ACETAMIPRID

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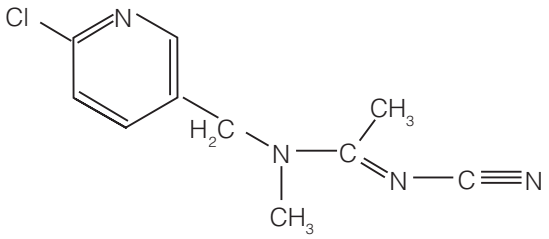
Explanation

Acetamiprid is the International Organization for Standardization (ISO)–approved name for (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methyl acetamidine (International Union of Pure and Applied Chemistry). Its Chemical Abstracts Service number is 135410-20-7. Acetamiprid is a neonicotinoid insecticide that is used for the control of sucking-type insects on leafy vegetables, fruiting vegetables, cole crops, citrus fruits, pome fruits, grapes, cotton and ornamental plants and flowers. Acetamiprid is being reviewed for the first time by the Joint FAO/WHO Meeting on Pesticide Residues at the request of the Codex Committee on Pesticide Residues.

All critical studies contained statements of compliance with good laboratory practice (GLP).

The chemical structure of acetamiprid is shown in Figure 1.

Figure 1. Chemical structure of acetamiprid



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, elimination and pharmacokinetics

(a) Oral route

To obtain information on the absorption, distribution, rate and route of elimination, metabolism and pharmacokinetics of acetamiprid, a study was performed in adult Sprague-Dawley rats (body weight 154–193 g for males, 134–152 g for females; aged 5–6 weeks at the start of dosing; dosing for 15 days) using [^{14}C]acetamiprid. The radiolabelled test substance (batch No. CFQ8019, chemical purity > 99.9%, radiochemical purity 97.1–97.2%) was sent by the sponsor to the contract research organization. The non-labelled test substance was from lot No. NNI-01, with a chemical purity of greater than 99.9%.

The studies were conducted after oral administration of the test substance for 15 days. In total, five treatment groups (groups I, II, III, IV and V), consisting of 6 rats (3 males and 3 females) in each of the first three groups and 10 rats (5 males and 5 females) in each of the two remaining groups, were used. A single control group (group VI), consisting of four rats (two males and two females), was used.

Groups I, II and III received oral doses of [^{14}C]acetamiprid in 0.9% saline for 15 days at a target dose rate of 1.0 mg/kg body weight (bw). Groups IV and V received oral doses of acetamiprid in 0.9% saline for 14 days followed by a single oral dose of [^{14}C]acetamiprid in 0.9% saline on day 15. The actual dose rate was 0.97–1.01 mg/kg bw for the rats in all five groups. The radiochemical purity of [^{14}C]acetamiprid in the dose solution was determined to be 97.9% by high-performance liquid chromatographic (HPLC) analysis. The dose solution was stable under refrigerated conditions for at

Table 1. Group designation and dose level

Group No.	No. of males/ no. of females	Nominal dose		Frequency	Sacrifice (h)
		mg/kg bw	ml/kg bw		
I	3/3	1.0	1	Daily for 15 days	1
II	3/3	1.0	1	Daily for 15 days	10
III	3/3	1.0	1	Daily for 15 days	96
IV	5/5	1.0	1	Daily for 15 days	96
V	5/5	1.0	1	Daily for 15 days	48
VI	2/2	0	1	Daily for 15 days	96

From Premkumar, Guo & Vegurlekar (1995)

Table 2. [^{14}C]Acetamiprid concentration in blood collected 1 hour post-dosing on days 1, 3, 7 and 15 from rats of group III

Sex of rat		[^{14}C]Acetamiprid concentration in blood (µg/ml)			
		Day 1	Day 3	Day 7	Day 15
Male	Mean	0.590	0.747	0.477	0.606
	± SD	0.130	0.211	0.135	0.073
Female	Mean	0.465	0.491	0.511	0.698
	± SD	0.043	0.060	0.099	0.065

From Premkumar, Guo & Vegurlekar (1995)
SD, standard deviation

least 15 days. The specific activity of the radiolabelled dose solution was determined to be 1.85×10^3 Bq/µg. Group VI was dosed with 0.9% saline only.

Rats of groups I, II and III were sacrificed 1, 10 and 96 hours, respectively, after dosing of [^{14}C]acetamiprid for 15 days. Rats of group IV were sacrificed 96 hours after a single dose of [^{14}C]-acetamiprid for tissue and organ collection. Group V was used only for blood pharmacokinetic analysis (Table 1).

Whole blood was drawn from each rat of group III approximately 1 hour post-dosing on days 1, 3, 7 and 15 to determine the [^{14}C]acetamiprid concentration in blood. The average concentration in blood was in the range of 0.477–0.747 µg/ml in the males and 0.465–0.698 µg/ml in the females. Variation between animals was observed. These results indicate that the blood concentration at 1 hour post-dosing was consistent during the entire dosing period (Table 2).

Whole blood was drawn from each rat of group V at approximately 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24 and 48 hours to determine the [^{14}C]acetamiprid concentration in blood. The mean values for peak concentration (C_{max}), time to C_{max} (T_{max}), absorption half-life ($t_{½\,(ka)}$) and area under the concentration versus time curve at infinity (AUC_{∞}) for the male rats were 0.798 ± 0.111 µg/ml, 2.80 ± 0.637 hours, 1.35 ± 0.825 hours and 8.35 ± 1.12 µg eq·h/ml, respectively. Values for the same parameters in female rats averaged 0.861 ± 0.132 µg/ml, 2.81 ± 0.894 hours, 1.18 ± 0.868 hours and 10.3 ± 2.90 µg eq·h/ml, respectively. The elimination half-lives ($t_{½\,(k)}$) for the male and female rats were 4.42 ± 1.10 hours and 5.56 ± 1.93 hours, respectively. The pharmacokinetic parameters for both sexes did not differ considerably. The T_{max} values in both sexes indicated that the rate of absorption of acetamiprid was rapid, and a maximum blood concentration to possible saturation was achieved in approximately 2–3 hours (Table 3).

Table 3. Mean whole blood pharmacokinetic parameters in rats in group V (dosed for 14 days with non-labelled acetamiprid followed by labelled acetamiprid on day 15)

Sex of rat		C_{max} (µg/ml)	T_{max} (h)	$t_{½\,(ka)}$ (h)	$t_{½\,(k)}$ (h)	AUC_{∞} (µg eq·h/ml)
Male	Mean	0.798	2.80	1.35	4.42	8.35
	± SD	0.111	0.637	0.825	1.10	1.12
Female	Mean	0.861	2.81	1.18	5.56	10.3
	± SD	0.132	0.894	0.868	1.93	2.90

From Premkumar, Guo & Vegurlekar (1995)
eq, equivalent; SD, standard deviation

Table 4. Recovery of administered dose in faeces, urine and cage rinse[a]

Sacrifice time (h)	Group	Sex		% of total administered dose eliminated in			
				Faeces	Urine	Cage rinse	Total
1	I	Male	Mean	31.0	53.4	7.57	92.0
			± SD	0.56	5.24	2.97	2.89
		Female	Mean	21.9	58.0	10.7	90.6
			± SD	2.43	5.39	1.42	5.08
10	II	Male	Mean	29.8	56.6	7.32	93.7
			± SD	3.12	6.90	1.95	3.56
		Female	Mean	25.2	59.3	6.98	91.5
			±SD	6.00	4.11	2.21	0.92
96	III	Male	Mean	32.0	61.4	3.92	97.4
			± SD	4.08	0.64	0.73	4.87
		Female	Mean	27.5	56.0	7.93	91.4
			± SD	1.42	2.45	2.67	1.51
96	IV	Male	Mean	35.3	64.8	5.86	106
			± SD	5.97	6.99	2.81	5.44
		Female	Mean	28.7	62.1	11.3	102
			± SD	4.30	5.32	3.72	5.31

From Premkumar, Guo & Vegurlekar (1995)
SD, standard deviation
[a] Animals of groups I, II and III were treated with [^{14}C]acetamiprid for 15 days and sacrificed 1, 10 and 96 hours after the administration of the 15th dose. Animals of group IV were treated with acetamiprid for 14 days, and on the 15th day, a single dose of [^{14}C]acetamiprid was given, 96 hours after which the animals were sacrificed.

The elimination results indicate that most acetamiprid (53–65%) was excreted in the urine. The excretion in urine and cage rinse combined amounted to 61–73%. The results also indicate that acetamiprid was absorbed rapidly (within 1 hour) from the gastrointestinal tract, as greater than 90% of the administered dose was eliminated from the gastrointestinal tract within 1 hour after dosing. No difference was observed in elimination of test substance between chronic administration of acetamiprid for 14 days followed by a single administration of radiolabelled acetamiprid on day 15 (group IV) and chronic administration of radiolabelled acetamiprid for 15 days (groups I, II and III). The amount of administered radioactivity eliminated in faeces was lower for females (22–29%) than for males (30–35%) (Table 4).

The whole blood, liver, kidney, lung, pancreas, spleen, heart, brain, testes (male), ovary (female), skeletal muscles, inguinal fat (white), skin with hair, thyroid, bone, adrenal glands, gastrointestinal tract with contents, cage rinses and residual carcasses were collected from each rat of groups I, II, III and IV. All collected samples were not composited but kept and analysed separately to account for the material balance for each rat.

Radioactivity, after administration of the last chronic dose, was detected at the earliest sampling point (1 hour) in all the tissues collected from each rat. The radioactivity in most tissues was the highest at 1 hour post-dosing and declined rapidly thereafter (groups II and III). The T_{max} for [^{14}C]acetamiprid in the male and female rats indicated that the rate of absorption was rapid, and a maximum blood concentration (~0.8 µg/ml) to possible saturation was achieved in approximately 2–3 hours. The levels of [^{14}C]acetamiprid residue in tissues collected at 1 hour post-dosing confirm the results obtained from the pharmacokinetic analysis.

[^{14}C]Acetamiprid residue levels seen in tissues collected 10 hours post-dosing (group II) were found to be substantially lower than residue levels in tissues collected 1 hour post-dosing. The elimination half-life ($t_{½(k)}$) for both sexes indicated that the rate of elimination was rapid. The levels of [^{14}C]acetamiprid residues in tissues collected at 10 hours post-dosing confirm the results obtained from the pharmacokinetic studies.

[^{14}C]Acetamiprid residue levels seen in tissues collected 96 hours post-dosing (group III) were found to be very low compared with the levels observed in the tissues collected at 1 hour and 10 hours post-dosing. The elimination half-life ($t_{½(k)}$) for both sexes was between 4 and 6 hours post-dosing, indicating that the rate of elimination was rapid and that retention of residue in tissues after chronic administration was minimal.

The highest radioactivity levels were observed in the gastrointestinal tract, liver and kidney in both sexes at all sacrifice times. The lowest concentration was observed in bone and white fat. The residue levels observed were higher in all tissues of rats chronically treated with [^{14}C]acetamiprid for 15 days (group III) compared with the rats in group IV, which received a single final dose of [^{14}C]-acetamiprid following 14 days of non-labelled acetamiprid doses. The residue levels observed in the tissues of rats sacrificed 96 hours after the last dose were very low (0.01–0.1 part per million [ppm]), as most of the administered dose (> 90%) was eliminated through the urine and faeces (Table 5).

The total administered radioactivity recovered in groups I, II, III and IV was in the range of 91.7–106%, whereas recovery in group V (the pharmacokinetics group) was 71.7% and 85.6% in males and females, respectively (Table 6). The loss of urine samples during a series of bleeding procedures is a possible explanation for the low recovery in group V.

The study described in this report was conducted in compliance with GLP. A quality assurance (QA) statement was attached (Premkumar, Guo & Vegurlekar, 1995).

To ascertain the effect of administration of acetamiprid in single low and high doses, the absorption, distribution, metabolism and excretion of acetamiprid in rats were investigated. [Pyridine-2,6-^{14}C]acetamiprid was intravenously or orally administered to five male and five female rats in groups A, B and D at dose levels of 1.0, 1.0 and 50 mg/kg bw, respectively. In group CN-B, the metabolism study of [cyano-^{14}C]acetamiprid was performed at a dose level of 1.0 mg/kg bw. Group A was for the determination of the absorption rate by calculation from the excretion rate and metabolite analysis. Groups B, D and CN-B were for blood levels, tissue distribution, metabolite analysis and excretion rate. The chemical structure and position of the label on the test substance are as shown in Figure 2. The study design is as described in Table 7.

In groups B and D, the absorption in the rats was rapid. The maximum concentrations in the blood were observed at 0.5–2 hours after administration at 0.91 mg/kg bw for males and 1.01 mg/kg bw for females (low dose: ring label, group B) and at 3–7 hours after administration at 40.50 mg/kg bw for males and 31.46 mg/kg bw for females (high dose: ring label, group D).

Table 5. Distribution of [^{14}C]acetamiprid residues in various tissues collected from rats at 1, 10 and 96 hours post-dosing (dosed with [^{14}C]acetamiprid for 15 days)

Tissues	Sex		Concentration of residues (ppm)		
			1 h post-dosing, group I	10 h post-dosing, group II	96 h post-dosing, group III
Gastrointestinal tract	Male	Mean	4.48	2.40	0.010
		± SD	0.82	0.14	0.002
	Female	Mean	3.79	1.91	0.011
		± SD	0.59	0.40	0.001
Liver	Male	Mean	1.62	0.78	0.014
		± SD	0.05	0.28	0.003
	Female	Mean	1.86	0.69	0.011
		± SD	0.09	0.39	0.000
Kidney	Male	Mean	1.43	0.79	0.028
		± SD	0.05	0.26	0.005
	Female	Mean	1.48	0.66	0.023
		± SD	0.09	0.32	0.001
Heart	Male	Mean	0.81	0.39	0.006
		± SD	0.05	0.13	0.001
	Female	Mean	1.00	0.38	0.006
		± SD	0.07	0.26	0.001
Lung	Male	Mean	0.76	0.39	0.009
		± SD	0.06	0.13	0.001
	Female	Mean	0.98	0.38	0.008
		± SD	0.08	0.26	0.003
Blood	Male	Mean	0.75	0.36	0.015
		± SD	0.04	0.10	0.008
	Female	Mean	0.92	0.33	0.007
		± SD	0.04	0.20	0.000
Thyroid	Male	Mean	0.80	0.39	0.000
		± SD	0.04	0.13	0.000
	Female	Mean	0.94	0.37	0.000
		± SD	0.08	0.25	0.000
Spleen	Male	Mean	0.66	0.31	0.004
		± SD	0.04	0.11	0.001
	Female	Mean	0.81	0.31	0.004
		± SD	0.05	0.21	0.001
Adrenals	Male	Mean	0.64	0.35	0.010
		± SD	0.09	0.19	0.002
	Female	Mean	1.23	0.42	0.015
		± SD	0.07	0.26	0.007
Muscle	Male	Mean	0.63	0.31	0.006
		± SD	0.05	0.10	0.001
	Female	Mean	0.81	0.35	0.007
		± SD	0.06	0.22	0.001

Table 5 (continued)

Tissues	Sex		Concentration of residues (ppm)		
			1 h post-dosing, group I	10 h post-dosing, group II	96 h post-dosing, group III
Testes	Male	Mean	0.60	0.30	0.003
		± SD	0.04	0.10	0.001
Ovaries	Female	Mean	0.46	0.23	0.008
		± SD	0.13	0.14	0.004
Skin	Male	Mean	0.58	0.31	0.106
		± SD	0.07	0.10	0.033
	Female	Mean	0.73	0.33	0.067
		± SD	0.05	0.20	0.002
Pancreas	Male	Mean	0.70	0.26	0.002
		± SD	0.17	0.07	0.001
	Female	Mean	0.83	0.25	0.002
		± SD	0.05	0.17	0.001
	Male	Mean	0.59	0.25	0.002
Brain		± SD	0.04	0.07	0.001
	Female	Mean	0.75	0.24	0.002
		± SD	0.02	0.18	0.001
Bone	Male	Mean	0.45	0.17	0.003
		± SD	0.05	0.05	0.001
	Female	Mean	0.58	0.20	0.004
		± SD	0.07	0.14	0.001
Fat	Male	Mean	0.27	0.11	0.007
		± SD	0.02	0.02	0.002
	Female	Mean	0.47	0.17	0.010
		± SD	0.08	0.11	0.002

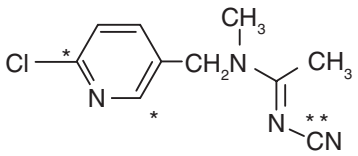
From Premkumar, Guo & Vegurlekar (1995)
SD, standard deviation

Table 6. Mass balance: total average percentage of administered radioactivity dose recovered in samples collected in various groups

Group of animals	Timing of sample collection (h)	Average % of administered dose	
		Males	Females
I	1	101	99.7
II	10	98.5	95.5
III	96	97.6	91.7
IV	96	106.1	103
V	48	71.7	85.6

From Premkumar, Guo & Vegurlekar (1995)

Figure 2. Chemical structure and label position of test substance



(* labelled position for ring -^{14}C-NI-25)
(** labelled position for CN-^{14}C-NI-25)

Table 7. Study design

Group	Mode of administration	Target dose level (mg/kg bw)	Experiment targeted	Test substance	Number of animals	
					Male	Female
A	Single intravenous	1.0	1. Excretion rate 2. Quantitative analysis of metabolites	Ring-^{14}C-acetamiprid	5	8
B	Single oral (low dose)	1.0	1. Blood levels 2. Excretion rate 3. Quantitative analysis of metabolites 4. Tissue distribution 5. Biliary excretion	Ring-^{14}C-acetamiprid	5 9 3	5 (for each of experiments 1, 2 and 3) 9 (for experiment 4) 3 (for experiment 5)
D	Single oral (high dose)	50	1. Blood levels 2. Excretion rate 3. Quantitative analysis of metabolites 4. Tissue distribution	Ring-^{14}C-acetamiprid	5 9	5 (for each of experiments 1, 2 and 3) 9 (for experiment 4)
CN-B	Single oral (low dose)	1.0	1. Blood levels 2. Excretion rate 3. Quantitative analysis of metabolites	CN-^{14}C-acetamiprid	5	5

From Tanoue & Mori (1997a)

The absorption rate of acetamiprid following oral administration was calculated using the following equation, based on urinary excretion rates in oral and intravenous administrations at the low dose:

$$\text{Absorption rate} = \frac{\text{Urinary excretion rate following oral administration}}{\text{Urinary excretion rate following intravenous administration}} \times 100$$

The calculations are shown in Table 8. Thus, the absorption rates were more than 95%. This shows that acetamiprid is easily absorbed in rats.

Table 8. Calculation of absorption rates

Period	Male	Female
Days 0–1	(76.28 ÷ 78.92) × 100 = 96.7%	(73.16 ÷ 76.00) × 100 = 96.3%
Days 0–4	(81.07 ÷ 81.59) × 100 = 99.4%	(79.33 ÷ 79.73) × 100 = 99.5%

From Tanoue & Mori (1997a)

Table 9. Blood concentration of parent substance equivalents and half-life in rats after oral administration of acetamiprid

Group	Blood concentration at the time (h) after administration (mg/kg)											
	0.25	0.5	1	2	3	4	5	7	9	12	24	48
Males												
B	0.55	0.78	0.88	0.81	0.74	0.66	0.58	0.40	0.27	0.14	0.02	< 0.02
C	16.2	23.5	31.4	38.4	39.3	39.9	38.1	33.6	29.0	23.0	5.2	0.3
CN-B	0.64	0.89	0.97	0.95	0.89	0.86	0.79	0.62	0.47	0.30	0.06	0.01
Females												
B	0.79	1.00	1.00	0.88	0.80	0.72	0.63	0.46	0.32	0.20	0.04	< 0.02
C	8.1	15.5	22.4	25.6	28.7	30.3	29.0	27.2	23.6	21.6	9.0	0.4
CN-B	0.53	0.86	0.97	0.96	0.91	0.86	0.78	0.59	0.43	0.26	0.05	0.01

From Tanoue & Mori (1997a)

Table 10. Average C_{max}, range in T_{max} and half-life values of acetamiprid in rats

Group	Sex	$t_{½}$ (h)	C_{max} [a] (mg/kg)	T_{max} [b] (h)
B	Male	7.11	0.91	0.5–2.0
	Female	5.84	1.01	0.5–1.0
D	Male	8.07	40.50	3.0–5.0
	Female	15.03	31.46	3.0–7.0
CN-B	Male	5.90	0.97	1.0
	Female	11.29	0.97	1.0–2.0

From Tanoue & Mori (1997a)
[a] Average C_{max} of five individual values.
[b] Range in T_{max} of five individual values.

After acetamiprid reached its maximum concentrations in the blood, its levels decreased linearly and rapidly. The half-lives of the radioactivity were 5.84–7.11 hours for group B and 8.07–15.03 hours for group D. Similar to group B, the absorption in group CN-B was rapid, and the maximum concentrations in the blood were 0.97 mg/kg for both sexes at 1–2 hours after administration. The blood levels then decreased linearly and rapidly, with half-lives of the radioactivity of 5.90–11.29 hours (Tables 9 and 10).

In group A, the rates of excretion in the urine and faeces 1 day after dosing were 76.00–78.92% and 11.44–11.90% of the initially administered radioactivity, respectively. Rates of excretion in the urine and faeces 1 day after dosing in group B were 73.16–76.28% and 9.91–11.10%, respectively, whereas those in group CN-B were 75.15–79.24% and 4.12–4.56%, respectively. In group D, 72.84% and 56.39% of the total radioactivity were excreted in the urine of males and females, respectively, 1 day after dosing, and 6.13% and 10.20% of the total radioactivity were excreted into the faeces of males and females, respectively. In all of the groups, total excretion rates (i.e. the sum of the excretion

Table 11. Excretion rate of radioactivity in rats after administration of acetamiprid

Group	% of initially administered radioactivity									
	Urine				Faeces				Residual in body	Sum
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4		
Males										
A	78.92	1.89	0.56	0.22	11.44	3.32	0.67	0.12	0.63	97.78
B	76.28	4.09	0.46	0.23	9.91	1.53	0.15	0.05	0.42	93.13
D	72.84	11.98	1.17	0.44	6.13	6.39	0.36	0.08	0.74	100.13
CN-B	79.24	9.58	1.05	0.40	4.56	0.63	0.05	0.05	0.96	96.55
Females										
A	76.00	2.40	0.99	0.35	11.90	4.04	0.88	0.22	0.48	97.26
B	73.16	4.63	0.90	0.64	11.10	2.21	0.22	0.26	0.52	93.64
D	56.39	15.20	1.33	0.92	10.20	6.61	0.43	0.09	0.58	91.74
CN-B	75.15	10.93	1.37	0.90	4.12	0.88	0.13	0.06	0.84	94.38

From Tanoue & Mori (1997a)

in urine and faeces) were more than 90% in a 4-day period, and the residual radioactivity in the body was less than 1% of the dose. Because faecal excretion of radioactivity was also observed in group A with intravenous dosing, biliary excretion was suggested. The absorption rates of acetamiprid were all more than 95%, as calculated from the urinary excretion rates in groups A and B (Table 11).

Tissue concentrations in groups B and D were investigated 1, 5, 10 and 96 hours and 5, 14, 24 and 96 hours after administration, respectively, and the half-lives of radioactivity in the tissues were calculated. In a short time after dosing, the radioactivity was widely distributed in the body, but the concentrations in bone and fat were clearly low compared with the blood concentration. In contrast, the adrenal (group B: 1.344–2.409 mg/kg at 1 hour; group D: 51.88–62.87 mg/kg at 5 hours), thyroid (group B: 1.345–1.493 mg/kg at 1 hour; group D: 64.72–68.13 mg/kg at 5 hours), liver (group B: 1.651–1.711 mg/kg at 1 hour; group D: 61.34–62.02 mg/kg at 5 hours) and kidney (group B: 1.458–1.777 mg/kg at 1 hour; group D: 52.55–55.66 mg/kg at 5 hours) had higher concentrations than the blood (group B: 0.771–0.803 mg/kg at 1 hour; group D: 31.46–34.77 mg/kg at 5 hours). The rate of disappearance of radioactivity in the tissues was nearly the same as that in the blood. The blood concentrations in groups B and D were 0.001 mg/kg and 0.07 mg/kg, respectively, at 96 hours after dosing. The half-lives of the radioactivity in the tissues ranged from 2.9 to 7.9 hours (group B) and from 6.0 to 8.5 hours (group D). There were no tissues that were presumed to have accumulated the substance. Similarly to groups B and D, the tissue concentrations in groups A and CN-B were low 96 hours after dosing (Tables 12–15).

In summary, acetamiprid orally dosed in rats was rapidly absorbed and widely distributed into the tissues via blood. The majority of the radioactivity was excreted in the urine through the kidney and in the faeces via bile. The disappearance of the radioactivity from the body of the rat was rapid, and there were no tissues that are presumed to accumulate the compound. No differences in the sexes were observed. This study meets the requirements for GLP, and a QA statement was attached (Tanoue & Mori, 1997a).

A biliary excretion study was conducted using Sprague-Dawley bile duct–cannulated rats approximately 10–12 weeks old at dosing. Four male and four female bile duct–cannulated rats received single doses of [^{14}C]acetamiprid in 0.9% saline through an intragastric cannula. The average dose rates were 1.02 and 1.07 mg/kg bw for the male and female rats, respectively. The radiochemical

Table 12. Tissue concentration of parent substance equivalents in rats after oral administration of acetamiprid (ring label, group B)

	Mean tissue concentration (mg/kg)			
	1 h	5 h	10 h	96 h
Males				
Spleen	0.750	0.438	0.217	0.001
Heart	0.828	0.507	0.255	0.001
Bone	0.470	0.285	0.146	0.001
Lung	0.864	0.507	0.254	0.001
Adrenal	1.344	0.784	0.394	0.001
Sciatic nerve	0.786	0.502	0.252	0.001
Pancreas	0.858	0.503	0.251	0.001
Thyroid	1.493	0.556	0.251	0.002
Whole blood	0.771	0.458	0.221	0.001
Brain	0.677	0.383	0.179	0.001
Liver	1.711	0.918	0.409	0.002
Kidney	1.777	1.268	0.521	0.003
Muscle	0.755	0.467	0.219	0.001
Fat	0.359	0.216	0.093	0.003
Testis	0.754	0.430	0.215	0.000
Skin	0.711	0.430	0.226	0.002
Carcass	0.874	0.516	0.304	0.009
Females				
Spleen	0.801	0.500	0.185	0.001
Heart	0.901	0.616	0.217	0.001
Bone	0.461	0.302	0.209	0.001
Lung	0.858	0.547	0.213	0.002
Adrenal	2.409	1.361	0.296	0.002
Ovary	0.822	0.519	0.183	0.001
Sciatic nerve	0.685	0.570	0.226	0.002
Pancreas	0.914	0.578	0.209	0.001
Thyroid	1.345	0.840	0.467	0.004
Whole blood	0.803	0.505	0.190	0.001
Brain	0.712	0.437	0.150	0.001
Liver	1.651	0.960	0.355	0.002
Kidney	1.458	0.965	0.392	0.003
Muscle	0.800	0.541	0.184	0.001
Fat	0.365	0.219	0.083	0.001
Skin	0.762	0.494	0.184	0.002
Carcass	0.818	0.612	0.262	0.016

From Tanoue & Mori (1997a)

Table 13. Distribution of parent substance equivalent radioactivity in tissues in rats after oral administration of acetamiprid (ring label, group B)

	% of initially administered radioactivity			
	1 h	5 h	10 h	96 h
Males				
Spleen	0.18	0.12	0.05	0.00
Heart	0.34	0.20	0.09	0.00
Lung	0.39	0.24	0.12	0.00
Adrenal	0.03	0.02	0.01	0.00
Pancreas	0.28	0.15	0.08	0.00
Thyroid	0.01	0.01	0.00	0.00
Brain	0.63	0.34	0.17	0.00
Liver	5.59	3.43	1.70	0.01
Kidney	1.66	1.31	0.51	0.00
Testis	0.74	0.43	0.22	0.00
Carcass[a]	73.04	46.14	28.87	0.40
Females				
Spleen	0.21	0.11	0.05	0.00
Heart	0.37	0.22	0.08	0.00
Lung	0.44	0.32	0.11	0.00
Adrenal	0.09	0.06	0.01	0.00
Ovary	0.05	0.04	0.01	0.00
Pancreas	0.32	0.17	0.05	0.00
Thyroid	0.01	0.01	0.00	0.00
Brain	0.86	0.53	0.18	0.00
Liver	6.16	3.92	1.57	0.01
Kidney	1.42	0.94	0.40	0.00
Carcass[a]	73.60	56.24	23.99	0.50

From Tanoue & Mori (1997a)

[a] "Carcass" includes residual tissues after necropsy (bone, nervous tissue, blood, muscle, fat and skin).

purity of [^{14}C]acetamiprid in the dose solution was determined to be 97.1% by HPLC analysis. One male and one female rat were dosed with placebo (0.9% saline, containing no test substance).

A steady increase in [^{14}C]acetamiprid residue level was observed in bile from 3 to 12 hours post-dosing, with the highest amount (percentage of administered dose) at 12 hours post-dosing in both male and female rats. The average recovery of the administered dose in bile over a 48-hour period was 19.9% ± 1.47% in the male rats and 18.6% ± 0.62% in the female rats. Recovery of the [^{14}C]acetamiprid residues excreted in bile accounted for less than 20% of the total administered dose, suggesting that bile is not a predominant excretory pathway in either the male or the female rats. The absorption of the test substance and the extent of first-pass metabolism/presystemic elimination were not significantly different between the sexes.

The average recovery of the administered dose in faeces over a 48-hour period was 6.72% ± 3.36% in the male rats and 5.84% ± 0.86% in the female rats. The average recovery of the administered dose in urine over a 48-hour period was 24.3% ± 5.22% in the male rats and 36.9% ± 3.80% in the female rats. In the male and female rats, the sum of urine plus cage rinses, 60.2% ± 5.20% and 64.4% ± 2.86%, respectively, accounted for the major residues, suggesting that most of the administered dose was excreted in urine.

The average recovery of the administered dose in liver at 48 hours post-dosing was 0.22% ± 0.13% in the male rats and 0.18% ± 0.18% in the female rats. The average recovery of the administered

Table 14. Tissue concentration of parent substance equivalents in rats after oral administration of acetamiprid (ring label, group D)

	Tissue concentration (mg/kg)			
	5 h	14 h	24 h	96 h
Males				
Spleen	35.67	15.35	4.83	0.04
Heart	37.70	17.57	5.77	0.06
Bone	22.12	10.24	3.51	0.04
Lung	43.65	17.38	5.45	0.06
Adrenal	62.87	24.22	7.87	0.15
Sciatic nerve	47.18	14.15	6.55	0.08
Pancreas	36.83	15.96	5.27	0.04
Thyroid	68.13	49.85	7.89	0.13
Whole blood	31.46	15.45	5.05	0.07
Brain	27.80	12.28	3.60	0.03
Liver	61.34	25.84	9.70	0.16
Kidney	52.55	27.02	11.25	0.21
Muscle	33.37	14.77	4.90	0.06
Fat	15.97	7.41	2.05	0.10
Testis	30.54	13.88	4.62	0.06
Skin	30.26	14.90	5.95	0.18
Carcass	39.01	22.49	7.43	0.74
Females				
Spleen	34.49	11.85	4.93	0.04
Heart	37.39	13.76	5.54	0.05
Bone	20.91	8.13	3.09	0.07
Lung	40.39	13.40	5.34	0.05
Adrenal	51.88	17.44	8.82	0.05
Ovary	34.56	12.24	5.14	0.03
Sciatic nerve	50.84	15.78	6.51	0.01
Pancreas	39.40	13.77	5.69	0.03
Thyroid	64.72	18.42	7.83	0.08
Whole blood	34.77	10.71	5.30	0.07
Brain	28.89	9.64	3.92	0.06
Liver	62.02	21.16	9.20	0.12
Kidney	55.66	23.37	11.32	0.18
Muscle	34.36	13.97	5.15	0.07
Fat	17.73	6.00	3.25	0.15
Skin	31.61	11.70	5.33	0.16
Carcass	39.07	21.99	7.43	0.96

From Tanoue & Mori (1997a)

Table 15. Distribution of parent substance equivalent radioactivity in tissues of rats after oral administration of acetamiprid (ring label, group D)

	% of initially administered radioactivity			
	5 h	14 h	24 h	96 h
Males				
Spleen	0.17	0.08	0.02	0.00
Heart	0.33	0.15	0.05	0.00
Lung	0.78	0.16	0.05	0.00
Adrenal	0.03	0.01	0.01	0.00
Pancreas	0.22	0.09	0.04	0.00
Thyroid	0.01	0.01	0.00	0.00
Brain	0.53	0.23	0.07	0.00
Liver	4.30	2.34	1.16	0.02
Kidney	0.96	0.54	0.23	0.00
Testis	0.57	0.20	0.09	0.00
Carcass[a]	66.47	44.06	14.83	0.71
Females				
Spleen	0.19	0.06	0.02	0.00
Heart	0.35	0.11	0.04	0.00
Lung	0.45	0.15	0.06	0.00
Adrenal	0.04	0.01	0.01	0.00
Ovary	0.05	0.01	0.01	0.00
Pancreas	0.28	0.10	0.04	0.00
Thyroid	0.01	0.00	0.00	0.00
Brain	0.70	0.21	0.09	0.00
Liver	4.60	2.25	1.05	0.02
Kidney	1.15	0.50	0.23	0.00
Carcass[a]	69.72	43.08	14.70	0.56

From Tanoue & Mori (1997a)

[a] "Carcass" includes residual tissues after necropsy (bone, nervous tissue, blood, muscle, fat and skin).

dose in the gastrointestinal tract at 48 hours post-dosing was 0.46% ± 0.34% in the male rats and 0.33% ± 0.23% in the female rats. These results indicate that an insignificant amount of acetamiprid (< 1% in the collected tissues) was absorbed into the liver or remained in the gastrointestinal tract in both the male and female rats.

The total recoveries of the administered dose in the three male rats were 93.2%, 92.8% and 89.6%, respectively. The total recoveries of the administered dose in the three female rats were 94.9%, 93.5% and 91.2%, respectively.

The study described in this report was conducted in compliance with GLP. A QA statement was attached (Premkumar & Guo, 1995).

(b) Dermal route

The extent of absorption of acetamiprid was studied following application of 70% wettable powder containing [^{14}C]acetamiprid (purity 97.5%) to the skin of male Crl: CD(SD)BR rats. The animals were approximately 8 weeks old upon arrival and weighed 176–216 g (preliminary phase) and

Table 16. Dose administration

Phase	Group	Mean dose levels	
		mg/animal	μg/cm^2
Preliminary	1	0.0128	1.03
Preliminary	2	1.26	101
Definitive	4	0.0136	1.09
Definitive	5	0.119	9.53
Definitive	6	1.13	90.2

From Cheng (1997)

143–203 g (definitive phase). Target dose levels were 1, 10 and 100 μg/cm^2. Actual dose levels were 0.0136 mg/animal (1.09 μg/cm^2), 0.119 mg/animal (9.53 μg/cm^2) and 1.13 mg/animal (90.2 μg/cm^2).

A preliminary phase, consisting of two groups of four animals each, was conducted to evaluate and establish test material application and skin washing techniques. In the preliminary phase, male rats were dermally dosed at two levels (0.0128 mg/animal and 1.26 mg/animal) (Table 16).

In the definitive phase, three groups of 24 rats per group were dermally dosed with [^{14}C]-acetamiprid at three dose levels (Table 16). A control group of two rats received only the vehicle (1% carboxymethylcellulose aqueous solution). Urine and faeces were collected from each rat. Immediately before sacrifice, the skin at the application site was washed. Four rats per time point from each dose group were sacrificed at 0.5, 1, 2, 4, 10 and 24 hours; the control rats were sacrificed at 24 hours. At sacrifice, blood was collected by cardiac puncture.

Among the treated groups, the mean total recovery of radioactivity ranged from 96.6% to 102%, with most of the radioactivity (63.9–87.5%) in the skin wash. Radioactivity in the skin at the application site accounted for 10.2–32.2% of the applied radioactivity. Radioactivity in blood, excreta and carcasses accounted for less than 6.50% of the applied radioactivity.

The amounts of radioactivity found in the blood, eliminated in the excreta and retained in the carcass were considered to result from direct dermal absorption of [^{14}C]acetamiprid. Within groups, amounts of dermal absorption increased with increasing exposure time. The highest absorption was detected at the longest exposure time, 24 hours post-dosing, and accounted for 4.27% (0.581 μg), 6.34% (7.54 μg) and 2.82% (31.9 μg) for the 1.09, 9.53 and 90.2 μg/cm^2 dose groups, respectively. The sum of direct absorption and amount of radioactivity remaining in the skin at the application site was considered to be indirect absorption. The amounts of indirect absorption were 3–5 μg, 25–37 μg and 118–197 μg for the 1.09, 9.53 and 90.2 μg/cm^2 dose groups, respectively. The highest concentration of radioactivity in blood was 0.001 ppm for the 1.09 μg/cm^2 dose group at 24 hours post-dosing, 0.019 ppm and 0.010 ppm for the 9.53 μg/cm^2 dose group at 10 and 24 hours post-dosing, respectively, and 0.041 ppm for the 90.2 μg/cm^2 dose group at 24 hours post-dosing. The amount of direct absorption of acetamiprid in rats was proportional at the two lower dose levels and appeared to reach saturation at the highest dose level.

The study complied with GLP, and a QA statement was attached (Cheng, 1997).

1.2 Biotransformation

In order to undertake the qualitative and quantitative analysis of metabolites, the group IV animals of the Premkumar, Guo & Vegurlekar (1995) study described above were used. This group IV corresponds to group C of United States Environmental Protection Agency (USEPA) guidelines.

In this group, five males and five females were orally administered a daily dose of non-labelled acetamiprid for 14 days followed by a single dose of radiolabelled acetamiprid on day 15. The urine and faeces were collected once on day 14 and then at 24-hour intervals after administration of the [^{14}C]acetamiprid dose solution until sacrifice.

Qualitative analysis of metabolites was performed by thin-layer co-chromatography with unlabelled reference substances. The unknown metabolite was identified by liquid chromatography–tandem mass spectrometry (LC-MS/MS) as the glycine conjugate of IC-O (abbreviated as IC-O-Gly).

The major radioactive compounds in the excreta of rats were acetamiprid itself (males: 5.21%; females: 7.41%), demethylated compound IM-2-1 (males: 15.48%; females: 20.39%), nicotinic acid derivative IC-O (males: 11.12%; females: 8.01%) and IC-O glycine conjugate IC-O-Gly (males: 10.10%; females: 10.32%). In addition, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 were detected, but they accounted for less than 2% of the dose. There were several unknown compounds in urine, and the maximum abundance of an unknown compound in the "others" fraction was 1.0%.

It was considered that the major metabolic routes of acetamiprid in rats are the production of IM-2-1 by *N*-demethylation, the production of IC-O by detachment of the cyanoacetamide side-chain from IM-2-1, and the production of IS-1-1 and IS-2-1 by detachment of the cyanoacetamide side-chain from acetamide and IM-2-1, respectively.

The study described in this report was conducted in compliance with GLP. A QA statement was attached (Premkumar, Guo & Vegurlekar, 1995).

A similar picture of metabolites was also observed in the study of Tanoue & Mori (1997a) described above. In that study, radioactive compounds in the excreta of rats were identified and analysed quantitatively. The major compounds identified were acetamiprid itself (males: 6.10%; females: 5.63%), demethylated compound IM-2-1 (males: 19.51%; females: 19.00%) and nicotinic acid derivative IC-O (males: 28.19%; females: 25.52%) in group B; acetamiprid (males: 7.75%; females: 7.34%), IM-2-1 (males: 24.48%; females: 21.37%) and IC-O (males: 27.11%; females: 27.63%) in group D; and acetamiprid (males: 4.16%; females: 6.12%), IM-2-l (males: 13.39%; females: 18.98%) and IC-O (males: 28.13%; females: 24.73%) in group A. Acetamiprid (males: 3.98%; females: 4.51%), IM-2-1 (males: 16.95%; females: 16.56%), IS-1-1 (males: 13.15%; females: 16.45%) and IS-2-1 (males: 35.61%; females: 30.23%) were detected as the main compounds in group CN-B. IS-1-1 and IS-2-1 were thought to be generated by cleavage of the side-chains of acetamiprid and IM-2-1. In addition, IC-O-Gly, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 were detected in groups A, B and D, but each at less than 4% of the dose.

The main metabolic pathways of acetamiprid in rats were the transformation to IM-2-1 by demethylation and further to IC-O after cleaving IS-1-1 and IS-2-1 from acetamiprid and IM-2-1, respectively (Tanoue & Mori, 1997a).

The study described in this report was conducted in compliance with GLP. A QA statement was attached (Tanoue & Mori, 1997b).

Another metabolism study of acetamiprid in rats was performed to determine whether IM-1-5, a metabolite, was found in excreta. In each group, three male rats 7 weeks of age were dosed orally with [pyridine-2,6-^{14}C]acetamiprid by a single gavage at a low dose of 1 mg/kg bw or a high dose of 51 mg/kg bw. The excretion balance was also investigated until 96 hours after dosing.

Excretion was rapid, and most of the radioactivity (85.7% of the initially administered radioactivity for the low dose within 24 hours and 90.4% of the initially administered radioactivity for the high dose within 48 hours) was eliminated, especially in the urine.

The quantification of urinary and faecal metabolites was carried out by HPLC for each specimen collected during the 24 hours after dosing. The amount of IM-1-5 was estimated, and it account-

ed for 4.5% and 0.4% of the initially administered radioactivity in the low-dose and high-dose urine, respectively. In the faeces of both doses, no IM-1-5 was detected. The major metabolite in the excreta was IC-O, which accounted for 35.9% and 33.6% of the initially administered radioactivity at the low dose and high dose, respectively. IM-2-1 was the second major metabolite, with 18.5% and 9.3% of the initially administered radioactivity at the low dose and high dose, respectively. Acetamiprid was detected in amounts of 5.2% of the initially administered radioactivity at the low dose and 4.5% of the initially administered radioactivity at the high dose. Similar metabolite profiles were observed for the two doses.

The study was conducted as per GLP, and a QA statement was attached (Saito, 2003).

The proposed metabolic pathway is shown in Figure 3.

2. Toxicological studies

2.1 Acute toxicity

The oral median lethal dose (LD_{50}) of acetamiprid was 198 and 184 mg/kg bw in male and female mice, respectively. In different rat strains, the LD_{50} was in the range of 140–417 mg/kg bw. These studies demonstrated dose-related reversible toxic signs, such as crouching, tremor and convulsion, mydriasis and sensitivity (e.g. lateral position, salivation and ataxia), appearing within 10 minutes to 3 hours after administration and disappearing after 1 day. The dermal LD_{50} in rats was greater than 2000 mg/kg bw, with a dose of 2000 mg/kg bw causing neither mortality nor systemic toxicity. No local skin reaction was observed at the application site. When acetamiprid was administered to rats by inhalation through nose-only exposure, the median lethal concentration (LC_{50}) was greater than 1.15 mg/l of air (4-hour exposure), with a mass median aerodynamic diameter (MMAD) of 8 µm, the highest concentration tested, without any noted clinical signs. However, when rats were exposed whole body to acetamiprid with an MMAD of 5 µm, the LC_{50} was greater than 0.30 mg/l, the highest dose tested. Mydriasis in many rats and tremor and convulsion in a few rats were observed, which disappeared after 1 day. Acetamiprid was not an irritant in a study of ocular and dermal irritation in rabbits or a dermal sensitizer in the Magnusson and Kligman maximization test in guinea-pigs.

The results of acute toxicity studies with acetamiprid are summarized in Table 17. All the studies were conducted as per Organisation for Economic Co-operation and Development (OECD), USEPA and Japanese Ministry of Agriculture, Forestry and Fisheries guidelines and complied with GLP.

2.2 Short-term studies of toxicity

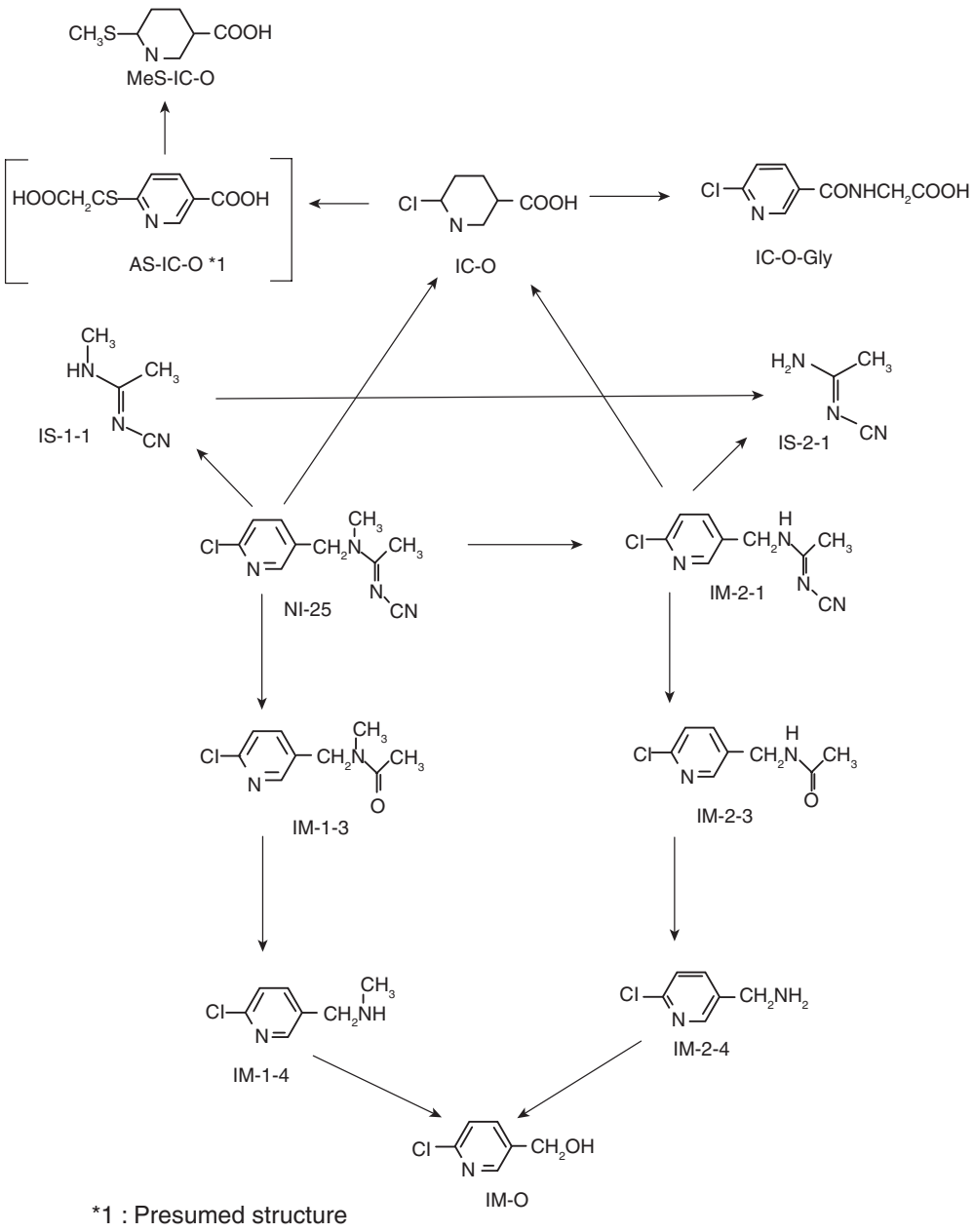
Short-term studies of oral toxicity in mice, rats and dogs were conducted.

(a) Oral administration

Mice

The subchronic toxicity of acetamiprid (lot No. 5910017-(Tox-470), purity 99.2%) in Cij: CD-1(ICR) mice (7 weeks of age) was assessed. The test compound was offered in the diet to 100 mice (10 of each sex per group) at a dose level of 0, 400, 800, 1600 or 3200 ppm for a period of 13 weeks. On the day of study initiation, the weights of animals were 34.4 ± 1.5 g (mean ± standard deviation [SD]) (range 31.2–37.8 g) for males and 25.8 ± 1.2 g (23.5–28.1 g) for females. Mean test compound consumptions for the 400, 800, 1600 and 3200 ppm groups were 53.2, 106.1, 211.1 and 430.4 mg/kg bw per day in males and 64.6, 129.4, 249.1 and 466.3 mg/kg bw per day in females, respectively.

Figure 3. Proposed metabolic pathway of acetamiprid in rats



The five females of the 3200 ppm group showed tremor at weeks 4–13, and two of them died, one at week 8 and the other at week 10. Two females, one each from the control and 800 ppm groups, died as a result of sampling accidents during the haematological examination performed at week 13. Two males of the 3200 ppm group died at week 12; one of them was euthanized in extremis because of decreased body weight compared with the initial body weight of this animal. These male animals did not show any tremor during in-life observations.

Decreased body weights were noted in both sexes of the 1600 and 3200 ppm groups at the study termination, and mean body weights of these groups were 87% and 66% of control values in males and 82% and 64% of control values in females, respectively (Table 18). Decreases in feed

Table 17. Summary of acute toxicity studies with acetamiprid

Species	Strain	Sex	Route	Batch No.; purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Results	Reference
Mouse	Crj:ICR,SPF	M + F	Oral	NNI-02; 99.46	M: 198 F: 184	—	[a]	Mochizuki & Goto (1992)
Rat	Crj:CD(SD), SPF	M + F	Oral	NNI-02; 99.46	M: 217 F: 146	—	[b]	Mochizuki & Kanaguchi (1992)
Rat	Crj:CD(SD), SPF	M + F	Oral	NFG-02; 99.9	M: 417 F: 314	—	[c]	Takaori (1997b)
Rat	Crj:CD(SD), IGS, SPF	M + F	Oral	NKP-194-07; 99.9 (suspended in corn oil)	M: 195 F: 140–200	—	[d]	Fujii (2002a)
Rat	Crj:CD(SD), SPF	M + F	Dermal	NNI-02; 99.46	> 2000	—	[e]	Mochizuki & Fujii (1998)
Rat	Crj:CD(SD), SPF	M + F	Dermal	NFG-02; 99.9	> 2000	—	[e]	Takaori (1997a)
Rat	Crj:CD(SD)	M + F	Inhalation 4 h (whole-body exposure)	NNI-03; 99.57	—	> 0.30 (dust; MMAD 5 µm)	[f]	Saika (1994)
Rat	Sprague-Dawley	M + F	Inhalation 4 h (nose-only exposure)	NFG-02; 99.9	—	> 1.15 (dust; MMAD 8 µm)	[g]	Jackson (1997)
Rabbit	New Zealand White	M	Primary dermal irritation	NNI-02; 99.46	—	—	Non-irritant	Mochizuki & Goto (1993a)
Rabbit	New Zealand White	M	Eye irritation	NI-25; 99.46	—	—	Non-irritant	Mochizuki & Goto (1993b)

Table 17 (continued)

Species	Strain	Sex	Route	Batch No.; purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Results	Reference
Guinea-pig	Dunkin/ Hartley	F	Skin sensitization effects (guinea-pig maximization)	NNI-02; 99.46	—	—	Non-sensitizer	Mochizuki (1994a)
Guinea-pig	Hartley	M + F	Skin sensitization effects (delayed contact hypersensitivity)	NFG-02; 99.9	—	—	Non-sensitizer	Coleman (1997)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; MMAD, mass median aerodynamic diameter

[a] At a dose of 100 mg/kg bw, crouching was observed for 20 minutes to 3 hours in males and for 20 minutes to 1 hour in females after administration. At 150–400 mg/kg bw in both sexes, most mice showed tremors for 10 minutes to 3 hours after administration. Additionally, in 150–400 mg/kg bw males and 290–400 mg/kg bw females, a few mice showed convulsion for 20 minutes to 1 hour after administration. All toxic signs disappeared within 1 day after the administration. In some surviving females of the two highest doses, the body weight decreased on day 1 and recovered afterwards. Six out of 27 dead mice revealed dark-reddish lung on necropsy.

[b] No toxic signs were observed in 100 mg/kg bw males and 80 mg/kg bw females. In 150–304 mg/kg bw males and in 100–230 mg/kg bw females, most rats showed crouching for 3 hours to 1 day after administration. In 150–510 mg/kg bw males and 100–510 mg/kg bw females, most rats showed tremors for 3 hours to 1 day after administration. A few rats showed low sensitivity, lateral position, prone position, salivation, urinary incontinence and ataxia for 60 minutes to 1 day. All toxic signs disappeared within 2 days after administration. Three rats out of 37 dead revealed dark-reddish lung on necropsy.

[c] Clinical signs noted in the treated rats were lacrimation (1 rat in 100 mg/kg bw group), mydriasis, tremor, clonic convulsion, prone position and lateral position. These signs appeared shortly after administration, and their incidences reached a maximum at 60 or 180 minutes. No abnormality was observed at gross necropsy.

[d] Mydriasis and tremor were observed in all dose groups. Clonic convulsions were observed in males at 200, 280 and 560 mg/kg bw and in females at 280, 400 and 560 mg/kg bw. These signs appeared shortly after administration and reached a maximum at 60 or 180 minutes. All deaths occurred within 1 day after administration. There were no treatment-related macroscopic observations.

[e] No toxic signs were observed, and no deaths occurred.

[f] Mydriasis in many rats and tremor and convulsion in a few rats were observed. These toxic signs disappeared after 1 day. Alopecia and crust were observed in a few rats after 1–4 days.

[g] Highest concentration tested. No clinical signs were noted during exposure.

Table 18. Mean body weight and comparison with control values

Dietary concentration (ppm)	Mean body weight (g) and comparison with control values (%)					
	Males			Females		
	Week 0	Week 6	Week 13	Week 0	Week 6	Week 13
0	34.26 (100)	39.30 (100)	41.22 (100)	25.78 (100)	30.44 (100)	33.64 (100)
400	34.45 (100)	38.55 (98)	41.71 (101)	25.77 (100)	29.47 (97)	30.73 (91)
800	34.20 (100)	38.30 (97)	40.13 (97)	25.89 (100)	29.92 (98)	31.37 (93)
1600	34.48 (99)	35.14 (89)a	35.83 (87)b	25.84 (100)	26.93 (88)b	27.45 (82)a
3200	34.46 (100)	26.95 (69)b	27.34 (66)c	25.73 (100)	21.43 (70)b	21.61 (64)c

From Nukui & Ikeyama (1992a)
Significantly different from the control group: "a" $P < 0.05$; "b" $P < 0.01$; "c" $P < 0.001$ (multiple comparison procedure)

consumption values (grams per animal per day) were noted in both sexes at 3200 ppm and in females at 1600 ppm. No effect of test compound treatment on feed consumption value per unit body weight (grams per kilogram body weight per day) was evident, except for a statistically significant decrease at week 1 and an increase at week 9 in the 3200 ppm males (Table 19). Feed efficiency values in both sexes of the 3200 ppm group were decreased from the control group and attained statistical significance occasionally throughout the study, except that the values increased over the control group at week 13.

All animals received ophthalmological examinations prior to study initiation and at week 12 of the study. No test compound treatment–related effects were evident in treated groups at the week 12 examination.

The haematological examination was not performed on the 3200 ppm group because of marked growth depression in both sexes. No effects of test compound treatment were evident in treated groups, except for a statistically significant decrease in haemoglobin concentration seen in the 1600 ppm group females.

Statistically significant decreases in total cholesterol concentration were seen in the females of the 800, 1600 and 3200 ppm groups at study termination. There was also a decrease in total cholesterol concentration in males at 3200 ppm. Glucose concentration was decreased in both sexes at 3200 ppm and in males only at 1600 ppm. Statistically significant increases were noted in blood urea nitrogen level in males and females at the high dose (3200 ppm); however, no effects on creatinine levels were seen at this dose (Table 20).

No statistically significant changes were noted in creatinine, total bilirubin, total protein, albumin, albumin to globulin ratio, sodium, potassium, chloride, calcium, phosphorus, alkaline phosphatase, lactate dehydrogenase or creatine kinase. Considering that proteinuria and renal lesions were not seen in these groups, the cause of the increased blood urea nitrogen may be prerenal.

A statistically significant decrease in urinary pH was found in the 3200 ppm group males at the week 12 examination. The mechanism of this decrease was not apparent.

Statistically significant increases were noted in the liver to body weight ratios of males and females at 800 ppm and above. In the high-dose (1600 and 3200 ppm) groups, decreases in organ weights were found for many organs, which were considered to be attributed to the decreased body weights of the groups (Table 21).

Mean relative liver weight ratios were increased. The liver lesion having a test compound relationship in both sexes was centrilobular hepatocellular hypertrophy. This hypertrophy could be due to induction of microsomal enzymes.

Table 19. Mean feed consumption and mean acetamiprid consumption

Dietary concentration (ppm)	Mean feed consumption (weeks 1–13)				Mean acetamiprid consumption (weeks 1–13)	
	g/animal per day		g/kg bw per day		mg/animal per day	
	Males	Females	Males	Females	Males	Females
0	5.0	4.8	129.7	159.1	0.0	0.0
400	5.2	4.7	132.9	161.6	53.2	646
800	5.1	4.8	132.6	161.8	106.1	129.4
1600	4.6	4.1	132.0	155.7	211.1	249.1
3200	3.6	3.1	134.5	145.7	430.4	466.3

From Nukui & Ikeyama (1992a)

Table 20. Statistically significant changes in blood chemistry examination

	Parameters	Dietary concentration (ppm)			Sex
Decrease	Glucose		1600	3200	Male
				3200	Female
	Total cholesterol			3200	Male
		800	1600	3200	Female
Increase	Urea nitrogen			3200	Male + female
	Alanine aminotransferase			3200	Male + female
	Aspartate aminotransferase			3200	Male
	Cholinesterase			3200	Male

From Nukui & Ikeyama (1992a)

Table 21. Statistically significant changes in organ weights

	Organ	Measurement[a]	Dietary concentration (ppm)			Sex
Decrease	Brain	Absolute		1600	3200	Female
	Thymus	Absolute			3200	Male + female
	Lung	Absolute			3200	Male + female
	Spleen	Absolute, relative			3200	Male + female
		Absolute		1600		Male
	Kidney	Absolute			3200	Male
		Absolute		1600	3200	Female
	Adrenal	Absolute			3200	Female
	Ovary	Absolute, relative			3200	Female
Increase	Brain	Relative			3200	Male + female
	Lungs	Relative			3200	Male + female
	Liver	Relative	800	1600	3200	Male + female
	Adrenal	Relative			3200	Male
	Testis	Relative		1600	3200	Male

From Nukui & Ikeyama (1992a)
[a] Absolute organ weight or organ weight relative to body weight.

Necropsy revealed no compound-related lesions. Histologically, dose-related centrilobular hepatocellular hypertrophy was seen in males and females of the 3200 ppm groups. In animals that died during the study, pulmonary congestion and thymic atrophy were observed, along with some lesions seen in terminally sacrificed animals.

Based on the results mentioned above, the effects of acetamiprid offered in the diet to Crj:CD-1(ICR) mice were tremor, decreased body weight gain, decreased feed consumption, decreased haemoglobin concentration, decreased serum total cholesterol and glucose levels, decreased urinary pH, increased liver to body weight ratios and centrilobular hepatocellular hypertrophy. The no-observed-adverse-effect level (NOAEL) was considered to be 400 ppm (equal to 53.2 mg/kg bw per day), based on a significant decrease in total cholesterol level in females at 800 ppm (equal to 106.1 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Nukui & Ikeyama, 1992a).

Rats

In a 13-week dietary study, the subchronic toxicity of acetamiprid (lot No. 31-0023-HY(Tox-447), purity > 99%) in Crj:CD(SD) rats (6 weeks of age) was assessed. The test compound was offered in the diet to 120 rats (10 of each sex per group) at a dose level of 0, 50, 100, 200, 800 or 1600 ppm for 13 weeks. On the day of study initiation, 60 males weighing 178.5 ± 8.5 g (mean ± SD) (range 157.5–190.5 g) and 60 females weighing 147.6 ± 7.0 g (range 137.3–161.1 g) were assigned to one of the six groups by a computerized randomization procedure. Mean test compound consumptions of the 50, 100, 200, 800 and 1600 ppm groups were 3.1, 6.0, 12.4, 50.8 and 99.9 mg/kg bw per day in males and 3.7, 7.2, 14.6, 56.0 and 117.1 mg/kg bw per day in females, respectively.

There were no signs of reaction to treatment in any treated animals. All animals survived throughout the study. Mean weights for high-dose (800 and 1600 ppm) males and females were significantly less than those of the control group throughout the study. Mean body weights of these groups were 91% and 87% of control values in males and 89% and 79% of control values in females, respectively (Table 22). Feed consumption values of these groups were lower than control values. Feed efficiency values of the 1600 ppm animals were occasionally decreased.

Ophthalmological examination did not reveal any effects of test compound treatment in the 1600 ppm group animals at week 12–13 examinations. No effects of test compound treatment were found in the haematological parameters in the treated group animals at the 13-week examination. Increases in total cholesterol concentration were seen in the 1600 ppm group males and females at study termination, reaching statistical significance in the males. On urinalysis, although a statistically significant decrease in urinary ketone body was found in the 1600 ppm males, it was considered to be an occasional occurrence.

Statistically significant increases were noted in the liver to body weight ratios of the 800 and 1600 ppm males and females. Mean absolute thyroid weights and thyroid to body weight ratio were increased to a statistically significant degree only in the males of the two highest dose groups (800 and 1600 ppm), but not in females. Because of the decreases in final body weight in the high-dose groups, statistically significant increases in organ weight ratios (testis, brain, lung, heart and kidney) and decreases in absolute organ weights (adrenal, heart and kidney) were noted in the high-dose groups; however, no microscopic findings were correlated with the weight changes (Table 23).

Necropsy revealed no compound-related lesions. Histologically, dose-related centrilobular hepatocellular hypertrophy was seen in the males and females of the 800 ppm groups and above. Although the thyroid weights were increased in males of the high-dose group, there were no correlating microscopic observations to explain the weight increase. Other microscopic changes were occasionally seen in the control and treated groups, but they were unrelated to the treatment of test compound. One mammary adenocarcinoma, which is considered to be spontaneous, was seen in a female of the 200 ppm group. Other lesions were occasionally seen in the liver (microgranuloma), stomach

Table 22. Mean body weight and comparison with control values

Dietary concentration (ppm)	Mean body weight (g) and comparison with control values (%)					
	Males			Females		
	Week 0	Week 6	Week 13	Week 0	Week 6	Week 13
0	178.5 (100)	424.6 (100)	504.9 (100)	147.7 (100)	267.0 (100)	307 (100)
50	178.4 (100)	417.6 (98)	498.5 (98)	147.9 (100)	261.7 (98)	295.0 (96)
100	178.4 (100)	414.6 (98)	507.6 (100)	147.5 (100)	263.0 (99)	304.9 (99)
200	178.6 (100)	427.8 (101)	525.3 (104)	147.7 (100)	265.5 (99)	315.0 (102)
800	178.5 (100)	384.2 (90)a	463.7 (91)	147.5 (100)	240.7 (90)a	273.6 (89)
1600	178.8 (100)	364.4 (86)b	441.4 (87)a	147.4 (100)	209.6 (79)b	242.5 (79)b

From Nukui & Ikeyama (1992b)
Significantly different from the control group: a, $P < 0.05$; b, $P < 0.01$ (multiple comparison procedure)

Table 23. Statistically significant changes in organ weights

Change	Organ	Measurement[a]	Dietary concentration (ppm)		Sex
Increase	Thyroid	Absolute, relative	800	1600	Male
	Liver	Relative	800	1600	Male + female
	Testis	Relative	800	1600	Male
	Brain, lungs, kidney (left)	Relative		1600	Male + female
	Kidney (right)	Relative	800	1600	Female
	Heart	Relative		1600	Female
Decrease	Adrenal	Absolute		1600	Female
	Heart	Absolute		1600	Female
	Kidney (left)	Absolute		1600	Female

From Nukui & Ikeyama (1992b)
[a] Absolute organ weight or organ to body weight ratio.

(haemorrhage), kidney (lymphocytic inflammatory infiltrate, calcium deposition and regenerative tubules), prostate (lymphocytic inflammatory infiltrate), thymus (haemorrhage), conjunctiva (cell infiltrate), skin (follicular atrophy), spleen (microgranuloma) and lung (cell infiltrate).

Based on the results mentioned above, the effects of acetamiprid offered in the diet to rats were decreased body weight gain, decreased feed consumption, increased serum total cholesterol level, increased liver to body weight ratio and centrilobular hepatocellular hypertrophy in the 800 and 1600 ppm groups (equal to 50.8 and 99.9 mg/kg bw per day, respectively). The NOAEL was 200 ppm (equal to 12.4 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Nukui & Ikeyama, 1992b).

Dogs

To assess potential toxicity, acetamiprid (lot No. NNI-02, purity 99.46%) was administered via dietary admixture to 16 Beagles (two of each sex per group) at a dose level of 250, 500, 1000 or 3000 ppm for a period of 28 days. Control animals (two of each sex per group) received standard laboratory diet. Animals receiving 3000 ppm started with a dose of 125 ppm daily up to test day 12, after which the dose was increased to 3000 ppm, as it was decided that an increase in the low dose level was necessary because of a lack of significant toxicity at the 1000 ppm dose level. After the concentration was increased to 3000 ppm, animals in this group continued to receive the 3000 ppm

Table 24. Test substance intake

Group	Dietary concentration (ppm)	Test substance intake (mg/kg bw per day)	
		Males	Females
II	125	3–5	4–6
III	250	8–9	6–10
IV	500	16–18	15–22
V	1000	22–34	28–43
II	3000	21–81	23–82

From Auletta (1992)

dose level for a 28-day period. At the initiation of the study, the animals were approximately 6 months old, and the mean weights were 11 kg (range 9.8–12.6 kg) for males and 9 kg (range 7.3–8.6 kg) for females.

Physical observations, body weight and feed consumption measurements, haematology and biochemistry studies were performed on all animals pretest and at the termination of the treatment period. After at least 28 days of treatment, all survivors were sacrificed, selected organs were weighed and organ to body weight and organ to brain weight ratios were calculated. Complete gross postmortem examinations were conducted on all animals.

Analysis of preliminary batches of diet confirmed that the mixing procedure used produced homogeneous mixtures of acetamiprid in the diet and that this material was stable in the diet when stored at room temperature for at least 14 days after preparation. Analyses confirmed that diets administered were of the appropriate concentrations. Individual test substance intake values, based on feed consumption values and nominal dietary concentrations, ranged as shown in Table 24. The wide range of values for the group receiving 3000 ppm reflects the wide variability of feed consumption values for this group.

All animals survived throughout the study. Acetamiprid administration produced marked weight losses (11–19% of pretest weights), correlating with decreased feed consumption and an emaciated appearance, in dogs receiving 3000 ppm and possible slight decreases in body weight gain and/or feed consumption in animals receiving 1000 ppm. Body weight and feed consumption patterns for animals receiving dietary concentrations of 125, 250 or 500 ppm were comparable to patterns seen in control animals.

No effect of acetamiprid administration on haematology or biochemistry parameters was evident at any dose level. Organ weights were comparable for control and treated groups or exhibited differences consistent with body weight losses; macroscopic postmortem examinations were unremarkable.

Based on the body weight losses seen in animals receiving a dietary concentration of 3000 ppm and the absence of a definitive effect at 1000 ppm, the NOAEL for dietary administration of acetamiprid to dogs for 4 weeks under the conditions of this study is 1000 ppm (equal to a range of 22–43 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Auletta, 1992).

In a second dog study, acetamiprid (lot No. NNI-01, purity 99.36%) was administered orally via dietary admixture to 24 Beagle dogs (four of each sex per group) at a dose level of 320, 800 or 2000 ppm (equal to 13, 32 and 58 mg/kg bw per day for males and 14, 32 and 64 mg/kg bw per day for females, respectively) for a period of at least 3 months (Table 25). Control animals (four of each sex per group) received untreated standard laboratory diet. At the initiation of the study, the animals

Table 25. Test substance intake

Group	Dietary concentration (ppm)	Mean test substance intake (mg/kg bw per day)			
		Males		Females	
		Range	Average	Range	Average
II	320	12–14	13	13–14	14
III	800	30–34	32	28–35	32
IV	2000	35–81	58	42–85	64

From Auletta (1994a)

were approximately 6 months old, and the mean weights were 8.6 kg (range 7.6–10 kg) for males and 8.0 kg (range 7.3–8.6 kg) for females.

Physical observations, ophthalmoscopic examinations, body weight and feed consumption measurements, haematology, clinical chemistry and urinalyses were performed on all animals pretest and at selected intervals during the treatment period. After at least 3 months of treatment, all survivors were sacrificed, selected organs were weighed and organ to body weight and organ to brain weight ratios were calculated. Complete gross postmortem examinations and histopathological evaluation of selected tissues were conducted on all animals.

Analysis of preliminary mixes confirmed that the preparation procedure used for this study produced homogeneous mixtures and that the test substance was stable in the diet for at least 14 days. Analyses conducted during the treatment period confirmed that diets of appropriate concentrations were administered.

All animals survived throughout the study. Dose-related effects on body weights were evident early in the study in the groups receiving the 800 and 2000 ppm concentrations. All high-dose (2000 ppm) dogs lost weight during the 1st week of study, and all but one lost weight between the 1st and 2nd weeks. Little or no weight change occurred in these groups during the subsequent weeks. Mean weights and weight gains for mid-dose (800 ppm) dogs were slightly lower than control values during the first 4 weeks and generally close to control values thereafter. Body weights and body weight gains for low-dose (320 ppm) males and females were comparable to concurrent control values throughout the study (Figures 4 and 5).

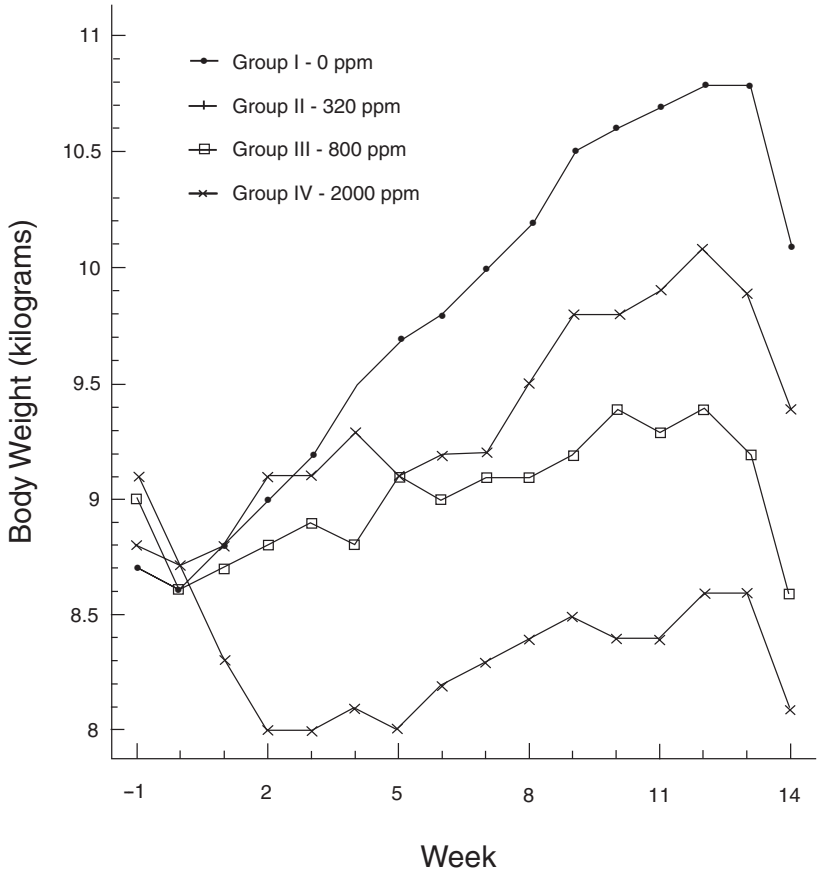
Feed consumption alterations were consistent with body weight patterns. Feed consumption values for high-dose males were lower than control values during the first 6 weeks of treatment; values for high-dose females were lower than control values through week 10. Values for mid-dose females were slightly lower than control values for weeks 1 through 3, but generally comparable to control values thereafter. Values for mid-dose males and low-dose males and females were comparable to control values.

No effect of acetamiprid administration was evident from clinical observations or ophthalmological examinations. No clear effects were seen in clinical laboratory studies (haematology, biochemistry and urinalysis).

Alterations suggestive of an effect of acetamiprid administration on organ weights consisted of a dose-related, statistically significant decrease in mean thyroid/parathyroid weights, relative to control, for all three treated groups of females. Mean thyroid/parathyroid to body weight ratios for all groups and the mean thyroid/parathyroid to brain weight ratio for the high-dose group were also statistically significantly lower than the control values. Similar differences were not apparent in males treated at any dose level.

Although decreased thyroid/parathyroid weights were seen for females at all dose levels (320, 800 and 2000 ppm), the toxicological significance of this finding, in the absence of morphological

Figure 4. Group mean body weight values in male dogs



Source: Auletta (1994a)

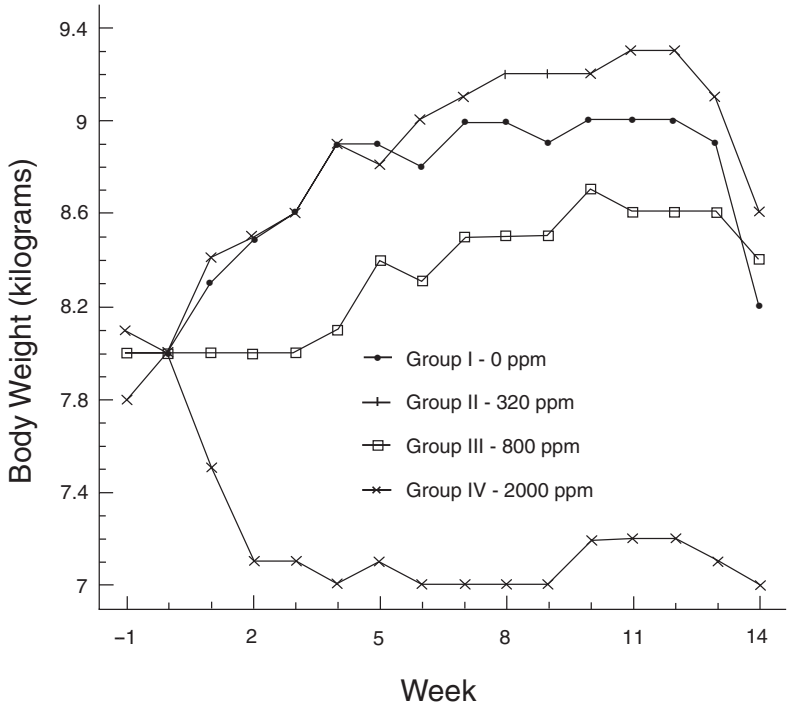
alterations, is not clear. Based on all other evaluations, the NOAEL for dietary administration of acetamiprid to dogs for 3 months under the conditions of this study was 800 ppm (equal to 32 mg/kg bw per day), based on the decrease in body weight at 2000 ppm (equal to 58 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Auletta, 1994a).

In the final dog study, acetamiprid (lot No. NNI-03, purity > 99%) was administered orally via dietary admixture to 24 Beagle dogs (four of each sex per group) at a dose level of 240, 600 or 1500 ppm (equal to 9, 20 and 55 mg/kg bw per day for males and 9, 21 and 61 mg/kg bw per day for females, respectively) for a period of at least 12 months. Control animals (four of each sex per group) received untreated standard laboratory diet. At the initiation of the study, the animals were approximately 6 months old, and the mean weights were 9.3 kg (range 7.6–10.3 kg) for males and 8.3 kg (range 7.6–10.1 kg) for females.

Physical observations, ophthalmoscopic examinations, body weight and feed consumption measurements, haematology, biochemistry and urinalyses were performed on all animals pretest and at selected intervals during the treatment period. After at least 12 months of treatment, all animals were sacrificed, selected organs were weighed and organ to body weight and organ to brain weight ratios were calculated. Complete gross postmortem examinations and histopathological evaluation of selected tissues were conducted on all animals.

Figure 5. Group mean body weight values in female dogs



Source: Auletta (1994a)

All animals survived throughout the study. No significant abnormalities were noted during weekly examinations, and daily observations were generally unremarkable.

Terminal ophthalmological examinations revealed no evidence of acetamiprid-related changes.

Moderate mean weight losses (0.5 kg) occurred in high-dose (1500 ppm) females during weeks 1 and 2 and in high-dose males between weeks 2 and 3.

Body weights stabilized after these initial decreases, although mean weights remained lower than concurrent control values for high-dose males and females for the remainder of the study. Mean weights at study termination for high-dose males and females were 16% and 20%, respectively, lower than control values.

Mean body weights and body weight gains for low-dose (240 ppm) and mid-dose (600 ppm) males and females were generally comparable to or higher than control values throughout the study. The initial weight losses seen in high-dose males and females correlated with statistically significant decreases in feed consumption, relative to control values, at weeks 1 and 2. Feed consumption values after week 3 were close to control values for test material–treated males and comparable to or slightly lower than control values for test material–treated females.

Test material intake, based on feed consumption data and nominal dietary concentrations, ranged during the study as shown in Table 26.

Haematology, blood biochemistry and urinalysis evaluations performed periodically throughout the study revealed no evidence of toxicity associated with acetamiprid administration.

No adverse effects of acetamiprid administration on organ weights at study termination were evident.

All the animals survived the experiment and were killed at the end of 12 months. Few gross lesions were observed at the terminal necropsy. The gross lesions occurred sporadically among the

Table 26. Mean test material intake

Group	Dietary concentration (ppm)	Mean test material intake (mg/kg bw per day)			
		Males		Females	
		Range	Average	Range	Average
II	240	8–10	9	7–11	9
III	600	18–23	20	17–25	21
IV	1500	39–65	55	36–75	61

From Auletta (1994b)

Table 27. Comparative NOAELs and LOAELs of three dog studies

Period of study	NOAEL		LOAEL		Critical end-points
	ppm	mg/kg bw per day	ppm	mg/kg bw per day	
28 days	1000	22–34	3000	21–81	Decrease in body weight (11–19% of pretest weight)
3 months	800	32	2000	58	Decrease in body weight
12 months	600	20	1500	55	Initial body weight losses and decreased body weight gains during the study in males and females

control and the treated groups. There were no compound-related lesions among the males and females in this study. There were no compound-related microscopic changes among males and females of this study. The microscopic findings either occurred sporadically or otherwise showed similar incidence between the control and the treated groups.

Based on the initial body weight losses and decreased body weight gains during the study in males and females receiving the highest dietary concentration (1500 ppm, equal to 55 mg/kg bw per day) of acetamiprid, the NOAEL for dietary administration of this material to dogs for 1 year under the conditions of this study was 600 ppm (equal to 20 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Auletta, 1994b).

When all three dog studies (28 days, 90 days and 1 year) described above are considered together, it is observed that the common critical toxicological effect is growth retardation, represented by a decrease in body weight. In view of this, there is a need for prescription of an overall NOAEL, which is set at 800 ppm (equal to 32 mg/kg bw per day). Table 27 compares the NOAELs and lowest-observed-adverse-effect levels (LOAELs) of these three dog studies.

(b) Dermal application

Rabbits

Acetamiprid (batch No. NFG-02, purity 99.9%) was evaluated for dermal irritation and systemic toxicity when applied to the dorsal intact skin of male and female New Zealand White rabbits (Hra:[NZW] SPF) (five of each sex per group) 5 days/week for at least 3 consecutive weeks. Dose levels were 100, 500 and 1000 mg/kg bw per day. A fourth group of five male and five female rabbits served as the control and was sham treated. The body weight of animals at the initiation of the study was approximately 2–3 kg.

Survival, clinical signs, body weight and feed consumption data, gross signs of dermal irritation (graded according to Draize), clinical pathology studies (haematology and serum chemistry),

absolute and relative (to body weight) organ weight data, and necropsy and microscopic pathological findings were evaluated.

All animals survived until study termination. Clinical observations, body weight data and feed consumption measurements provided no evidence of toxicity. No signs of compound-induced dermal irritation were observed. There was no evidence of an effect of administration of the test material in the haematology, serum chemistry, necropsy or organ weight data. No compound-related histomorphological findings were observed in tissues that were examined microscopically (liver, kidney and treated and untreated skin).

In conclusion, under the conditions of this study, dermal application of acetamiprid at dose levels of 100, 500 and 1000 mg/kg bw per day for at least 3 consecutive weeks to rabbits of both sexes did not cause systemic toxicity, dermal irritation or histomorphological lesions in any of the tissues examined. In view of the above, the no-observed-effect level (NOEL) is 1000 mg/kg bw per day, the highest dose tested.

The study complied with GLP, and a QA statement was attached (Trutter, 1997).

2.3 Long-term studies of toxicity and carcinogenicity

Long-term studies of oral toxicity in mice and rats were conducted using acetamiprid.

Mice

In an 18-month oncogenicity study, acetamiprid (lot No. NNI-01, purity 99.38%) was offered to 60 male and 60 female Crl:CD-I (ICR)BR mice in the diet at a dose level of 130, 400 or 1200 ppm. The control group (60 animals of each sex) received untreated diet as obtained from the supplier. At the initiation of the study, the mice were 4 weeks old and weighed 23–28 g (males at randomization) and 19–26 g (females at randomization).

Each mouse was observed 3 times daily (twice daily on weekends and holidays) for mortality and overt signs of toxicity. Detailed observations were performed at least once weekly. Individual body weights and feed consumption values were recorded weekly for the first 16 weeks of the study and once every 4 weeks thereafter, and compound consumption was calculated from these values. Feed efficiency was calculated weekly for the first 16 weeks of the study. Average feed consumption and compound consumption are shown in Table 28.

Haematological evaluations were conducted on 10 mice of each sex per group at 12 and 18 months of the study. At the termination of the study, all mice were euthanized. A thorough post-mortem examination was conducted, and selected organ weights were determined at the interim and terminal sacrifices. Protocol-specified tissues were processed histologically, and a microscopic examination was conducted. Photomicrographs of representative lesions seen in microscopy were taken. A peer review was performed on all target organs and neoplasms and on all tissues from 10% of the mice randomly selected from the control and 1200 ppm groups.

No test article effect on survival was noted. Decreased defecation, decreased body weight and decreased feed consumption were observed in males and females at the 1200 ppm dose level. Body weights were slightly decreased in males at 400 ppm, and statistical significance was attained sporadically. The body weight gain of males at 400 ppm was statistically significantly decreased compared with controls through 13 weeks of study but was comparable to that of controls during the remainder of the study. In both males and females at 130 ppm, feed consumption was slightly increased over controls. No differences between the groups were noted for the haematological parameters. There were no treatment-related observations at necropsy, but at the end of 18 months, mean liver to body weight ratios were increased in males and females receiving 1200 ppm and also in females receiving 400 ppm. On microscopic examination, treatment-related hepatocellular hypertrophy was seen

Table 28. Average feed consumption and compound consumption

Dietary concentration (ppm)	Feed consumption (g/kg bw per day)		Compound consumption (mg/kg bw per day)	
	Males (%)[a]	Females (%)	Males	Females
0 (control)	157 (NA)	185 (NA)	NA	NA
130	156 (−0.6)	194 (+4.9)	20.3	25.2
400	164 (+4.4)	190 (+2.7)	65.6	75.9
1200	155 (−1.3)	179 (−3.2)	186.3	214.6

From Goldenthal (1994)
NA, not applicable
[a] Per cent difference from control.

in male and female mice receiving 1200 ppm at the 12-month interim sacrifice and at 18 months. An extremely low incidence of hepatocellular hypertrophy was present in males and females at 400 ppm. Hepatocellular hypertrophy is generally considered to be a pharmacologically adaptive response of the liver to exposure to a xenobiotic.

The only other statistically significant finding was an increased incidence of myeloid hyperplasia in the bone marrow of the femur in 1200 ppm males euthanized at the interim sacrifice. This change was considered spurious, as the increase was small, it was not significant when both mice at interim sacrifice and mice that died or were sacrificed in extremis were considered together, and the incidence in the bone marrow of the sternum was not statistically significantly increased.

There were no other test article–related effects noted.

Statistical analysis of tumour incidence showed no positive trends or differences.

Based on transient decreased body weight observed at 400 ppm (equal to 65.6 mg/kg bw per day) in males, the NOAEL of acetamiprid was considered to be 130 ppm (equal to 20.3 mg/kg bw per day). There was no evidence of an oncogenic effect in mice.

The study complied with GLP, and a QA statement was attached (Goldenthal,1994; Cunny, 2000a; Gopinath, 2001).

Rats

To evaluate the oncogenic potential as well as the general toxicity of acetamiprid, acetamiprid (lot No. NN I-01, purity 99.7%) was offered to groups of Crl: CD BR rats (60 of each sex per group) in the diet at a concentration of 160, 400 or 1000 ppm (equal to 7.1, 17.5 and 46.4 mg/kg bw per day for males and 8.8, 22.6 and 60 mg/kg bw per day for females, respectively) for 2 years. The control group (60 rats of each sex) was given untreated diet as obtained from the supplier. At the initiation of the study, the rats were 6 weeks old and weighed 164–208 g and 137–157 g for males and females, respectively, at randomization. Fresh diet was offered weekly.

Each rat was observed twice daily for mortality and signs of toxicity. Detailed clinical examinations were conducted weekly. Individual body weights and feed consumption were determined weekly for the first 14 weeks of the study and every 2 weeks thereafter. Feed efficiency was calculated weekly for the first 14 weeks of the study. An ophthalmoscopic examination was performed on all rats pretest and at 6, 12 and 24 months. Clinical pathology studies were conducted on 10 randomly selected male and female rats from each group at 3, 6, 12, 18 and 24 months, and, when possible, the same rats were used at each time interval. Complete necropsy examinations were conducted on rats that died on study or were euthanized in extremis, on 10 rats of each sex per group at the 12-month interim sacrifice and on all surviving rats at study termination. Selected organ weights were determined at the interim and terminal sacrifices. Protocol-specified tissues were processed histologically, and microscopic examinations were conducted and peer-reviewed.

Increased incidences of clinical signs were observed in males, such as rales, hunched posture, laboured breathing and red/brown material around the nose; hunched posture and laboured breathing were also observed in the females, particularly in the latter part of the study. These increased incidences occurred mainly in the 400 and 1000 ppm groups. Other in-life observations included low weekly/biweekly mean body weights and body weight gains and high mean feed consumption values (1000 ppm males; 400 and 1000 ppm females), increased mean water consumption secondary to high feed consumption for the 1000 ppm males (week 24) and females (week 76) and, possibly, low mean serum triglyceride concentrations for the 1000 ppm females.

Average feed and compound consumption values (weeks 1–104) are summarized in Table 29.

Table 30 summarizes the body weight data at study termination (104 weeks).

There were no apparent test article–related changes in haematological parameters. As regards the serum biochemical values, at 12 and 18 months and at study termination, females in the 1000 ppm group had lower triglyceride values than controls. These differences were statistically significant at 12 and 18 months and were considered to be possible effects of acetamiprid administration. These changes may be secondary to altered lipid metabolism, as the rats had higher feed consumption, but lower body weights, than controls. Some biochemical parameters were occasionally statistically significantly different from control values; however, these were considered incidental and not associated with test article administration.

Test article–related pathological changes included trace to mild centrilobular hepatocellular hypertrophy in the 400 and 1000 ppm males and the 1000 ppm females; trace to moderate hepatocellular vacuolation in the 400 and 1000 ppm males; and trace to severe microconcretions (calculi) in the renal papillae of the 1000 ppm males (Tables 31 and 32).

The incidences of hepatocellular hypertrophy and hepatocellular vacuolation were statistically significantly increased in the 400 and 1000 ppm male treatment groups when only the animals of the 12 month to termination study interval were analysed (Table 32), and also when the combined incidences (all animals from both study intervals) were analysed. Both findings were considered to be test article related in males in these treatment groups. In females, the incidence of hepatocellular hypertrophy was statistically significant ($P \leq 0.01$) only when all animals from both sacrifice intervals were combined and only in the 1000 ppm treatment group; hepatocellular hypertrophy was also considered test article related in the 1000 ppm female treatment group. The incidence of hepatocellular vacuolation did not achieve statistical significance in any female treatment group, regardless of the manner in which the animals were grouped for analysis, and was therefore not considered a test article–related effect in this sex. There were no macroscopic observations or organ weight changes that correlated with these findings.

At the time of the terminal sacrifice, compound-related non-proliferative microscopic changes were identified in the kidney of the 1000 ppm group males. Trace to severe microconcretions of the renal papilla were seen with increased incidence and severity in the 1000 ppm group males and were considered to be compound related in that treatment group (Table 33).

The incidence of microconcretions in all male groups (range 35.4–75.5%) exceeded MPI Research historical control values (range 0–14.82%; calculus/calculi used synonymously with microconcretions) and was statistically significantly increased ($P \leq 0.01$) in the 1000 ppm group when the animals of the 12 months to termination study interval were analysed, and also when the combined incidence (all animals from both sacrifice intervals) was analysed. Microconcretions of the renal papilla were not increased significantly in incidence or severity in females. There were no compound-related macroscopic or organ weight findings that correlated with these microscopic observations.

There were no proliferative lesions in any treatment group of either sex that were considered to be definitively test article related. In females, there was an increased incidence of hyperplasia of the mammary gland observed in the 1000 ppm group, which attained statistical significance. The incidences of these findings are presented in Table 34.

Table 29. Average feed consumption and compound consumption

Dietary concentration (ppm)	Feed consumption (g/kg bw per day)		Compound consumption (mg/kg bw per day)	
	Male (%)[a]	Female (%)[a]	Male	Female
0 (control)	43.9 (NA)	55.3 (NA)	NA	NA
160	44.3 (+0.9)	55.3 (−0.4)	7.1	8.8
400	43.9 (0.0)	56.5 (+2.2)	17.5	22.6
1000	46.4 (+5.7)	60.0 (+8.5)	46.4	60.0

From Hatch (1994)
NA, not applicable
[a] Per cent difference from control.

Table 30. Body weight at study termination (104 weeks)

Dietary concentration (ppm)	Body weight			
	Males		Females	
	g	%[a]	g	%[a]
0 (control)	667	NA	433	NA
160	668	+0.1	453	+4.6
400	673	+0.9	388	−10.4
1000	578	−13.3	367	−15.2

From Hatch (1994)
NA, not applicable
[a] Per cent difference from control.

Table 31. Test article–related observations (0–12 months interim)

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*10*	*11*	*13*	*11*
Males				
Liver hypertrophy	0	0	5*	10**
- trace	0	0	5	8
- mild	0	0	0	2
Hepatocyte vacuolation	2	4	10**	10**
- trace	2	3	9	8
- mild	0	0	1	2
- moderate	0	1	0	0
Females				
Liver hypertrophy	0	0	0	4
- trace	0	0	0	4*

From Hatch (1994)
* $P \leq 0.05$; ** $P \leq 0.01$

Table 32. Test article–related observations in males (12 months to termination)[a]

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*48*	*50*	*48*	*49*
Liver hypertrophy	0	0	15**	34**
- trace	0	0	15	22
- mild	0	0	0	12
Hepatocyte vacuolation	10	9	22*	29**
- trace	7	6	17	18
- mild	2	3	5	10
- moderate	0	0	0	1
- severe	1	0	0	0

From Hatch (1994)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] The changes were not observed in females.

Table 33. Compound-related observations in male kidney, 12 months to termination

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*48*	*50*	*48*	*49*
Microconcretion of papilla	17	23	23	37*
- trace	16	21	17	26
- mild	1	2	4	8
- moderate	0	0	2	2
- severe	0	0	0	1

From Hatch (1994)
* $P \leq 0.01$

However, the severity of mammary gland hyperplasia was trace to mild in most cases, and there was little, if any, significant increase in severity in a dose-related manner. No increase in incidence or severity of this finding was evident in any female treatment group at the 12-month interim necropsy interval. When all females on study were analysed as a group, the incidence of hyperplasia in the 1000 ppm group (26/60; 43.3%) did not exceed the range of historical control values for this finding at MPI Research (range 0–58.6%), and in no test article treatment group did the incidence of mammary gland hyperplasia attain statistical significance when compared with the incidence in the control group.

Because of the above considerations, the toxicological significance of mammary hyperplasia in the 1000 ppm treatment group is uncertain.

Although the incidence of mammary gland adenocarcinoma was statistically significant ($P \leq 0.05$) when analysed by the Cochran Armitage Trend Test and Peto Test, adenocarcinomas of the mammary gland were not considered to be a test article–related finding. Historical control data maintained at MPI Research show a range of incidences for mammary gland adenocarcinoma from 13.3% to 28.6%; the incidence of mammary gland adenocarcinoma in 400 ppm females of this study (16/60; 26.6.3%) and in 1000 ppm females of this study (17/60; 28.3%) is within the range of historical values, albeit just barely. In no case was the incidence of mammary gland adenocarcinoma in

Table 34. Incidence of hyperplasia/neoplasia of the mammary gland (all female animals combined)

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*59*	*60*	*60*	*60*
Mammary gland				
Hyperplasia	17	13	16	26*
- trace	12	10	12	19
- mild	5	3	3	6
- moderate	0	0	1	1
Fibroadenoma	17	15	10	15
Adenoma	1	0	4	3
Benign tumour (adenoma/fibroadenoma)	18	15	14	18
Adenocarcinoma	10	11	16	17**
Any mammary tumour	24	21	24	29

From Hatch (1994)
* $P \leq 0.01$ by Fisher's exact test; ** $P \leq 0.05$ by Cochran Armitage Trend Test and Peto Test

any test article treatment group significantly different from the incidence in the control group when analysed using the Fisher Exact Test. The incidences of animals with any mammary tumours (adenoma, fibroadenoma and/or adenocarcinoma) in the test article–treated group were not substantially different from the control group.

There were no test article–related proliferative lesions or neoplasms observed in this study.

An MPI Research Toxicology Working Group was convened to examine the increased incidences of certain clinical signs, particularly rales in the 160 ppm males. The Toxicology Working Group concluded that rales were not test article related in this group. As rales were the only potentially dose-related observation made in the 160 ppm group, the NOAEL of acetamiprid in this study was 160 ppm (equal to 7.1 mg/kg bw per day), based on trace centrilobular liver hypertrophy and hepatocyte vacuolation at 400 ppm (equal to 17.5 mg/kg bw per day).

Acetamiprid was not oncogenic in rats.

The study complied with GLP, and a QA statement was attached (Hatch, 1994; Pallen, 1999; Cunny, 2000b).

2.4 Genotoxicity

Acetamiprid was tested for genotoxicity in nine genotoxicity studies, of which six were in vitro studies and three were in vivo studies (Table 35). Acetamiprid gave negative results in all in vivo studies and four in vitro studies. In studies of chromosomal aberrations (mouse lymphoma forward mutation assay) in vitro, although acetamiprid was found to be negative without metabolic activation, it was questionable for inducing forward mutation with metabolic activation. In another study of chromosomal aberrations (in Chinese hamster ovary cells) in vitro, acetamiprid induced chromosomal aberration with metabolic activation and weakly without metabolic activation.

All the studies complied with GLP, and QA statements were attached.

On the basis of these studies, it is concluded that acetamiprid is unlikely to be genotoxic.

Table 35. Results of genotoxicity studies with acetamiprid

Genotoxicity study	Strain/species	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation study	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	99.2	Negative with S9 mix Negative without S9 mix	Kanaguchi (1993a)
DNA repair test on bacteria (rec assay)	*Bacillus subtilis* M45(rec−) and H17(rec+)	1359, 2718, 5435, 10 870, 21 740 µg/disc without metabolic activation, 679.4, 1359, 2718, 5435, 10 870 µg/disc with metabolic activation	99.46	Negative	Kanaguchi (1992b)
Gene mutation, mammalian cells[a]	CHO cells (*HPRT* locus)	*Without S9 mix* Test 1: 500, 1000, 2000, 2500, 3000, 3500, 4000 µg/ml Test 2: 1000, 2000, 2500, 3000, 3500, 4000 µg/ml *With S9 mix* Test 1: 250, 500, 1000, 1500, 2000, 3000, 3500, 4000 µg/ml Test 2: 500, 1000, 1500, 2000, 2250, 2500, 2750 µg/ml	Not given	Negative	Adams (1997)
Gene mutation/chromosomal aberration, mammalian cells[b]	Mouse lymphoma forward mutation assay, L5178Y Tk+/− cells (*TK* locus)	63.5–2000 µg/ml	99.57	Negative without metabolic activation Questionable for inducing forward mutation with metabolic activation	Cifone (1994)
Chromosomal aberration, mammalian cells[c]	CHO cells	17.0, 33.9, 67.8, 135.6, 271.3, 542.5, 1085, 2170 µg/ml	99.2	Induced chromosomal aberration with metabolic activation and weakly without metabolic activation	Kanaguchi (1992a)
Unscheduled DNA synthesis[d]	Rat primary hepatocytes	10.1–505 µg/ml	99.57	Negative	Ham (1994)

Table 35 (continued)

Genotoxicity study	Strain/species	Concentration	Purity (%)	Result	Reference
In vivo					
Micronucleus test[e]	CD-1 (ICR) mouse	Oral (gavage, one application) 20, 40, 80 mg/kg bw	99.57	Negative	Murli (1994a)
Chromosomal aberration test (bone marrow cells)[f]	Sprague-Dawley CD strain rat	Single oral dose of 250 mg/kg bw (gavage)	99.46	Non-clastogenic	Durward (1993)
UDS[g]	Sprague-Dawley strain rat (liver cell)	75, 150, 300 mg/kg bw	99.9	Negative	San & Sly (1997)

CHO, Chinese hamster ovary; DNA, deoxyribonucleic acid; HPRT, hypoxanthine-guanine phosphoribosyl transferase; S9, 9000 × *g* rat liver supernatant; TK, thymidine kinase; UDS, unscheduled DNA synthesis

[a] Acetamiprid was tested for its ability to induce forward mutation at the functionally hemizygous *HPRT* locus in CHO cells in vitro in both the presence and the absence of exogenous metabolic activation in the form of Aroclor 1254–induced rat liver S9. Toxicity was observed after treatment with acetamiprid in all of the tests, in both the absence and the presence of S9 mix. No significant increases in mutant frequency were observed in cultures treated with acetamiprid in any of the tests in either the absence or the presence of S9 mix. The positive controls induced highly significant increases in mutant frequency in all of the tests in both the absence and the presence of S9 mix. It was concluded that acetamiprid did not demonstrate mutagenic potential in this in vitro mammalian cell gene mutation assay.

[b] The assay was to evaluate the ability of acetamiprid to induce forward mutations at the *TK* locus in the L5178Y mouse lymphoma cell line. The test material was soluble in dimethyl sulfoxide at 500 mg/ml. In the preliminary cytotoxicity assay, cells were exposed to the test material at concentrations from 1.95 to 1000 µg/ml for 4 hours in the presence and absence of rat liver S9 metabolic activation. The test material remained in solution in culture medium at all concentrations tested. The test material was weakly to moderately cytotoxic with and without metabolic activation at 1000 µg/ml, and lower concentrations were non-toxic. The mutation assays were initiated with treatments up to about 5070 µg/ml in an attempt to obtain more cytotoxic dose levels. Four non-activation and four S9 metabolic activation mutation assays were initiated. The studies were repeated several times in an attempt to clarify a response and because shifts in cytotoxicity occurred. The test material produced dose-related increases in toxicity in all mutation trials. In the non-activation trials, the test material was lethal or excessively toxic between 1500 µg/ml and about 3000 µg/ml. Treatments that induced less than 10% relative growth were not used in the analysis, as these results are considered unreliable. None of the remaining treatments induced mutant frequencies that exceeded the minimum criterion for a positive response. In the presence of metabolic activation, treatments from 63.5 to about 2000 µg/ml were evaluated. Small increases that just exceeded the minimum criterion for a positive response were observed in two trials. One trial did not have highly toxic treatments, and the results were inconclusive. The last trial had one treatment that approached, but did not meet, the minimum criterion for a positive response. The test material was therefore considered to have questionable activity with activation. Acetamiprid was therefore evaluated as negative without metabolic activation and questionable for inducing forward mutations at the *TK* locus in L5178Y mouse lymphoma cells in the presence of S9 metabolic activation and under the conditions used in this study.

[c] In the direct method, chromosomal aberration was slightly increased ($P < 0.05$) at 175 and 700 µg/ml in comparison with that in the solvent control. In the metabolic activation method, chromosomal aberration was significantly increased with a dose–response relationship at the middle and high test concentrations (675 and 1350 µg/ml). The frequencies of chromosomal aberration did not increase in the metabolic activation without S9 mix (reference test). Benzo(*a*)pyrene, which requires metabolic activation for expression of mutagenicity, increased chromosomal aberrations in the presence of S9 mix, but not in the absence of S9 mix. Through both methods, chromosomal aberration frequencies in the negative and the solvent controls were in the range of historical background data. On the basis of the results, the author considered that acetamiprid induced chromosomal aberration with metabolic activation, and its activity was lower without metabolic activation.

[d] In the assay for UDS in rat liver primary cell cultures, the test material, acetamiprid, did not induce significant increases in UDS in two independent trials. In each trial described in Ham (1994), freshly prepared rat hepatocytes were exposed to acetamiprid at concentrations ranging from 5000 to 0.500 µg/ml in the presence of 0.37 MBq/ml ^{3}H-methyl thymidine (1554 GBq/mmol). In Trial 1, 15 treatments from 5000 to 0.500 µg/ml were initiated. The test material was insoluble in media at concentrations of 5000 and 4000 µg/ml, with apparent solubility occurring at 3000 µg/ml. Five treatments from 5000 to 1000 µg/ml were not analysed for nuclear labelling as a result of high toxicity. Six treatments from 500 to 10.0 µg/ml covered a good range of toxicity (53.2–98.4% survival) and were selected for analysis of nuclear labelling. None of the criteria used to indicate UDS were approached by the chemical treatments in Trial 1, and no dose-related response was observed. A second trial was initiated to confirm these results. Based upon cytotoxicity information obtained in Trial 1, 12 dose levels from 2020 to 0.505 µg/ml were initiated in Trial 2. Treatments from 2020 to 1010 µg/ml were not analysed as a result of high toxicity. Six treatments from 505 to 10.1 µg/ml covered a good range of toxicity (64.4–107.5% survival) and were selected for analysis of nuclear labelling. None of the criteria used to indicate UDS were approached by the chemical treatments in Trial 2. The data confirmed the results from Trial 1, and acetamiprid was evaluated as inactive in both trials of the rat primary hepatocyte UDS assay.

[e] In this mouse micronucleus assay, animals were dosed at 20, 40 and 80 mg/kg bw. Ten animals (5 males and 5 females) were randomly assigned to each dose/harvest time group. Positive control groups euthanized approximately 24 hours after dosing were included in the assay. The animals dosed with the test article and the vehicle control were euthanized approximately 24, 48 and 72 hours after dosing for extraction of bone marrow. The test material, acetamiprid, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay.

[f] The study was performed to assess the potential of acetamiprid to produce damage to chromosomes or the mitotic apparatus of rats when administered by the oral route. The method used followed that described in the OECD guidelines. Following a preliminary range-finding study to confirm the oral toxicity of the test material, the chromosomal aberration study was conducted using acetamiprid at the maximum tolerated dose of 250 mg/kg bw. In the study, groups of 10 rats (5 males and 5 females) were given a single oral dose of acetamiprid at the maximum tolerated dose (250 mg/kg bw). Animals were killed 6, 24 or 48 hours later, the bone marrow was extracted and slide preparations were made and stained. Bone marrow cells were scored for the presence of chromosomal aberrations. Further groups of rats were treated with arachis oil BP or cyclophosphamide, to serve as vehicle and positive controls, respectively. There was no evidence of an increase in the incidence of chromosomal aberrations in animals treated with acetamiprid when compared with the vehicle control groups. The positive control material produced a marked increase in the frequency of chromosomal aberrations. Acetamiprid was considered to be non-clastogenic under the conditions of the test.

[g] Tested in the UDS test with mammalian liver cells in vivo. The assay was performed in two phases. The first phase, the initial and repeated dose range–finding assays, was a toxicity study used to aid in the selection of dose levels to be used in the UDS assay. The second phase, the UDS assay, was used to evaluate the potential of the test article to induce UDS in hepatocytes of exposed male rats. In all phases of the study, test and control articles were administered at a constant volume of 10 ml/kg bw by a single oral gavage injection. In the initial dose range–finding assay, male rats were exposed to 50, 100, 200, 400 or 1250 mg of test article per kilogram body weight. All five dose preparations were prepared independently, vortexed upon preparation and vortexed again immediately before dosing the animals. Weight loss was observed at all dose levels, with only the animals in the 50 and 100 mg/kg bw dose groups regaining body weight on day 3. As the LD_{50} (1640 mg/kg bw) calculated in this study was different from that of the earlier study (217 mg/kg bw), the dose range–finding assay was repeated. In the repeated dose range–finding assay, male rats were exposed to 50, 100, 200, 400 or 1250 mg of test article per kilogram body weight. Weight loss was observed in animals of the 200 and 400 mg/kg bw dose groups, whereas the 100 mg/kg bw animals exhibited weight gain on day 1 and weight loss on day 3. The 50 mg/kg bw animals showed minimal weight gain on days 1 and 3. The 1250 mg/kg bw animals were found dead before a weight determination could be made on day 1. Body weights were determined prior to treatment, on day 1 and day 3. In the UDS assay, male rats were exposed to 75, 150 and 300 mg of test article per kilogram body weight. The selection of 300 mg/kg bw as the high dose was based on the clinical signs and weight loss observed at 400 mg/kg bw and weight loss observed at 200 mg/kg bw (in the repeated dose range–finding assay). In the UDS assay, no mortality was observed in any treated or control rats. Clinical signs were normal in all dose groups following dose administration and prior to harvesting of the hepatocytes after exposures for 2–4 hours and 12–16 hours, with the exception of one animal in the 300 mg/kg bw dose group exposed for 2–4 hours, which exhibited lethargy and tremors. However, this animal was not used in the hepatocyte harvest. Upon perfusion, the livers of the 300 mg/kg bw animals exposed for 2–4 hours and 12–16 hours were observed to be much darker than the livers from any of the other dose groups. The test article, acetamiprid, did not induce a significant increase in the mean number of net nuclear grain counts (i.e. an increase of at least five counts over the negative control group) in hepatocytes isolated either 2–4 hours or 12–16 hours after dose administration. Acetamiprid was concluded to be negative in the UDS test with mammalian liver cells in vivo.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

A range-finding study was undertaken to determine the dose levels to be employed in a multigeneration reproduction study in rats. Charles River Crl:CD VAF/Plus rats were randomly assigned to one control and four treatment groups of 10 animals each (5 males and 5 females). The age of the rats at the time of initiation of the study was 71 days for both males and females. The male rats weighed between 343 and 374 g, and the females weighed between 211 and 239 g.

Acetamiprid (lot No. NNI-01, purity 99.38%) was administered orally via the diet to four groups of rats (males and females) beginning 14 days prior to mating and continuing until euthanasia. Animals in the treatment groups received the test material in the diet at concentrations of 125, 250, 500 and 1000 ppm (average equal to 8.3, 16.6, 31.4 and 59.7 mg/kg bw per day for males and 9.0, 18.0, 33.7 and 63.6 mg/kg bw per day for females, respectively). The control group received only the ground basal laboratory diet on a comparable regimen.

The mated females were allowed to deliver. The litters were evaluated for survival and growth. The dams and their litters were euthanized on lactation day 4.

The death of a single male rat was observed at the 250 ppm level. This death was not considered to be related to treatment, as no signs of overt toxicity were observed prior to death and no other deaths were observed at higher dose levels.

No clinical signs of toxicity or gross necropsy findings were observed for any animal that survived to scheduled euthanasia.

Treatment-related reductions in body weight and feed consumption were observed at dose levels of 500 and 1000 ppm when compared with the control groups. These reductions were observed for males and females. Reductions observed for females during weekly measurements often continued through the gestation and lactation periods as well.

There was no indication of impaired fertility as a result of treatment. Male and female copulatory and fertility indices of the treatment groups were generally comparable with the corresponding control indices. Offspring growth was inhibited (as evidenced by reduced litter weights, relative to the control values) by treatment of the F_0 dams at dose levels of 500 and 1000 ppm. Development of the F_1 offspring was also adversely affected by treatment of the dams, as evidenced by the reduction in the number of implantation sites at the 1000 ppm level when compared with the control group.

The study complied with GLP, and a QA statement was attached (Schardein, 1991).

In a two-generation reproduction study in Charles River Crl:CD VAF/Plus rats, the test article, acetamiprid (lot No. NNI-01, purity 99.38%), was administered orally in the ground diet and was available ad libitum. Dietary concentrations of 100, 280 and 800 ppm were evaluated. The control group received the basal laboratory diet on an identical regimen. Each group (F_0 parents) consisted of 26 males and 26 females; these animals were treated beginning at 37 days of age for 59 days prior to the F_1 mating. The F_0 parents were mated to produce the F_1 litters. From the F_1 litters, 26 males and 26 females were selected to become F_1 parents. These F_1 parental animals were treated beginning at 22 days of age for a minimum of 64 days prior to mating to produce the F_2 generation. Treatment continued through mating, gestation and lactation. The control group received the basal laboratory diet on the same regimen.

The F_0 animals were approximately 14 weeks old at the time of pairing; males and females weighed 137–158 g and 125–152 g, respectively, at randomization on the day prior to the 1st day of treatment.

The average acetamiprid consumptions of the F_0 animals are shown in Table 36.

The average acetamiprid consumptions of the F_1 animals are shown in Table 37.

All parental animals and offspring were observed for mortality and overt toxicity twice each day, 7 days/week. Detailed clinical observations of the adults were recorded at least once weekly. Body weights for the parental males were recorded weekly, as was feed consumption, except during the mating periods. Body weights for the females were recorded weekly until evidence of copulation; feed consumption was recorded weekly until the initiation of the mating period. Maternal body weights and feed consumption were recorded at specified intervals during gestation and lactation. The F_0 and F_1 parental reproductive performance was assessed, and the F_1 and F_2 litters were evaluated for viability and growth.

All parental animals and offspring were subjected to gross necropsy at scheduled sacrifice or time of death. Selected organs from the parental animals were preserved, and the tissues were examined microscopically. The weights of the testes were recorded, and an evaluation of spermatogenesis was conducted at necropsy for any adult male that failed to sire a litter.

At the high dose level (800 ppm), statistically significant decreases in weekly body weights relative to the control values were noted throughout the study for the males in the F_0 generation (Table 38), but not for females (Table 39). Significantly reduced maternal body weight gain compared with that of the control group occurred early in the gestation period for the F_0 females in this treated group, and some reduction of body weight was present thereafter (Tables 40 and 41).

The mean maternal body weight gains for the F_0 high-dose females were also reduced relative to those of the control group during the lactation period (Tables 42 and 43).

Similarly, significant inhibition of growth of F_1 offspring in comparison with the control values occurred at this dose level, and depressed body weights continued to be evident for the F_1 adults (Tables 44 and 45). Significantly decreased body weight and body weight gain were also seen in the high-dose group in comparison with the control group of the F_1 generation during gestation and lactation (Tables 46–49).

Reductions in F_2 offspring body weights relative to the control values were also observed in the high-dose group (Table 50).

In both the F_0 and F_1 generations, there were reductions in parental feed consumption values during the first few weeks of the study for males and females in the high-dose group in comparison with the control values. Decreased feed consumption was also evident for the high-dose dams in both generations during gestation and lactation. At the intermediate dose level (280 ppm), reductions in F_0 parental feed consumption were noted in the first 2 weeks of the study and during gestation, but the difference from the control group data was less pronounced than that at the high dose level.

At the low dose level (100 ppm), statistically significant reductions in feed consumption were noted for the F_0 males in the first 2 weeks of the study. These reductions, as well as those seen at other treatment levels during these weeks, may have been due to the rats initially finding the diet unpalatable. Slight reductions in feed consumption during the gestation period were also evident for the F_1 females in the low-dose group but were not considered adverse, as they did not occur in the premating or lactational periods and did not result in statistically significantly reduced body weights. These variations in feed consumption did not represent significant toxicity (Tables 51–54).

Treatment-related microscopic changes in the liver consisting of hepatocyte hypertrophy (males and females, F_0 and F_1 generations) and vacuolar change in hepatocytes (F_1 males) were noted in the high-dose group.

No adverse effects on F_1 offspring survival were observed in the high-dose group. However, biologically and/or statistically significant reductions in the F_2 offspring survival indices at lactation days 0 and 4 were observed in the high-dose litters in comparison with the control values. The

Table 36. Average acetamiprid consumption by F_0 animals

Dietary concentration (ppm)	Average F_0 acetamiprid consumption (mg/kg bw per day)		
	Males (weekly)	Females (weekly)	Females (gestation)
0	0	0	0
100	6.67	8.42	5.04
280	18.9	23.1	13.9
800	54.6	66.5	38.7

From York (1994)

Table 37. Average acetamiprid consumption by F_1 animals

Dietary concentration (ppm)	Average F_1 acetamiprid consumption (mg/kg bw per day)		
	Males (weekly)	Females (weekly)	Females (gestation)
0	0	0	0
100	7.60	9.40	5.67
280	23.3	27.0	15.1
800	65.0	87.1	45.7

From York (1994)

Table 38. Males: Summary of body weight values, F_0 weekly

Week of study	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	148	4.6	26	147	5.4	26	149	5.5	26	145	5.1	26
1	211	5.9	26	208	8.1	26	211	9.4	26	194*	7.5	26
2	259	8.7	26	255	11.2	26	259	16.0	26	239**	11.9	26
3	301	12.9	26	299	16.2	26	303	17.1	26	280**	15.5	26
4	330	16.3	26	328	19.7	26	333	21.6	26	309**	18.6	26
5	359	19.5	26	359	23.3	26	363	24.6	26	335**	20.6	26
6	381	24.7	26	382	26.4	26	388	26.8	26	359**	22.8	26
7	405	26.5	26	406	30.5	26	413	30.1	26	381**	22.6	26
8	422	31.3	26	417	28.3	26	428	29.9	26	394**	23.4	26
9	425	31.9	26	425	29.4	26	439	33.7	26	401**	22.9	26
10	445	33.9	26	443	30.9	26	458	35.8	26	420*	24.2	26
11	462	36.7	26	463	33.8	26	476	36.6	26	437*	24.7	26
12	468	35.9	26	469	37.9	26	478	36.5	26	440*	25.3	26
13	484	40.8	26	487	38.6	26	494	40.8	26	453*	32.1	26
14	485	42.9	26	486	37.3	26	498	42.0	26	455*	35.3	26
15	500	42.6	26	500	37.3	26	512	44.2	26	467*	35.1	26
16	508	43.8	26	507	38.0	26	517	46.1	26	472**	37.2	26
17	512	44.8	26	516	40.0	26	524	48.0	26	483*	35.5	26
18	521	44.5	26	523	40.4	26	534	49.4	26	488*	39.9	26

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 39. Females: Summary of body weight values, F_0 weekly[a]

Week of study	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	N
0	141	7.9	26	140	6.8	26	139	7.8	26	142	7.3	26
1	181	10.9	26	183	8.8	26	179	7.8	26	177	8.7	26
2	206	14.2	26	205	10.9	26	200	9.2	26	200	11.0	26
3	227	17.7	26	230	15.5	26	223	11.5	26	219	14.8	26
4	241	17.5	26	244	15.2	26	238	13.1	26	233	13.5	26
5	256	19.9	26	266	17.5	26	256	12.8	26	247	13.5	26
6	268	19.6	26	275	18.3	26	265	15.9	26	259	14.6	26
7	282	22.0	26	289	21.9	26	278	15.8	26	272	17.5	26
8	286	22.5	26	295	22.2	26	284	17.4	26	277	18.1	26
9	314	18.6	4	311	22.6	6	306	11.7	3	300	9.5	3
10	345	19.1	2	329	16.3	2	299	NA	1	318	NA	1
11	376	NA	1	363	NA	1	292	NA	1	NA	NA	0
12	448	NA	1	400	NA	1	297	NA	1	NA	NA	0
13	NA	NA	0	NA	NA	0	297	NA	1	NA	NA	0
14	NA	NA	0	NA	NA	0	301	NA	1	NA	NA	0
15	NA	NA	0	NA	NA	0	278	NA	1	NA	NA	0

From York (1994)
N, number of animals; NA, not applicable; SD, standard deviation
[a] No statistical significance observed.

Table 40. Females: Summary of body weight values, F_0 gestation

Days	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	293	23.3	25	299	21.1	22	289	21.9	20	281	18.2	21
6	329	24.2	25	332	24.1	25	320	22.9	25	306**	18.8	25
15	369	24.9	25	373	30.4	25	357	31.2	25	344**	27.2	25
20	436	39.7	25	438	48.3	25	420	55.9	25	404	50.4	25

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 41. Females: Summary of body weight changes, F_0 gestation

Days	Body weight changes (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	36	10.0	25	34	9.4	22	33	5.9	20	27**	7.4	21
6–15	40	12.7	25	41	16.0	25	38	20.9	25	38	17.4	25
15–20	67	21.0	25	65	26.3	25	63	30.5	25	60	27.5	25
0–20	143	32.5	25	154	21.9	25	156	15.6	20	143	14.4	21

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 42. Females: Summary of body weight values, F_0 lactation

Day of lactation	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	342	25.9	23	347	21.1	23	338	20.2	20	326*	15.7	21
7	360	20.0	24	361	19.9	23	354	29.3	20	340*	21.8	21
14	387	22.6	24	384	23.5	23	376	28.8	20	355**	20.0	21
21	357	22.2	24	348	22.2	23	356	30.9	20	339	25.0	21

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 43. Females: Summary of body weight changes, F_0 lactation[a]

Days of lactation	Body weight changes (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	19	12.0	23	14	19.1	23	16	14.7	20	14	15.8	21
7–14	27	15.4	24	23	14.2	23	22	17.3	20	15	16.7	21
14–21	−31	17.5	24	−36	23.8	23	−21	16.6	20	−16	21.5	21
0–21	14	19.9	23	1	27.7	23	18	20.1	20	14	21.1	21

From York (1994)
N, number of animals; SD, standard deviation
[a] No statistical significance observed.

Table 44. Males: Summary of body weight values, F_1 weekly

Week of age	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
4	103	6.9	26	105	11.8	26	103	11.2	26	91**	10.7	26
5	164	10.5	26	167	16.2	26	164	14.4	26	141**	16.5	26
6	223	12.6	26	227	17.6	26	225	19.6	26	194**	20.4	26
7	279	15.4	26	287	20.3	26	283	23.5	26	245**	23.5	26
8	336	19.3	26	350	25.9	26	343	30.7	26	298**	27.3	26
9	381	22.4	26	396	30.8	26	390	35.4	26	344**	32.4	26
10	417	26.0	26	435	35.6	26	425	38.9	26	374**	36.7	26
11	454	29.5	26	466	36.7	26	460	43.3	26	406**	40.4	26
12	469	28.1	26	491	37.9	26	481	46.6	26	428**	44.8	26
13	495	34.9	26	515	41.6	26	505	46.4	26	450**	46.9	26
14	515	42.4	26	540	48.5	26	526	46.9	26	467**	51.8	26
15	525	43.4	26	550	48.2	26	539	50.2	26	480**	51.7	26
16	537	41.8	26	564	52.2	26	554	49.8	26	493**	56.2	26
17	548	45.4	26	574	54.9	26	558	43.8	26	496**	57.9	26
18	553	49.5	26	580	58.9	26	566	44.5	26	507**	59.9	26
19	562	57.6	26	601*	63.7	26	583	48.0	26	522*	61.7	26
20	574	54.9	26	609	67.0	26	591	48.0	26	529*	66.2	26

Table 44 (continued)

Week of age	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
21	586	58.5	26	624	66.9	26	604	54.5	26	539*	66.6	26
22	596	60.9	26	633	68.1	26	609	52.9	26	551*	66.2	26
23	601	61.0	25	649*	75.7	25	616	49.2	26	557*	72.0	26
24	614	62.0	24	663*	78.6	23	631	53.3	25	557*	72.3	23

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 45. Females: Summary of body weight values, F_1 weekly

Week of age	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	N
4	99	7.2	25	96	9.2	26	96	7.4	26	84**	7.8	25
5	145	10.6	25	141	12.1	26	143	8.6	26	127**	10.4	25
6	177	12.9	25	170	19.7	26	176	12.6	26	160**	10.8	25
7	198	16.0	25	191	24.4	26	198	15.1	26	183**	10.9	25
8	222	20.2	25	219	22.7	26	222	19.0	26	204**	13.4	25
9	240	22.6	25	233	27.4	26	240	21.0	26	220**	14.1	25
10	256	24.4	25	250	26.3	26	253	22.5	26	233**	14.6	25
11	267	26.8	25	264	32.5	26	268	25.3	26	245**	16.3	25
12	276	27.3	25	271	31.4	26	278	30.3	26	256*	16.8	25
13	285	28.9	24	282	32.8	25	285	30.4	26	264*	19.8	25
14	293	30.3	21	290	35.0	22	293	28.7	24	268*	16.8	21
15	308	36.7	6	309	27.7	10	306	24.3	10	279	17.0	3
16	333	27.4	3	329	28.5	8	337	24.6	6	307	43.1	2
17	341	43.1	3	330	42.8	5	357	40.4	5	396	NA	1
18	369	49.7	3	342	64.6	3	314	23.9	3	NA	NA	0
19	341	39.6	2	311	28.3	2	324	17.1	3	NA	NA	0
20	345	45.3	2	318	35.4	2	325	28.1	3	NA	NA	0

From York (1994)
N, number of animals; NA, not applicable; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 46. Females: Summary of body weight values, F_1 gestation

Day of study	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	292	26.9	18	286	34.4	19	296	26.8	16	267*	20.1	20
6	326	26.5	18	314	33.8	19	317	22.4	16	290**	18.8	20
15	372	28.0	18	356	31.3	19	357	23.0	16	326**	22.2	20
20	444	30.6	18	423	34.5	19	434	29.9	16	385**	28.9	20

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 47. Females: Summary of body weight changes, F_1 gestation

Days of study	Body weight change (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	34	10.9	18	28	8.5	19	21**	11.5	16	23**	8.4	20
6–15	46	9.2	18	42	8.6	19	40	8.2	16	36**	9.6	20
15–20	72	11.4	18	68	17.5	19	77	14.3	16	59*	18.6	20
0–20	152	16.8	18	137	27.5	19	138	19.9	16	117**	21.1	20

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 48. Females: Summary of body weight values, F_1 lactation

Day of lactation	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	343	27.9	19	330	28.1	22	329	20.3	18	294**	21.5	21
7	357	27.6	19	349	23.9	22	345	24.0	18	315**	21.6	21
14	376	21.9	19	363	26.8	22	361	22.5	18	327**	21.9	21
21	351	22.2	19	347	24.0	22	343	17.7	18	335	20.1	21

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 49. Females: Summary of body weight changes, F_1 lactation

Days of lactation	Body weight change (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	13	14.1	19	19	15.0	22	16	20.1	18	22	18.1	21
7–14	19	14.5	19	14	18.5	22	16	15.3	18	11	9.6	21
14–21	−24	12.4	19	−16	19.1	22	−18	17.6	18	8**	24.5	21
0–21	8	19.0	19	17	14.7	22	14	18.0	18	41**	28.0	21

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 50. Summary of F_2 offspring growth

Dietary concentration (ppm)		Group mean body weight of live offspring during lactation (g)											
		Day of age											
		0		4BR		4AR		7		14		21	
		M	F	M	F	M	F	M	F	M	F	M	F
0	Mean	6.7	6.1	10.8	10.0	10.7	10.0	18.1	16.7	37.5	35.2	59.6	55.1
	SD	0.61	0.50	1.62	1.41	1.63	1.43	2.32	2.01	3.22	3.54	5.94	5.21
100	Mean	6.6	6.2	10.8	10.2	10.8	10.2	18.2	17.0	37.2	35.4	58.6	55.3
	SD	0.72	0.73	1.63	1.70	1.59	1.70	1.89	2.14	3.17	3.52	5.36	5.37

Table 50 (continued)

Dietary concentration (ppm)		Group mean body weight of live offspring during lactation (g)											
		Day of age											
		0		4BR		4AR		7		14		21	
		M	F	M	F	M	F	M	F	M	F	M	F
280	Mean	6.3	6.0	10.4	9.9	10.4	9.9	17.1	16.4	35.6	34.0	54.7	51.6
	SD	0.64	0.62	1.44	1.42	1.41	1.52	1.98	2.11	2.80	2.92	4.56	4.20
800	Mean	6.1	5.8	9.4	8.9	9.4	8.9	15.6*	14.6	31.6*	29.8*	48.7*	45.5*
	SD	0.74	0.80	1.75	1.55	1.77	1.53	2.69	2.64	3.61	4.55	4.60	5.94

AR, after reduction; BR, before reduction; F, females; M, males; SD, standard deviation
* Significantly different from control group, $P \leq 0.05$

Table 51. Females: Summary of feed consumption values, F_0 gestation

Days of study	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	18.8	1.87	25	18.8	1.87	25	17.5*	1.98	25	15.8**	1.39	25
6–15	31.0	3.86	25	30.8	3.28	25	28.2*	3.80	25	27.5**	3.35	25
15–20	18.2	3.20	25	18.1	2.51	25	17.7	2.66	24	16.5	2.42	25
0–20	22.6	2.7	50	22.5	2.03	50	21.2**	2.40	50	19.9**	2.04	51

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 52. Females: Summary of feed consumption values, F_0 lactation

Days of lactation	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	41.8	3.77	24	39.2	4.02	23	40.4	3.24	19	38.2*	4.9	21
7–14	67.9	17.19	23	61.1	7.52	23	61.9	3.89	19	56.8*	6.57	21
14–21	77.4	5.35	24	74.7	5.29	23	78.3	11.62	20	72.2*	4.48	20
0–21	62.2	7.4	24	58.4	4.65	23	60.7	5.83	20	55.4**	5.47	21

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 53. Females: Summary of feed consumption values, F_1 gestation

Days of gestation	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	23.7	2.67	18	21.6*	2.92	19	20.7**	2.38	16	19.5**	2.54	20
6–15	26.8	2.20	18	24.5**	2.32	19	23.7**	1.77	16	22.4**	2.73	20
15–20	26.9	2.57	18	26.0	2.64	19	25.7	3.19	16	23.8**	3.29	20
0–20	25.9	2.1	18	24*	2.14	19	23.3**	1.85	16	21.9**	2.15	20

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 54. Females: Summary of feed consumption values, F_1 lactation

Days of lactation	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	39.0	5.24	19	38.9	5.53	22	36.9	5.09	17	36.5	6.94	21
7–14	61.5	6.43	18	58.0	12.29	21	56.2*	3.95	18	51.6**	7.6	20
14–21	72.0	7.80	19	70.4	13.39	22	68.4	5.67	15	62.6*	11.66	20
0–21	57.5	5.9	19	55.9	10.02	22	53.1	6.46	18	49.8**	8.29	21

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

weaning index, which measures offspring survival between lactation day 4 after culling and lactation day 21, was also reduced in this treated group when compared with that of the control group.

The treatment-related microscopic changes in the liver noted in the high-dose group, hepatocyte hypertrophy (F_0 and F_1 generations) and vacuolar change in hepatocytes (F_1 generation), were also observed for the mid-dose males. The severity of these changes was less in the mid-dose group than in the high-dose group (Tables 55–58).

There were no adverse effects on offspring development in litters in the mid-dose group in either generation.

There were apparent reductions in the F_0 male and female fertility indices and in the pregnancy index in the mid- and high-dose groups in comparison with the concurrent control group, with the difference being statistically significant in the mid-dose group. However, there were no dose-related differences in the F_1 male and female fertility indices and in the pregnancy index between the control and these treated groups. In the F_0 generation, the control group had values of 100% for these indices, whereas in the F_1 generation, there were values of 76% and 82% for the fertility and pregnancy indices, respectively, in the control group. All of these values, and the values in the treated groups in both generations, were within the range of values in the historical control data. It is therefore questionable if the variations between the control and mid- and high-dose groups in the F_0 generation represent an adverse test article–related effect on reproductive performance.

For the F_0 males that failed to sire a litter in the treated groups, the sperm were generally at least 50% motile, and a minimum of 92% of the sperm examined from each male were morphologically normal. As spermatogenesis was not evaluated for any control males (all sired litters) or for any fertile males, assessment of sperm concentration values of the non-fertile treated males for adverse effects is difficult. However, only one male in the mid-dose group had a concentration sufficiently low that morphological assessment could not be made. The values are presented in Table 59.

No adverse effects on reproductive performance or offspring development were evident at the low dose level.

A supplementary reproductive assessment was done. As the epididymides of F_0 high-dose males were autolysed due to an error, a complete reproductive assessment was not obtained for that dose (800 ppm) in the two-generation study. In this supplementary study, only two groups of male rats were taken (group I, control; group II, 800 ppm) for study, and acetamiprid was administered through the diet at a dose of 800 ppm to group II animals for 20 weeks, a duration comparable to that for the F_0 males in the two-generation study. Group I animals were given food ad libitum.The males were monitored for clinical signs, body weight changes, feed consumption and general condition throughout the study. At termination, organ weights were obtained, and a reproductive capacity evaluation, which included total testicular and epididymal sperm counts and sperm morphology, was perfomed.

Table 55. Incidence of microscopic observations in liver, terminal sacrifice of F_0 rats (males)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*0*	*26*	*0*	*26*	*0*	*26*	*0*	*26*
Number of animals within normal limits	*0*	*23*	*0*	*25*	*0*	*5*	*0*	*0*
Extramedullary haematopoiesis, trace	0	1	0	0	0	0	0	1
Inflammation, trace	0	0	0	1	0	0	0	1
Necrosis, mild	0	1	0	0	0	0	0	0
Vacuolar change	0	1	0	0	0	2	0	3
- trace	0	1	0	0	0	2	0	2
- mild	0	0	0	0	0	0	0	1
Hepatocyte hypertrophy	0	0	0	0	0	21**	0	26**
- trace	0	0	0	0	0	21	0	0
- mild	0	0	0	0	0	0	0	16
- moderate	0	0	0	0	0	0	0	10

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 56. Incidence of microscopic observations in liver, terminal sacrifice of F_0 rats (females)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*0*	*26*	*0*	*26*	*0*	*26*	*0*	*26*
Number of animals within normal limits	*0*	*25*	*0*	*25*	*0*	*25*	*0*	*4*
Extramedullary haematopoiesis, trace	0	0	0	0	0	1	0	1
Inflammation	0	1	0	0	0	0	0	2
- trace	0	1	0	0	0	0	0	1
- mild	0	0	0	0	0	0	0	1
Necrosis, trace	0	0	0	1	0	0	0	1
Hepatocyte hypertrophy	0	0	0	0	0	0	0	22**
- trace	0	0	0	0	0	0	0	6
- mild	0	0	0	0	0	0	0	16

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 57. Incidence of microscopic observations in liver, day 0 to termination, F_1 rats (males)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*0*	*26*	*0*	*26*	*0*	*26*	*0*	*26*
Number of animals within normal limits	*0*	*25*	*0*	*25*	*0*	*1*	*0*	*0*
Extramedullary haematopoiesis, trace	0	0	0	0	0	0	0	1
Inflammation, trace	0	1	0	0	0	0	0	0
Necrosis, mild	0	0	0	0	0	1	0	0
Vacuolar change	0	0	0	0	0	2	0	12**

Table 57 (continued)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
- trace	0	0	0	0	0	2	0	9
- mild	0	0	0	0	0	0	0	3
Hepatocyte hypertrophy	0	0	0	1	0	25**	0	26**
- trace	0	0	0	1	0	21	0	0
- mild	0	0	0	0	0	4	0	11
- moderate	0	0	0	0	0	0	0	15

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 58. Incidence of microscopic observations in liver day 0 to termination, F_1 rats (females)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*1*	*25*	*0*	*26*	*0*	*26*	*1*	*25*
Number of animals within normal limits	*1*	*21*	*0*	*26*	*0*	*26*	*1*	*5*
Extramedullary haematopoiesis, trace	0	4	0	0	0	0	0	3
Hepatocyte hypertrophy	0	0	0	0	0	0	0	20**
- trace	0	0	0	0	0	0	0	13
- mild	0	0	0	0	0	0	0	7

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 59. Summary of F_0 male spermatogenesis data

	100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Sperm motility (%)	71	13.6	3	61	33.4	6	53	35.6	5
Sperm concentration (number/g caudal tissue)	761.1	442.94	3	797.2	492.29	6	716.7	523.17	5
% abnormal	2.0	1.5	3	1	0.7	5	3	3.1	5

From York (1994)
N, number of animals; SD, standard deviation

There were no deaths attributable to administration of the test material. One 800 ppm male was sacrificed in extremis during week 16. No compound-related effects were seen.

Mean body weight and feed consumption values were consistently lower in the 800 ppm group throughout the study. The lower body weights of the test group males relative to control values correlated to their lower feed consumption. Feed efficiency was similar between control and test groups. There were no compound-related effects in the evaluation of organ weights of the 800 ppm males. Also, no effects were observed in the evaluation of testicular and epididymal sperm count and sperm morphology in this group.

In conclusion, dietary exposure of male rats to acetamiprid at a dose level of 800 ppm for at least 20 weeks did not result in effects on reproductive capacity (Trutter, 1999).

Keeping in view the above, the NOAEL for systemic adult toxicity when acetamiprid was administered to Charles River Crl:CD VAF/Plus rats was 100 ppm (equal to 6.67 mg/kg bw per

day), on the basis of a decline in body weights and feed consumption and an increased incidence of hepatocyte hypertrophy and vacuolation in the liver at 280 ppm (equal to 18.9 mg/kg bw per day) and above. The NOAEL for offspring toxicity was 280 ppm (equal to 13.9 mg/kg bw per day), on the basis of decreases in body weight gain in both generations and reduced postnatal survival in the F_2 offspring at 800 ppm (equal to 38.7 mg/kg bw per day). The NOAEL for reproductive toxicity was 800 ppm (equal to 38.7 mg/kg bw per day), the highest dose tested.

This study was conducted in compliance with GLP, and a QA statement was attached (York, 1994, 1995).

(b) Developmental toxicity

Rats

A study was conducted to assess the potential maternal toxicity, fetal toxicity and teratogenicity of acetamiprid in pregnant rats. Acetamiprid (lot No. NNI-02, purity 99.46%) suspensions in vehicle (5% gum arabic and 0.01% Tween 80 in water) were administered orally by gastric intubation to Cij:CD(SD) rats (24 mated females per group) at dose levels of 5, 16 and 50 mg/kg bw per day for a period of 10 days from gestation days 6 to 15. Control animals received vehicle (0.2 ml/100 g bw per day) only, in the same procedure as treated animals.

The mated females were observed for clinical signs once a day from the first mating to necropsy. Individual body weights (on gestation days 0 and 6–21) and feed consumption (on days 6–21) were measured once a day.

On gestation day 21, all mated females were sacrificed, and their organ weights (liver, spleen, kidney, adrenal and ovary) were measured. The number of corpora lutea was counted. After the gravid uterus was weighed, the numbers of total implantations, viable fetuses, dead fetuses and resorbed fetuses were counted. Viable fetuses were examined for external malformations and variations, and their body weight and placental weight were recorded. One half of the viable fetuses in each litter were used for visceral evaluation using the method of Wilson and Nishimura, and the remaining fetuses were used for skeletal evaluation (McLeod method).

Acetamiprid did not cause toxic signs or deaths in any groups. Pregnancy rates for the control, 5, 16 and 50 mg/kg bw per day groups were 95.8%, 95.8%, 100% and 95.8%, respectively.

Effects of acetamiprid treatment on body weight, feed consumption and liver weights were seen in the high-dose (50 mg/kg bw per day) group. Mean body weights for the high-dose animals were lower than control values throughout the treatment period; differences were statistically significant ($P < 0.05$ or $P < 0.01$) on gestation days 8–16. Their cumulative body weight gains during the treatment period were significantly lower ($P < 0.01$, reduced by 41%) than control values. Feed consumption of the high-dose group was lower (reduced by 2.45%) than control values throughout the study; differences were statistically significant ($P < 0.01$) on gestation days 7–11. Increases in liver weights ($P < 0.05$) and liver to body weight ratios ($P < 0.01$) of the high-dose group were statistically significant.

No effects of acetamiprid treatment on the numbers of total implantation sites, corpora lutea and viable fetuses were evident.

Macroscopically, no adverse effect of acetamiprid treatment was evident. The following two lesions were seen in one animal each, but they were not considered to be related to treatment: splenodiaphragmatic adhesion in a 16 mg/kg bw per day animal and splenic hypertrophy in a 50 mg/kg bw per day animal.

Observations of the maternal reproductive system revealed no adverse effect of acetamiprid treatment. No immature delivery or abortion was seen in any treated group, including the 50 mg/kg bw per day group. The number of resorbed fetuses (early death) and post-implantation loss values for the 16 mg/kg bw per day group were significantly increased, relative to control values, but were

not considered to be related to acetamiprid treatment, because there was no treatment relationship ($P = 0.12$ and $P = 0.11$, not significant). There was no adverse effect of acetamiprid treatment on the uterine weight.

No significant change, relative to control values, was noted in the numbers of male and female fetuses per pregnant female, sex ratio or fetal placental weight.

The only external malformation noted was short tail in one fetus from the litter of one control female (incidence 0.3%). The external variations seen were subcuticular and placental haemorrhage. Based on litter analysis, the incidences of subcuticular haemorrhage for the control, 5, 16 and 50 mg/kg bw per day groups were 8.7% (2/23 litters), 4.3% (1/23 litters), 4.2% (1/24 litters) and 17.4% (4/24 litters). The placental haemorrhage was seen in three fetuses from two litters of the 50 mg/kg bw per day group (0.9% for the fetal incidence and 8.7% for the maternal incidence). The incidences in each group were not statistically significantly different either for the placental haemorrhage or for the fetal subcuticular haemorrhage. After reanalysis of values of the low and intermediate doses only for a trend effect, there was no dose-dependent incidence of these parameters. Acetamiprid treatment was not considered to cause external modifications of fetuses.

Acetamiprid treatment did not increase the incidences of fetuses with visceral malformations and/or variations.

There were no visceral malformations in the control and treated groups.

The incidences of fetuses with visceral variations for the control, 5, 16 and 50 mg/kg bw per day groups were 12.6% (22/174 fetuses), 8.6% (15/175 fetuses), 13.3% (24/180 fetuses) and 8.0% (13/162 fetuses), respectively; the incidences, relative to control values, were not statistically significant. The main visceral variations seen in fetuses of the control and treated groups were dilatation of the renal pelvis and thymic remnants in the neck. The incidences of fetuses with dilatation of the renal pelvis for the control, 5, 16 and 50 mg/kg bw per day groups were 7.5% (13/174 fetuses), 4.6% (8/175 fetuses), 10.6% (19/180 fetuses) and 6.2% (10/162 fetuses), respectively. The incidences of fetuses with thymic remnants in the neck were 4.6% (8/174 fetuses), 4.0% (7/175 fetuses), 2.8% (5/180 fetuses) and 1.9% (3/162 fetuses), respectively. No statistical significance was attained in the treated groups, compared with control values. The low incidences of other visceral variations seen in the treated groups were not considered to be indicative of treatment-related responses. These results suggest that acetamiprid did not affect visceral variations.

Acetamiprid treatment did not cause skeletal malformations, variations or retarded ossification in fetuses. The incidences of fetuses with skeletal malformations for the control, 5, 16 and 50 mg/kg bw per day groups were 1.8% (3/171 fetuses), 0% (0/165 fetuses), 1.1% (2/175 fetuses) and 0.6% (1/162 fetuses), respectively. The incidences of litters containing fetuses with skeletal malformations for these same groups were 13% (3/23 litters), 0% (0/23 litters), 8.3% (2/24 litters) and 4.5% (1/22 litters), respectively. There were no statistically significant differences in the incidences of skeletal malformations, on either a per fetus or per litter basis, when compared with control values. The main skeletal malformations seen in fetuses of the control and treated groups were defect of post-cervical vertebrae, partial hypertrophy, fusion and bifurcation of the ribs and fusion of the sternums. Partial hypertrophy of the rib was seen in one fetus from each of the control, 16 and 50 mg/kg bw per day groups. In the control group, three fetuses had defect of post-cervical vertebrae, fusion or bifurcation of the ribs. Fusion of the sternums was seen in one fetus from the 16 mg/kg bw per day group. These incidences of fetuses with skeletal malformations were considered comparable to control values. These results suggest that there was no effect of acetamiprid treatment on skeletal malformation.

The incidences of fetuses with skeletal variations for the control, 5, 16 and 50 mg/kg bw per day groups were 21.6% (37/171 fetuses), 28.5% (47/165 fetuses), 25.1% (44/175 fetuses) and 29.0% (47/162 fetuses), respectively. The main skeletal variations seen in fetuses of the control and treated groups were bibbed shape of the thoracic vertebral body, splitting of the cervical vertebral body,

shortening of the 13th rib, 14th rudimentary rib and asymmetry of the sternum. The incidences of fetuses with bibbed shape of the thoracic vertebral body, splitting of the cervical vertebral body, 14th rudimentary rib and asymmetry of the sternum in the treated groups were comparable to or less than control values. The incidence of fetuses with 14th rudimentary rib from the 50 mg/kg bw per day group (0/162 fetuses) was lower than the control value (8/171 fetuses) and was inversely dose dependent ($P < 0.01$). The incidences of fetuses (and litters) with shortening of the 13th rib for the control, 5, 16 and 50 mg/kg bw per day groups were 0.6% (1/171 fetuses, 1/23 litters), 3.6% (6/165 fetuses, 3/23 litters), 0.6% (1/175 fetuses, 1/24 litters) and 9.3% (15/162 fetuses, 8/22 litters), respectively. Statistical significance was observed at 50 mg/kg bw per day on both a fetus ($P < 0.01$) and litter ($P < 0.01$) basis for the shortening of the 13th rib compared with the control group.

There was no effect of acetamiprid treatment on the ossification process.

No effects of acetamiprid treatment were evident in the numbers of total implantations.

The NOAEL for maternal toxicity in rats was 16 mg/kg bw per day, based on decreases in feed consumption and body weight gain during the treatment period noted in maternal rats in the 50 mg/kg bw per day group at scheduled sacrifice. The developmental NOAEL in rats was 16 mg/kg bw per day, based on the increased incidences of fetuses with shortening of the 13th rib at 50 mg/kg bw per day.

This study was conducted in compliance with GLP, and a QA statement was attached (Nukui & Fujii, 1994a).

Rabbits

In a developmental toxicity study, acetamiprid (lot No. NNI-02, purity 99.46%) suspended in vehicle (5% gum arabic and 0.01% Tween 80 in water) was administered orally by gastric intubation to Kbs: NZW rabbits (17 mated females per group) at dose levels of 7.5, 15 and 30 mg/kg bw per day for a period of 13 days from gestation days 6 to 18. Control animals received vehicle (4 mg/kg bw per day) only in the same procedure as treated animals.

The age of the animals was 5 months at the time of receipt, and the mean weight of males was 4111.4 ± 248.9 g (range 3547.9–4513.7 g) and of females was 3659.8 ± 265.0 g (range 3193.8–4298.3 g).

The mated females were observed for clinical signs once a day from the first mating to necropsy. Individual body weights (on gestation days 0, 6, 7, 8, 10, 12, 14, 16, 18, 19, 20, 22, 24 and 28) and daily feed consumption (on days 6, 7, 8, 10, 12, 14, 16, 18, 19, 20, 22, 24 and 28) were measured.

On gestation day 28, all mated females were sacrificed, and their organ weights (liver, spleen, kidney, adrenal and ovary) were measured. The number of corpora lutea was counted. After the gravid uterus was weighed, the numbers of total implantations and viable, dead and resorbed fetuses were counted. Viable fetuses were examined for external malformations and variations, and their body weights and placental weights were recorded. After fixation with ethanol, each fetus was used for visceral and skeletal evaluation.

Acetamiprid did not cause death in any treated animal. As a result of inadequate treatment or restraint, three, two and one animal from the 7.5, 15 and 30 mg/kg bw per day groups, respectively, died. The mortalities for the control, 7.5, 15 and 30 mg/kg bw per day groups were 0% (0/17 dams), 17.6% (3/17 dams), 11.8% (2/17 dams) and 5.9% (1/17 dams), respectively. Pregnancy rates for the control, 7.5, 15 and 30 mg/kg bw per day groups were 70.6%, 88.2%, 82.4% and 82.4%, respectively.

One control animal and one treated animal of the 7.5 mg/kg bw per day group showed prone position and abnormal gait (lumber paralysis), respectively. These signs were not considered to be related to treatment, because the former animal had lung lesions, and the latter died from inadequate restraint.

Treatment-related effects on maternal body weight were evident. Following the first treatment, maternal growth in the control, 15 and 30 mg/kg bw per day groups was depressed. Maternal body

weights for the control and 15 mg/kg bw per day groups were temporarily decreased from the first treatment to day 10 or 12 and recovered to the pretreatment level by gestation day 14 or 16. In the 30 mg/kg bw per day group, maternal body weights on day 19 were still lower than the pretreatment level, and body weight gain during the treatment period (gestation days 6–19) was −7.3 g (35.2 g in the control group).

During the post-treatment period (gestation days 19–28), body weight gains for the 7.5 and 15 mg/kg bw per day groups were lower than those for the control group. However, they were not considered to be related to the treatment, because no statistical significance was noted in these same groups, and body weight gains for the 30 mg/kg bw per day group were comparable to those for the control group.

Treatment-related effects on maternal feed consumption were evident. Oral administration of acetamiprid at a dose level of 30 mg/kg bw per day resulted in a tendency to reduced mean feed consumption during the treatment period. Statistically significant reductions in feed consumption, relative to control values, were noted for the 30 mg/kg bw per day group on gestation days 6–7 and 7–8. In the 30 mg/kg bw per day group, decreased body weights were noted simultaneously, and the decreased feed consumption was considered to be related to acetamiprid treatment. During the post-treatment period, mean feed consumption for the 30 mg/kg bw per day group was comparable to that for the control group. In other groups, feed consumption during the pretreatment, treatment and post-treatment period was comparable to the control group values.

No effect of acetamiprid on maternal organ weights was evident.

Macroscopically, no treatment-related effect was evident. In animals that died accidentally, the following lesions were seen: perforation, dark red areas, red patches in the lung and red fluid on the tracheal mucosa and in the thoracic cavity. These lesions were attributable to improper treatment. In the dams that survived until gestation day 28, pale-brown areas and black patches in the lung, red fluid on the tracheal mucosa and prominent hepatic lobules were seen occasionally in each group, including the control group.

No adverse effect of acetamiprid treatment on the maternal reproductive system was evident. No premature delivery or abortion was seen in any treated group, including the 30 mg/kg bw per day group. No adverse effect on the numbers of total implantations, corpora lutea, or viable and non-viable fetuses or on the values of pre-implantation and post-implantation losses was noted.

No significant changes, relative to control values, were noted in fetal body weights, placental weights and numbers of male and female fetuses per pregnant female for the treated groups. The incidences of underdeveloped fetuses in the treated groups were higher than the control values, but there was no statistical significance.

External malformations, open eyelid and manus varus, were seen in one fetus from the litter of one 30 mg/kg bw per day female (incidence 1.2%, 1/86 fetuses). External variations were not seen in any group. The external malformations seen in the 30 mg/kg bw per day group were not considered to be related to treatment because the incidence was not statistically significant.

No effects of treatment on visceral malformation or variation were seen in fetuses. The incidences of fetuses with visceral malformations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 1.1% (1/93 fetuses), 1.0% (1/99 fetuses), 0% (0/94 fetuses) and 1.2% (1/86 fetuses), respectively. The incidences of dams containing fetuses with visceral malformations for these same groups were 8.3% (1/12 dams), 8.3% (1/12 dams), 0% (0/12 dams) and 7.7% (1/13 dams), respectively. There were no statistically significant differences in the incidences of visceral malformations, on either a per fetus or per dam basis, when compared with control data. The visceral malformations seen in fetuses of the control and treated groups were microphthalmia (30 mg/kg bw per day), abnormal origin of right subclavian artery (control) and bifid apex of heart (7.5 mg/kg bw per day).

The incidences of fetuses with visceral variations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 3.2% (3/93 fetuses), 3.0% (3/99 fetuses), 5.3% (5/94 fetuses) and 2.3% (2/86 fetuses), respectively. These incidences were not statistically significant compared with control values. The visceral variations seen in fetuses from the control and treated groups were dilatation of renal pelvis and thymic remnant in the neck.

Acetamiprid did not cause fetal skeletal malformations, variations or ossification defects. The incidences of fetuses with skeletal malformations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 4.3% (4/93 fetuses), 4.0% (4/99 fetuses), 3.2% (3/94 fetuses) and 4.7% (4/86 fetuses), respectively. The incidences of dams containing fetuses with skeletal malformations for these same groups were 33.3% (4/12 dams), 25.0% (3/12 dams), 25.0% (3/12 dams) and 30.8% (4/13 dams), respectively. There were no statistically significant differences in the incidences of skeletal malformations, on either a per fetus or per dam basis, when compared with control data. The main skeletal malformations seen in fetuses of the control and treated groups were absence and fusion of thoracic vertebral arches, absence of thoracic vertebral body, fusion of ribs, bifurcation of rib, fusion of sternebrae and absence of lumbar vertebral arch. The fusion of thoracic vertebral arches was seen only in the 30 mg/kg bw per day group (2/86 fetuses), with a dose–response relationship ($P < 0.05$). However, this effect was not considered to be related to the treatment, because the incidence was not statistically significant when compared with the control values.

The incidences of fetuses with skeletal variations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 71.0% (66/93 fetuses), 64.6% (64/99 fetuses), 45.7% (43/94 fetuses) and 69.8% (60/86 fetuses), respectively. The skeletal variations seen in fetuses from the control and treated groups were cervical rib, asymmetry, splitting and bilobed shape of the sternebrae, shortened 13th rib, nodulated rib, 13th rib and floating rib. The most frequent variation was the 13th rib. Morita et al. (1987) reported that the incidences of the 13th rib in New Zealand White rabbits were higher than those of other rabbit strains. The 13th ribs seen in this study were also considered to be spontaneous. The incidence of the 13th rib in the 15 mg/kg bw per day group was significantly lower than the control incidence. However, this is not considered to be related to acetamiprid treatment, because no statistical significance was noted in the higher dose group. The incidence of floating rib in the 30 mg/kg bw per day group (4/86 fetuses) was significantly lower than the control incidence (14/93 fetuses), with a negative dose–response relationship ($P < 0.05$). The incidence of shortened 13th rib in the 7.5 mg/kg bw per day group (16.2%, 16/99 fetuses) was significantly higher than the control incidence (3.2%, 3/93 fetuses). However, this was not considered to be related to acetamiprid treatment, because there was no dose–response relationship ($P = 0.68$, not significant). The incidences of other variations lacked statistical significance compared with the control group. Based on the findings mentioned above, it was considered that acetamiprid treatment did not cause fetal skeletal variations. Acetamiprid treatment did not cause retarded ossification in fetuses of any treated group.

The NOAEL for maternal toxicity in rabbits was 15 mg/kg bw per day, based on the decreases in feed consumption and body weight gain during the treatment period noted at 30 mg/kg bw per day. The developmental NOAEL was 30 mg/kg bw per day, the highest dose tested.

This study was conducted in compliance with GLP, and a QA statement was attached (Nukui & Fujii, 1994b).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity

A dose range–finding study was undertaken for the assessment of behavioural changes and time for peak effect for these changes following treatment with acetamiprid. Three groups of three

male and three female Crl:CD(SD)BR rats were dosed with acetamiprid (batch No. NFG-02, purity 99.9%) on a single occasion at a dose of 10, 50 or 100 mg/kg bw. The animals were 35 days old with weights of approximately 15 g at the time of receipt and acclimatized for 6 days before initiation of the study. A functional observational battery was performed prior to treatment and 0.5, 2 and 5 hours after dosing. Body weight was recorded on a daily basis after treatment. Animals were maintained for 14 days after treatment and then killed and subjected to routine macroscopic examination.

At 100 mg/kg bw, there were clinical signs of dilated pupil (females only) and tremors and an initial impairment in body weight gain for females on day 1. Neurobehavioural observations included body tremors, chewing (females only), hunched posture and clear reduction in body temperature. At 50 mg/kg bw, neurobehavioural observations included body tremors and tail tremors (females only). There were no clear effects of treatment at 10 mg/kg bw. The time for peak effect was between 5 and 6 hours post-dosing.

In view of the above, a dose of 100 mg/kg bw was suggested to be a suitable highest dose for the subsequent acute neurotoxicity study, with post-dosing observations being performed at approximately 6 hours after dosing.

This study was not conducted in compliance with GLP (Hughes, 1997a).

Assessment of the potential neurotoxicity of acetamiprid in male and female rats was studied. Three groups of 10 male and 10 female Crl:CD BR rats (approximately 35 days old for males and 28 days old for females and weighing approximately 15 g for each sex) were given acetamiprid (batch No. NFG-02, purity 99.9%) as a single oral (by gavage in 0.5% sodium carboxymethylcellulose) dose at levels of 10, 30 and 100 mg/kg bw. A similar size group was given the vehicle and acted as control.

Throughout the study, clinical signs, body weight and feed consumption were monitored. All animals were subjected to a functional observational battery prior to treatment, at 6 hours post-dosing and on days 7 and 14 post-dosing. The motor activity of each animal was also quantitatively assessed prior to treatment, at 6 hours post-dosing and on days 7 and 14 post-dosing. At the end of the treatment period, all animals were killed using whole-body perfusion. Examination was confined to designated tissues of the nervous system, which were subsequently examined microscopically.

With respect to the clinical signs, signs of reaction to treatment were confined to the day of dosing at 100 mg/kg bw and were observed 7–8 hours after dosing only. Signs included tremor, hunched posture, unsteadiness and coldness to touch. These were the same signs that were noted during the performance of the functional observational battery (Table 60).

Feed consumption among males of the 100 mg/kg bw group was significantly lower than that of controls in the 1st week following administration. The gains in week 2 were comparable to those of controls. No such findings could be seen among females of the highest-dose group (Table 61).

Body weight gains for males at 100 mg/kg bw were statistically significantly lower compared with controls in the 1st week following the acute administration. The gains in week 2 were comparable with those for controls. There were no similar findings among females at 100 mg/kg bw. At lower doses, there was no effect on weight gain (Table 62).

There was no difference in feed conversion ratios between treated and control groups.

Functional observational data revealed no remarkable differences between the groups either in terms of the observational end-points or in terms of the continuous measurements prior to dosing.

Six hours post-dosing, a range of effects was observed. Tremors were observed among males and females treated at 100 mg/kg bw. A few animals were observed with tremors in the cage (females only) or on removal from the cage (males and females). Although these observations of tremor were not always supported by statistically significant differences, the occurrences of tremors in the cage and in the hand has never been seen in control animals, and it was considered to be attributable to treatment. Most of the tremors were observed once the animals were placed into the arena. Tremors

Table 60. Principal clinical signs associated with treatment

Signs observed	Number of animals in groups showing signs							
	Males				Females			
	Dose (mg/kg bw)							
	Control	10	30	100	Control	10	30	100
Tremor	0	0	0	3	0	0	0	4
Unsteady	0	0	0	2	0	0	0	1
Reluctant to move	0	0	0	0	0	0	0	1
Hunched	0	0	0	1	0	0	0	3
Cold to touch	0	0	0	0	0	0	0	2
Nasal staining	0	0	0	0	0	0	0	1

From Hughes (1997b)

Table 61. Feed consumption, group mean values

Week	Feed consumption, mean ± standard deviation (g)			
	Dose (mg/kg bw)			
	Control	10	30	100
Males				
−1	205	207	205	199
1	233 ± 12.7	237 ± 17.9	234 ± 22.1	211* ± 24.6
2	231 ± 8.4	231 ± 17.1	235 ± 20.5	223 ± 21.5
Females				
−1	139	137	143	141
1	173 ± 10.8	180 ± 12.7	182 ± 9.2	172 ± 6.9
2	175 ± 18.3	176 ± 11.0	175 ± 12.9	179 ± 11.5

From Hughes (1997b)
* $P < 0.05$

ranged from moderate to marked and affected the body, head, limbs and tail. They appeared to be exacerbated by the animal moving, and on some occasions, for some females, the animals appeared to collapse for a brief period following a bout of tremor. The incidence of tremors in the arena was statistically significantly increased among females at 100 mg/kg bw. Although not statistically significant, the occurrence of marked tremors in males at 100 mg/kg bw was attributed to treatment (Table 63).

Chewing movements of the mouth were observed in one male at 100 mg/kg bw, one female at 10 mg/kg bw and five females at 100 mg/kg bw. The latter incidence was statistically significant. Males at 100 mg/kg bw were statistically significantly more difficult to handle on removal from their cage. Some males and females at 100 mg/kg bw were cold to the touch, with the incidence among females attaining statistical significance.

Pupils were observed as being dilated just prior to the pupil reflex in three males and six females at 100 mg/kg bw. These same animals were assessed as having medium to large pupil size under normal light. These incidences were statistically significant.

There was an indication that frequency of urination was increased among males treated at 30 and 100 mg/kg bw, with the incidence among males at 100 mg/kg bw attaining statistical significance.

Table 62. Body weight, group mean values

Weeks	Dose (mg/kg bw)			
	Control	10	30	100
Males				
Body weight (g)				
−1	172	173	172	172
0	201	205	201	199
1	278	283	281	265
2	322	322	322	305
Body weight gain, mean ± standard deviation (g/rat per week)				
0–1	77 ± 6.3	78 ± 5.4	80 ± 8.5	66* ± 14.4
1–2	43 ± 5.5	39 ± 7.5	41 ± 8.6	41 ± 7.9
0–2	121 ± 10.7	117 ± 12.1	121 ± 15.6	106* ± 20.2
% of control				
0–1	—	101	104	86
1–2	—	91	95	95
0–2	—	97	100	88
Females				
Body weight (g)				
−1	153	154	155	155
0	154	155	157	158
1	193	199	200	200
2	215	221	220	224
Body weight gain, mean ± standard deviation (g/rat per week)				
0−1	39 ± 6.3	45 ± 5.0	43 ± 5.8	42 ± 6.8
1−2	22 ± 5.4	21 ± 6.2	20 ± 7.9	24 ± 3.9
0−2	61 ± 7.0	66 ± 5.6	63 ± 7.7	66 ± 7.6
% of control				
0−1	—	115	110	108
1−2	—	95	91	109
0−2	—	108	103	108

From Hughes (1997b)
* $P < 0.05$

There was hunched posture in males treated at 100 mg/kg bw. A low incidence of hunched posture was observed in all treated females, with the highest incidence occurring among females treated at 100 mg/kg bw. This incidence was statistically significantly increased compared with controls. An increased incidence of walking on toes was noted in males treated at 100 mg/kg bw and in all treated female groups. The incidence among males was statistically significantly increased.

Slipping of hindlimbs was observed in one male and three females at 100 mg/kg bw. The incidence among females was statistically significantly increased.

Two females at 100 mg/kg bw were unable to lift their front feet and were recorded as "front feet dragging". A female was assessed as having impaired mobility due to the changes in gait.

Table 63. Functional observational data (tremor)

	Incidence of tremor															
	Pre-dosing								6 h post-dosing							
	Males				Females				Males				Females			
	Dose (mg/kg bw)															
	0	10	30	100	0	10	30	100	0	10	30	100	0	10	30	100
Number of animals examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Home cage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Removal from cage	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1
In the arena	0	0	0	0	1	0	0	0	5	4	8	6	4	3	5	7*

From Hughes (1997b)
* $P = 0.007$

On day 7, the incidence of defecation was statistically significantly increased among males at 100 mg/kg bw. In the absence of other effects, this change was not considered attributable to treatment. On day 14, there was no remarkable difference between the groups.

There were no statistically significant differences in activity counts or rearing counts between the observation days.

Six hours after dosing, there were statistically significantly increased values recorded for forelimb grip strength among males treated at 100 mg/kg bw. Forelimb grip strength among females at 100 mg/kg bw was slightly increased from controls, but differences failed to attain statistical significance. There were no statistically significant differences at lower doses. Hindlimb grip strength was also increased among males treated at 100 mg/kg bw, with differences just failing to attain statistical significance. Among females, values were comparable between treated and control groups. There were no statistically significant differences at lower doses 6 hours after dosing as well as on day 7 or 14 (Tables 64 and 65).

Only 5 hours after dosing, there was a tendency for lower splay values among males treated at 100 mg/kg bw. Among females treated at 100 mg/kg bw, mean splay values were statistically significantly lower compared with controls (Table 66). At the same time, the body temperature values of males and females of the same group were statistically significantly lower than those of the controls (Table 67). At lower doses, values were considered comparable with those of controls. There was no statistically significant difference on days 7 and 14.

Six hours after dosing, Coulbourn activity was statistically significantly lower among males treated at 30 and 100 mg/kg bw and among females treated at 100 mg/kg bw. At 100 mg/kg bw, there was basically no activity. For males at 30 mg/kg bw, the initial level of activity was lower compared with controls (Table 68). The pattern of activity over time was similar to controls. There were no statistically significant differences in activity between treated and control groups on day 7 or 14.

There were no microscopic findings that were considered to be related to the administration of acetamiprid.

In view of the above findings, it can be concluded that a single acute dose of acetamiprid at 10, 30 or 100 mg/kg bw was associated with clinical signs of decreased weight gain and lower feed consumption among males treated at 100 mg/kg bw during the week following treatment.

Behavioural changes were confined to the day of treatment. Six hours after an acute dose of acetamiprid, a range of effects was observed, particularly at 100 mg/kg bw. Behavioural observations

Table 64. Group mean forelimb grip strength, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Forelimb grip strength (kg)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	0.71	0.157	0.77	0.108
10	0.81	0.085	0.75	0.165
30	0.82	0.127	0.81	0.104
100	0.94**	0.110	0.86	0.062

From Hughes (1997b)
** $P < 0.05$

Table 65. Group mean hindlimb grip strength, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Mean hindlimb grip strength (kg)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	0.64	0.151	0.75	0.149
10	0.70	0.193	069	0.177
30	0.71	0.191	0.76	0.171
100	0.81	0.174	0.73	0.115

From Hughes (1997b)

at 100 mg/kg bw included observations of tremor, dilated pupils, increased urination (among males) and altered patterns of gait and posture. There were clear effects on temperature, which was reduced. Grip strength was increased, and landing foot splay was decreased. The level of locomotor activity was reduced.

At 30 mg/kg bw, there was evidence of tremor among males (one animal showed continuous tremor), and one female showed tail tremor. There was also an indication of increased urination among males. There was a statistically significant reduction of locomotor activity among males.

At 10 mg/kg bw, there were no effects of treatment. The singular occurrence of chewing behaviour in one female in the absence of other changes was not considered clearly related to treatment.

The main effect of treatment would appear to be on the central nervous system, with the observation of tremors. There were some effects on the autonomic nervous system, as indicated by observations of increased urination and increased pupil diameter/dilation. The changes in grip strength (increased) and foot splay (decreased) would appear to be enhanced performance and are suggestive of an increase in muscle tone. There were no apparent effects on sensory systems. There was no evidence of neuropathology.

Based on these findings, the NOAEL was considered to be 10 mg/kg bw, based on evidence of increased urination frequency (males) and a statistically significant reduction of locomotor activity (males) at 30 mg/kg bw.

The study described in this report was conducted in compliance with GLP, and a QA statement was attached (Hughes, 1997b).

Subchronic neurotoxicity

Four groups of 10 male and 10 female Crl:CD BR rats were given acetamiprid (batch No. NFG-02, purity 99.9%) by dietary admixture at a level of 100, 200, 800 or 1600 ppm (equivalent to

Table 66. Group mean splay values, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Splay values (cm)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	7.9	1.74	8.6	2.11
10	9.2	2.30	8.0	1.60
30	7.5	2.02	7.2	2.30
100	6.7	1.02	6.1**	1.15

From Hughes (1997b)
** $P < 0.01$

Table 67. Group mean temperature, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Mean temperature (°C)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	37.1	0.37	37.4	0.66
10	37.3	0.44	37.5	0.58
30	37.1	0.33	37.4	0.50
100	35.4**	1.22	34.9**	2.13

From Hughes (1997b)
** $P < 0.01$

Table 68. Coulbourn activity monitoring, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Mean large movements (in sets) during 1 h observation period			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	256	83.4	258	102.0
10	235	153.1	250	92.3
30	154*	40.5	219	66.6
100	45**	54.2	33**	25.2

From Hughes (1997b)
* $P < 0.05$; ** $P < 0.01$

7.4, 14.8, 59.7 and 118 mg/kg bw per day for males and 8.5, 16.3, 67.6 and 134 mg/kg bw per day for females, respectively) for 13 weeks. Dietary inclusion levels remained constant throughout the study. A similar sized group was given untreated diet and acted as controls.

All animals were subjected to a functional observational battery prior to treatment and after 4, 8 and 13 weeks of treatment. The motor activity of each animal was also quantitatively assessed at the same intervals. Throughout the study, clinical signs, body weights and feed consumption were monitored. At the end of the treatment period, all animals were killed using whole-body perfusion. Examination was confined to designated tissues of the nervous system, which were subsequently examined microscopically.

There were no mortalities. There were no clinical signs associated with treatment. Body weights at 800 and 1600 ppm, for males and females, were statistically significantly lower than those of controls. At 800 ppm, body weight gains were 80% and 66% of control values for males and females, respectively. At 1600 ppm, gains were 56% and 51% of control values for males and females, respectively (Table 69). The cumulative intake of food was statistically significantly lower among males and females treated at 800 or 1600 ppm. At 800 ppm, cumulative values were 89% and 87% of control values for males and females, respectively. At 1600 ppm, cumulative values were 77% and 81% of control values for males and females, respectively (Table 70). Utilization of feed was impaired for males at 1600 ppm and for females at 800 and 1600 ppm.

There were no changes that were considered indicative of neurotoxicity. Quantitative assessment of locomotor activity showed that it was unaffected by treatment. There were no differences in brain parameters between the groups. There were no findings in neuropathology that were attributable to treatment.

In view of the above, it can be concluded that the treatment with acetamiprid for 13 weeks at dietary levels of 100, 200, 800 or 1600 ppm was associated with effects that were limited to 800 and 1600 ppm. At 800 and 1600 ppm, treatment was associated with lower body weights and lower feed consumption values. Feed efficiency was also affected, with impaired efficiency observed at 1600 ppm for males and 800 and 1600 ppm for females. There were no behavioural changes that were considered to be indicative of neurotoxicity, nor were there any neuropathological findings that were attributed to treatment.

The NOAEL was established as 200 ppm (equivalent to 14.8 mg/kg bw per day for males and 16.3 mg/kg bw per day for females), on the basis of lower body weights and feed consumption at the next higher dose.

The study was compliant with GLP, and a QA statement was attached (Hughes, 1997c).

Developmental neurotoxicity

A range-finding study was designed with the objective to provide acetamiprid dose selection information for a developmental neurotoxicity study and to define levels inducing toxic effects from maternal exposure during organogenesis on pregnancy, parturition and lactation and on the growth, viability and development of the F_1 neonates.

Acetamiprid (batch No. NNI-03, purity 100%) was administered orally by gavage to four groups of 12 bred Crl:CD(SD)IGS BR rats (84 days old at the initiation of administration, a minimum weight of 220 g) once daily from gestation day 6 through lactation day 10, inclusively. Dose levels were 5, 10, 20 and 40 mg/kg bw per day administered at a dose volume of 5 ml/kg bw. For comparative purposes, a concurrent control group of identical design received the vehicle, 5% gum arabic with 0.01% Tween 80, on a comparable regimen at 5 ml/kg bw. All animals were observed twice daily for appearance and behaviour. Maternal body weights and feed consumption were recorded on gestation days 0, 3, 6, 9, 12, 15 and 20 as well as on lactation days 1, 3, 7, 10, 16 and 21. All of the F_0 females were allowed to deliver and rear their pups to weaning (lactation day 21). The offspring were potentially exposed to the test article in utero (placental transfer) and through nursing during lactation. F_0 females that failed to deliver were necropsied on post-mating day 25. All surviving maternal animals were euthanized on lactation day 21 and subjected to a gross necropsy.

All females survived to the scheduled necropsy. No treatment-related clinical signs or internal findings were noted at any dose level.

In the control, 5, 10, 20 and 40 mg/kg bw per day groups, 11, 12, 11, 12 and 12 females, respectively, delivered litters. The duration of gestation was unaffected by treatment at all dose levels. Mean body weight gains in the 20 and 40 mg/kg bw per day groups were reduced during gestation days 6–9 and 9–12 compared with the control group. Mean body weight gain in the 40 mg/kg bw per day group was slightly reduced compared with the control group during gestation days 15–20. When

Table 69. Body weight gain

Weeks	Body weight gain (g)									
	Males					Females				
	Dietary concentration (ppm)									
	Control	100	200	800	1600	Control	100	200	800	1600
0–4	166	156	148	120**	74**	79	75	77	50**	35**
0–8	261	246	236	202**	139**	120	116	114	80**	59**
0–13	333	327	313	265**	186**	152	144	139	100**	78**
SD										
0–4	18.3	24.9	23.3	23.4	15.6	21.8	12.3	9.9	9.0	9.5
0–8	23.9	34.5	31.8	34.0	29.2	25.2	17.0	17.1	15.3	16.0
0–13	25.5	44.4	40.0	41.0	32.1	39.3	19.9	22.7	12.6	19.7
% of control										
0–4	—	94	89	72	45	—	95	97	63	44
0–8	—	94	90	77	53	—	97	95	67	49
0–13	—	98	94	80	56	—	95	91	66	51

From Hughes (1997c)
SD, standard deviation; ** $P < 0.01$

Table 70. Cumulative feed consumption, group mean values

	Cumulative feed consumption (g)									
	Males					Females				
	Dietary concentration (ppm)									
	Control	100	200	800	1600	Control	100	200	800	1600
Weeks 1–13	2801	2719	2718	2493**	2146**	2039	2014	1944	1782**	1659**
SD	132.1	209.9	133.5	173.7	126.1	237.9	168.4	105.1	143.7	217.1
% of control	—	97	97	89	77	—	99	95	87	81

From Hughes (1997c)
SD, standard deviation; ** $P < 0.01$

the entire gestational treatment period (gestation days 6–20) was evaluated, mean body weight gains in the 20 and 40 mg/kg bw per day groups were reduced (7.8% and 17%, respectively) compared with the control group. Mean body weights in the 20 mg/kg bw per day group were similar to those of the control group throughout gestation and lactation; however, the 40 mg/kg bw per day group values were slightly reduced during gestation days 12, 15 and 20 and throughout lactation (lactation days 1, 3, 7, 10, 16 and 21). Mean body weight gains in the 20 and 40 mg/kg bw per day groups were unaffected by treatment during lactation. Mean body weights and body weight gains in the 5 and 10 mg/kg bw per day groups were unaffected by treatment during gestation and lactation.

In the 40 mg/kg bw per day group, feed consumption was reduced relative to the control group for gestation days 6–9, 9–12, 12–15 and 15–20 and when the entire gestational treatment period (gestation days 6–20) was evaluated. In the 20 mg/kg bw per day group, feed consumption was reduced during gestation days 6–9. When the entire gestational treatment period (gestation days 6–20) was evaluated, feed consumption in the 20 mg/kg bw per day group was reduced compared with the control group. Feed consumption in the 20 and 40 mg/kg bw per day groups was unaffected by treatment

during lactation. Feed consumption in the 5 and 10 mg/kg bw per day group was unaffected by treatment throughout the study. The mean numbers of pups born, mean live litter size and percentage of males per litter at birth were unaffected by treatment in the 5, 10, 20 and 40 mg/kg bw per day groups. Postnatal survival in the treated groups was similar to that in the control group throughout the postnatal period. The general physical condition of the F_1 pups in the 5, 10, 20 and 40 mg/kg bw per day groups was similar to that of the control group. Mean pup weights in the 40 mg/kg bw per day group were reduced relative to the control group values during postnatal days 4 (pre- and post-selection), 7 and 10. Mean pup weights in the 5, 10 and 20 mg/kg bw per day groups were generally similar to the control group values throughout the postnatal period. The general physical condition of the F_1 pups during the postnatal period was unaffected by treatment at all dose levels. Necropsy findings for pups that were found dead or at the scheduled euthanasia on postnatal day 21 were not suggestive of any correlation with maternal treatment.

In conclusion, there were no observations indicative of neurotoxicity in either the maternal animals or the F_1 offspring. Maternal toxicity, however, was exhibited at dose levels of 20 and 40 mg/kg bw per day as reductions in mean body weight gain and feed consumption. Gestation length was unaffected by treatment at all dose levels. Neonatal toxicity was exhibited in the 40 mg/kg bw per day group as reduced mean pup weights. Based on the results of this study, dose levels of 2.5, 10 and 45 mg/kg bw per day were selected for a developmental neurotoxicity study of acetamiprid in rats.

This study was conducted in compliance with GLP (Nemec, 2004).

An oral developmental toxicity study was designed to determine the potential of the test article, acetamiprid, to induce functional and/or morphological changes in the nervous system, which may arise in the offspring from exposure of the mother during pregnancy and lactation. Acetamiprid (lot No. NNI-03, purity > 99%) was administered orally by gavage to three groups of 25 bred Crl:CD(SD) IGS BR rats (75 days old at the initiation of the study, weight 210–270 g) once daily from gestation day 6 through lactation day 21, inclusively. Dose levels were 2.5, 10 and 45 mg/kg bw per day administered at a dose volume of 5 ml/kg bw. For comparative purposes, a concurrent control group of identical design received the vehicle, 5% gum arabic with 0.01% Tween 80, on a comparable regimen at 5 ml/kg bw.

All animals were observed twice daily for appearance and behaviour. Clinical observations were performed on all females daily. In addition, detailed clinical observations were performed on 10 females per group on gestation days 6 and 12 and on lactation days 4 and 7. Maternal body weights and feed consumption were recorded on gestation days 0, 3, 6, 9, 12, 15 and 20, as well as on lactation days 1, 4, 7, 10, 16 and 21. All of the F_0 females were allowed to deliver and rear their pups to weaning (lactation day 21). The offspring were potentially exposed to the test article in utero (placental transfer) and through nursing during lactation. F_0 females that failed to deliver were necropsied on post-mating day 25. F_0 females that delivered were allowed to rear their offspring to lactation day 21 and were then necropsied. F_0 females with litters that did not meet the sex ratio criteria were necropsied with their litters on postnatal day 4. Following culling on postnatal day 4, 10 pups of each sex per group were randomly assigned to one of the following tests: learning and memory (different pups for each interval), locomotor activity and acoustic startle. Additional pups were assigned to neuropathological, brain weight and brain morphometric evaluations on postnatal day 11 (one pup of each sex per litter) and postnatal day 72 (10 pups of each sex per group). Detailed clinical observations were performed on 10 pups of each sex per group on postnatal days 4, 11, 21, 35, 45 and 60. Indicators of physical development (balanopreputial separation and vaginal patency) were evaluated for all pups. All pups not selected for behavioural evaluations were euthanized and necropsied on postnatal day 28.

One female in the 45 mg/kg bw per day group died during parturition on gestation day 23, following delivery of one pup. This dystocia-related death was considered potentially treatment related.

All other females survived to the scheduled necropsies. No adverse clinical signs of toxicity were noted. Pregnancy rates were 92.0%, 92.0%, 92.0% and 100% in the control, 2.5, 10 and 45 mg/kg bw per day groups, respectively. A mean body weight loss was observed in the 45 mg/kg bw per day group during the first 3 days of dosing (gestation days 6–9). Mean gestation body weights in this group were lower than the control group values during the majority of gestation. Mean body weight in this group was reduced compared with the control group on lactation day 1. Mean lactation body weight gain in the 45 mg/kg bw per day group was increased on lactation days 1–4. Mean gestation and lactation body weights in the 2.5 and 10 mg/kg bw per day groups were similar to the control group values.

Feed consumption in the 45 mg/kg bw per day group was reduced during gestation days 6–9 and 9–12. During lactation, feed consumption in this group was similar to that in the control group. Feed consumption in the 2.5 and 10 mg/kg bw per day groups was similar to the control group values during gestation and lactation.

No treatment-related internal findings were noted at the scheduled necropsies of the F_0 females. The mean numbers of implantation sites and sites that were unaccounted for in the treated groups were similar to those in the control group. Mean live litter size in the 45 mg/kg bw per day group was 7.3% lower than the control group value. Mean live litter size in the 2.5 and 10 mg/kg bw per day groups was similar to that in the control group. Postnatal survival in the 45 mg/kg bw per day group was reduced on postnatal day 0 and postnatal days 0–1. Three females in this group had total litter loss on postnatal day 1. Postnatal survival in the 2.5 and 10 mg/kg bw per day groups was not affected by F_0 maternal test article administration.

The general physical condition of the F_1 animals during the pre-weaning and post-weaning periods was similar in all groups, including the control group. Mean offspring body weight gains in the treated groups were similar to the control group values during the pre-weaning period. Mean body weights in the 45 mg/kg bw per day group males and females, however, were statistically significantly reduced on postnatal day 1 and were numerically reduced throughout the remainder of the pre-weaning period. Mean body weight gains in the 45 mg/kg bw per day group males were reduced during postnatal days 35–72. Mean body weight gains in the 45 mg/kg bw per day group females were reduced on postnatal days 28–35 and 49–56. Mean body weights of these males and females were reduced throughout the post-weaning period. Mean body weights and body weight gains in the 2.5 and 10 mg/kg bw per day groups were not affected by F_0 maternal test article administration. The mean numbers of days to acquisition of balanopreputial separation and vaginal patency in the treated group males and females were not affected by F_0 maternal test article administration. Mean body weights in the treated groups on the day of acquisition of balanopreputial separation were similar to the control group value. Mean body weight in the 45 mg/kg bw per day group females was reduced on the day of vaginal patency acquisition. Mean body weights on the day of acquisition of vaginal patency in the 2.5 and 10 mg/kg bw per day group females were similar to the control group value.

For auditory startle response, the maximum response amplitudes (V_{max}) and average response amplitudes (V_{avg}) were reduced on postnatal days 20 and 60 in the 45 mg/kg bw per day group males. V_{max} and V_{avg} were reduced in the 45 mg/kg bw per day group females on postnatal day 20. V_{max} and V_{avg} in the 45 mg/kg bw per day group females were similar to the control group values on postnatal day 60. Auditory startle response in the 2.5 and 10 mg/kg bw per day group males and females was unaffected by F_0 maternal test article administration.

Locomotor activity and learning and memory in the 2.5, 10 and 45 mg/kg bw per day group offspring were unaffected by F_0 maternal test article administration. Necropsy findings for F_1 pups that were found dead, euthanized in extremis and euthanized due to failure to meet sex ratio criteria during the pre-weaning period were not suggestive of any correlation with maternal treatment. At the scheduled necropsy of pups that were not selected for behavioural evaluations (postnatal day 28), the postnatal day 72 necropsies of offspring not selected for neuropathology and brain weight

measurements and the postnatal day 11 and postnatal day 72 necropsies of offspring selected for neuropathology and brain weight measurements, no internal findings were observed that could be attributed to maternal test article administration. There were no test article–related microscopic findings in the central and peripheral nervous system tissues examined from the selected postnatal day 11 and postnatal day 72 pups. Mean absolute brain weights, brain weights relative to final body weights, brain lengths, brain widths and F_1 brain morphometry at postnatal day 11 and postnatal day 72 in the treated group males and females were not affected by F_0 maternal test article administration.

In conclusion, F_0 maternal toxicity was expressed at a dose level of 45 mg/kg bw per day by a single mortality and reductions in body weight gain and feed consumption. No maternal toxicity was exhibited at dose levels of 2.5 and 10 mg/kg bw per day. F_1 developmental toxicity was expressed at a dose level of 45 mg/kg bw per day by early postnatal mortality and reduced post-weaning body weights. No developmental toxicity was exhibited at dose levels of 2.5 and 10 mg/kg bw per day.

Deficits in auditory startle response occurred in the 45 mg/kg bw per day group F_1 males and females without concomitant effects in other functional end-points (functional observational battery), neuropathology or brain morphometry. For this reason, as auditory startle response amplitude is affected by treatment, the conclusion could be that some part of the pathway is affected. The affected component could be sensory or it could be motor, but some part of the nervous system (or muscle fibre activity) is affected. However, the startle response may not be free from central involvement, as there is ample evidence for descending inhibitory activity, which may, in fact, be the basis for habituation (Tyl et al., 2008).

Based on the results of this study, the NOAEL for maternal toxicity, developmental toxicity and developmental neurotoxicity was considered to be 10 mg/kg bw per day.

This study was conducted in compliance with GLP, and a QA statement was attached (Nemec, 2003).

Acute delayed neurotoxicity

To determine the acute oral toxicity of acetamiprid in adult hens, six groups of 10 birds (hybrid brown laying strain *Gallus gallus domesticus* approximately 15 months old, weight range 1930–2295 g) were given a single dose of acetamiprid (batch No. NNI-03, purity > 99%) and observed for 14 days. The LD_{50} value was calculated to be 129 mg/kg bw.

To investigate any delayed neurotoxic effects of acetamiprid, the doses used in the neurotoxicity assessment were based on the LD_{50} value. The study involved a group of 32 birds (hybrid brown laying strain *Gallus gallus domesticus* approximately 14 months old, weight range 2090–2395 g). Negative and positive control groups were also dosed, with 12 birds allocated to each. Observations included mortality, adverse clinical signs, assessment of delayed locomotor ataxia and body weight. Forty-eight hours after dosing, predetermined birds were examined for brain acetylcholinesterase and brain and spinal cord neuropathy target esterase activities. At the end of the observation period, additional designated birds were sent for histopathological examination. During the neurotoxicity assessment, all birds treated with acetamiprid either died or showed clinical signs of toxicity, which included unsteadiness and subdued behaviour. There were no signs of delayed locomotor ataxia in any bird treated with acetamiprid. One bird in the positive control group showed slight incoordination. Brain acetylcholinesterase and brain and spinal cord neuropathy target esterase activities in birds treated with acetamiprid were considered to be normal. Oral administration of a single dose of acetamiprid at the LD_{50} dose level of 129 mg/kg bw did not produce any clinical signs of delayed neurotoxicity when assessed in terms of ataxia. There was no histological evidence of acute delayed neurotoxicity. No other treatment-related changes were detected in this examination. As expected, given the absence of any other indication of delayed neurotoxicity, there were no significant reductions in brain acetylcholinesterase levels or neuropathy target esterase levels in the brain and spinal cord.

This study was conducted in compliance with GLP, and a QA statement was attached (Redgrave, 1994).

(b) Immunotoxicity

Mice

To assess the functional responsiveness of specified elements of the immune system using a modification of the Jerne plaque-forming cell assay in the CD-1 mouse, three groups of mice, each comprising 10 males and 10 females, were administered acetamiprid (lot No. NFG-02, purity 99.9%) in the diet at a concentration of 100, 300 or 900 ppm (equal to 15.3, 47.0 and 128 mg/kg bw per day for males and 20.2, 54.1 and 157 mg/kg bw per day for females, respectively) for a period of 4 weeks. A similarly constituted control group received the vehicle, basal diet. The age of animals at the start of the study was between 47 and 56 days, and their body weights were in the range of 29.2–38.9 g for males and 22.7–33.0 g for females. A further eight males and eight females received cyclophosphamide (a positive control) at 20 mg/kg bw, administered as five daily oral (gavage) doses on days 22–26. During the study, clinical condition, body weight, feed and water consumption, organ weight, macropathology and plaque-forming cell assays were undertaken.

There were no unscheduled deaths. Noisy breathing (rales) was reported in week 4 in 2 of 10 males and 3 of 10 females receiving 900 ppm.

Body weight gains were low during the 4-week treatment period for males and females receiving 900 ppm, with the magnitude of the effect being greatest on days 1–7, when several animals experienced weight loss (Table 71).

Feed intake at 900 ppm was reduced in weeks 1 and 2, and water intake was slightly lower than that of controls throughout the treatment period in males and in week 1 in females. Females receiving 300 ppm had low body weight gain from the end of week 1 and low feed consumption in weeks 1 and 2 (Table 72).

Low spleen weights were reported after 4 weeks in females receiving 900 ppm. There were no treatment-related effects on thymus weights or macropathology.

There was considered to have been no effect of treatment on the immune system. Female mice given acetamiprid at 900 ppm showed a statistically significant reduction in the observed number of plaque-forming cells per spleen. This finding correlates with a general reduction in total cell numbers per spleen, showing that the reduced response was due to a reduction in cell number, rather than the immune function of the cells. There was no similar trend in males (Table 73).

It is concluded that dietary administration of acetamiprid to CD-1 mice at dietary concentrations up to 900 ppm for 4 weeks caused a nonspecific toxic response at 300 ppm in females and at 900 ppm in both sexes, but there was no effect on immune function, as assessed by the measurement of antigen-specific, T cell–dependent antibody formation. The NOEL for immunotoxicity by acetamiprid in mice was therefore greater than 900 ppm (i.e. > 128 mg/kg bw per day in males and > 157 mg/kg bw per day in females), the highest dose tested.

This study was conducted in compliance with GLP, and a QA statement was attached (Bown, 2010).

Rats

A study was conducted to assess the functional responsiveness of specified elements of the immune system, using a modification of the Jerne plaque-forming cell assay, in the Sprague-Dawley rat following dietary administration for 4 weeks. Three groups of Sprague-Dawley rats (Crl:CD (SD) strain), each comprising 10 males and 10 females, received acetamiprid (lot No. NFG-02, purity 99.9%) in the diet at a concentration of 100, 300 or 900 ppm (equivalent to 7.4, 21.6 and 62.9 mg/kg bw per day for males and 8.6, 24.3 and 67.7 mg/kg bw per day for females). A similarly constituted

Table 71. Summary of body weight gain

Days	Body weight gain (g)							
	Males				Females			
	Dietary concentration (ppm)							
	Control	100	300	900	Control	100	300	900
1–7	1.8	0.7	1.4	−0.3**	1.0	0.9	1.0	−0.2*
8–29	2.8	3.6	3.1	2.3	2.2	3.1	1.8	1.7
1–29	4.6	4.3	4.5	2.0**	3.2	4.0	2.8	1.5
% of control (days 1–29)	—	93	98	44	—	125	88	48

From Bown (2010)
* $P < 0.05$; ** $P < 0.01$

Table 72. Summary of feed consumption

Weeks	Feed consumption (g/week)							
	Males				Females			
	Dietary concentration (ppm)							
	Control	100	300	900	Control	100	300	900
1–2	39[a]	36	42	31**	37	38	33	31*
3–4	38	41	41	38	41	45	39	37
1–4	39[a]	38	42	34	39	41[a]	36	34

From Bown (2010)
* $P < 0.05$; ** $P < 0.01$
[a] Feed consumption value includes an estimate of a missing value.

control group received the vehicle, basal diet. A further eight males and eight females received a single intraperitoneal dose of cyclophosphamide (a positive control) at 50 mg/kg bw 2 days prior to necropsy (i.e. on day 27). The age of those animals selected for the study at the start of treatment was 48–57 days, and their body weights were in the range of 262–330 g for males and 184–232 g for females. During the study, clinical condition, body weight, feed and water consumption, organ weights, macropathology and plaque-forming cell assays were undertaken.

There were no unscheduled deaths or any treatment-related clinical signs. Body weight gains and feed consumption (Tables 74 and 75) were low during the 4-week treatment period for males and females receiving 900 ppm, with the magnitude of the effect on body weight being greatest on days 1–3 and the effect on feed consumption being greatest in weeks 1 and 2 for males. Males receiving 300 ppm had low body weight gain during days 1–3 and low feed consumption in week 1.

Water consumption was low for males receiving 300 or 900 ppm. There were no treatment-related effects on spleen and thymus weights or macropathology. There were no statistically significant changes in the numbers of cells per spleen, plaque-forming cells per 10^6 cells or plaque-forming cells per spleen for any of the groups that received acetamiprid.

It is concluded that dietary administration of acetamiprid to Sprague-Dawley rats at dietary concentrations up to 900 ppm for 4 weeks caused a nonspecific toxic response at 300 ppm in males and 900 ppm in both sexes, but there was no effect on immune function, as assessed by the measurement of antigen-specific, T cell–dependent antibody formation.

The NOEL for immunotoxicity by acetamiprid in rats was therefore greater than 900 ppm (i.e. > 62.9 mg/kg bw per day in males and > 67.7 mg/kg bw per day in females), the highest dose tested.

Table 73. Summary of immunotoxicity findings

	Dietary concentration (ppm)				Cyclophosphamide[a]
	Control	100	300	900	
Males					
Cells/spleen (× 10^7)	6.65	5.64	7.04	6.73	3.06**
PFC/10^6 cells	1611.5	1076.5	1062.5	1350.5	186.6***
PFC/spleen	104 208	59 182	78 226	101 745	8098***
Females					
Cells/spleen (× 10^7)	9.46	7.68	7.33	6.08*	4.53**
PFC/10^6 cells	1740.0	1333.8	1737.3	1525.5	320.6***
PFC/spleen	170 060	105 397	116 812	93 878*	17 073***

From Bown (2010)
PFC, plaque-forming cells
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
[a] 20 mg/kg bw per day administered as five daily oral doses on days 22–26.

The study was conducted in compliance with GLP, and a QA statement was attached (Moore, 2010).

(c) Pharmacological studies

The general pharmacological properties of acetamiprid (lot No. NNI-02, purity 99.46%) were investigated in mice, rats, guinea-pigs and rabbits.

Animals used in this study were as follows:

- male Crj:ICR mice (5–6 weeks old, weight 27.7–31.9 g);
- male Crj:CD(SD) rats (7 weeks old, weight 262.4–323.2 g);
- male Std:Hartley guinea-pigs (4–6 weeks old, weight 284.2–416.7 g);
- male Kbs:NZW rabbits (11–13 weeks old, weight 2.48–2.84 kg).

Dosage and dose volume

Based on the results of the study on general activity and behaviour in mice (1, 3, 5, 10, 20, 30 and 60 mg/kg bw intraperitoneally), three doses (5, 10 and 20 mg/kg bw) at a common ratio of 2 were selected for the intraperitoneal experiments. Three doses (10, 20 and 40 mg/kg bw) at a common ratio of 2 were selected for the oral experiments. In the study on general activity and behaviour in rabbits, doses of 10, 30 and 60 mg/kg bw were administered. In this study using anaesthetized rabbits, doses of 1, 3 and 10 mg/kg bw were administered. In the in vitro experiment, concentrations of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} g/ml at a common ratio of 10 were selected. The solvent used to dissolved the test substance was used for the corresponding vehicle group in each study.

Dose volumes were 10 ml/kg bw for oral and intraperitoneal administration and 3 ml/kg bw for intravenous injection in the study using conscious rabbits and 1 ml/kg bw for intravenous injection in the study using anaesthetized rabbits.

Results

Effects on general activity and behaviour. In the study of general activity and behaviour in mice, lower doses of acetamiprid (1, 3 and 5 mg/kg bw) (intraperitoneal) showed no clinical signs. One out

Table 74. Summary of body weight gain

	Body weight gain (g)							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	300	900	0	100	300	900
Days 1–3	23	21	17*	1**	4	7	4	−2**
Days 4–29	125	122	120	98**	44	37	42	25**
Days 1–29	148	144	137	99**	48	44	47	23**
% of control (days 1–29)	—	97	93	67		92	97	48

From Moore (2010)
* $P < 0.05$; ** $P < 0.01$

Table 75. Summary of feed consumption

	Feed consumption (g/week)							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	300	900	0	100	300	900
Weeks 1–2	199	192	177	158	135	142	129	115
Weeks 3–4	194	193	186	174	140	137	138	120
Weeks 1–4	196	192	181	166	137	139	133	117

From Moore (2010)

of the three mice at 10 mg/kg bw showed vocalization and slight depression of spontaneous motor activity. All mice of the 20 mg/kg bw group showed the following signs: a slight to moderate decrease in alertness, grooming, reactivity and spontaneous activity, a slight decrease in grip strength, tremor, and abnormality of body and limb position. At 30 mg/kg bw, a slight decrease in alertness, grooming, touch response, pain response, passivity righting reflex, limb tone, pinna reflex and ipsilateral flexor reflex, a severe decrease in reactivity, spontaneous activity and grip strength, an abnormality of body position, tremor and convulsion were observed. One out of three mice died within 120 minutes of the administration. At 60 mg/kg bw, all mice showed a severe decrease in reactivity and spontaneous activity, passivity, tremor, etc. and died within 30 minutes of the administration.

In the study of general activity and behaviour in rabbits, no clinical signs were noted in the vehicle control and the 10 mg/kg bw group (intravenous). At 30 mg/kg bw, one or two out of the three rabbits showed a slight decrease in spontaneous activity, alertness, limb tone, abdominal tone and papillary reflex, a slight increase in respiratory rate and convulsion, motor incoordination and mydriasis from 5 to 180 minutes after the administration. All of these clinical signs disappeared 1 day after the administration. At 60 mg/kg bw, the rabbits showed a severe decrease in spontaneous activity and muscle tone, convulsion, mydriasis, abnormal respiration and cyanosis, etc., and all animals died within 60 minutes of the administration.

Effects on the central nervous system. The test substance elicited no obvious effects on anticonvulsant activity and pain response in mice and on body temperature in rats in the range of 5–20 mg/kg bw (intraperitoneal). In contrast, decreases in motor activity and hypnotic prolongation action were recognized with a dose of 20 mg/kg bw (intraperitoneal).

Effects on the somatic nervous system. No muscle relaxation effects in mice were observed at 5 or 10 mg/kg bw (intraperitoneal). At 20 mg/kg bw, muscle tone tended to be reduced 60–90 minutes after the injection, without statistical significance.

Effects on the autonomic nervous system and smooth muscle (isolated guinea-pig ileum). No direct action was noted at 10^{-6} or 10^{-5} g/ml of the test substance. However, at higher doses (10^{-4} and 10^{-3} g/ml), a transient contraction followed by relaxation was noted. The contraction caused by each agonist was not affected by the test substance (10^{-6} to 10^{-4} g/ml).

At 10^{-3} g/ml, the test substance significantly inhibited the contraction caused by each agonist.

Effects on the respiratory and cardiovascular systems (rabbits). At 1 mg/kg bw, no significant effect was noted on the parameters determined. At 3 and 10 mg/kg bw, a decrease in blood pressure and a slight and transient increase in respiratory rate were noted. The heart rate was not altered by the treatment at any dose.

Effects on the digestive system (mice). Gastrointestinal transport was not affected at 10 or 20 mg/kg bw. However, a statistically significant decrease in gastrointestinal transport was noted at 40 mg/kg bw.

Effects on water and electrolyte metabolism (rats). At 5 and 10 mg/kg bw, the test substance did not alter the urine volume or urinary sodium, potassium and chloride concentrations. However, a statistically significant decrease in urine volume and urinary sodium and chloride concentrations was noted at 20 mg/kg bw.

Effects on blood (rats). No significant changes were noted in blood clotting or haemolysis in rats at 5–20 mg/kg bw.

Effects on plasma cholinesterase activity. Plasma cholinesterase activity was not altered by the intraperitoneal administration of acetamiprid (5–20 mg/kg bw) in rats.

Summary. From the experiments described above, the effect levels of acetamiprid for intraperitoneal, intravenous and oral administration and in vitro tests were 10 mg/kg bw, 3 mg/kg bw, 40 mg/kg bw and 10 g/ml, respectively.

The study was GLP compliant, and a QA statement was attached (Mochizuki & Fujii, 1993).

(d) Studies on impurities

The results of five batch analyses submitted are shown in Table 76.

From Table 76, it can be seen that the impurities present in acetamiprid are AM-1, AM-2, AM-3 and AM-4. The toxicological studies on these impurities that have been submitted are summarized in Tables 77 and 78. All studies were GLP compliant.

(e) Toxicity of metabolites

There are nine compounds identified as plant metabolites. These are IM-1-3, IM-1-4, IM-2-1, IM-2-3, IM-2-4, IM-O, IC-O, IS-1-1 and IS-2-1. The acute toxicity and genotoxicity of these compounds are summarized in Tables 79 and 80. Subchronic toxicity studies on IM-1-4 and IM-O are also described. All these studies were GLP compliant.

Subchronic toxicity study on IM-1-4

The subchronic toxicity of IM-l-4 administered in the diet to Sprague-Dawley Crl:CD BR rats for 13 weeks was evaluated in this study. Male and female rats were assigned to five groups (10 of each sex per group). Each group received IM-1-4 in the diet at the following concentrations: 0, 200, 600, 1800 or 5400 ppm (equal to 0, 12.8, 36.5, 112.2 and 319.3 mg/kg bw per day for males and 0, 15.6, 44.6, 135.6 and 356.1 mg/kg bw per day for females, respectively; however, the compound intake corresponding to 5400 ppm for females related to mean intake between 8 and 13 weeks, as the week 6 feed consumption data were lost for this group). At the initiation of dosing, the animals were approximately 6 weeks old, with body weights ranging from 211 to 270 g for the males and from 154 to 192 g for the females.

Table 76. Analysis of impurities

Lot No.	Contents (% w/w)						
	Acetamiprid	Impurities				Volatiles	Total
		AM-1	AM-2	AM-3	AM-4		
NJLP-18	100.1	0.01	0.05	0.05	0.06	0.1	100.3
NJLP-26	100.0	0.01	0.05	0.04	0.06	< 0.1	100.2
NAAP-24	99.9	0.03	0.05	0.05	0.06	< 0.1	100.1
NAAP-29	100.1	0.03	0.06	0.06	0.06	< 0.1	100.3
NABP-08	100.0	0.03	0.05	0.04	0.06	< 0.1	100.1
Minimum	99.9	0.01	0.05	0.04	0.06	< 0.1	100.1
Maximum	100.1	0.03	0.06	0.06	0.06	0.1	100.3
Mean	100.01	0.02	0.05	0.05	0.06	—	100.2
Standard deviation	0.09	0.01	0.00	0.01	0.00	—	—
Mean + 3 × standard deviation	99.56	0.05	0.07	0.08	0.06	—	—
Specification	99.0	0.2	0.2	0.2	0.2	0.5	—

From five batch analysis reports submitted to WHO
w/w, weight per weight

Table 77. Acute toxicities of impurities

Impurity	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
AM-1	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki (1994b)
			Female	Oral	4811	
AM-2	Rat	Crj:CD(SD)	Male	Oral	603	Mochizuki (1994c)
			Female	Oral	806	
AM-3	Rat	Crj:CD(SD)	Male	Oral	2543	Mochizuki & Goto (1994d)
(=IM-2-1)			Female	Oral	1762	
AM-4	Rat	Crj:CD(SD)	Male	Oral	924	Mochizuki (1994d)
			Female	Oral	1121	

LD_{50}, median lethal dose

Table 78. Results of genotoxicity studies on impurities

Impurity	End-point	Test object	Concentration (µg/plate)	Result	References
AM-1	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Kanaguchi (1994a)
AM-2	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Kanaguchi (1994b)
AM-3 (=IM-2-1)	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994f)
AM-4	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Kanaguchi (1994c)

Table 79. Results of acute toxicity studies on plant metabolites

Plant metabolites	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
IM-1-3	Rat	Crj:CD(SD)	Male	Oral	1142	Mochizuki & Goto (1994b)
			Female	Oral	900–1000	
IM-1-4	Rat	Crj:CD BD	Male	Oral	1223.7	Wakefield (1998b)
			Female	Oral	962.84	
	Rat	Crj:CD(SD)	Male	Oral	1259	Mochizuki & Goto (1994c)
			Female	Oral	1176	
	Rat	Crj:CD BD	Male	Dermal	> 2000	Wakefield (1998a)
			Female	Dermal	> 2000	
IM-2-1 (=AM-3)	Rat	Crj:CD(SD)	Male	Oral	2543	Mochizuki & Goto (1994d)
			Female	Oral	1762	
IM-2-3	Rat	Crj:CD(SD)	Male	Oral	1378	Mochizuki & Goto (1994e)
			Female	Oral	900–1000	
IM-2-4	Rat	Crj:CD(SD)	Male	Oral	1592	Mochizuki (1994e)
			Female	Oral	1381	
IM-O	Rat	Crj:CD(SD)	Male	Oral	1842	Mochizuki & Goto (1993d)
			Female	Oral	1483	
IC-O	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki & Goto (1993c)
			Female	Oral	> 5000	
IS-1-1	Rat	Crj:CD(SD)	Male	Oral	2662	Mochizuki & Goto (1994f)
			Female	Oral	2420	
IS-2-1	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki & Goto (1994g)
			Female	Oral	> 5000	

LD_{50}, median lethal dose

Diet and water were provided ad libitum. The animals were observed twice daily (morning and afternoon) for mortality and moribundity, and daily cage-side observations (cage was opened) were performed for obvious indications of a toxic effect. At least once each week, each animal was removed from its cage and examined for abnormalities and signs of toxicity. Body weights and feed consumption data were collected weekly.

Ophthalmic examinations were conducted prior to treatment and during week 13. Blood and urine samples were collected for haematology and coagulation, serum chemistry and urinalysis tests from all animals prior to termination. At necropsy, macroscopic observations were recorded, selected organs were weighed and selected tissues were collected and preserved. Microscopic examinations were done on tissues from each animal in the control and high-dose groups. The lungs, liver, kidney, spleen and gross lesions were also examined microscopically from each animal in the low- and mid-dose groups.

M-l-4 consumption (milligrams per kilogram body weight per day) was greater in the females than in the males at all dose levels for the duration of the study. There were no compound-related deaths, clinical or macroscopic pathology findings or effects on ophthalmology, haematology and coagulation, urinalysis or organ weights. Significant compound effects on mean feed consumption and body weights that were likely related to reduced diet palatability were observed in the males and females that received the 5400 ppm diet. In the males, mean feed consumption was also significantly reduced below the controls during multiple weeks at both the 600 and 1800 ppm dose levels, although a corresponding significant reduction in body weight was not observed. Female mean body weight

Table 80. Results of genotoxicity studies on plant metabolites

Plant metabolites	End-point	Test object	Concentration	Result	References
IM-1-3	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994d)
IM-1-4	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994e)
	Forward mutation assay	*HGPRT* locus in Chinese hamster ovary cells	250, 500, 1000, 2000, 2500, 3000 µg/ml	Negative both with and without metabolic activation	Cifone (1998)
	In vivo mouse micronucleus assay	Crl:CD-1(ICR)BR mouse bone marrow	175, 350, 700 mg/kg bw	Negative	Curry (1998)
IM-2-1 (=AM-3)	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994f)
IM-2-3	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994g)
IM-2-4	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Kanaguchi (1993b)
IM-O	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994b)
	Chromosomal aberration	Chinese hamster lung cells	*Direct method*: 1, 1.5, 2, 3 mg/ml (24 h) 0.6, 0.8, 1, 1–2 mg/ml (48 h) *Metabolic activation method*: 2, 3, 4, 5 mg/ml	Clastogenic	Kanaguchi (1994d)
	In vivo micronucleus	CD-1(ICR) mice	325, 650, 1300 mg/kg bw	Negative	Murli (1994b)

Table 80 (continued)

Plant metabolites	End-point	Test object	Concentration	Result	References
IC-O	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994a)
IS-1-1	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994h)
IS-2-1	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994i)

and total feed consumption were not significantly affected at the 200–1800 ppm doses. The slight reduction in total protein and globulin in the high-dose animals and the slight changes in several urinalysis parameters in the high-dose females are considered to be related to the observed decreases in body weight and feed consumption in these animals. IM-l-4-related effects were observed in the spleen (increased pigment in the splenic sinusoids) of treated males at 1800 and 5400 ppm and in the spleen of treated females at 5400 ppm.

Based on the results of this study, the NOAEL is considered to be 600 ppm (equal to 36.5 mg/kg bw per day) for males and 1800 ppm (equal to 135.6 mg/kg bw per day) for females.

This study was conducted in compliance with GLP, and a QA statement was attached (Ivett, 1999).

Subchronic toxicity study on IM-O

This study was conducted to assess the subchronic toxicity of compound IM-O in Crj:CD(SD) rats. The test compound (lot No. NK-3266(Tox-563), purity 98.94%) was offered in the diet to 100 rats (10 of each sex per group) at a dose level of 0, 160, 800, 4000 or 20 000 ppm (equal to 0, 9.9, 48.9, 250.1 and 1246.6 mg/kg bw per day in males and 0, 11.1, 55.9, 275.9 and 1173.7 mg/kg bw per day in females) for a period of 13 weeks. The rats used were about 6 weeks old and weighed 108.5–207.3 g for males and 137.2–163.9 g for females, respectively. The results and conclusions are as follows:

1. *Clinical observation and mortality*: There were no signs of reaction to treatment in any treated groups of animals. All animals survived throughout the study.
2. *Body weight and feed consumption*: Mean body weights for the highest-dose (20 000 ppm) males and females were significantly less than those of the respective control groups throughout the study. Mean body weights of the high-dose groups were 78% of control values in males and 77% of control values in females. Mean body weight gains of the high-dose groups were 67% of control values in males and 57% of control values in females. Feed consumption values of these groups were lower than control values at examinations at weeks 1–4, 6, 9 and 13 (days 7–28, 42, 63 and 91) in males and at all weeks in females. Feed efficiency values of the 20 000 ppm animals were significantly decreased at weeks 1 and 10 (days 7 and 70) in males and at week 1 (day 7) in females.
3. *Ophthalmological examinations*: No effects of test compound treatment were evident at the week 12 examinations.
4. *Haematology*: No effects of test compound treatment were evident in the treated groups of animals at study termination.
5. *Biochemistry*: The statistically significant increases in serum alkaline phosphatase activity were seen only in the 20 000 ppm group females at study termination.
6. *Urinalysis*: No effects of test compound treatment were evident in the treated groups of animals at the week 13 examinations.
7. *Organ weights*: Statistically significant decreases in mean absolute weights of lung (males only) and liver (males only) and statistically significant increases in mean relative organ weight ratios of brain (both sexes), lung (females only), liver (females only), kidney (both sexes) and testis (right side only) were seen only in the 20 000 ppm groups. However, these changes were attributed to the decreased body weights in these groups (reduction rates, 21% in males, 22% in females).
8. *Necropsy and microscopic observations*: Necropsy revealed no compound-related lesions. Histologically, dose-related eosinophilic intranuclear inclusions were seen in the proximal tubular epithelium of kidneys for 20 000 ppm males and females and 4000 ppm males. Other microscopic changes were occasionally seen in the control and treated groups, but they were unrelated to the treatment of test compound.

Based on the results mentioned above, the effects of the test compound (IM-O), when offered in the diet to Crj:CD(SD) rats for 13 weeks, were decreased body weight gains, decreased feed consumption values, increased serum alkaline phosphatase activity and eosinophilic intranuclear inclusions in the proximal tubular epithelium of kidney. The NOAEL is 800 ppm (48.9 mg/kg bw per day) in males and 4000 ppm (275.9 mg/kg bw per day) in females.

This study was conducted in compliance with GLP, and a QA statement was attached (Nukui & Ikeyama, 1997).

Toxicity of other metabolites

The acute toxicity and genotoxicity of other metabolites of acetamiprid are summarized in Tables 81 and 82, respectively.

3. Observations in humans

No adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to acetamiprid have been reported.

3.1 Medical surveillance of manufacturing plant personnel

Nippon Soda started the commercial production of the technical material of acetamiprid at its Nihongi plant in Japan in April 1995. The workers were also engaged in the production of other chemicals. The production of acetamiprid was carried out from 8 to 30 April 1996, 7 to 17 May 1996, 28 June to 21 July 1996, 19 August to 20 September 1996 and 28 February to 10 April 1997.

During the production of acetamiprid, the following protective measures were taken to avoid exposures to chemicals and to ensure physical safety: helmet, safety goggles, safety shoes, filter respirator and rubber gloves.

Once a year, a health examination was performed on all employees in the plant. This health examination was compulsory under the Japanese occupational safety and health law regulations. The health examination consisted of physical examination, haematology, urinalysis and blood chemistry. Haematology includes haemoglobin (g/dl), haematocrit (%), erythrocytes and white blood cells. Urinalysis includes protein (mg/dl), glucose (mg/dl) and urobilinogen (mg/dl). Blood chemistry includes aspartate aminotransferase and alanine aminotransferase activities (IU/l), alkaline phosphatase activity, gamma-glutamyltransferase and total cholesterol measurements.

The data from this annual health examination on all employees involved in acetamiprid production have been presented to the sponsor. The individual data showed that although the health examination had been performed only twice since the commercial production was started in 1995, there were no significant changes in the parameters examined.

In addition to the clinical observations during the production of the technical material, there were no reports of any acute poisoning from exposure or any skin or eye irritation (Takashiba, 1997).

3.2 Direct observation (e.g. clinical cases and poisoning incidents)

As the toxicity of acetamiprid to insect pests is higher than that to humans, cases of acetamiprid poisoning are rare. However, Todani et al. (2008) reported a case of acute acetamiprid poisoning and measured the blood concentration of acetamiprid. A 79-year-old man had ingested acetamiprid and received medical attention 2 hours after ingestion. On arrival, he had consciousness disturbance (GCS-8), hypotension, nausea, vomiting and hyperglycaemia, but he had neither constricted pupils nor mucous supersecretions, which are characteristic of organophosphate poisoning. Gastric lavage was performed, and activated charcoal and laxative were administered. Paroxysmal atrial fibrillation persisted until 11

Table 81. Acute toxicity of other metabolites

Metabolite	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
IM-1-5	Rat	Crj:CD(SD)IGS	Male	Oral	119	Goto (1997)
			Female	Oral	104	
IM-1-5	Rat	Crj:CD(SD)IGS	Male	Oral	141	Fujii (2002b)
			Female	Oral	132	
IM-1-2	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki & Goto (1994a)
			Female	Oral		
IB-1-1	Rat	Crj:CD(SD)IGS	Male	Oral	> 2000	Kanaguchi (1999a)
			Female	Oral		

LD_{50}, median lethal dose

Table 82. Genotoxicity of other metabolites

Metabolite	End-point	Test object	Concentration	Result	References
IM-1-5	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Kanaguchi (1997)
IM-1-2	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994c)
IB-1-1	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Kanaguchi (1999b)

hours after ingestion. The next day, his symptoms with regard to the effects of acetamiprid improved, and he was discharged from the hospital without complication. The blood concentration of acetamiprid on arrival, approximately 2 hours after ingestion, was 21.1 µg/ml (Todani et al., 2008).

Imamura et al. (2010) also described two cases of acute poisoning with an insecticide formulation containing acetamiprid for suicidal purposes. Both cases experienced severe nausea and vomiting, muscle weakness, hypothermia, convulsions and clinical manifestations, including tachycardia, hypotension, electrocardiogram changes, hypoxia and thirst in the case with the higher serum concentration of acetamiprid. The symptoms were partially similar to those of acute organophosphate intoxication. Supportive treatments for a variety of symptoms were sufficient for recovery, and both individuals were discharged without any complications 2 days after ingestion.

4. Literature review

A search of literature databases for published toxicological data on acetamiprid was performed. Only one study regarding the effect of acetamiprid on the immune system in female rats was found. A subacute toxicity study of acetamiprid was undertaken in 72 female Wistar rats in four groups (18 in each group). Three different concentrations of acetamiprid (25, 100 and 200 mg/kg bw) were administered orally to rats. Untreated rats served as controls. The antibody titre of sheep red blood cells in all the treated groups and a decrease in cell-mediated immune response were evaluated by delayed-type hypersensitivity reaction to dinitrofluorobenzene. The results revealed that in rats treated with 200 mg/kg bw, there were significant ($P \leq 0.01$) decreases in mean values of total leukocyte count and relative lymphocyte count. Globulin was also decreased significantly ($P \leq 0.01$) in acetamiprid-treated rats compared with controls. There was a significant decrease ($P \leq 0.01$) in spleen weight in rats treated

with the 200 mg/kg bw dose compared with controls. Histopathological examination of spleen revealed depletion of lymphocytes from Malpighian corpuscles in all treated groups in a dose-dependent manner. The results indicated that acetamiprid suppressed both cell-mediated immune response and antibody-forming ability of lymphocytes at dose levels above the maximum tolerated dose. Further, the substance used in the experiment was a 20% formulation of which the detailed identification is lacking. The study does not comply with guidelines and also suffers from a lack of reproducibility. For these reasons, this study was not taken into consideration in the current evaluation (Mondal et al., 2009).

Comments

Biochemical aspects

Acetamiprid is rapidly absorbed, with a maximum concentration in blood being achieved in approximately 2–3 hours. The extent of absorption was more than 90% of the administered radioactivity. Acetamiprid is widely distributed in the tissues, with highest concentrations being found in the adrenal gland, liver and kidney following oral administration to the rat. The concentration of radioactivity in the brain was lower than the concentration in blood at all time points. No sex differences were observed. The major route of elimination was via urine (53–65%). The recovery of the radioactivity excreted in the bile was less than 20% of the administered dose, which suggests that the bile is not a major route of excretion. The disappearance of radioactivity from the body of the rat was rapid, and there was no indication of accumulation in any tissue. Less than 1% of the administered radioactivity remained in the tissues by day 4 following dosing. The major radioactive compounds in the excreta of rats were acetamiprid (~5–7%), the demethylated compound IM-2-1 (~15–20%), the nicotinic acid derivative IC-O (~8–11%) and the IC-O glycine conjugate IC-O-Gly (~10%). In addition, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 were detected, each at less than 2% of the dose. There were several unknown compounds in the urine, with a maximum abundance of 1%. The main metabolic pathway of acetamiprid in rats is the transformation to IM-2-1 by demethylation. IM-2-1 is further metabolized to IC-O, with the release of IS-1-1 and IS-2-1 after cleavage from the side-chains of NI-25 (parent compound) and IM-2-1.

Toxicological data

In mice and rats, the oral LD_{50} was in the range of 140–417 mg/kg bw. Dose-related reversible toxic signs (crouching, tremor, convulsion and mydriasis) were observed. The dermal LD_{50} in rats was greater than 2000 mg/kg bw. When acetamiprid was administered by inhalation through nose-only exposure, the LC_{50} was greater than 1.15 mg/l of air. Mydriasis in many rats and tremor and convulsion in a few rats were observed when acetamiprid was administered through inhalation (whole-body exposure), and these effects disappeared after 1 day. Acetamiprid was not an irritant in studies of ocular or dermal irritation in rabbits or a dermal sensitizer in the Magnusson and Kligman maximization test in guinea-pigs.

Short-term studies of oral toxicity in mice, rats and dogs were conducted using acetamiprid. These studies are characterized by similar toxic responses, such as decreased feed consumption and body weight.

In a 13-week study in mice, the NOAEL was 400 ppm (equal to 53.2 mg/kg bw per day), on the basis of a significant decrease in total cholesterol level in females at 800 ppm (equal to 106.1 mg/kg bw per day). Tremor, decreased body weight gain, decreased feed consumption, decreased haemoglobin concentration, decreased serum total cholesterol and glucose levels, decreased urinary pH, increased liver to body weight ratio and centrilobular hypertrophy were observed at higher doses.

In a 13-week study of oral toxicity in rats, the NOAEL was 200 ppm (equal to 12.4 mg/kg bw per day), on the basis of decreased body weight gain, decreased feed consumption and increased serum total cholesterol levels at 800 ppm (equal to 50.8 mg/kg bw per day).

In three oral dog studies (4 weeks, 90 days and 1 year), initial body weight losses and decreased body weight gains were observed in males and females receiving the highest dietary concentrations of acetamiprid. In the 4-week study, the NOAEL was 22 mg/kg bw per day. However, an overall NOAEL for the other two oral dog studies was 800 ppm (equal to 32 mg/kg bw per day).

In an 18-month study of toxicity and carcinogenicity in mice, decreased feed consumption was observed in males and females at 1200 ppm. At 400 ppm in males, body weights were decreased, and the body weight gain was statistically significantly decreased compared with controls through 13 weeks of study. At the end of 18 months, mean relative liver weights were increased in males and females receiving 1200 ppm and also in females receiving 400 ppm. On microscopic examination, treatment-related hepatocellular hypertrophy was seen in male and female mice receiving 1200 ppm after 12 and 18 months of treatment. These microscopic findings are considered to be an adaptive response of the liver to exposure to acetamiprid. The NOAEL was 130 ppm (equal to 20.3 mg/kg bw per day), based on transient decreased body weight observed at 400 ppm (equal to 65.6 mg/kg bw per day) in males. There was no evidence of any carcinogenic effect in mice.

A 2-year study of toxicity and carcinogenicity in rats demonstrated an increased incidence of clinical signs, such as rales, hunched posture and laboured breathing, in the 400 and 1000 ppm dose groups. The body weights of the 1000 ppm males and females and the 400 ppm females (until week 100) were statistically significantly lower than those of controls during the study. Trace to mild centrilobular hepatocellular hypertrophy and vacuolation were seen at 400 ppm and above. Incidences of mammary gland adenocarcinomas and hyperplasias were increased in females at 1000 ppm (equal to 60 mg/kg bw per day); however, incidence levels were within normal limits for ageing Crl:CD rats, and therefore these lesions are considered to be unlikely to be due to an endocrine or carcinogen effect of acetamiprid. Because the observation of rales at 160 ppm was not correlated to the other clinical signs, such as laboured breathing, moribundity, hunched posture and decreased activity, the NOAEL in this study was 160 ppm (equal to 7.1 mg/kg bw per day), based on hepatocyte vacuolation at 400 ppm (equal to 17.5 mg/kg bw per day). Acetamiprid was not carcinogenic in rats.

Acetamiprid was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No significant result is obtained in these tests, except for chromosomal aberration induction in vitro. In vivo, there was no confirmation of chromosomal aberration in a number of tests, and there was no evidence of induction of DNA damage.

The Meeting concluded that acetamiprid is unlikely to be genotoxic in vivo.

In view of the lack of genotoxicity in vivo and the absence of carcinogenicity in rats and mice, the Meeting concluded that acetamiprid is unlikely to pose a carcinogenic risk to humans.

In a two-generation study in rats, the NOAEL for systemic parental toxicity was 100 ppm (equal to 6.67 mg/kg bw per day), on the basis of a decline in body weights and feed consumption and an increased incidence of hepatocellular hypertrophy and vacuolation at 280 ppm (equal to 18.9 mg/kg bw per day) and above. The NOAEL for offspring toxicity was 280 ppm (equal to 13.9 mg/kg bw per day), on the basis of decreases in body weight gain in both generations and reduced postnatal survival in the F_2 offspring at 800 ppm (equal to 38.7 mg/kg bw per day). However, there are no effects on reproduction with treatment up to 800 ppm (equal to 38.7 mg/kg bw per day), the highest dose tested.

In a study of developmental toxicity in rats, the NOAEL for maternal toxicity was 16 mg/kg bw per day, based on decreased feed consumption and body weight gain during the treatment period in maternal rats in the 50 mg/kg bw per day group at scheduled sacrifice. The developmental NOAEL in rats was 16 mg/kg bw per day, based on the increased incidence of fetuses with shortening of the 13th rib at 50 mg/kg bw per day.

In a study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 15 mg/kg bw per day, based on decreased feed consumption and body weight gain during the treatment period at 30 mg/kg bw per day. The developmental NOAEL was 30 mg/kg bw per day, the highest dose tested.

The Meeting concluded that acetamiprid was not teratogenic in rats or rabbits.

In an acute oral neurotoxicity study, increased urination frequency and reduced locomotor activity were observed at doses of 30 mg/kg bw and above. Other clinical signs of neurotoxicity (e.g. hunching, tremors) were observed at higher doses. No apparent effects on sensory systems or evidence of neuropathology was seen. The NOAEL was 10 mg/kg bw, based on evidence of increased urination frequency (males) and a statistically significant reduction of locomotor activity (males) at 30 mg/kg bw.

A 13-week dietary neurotoxicity study in rats did not result in any changes that were considered indicative of neurotoxicity. The NOAEL was 200 ppm (equal to 14.8 mg/kg bw per day), on the basis of lower body weights and feed consumption at 800 ppm (equal to 59.7 mg/kg bw per day).

A developmental neurotoxicity study in rats revealed the NOAEL for maternal toxicity, developmental toxicity and developmental neurotoxicity to be 10 mg/kg bw per day, based on a reduction in body weight gain in dams during the first 3 days of dosing (gestation days 6–9), decreased feed consumption in F_0 animals, early postnatal mortality, reduced post-weaning body weights and deficits in auditory startle response without neuropathology or changes in brain morphometry in F_1 animals at 45 mg/kg bw per day.

Acetamiprid did not cause delayed neuropathy in hens.

Studies for immunotoxicity in mice (highest dose tested was 157 mg/kg bw per day) and rats (highest dose tested was 67.7 mg/kg bw per day) indicated no specific effect on immune function as assessed by the measurement of antigen-specific T cell–dependent antibody formation.

Toxicological data on impurities and metabolites

Acute toxicity studies and studies of genotoxicity have been undertaken for four compounds that are present as impurities in technical acetamiprid. None of them were genotoxic in a number of assays, and they had acute oral LD_{50} values in rats between 603 and greater than 5000 mg/kg bw. Nine compounds identified as plant metabolites are IM-1-3, IM-1-4, IM-2-1, IM-2-3, IM-2-4, IM-O, IC-O, IS-1-1 and IS-2-1. None were genotoxic in a number of assays, and they had acute oral LD_{50} values in rats between 900 and greater than 5000 mg/kg bw. The NOAEL following repeated exposure of rats to diets containing IM-1-4 for 13 weeks was 600 ppm (equal to 36.5 mg/kg bw per day), based on effects on spleen (increased pigments in splenic sinusoids) at 1800 ppm (112.2 mg/kg bw per day) in treated males. The NOAEL for IM-O in a 13-week study in rats was 800 ppm (equal to 48.9 mg/kg bw per day), on the basis of eosinophilic intranuclear inclusions seen in proximal tubular epithelium of kidneys at 4000 ppm (250.1 mg/kg bw per day) in males. All the impurities and metabolites were of lesser toxicity than the parent (acetamiprid).

No adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to acetamiprid have been reported.

Three cases of intentional poisoning with acetamiprid formulation have been reported. In one case, the concentration of acetamiprid in blood was measured at the time of reporting for treatment. In all cases, some signs similar to those associated with acute organophosphate intoxication were reported. Supportive treatments for a variety of signs were sufficient for recovery, and all recovered within 24–48 hours of the initiation of treatment.

The Meeting concluded that the existing database on acetamiprid was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.07 mg/kg bw on the basis of the NOAEL of 7.1 mg/kg bw per day from the 2-year study of toxicity and carcinogenicity in rats, based on clinical signs and hepatocyte vacuolation seen at 17.5 mg/kg bw per day. A safety factor of 100 was applied. This ADI was supported by the NOAEL of 6.67 mg/kg bw per day observed in a

two-generation study of reproductive toxicity in rats on the basis of decreased parental body weight gain and feed consumption and hepatocyte vacuolation at 18.9 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.1 mg/kg bw on the basis of a NOAEL of 10 mg/kg bw in an acute neurotoxicity study in rats, based on evidence of neurotoxicity, decreased locomotor activity and increased urination frequency. This ARfD was supported by the NOAEL for maternal toxicity in the developmental neurotoxicity study of 10 mg/kg bw per day, based on reduced body weight gain in dams during the first 3 days of dosing (gestation days 6–9).

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	130 ppm, equal to 20.3 mg/kg bw per day	400 ppm, equal to 65.6 mg/kg bw per day
		Carcinogenicity	1200 ppm, equal to 214.6 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	160 ppm, equal to 7.1 mg/kg bw per day	400 ppm, equal to 17.5 mg/kg bw per day
		Carcinogenicity	1000 ppm, equal to 60 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Offspring toxicity	280 ppm, equal to 13.9 mg/kg bw per day	800 ppm, equal to 38.7 mg/kg bw per day
		Reproductive toxicity	800 ppm, equal to 38.7 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 6.67 mg/kg bw per day	280 ppm, equal to 18.9 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	16 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	16 mg/kg bw per day	50 mg/kg bw per day
	Acute neurotoxicity study[c]	Acute neurotoxicity	10 mg/kg bw	30 mg/kg bw
	Developmental neurotoxicity study[c]	Developmental neurotoxicity	10 mg/kg bw per day	45 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	15 mg/kg bw per day	30 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day[b]	—
Dog	Ninety-day and 1-year studies of toxicity[a,d]	Toxicity	800 ppm, equal to 32 mg/kg bw per day	1500 ppm, equal to 55 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two studies combined.

Estimate of acceptable daily intake for humans

0–0.07 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to acetamiprid

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and almost completely absorbed (> 90%)
Distribution	Widely distributed; highest concentrations in adrenal, liver and kidney
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid, more than 90% within 96 h, mainly via urine
Metabolism in animals	Moderately metabolized; the major radioactive compounds in the excreta of rats were acetamiprid itself and IC-O glycine conjugate
Toxicologically significant compounds (animals, plants and the environment)	Acetamiprid (parent compound)
Acute toxicity	
Rat, LD_{50}, oral	140–417 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 0.30 mg/l (whole-body exposure) > 1.15 mg/l (nose-only exposure)
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Non-irritant
Guinea-pig, dermal sensitization (Magnusson and Kligman test)	Non-sensitizer
Short-term studies of toxicity	
Target/critical effect	Increased cholesterol, decreased body weight, decreased feed consumption
Lowest relevant oral NOAEL	53.2 mg/kg bw per day (13-week study in mice)
Genotoxicity	
	Not genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increased clinical signs; hepatic vacuolation
Lowest relevant NOAEL	7.1 mg/kg bw per day (rats)
Carcinogenicity	Not carcinogenic in rats or mice
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	38.7 mg/kg bw per day, highest dose tested
Developmental target/critical effect	Skeletal anomalies
Lowest relevant developmental NOAEL	16 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity target/critical effect	Motor activity and increased frequency of urination
Lowest relevant acute neurotoxic NOAEL	10 mg/kg bw
Subchronic neurotoxicity target/critical effect	Not neurotoxic (rats)
Developmental neurotoxicity target/critical effect	Deficits in auditory startle response
Lowest relevant developmental neurotoxic NOAEL	10 mg/kg bw per day (rat)

Immunotoxicity	
28-day immunotoxicity	Not immunotoxic (mice and rats)
Medical data	
	No significant health effects were reported among manufacturing personnel; however, three cases of intentional poisoning have been reported with some signs similar to those of acute organophosphate poisoning

Summary

	Value	Study	Safety factor
ADI	0–0.07 mg/kg bw	Two-year rat study (supported by parental toxicity in the multigeneration rat reproduction study)	100
ARfD	0.1 mg/kg bw	Acute neurotoxicity, rat (supported by maternal toxicity in the developmental neurotoxicity rat study)	100

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