(8-d)

EXPOSURE OF MALE LONG-EVANS RATS TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN USING LOADING-DOSE/MAINTENANCE-DOSE REGIMEN DOES NOT ALTER THE SEX RATIO OF THEIR OFFSPRING

J. Yonemoto [1, 3], M. Inouye[4], R. Nagata[4], T. Takei[5] and C. Tohyama[1, 2, 3]

[1]Endocrine Disruptors and Dioxin Research Project and [2]Environmental Health Sciences Division, National Institute for Environmental Studies (NIES), Tsukuba 305-8506, Japan. [3]CREST, Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan. [4]Shin Nippon Biomedical Laboratories (SNBL), Tokyo 100-0006, Japan. [5]Ministry of the Environment Japan, Tokyo 100-8975, Japan.

Introduction

A skewed offspring sex ratio (65% girls) was reported in families with both parents exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) following the explosion at a chemical plant in Seveso, Italy in 1976[1)]. A subsequent study of the Seveso cohort revealed that this skewness was associated with paternal, not maternal, exposure[2)]. Several epidemiological studies, such as Russian pesticide worker cohort[3)], Australian chloracne cohort[4)] and Yu-chen cohort[5)], also suggested the association of paternal exposure to dioxin with a lowered sex ratio. On the other hand, no alteration in sex ratios of offspring was found in workers in the factories produced Agent Orange[6)], a TCDD-containing herbicide, and in Vietnam veterans engaged in "Operation Ranch Hand", the unit responsible for spraying Agent Orange[7)]. To test the possibility that paternal TCDD exposure is associated with a lowered sex ratio in the offspring, in the present study, we exposed male Long-Evans rats to TCDD for 9 weeks and examined the sex ratio and other toxicological parameters.

Materials and Methods

Animals and treatment

Rats were handled with care according to the guidelines for animal experiments at SNBL. Two hundred 5-week-old male Long-Evans rats (Charles River Laboratories) were divided into one control and three treatment groups. We adopted a loading dose/maintenance dose regimen to expose the animals to a rather constant level of TCDD[8)]. Rats were given one initial dose of 0, 0.1, 1, or 5 µg TCDD/kg by gavage followed by a weekly maintenance dose (20% of initial dose) for 9 weeks. After the 9-week exposure period, each male was mated 1:1 with an intact female rat of the same strain. A female that had a vaginal plug the following

morning was designated as Day 0 of gestation (GD0). This plug-positive female was then removed and replaced by another female after a minimum two-day interval until each male had two plug-positive females. Mating performance and male fertility were evaluated. After the inspection of dams and pups, all males were sacrificed under light diethylether anesthesia for tissue collection. The left testis and epididymis were fixed in Bouin's solution followed by 10% buffered formalin for histopathological inspection. The right testis and epididymis were immediately frozen and stored at –80°C for further investigation.

Eighty dams per group were sacrificed on GD20, and pregnancy outcome was evaluated. Number of viable fetuses, dead fetuses, resorptions, and corpora lutea were counted. Fetuses were weighed and sexed, and their external morphology and internal genital organs were examined under a dissecting microscope.

Twenty dams per group were allowed to deliver, and the number of pups (alive or dead) as well as their sex was recorded. The pups (F1) were culled to 8 per litter (4 of each sex, if possible, on postnatal day (PND) 4 (day of birth=PND0)). At weaning (PND22), 4 pups (2 of each sex) were necropsied. The following developmental landmarks were monitored: anogenital distance (PND4 and 91), preputial separation (male: PND40, 45, and 50), and vaginal opening (female: PND30, 35, and 40).

The animals (91- 112 days old, 2 of each sex per litter) were mated with the opposite sex of another litter of the same group for up to 2 weeks. Half of the dams were sacrificed on GD15, and the number of corpora lutea, implantation sites, and live fetuses was counted. The rest of the dams were sacrificed on GD20, the fetuses (F2) were sexed, and their gross morphology was inspected.

Statistical methods

The unit of comparison was the individual animal or the litter, as appropriate. Homogeneity of variance was evaluated by Bartlett's test. Copulatory rate and fertility rate were analyzed by Fisher's exact test. Other data were analyzed by Dunnett's multicomparison test.

Results and Discussion

Neither death nor abnormality in general condition of rats was observed during the study period. The body weight gain of males was significantly suppressed in the highest-dose group (Loading-dose/Maintenance-dose: 5/1µg TCDD/kg). The copulatory rate and fertility rate were not affected by the treatment.

The sex ratio of the fetuses was slightly higher in both the control and exposed groups (Fig. 1). Paternal TCDD exposure had no effects on the sex ratio of their offspring, which did not allow us to speculate the mechanisms involved in the male-mediated skewed sex ratio reported by the epidemiological studies. James proposed a hormonal hypothesis that high gonadotropin and low testosterone levels injured and skewed the Y-bearing gametes before conception[9]. Jongbloet et al. proposed a hypothesis of post- and pre-ovulatory over-ripeness of the oocyte, which leads to preferential loss of male fetuses and preferential insemination by Y-bearing spermatocytes, respectively [10]. They claimed that their hypothesis explained both

a significant low sex ratio in exposed man-unexposed woman couples and a tendency to higher（0.545） sex ratio in unexposed man-exposed woman couples in the Seveso cohort[2]. It would be worth while to test this ovopathy concept in animal models.

Fetal mortality was significantly elevated only in the lowest-exposure group (Fig. 2) without a dose-dependent tendency. Since dose–response fallacy was often observed in many reproductive end points [11], confirmation of this observation is now in progress.

Paternal TCDD exposure had no effects on the development of naturally delivered F1 offspring. No TCDD effect was observed in the copulatory rate and fertility of F1 or in the development and sex ratio of their offspring (F2). Vehicle-treated control animals showed no significant effects for the weight of the testes and the epididymides, suggesting the absence of treatment effects. No abnormal finding was observed in histopathological examination of testes and epididymides of rats of the highest-dose group.

In summary, TCDD exposure to male rats using loading-dose/maintenance-dose regimen suppressed body weight gain, but no effect was observed in either the fertility of the male rats or the sex ratio of their offspring. Fetal mortality was significantly elevated in the lowest-dose group.

References

1 Mocarelli, P., Brambilla, P., Gerthoux, P. M., Patterson, D. G. J. and Needham, L. L. (1996) Lancet **348**, 409

2 Mocarelli, P., Gerthoux, P. M., Ferrari, E., Patterson, J., D. G., Kieszak, S. M., Brambilla, P., Vincoli, N., Signorini, S., Tramacere, P., Carreri, V., Sampson, E. J. and Turner, W. (2000) Lancet **355**, 1858-1863

3 Ryan, J. J., Amirova, Z. and G., C. (2002) Environ. Health Perspect. **110**, A669-A701

4 Moshammer, H. and Neuberger, M. (2000) Lancet **356**, 1271-1272

5 Gomez, L. D., Marshall, T., Tsai, P., Shao, Y.-S. and Guo, Y. L. (2002) Lancet **360**, 143-144

6 Schnorr, T. M., Lawson, C. C., Whelan, E. A., Dankovic, D. A., Deddens, J. A., Piacitelli, L. A., Reefhuis, J., Sweeney, M. H., Connally, L. B. and Fingerhut, M. A. (2001) Environ.Health Perspect. **109**, 1127-1132.

7 Michalek, J. E., Rahe, A. J. and Boyle, C. A. (1998) Epidemiology **9**, 474-475

8 Krowke, R., Chahoud, I., Baumann-Wilschke, I. and Neubert, D. (1989) Arch. Toxicol. **63**, 356-360.

9 James, W. H. (1990) J. Theor. Biol. **143**, 555-564.

10 Jongbloet, P. H. and Groenewoud, H. M. M. (2002) Environ. Health Perspect. **110**, 1-3.

11 Selevan, S. G. and Lemasters, G. K. (1987) J. Occup. Med. **29**, 451-454.

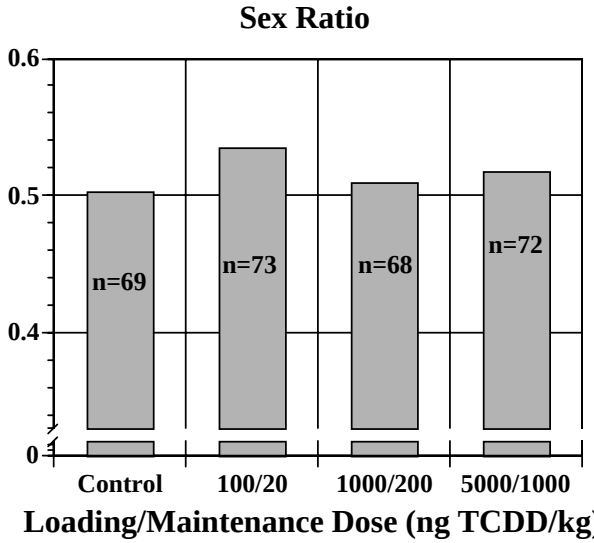

Fig. 1. Effect of paternal TCDD exposure using loading/maintenance dose regimen on sex ratio of offspring.

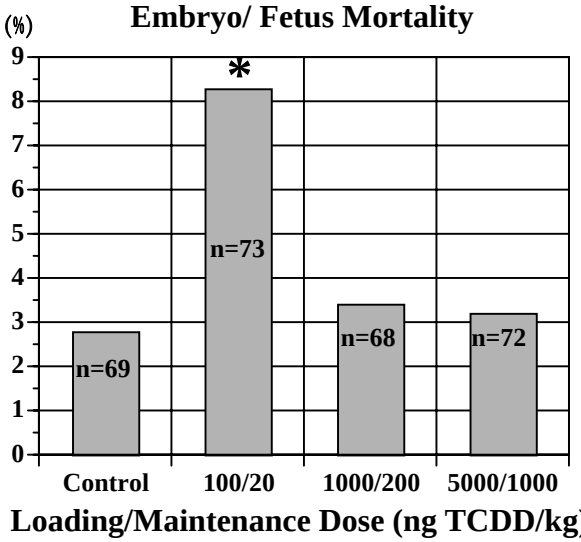

Fig. 2. Effect of paternal TCDD exposure using loading/maintenance dose regimen on embryo/fetus mortality.
* significantly different from control, $p < 0.05$ by Dunnett's multicomparison test.