Cancer Chemother Pharmacol (1997) 40: 239–244

ORIGINAL ARTICLE

Gulan Sun · Stephen Safe

Antiestrogenic activities of alternate-substituted polychlorinated dibenzofurans in MCF-7 human breast cancer cells

Received: 26 July 1996 / Accepted: 16 November 1996

Abstract *Purpose*: 1,3,6,8-Substituted alkyl polychlorinated dibenzofurans (PCDFs), typified by 6-methyl-1,3,8-triCDF (MCDF), inhibit 17β-estradiol (E2)-induced responses in the rodent uterus and human breast cancer cells. The major purpose of the experiments reported here was to determine the structure-dependent antiestrogenic activities of several alternate-substituted (1,3,6,8- and 2,4,6,8-) PCDFs. *Methods*: The antiestrogenic activities were determined in MCF-7 human breast cancer cells using two assays, that is E2-induced cell proliferation and induction of chloramphenicol acetyl transferase (CAT) activity in cells transiently transfected with the E2-responsive Vit-CAT plasmid. *Results*: MCDF (10^{-5} *M*), 6-isopropyl-1,3,8-triCDF, 6-ethyl-1,3,8-triCDF, 3-isopropyl-6-methyl-1,8-diCDF, and 6-methyl-2,4,8-triCDF, inhibited both E2-induced cell proliferation and CAT activity in MCF-7 cells. All of the remaining ten congeners inhibited either E2-induced cell proliferation or CAT activity, but not both responses. *Conclusions*: The antiestrogenic activity of the alternate-substituted PCDFs involves interactions between the aryl hydrocarbon and estrogen receptor signaling pathways. Although these compounds exhibited antiestrogenic activity in MCF-7 cells, the effects of individual congeners were response-specific, and there were no apparent structure-activity relationships.

Key words Alternate-substituted PCDFs · Antiestrogens

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatic hydrocarbons are industrial or combustion byproducts which have been identified as contaminants in the environment and in human adipose tissue, serum and milk [33]. TCDD induces a diverse spectrum of biochemical and toxic responses in laboratory animals and mammalian cells including a wasting syndrome, immunotoxicity, porphyria, reproductive and developmental toxicity, carcinogenicity and modulation of several enzyme activities [30, 33, 40]. The mechanism of action of TCDD and related compounds has been extensively investigated and the aryl hydrocarbon (Ah) receptor has been identified as the intracellular protein that mediates responses induced by these compounds [30, 35, 37, 40]. The proposed mechanism of Ah receptor-mediated responses was initially derived from studies on the induction of CYP1A1 gene expression by aromatic hydrocarbons. The inducer passively diffuses into target cells and initially binds to the cytosolic Ah receptor; the ligand-bound receptor translocates into the nucleus and forms a heterodimer complex with the Ah receptor nuclear translocator (Arnt) protein. The nuclear Ah receptor complex acts as a ligand-induced transcription factor which interacts with *cis*-acting dioxin responsive elements (DREs) in the 5′-promoter regions of the Ah-responsive gene thereby triggering transactivation [35, 37, 40].

TCDD and related compounds also inhibit the expression of several genes, and research in this laboratory and others has focused on the antiestrogenic activity of Ah receptor agonists [35]. TCDD and related compounds inhibit a diverse spectrum of estrogen (E2)-induced responses in rodent uterus, rodent mammary and human breast cancer cell lines. For example, in the rodent uterus, TCDD inhibits E2-induce uterine wet weight increase, progesterone receptor (PR) binding, peroxidase activity, epidermal growth factor receptor (EGFR) binding, c-*fos* and EGFR mRNA levels [1, 3, 6, 7, 11, 13, 15, 31, 32, 38, 39]. In estrogen receptor$^+$ (ER$^+$)/Ah receptor$^+$ MCF-7 cells, TCDD inhibits E2-induced proliferation, [^{3}H]thymidine uptake, secretion or formation of pS2, cathepsin D, procathepsin D and tissue-plasminogen activator activity and PR levels as

G. Sun · S. Safe (✉)
Veterinary Physiology and Pharmacology,
Texas A&M University,
College Station, TX 77843-4466, USA
Tel 409-845-5988; Fax 409-862-4929

well as PR, pS2 and cathepsin D gene expression [10, 16–19, 24, 25, 27, 43].

Although TCDD is a potent antiestrogen and inhibits mammary tumor formation and growth in rodent models [18, 21, 23], the high toxicity of this compound precludes its use as an antineoplastic agent. However, several studies have shown that alternate-substituted 6-alkyl-substituted polychlorinated dibenzofurans (PCDFs) are relatively nontoxic Ah receptor agonists which have retained their antiestrogenic activity [1, 2, 4, 5, 8, 13, 34, 41, 42]. 6-Methyl-1,3,8-trichlorodibenzofuran (MCDF) has been used as a prototype for the alkyl PCDF and several studies have demonstrated the low toxicity of this compound [1, 8, 20, 35, 41] whereas MCDF elicits the same broad spectrum of antiestrogenic responses previously reported for TCDD [1, 4, 13, 19, 42]. This study reports the antiestrogenic activity of a series of alternate-substituted (1,3,6,8- and 2,4,6,8-) PCDFs (Fig. 1) in MCF-7 cells using two well-characterized E2-induced responses, that is cell proliferation and induction of chloramphenicol acetyl transferase (CAT) activity in cells transiently transfected with a Vit-CAT plasmid which contains an E2-responsive promoter fragment (-821 to -87) from the vitellogenin A2 gene linked to a thymidine kinase promoter and a bacterial CAT reporter gene [22]. The results showed that all

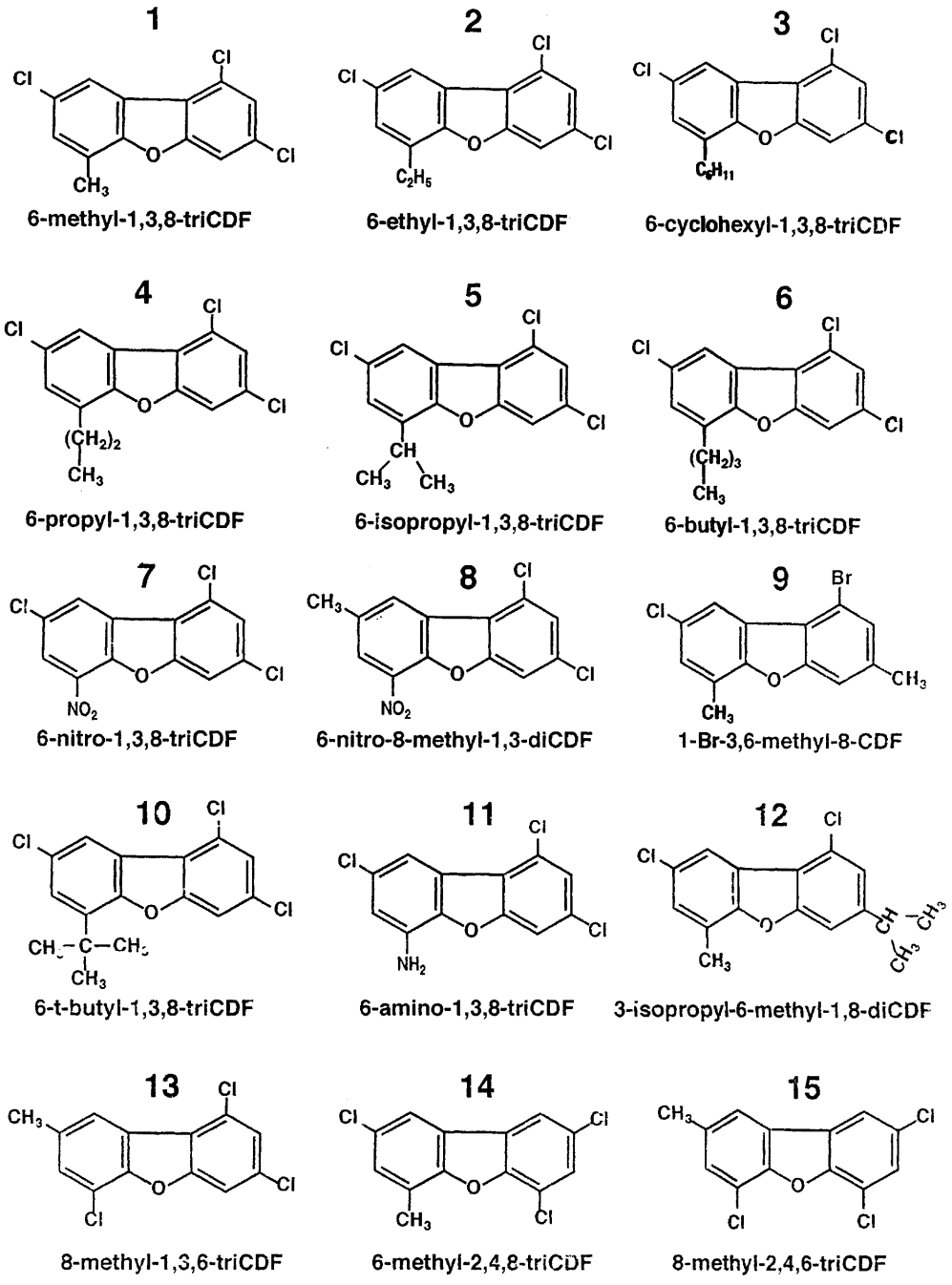


Fig. 1 Structures of alternate-substituted PCDFs

of the congeners inhibited at least one E2-induced response but only five compounds were active in both assays.

Materials and methods

Chemicals

The substituted dibenzofurans, TCDD and ethoxyresorufin used in these studies were synthesized to greater than 97% purity as determined by gas-liquid chromatography by Dr. S. Safe. The synthesis of compounds 1 through 8, 10, 11 and 13 have previously been reported [4, 12, 13]. The remaining alternate-substituted PCDFs (Fig. 1) were also synthesized by coupling a substituted aromatic amine and phenol with isoamyl nitrite to give the corresponding substituted biphenylol. Ring closure after heating with DMSO and anhydrous sodium carbonate gave the alternate-substituted PCDF which was purified by column and thin-layer chromatography and crystallization from methanol as previously described [4, 12, 13, 36]. The aromatic amines and phenols used to synthesize the remaining compounds included the following: 1-bromo-3,6-dimethyl-CDF [9], 2,6-dibromo-*p*-toluidine (Aldrich Chemical Co., Milwaukee, Wis.)/4-chloro-2-methylphenol; 3-isopropyl-6-methyl-1,8-diCDF [12], 2,6-dichloro-4-isopropylaniline (Maybridge Chemical Co., Trevillett Tintagel, UK)/4-chloro-2-methylphenol; 6-methyl-2,4,8-triCDF [14], 2,3,5-trichloroaniline (Wellington Science, Guelph, Canada)/4-chloro-2-methylphenol; and 8-methyl-2,4,6-triCDF [15], 2,3,5-trichloroaniline (Wellington Science) 4-methyl-2-chlorophenol. The phenolic compounds were all purchased from Aldrich Chemical Co. All of these compounds were >97% pure as determined by chromatographic and spectrometric studies [4, 12, 13, 36]

Biochemicals

The resorufin was obtained from Eastman Kodak (Rochester, N.Y.). The tetrasodium salts of NADH and NADPH were obtained from United States Biochemicals (Cleveland, Ohio). All other biochemicals were obtained from Sigma Chemical Company (St. Louis, Mo.) or were the highest quality available from commercial sources. The Vit-CAT plasmid contained the -821/-87 5′-promoter region from the vitellogenin A2 gene linked to a thymidine kinase and a bacterial CAT reporter gene. This construct was kindly provided by Drs. Ryffel and Klein-Hitpass (University of Essen, Essen, Germany). The human ER (hER) expression plasmid was kindly provided by Dr. Ming Jer Tsai (Baylor College of Medicine, Houston, Tx.). MCF-7 cells were obtained from the American Type Culture Collection.

Cell culture proliferation

MCF-7 cells were maintained in MEM medium with 10% fetal bovine serum (FBS) plus 10 ml antibiotic/antimycotic solution and 10 μg insulin. Near confluent MCF-7 cells were seeded into 35-mm wells (7.5×10^4 cells/well) in 2 ml DME/F12 (without phenol red) medium supplemented with 5% FBS treated with dextran-coated charcoal as previously described [14, 38], 1.2 g/l $NaHCO_3$ and 10 ml/l antibiotic/antimycotic solution. After 20 h, the cells were treated with the appropriate chemicals for 10 days. The medium was changed and the cells were redosed every 48 h. The cells were harvested and counted using a Coulter Z1 cell counter.

Ethoxyresorufin *O*-deethylase (EROD) activity

Cells were seeded into 35-mm wells. TCDD (10^{-9} *M*) and 1,3,6,8- or 2,4,6,8- alternate-substituted PCDFs (10^{-6} *M*) dissolved in DMSO were added to the culture dishes when the cells reached 70% confluency. Cells were harvested 24 h after chemical treatment and EROD activity was determined by the fluorimetric method of Pohl and Fouts [29].

Estrogenic and antiestrogenic activities using the Vit/CAT reporter plasmid

Cultured MCF-7 cells were cotransfected with 5 and 1 μg of the Vit-CAT and hER constructs, respectively, using the calcium phosphate method. Cells were dosed with the appropriate compounds for 48 h and assayed for CAT activity as previously described [22, 28].

Statistics

All experiments were carried out in triplicate and the results are expressed as means ± SD. The data were analyzed by ANOVA and Scheffe's test.

Results

The Ah-responsiveness of 15 alternate-substituted PCDFs (Fig. 1) was assessed by determining their induction of CYP1A1-dependent EROD activity in MCF-7 human breast cancer cells. EROD activity in MCF-7 cells treated with 10^{-9} *M* TCDD for 24 h was 234 ± 16.8 pmol/min per mg. In contrast, the activity of the alternate-substituted PCDFs was nondetectable at a concentration of 10^{-7} *M* (data not shown) whereas variable induction responses were observed after treating MCF-7 cells with these compounds at a concentration of 10^{-6} *M* (Table 1). At this concentration, all of the compounds induced < 50% of the response observed with 10^{-9} *M* TCDD. The effects of the alternate-substituted PCDFs on the growth of MCF-7 human breast cancer cells was also investigated. The effects of the compounds alone (10^{-6} and 10^{-7} *M*) on cell proliferation were initially investigated and, at a concentration of 10^{-7} *M*, only three of the congeners (4, 5 and 6) significantly induced cell proliferation (383 ± 41%, 419 ± 38% and 387 ± 47% in relation to control DMSO-treated cells, respectively). At the highestconcentration (10^{-6} *M*), compounds 2, 3, 4, 5 and 6 increased cell proliferation by 255 ± 34.7%, 344 ± 15.6%, 423 ± 29%, 341 ± 11.3% and 914 ± 109% compared with control cells, respectively. In these same experiments, 10^{-9} *M* E2 increased cell proliferation by 1617 ± 151% to 2666 ± 283%.

The effects of alternate-substituted PCDFs on E2-induced proliferation of MCF-7 cells is summarized in Table 1. Compared to treatment with 10^{-9} *M* E2 alone (100%), there was a significant ($P < 0.05$) 28% and 26% decrease in proliferation of MCF-7 cells cotreated with 10^{-9} *M* E2 and compounds 3 and 4 at a concentration of 10^{-7} *M*, respectively. In cells cotreated with 10^{-9} *M* E2 plus the alternate-substituted PCDFs at a concentration of 10^{-6} *M*, a significant ($P < 0.05$) inhibition of cell proliferation was observed for compounds 1 through 5, 12, 14 and 15 (43%, 34%, 13%, 24%, 36%, 31%, 21% and 19% decrease in E2-induced cell growth, respectively; Table 1). In contrast, combined treatment of alternate-substituted PCDFs 6 through 9 (10^{-7} and 10^{-6} *M*) plus E2 gave proliferative responses higher than those observed after treatment with 10^{-9} *M* E2

Table 1 Effects of alternate-substituted PCDFs on EROD activity and E2-induced responses in MCF-7 human breast cancer cells. To determine EROD activity, MCF-7 cells were treated for 24 h with the test compounds (10^{-9} *M* TCDD and 10^{-6} *M* alternate-substituted PCDFs). The EROD activity was determined fluorimetrically as described in Materials and Methods. The activity observed for TCDD was 234 ± 16.8 pmol/min per mg. To determine CAT activity, MCF-7 cells were transiently transfected with the Vit-CAT and hER plasmids (5 and 1 μg, respectively) and treated with the appropriate chemicals (10^{-9} *M* E2 plus 10^{-9} *M* TCDD or 10^{-6} *M* alternate-substituted PCDFs). CAT activity was determined as described in Materials and methods. To determine cell proliferation, cells were treated every other day with the appropriate chemicals (10^{-9} *M* E2 plus 10^{-9} *M* TCDD or 10^{-6} *M* alternate-substituted PCDFs) and counted after 10 days as described in Materials and methods. The values are means ±SD for three separate determinations for each treatment group (ND, nondetectable)

Compound	Relative EROD activity (pmol/min/mg)	CAT activity (% of E2-induced)	Cell proliferation (% of E2-induced)
DMSO (control)	ND	28.2 ± 2.9**	5.3 ± 0.3**
TCDD	100*	42.1 ± 2.6**	66.2 ± 5.8**
1	41.5 ± 4.0*	56.9 ± 7.9**	56.7 ± 7.9**
2	19.1 ± 1.4*	65.4 ± 10**	66.2 ± 1.3**
3	ND	111 ± 9.9	86.9 ± 4.3**
4	6.0 ± 1.9*	149 ± 22.8	75.8 ± 1.6**
5	14.0 ± 1.1*	82.7 ± 0.9**	64.4 ± 4.1**
6	ND	38.7 ± 5.8**	136 ± 10
7	ND	31.6 ± 2.8**	134 ± 4.4
8	7.2 ± 0.6*	30.3 ± 2.4**	133 ± 18
9	16.0 ± 1.9*	29.7 ± 2.1**	129 ± 12
10	ND	34.6 ± 1.7**	95 ± 9.4
11	2.4 ± 2.8	41.2 ± 7.6**	109 ± 5.3
12	29.9 ± 2.5*	37.6 ± 1.3**	69.0 ± 7.9**
13	8.4 ± 1.8*	41.4 ± 2.7**	98.7 ± 4.7
14	18.8 ± 3.0*	35.2 ± 7.3**	79.1 ± 8.2**
15	2.9 ± 1.3*	111 ± 22	80.7 ± 8.5**

*$P < 0.05$ vs DMSO-treated cells, **$P < 0.05$ vs E2-treated cells

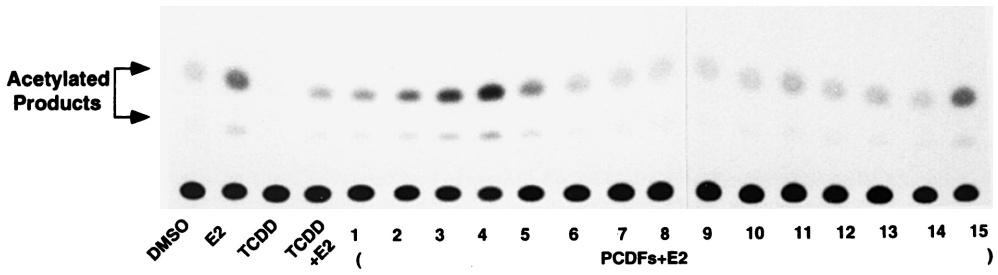
alone. In parallel experiments, 10^{-9} *M* TCDD decreased E2-induced proliferation of MCF-7 cells by 39%.

The antiestrogenic activities of these compounds were also investigated in MCF-7 cells transiently transfected with the E2-responsive Vit-CAT plasmid (Table 1, Fig. 2). With the exception of compound 4, the alternate-substituted PCDFs at a concentration of 10^{-6} *M* and 10^{-9} *M* TCDD alone did not significantly increase CAT activity, whereas 10^{-9} *M* E2 caused a >3.5-fold induction in CAT activity. In contrast, compound 4 induced CAT activity (2.5-fold) compared with DMSO-treated cells. In cells cotreated with 10^{-9} *M* TCDD or the alternate-substituted PCDFs at a concentration of 10^{-6} *M* (compounds 1, 2, and 5 through 14) plus 10^{-9} *M* E2, there was a 58%, 43%, 35%, 17%, 61%, 68%, 70%, 70%, 65%, 59%, 62%, 59% and 65% decrease in the E2-induced response, respectively. Increased induction of CAT activity in the cotreated cells (compared with 10^{-9} *M* E2 alone) was observed only for PCDFs 3, 4 and 15. Thus, the alternate-substituted PCDFs inhibited at least one of two E2-induced responses in MCF-7 cells and the antiestrogenic activities of these compounds was confirmed.

Fig. 2 Antiestrogenic activities of alternate-substituted PCDFs in MCF-7 cells. MCF-7 cells were transiently transfected with the Vit-CAT (5 μg) and hER (1 μg) plasmids, treated with 10^{-9} *M* E2, 10^{-9} *M* TCDD, 10^{-9} *M* E2 plus 10^{-9} *M* TCDD, and 10^{-9} *M* E2 plus 10^{-9} *M* alternate-substituted PCDFs. CAT activity was determined as described in Materials and methods. The thin-layer chromatogram illustrates the results reported in Table 1

Discussion

Previous studies with alternate-substituted alkyl PCDFs, typified by MCDF have shown that these compound are relatively weak inducers of CYP1A1-dependent hepatic EROD activity in rodents and this parallels their Ah receptor-mediated toxicities such as immunotoxicity, and those that cause body weight loss, thymic atrophy,



fetal cleft palate formation, and porphyria [1, 2, 5, 8, 41]. Many of these responses were not observed at the highest dose of MCDF, and in rodents cotreated with MCDF plus a toxic dose of TCDD, there was partial antagonism of some TCDD-induced toxic responses. In contrast, MCDF and several 6-alkyl-1,3,8-trichlorodibenzofurans exhibit antiestrogenic activity in the rodent uterus [1, 4, 13] and MCDF is also antiestrogenic in MCF-7 cells [19, 42]. Recent studies have shown that relatively low doses of MCDF (1 mg/kg) significantly inhibit growth of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors in adult female Sprague-Dawley rats (McDougal, unpublished results) and therefore the antiestrogenic activities of several alternate-substituted PCDFs was determined in MCF-7 cells to identify additional active compounds for in vivo studies.

With the exception of compounds 3, 6, 7 and 10, all of the alternate-substituted PCDFs induced EROD activity in MCF-7 cells. All of the noninducers were 1,3,6,8-substituted PCDFs containing cyclohexyl, *n*-butyl, nitro and *t*-butyl substituents at C-6 of the dibenzofuran ring, and previous in vivo studies have also shown that these compounds do not induce hepatic microsomal EROD activity in immature Long Evans rats [12, 13].

The estrogenic and antiestrogenic activity of the alternate-substituted PCDFs was determined using two assays, that is the induction of cell proliferation and CAT activity in MCF-7 cells transiently transfected with the E2-responsive Vit-CAT plasmid and treated with 10^{-9} *M* E2. Treatment of MCF-7 cells with 10^{-7} or 10^{-6} *M* concentrations of the 6-alkyl-substituted PCDFs (2–6) showed that these compounds induced cell proliferation and were weakly estrogenic. Previous in vitro studies in the immature female rat uterus have shown that with one exception these congeners do not induce estrogenic responses [1, 4, 13]. 6-Isopropyl-1,3,8-triCDF (5) causes a small but significant (20%) increase in uterine wet weight [13]. However, in rats cotreated with E2 plus compounds 2, and 4 through 6, there is significant inhibition of several E2-induced responses [1, 4, 13]. Compound 3 has been shown to be inactive as an estrogen or an antiestrogen in in vivo studies [4]. Only compound 4 exhibited estrogenic activity (2.5-fold induction) at a concentration of 10^{-6} *M* in the transient transfection assay whereas the remaining 14 compounds were inactive at this concentration. These results indicate that for some of the alkyl-substituted PCDFs there was no concordance between the in vivo results [1, 4, 13] and the in vitro data reported here. In addition, relatively weak estrogenic activity was observed for only one compound (40) in both the cell proliferation and CAT assays. The reason for the assay-specific differences in the weakly estrogenic activity of the alkyl-substituted PCDFs is unknown and is currently being investigated.

In the cell proliferation assay compounds 6–11 and 13 did not significantly inhibit E2-induced cell proliferation. All of these compounds were 1,3,6,8-substituted PCDFs with variable substituent groups, and previous in vivo studies have shown that compounds 6, 10, 11 and 13 exhibit antiestrogenic activity in the immature female rat uterus [1, 4, 12, 13]. In MCF-7 cells treated with 10^{-9} *M* E2 and transiently transfected with the Vit-CAT plasmid, antiestrogenic activity was observed for all the alternate-substituted compounds except compounds 3, 4 and 15. These results demonstrate that all of the congeners 1 through 15 inhibited at least one of the in vitro estrogenic responses in MCF-7 cells, but only compounds 1, 2, 5, 12 and 14 were antiestrogenic in both assays. Thus, in two different assays, which are routinely used to measure estrogenic and antiestrogenic activities, there was not a response- or structure-dependent correlation for the antiestrogenic effects of, the alternate-substituted PCDFs. Moreover, the effects of structurally related isomers could be highly variable. For example, compounds 14 and 15 are 2,4,6,8-substituted isomers in which the chloro- and methyl-substituents at positions 6 and 8 have been interchanged. Both compounds inhibited E2-induced cell proliferation (19–21%) whereas only compound 14 inhibited E2-induced CAT activity. Compounds 1 and 13 are 1,3,6,8-substituted isomers which also differ only in the placement of chloro and methyl groups at positions 6 and 8. Both of these isomers significantly inhibited E2-induced CAT activity (43–59%) whereas only compound 1 (MCDF) inhibited E2-induced cell proliferation.

In summary, the results of this study demonstrate that several alternate-substituted PCDFs exhibit antiestrogenic activity in MCF-7 cells, thus confirming the results of previous in vivo and/or in vitro studies. Their structure-dependent antiestrogenic activities were response-specific and the inhibition of estrogen-responsive reporter gene activity did not correlate with the inhibition of cell proliferation, which is a more complex process involving multiple genes. Therefore, additional studies are required to establish specific in vitro bioassays which are predictive of the in vivo antiestrogenic/antitumorigenic activities of the alternate-substituted PCDFs.

Acknowledgements The financial assistance of the National Institutes of Health (CA64081) and the Texas Agricultural Experiment Station is gratefully acknowledged. S. Safe is a Sid Kyle Professor of Toxicology.

References

1. Astroff B, Safe S (1988) Comparative antiestrogenic activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 6-methyl-1,3,8-trichlorodibenzofuran in the female rat. Toxicol Appl Pharmacol 95: 435
2. Astroff B, Safe S (1989) 6-Substituted-1,3,8-trichlorodibenzofurans as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonists in the rat: structure-activity relationships. Toxicology 59: 285
3. Astroff B, Safe S (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin as an antiestrogen: effect on rat uterine peroxidase activity. Biochem Pharmacol 39: 485
4. Astroff B, Safe S (1991) 6-Alkyl-1,3,8-trichlorodibenzofurans as antiestrogens in female Sprague-Dawley rats. Toxicology 69: 187
5. Astroff B, Zacharewski T, Safe S, Arlotto MP, Parkinson A,Thomas P, Levin W (1988) 6-Methyl-1,3,8-trichlorodiben-

zofuran as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist: inhibition of the induction of rat cytochrome P-450 isozymes and related monooxygenase activities. Mol Pharmacol 33: 231
6. Astroff B, Rowlands C, Dickerson R, Safe S (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inhibition of 17β-estradiol-induced increases in rat uterine EGF receptor binding activity and gene expression. Mol Cell Endocrinol 72: 247
7. Astroff B, Eldridge B, Safe S (1991) Inhibition of 17β-estradiol-induced and constitutive expression of the cellular protooncogene c-*fos* by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the female uterus. Toxicol Lett 56: 305
8. Bannister R, Biegel L, Davis D, Astroff B, Safe S (1989) 6-Methyl-1,3,8-trichlorodibenzofuran (MCDF) as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6 mice. Toxicology 54: 139
9. Bertazzi PA, Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C (1993) Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Epidemiology 4: 398
10. Biegel L, Safe S (1990) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on cell growth and the secretion of the estrogen-induced 34-, 52- and 160-kDa proteins in human breast cancer cells. J Steroid Biochem Mol Biol 37: 725
11. DeVito MJ, Thomas T, Martin E, Umbreit TH, Gallo MA (1992) Antiestrogenic action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: tissue-specific regulation of estrogen receptor in CD1 mice. Toxicol Appl Pharmacol 113: 284
12. Dickerson R, Safe S (1992) The effects of 6-nitro-1,3,8-trichlorodibenzofuran as a partial estrogen in the female rat. Toxicol Appl Pharmacol 113: 55
13. Dickerson R, Howie-Keller L, Safe S (1995) Alkyl polychlorinated dibenzofurans and related compounds as antiestrogens in the female rat uterus: structure-activity studies. Toxicol Appl Pharmacol 135: 287
14. Fernandez P, Burghardt R, Smith R, Nodland K, Safe S (1994) High passage T47D human breast cancer cells: altered endocrine and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin responsiveness. Eur J Pharmacol 270: 53
15. Gallo MA, Hesse EJ, MacDonald GJ, Umbreit TH (1986) Interactive effects of estradiol and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic cytochrome P-450 and mouse uterus. Toxicol Lett 32: 123
16. Gierthy JF, Lincoln DW (1988) Inhibition of postconfluent focus production in cultures of MCF-7 breast cancer cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Breast Cancer Res 12: 227
17. Gierthy JF, Lincoln DW, Gillespie MB, Seeger JI, Martinez HL, Dickerman HW, Kumar SA (1987) Suppression of estrogen-regulated extracellular plasminogen activator activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cancer Res 47: 6198
18. Gierthy JF, Bennett JA, Bradley LM, Cutler DS (1993) Correlation of *in vitro* and *in vivo* growth suppression of MCF-7 human breast cancer by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cancer Res 53: 3149
19. Harper N, Wang X, Liu H, Safe S (1994) Inhibition of estrogen-induced progesterone receptor in MCF-7 human breast cancer cells by aryl hydrocarbon (Ah) receptor agonists. Mol Cell Endocrinol 104: 47
20. Harris M, Zacharewski T, Astroff B, Safe S (1989) Partial antagonism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated induction of aryl hydrocarbon hydroxylase by 6-methyl-1,3,8-trichlorodibenzofuran: mechanistic studies. Mol Pharmacol 35: 729
21. Holcomb M, Safe S (1994) Inhibition of 7,12-dimethylbenzanthracene-induced rat mammary tumor growth by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cancer Lett 82: 43
22. Klein-Hitpass L, Schorpp M, Wagner U, Ryffel GU (1986) An estrogen-responsive element derived from the 5′-flanking region of the xenopus vitellogenin A2 gene functions in transfected human cells. Cell 46: 1053
23. Kociba RJ, Keyes DG, Beger JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CL, Barnard SD, Hummel RA, Humiston CG (1978) Results of a 2-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Toxicol Appl Pharmacol 46: 279
24. Krishnan V, Safe S (1993) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) as antiestrogens in MCF-7 human breast cancer cells: quantitative structure-activity relationships. Toxicol Appl Pharmacol 120: 55
25. Krishnan V, Porter W, Santostefano M, Wang X, Safe S (1995) Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in MCF-7 cells. Mol Cell Biol 15: 6710
26. Mason G, Sawyer T, Keys B, Bandiera S, Romkes M, Piskorska-Pliszczynska J, Zmudzka B, Safe S (1985) Polychlorinated dibenzofurans (PCDFs): correlation between *in vivo* and *in vitro* structure-activity relationships. Toxicology 37: 1
27. Merchant M, Krishnan V, Safe S (1993) Mechanism of action of α-naphthoflavone as an Ah receptor antagonist in MCF-7 human breast cancer cells. Toxicol Appl Pharmacol 120: 179
28. Moore M, Wang X, Lu Y-F, Wormke M, Craig A, Gerlach J, Burghardt R, Safe S (1994) Benzo[*a*]pyrene resistant (BaP^R) human breast cancer cells: a unique aryl hydrocarbon (Ah)-nonresponsive clone. J Biol Chem 269: 11751
29. Pohl RJ, Fouts JR (1980) A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. Anal Biochem 107: 150
30. Poland A, Knutson JC (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons. Examinations of the mechanism of toxicity. Annu Rev Pharmacol Toxicol 22: 517
31. Romkes M, Safe S (1988) Comparative activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and progesterone on antiestrogens in the female rat uterus. Toxicol Appl Pharmacol 92: 368
32. Romkes M, Piskorska-Pliszczynska J, Keys B, Safe S, Fujita T (1987) Quantitative structure-activity relationships: analysis of interactions of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2-substituted analogues with rat, mouse, guinea pig and hamster cytosolic receptor. Cancer Res 47: 5108
33. Safe S (1990) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). C R C Crit Rev Toxicol 21: 51
34. Safe S (1992) MCDF, 6-methyl-1,3,8-trichlorodibenzofuran. Drugs Future 17: 564
35. Safe S (1995) Modulation of gene expression and endocrine response pathways by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds. Pharmacol Ther 67: 247
36. Safe S, Safe L (1984) Synthesis and characterization of twenty-two purified polychlorinated dibenzofuran congeners. J Agric Food Chem 32: 68
37. Swanson HI, Bradfield CA (1993) The Ah-receptor: genetics, structure and function. Pharmacogenetics 3: 213
38. Umbreit TH, Gallo MA (1988) Physiological implications of estrogen receptor modulation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Lett 42: 5
39. Umbreit TH, Hesse EJ, MacDonald GJ, Gallo MA (1988) Effects of TCDD-estradiol interactions in three strains of mice. Toxicol Lett 40: 1
40. Whitlock JP Jr (1993) Mechanistic aspects of dioxin action. Chem Res Toxicol 6: 754
41. Yao C, Safe S (1989) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced porphyria in genetically inbred mice: partial antagonism and mechanistic studies. Toxicol Appl Pharmacol 100: 208
42. Zacharewski T, Harris M, Biegel L, Morrison V, Merchant M, Safe S (1992) 6-Methyl-1,3,8-trichlorodibenzofuran (MCDF) as an antiestrogen in human and rodent cancer cell lines: evidence for the role of the Ah receptor. Toxicol Appl Pharmacol 13: 311
43. Zacharewski T, Bondy K, McDonell P, Wu ZF (1994) Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on 17β-estradiol-induced pS2 expression. Cancer Res 54: 2707