Teratogenicity Assessment of Ethylenebis (isothiocyanate) in the Rat

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The ethylenebis(dithiocarbamate) fungicides, which are used on food crops, undergo decomposition to several intermediate products. Among these intermediates, ethylenethiourea (ETU) was reported to be teratogenic following multiple (KHERA, 1973) and single oral dosages (RUDDICK and KHERA, 1975). Its metabolism in the rat following a teratogenic dose was also reported (RUDDICK et al. 1976a). Other breakdown intermediates were also tested for teratogenicity (RUDDICK et al. 1976b) and they were not found to produce anomalies following a single oral dose. This report presents the teratologic evaluation of ethylenebis(isothiocyanate) (EBI), (THORN and LUDWIG, 1962 and VONK, 1975), which is thought to arise from another dithiocarbamate intermediate, ethylenethiuram monosulfide (LUDWIG et al. 1955). NEWSOME (1975) reported a method for the quantitative determination of EBI in food crops and has found EBI in food commodities (unpublished data).

Materials and Methods

Ethylenebis(isothiocyanate) was synthesized from the ethoxycarbonyl derivative of disodium ethylenebis(dithiocarbamate) (NEWSOME 1975). Before doing the teratologic evaluation, an estimation of the oral LD_{50} of EBI was carried out and calculated according to the procedure described by WEIL (1952). Single oral dosages of 50, 100, 200 and 400 mg EBI per kg body weight, dissolved in corn oil, were administered at 1 ml/200 g body weight by stomach intubation to six female rats (200-250 g) at each dose. The treated females had free access to food (Purina cubes) and water, and were kept under observation for five days.

For the teratology study, primiparous Wistar rats (Woodlyn Farms, Guelph, Ontario), 175-200 g were paired overnight with proven sires. A sperm positive smear on the following morning placed the female in day 1 of pregnancy. The mated females were caged individually and they were provided with Purina rat cubes and water ad libitum. Four dosages based on the oral LD_{50} were chosen; 0, 12.5, 25.0 and 50.0 mg EBI per kg body

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weight and administered orally by stomach intubation to 15 mated females at each dose. The EBI was dissolved in corn oil and given at 1.0 ml/200 g body weight on days 6 through to 15 of gestation. Necropsies were carried out on day 22 of gestation to provide the following information: litter size, number of deciduomas, individual litter weights and maternal weight gain. The maternal weight gain was determined by subtracting from the female's weight on day 1 of gestation her weight at necropsy after removing the pups and uterine horns. Only live fetuses were counted in the litter size. Approximately two-thirds of the fetuses from each litter were processed for skeleton examination while the remainder was fixed in Bouin's fluid for visceral investigation.

Subsequent to obtaining these results, a repeat of the 50.0 mg/kg dose was carried out on 15 mated females along with 5 control females. The data was collated, analyzed with the "t" test and is presented in Table 1.

Results and Discussion

The median oral LD_{50} was calculated to be 112.3 mg/kg with the 95% confidence interval being 76.3 to 165.2 mg/kg.

Table I

Effects of ethylenebis(isothiocyanate) on maternal weight gain and embryos in the rat

Dose (mg/kg)	No. on test	Litters	maternal weight gain (g, mean ± S.E.)	litter size (mean ± S.E.)	fetal weight (g, mean ± S.E.)
0	19	16	47.3 ± 3.9	12.9 ± 0.7	5.1 ± 0.1
12.5	15	10	41.3 ± 5.1	12.4 ± 0.9	5.2 ± 0.1
25.0	14	11	32.4 ± 5.1*	12.9 ± 0.4	4.9 ± 0.2
50.0	30	9	23.1 ± 6.7*	12.3 ± 0.8	4.7 ± 0.1*

* $P > 0.05$

No skeleton or viscera anomalies were observed in the teratology study of EBI treatments. Dosages of 25.0 and 50.0 mg/kg decreased significantly ($P < 0.05$) the mean maternal weight gain when compared to the control value (Table 1). An effect was also observed on the mean fetal weight with the 50.0 mg/kg dose.

Accompanying the significant maternal weight loss at 50.0 mg/kg, was a very sudden drop in the number of mated dams that had no litter; 11/15 as compared to 3/15 in the control. The uteri of these treated females appeared normal except for two females which had a slight uterine swelling. A repeat of the 50.0 mg/kg dose revealed that 10/15 females did not have any pups. Two of the females, again, had fluid-filled uteri while another had 12 deciduomas. A total of nine of the 50.0 mg/kg treated females did have litters. The treatment had no effect statistically on their litter size (Table 1) but did effect the fetal weight when compared to the control value.

If the maternal weight loss and increase in the number of dams which had no litter are mutually exclusive, EBI is embryocidal at 50.0 mg/kg, killing the embryo soon after implantation. It cannot be determined in this experiment whether the maternal weight loss alone could explain the significant reduction in the number of litters. Using weight gain as a standard, a maternal toxic effect was noted at 50.0 and 25.0 mg/kg treatments while only the 50.0 mg/kg dose effected the mean fetal weight.

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References

KHERA, K.S. Ethylenethiourea: teratogenicity study in rats and rabbits. Teratology 7, 243 (1973).

LUDWIG, R.A., G.D. THORN and C.H. UNWIN. The mechanism of fungicidal action of metallic ethylenebis(dithiocarbamates). Can. J. Bot. 33, 42 (1955).

NEWSOME, W.H. A method for the determination of ethylenebis(isothiocyanate) on food crops. J. Agr. Food Chem. (In press) 1975.

RUDDICK, J.A. and K.S. KHERA. Pattern of anomalies following single doses of ethylenethiourea to pregnant rats. Teratology 12: 277 (1975).

RUDDICK, J.A., D.T. WILLIAMS, K.S. KHERA and L. HIERLIHY. [^{14}C]-Ethylenethiourea: distribution, excretion and metabolism in pregnant rats. Teratology (In press) 1976a.

RUDDICK, J.A., W.H. NEWSOME and L. NASH. Correlation of teratogenicity to molecular structure: ethylenethiourea and related compounds. Teratology (submitted) 1976b.

THORN, G.D. and R.A. LUDWIG. The dithiocarbamates and related compounds. Elsevier, Amsterdam, Holland 1962.

VONK, J.W. Chemical decomposition of bisdithiocarbamate fungicides and their metabolism by plants and microorganisms. Ph.D. Thesis, Elinkwijk Bv., Utrecht, Holland 1975.

WEIL, C.S. Tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics 8, 250 (1952).