Estrogenic Activity, and Developmental Toxicity Studies of Pyrethroid Insecticides

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It is well known that many pesticides possess hormonal activity, and affect the developments of wildlife and mammals including human. Currently, pyrethroid insecticides are in worldwide use to control in and outdoor pests, providing potential for environmental exposure. Hormonal activities of these pyrethroid insecticides, however, have been little studied, and the developmental effects of them were no reported. Therefore, we firstly examined the potential estrogenic activities of some pyrethroid insecticides (permethrin, cypermethrin, tetramethrin, deltamethrin, sumithrin, fenvalerate and bioallethrin) by immature rat uterotrophic assay, luciferase reporter gene assay and Calbindin-D_{9k} (CaBP-9k) gene expression assay. Uterine wet weights were increased by permethrin, and the permethrin-induced weights were inhibited by ICI 182780 in the uterotrophic assay. On the other hand, tetramethrin significantly reduced uterine and vaginal wet weights, and also inhibited the E2-induced weight increases at all doses tested. Cypermethrin and sumithrin had a tendency to increase uterine weights, although not statistically significant. Permethrin and cypermethrin dose-dependently increased the luciferase activity in reporter gene assay. Northern blot analysis showed that permethrin induced CaBP-9k mRNA expression whereas tetramethrin inhibted. Subsequent studies were conducted to investigate the possible developmental effects of four pyrethroid insecricides (permethrin, cypermethrin, sumithrin and teramethrin). Either diethlbestrol (DES) or 17β -estradiol (E2) was used as a reference control in this study. Pyrethroid insecticides were administered to Sprague Dawley rats via subcutaneous injection at 6 to 18 days of gestation or 1 to 5 days after birth. In utero treatment of permethrin (10㎎/kg/day) in female rat resulted in significant increases in uterine and ovarian weights while significant decreases in serum E2 concentration, uterine and ovarian ERα mRNA levels. Sumithrin and permethrin led to acceleration in vaginal opening of female rat, while delay in preputial separation of male after neonatal treatment. Anogenital distances of PND 18 were significantly reduced in sumthrin-treated, and permerhrin-treated male rats after neonatal treatment. All the pyrethroid insecticides tested caused significant increases in uterine weights on PND 18, while significant reductions in the first diestrus phase when neonataly treated. In addition, exposure to pyrethroids in neonatal period led to significant reduction in relative brain weight in female rat on PND 18, but its weight was recovered in diestrus phase. In summary, Our experimental data demonstrate the possibilities of developmental effects of pyrethroid insecticides via estrogenic or antiestrogenic activity.

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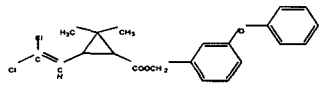
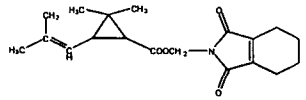
Introduction

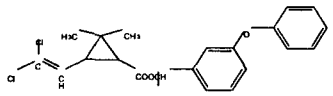
- **Pyrethroid insecticides are synthetic derivatives of naturally occurring pyrethrins**



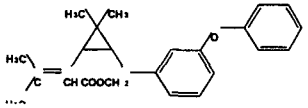
3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid (3-phenoxyphenyl) methyl ester (permethrin)



2,2-Dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylic acid (1,3,4,5,6,7-hexaydro- 1,3-dioxo -2H-isoindol-2-yl)methyl ester (tetramethrin)



3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid cyano(3-phenoxyphenyl)-methyl ester (cypermethrin)



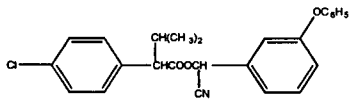
2,2-Dimethyl-3-(2-methyl 1-propenyl) cyclopropanecarboxylic acid (sumithrin)

Fig. 1. Structures of pyrethroid insecticides tested

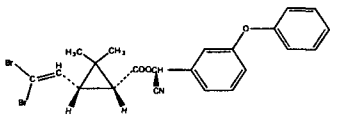
Background

- **Pyrethroids were developed as insecticide due to their high insecticidal potency and low mammalian toxicity relative to organochlorine and organophosphorus insecticides**
- **Currently, these pyrethroids are in worldwide use to control in and outdoor pests, providing potential for environmental exposure**
- **Hormonal activities of these pyrethroid insecticides, however, have been little studied, and the developmental effects of them were no reported**

→ **Therefore, This study investigated the potential estrogenic act ivity, and the developmental effects of some pyrethroid insecticides**



Cyano(3 -phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl) benzeneacetate (fenvalerate)



3-(2,2-Dibromoethenyl) –2,2-dimethyl cyclopropanecarboxylic acid cyano (3-phenoxyphenyl)-methyl ester (deltamethrin)

Materials & Methods

Animals and test chemicals

• Animals

Immature (18-day old) female, and sexually matured (10 to 11-weeks old) male and female Sprague Dawley rats bred in National Institute of Toxicological Research, KFDA were used

• Test chemicals

Diethylstilbestrol	56-53-1	99.0	Sigma, USA
17β-estradiol	50-28-2	98.0	Sigma, USA
Permethrin	52645-53-1	96.6	Reidel, Germany
Cypermethrin	52315-07-8	98.0	Supelco, USA
Tetramethrin	7696-12-01	98.7	Reidel, Germany
Sumithrin	26002-80-2	97.0	Supelco, USA
Bioallethrin	28057-48-9	97.0	Supelco, USA
Deltamethrin	05291-86-35		Supelco, USA
Fenvalerate	0051-630-58		Reidel, Germany

Materials & Methods

Experimental design I

Estrogenic activity test

- Immature rat Uterotrophic assay
- Ruciferase reporter gene assay
- Calbindin-D9k (CaBP-9k) gene expression assay

Materials & Methods

Experimental design II

Developmental toxicity study

- Treatment
  - Subcutaneous injection at 6 to 18 days of gestation or 1 to 5 days after birth
- Developmental parameters tested
  - Postnatal body weight changes
  - Pubertal onset
    (preputial separation in male rat, and vaginal opening in female)
  - Anogenital distance in male rat
  - General organ (brain and liver) weights in pre, and postpubertal periods
  - Reproductive organ weights in pre, and postpubertal periods
  - Serum sex hormone concentrations in pre, and postpubertal periods
  - ER expression in female reproductive organs in pre, and postpubertal periods
  - *Hox*-a 10 expression in female mice reproductive tract in neonatal period

Results I

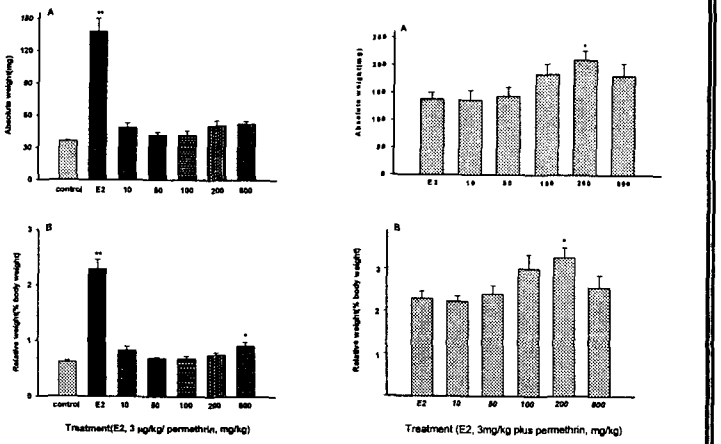


Fig. 2. Effects of permethrin on absolute (A) and relative (B) uterine wet weights.

Fig. 3. Effects of permethrin on E2-induced absolute (A) and relative (B) uterine wet weights.

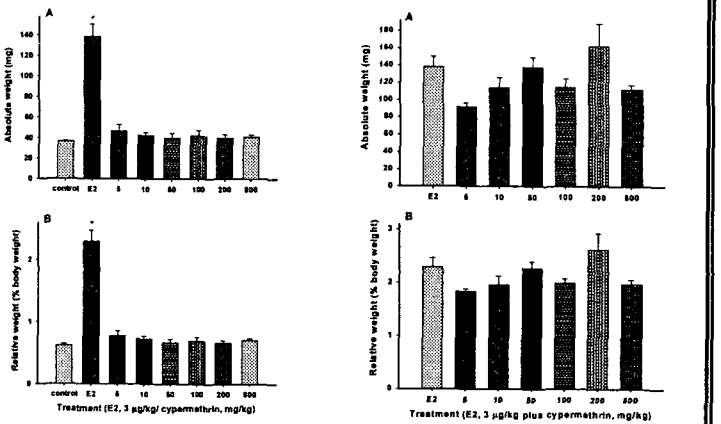


Fig. 4. Effects of cypermethrin on absolute (A) and relative (B) uterine wet weights.

Fig. 5. Effects of cypermethrin on E2-induced absolute (A) and relative (B) uterine wet weights.

Table 1. Effects of ICI 182780 on uterine and vaginal weights in permethrin-treated female rats

Treatment	Body weight	Uterine weight		Vaginal weight	
	(bw, g)	Absolute (mg)	Relative (% of bw)	Absolute (mg)	Relative (% of bw)
	48.43±3.477	53.180±7.089	1.1089±0.216	42.580±5.861	0.8802±0.109
Permethrin 10	48.43±3.477	53.180±7.089	1.1089±0.216	42.580±5.861	0.8802±0.109
Permethrin 10 + ICI	42.01±5.508	48.340±4.844	1.1634±0.154	35.040±3.865*	0.8498±0.176
Permethrin 50	45.01±3.503	49.700±10.055	1.1049±0.213	38.280±4.452	0.8541±0.110
Permethrin 50 + ICI	39.02±6.480	26.220±6.968**	0.6644±0.073**	27.580±8.621*	0.6941±0.107*
Permethrin 100	45.33±1.331	33.120±1.810	0.7311±0.046	31.300±4.457	0.6928±0.116
Permethrin 100 + ICI	42.28±7.355	36.475±9.053	0.8637±0.147	31.900±13.557	0.7339±0.238
Permethrin 200	40.76±6.865	33.340±6.594	0.8315±0.205	28.750±9.717	0.7329±0.278
Permethrin 200 + ICI	35.21±7.746	27.400±2.826	0.7937±0.096	24.540±7.853	0.6950±0.156
Permethrin 800	45.89±2.538	29.950±1.008	0.6543±0.045	38.100±2.906	0.8326±0.083
Permethrin 800 + ICI	38.79±9.859	22.320±3.832**	0.5909±0.101	18.440±4.758**	0.4833±0.090**

Data are presented as mean ± SEM. The statistical significances were evaluated between permethrin and permethrin plus ICI 182780 - treated groups. *P<0.05 when compared to the corresponding permethrin-treated group.
**P<0.01 when compared to the corresponding permethrin-treated group.

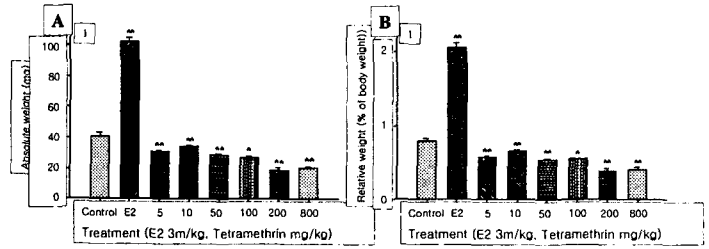


Fig. 6. Effects of tetramethrin on absolute (A) and relative (B) uterine wet weight in immature female rats. Data are presented as mean ± SEM. Significantly different from control, *P<0.05,P<0.01.**

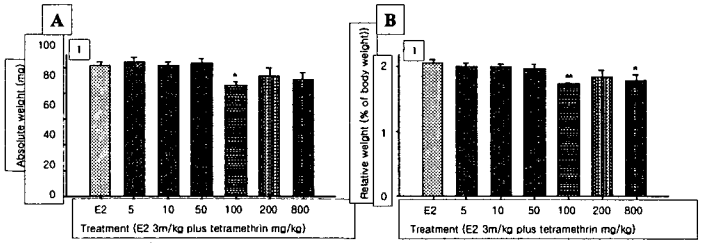


Fig. 7. Effects of tetramethrin on E2-induced absolute (A) and relative (B) uterine wet weights in immature female rats. Data are presented as mean ± SEM. Significantly different from E2, *P 〈0.05, **P 〈0.01.

Table 2. Effects of tetramethrin on body and vaginal weights in immature rats

Treatment (㎎/kg)	No. of animals	Body weight (g)	Vaginal weight	
			Absolute (㎎)	Relative (% body weight)
Control	8	52.28 ± 1.65	35.38 ± 2.28	0.68 ± 0.04
17β–estradiol (E2, 3 ㎍/kg)	9	51.28 ± 1.14	54.27 ± 4.60*	1.07 ± 0.09*
Tetramethrin (5)	12	54.08 ± 1.11	31.04 ± 1.08	0.58 ± 0.03
Tetramethrin (10)	14	52.83 ± 1.45	30.80 ± 1.96	0.59 ± 0.04
Tetramethrin (50)	12	53.57 ± 1.19	29.50 ± 1.45	0.55 ± 0.03
Tetramethrin (100)	9	48.82 ± 1.91	27.89 ± 1.94	0.58 ± 0.04
Tetramethrin (200)	12	48.52 ± 1.22	18.76 ± 1.92*	0.39 ± 0.04*
Tetramethrin (800)	9	51.26 ± 1.58	26.16 ± 1.92	0.52 ± 0.04
E2 + Tetramethrin (5)	10	52.57 ±3.51	49.52 ± 2.55	0.94 ± 0.04
E2 + Tetramethrin (10)	10	50.82 ± 1.39	45.88 ± 3.87	0.91 ± 0.08
E2 + Tetramethrin (50)	11	52.19 ± 1.89	54.24 ± 2.74	1.04 ± 0.03
E2 + Tetramethrin (100)	11	49.81 ± 1.44	46.58 ± 2.25	0.94 ± 0.05
E2 + Tetramethrin (200)	10	50.89 ± 1.46	48.75 ± 1.74	0.96 ± 0.02
E2 + Tetramethrin (800)	6	50.82 ± 1.12	52.15 ± 1.80	1.03 ± 0.03

Data are presented as mean ± SEM. Significantly different from control, *P 〈0.01

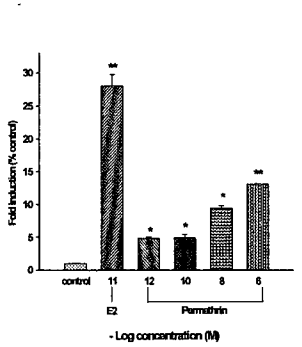


Fig. 8. The dose response of permethrin on the luciferase activity in MCF-7-ERE cells.

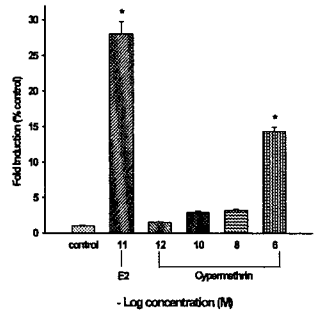


Fig. 9. The dose response of cypermethrin on the luciferase activity in MCF-7-ERE cells.

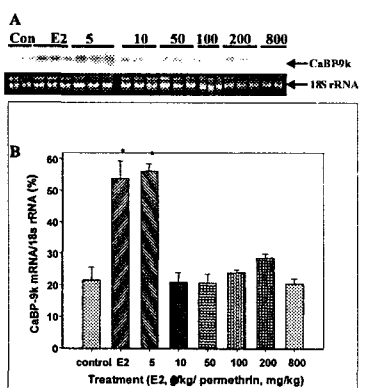


Fig. 10. (A) Northern blot analysis and (B) Schematic representation of CaBP-9k mRNA expression in the uteri of immature rats treated with permethrin.

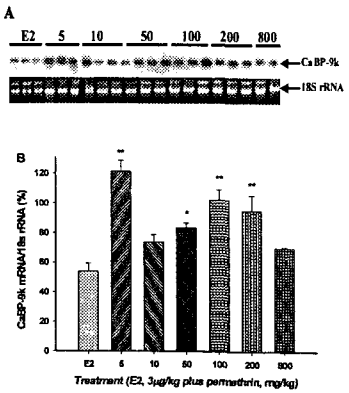


Fig. 11. (A) Northern blot analysis and (B) Schematic representation of CaBP-9k mRNA expression in the uteri of immature rats treated with E2 plus permethrin.

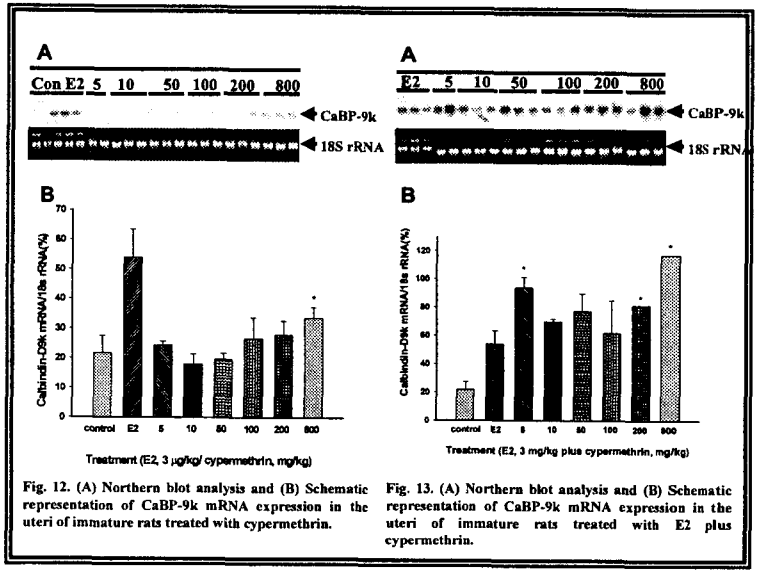


Fig. 12. (A) Northern blot analysis and (B) Schematic representation of CaBP-9k mRNA expression in the uteri of immature rats treated with cypermethrin.

Fig. 13. (A) Northern blot analysis and (B) Schematic representation of CaBP-9k mRNA expression in the uteri of immature rats treated with E2 plus cypermethrin.

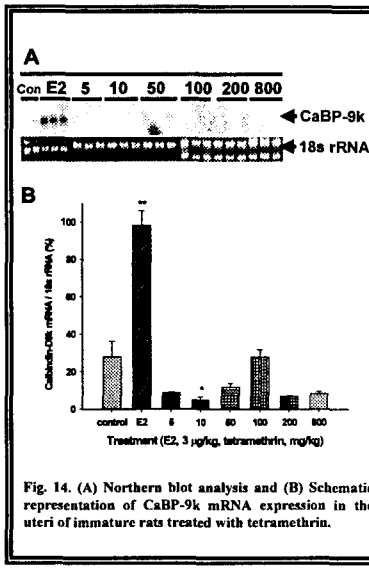


Fig. 14. (A) Northern blot analysis and (B) Schematic representation of CaBP-9k mRNA expression in the uteri of immature rats treated with tetramethrin.

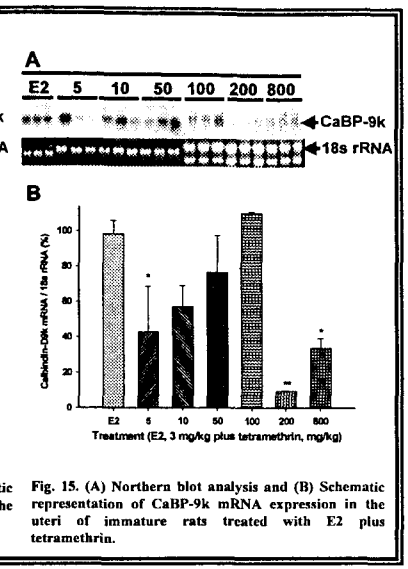


Fig. 15. (A) Northern blot analysis and (B) Schematic representation of CaBP-9k mRNA expression in the uteri of immature rats treated with E2 plus tetramethrin.

Summarys I

Uterotrophic assay

- **E2 (3 /g/kg) caused about 3.7 and 3.8 fold increases in absolute and relative uterine wet weights compared to the control, respectively**
- **Permethrin increased absolute and relative uterine wet weights, and E2-induced their weights, while tetramethrin led to statistically significant decreases in their weights , and also inhibited the E2-induced their weights**

Reporter gene assay

- **MCF-7 cells exhibited about 20 to 25 fold increase of luciferase activity on addition of 0.01nM E2 compared to the control**
- **Permethrin, and cypermethrin dose-dependently induced the luciferase activity in MCF-7 cells**

Calbindin-D9k expression

- **Permethrin induced the uterine CaBP-9k mRNA expression, while tetramethrin inhibited**

→ **Based on our experimental data, it appears thatpermethrin and cypermethrin have estrogenic activity, whereas tetramethrin has antiestrogenic activity**

Results II

Table 3. Effects of pyrethroid insecticides on reproductive parameters in rat

Treatment	No. of dam	No. of implantation	No. of fetal death		No. of live fetuses/dam	% of Male offspring	Average male+female pup weights (g, PND 0)
			early	late			
Control	5	16.80± 1.30	0.40± 0.55	0	16.40± 1.14	50.60± 9.50	3.64± 0.15
DES (㎍/kg) 0.002	5	13.60± 3.29	1.40± 1.14	0.20± 0.45	12.00± 3.74*	54.90± 20.6	3.64± 0.44
0.02	5	16.40± 1.82	2.60± 2.88	2.60± 2.88	13.80± 1.92	45.2± 16.8	3.67± 0.20
Permethrin 0.5	5	16.75± 0.96	1.25± 0.96	0	15.00± 2.58	67.10± 11.41	3.54± 0.30
(mg/kg) 5	5	17.60± 0.89	0.60± 0.55	0.80± 0.84	15.40± 0.55	61.30± 23.88	3.57± 0.26
10	5	13.00± 2.21*	0.20± 0.45	1.80± 4.03	11.0± 4.06*	5550± 13.30	3.58± 0.37
Cypermethrin 0.5	5	14.67± 3.14	0.17± 0.41	2.50± 3.99	12.20± 5.19	53.20± 8.18	3.17± 0.69
(mg/kg) 5	5	15.00± 0	0.60± 0.89	2.00± 3.46	12.80± 3.35	55.20± 5.55	3.75± 0.31
10	5	14.80± 2.59	0.20± 0.45	0.60± 0.55	14.00± 2.45	45.50± 6.92	3.47± 0.15
Tetramethrin 0.5	5	16.00± 0.71	0.80± 1.30	0.80± 1.30	16.80± 1.30	52.60± 7.76	3.34± 0.34
(mg/kg) 5	5	17.50± 1.29	2.00± 2.83	1.00± 1.16	14.50± 1.29	44.60± 6.25	3.54± 0.33
10	5	15.00± 2.00	0.20± 0.45	1.40± 2.07	13.40± 1.14	55.10± 14.40	3.66± 0.26

Pregnant rats were treated with pyrethroid insecticides at 6 to 18 days of gestation, and subjected to caesarean section on gastation day 20. Data are presented as mean ±SD. Significantly different from control, *P<0.05

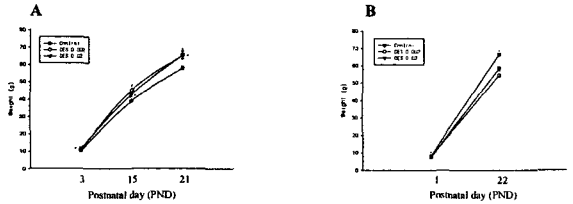


Fig.16. Body weight changes in male (A) and female (B) offsprings, treated with DES (mg/kg/day) in utero. Data are presented as mean ± SEM. Significantly different from control, *P<0.01

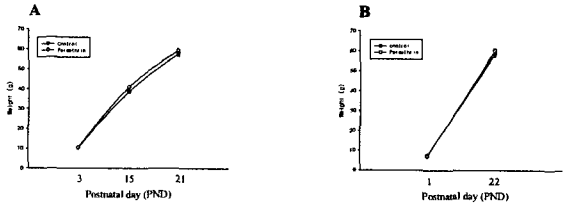


Fig. 17. Body weight changes in male (A) and female (B) offsprings, treated with permethrin (10 mg/kg/day) in utero. Data are presented as mean ± SEM.

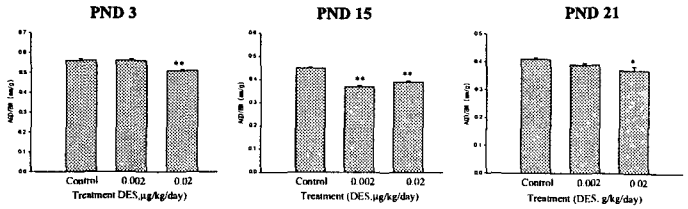


Fig. 18. Anogenital distances in male offsprings on PND 3, 15 and 21, treated with DES (mg/kg/day) in utero. Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

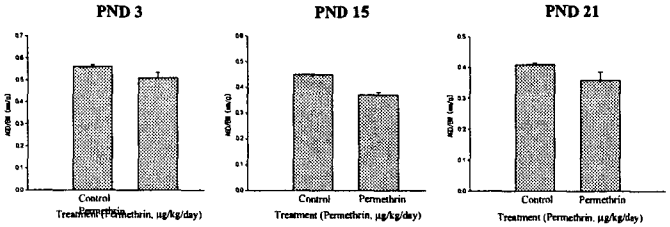


Fig. 19. Anogenital distances in male offsprings on PND 3, 15 and 21, treated with permethrin (10mg/kg/day) in utero. Data are presented as mean ± SEM.

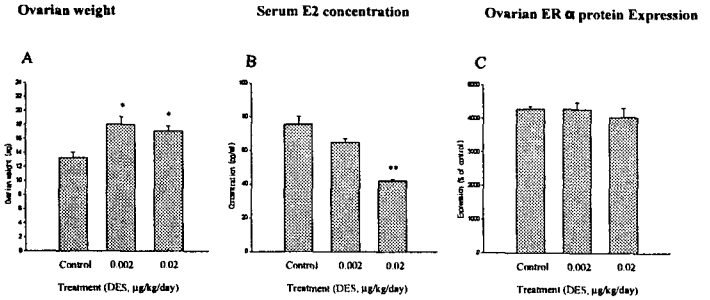


Fig. 20. Ovarian weight (A), serum E2 concentration (B) and ovarian ER α protein expression (C) in female offsprings treated with DES in utero on PND 22. Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

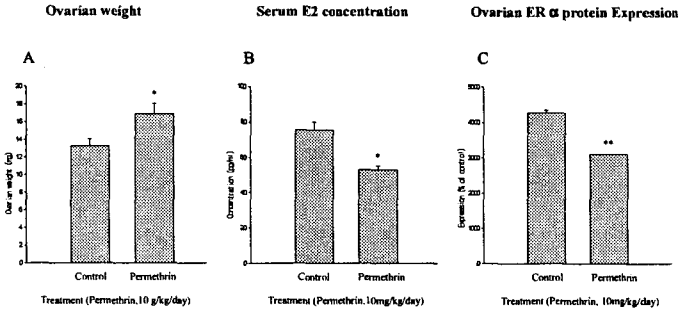


Fig.21. Ovarian weight (A), serum E2 concentration (B) and ovarian ER α protein expression (C) in female offsprings treated with permethrin in utero on PND 22 . Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

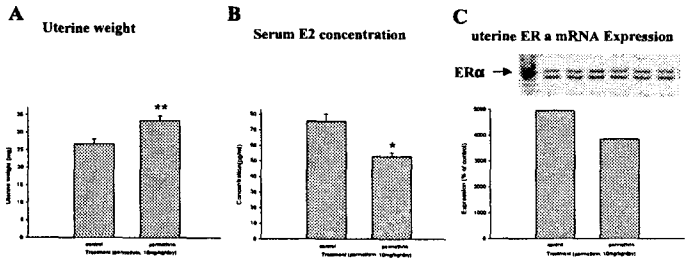


Fig. 22. Uterine weight (A), serum E2 concentration (B) and uterine ER a mRNA expression (C) on PND 22 in female offsprings treated with permethrin in utero. Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

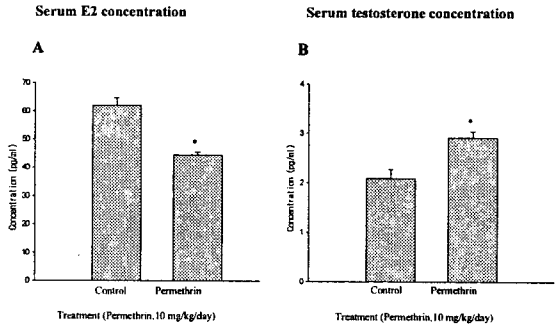


Fig. 23. Serum E2 (A) and testosterone (B) concentrations on PND 49 in male offsprings treated with permethrin in utero. Data are presented as mean ± SEM. Significantly different from control, *P<0.01.

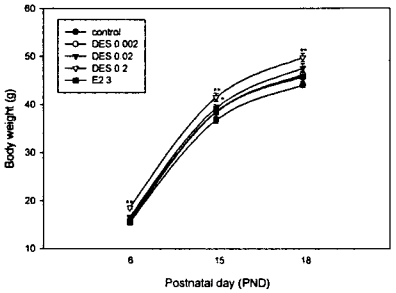


Fig. 24. Body weight changes in male rats, treated with DES or E2 (μg/kg/day). Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

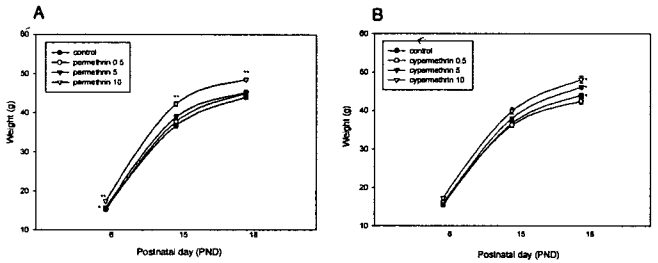


Fig.25. Body weight changes in male rat after neonatal treatment of permethrin (A) and cypermethrin (B) (mg/kg/day). Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

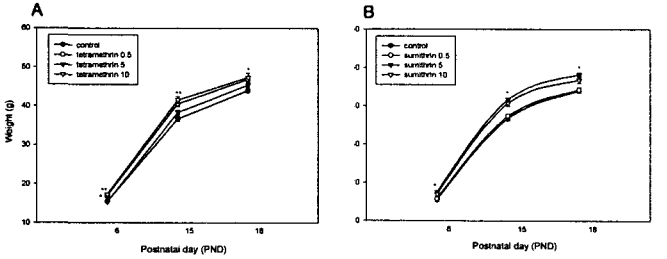


Fig. 26. Body weight changes in male rat after neonatal treatment of tetramethrin (A) and sumithrin (B) (mg/kg/day). Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

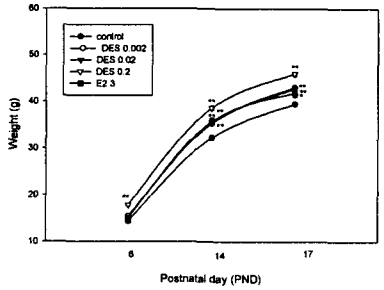


Fig. 27. Body weight changes in female rat after neonatal treatment of DES or E2 (μg/kg/day). Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

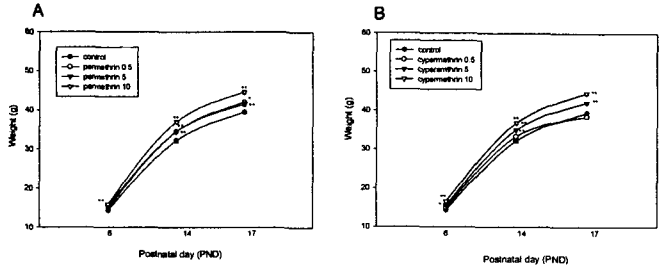


Fig. 28. Body weight changes in female rat after neonatal treatment of permethrin (A) and cypermethrin (B) (mg/kg/day). Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

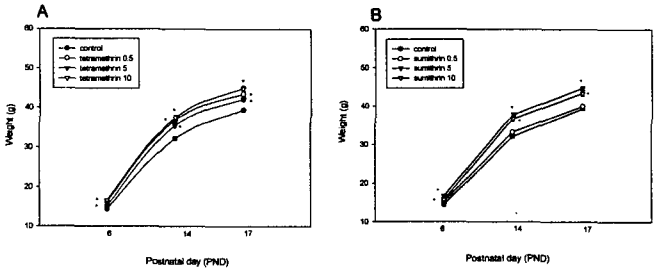


Fig. 29. Body weight changes in female rat after neonatal treatment of tetramethrin (A) and sumithrin (B) (mg/kg/day). Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

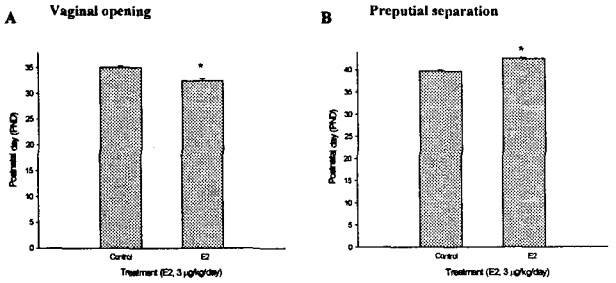


Fig. 30. Vaginal opening in female (A) and preputial separation in male (B) rats, treated with E2 at 1 to 5 days after birth. Data are presented as mean ± SEM. Significant different from control, *P<0.01.

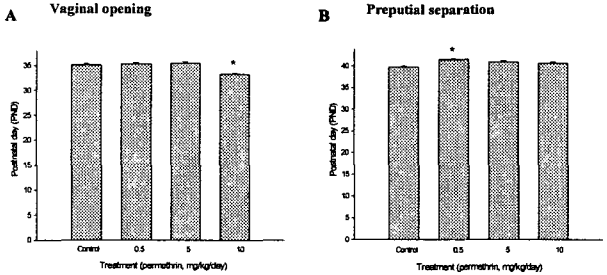


Fig. 31. Vaginal opening in female (A) and preputial separation in male (B) rats, treated with permethrin at 1 to 5 days after birth. Data are presented as mean ± SEM. Significant different from control, *P<0.01.

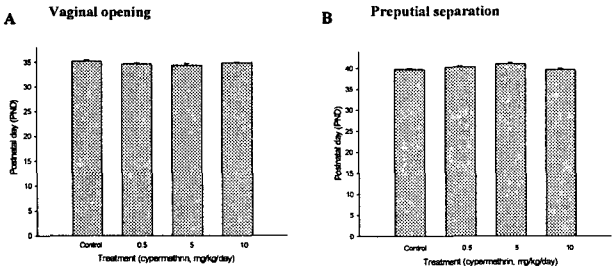


Fig. 32. Vaginal opening in female (A) and preputial separation in male (B) rats, treated with cypermethrin at 1 to 5 days after birth. Data are presented as mean ± SEM.

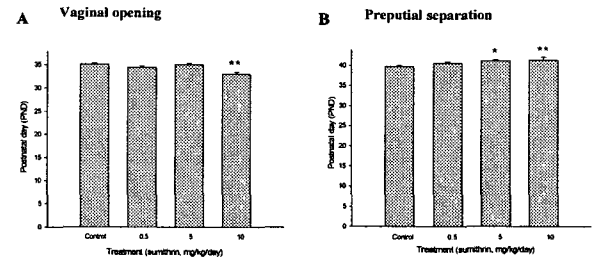


Fig. 33. Vaginal opening in female (A) and preputial separation in male (B) rats, treated with sumithrin at 1 to 5 days after birth. Data are presented as mean ± SEM. Significant different from control, *P<0.05, **<0.01.

Table 4. Reproductive organ weights on PND18 in female rats treated treated with pyrethroid insecticides at 1-5 days after birth

Treatment 70		Body weight (g)	Ovary		Uterus	
			Absolute weight (g)	Relative weight (g)	Absolute weight (g)	Relative weight (g)
Control		39.48±1.309	0.0050±0.00046	0.00013 ± 0.000011	0.0199 ± 0.00041	0.00050 ± 0.000016
Des (μg/kg/day)	0.002	46.78 ± 0.602**	0.0035 ± 0.00081	0.00007 ± 0.000018	0.0169 ± 0.00041	0.00040 ± 0.000012 *
	0.02	42.56 ± 1.495	0.0029 ± 0.00023	0.00007 ± 0.000006	0.0150 ± 0.00081**	0.00035 ± 0.000017 **
	0.2	42.99 ± 2.266	0.0048 ± 0.00105	0.00011 ± 0.000026	0.0150 ± 0.00053**	0.00040 ± 0.000015 *
E2 (mg/kg/day)	3	48.85 ± 0.510 **	0.0073 ± 0.00200	0.00015 ± 0.000024	0.0262 ± 0.00088**	0.00054 ± 0.000019
Permethrin (mg/kg)	0.5	42.86 ± 0.980	0.0069 ± 0.00039	0.00016 ± 0.000008	0.0250 ± 0.00069**	0.00058 ± 0.000012
	5	43.53 ± 0.859	0.0074 ± 0.00082	0.00017 ± 0.000017	0.0251 ± 0.00168**	0.00058 ± 0.000037
	10	46.01 ± 0.962 **	0.0082 ± 0.00072*	0.00020*	0.0271 ± 0.00078**	0.00060 ± 0.000018 *
Sumithrin (mg/kg)	0.5	41.01 ± 0.800	0.0058 ± 0.00077	0.00018 ± 0.000016	0.0252 ± 0.00104**	0.00061 ± 0.0000002 **
	5	47.85 ± 0.378 **	0.0080 ± 0.00076	0.00017 ± 0.000017	0.0283 ± 0.00087**	0.00058 ± 0.000018
	10	48.94 ± 1.382 **	0.0088 ± 0.00067**	0.00018 ± 0.000012	0.0271 ± 0.00068**	0.00058 ± 0.3000023
Cypermethrin (mg/kg/day)	0.5	41.53 ± 0.638	0.0070 ± 0.00054	0.00017 ± 0.000014	0.0224 ± 0.00048	0.00050 ± 0.000009
	5	44.48 ± 0.800 *	0.0075 ± 0.00065	0.00017 ± 0.000015	0.0270 ± 0.00103**	0.00060 ± 0.000022 *
	10	44.27 ± 1.200	0.0073 ± 0.00035	0.00017 ± 0.000009	0.0234 ± 0.00069	0.00053 ± 0.000015
Tetramethrin (mg/kg)	0.5	42.29 ± 1.380	0.0054 ± 0.00068	0.00013 ± 0.000014	0.0225 ± 0.00078	0.00050 ± 0.000010
	5	41.84 ± 0.644	0.0079 ± 0.00050	0.00019 ± 0.000013	0.0244 ± 0.00069*	0.00060 ± 0.000035 *
	10	46.32 ± 1.052 **	0.0065 ± 0.00044	0.00014 ± 0.000007	0.0236 ± 0.00076	0.00050 ± 0.000013

Data are presented as mean ±SEM (N=6-10). Significantly different from control, *P<0.05,**<0.01.

Table 5. Serum hormone concentrations on PND18 in female rats treated treated with pyrethroid insecticides at 1-5 days after birth

Treatment	Dose	E2(pg/ml)	T3(ng/dL)	T4(μg/dL)
Control		68.624±2.2620	68.414±38.0038	3.821±0.0335
DES(μg/kg/day)				
	0.002	41.195±3.5970**	25.758±6.3857	2.388±0.2330**
	0.02	30.745±4.5330**	27.096±3.8985	2.547±0.2790*
	0.2	24.398±3.4700**	31.756±11.7244	2.238±0.0644**
E2(3μg/kg/day)		24.398±2.8340**	83.652±9.6752	2.031±0.3080**
Permethrin(mg/kg/day)				
	0.5	24.654±0.1270**	55.988±3.0480	2.736±0.1750*
	5	20.121±0.0315**	59.454±16.0195	2.554±0.2100*
	10	20.124±1.4570**	89.231±7.1635	3.419±0.0784
Sumithrin(mg/kg/day)				
	0.5	16.490±1.3160**	30.571±9.5823	2.401±0.1350**
	5	13.291±1.1980**	48.057±23.3150	2.408±0.3090*
	10	12.925±1.1220**	20.950±16.7509	2.309±0.0347**
Cypermethrin(mg/kg/day)				
	0.5	24.031±2.1720**	54.743±53.8995	1.962±0.1620**
	5	21.113±1.7300**	46.106±28.9342	2.643±0.1300*
	10	23.144±0.9650**	81.643±44.4713	2.483±0.0793*
Tetramethrin(mg/kg/day)				
	0.5	17.391±1.4080**	48.749±19.1929	2.310±0.1770**
	5	14.825±1.1990**	52.016±14.6280	3.099±0.3700*
	10	16.753±2.1880**	17.662±3.2734	1.797±0.0902**

Data are presented as mean ±SEM. Significantly different from control, *P<0.05, **<0.01.

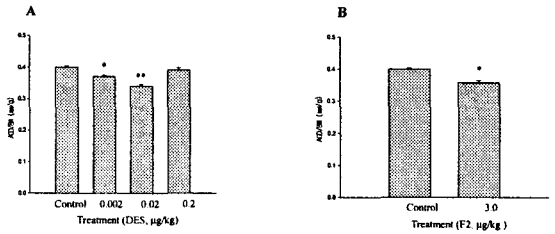


Fig. 34. Anogenital distances on PND 18 in male rats treated with DES (A) and E2 (B), Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

Table 6. Reproductive organ weights in diestrus in female rats treated with pyrethroid insecticides at 1-5 days after birth.

Treatment		Body weight (g)	Ovary		Uterus	
			Absolute weight (g)	Relative weight (g)	Absolute weight (g)	Relative weight (g)
Control		158.447 ± 2.525	0.0493 ± 0.00317	0.00030 ± 0.000019	0.372 ± 0.0500	0.00240 ± 0.000341
Des (μg/kg/day)	0.002	167.500 ± 2.947	0.0527 ± 0.00326	0.000360 ± 0.000018	0.215 ± 0.0088 **	0.00130 ± 0.000055 **
	0.02	164.833 ± 3.300	0.0642 ± 0.00488	0.00040 ± 0.000028	0.272 ± 0.0184 **	0.00160 ± 0.000098 **
	0.2	158.925 ± 2.408	0.0502 ± 0.00133	0.00030 ± 0.000011	0.201 ± 0.0068 **	0.00130 ± 0.000051 **
E2 (mg/kg/day)	3	174.362 ± 2.616	0.0464 ± 0.00480	0.00030 ± 0.000028 *	0.254 ± 0.0143 **	0.00150 ± 0.000075 **
Permethrin (mg/kg)	0.5	160.495 ± 3.600	0.0507 ± 0.00382	0.00030 ± 0.000023	0.247 ± 0.0198 **	0.00150 ± 0.000122 **
	5	152.143 ± 5.238	0.0445 ± 0.00377	0.00030 ± 0.000022	0.209 ± 0.0129 **	0.00140 ± 0.000080 **
	10	161.161 ± 5.381	0.0550 ± 0.00315	0.00030 ± 0.000013	0.255 ± 0.0155 **	0.00160 ± 0.000121 **
Sumithrin (mg/kg)	0.5	155.014 ± 3.317	0.0520 ± 0.00340	0.00033 ± 0.000033	0.224 ± 0.0096 **	0.00145 ± 0.000067 **
	5	165.878 ± 3.336	0.0574 ± 0.00351	0.00030 ± 0.000041	0.228 ± 0.0043 **	0.00140 ± 0.000000 **
	10	162.067 ± 6.156	0.0524 ± 0.00432	0.00032 ± 0.000018	0.234 ± 0.0086 **	0.00100 ± 0.000051 **
Cypermethrin (mg/kg/day)	0.5	151.500 ± 5.367	0.0485 ± 0.00432	0.00030 ± 0.000027	0.262 ± 0.0210 **	0.00170 ± 0.000133 **
	5	151.890 ± 3.947	0.0540 ± 0.00290	0.00040 ± 0.000014 *	0.245 ± 0.0145 **	0.00160 ± 0.000125 **
	10	170.333 ± 4.201	0.0545 ± 0.00322	0.00030 ± 0.000016	0.293 ± 0.0215 *	0.00170 ± 0.000154 **
Tetramethrin (mg/kg)	0.5	157.650 ± 5.151 **	0.0448 ± 0.00235	0.00030 ± 0.000011	0.195 ± 0.0080 **	0.00140 ± 0.000048 **
	5	169.244 ± 2.300	0.0524 ± 0.00328	0.00030 ± 0.000017	0.220 ± 0.0195 **	0.00130 ± 0.000105 **
	10	167.105 ± 4.909	0.0491 ± 0.00205	0.00030 ± 0.000010	0.225 ± 0.169 **	0.00150 ± 0.000097 **

Data are presented as mean ± SEM. Significantly different from control, *P<0.05,**<0.01.

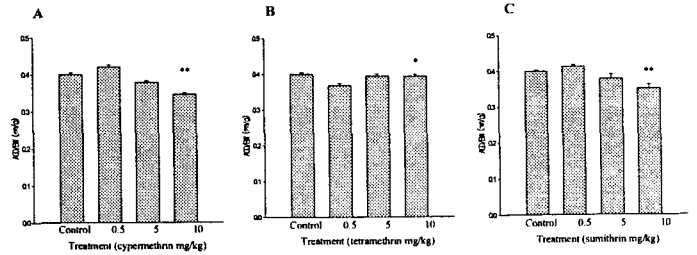


Fig. 35. Anogenital distances on PND 18 in male rats treated with cypermethrin (A), tetramethrin (B) and sumithrin (C). Data are presented as mean ± SEM. Significantly different from control, *P<0.05, **<0.01.

Summarys II

In utero exposure

- Permethrin-treated rats had significant decreases in implantation sites and live fetuces
- Permethrin led to statistically significant increases in uterine and ovari an weights, while reductions in serum E2 concentration, and uterine and ovarian ERα mRNA Expression in female offspring on PND 22

Ineonatal exposure

- Permethrin and sumithrin significantly accelerated vaginal opening in female rat, whereas extended preputial separation in male
- All pyrethroids tested significantly increased uterine weight, but reduced in diestrus phase. In these rats, serum E2 concentrations were reduced on PND 18, but increased in diestrus phase
- Cypermethrin and sumithrin significantly reduced anogenital distances on PND 18

→ These results demonstrate the possibilities of reproductive and developmental effects of pyrethroid insecticides via estrogenic or antiestrogenic activity

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