によって児動物の体重抑制を生じることから (Wise et al., Neurotoxicol Teratol. 1995)、実験 1 で出生直後に認められた児動物の体重抑制には、胎児期に投与された ACR の発達毒性作用が関与した可能性が考えられた。実験 2 では、逆に生後 2 日目の児動物体重が一部で高値を示したが、これは一腹当たりの産児数や妊娠期間の偏りに起因し、毒性学的意義は乏しいと考えられた。授乳期およびその後の体重低値と発達遅延には、母動物の神経症状進行に伴う一般状態悪化が大きく影響していると推測された。

精巣では、飲水投与群および腹腔内投与群ともに精細管上皮の発達遅延がみられたが、両群とも性細胞に対する障害作用は確認されなかった。飲水投与群では、神経毒性と同様に、乳汁を介した ACR 曝露量が精巣毒性を生じるのに不十分であった可能性が高い。腹腔内投与群については、成熟動物で同じプロトコールの報告がないため一概に比較はできないが、成熟雄ラットに ACR 60 mg/kg/day を 5 日間強制経口投与した場合に、多核巨細胞の出現や性細胞の消失等が報告されている (Yang et al., Reprod Toxicol., 2005)。ACR の精巣毒性には、減数分裂後の精上皮細胞に対する DNA 付加体形成が関与すると考えられており、精子形成が始まる以前では ACR に対して感受性を示さない可能性が考えられた。一方、発育期曝露では、精子細胞を主体とした障害が顕著であった。成熟動物に比べ ACR 摂取量に対して著しく重度で多様な病変が観察されたことから、性成熟前後の動物は成熟期と比べて ACR の精巣毒性に高い感受性を持つことが示唆された。ACR はキネシン等のモーター蛋白を障害することが知られており、これが精子細胞の障害に関与した可能性が考えられる。しかし、精子形成開始以前の精巣における感受性の有無や、発育期と成熟期における感受性差については、さらに検討が必要である。

E. 結論

ACR の神経毒性作用に対して、離乳前児動物は感受性を有するが、母動物に対する飲水投与では経乳暴露の程度が低く、児動物に神経障害を生じるには不十分であることが示された。また、発育期と成熟期の感受性差は乏しく、その障害の程度は体重当たりの ACR 摂取量に相関するものと考えられた。精巣毒性に対する感受性は、離乳前では低く発育期では高い可能性が示唆された。

F. 研究発表

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G. 知的所有権の取得状況

特になし。

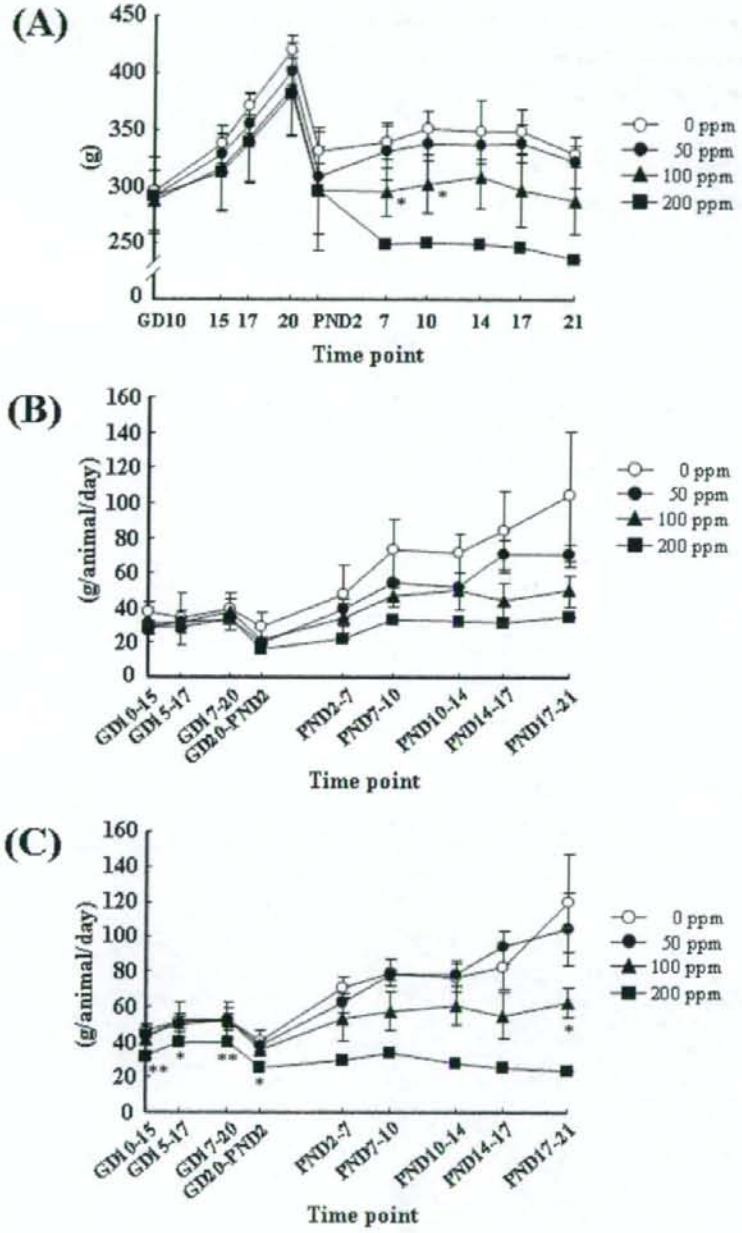

Fig. 1 Time course of body weight (A), food consumption (B) and water consumption (C) of dams given ACR in the drinking water for the gestation and lactation periods (Experiment 1).
Data are mean ± SD. n=3 (0, 50 and 100 ppm group), n=3 or 2 (200 ppm group).
*, **: $p<0.05$ and $p<0.01$ vs. 0 ppm. Abbreviation: GD, gestation day; PND, postnatal day.

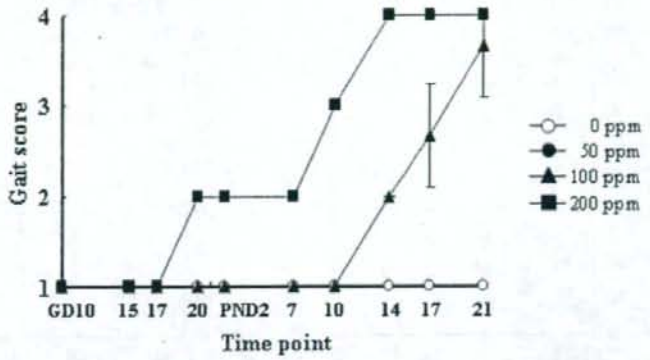

Fig. 2 Scores for gait abnormalities of dams given ACR in the drinking water for the gestation and lactation periods (Experiment 1).
Data are mean ± SD. n=3 (0, 50 and 100 ppm group), n=3 or 2 (200 ppm group).
Abbreviation: GD, gestation day; PND, postnatal day.

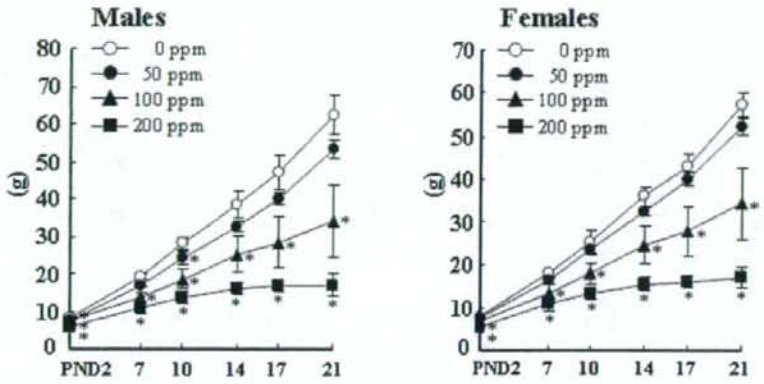

Fig. 3 Body weight changes of offspring before weaning (Experiment 1).
Data are mean ± SD. n=6 (0, 50 and 100 ppm group), n=4 (200 ppm group).
*: $p<0.01$ vs. 0 ppm group.
Abbreviation: PND, postnatal day.

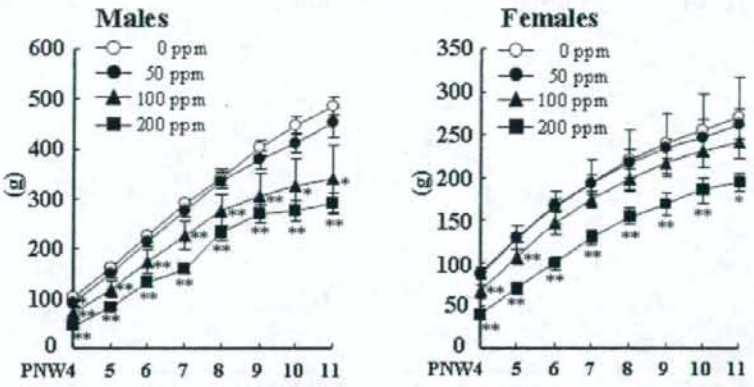

Fig. 4 Postweaning body weight changes of offspring (Experiment 1).
Data are mean ± SD. n=6 (0, 50 and 100 ppm group), n=4 or 3 (200 ppm group).
*, **: $p<0.05$, $p< 0.01$ vs. 0 ppm group. Abbreviation: PNW, postnatal week.

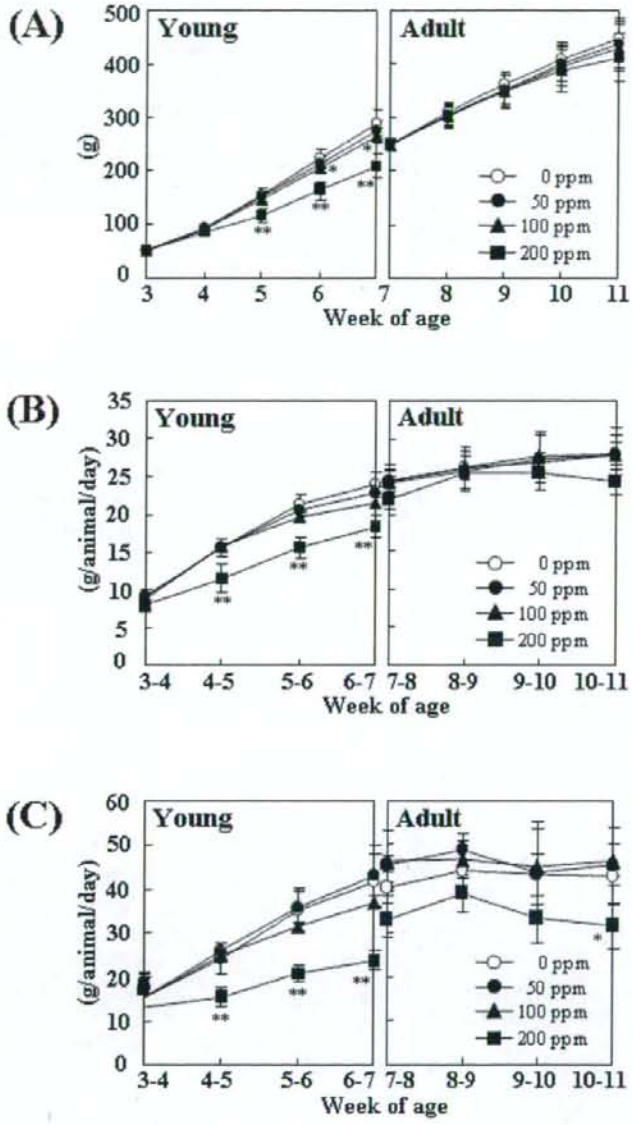

Fig. 5 Time course of body weight (A), food consumption (B) and water consumption (C) in young and adult rats given ACR in the drinking water for 4 weeks (Experiment 3). Data are mean ± SD. n=10. *, **: $p<0.05$ and $p<0.01$ vs. 0 ppm.

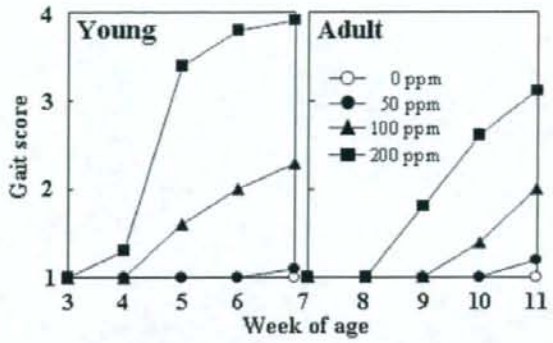

Fig. 6 Scores for gait abnormalities of young and adult rats given ACR in the drinking water for 4 weeks (Experiment 3). n=10.

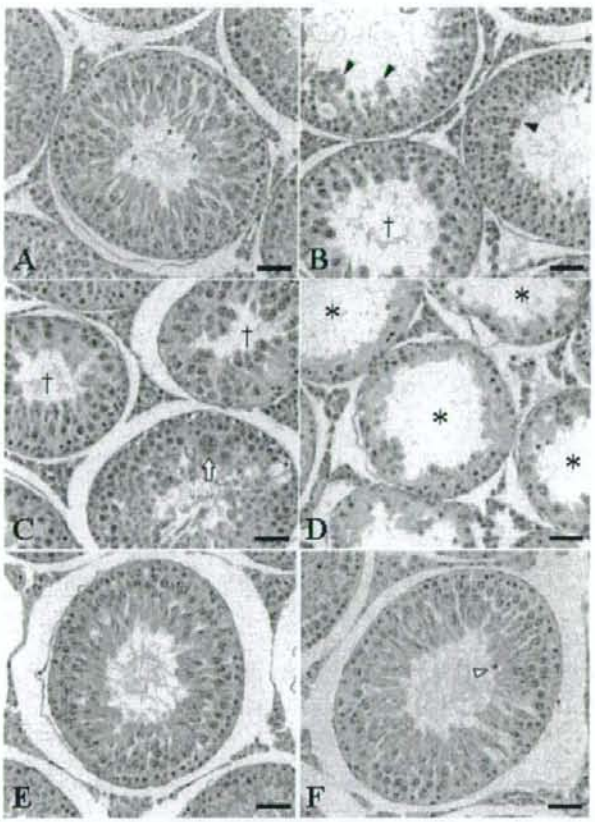

Fig. 7 Histopathology of the testis of young and adult rats given ACR at 0 or 200 ppm for 4 weeks (Experiment 3). (A) Normal seminiferous tubules of a young rat from 0 ppm group. (B-D) Degeneration of spermatids (black arrowheads), loss or decreased of elongated spermatid (†) and multinucleated giant cells (arrow) are apparent in a young rat at 200 ppm. In severely affected cases, many seminiferous tubules showed marked germ cell depletion (*). (E) Normal seminiferous tubules of an adult rat from 0 ppm group. (F) In adults, only small number of exfoliated germ cells (white arrowhead) was found in lumen of tubules at 200 ppm. Hematoxylin and eosin All bars=50 μm.

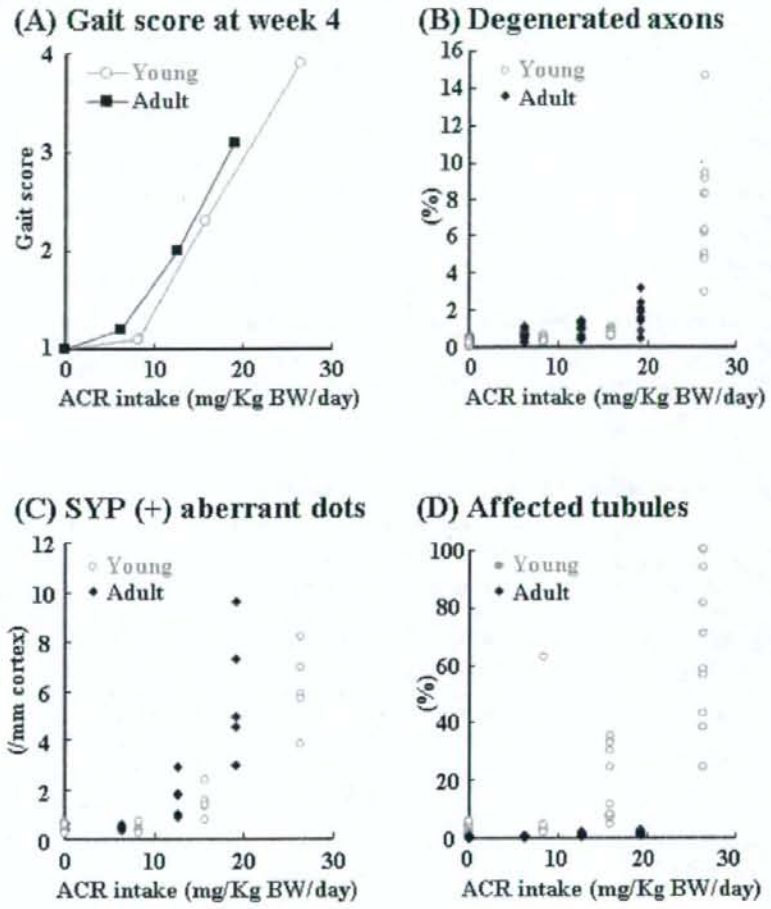

Fig. 8 Relationship between ACR intake per body weight and changes in neurotoxicity and testicular toxicity parameters in young and adult rats (Experiment 3).

Table 1 Reproductive data (Experiment 1).

		ACR in the drinking water (ppm)			
		0	50	100	200
No. of animals examined		3	3	3	3
Gestational length	(days)	22.0 ± 0.0 [a]	22.0 ± 0.0	21.3 ± 0.6	21.7 ± 0.6
No. of implantations/dam		13.3 ± 0.6	13.7 ± 1.2	13.7 ± 2.1	13.3 ± 2.1
Live birth ratio	(%)[b]	100	100	100	94
No. of live pups/litter[c]		12.7 ± 1.2	12.3 ± 2.5	11.3 ± 1.2	11.3 ± 3.8
No. of male pups[c]		8.7 ± 2.5	6.0 ± 4.6	5.0 ± 1.0	5.3 ± 2.3
No. of female pups[c]		4.0 ± 1.7	6.3 ± 3.2	6.3 ± 2.1	6.0 ± 3.6
Male pup ratio	(%)	67.9 ± 15.6	46.7 ± 28.5	45.0 ± 13.6	49.1 ± 17.9
Male pup weight[c]	(g)	8.27 ± 0.45	7.58 ± 0.66 *	7.27 ± 0.65 *	6.01 ± 0.39 *
Female pup weight[c]	(g)	7.97 ± 0.35	7.52 ± 0.43	6.67 ± 0.74 *	5.59 ± 0.47 *

[a]: Mean ± SD.

[b]: Live birth (%) = number of live pups delivered/total number of pups delivered×100.

[c]: Measured at PND 2.

*: $p<0.01$ vs. 0 ppm group.

Table 2 Body and organ weights of dams at weaning (Experiment 1).

		Acrylamide in the drinking water (ppm)			
		0	50	100	200
No. of animals examined		3	3	3	2
Body weight	(g)	323.6 ± 14.8 [a]	318.1 ± 23.9	284.3 ± 26.4	235.5
Brain	(g)	1.90 ± 0.08	2.04 ± 0.04	1.86 ± 0.09	1.89
	(g%)	0.59 ± 0.05	0.64 ± 0.05	0.66 ± 0.03	0.82
Liver	(g)	13.6 ± 0.7	13.95 ± 1.13	11.41 ± 1.69	8.67
	(g%)	4.20 ± 0.07	4.38 ± 0.06 *	4.01 ± 0.46	3.75
Spleen	(g)	0.62 ± 0.05	0.61 ± 0.06	0.51 ± 0.06	0.52
	(g%)	0.19 ± 0.02	0.19 ± 0.02	0.18 ± 0.00	0.22
Kidneys	(g)	2.28 ± 0.1	2.32 ± 0.18	2.10 ± 0.32	1.82
	(g%)	0.71 ± 0.01	0.73 ± 0.01	0.74 ± 0.04	0.78

[a]: Mean ± SD.

*: $p<0.05$ vs. 0 ppm group.

Table 3 Data for histopathology and morphometry of lesions developing in the nervous system of dams (Experiment 1).

		Acrylamide in the drinking water (ppm)			
		0	50	100	200
No. of animals examined		3	3	3	3
Trigeminal nerve					
Central chromatolysis (+/++/+++) [a]		0	3 (3/0)	3 (0/3)	3 (0/3)
Sciatic nerve (distal portion)					
Density	(/100 μm²)	1.89 ± 0.31 [b]	2.10 ± 0.11	1.96 ± 0.07	1.59 [c]
Degenerated axons	(%)	1.04 ± 1.56	0.94 ± 0.20	2.43 ± 0.50 *	5.78 [c]
Myelinated axons, <3 μm in diameter	(%)	7.82 ± 1.57	10.45 ± 2.80	13.02 ± 1.59 *	14.55 [c]
Cerebellar cortex, molecular layer					
SYP-immunoreactive aberrant dots	(/mm cortex)	0.44 ± 0.11	0.75 ± 0.27	3.18 ± 0.45 **	4.57 [c]

[a]: Grade of change: +; mild, ++; moderate.

[b]: Mean ± SD.

[c]: One dam was killed due to inability to deliver.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group.

Abbreviation: SYP, synaptophysin.

Table 4 Body and organ weights of F1 offspring at weaning (Experiment 1).

		Acrylamide in the drinking water (ppm)			
		0	50	100	200
Males	No. of animals examined	6	6	6	4
Body weight	(g)	59.73 ± 5.84 [a]	52.03 ± 2.20	33.65 ± 9.71 *	15.38 ± 1.38 **
Brain	(g)	1.46 ± 0.04	1.52 ± 0.06	1.34 ± 0.09 *	1.14 ± 0.02 **
	(g%)	2.46 ± 0.21	2.92 ± 0.10	4.25 ± 1.11 *	7.46 ± 0.74 **
Liver	(g)	2.23 ± 0.15	1.96 ± 0.19	1.12 ± 0.30 **	0.46 ± 0.10 **
	(g%)	3.74 ± 0.20	3.76 ± 0.24	3.38 ± 0.21	2.99 ± 0.374 **
Spleen	(g)	0.27 ± 0.06	0.21 ± 0.04	0.10 ± 0.05 **	0.02 ± 0.01 **
	(g%)	0.45 ± 0.10	0.40 ± 0.07	0.28 ± 0.07 **	0.13 ± 0.06 **
Kidneys	(g)	0.64 ± 0.08	0.58 ± 0.04	0.37 ± 0.07 **	0.22 ± 0.04 **
	(g%)	1.08 ± 0.04	1.11 ± 0.05	1.12 ± 0.14	1.43 ± 0.15 **
Testes	(g)	0.25 ± 0.02	0.22 ± 0.03	0.14 ± 0.04 *	0.07 ± 0.00 **
	(g%)	0.42 ± 0.04	0.41 ± 0.04	0.43 ± 0.03	0.43 ± 0.03
Epididymides	(g)	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.01 **	0.01 ± 0.00 **
	(g%)	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.09 ± 0.02
Females	No. of animals examined	6	6	6	4
Body weight	(g)	56.00 ± 1.24	50.63 ± 1.34	34.03 ± 7.98 *	16.30 ± 2.24 **
Brain	(g)	1.44 ± 0.04	1.44 ± 0.03	1.30 ± 0.10	1.11 ± 0.05 *
	(g%)	2.57 ± 0.06	2.85 ± 0.12	3.97 ± 0.80 *	6.85 ± 0.71 **
Liver	(g)	2.14 ± 0.08	1.92 ± 0.13	1.18 ± 0.27 *	0.55 ± 0.09 **
	(g%)	3.83 ± 0.13	3.79 ± 0.21	3.47 ± 0.13 *	3.40 ± 0.49
Spleen	(g)	0.24 ± 0.04	0.21 ± 0.02	0.10 ± 0.04 **	0.03 ± 0.01 **
	(g%)	0.43 ± 0.07	0.41 ± 0.04	0.27 ± 0.07 **	0.20 ± 0.04 **
Kidneys	(g)	0.63 ± 0.04	0.58 ± 0.05	0.39 ± 0.08 **	0.24 ± 0.019 **
	(g%)	1.12 ± 0.06	1.14 ± 0.10	1.17 ± 0.11	1.46 ± 0.16 **

[a]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group

Table 5 Data from histopathological analysis of F1 offspring at weaning (Experiment 1).

	Males				Females			
	Acrylamide in the drinking water (ppm)				Acrylamide in the drinking water (ppm)			
Organs and findings	0	50	100	200	0	50	100	200
No. of animals examined	6	6	6	4	6	6	6	4
Cerebellum								
Increase of external granular cells (+/++/+++)[a]	6 (6/0/0)	6 (4/2/0)	6 (2/4/0)	4 (0/2/2)††	6 (5/1/0)	6 (5/1/0)	6 (1/4/1)	4 (0/2/2)†
Liver								
Extramedullary hematopoiesis (±/+/++)[a]	6 (0/0/6)	6 (0/2/4)	6 (1/5/0)††	2 (2/0/0)††	6 (0/3/3)	6 (0/1/5)	5 (1/3/1)	4 (4/0/0)††
Loss of cytoplasmic glycogen vacuoles, hepatocytes	0	0	0	3	0	0	0	2
Spleen								
Extramedullary hematopoiesis (±/+/++/+++)[a]	6 (0/0/0/6)	6 (0/0/2/4)	6 (0/3/3/0)††	4 (4/0/0/0)††	6 (0/0/5/1)	6 (0/0/6/0)	6 (1/3/2/0)	4 (3/1/0/0)††
Testes								
Retardation of spermatogenesis (+/++/+++)[a]	0	0	6 ## (4/2/0)††	4 ## (0/2/2)††	-	-	-	-

[a]: Grade of change: ±: slight; +; mild, ++; moderate, +++: severe.

-: Not available.

##: $p<0.01$ vs. 0 ppm group (Fisher's exact test).

†, ††: $p<0.05$, $p<0.01$ vs. 0 ppm group (Mann-Whitney's U-test).

Table 6 Data for morphometry of lesions developing in the nervous systems of offspring at weaning and postnatal week 11 (Experiment 1).

		Acrylamide in the drinking water (ppm)			
		0	50	100	200
Weaning					
Males	No. of animals examined	6	6	6	4
Sciatic nerve (distal portion)					
Density	(/100 μm²)	4.08 ± 0.34[a]	4.40 ± 0.22	4.47 ± 0.64	5.42 ± 0.37 **
Degenerated axons	(%)	0.85 ± 0.17	0.67 ± 0.17	0.76 ± 0.20	0.72 ± 0.18
Myelinated axons, <3 μm in diameter	(%)	18.62 ± 2.32	19.11 ± 2.50	22.04 ± 5.08	25.51 ± 1.87 *
Cerebellar cortex, molecular layer					
SYP-immunoreactive aberrant dots	(/mm cortex)	0.29 ± 0.09	0.26 ± 0.11	0.32 ± 0.16	0.44 ± 0.09
Females	No. of animals examined	6	6	6	4
Sciatic nerve (distal portion)					
Density	(/100 μm²)	4.26 ± 0.21	4.14 ± 0.48	4.73 ± 0.70	5.28 ± 1.27
Degenerated axons	(%)	0.82 ± 0.33	0.71 ± 0.18	0.81 ± 0.31	0.86 ± 0.38
Myelinated axons, <3 μm in diameter	(%)	20.93 ± 1.64	20.1 ± 2.55	22.37 ± 2.48	23.92 ± 8.21
Cerebellar cortex, molecular layer					
SYP-immunoreactive aberrant dots	(/mm cortex)	0.55 ± 0.17	0.46 ± 0.17	0.55 ± 0.27	0.64 ± 0.33
Postnatal week 11					
Males	No. of animals examined	6	6	6	4
Sciatic nerve (distal portion)					
Density	(/100 μm²)	1.87 ± 0.19	1.94 ± 0.19	1.83 ± 0.39	1.89 ± 0.16
Degenerated axons	(%)	1.06 ± 0.45	1.01 ± 0.55	0.93 ± 0.23	1.04 ± 0.19
Myelinated axons, <3 μm in diameter	(%)	9.50 ± 2.19	11.82 ± 1.75	11.38 ± 3.68	12.88 ± 1.03
Cerebellar cortex, molecular layer					
SYP-immunoreactive aberrant dots	(/mm cortex)	0.58 ± 0.17	0.63 ± 0.28	0.53 ± 0.20	0.51 ± 0.12
Females	No. of animals examined	6	6	6	3
Sciatic nerve (distal portion)					
Density	(/100 μm²)	2.22 ± 0.14	2.01 ± 0.27	2.08 ± 0.09	1.99 ± 0.11
Degenerated axons	(%)	1.11 ± 0.84	0.89 ± 0.40	1.08 ± 0.71	1.30 ± 0.23
Myelinated axons, <3 μm in diameter	(%)	11.47 ± 0.76	10.99 ± 2.01	11.41 ± 1.90	12.03 ± 2.39
Cerebellar cortex, molecular layer					
SYP-immunoreactive aberrant dots	(/mm cortex)	0.74 ± 0.27	0.59 ± 0.36	0.61 ± 0.29	0.67 ± 0.38

[a]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group.

Abbreviation: SYP, synaptophysin.

Table 7 Body and organ weights of F1 offspring at postnatal week 11 (Experiment 1).

		Acrylamide in the drinking water (ppm)			
		0	50	100	200
Males	No. of animals examined	6	6	6	4
Body weight	(g)	473.5 ± 16.7[a]	441.8 ± 28.2	342.4 ± 60.6 *	299.6 ± 18.4 **
Brain	(g)	2.12 ± 0.06	2.12 ± 0.09	1.98 ± 0.11 **	1.86 ± 0.07 **
	(g%)	0.45 ± 0.00	0.48 ± 0.04	0.58 ± 0.08 **	0.62 ± 0.05 **
Liver	(g)	17.59 ± 1.48	16.61 ± 1.05	11.62 ± 3.87 *	10.65 ± 1.41 *
	(g%)	3.71 ± 0.20	3.76 ± 0.12	3.34 ± 0.56	3.55 ± 0.26
Spleen	(g)	0.81 ± 0.05	0.79 ± 0.15	0.60 ± 0.07 *	0.54 ± 0.03 *
	(g%)	0.17 ± 0.01	0.18 ± 0.03	0.18 ± 0.02	0.18 ± 0.01
Kidneys	(g)	3.24 ± 0.17	3.13 ± 0.14	2.50 ± 0.49	2.16 ± 0.21 *
	(g%)	0.68 ± 0.03	0.71 ± 0.03	0.73 ± 0.08	0.72 ± 0.06
Testes	(g)	3.62 ± 0.27	3.19 ± 0.21	2.91 ± 0.45 **	2.78 ± 0.22 **
	(g%)	0.76 ± 0.06	0.73 ± 0.07	0.86 ± 0.14	0.93 ± 0.07 *
Epididymides	(g)	1.08 ± 0.06	0.93 ± 0.05 **	0.90 ± 0.11 **	0.81 ± 0.05 **
	(g%)	0.23 ± 0.01	0.21 ± 0.02	0.27 ± 0.03	0.27 ± 0.00
Females	No. of animals examined	6	6	6	3
Body weight	(g)	268.3 ± 44.5	261.7 ± 17.1	240.5 ± 18.8	191.8 ± 10.7 **
Brain	(g)	1.92 ± 0.06	1.98 ± 0.07	1.85 ± 0.05	1.71 ± 0.11 **
	(g%)	0.73 ± 0.12	0.76 ± 0.03	0.77 ± 0.04	0.89 ± 0.07
Liver	(g)	9.34 ± 1.80	9.13 ± 0.67	8.39 ± 1.02	6.83 ± 0.35 *
	(g%)	3.47 ± 0.21	3.50 ± 0.25	3.48 ± 0.18	3.56 ± 0.07
Spleen	(g)	0.51 ± 0.09	0.51 ± 0.10	0.55 ± 0.05	0.41 ± 0.02
	(g%)	0.19 ± 0.01	0.19 ± 0.03	0.23 ± 0.03*	0.21 ± 0.02
Kidneys	(g)	2.00 ± 0.34	1.88 ± 0.10	1.73 ± 0.18	1.55 ± 0.12 *
	(g%)	0.75 ± 0.06	0.72 ± 0.02	0.72 ± 0.03	0.81 ± 0.04

[a]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group

Table 8 Reproductive data (Experiment 2).

		Acrylamide in the drinking water (ppm)				Acrylamide i.p.
		0	25	50	100	
No. of animals examined		4	4	4	4	2
Gestational period	(days)	21.5 ± 0.6 [a]	21.8 ± 0.5	21.8 ± 0.5	22.0 ± 0.0	21.5
No. of implantation/dam		15.5 ± 3.4	14.5 ± 1.3	14.0 ± 0.8	14.8 ± 0.5	15.0
Live birth	(%) [b]	100	100	100	100	100
No. of live pups/litter [c]		13.5 ± 4.2	12.8 ± 1.0	13.8 ± 1.0	14.0 ± 1.2	14.0
No. of male pups [c]		7.0 ± 1.6	6.5 ± 1.3	6.8 ± 2.6	7.0 ± 2.7	6.0
No. of female pups [c]		6.5 ± 2.6	6.3 ± 0.5	7.0 ± 2.2	7.0 ± 2.2	8.0
Male ratio	(%)	53.3 ± 6.8	50.7 ± 6.6	48.6 ± 17.8	49.5 ± 16.2	42.9
Male pup body weight [c]	(g)	6.35 ± 0.56	7.27 ± 0.71 **	6.96 ± 0.91 **	7.00 ± 0.48 **	6.73 ± 0.47
Female pup body weight [c]	(g)	6.32 ± 0.68	7.01 ± 0.60 **	6.68 ± 0.84	6.56 ± 0.55	6.50 ± 0.52

[a]: Mean ± SD.

[b]: Live birth (%)=No. of live pups delivered/Total no. of pups delivered×100

[c]: Measured at PND2

**: $p<0.01$ vs. 0 ppm group

Table 9 Body and brain weights of dams at weaning (Experiment 2).

		Acrylamide in the drinking water (ppm)				Acrylamide
		0	25	50	100	i.p.
No. of animals examined		4	4	4	4	2
Body weight	(g)	316.3 ± 18.6[a]	323.9 ± 21.2	315.9 ± 26.2	291.0 ± 18.3	317.5
Brain	(g)	1.96 ± 0.09	1.92 ± 0.11	1.92 ± 0.06	1.85 ± 0.06	1.95
	(g%)	0.62 ± 0.05	0.60 ± 0.04	0.61 ± 0.06	0.64 ± 0.05	0.62

[a]: Mean ± SD.

Table 10 Body and organ weights of F1 offspring at weaning (Experiment 2).

		Acrylamide in drinking water (ppm)				Acrylamide
		0	25	50	100	i.p.
Males No. of animals examined		3	4	8	5	4
Body weight	(g)	57.6 ± 4.9[a]	58.2 ± 9.1	52.7 ± 7.3	33.3 ± 2.6**	31.3 ± 9.0**
Brain	(g)	1.45 ± 0.08	1.46 ± 0.03	1.48 ± 0.04	1.33 ± 0.06*	1.10 ± 0.07**
	(g%)	2.53 ± 0.08	2.55 ± 0.40	2.85 ± 0.35	4.00 ± 0.23**	3.71 ± 0.90
Testes	(g)	0.22 ± 0.02	0.22 ± 0.05	0.20 ± 0.02	0.14 ± 0.01*	0.14 ± 0.04
	(g%)	0.38 ± 0.02	0.38 ± 0.04	0.38 ± 0.05	0.42 ± 0.03	0.46 ± 0.04*
Epididymides	(g)	0.035 ± 0.004	0.036 ± 0.003	0.029 ± 0.002*	0.026 ± 0.004**	0.022 ± 0.003
	(g%)	0.060 ± 0.000	0.063 ± 0.010	0.055 ± 0.008	0.076 ± 0.011	0.075 ± 0.017
Females No. of animals examined		7	6	6	3	5
Body weight	(g)	58.8 ± 3.9	49.6 ± 18.3	53.5 ± 7.2	31.9 ± 1.7*	36.8 ± 4.6*
Brain	(g)	1.44 ± 0.06	1.37 ± 0.15	1.43 ± 0.08	1.32 ± 0.02	1.13 ± 0.03*
	(g%)	2.46 ± 0.13	3.19 ± 1.47	2.71 ± 0.36	4.13 ± 0.20*	3.10 ± 0.34

[a]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group.

Table 11 Data from histopathological analysis of dams and offspring at PND 21 (Experiment 2).

Organs and findings		Acrylamide in the drinking water (ppm) 0	25	50	100	Acrylamide i.p.
Dams	No. of animals examined	4	4	4	4	2
Trigeminal nerve						
Central chromatolysis (+/++/+++) [a]		0	0	4 (3/1/0)	4 (0/3/1)	0
Offspring						
Males	No. of animals examined	3	4	8	5	4
Cerebellum						
Retained external granule layer (+/++/+++)		3 (3/0/0)	4 (3/1/0)	8 (1/7/0)	5 (1/3/1)	4 (0/3/1)
Trigeminal nerve						
Central chromatolysis (+/++/+++)		0	0	0	0	4 (3/1/0)
Testis						
Retardation of spermatogenesis (+/++/+++)		0	0	0	5 (3/2/0)	4 (2/2/0)
Females	No. of animals examined	7	6	6	3	5
Cerebellum						
Retained external granule layer (+/++/+++)		7 (5/2/0)	6 (3/3/0)	6 (3/3/0)	3 (0/3/0)	5 (1/3/1)
Trigeminal nerve						
Central chromatolysis (+/++/+++)		0	0	0	0	5 (4/1/0)

[a]: Grade of change: +, mild; ++, moderate; +++, severe.

Table 12 Data from morphometry of lesions developing in the nervous tissues of dams (Experiment 2).

		Acrylamide in the drinking water (ppm) 0	100
No. of animals examined		4	4
Sciatic nerve (distal portion)			
Density	(/100 μm^2)	1.82 ± 0.04 [a]	1.97 ± 0.12
Degenerated axons	(%)	0.39 ± 0.09	1.93 ± 0.64 *
Myelinated axons, <3 μm in diameter	(%)	10.41 ± 1.14	13.03 ± 1.32 *
Cerebellar cortex, molecular layer			
SYP-immunoreactive aberrant dots	(/mm cortex)	0.38 ± 1.50	1.50 ± 0.56 *

[a]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group (Student's *t*-test).

Abbreviations: SYP, synaptophysin.

Table 13 Data from morphometry of lesions developing in the nervous tissues of offspring (Experiment 2).

		Acrylamide in drinking water (ppm)		Acrylamide
		0	100	i.p.
Males	No. of animals examined	3	5	4
Sciatic nerve (distal portion)				
Density	(/100 μm²)	4.35 ± 0.34	4.21 ± 0.39	4.00 ± 0.27
Degenerated axons	(%)	0.59 ± 0.53	0.44 ± 0.21	2.99 ± 0.63 **
Myelinated axons, <3 μm in diameter	(%)	28.45 ± 4.34	31.23 ± 4.17	48.97 ± 4.34 **
Cerebellar cortex, molecular layer				
SYP-immunoreactive aberrant dots	(/mm cortex)	0.31 ± 0.12 [a]	0.31 ± 0.08	0.50 ± 0.24
Females	No. of animals examined	5	3	5
Sciatic nerve (distal portion)				
Density	(/100 μm²)	4.46 ± 0.19	4.32 ± 0.18	4.39 ± 0.50
Degenerated axons	(%)	0.53 ± 0.27	0.33 ± 0.01	2.02 ± 0.22 **
Myelinated axons, <3 μm in diameter	(%)	30.19 ± 3.62	28.03 ± 2.72	44.24 ± 4.87 **
Cerebellar cortex, molecular layer				
SYP-immunoreactive aberrant dots	(/mm cortex)	0.23 ± 0.06	0.18 ± 0.06	0.26 ± 0.13

[a]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group (Student's *t*-test).

Abbreviations: SYP, synaptophysin.

Table 14 Measurement of ACR-Hb adducts in dams and offspring after maternal exposure to ACR (Experiment 2).

		Acrylamide in the drinking water (ppm)				R[d]
		0	25	50	100	
Dams	No. of animals examined	4	4	4	4	
ACR adducts/Hb	(μmol/Hb g)	0.19 ± 0.05 [b,c]	3.34 ± 0.16	4.68 ± 0.55	5.74 ± 1.15 *	0.862
ACR concentration /RBC [a]	(μmol/g)	0.04 ± 0.01	0.88 ± 0.09	1.18 ± 0.32 *	1.15 ± 0.18 *	0.798
Offspring	No. of animals examined	5	6	7	8	
ACR adducts/Hb	(μmol/Hb g)	0.12 ± 0.05	0.22 ± 0.08	0.28 ± 0.12 *	0.34 ± 0.08 **	0.669
ACR concentration /RBC [a]	(μmol/g)	0.02 ± 0.01	0.06 ± 0.01	0.07 ± 0.03 *	0.08 ± 0.04 *	0.616

[a]: Calculated as ACR monomer per 1 g red blood cell.

[b]: Mean±SD.

[c]: Measurable level of Hb adduct was observed in untreated animals (0.15 (0.14-0.17) μmol/Hb g, Bergmark, E. et al., Toxicol. Appl. Pharmacol., 1991).

[d]: Correlation coefficients between the mean daily intake of ACR by dams and the levels of ACR adducts or ACR concentration per red blood cells.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group.

Abbreviations: ACR, acrylamide; Hb, hemoglobin; RBC, red blood cell.

Table 15 Mean daily intake of acrylamide in young and adult rats (Experiment 3).

		Acrylamide in the drinking water (ppm)			
Group		0	50	100	200
	No. of animals examined	10	10	10	10
Young	(mg/kg/day)	0 ± 0 [a]	8.27 ± 0.32	15.73 ± 1.51	26.37 ± 3.51
Adult	(mg/kg/day)	0 ± 0	6.26 ± 1.10	12.63 ± 1.97	19.07 ± 3.46

[a]: Mean ± SD.

Table 16 Body and organ weights of young and adult rats given acrylamide in the drinking water for 4 weeks (Experiment 3).

		Acrylamide in the drinking water (ppm)			
		0	50	100	200
Young	No. of animals examined	10	10	10	10
Body weight	(g)	287.4 ± 24.6 [a]	273.5 ± 15.7	263.7 ± 14.4 *	210.4 ± 24.3 **
Brain	(g)	1.97 ± 0.05	1.94 ± 0.09	1.83 ± 0.09 *	1.66 ± 0.04 **
	(g%)	0.69 ± 0.06	0.71 ± 0.05	0.69 ± 0.03	0.80 ± 0.08 **
Testes	(g)	2.57 ± 0.15	2.44 ± 0.22	2.39 ± 0.19	1.87 ± 0.36 **
	(g%)	0.90 ± 0.07	0.90 ± 0.09	0.91 ± 0.07	0.89 ± 0.12
Epididymides	(g)	0.40 ± 0.04	0.35 ± 0.02 **	0.37 ± 0.04	0.30 ± 0.02 **
	(g%)	0.14 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.15 ± 0.02
Adult					
Body weight	(g)	444.3 ± 38.0	433.0 ± 42.0	426.7 ± 42.1	409.2 ± 45.5
Brain	(g)	2.07 ± 0.06	2.08 ± 0.11	2.02 ± 0.09	1.99 ± 0.07
	(g%)	0.47 ± 0.03	0.48 ± 0.04	0.48 ± 0.04	0.49 ± 0.06
Testes	(g)	3.30 ± 0.26	3.39 ± 0.39	3.25 ± 0.20	3.19 ± 0.24
	(g%)	0.74 ± 0.07	0.78 ± 0.08	0.77 ± 0.08	0.79 ± 0.09
Epididymides	(g)	0.97 ± 0.05	1.04 ± 0.09	0.97 ± 0.07	0.84 ± 0.06 **
	(g%)	0.22 ± 0.02	0.24 ± 0.02	0.23 ± 0.03	0.21 ± 0.02

[a]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group.

Table 17 Data for histopathology and morphometry of lesions developed in the nervous system (Experiment 3).

		Acrylamide in the drinking water (ppm)			
		0	50	100	200
Young	No. of animals examined	10	10	10	10
Trigeminal nerve					
Central chromatolysis (+/++/+++) [a]		0	3 (3/0/0)	10 ## (0/5/5)	10 ## (0/0/10)
Sciatic nerve (distal portion)					
Density	(/100 μm²)	2.56 ± 0.32 [b]	2.73 ± 0.17	2.92 ± 0.25 **	2.42 ± 0.25
Degenerated axons	(%)	0.28 ± 0.15	0.39 ± 0.14	0.82 ± 0.19 **	7.51 ± 3.25 **
Myelinated axons, <3 μm in diameter	(%)	18.01 ± 3.45	16.74 ± 2.79	18.80 ± 2.73	21.57 ± 4.07
Cerebellar cortex, molecular layer					
SYP-immunoreactive aberrant dots	(/mm cortex)	0.50 ± 0.20	0.41 ± 0.18	1.49 ± 0.59	6.09 ± 1.62 *
Adult					
Trigeminal nerve					
Central chromatolysis (+/++/+++) [a]		0	3 (3/0/0)	10 ## (3/7/0)	10 ## (0/3/7)
Sciatic nerve (distal portion)					
Density	(/100 μm²)	2.10 ± 0.23	2.03 ± 0.15	2.10 ± 0.24	2.15 ± 0.24
Degenerated axons	(%)	0.39 ± 0.16	0.65 ± 0.27	0.96 ± 0.37 *	1.74 ± 0.77 **
Myelinated axons, <3 μm in diameter	(%)	13.96 ± 2.75	12.30 ± 2.39	13.45 ± 2.68	14.16 ± 2.82
Cerebellar cortex, molecular layer					
SYP-immunoreactive aberrant dots	(/mm cortex)	0.54 ± 0.12	0.47 ± 0.09	1.71 ± 0.81	5.88 ± 2.61 *

[a]: Grade of change: +; mild, ++; moderate, +++: severe.

[b]: Mean ± SD.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group.

##: $p<0.01$ vs. 0 ppm group (Fisher's exact test).

Abbreviation: SYP, synaptophysin.

Table 18 Histopathological data of the testis of young and adult rats given acrylamide in the drinking water for 4 weeks (Experiment 3).

Findings (%) [a]	Acrylamide in the drinking water (ppm)			
	0	50	100	200
Young No. of animals examined	10	10	10	10
Affected tubules [b]	3.51 ± 1.68 [c]	9.03 ± 18.81	16.93 ± 12.23 *	66.59 ± 26.96 **
Exfoliation of germ cells	3.39 ± 1.61	3.93 ± 3.07	9.80 ± 6.22 *	10.44 ± 9.87
Multinucleated gaint cells	0.02 ± 0.06	0.07 ± 0.14	0.57 ± 0.71	1.67 ± 3.06 **
Degeneration of spermatids	0.10 ± 0.18	0.84 ± 2.54	3.95 ± 6.47	20.90 ± 13.37 **
Loss or decrease of elongated spermatids	0 ± 0	4.99 ± 15.74 *	5.62 ± 8.87 **	20.43 ± 14.61 **
Loss or decrease of round spermatids	0.02 ± 0.06	0 ± 0	1.51 ± 3.19	12.68 ± 10.97 **
Degenerated tubules [d]	0 ± 0	0.17 ± 0.54 **	0.12 ± 0.38 **	24.03 ± 30.83 **
Sertoli cell vacuolation	0.60 ± 0.57	1.07 ± 0.55	0.99 ± 0.62	1.06 ± 0.92
Adult				
Affected tubules [b]	0.47 ± 0.30	0.58 ± 0.23	1.17 ± 0.60 *	1.53 ± 0.67 **
Exfoliation of germ cells	0.45 ± 0.30	0.56 ± 0.23	1.17 ± 0.60 *	1.46 ± 0.71 **
Multinucleated gaint cells	0 ± 0	0 ± 0	0 ± 0	0.07 ± 0.17
Degeneration of spermatids	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Loss or decrease of elongated spermatids	0.02 ± 0.06	0.02 ± 0.07	0 ± 0	0 ± 0
Loss or decrease of round spermatids	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Degenerated tubules [d]	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sertoli cell vacuolation	0.62 ± 0.45	0.53 ± 0.50	0.72 ± 0.43	0.81 ± 0.53

[a]: Approximately 400-650 tubules/rat were examined.

[b]: Affected tubules represent total tubules with below findings, except for the tubules showing only Sertoli cell vacuolation.

[c]: Mean ± SD.

[d]: Degenerated tubules are those showing marked germ cell depletion.

*, **: $p<0.05$, $p<0.01$ vs. 0 ppm group.

（資料３）

厚生労働科学研究費補助金（食品の安心・安全確保推進研究事業）
分担研究報告書（平成 18-20 年度 総合研究報告書）

ライフステージによるアクリルアミドの発がん感受性に関する研究

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研究要旨

ラットの乳幼児期にアクリルアミド（AA）を投与することにより、その後に投与する *N*-メチル-*N*-ニトロソ尿素（MNU）誘発性発がんに対して相加作用を示すか否かを検討するため、ラット多臓器中期発がん実験を行った。すなわち、ラットの 0～6 週齢時に AA を 0（対照）、20、40 及び 80 ppm 濃度で飲水投与し、7 週齢時に多臓器に対して標的性を示す発がん物質である MNU を単回腹腔内投与し、その後 50 週齢時まで基礎飼料及び脱イオン水あるいは抗甲状腺剤のスルファジメトキシンを 125 ppm 濃度で混じた飲料水を自由摂取させた。AA の投与濃度は、本実験に先立って実施したラットの 0～12 週齢時に AA を投与した予備実験の結果に基づいて決定した。本実験では、病理組織学的に AA の標的臓器である甲状腺、乳腺及び精巣鞘膜を含む諸臓器、組織において前がん病変あるいは腫瘍の発生がみられたが、それらの発生率に群間の明らかな差は認められなかった。以上、AA の乳幼児期投与は、MNU 誘発性ラット多臓器発がんに対して明らかな影響を示さなかった。

A. 研究目的

加工食品中に種々の濃度で含まれていることが明らかにされたアクリルアミド（AA）については、実験的に神経毒性、精巣毒性、遺伝毒性及び発がん性を示すことが知られ、中でも遺伝毒性を伴う発がん性のヒトへのリスクが懸念されている。一方、AA の職業暴露に対するリスク評価を主な目的とした実験データは多数報告されているものの、食品を介する暴露を想定した毒性データは十分ではない。そこで、米国を中心に AA あるいはその代謝物で生体内活性の高いグリシドアミド（GA）の発がん性試験及び発達期神経毒性試験により、低用量長期間投与による毒性評価を目的とした研究が行われており、それらの結果が明らかにされた際に再度リスク評価をすべきであるとされている。また AA の発がん性については、乳腺、甲状腺、副腎、子宮などに標的性を示すことから、遺伝毒性のみならず、内分泌系に対する影響の関与している可能性が指摘されている。第 47 回米国トキシコロジー学会（2008 年 3 月、シアトル）では、AA の甲状腺発がんに関する機序解析の結果が発表された。即ち、AA を 2.5-50 mg/kg 体重/日の用量で 14 日間飲水投与し、甲状腺の病理組織学的、細胞動態学的あるいは遺伝子発現解析及び血清ホルモン値の測定を行った結果、甲状腺における発がん性の機序を説明し得る変化は認められなかったとする報告がされた（Deorge