Supplemental Material

Christiansen et al, **Synergistic disruption of external male sex organ development by a mixture of four anti-androgens**

Supplemental Material, Table 1. Statistical dose-effect descriptors for pooled single and mixture exposures – nipple retention and normalized AGD index

Substance	Dose Response Function						Effect doses (mg/kg/day)				Max. observed
	RM [a]	$\hat{\theta}_1$	$\hat{\theta}_2$	$\hat{\theta}_3$	θ_{min}	$\hat{\theta}_{max}$	medium		low		mean effect
Nipple retention							ED6 [b]	[CI]	ED3 [b]	[CI]	
vinclozolin	G.Logit	-5.72	4.97	7.80	0	12	42.5	[36.0;45.9]	30.2	[25.9;33.2]	11.8
finasteride	Logit	3.25	2.09	-	0	12	0.028	[0.016;0.047]	0.008	[0.004;0.02]	9.5
DEHP	Logit	-4.22	1.32	-	0	12	1598	[612;11680]	234	[127;583]	5.2
prochloraz	BC-logit	-2.81	0.20	0.32	0	12	199	[99;714]	60.4	[34.0;93.1]	4.4
mixture	Logit	-4.38	3.09	-	0	13	23.3	[17.0;29.4]	10.6	[6.5;15.3]	11.6
AGD index (normalized to the controls)							ED60 [c]	[CI]	ED90 [c]	[CI]	
vinclozolin	Weibull	-9.97	4.94	-	0	1	76.1	[65.0;86.6]	36.5	[22.1;52.8]	25%
finasteride	Logit	-0.99	0.62	-	0	1	8.63	[1.83;94.7]	0.34	[0.19;0.57]	60%
DEHP	Weibull	-6.85	1.89	-	0	1	1945	[913; n.d.]	275	[41;1639]	86%
prochloraz	Logit	-12.00	4.95	-	0	1	219	[151;6314]	95.4	[39.2;147.7]	80%
mixture	Logit	-10.51	1.70	-	0	592	77.1	[59.4;99.8]	11.8	[4.3;34.7]	37%

[a] RM – Regression models as defined in (Scholze et al. 2001); $\hat{\theta}_1, \hat{\theta}_2, \hat{\theta}_3$ – Statistical estimates of model parameters, given for doses expressed as mg/kg/day (rounded values); $\hat{\theta}_{max}$ – upper model asymptote; θ_{min} – fixed lower model asymptote; [b] ED6, ED3 – Effect doses for 6 and 3 nipples, calculated from the respective dose response function; [c] ED60, ED90 – Effect doses for 60 % and 90 % normalized AGD index, calculated from the respective dose response function; CI – 95 % confidence intervals for mean effect doses given in mg/kg/day.

Supplemental Material, Table 2. Statistical dose-effect descriptors for pooled single and mixture exposures - organ weights and occurrences of malformation from male PND16 rat pups

Substance	Dose Response Function						Effect doses (mg/kg/day)				Max observed
	RM [a]	$\hat{\theta}_1$	$\hat{\theta}_2$	$\hat{\theta}_3$	θ_{min}	$\hat{\theta}_{max}$	ED60 [b]	[CI]	ED90 [b]	[CI]	mean effect
Ventral Prostate (mg, normalized to the controls)											
vinclozolin	logit	-7.96	3.94	-	0	0.99	81.1	[66.9;97.4]	27.2	[14.4;47.7]	33%
finasteride	logit	-2.02	0.69	-	0	1.00	216.7	[69.3;1965.6]	0.54	[0.02;6.28]	67%
DEHP	G.logit I	-9.93	2.44	0.996	0	1.00	2169	[n.d;n.d.]	114.1	[12.7;426.2]	71%
prochloraz	logit	-8.58	3.31	-	0	0.99	292	[n.d.;n.d.]	79.2	[4.9;531.8]	80%
mixture	logit	-10.18	4.85	-	0	1.00	103.7	[78.9;130.5]	44.7	[13.9;79.3]	48%
LABC[c] (mg, normalized to the controls)											
vinclozolin	logit	-8.32	3.80	-	0	0.99	120.1	[95.4;190.8]	39.4	[16.0;73.6]	47%
finasteride	logit	-1.64	0.78	-	0	0.99	38.6	[10.8;651.8]	0.18	[0.01;7.86]	64%
DEHP	logit	-3.82	0.59	-	0	1.00	604000	[n.d.;n.d.]	619	[11;13337]	75%
prochloraz	logit	-9.55	4.13	-	0	1.00	164	[n.d;n.d.]	60.1	[15.8;113.7]	79%
mixture	logit	-9.76	4.53	-	0	1.02	118.9	[93.9;152.1]	51.0	[19.3;81.9]	56%
Malformation[d] (likelihood, %) at PND16							ED50 [e]	[CI]	ED10 [e]	[CI]	
vinclozolin	logit	-21.98	11.3	-	0	1	87.7	[78.5;103.2]	56.1	[45.8;74.4]	78%
finasteride	logit	-2.27	1.78	-	0	1	18.8	[7.2;62.5]	1.1	[0.3;7.5]	70%
mixture	Weibull	-14.68	7.39	-	0	1	37.5	[32.5;44.6]	25.5	[21.0;31.2]	92%

[a] RM – Regression models as defined in (Scholze et al. 2001); $\hat{\theta}_1, \hat{\theta}_2, \hat{\theta}_3$ – Statistical estimates of model parameters, given for doses expressed as mg/kg/day (rounded values); $\hat{\theta}_{max}$ – upper model asymptote; θ_{min} – fixed lower model asymptote; [b] ED60, ED90 – Effect doses for 60 % and 90 % normalized organ weights, calculated from the respective dose response function; CI – 95 % confidence intervals for mean effect doses given in mg/kg/day; n.d. – not determined, [c] Levator ani/bulbocavernosus muscles; [d] enlarged preputial clefts and hypospadias; [e] ED10, ED50 – Effect doses for a 10 % and 50 % likelihood of a malformation occurring, calculated from the respective dose response function.

Supplemental Material, Table 3. Occurrences of genital malformations at PND16 in male rat pups after administration of vinclozolin, finasteride, DEHP and prochloraz to pregnant rats from GD7 to PND16. Results from 2-3 independent studies are shown (number of pups and litters examined in brackets).

		Dose (mg/kg/day)								
vinclozolin	Controls	5	10	20	24.5	40	50	80	95.9	160
Study1	0% (40;13)	0% (24;5)	0% (21;6)	0% (32;7)	--	0% (19;7)	--	30.3% (33;8)	--	75% (12;6)
Study 2	0% (36;13)	--	--	--	0% (37;13)	--	--	--	77.8% (36;11)	--
Mixture study[a]	0% (31;13)	0% (18;5)	--	--	--	--	4.3% (23;7)	--	--	--

		Dose (mg/kg/day)					
finasteride	Controls	0.001	0.01	0.1	1	10	50
Study 1	0% (46;14)	0% (23;7)	0% (29;7)	5.6% (18;8)	8.7% (23;8)	35.3% (17;8)	69.6% (23;6)
Mixture study	0% (31;13)	--	0% (17;5)	0% (15;5)	--	--	--

		Dose (mg/kg/day)							
DEHP	Controls	3	10	15	30	100	300	600	900
Study1	0% (46;14)	--	0% (28;7)	--	0% (26;6)	0% (23;7)	4.8% (21;6)	0% (23;6)	0% (26;6)
Mixture study	0% (31;13)	0% (15;4)	--	0% (26;7)	0% (22;6)	--	--	--	--

		Dose (mg/kg/day)						
prochloraz	Controls	5	10	25	30	50	100	150
Study1	0% (40;13)	--	--	--	--	0% (29;8)	--	9.5% (21;6)
Study 2	0% (37;15)	0% (28;7)	0% (27;8)	0% (19;7)	--	0% (19;6)	0% (24;5)	--
Mixture study	0% (31;13)	0% (22;7)	--	0% (14;5)	--	0% (15;6)	--	--

a Doses run in parallel with the mixture experiment, study No 4 (see Table 1)

-- Doses not examined

Supplemental Material, Table 4. Summary of the outcome of the mixture experiment, and the responses to the single chemicals tested in parallel with the mixture.

Group (dose in mg/kg/day)	# of litters	AGD index	Nipple retention	Right testis (mg)	Left Testis (mg)	Epididy-mides (mg)	Ventral Prostate (mg)	Seminal vesicle (mg)	LABC[e] (mg)	Bulbour Glands[f] (mg)	Adrenals (mg)	Kidneys (mg)	Liver (mg)	Thyroid Gland (mg)	malformation (%) PND16[g]	malformation (%) PND47[h]
Control	13	11.3±0.2	1.6 [0.9;2.5]	65.9±1.9	66.7±1.6	24.9±0.4	15.1±1.2	18.1±0.8	25.3±1.2	1.6±0.1	10.8±0.6	372±19	949±49	5.0±0.3	0[c]	0[c]
Mix1 (13.01)	16	**10.5±0.1**[b]	**3.7 [2.6;5.1]**[a]	63.9±1.6	64.7±1.6	23.2±0.7	14.3±0.5	17.2±1.3	24.9±0.9	1.7±0.2	10.5±0.3	348±11	878±26	4.5±0.2	0[c]	0[c]
Mix2 (65.05)	10	**9.7±0.3**[b]	**10.1 [9.3;10.7]**[b]	65.9±2.2	65.0±2.1	**22.0±0.9**[a]	**12.2±0.8**[a]	17.8±1.0	**18.5±2.6**[a]	**1.0±0.1**[a]	10.6±0.3	362±11	942±21	4.7±0.4	25.0 [13.6;41.5]	21.4 [7.1;49.4]
Mix3 (130.1)	12	**8.0±0.2**[b]	**11.6 [11.1;12.0]**[b]	60.0±2.3	57.2±2.5	**21.2±1.0**[b]	**6.7±0.7**[b]	**12.2±1.8**[a]	**13.1±0.8**[b]	**0.5±0.2**[b]	10.3±0.4	335±20	870±32	4.4±0.3	92.1 [78.2;97.4]	100[c]
VZ (5)	6	11.1±0.5	2.7 [1.6;4.3]	58.6±1.4	59.5±1.1	21.9±2.1	12.6±0.9	15.7±1.3	21.4±2.4	1.1±0.3	10.5±0.4	356±26	826±49	4.4±0.1	0[c]	0[c]
VZ (50)	8	**10.3±0.3**[b]	**8.4 [6.9;9.6]**[b]	63.7±4.4	63.8±3.9	**21.9±1.3**[a]	12.9±1.0	16.2±1.6	25.5±3.1	**0.5±0.1**[a]	12.0±1.1	373±43	993±95	4.6±0.5	4.3 [0.6;25.2]	12.5 [1.7;53.7]
FIN (0.01)	5	10.8±0.2	**4.4 [2.8;6.2]**[a]	65.0±2.5	65.7±2.7	24.6±0.6	14.4±0.8	17.0±1.4	24.4±0.5	1.1±0.2	10.2±0.4	362±16	889±35	4.7±0.3	0[c]	0[c]
FIN (0.1)	6	**10.3±0.2**[b]	**8.7 [7.5;9.7]**[b]	61.3±2.4	60.4±2.6	**22.1±0.9**[a]	15.0±2.0	16.4±1.4	25.6±1.8	0.8±0.3	10.0±0.6	364±23	869±39	4.7±0.5	0[c]	0[c]
DEHP (3)	5	11.1±0.6	1.8 [0.6;4.7]	61.8±1.1	61.9±1.7	22.8±1.5	13.7±0.9	17.0±1.7	24.0±1.1	1.4±0.3	10.1±0.5	326±7	852±34	5.3±0.3	0[c]	0[c]
DEHP (15)	7	11.2±0.2	1.7 [0.9;3.0]	63.2±2.2	63.3±2.0	24.0±1.0	15.1±0.8	14.3±1.1	23.8±1.1	1.6±0.1	10.7±0.7	358±22	910±36	5.0±0.2	0[c]	0[c]
DEHP (30)	7	11.5±0.3	3.1 [2.0;4.6]	68.3±1.9	68.5±1.9	27.0±0.7	17.0±1.0	17.3±1.9	24.5±1.3	1.8±0.2	10.9±0.7	378±13	1005±27	5.0±0.4	0[c]	0[c]
PZ (5)	7	11.5±0.2	2.9 [1.8;4.5]	65.4±2.9	65.3±2.2	23.6±0.9	15.1±1.4	20.3±1.3	25.2±0.7	1.4±0.2	10.5±0.6	352±17	922±40	4.5±0.2	0[c]	0[c]
PZ (25)	5	11.5±0.2	3.2 [2.3;4.4]	62.9±0.6	64.7±0.5	23.4±0.6	13.6±0.8	18.0±2.6	25.9±1.2	1.1±0.2	11.3±0.3	346±11	897±29	4.5±0.3	0[c]	0[c]
PZ (30)	7	11.3±0.4	**3.6 [2.2;5.4]**[a]	73.3±4.5	73.5±3.6	26.3±2.2	15.4±1.2	15.1±1.1	23.7±1.9	1.3±0.2	11.9±0.7	377±13	989±53	5.8±0.8	0[c]	0[c]

Mix1, Mix2, Mix3: combinations of vinclozolin, finasteride, prochloraz and DEHP at the ratio of 5 : 0.01 : 5 : 3. VZ: vinclozolin, FIN: finasteride, PZ: prochloraz. Data represent means ± SEM, in the case of nipple retention and hypospadia data are means ± 95%CI

[a, b]: Statistically significantly different relative to controls ($p < 0.05$) and ($p < 0.01$), respectively. All statistically significant numbers are shown in bold.

[c]: Statistical analysis was not possible as all individual responses had the same value.

[e]: Levator ani/bulbocavernosus muscles; [f]: Bulbourethral glands; [g]: enlarged preputial clefts and hypospadias; [h]: severe hypospadias including exposure of the os penis and blind vaginal opening.

Supplemental Material, Table 5. Comparison of NOAELs estimated for nipple retention in the present study with those currently used for regulatory purposes in human risk assessment

Compound	Our NOAEL (NR) mg/kg/day	Regulatory NOAEL mg/kg/day	Comments	References
DEHP	3	5	Based on effects on testes	EU RAR 2004
		Between 1 and 10	Based on effects on Leydig cells	NTP –CEHR 2005
Vinclozolin	5	4	Study of reproductive toxicity	RAR from http://www.inchem.org
		4.9	2. Generation study in rats	BASF Study
Prochloraz	5	3.7	Multigenerational reproductive toxicity in rats	JMPR 2001
Finasteride	0.01	0.10	Based on hypospadias	MERCK Propecia® (Finasteride) Prescribing information
		0.03	Based on nipple retention	
		0.003	Based on AGD	

References

EU RAR. EU-risk assessment of bis(2-ethylhexyl) phthalate (DEHP). Consolidated final report March 2004.

NTP-CERHR. Expert panel update on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate 2005.

BASF Studies Reproductive Toxicity Testing Two-Generation Reproduction RAR from http://www.inchem.org

JMPR (Joint Meeting on Pesticide Residues) 2001

Link to prescribing information for finasteride from the producer MERCK: http://www.merck.com/product/usa/pi_circulars/p/proscar.html