DIFFERENT EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) AND NATURAL MIXTURE OF PCDDS/PCDFS ON HUMAN PLACENTAL AROMATASE ACTIVITY.

Augustowska, K.[1], Milewicz, T.[2], Grochowalski, A.[3], Chrząszcz, R.[3], Gregoraszczuk, EL.[1]

[1] Laboratory of Physiology and Toxicology of Reproduction, Dept of Animal Physiology, Institute of Zoology, Jagiellonian University, Krakow, Poland.
[2] Department of Endocrinology and Fertility, Collegium Medicum, Jagiellonian University, Krakow, Poland.
[3] Department of Chemistry, Krakow, University of Technology, Krakow, Poland.

Introduction

TCDD and related compounds elicit a diverse spectrum of toxic responses. They are able to pass through the human placenta (1). Taking into account solubility in fat of these compounds and the maternal origin of 10 to 20% of foetal fatty acids, polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) may impair foetal development (2). The human placenta expresses high levels of aromatase activity and thus regulates the balance of estrogens in the uterus. An alteration in aromatase function in the uterus has been shown to permanently affect human embryos (3). Studies reporting the effects of xenobiotics on aromatase activity have been much more limited. Moreover, so far, in many experiments concerning endocrine disruption effects or other undesired actions of dioxins and furans congeners were investigated in respect to particular congeners. Taking into account that in the environment PCDDs and PCDFs are present in the mixed form of all 17 toxic congeners as well as non-toxic congeners, we decided to experiment with the real standard DMSO solution obtained from the extraction and clean-up of a real fly ash, which is spread-out into the atmosphere from thermal, industrial processes. Therefore, the study should be focused on natural PCDDs/PCDFs mixtures which may be found in the human environment, to evaluate their potential action. This will help us understood their action in a natural mixture rather than of an individual congener, which never occurs in nature.

Material and Methods

1. Reagents

Medium M199, penicillin, trypsin, and calf serum were purchased from the Laboratory of Vaccines, Lublin, Poland. Testosterone, dehydroepiandrosterone, Antibiotic antymycotic solution (100x)- were obtained from Sigma Chemical Co., St. Louis, MO, USA. 2,3,7,8-TCDD solutions were prepared by the dilution of evaporated, concentrated toluene standard (Promochem) in DMSO. PCDDs and PCDFs natural congener mixture in DMSO was prepared by toluene Soxhlet extraction of 10 g of fly ash sample collected from the hospital waste incinerator and Alumina column cleaned-up according to Grochowalski (4). Concentrations of all 17 toxic congeners was reported and toxic equivalent (TEQ) was calculated as a 27,7 µg-TEQ/kg of fly ash. After extract clean-up using standard procedure on Alumina, the solvent was exchanged to DMSO to obtain the stock solution of a concentration of 10 ng-TEQ/ml. Work solutions were prepared by DMSO dilution of the stock solution to obtain appropriate PCDDs/PCDFs concentration just before adding to the culture medium.

Table 1. Individual PCDD/PCDF congener concentrations in the stock solution.

No:	Congeners	m_i [ng/ml]	m_i x TEF_i [ng/ml]
1	2,3,7,8-TCDD	0,28	0,277
2	1,2,3,7,8-PeCDD	1,74	0,871
3	1,2,3,4,7,8-HxCDD	2,93	0,293
4	1,2,3,6,7,8-HxCDD	4,53	0,453
5	1,2,3,7,8,9-HxCDD	5,03	0,503
6	1,2,3,4,6,7,8-HpCDD	27,58	0,276
7	OCDD	27,18	0,027
8	2,3,7,8-TCDF	1,14	0,114
9	1,2,3,7,8-PeCDF	3,54	0,177
10	2,3,4,7,8-PeCDF	6,69	3,346
11	1,2,3,4,7,8-HxCDF	9,20	0,920
12	1,2,3,6,7,8-HxCDF	8,98	0,898
13	2,3,4,6,7,8-HxCDF	13,64	1,364
14	1,2,3,7,8,9-HxCDF	0,83	0,083
15	1,2,3,4,6,7,8-HpCDF	33,41	0,334
16	1,2,3,4,7,8,9-HpCDF	5,70	0,057
17	OCDF	11,92	0,012
Summary concentration in ng-TEQ/ml		10,00	

2. Tissue

Placental tissues were collected in Kraków, Poland where the clinical information on pregnancy outcomes was gathered. Placental cotyledons were harvested immediately after expulsion of the placenta, placed in ice-cold PBS and transported to the laboratory.

The cells were prepared by a modification of Lobo et al., (5) method. Cells suspension were obtained by digesting pieces of placental tissue (20 g/100ml) in PBS containing 0.25 % trypsin, at 37 °C five times for 15-min. Pellets of cells after centrifugation were incubated with 500 U of DNAse for about 7 min at 37 °C. Finally, the cells were spun and resuspended in 24-36 ml of M-199 medium supplemented with 10 % calf serum, and plated one ml/well in 24 well plastic cell-culture plates (Falcon, Lincoln Park, NJ). The cultures were maintained at 37 °C in a humidified atmosphere of 5 % CO_2.

3. Experimental procedure

The activity of $P450_{arom}$ was measured by the conversion of testosterone (T; 10^{-7} M) or dehydroepiandrosterone (DHEA; 1 ng/ml) to estradiol. The net synthesis and secretion of estradiol to the culture medium was used as the indicator of aromatase activity. TCDD and natural PCDDs/PCDFs mixtures was added in doses of 10, 20, 40, 60 and 80 pg-TEQ/ml to the control, T or DHA treated cells. 48h later media were collected and frozen for estradiol estimation.

Estradiol concentration was determined radioimmunologically using Spectra kits (Orion, Diagnica, Finland), supplied by Polatom (Œwierk, Poland).

4. Statistical analysis

Significance of differences between the concentrations of steroids in the control and experimental cultures were compared by analysis of variance and by using Duncan's new multiple range test.

Results and Discussion

In the presented paper aromatase activity was measured by conversion of dehydroepiandrosterone (DHEA) to estradiol (E2) and testosterone (T) to estradiol (E2).

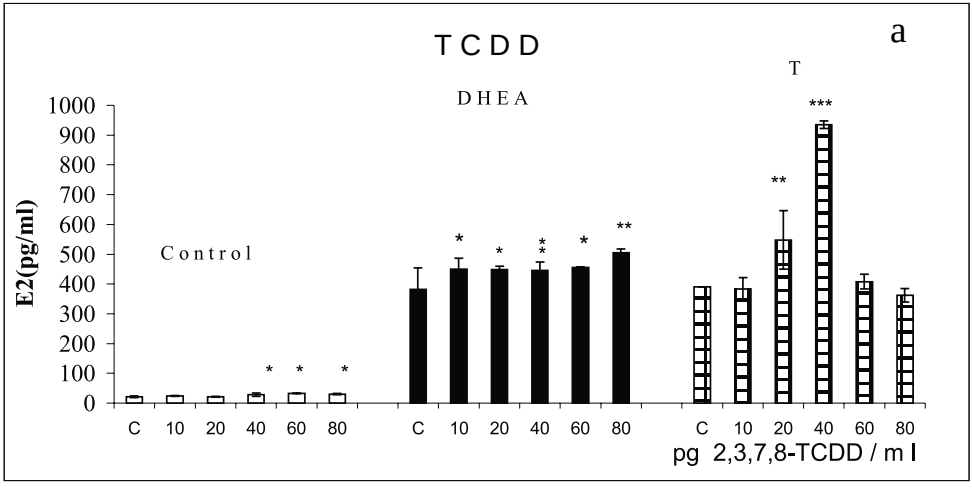

Figure 1a. The influence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the conversion of dehydroepiandrosterone (DHEA) and testosterone (T) to estradiol 17 ß (E2). * P<0.05

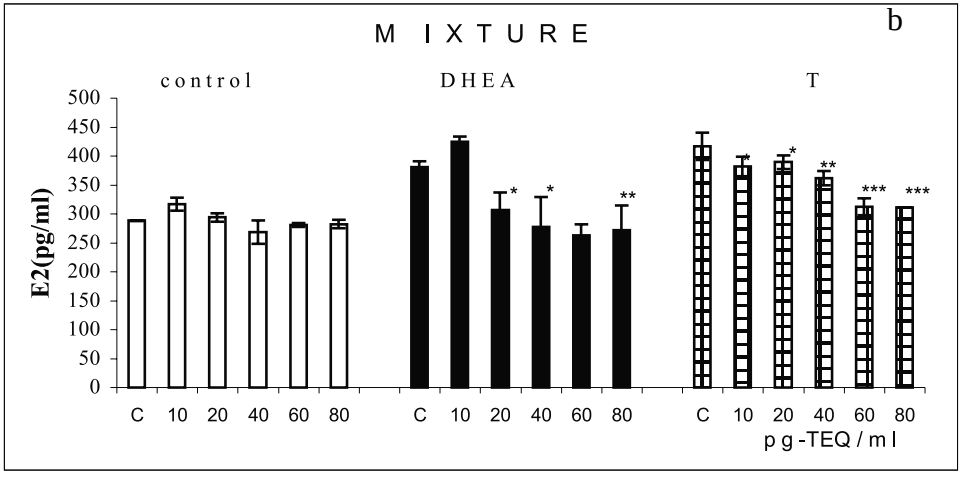

Figure 1b. The influence of mixture of dioxins and furans on the conversion of dehydroepiandrosterone (DHEA) and testosterone (T) to estradiol 17 ß (E2). * P<0.05.

DHEA is a substrate for E2 synthesis and derives from the foetal adrenal (6) due to the inability of the placenta to synthesise androgen. The present data showed different action of natural PCDDs/ PCDFs mixtures and pure 2,3,7,8-tetrachlorodibezo-p-dioxin on E2 secretion.

Humans are exposed to many chemical compounds, such as a mixture of dioxins and furans, rather than to individual chemicals. From a public health point of view, it is most relevant to answer the question of whether or not the components in a mixture interact in a way that results in an increase in their overall effect compared with the sum of the effects of the individual components. In the present data we noted different action of natural PCDDs/PCDFs mixtures on E2 secretion by the placental tissue.

TCDD in all doses used had small stimulatory effect on the conversion of DHEA to E2 and in doses 10, 20 and 40 pg/ml high stimulatory effect on the conversion of T to E2 by the placental tissue (Fig. 1a). Inhibition of both conversion of DHEA to E2 and T to E2 was noted under the influence of higher doses of the mixture. Taking into consideration that the regulation of placental E2 formation is a multifactoral process and different enzymes are involved: DHEA to A4 - 3â-HSD; A4 to T- 17 ß-HSD, and in the final step T to E2 - P450 arom, the presented result suggests possible inhibition not only of P450 arom but also of 3ß-HSD and 17ß-HSD under the influence of the natural PCDDs/PCDFs mixtures. Small influence of TCDD on DHEA conversion to E2 and high stimulatory effect on the conversion of T to E2 observed in the present study under the influence of TCDD suggested its action mainly through to the activation of P450 arom activity.

In light of data of Genti-Raimondi et al., (7) who showed that physiological doses of E2 had stimulatory effect on the conversion of pregnenolone to P4, however supraphysiological doses exhibited an inhibitory effect, both estrogenic action of TCDD and antiestrogenic action of the natural PCDDs/ PCDFs mixtures can be responsive for adverse pregnancy outcomes including intrauterine growth retardation (IUGR), congenital structural anomalies and cognitive developmental deficits. McMurry and Dickerson, (8) showed that a mixture of six different endocrine disrupters exerts very different effects from either or both mixture components, indicating the lack of predictability of chemicals when combined in a mixture. Chu et al. (9) indicated that the action of the mixture of PCBs and TCDD may be additive or antagonistic depending on the dose and endpoints measured. For this purpose of predicting mixture effects, knowledge of the mechanism of action and toxicokinetics is required.

Acknowledgements

The authors wish to thank Dr M Mika from the Department of Physiology, Academy of Agriculture, Kraków for radioimmunological determinations of steroid hormones. This work was supported partly by DS./IZ/2002.

References

1. Koppe J.G, Olie K, van Wijnen J. (1992). Dev Pharmacol Ther 18: 9-13.
2. Manchester DK, Jacobsy E. (1984). Teratology 30: 31-37.
3. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM (2001) Chemosphere 37: 2279-2291
4. Grochowalski A. (1998) Chemosphere 37: 2279-2291
5. Lobo JO, Bellino FL, Bankert L. (1985) Endocrinology 116:889-895
6. Somson ER, Mahendroo MS, Means GD, et al. (1981) Endoc Rev 15: 342-355
7. Genti-Raimondi S, Patrito LC, Flury A. (1983) Steroids 41:467-474
8. McMurry CS, Dickerson RL. (2001) Chemosphere 43: 829-837
9. Chu I, Lecavalier P, Hakansson H, Yagminas A, Valli V, Poon P, Feeley M.(2001) Chemosphere 43:807-14