Time Course of Ethylene Thiourea in Maternal Plasma, Amniotic Fluid and Embryos in Rats Following Single Oral Dosing

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ABSTRACT. Ethylene thiourea (ETU) was administered once orally to pregnant rats on gestation day 12 at a dose of 200 mg/kg, and its concentration-time courses in the maternal plasma, amniotic fluid and embryos were investigated. The ETU concentrations in the maternal plasma and amniotic fluid reached the peak level about 2 hr after dosing, then declined gradually and had disappeared by 48 hr. In embryos, the concentration of ETU peaked at 30 min after dosing and disappeared at 48 hr. The prolonged exposure of the embryos to the high concentration of ETU in the amniotic fluid could be partially responsible for the teratogenic effect of ETU. — **KEY WORDS:** concentration-time curve, ethylene thiourea.

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Ethylenebisthiocarbamate fungicides are used in fruits and vegetables. Ethylene thiourea (ETU) is their degradation product in the environment, and their metabolite in plants and animals [1–4]. Ethylenebisthiocarbamate fungicides such as zineb, maneb and macozeb are known to induce fetal malformations when they are given orally to pregnant rats at a high dose [6, 9, 12], and ETU is considered to be responsible for their teratogenic potential [7].

It has been reported that, after administration of a single oral dose of ¹⁴C-labeled ETU to pregnant rats, radioactivity was detected in the maternal blood, tissues, and urine and fetal tissues, and disappeared by 24 hr [5, 13, 14]. However, there are no detailed reports about the rate of transfer of ETU to maternal blood, amniotic fluid and fetal tissues, and the time of its appearance and subsequent kinetic behavior in these tissues after maternal p.o. administration.

In the present study, therefore, a teratogenic dose of ETU [15] was administered once orally to rat dams on gestation day 12, and the concentration-time profiles of ETU in the maternal plasma, amniotic fluid and embryos were investigated.

Wistar Imamichi rats were purchased from the Institute of Animal Reproduction on gestation days 5–7 and used in the experiment. The day of finding sperms in the vaginal smear was defined as day 0 of gestation. The animals were kept in an air-conditioned room at a constant temperature of 22 ± 2°C with a 12 hr light/dark cycle, and *ad libitum* access to food and tap water. Ethylene thiourea (ETU, Wako Pure Chemical, Osaka, Japan) was suspended in a 0.5% tragacanth gum solution before use, and administered to the pregnant rats at a dose of 200 mg/kg by oral gavage using a dosing volume of 5 ml/kg.

ETU was administered once orally at a dose of 200 mg/kg at 10:00 in the morning of gestation day 12. At 5, 10, 15 and 30 min, and 2, 6, 12, 24, 36 and 48 hr after dosing, blood was drawn from three to five dams under ether anesthesia. The dams were then killed, and amniotic fluid was immediately collected under a dissecting microscope using a 25G needle. The embryos were subsequently

removed from the uteri. Maternal blood was drawn into heparinized 500- μ l tubes. The samples of amniotic fluid and maternal blood were centrifuged at 12,000 rpm for 1 min, and the supernatant or plasma was used for the concentration analysis. Individual embryos were weighed and stored at –40°C in a freezer until assayed. The ETU concentrations in each sample were determined according to the method of Kobayashi *et al.* [8] with the following modifications. Fifty μ l of plasma or amniotic fluid was added to 100 μ l of methanol and centrifuged at 8,000 rpm for 2 min, and the supernatant was passed through a 0.45- μ m filter. Fifty μ l of the filtrate was added to 250 μ l of purified water and stirred, and 50 μ l of the mixture was injected into HPLC. In the recovery test, the recovery rate of ETU by this method was 92 ± 3.7%. The ETU concentration in the amniotic fluid was determined using six amniotic fluid samples (three conceptuses each from the bilateral uterine horns) per litter, and the mean of the six samples was used as the litter mean.

The embryos were thawed at 4°C and quickly washed with physiological saline to remove the amniotic fluid remaining on the fetal surface. After addition of 1 ml of water, 1.5 g of potassium fluoride and 1.5 ml of 75% MeOH per 100 mg of the fetal tissue, the mixture was homogenized in an ultrasonic-high speed homogenizer for 2 min. The homogenate was then added to 0.5 ml of 75% MeOH and extracted by the original method to obtain the specimens for HPLC analysis. A 10- μ l aliquot was injected into the HPLC system. In the recovery test, the recovery rate of ETU of this method was 89 ± 9.0 (S.D.)%.

HPLC (LC6A, Shimadzu Co., Ltd., Tokyo, Japan) was equipped with a YMC-Pak AQ303 column (250 × 4.6 mm I. D., YMC Co., Ltd.) and operated at a flow rate of 1 ml/min, using a water-methanol (95:5, v/v) mixture as the mobile phase. The UV detector was operated at 240 nm. The detection limit of this analysis is 0.01 ppm.

Figure 1 shows the concentration-time curves of ETU in the maternal plasma, amniotic fluid and fetuses. ETU was detected in maternal plasma and amniotic fluid as early as 5

Fig. 1. Concentration-time curve of ETU in maternal plasma, amniotic fluid and embryo after oral administration of 200 mg/kg ETU to dams on day 12 of gestation. Upper bars indicates S.E.M.

min after dosing. At 30 min, the ETU concentration in the amniotic fluid almost equilibrated with that in plasma, and the concentration of ETU in these body fluids then increased with similar time profiles, peaking at 2 hr, then decreased gradually to an undetectable level at 48 hr. In embryos from the treated dams, the ETU concentration peaked at 30 min and then gradually decreased to below the detection limit at 48 hr.

In an earlier study, Kato *et al.* [5] measured radioactivity in the amniotic fluid, maternal blood and various maternal organs and tissues at 2, 6 and 24 hr after maternal administration of ^{14}C -ETU 100 mg/kg, p.o., on gestation day 12 and reported that ETU is rapidly absorbed from the maternal intestine and transferred into maternal plasma and amniotic fluid. In our study we obtained more detailed information about the early phase distribution kinetics of ETU in the maternal plasma, embryos and amniotic fluid immediately after oral administration to dams.

After oral administration to dams, ETU is naturally thought to be transferred to embryos via the placenta during

the early distribution phase. However, the almost complete disappearance of ETU from the embryos, within as early as about 12 hr after dosing, suggests that ETU in the embryos is excreted into the maternal tissue via the transplacental pathway without any appreciable uptake by the embryonal tissues. Pharmacokinetics models for clearance in the maternal-fetal amniotic fluid system of rats and humans showed that drugs were transferred through the placenta and the fetus to the amniotic fluid and excreted through the reverse course [10, 11]. It is also possible that ETU in the embryos is excreted into the amniotic fluid, and in addition, some fraction of ETU penetrating the embryo may be secreted into amniotic fluid through the amnion and remain there over a much longer period than in the embryos.

It is well known that ETU ingested by dams induces malformations in fetuses [7]. The teratogenic effect of ETU is clearly attributable to the fraction taken up by the embryos via the transplacental transfer, when the concentration-time curves of ETU in the various tissues obtained in the present study are considered, but it is also likely that the prolonged exposure of the embryos to the much higher concentration of ETU in the amniotic fluid, than that in the embryos per se, contributes to the induction of fetal malformations. To confirm this possibility, further studies using *in vitro* systems will be needed to exclude the maternal effects.

REFERENCES

1. Autio, K. and Pyysalo, H. 1983. *J. Agric. Food Chem.* 31: 568-571.
2. Brocker, E. R. and Schlatter, C. 1980. *Toxicol. Letters* 6: 221-224.
3. Czegldi-Jank, G. 1967. *J. Chromatog.* 31: 89-95.
4. Jordan, L. W. and Neal, R. A. 1979. *Bull. Environ. Contam. Toxicol.* 22: 271-277.
5. Kato, Y., Odanaka, Y., Teramoto, S., and Matano, O. 1976. *Bull. Environ. Contam. Toxicol.* 16: 546-555.
6. Khera, K. S. 1973. *Teratology* 7: 243-252.
7. Khera, K. S. 1987. *CRC Critic. Rev. Toxicol.* 18: 129-139.
8. Kobayashi, H., Matano, O., and Goto, S. 1986. *J. Pesticide Sci.* 11: 81-84.
9. Larsson, K. S., Arnander, C., Cekanova, E., and Kjellberg, M. 1976. *Teratology* 14: 171-184.
10. Luecke, R. H., Wosilait, W. D., Pearce, B. A., and Young, J. F. 1994. *Teratology* 49: 90-103.
11. Nanba, T. 1988. *Toxicol. Appl. Pharmacol.* 92: 381-389.
12. Peterova-Vergieva, T. and Ivanova-Tchemishanska, L. 1973. *Fd. Cosmet. Toxicol.* 11: 239-244.
13. Ruddick, J. A., Williams, D. T., Hierlihy, L., and Khera, K.S. 1976. *Teratology* 13: 35-39.
14. Ruddick, J. A., Newsome, W. H., and Iverson, F. 1977. *Teratology* 16: 159-162.
15. Teramoto, S., Harada, T., and Kaneda, M. 1978. *Cong. Anom.* 18: 241-249.