162.1°C) was dissolved in corn oil and administered in amounts of 1 ml/200 g body weight.

On day 22 of gestation, all females were necropsied and internal organs were examined. The following fetal and maternal parameters were determined; the number of corpora lutea, live fetuses and deciduomas, live fetal weight, and maternal weight (with and without the uterine content). The maternal weight gain was derived by comparing the weight at the initiation of

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gestation to that at the end of gestation without the uterine contents. Two-thirds of the fetuses from each litter were processed for skeleton examination; the remainder were fixed in Bouin's fluid for visceral examination. The visceral anomalies were searched for by dissecting and razor sectioning the fetuses; the skeleton anomalies by stereoscopic examination of stained skeletons.

The number of anomalous fetuses versus total numbers examined for each experimental group constituted the basis for teratogenic evaluation. For evaluating fetal survival a Chi-square test was used on ratio of deciduomas divided by total implants. For the reproductive effect in table 1, a "t" value was used for test and control group comparisons.

Results and Discussion

None of the dosages produced any apparent signs of maternal toxicity during the gestation period, nor were there any noticable lesional changes in the maternal viscera at necropsy. A statistically significant loss in maternal weight gain was observed in the 10 mg/kg group in comparison with the control group (Table 1). At this dose the mean fetal weight was significantly ($P < 0.05$) below the control value.

The incidence of deciduomas was slightly increased for the 10 mg/kg group (Table 1), but the increase was not statistically significant ($P = 0.05$).

TABLE 1

Maternal and fetal effects of zearalenone administered orally during days 6-15 of gestation to the rat.

dose (mg/kg)	litters	maternal weight gain (g) (mean ± SE)	litter size (mean ± SE)	fetal weight (g) (mean ± SE)	deciduomas (percent)[1]
0	10	53.3±4.6	12.9±0.35	4.9±0.09	6.5
1	10	56.2±3.9	12.7±0.44	4.5±0.25	5.2
5	9	50.0±4.6	11.6±0.57	4.9±0.13	5.4
10	10	38.7±2.4*	11.3±0.69	4.3±0.09*	10.3

* $P < 0.05$

[1] $\frac{\text{No. deciduomas} \times 100}{\text{total implants}}$

The incidence of delayed or absent skeleton ossification increased with increasing dosage; the rib, sternum, tarsal bones and the parietal bone were those commonly affected.

At 1 mg/kg, the incidence of skeleton defects was 12.8% (11/86). These included missing or mal-appositioned sternal plates, short ribs, extra or 14th rib and wavy ribs. In addition to these, at 5 mg/kg, some tarsus were missing while the total incidence of skeletal defects had increased to 26.1% (18/69). At 10 mg/kg, the incidence was the highest or 36.8% (28/76) and arrested parietal ossification was, beside the above retarded ossification, a prominent anomaly. In the control group the arrested ossification was generally not observed; two of the 85 control fetuses (2.4%) had uni- or bilateral wavy ribs.

The visceral examination revealed no apparent difference between the control and experimental groups.

Zearalenone suppressed maternal weight gain at 10 mg/kg. The observed fetal skeleton anomalies were present at a much higher incidence than generally observed (5%) for this strain of rat. Lower dosages of 0.075 -0.30 mg/kg did not result in any of these alterations (unpublished data). Aside from zearalenone's estrogenic effect, there appears to be a fetal esteogenic effect manifested by the skeleton anomalies in the rat and the previously reported "splayleg" condition in pigs (MILLER et al., 1973).

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