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Environmental toxicants and effects on female reproductive function

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Abstract

One of the most toxic substances known to humans, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin), is also highly pervasive in the environment. It is created naturally in volcanic eruptions and forest fires, and anthropogenically in waste incineration, chlorination processes and certain plastics manufacture. From reports of large industrial and other accidents, or from experimental studies, dioxin exposure has been correlated in animal models and/or humans with chloracne of the skin, organ cancers, hepatotoxicity, gonadal and immune changes, pulmonary and other diseases such as diabetes, skewing of the sex ratio, and infertility. We have demonstrated that the aromatic hydrocarbon receptor (AHR) that binds dioxin in tissues is localized in zebrafish, rat and rhesus monkey (*Macaca mulatta*) ovaries and in rat and human luteinizing granulosa cells (GC) (among other tissues), that labeled dioxin is specifically localized to granulosa cells of the ovarian follicle as observed by autoradiography, and that incubations of GC or ovarian fragments with environmentally relevant concentrations (fM to nM) of dioxin inhibit estradiol secretion significantly. Our experiments show that in human, non-human primate, rat, trout, and zebrafish ovarian tissues, dioxin inhibits estrogen synthesis at some level of the steroid biosynthetic pathway, most likely by inhibiting transcription of mRNAs for or activity of side-chain cleavage (*Cyp11a1* gene) and/or aromatase (*Cyp19a1* gene) enzymes, or conceivably other steroidogenic enzymes/factors. Such an untoward effect on estrogen synthesis in females exposed to dioxin environmentally may predispose them to defects in aspects of their fertility.

Keywords

dioxin; environment; estrogen; female; ovary; reproduction; steroid synthetic enzymes; xenobiotic

INTRODUCTION

The premise that there exist man-made substances that accumulate in the environment and which are responsible for reproductive and developmental deficits observed in animals was brought to our attention vividly in the early 1960's by Rachel Carson in her ground-breaking book, *Silent Spring* [1]. The field of reproductive toxicology was ultimately created as a result of this and other work, and has advanced rapidly in recent years especially because of the

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widening public interest in environmental pollutants that are endocrine disrupting or that otherwise modulate in some way the reproductive system of animals and humans. Particularly in the past 10 years, researchers from various disciplines have lent their expertise to reproductive toxicology, hastening the development of this area. Physiologists, endocrinologists, molecular biologists, biochemists and others that work in the field of reproduction have begun to evaluate xenobiotic compounds and their effects on the reproductive axis (hypothalamus, anterior pituitary, testis and ovary, uterus and male sex glands). This review will focus on effects of environmental pollutants on female reproductive function.

Reproductive hazards in the environment

Reproductive deficits induced by estrogenic substances in the environment constitute one such recent concern. We have incurred large estrogenic exposures due to diethylstilbestrol (DES), plant or phytoestrogens, and other man-made estrogen-like chemicals [2]. There have been numerous reports that revealed high concentrations of contraceptive or menopausal estrogens in wastewater effluent in English rivers; and these may be responsible for alterations in fish sex ratios [3,4]. In addition, human populations carry PCBs due to diets high in fish heavily laden with these compounds [5]. Certain PCBs, although only loosely linked to diminished fertility rates in humans, are quite strongly documented with regard to reproductive impairment in birds, fish and mammals [6]. The pesticide DDT, which is still used in developing countries, has been shown to exert deleterious effects on wildlife populations, and might be linked to breast and other cancers in women exposed to this pesticide as long as 20 years ago [7–9].

Some Great Lakes' fish show reduced gonadal weights, sex steroid hormone concentrations, and survivability [10], due potentially to the wood-derived phytoestrogen β -sitosterol, dioxins, PCBs, *etc.*[11]. Whether these effects are the result of endocrine disruption is not yet clearly established, as lake trout contaminated with PCBs were fed to rats, resulting in few endocrine changes except for subtle alterations in aromatase activity (which converts androgens to estrogens) [12].

Regarding other reproductive toxicants, Baldrige showed reduced numbers of small preantral follicles and of mid- and large-sized antral follicles with the PCB mixture Aroclor 1016; and that this effect was negated with thyroxine supplementation only in the preantral follicles, and “reversed” signs of atresia/apoptosis in the smallest antral follicles [13]. Similar effects were observed with ammonium perchlorate (found in explosives and rocketry materials) [14]. Dioxins and β -naphthoflavone (BNF, both AHR agonists) reduced vitellogenin synthesis in fish primary hepatocyte cultures [15] and demonstrated a negative correlation between cytochrome P450 1A1 (Cyp1a1)-inducing capability (a prominent biomarker of dioxin/AHR agonist action) and anti-estrogenicity. This fits with our results where presumed greater Cyp1a1 activity with increasing BNF dosage administered to rainbow trout resulted in reduced circulating E₂ concentrations in a dose-dependent manner at 48 h of exposure [16], but without affecting genes involved in immune regulation (a typical sequela of AHR agonism) [17].

It is tempting to overstate the contention that all environmental compounds exert a negative effect on physiologic systems in animals. However, the effects observed are complex and dependent upon the compound evaluated, whether it is in a binary or other mixture, whether its concentration is environmentally relevant (*i.e.*, similar to that found naturally), the route of exposure/administration, the animal model evaluated, the experimental paradigm used (*e.g.*, *in vitro*, *in vivo*), *etc.*

Although our laboratories have investigated effects of endogenous and xenobiotic estrogens for almost 25 years, we have since about 1993 evaluated other environmental molecules and toxicants that appear to alter the ER-signaling pathway (*e.g.*, dioxin) and thereby modulate

ovarian function and female reproduction generally. Herewith, we have attempted to discern a role(s) for alterations in ER signal transduction or in estrogen biosynthesis in the untoward effects observed on fertility and more subtle indices of reproductive capacity. A drop in circulating estrogen or in its action may be responsible for the diminution in fertility that is characteristic of captive and wild animals exposed to certain toxicants such as TCDD [18].

TCDD has been referred to as the most toxic substance known to man and is produced as a by-product of waste incineration (personal now more so than municipal), herbicide overuse, paper chlorination, polyvinylchloride plastics production, *etc.*, and we are exposed to dioxin-containing animal products via our daily diet [18,19]. Aside from acting as a teratogen, embryotoxin, carcinogen (the Report on Carcinogens [20] listed TCDD as “known to be a human carcinogen”), tumor progressor, and innate and adaptive immune function suppressor, TCDD is a known endocrine disrupter in laboratory species, wildlife, and from the data from several accidental exposures, in humans [18]. We have demonstrated attenuated secretion of ovarian estrogen, reduced numbers of large preovulatory ovarian follicles and altered expression of ovarian mRNAs for ER and AHR in female peri-pubertal rat pups after *in-vivo* administration of TCDD to pregnant dams [21,22]. These results on reduced E₂ secretion and reduced antral follicle numbers are now corroborated by several recent reports [23–25]. We have more recently demonstrated that TCDD reduces *Cyp11a1* and *Cyp19a1* mRNA copy numbers in rat after equine chorionic gonadotropin (eCG)-induced ovarian superstimulation using competitive RT-PCR [26]; although another group has suggested little effect of TCDD on human GC *CYP19a1* [24,27]. Regardless, the available evidence (especially *in vitro*) strongly supports the ovary as a major target of TCDD action [18,28,29]. The putative mechanism for this action is described below.

Mechanism for TCDD action

Most of the biologic effects of TCDD are mediated upon binding to a high-affinity cytoplasmic receptor, the AHR (refer to Figure 1 below) [30]. Binding of ligand to cytosolic AHR (roughly 800 amino acids long, a ligand-inducible transcription factor and member of the PER-ARNT-SIM [PAS] transcription factor family) induces allosteric modifications in the AHR, and the receptor-ligand complex undergoes conformational changes including dissociation of two heat-shock proteins (HSP-90s) and an AHR-interacting protein (AIP), and/or other protein(s), depending upon cellular paradigm. Ligand-bound AHR is then translocated to the nucleus where it further complexes with the resident ARNT (or hypoxia-inducible factor [HIF] 1 β) protein and, in certain cell types, the retinoblastoma protein (RbP, [31]). This heterodimer forms a transcriptional complex at the AHRE (TNGCGTG DNA motifs, consensus binding sequence underlined) in the regulatory region of several target genes (*e.g.*, cytochromes P450 *Cyp1a1*, *Cyp1a2*, *Cyp1b1*) and some phase II detoxifying enzymes, inducing gene transcription. White and Gasiewicz [32] reported that the ER structural gene (*Esr*) contains several AHREs, and therefore may be a site of interaction (“crosstalk”) between signaling pathways; *e.g.*, Ohtake *et al.* have shown that unliganded ER can be affected by liganded AHR agonistically [33]. To complicate matters further, it appears that estrogen also modulates the cellular response to TCDD. In MCF-7 cells (human breast carcinoma), TCDD inhibited estrogen-induced cathepsin D (*CTSD*) expression whereas TCDD induction of *CYP1A1* was inhibited by estrogen [34]. Tian *et al.* [35] reported that TCDD down-regulates *Esr1* mRNA in both the liver and ovary of female CD-1 mice with the greatest effect in the ovary. Therefore, there does appear to exist reciprocal receptor crosstalk. This cross-talk may predispose effects on fertility.

Dioxin and female fertility

We have chosen to focus our energies therefore on the reproductive effects of dioxins. Recent reports have concentrated on TCDD’s reproductive and antiestrogenic effects. In rats, TCDD

has been shown to interfere with the maintenance of pregnancy, fetal growth, and development, and fecundity and fertility parameters [36–38]. In primates, TCDD exposure resulted in significant hormonal alterations, failure of female rhesus monkeys to conceive, and was correlated with increased severity of endometriosis [39,40]. Umbreit and Gallo suggested that TCDD's effects on reproductive functions are due to its effects on the actions of estrogens [41]. The antiestrogenic effects of TCDD were observed as a reduction of ER levels in immature mice [42]; TCDD reduced basal and E₂-stimulated ER in rats [43–45]. In MCF-7 cells, TCDD has also been noted to reduce ER levels [46]. Others, however, have shown that the effects of TCDD on ER are both species and tissue specific, and dependent upon the age and hormonal status of the animal [47,48].

Petroff *et al.* [49] using an immature rat model showed *in vivo* that E₂ administered locally antagonized the TCDD effect of modulating ovulation. And it now appears that TCDD *in vivo* reduces the number of large preantral/antral ovarian follicles, and that this may be the reason for the reduced peripheral E₂ concentrations [21,22]. (Interestingly, this effect is also shown with AHR null or “knockout” mice; *i.e.*, a 50% drop in the number of pre-antral/antral follicles in the ovaries of deficient mice at 53 days of age [23]). Genetic inactivation of AHR may emulate administration of TCDD, as we observe a down-regulation of *Ahr* mRNA with TCDD administration [50]. And although 2 µg/kg TCDD was shown to increase the number of days to first litter in Siberian hamsters [51], no long-term fertility defect was observed in this model. Finally, nM TCDD significantly reduced P₄ accumulation into culture medium by porcine luteal cells [52]. Recent work showed an overall reduction in ovarian cAMP accumulation with TCDD *in vivo*, and a reduced binding of FSH and hCG to rat GC [25]. This implies a local inhibition by TCDD in reducing ovarian response, which is further supported by the attenuated follicular development and diminished pre-antral/antral follicle numbers noted above and observed by ourselves and others [21,22,25].

In studies that followed up those of Peterson and others [53], we have demonstrated a reduction in circulating estrogen by about 50% in female rat pups after maternal oral administration of 1 µg TCDD/kg body mass [21]. This is accompanied by an increase in pup ovarian and uterine *Esr1* mRNA, some diminution in mRNA for β-subunit of FSH (*Fshb*), and changes in ER and presumably in estrogen-signaling pathway function observed in hypothalamus and pituitary. Dasmahapatra *et al.* showed increased steady-state *Esr2* mRNA with TCDD in rat GC, and some augmented metabolism (hydroxylation) was expected because of increases observed in *Cyp1b1* [54]. We have shown that the presence and expression of *Ahr* and *Arnt* mRNAs correlated with changes in the rat estrous cycle [50]; and have localized them to monkey ovary and hLGC [55]. An index of AHR activation is increased *Cyp1a1* and *Cyp1b1* messages and proteins [54,56]. We have also very recently demonstrated topographic localization of presumed dioxin effect by using autoradiography of rhesus monkey ovaries. The ovaries bound radiolabeled dioxin in the granulosa cells and oocytes of their antral follicles, and this specific binding could be blocked by the addition of 200-fold excess of the AHR antagonist, α-naphthoflavone [57]. TCDD also appeared to inhibit mid-sized ovarian follicle growth, but did not accomplish this by enhancing GC apoptosis in the rat [22]. And with regard to effects on embryonic development, TCDD did not retard the development of early mouse morulae to blastocysts, and did not alter degree of apoptosis of these early pre-implantation embryos [58]. Other types of *in-vitro studies* allowed for more profound experimental manipulation.

Similar to the studies with incubated rat GC, we used cultures of GC aspirated from ovarian follicles in women participating in *in-vitro* fertilization protocols [59,60], and attempted to delve deeper into the mechanism(s) whereby ovarian estrogen secretion was reduced. Heimler [59] showed a dramatic reduction in GC secretion of E₂ *in vitro* by 8 h of culture with as little as 3.1 pM TCDD. This reduction was prevented with the inclusion of an aromatizable substrate, A₄, at 10⁻⁷ M, suggesting that aromatase activity *per se* was not affected, but rather provision

of the androgen precursor (*cf.* Figure 2). More recently, we have demonstrated that fM concentrations of TCDD (the lowest yet reported) substantially reduced E₂ output (Baldrige *et al.*, unpublished observations; Figure 3) by hLGC and increased inhibin A secretion [60]; this may be the reason why we and others have observed a diminution in *Fshb* mRNA [61] or in its receptor (indicative of FSH responsiveness) [29], as inhibins feed back negatively on pituitary FSH secretion. Moran *et al.* showed in 2000 and 2003 that indeed in hLGC cultured with hCG long term, CYP17A1 activity (which provisions androgens) was compromised, and that this is a locus of major effect in the steroidogenic pathway in their system [24,27]. However, it certainly appears that the effect is model dependent, as we have shown a diminution in *Cyp19a1* mRNA and activity and *Cyp11a1* mRNA in rat [26]. Therefore, it is clear that there is species dependence in dioxin effects, although the diminution in ovarian estrogen secretion is a common thread. We have now shown repeatedly that estrogen is attenuated in several animal systems: human LGC [59], rat [21,26], trout [16] and zebrafish [62]; and we showed recently that cultures of ovarian fragments from rhesus monkey ovary manifested reduced secretion of E₂ after 48 h of culture [63] (Figure 4). We are currently further evaluating in rat the promoter regions within the genes of the two cytochromes involved in order to understand the potential regulation of steroidogenesis by TCDD [64,65]. To bring our *in-vitro* studies full circle, we have also been developing an *in-vivo* zebrafish model.

We instituted the use of an *in-vivo* model, that of the genetically well-characterized zebrafish. A major advantage here of this relatively simple *in-vivo* model is the ability to apply sublethal exposure over the lifetime of the animal. We orally exposed adult female zebrafish to 0.08 to 2.16 ng TCDD/fish/day and observed accumulation of TCDD in a dose- and time-dependent manner in all tissues investigated but brain (1.1 – 36 ng/g) [66]. The ratio of ovarian to body mass (ovosomatic index) was reduced with TCDD concentrations of 0.6 ng/g fish or above; and 10% of females showed ovarian necrosis with 3 ng/g. The lowest observed adverse effects level (LOAEL) for female zebrafish was 0.6 ng/g fish, and the LOAEL for effects in their offspring was 1.1 ng/g fish. The maternal transfer of the TCDD induced larval toxicity similar to blue sac syndrome, which is a disorder of the nutrient-rich yolk sac of developing fish. Although zebrafish are overall less sensitive to dioxin than are other fish, their sensitivity may actually be more similar to that of mouse, rat, and human [66].

Egg production in zebrafish was decreased by over 50% following exposure to 40 and 100 ng/g in the diet for 5 days, and spawning success (females able to reproduce) was decreased by up to 96% [62]. Serum E₂ was also reduced more than two-fold, which is very similar to that observed for other animal/human/*in-vitro* model systems. Following 15 days of dietary exposure to 0.08 to 0.8 ng TCDD/fish/day, we also evaluated the expression of genes important in estrogen synthesis and signaling. TCDD exposure decreased significantly mRNA abundance for FSH receptor, LH receptor, *Esr1*, *Esr2a* and *Esr2b*, and greatly diminished *Star* (which translocates cholesterol to the mitochondrion), *Cyp19a1*, activins and epidermal growth factor receptor. There was a reduction in the total numbers of follicles after 20 days at 100 ng/g TCDD and a dose-dependent increase in atretic follicles. Fewer large or vitellogenic follicles were seen with treatment and there were significantly fewer secondary growth follicles; vitellogenic follicles only remained in the lowest treatment group. Although large deficits were incurred in the ovary with TCDD, whether the defect is entirely at this level is controversial.

We expect that to a great extent the steroidogenic defect is at the ovarian level, but our and others' studies do not preclude effects at additional loci within the reproductive axis [49,61, 67]. Therefore, effects could be exerted at multiple levels. To this end we have shown subtle effects on *Fshb* mRNA in pituitary but not on dimeric LH or FSH in circulating blood [61]. We have to date not observed effects of TCDD *in vitro* on pulsatile release of gonadotropin-releasing hormone (GnRH) from hypothalamic explants in rat, nor on LH or FSH secretion from hemi-pituitary cultures [68,69]. Others have shown that TCDD alters pituitary

responsiveness to GnRH from the hypothalamus [70]; and may modulate action of Star [71], and/or alter Cyp11a1 enzyme action [72]. Mutoh *et al.* [73] recently supported this by demonstrating that dioxin inhibited cholesterol translocation and steroidogenesis in rat by reducing synthesis of fetal brain LH and expression of testicular *Star*. In males this may be one mechanism of TCDD action at the steroidogenic pathway. Whether this holds for females also is unknown presently. Although several loci may be affected in subtle fashion by TCDD, it is obvious that ovarian steroidogenesis is greatly altered--we believe directly. A brief outline of steroidogenesis in the ovary follows.

Steroidogenic pathway

It is known that inner mitochondrial membrane of ovarian follicle thecal cells is the location of the Cyp11a1 and that cholesterol is transported there by Star through the peripheral benzodiazepine receptor channel in the outer mitochondrial membrane, and cholesterol is then cleaved to P₅ (refer to Figure. 2). TCDD inhibited rat testis steroidogenesis by diminishing mobilization of cholesterol to Cyp11a1, a clear indication that a TCDD effect on Star protein is implicated in this model [71, 73]. Thecal smooth endoplasmic reticulum (microsomal fraction) is then the site for Hsd3b conversion of P₅ to P₄, and dehydroepiandrosterone (DHEA) to A₄; and for the conversion of P₅ to DHEA under Cyp17a1. A₄ ultimately diffuses across the basement membrane (*basal lamina*) to layers of mural granulosa cells (*membrana granulosa*), where it is aromatized by Cyp19a1 to estrone and interconverted with E₂ via Hsd17b1 and Hsd17b2.

Hypothesis for TCDD action

Our overall hypothesis has been that TCDD exerts an anti-reproductive effect by binding AHR and affects ovarian steroidogenesis at two loci in the steroid biosynthetic pathway; *i.e.*, at the level of Cyp11a1 and Cyp19a1, specifically. We have previously evaluated several of the mRNAs/enzymes involved in the steroidogenic pathway outlined below in Figure 2.

We have observed no effects of TCDD on *Cyp17a1* or *Hsd3b1* mRNA copy number in rat GC (refer to Fig. 2), but possess unique data that *Cyp11a1* and *Cyp19a1* mRNA copy numbers are drastically reduced in GC from rats that are either unstimulated (basal condition) or stimulated with exogenous gonadotropins (*e.g.*, FSH) in the presence of environmentally relevant concentrations of TCDD [26]. This is supported by our very recent zebrafish data, where both *Cyp11a1* and *Cyp19a1* mRNAs were also reduced with TCDD *in vivo* as ascertained using real-time RT-PCR [64]. Our aims have been to show effects using low-dose TCDD, and to dissect these effects at the molecular level.

Potential transcriptional effects

It is known that AHRE sequences function as transcriptional enhancers at the *Cyp11a1* gene [74,75]; however, AHRE-like sequences are also implicated in gene suppression in genes such as *pS2*, *Ctsd*, and *c-fos*. In each case, the AHRE sequence contains the four invariant nucleotides (the CGTC "core") required for AHR binding [5'-ACGTGNN(A/T)NNN(C/G)-3'], but contains one or more mismatches with the consensus sequence for functional activity (*i.e.*, dioxin responsiveness, [5'-(T/G)NGCGTG (A/C)(G/C)A-3']), and additional flanking sites (creating a 9-total-bp sequence) may play a role. Binding of the agonist-bound AHR to these "inhibitory" or "iAHREs" is thought to interfere with the binding of other positive transcriptional activating factors to nearby response elements (*e.g.*, steroid receptor co-activator [SRC]-1 to transactivation function [AF] sites, [76]), thus resulting in down-regulation of gene expression. Previous work also shows that AHR cooperates with an orphan nuclear receptor, Ad4BP/SF-1, to activate *Cyp11a1* gene transcription in ovarian GC [77], thereby providing more evidence of transcriptional effects of TCDD. We are currently evaluating in our zebrafish model the *in-vivo* effects of dioxin and searching steroidogenic

enzyme promotor regions for these (i)AHRE's. Binding of dioxin/AHR/ARNT to these (i) AHREs may constitute a dosage effect and result in gene activation or repression, but would not preclude other mechanism(s) of TCDD action on estrogen secretion.

The above findings are primarily descriptive and much research is still needed before we understand the molecular regulation of reproductive function by dioxin and other AHR agonists. We summarize our overall recent findings as follows.

SYNOPSIS

The ovaries of several species bind TCDD via the AHR in the GC primarily although certainly not exclusively. Ovarian E₂ secretion is diminished following TCDD (or other TCDD-like molecule) exposure *in vivo* (rat, trout and zebrafish) and *in vitro* (human and rat GC and monkey ovarian fragments). Biochemically, this deficit is manifested in the disruption of the steroidogenic pathway, resulting in attenuated secretion of estrogen particularly. *In-vitro* work suggests less of an effect at the pituitary or hypothalamus, but rather a direct, local, *i.e.*, ovarian, effect. In several model systems, this impairment is at the level of transcription of several steroid-synthetic enzymes, *Cyp11a1* and *Cyp19a1*, for example. While the biochemical effects can certainly differ with respect to dose, duration, animal, or model system, we believe that the resultant inhibition of estrogen synthesis is then responsible for the reduced fertility of animals or humans exposed to dioxins in the environment.

The significance of this recent work is the focus on very low levels of the "real-life" toxin TCDD to which animals and women are exposed during their reproductive years; encountering TCDD in their surrounding environment from food, air, water, chemicals, accidental exposures, *etc.* Evidence suggests that environmental pollutants such as dioxins can engender fertility-disrupting deficits in animals and humans. Further research is expected to continue to address the significant problem of potential reproductive hazards posed to women and animals by TCDD and other noxious environmental chemicals.

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ABBREVIATIONS

A₄	androstenedione
AHR	aromatic hydrocarbon receptor
AHRE	AH-responsive element
ARNT	AHR nuclear translocator protein
<i>Cyp</i>	rat (lower case italicized)
human	upper case italicized) cytochrome P450 gene, cDNA, and mRNA
<i>Cyp11a1</i>	

P450 C_{20,22}-side-chain cleavage gene, cDNA, and mRNA

Cyp11a1 (non italicized)

enzyme protein

Cyp19a1

P450 aromatase gene, cDNA, and mRNA

Cyp19a1

enzyme protein

Cyp17a1

P450 C_{17,20}-lyase gene, cDNA, and mRNA

E₁

estrone

E₂

estradiol-17 β

Esr1

Esr2a, *Esr2b*, ER, estrogen receptor (α , β 1, β 2) genes, protein

ERE

estrogen-responsive element

Fshb

follicle- stimulating hormone gene

GC

granulosa cells

h

hours

hCG

human chorionic gonadotropin

hLGC

human luteinizing granulosa cells

Hsd3b

Hsd3b, 3 β -hydroxysteroid dehydrogenase

Hsd17b

Hsd17b, 17 β -hydroxysteroid dehydrogenase

PCB

polychlorinated biphenyl

P₅

pregnenolone

RT-PCR

reverse transcriptase-polymerase chain reaction

Star

Star, steroid acute regulatory protein gene, protein

TCDDtetrachlorodibenzo- *p*-dioxin**fM**

pM, nM, femto-, pico-, nanoMolar

References

1. Carson, RL. Silent Spring. Boston, MA: Houghton-Mifflin; 1962.
2. Whitten, P.; Naftolin, F. Prenatal Exposure to Environmental Toxicants: Developmental Consequences. Nedleman, HL.; Bellinger, D., editors. Baltimore: Johns Hopkins Univ. Press; 1994.
3. McLachlan JA, Korach KS. Proceedings of the Meeting: Estrogens in the Environment, III: Global Health Implications; Washington DC. Environ Health Perspect 1995;103(suppl 7):1.
4. White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Endocrinology 1994;135:175. [PubMed: 8013351]
5. Gerstenberger SL, Tavis DR, Hansen LK, Pratt-Shelley J, Dellinger JA. J Toxicol Clin Toxicol 1997;35:377. [PubMed: 9204098]
6. Hileman B. Chem Eng News 1993;19:11.
7. Milne D. J Natl Cancer Inst 1992;84:834. [PubMed: 1593649]
8. Taubes G. Science 1994;264:499. [PubMed: 8160007]
9. Safe SH. New Engl J Med 1997;337:1303. [PubMed: 9345081]
10. Van Der Kraak GJ, Munkittrick KR, McMaster ME, Porte CB, Chang. Toxicol Appl Pharmacol 1992;115:224. [PubMed: 1641856]
11. Cook PM, Butterworth BC, Walker MK, Hornung MW, Zabel EW, Peterson RE. Proc Soc Environ Toxicol Chem 1994;15:58.
12. Gerstenberger SL, Heimler I, Smies R, Hutz RJ, Dasmahapatra AK, Dellinger JA, Tripoli V. Arch Environ Contam Toxicol 2000;38:371. [PubMed: 10667936]
13. Baldrige MG, Stahl RL, Gerstenberger SL, Tripoli V, Hutz RJ. Reprod Toxicol 2003;17:567. [PubMed: 14555195]
14. Baldrige MG, Gerstenberger SL, Tripoli V, Stahl R, Hutz RJ. Reprod Toxicol 2004;19:155. [PubMed: 15501380]
15. Anderson MJ, Miller MR, Hinton DE. Aquat Toxicol 1996;34:327.
16. Hutz RJ, Wimpee BAB, Dasmahapatra AK, Weber DN, Heimler I, Chaffin CL. Zool Sci 1999;16:161.
17. Dasmahapatra AK, Wimpee BAB, Budberg KJ, Dorschner M, Phillips R, Hutz RJ. Marine Env Res 2000;50:147.
18. Hutz RJ. J Reprod Develop 1999;45:1.
19. Safe S, Krishnan V. Toxicol Lett 1995;82:731. [PubMed: 8597135]
20. Report on Carcinogens. Vol. 11. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program; 2005.
21. Chaffin CL, Peterson RE, Hutz RJ. Biol Reprod 1996;55:62. [PubMed: 8793059]
22. Heimler I, Trewin AL, Chaffin CL, Rawlins RG, Hutz RJ. Reprod Toxicol 1998;12:69. [PubMed: 9431574]
23. Benedict JC, Lin TM, Loeffler IK, Peterson RE, Flaws JA. Toxicol Sci 2000;56:382. [PubMed: 10910997]
24. Moran FM, Conley AJ, Corbin CJ, Enan E, VandeVoort C, Overstreet JW, Lasley BL. Biol Reprod 2000;62:1102. [PubMed: 10727284]
25. Roby KF. Endocrinology 2001;142:2328. [PubMed: 11356679]
26. Dasmahapatra AK, Wimpee BAB, Trewin AL, Wimpee CF, Ghorai JK, Hutz RJ. Mol Cell Endocrinol 2000;164:5. [PubMed: 11026553]
27. Moran FM, VandeVoort CA, Overstreet JW, Lasley BL, Conley AJ. Endocrinology 2003;144:467. [PubMed: 12538606]

28. Son DS, Ushinohama K, Gao X, Taylor CC, Roby KF, Rozman KK, Terranova PF. *Reprod Toxicol* 1999;13:521. [PubMed: 10613400]
29. Hirakawa T, Minegishi T, Abe K, Kishi H, Inoue K, Ibuki Y, Miyamoto K. *Endocrinology* 2000;141:1470. [PubMed: 10746652]
30. Wilson CL, Safe S. *Toxicol Pathol* 1999;26:657. [PubMed: 9789953]
31. Ge NL, Elferink CJ. *J Biol Chem* 1998;273(22):708.
32. White EK, Gasiewicz TA. *Biochem Biophys Res Comm* 1993;193:956. [PubMed: 8391813]
33. Ohtake F, Takeyama K, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, Tohyama C, Krust A, Mimura J, Chambon P, Yanagisawa J, Fujii-Kuriyama Y, Kato S. *Nature* 2003;423:545. [PubMed: 12774124]
34. Kharat I, Saatcioglu F. *J Biol Chem* 1996;271:10533. [PubMed: 8631852]
35. Tian Y, Ke S, Thomas T, Meeker RJ, Gallo MA. *J Biochem Mol Toxicol* 1998;12:71. [PubMed: 9443063]
36. Birnbaum LS, Tuomisto J. *J Food Addit Contam* 2000;17:275.
37. Kleeman JM, Moore RW, Peterson RE. *Toxicol Appl Pharmacol* 1990;106:112. [PubMed: 2251676]
38. Giavani E, Prati M, Vismara C. *Environ Res* 1983;31:105. [PubMed: 6851974]
39. Barsotti DA, Abrahamsom LJ, Allen JR. *Bull Environ Contam Toxicol* 1979;21:463. [PubMed: 109152]
40. Rier SE, Turner WE, Martin DC, Morris R, Lucier GW, Clark GC. *Toxicol Sci* 2001;59:147. [PubMed: 11134554]
41. Umbreit TH, Gallo MA. *Toxicol Lett* 1988;42:5. [PubMed: 2838937]
42. DeVito MJ, Thomas T, Martin E, Umbreit TH, Gallo MA. *Toxicol Appl Pharmacol* 1992;113:284. [PubMed: 1561637]
43. Romkes M, Piskorska J, Safe S. *Toxicol Appl Pharmacol* 1987;87:306. [PubMed: 3029898]
44. Astroff B, Safe S. *Toxicol Appl Pharmacol* 1988;95:435. [PubMed: 2847362]
45. Romkes M, Safe S. *J Appl Pharmacol* 1988;92:368.
46. Wang X, Porter W, Krishnan V, Narasimhan TR, Safe S. *Mol Cell Endocrinol* 1993;96:159. [PubMed: 8276131]
47. Hruska RE, Olson JR. *Toxicol Lett* 1989;48:289. [PubMed: 2781598]
48. White TEK, Rucci G, Liu Z, Gasiewicz TA. *Toxicol Appl Pharmacol* 1995;133:313. [PubMed: 7645028]
49. Petroff BK, Gao X, Rozman KK, Terranova PF. *Reprod Toxicol* 2000;14:247. [PubMed: 10838126]
50. Chaffin CL, Trewin AL, Hutz RJ. *Chem Biol Interact* 2000;124:205. [PubMed: 10728779]
51. Yellon SM, Singh D, Garrett TM, Fagoaga OR, Nehlsen-Cannarella SL. *Biol Reprod* 2000;63:538. [PubMed: 10906062]
52. Gregoraszuk EL, Wojtowicz AK, Zabelny E, Grochowalski A. *J Physiol Pharmacol* 2000;51:127. [PubMed: 10768856]
53. Mably TA, Moore RW, Peterson RE. *Toxicol Appl Pharmacol* 1992;114:97. [PubMed: 1585378]
54. Dasmahapatra AK, Wimpee BAB, Trewin AL, Hutz RJ. *Mol Cell Endocrinol* 2001;182:39. [PubMed: 11500237]
55. Chaffin CL, Heimler I, Wimpee B, Sommer C, Rawlins RG, Hutz RJ. *Endocrine* 1997;5:315.
56. Dasmahapatra AK, Trewin AL, Hutz RJ. *Comp Bioch Physiol Part B, Biochem Molec Biol* 2002;133:127.
57. Hutz, RJ.; Baldrige, MG.; Stahl, RL.; Hempeck, N.; Young, C. *Proc Congress Int'l Primatol Soc.* Torino, Italy: 2004.
58. Matthews M, Heimler I, Fahy M, Radwanska E, Hutz R, Trewin A, Rawlins R. *Fertil Steril* 2001;75:1159. [PubMed: 11384643]
59. Heimler I, Rawlins RG, Owen H, Hutz RJ. *Endocrinology* 1998;139:4373. [PubMed: 9751521]
60. Ho HM, Oshima K, Tompa D, Watanabe G, Taya K, Strawn EY, Rawlins RG, Hutz RJ. *J Reprod Develop* 2006;52:523.

61. Chaffin CL, Trewin AL, Watanabe G, Taya K, Hutz RJ. *Biol Reprod* 1997;56:1498. [PubMed: 9166703]
62. King Heiden T, Carvan MJ III, Hutz RJ. *Toxicol Sci* 2006;90:490. [PubMed: 16387744]
63. Conley, LK.; White, G.; Hutz, RJ. *Proc Soc Study Reprod*. Quebec City; Quebec, Canada: 2005.
64. King Heiden, T.; Struble, C.; Hessner, M.; Hutz, RJ.; Carvan, MJ, III. *Proc Soc of Toxicology*. San Diego, C. A., USA: 2006.
65. King, Heiden T.; Struble, CA.; Rise, ML.; Hessner, M.; Hutz, RJ.; Carvan, MJ. 7th Int'l. Conference on Zebrafish Development and Genetics; Madison, WI, USA. 2006.
66. King Heiden T, Hutz RJ, Carvan MJ III. *Toxicol Sci* 2005;87:497. [PubMed: 15901917]
67. Gao X, Petroff BK, Rozman KK, Terranova PF. *Toxicology* 2000;147:15. [PubMed: 10837928]
68. Trewin, AL.; Woller, M.; Ho, HM.; Hutz, RJ. *Proc Soc Study Reprod*. Ottawa, ON, Canada: 2001.
69. Trewin, AL.; Conley, LK.; Woller, M.; Hutz, RJ. *Proc Soc Study Reprod*. Baltimore, MD, USA: 2002.
70. Bookstaff RC, Kamel F, Moore RW, Bjerke DL, Peterson RE. *Toxicol Appl Pharmacol* 1990;105:78. [PubMed: 2168101]
71. Moore RW, Jefcoate CR, Peterson RE. *Toxicol Appl Pharmacol* 1991;109:85. [PubMed: 1645482]
72. diBartolomeis MJ, Moore RW, Peterson RE, Jefcoate CR. *Toxicol Appl Pharmacol* 1986;85:313. [PubMed: 3764917]
73. Mutoh J, Taketoh J, Okamura K, Kagawa T, Ishida T, Ishii Y, Yamada H. *Endocrinology* 2006;147:927. [PubMed: 16254025]
74. Bhatena A, Lee C, Riddick DS. *Drug Metab Dispos* 2002;30:1385. [PubMed: 12433808]
75. Baba T, Mimura J, Nakamura N, Harada N, Yamamoto M, Morohashi K, Fujii-Kuriyama Y. *Mol Cell Biol* 2005;25:10040. [PubMed: 16260617]
76. Beischlag TV, Wang S, Rose DW, Torchia J, Reisz-Porszasz S, Muhammad K, Nelson WE, Probst MR, Rosenfeld MG, Hankinson O. *Mol Cell Biol* 2002;22:4319. [PubMed: 12024042]
77. Morohashi K, Zanger UM, Honda S, Hara M, Waterman MR, Omura T. *Mol Endocrinol* 1993;7:1196. [PubMed: 8247022]

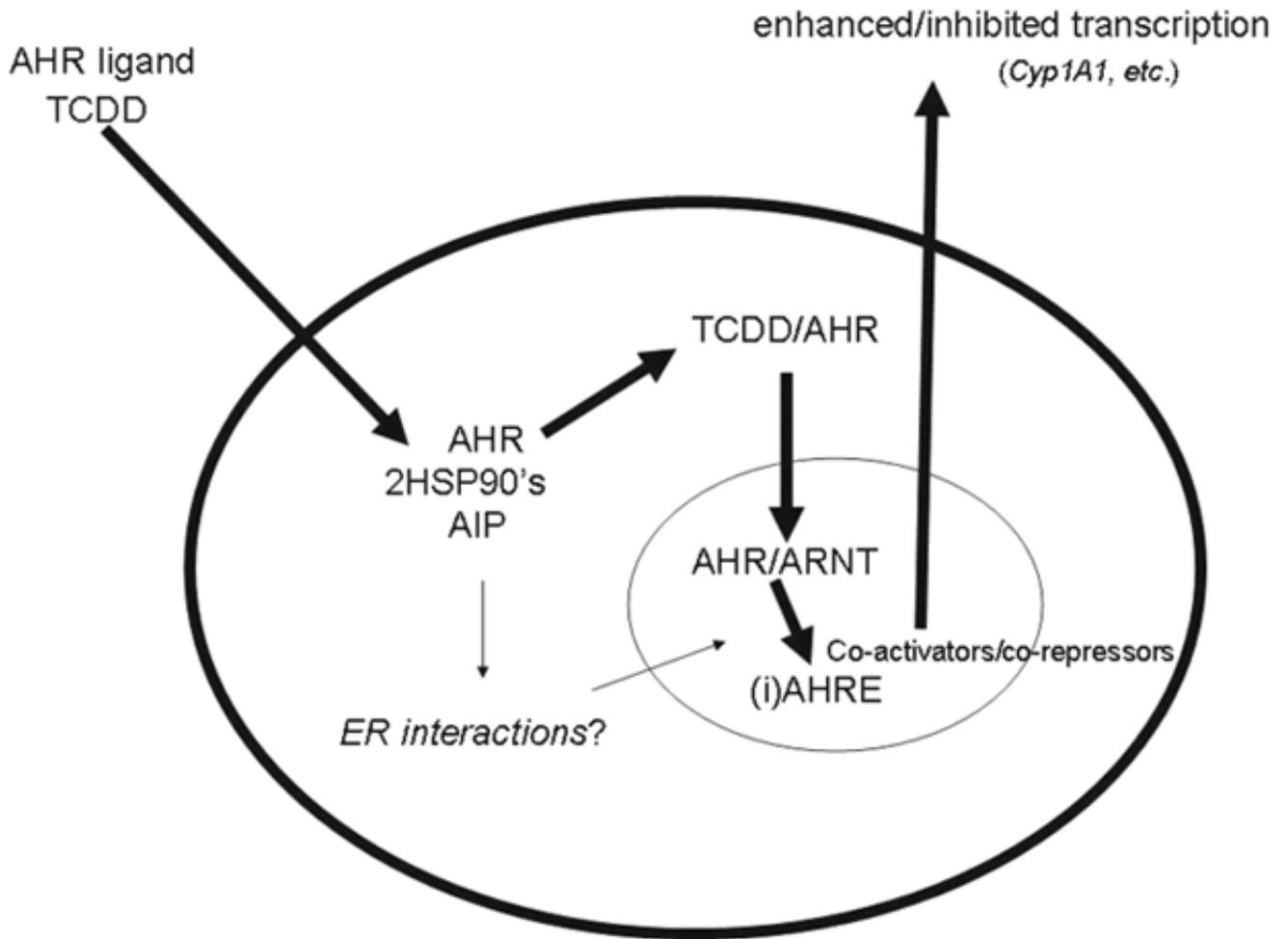


Figure 1. Simplified schematic of AHR activation by TCDD

TCDD binds the AHR in the cytoplasm, which displaces heat shock proteins and an AHR-interacting protein. TCDD-AHR translocates to the nucleus and is complexed with ARNT. This heterotrimeric complex binds AHREs or iAHREs and interacts with co-activators or co-repressors at the enhancer regions of DNA to achieve biologic effect (transcriptional activation or inhibition). Interactions with the ER may occur in multiple regions of the cell and this may represent a potential site of cross-talk between AHR- and ER-signaling pathways.

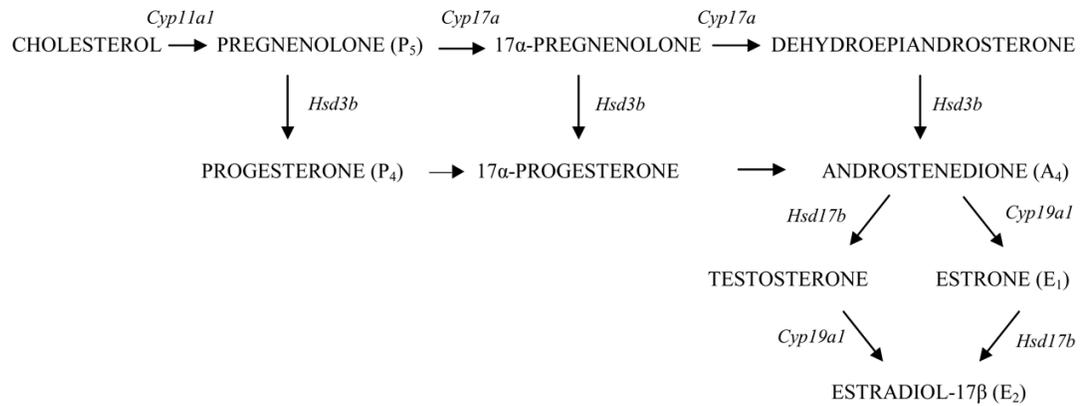


Figure 2. Brief schematic of the steroidogenic pathway to estrogens in ovarian GC

Cholesterol is transported to the inner mitochondrial membrane of ovarian follicle thecal cells by Star through the peripheral benzodiazepine receptor channel in the outer mitochondrial membrane, and cleaved to P₅. Thecal smooth endoplasmic reticulum is then the site for Hsd3b isozyme conversion of P₅ to P₄ and DHEA to A₄; and for the conversion of P₅ to DHEA under Cyp17a. A₄ ultimately diffuses across the basement membrane to layers of mural granulosa cells where it is aromatized by Cyp19a1 to estrone; and interconverted with E₂ via Hsd17b isozymes, as occurs between A₄ and testosterone.

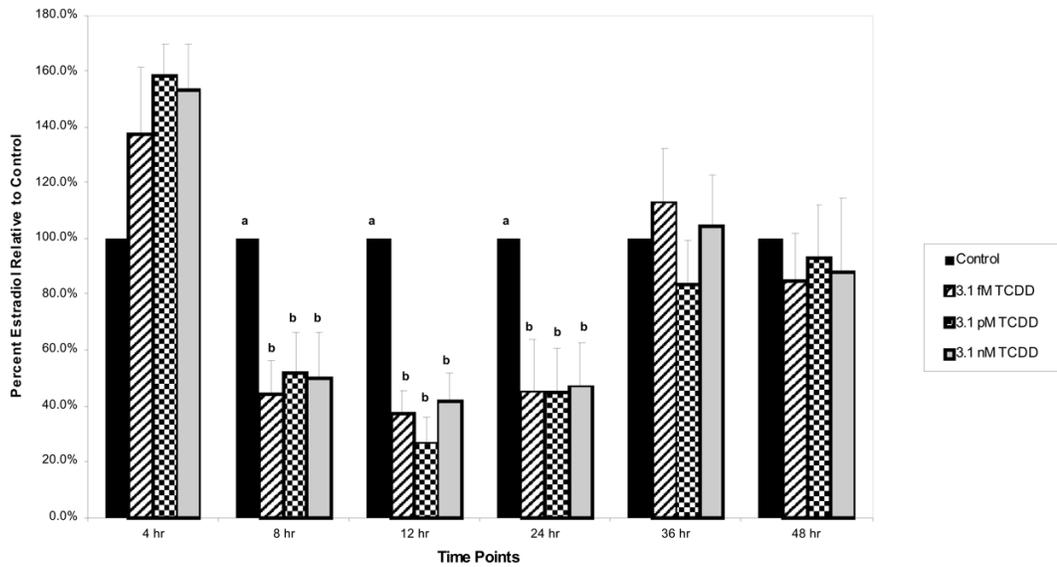


Figure 3. Effects of TCDD on E₂ secretion by human LGC

TCDD at fM, pM and nM concentrations caused a significant decrease in E₂ secretion at 8, 12 and 24 h in all treatment concentrations versus control. Data are presented as mean \pm S.E.M. Letters (a, b) denote significance among treatment groups, based on Tukey's *post-hoc* test ($P < 0.05$). Treatments sharing the same letter did not differ significantly. $N = 8$ for each treatment group.

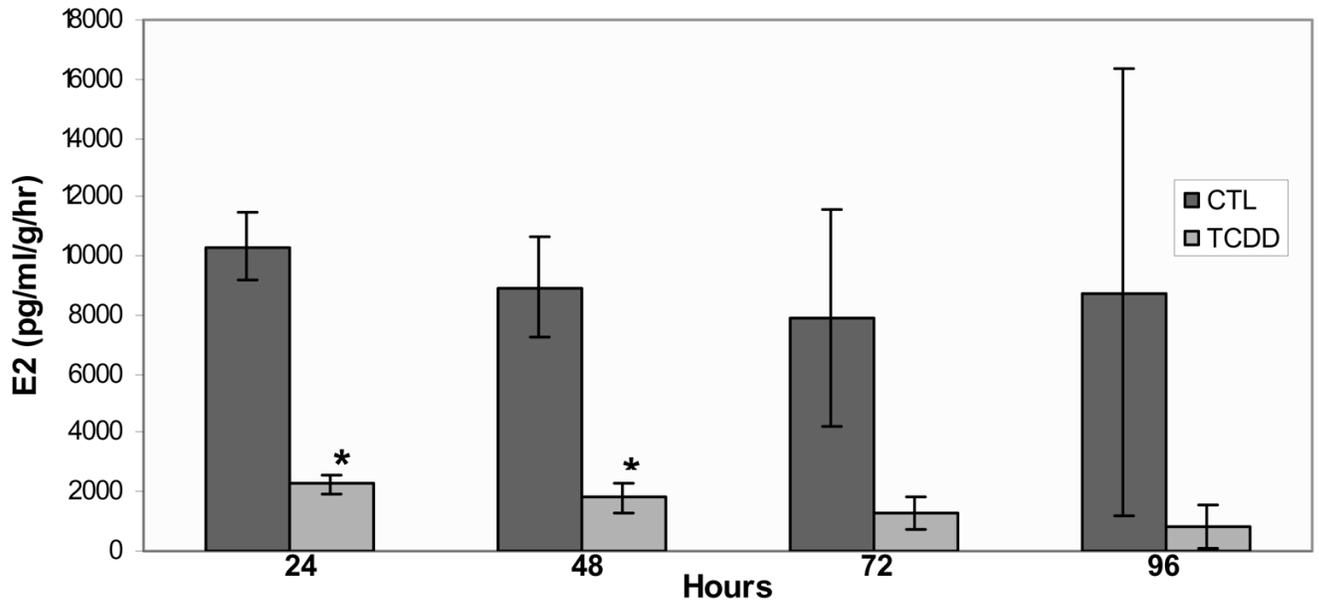


Figure 4. Comparison of average E₂ release noted in monkey ovarian fragments cultured with either control medium (CTL) or medium containing pM TCDD (TCDD)
 TCDD exposure at this environmentally relevant concentration markedly suppressed E₂ release at 24 (*, $p < 0.003$) and 48 (*, $p < 0.02$) h. Although marked suppression in E₂ release was also suggested at 72 and 96 h, differences between the CTL and TCDD cultures were not found to be statistically significant ($p = 0.07$ and $p = 0.20$, respectively). Rather, variability of response at these latter times increased dramatically. Data represent means \pm SE. “n” = 3 at 24, 48, and 72 h; “n” = 2 at 96 h.