In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens.

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The antimicrobial agents triclocarban (TCS; 2,4,4´-trichloro-2´-hydroxydiphenyl ether) and triclosan (TCC; 3,4,4´-trichlorocarbanilide) are high-production-volume chemicals that are widely used as “value added” chemicals in personal care products. In a limited retail survey, approximately 45% of liquid and bar soaps on the market contained these antimicrobials; TCS and TCC were the predominant antimicrobials in liquid soaps and bar soaps, respectively (Perencevich et al. 2001). According to the U.S. Environmental Protection Agency (2007), U.S. consumers spend nearly $1 billion/year on these products.

TCS is a broad-spectrum bacteriostatic germicide that is now used in consumer products such as liquid hand soap, toothpaste, mouth rinse, cosmetics, pharmaceutical products, fabrics, plastics, textiles, and plastic kitchenware since its original introduction as an active ingredient in a surgical scrub for professional health care in 1972 (Tienro 1999). It is a powerful antibacterial agent that inhibits the activity of the enzyme enoyl-acyl carrier-protein reductase, which catalyzes an essential step in membranes of many bacteria and fungi (Heath et al. 1999; McMurry et al. 1998b). TCC, another antimicrobial agent, is more often added to consumer bar soaps and deodorants, and it is active predominantly against gram-positive bacteria (McDonnell and Russell 1999). The carbanilide analog 3-trifluoromethyl-4,4´-dichlorocarbanilide (TFC) is also used as an antibacterial agent (Jeffcoat et al. 1977).

Although these compounds are broadly classified as halogenated aromatic hydrocarbons, TCS has functional moieties representative of phenols, diphenyl ethers, and polychlorinated biphenyls (PCBs), whereas TCC is structurally related to carbanilide compounds, including some drugs and pesticides, and sterically and electronically related to a variety of other chemicals (Figure 1). Both TCS and TCC have been detected at microgram per liter levels in waterways in the United States and Switzerland, indicating extensive contamination of aquatic ecosystems (Halden and Paull 2005; Kolpin et al. 2002; Lindstrom et al. 2002). The potential of these compounds for bioaccumulation has raised public concern regarding their possible effects on human health (Coogan et al. 2007; Daughton and Ternes 1999) and microbial resistance (McMurry et al. 1998a). Recent reports note that TCS levels as high as 2,000 µg/kg lipid have been detected in human breast milk (Dayan 2007), and concentrations in human fluids such as plasma and milk are positively correlated to levels of exposure (Adolfson-Erici et al. 2002; Almyr et al. 2006; Dayan 2007; Hovander et al. 2002).

Because of these concerns, we screened TCS, TCC, and a series of TCC analogs for biological activity in several mechanistically derived cell-based assay systems. Mammalian ligand-dependent nuclear receptors serve as biomarkers that evaluate the potential of an environmental toxicant to affect endocrine and non–endocrine-signaling systems. One set of assays used in the present study is based on the chemically activated luciferase gene expression (CALUX) biosays. The recombinant cells used in the CALUX biosays include a stably transfected aryl hydrocarbon receptor (AhR)- and androgen receptor (AR)-, or estrogen receptor (ER)-responsive firefly luciferase reporter gene that responds to chemicals that can bind to and/or activate the respective receptor, leading to the induction of luciferase reporter gene expression.

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AhR is a transcription factor that activates gene expression in a ligand-dependent manner. Exposure to the most potent ligand, 2,3,7,8-tetrachlorodibenzop-\(\beta\)-dioxin (TCDD), results in a variety of toxic and biological responses, most of which are AhR dependent, such as birth defects, immunotoxicity, tumor production, changes in metabolism, and lethality. Dioxin-like PCBs, polychlorinated dibenzofurans, and related chemicals that mimic the action of TCDD at the level of the AhR are detected by measurement of their ability to stimulate AhR-dependent gene expression in the CALUX bioassay (Garrison et al. 1996; Han et al. 2004).

Steroid hormones control reproduction, metabolism, and ion balance in vertebrates. ER and AR are nuclear receptors for estrogenic and androgenic chemicals such as estradiol (E\(\text{2}\)) and testosterone, respectively, that function as transcription factors to regulate female and male reproduction, sexual development, and bone structure. Chemicals acting as endocrine-disrupting compounds (EDCs) affect these receptors and lead to activation/inhibition of hormone-dependent gene expression. However, EDCs may also alter hormone receptor function simply by changing phosphorylation of the receptors (activating them) without the responsible chemical or natural ligand ever binding to the receptor (Weigel et al. 2006). Recently, Chen et al. (2008) reported data indicating a mechanism of endocrine disruption that involves the receptor but that does not appear to act through competition with the receptor’s primary binding site. Instead, they observed an amplification or enhancement of the ability of the chemical to stimulate gene expression in concert with the natural ligand, possibly indicating a new type of EDC that may not share the basic qualities of previously defined EDCs.

An increasing number of studies report that chemicals in the environment, mimicking natural estrogen, interact with or affect the ER in cells and thereby disrupt normal endocrine function, raising public concerns about the biological/ reproductive effects of these chemicals (Darbre 2006). Reproductive health concerns regarding androgenic EDCs that may reduce sperm production, alter genital development, and contribute to neurologic syndromes in males have been proposed (Chen et al. 2007; Larsson et al. 2006; Sonneveld et al. 2005). In relation to the work reported here, TCS may have cytotoxic effects on breast cancer cells (Liu et al. 2002) and have endocrine-disrupting properties (antiandrogenic activity and thyroid-hormone-like activity) in aquatic species and human recombinant cells in culture (Chen et al. 2007; Foran et al. 2000; Veldhoen et al. 2006).

The ryanodine (Ry) receptor type 1 (RyR1)-based bioassay is used for screening potential compounds exhibiting biological activity that alters Ca\(^{2+}\) homeostasis (Pessah et al. 2006). RyR\(\text{2}^{+}\) function as high-conductance Ca\(^{2+}\) channels broadly expressed in animal cells, including muscle (skeletal, cardiac, smooth), neurons, and immune cells. Chemicals that enhance or inhibit RyR channel activity, such as caffeine and Ry, have been demonstrated to influence Ca\(^{2+}\) signaling events and Ca\(^{2+}\)-dependent processes in a number of cell types. Noncoplanar, ortho-substituted PCB congeners that exhibit weak AhR activity enhance the sensitivity of RyRs to activation by endogenous ligands in a manner requiring the immunophilin FKBP12–RyR complex (Wong et al. 1997). Disruption of Ca\(^{2+}\) homeostasis in the affected regions of the brain by compounds altering RyR may contribute to alteration of neurodevelopment and neuroplasticity function (Gafni et al. 2004; Wong et al. 2001).

The screening assays used in this study are part of a library of techniques developed by the University of California, Davis Superfund Basic Research Program, whose aim is to identify biomarkers of exposure and effect of toxic substances. The goal of this study was to demonstrate that such mechanistic, nuclear-receptor–based screening assays can rapidly provide useful information on environmental chemicals, and to assess the potential for the antimiocribs TCC, its analogs, and TCS to produce specific toxic effects that would warrant further study.

### Materials and Methods

#### Chemicals

Compounds, identified by Roman numerals in the text, are listed in Figure 1; compounds were either purchased or synthesized in this laboratory. The carbanilide compounds, carbanilide (I; reported purity, 99.9%), TCC (III; reported purity, 99.3%), and 1,3-dicyclohexylurea (VI; reported purity, > 98%) were purchased from Aldrich (St. Louis, MO); 3-trifluoromethyl-4,4\(^{\text{\prime}}\)-dichloro-carbanilide (VII; reported purity, > 99%) was purchased from Chembridge (San Diego, CA). We synthesized structurally related carbanilide compounds (II, IV, and V; purity, > 99%) by condensing the appropriate isocyanate and amine according to previously published reports (Moriseseau et al. 1999; Newman et al. 2001). We purchased TCS (VIII; reported purity of 99.8%) from Fluka (St. Louis, MO). The commercial TCC (III) and TCS (VIII) compounds were further purified to approximately 100% purity by recrystallization three times from ethanol and petroleum ether, respectively. We obtained TCDD from S. Safe (Texas A&M University, College Station, TX). We purchased dimethyl sulfoxide (DMSO), 17\(^{\beta}\)-E\(\text{2}\), and phenol red-free Dulbecco’s modified Eagle medium (DMEM) from Sigma Chemical Co. (St. Louis, MO); cell culture reagents and media from Gibco/BRL (Grand Island, NY); and 17\(^{\beta}\)-testosterone from Alltech (State College, PA). All test compounds, except for the steroids, were dissolved in DMSO; steroids were dissolved in absolute ethanol. We

| Compound | Common name | Chemical structure | Remark (production) |
|----------|-------------|--------------------|---------------------|
| I        | Carbanilide | ![Carbanilide Structure](image1) | Possible by-product in the synthesis of the herbicide siduron |
| II       | 4,4\(^{\text{\prime}}\)-Dichlorocarbanilide | ![4,4\(^{\text{\prime}}\)-Dichlorocarbanilide Structure](image2) | Possible by-product in the synthesis of triclofen and the insecticide diflubenzuron |
| III      | 3,3,4,4\(^{\text{\prime}}\)-Tetrachloro-carbanilide, triclofen (TCC) | ![3,3,4,4\(^{\text{\prime}}\)-Tetrachloro-carbanilide Structure](image3) | Antimicrobial agent |
| IV       | 3,3,4,4\(^{\text{\prime}}\)-Tetramethylene-5-carbanilide | ![3,3,4,4\(^{\text{\prime}}\)-Tetramethylene-5-carbanilide Structure](image4) | Possible by-product in the synthesis of triclofen and the insecticide diflubenzuron |
| V        | 4\(^{\prime}\)-Methoxy-3,4-dichloro-carbanilide | ![4\(^{\prime}\)-Methoxy-3,4-dichloro-carbanilide Structure](image5) | Structurally related carbanilide compound |
| VI       | 1,3-Dicyclohexylurea | ![1,3-Dicyclohexylurea Structure](image6) | By-product in the synthesis of the peptide |
| VII      | 3-Trifluoromethyl-4,4\(^{\text{\prime}}\)-dichloro-carbanilide (TCC) | ![3-Trifluoromethyl-4,4\(^{\text{\prime}}\)-dichloro-carbanilide (TCC) Structure](image7) | Antimicrobial agent |
| VIII     | 2,4,4\(^{\text{\prime}}\)-Trichloro-2\(^{\prime}\)-hydroxydiphenyl ether, triclosan (TCS) | ![2,4,4\(^{\text{\prime}}\)-Trichloro-2\(^{\prime}\)-hydroxydiphenyl ether, triclosan (TCS) Structure](image8) | Antimicrobial agent |

*Figure 1. Chemical structures and use of TCC, its analogs, and TCS.*
purchased [3H]Ry (60–90 Ci/mmol; > 99% pure) from Perkin-Elmer New England Nuclear (Wilmington, DE) and unlabeled Ry (> 99% by ultraviolet-HPLC from Calbiochem (San Diego, CA).

Cell-based AhR-mediated bioassay. Recombinant rat hepatoma (H4L1.1c4) cells were grown and maintained as previously described (Garrison et al. 1996). These cells contain the stably integrated, dioxin-responsive-element (DRE)-driven firefly luciferase reporter gene plasmid pGudLuc1.1. Transcriptional activation of the plasmid occurs in a ligand-, dose-, and AhR-dependent manner. Cells were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and allowed to attach for 24 hr. Cells were incubated with carrier solvent DMSO (1% final solvent concentration), TCDD (1 nM), the indicated compound (for measurement of agonist activity), or the indicated compound plus 1 nM TCDD (for measurement of antagonist activity) for 4 hr at 37°C. We measured luciferase activity as described above; activity in each well is expressed relative to that maximally induced by TCDD. For comparison, we present data from a previously published study on the effect of these chemicals on AR in human embryonic kidney (HEK) 293T cells that lack key steroid-metabolizing enzymes (Chen et al. 2008).

RyR1-mediated bioassay. Preparation of primary cultures of skeletal myoblasts from wild-type mice has been previously described (Rando and Blau 1994). Wild-type myoblasts were cultured in treated dishes coated with calf skin collagen with F-10 nutrient medium containing 20% (vol/vol) fetal bovine serum, 2 mM l-glutamine, 4 ng/mL fibroblast growth factor, 100 units/mL penicillin-G, and 100 μg/mL streptomycin sulfate at 37°C in 10% CO2/5% O2. For fura-2 imaging, cells were plated onto 96-well μ-clear plates (Greiner Bio-One GmbH, Monroe, NC) with automatic injection of Promega stabilized luciferase reagent. Luciferase activity in each well was expressed relative to that maximally induced by TCDD.

Cell-based ER-mediated bioassay. Recombinant human ovarian cancer cells (BG1LucER2, ER-α-positive) were grown and maintained as previously described (Rogers and Denison 2000). These cells contain a stably integrated, ER-responsive firefly luciferase reporter plasmid, pGudLuc7ERE. Cells were maintained in estrogen-stripped media for 5 days before they were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and allowed to attach for 24 hr. Cells were then incubated with carrier solvent (ethanol; 1% final solvent concentration), E2 (1 nM), the indicated compound (for measurement of agonist activity), or the indicated compound plus 1 nM E2 (for measurement of antagonist activity) for 24 hr at 37°C. We measured luciferase activity as described above; activity is expressed relative to that maximally induced by E2.

Cell-based AR-mediated bioassays. For the cell-based human AR-responsive bioassay, recombinant human cells [T47D-androgen-responsive element (ARE)] were grown and maintained as described above for H4L1.1c4 cells. The T47D-ARE cells contain a stably integrated AR-responsive firefly luciferase reporter gene plasmid, pGudLuc7ARE (Rogers and Denison 2002). Cells were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and allowed to attach for 24 hr. Cells were incubated with carrier solvent (ethanol; 1% final solvent concentration), 10 μM testosterone, the indicated compound (for measurement of agonist activity), or the indicated compound plus the indicated concentration of testosterone (for measurement of antagonist activity) for 24 hr at 37°C. We measured luciferase activity as described above; activity in each well is expressed relative to that maximally induced by testosterone. For comparison, we present data from a previously published study on the effect of these chemicals on AR in human embryonic kidney (HEK) 293T cells that lack key steroid-metabolizing enzymes (Chen et al. 2008).

Data analysis. We measured luciferase activity per well as well as relative light units. We calculated luciferase induction as a percentage of TCDD (AhR bioassay), E2 (ER bioassay), or testosterone (AR bioassay) activity by setting the maximal induction by 1 nM TCDD, 1 nM E2, or 10 μM testosterone at 100%. Background activity present in the vehicle control was subtracted from treated cells. For antagonistic effects in the AhR bioassay, the induction at 1 nM concentration of TCDD was set at 100%. When evaluating for enhancement effects of the hormone receptors in the CALUX bioassays, the induction at a concentration of 1 nM E2 (ER bioassay) or 10 μM testosterone (AR bioassay) was set at 100%, and the degree of enhancement by each compound tested was calculated as the ratio of the luciferase reporter gene induction value of each compound when combined with E2 or testosterone relative to that of the hormone alone.

We calculated the fold increase of [3H]Ry binding by TCS in skeletal muscle sarcoplasmic reticulum vesicles by dividing the mean value of [3H]Ry binding triggered by TCS with that triggered by the solvent control (DMSO); the solvent had none of the effects seen with TCS. Values shown are mean ± SD from three independent experiments for each dose tested, with vehicle control values subtracted. We analyzed data by one-way analysis of variance, followed by a multiple comparisons test when appropriate, using SigmaStat version 3.5 (Systat Software, San Jose, CA). We set the level of significance at p < 0.05.

Molecular modeling of TCS. We performed molecular modeling using the CS ChemOffice 2005 software package (CambridgeSoft Corp., Cambridge, MA). We compared the optimized geometries of 2,2′,3′,5′-4-pentachlorobiphenyl (PCB-95) and TCS at their minimum energy levels with a minimum root mean square gradient of 0.1 computed by MM2 force fields. We measured the dihedral angles formed by two phenyl rings in the structures of PCB-95 and TCS after molecular modeling; three-dimensional projections of the structures of TCS and PCB-95 were simulated using ChemIDplus (National Library of Medicine 2008).

Results

Cell-based AhR-mediated bioassay. We evaluated the activity of AhR-mediated cells by measuring luciferase activity induced by test compounds compared with that of the solvent control (DMSO) or TCDD as positive control. As shown in Table 1, no carbonilide compounds tested (I–VII) exhibited induction except 1,3-dicyclohexyleuore (VI), which induced reporter gene activity to 51% of that induced by TCDD. Interestingly, induction
by compound VI was lower at the higher concentration, suggesting that it may be toxic to the cells, although we observed no overt cell toxicity by visual inspection. Except for compound VI, all carbanilides at higher concentrations (10 μM) inhibited TCDD-dependent luciferase gene expression between 20% and 70%, suggesting that these chemicals may act as weak AhR antagonists.

We tested TCS (compound VIII) in the AhR bioassay because of its structural similarity to hydroxylated metabolites of the polybrominated diphenyl ethers 2,4,4’-tribromodiphenyl ether [bromodiphenyl ether-28 (BDE-28)], and 2,2’,4,4’-tetrabromodiphenyl ether (BDE-47). TCS, at 10 μM, not only induced luciferase expression to 40% of that of TCDD induction but also inhibited the induction of luciferase expression by TCDD by approximately 30%. These agonist/antagonist results are consistent with TCS being a partial agonist of the AhR.

Table 1. Induction or inhibition of AhR-dependent luciferase reporter gene expression in H4L1.1c4 cells.

| Compound | 1 μM of compound | 10 μM of compound |
|-----------|------------------|-------------------|
| Induction of luciferase | | |
| TCDD | 100 ± 8 | 100 ± 8 |
| I | 2.0 ± 0.1 | 1.8 ± 0.2 |
| II | 0.1 ± 1.7a | 0.6 ± 0.1a |
| III (TCC) | 0.8 ± 0.4a | 1.2 ± 0.3a |
| IV | 0.1 ± 0.2a | 0.1 ± 0.5a |
| V | 0.5 ± 0.7 | 1.3 ± 0.4 |
| VI | 50.9 ± 1.5* | 13.6 ± 1.9 |
| VII | 85 ± 0.6 | 3.6 ± 0.2a |
| VIII (TCS) | 6.0 ± 1.5 | 40.5 ± 6.1* |
| Inhibition of TCDD induction of luciferase | | |
| DMSO | 100 ± 8 | 100 ± 8 |
| I | 85.9 ± 4.4* | 80.3 ± 0.6a |
| II | 61.4 ± 0.7a | 81.3 ± 4.2a |
| III (TCC) | 64.6 ± 3.2a | 50.7 ± 2.8a |
| IV | 75 ± 2.5a | 33.4 ± 4.1a |
| V | 96.2 ± 3.2 | 79.3 ± 4.6a |
| VI | 103.2 ± 2 | 112 ± 3 |
| VII | 97.1 ± 2.0 | 32.5 ± 1.6* |
| VIII (TCS) | 98.3 ± 5.5 | 70.4 ± 2.1* |

Values are expressed as a percentage of that induced by 1 nM TCDD and represent the mean ± SD of triplicate determinations of luciferase activity.

*Data from Zhao et al. (2006). aSignificantly different from the DMSO-treated controls or TCDD-treated samples (p < 0.05).

Figure 2. Results of ER- and AR-mediated bioassays showing the effects of 1 μM TCC on gene expression of ER (A) and AR (B) induced by E2 in BG1-ERE cells and testosterone (T) in T47D-ARE cells, respectively. Luciferase activity (mean ± SD) is expressed relative to that maximally induced by E2 and T in (A) and (B), respectively.

*Significantly greater than E2 or T positive control groups (p < 0.05).
HEK 293Y-ARE cells; however, when combined with testosterone at a physiologically relevant concentration of 0.1 nM in HEK 293Y-ARE cells (Figure 4B), the 1-µM concentration of the carbanilide compounds, except for TFC (VII), exhibited a range of 20–100% amplification of testosterone-induced AR activity. Carbanilide VI and VII did not alter the signal significantly. Taken together, the results observed in the two cell lines strongly support the ability of a number of the carbanilide compounds to interact with the AR or AR-signal transduction pathway, leading to enhancement of AR-dependent gene expression.

The above results indicate the ability of TCC and other compounds to enhance hormone-, ER-, and AR-dependent reporter gene expression. Given the environmental and exposure relevance of TCC, we examined the concentration-dependent nature of the TCC-dependent enhancing effect in both cell bioassay systems. We examined the effect of increased concentrations of TCC in the cell incubation medium (up to a maximum of 1 µM; we observed toxicity at higher concentrations) on ER- and AR-dependent gene expression levels using maximally inducing concentrations of E2 or testosterone, respectively. These analyses reveal a TCC concentration-dependent enhancement of E2- and testosterone-dependent reporter gene expression (Figure 5). In contrast, coincubation with TCS (VIII) resulted in a TCS concentration-dependent decrease of E2-dependent reporter gene expression, with 50% inhibition observed at a concentration of 1 µM TCS (Figure 6). TCS did not exhibit estrogenic activity at any concentration shown in the absence of E2.

RyR-responsive bioassay. TCS significantly increased the amount of [3H]Ry binding to microsomes enriched in RyR1 from skeletal muscle (Figure 7). We measured net changes in intracellular Ca2+ concentration in myotubes with the fluorometric dye fura-2. The initial rate of Ca2+ increase in the cytoplasm in resting myotubes depends on the concentration of TCS (0.5–10 µM) perfused in the medium (Figure 8A). As shown in Figure 8B, TCS increased cytosolic Ca2+ concentrations even in the presence of a buffer containing nominally free extracellular Ca2+ (~ 7 µM), indicating that TCS can mobilize Ca2+ to the cytoplasm from endoplasmic reticulum/sarcoplasmic reticulum and/or mitochondrial intracellular stores (Figure 8B). The carbanilide compounds, including TCC, showed no significant perturbation of resting Ca2+ concentration when perfused at ≤ 10 µM (data not shown). TCS has a noncoplanar configuration and substitutions at the ortho position similar to PCB-95, one of the most active congeners toward RyR1 (Pessah et al. 2006; Wong and Pessah 1996).
(Figure 9). However, TCS is significantly less hydrophobic than PCB-95 (log P = 4.76 vs. 6.55, respectively). Also, because of the other linkage, TCS is significantly longer and more flexible than PCB-95. TCS activity at RyR may therefore reflect its ability to assume a conformation like those of noncoplanar PCBs.

Results from molecular modeling indicate that TCS has around 67° and 100° dihedral angles formed by the two phenyl rings, similar to the configuration of PCB-95, which has around 78° and 113° dihedral angles, supporting the importance of noncoplanarity for RyR activity.

Discussion

We have examined the biological activity of the antimicrobial TCC, its carbanilide analogs, and TCS in in vitro and cell-based AhR, ER, AR, and RyR bioassays. Although TCC and other carbanilide compounds exhibited no significant or weak agonist activity in the ER and AR cell bioassays, most of the compounds enhanced the ability of the steroid hormones E2 and testosterone to induce AR-dependent reporter gene expression. These results indicate that these chemicals exhibit distinct mechanisms of action on these distinctly different ligand-dependent nuclear receptor signaling pathways.

The signal enhancement activities by the carbanilide compounds were not involved in cell proliferation. At concentrations < 1 μM, TCC, its analogs, and TCS showed no significant effect on ATP levels for cell proliferation/cytotoxicity by the ViaLight kit in HEK-293 cells relative to the solvent control (data not shown). ViaLight cell proliferation and cytotoxicity assay was performed according to manufacturer instructions (Cambrex, East Rutherford, NJ). Similarly, < 1 μM, TCC showed no significant effect on methylthiazol tetrazolium activity in the HEK 293Y-ARE cell proliferation assay (Chen et al. 2008). Some compounds exhibited cytotoxic effects at concentrations > 0.1 μM in HEK-293Y-ARE, T47D-ARE, and BG1-ERE cell lines.

Because relatively small quantities of impurities may obscure the results for TCC, we further purified commercial TCC purchased at a purity of 99.8% by recrystallization, and we estimated higher purity (> 100%) of TCC at 270 nm by HPLC. We evaluated the recrystallized TCC for the enhancement effect in the ER- and AR-mediated bioassays. The results were not significantly different from those in the bioassays using the commercial TCC (data not shown), so we conclude that the steroid-enhancing activity is mediated by the chemicals themselves and not by a contaminant(s).

Although the trend of the enhancing activity produced by several carbanilides in the presence of testosterone in androgen-responsive recombinant T47D breast cancer cells and HEK-293Y cells is similar, we demonstrated the testosterone concentrations at which we observed enhanced induction to be distinctly different. The different outcomes may result from a cell-type-specific biological response for the testosterone-induced AR-dependent luciferase reporter gene expression and from differences between the endogenous AR in T47D cancer cells and the exogenous transformed AR in HEK-293Y cells, as well as differences in the specific interlaboratory protocols.

Enhanced gene expression by carbanilide compounds in the presence of endogenous steroids in the AR- and ER-mediated reporter gene bioassays (present study) and the increased expression of AR protein by TCC and testosterone in MDA-kb2 human breast cancer cells (Chen et al. 2008) suggest that the carbanilides may sensitize the complex of
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Table 2. The biological activity of TCC, its analogs, and TCS in the receptor bioassay screens.

| Compound | AhR (H4L1.1c4-DRE cells) | ER (B51-ERE cells) | AR (T47D-ARE or HEK2933Y-ARE cells) | RRA (lH)H(1 binding) |
|----------|--------------------------|-------------------|-----------------------------------|---------------------|
|          | Compound alone | With 1 nM TCC | Compound alone | With 1 nM E2 | Compound alone | With 0.1 nM or 10 μM T |
| I        | –             | + (ant)         | + (ag)           | + (lamp)         | + (ag)* | + (amp)* |
| II       | –             | + (ant)         | + (ag)           | + (lamp)         | + (ag)* | + (amp)* |
| III (TCC) | –             | + (ant)         | + (ag)           | + (lamp)         | –       | + (amp)* |
| IV       | –             | + (ant)         | + (ag)           | –               | –       | + (amp)* |
| V        | –             | + (ant)         | + (ag)           | + (lamp)         | – (ag)* | + (amp)* |
| VI       | + (ag)         | –               | + (ag)           | + (lamp)         | –       | + (amp)* |
| VII      | + (ag)         | –               | + (ag)           | + (lamp)         | –       | + (amp)* |
| VIII (TCS) | + (ag)     | + (ant)         | –               | + (ant)          | –       | + (amp)* |

Abbreviations: –, no effect; +, positive effect; ag, agonistic; amp, amplification; ant, antagonistic.

*Data from T47D-ARE bioassays. *Data from HEK 2933Y-ARE bioassay.
bioassay. Similar to noncoplanar ortho-substituted PCBs, the noncoplanar antimicrobial compound TCS exhibited weak AhR activity but was a potent antagonist in both ER-mediated and AR-mediated bioassays and a potent channel sensitizer in an Ryr2-mediated bioassay and dysregulator of cell Ca2+ homeostasis. These observations have potentially significant implications regarding human and animal health because exposure may be directly through dermal contact or indirectly through the food chain. These screening studies revealed that further investigations into the biological and toxicologic effects of TCS, its carbamidane analogs, and TCS are urgently needed.

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