Reproductive Sciences
18(2) 119-127

DOI: 10.1177/1933719110382581
http://rs.sagepub.com

# Effects of Triclocarban on Intact Immature Male Rat: Augmentation of Androgen Action

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**Abstract**

Triclocarban (TCC; 3,4,4′-trichlorocarbanilide) is an antimicrobial agent used widely in various personal hygiene products including soaps. Recently, TCC has been shown to enhance testosterone-induced effects in vitro and to enlarge accessory sex organs in castrated male rats. This study was designed to evaluate the effects of TCC on intact age-matched male rats and on human prostate LNCaP and C4–2B cells. Seven-week-old male Sprague-Dawley rats received either a normal diet or a diet supplemented with TCC (0.25% in diet) for 10 days. Triclocarban induced hyperplasia of accessory sex organs in the absence of significant qualitative histological changes. Serum luteinizing hormone (LH) and testosterone were not significantly altered by TCC treatment. In prostate cancer-derived LNCaP and C4–2B cells, TCC potentiated androgen actions via androgen receptor-dependent actions. In conclusion, TCC significantly affects intact male reproductive organs and potentiates androgen effects in prostate cancer cells.

**Keywords**

triclocarban, androgen receptor, luteinizing hormone, testosterone, endocrine-disrupting substance

## Introduction

Triclocarban (TCC; 3,4,4′-trichlorocarbanilide) is a topical antimicrobial agent used in a wide range of personal hygiene products including many brands of soap, toothpaste, and shampoo. Environmental contamination by TCC is significant, as this compound is incompletely removed by wastewater treatment, resistant to transformation, and it accumulates at relatively large concentrations in municipal sludge and biosolids-amended soils.[1-6] In a recent study, TCC was detected in all samples of water collected downstream of a wastewater treatment plant in the United States at concentrations averaging over 80 ng/L, these concentrations were over 6-fold greater than upstream of the plant.[5] The average TCC content in the sludge from the same plant was nearly 20 mg per kg of dry mass. A study performed at a typical US wastewater treatment plant has demonstrated that approximately three quarters of TCC disposed by the consumers is released to the environment in the form of sludge, which is often used in agriculture.[1,3,6] Potential human health risks resulting from TCC exposures were addressed in the literature over two decades ago.[7] However, at that time it was not recognized that TCC could act as an endocrine-disrupting substance (EDS) at exposure levels that are a fraction of those required for traditionally recognized toxic effects.

Recently, we have demonstrated that TCC possesses unique and potent androgen-augmenting activity.[8] In vitro, TCC effects were evaluated in a cell-based human androgen receptor

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(AR)-mediated bioassay system.[9] Triclocarban alone had no effect on AR-mediated transcriptional activity; however, it augmented testosterone (T)-induced effects.[8] In castrated peripubertal male rats, TCC also augmented androgen-induced weight gain of accessory sex organs, but it had no such effect when given to these animals in the absence of exogenous androgen.[8]

Exposures to EDS during early life are perhaps the prime suspects causing irreversible adverse effects because of the crucial role of hormones in directing the development and programming of cells for later life.[10-14] In view of the widespread and multifaceted exposure to TCC as well as potential adverse effects on androgen-dependent tissue, several new questions arose. Does TCC adversely affect the peripubertal intact (ie, noncastrated) male? Are the effects of TCC on accessory sex organs' weight related to water imbibition? Does TCC exposure lead to hyperplasia in steroid hormone-sensitive tissues?

To address these questions, this report presents a new study on intact male rat evaluating the effects of TCC on accessory sex organs, T levels, and luteinizing hormone (LH) levels. In addition, the mechanism of TCC action was investigated using 2 human prostate cancer cell lines with different levels of AR expression. The results of these studies demonstrate for the first time that TCC induces androgen-augmenting effects on intact peripubertal animal without significant changes of circulating LH or T levels and that action of TCC on prostate cancer cells is androgen-receptor dependent.

## Materials and Methods

### Chemicals

Bovine serum albumin protein standard and Bio-Rad protein assay dye reagent were obtained from Bio-Rad Laboratories (Hercules, California). Triclocarban (99.3% pure), double-stranded deoxyribonucleic acid, and other reagents were purchased from Sigma-Aldrich (St. Louis, Missouri). The reference standard for LH assays was rat LH-RP-2 provided by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

### Animals and Specimen Collection

Twenty-four Sprague-Dawley rats (at the age of 48-52 days) were randomly assigned to 2 treatment groups, each consisting of 12 animals: control group (standard diet) and TCC group (0.25% TCC by weight in diet of standard rat chow). The dose of TCC was based on our previous in vitro work and previous study evaluating effects of oral exposure to TCC in castrated animals.[8] Food intake was not monitored. After 10 days of treatment, the animals were anesthetized and blood was collected by cardiac puncture prior to euthanasia. Necropsies and blood sample collections were conducted at the same time of the day (9 AM to 11 AM) for both treated and control groups. Blood was allowed to clot at room temperature before serum was obtained by centrifugation. There was no noticeable difference in visceral fat between the groups. Liver, kidney, adrenal glands, testes, levator ani-bulbocavernosus muscle (LABC), glans penis, ventral prostate, and seminal vesicles were surgically removed and weighed. Organs of half of the animals from each group were fixed and assessed histologically. Organs of the other half of the animals were freeze-dried using a Virtis 50-SRC freeze dryer (Virtis Co, Inc, New York), weighed, and protein and DNA content determined.

All experiments were conducted in accordance with regulations of the Animal Care and Use Committee of University of California Davis, in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

### Histology and Immunohistochemistry

Evaluations were performed by a board-certified histopathologist who was blinded to treatment groups. Sections were examined with routine hematoxylin-eosin staining. Immunohistochemistry for ARs was also conducted on sections after antigen retrieval as previously described with antisera raised in rabbits against human AR (Santa Cruz Biotechnology, Santa Cruz, California).[15] The primary antisera was used at both 1:100 and 1:1000 dilutions and incubated with sections overnight at 4°C, followed by addition of biotinylated secondary antibody and avidin–biotin horseradish peroxidase complex (ABC reagent, Vector Laboratories, Burlingame, California). Immunorecognition was visualized using 3-amino-9-ethylcarbazol (AEC).

### Protein and DNA Determinations

Frozen tissues were homogenized in 6 to 20 volumes of Dulbecco's buffered saline and protein concentration determined by the Bio-Rad (Bradford) protein assay (BioRad Laboratories, Hercules, California). Total DNA of tissue homogenates was assessed by the diphenylamine assay.[16] Briefly, 0.2 mL of tissue homogenates was mixed with 0.8 mL of 10% TCA and centrifuged 2 minutes at 2000*g*. The pellet was resuspended in 0.5 mL of 5% Trichloroacetic acid (TCA) and incubated 15 minutes at 90°C to degrade nucleic acids. Following 2 minutes centrifugation at 2000*g*, 0.4 mL of the supernatant fraction was mixed with 0.8 mL of diphenylamine reagent (1.5 g diphenylamine, 100 mL acetic acid, and 1.5 mL sulfuric acid). The diphenylamine-deoxyribose sugar colorimetric reaction was attained by for boiling 10 minutes in a water bath followed by overnight incubation at room temperature and absorbance reading at 595 nm. Stock DNA solution for the standard curve was prepared using a commercial double-stranded DNA (Sigma-Aldrich).

### Luteinizing Hormone and T Assays

The serum samples were stored at −70°C. Luteinizing hormone was assayed by radioimmunoassay (RIA) using monoclonal antibody 518B7 to bovine LHβ[17] and labeled ovine LH (AFP86148; NHPP-NIDDK) as tracer. Reference standard was rat LH-RP-2 obtained from NIDDK. The intra- and inter-assay coefficients of variation for a pool of medium (obtained from GnRH-stimulated rat pituitary cells in culture) containing

5.1 ± 0.14 ng LH/mL (n = 4 assays) were 4.7% and 5.5%. Serum T was measured using a commercially available competitive chemiluminescent immunoassay kit (Siemens Diagnostics, Tarrytown, New York) on the ACS-180 automated platform. The intra- and interassay coefficients of variation were 6.0 and 8.7%, respectively. The analytical sensitivity of the assay for T was 0.1 ng/mL.

### Detection and Quantification of TCC

Serum (10 μL) was diluted with 50 μL of water followed by 200 μL ethyl acetate and 10 μL of surrogate 13C-triclocarban (50 ng/mL dissolved in acetonitrile, AcN). The mixture was vigorously shaken for 30 seconds on a vortex mixer and then centrifuged at 10 000 rpm for 5 minutes. The ethyl acetate layer was then aspirated and the aqueous layer reextracted with 200 μL of ethyl acetate. The combined ethyl acetate extractants were dried with high purity nitrogen and reconstituted in methanol and taken directly to the LC-MS for analysis. Chromatographic separation was performed using an Agilent 1100 HPLC (Palo Alto, California) equipped with a 100 × 2 mm 5 um C18 Prodigy column (Phenomenex, Torrance, California) held at 40°C. A solvent system consisting of $H_2O$ (solvent A) and AcN (solvent B) was used in a gradient system. The analytes were separated using a gradient program (0.3 mL/min) starting with a solvent composition of 40% AcN, ramped linearly for 10 minutes to 100% AcN, and then held for 6 minutes. An injection volume of 10 μL extracted sample was used. Following chromatographic separation, the target analytes were detected by electrospray ionization in negative mode, ion trap mass spectrometry in multiple reaction monitoring mode (MRM) using a Brukerion trap mass spectrometer (Agilent). Nitrogen gas provided a nebulizer pressure of 35 psi and a drying gas flow of 12 L/min. Electrospray ionization used a capillary voltage fixed at 5 kV and a drying temperature of 350°C.

### Cell Culture and Transfection

The human prostate cancer cell line LNCaP and C4–2B were obtained from the American Type Culture Collection (ATCC, Manassas, Virginia). Cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 media supplemented with 10% FBS and supplemented with 100 U penicillin/streptomycin at 37 °C and 5% CO2. Luciferase reporter plasmids containing probasin or three repeats of the androgen response element ligated in tandem to the luciferase reporter (ARE-luc) were described previously[18,19]

For luciferase reporter assays, cells were seeded in 24-well plates ($5 \times 10^4$ cells per well) and grown to about 50% to 60% confluence. Each of the relevant promoter–luciferase constructs (Probasin-luc or ARE-luc) was transfected into LNCaP or C4–2B cells. Transfected cells were treated with T, DHT, TCC, or the combination of TCC and alpha dihydrotestosterone (DHT) or TCC and T. In addition, the AR inhibitor, bicalutamide, was added to test the independency of the signal transduction via AR. After 24 hours of treatment, the cells were processed for luciferase assays. Cell lysates (25 μL/well) were used for measurement of luciferase activity in a luminometer. Data were represented as relative light units (RLU).

### Statistical Analysis

All data were expressed as mean ± SE. Data were analyzed, as appropriate, either by Student *t* test or, in the absence of normal distribution, by Wilcoxon rank-sum test using JMP statistical package for Macintosh computer.

## Results

### Effect of TCC on Animal and Organ Weights, Water Content, Protein, and DNA

Table 1 summarizes effects of TCC on animal body weight and on the total weight of individual organs. Pretreatment body weights were comparable in both groups; however, during the 10-day course of the study, animals exposed to TCC gained significantly more weight than control animals (on average, 85.6 vs 67.0 g). This resulted in an average 5.1% greater terminal weight in the TCC group. At the end of the treatment, livers in the TCC group were also bigger, weighing on average 13.3% more than in the control group. Kidneys, adrenals, and testes in both groups had comparable weight. In contrast, all accessory sex organs were significantly enlarged in TCC group, with seminal vesicles weighing 42% more, ventral prostate 37% more, LABC 136% more, and glans penis 35% more. To determine whether the differences in organ weight persisted after accounting for greater total body weight in the TCC group, organ weights were also expressed as percentage of terminal body weight (Table 1, columns on the right). Following this calculation, liver, seminal vesicles, ventral prostate, LABC, and glans penis remained significantly larger in the TCC group compared to control group.

Since total organ weight may be affected by water imbibition, individual organs from half of the animals were also weighed after freeze-drying. Table 2 summarizes effects of TCC on dry weight and water content. It is apparent that TCC treatment significantly affected dry weight of accessory sex organs but had no effect on the dry weight of testes, liver, kidneys, or adrenal glands. Water content remained comparable in TCC and control group with the exception of LABC, which was significantly greater in TCC group by 11%.

Table 3 summarizes protein and DNA content of individual organs in TCC and control groups. Significantly greater protein and DNA content was noted in TCC group in ventral prostate, LABC, and glans penis.

### Luteinizing Hormone, T, and TCC Levels

No significant differences in either of circulating LH or T were noted between TCC and control groups (Figure 1). When the levels of TCC in serum were compared, treated animals showed significantly higher levels of TCC compared to controls (43.1 ± 4.2 vs 3.6 ± 0.5 ng/mL respectively, $P < .05$).

**Table 1.** Effect of TCC on Weights of Animals and Individual Organs[a]

| Variable | Weight (g) | | | Weight as Percent of Terminal Body Weight | | |
|---|---|---|---|---|---|---|
| | Control | TCC | *P* Value | Control | TCC | *P* Value |
| Pre-treatment body weight | 226.7 ± 20.9 | 223.2 ± 14.9 | .64 | | | |
| Post-treatment body Weight | 293.7 ± 11.7 | 308.8 ± 18.8 | .028 | | | |
| Liver | 11.7 ± 1.1 | 13.3 ± 1.2 | .002 | 3.99 ± 0.34 | 4.31 ± 0.38 | .004 |
| Kidney | 2.74 ± 0.27 | 2.94 ± 0.26 | .08 | 0.93 ± 0.07 | 0.95 ± 0.10 | .54 |
| Adrenal | 0.080 ± 0.016 | 0.081 ± 0.012 | .82 | 0.027 ± 0.005 | 0.026 ± 0.004 | .73 |
| Testes | 2.82 ± 0.19 | 2.82 ± 0.20 | .99 | 0.96 ± 0.07 | 0.92 ± 0.08 | .16 |
| Seminal vesicle | 0.625 ± 0.11 | 0.894 ± 0.153 | <.0001 | 0.21 ± 0.04 | 0.29 ± 0.04 | <.0001 |
| Ventral prostate | 0.342 ± 0.082 | 0.468 ± 0.085 | .001 | 0.12 ± 0.03 | 0.15 ± 0.02 | .002 |
| LABC | 0.226 ± 0.048 | 0.532 ± 0.186 | <.0001 | 0.077 ± 0.016 | 0.174 ± 0.063 | <.0001 |
| Glans penis | 0.103 ± 0.014 | 0.138 ± 0.016 | <.0001 | 0.035 ± 0.005 | 0.045 ± 0.004 | <.0001 |

Abbreviations: LABC, levator ani-bulbocavernosus muscle; TCC, 3,4,4′-trichlorocarbanilide.
[a] Each value represents mean ± SD of 12 rats per group.

**Table 2.** Effect of TCC on Dry Weight and Water Content of Individual Organs[a]

| Variable | Dry weight (g/organ) | | | Water content (%) | | |
|---|---|---|---|---|---|---|
| | Control | TCC | *P* Value | Control | TCC | *P* Value |
| Liver | 3.46 ± 0.24 | 3.75 ± 0.33 | .11 | 71.1 ± 1.2 | 71.0 ± 1.4 | .81 |
| Kidney | 0.69 ± 0.04 | 0.77 ± 0.08 | .05 | 74.6 ± 1.3 | 75.0 ± 2.7 | .75 |
| Adrenal | 40.9 ± 17.3 | 33.2 ± 14.2 | .42 | 59.7 ± 14.2 | 57.4 ± 19.3 | .84 |
| Testes | 0.40 ± 0.04 | 0.41 ± 0.04 | .74 | 85.6 ± 0.9 | 85.1 ± 0.6 | .31 |
| Seminal vesicle | 157 ± 36 | 216 ± 34 | .02 | 76.9 ± 2.6 | 75.8 ± 2.4 | .43 |
| Ventral prostate | 107 ± 21 | 121 ± 20 | .27 | 72.9 ± 4.9 | 74.2 ± 3.5 | .63 |
| LABC | 62 ± 10 | 143 ± 32 | .0001 | 68.9 ± 4.6 | 76.8 ± 5.0 | .02 |
| Glans penis | 25 ± 4 | 38 ± 6 | .001 | 75.3 ± 2.7 | 73.1 ± 3.7 | .27 |

Abbreviations: LABC, levator ani-bulbocavernosus muscle; TCC, 3,4,4′-trichlorocarbanilide.
[a] Each value represents mean ± SD of 6 rats per group.

**Table 3.** Effect of TCC on Protein and DNA Content of Individual Organs; Each Value Represents Mean ± SD of 6 Rats Per Group

| Variable | Protein (mg/organ) | | | DNA (mg/organ) | | |
|---|---|---|---|---|---|---|
| | Control | TCC | *P* Value | Control | TCC | *P* Value |
| Liver | 1,942 ± 368 | 2,073 ± 402 | .56 | 27.6 ± 4.6 | 28.5 ± 6.9 | .81 |
| Kidney | 529 ± 135 | 620 ± 53 | .15 | 8.8 ± 2.0 | 9.5 ± 2.0 | .59 |
| Adrenal | 18.5 ± 2.7 | 15.1 ± 3.6 | .09 | 0.61 ± 0.18 | 0.46 ± 0.11 | .10 |
| Testes | 332 ± 46 | 321 ± 55 | .69 | 8.6 ± 1.3 | 8.0 ± 1.2 | .47 |
| Seminal vesicle | 66.0 ± 21.3 | 73.0 ± 12.0 | .49 | 0.75 ± 0.09 | 0.95 ± 0.24 | .12 |
| Ventral prostate | 58.9 ± 9.9 | 75.4 ± 7.4 | .008 | 0.93 ± 0.36 | 1.44 ± 0.39 | .04 |
| LABC | 21.2 ± 5.9 | 75.0 ± 16.9 | <.0001 | 0.37 ± 0.17 | 0.75 ± 0.23 | .008 |
| Glans penis | 13.4 ± 4.1 | 22.5 ± 5.4 | .01 | 0.30 ± 0.16 | 0.51 ± 0.16 | .02 |

Abbreviations: LABC, levator ani-bulbocavernosus muscle; TCC, 3,4,4′-trichlorocarbanilide.

## *Histology and ARs*

There were no visible abnormalities of any of the accessory sex glands, penis or testes, in treated animals and no histologically distinguishable difference between specimens from the control and treated animals. The vesicular glands were variably distended with fluid, the epithelium was simple or pseudo-stratified and thrown into numerous, complex, primary and secondary folds extending into and sometimes obliterating the lumen. Lobes were surrounded by connective tissue and a thick layer of smooth muscle but appeared similar in treated and control tissues (Figure 2A). The acini of the prostate gland were also distended, lined by a simple epithelium, and surrounded by a thin connective tissue and smooth muscle layer (Figure 2 C). As for the vesicular glands and prostate gland, AR expression was higher in the nuclei of the epithelial cells than those of the smooth muscle (Figure 2B and D). Although immunostaining

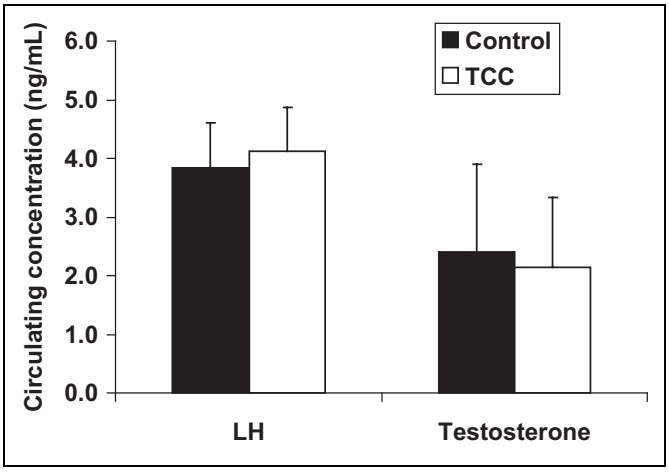


**Figure 1.** Circulating levels of luteinizing hormone (LH) and total testosterone at the end of the experiment; each bar represents mean ± SE.

was far less intense at the 1:1000 dilution, still no difference was obvious in levels of AR expression in neither vesicular nor prostate gland from treated and control animals. Overall, all tissues were histologically normal and there were no consistent histological differences among any animals examined from treated and control groups.

### *Effect of TCC on Androgen Induced Transcriptional Activity in Human Prostate Cells*

Testosterone and DHT treatments induced luciferase activity in LNCaP cells transfected with probasin (Figure 3A) or simple ARE promoters (Figure 3C). Cotreatment of androgen with TCC (1.0 nmol/L) further increased luciferase activity by 221% (Probasin promoter) and 175% (ARE promoter) in LNCaP cells compared to androgen treatment alone ($P < .01$, Figure 3A and C). Similarly, in C4–2B cells, TCC further potentiated androgen-induced luciferase activity by 25.9% (Probasin promoter, Figure 3B) and 38.5% (ARE promoter, Figure 3D), compared to androgen treatment alone, although the amplification was less substantial than that observed in LNCaP cells, which have higher expression of AR ($P < .05$, Figure 3B and D). In both cell lines, the amplification enhanced by TCC was significantly suppressed by the strong AR binding inhibitor, bicalutamide.

## Discussion

The relevance of endocrine disruptors is being increasingly recognized.[20] This is the first report demonstrating that a novel endocrine disruptor, TCC, induces androgen-like effects in intact immature males. Key findings in this study include demonstrations that exposure to TCC (1) results in an increase of accessory sex organ weight, protein content, and DNA content, (2) has no significant effect on histological appearance of these organs, and (3) has no significant effect on circulating LH levels, circulating T levels, and on testicular size or histological appearance of testes.

Previously, our group demonstrated that TCC amplified the androgenic action of exogenous androgens with respect to the weight of accessory sex organs. The data presented here demonstrate that TCC induced a prominent increase of weight in all accessory sex organs (Tables 1 and 2) in age-matched intact male rats (48-52 days old). These tissues rely on androgenic stimulation to develop, grow, and maintain function. In addition, but to a lesser degree, TCC exposure resulted in modest but statistically significant increases of body weight and liver size, and these effects are consistent with the proposed androgen-augmenting actions of TCC and not to direct toxic effects of TCC. Anabolic effects of androgens are well recognized; for example, a previous study using the Hershberger assay has shown that testosterone propionate (TP) injections alone in castrated rat increases both total body weight and liver weight.[21] An increase of the body weight in the TCC-treated animals may be related to changes of androgen-binding protein (ABP) and/or effects of TCC on insulin action; future studies should address this issue.

In this study (in the presence of endogenous androgens) and our previous report (in the presence of exogenous androgens),[8] TCC treatment consistently induced increases of accessory sex organs that were disproportionately greater than its effects on body and liver weight. These effects persist, when weights of accessory sex organs are expressed as percentage of terminal body weight (Table 1, columns on the right) or percentage of liver weight (not shown). Since the weights of accessory sex organs may be affected by their water content, as well as the size and the number of cells, this study also collected data regarding dry organ weight, percentage of water content, protein content, and DNA content (Table 3). Only the LABC had a significant increase in water content following treatment with TCC. However, all accessory sex organs, including LABC, exhibited an increase in weight suggestive of either cell hypertrophy or hyperplasia. Ventral prostate, LABC and glans penis had significantly increased protein and DNA content (Table 3); hence, in view of the latter finding, exposure to TCC resulted in increased number of cells per organ, that is, hyperplasia.

Despite the clear differences in organ weights in this study, there was no defining histological difference between accessory sex glands from treated and control rats. This suggests that the increased growth associated with TCC in this short exposure period was proportional in the epithelium and surrounding parenchyma of each organ. Androgen receptor expression was higher in the epithelial cells of both the vesicular glands and the prostate than in the surrounding smooth muscle, as reported previously for rats and mice.[22,23] This difference in AR concentration did not result in an exaggerated or unbalanced growth response within the organ under the experimental conditions used. Tissue growth was apparently normal and organized, unlike that observed after neonatal exposure to estradiol.[22]

In our previous report,[8] effects of TCC were studied in vitro using the cell-based human AR-mediated bioassay developed by Chen and associates.[9] Triclocarban alone had no effect on

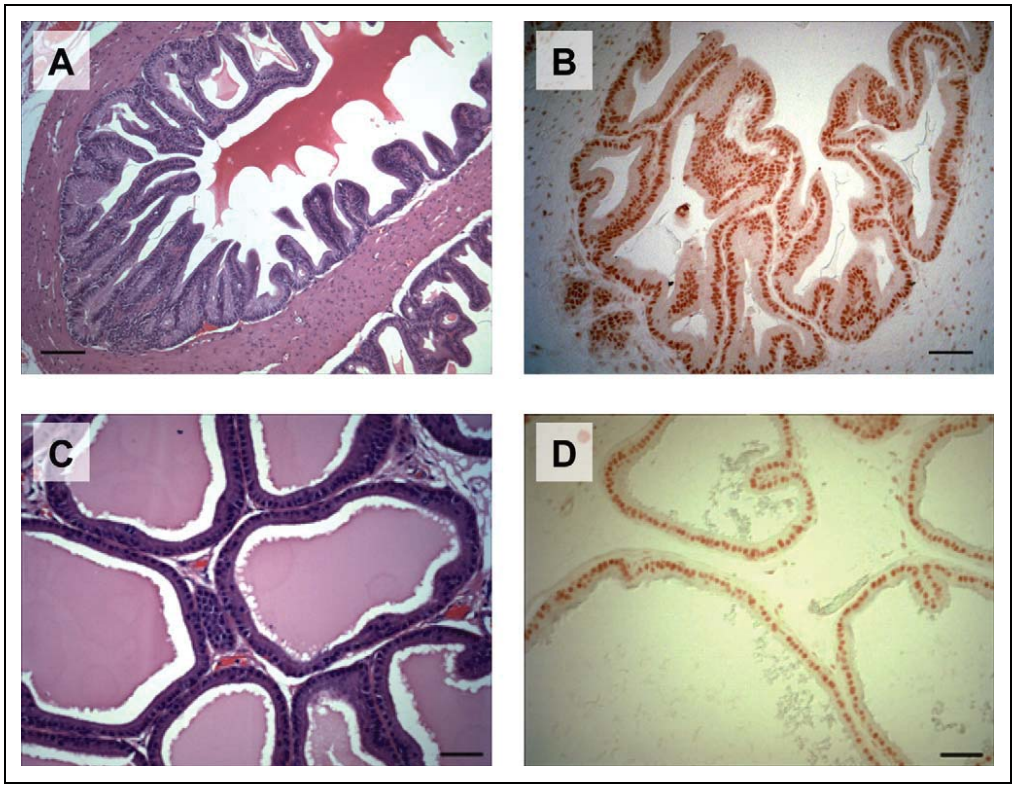


**Figure 2.** Triclocarban (TCC)-treated rat vesicular glands (A, B) and prostate (C, D) stained routinely with hematoxylin and eosin (A, C) and immunostained for androgen receptor (B, D). Note more heavily staining of androgen receptor expression in epithelial cell nuclei lining both the vesicular lobes and prostatic acini compared to staining in the nuclei of smooth muscle cells in both tissues. Bars = 100 μm (A, B) and 50 μm (C, D).

AR-mediated transcriptional activity, but it significantly amplified effects induced by T.[8] Furthermore, in that study, using the castrated immature rat model, TCC alone had no effect on accessory sex organs, but in the presence of TP, TCC significantly increased weight of all accessory sex organs above and beyond the effect of TP alone.[8,21] Previously, we have shown the lack of effect of TCC in cells with no AR expression.[8]

To further investigate the AR-dependent mode of action of TCC, 2 types of human prostate cancer cells with different levels of AR expression were transiently transfected with reporter gene driven by various androgen response elements. Human LNCaP is a well-established model for studies on androgen-dependent prostate growth.[24,25] C4-2 is a second generation of LNCaP subline.[26] It was derived from a chimeric tumor induced by co-inoculating castrated mouse with C4 cells and a bone stromal cell line. Although having chromosomal markers similar to those of the parental LNCaP cells, C4–2B expressed lower steady-state levels of AR protein and messenger RNA (mRNA) transcript and the cells is less responsive to androgens.[26] In this report, TCC and androgen (either T or DHT) cotreatment significantly increased luciferase activity compared to androgen alone in both LNCaP and C2–4B cells, although the effect was less pronounced in C4–2B (Figure 3); this may be due to its relative low level of AR expression. In both models, the enhancement of signal by TCC was suppressed by biclutamide, indicating the potentiation effect of TCC is AR-dependent. Hence, both in vitro and in vivo data point at TCC acting as a novel and unique endocrine disruptor with no intrinsic agonist or antagonist properties, but only potentiating androgenic effects at the level of the target cell. This report verifies these effects and demonstrates that exposure to TCC in the presence of physiological levels of endogenous androgens increases androgenic effects.

Another interesting observation was the absence of significant effect of TCC on LH and T levels, as well as no significant change in size or histology of testes. Since TCC exerted androgen-potentiating actions in vitro and in castrated rats, one could expect that TCC would potentiate androgen-induced negative feedback on hypothalamo-pituitary action, reduce LH, and ultimately decrease testicular androgen production. Several possibilities could attribute to the lack of the effect. Although the blood of both treated and control animals was collected at the same time of the day, LH is secreted in pulses varying in frequency and amplitude, which also stimulates T secretion in a pulsatile manner.[27] Consequently, the interpretation of the data regarding LH and T levels from a single blood collection should be cautious and future studies with serial blood collection during the treatment are warranted.

Peripubertal animals (48-52 days) were used in both castrated[8] and current intact male study for TCC exposure. The use of immature castrated male rats in our previous study was based on Hershberger's protocol.[28] Consequently, the present

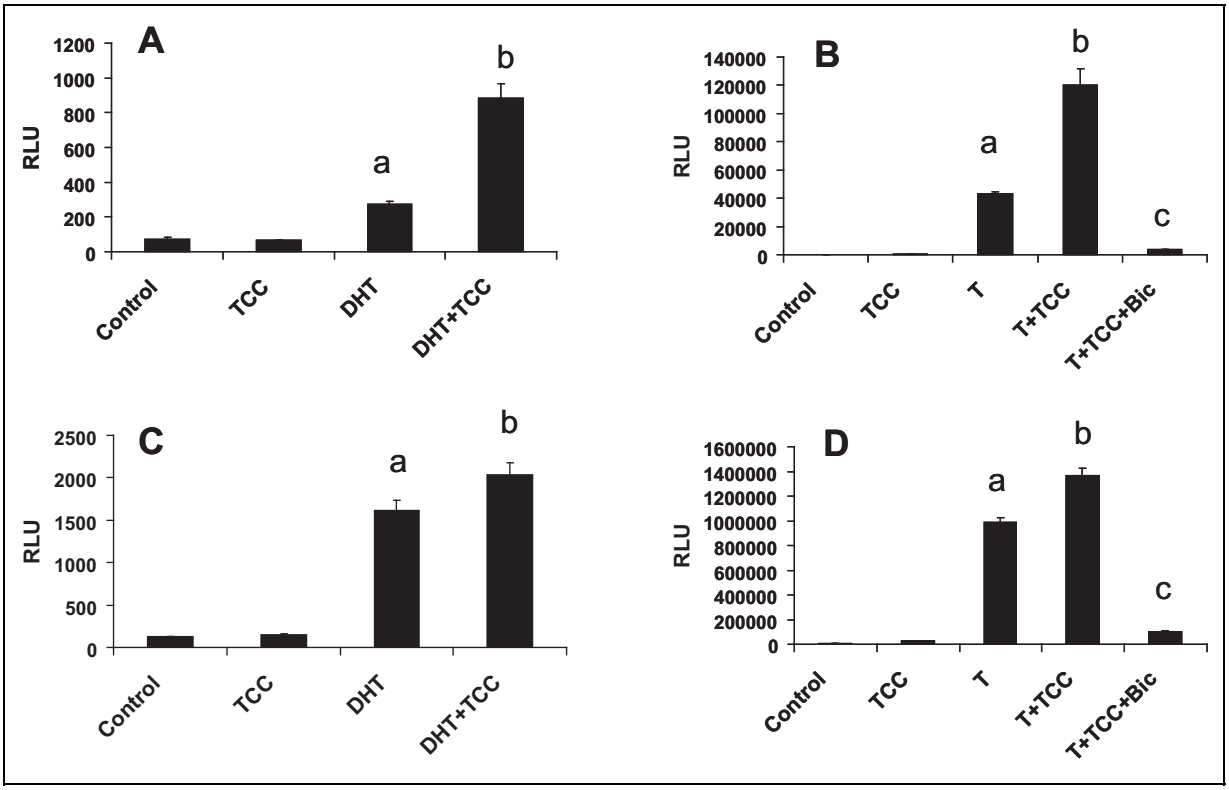


**Figure 3.** Effect of triclocarban (TCC) on androgen receptor (AR)-dependent transactivation activity induced by androgens in LNCaP (A and B) and C4–2B (C and D) cells. Cells were treated with 1.0 μmol/L of TCC in the presence and absence of 1.0 nmol/L of androgen (A and C) or in the presence and absence of 5.0 μmol/L of biclutamide (Bic; B and D). Data were expressed as relative light unit (RLU). Each bar represents mean ± SE; a, significantly different from vehicle control and TCC; b, significantly different from vehicle control and from androgen treatment; c, significantly different from androgen treatment and from TCC plus androgen treatment.

report was designed to assess the effect of TCC exposure on age-matched intact males. It is known that in the rat, production of T is high during late gestation, decreases dramatically in the neonate, reaching a nadir at about 2 weeks after birth, and increases again to attain adult levels by 60 days of age[29,30] accompanied by a shift from predominately androstenedione, observed in prepubertal rats, to testosterone seen in mature rats.[31] This changing androgen secretion pattern may be critical for T to reach adult levels with decreasing sensitivity of the LH negative feedback system being a component of the underlying mechanism.[31] It is therefore possible that the fully mature long-loop feedback has not yet been established in our rat model. Alternatively, the mechanism(s) by which TCC augments androgen action on growth of accessory sex organs may involve different AR coregulators than those involved in mediating T actions on the hypothalamus and pituitary. Thus, for example, TCC effects may be limited to direct AR-dependent mechanisms and may not influence the long-loop negative feedback, which requires aromatization and estrogen receptor signal transduction at the central level.[32-35] Furthermore, it is not known whether TCC crosses the blood-brain barrier. Nevertheless, the absence of compensatory effects on LH in immature rats indicates that TCC exposure may be of potential clinical concern and warrants more investigation on interaction of age, dose, and length of exposure.

Another interesting point is the observation that the serum of control animals contained low but detectable levels of TCC. Hence, one may speculate that water or food may have contained TCC. However, the level of TCC in water and rat chow was not measured in this study.

The present observations raise concerns regarding potential significant health risks related to exposure to TCC.[1-6] Indeed, widespread use of TCC-containing soaps and other personal hygiene products over several decades has led to repeated exposures, which may have induced important long-term effects. Some of these potential adverse effects including in utero and peripubertal period may be far-reaching and may have irreversible consequences because of the crucial role of hormones in directing the development and programming cells for later life.[10-14] Furthermore, since TCC is remarkably stable and resistant to biological and chemical treatments, there is also a potential for exposure by ingestion of contaminated water and/or agricultural products exposed to TCC-containing sludge.[1-6] Notably, this study only evaluated the effects of oral exposure to TCC, whereas a dominant route of human exposure is likely dermal due to the use of TCC-containing soaps. However, human oral exposure is also possible in view of the presence of TCC in municipal sludge and water.[1-6] Further studies will need to include assessment of TCC absorption following use of TCC-containing products and determination of TCC content in drinking water and food.

## Declaration of Conflicting Interests

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

## Funding

The authors disclosed receipt of the following financial support for the research and/or authorship of this article: NIEHS Superfund Basic Research Program (P42ES04699)

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