Supplemental Tables

Supplemental Table S1. Effect of Technical Toxaphene and Toxaphene Congeners on Cytotoxicity in Male and Female B6C3F1 Hepatocytes after 24 Hours of Exposure

		Cell viability (% control)	
Exposure (ppm)		**Male**	**Female**
DMSO (%)	0.1	100.0	100.0
	0.25	100.0	97.3
TT (µg/ml)	1.0	94.3	99.9
	2.5	95.4	98.3
	5.0	89.8	88.4
	10	84.2*	74.8*
	15	70.9*	64.8*
	25	65.6*	52.1*
	50	47.8*	12.7*
	100	6.0*	5.9*
p-26 (µg/ml)	1.0	103.3	98.7
	2.5	100.0	98.1
	5.0	92.5	94.3
	10	90.4	90.1
	15	82.2*	78.6*
	25	78.4*	68.2*
	50	35.4*	61.8*
p-50 (µg/ml)	1.0	97.3	100.0
	2.5	95.1	96.4
	5.0	95.0	90.1*
	10	96.4	87.8*
	15	83.6*	86.2*
	25	77.9*	81.2*
	50	44.7*	50.3*
p-62 (µg/ml)	1.0	101.4	99.7
	2.5	91.1	91.9
	5.0	84.8	84.1
	10	79.2*	75.9*
	15	75.4*	72.2*
	25	66.9*	64.6*
	50	29.3*	47.1*
Hx-Sed (µg/ml)	1.0	102.4	105.3
	2.5	88.6	95.0
	5.0	82.0	89.4
	10	73.6*	87.4*
	15	68.8*	77.2*
	25	57.0*	69.7*
	50	54.3*	62.8*
Hp-Sed (µg/ml)	1.0	98.6	100.0
	2.5	95.0	90.4
	5.0	91.8	93.1
	10	95.5	109.2
	15	93.5	107.4
	25	91.1	86.7
	50	22.7*	49.4*

		Cell viability (% control)	
Exposure (ppm)		**Male**	**Female**
Mixture 1 (ug/ml)	0.8	90.9	85.0
	2	98.8	90.5
	4	112.1	81.4
	8	113.4	92.9
	20	116.1	91.1
	40	69.3*	87.4
	80	58.9*	92.9
Mixture 2 (ug/ml)	1	88.2	92.5
	2.5	89.5	80.3
	5	83.0	86.5
	10	92.6	79.7
	25	87.8	94.8
	50	58.7*	96.6
	100	49.9*	89.8

TT=Technical toxaphene.
*Significantly different from control value at $p < 0.05$ (one-way ANOVA with Holm-Sidak post hoc). $n \geq 3$.

Supplemental Table S2: Effect of Technical Grade Toxaphene on GJIC in Male and Female B6C3F1 Mouse Hepatocytes

		3-hour		24-hour	
Male	**Toxaphene (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	94.3	100.0	92.0	100.0
	PB (2mM)	72.8*	77.2* (22.8)	86.0	93.5 (6.5)
	0.2 ppm	92.2	97.7 (2.3)	88.6	96.3 (3.7)
	1 ppm	86.4	91.6 (8.4)	86.3	93.8 (6.2)
	5 ppm	77.8*	82.5* (17.5)	67.9*	73.9* (26.1)
Female	**Toxaphene (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	91.2	100.0	91.8	100.0
	PB (2mM)	77.5*	85.0* (15.0)	87.7	95.5 (4.5)
	0.2 ppm	92.4	101.3 (-1.3)	89.5	97.5 (2.5)
	1 ppm	85.6	93.8 (6.2)	89.2	97.2 (2.8)
	5 ppm	76.6*	84.1* (15.9)	79.7*	86.8* (13.2)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of technical toxaphene. PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

Supplemental Table S3: Reversibility of GJIC Inhibited by Technical Grade Toxaphene in Male and Female B6C3F1 Mouse Hepatocytes

		2-hour		4-hour		24-hour	
Male	**Treatment**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	94.0	100.0	91.0	100.0	86.2	100.0
	PB (2mM)	81.4*	86.6* (13.4)	80.2*	88.1* (11.9)	90.1	104.6 (-4.6)
	TT+	74.0*	78.7* (21.3)	78.4*	86.2* (13.8)	71.1*	82.6* (17.4)
	TT-	70.2*	74.7* (25.3)	77.2*	84.9* (15.1)	86.3	100.2 (-0.2)
Female	**Treatment**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	93.3	100.0	89.4	100.0	89.5	100.0
	PB (2mM)	73.8*	79.1* (20.9)	80.0	89.5 (10.5)	87.4	97.6 (2.4)
	TT+	69.4*	74.4* (25.6)	76.3*	85.3* (14.7)	71.8*	80.2* (19.8)
	TT-	74.1*	79.4* (20.6)	72.2*	80.8* (19.2)	84.7	94.7 (5.3)

Cells were exposed to TT at 5 ppm
"TT+" = Samples without TT being removed via fresh Media
"TT-"= Samples with TT removed after 4 hours, washed, and replaced by fresh media
*Statistically significant compared to control ($p<0.05$, Fisher's Exact Test)

Supplemental Table S4: Effect of the p-26 Congener on GJIC in B6C3F1 Mouse Hepatocytes

		3-hour		24-hour	
Male	**p-26 (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	91.8	100.0	91.5	100.0
	PB (2mM)	74.8*	81.4* (18.6)	90.3	98.7 (1.3)
	2.5 ppm	93.4	101.7 (-1.7)	90.4	98.8 (1.2)
	5 ppm	92.2	100.5 (-0.5)	90.4	98.8 (1.2)
	10 ppm	92.2	100.4 (-0.4)	85.1	93.0 (7.0)
Female	**p-26 (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	87.6	100.0	92.0	100.0
	PB (2mM)	69.6*	79.4* (20.6)	80.4	87.4 (12.6)
	2.5 ppm	84.8	96.8 (3.2)	80.4	87.5 (12.6)
	5 ppm	84.4	96.3 (3.7)	80.2	87.2 (12.8)
	10 ppm	79.8	91.1 (8.9)	70.7*	76.9* (23.1)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of the p-26 toxaphene congener.
PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

Supplemental Table S5: Effect of the p-50 Congener on GJIC in B6C3F1 Mouse Hepatocytes

		3-hour		24-hour	
Male	**p-50 (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	91.8	100.0	90.5	100.0
	PB (2mM)	76.3*	83.1* (16.9)	91.3	100.9 (-0.9)
	1 ppm	88.3	96.2 (3.8)	90.7	100.3 (-0.3)
	2.5 ppm	84.7	92.2 (7.8)	88.6	97.9 (2.1)
	5 ppm	77.5*	84.3* (15.7)	81.7*	90.3* (9.7)
Female	**p-50 (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	87.5	100.0	91.1	100.0
	PB (2mM)	71.8*	82.1* (17.9)	79.6	87.4 (12.6)
	0.5 ppm	86.7	99.1 (0.9)	85.3	93.6 (6.4)
	1 ppm	88.7	101.3 (-1.3)	80.2	88.1 (11.9)
	2.5 ppm	80.6	92.1 (7.9)	73.7*	80.9* (19.1)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of the p-50 toxaphene congener.
PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

Supplemental Table S6: Effect of the p-62 Congener on GJIC in B6C3F1 Mouse Hepatocytes

		3-hour		24-hour	
Male	**p-62 (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	90.9	100.0	89.4	100.0
	PB (2mM)	76.5*	84.2* (15.8)	90.7	101.5 (-1.5)
	1 ppm	88.5	97.4 (2.6)	90.7	101.4 (-1.4)
	2.5 ppm	81.6	89.7 (10.3)	84.5	94.5 (5.5)
	5 ppm	70.0*	77.0* (23.0)	77.3	86.5 (13.5)
Female	**p-62 (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	86.4	100.0	90.7	100.0
	PB (2mM)	63.9*	73.9* (26.1)	76.0*	83.8 (16.2)
	1 ppm	88.7	102.6 (-2.6)	88.0	97.0 (3.0)
	2.5 ppm	87.1	100.8 (-0.8)	76.7*	84.5* (15.5)
	5 ppm	80.4*	93.1* (6.9)	70.1*	77.3* (22.7)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of the p-62 toxaphene congener.
PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

Supplemental Table S7: Effect of the Hx-Sed Congener on GJIC in B6C3F1 Mouse Hepatocytes

		3-hour		24-hour	
Male	**Hx-Sed (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	94.6	100.0	94.3	100.0
	PB (2mM)	80.2*	84.8* (15.2)	88.7	94.0 (6.0)
	1 ppm	95.3	100.8 (-0.8)	91.6	97.1 (2.9)
	2 ppm	90.5	95.6 (4.4)	88.6	93.9 (6.1)
	5 ppm	90.4	95.5 (4.5)	87.4	92.6 (7.4)
Female	**Hx-Sed (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	91.9	100.0	93.1	100.0
	PB (2mM)	71.4*	77.7* (22.3)	87.5	93.9 (5.9)
	1 ppm	90.1	98.0 (2.0)	83.5	89.6 (10.4)
	2.5 ppm	85.4	93.0 (7.0)	84.8	91.0 (9.0)
	5 ppm	79.2*	86.2* (13.8)	69.7*	74.8* (25.2)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of the Hx-Sed toxaphene congener.
PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

Supplemental Table S8: Effect of the Hp-Sed Congener on GJIC in B6C3F1 Mouse Hepatocytes

		3-hour		24-hour	
Male	**Hp-Sed (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	94.4	100.0	93.2	100.0
	PB (2mM)	83.0*	87.9* (12.1)	92.2	99.0 (1.0)
	10 ppm	96.2	101.8 (-1.8)	85.6	91.8 (8.2)
	15 ppm	88.5	93.7 (6.3)	84.8	90.9 (9.1)
	25 ppm	*82.2**	87.0* (13.0)	77.7*	83.3* (16.7)
Female	**Hp-Sed (µg/mL)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	95.6	100.0	95.3	100.0
	PB (2mM)	78.9*	82.5* (17.5)	92.5	97.0 (3.0)
	10 ppm	93.4	97.7 (2.3)	89.5	94.0 (6.0)
	15 ppm	86.8	90.8 (9.2)	84.8*	89.0* (11.0)
	25 ppm	78.9*	82.5* (17.5)	75.0*	78.7* (21.3)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of the Hp-Sed toxaphene congener.
PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

Supplemental Table S9: Effect of Congener Mix 1 (p-26 + p-50 + p-62) on GJIC in B6C3F1 Mouse Hepatocytes

		3-hour			24-hour		
Male	**Mixture 1 (% mix in DMSO) [a]**	**Dye Coupling %**	**% of Control "()"= Difference from Control**		**Dye Coupling %**	**% of Control "()"= Difference from Control**	
	DMSO (0.1%)	94.4	100.0		93.2	100.0	
	PB (2mM)	83.0*	87.9*	(12.1)	92.2	99.0	(1.0)
	5 ppm	91.6	97.0	(3.0)	91.6	98.3	(1.7)
	10 ppm	93.3	98.8	(1.2)	87.5	93.9	(6.1)
	25 ppm	87.9	93.0	(7.0)	86.0	92.3	(7.7)
Female	**Mixture 1 (% mix in DMSO)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**		**Dye Coupling %**	**% of Control "()"= Difference from Control**	
	DMSO (0.1%)	95.6	100.0		95.3	100.0	
	PB (2mM)	78.9*	82.5*	(17.5)	92.5	97.0	(3.0)
	5 ppm	95.2	99.6	(0.4)	93.5	98.1	(1.9)
	10 ppm	92.3	96.5	(3.5)	85.3	89.5	(10.5)
	25 ppm	88.8	92.9	(7.1)	77.5*	81.3*	(18.7)
	50 ppm	85.7	89.6	(10.4)	76.5*	80.3*	(19.7)
	100 ppm	75.7*	79.2*	(20.8)	66.0*	69.3*	(30.7)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of the toxaphene congener Mix 1.
PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

[a] % represents the fraction of each congener present in the mixture based on the concentrations for each congener shown in Table 1.

Supplemental Table S10: Effect of Congener Mix 2 (p-26 + p-60 + p-62 + Hx-Sed) on GJIC in B6C3F1 Mouse Hepatocytes

		3-hour		24-hour	
Male	**Mixture 2 (% mix in DMSO) [a]**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	94.4	100.0	93.2	100.0
	PB (2mM)	83.0*	87.9* (12.1)	92.2	99.0 (1.0)
	5 ppm	94.3	99.8 (0.2)	90.8	97.4 (2.6)
	10 ppm	87.6	92.8 (7.2)	88.3	94.7 (5.3)
	25 ppm	84.2	89.1 (10.9)	78.0*	83.7* (16.3)
Female	**Mixture 2 (% mix in DMSO)**	**Dye Coupling %**	**% of Control "()"= Difference from Control**	**Dye Coupling %**	**% of Control "()"= Difference from Control**
	DMSO (0.1%)	95.6	100.0	95.3	100.0
	PB (2mM)	78.9*	82.5* (17.5)	92.5	97.0 (3.0)
	5 ppm	92.5	96.7 (3.3)	91.4	96.0 (4.0)
	10 ppm	92.2	96.5 (3.5)	85.8	90.1 (9.9)
	25 ppm	85.7	89.6 (10.4)	76.4*	80.2* (19.8)
	50 ppm	79.6*	83.3* (16.7)	74.0*	77.7* (22.3)
	100 ppm	72.8*	76.2* (23.8)	68.3*	71.6* (28.4)

Gap junctional intercellular communication inhibition as measured by the dye coupling in B6C3F1 mouse hepatocytes exposed to non-cytotoxic concentrations of the toxaphene congener Mix 2.
PB=Phenobarbital.
*Significantly different from control value at $p < 0.05$ (Fisher exact test).

[a] % represents the fraction of each congener present in the mixture based on the concentrations for each congener shown in Table 1.

Supplemental Table S11. Modeled Effect of Technical Toxaphene and Toxaphene Congeners on Cytotoxicity in Male and Female B6C3F1 Hepatocytes after 24 Hours of Exposure

Congener[a]	Sex	df	R^2	EC50 (ppm)	SE (ppm)	EC20 (ppm)	SE (ppm)
TT	M	8[b]	0.978	45.3	1.49	9.99	0.547
TT	F	9	0.983	20.5	1.97	8.97	0.774
p-26	M	5	0.826	39.5	4.07	18.7	1.52
p-26	F	5	0.629	75.3	13.0	16.1	1.14
p-50	M	5	0.760	45.8	4.04	20.4	1.29
p-50	F	5	0.683	58.4	10.7	19.1	1.73
p-62	M	5	0.917	32.5	5.16	10.3	1.21
p-62	F	5	0.800	46.8	4.18	8.30	0.470
Hx-Sed	M	5	0.696	49.3	10.1	6.64[c]	0.710
Hx-Sed	F	5	0.608	87.1	22.5	14.7	1.33
Hp-Sed	M	5	0.777	38.9	2.19	29.3	1.75
Hp-Sed	F	5	0.603	49.2	5.04	31.5	3.00
Mix 1[d]	M	5	0.250	87.3	28.1	44.1	8.01
Mix 1	F	5	NS	–	–	–	–
Mix 2	M	5	0.636	81.1	37.8	12.8	2.49
Mix 2	F	5	NS	–	–	–	–

[a] Congener concentrations in Mixtures 1 & 2 defined in Table 1. Exposure duration = 24 hours. df = degrees of freedom. R^2 = coefficient of determination = fraction of response variance explained by lognormal regression model. NS = response did not differ significantly ($p > 0.05$) by Fisher exact test from that of unexposed cells tested at any concentration. EC50 (or EC20) = benchmark concentration in ppm at which a 50% (or 20%) relative decline in B6C3F1 mouse hepatocyte viability is predicted by the fitted model. SE = standard error of estimate in column to the left of same row.

[b] Response at highest dose deleted as outlier using an F-test.

[c] Significantly less than male TT EC20, by 2-tail t-test ($p < 10^{-6}$).

[d] If more refined data on Mix 1 and 2 concentrations used in the assay become available, modeling of the EC50 and E20 will be performed.