Table 5 (*Continued*)

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
	Flutamide+TP	285.7±17.8	5.9±1.0**	12.9±2.7**	47.7±5.9**	12.2±1.1**	1.4±0.7**
Steroids							
Norethindrone	Vehicle control	280.8±6.6	5.2±1.1	9.9±0.6	48.2±5.8	10.3±1.0	1.3±0.4
	0.5	279.5±15.2	5.5±1.5	10.6±2.1	50.6±3.2	11.4±1.9	1.5±0.2
	2	278.7±7.1	5.2±0.9	10.8±2.3	50.3±7.5	10.4±2.1	1.4±0.2
	10	255.7±7.5**	5.3±0.9	10.4±1.7	47.9±3.2	11.1±1.3	1.4±0.4
	Vehicle+TP	293.2±10.9	29.6±4.0	56.9±13.1	105.3±14.9	22.7±2.0	5.8±1.4
	0.5+TP	287.3±10.0	31.0±3.6	57.1±5.3	113.8±10.2	22.4±1.4	5.8±0.9
	2+TP	284.3±11.1	33.2±2.9	71.9±15.7	110.8±6.8	23.9±1.6	5.8±1.3
	10+TP	263.8±11.1**	31.5±4.3	76.7±7.4	120.7±11.6	25.7±1.6*	7.6±1.6
	Flutamide+TP	283.1±8.2	7.1±1.2**	11.8±1.8**	57.6±8.6**	12.9±1.5**	1.3±0.3**
Norgestrel	Vehicle control	276.8±9.5	6.0±0.8	10.3±2.0	48.5±3.6	10.7±1.9	1.5±0.4
	10	275.6±12.5	6.8±1.9	11.2±1.3	56.4±8.2	12.3±1.3	1.4±0.4
	30	267.1±11.7	11.4±3.3**	14.9±2.5**	67.6±17.5*	13.7±3.2	1.7±0.4
	100	262.4±9.9*	20.9±9.9*	35.3±13.9**	94.3±22.3**	17.8±2.6**	2.7±1.8
	Vehicle+TP	286.3±17.0	33.9±3.8	85.9±16.5	121.9±15.8	23.5±0.9	6.9±1.3
	10+TP	285.0±6.2	33.8±5.8	76.2±18.6	112.3±13.9	22.1±2.4	7.0±1.3
	30+TP	279.5±18.7	32.0±3.8	83.9±27.3	133.6±20.4	24.8±3.4	7.6±1.5
	100+TP	259.6±13.7*	45.3±8.9*	117.4±28.5*	151.9±18.0*	26.5±1.9**	8.6±1.6
	Flutamide+TP	285.5±11.5	6.3±0.9**	10.3±1.3**	52.7±6.7**	11.5±1.9**	1.2±0.3**
Ethynyl estradiol	Vehicle control	278.0±18.1	4.9±1.2	10.7±1.2	48.7±6.5	10.5±0.9	1.5±0.7
	10	270.6±11.8	5.7±0.9	10.8±2.5	48.8±6.2	10.7±1.7	1.5±0.3
	50	255.4±14.0*	6.2±0.7*	12.6±1.7*	50.8±4.1	12.6±1.9*	1.5±0.2
	200	245.6±8.7**	5.7±0.7	12.7±1.9	44.0±3.0	12.6±1.1**	1.3±0.2
	Vehicle+TP	283.2±10.0	34.5±4.4	80.9±14.1	118.9±6.0	23.7±1.2	7.5±1.2
	10+TP	282.6±13.7	35.1±6.5	80.3±12.3	124.9±14.6	24.2±1.8	6.6±1.2
	50+TP	266.6±15.9	39.0±4.6	107.4±9.8**	129.1±10.0	25.4±1.7	7.5±1.4
	200+TP	259.8±14.7**	35.0±7.5	108.6±18.2*	125.2±11.8	26.2±2.2*	8.8±1.5
	Flutamide+TP	279.1±15.2	6.8±1.3**	10.2±0.9**	50.9±8.7**	10.5±1.9**	1.5±0.4**
Alkyl phenol							
Bisphenol A	Vehicle control	280.8±10.1	5.5±1.0	10.8±1.9	52.3±7.1	10.6±1.1	1.2±0.2
	50	280.2±13.5	5.4±1.0	12.0±2.6	46.1±6.1	11.6±2.0	1.1±0.4
	200	273.3±12.6	5.8±1.0	12.3±2.2	51.6±2.4	12.1±2.0	1.1±0.3
	600	272.8±13.9	5.9±1.0	12.8±1.6	53.0±9.1	13.1±1.1**	1.3±0.3
	Vehicle+TP	285.1±14.7	39.2±8.5	84.0±7.5	113.6±10.3	23.9±0.7	7.0±1.5
	50+TP	286.5±13.4	40.0±7.5	74.0±12.1	121.0±15.1	24.1±1.2	6.9±0.7
	200+TP	280.9±9.9	33.6±4.5	84.4±17.5	121.5±14.0	24.2±2.3	7.9±2.1
	600+TP	271.1±13.6	39.8±3.0	91.0±14.8	119.1±10.4	24.7±1.3	8.3±1.6
	Flutamide+TP	280.8±12.6	6.7±1.1**	12.6±2.6**	57.5±8.8**	11.9±1.9**	1.4±0.2**
Bisphenol B	Vehicle control	274.4±14.6	5.5±0.9	11.2±1.7	58.6±3.2	11.6±2.4	1.5±0.5

Table 5 (*Continued*)

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
	50	263.2±9.2	5.7±0.5	10.8±1.9	48.8±9.5	11.0±1.9	1.2±0.2
	200	260.1±9.4	6.1±0.8	12.9±1.3	47.8±8.0*	13.4±2.9	1.6±0.2
	600	251.6±7.6**	6.1±0.8	12.4±2.3	47.8±9.8*	12.5±2.1	1.3±0.3
	Vehicle+TP	281.1±12.7	29.2±3.7	72.2±20.3	112.1±11.1	23.3±2.2	6.8±0.9
	50+TP	273.8±12.8	30.4±5.6	68.8±19.8	109.6±9.0	22.5±2.4	6.9±1.1
	200+TP	270.5±10.4	35.3±3.8*	72.6±14.4	116.7±13.5	22.6±2.8	7.2±1.9
	600+TP	259.3±18.2*	45.6±5.6**	113.4±13.5**	142.8±17.0**	26.3±1.9*	8.7±1.4*
	Flutamide+TP	278.9±9.3	6.4±1.1**	13.7±4.8**	55.8±5.7**	12.5±2.3**	1.6±0.8**
Bisphenol F	Vehicle control	273.3±9.8	5.2±0.9	9.7±0.8	47.3±3.8	10.7±2.0	1.2±0.3
	50	268.5±10.1	5.2±1.3	10.9±1.1	47.7±7.9	11.8±1.5	1.2±0.3
	200	265.5±9.9	4.7±1.6	10.6±1.8	48.2±6.7	11.0±2.5	1.3±0.5
	1000	253.2±12.4*	5.7±0.4	10.7±1.3	47.9±4.6	10.9±2.4	1.4±0.3
	Vehicle+TP	282.1±13.1	31.3±6.4	75.9±21.1	115.3±19.6	23.2±1.8	5.8±1.4
	50+TP	276.9±8.2	33.6±4.7	76.7±22.4	105.3±19.2	23.6±1.1	6.9±0.9
	200+TP	266.0±11.2*	35.9±7.3	81.2±13.1	123.7±7.0	24.9±1.2	8.0±1.2*
	1000+TP	263.3±11.5*	34.9±4.8	85.9±23.1	114.6±14.5	23.2±2.8	7.6±2.3
	Flutamide+TP	282.2±7.9	6.4±0.9**	10.1±1.1**	51.4±9.8**	11.0±1.6**	1.1±0.3**
Alkyl phenol							
4-Tert-octylphenol	Vehicle control	281.9±7.9	5.2±0.7	10.2±1.2	50.0±5.4	11.6±1.3	1.2±0.4
	50	278.7±9.5	5.9±0.9	11.6±0.7*	50.8±6.7	12.8±1.4	2.1±1.1
	200	256.7±15.3**	5.8±0.9	10.4±1.9	49.4±7.8	12.3±2.1	1.7±0.4
	600(400)[a]	–	–	–	–	–	–
	Vehicle+TP	285.0±14.4	36.4±4.6	76.9±18.3	113.5±12.9	24.0±2.6	6.8±0.9
	50+TP	281.5±13.0	36.2±6.2	83.5±13.8	106.0±9.3	23.2±0.8	6.2±1.2
	200+TP	277.6±18.7	42.9±5.7	93.3±19.5	119.6±6.2	24.9±1.0	8.2±1.1*
	600(400)+TP	–	–	–	–	–	–
	Flutamide+TP	283.1±7.5	6.7±0.9**	10.8±1.7**	50.2±4.2**	11.3±2.6**	1.3±0.3**
p-Cumyl phenol	Vehicle control	279.9±17.4	5.7±1.2	10.5±2.8	56.3±6.8	11.1±2.3	1.5±0.7
	50	275.8±11.3	4.8±0.8	10.1±2.1	55.1±8.6	11.8±2.6	1.5±0.5
	200	274.8±14.3	6.0±0.4	10.5±2.1	51.8±6.5	12.0±2.4	1.5±0.4
	600	254.6±6.9*	5.7±1.2	12.4±2.6	51.6±4.8	12.9±3.0	1.4±0.4
	Vehicle+TP	287.1±7.9	33.9±7.2	71.7±12.0	116.6±12.4	23.1±1.5	6.9±0.9
	50+TP	289.0±14.6	36.6±7.9	67.6±16.8	109.8±8.4	22.5±0.4	6.5±1.0
	200+TP	286.5±11.7	35.7±2.2	79.7±15.9	122.0±13.6	23.6±2.2	5.7±1.5
	600+TP	271.5±13.8*	34.1±6.4	80.7±10.3	123.9±8.5	24.1±2.1	7.9±1.9
	Flutamide+TP	281.5±11.9	6.5±1.2**	10.2±1.9**	50.7±6.3**	11.4±2.4**	1.5±0.3**
Nonylphenol	Vehicle control	285.3±17.3	5.0±0.8	10.0±1.7	54.3±7.1	11.4±2.6	1.4±0.2
	10	279.8±16.8	5.4±0.3	10.3±1.0	49.0±6.2	10.8±1.2	1.1±0.4
	50	282.9±13.1	5.7±1.1	10.1±1.3	50.1±8.3	11.2±2.0	1.5±0.4
	200	251.4±23.6*	5.8±0.9	11.1±1.4	56.6±9.1	13.8±1.9	1.7±0.5

Table 5 (*Continued*)

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
	Vehicle+TP	288.8±18.4	35.9±3.2	91.2±12.5	117.2±8.2	23.5±1.1	7.0±1.0
	10+TP	290.1±7.3	34.1±7.2	78.1±19.8	119.9±14.1	23.8±0.7	6.9±1.0
	50+TP	287.9±14.4	40.0±6.0	85.6±19.9	123.4±16.4	24.7±2.2	7.1±1.6
	200+TP	270.9±22.5	37.4±5.6	76.6±9.4*	117.0±9.1	24.5±1.3	8.0±1.1
	Flutamide+TP	288.2±14.8	6.5±0.6**	10.2±1.2**	52.3±6.6**	11.7±1.8**	1.4±0.3**

[a] Numbers in parenthesis are reduced dose because toxic signs were observed during the study.
* Significantly different from vehicle control or vehicle plus TP at $P < 0.05$.
** Significantly different from vehicle control or vehicle plus TP at $P < 0.01$.
–, no data because animals died during the study.

contain estrogen receptors, which may mediate the weight changes observed in the rats given these chemicals. The seminal vesicle weight in rats given diphenyl-p-phenylenediamine and the glans penis weight in rats given 4,4′-(hexafluoroisopropylidene)diphenol, EE, and bisphenol A increased in the high-dose group and the middle- and high-dose groups, respectively. However, the control values for these organs in all of the studies varied considerably, and some of the accessory sex organ weight values for the groups tested with these chemicals are within the control ranges. Therefore, whether these chemicals exhibit an androgen agonistic property could not be determined. On the other hand, the seminal vesicle weight in rats given nonylphenol decreased in the high-dose plus TP group, but an apparent dose dependency was not observed. Furthermore, the seminal vesicle weight in this group was within the control ranges of other studies. Thus, nonylphenol was also not classified as an androgen antagonistic chemical. Further supportive data using in vitro assays are needed to determine the androgen agonistic or antagonistic effects of the chemicals examined in the Hershberger assay.

We did not classify chemicals as having an androgen agonistic effect or as inhibitors of the agonistic effect of TP if the organ weight of rats given the chemical increased without any clear dose-dependent relationship or if the organ weight of rats given the chemical decreased but did not decrease in rats given the chemical plus TP. On the other hand, the organ weights of rats given a particular chemical plus TP increased, but the same change was not clearly observed in rats given only chemical alone. This phenomenon is very interesting and suggests that the administration of these chemicals increases the availability or increases the action of TP.

Some chemicals have been found to have both estrogenic and androgenic effects in in vivo or in vitro assays (Cupp and Skinner, 2001; Re et al., 2001; Waters et al., 2002; Raun Andersen et al., 2002). Morphological testicular changes were detected in toxicological studies of estrogen agonistic compounds, such as EE or diethylstilbestrol (Atanassova et al., 1999; Yamasaki et al., 2002a), and the testicular changes and weight changes of

male accessory sex organs are thought to be attributable to increased secretion of FSH by the hypophysis following administration of estrogenic compounds for long periods (Atanassova et al., 1999). On the other hand, a decrease in ovary weight, increase in number of immature follicles in the ovaries, and increase in mammary gland secretion were detected in a repeated-dose oral toxicity study of the androgenic compound methyltestosterone (Okazaki et al., 2001). In the present study, uterotrophy by the androgen derivatives testosterone enanthate and methyltestosterone and increased accessory organ weights by estrogenic compounds equilin, norgestrel and estrone were detected. Estrogen and androgen receptors are said to be present in the accessory sex organs of male rats and mice (Re et al., 2001; Weihua et al., 2001; Williams et al., 2001), and specific androgen receptors have been demonstrated in the uterus of rats and mice (Beri et al., 1998). In our reporter gene assay, the PC10 values for ER-alpha of testosterone enanthate and methyltestosterone were 17 140 and 173 235, respectively, compared with values for EE and bisphenol A of <30 and 602 983 (Yamasaki et al., 2002b), respectively. Thus, the affinity for estrogen receptor alpha of testosterone enanthate and methyltestosterone in the reporter gene assay was higher than that of bisphenol A. The uterotrophy induced by androgen derivatives in the uterotrophic assay and increase in male accessory sex organ weights by estrogen compounds in the Hershberger assay may be receptor-mediated changes. Further studies using the receptor binding assays and reporter gene assays for androgen receptors as well as an aromatase assay are needed.

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