

Fig. 45. Distribution of PCNA-immunoreactive proliferating cells and Cresyl Violet -positive apoptotic bodies in the SGZ of pups at PND 21 in Experiment 1 of the acrylamide study.

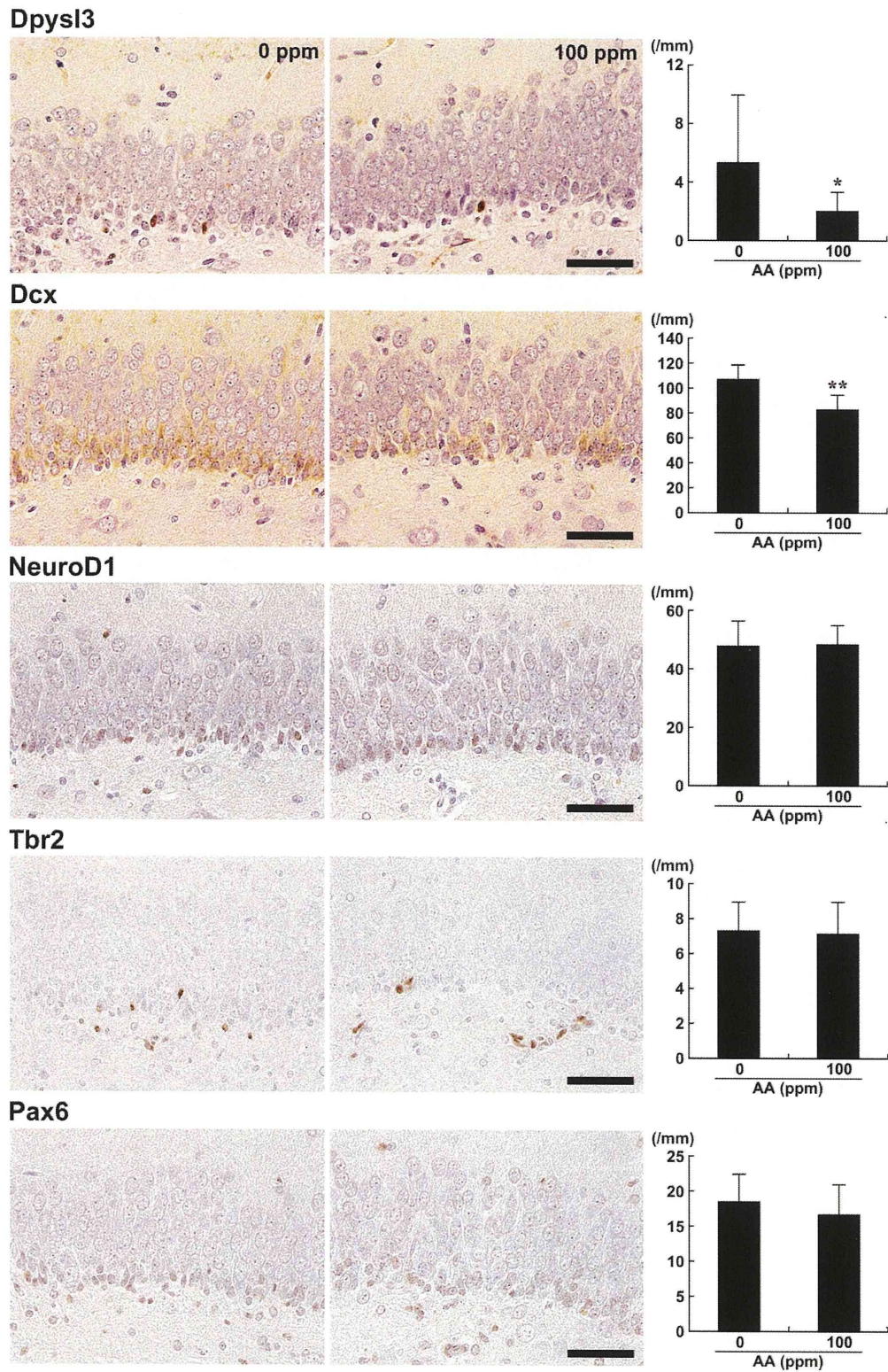


Fig. 46. Distribution of immunoreactive cells for neuronal stage-defining markers in the SGZ of pups at PND 21 in Experiment 1 of the acrylamide study.

Table 1 List of animal groups examined for immunohistochemistry and apoptosis assays in experiments of acrylamide

	Experiment 1				Experiment 2	
	AA in the drinking water (ppm)				Intraperitoneal injections	
	0 (control)	4	20	100	Saline (control)	AA
PND 21, male offspring						
reelin	T	T	T	T	T	T
NeuN	T	T	T	T	T	T
PCNA	T	T	T	T	T	T
Dpysl3	T	N.E.	N.E.	T	N.E.	N.E.
Dcx	T	N.E.	N.E.	T	N.E.	N.E.
NeuroD1	T	N.E.	N.E.	T	N.E.	N.E.
Tbr2	T	N.E.	N.E.	T	N.E.	N.E.
Pax6	T	N.E.	N.E.	T	N.E.	N.E.
Cresyl Violet staining	T	T	T	T	T	T
TUNEL-assay	S	S	S	S	S	S
PND 77, male offspring						
reelin	T	T	T	T	T	T
NeuN	T	T	T	T	T	T
PCNA	T	T	T	T	T	T
Dpysl3	S	N.E.	N.E.	S	N.E.	N.E.
Dcx	S	N.E.	N.E.	S	N.E.	N.E.
Cresyl Violet staining	T	T	T	T	T	T
PND 4, female offspring						
reelin	S	S	S	S	S	S
Dams						
reelin	T	N.E.	N.E.	T	N.A.	N.A.
PCNA	T	N.E.	N.E.	T	N.A.	N.A.

T: Stained three different sections with approximately 250 μ m interval using serial sections in each brain.

S: Stained one section in each brain.

N.E.: Not examined.

N.A.: Not applicable.

Table 2. Body weights of dams fed either a normal or low protein diet from GD 10 to PND 21 in rats.

Casein level	Normal protein		Low protein	
		20%		10%
No. of dams examined		8		8
Body weight (g)				
GD 10		287.4 ± 16.1 ^a		293.1 ± 23.5
GD 11		297.8 ± 16.4		299.5 ± 20.0
GD 12		303.8 ± 17.2		304.0 ± 20.9
GD 13		309.8 ± 18.9		308.5 ± 21.8
GD 14		317.4 ± 17.9		318.8 ± 22.5
GD 15		323.4 ± 18.0		324.0 ± 21.7
GD 16		335.1 ± 17.2		336.5 ± 22.4
GD 17		348.3 ± 18.6		348.1 ± 22.6
GD 18		362.0 ± 19.1		360.9 ± 24.6
GD 19		378.0 ± 19.7		374.5 ± 26.8
GD 20		393.3 ± 21.0		384.3 ± 26.8
PND 0		318.0 ± 27.9		309.5 ± 19.7
PND 4		317.3 ± 22.4		297.6 ± 28.2
PND 7		330.1 ± 20.9		303.8 ± 29.5
PND 11		333.1 ± 16.0		294.8 ± 33.1*
PND 14		327.1 ± 22.5		290.3 ± 36.6*
PND 17		322.5 ± 22.3		283.6 ± 35.7*
PND 21		307.4 ± 18.3		266.5 ± 38.4*

^aMean±SD.* Significantly different from the normal protein group (**P*<0.05).

Abbreviations: GD, gestation day; PND, postnatal day.

Table 3. Food consumption of dams fed either a normal or low protein diet from GD 10 to PND 21 in rats.

Casein level	Normal protein		Low protein	
		20%		10%
No. of dams examined		8		8
Food consumption (g/animal/day)				
GD 9-10		24.8 ± 1.8 ^a		26.4 ± 3.0
GD 10-14		30.3 ± 3.5		31.1 ± 1.6
GD 14-17		28.6 ± 2.6		29.5 ± 1.9
GD 17-20		24.8 ± 3.7		23.5 ± 2.7
PND 0-4		32.9 ± 3.7		28.5 ± 4.6
PND 4-7		37.8 ± 3.1		34.1 ± 2.6*
PND 7-11		44.6 ± 2.7		35.8 ± 3.7**
PND 11-14		51.1 ± 2.6		39.0 ± 5.0**
PND 14-17		50.8 ± 4.6		39.8 ± 5.3**
PND 17-21		56.6 ± 5.4		42.0 ± 3.1**
Food consumption (g/kg body weight/day)				
GD 9-10		86.2 ± 5.1		89.9 ± 4.9
GD 10-14		95.2 ± 8.0		97.8 ± 3.8
GD 14-17		82.2 ± 6.2		85.0 ± 6.3
GD 17-20		62.8 ± 7.3		61.2 ± 6.3
PND 0-4		104.3 ± 15.8		96.1 ± 14.8
PND 4-7		114.8 ± 11.9		112.7 ± 6.7
PND 7-11		134.3 ± 11.6		121.6 ± 8.8*
PND 11-14		157.2 ± 16.9		129.5 ± 11.2**
PND 14-17		158.0 ± 18.2		140.2 ± 7.6*
PND 17-21		185.2 ± 24.7		159.4 ± 17.7*

^aMean±SD.*** Significantly different from the normal protein group (**P*<0.05, ***P*<0.01).

Abbreviations: GD, gestation day; PND, postnatal day.

Table 4. Body weight of offspring after maternal protein restriction during the period from GD 10 to PND 21 in rats.

Casein level	Males		Females	
	Normal protein	Low protein	Normal protein	Low protein
	20%	10%	20%	10%
No. of offspring examined	16	16	16	16
Body weight (g)				
PND 0	6.7 ± 0.6 ^a	6.3 ± 0.4**	6.2 ± 0.4	5.9 ± 0.5*
PND 4	10.9 ± 1.4	9.0 ± 1.1**	10.3 ± 1.5	8.5 ± 1.0**
PND 7	17.6 ± 2.3	13.7 ± 1.9**	16.4 ± 2.6	12.4 ± 1.8**
PND 11	29.0 ± 3.6	21.4 ± 1.6**	27.0 ± 3.7	19.2 ± 2.3**
PND 14	38.4 ± 4.4	26.6 ± 1.5**	36.1 ± 3.9	24.5 ± 2.3**
PND 17	46.5 ± 5.5	31.6 ± 1.9**	43.9 ± 4.5	29.2 ± 2.5**
PND 21	61.4 ± 7.0	40.4 ± 2.4**	58.0 ± 6.1	38.0 ± 3.6**
PND 28	102.8 ± 10.9	75.4 ± 4.7**	90.1 ± 9.6	70.8 ± 4.1**
PND 35	174.5 ± 16.3	136.5 ± 8.1**	142.3 ± 12.3	121.4 ± 6.6**
PND 42	253.0 ± 19.6	203.1 ± 9.6**	181.3 ± 12.4	160.6 ± 8.4**
PND 49	322.8 ± 21.7	263.2 ± 13.6**	206.2 ± 13.6	187.5 ± 12.1**
PND 56	382.8 ± 22.1	318.6 ± 15.0**	228.7 ± 14.4	207.2 ± 15.1**
PND 63	429.4 ± 25.9	358.9 ± 20.5**	255.4 ± 19.1	231.1 ± 18.8**
PND 70	474.5 ± 28.0	398.3 ± 23.1**	276.8 ± 23.3	247.4 ± 20.4*
PND 77	508.3 ± 30.6	427.0 ± 26.9**	285.3 ± 22.5	254.9 ± 22.6*

^aMean±SD.*** Significantly different from the normal protein group (* $P < 0.05$, ** $P < 0.01$).

Abbreviations: PND, postnatal day.

Table 5. Food consumption of offspring after maternal protein restriction during the period from GD 10 to PND 21 in rats.

Casein level	Males		Females	
	Normal protein	Low protein	Normal protein	Low protein
	20%	10%	20%	10%
No. of offspring examined	16	16	16	16
Food consumption (g/animal/day)				
PND 35	19.6 ± 1.7	17.1 ± 0.7**	16.6 ± 1.7	15.5 ± 0.9
PND 42	27.3 ± 2.0	23.4 ± 1.2**	19.9 ± 1.6	19.1 ± 1.1
PND 49	30.4 ± 2.2	25.9 ± 1.4**	20.2 ± 1.2	19.0 ± 1.2
PND 56	32.3 ± 1.9	28.0 ± 1.6**	20.6 ± 1.3	19.1 ± 1.4*
PND 63	31.6 ± 2.0	27.9 ± 1.8**	20.9 ± 1.5	19.2 ± 1.4*
PND 70	32.1 ± 2.1	27.9 ± 2.0**	22.2 ± 1.9	20.3 ± 1.3*
PND 77	31.3 ± 2.1	27.4 ± 2.4**	20.7 ± 1.9	18.9 ± 1.5*
Food consumption (g/kg body weight/day)				
PND 35	141.7 ± 5.1	161.4 ± 9.0**	143.1 ± 8.3	161.1 ± 6.4**
PND 42	127.6 ± 3.4	137.8 ± 5.7**	122.9 ± 7.8	135.0 ± 4.7**
PND 49	105.8 ± 5.2	111.0 ± 4.0**	104.3 ± 6.6	109.1 ± 4.3*
PND 56	91.5 ± 4.6	96.3 ± 4.6**	94.6 ± 4.7	96.6 ± 3.4
PND 63	77.9 ± 3.9	82.3 ± 4.1**	86.5 ± 3.9	87.7 ± 5.4
PND 70	71.1 ± 4.0	73.6 ± 3.7	83.3 ± 4.6	84.8 ± 3.2
PND 77	63.8 ± 3.2	66.3 ± 3.5*	73.6 ± 4.0	75.3 ± 3.9

^aMean±SD.*** Significantly different from the normal protein group (* $P < 0.05$, ** $P < 0.01$).

Abbreviations: PND, postnatal day.

Table 6. Organ weights of offspring after maternal protein restriction during the period from GD 10 to PND 21 in rats.

	Casein level	Males		Females	
		Normal protein	Low protein	Normal protein	Low protein
		20%	10%	20%	10%
PND 21					
No. of offspring examined		10	10	10	10
Brain (g)		1.55 ± 0.06 ^a	1.44 ± 0.04**	1.45 ± 0.05	1.33 ± 0.09**
(g/100g BW)		2.42 ± 0.23	3.68 ± 0.36**	2.53 ± 0.20	4.17 ± 0.80**
Liver (g)		2.51 ± 0.38	1.64 ± 0.31**	2.39 ± 0.25	1.37 ± 0.41**
(g/100g BW)		3.87 ± 0.24	4.16 ± 0.35*	4.14 ± 0.21	4.04 ± 0.41
PND 77					
No. of offspring examined		10	10	10	10
Brain (g)		2.06 ± 0.06	2.03 ± 0.08	1.90 ± 0.06	1.86 ± 0.08
(g/100g BW)		0.41 ± 0.02	0.48 ± 0.04**	0.67 ± 0.08	0.73 ± 0.06
Liver (g)		18.85 ± 2.70	15.33 ± 1.90**	10.06 ± 1.83	9.27 ± 1.45
(g/100g BW)		3.75 ± 0.31	3.60 ± 0.19	3.47 ± 0.20	3.63 ± 0.24
Kidneys (g)		3.21 ± 0.47	2.86 ± 0.24*	2.07 ± 0.27	1.85 ± 0.14*
(g/100g BW)		0.64 ± 0.07	0.67 ± 0.05	0.72 ± 0.06	0.73 ± 0.05
Testes (g)		3.62 ± 0.30	3.20 ± 0.47*	-	-
(g/100g BW)		0.72 ± 0.05	0.75 ± 0.09	-	-
Ovaries (mg)		-	-	95.7 ± 17.5	85.3 ± 14.8
(mg/100g BW)		-	-	33.3 ± 4.6	33.7 ± 6.0

^aMean±SD.

- Not applicable.

*** Significantly different from the normal protein group (**P*<0.05, ***P*<0.01).

Abbreviations: BW, body weight; PND, postnatal day.

Table 7. Distribution of immunoreactive cells for Reelin, NeuN, Calb-D-28K, GAD67 and FoxG1 in the hilus of the hippocampal dentate gyrus in male rat offspring at PND 21 after maternal protein restriction from GD 10 to PND 21.

Casein level	Normal protein	Low protein
	20%	10%
No. of dams examined	10	10
Reelin (+) cell count (/mm ²)	72.2 ± 16.4 ^a	77.9 ± 19.4
NeuN (+) cell count (/mm ²)	250.3 ± 46.8	272.4 ± 72.2
Calb-D-28K (+) cell count (/mm ²)	362.7 ± 53.6	392.7 ± 44.9
GAD67 (+) cell count (/mm ²)	36.7 ± 13.6	36.7 ± 13.2
FoxG1 (+) cell count (/mm ²)	337.4 ± 31.0	375.9 ± 56.5

^aMean±SD.

Table 8. Distribution of immunoreactive cells for Reelin, NeuN, Calb-D-28K, GAD67 and FoxG1 in the hilus of the hippocampal dentate gyrus in male rat offspring at PND 77 after maternal protein restriction from GD 10 to PND 21.

Casein level	Normal protein	Low protein
	20%	10%
No. of dams examined	10	10
Reelin (+) cell count (/mm ²)	37.6 ± 10.2 ^a	39.2 ± 12.6
NeuN (+) cell count (/mm ²)	148.4 ± 19.3	140.1 ± 26.6
Calb-D-28K (+) cell count (/mm ²)	266.1 ± 30.2	227.3 ± 57.8
GAD67 (+) cell count (/mm ²)	22.8 ± 12.3	20.1 ± 12.0
FoxG1 (+) cell count (/mm ²)	227.6 ± 27.4	207.1 ± 27.1

^aMean±SD.

Table 9. Distribution of apoptotic cells and proliferating cells in the dentate subgranular zone of male rat offspring at both PND 21 and PND 77 after maternal protein restriction from GD 10 to PND 21.

	Casein level	Normal protein	Low protein
		20%	10%
PND 21			
	No. of dams examined	10	10
	PCNA (+) cell count (/mm)	2.33 ± 1.08 ^a	2.39 ± 0.99
	Apoptotic cell count (/mm)	0.091 ± 0.202	0.140 ± 0.299
PND 77			
	No. of dams examined	10	10
	PCNA (+) cell count (/mm)	1.25 ± 0.95	1.19 ± 0.60
	Apoptotic cell count (/mm)	0.034 ± 0.107	0.000 ± 0.000

^aMean±SD.

Table 10. Histopathological examination of the cerebellum of male rat offspring at PND 21 after maternal protein restriction from GD 10 to PND 21.

	Casein level	Normal protein	Low protein
		20%	10%
Cerebellum			
	No. of offspring examined	10	10
	Increase of external granular cells (±/+) ^a	1(1/0)	5(3/2) *

^aGrade of change: (±), minimal, (+), slight.

* Significantly different from the normal protein group by Mann-Whitney's *U*-test (**P*<0.05).

Table 11. Body weight of dams exposed to MnCl₂·4H₂O from day 10 of pregnancy to day 21 after delivery in rats.

	Control	MnCl ₂ ·4H ₂ O in diet		
		32 ppm	160 ppm	800 ppm
	No. of dams examined	8	8	8
Body weight (g)				
GD 10	287.5 ± 22.2 ^a	284.4 ± 23.1	277.0 ± 17.4	276.9 ± 24.6
GD 11	301.1 ± 26.6	296.0 ± 23.6	292.9 ± 20.6	294.0 ± 23.9
GD 12	309.4 ± 25.7	304.4 ± 24.9	301.4 ± 20.9	303.5 ± 25.2
GD 13	313.0 ± 26.6	308.4 ± 23.7	306.3 ± 20.5	309.3 ± 23.2
GD 14	321.5 ± 25.5	316.1 ± 23.2	312.1 ± 22.1	316.0 ± 23.9
GD 15	328.5 ± 27.3	323.6 ± 23.2	320.0 ± 21.2	325.9 ± 24.1
GD 16	340.5 ± 28.2	335.5 ± 24.6	331.1 ± 22.8	337.4 ± 23.9
GD 17	352.0 ± 27.8	349.4 ± 24.7	343.6 ± 22.7	349.0 ± 25.6
GD 18	362.5 ± 27.7	359.3 ± 26.0	356.4 ± 22.3	359.8 ± 26.9
GD 19	378.3 ± 27.0	375.3 ± 26.6	370.4 ± 27.2	377.8 ± 28.2
GD 20	398.4 ± 31.7	394.1 ± 27.6	390.9 ± 25.3	394.9 ± 28.9
PND 0	314.9 ± 22.3	309.0 ± 33.3	310.4 ± 21.1	312.9 ± 24.3
PND 4	338.8 ± 21.1	331.0 ± 25.3	334.1 ± 23.7	333.9 ± 21.3
PND 7	339.9 ± 18.4	329.9 ± 24.6	335.3 ± 21.9	336.9 ± 18.5
PND 11	334.9 ± 19.8	334.8 ± 23.9	343.9 ± 24.5	329.6 ± 14.5
PND 14	343.4 ± 14.4	331.1 ± 17.6	337.0 ± 19.9	332.0 ± 18.5
PND 17	326.5 ± 13.3	324.4 ± 21.9	328.3 ± 17.7	326.8 ± 19.1
PND 21	323.3 ± 21.1	314.9 ± 15.1	316.8 ± 21.6	318.3 ± 14.1

^aMean±SD.

Abbreviations: GD, gestation day; PND, postnatal day.

Table 12. Food consumption of dams exposed to $MnCl_2 \cdot 4H_2O$ from day 10 of pregnancy to day 21 after delivery in rats.

	No. of dams examined	$MnCl_2 \cdot 4H_2O$ in diet			
		Control	32 ppm	160 ppm	800 ppm
	8	8	8	8	
Food consumption (g/animal/day)					
GD 10		21.5±3.0 ^a	22.0 ± 2.7	16.6 ± 5.9	15.8 ± 9.1
GD 14		24.1±2.4	24.4 ± 2.1	24.0 ± 2.0	24.0 ± 2.0
GD 17		24.5±1.8	24.5 ± 1.8	24.1 ± 2.1	24.4 ± 1.7
GD 20		24.4±2.1	24.1 ± 2.2	25.0 ± 1.6	24.4 ± 1.8
PND 4		34.9±2.9	36.5 ± 3.1	36.6 ± 5.3	37.1 ± 3.2
PND 7		40.9±2.0	40.3 ± 2.8	42.4 ± 2.9	42.4 ± 2.8
PND 11		45.1±3.8	47.5 ± 3.3	50.3 ± 3.8*	49.6 ± 3.4*
PND 14		52.6±2.8	50.3 ± 3.0	51.6 ± 3.1	56.4 ± 3.5
PND 17		51.1±2.9	51.5 ± 3.0	55.5 ± 4.0	54.5 ± 4.8
PND 21		66.0±3.2	66.1 ± 3.1	68.0 ± 4.3	70.1 ± 4.5
Food consumption (g/kg body weight/day)					
GD 10		74.7±8.4	77.5 ± 8.0	60.6 ± 22.2	55.2 ± 31.4
GD 14		75.0±3.7	77.2 ± 4.4	76.9 ± 3.4	76.0 ± 4.3
GD 17		69.7±3.5	70.2 ± 2.8	70.2 ± 3.3	70.0 ± 5.5
GD 20		61.3±3.6	61.2 ± 2.7	64.0 ± 3.0	62.4 ± 4.7
PND 4		103.3±11.0	110.7 ± 11.7	109.6 ± 13.8	111.5 ± 11.6
PND 7		120.5±8.2	122.5 ± 10.9	126.5 ± 6.5	125.9 ± 8.1
PND 11		134.9±11.5	142.6 ± 15.2	146.3 ± 8.7	150.7 ± 10.8*
PND 14		153.4±8.8	152.1 ± 11.2	153.6 ± 11.5	170.3 ± 14.7*
PND 17		156.8±11.1	159.3 ± 12.0	169.1 ± 8.1	167.0 ± 13.7
PND 21		205.0±17.3	210.4 ± 13.2	215.3 ± 16.5	220.7 ± 17.0

^aMean±SD.

* Significantly different from the control group (* $P < 0.05$).

Abbreviations: GD, gestation day; PND, postnatal day.

Table 13. Body weight of offspring exposed maternally to $MnCl_2 \cdot 4H_2O$ from GD 10 to PND 21 in rats.

	$MnCl_2 \cdot 4H_2O$ in diet			
	Control	32 ppm	160 ppm	800 ppm
Body weight (g)				
Males				
No. of offspring examined	16	16	16	16
PND 0	6.6 ± 0.5 ^a	6.7 ± 0.4	6.8 ± 0.4	6.7 ± 0.6
PND 4	10.6 ± 0.9	10.4 ± 1.0	11.6 ± 0.8	11.0 ± 1.3
PND 7	17.2 ± 1.8	17.2 ± 1.3	18.9 ± 1.4	18.2 ± 1.6
PND 11	26.4 ± 2.5	26.5 ± 1.8	29.0 ± 2.3	28.1 ± 2.6
PND 14	33.6 ± 2.9	33.7 ± 2.3	36.4 ± 3.0	35.7 ± 2.9
PND 17	41.3 ± 3.7	41.4 ± 2.7	44.9 ± 3.7	42.9 ± 2.8
PND 21	58.4 ± 5.4	58.8 ± 2.6	62.1 ± 4.9	60.5 ± 4.1
PND 28	99.7 ± 7.6	101.1 ± 3.4	104.9 ± 9.8	101.1 ± 7.2
PND 35	169.2 ± 10.4	170.8 ± 5.6	173.9 ± 15.9	169.4 ± 11.6
PND 42	244.3 ± 11.0	246.4 ± 7.8	249.9 ± 21.5	244.0 ± 16.0
PND 49	313.3 ± 12.1	317.6 ± 10.5	317.4 ± 22.7	314.1 ± 22.1
PND 56	373.9 ± 12.7	378.9 ± 15.8	381.7 ± 31.6	377.6 ± 27.0
PND 63	417.9 ± 12.0	425.9 ± 17.9	427.8 ± 38.0	423.6 ± 33.7
PND 70	455.4 ± 11.5	467.7 ± 20.3	468.0 ± 43.7	463.3 ± 38.4
PND 77	489.0 ± 15.4	501.9 ± 23.9	501.9 ± 47.2	499.2 ± 43.4
Females				
No. of offspring examined	16	16	16	16
PND 0	6.3 ± 0.4	6.3 ± 0.3	6.4 ± 0.5	6.4 ± 0.4
PND 4	10.0 ± 0.7	10.1 ± 0.9	11.0 ± 1.0	10.5 ± 0.8
PND 7	16.7 ± 1.1	16.9 ± 1.5	18.2 ± 1.3	17.4 ± 1.1
PND 11	25.9 ± 1.6	26.3 ± 2.3	28.4 ± 2.1	27.0 ± 2.1
PND 14	32.9 ± 2.0	33.4 ± 2.7	35.4 ± 2.4	34.6 ± 2.3
PND 17	40.4 ± 2.3	40.8 ± 3.0	43.6 ± 2.7	41.9 ± 2.8
PND 21	56.2 ± 3.5	57.3 ± 3.3	60.0 ± 3.4	58.9 ± 4.0
PND 28	92.5 ± 5.8	91.7 ± 5.7	95.1 ± 6.6	93.6 ± 6.2
PND 35	146.1 ± 7.6	141.9 ± 6.1	148.3 ± 10.7	144.8 ± 9.1
PND 42	186.7 ± 8.2	181.3 ± 8.6	189.0 ± 16.3	184.7 ± 12.8
PND 49	216.0 ± 7.9	211.9 ± 12.4	214.0 ± 16.1	214.8 ± 17.5
PND 56	238.5 ± 10.3	235.8 ± 12.5	239.9 ± 22.0	238.9 ± 19.1
PND 63	263.4 ± 13.6	259.3 ± 13.5	261.1 ± 21.3	264.6 ± 19.2
PND 70	276.1 ± 13.8	274.9 ± 14.0	274.8 ± 22.2	282.6 ± 17.5
PND 77	290.1 ± 17.0	289.0 ± 12.7	287.0 ± 23.3	294.9 ± 17.7

^aMean±SD.

Abbreviations: PND, postnatal day.

Table 14. Food consumption of offspring exposed maternally to MnCl₂·4H₂O from GD 10 to PND 21 in rats.

	MnCl ₂ ·4H ₂ O in diet			
	Control	32 ppm	160 ppm	800 ppm
Males				
No. of offspring examined	16	16	16	16
Food consumption (g/animal/day)				
PND 35	18.5±1.1 ^a	18.4 ± 1.0	18.8 ± 1.4	18.9 ± 1.2
PND 42	25.4±0.9	25.3 ± 1.3	25.4 ± 1.6	26.0 ± 1.5
PND 49	28.3±0.8	28.9 ± 1.6	28.6 ± 1.8	28.9 ± 2.0
PND 56	29.7±1.0	31.0 ± 2.1	30.1 ± 2.1	30.7 ± 2.7
PND 63	30.9±1.0	32.0 ± 2.4	31.2 ± 2.5	31.8 ± 3.1
PND 70	31.0±1.0	32.5 ± 2.5	31.4 ± 2.9	32.1 ± 2.6
PND 77	29.9±1.2	31.7 ± 2.6	30.8 ± 2.6	31.2 ± 2.9
Food consumption (g/kg body weight/day)				
PND 35	109.4±3.2	107.9 ± 3.0	108.4 ± 3.4	111.5 ± 4.6
PND 42	104.2±2.4	102.4 ± 3.5	101.8 ± 4.2	106.6 ± 2.5
PND 49	90.2±2.4	91.1 ± 3.7	90.1 ± 3.5	92.2 ± 3.1
PND 56	79.4±1.8	81.8 ± 3.8	78.9 ± 2.9	81.3 ± 3.4
PND 63	73.9±2.2	75.1 ± 4.0	73.0 ± 2.4	74.9 ± 3.7
PND 70	68.1±1.4	69.5 ± 3.6	67.1 ± 2.0	69.4 ± 2.6
PND 77	61.2±1.1	63.1 ± 3.6	61.5 ± 2.1	62.5 ± 2.7
Females				
No. of offspring examined	16	16	16	16
Food consumption (g/animal/day)				
PND 35	16.2±1.2	15.9 ± 1.0	16.2 ± 0.9	16.3 ± 1.2
PND 42	19.8±0.8	19.1 ± 1.1	19.7 ± 1.9	19.9 ± 1.1
PND 49	19.7±0.7	20.1 ± 1.6	19.3 ± 1.5	20.1 ± 1.3
PND 56	20.2±1.0	20.6 ± 1.3	19.8 ± 1.6	20.6 ± 1.4
PND 63	21.9±1.3	21.7 ± 1.0	20.9 ± 1.3	21.9 ± 1.5
PND 70	21.6±1.5	21.9 ± 1.1	21.6 ± 1.6	22.3 ± 1.2
PND 77	21.1±1.8	20.9 ± 0.9	20.3 ± 1.9	21.1 ± 1.5
Food consumption (g/kg body weight/day)				
PND 35	110.7±4.2	112.3 ± 5.1	109.3 ± 3.4	112.7 ± 4.2
PND 42	106.2±3.0	105.5 ± 4.2	104.1 ± 2.8	107.7 ± 2.5
PND 49	91.2±2.0	94.9 ± 4.7	90.3 ± 3.3	94.0 ± 5.7
PND 56	84.7±3.6	87.2 ± 3.5	82.4 ± 2.6	86.5 ± 4.8
PND 63	83.1±3.7	83.7 ± 2.8	80.1 ± 2.9	83.1 ± 4.8
PND 70	78.3±3.8	79.6 ± 3.3	78.5 ± 2.3	79.0 ± 6.1
PND 77	72.8±3.4	72.5 ± 3.5	70.8 ± 2.2	71.6 ± 5.7

^aMean±SD.

Abbreviations: PND, postnatal day.

Table 15. Organ weight of offspring exposed maternally to MnCl₂ · 4H₂O from GD 10 to PND 21 in rats.

	MnCl ₂ · 4H ₂ O in diet			
	Control	32 ppm	160 ppm	800 ppm
PND 21				
Males				
No. of offspring examined	10	10	10	10
Brain (g)	1.51 ± 0.05 ^a	1.56 ± 0.05	1.48 ± 0.05	1.51 ± 0.04
(g/100g BW)	2.70 ± 0.30	2.74 ± 0.23	2.54 ± 0.19	2.47 ± 0.17
Liver (g)	2.15 ± 0.30	2.17 ± 0.19	2.16 ± 0.17	2.21 ± 0.23
(g/100g BW)	3.79 ± 0.17	3.79 ± 0.17	3.67 ± 0.22	3.59 ± 0.17
Kidneys (g)	0.64 ± 0.07	0.64 ± 0.06	0.66 ± 0.04	0.66 ± 0.06
(g/100g BW)	1.13 ± 0.07	1.13 ± 0.04	1.12 ± 0.07	1.08 ± 0.05
Testes (g)	0.26 ± 0.02	0.24 ± 0.03	0.26 ± 0.02	0.25 ± 0.03
(g/100g BW)	0.45 ± 0.04	0.42 ± 0.04	0.45 ± 0.04	0.41 ± 0.02
Females				
No. of offspring examined	10	10	10	10
Brain (g)	1.48 ± 0.05	1.54 ± 0.06	1.50 ± 0.04	1.47 ± 0.04
(g/100g BW)	2.75 ± 0.24	2.75 ± 0.18	2.56 ± 0.19	2.55 ± 0.25
Liver (g)	2.06 ± 0.19	2.08 ± 0.18	2.23 ± 0.20	2.10 ± 0.27
(g/100g BW)	3.82 ± 0.20	3.71 ± 0.15	3.81 ± 0.20	3.60 ± 0.16
Kidneys (g)	0.63 ± 0.07	0.64 ± 0.05	0.67 ± 0.05	0.67 ± 0.07
(g/100g BW)	1.17 ± 0.08	1.14 ± 0.06	1.15 ± 0.05	1.14 ± 0.06
Ovaries (mg)	16.68 ± 1.70	16.94 ± 1.89	17.01 ± 3.52	16.14 ± 2.22
(mg/100g BW)	30.94 ± 2.41	30.35 ± 4.36	29.30 ± 7.29	27.87 ± 4.08
PND 77				
Males				
No. of offspring examined	10	10	10	10
Brain (g)	2.04 ± 0.06	2.07 ± 0.06	2.00 ± 0.07	2.03 ± 0.06
(g/100g BW)	0.42 ± 0.03	0.42 ± 0.03	0.41 ± 0.04	0.42 ± 0.03
Liver (g)	18.09 ± 2.16	18.81 ± 2.85	18.11 ± 2.65	17.48 ± 2.14
(g/100g BW)	3.74 ± 0.28	3.77 ± 0.33	3.69 ± 0.26	3.59 ± 0.24
Kidneys (g)	3.08 ± 0.35	3.20 ± 0.38	3.18 ± 0.18	3.22 ± 0.29
(g/100g BW)	0.64 ± 0.05	0.64 ± 0.05	0.65 ± 0.05	0.66 ± 0.03
Testes (g)	3.46 ± 0.30	3.16 ± 0.21	3.28 ± 0.32	3.36 ± 0.27
(g/100g BW)	0.72 ± 0.08	0.64 ± 0.05	0.68 ± 0.09	0.69 ± 0.07
Females				
No. of offspring examined	10	10	10	10
Brain (g)	1.90 ± 0.05	1.90 ± 0.06	1.84 ± 0.05	1.87 ± 0.08
(g/100g BW)	0.67 ± 0.07	0.66 ± 0.07	0.66 ± 0.07	0.65 ± 0.04
Liver (g)	9.70 ± 1.43	9.67 ± 1.41	9.25 ± 1.52	9.66 ± 0.55
(g/100g BW)	3.38 ± 0.27	3.33 ± 0.27	3.28 ± 0.22	3.38 ± 0.15
Kidneys (g)	1.90 ± 0.14	1.88 ± 0.25	1.81 ± 0.14	1.91 ± 0.13
(g/100g BW)	0.67 ± 0.05	0.65 ± 0.05	0.65 ± 0.07	0.67 ± 0.05
Ovaries (mg)	84.18 ± 11.37	81.06 ± 12.56	88.98 ± 17.50	93.70 ± 8.69
(mg/100g BW)	29.46 ± 3.16	28.06 ± 3.42	31.58 ± 3.91	32.83 ± 3.01

^aMean±SD.

Abbreviations: BW, body weight; PND, postnatal day.

Table 16. Manganese concentrations in the cerebellum of offspring and dams exposed to $MnCl_2 \cdot 4H_2O$ from GD 10 to PND 21 in rats.

	MnCl ₂ ·4H ₂ O in diet			
	Control	32 ppm	160 ppm	800 ppm
Dams				
No. of dams examined	8	8	8	8
manganese concentration (µg Mn/g tissue)	0.468 ± 0.023 ^a	0.432 ± 0.045	0.418 ± 0.014	0.433 ± 0.045
Offspring				
PND 21				
No. of offspring examined	6	6	6	6
manganese concentration (µg Mn/g tissue)	0.488 ± 0.022	0.553 ± 0.020	0.641 ± 0.092*	0.610 ± 0.128*
PND 77				
No. of offspring examined	6	6	6	6
manganese concentration (µg Mn/g tissue)	0.350 ± 0.014	0.387 ± 0.048	0.400 ± 0.033	0.440 ± 0.043

^aMean±SD.

* Significantly different from the control group (**P*<0.05).

Abbreviations: PND, postnatal day.

Table 17. Quantitative analysis of Tbr2-, DCX- or GFAP-immunoreactive cells in the subgranular zone of the dentate gyrus of the male offspring at PND 21 and 77 exposed to $MnCl_2 \cdot 4H_2O$ during the 2nd half of gestation and lactation periods in rats.

	MnCl ₂ ·4H ₂ O in the diet			
	Control	32 ppm	160 ppm	800 ppm
PND 21				
No. of offspring examined ^a	10	10	10	10
Tbr2 (+) cell count (/mm)	7.3 ± 3.1 ^b	7.9 ± 3.0	8.2 ± 2.3	8.4 ± 1.8
DCX (+) cell count (/mm)	129.9 ± 21.7	142.7 ± 32.0	156.0 ± 19.6	162.2 ± 23.0*
GFAP(+) cell count (/mm)	2.5 ± 1.2	2.5 ± 0.7	2.6 ± 0.8	2.3 ± 0.9
PND 77				
No. of offspring examined ^a	10	10	10	10
Tbr2 (+) cell count (/mm)	3.6 ± 1.6	4.0 ± 1.7	3.8 ± 1.6	3.3 ± 1.4
DCX (+) cell count (/mm)	17.8 ± 4.5	18.6 ± 3.9	20.7 ± 4.9	16.4 ± 3.4
GFAP(+) cell count (/mm)	3.8 ± 1.4	4.1 ± 1.5	4.0 ± 1.4	4.0 ± 1.4

* Significantly different from the control group by Dunnett's test (**P*<0.05).

^aAll identical 10 male offspring from 8 dams (one or two animals per dam) were subjected to immunohistochemical analyses in each group. Statistical analysis was performed using the litter as the experimental unit, and litter mean values were subjected to analysis on two offspring samples from the same dam.

^bMean±SD.

Table 18. Quantitative analysis of proliferating cells as detected by nuclear immunoreactivity of PCNA and apoptotic cells as detected by TUNEL method in the subgranular zone of the dentate gyrus of the male offspring at PND 21 and 77 exposed to $MnCl_2 \cdot 4H_2O$ during the 2nd half of gestation and lactation periods in rats.

	MnCl ₂ ·4H ₂ O in the diet			
	Control	32 ppm	160 ppm	800 ppm
PND 21				
No. of offspring examined ^a	10	10	10	10
PCNA (+) cell count (/mm)	3.1 ± 0.8 ^b	3.2 ± 1.4	3.2 ± 1.3	3.0 ± 1.0
Apoptotic cell count (/mm)	0.65 ± 0.62	0.43 ± 0.67	0.83 ± 1.10	0.79 ± 0.86
PND 77				
No. of offspring examined ^a	10	10	10	10
PCNA (+) cell count (/mm)	1.1 ± 0.5	1.4 ± 1.0	1.3 ± 0.8	1.4 ± 0.9
Apoptotic cell count (/mm)	0.06 ± 0.13	0.10 ± 0.22	0.06 ± 0.13	0.03 ± 0.10

* Significantly different from the control group by Dunnett's test (**P*<0.05).

^aAll identical 10 male offspring from 8 dams (one or two animals per dam) were subjected to immunohistochemical analyses in each group. Statistical analysis was performed using the litter as the experimental unit, and litter mean values were subjected to analysis on two offspring samples from the same dam.

^bMean±SD.

Table 19. Quantitative analysis of reelin-, GAD67-, NeuN-, Iba1- or Cox2-immunoreactive cells in the hilus of the dentate gyrus of the male offspring at PND 21 and 77 exposed to MnCl₂4H₂O during the 2nd half of gestation and lactation periods in rats.

	MnCl ₂ 4H ₂ O in the diet			
	Control	32 ppm	160 ppm	800 ppm
PND 21				
No. of offspring examined ^a	10	10	10	10
Reelin (+) cell count (/mm ²)	68.7 ± 26.8 ^b	83.4 ± 12.5	88.8 ± 19.3	92.7 ± 12.9*
GAD67 (+) cell count (/mm ²)	32.0 ± 7.7	39.5 ± 11.1	39.4 ± 15.5	34.5 ± 10.1
NeuN (+) cell count (/mm ²)	314.5 ± 41.4	332.0 ± 35.3	322.7 ± 43.6	291.2 ± 53.8
Iba1(+) cell count (/mm ²)	52.6 ± 17.7	84.9 ± 19.2*	72.8 ± 13.6*	81.8 ± 19.4*
Cox2(+) cell count (/mm ²)	43.8 ± 9.5	56.4 ± 12.0	59.2 ± 15.5*	54.7 ± 15.0
PND 77				
No. of offspring examined ^a	10	10	10	10
Reelin (+) cell count (/mm ²)	24.7 ± 5.6	26.0 ± 9.9	20.3 ± 4.6	23.6 ± 6.2
GAD67 (+) cell count (/mm ²)	12.1 ± 5.6	12.2 ± 5.4	9.5 ± 4.9	12.1 ± 7.5
NeuN (+) cell count (/mm ²)	192.9 ± 30.3	201.2 ± 46.3	214.6 ± 28.2	218.6 ± 22.8
Iba1(+) cell count (/mm ²)	64.2 ± 15.5	62.4 ± 15.3	60.5 ± 21.3	60.2 ± 14.0
Cox2 (+) cell count (/mm ²)	43.1 ± 13.8	47.2 ± 12.4	53.6 ± 15.9	44.8 ± 11.2

* Significantly different from the control group by Dunnett's test (**P*<0.05).

^aAll identical 10 male offspring from 8 dams (one or two animals per dam) were subjected to immunohistochemical analyses in each group. Statistical analysis was performed using the litter as the experimental unit, and litter mean values were subjected to analysis on two offspring samples from the same dam.

^bMean±SD.

Table 20. Real-time RT-PCR analysis in the hippocampus of male offspring at PND 21 exposed to MnCl₂4H₂O during the 2nd half of gestation and lactation periods in rats.

	MnCl ₂ 4H ₂ O in the diet			
	Control	32 ppm	160 ppm	800 ppm
No. of offspring examined ^a	6	6	6	5
<i>Cox2</i>	1.02 ± 0.20 ^b	1.23 ± 0.18	1.16 ± 0.15	0.85 ± 0.23
<i>Il1a</i>	1.02 ± 0.18	0.93 ± 0.25	1.58 ± 0.49	2.41 ± 1.37**
<i>Il1β</i>	1.01 ± 0.14	1.01 ± 0.18	1.01 ± 0.10	1.10 ± 0.17
<i>Il6</i>	1.01 ± 0.16	0.87 ± 0.24	1.41 ± 0.39	2.31 ± 1.17**
<i>Nos2</i>	1.07 ± 0.49	0.93 ± 0.30	1.35 ± 0.55	2.60 ± 1.81*
<i>Tnfa</i>	1.05 ± 0.36	0.82 ± 0.33	1.42 ± 0.26	2.06 ± 1.04*
<i>DCX</i>	1.02 ± 0.25	1.06 ± 0.32	0.93 ± 0.11	0.87 ± 0.14
<i>NeuroD1</i>	1.00 ± 0.10	1.03 ± 0.18	1.08 ± 0.13	1.23 ± 0.26
<i>Pax6</i>	1.03 ± 0.28	0.76 ± 0.12	1.05 ± 0.26	1.24 ± 0.41
<i>TUC4</i>	1.01 ± 0.18	0.98 ± 0.17	0.88 ± 0.09	0.86 ± 0.13
<i>Reelin</i>	1.03 ± 0.29 ^b	1.04 ± 0.12	0.91 ± 0.21	0.86 ± 0.17
<i>VLDLR</i>	1.02 ± 0.26	1.12 ± 0.25	0.88 ± 0.18	0.83 ± 0.07
<i>ApoER2</i>	1.03 ± 0.30	0.99 ± 0.15	0.93 ± 0.15	0.88 ± 0.14
<i>Dabl</i>	1.02 ± 0.21	0.96 ± 0.12	1.03 ± 0.36	0.96 ± 0.28

*** Significantly different from the control group by Dunnett's test or Steel's test (**P*<0.05, ***P*<0.01).

Abbreviations: *Cox*, cyclooxygenase; *Il*, interleukin; *Nos2*, nitric oxide synthase 2, inducible; *Tnf*, tumor necrosis factor; *DCX*, doublecortin; *NeuroD1*, neurogenic differentiation 1; *Pax6*, paired box 6; *TUC4*, dihydropyrimidinase-like 3; *VLDLR*, very low density lipoprotein receptor; *ApoER2*, low density lipoprotein receptor-related protein 8; *Dabl*, disabled homolog 1.

^aOne male offspring per dam (n = 5 or 6/group) was subjected to analysis.

^bMean±SD.

Table 21. Serum levels of thyroid-related hormones of male offspring and dams exposed to MnCl₂ · 4H₂O during the 2nd half of gestation and lactation periods in rats.

	MnCl ₂ 4H ₂ O in the diet		
	Control	800 ppm	1600 ppm
Dams			
No. of dams examined	6	6	6
T ₃ (ng/mL)	1.02 ± 0.06 ^b	0.96 ± 0.11	1.07 ± 0.15
T ₄ (µg/dL)	5.97 ± 1.96	5.87 ± 0.79	5.55 ± 0.88
TSH (ng/mL)	2.79 ± 1.80	3.84 ± 1.51	2.83 ± 1.88
Offspring			
PND 21			
No. of offspring examined ^a	10	10	10
T ₃ (ng/mL)	1.47 ± 0.16	1.27 ± 0.14*	1.27 ± 0.20*
T ₄ (µg/dL)	6.35 ± 0.61	5.44 ± 0.96*	4.77 ± 0.73**
TSH (ng/mL)	2.46 ± 0.93	4.29 ± 2.17*	2.92 ± 1.23
PND 77			
No. of offspring examined ^a	10	10	10
T ₃ (ng/mL)	1.12 ± 0.21	1.04 ± 0.12	1.11 ± 0.12
T ₄ (µg/dL)	6.19 ± 1.27	5.64 ± 1.44	6.03 ± 1.09
TSH (ng/mL)	5.02 ± 2.55	4.11 ± 1.83	2.83 ± 1.62

* ** Significantly different from the control group by Dunnett's test or Steel's test (**P*<0.05, ***P*<0.01).

Abbreviations: PND, postnatal day; T₃, triiodothyronin; T₄, thyroxin; TSH, thyroid-stimulating hormone

^a One male offspring of each sex per dam (n = 6/group) were subjected to measurement at each time point.

^b Mean±SD.

Table 22. Real-time RT-PCR analysis in the hippocampus of offspring at PND 21 exposed to MnCl₂ · 4H₂O during the 2nd half of gestation and lactation periods in mice.

	0 ppm MnCl ₂ 4H ₂ O		800 ppm MnCl ₂ 4H ₂ O	
	Relative transcript level normalized by		Relative transcript level normalized by	
	<i>Hprt</i>	<i>Gapdh</i>	<i>Hprt</i>	<i>Gapdh</i>
No. of offspring examined ^a	3	3	3	3
<i>Reelin</i>	1.02 ± 0.23 ^b	1.23 ± 0.75	2.76 ± 0.35**	4.09 ± 0.97**
<i>ApoER2</i>	1.01 ± 0.14	1.08 ± 0.47	1.63 ± 0.29**	2.41 ± 0.39**
<i>VLDLR</i>	1.00 ± 0.05	1.10 ± 0.52	0.93 ± 0.09	1.38 ± 0.10
<i>GAD67</i>	1.00 ± 0.04	1.13 ± 0.58	1.86 ± 0.06**	2.76 ± 0.45**
<i>Pax6</i>	1.05 ± 0.40	1.03 ± 0.26	1.13 ± 0.40	1.66 ± 0.54
<i>Tbr2</i>	1.06 ± 0.47	1.02 ± 0.21	1.22 ± 0.48	1.80 ± 0.66
<i>TUC4</i>	1.00 ± 0.06	1.12 ± 0.06	0.47 ± 0.04**	0.69 ± 0.03**

* ** Significantly different from the control group by Student's or Aspin-Welch's *t*-test (***P*<0.01).

Real-time PCR analysis of *Hprt* and *Gapdh* was performed in the analysis of each target gene.

Abbreviations: *Hprt*, Hypoxanthine-guanine phosphoribosyltransferase; *Gapdh*, Glyceraldehyde 3-phosphate dehydrogenase; *ApoER2*, Apolipoprotein E receptor 2; *Vldlr*, very-low-density. lipoprotein receptor; *Pax6*, paired box 6; *Tbr2*, t box brain 2; *TUC4*, dihydropyrimidinase-like 3.

^a One male offspring per dam (n = 3/group) was subjected to analysis.

^b Mean±SD.

Table 23. Hypermethylated genes, CpG island located in the transcription site, subjected to methylation-specific qPCR analysis in the hippocampus of offspring at PND 21 exposed to MnCl₂ · 4H₂O during the 2nd half of gestation and lactation periods in mice.

Probe No	Genome location	Feature	Description	Accession No.
CHR15FS078031394	Chr 15: 78031394	Pvalb	parvalbumin	MGI:97821
CHR05FS137798762	Chr 05: 137798762	Actl6b	actin-like 6B	MGI:1933548
CHR15FS102818276	Chr 15: 102818276	Hoxc8	homeo box C8	MGI:96198
CHRXFS165333432	Chr X: 165333432	Mid1	midline 1	MGI:1100537
CHR07FS024715229	Chr 7: 24715229	Atpla3	ATPase, NA+/K+ transportation, alpha 3 polypeptide	MGI:88107
CHR16FS064793409	Chr 16: 64793409	Cggbp1	CGG triplet repeat binding protein 1	MGI:2146370
CHR13FS078662694	Chr 13: 78662694	Nr2f1	nuclear receptor subfamily 2, group F, member 1	MGI:135245

Table 24. Serum levels of thyroid-related hormones of male offspring at PND 21 to MnCl₂ · 4H₂O during the 2nd half of gestation and lactation periods in mice.

	MnCl ₂ ·4H ₂ O in the diet	
	Control	800 ppm
No. of pups examined ^a	40 (12)	40 (12)
T ₃ (ng/dL)	53.7 ± 7.0 ^b	48.9 ± 6.8
T ₄ (µg/dL)	3.62 ± 0.70	3.16 ± 0.48*
TSH (ng/dL)	0.011 ± 0.006	0.013 ± 0.004

* ** Significantly different from the control group by Student's or Aspin-Welch's *t*-test (**P*<0.05).

Abbreviations: PND, postnatal day; T₃, triiodothyronin; T₄, thyroxin; TSH, thyroid-stimulating hormone

^a Number in parenthesis represents that of pooled samples: 3-4 animal sera/sample.

^b Mean±SD.

Table 25. Body weight of dams exposed to chlorpyrifos during the 2nd half of gestation and lactation periods in rats.

	Chlorpyrifos in the diet			
	Control	2.8 ppm	14 ppm	70 ppm
No. of dam examined	8	8	8	8
Body weight (g)				
GD 10	283.4 ± 24.3 ^a	289.4 ± 17.2	290.1 ± 17.0	289.6 ± 14.2
GD 11	291.5 ± 25.4	298.3 ± 17.0	298.4 ± 15.8	296.5 ± 12.9
GD 12	297.3 ± 24.3	305.3 ± 16.9	306.4 ± 17.5	303.1 ± 14.4
GD 13	301.3 ± 23.7	310.4 ± 17.2	311.4 ± 18.2	308.5 ± 15.2
GD 14	307.0 ± 23.8	315.6 ± 17.0	317.9 ± 18.1	316.3 ± 14.5
GD 15	314.5 ± 27.4	324.4 ± 19.2	326.9 ± 17.6	325.9 ± 16.0
GD 16	324.0 ± 26.8	335.0 ± 16.9	336.5 ± 16.7	335.3 ± 14.5
GD 17	337.9 ± 29.6	347.9 ± 18.2	349.9 ± 18.5	347.3 ± 15.3
GD 18	350.0 ± 29.2	361.9 ± 18.2	365.4 ± 16.5	360.9 ± 16.7
GD 19	368.1 ± 29.2	377.6 ± 17.7	382.6 ± 16.8	379.3 ± 16.7
GD 20	383.4 ± 33.8	392.8 ± 18.8	401.3 ± 17.8	393.5 ± 17.5
PND 0	291.8 ± 21.9	312.9 ± 25.2	309.3 ± 17.7	300.3 ± 14.2
PND 4	311.5 ± 26.0	330.6 ± 21.8	325.4 ± 17.1	324.3 ± 15.1
PND 7	319.8 ± 20.9	328.6 ± 22.0	330.4 ± 16.8	322.5 ± 8.3
PND 11	303.6 ± 13.9	327.9 ± 26.7*	325.4 ± 9.2*	326.0 ± 13.2*
PND 14	317.4 ± 18.8	328.6 ± 23.6	322.6 ± 8.2	315.9 ± 12.7
PND 17	322.0 ± 22.0	326.0 ± 22.8	331.8 ± 12.9	316.3 ± 18.5
PND 21	308.0 ± 24.0	315.5 ± 17.3	312.6 ± 10.7	303.9 ± 17.8

* Significantly different from the control group (**P*<0.05).

^aMean±SD.

Abbreviations: GD, gestation day; PND, postnatal day.

Table 26. Food consumption of dams exposed to chlorpyrifos during the 2nd half of gestation and lactation periods in rats.

	Chlorpyrifos in the diet			
	Control	2.8 ppm	14 ppm	70 ppm
No. of dam examined	8	8	8	8
Food consumption (g/animal/day)				
GD 10	19.6±2.6 ^a	20.3 ± 2.6	20.0 ± 2.9	20.0 ± 1.3
GD 14	22.8±1.6	23.9 ± 1.6	24.6 ± 1.4*	24.1 ± 1.1
GD 17	23.4±1.3	24.6 ± 1.6	25.4 ± 1.6*	25.4 ± 1.8*
GD 20	24.5±1.1	24.9 ± 1.8	26.4 ± 1.3*	25.6 ± 1.4
PND 4	34.5±4.9	35.8 ± 2.4	36.9 ± 2.4	37.8 ± 2.8
PND 7	41.3±2.7	40.6 ± 2.6	43.3 ± 2.3	41.6 ± 1.9
PND 11	45.4±1.5	47.8 ± 2.9	48.4 ± 3.4	47.9 ± 3.1
PND 14	53.4±4.6	54.5 ± 4.0	54.5 ± 3.2	52.1 ± 2.7
PND 17	58.1±3.8	57.1 ± 5.3	59.6 ± 4.7	55.1 ± 4.7
PND 21	68.3±3.0	69.0 ± 3.5	71.4 ± 3.9	68.0 ± 4.5
Food consumption (g/kg body weight/day)				
GD 10	69.4±8.7	74.0 ± 13.7	73.0 ± 15.2	69.0 ± 2.8
GD 14	74.3±5.0	75.7 ± 4.0	77.5 ± 1.5	76.3 ± 1.7
GD 17	69.4±3.8	70.8 ± 3.9	72.6 ± 3.7	73.0 ± 2.8
GD 20	64.2±4.0	63.4 ± 3.7	65.8 ± 4.0	65.2 ± 3.2
PND 4	110.7±12.2	108.8 ± 13.1	113.6 ± 9.8	116.7 ± 11.1
PND 7	129.2±8.4	124.4 ± 12.9	131.3 ± 10.4	129.2 ± 8.7
PND 11	149.7±9.2	146.5 ± 15.1	148.6 ± 9.1	147.1 ± 11.4
PND 14	168.3±12.0	167.2 ± 22.9	169.0 ± 10.2	165.4 ± 12.9
PND 17	180.7±7.3	176.1 ± 21.9	179.7 ± 12.1	174.4 ± 11.9
PND 21	222.6±17.8	219.6 ± 20.4	228.4 ± 12.5	224.6 ± 22.3

* Significantly different from the control group (**P*<0.05).

^aMean±SD.

Abbreviations: GD, gestation day; PND, postnatal day.

Table 27. Body weight of offspring exposed to chlorpyrifos during the 2nd half of gestation and lactation periods in rats.

	Chlorpyrifos in the diet			
	Control	2.8 ppm	14 ppm	70 ppm
Body weight (g)				
Males				
No. of offspring examined ^a	16	16	16	16
PND 0	6.6 ± 0.3 ^b	6.9 ± 0.5	6.9 ± 0.4	6.7 ± 0.2
PND 4	10.7 ± 1.1	10.9 ± 0.9	11.4 ± 0.5	10.9 ± 0.7
PND 7	17.2 ± 1.6	17.6 ± 1.8	18.8 ± 0.5*	18.0 ± 0.6
PND 11	26.2 ± 1.3	27.4 ± 3.0	29.1 ± 1.1**	27.4 ± 1.2
PND 14	33.2 ± 2.1	34.8 ± 3.1	36.6 ± 1.4**	34.3 ± 1.5
PND 17	41.0 ± 2.7	42.5 ± 3.5	44.3 ± 2.1*	41.1 ± 1.9
PND 21	57.8 ± 3.1	60.5 ± 4.3	63.1 ± 2.8*	57.2 ± 3.1
PND 28	100.6 ± 4.4	103.9 ± 5.1	107.1 ± 7.4	99.4 ± 4.8
PND 35	171.1 ± 8.7	174.5 ± 6.3	179.3 ± 11.5	168.3 ± 8.4
PND 42	245.1 ± 14.7	251.3 ± 9.9	256.4 ± 17.5	243.1 ± 12.2
PND 49	315.6 ± 18.6	321.9 ± 13.1	327.3 ± 23.5	314.9 ± 16.8
PND 56	375.4 ± 21.2	384.7 ± 19.5	391.4 ± 28.3	380.6 ± 19.1
PND 63	422.4 ± 21.6	435.1 ± 26.8	440.3 ± 28.8	429.3 ± 21.1
PND 70	464.5 ± 23.5	477.3 ± 31.3	485.0 ± 33.0	474.1 ± 23.2
PND 77	496.6 ± 25.9	514.7 ± 34.6	520.5 ± 33.6	508.3 ± 25.9
Females				
No. of offspring examined ^a	16	16	16	16
PND 0	6.2 ± 0.3	6.5 ± 0.7	6.6 ± 0.4	6.3 ± 0.2
PND 4	9.9 ± 1.2	10.4 ± 1.0	11.1 ± 0.8*	10.1 ± 0.9
PND 7	15.9 ± 1.8	16.8 ± 1.9	17.8 ± 1.2*	16.6 ± 0.9
PND 11	24.3 ± 2.0	26.1 ± 2.6	27.5 ± 1.8**	25.6 ± 1.5
PND 14	31.2 ± 2.9	33.3 ± 2.6	34.8 ± 2.0*	32.5 ± 1.7
PND 17	38.6 ± 3.1	40.4 ± 3.1	42.3 ± 2.5*	39.0 ± 2.4
PND 21	54.3 ± 4.0	57.1 ± 3.5	58.6 ± 3.6	54.2 ± 3.6
PND 28	88.0 ± 7.2	91.6 ± 3.3	95.3 ± 9.5	89.6 ± 5.0
PND 35	138.3 ± 8.9	141.8 ± 6.0	143.6 ± 11.9	142.8 ± 7.2
PND 42	176.6 ± 13.1	180.5 ± 7.6	183.4 ± 14.7	183.4 ± 6.6
PND 49	202.9 ± 13.7	208.9 ± 10.5	213.5 ± 15.3	215.4 ± 8.2
PND 56	226.4 ± 17.5	233.0 ± 14.3	239.3 ± 18.9	241.3 ± 10.0
PND 63	251.4 ± 20.3	255.8 ± 16.5	260.4 ± 22.5	265.8 ± 9.9
PND 70	268.9 ± 22.8	273.1 ± 16.8	281.4 ± 22.8	286.0 ± 10.1
PND 77	279.8 ± 23.8	287.3 ± 15.9	296.8 ± 22.6	300.6 ± 12.3

*** Significantly different from the control group by Dunnett's test or Steel's test (* $P < 0.05$, ** $P < 0.01$).

Abbreviations: PND, postnatal day.

^aIdentical two male and two female offspring per dam ($n = 8$ /group) were used for body weight measurement throughout the experiment. Statistical analysis was performed using the litter as the experimental unit.

^bMean ± SD.

Table 28. Food consumption of offspring exposed to chlorpyrifos during the 2nd half of gestation and lactation periods in rats

	Chlorpyrifos in the diet			
	Control	2.8 ppm	14 ppm	70 ppm
Males				
No. of offspring examined ^a	16	16	16	16
Food consumption (g/animal/day)				
PND 35	19.5±0.8 ^b	19.7 ± 1.1	20.3 ± 1.6	19.1 ± 1.3
PND 42	25.8±1.2	26.4 ± 1.3	27.1 ± 2.2	26.5 ± 2.1
PND 49	28.9±1.7	29.6 ± 1.8	30.3 ± 2.6	29.3 ± 2.1
PND 56	30.6±1.5	31.2 ± 2.3	31.9 ± 2.6	31.1 ± 1.8
PND 63	30.8±1.2	31.8 ± 2.4	32.6 ± 2.7	31.1 ± 2.2
PND 70	31.0±1.6	32.1 ± 2.4	32.6 ± 2.2	31.4 ± 1.8
PND 77	30.4±1.8	31.4 ± 2.5	32.3 ± 2.5	31.3 ± 1.6
Food consumption (g/kg body weight/day)				
PND 35	114.1±4.1	112.8 ± 3.8	113.2 ± 3.9	113.2 ± 4.3
PND 42	105.1±3.0	105.2 ± 3.2	105.5 ± 3.8	109.0 ± 6.4
PND 49	91.7±2.5	91.8 ± 3.4	92.4 ± 3.6	93.0 ± 2.1
PND 56	81.5±2.2	81.2 ± 7.0	81.6 ± 2.4	81.8 ± 1.9
PND 63	73.0±2.3	73.1 ± 2.5	74.0 ± 2.1	72.5 ± 3.0
PND 70	66.8±2.3	67.2 ± 1.7	67.3 ± 2.0	66.2 ± 1.5
PND 77	61.3±2.3	60.9 ± 2.2	61.9 ± 1.9	61.5 ± 1.6
Females				
No. of offspring examined ^a	16	16	16	16
Food consumption (g/animal/day)				
PND 35	16.3±0.7	16.4 ± 0.7	16.2 ± 1.9	16.8 ± 1.1
PND 42	19.3±1.3	19.5 ± 0.8	19.3 ± 1.6	20.1 ± 1.3
PND 49	19.4±1.1	19.9 ± 1.3	20.3 ± 2.0	21.3 ± 1.6
PND 56	20.2±1.4	21.1 ± 2.0	21.1 ± 2.0	21.5 ± 1.6
PND 63	20.5±1.9	21.2 ± 1.8	21.1 ± 2.5	21.4 ± 1.5
PND 70	21.1±1.6	21.4 ± 1.9	21.8 ± 1.9	22.1 ± 1.9
PND 77	19.6±1.6	20.5 ± 1.6	21.1 ± 1.6	21.1 ± 2.1
Food consumption (g/kg body weight/day)				
PND 35	117.7±3.1	116.0 ± 3.9	112.6 ± 5.7	117.8 ± 6.2
PND 42	109.5±4.1	108.1 ± 1.9	105.6 ± 8.7	109.8 ± 6.4
PND 49	95.9±3.1	95.4 ± 2.3	94.8 ± 5.3	98.6 ± 5.4
PND 56	89.3±4.7	90.5 ± 4.5	88.3 ± 4.2	89.1 ± 4.9
PND 63	81.5±2.3	82.7 ± 2.0	80.7 ± 3.7	80.6 ± 4.0
PND 70	78.4±2.8	78.2 ± 2.7	77.5 ± 2.8	77.3 ± 5.0
PND 77	70.2±2.4	71.3 ± 2.1	71.3 ± 3.4	70.2 ± 4.6

No significant difference in any treated group from the control group.

Abbreviations: PND, postnatal day.

^aIdentical two male and two female offspring per dam (n = 8/group) were used for food consumption measurement after weaning. Statistical analysis was performed using the litter as the experimental unit.

^bMean±SD.

Table 29. Organ weight of offspring exposed to chlorpyrifos during the 2nd half of gestation and lactation periods in rats

	Chlorpyrifos in the diet			
	Control	2.8 ppm	14 ppm	70 ppm
PND 21				
Males				
No. of offspring examined ^a	16	16	16	16
Brain (g)	1.50 ± 0.06 ^b	1.53 ± 0.06	1.53 ± 0.07	1.50 ± 0.06
(g/100g BW)	2.66 ± 0.12	2.60 ± 0.19	2.49 ± 0.18	2.70 ± 0.13
Liver (g)	2.14 ± 0.22	2.21 ± 0.25	2.29 ± 0.20	1.96 ± 0.21
(g/100g BW)	3.77 ± 0.21	3.72 ± 0.12	3.70 ± 0.21	3.50 ± 0.15*
Kidneys (g)	0.66 ± 0.03	0.64 ± 0.08	0.69 ± 0.04	0.65 ± 0.06
(g/100g BW)	1.14 ± 0.06	1.08 ± 0.03*	1.12 ± 0.03	1.14 ± 0.05
Testes (g)	0.23 ± 0.04	0.24 ± 0.04	0.24 ± 0.04	0.24 ± 0.05
(g/100g BW)	0.40 ± 0.07	0.41 ± 0.05	0.39 ± 0.06	0.42 ± 0.07
Salivary glands (g)	0.19 ± 0.01	0.20 ± 0.04	0.19 ± 0.01	0.19 ± 0.02
(g/100g BW)	0.33 ± 0.02	0.35 ± 0.04	0.31 ± 0.01	0.34 ± 0.02
Females				
No. of offspring examined ^a	16	16	16	16
Brain (g)	1.44 ± 0.05	1.44 ± 0.04	1.47 ± 0.04	1.44 ± 0.05
(g/100g BW)	2.76 ± 0.20	2.58 ± 0.21	2.60 ± 0.18	2.69 ± 0.18
Liver (g)	2.01 ± 0.19	2.08 ± 0.17	2.06 ± 0.20	1.87 ± 0.22
(g/100g BW)	3.81 ± 0.10	3.69 ± 0.07	3.64 ± 0.26	3.46 ± 0.19*
Kidneys (g)	0.62 ± 0.04	0.62 ± 0.05	0.65 ± 0.03	0.67 ± 0.06
(g/100g BW)	1.15 ± 0.05	1.13 ± 0.05	1.14 ± 0.04	1.20 ± 0.06
Ovaries (mg)	19.44 ± 4.85	17.91 ± 2.68	17.39 ± 1.87	15.11 ± 3.53*
(mg/100g BW)	36.20 ± 8.02	32.65 ± 5.34	30.23 ± 2.32	26.98 ± 5.49*
Salivary glands (g)	0.18 ± 0.01	0.18 ± 0.02	0.19 ± 0.01	0.19 ± 0.02
(g/100g BW)	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.34 ± 0.02
PND 77				
Males				
No. of offspring examined ^a	16	16	16	16
Brain (g)	2.02 ± 0.04	2.11 ± 0.06	2.05 ± 0.11	2.03 ± 0.09
(g/100g BW)	0.41 ± 0.02	0.41 ± 0.03	0.39 ± 0.01	0.40 ± 0.02
Liver (g)	17.63 ± 1.61	17.99 ± 2.05	19.12 ± 2.19	18.22 ± 2.41
(g/100g BW)	3.54 ± 0.20	3.49 ± 0.26	3.66 ± 0.23	3.57 ± 0.33
Kidneys (g)	3.12 ± 0.21	3.19 ± 0.26	3.37 ± 0.20	3.39 ± 0.34
(g/100g BW)	0.63 ± 0.03	0.62 ± 0.05	0.65 ± 0.03	0.67 ± 0.05
Testes (g)	3.37 ± 0.26	3.54 ± 0.25	3.54 ± 0.29	3.67 ± 0.26
(g/100g BW)	0.68 ± 0.05	0.69 ± 0.06	0.68 ± 0.06	0.72 ± 0.06
Salivary glands (g)	0.68 ± 0.06	0.73 ± 0.03	0.74 ± 0.08	0.70 ± 0.07
(g/100g BW)	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.02	0.14 ± 0.01
Females				
No. of offspring examined ^a	16	16	16	16
Brain (g)	1.88 ± 0.08	1.92 ± 0.04	1.92 ± 0.06	1.89 ± 0.04
(g/100g BW)	0.68 ± 0.04	0.67 ± 0.03	0.65 ± 0.05	0.63 ± 0.02*
Liver (g)	9.32 ± 1.05	9.49 ± 0.78	9.95 ± 1.06	10.13 ± 0.83
(g/100g BW)	3.32 ± 0.14	3.29 ± 0.13	3.35 ± 0.15	3.37 ± 0.20
Kidneys (g)	1.83 ± 0.17	1.90 ± 0.13	2.01 ± 0.15	2.04 ± 0.18*
(g/100g BW)	0.66 ± 0.02	0.66 ± 0.03	0.68 ± 0.05	0.68 ± 0.04
Ovaries (mg)	100.71 ± 17.41	92.26 ± 11.07	99.42 ± 12.40	100.99 ± 9.97
(mg/100g BW)	36.05 ± 5.56	32.21 ± 3.68	33.58 ± 4.00	33.56 ± 2.62
Salivary glands (g)	0.43 ± 0.04	0.47 ± 0.02	0.46 ± 0.05	0.47 ± 0.05
(g/100g BW)	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.02	0.16 ± 0.01

* Significantly different from the control group by Dunnett's test or Steel's test (* $P < 0.05$).

Abbreviations: BW, body weight; PND, postnatal day.

^a Two offspring of each sex per dam (n = 8/group) were subjected to autopsy and following organ weight measurement at each time point. Statistical analysis was performed using the litter as the experimental unit, and mean values were estimated as a litter value when two offspring were examined from the same dam.

^b Mean ± SD.

Table 30. Cholinesterase (ChE) activity male offspring and dams exposed to chlorpyrifos during the 2nd half of gestation and lactation periods in rats

	Chlorpyrifos in the diet			
	Control	2.8 ppm	14 ppm	70 ppm
Dams				
No. of dams examined	8	8	8	8
plasma (IU/L)	826 ± 188 ^b	650 ± 103*	345 ± 50**	194 ± 34**
RBC (IU/L)	2475 ± 221	2175 ± 259*	848 ± 129**	540 ± 108**
frontal cortex (IU/g tissue)	19.2 ± 1.6	20.0 ± 1.3	18.4 ± 1.5	3.2 ± 0.7**
Offspring				
PND 21				
No. of offspring examined ^a	6	6	6	6
plasma (IU/L)	582 ± 104	624 ± 60	426 ± 73**	198 ± 28**
RBC (IU/L)	3311 ± 469	3139 ± 150	3011 ± 339	1184 ± 248**
frontal cortex (IU/g tissue)	13.4 ± 0.9	14.0 ± 0.5	12.8 ± 0.6	6.9 ± 2.1**
PND 77				
No. of offspring examined ^a	6	6	6	6
plasma (IU/L)	417 ± 121	484 ± 111	344 ± 54	414 ± 74
RBC (IU/L)	2553 ± 97	2635 ± 128	2558 ± 180	2584 ± 187
frontal cortex (IU/g tissue)	18.6 ± 2.2	17.3 ± 2.9	19.7 ± 0.9	18.5 ± 2.3

*** Significantly different from the control group by Dunnett's test or Steel's test (* $P < 0.05$, ** $P < 0.01$).

Abbreviations: ChE, cholinesterase; PND, postnatal day; RBC, red blood cell

^aOne male offspring per dam (n = 6/group) was subjected to measurement.

^bMean ± SD.

Table 31. Plasma levels of thyroid-related hormones of male offspring and dams exposed to chlorpyrifos during the 2nd half of gestation and lactation periods in rats

	Chlorpyrifos in the diet			
	Control	2.8 ppm	14 ppm	70 ppm
Dams				
No. of dams examined	8	8	8	8
T ₃ (ng/mL)	0.52 ± 0.23 ^b	0.64 ± 0.09	0.72 ± 0.16*	0.64 ± 0.11
T ₄ (µg/dL)	4.88 ± 0.87	5.04 ± 1.68	4.31 ± 0.78	3.96 ± 0.82
TSH (ng/mL)	4.95 ± 3.16	4.57 ± 0.89	2.74 ± 1.13	4.40 ± 1.80
Offspring				
PND 21				
No. of offspring examined ^a	6	6	6	6
T ₃ (ng/mL)	0.91 ± 0.16	0.87 ± 0.17	0.77 ± 0.11	1.04 ± 0.17
T ₄ (µg/dL)	5.42 ± 1.47	5.37 ± 1.00	4.32 ± 0.58	4.82 ± 0.58
TSH (ng/mL)	2.87 ± 0.56	2.53 ± 1.05	2.62 ± 0.89	2.81 ± 0.91
PND 77				
No. of offspring examined ^a	6	6	6	6
T ₃ (ng/mL)	0.72 ± 0.20	0.73 ± 0.12	0.78 ± 0.15	0.87 ± 0.14
T ₄ (µg/dL)	6.27 ± 1.32	5.73 ± 1.39	5.88 ± 0.84	6.38 ± 0.94
TSH (ng/mL)	4.08 ± 1.42	5.24 ± 1.55	7.80 ± 3.58	11.24 ± 9.68

Significantly different from the control group by Dunnett's test or Steel's test ($P < 0.01$).

Abbreviations: ChE, cholinesterase; PND, postnatal day; RBC, red blood cell

^aOne male offspring per dam (n = 6/group) was subjected to measurement.

^bMean ± SD.