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Embryotoxicity Study on Cyclopiazonic Acid in Mice

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Cyclopiazonic acid, an indole tetramic acid formed by several species of Penicillium and Aspergillus including strains of P. camemberti and P. caseicolum and A. oryzae used as starter cultures in the manufacture of enzyme and mold ripened cheese (Orth 1977; Scott 1981). Cyclopiazonic acid levels as high as 4 µg/g (Still et al. 1978) and 1.5 µg/g (LeBars 1979) have been detected in the crust of Camembert cheese. It has also been identified in peanuts contaminated with A. flavus (Lansden and Davidson 1983) and corn (Gallagher et al. 1978).

Only limited information is available on the toxicity of cyclopiazonic acid. Isaacson (1966) has suggested that this mycotoxin may be responsible for liver cancer in Bantu tribes of Africa. Oral administration to rats results in death in 1-5 days and acute LD_{50} values of 36 mg/kg for male and 63 mg/kg for female rats have been reported (Purchase 1971). Broiler chickens given high levels of cyclopiazonic acid in the feed had severe liver lesions (Dorner et al. 1983). Since there is a distinct human exposure, cyclopiazonic acid was tested for its teratogenic potential in mice and the results are described here.

METHODS AND MATERIALS

Female Swiss-Webster mice weighing about 30 g were paired overnight with proven males. The morning that a vaginal plug was observed, was counted as day one of pregnancy. Fifteen to 20 mated females per group were assigned by random selection to the three test groups and one control group. Test doses of cyclopiazonic acid were 4,8 or 16 mg/kg bw and were selected from the results of a preliminary study in which a single oral dose of 32 mg/kg bw caused tremors and diarrhea and killed two of five treated dams. Cyclopiazonic acid (purity 98% based upon UV extinction coefficient at 284 nm) was dissolved in 1N sodium bicarbonate aqueous solution (vehicle) and administered once daily by esophageal intubation for four consecutive days from day 9 until day 12 of pregnancy. The volume of the test solution administered was 6,3 or 1.5 ml/100g bw for the 16,8 or 4 mg/kg cyclopiazonicacid dose levels, respectively. Control females were administered 6 ml of vehicle/100g bw, and were otherwise treated similarly as the test females. All female mice were weighed on pregnancy day 1, 9,10,11,12 and 19 and at necropsy without uterine contents. On the 19th day of pregnancy, the dams were killed with CO_2, their uterine contents removed and necropsies performed. The number of early resorptions and

fetuses dying late in pregnancy were recorded. Live fetuses were weighed and examined for external malformations. Two-thirds of the live fetuses from each litter were examined for skeleton abnormalities after alizarine staining. The remaining fetuses were fixed in Bouin's fluid and dissected for study of possible visceral defects.

RESULTS AND DISCUSSION

Cyclopiazonic acid at daily doses of up to 16 mg/kg administered for four consecutive days did not produce any overt sign of toxicity or body weight suppression in dams during pregnancy or at necropsy (Table 1). A slightly decreased incidence of pregnancy was observed at all test doses but the decrease was neither dose-related nor statistically significant.

TABLE 1 Maternal body weight of mice dosed orally with cyclopiazonic acid on days 9 - 12 of pregnancy

Dose Group (mg/kg)	No. of dams pregnant	Body Weight, g (mean) Days of pregnancy 1	9	10	11	12	19	Less uterine content at necropsy
Control	20	29.5	35.3	36.7	38.0	40.4	61.5	37.6
4	16	29.9	35.8	37.3	38.5	40.7	63.6	38.0
8	18	29.7	35.6	36.7	38.4	40.4	60.8	37.9
16	15	30.9	36.5	37.5	39.5	40.9	64.7	39.1

The incidences of live, runted and dead fetuses, resorptions and male/female fetuses at all test doses of cyclopizonic acid were within the control range (Table 2). The mean fetal weight failed to show a dose-related effect since it was reduced at the 4 and 8 mg/kg doses ($P < 0.05$) but was not affected at the 16 mg/kg dose. The incidence of malformations and aberrations in fetuses of all treated groups were not statistically different from control values.

In a preliminary study, not reported here, oral treatment with a single 32 mg/kg dose of cyclopiazonic acid caused tremors and diarrhea in all five pregnant mice, of which two died within 1.5 hours after dosing. The finding suggested that the mycotoxin is rapidly absorbed from the gastro-intestinal tract and is neurotoxic. It is interesting that oral treatment of ducklings produced nervous symptoms and death within 30 minutes (Purchase 1971) suggesting quick absorption from the crop. However, in rats, oral treatment with cyclopiazonic acid failed to produce an immediate effect, because it was not quickly absorbed (Purchase 1971). A rapid absorption from the gastrointestinal tract and neurotoxic response in mice tend to suggest the suitability of this species for teratologic evaluation of cyclopiazonic acid.

Daily human intake of cyclopiazonic acid calculated for a 50 kg person consuming 200 g of cheese containing 4 µg/g of cyclopiazonic acid will be

TABLE 2 **Fetal and maternal values from mice orally dosed with cyclopiazonic acid on day 9 - 12 of pregnancy**

	Dose group, mg/kg/day			
	Control	**4**	**8**	**16**
Number of females pregnant/ number initiated on test	20/20	16/18	18/20	15/19
Implants per pregnancy (mean)	12.9	14.4	13.7	14.0
Number of dams with resorptions	7	5	8	8
Number of resorptions ÷ total implants x 100	5.4	3.5	7.3	6.2
Number of dead fetuses	3	1	5	2
Number of runted fetuses	1	1	1	1
Live fetuses per pregnancy	12.2	13.9	12.7	13.1
Male/female, number of live fetuses	121/123	114/108	102/126	103/94
Fetal weight, g (mean)	1.4	1.3*	1.3*	1.4
I EXTERNAL MALFORMATIONS	— (percent fetal incidence) —			
Number of fetuses examined	224	222	228	197
Cleft palate	1	0	0	1
Unbilical hernia	0	1	0	0
II VISCERAL MALFORMATIONS				
Number of fetuses examined	74	69	69	58
Number of fetuses malformed	0	0	0	0
III SKELETAL ABERATIONS	— (percent fetal incidence) —			
Number fetuses examined	170	153	159	139
Retarded ossifications:				
calvarium	1	4	4	2
sternebrae	0	1	2	4
13th rib, rudimentary	1	7	8	9
14th rib, present	0	1	1	2

*Significantly different from control, $P < 0.5$ (student's t test)

about 16 µg/kg. Since a thousand-fold higher dose of 16 mg/kg failed to show any adverse effect on fetal development in mice, no significant concern regarding current levels of human exposure to cyclopiazonic acid seems warranted at present.

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