The Transfer of 2,3,7,8-Tetrachlorodibenzo-p-dioxin into Eggs and Chicks following Exposure to Hens

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Introduction

Dioxins have been shown to exert reproductive and teratogenic effects in several strains of mice, rats, and chickens. We reported that *in ovo* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at less than 7.5 ng/egg on day 0 did not influence hatchability**,** whereas more than 10 ng/egg completely inhibited hatching. We also reported that maternal exposure to TCDD in Barred Plymouth Rock hens induced a reversible inhibition of egg laying. The hatchability of the eggs from TCDD exposed hens was significantly decreased and eggshell thickness was thicker than that from control hens 1. These results suggested that the TCDD in maternally exposed hens was transferred into eggs and induced embryo toxicity. Transfer of TCDD in eggs has been reported previously in foraging chickens 2,3 and ring-necked pheasants 4,5. The TCDD concentration in chicken eggs related to environmental exposure, especially contact with soil. The measurement of dioxins in eggs is important for assessing environmental contamination by dioxins and for humans because chicken eggs are one of the most popular food for humans. Measurement of TCDD concentration is generally performed by GC/MS method which is expensive and requires special equipment. Recently, a simple method for TCDD assay using enzyme-linked immunosorbent assay (ELISA) 6 and CALUX bioassay 7 has been reported.

The objectives of this study were, first, to determine the TCDD concentration in eggs by ELISA. Second, the transfer of maternally exposed TCDD into the egg, embryo and chicks was examined.

Materials and Methods

Materials and eggs:

TCDD in nonane (containing 10% toluene) was obtained from Wellington Laboratories (Ontario, Canada). Solvents were removed by evaporation and TCDD was dissolved in DMSO.

White Leghorn chickens were obtained from Hoshino Poultry Breeding Farm (Shizuoka, Japan) and maintained in Shizuoka Swine & Poultry Experiment Station under natural sun light (approximately 11 hr light-13 hr dark). To obtain fertile eggs, hens were crossed by artificial insemination.

Maternal exposure:

TCDD in DMSO diluted with corn oil (final DMSO concentration was 0.1%) were intramuscularly administered to White leghorn hens dosed at 10 ng/kg (10-ng group), 50 ng/kg (50-ng group) or 200 ng/kg (200-ng group) once a week from 14 to 23 weeks of age. In control, the vehicle (corn oil with 0.1% DMSO) was administered. Laid eggs were collected at 19, 21, 23, 26 and 29 weeks of age. Blood were collected from a wing vein at 19 and 23 weeks of age. Some of the hens were euthanized and abdominal adipose tissue was excised at 23, 26 and 29 weeks of age. Egg production (%) and body weight were measured during the experimental period.

In ovo exposure:

TCDD in DMSO was diluted with propylene glycol (final DMSO concentration was 0.1%). TCDD (2.5 ng/egg) or the vehicle was injected into the fertile egg white using 1 ml syringe with a 23 gauge needle on day 0, and incubated at 37.6°C with a relative humidity of 53% in a SHYOWA FURANKI incubator (model AH3)**.** The eggs were automatically turned once per hour. On embryo day 6 and 12, TCDD concentration in embryo and egg yolk was measured as described below.

Assay for TCDD concentration:

Boiled egg yolk (1-3 g) was dried for 120 min at 100°C. The fat was extracted using a Soxhlet extractor (MITAMURA RIKEN Ltd.) with diethyl ether (70 ml) by boiling for 36 min and recycling for 60 min. Fat extract, adipose tissue (1-2 g) and plasma (1 ml) were decomposed with 1 M KOH/EtOH (10 ml) overnight and TCDD was extracted with 40 ml of n-hexane. Hexane layer was applied on the three-layer silica-gel column (Na2SO4, Silica-gel, 44% H2SO4 Silica-gel) and TCDD was eluted with n-hexane. Hexane was removed under N2

and residue was dissolved with sample buffer included in ELISA kit. TCDD concentration was measured using ELISA kit (Immunoeco DXN, COSMO BIO Ltd., Japan) with 2,3,7-trichloro-8-methyldibenzo-p-dioxin (TMDD) as a standard.

Results and Discussion

Extraction of TCDD from egg yolk:

To determine TCDD concentration in egg yolk, TCDD was extracted with diethyl ether and cleaned up by three-layer silica-gel column. All extraction procedures were done in one day. The recovery of TCDD (100 pg/g-yolk, 1 μg/g-yolk) was over 80%. A limit of detection was 1.7 pg/g-yolk. This extraction method seems to be more simple and certain than previous methods. Sugawara et al. reported that a fairly good correlation between immunoassay using Immunoeco DXN, and GC/MS was achieved for human milk by comparing TEQ values 6. We will examine the correlation by TEQ values between dioxin egg concentrations determined by ELISA and those determined by GC/MS.

Maternal exposure:

During the exposure period, each hen was administered a cumulative dose of 132, 660 and 2,560 ng TCDD in 10-ng, 50-ng and 200-ng groups, respectively. The body weight in TCDD treated groups tended to be higher than that in the control group but not significant. Egg laying started at 15 weeks of age in all groups. The onset of 50% egg production in the control group was 18 weeks of age. TCDD exposed hens were premature in a dose-dependent manner. The onset of 50% egg production in 200-ng group was 16 weeks of age. However, there was no significant difference in the change of egg production (hen day %) by age in all groups. Absorbed TCDD will be incorporated in very low density lipoprotein (VLDL), the major lipoprotein in chicken plasma. VLDL has crucial roles in the development of yolk. It is unclear whether VLDL containing TCDD affects yolk development.

To determine the TCDD concentration in egg from TCDD exposed hens, values measured by the ELISA kit were corrected by dividing with 1.74, because cross reactivity of TCDD with TMDD was 174%. In control, the TCDD concentration in eggs was 0.038 ng/yolk. TCDD concentration in eggs from the 10-ng, 50-ng and 200-ng group hens at 23 weeks of age was 0.82, 3.18 and 8.32 ng/yolk, respectively. TCDD concentration in eggs was increased by TCDD

exposure in a dose-dependent manner. However, the TCDD concentration in laid eggs did not change by age (from 19 to 29 weeks) in laying hens of each group (Figure 1). Both plasma and abdominal adipose tissue TCDD concentration from hens at 23 weeks of age was increased by TCDD exposure in a dose-dependent manner. TCDD concentration in abdominal adipose tissue at 23 weeks of age was 0.53, 3.51 and 6.55 ng/g-fat in 10-ng, 50-ng and 200-ng group, respectively.

These results suggest that TCDD exposed in hens was stored in adipose tissue and transferred into the eggs for a long period at a constant concentration.

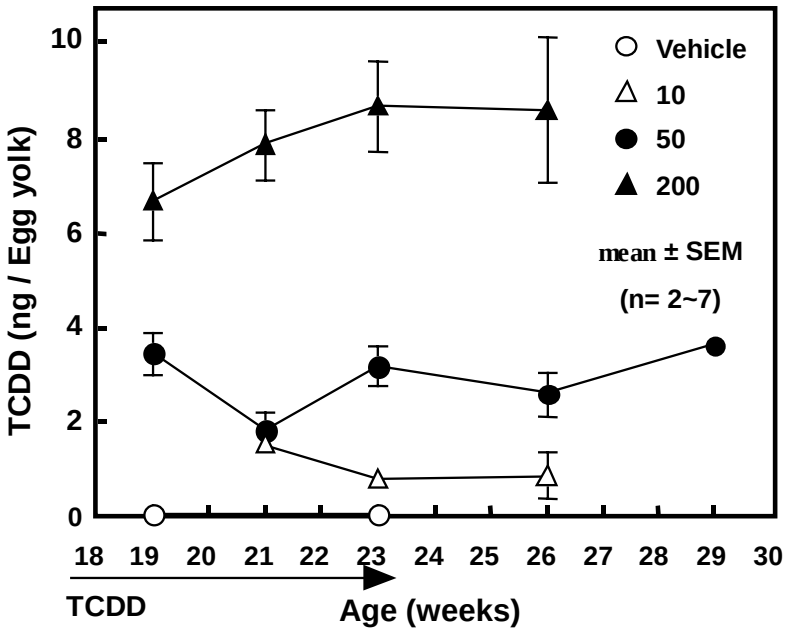

Figure 1 TCDD concentrations in the yolk of eggs layed by TCDD-exposed hens
White Leghorn hens were administered to TCDD (*i.m.*) from 14 to 23 weeks of age. TCDD concentration in the egg yolk was determined by ELISA.

In ovo exposure:

TCDD 2.5 ng was injected into the fertilized egg on day 0. TCDD concentration in egg yolk at embryo day 6 was 647 pg/yolk (26% of dosed TCDD). TCDD concentration in the embryo was 48.5 pg (1.9%) and 93.1 pg

(3.7%) at embryo day 6 and 12, respectively. These results suggest that *in ovo* injected TCDD enters the egg yolk slowly and is transferred to embryo gradually from early stage of development. In other experiments, we observed that about 50% of *in ovo* injected TCDD on day 0 remained in the chick abdominal egg yolk at hatching. This TCDD will be absorbed into the chick within 2-3 days after hatching and can influence development of chick.

Conclusion

In conclusion, a simple method for the assay of TCDD in chicken eggs was established. This method could be useful for the detection of egg contaminated by dioxins. Further, maternally exposed TCDD was stored in adipose tissue and transferred into the egg yolk for a long period, and then into the embryo and chick gradually.

References

1. Matsushita S., Ikeda M., Yamashita J., Suzuki C., Tamura M., Setani K., Ikeya M., Tomita T. (2002) Organohalogen Compounds. 55 409.
2. Schuler F. and Schlatter C. (1997) Chemosphere. 34, 711.
3. Traag W., Portier L., Bovee T., Weg G., Onstenk C., Elghouch N., Coors R., Kraats C., Hoogenboom R. (2002) Organohalogen Compounds. 57, 245.
4. Nosek J. A., Craven S.R., Sullivan J.R., Olson J.R., Peterson R.E. (1992) J. Toxicol. Environ. Health. 35, 153.
5. Nosek J. A., Craven S.R., Sullivan J.R., Hurley S.S., Peterson R.E. (1992) J. Toxicol. Environ. Health. 35, 187.
6. Sugawara Y., Saito K., Ogawa M., Kobayashi S., Shan G., Sanborn J.R., Hammock B.D., Nakazawa H., Matsui Y. (2002) Chemosphere. 46,1471
7. Hoogenboom R., Portier L., Onstenk C., Polman Th., Hamers A., Traag W.A. (2002) Organohalogen Compounds. 45, 180.