CYROMAZINE

First draft prepared by
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Explanation

Cyromazine, the International Organization of Standardization (ISO) approved name for ***N***-cyclopropyl-[1,3,5] triazine-2,4,6-triamine, is a selective insecticide used on a broad range of vegetable crops. It acts by inhibiting the moulting process, particularly in dipterian insects.

Cyromazine was first evaluated by the 1990 JMPR, when an acceptable daily intake (ADI) of 0–0.02 mg/kg bw was established on the basis of a no-observed-adverse-effect level (NOAEL of 1.8 mg/kg bw per day for body-weight changes in a 2-year dietary study in rats and a NOAEL of 2 mg/kg bw per day in a two-generation study of reproductive toxicity in rats, with a safety factor of 100.

Cyromazine was considered by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). The Meeting reviewed new data on cyromazine, including studies of toxicokinetics, metabolism, dermal absorption, acute toxicity after inhalation, skin sensitization, a 1-year study of toxicity in dogs and a 3-week study of dermal toxicity in rabbits, as well as data on mutagenesis and toxicity for the metabolite, melamine. Relevant data from the previous evaluation were also considered.

All pivotal studies with cyromazine were certified as complying with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

The excretion and tissue distribution of radioactivity was investigated in male and female Charles River albino rats given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw (specific activity, 1.395 MBq/mg; radiochemical purity, > 99%) in aqueous Carbowax 400 formulation. Exhaled volatile metabolites were collected from one male and one female housed individually in glass metabolism cages. One female and two males were housed singly in metal metabolism cages for 72 h for the collection of urine and faeces. Seventy-two hours after dosing, the rats were killed and a range of tissues was taken for the measurement of radioactivity. This comprised brain, fat, heart, kidneys, liver, lungs, muscle, ovaries, spleen, testes and whole blood. All samples were counted for radioactivity by liquid scintillation counting (LSC), either directly or after sample oxidation. The study was performed before the introduction of GLP and prior to OECD TG 417 (1984), but complied to a great extent with these requirements.

The predominant route of excretion was via the kidneys, with approximately 94% or more of the administered dose excreted in the urine (Table 1). Hence, after an oral dose at 0.5 mg/kg bw, absorption appeared to be almost quantitative. Faecal elimination accounted for just 4% and 1% of the administered dose in males and females respectively. Virtually no radioactivity was recovered from exhaled air. Excretion was rapid since greater than 97% of the administered dose was excreted within 24 h. The urinary and faecal excretion data were derived from the rats housed in metal metabolism cages. The exhaled volatile radioactivity measurements were derived from the rats housed in glass metabolism cages.

Table 1. Excretion profiles over 72 h in rats given [U-^{14}C triazine]cyromazine as a single oral dose at 0.5 mg/kg bw

Excretion	Percentage of administered dose	
	Male (n = 2)	Female (n = 1)
Urine		
0–24 h	93.8	96.6

24–48 h	< 0.3	0.5
48–72 h	< 0.2	0.3
Subtotal	93.8	97.4
Faeces		
0–24 h	3.8	0.6
24–48 h	< 0.1	0.4
48–72 h	< 0.1	< 0.1
Subtotal	3.8	1.0
Cage wash	< 0.1	< 0.1
Volatile metabolites	< 0.1	< 0.1
Exhaled carbon dioxide	< 0.1	< 0.1
Tissues	< 0.1	< 0.1
Blood	< 0.1	< 0.1
Intestinal tract	< 0.1	< 0.1
Total recovery	97.5	98.4

From Simoneaux & Cassidy (1978)

Three days after oral dosing, tissue residues were very low, with < 0.007 ppm in the liver and < 0.003 ppm in all other tissues. This was consistent with the rapid and extensive excretion of the administered dose. The tissue residue data tabulated were derived from the rats housed in metal metabolism cages.

A single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw was almost quantitatively absorbed and was rapidly excreted, almost exclusively in the urine. More than 97% of the administered dose was eliminated within 24 h after dosing and 72 h after dosing, tissue residues were very low (Simoneaux & Cassidy, 1978).

The excretion and tissue distribution of [U-^{14}C triazine]cyromazine was investigated in male and female Sprague Dawley rats (Table 2).

Table 2. Design of a study to investigate excretion and tissue distribution of [U-^{14}C triazine] cyromazine in male and female rats

Group	Number and sex	Route and dose of [U-^{14}C triazine]cyromazine
1	One male and one female	Single intravenous dose of vehicle (carboxymethylcellulose plus Hi Sil)
2	Five males and five females	Single intravenous dose at approx. 3 mg/kg bw
3	Five males and five females	Single oral dose at approx. 3 mg/kg bw
4	Five males and five females	Single oral dose at approx. 3 mg/kg bw. Rats pre-conditioned with 14 daily non-radiolabelled doses at 3 mg/kg bw.
5	Five males and five females	Single oral dose at approx. 300 mg/kg bw
6	One male and one female	Single oral dose of vehicle
7	One male and one female	Rats dosed with vehicle each time group 4 received a dose.
8	One male and one female	Single oral dose of dose vehicle

From Capps (1990)
Approx., approximately.

After each dose of [U-^{14}C triazine]cyromazine (specific activity, 9.8 μCi/mg for the lowest dose and 0.8 μCi/mg for the highest dose; radiochemical purity, 97.2%), urine and faeces were collected at intervals over 7 days from rats in groups 2, 3, 4 and 5. At termination, samles of a range of tissues

were taken for the measurement of radioactivity present. These comprised bone (femur), brain, carcass, fat, heart, kidneys, liver, lungs, muscle, ovaries, plasma, erythrocytes, spleen, testes and uterus. Radioactivity was measured by LSC, either directly or after sample combustion. The study was conducted according to the principles and practices of GLP (including certification for quality assurance, QA) and the protocol was in accordance with OECD TG 417 (1984).

The predominant route of excretion was via the kidneys, irrespective of the route of administration. The inclusion of radioactivity from cage rinses and washes with urine shows that 82–92% of the administered dose was attributable to urinary excretion over 7 days (Table 3). Hence, absorption of an oral dose of cyromazine was extensive. Faecal elimination over the same period accounted for between 3% and 8% of the dose. There was no apparent sex difference in excretion profiles, although there were some minor differences in the rates of elimination. Excretion of radioactivity in urine and faeces over the first 24 h after dosing was slightly faster in rats given a single oral dose at 3 mg/kg bw compared with rats given a single oral dose at 300 mg/kg bw.

Table 3. Excretion data in rats given [U-^{14}C triazine]cyromazine as a single oral dose at 3 or 300 mg/kg bw

Sample	Percentage of administered dose							
	Group 2		Group 3		Group 4		Group 5	
	Single intravenous dose		Single oral dose		Single oral dose (pre-treated for 14 days)		Single oral dose	
	Male	Female	Male	Female	Male	Female	Male	Female
	Dose (mg/kg bw)							
	3		3		3		300	
Urine								
0–24 h	78.14	72.38	65.22	56.87	77.82	58.55	51.81	55.64
24–48 h	1.58	0.90	1.22	2.48	0.80	2.53	10.10	9.75
48–168 h	1.09	1.50	1.70	3.53	2.20	2.13	3.82	2.52
Subtotal	80.81	74.78	68.14	62.88	80.82	63.21	65.73	67.91
Cage rinse								
24 h	1.71	8.79	8.38	14.8	9.36	24.9	15.20	14.60
Cage wash								
168 h	2.96	1.83	4.24	6.40	0.81	0.93	1.56	2.89
Cage wipe								
168 h	1.08	1.09	1.61	2.34	0.89	1.05	1.02	1.04
Subtotal	5.75	11.71	14.23	23.54	11.06	26.88	17.78	18.53
Faeces								
0–24 h	2.62	4.80	3.24	2.04	1.40	1.34	3.96	2.73
24–48 h	1.16	0.68	0.35	0.79	0.33	0.34	2.20	1.69
48–168 h	1.39	0.96	0.47	0.92	1.57	1.02	1.36	1.93
Subtotal	5.17	6.44	4.06	3.75	3.30	2.70	7.52	6.35
Excretion summary								
0–24 h	82.47	85.97	76.84	73.71	88.58	84.79	70.97	72.97
24–48 h	2.74	1.58	1.57	3.27	1.13	2.87	12.30	11.44
Total excretion	*91.73*	*92.93*	*86.43*	*90.17*	*95.18*	*92.79*	*91.03*	*92.79*

From Capps (1990)
Values are expressed as percentage of dose

Seven days after an oral dose of [U-^{14}C triazine]cyromazine, tissue residues were detectable only in carcass (at 0.004–0.017 ppm after the lowest dose and 0.565–0.782 ppm after the highest dose), liver (at 0.004–0.011 ppm after the lowest dose and 0.455–0.601 ppm after the highest dose) and erythrocytes (at < 0.001–0.001 ppm after the lowest dose and 0.153–0.164 ppm after the highest dose) of rats at 3 or 300 mg/kg bw and in the spleen (< 0.001 ppm) of female rats at 300 mg/kg bw. Tissue residue concentrations were very low, < 0.017 ppm at 3 mg/kg bw and < 0.79 ppm at 300 mg/kg bw.

A single oral dose of [U-^{14}C triazine]cyromazine at 3 mg or 300 mg/kg bw was extensively and rapidly absorbed in male and female rats. At both doses, excretion was rapid and predominately in the urine. Seven days after dosing, tissue residues were very low and detectable only in carcass, liver, erythrocytes and spleen. There were no pronounced differences between the sexes and relatively minor differences between the doses. The pretreatment of rats with unlabelled cyromazine (3 mg/kg bw per day) for 14 days before a single radiolabelled dose of 3 mg/kg bw had no marked effect on its absorption or excretion (Capps, 1990).

The blood kinetics, tissue distribution and tissue depletion of radioactivity were studied in male and female Sprague-Dawley rats given [U-^{14}C triazine]cyromazine as a single oral dose at 3 mg or 300 mg (specific activity, 1.87 MBq/mg for the lowest dose and 36.67–46.14 kBq/mg for the highest dose; radiochemical purity, > 97%)/kg bw in ethanol : PEG 200 : water (2 : 5 : 3 v/v).

Table 4. Design of a study to investigate blood kinetics, tissue distribution and tissue depletion in rats given [U-^{14}C triazine]cyromazine as a single oral dose

Group	Number and sex	Regime
E1	Three males	Approximately 3 mg/kg bw with collection of blood over a time course
E2	Three males	Approximately 300 mg/kg bw with collection of blood over a time course
E3	Three females	Approximately 3 mg/kg bw with collection of blood over a time course
E4	Three females	Approximately 300 mg/kg bw with collection of blood over a time course
F1	Twelve males	Approximately 3 mg/kg bw with collection of tissues over a time course
F2	Twelve males	Approximately 300 mg/kg bw with collection of tissues over a time course

From Paul & Dunsire (1994)

Blood was collected from rats in groups E1 to E4 at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 32, 48, 72, 96 and 120 h after dosing. Based upon the profiles determined for groups E1 and E3, rats in groups F1 and F2 were killed in groups of three over a time course (F1, 0.5, 3.5, 5.5 and 24 h after dosing; F2, 8, 21, 27 and 48 h after dosing) and samples of a range of tissues were taken for the measurement of radioactivity present. These comprised heart, lungs, spleen, liver, abdominal fat, skeletal muscle, brain, bone, testes, kidney, urinary bladder, plasma, erythrocytes, gastrointestinal tract and contents and residual carcass. Radioactivity was measured by LSC, either directly or after sample combustion. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 417 (1984).

Blood kinetics data show that after a dose at 3 mg/kg bw, concentrations of radiolabel rose quickly, reaching a maximum at 0.5 h after dosing (Table 5). By 8 h after dosing, blood concentrations had declined markedly and by 24 h approached background values. Elimination appeared to be biphasic, with an initial rapid phase of 2–12 h, followed by a slower phase. In males given a dose at 300 mg/kg bw, two peaks were present, at 0.5 and 8 h. After 24 h, [U-^{14}C triazine] concentrations declined rapidly. In female rats at 300 mg/kg bw, concentrations of the radiolabel rose to a broad plateau between 1 h and 4 h, declining quickly after 12 h. The plasma data showed that the area under the curve of concentration-time (AUC, 0–24 h) increased approximately 150-fold after a 100-fold increase in dose, reflecting the sustained high blood concentrations, particularly at the

highest dose, rather than indicating that the extent of absorption was dose-dependent. Similarly, there was no evidence to suggest that blood kinetics were sex-dependent, hence only male rats were used to investigate the distribution and elimination of radioactivity from tissues.

Table 5. Group mean blood kinetics in rats given [U-^{14}C triazine]cyromazine as a single oral dose

Time (h)	Radiolabel (ppm cyromazine equivalents)			
	Dose (mg/kg bw)			
	Approx. 3	Approx. 300	Approx. 3	Approx. 300
	Group E1[a]	Group E2[a]	Group E3[a]	Group E4[a]
	Male	Male	Female	Female
0.25	0.743	20.4	0.967	31.0
0.50	1.151	23.5	1.059	35.9
1	1.025	21.3	0.897	38.5
2	0.775	19.1	0.757	45.4
4	0.469	27.3	0.373	42.7
8	0.040	34.8	0.057	34.0
12	0.007	28.4	0.015	31.7
24	0.005	13.9	0.008	10.5
32	0.005	0.5	0.004	1.0
48	0.004	0.3	0.005	0.3
72	0.002	0.4	0.001	0.2
96	0.003	0.3	0.004	0.2
120	0.002	0.2	0.002	0.1
AUC 0–24 h[b]	4.2	590	3.9	697

From Paul & Dunsire (1994)

[a] $n = 3$.

[b] AUC 0–24 h expressed as ppm × h.

At the first three time-points, the highest concentrations of radioactivity to male rats given a single oral dose of[U-^{14}C triazine]cyromazine at 3 mg/kg bw were present in the gastrointestinal tract and its contents (21.352, 5.379 and 0.962 ppm, respectively), the bladder (2.468, 3.523 and 2.359 ppm, respectively), kidneys (1.955, 2.090 and 0.790 ppm, respectively) and liver (0.859, 0.913 and 0.409 ppm, respectively). The radiolabel found in bladder was attributed to residues in urine, since it has been established that urine is an important route of elimination of cyromazine metabolites. Twenty-four hours after dosing, all tissue residues were very low and, with the exception of the gastrointestinal tract and its contents (0.010 ppm), liver (0.061 ppm), kidney (0.002 ppm) and erythrocytes (0.005 ppm), were approaching background values. Assuming first-order kinetics, the elimination half-life between 3.5 and 24 h was approximately 6 h for liver, compared with approximately 2 h for other tissues. The Meeting concluded that there was no tissue accumulation of radioactivity.

At the first three time-points, the highest concentrations of radioactivity in male rats given a single oral dose of [U-^{14}C triazine]cyromazine at 300 mg/kg bw were present in the gastrointestinal tract and its contents (1233, 128 and 22 ppm, respectively), the bladder (196, 223 and 23 ppm, respectively), kidneys (83, 51 and 16 ppm, respectively) and liver (49, 24 and 10 ppm, respectively). As for the lowest dose, the radiolabel found in bladder was attributed to residues in urine. Forty-eight hours after dosing, all tissue residues were very low, < 0.3 ppm, with the single exception of liver (2.23 ppm) and gastrointestinal tract and its contents (0.66 ppm). Assuming first-order

kinetics, the elimination half-life between 21 and 48 h was approximately 8 h for liver, compared with approximately 3–5 h for other tissues. The Meeting concluded that there was no tissue accumulation of radioactivity.

After a single oral administration of [U-^{14}C triazine]cyromazine at 3 mg or 300 mg/kg bw in male and female rats, absorption was very rapid. Overall, rapid elimination of residues from tissues occurred. Blood kinetic data in both sexes and tissue depletion data in males suggested that the rates of distribution and/or elimination were rate-limiting. It was assumed that blood kinetics and tissue distribution were independent of sex and dose (Paul & Dunsire, 1994).

The absorption, distribution and excretion of cyromazine was investigated in male rats given radiolabelled cyromazine as 14 consecutive doses at 3.0 mg/kg bw per day by oral gavage. A group of 16 male Hanlbm:WIST (SPF) rats (Group J1) was given [U-^{14}C triazine]cyromazine (specific activity, 2580 kBq/mg; radiochemical purity, ≥ 98.5%) dissolved in a mixture of ethanol/polyethylene glycol 200/water (2/5/3, v/v). The depletion of radioactivity was measured for 5 days after the last dose. At each time-point, three animals were selected and killed. The residual radioactivity in selected tissues (adrenals, blood, bone, brain, fat, heart, kidneys, liver, lungs, muscle, pancreas, plasma, spleen, testes, thymus, thyroids) was determined during the administration phase on days 1 (T1), 7 (T2), 14 (T3) after the first dose and during the depletion phase on day 18 (T4) after the first dose. Serial daily blood samples were collected from rats in the subgroup treated for 14 days. The excreted radioactivity was determined in the urine and faeces at daily intervals. Radioactivity was measured by LSC. Plasma and urine were measured directly. Aliquots of adrenals, brain, fat, heart, kidneys, liver, muscle, pancreas, spleen, testes, thymus, and thyroids were digested with Soluene 350 tissue-solubilizer, and neutralized with hydrochloric acid before measuring the radioactivity by LSC. Radioactivity in blood, bone, faeces, lung and carcass was measured by combustion analysis. The stability of the test substance in the administered solution was checked by thin-layer chromatography (TLC) at the time of the first, eighth and after the last dose. Cyromazine was stable over the dosing period and represented more than 96% of the radioactivity. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 417 (1984).

The test substance was rapidly absorbed from the gastrointestinal tract into the systemic circulation and the daily doses were rapidly and almost completely excreted within 24 h after dosing. After the start of dosing, a steady state in terms of excretion was reached within 1 day. Most of the daily administered dose was excreted via the urine (about 90%) and a smaller part was excreted via the faeces (about 4%). In total, 92.9% and 4.2% of the administered dose was excreted via the urine and the faeces, respectively (Table 6). One and five days after the last dose, only 0.01% and less than 0.01%, respectively, of the total administered dose remained in tissues and organs.

Table 6. Excretion and recovery of radiolabel in male rats given 14 consecutive daily oral doses of [U-^{14}C triazine]cyromazine at 3 mg/kg bw by gavage

Sample	Percentage of total administered dose	
	Day 18	
	Mean	Standard deviation
Urine	92.86	0.61
Faeces	4.19	0.71
Cage wash	0.16	0.06
Total excretion, 0–18 days	97.21	0.67

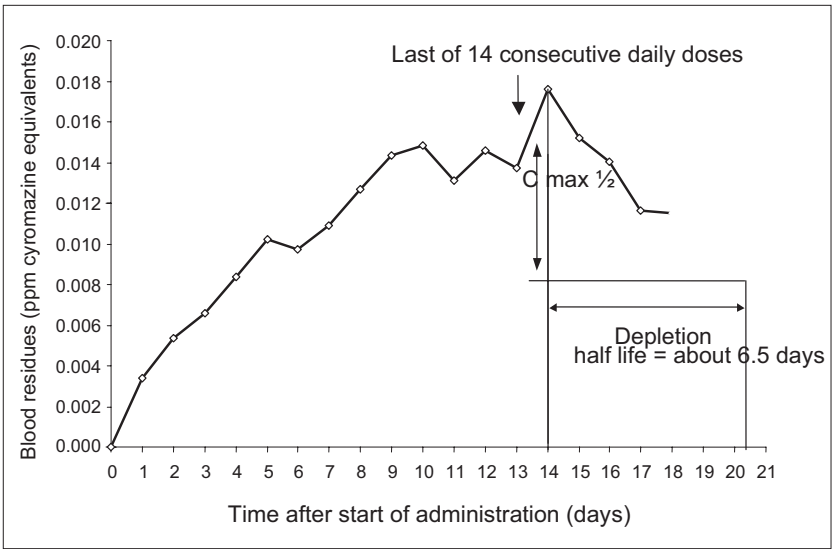
Tissue residues:		
Tissues[a]	< 0.01	< 0.01
Carcass	0.17	0.02
Subtotal	0.18	0.02
Total recovery	97.39	0.68

From Löffler (2003)

[a] Residues determined in the excised part of the tissues and organs.

Serial blood residue concentrations were determined at 24 h intervals, before dosing and starting 1 day after the first administration. The blood kinetics showed increasing residue values with ongoing administration, reaching a plateau of about 0.016 ppm expressed as cyromazine equivalents within about 9 days (Figure 1). After the last dose, the very low residues in whole blood decreased with a half-life of about 6.5 days.

Figure 1. Blood residue concentrations at different time-points in rats given radiolabelled cyromazine by gavage for 14 days



From Löffler (2003)

The very low concentrations of tissue residues determined at four different time-points during and after the dosing period showed increasing residue concentrations for all of the tissues and organs during the dosing period, reaching their maximum 1 day after the last dose. Apparently, the residues in liver reached a plateau within the dosing period, while the other tissues showed a slow increase with ongoing administration. Highest maximum residue concentrations were determined in liver (0.080 ppm) and kidneys (0.024 ppm), followed by whole blood (0.015 ppm), adrenals (0.015 ppm) and thyroids (0.014 ppm). All other tissues and organs showed very low maximum concentrations not exceeding 0.01 ppm expressed as cyromazine equivalents.

After reaching the maximum concentration, the residues declined in all tissues and organs to at least half of the maximum concentration within 3.4 days after the last dose, except for whole blood. The calculated half-life (t½) for the depuration ranged from 1.9 (plasma) to 6.4 days (whole blood).

The metabolite pattern for urine and faeces, investigated at three different time intervals during dosing, was not influenced by repeated doses. In total, about 85% of the daily dose was excreted as unchanged parent via urine (83%) and faeces (2%).

Cyromazine was rapidly absorbed and rapidly and almost completely excreted after repeated doses. As observed after a single dose, the tissue residue concentrations were very low. A plateau for tissue residues was reached only in liver. All other tissue residues increased with ongoing dosing and did not reach a plateau within the 14 day dosing period. The rates and routes of excretion after repeated doses were similar to those after a single dose (Löffler, 2003).

Monkeys

A study was performed to investigate the excretion pattern of cyromazine in monkeys. Two male and two female (*Macaca fasicicula*) monkeys were given a single oral dose of [U-^{14}C triazine] cyromazine at 0.05 mg/kg bw. An additional two male and two female monkeys were given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw. Each animal weighed approximately 3 kg. The doses were administered in capsules containing [U-^{14}C triazine]cyromazine (specific activity, 16.6 μCi/mg; radiochemical purity, > 97.5%) on ground corn-on-the-cob. Each monkey was housed in a metabolism cage to which it had been first acclimatized. Urine and faeces were collected for 24 h before dosing and at daily intervals after dosing for 4 days. Radioactivity in urine and in combusted samples of faeces was measured by LSC. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. Tissue residues were not investigated. The study was regarded as additional information for the evaluation owing to incomplete recoveries of radioactivity.

At both doses, cyromazine was rapidly and extensively absorbed, as most of the administered radioactivity was excreted in the urine within 1 day of dosing (Table 7). At 0.05 mg/kg bw, males excreted 73% of the administered radioactivity in the urine and approximately 1% in the faeces over 4 days; females excreted 82% in urine and 1% in faeces over the same period. At 0.5 mg/kg bw, males excreted 56% of administered radioactivity in the urine and 1.5% in faeces over 4 days; females excreted 80% in the urine and less than 2% in faeces over the same time interval. The incomplete recoveries of administered dose were attributed to losses in the cages as no cage washes were collected. No tissue residues were measured.

Table 7. Excretion data in monkeys given [U-^{14}C triazine]cyromazine as a single oral dose at 0.05 or 0.5 mg/kg bw

Time-point	Percentage of administered dose							
	Dose (mg/kg bw)							
	0.05		0.5		0.5		0.5	
	Male		Female		Male		Female	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
Day –1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	69.69	0.61	80.83	0.56	52.22	1.06	78.01	0.85
2	2.81	0.55	1.00	0.69	3.27	0.39	1.68	0.69
3	0.46	0.07	0.18	0.05	0.26	0.02	0.20	0.19
4	0.00	0.00	0.16	0.00	0.09	0.01	0.07	0.03
Subtotal	72.96	1.23	82.16	1.29	55.84	1.48	79.95	1.75
Total excretion	74.19		83.45		57.32		81.70	

From Staley (1986)

In male and female monkeys, a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw was rapidly and extensively absorbed and rapidly excreted, predominantly in the urine (Staley, 1986).

Because of the low total recovery of radioactivity in the above study, a second study was performed in one male and one female of the same strain of monkey and at the same doses. Each animal weighed approximately 3 kg. The doses were administered in capsules containing [U-^{14}C triazine]cyromazine (specific activity, 16.6 µCi/mg; radiochemical purity, > 97.5%) on ground corn-on-the-cob. Each monkey was housed in a metabolism cage to which it had been first acclimatized. Urine and faeces were collected for 24 h before dosing and at daily intervals after dosing for 2 days. Radioactivity in the urine and in combusted samples of faeces was measured by LSC. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. Tissue residues were not investigated. The study was regarded as additional information for the evaluation owing to incomplete recoveries of radioactivity.

At both doses, cyromazine was rapidly and extensively absorbed, as most of the administered radioactivity was excreted in the urine within 1 day of dosing (Table 8). At 0.05 mg/kg bw, the male excreted 34% of administered radioactivity in urine with an additional 10% in the cage wash and approximately 13% in the faeces over 2 days; the female excreted 50% in the urine with an additional 15% in the cage wash and 15% in the faeces over the same time interval. At 0.5 mg/kg bw, the male excreted 65% of administered radioactivity in the urine with an additional 11% in the cage wash and only 0.3% in the faeces over 2 days; the female excreted 51% in the urine with an additional 8% in the cage wash and less than 0.1% in the faeces over the same period. The incomplete recoveries of administered radioactivity could not be explained, particularly since cage washes were collected during this study. No tissue residues were measured.

In male and female monkeys, a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw was rapidly and extensively absorbed and rapidly excreted, predominantly in the urine (Staley & Simoneaux, 1986).

Table 8. Excretion data in monkeys given [U-^{14}C triazine]cyromazine as a single oral dose at 0.05 or 0.5 mg/kg bw

Time-point	Percentage of administered dose											
	Dose (mg/kg bw)											
	0.05						0.5			0.5		
	Male			Female			Male			Female		
	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash
Day –1	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	32.33	1.08	9.40	48.77	13.99	14.00	64.96	0.24	11.34	50.57	0.05	7.93
2	1.44	12.14	0.44	0.85	0.93	1.02	0.42	0.07	0.10	0.86	0.00	0.10
Subtotal	34.10	13.22	9.84	49.62	14.92	15.02	65.38	0.31	11.44	51.43	0.05	8.03
Total excretion	57.16			79.56			77.13			59.51		

From Staley & Simoneaux (1986)

Goats

Lactating goats received [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 4.6 and 48.4 ppm for 10 days.

Most of the administered dose of [U-^{14}C triazine]-cyromazine was excreted in the urine (82–90%) and 6–7% in faeces. A small proportion was found in blood (0.3%), tissues (0.8–1.8%),

gastrointestinal tract (1.1–1.3%) and milk (0.3–0.4%). Cyromazine was thus rapidly eliminated from goats over a 10-day feeding period and did not accumulate in body tissues or blood.

Radioactivity in blood and milk reached a plateau by the second day of feeding at both doses. The goat at the lowest dose had average values of 0.017 mg/kg (as cyromazine) in milk and 0.012 mg/kg (as cyromazine) in blood while the animal at the highest dose had average values of 0.318 mg/kg in milk and 0.136 mg/kg in blood, indicating an approximate proportionality between dose and residue concentration. Radioactivity in the tissues of the goat at the lowest dose was ≤ 0.01 mg/kg except for kidney (0.04 mg/kg) and liver (0.79 mg/kg); that in the tissues of the goat at the highest dose was ≤ 0.15 mg/kg, except for kidney (0.44 mg/kg) and liver (1.52 mg/kg).

In conclusion, at least 80% of the administered dose of cyromazine was absorbed from the gastrointestinal tract and rapid and complete excretion occurred mainly via the urine. As a consequence, low concentrations of residues were detected in milk and tissues, and there was no evidence of accumulation or retention (Simoneaux & Marco, 1984). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

Hens

Two hens received capsules containing [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 5.0 ppm for 7 days.

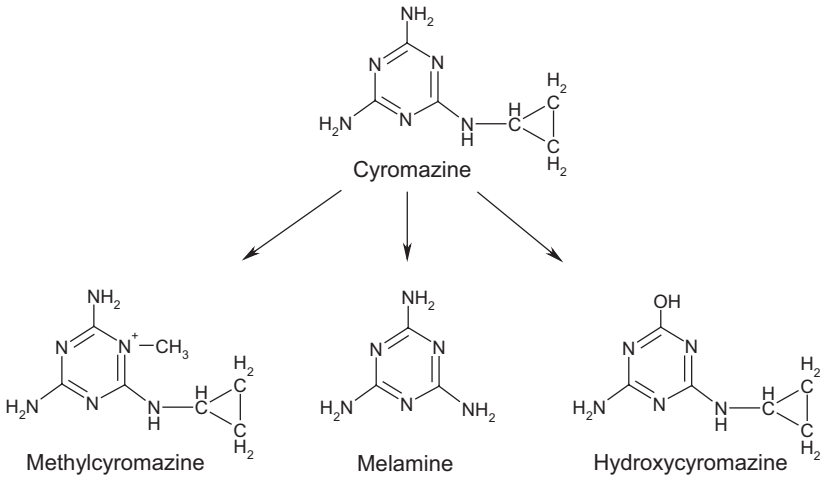
Most (99%) of the administered cyromazine dose was recovered from the excreta of the hens. Egg white and yolk contained 0.4% and 0.2% of the administered dose respectively. Tissue concentrations were low (≤ 0.05 mg/kg, as cyromazine) (Simoneaux & Cassidy, 1979). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

1.2 Biotransformation

Rats

A generalized metabolic pathway for cyromazine in rats is shown in Figure 2.

Figure 2. Proposed metabolic pathway of cyromazine in rats



Name	Description	Compound found in:	Denomination
Cyromazine	*N*-cyclopropyl-[1,3,5] triazine-2,4,6-triamine	Rat, monkey, celery, lettuce, tomatorotational celerygoathen eggs and liver	Parent compound
Melamine	*N*-cyclopropyl-[1,3,5] triazine-2,4,6-triamine	Rat, monkey, celery, lettuce, tomatorotational celerygoathen eggs and liver	Major metabolite
N-methyl cyromazine,1-methyl cyromazine	2,4-diamino-6-cyclo-propylamino-1-methyl-[1,3,5]triazin-1-ium salt	Rat, goat	—
Hydroxy-cyromazine	—	Rat	—

In the pharmacokinetic study in rats given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw, metabolite profiles in the urine and faeces were investigated by TLC. Faecal samples were extracted with acetonitrile : water (9 : 1), which extracted more than 90% of faecal radioactivity.

Urine contained predominantly unchanged parent, which accounted for approximately 80% of the dose and three minor metabolites each of which represented less than 5% of the administered dose. Faecal metabolite profiles appeared to be similar to those for urine, but from the low concentrations of faecal radioactivity, it was not possible to confirm that the metabolites corresponded to those excreted in urine.

Cyromazine was poorly metabolized since more than 80% of the administered dose was excreted unchanged (Simoneaux & Cassidy, 1978).

A study was performed to determine whether cyromazine (*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine) was metabolized to melamine (1,3,5-triazine-2,4,6-triamine) in rats. One male and one female fasted Sprague Dawley rat were fed diets containing cyromazine (purity, > 96.5%) at a concentration of 3000 ppm for 10 days. The rats were housed in metabolism cages from which urine and faeces were collected, although these samples were not analysed. The rats were then killed and the liver and kidneys were removed and analysed for cyromazine and melamine residues. Homogenates of both tissues were extracted with 90% acetonitrile : water, filtered and separated by ion exchange before high-performance liquid chromatography with ultraviolet light detector (HPLC-UV) determination of the relative proportions of cyromazine and melamine. Samples of treated diet were similarly analysed to determine the amount of melamine, originating from technical-grade cyromazine, which is known to contain melamine as a manufacturing impurity. The study was not performed according to GLP but it was an investigative study designed to complement the regulatory submission on metabolism.

The diet was shown to contain the correct amount of test substance. The ratio of cyromazine to melamine in diet was 120 : 1 (Table 9).

Table 9. Analysis of diet for cyromazine and melamine in a study in rats fed diets containing cyromazine at a concentration of 3000 ppm for 10 days

Sample	Cyromazine (ppm)	Melamine (ppm)	Ratio Cyromazine/melamine
Diet containing cyromazine on day 0	2585	25	103 : 1
Diet containing cyromazine on day 10	2933	21	140 : 1
Control diet	< 1.0	< 1.0	—

From Smith et al. (1983)

Residue concentrations of cyromazine and melamine were higher in the kidneys than in the liver and higher in males than in females (Table 10). The mean ratios of cyromazine : melamine between the sexes were approximately 30 : 1 for liver and approximately 40 : 1 for kidney. These ratios are quite different from those in the administered diet (120 : 1). Hence, these data indicate that rats metabolized cyromazine to melamine in liver and kidney.

Table 10. Analysis of liver and kidney for cyromazine and melamine in rats fed diets containing cyromazine at a concentration of 3000 ppm for 10 days

Sample	Cyromazine (ppm)	Melamine (ppm)[a]	Ratio
Liver			
Male liver at day 10 on control diet	< 0.05	< 0.05	—
Male liver at day 10 on diet containing cyromazine	31.3	0.96	33 : 1
Female liver at day 10 on diet containing cyromazine	13.2	0.51	26 : 1
Kidney			
Male kidney at day 10 on control diet	< 0.05	< 0.05	—
Male kidney at day 10 on diet containing cyromazine	62.4	1.3	48 : 1
Female kidney at day 10 on diet containing cyromazine	22.2	0.68	33 : 1

From Smith et al. (1983)

[a] Melamine residues have been converted to cyromazine equivalents.

Male and female rats were fed diets containing technical-grade cyromazine (containing melamine as a 0.8% impurity) at a concentration of 3000 ppm. After 10 days feeding, residues of cyromazine and melamine were higher in the kidneys than in the liver and higher in tissues in males than in females. The mean ratios of cyromazine : melamine between the sexes were approximately 30 : 1 for liver and approximately 40 : 1 for kidney, indicating that rats can metabolize cyromazine to melamine (Smith et al., 1983).

The biotransformation of [U-^{14}C triazine]cyromazine was investigated in male and female Sprague Dawley rats given doses as described in Table 11.

Table 11. Doses administered to male and female rats in a study on biotransformation of [U-^{14}C triazine]cyromazine

Group	Number and sex	Route and dose of [U-^{14}C triazine]cyromazine
1	One male and one female	Single intravenous dose of vehicle (carboxymethylcellulose plus Hi Sil)
2	Five males and five females	Single intravenous dose at approx. 3 mg/kg bw
3	Five males and five females	Single oral dose at approx. 3 mg/kg bw
4	Five males and five females	Single oral dose at approx. 3 mg/kg bw. Rats first conditioned with 14 daily non-radiolabelled doses at 3 mg/kg bw.
5	Five males and five females	Single oral dose at approx. 300 mg/kg bw
6	One male and one female	Single oral dose of vehicle
7	One male and one female	Rats dosed with vehicle each time group 4 received a dose.
8	One male and one female	Single oral dose of vehicle

From Capps (1990)

Approx., approximately.

After each dose of [U-^{14}C triazine]cyromazine (specific activity, 9.8 µCi/mg for the lowest dose and 0.8 µCi/mg for the highest dose; radiochemical purity, 97.2%), urine and faeces were collected at intervals over 7 days from rats in groups 2, 3, 4 and 5. Metabolite profiles in urine (collected over 0–24 h after a dose at 3 mg/kg bw and over 0–36 h after a dose at 300 mg/kg bw) and in solvent extracts of faeces (collections over 0–72 h) were investigated by TLC and liquid chromatography (LC). Structural assignments of resolved metabolites were confirmed by mass spectrometry. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 417 (1984).

The major component present in urine was cyromazine, accounting for 72% of urinary radioactivity (Table 12). An additional 7% was attributable to melamine, 9% to hydroxy-cyromazine and 2% to methyl-cyromazine, although there was no detectable amount of this latter metabolite in urine from rats at 300 mg/kg bw. Only 6% of [U-^{14}C triazine]-metabolites in urine remained uncharacterized and comprised minor metabolites, each of which represented less than 2% of urinary radioactivity. The urinary metabolite profiles were similar between the sexes and, apart from the absence of methyl-cyromazine in the group at the highest dose, there were no pronounced differences between doses.

Table 12. Quantification of urinary metabolites in rats given a single oral dose of [U-^{14}C triazine] cyromazine at 3 or 300 mg/kg bw

Metabolite	Percentage of administered radioactivity							
	Group 2		Group 3		Group 4		Group 5	
	Single intravenous dose		Single oral dose		Single oral dose (pre-treated for 14 days)		Single oral dose	
	Dose (mg/kg bw)							
	3		3		3		300	
	Male	Female	Male	Female	Male	Female	Male	Female
Methyl-cyromazine	1.98	2.93	2.15	2.02	1.63	1.59	ND	ND
Unidentified	5.19	6.11	6.54	6.76	6.60	6.09	2.59	1.73
Melamine	5.27	7.20	6.54	7.15	7.05	10.68	3.48	2.31
Hydroxy-cyromazine	6.76	5.69	14.02	8.47	8.32	4.06	4.94	4.86
Cyromazine	58.92	59.26	54.35	50.82	63.82	61.63	67.63	68.64

From Capps (1990)

ND, not detected.

The chromatographic profile of metabolites in faecal extracts was similar to that in urine with 71% of radioactivity corresponding to cyromazine and 7% to melamine (Table 13). An average of 8% co-chromatographed with methyl-cyromazine and hydroxy-cyromazine and 13% did not chromatograph with available standards and appeared to comprise several minor metabolites. The faecal metabolite profiles were similar for male and female rats.

Table 13. Quantification of faecal metabolites in rats given a single oral dose of [U-^{14}C triazine] cyromazine at 3 or 300 mg/kg bw

Metabolite	Percentage of administered radioactivity							
	Group 2		Group 3		Group 4		Group 5	
	Single intravenous dose		Single oral dose		Single oral dose (pre-treated for 14 days)		Single oral dose	
	Dose (mg/kg bw)							
	3		3		3		300	
	Male	Female	Male	Female	Male	Female	Male	Female
Unidentified	0.34	0.22	0.34	0.70	0.27	0.50	0.33	0.25
Melamine	0.38	0.51	0.47	0.16	0.22	0.15	0.33	0.32
Metabolite mixture[a]	0.38	0.50	0.92	0.21	0.20	0.13	0.43	0.24
Unidentified	0.13	0.10	IS	IS	0.12	IS	0.50	0.40
Unidentified	IS	IS	IS	IS	IS	IS	0.09	0.14
Cyromazine	3.77	4.84	2.78	2.59	2.26	1.83	5.76	4.95
Unidentified	0.14	0.19	0.04	0.06	0.12	0.07	0.07	0.05
Unidentified	0.04	0.08	< 0.01	0.04	0.11	0.02	< 0.01	< 0.01

From Capps (1990)

IS, Metabolite zones incompletely separated for samples from rats at the lowest dose, compared with those at the highest dose.

[a] Mixture of hydroxy-cyromazine, methyl-cyromazine and minor metabolites.

The predominant metabolic pathway for cyromazine in the rat involves biotransformation to hydroxy-cyromazine and methyl-cyromazine and then to melamine as illustrated in Figure 2.

In male and females rats given a single oral dose of [U-^{14}C triazine]cyromazine at 3 mg or 300 mg/kg bw, the absorbed dose was incompletely metabolized, with cyromazine being the predominant component of both urine and faeces. Cyromazine was metabolized to methyl-cyromazine, hydroxy-cyromazine and melamine. There were no pronounced differences between the sexes and relatively minor differences between the doses. The dosing of rats with unlabelled cyromazine at a dose of 3 mg/kg bw per day for 14 days before a single radiolabelled dose at 3 mg/kg bw had no marked effect on its biotransformation (Capps, 1990).

Monkeys

In a study performed to determine the excretion pattern of cyromazine in monkeys, metabolism was also investigated. Two male and two female *Macaca fasicicula* monkeys were given a single oral dose of [U-^{14}C triazine]-cyromazine at 0.05 mg/kg bw. An additional two male and two female monkeys were given a single oral dose of [U-^{14}C triazine]cyromazine at 0.5 mg/kg bw. The doses were administered in capsules. Each monkey was housed in a metabolism cage to which it had previously been acclimatized. Urine and faeces were collected for 24 h before dosing and at each day after dosing for 4 days. Radioactivity in urine and in combusted samples of faeces was measured by LSC. Aliquots of urine collected over the first 24 h after dosing were analysed by TLC against cyromazine and melamine reference standards. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. The study was regarded as additional information for the evaluation.

In all day-1 urine samples, more than 93% of the radioactivity present was characterized as cyromazine and the remainder as melamine (Table 14). There was considered to be no difference in metabolism between the doses or between the sexes.

In male and female monkeys given a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw, cyromazine accounted for more than 94% of urinary radioactivity, with the remainder attributable mainly to melamine (Staley, 1986).

Table 14. Relative proportions of cyromazine and melamine in samples of urine collected from monkeys over 24 h after a single oral dose of mg [U-^{14}C triazine]cyromazine at 0.05 or 0.5/kg bw

Sex	Dose (mg/kg bw)	Cyromazine (%)	Melamine (%)
Male	0.05	95.35	3.26
Female	0.05	94.10	5.90
Male	0.5	94.84	3.92
Female	0.5	94.90	3.37

From Staley (1986)

A second study was performed with one male and one female of the same strain of monkey and the same doses given by capsule as in Staley (1986). Each monkey was housed in a metabolism cage to which it had been first acclimatized. Urine and faeces were collected for 24 h before dosing and each day after dosing for 2 days. Radioactivity in urine and in combusted samples of faeces was measured by LSC. Aliquots of urine collected over the first 24 h after dosing were analysed by TLC against cyromazine and melamine reference standards. The study was not performed according to GLP, but it was an investigative study designed to complement the regulatory submission on metabolism. The study was regarded as additional information for the evaluation.

In all day-1 urine samples, approximately 95% or more of the radioactivity present was characterized as cyromazine and the remainder as melamine (Table 15). There was considered to be no difference in metabolism between the doses or between the sexes.

In male and female monkeys given a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw, cyromazine accounted for approximately 95% or more of urinary radioactivity with the remainder mainly attributable to melamine (Staley & Simoneaux, 1986).

Table 15. Relative proportions of cyromazine and melamine in samples of urine collected from monkeys over 24 h after a single oral dose of [U-^{14}C triazine]cyromazine at 0.05 or 0.5 mg/kg bw

Sex	Dose (mg/kg bw)	Cyromazine (%)	Melamine (%)
Male	0.05	100.00	0.00
Female	0.05	96.08	3.92
Male	0.5	94.99	3.73
Female	0.5	97.01	3.00

From Staley & Simoneaux (1986)

Goats

Lactating goats received [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 4.6 and 48.4 ppm for 10 days.

Cyromazine accounted for 43.7%, 35.9%, 0.2% and 32.5% of the extractable radioactivity in the urine, faeces, liver and milk, respectively at the lowest dose and 78.8%, 58.7%, 1.9% and 41.0% at the highest dose, respectively. Melamine concentrations in the urine, faeces, liver and milk were 11.9%, 14.3%, 1.7% and 9.2% at the lowest dose and 7.8%, 10.4%, 5.6% and 4.5% at the highest dose, respectively. 1-Methylcyromazine accounted for 44.4%, 17.4%, 92.7% and 1.0% of the extractable radioactivity at the lowest dose and 13.4%, 1.8%, 71.7% and 0.2% at the highest dose in urine, faeces, liver and milk, respectively (Simoneaux & Marco, 1984). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

Hens

Two hens received capsules containing [U-^{14}C triazine]cyromazine at a dose equivalent to a dietary concentration of 5.0 ppm for 7 days.

Most of the excreted radioactivity (75%) was unaltered cyromazine. A metabolite, incompletely characterized in this study but with the same retention characteristics as melamine, was found in egg white at 0.04 mg/kg and egg yolk at 0.01 mg/kg when the hens were fed diet containing cyromazine at 5 mg/kg.

The metabolism of cyromazine in hens appears to proceed by the same major pathway as in goat and rat, namely via dealkylation of the cyclopropyl group to yield the tri-amino-*s*-triazine, melamine (Simoneaux & Cassidy, 1979). This study had been evaluated by the 1990 JMPR and was not reviewed by the present Meeting.

Cow

In a feeding study in cows, 1-methylcyromazine was found at low levels of up to 0.11 ppm in the liver. It was below the level of quantification (LOQ) in milk, muscle, kidney, blood and fat. This metabolite was also found at a level of about 2% in urine of rats.

1.3 Dermal absorption

The dermal absorption of [U-^{14}C triazine]cyromazine (specific activity, 2590 kBq/mg, for the highest dose; the labelled test substance was diluted with non-labelled cyromazine to a specific activity of 70 kBq/mg; radiochemical purity, 98%), formulated as a wettable powder (WP) containing 7.5% active substance, was tested in groups of 16 rats at doses of 1.0 (lowest dose, P1) and 20 µg cyromazine/cm^2 (intermediate dose, P2), reflecting the range of typical concentrations recommended for the use in the field and 750 µg cyromazine/cm^2 (highest dose, P3) which represents the concentrate formulation (active substance, 75 g/l). Actual applied doses as cyromazine were 1.2, 23 and 864 µg/cm^2. The test substance was applied for 6 h to a shaved dorsal area of about 10 cm^2 of male rats (HanBrl: WIST (SPF). The application area was restricted by a non-absorbing 'O'-ring glued to the clipped skin using a cyanoacrylate adhesive. The application volume was 100 µl per animal. In order to prevent loss of the test substance, the 'O'-ring was covered with a non-occlusive cover tape. During the experiment, the rats were housed in all-glass metabolism cages. The depletion of the amount remaining in/on the skin after washing was determined on subgroups of four animals at four time-points, i.e. 6, 24, 48 and 72 h after application. The location within the skin of the skin-associated radioactivity was determined by removing the stratum corneum from the treated skin by tape stripping. After the 6-h exposure period, the cover was removed and retained for analysis. The unabsorbed material was removed from the application site by washing five times with a mild soap solution using cotton swabs. After each washing step, the skin was dried with a cotton swab. Finally a fresh cover was applied to the 'O'-ring. At necropsy, the 'O'-ring and the cover were carefully removed and extracted with methanol. Urine and faeces were collected from individual animals at 0–6, 6–24, 24–48, and 48–72 h after application. Blood was taken from the tail vein from each animal in the subgroup in which depletion was measured until 72 h after application at 0.5, 1, 2, 4, 6, 8, 24, and 48 h after application. In order to follow the fate of the applied test substance more closely, the treated skin was fractionated by tape-stripping into a fraction representing the stratum corneum and the remaining treated skin (epidermis and dermis). Radioactivity was determined in blood samples, urine, faeces, cage wash, skin wash, shavings, tape-strippings, residual skin of the application site, control skin, wash solution of 'O'-ring/cover and residual carcass. Additionally, TLC analysis of the dosing solution and of the skin wash was performed. The animals were checked for appearance and behaviour during acclimatization and at each sampling time-point. Some animals showed slight stress symptoms, i.e. chromodacryorrhoea and encrustations around the nostrils, during the first hour after administration. Also the slight weight loss of the animals was attributed to the

stress and discomfort during the experiment, e.g. the collar around the neck, the glued 'O'-ring on the dorsal area and the bandage. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 427 (2000).

The test substance was stable in the formulation, as indicated by the radiochemical purity of > 98%. TLC analysis of the skin wash solution revealed that more than 96% of the radioactivity was unchanged cyromazine.

The experimental recoveries were determined to be between 90% and 98% of the applied dose (subgroup mean). After an exposure time of 6 h, 3.20%, 1.93%, 2.04%, and 1.54% of the applied cyromazine were systemically absorbed within 6 h, 24 h, 48 h, and 72 h after application, indicating a moderate absorption of cyromazine through rat skin at the lowest dose (Table 16).

Table 16. Dermal absorption of [U-^{14}C triazine]cyromazine at an applied dose of 1.2 µg/cm² in rats[a]

Sample	Mean percentage of applied dose			
	Sacrifice time			
	6 h	24 h	48 h	72 h
Urine				
0–6 h	2.60	1.40	1.41	0.78
6–24 h	—	0.38	0.40	0.35
24–48 h	—	—	0.10	0.16
48–72 h	—	—	—	0.07
Subtotal	2.60	1.78	1.90	1.35
Faeces				
0–6 h	0.04	0.01	0.04	0.04
6–24 h	—	0.03	0.01	0.01
24–48 h	—	—	0.03	0.02
48–72 h	—	—	—	0.04
Subtotal	0.04	0.04	0.09	0.12
Cage wash	0.13	0.06	0.03	0.04
Total excretion	2.76	1.87	2.02	1.51
Residues				
Whole blood[b]	0.07	0.03	0.02	< 0.01
Skin non-treated[b]	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal tract	< 0.01	0.03	< 0.01	0.03
Remaining carcass	0.37	< 0.01	< 0.01	< 0.01
Subtotal	0.43	0.06	0.02	0.04
Systemic absorption	3.20	1.93	2.04	1.54
Skin stripping	11.25	16.54	16.62	17.70
Remaining treated skin	0.25	0.63	0.12	0.20
Application site	11.50	17.17	16.74	17.90
Skin wash	65.21	65.28	68.32	69.40
Cover and 'O'-ring	15.63	11.15	11.12	6.90
Dislodged dose	80.83	76.43	79.44	76.30
Total recovery	95.53	95.54	98.22	95.74

From Hassler (2002a)

[a] Corresponds to group P1.

[b] Residues determined in the portion of the specimen.

At the intermediate dose, the relative dermal penetration of cyromazine accounted for 2.14%, 1.71%, 2.51%, and 2.06% of the applied dose at 6, 24, 48, and 72 h (Table 17).

At the highest dose the relative dermal penetration of cyromazine accounted for 0.99, 0.97, 0.58, and 0.37% of the applied dose at 6, 24, 48, and 72 h (Table 18).

Table 17. Dermal absorption of [U-^{14}C triazine]cyromazine at an applied dose of 23 µg/cm^2 in rats[a]

Sample	Mean percentage of applied dose			
	Sacrifice time			
	(6 h)	(24 h)	(48 h)	(72 h)
Urine				
0–6 h	1.81	1.12	1.62	1.53
6–24 h	—	0.40	0.69	0.30
24–48 h	—	—	0.12	0.10
48–72 h	—	—	—	0.04
Subtotal	1.81	1.51	2.43	1.96
Faeces				
0–6 h	0.05	0.02	0.01	0.03
6–24 h	—	0.01	0.03	0.01
24–48 h	—	—	< 0.01	< 0.01
48–72 h	—	—	—	< 0.01
Subtotal	0.05	0.03	0.05	0.05
Cage wash	0.07	0.02	0.03	0.02
Total excretion	1.93	1.56	2.51	2.03
Residues				
Whole blood[b]	< 0.01	< 0.01	< 0.01	< 0.01
Skin, non-treated[b]	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal tract	0.02	< 0.01	< 0.01	< 0.01
Remaining carcass	0.18	0.15	< 0.01	0.02
Subtotal	0.21	0.15	< 0.01	0.03
Systemic absorption	2.14	1.71	2.51	2.06
Skin-stripping	12.01	13.68	12.88	15.53
Remaining treated skin	0.23	1.09	0.59	0.60
Application site	12.24	14.77	13.47	16.13
Skin wash	67.16	64.23	66.39	76.72
Cover and 'O'-ring	14.66	14.06	13.10	0.78
Dislodged dose	81.82	78.28	79.49	77.50
Total recovery	96.20	94.77	95.47	95.70

From Hassler (2002a)

[a] Corresponds to group P2

[b] Residues determined in the portion of the specimen.

Table 18. Dermal absorption of [U-^{14}C triazine]cyromazine at an applied dose of 864 µg/cm² in rats[a]

Sample	Mean percentage of applied dose			
	Sacrifice time			
	6 h	24 h	48 h	72 h
Urine				
0–6 h	0.72	0.79	0.42	0.21
6–24 h	—	0.12	0.10	0.08
24–48 h	—	—	0.02	0.03
48–72 h	—	—	—	0.01
Subtotal	0.72	0.91	0.54	0.34
Faeces				
0–6 h	< 0.01	0.02	< 0.01	< 0.01
6–24 h	—	< 0.01	< 0.01	< 0.01
24–48 h	—	—	< 0.01	< 0.01
48–72 h	—	—	—	< 0.01
Subtotal	< 0.01	0.02	< 0.01	0.02
Cage wash	0.23	0.02	< 0.01	0.01
Total excretion	0.95	0.95	0.56	0.36
Residues				
Whole blood[b]	< 0.01	< 0.01	< 0.01	< 0.01
Skin non-treated[b]	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal tract	< 0.01	< 0.01	< 0.01	< 0.01
Remaining carcass	0.04	0.02	0.02	< 0.01
Subtotal	0.04	0.02	0.03	< 0.01
Systemic absorption	0.99	0.97	0.58	0.37
Skin stripping	1.44	2.36	2.40	3.35
Remaining treated skin	0.07	0.32	0.02	0.02
Application site	1.51	2.68	2.43	3.37
Skin wash	82.96	86.28	84.86	85.98
Cover and 'O'-ring	5.10	0.45	2.13	1.08
Dislodged dose	88.06	86.73	86.99	87.06
Total recovery	90.57	90.38	90.00	90.79

From Hassler (2002a)

[a] Corresponds to group P3.

[b] Residues determined in the portion of the specimen.

The penetration rates during the 6-h exposure time, calculated from results of 6 h subgroups were 0.0064 µg cm^{-2} h^{-1} for the lowest dose (group P1), 0.0810 µg cm^{-2} h^{-1} for the intermediate dose (group P2), and 1.4326 µg cm^{-2} h^{-1} for the highest dose (group P3).

Because of the very low extent of absorption, the blood residues during and after dermal exposure at all doses were at or below the limit of determination.

About 76–81%, 77–82%, and 87–88% of the applied dose could be dislodged from the application site at the end of the exposure period at the lowest, intermediate and highest dose, respectively. The radioactivity that remained associated with the treated skin accounted for 11.5%, 12.2% and 1.5% of the lowest, intermediate and highest dose, respectively, at 6 h after application of the test substance and just after the skin-wash.

In order to follow the fate of cyromazine in the skin more closely, the skin was fractionated by tape-stripping the stratum corneum. At all doses the radioactivity remained in/on the treated skin area and was associated with the stratum corneum, i.e. 11–18% of the lowest dose, 12–16% of the intermediate dose, and 1–3% of the highest dose. The lower skin layers after skin-stripping, i.e. corium and subcutis, showed insignificant concentrations of radioactivity, indicating that the radioactivity present in the stratum corneum was not available for further penetration into the lower skin layers. The systemically absorbed dose was rapidly eliminated mainly via the urine. Elimination via the faeces was only a minor route of excretion (Hassler, 2002a).

Penetration of [U-^{14}C triazine]cyromazine (specific activity, 2590 kBq/mg, for the highest dose, the labelled test substance was diluted with non-labelled cyromazine to a specific activity of 70 kBq/mg; radiochemical purity, 98%), formulated as a WP containing 7.5% active substance, through rat and human epidermis was compared in vitro. The epidermal membranes were mounted to flow-through cells and each diffusion cell received a 6 µl aliquot of the application solution. Cyromazine was applied at a concentration of 1.1, 22 or 834 µg/cm^2 to rat or human epidermis for 6 h (Table 19). The two lowest doses reflected typical concentrations recommended for the use in the field, while the highest dose represented the concentrate formulation (active ingredient, 75 g/l).

Table 19. Dermal penetration of cyromazine through rat and human epidermis in vitro

Species	Dose	Applied dose		Concentration	
		mg/cell[a]	mg·cm^{-2}[a]	KBq/cell	(mg·cm^{-3})[a]
Rat	Lowest	0.7	1.1	1.9	0.12
	Intermediate	14	22	37	2.4
	Highest	534	834	37	89
Human	Lowest	0.7	1.1	1.9	0.12
	Intermediate	14	22	37	2.4
	Highest	534	834	37	89

[a] Expressed as cyromazine

From Hassler (2002b)

Rat epidermis was prepared from male HanBrl:WIST (SPF) rats aged about 9 weeks. Human epidermis was prepared from abdominal cadaver skin from Caucasian donors. Perfusates were collected at defined time-points. Twenty-four hours after application, the skin membrane surface was rinsed with ethanol (10 ml) and the radioactivity in the skin rinse was determined by LSC. The skin membrane was removed from the in-line cells and dissolved in tissue solubilizer before LSC. The receptor chamber was washed with ethanol and radioactivity determined by LSC. The study was conducted according to the principles and practices of GLP (with QA certificate) and the protocol was in accordance with OECD TG 428 (2000).

A TLC check for test substance stability of the formulated material revealed that [U-^{14}C triazine] cyromazine represented more than 98% of the radioactivity. The permeability check of the membranes revealed mean permeability constants (Kp) of tritiated water in the range of 0.54–0.98 × 10^{-3} cm/h and 0.53–0.96 × 10^{-3} cm/h for rat and human epidermis, respectively. At the end of the experiment, the skin rinse was analysed. In both groups, unchanged cyromazine amounted to more than 97% of the radioactivity present in the skin rinse. It was concluded that cyromazine remained unchanged for 24 h on the epidermis.

Within 24 h, 16.9% of the lowest dose, 19.1% of the intermediate dose, and 0.25% of the highest dose penetrated the rat epidermis membrane (Table 20), corresponding to penetration of 0.19 µg·cm^{-2}, 4.21 µg·cm^{-2}, and 2.07 µg·cm^{-2}, respectively. The flux, which reflects the penetration

rate under steady-state conditions, amounted to 0.018 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 0.560 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 0.370 $\mu g \cdot cm^{-2} \cdot h^{-1}$ at the lowest, intermediate and highest dose, respectively. While the concentration for the highest dose was 700-fold that at the lowest dose, the penetration rate at the highest dose was only 20-fold that at the lowest dose. Comparing the penetration rates of the highest dose with the intermediate dose, the calculated penetration rate remained almost constant while the concentration of the test substance at the highest dose was 40-times that at the intermediate dose. This observation was attributed to the limited solubility of the test substance in aqueous solution, i.e. 13 $mg \cdot cm^{-3}$. While the test substance at the lowest and intermediate dose formulations was dissolved, the concentration at the highest dose significantly exceeded the solubility of the test substance.

Within 24 h, 2.3% of the lowest dose, 1.1% of the intermediate dose, and 0.05% of the highest dose penetrated through the human skin membrane, corresponding to a penetration of 0.03 $\mu g \cdot cm^{-2}$, 0.25 $\mu g \cdot cm^{-2}$, and 0.40 $\mu g \cdot cm^{-2}$, respectively. The calculated flux, under steady-state conditions, accounted for 0.002 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 0.021 $\mu g \cdot cm^{-2} \cdot h^{-1}$, and 0.064 $\mu g \cdot cm^{-2} \cdot h^{-1}$ at the lowest, intermediate, and highest dose, respectively. However, based on the low specific activity and the very low penetration rate at the highest dose, most of the determined values were at or below the limit of determination. Therefore this calculated penetration rate (flux) was considered to be not very trustworthy. Again, the 700-times higher concentration of the highest dose led only to a 30-times higher penetration rate (flux) when compared with the lowest dose.

At the end of the experiments, the recovered radioactivity in the perfusate, skin rinse, skin membrane, and cell wash was measured (Table 21). Total recovery of radioactivity ranged from 98% to 103% and 96% to 103% of the applied radioactivity for rat and human skin membranes, respectively. Cyromazine amounted to more than 97% of the radioactivity present in the skin rinses for all doses and both species. Hence, it was concluded that the test substance remained essentially unchanged during the 24 h of exposure on the skin membrane.

Table 20. Dermal penetration of cyromazine through rat and human epidermis in vitro

Penetration	Applied dose ($\mu g \cdot cm^{-2}$)	Concentration ($mg \cdot cm^{-3}$)	Applied dose ($\mu g \cdot cm^{-2}$)	Concentration ($mg \cdot cm^{-3}$)	Applied dose ($\mu g \cdot cm^{-2}$)	Concentration ($mg \cdot cm^{-3}$)
	1.1	0.12	22	2.35	834	88.92
	Lowest dose		Intermediate dose		Highest dose	
	Percentage of dose	$\mu g \cdot cm^{-2}$	Percentage of dose	$\mu g \cdot cm^{-2}$	Percentage of dose	$\mu g \cdot cm^{-2}$
Rat epidermis						
Penetration at:						
6 h	6.26	0.07	7.93	1.75	0.16	1.37
12 h	10.64	0.12	12.07	2.66	0.20	1.68
24 h	16.85	0.19	19.09	4.21	0.25	2.07
Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	0.018		0.560		0.370	
Human epidermis						
Penetration at:						
6 h	0.47	0.01	0.28	0.06	0.02	0.15
12 h	0.87	0.01	0.54	0.12	0.03	0.24
24 h	2.27	0.03	1.14	0.25	0.05	0.40
Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	0.002		0.021		0.064	

From Hassler (2002b)

Table 21. Recovery of radioactivity in a study of dermal penetration with cyromazine in rat and human epidermis in vitro

Sample	Recovery (percentage of administered dose)					
	Rat epidermis			Human epidermis		
	Dose (µg·cm^{-2})					
	Lowest dose	Intermediate dose	Highest dose	Lowest dose	Intermediate dose	Highest dose
	1.1	22	834	1.1	22	834
Perfusates:						
0–24 h	16.85	19.09	0.25	2.27	1.14	0.05
Remaining dose:						
Cell wash	2.90	3.23	2.22	2.69	0.33	2.77
Skin rinse	35.47	59.47	71.43	62.57	91.53	84.09
Skin membrane	48.13	17.65	24.12	35.54	6.71	9.39
Subtotal	86.49	80.35	97.77	100.80	98.57	96.24
Total recovery	103.35	99.44	98.02	103.08	99.71	96.29

From Hassler (2002b)

Cyromazine, (formulated as a WP containing 7.5% active substance) penetrated at a faster rate and to a greater extent through rat than human split-thickness skin membranes, at all doses tested. The species difference was reflected in the flux constants determined as 0.018, 0.560 and 0.370 µg·cm^{-2}·h^{-1} through rat epidermis and 0.002, 0.021 and 0.064 µg·cm^{-2}·h^{-1} through human epidermis at the lowest, intermediate and highest dose, respectively. The resulting rat : human ratio of the flux constants was about 9 : 1, 27 : 1 and 6 : 1 at the lowest, intermediate and highest dose, respectively (Hassler, 2002b).

On the basis of the results of the dermal absorption study in rats in vivo and the comparative skin penetration study through rat and human skin in vitro, dermal penetration values for humans in vivo can be calculated by dividing the dermal absorption obtained in the rat in vivo by the ratio of the absorption rates between rat and human in vitro. In humans, the dermal absorption of cyromazine, formulated as a WP containing 7.5% active substance, was 0.1% for the concentrate and 0.1% for the highest and 0.3% for the lowest spray concentrations used in the field (Table 22).

Table 22. Calculation of dermal absorption of cyromazine[a] in humans

Parameter	Lowest dose 0.0011 mg/cm² (0.12 g/l)	Intermediate dose 0.022 mg/cm² (2.4 g/l)	Highest dose 0.834 mg/cm² (89 g/l)
Absorption in rats after 6 h (% of dose)	3.20	2.14	0.99
Absorption in rats after 72 h (% of dose)	1.54	2.06	0.37
Flux in rats (µg·cm-2·h^{-1})	0.018	0.560	0.370
Flux in humans (µg·cm-2·h^{-1})	0.002	0.021	0.064
Flux ratio (rat/human)	9	27	6
Absorption in humans (% of dose)	0.3	0.1	0.1

[a] Formulated as a wettable powder containing 7.5% active substance.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The acute toxicity of cyromazine after administration by the oral, dermal and inhalation routes is summarized in Table 23.

Table 23. Results of studies of acute toxicity with cyromazine

Species	Strain	Sex	Vehicle	LD_{50} (mg/kg bw; 95% CI or range) LC_{50} (mg/l air)	GLP or QA	Reference
Oral administration						
Rats	Tif:RAIf (SPF)	M & F	PEG 400	3387 (2524–4547)[a]	—	Sachsse & Bathe (1978a)
Inhalation						
Rats	HSD:SD	M & F	Aerosol, 4-h, nose-only	> 3.6 (maximum attainable concentration)[b]	GLP & QA	Holbert (1994)
Dermal administration						
Rats	Tif:RAIf (SPF)	M & F	PEG 400	> 3170[c]	—	Sachsse & Bathe (1978b)

F, female; GLP, good laboratory practice; M, male; PEG 400, polyethylene glycol; QA, quality assurance.

[a] Groups of five male and five female fasted rats received cyromazine (unknown purity) as a single gavage dose at 1000, 1670, 3590, 4640 or 6000 mg/kg bw as a suspension in PEG 400. The animals were observed for 14 days before sacrifice. Animals found dead and those surviving until the end of the study were subjected to macroscopic examination post mortem. The study was performed before the implementation of GLP and before EU or OECD guidelines had been enacted, but essentially conformed to OECD TG 401 (1987). Within 2 h of dosing, rats at all doses showed sedation, dyspnoea, exophthalmos, curved position and ruffled fur. Mortality occurred in zero, five (four on day 1 and one on day 7), two (day 1) and three (two on day 1 and one on day 2) males and in one (day 2), one (day 2), five (four on day 1 and one on day 2) and five (four on day 1 and one on day 2) females at 1000, 1670, 3590, 4640 and 6000 mg/kg bw, respectively. The surviving animals recovered within 9 to 12 days. There were no treatment-related macroscopic findings.

[b] Two groups of five male and five female rats were exposed nose-only for a single 4-h period to an aerosol generated from undiluted cyromazine technical (purity, 96.5%) at a mean concentration of 0.744 or 3.6 mg/l air. A control group of five males and five females was exposed to air only under similar experimental conditions. Clinical signs were recorded twice on the day of exposure and at least daily thereafter for up to 17 days. Body weights were measured before exposure, on day 7 and at the day of termination. At termination of the study, all animals were subjected to gross necropsy.

A maximum attainable exposure concentration of 3.6 mg/l was obtained, with a mean mass median aerodynamic diameter of 6.68 µm (3.92 µm at 0.744 mg/l). The study was performed according to Pesticide Assessment Guidelines, subdivision F, hazard Evaluation: Human and Domestic Animals, Series 81-3, EPA Publication, EPA 540/9-84-014, November 1984 and is in compliance with TM B2 of Dir. 92/69/EEC

None of the animals died before the scheduled termination. Prominent in-life observations included activity decrease, piloerection at both concentrations and additionally nasal discharge at the higher concentration. Clinical signs were no longer seen on day 2. Body-weight gain was largely unaffected. Two females at 3.6 mg/l and two females in the control group lost weight between days 0 and 7. Gross necropsy revealed discolouration of the lungs in animals of all groups and multiple red foci on lungs of males at 0.744 mg/l.

[c] Groups of five male and five female rats received a single 24-h dermal application of cyromazine (unknown purity) at 2150 or 3170 mg kg bw as a suspension in PEG 400. The animals were assessed for 14 days for any signs of systemic toxicity or skin irritation. At the end of the observation period all animals were killed and examined macroscopically. The study was performed before the implementation of GLP and before EU or OECD guidelines had been enacted, but essentially conformed to OECD TG 402 (1987).

None of the animals died. Within 24 h of treatment the rats in both groups showed dyspnoea, curved position and ruffled fur. No local skin irritation was seen. The animals recovered from systemic symptoms within 10 days. There were no treatment-related macroscopic findings.

Cyromazine is of low acute toxicity via the oral, dermal and inhalation routes. In rats, the oral median lethal dose (LD_{50}) for males and females combined was 3387 mg/kg bw; the dermal LD_{50} was greater than 3170 mg/kg bw and the inhalation LC_{50} (4-h exposure) was greater than 3.6 mg/l air, the highest achievable concentration. Symptoms of intoxication were sedation, dyspnoea, curved position and ruffled fur after oral or dermal administration. Animals recovered from systemic symptoms within 9–12 days. After inhalation, activity decrease, piloerection and nasal discharge were observed; these clinical signs were no longer seen on day 2.

(b) Dermal and ocular irritation and dermal sensitization

(i) Dermal irritation

A group of three male and three female Himalayan rabbits received a single 24-h occlusive application of approximately 0.5 g of cyromazine (unknown purity) to an abraded or non-abraded area of the shorn flank. The animals were assessed for any signs of skin irritation immediately and 48 h after removal of the dressings (24 h and 72 h after initiation of treatment). The Draize scale was used to assess the degree of erythema and oedema at the application sites. Mean erythema and oedema scores were calculated. The study was performed before the implementation of GLP and before EU or OECD guidelines had been enacted. Deviations to OECD TG 404 (1987) are considered not to compromise the scientific validity of the study.

On intact skin, very slight erythema (score 1) was noted in two animals at 24 h and very slight oedema (score 1) was recorded in four animals. All signs of skin irritation had disappeared 72 h after initiation of treatment. Mean scores for erythema and oedema were 0.17 and 0.3, respectively. On the basis of the these findings, cyromazine was not considered to be a skin irritant (Sachsse & Ullmann, 1978a).

(ii) Ocular irritation

Cyromazine (unknown purity) was not an irritant to the eye of Himalayan rabbits when assessed essentially in compliance with OECD TG 405 (1987) in a study that was performed before the implementation of GLP (Sachsse & Ullman, 1978b).

(iii) Dermal sensitization

The sensitization potential of technical cyromazine (purity, 97.4%) was assessed according to the maximization test of Magnusson & Kligman (1969). Groups of 10 male and 10 female young Himalayan Spotted (GOHI) guinea-pigs (test) and a control group of five males and five females were used for the main study. In test animals, the induction phase involved three intradermal injections of: (a) a 5% w/v preparation of cyromazine in the vehicle (0.5% carboxymethylcellulose sodium salt (CMC) and 0.1% Tween 80 in doubly-distilled water); (b) a 5% w/v preparation of cyromazine in a 1 : 1 preparation of saline : Freund's complete adjuvant (FCA); and (c) a 1 : 1 preparation of saline : FCA to a shorn area of the scapular region on day 1. This was followed 1 week later by a topical induction using the test substance (75% in 0.5% CMC and 0.1% Tween 80 in doubly-distilled water) under an occlusive dressing for 48 h. For guinea-pigs in the control group, the intradermal injections were saline : FCA and vehicle (0.5% CMC and 0.1% Tween 80 in doubly-distilled water) alone, and the topical applications were as for the test animals except that vehicle only was applied. Application sites were checked 24 h and 48 h after removal of the dressings. In the challenge phase, 2 weeks after completion of the induction phase, 50% cyromazine was applied to the shorn left flank and vehicle only was applied to the shorn right flank of test animals under an occlusive dressing for 24 h. Skin sites were examined approximately 2 h and 48 h after removal of the dressings. A study with a positive control was conducted using essentially the same methodology and using 2-mercaptobenzothiazole as

the test substance. The method used an intradermal induction of a 5% w/v preparation in mineral oil and in an emulsion of FCA : saline, 15% w/v preparation in mineral oil for the topical induction phase and 1% w/v preparation in mineral oil for the challenge phase. The study was conducted in compliance with the principles of GLP (with QA certificate) and according to OECD TG 406 (1992).

There were no skin reactions among the guinea-pigs in the control group. There were no positive skin reactions on the test flanks or control flanks of the test-group animals, corresponding to a sensitization rate of 0%. Therefore, cyromazine is not a skin sensitizer (Arcelin, 2000).

2.2 Short-term studies of toxicity

Rats

Groups of 20 male and 20 female Charles River CD rats were fed diets containing cyromazine (purity, 96.3%) at a concentration of 0, 30, 300, 1000 or 3000 ppm for up to 90 days. The doses were equal to a mean daily intake of 0, 2.4, 23, 79 and 232 mg/kg bw per day in males and 0, 2.6, 27, 88 and 264 mg/kg bw per day in females. An additional five rats of each sex per group were assigned to the control group and the group at 3000 ppm; they were kept for 4 weeks after the end of dosing to study recovery. Mortality and clinical signs were checked twice per day, body weights and food consumption were recorded weekly. Ophthalmological examinations were conducted in all animals before dosing, in week 13 and for recovery rats in week 16. Samples for laboratory investigations (haematology, clinical chemistry and urine analysis) were taken from 10 rats per dose and sex at intervals during the study (day 31, 58 and 86 for blood and day 31, 58, 83 and 89 for urine) and from recovery animals before termination. All animals were necropsied at terminal sacrifice, selected organs were weighed and selected organs and tissues were examined histopathologically. The study was performed before GLP had been formally adopted and before OECD TG 408 (1981) had been enacted, but complied to a great extent with these requirements.

There was no mortality and no treatment-related signs of toxicity were observed. Cyromazine triggered a slightly reduced body-weight gain at 1000 and 3000 ppm (reduction compared with controls of 5% and 8% in males and 7% and 8% in females, respectively). Body weight recovered during the 4-week recovery period. Food consumption remained unaffected. Ophthalmic examinations, haematological and biochemical tests and urine analyses revealed no compound-related effects.

There were no macroscopic or microscopic findings post mortem that could be attributed to treatment with cyromazine. Statistically significant differences in the relative liver weights were scattered over all dose groups. However, in the absence of any effects in clinical chemistry, macropathological or histopathological findings, these observations were considered to be of no biological relevance.

The NOAEL was 3000 ppm, equal to 232 mg/kg bw per day in males and 264 mg/kg bw per day in females, the highest dose tested considering that the slight reductions of body-weight gain observed at 1000 and 3000 ppm were not toxicologically relevant (Goldenthal, 1979).

In a study that complied with the principles of GLP (with QA certificate provided), groups of five male and five female Tif:RAIf (SPF) rats were exposed nose-only to cyromazine (purity not stated) in an aerosol at a concentration of 0, 0.058, 0.206 or 0.706 mg/l air for 4 h per day during 28 consecutive days. Additional groups of five males and five females treated at 0 and 0.706 mg/l air were maintained for a 3-week recovery period. Rats in the control group were exposed to filtered humidified air. Mortality and clinical signs were checked at least daily, body weights and food consumption were recorded weekly. Samples for laboratory investigations (haematology and blood clinical chemistry) were taken from all rats at the end of the treatment period, and from recovery animals after a 2-week treatment-free period. All animals were necropsied at terminal sacrifice,

selected organs were weighed, and selected organs and tissues were examined histopathologically. The study complied to a great extent with OECD TG 412 (1981), except that exposure was 4 h per day instead of 6 h per day.

There was no mortality. Treatment with cyromazine triggered clinical signs that included piloerection, dyspnoea, hunched posture, and reduced spontaneous activity in all treated groups. The time of onset and severity was concentration-dependent and subsided during the recovery period. There were no statistically significant, dose-related effects on body weights in either males or females; however, males in all exposure groups showed a slightly depressed body weight compared with controls at the end of the exposure period. The depression was independent of the dose and showed recovery by the end of the recovery period. There were no significant exposure-related effects on food consumption.

Minor effects noted in haematology were slightly higher values for erythrocyte parameters, specifically erythrocyte count, haemoglobin and erythrocyte volume fraction, in the males at the highest dose. At the end of the recovery period, the values for treated and control groups were comparable. There was no evidence that treatment with cyromazine had any influence on blood clinical chemistry parameters. Incidences of statistically significant differences between treated groups and control were sporadic and not dose-related.

Organ-weight analysis showed decreased pituitary weights in all treated males and increased liver weights at doses of 0.206 and 0.706 mg/l in treated females. However, pituitary weight in treated females was higher than that of controls, but did not reach statistical significance. In view of the small size of the pituitary and therefore the high variability in the weight, and in the absence of histopathological findings in this organ, the biological significance of this result is questionable. Pathology was essentially unremarkable. Microscopic examination revealed three females from the group at 0.706 mg/l and one female from the group at 0.058 mg/l with small foci of lymphocytic infiltration in the adrenal cortex. This change was not seen in females in the control group. All other changes were incidental in nature and not related to the test compound.

Daily exposure to cyromazine for 28 days resulted in slight, non-specific toxicity in rats. At the lowest concentration of 0.058 mg/l, equivalent to 9.3 mg/kg bw per day,[1] cyromazine triggered marginal effects in the rats' general condition only. In the absence of a substantial impairment of body-weight development, haematological and pathological parameters and clinical signs of only slight degree, is the Meeting considered this to be a no-observed-adverse-effect concentration (NOAEC). Exposure at 0.206 mg/l produced moderate signs of toxicity. Exposure at 0.706 mg/l produced moderate to severe signs of toxicity after 1 week of exposure (Hartmann, 1988).

Rabbits

In a study that complied with the principles of GLP (with QA certificate provided), groups of five male and five female New Zealand White rabbits were treated dermally with cyromazine (purity, 94.6%) 50, 500 or 2000 mg/kg bw per day and a vehicle control group received deionized water only. Application to the clipped dorsal surface lasted for 6 h per day, 5 days per week during 3 weeks. The test site, approximately 10% of the surface area, was moistened with 3.0 ml of deionized water and covered with a semi-occlusive dressing. One additional male was added to the group at 50 mg/kg bw and two males were added to the group at 500 mg/kg bw due to early non-treatment related deaths. Observations for mortality were made twice per day and the animals were assessed daily for any signs of systemic toxicity. Body weights and food consumption were recorded at intervals throughout the study. Twenty-four hours after the final application, the rabbits were killed and blood samples

[1] Twenty-four hour respiratory volume for rats is 0.096 m^3/kg bw (Zielhuis & Van der Kreek, 1979).

taken for haematological and biochemical analysis. Each rabbit was subjected to a macroscopic examination post mortem, selected organs weighed and selected tissues examined microscopically. The study complied with OECD TG 410 (1981).

Several deaths occurred with no dose–response relationship i.e. there were no deaths at the highest dose, while deaths occurred in the control group (one male), at 50 mg/kg bw (one female) and at 500 mg/kg bw (two males and two females). Clinical observations were generally minor. Changes in bowel and bladder function were observed in all groups and occurred more frequently during the first part of the study; these changes could have been in response to wrapping and handling of the rabbits. Other clinical observations included decreased activity, emaciation, lacrimation, yellow nasal discharge and ataxia; however, these effects were not dose-related and were considered not to be related to treatment. The 21-day dermal exposure to cyromazine produced no observable skin irritation. There were no dose-related effects on body weight or food consumption.

There were no dose-related differences in the clinical chemistry or haematological parameters measured in treated rabbits compared with those in the control group. There were no dose-related differences in organ weights, organ : body weight ratios, organ : brain weight ratios or histopathology in treated rabbits compared with those in the control group. A number of histopathological changes indicative of low-grade infection or parasite migration were observed in liver, kidneys and lungs taken at sacrifice from all four groups. These findings were considered not to be related to treatment.

In the absence of any treatment-related effect, the NOAEL was 2000 mg/kg bw per day, the highest dose tested (Kuhn, 1992).

Dogs

Groups of four male and four female beagle dogs were fed diets containing cyromazine (purity, 96.3%) at a concentration of 0, 30, 300, 1000 or 3000 ppm for up to 90 days. The doses were equal to a mean daily intake of 0, 1.2, 11, 36 and 100 mg/kg bw per day in males and 0, 1.1, 12, 33 and 96 mg/kg bw per day in females. An additional two dogs of each sex per group were assigned to the control group and the group at 3000 ppm; they were maintained for 4 weeks after the end of dosing to study recovery. Mortality and clinical signs were checked daily, body weights and food consumption were recorded weekly. Ophthalmological examinations were conducted in all dogs before dosing, in week 12 and for dogs in the recovery group in week 17. Samples for laboratory investigations (haematology, clinical chemistry and urine analysis) were taken from all dogs at intervals during the study (before dosing and after 4, 8 and 12 weeks) and from recovery animals before termination. All dogs were necropsied at terminal sacrifice, selected organs were weighed and selected organs and tissues were examined histopathologically. The study was performed before GLP had been formally adopted and before OECD TG 409 (1981) had been enacted but complied to a great extent with these requirements.

There was no mortality. Slightly relaxed nictitating membranes were quite frequently observed among treated dogs as was slightly dry nose among dogs at 1000 and 3000 ppm; in control dogs, relaxed nictitating membranes and dry nose were rarely encountered. Other findings in general behaviour, appearance and ophthalmoscopic examinations were similar for dogs in the control and test groups. Cyromazine triggered some reduction in body-weight gain in both sexes at 3000 ppm (reduction of 36% and 58% compared with controls in males and females, respectively) and in females at 1000 ppm (reduction of 38% compared with controls). During the recovery period, dogs (especially males) that had received cyromazine at 3000 ppm showed slightly greater increases in body weight than did dogs in the control group. Food consumption was slightly decreased for dogs at 3000 ppm when compared with dogs in the control group (decrease of 8.5% and 15.3% compared with controls in males and females, respectively). During the recovery period, food consumption for dogs previously treated at 3000 ppm exceeded that of dogs in the control group.

Cyromazine lowered erythrocyte parameters (total erythrocyte, haemoglobin and erythrocyte volume fraction values) in males at 3000 ppm (4–10%). These effects were considered to be physiologically significant but not pathological. They were reversible upon cessation of treatment. Biochemical tests and urine analyses revealed no compound-related effects.

There were no macroscopic or microscopic findings post mortem that could be attributed to treatment with cyromazine. Some variations of organ weights were considered to be of equivocal biological significance in the absence of any cyromazine-related morphological alterations.

The NOAEL was 1000 ppm, equal to 36 mg/kg bw per day, for males on the basis of decreases in body-weight gains, food consumption and some haematological parameters in dogs fed 3000 ppm. In females, the NOAEL was 300 ppm, equal to 12 mg/kg bw per day, on the basis of a decrease in body-weight gain at 1000 ppm (Jessup, 1979).

In a study that complied with the principles of GLP (with QA certificate provided), groups of four male and four female beagle dogs were fed diets containing cyromazine (purity, 97.5%) at a concentration of 0, 50, 200, 800 or 3500 ppm for at least 52 weeks. The doses were equal to a mean daily intake of 0, 1.4, 5.7, 23 and 94 mg/kg bw per day in males and 0, 1.5, 6.0, 25 and 110 mg/kg bw per day in females. Mortality, clinical signs of toxicity, body weights and food consumption were monitored throughout the study. Ophthalmological examinations were conducted in all animals before dosing and at the end of treatment. Laboratory investigations (haematology, clinical chemistry and urine analysis) were performed on all dogs at intervals during the study (before the start of the test and after 4, 8, 13, 26 and 52 weeks). At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Terminal samples of blood and bone marrow were taken, selected organs were weighed and specific tissues were taken for subsequent histopathological examination. The study complied with OECD TG 452 (1981).

One female in the group at 3500 ppm was found dead on day 21 (week 3) and one male in the group at 200 ppm had to be sacrificed prematurely on day 197 (week 29) due to behavioural changes (aggressiveness). In the female, the clinical signs before death included weakness of the hindlimbs and blood in vomitus. This animal exhibited histopathological changes in the liver, kidneys and intestine. Overt signs in the male consisted of multiple injuries on the hind legs and muzzle and skin lesions on the scrotum. This dog also showed pre-terminal reduced food intake and subsequently loss in body weight. The reason for this behavioural change was unknown but was considered not to be treatment-related owing to its single appearance, its absence at higher doses and in the absence of histopathological changes in this dog. There were no other mortalities. In-life clinical signs consisted of sporadic appearance of vomitus in the group at the highest dose, the effect being more pronounced in females. Diarrhoea of a variable degree was observed in control and treated animals, but no dose–response relationship was evident. There were also isolated findings of injuries, hair loss, skin changes and blood in faeces, but in the absence of a dose–response relationship, these findings were considered to be incidental. Mean body weight was slightly decreased in females at 3500 ppm during the first weeks of treatment, but reached values similar to those for controls after about 12 weeks. Mean food consumption was slightly depressed in the females of this group during the first 5 weeks of treatment only, with the lowest food intake during week 1. After this period, food consumption was comparable to that of controls. The ophthalmological examinations did not reveal any treatment-related changes.

Haematology changes were confined to a slight, hypochromic and microcytic anaemia, characterized by lower values for haemoglobin concentration, erythrocyte volume fraction, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), and a tendency to lower erythrocyte counts, seen in males and females at 3500 ppm. Similar findings but without changes to MCV and MCH values were also seen in the males at 800 ppm. In addition, males at 800 ppm and 3500 ppm exhibited platelet counts that were higher than those for controls; however, they did

not differ appreciably from pre-test values. Males and females at 800 and 3500 ppm and males at 200 ppm had higher values for concentrations of plasma proteins associated with higher values for globulin and lower albumin-to-globulin ratios. In addition, lower mean triglyceride values and lower creatine kinase activities were seen in males at 3500 ppm and females at 3500 ppm had higher plasma chloride concentrations at weeks 26 and 52. Before termination, one female of the group at the highest dose exhibited increased activities for alkaline phosphatase, alanine aminotransferase and gamma-glutamyl transpeptidase; however, the changes revealed no dose–response relationship and were not related to duration of treatment. Values for other blood chemistry parameters in treated animals were similar to those of controls and/or pre-test values and the differences were of insufficient magnitude to be toxicologically relevant. Urine analysis revealed no treatment-related changes.

Increased absolute and relative heart (19/19% in males and 25/33% in females) and liver (15/15% in males and 29/37% in females) weights were noted in males and females at 3500 ppm. Increased relative heart and liver weights were also recorded in females at 800 ppm (15% and 12%, respectively) (Table 24). Relative kidney weights in females at the highest dose were also increased (12.5% compared with controls). The changes in heart and kidney weights in the group at the highest dose correlated with histopathological findings.

Table 24. Organ weights, macroscopic and microscopic findings in beagle dogs fed diets containing cyromazine for 1 year

Finding	Dietary concentration (ppm)									
	0		50		200		800		3500	
	M	F	M	F	M	F	M	F	M	F
Organ weights (% change)										
Heart,										
Absolute	—	—	—	—	—	—	—	—	+19%	+25%
Relative	—	—	—	—	—	—	—	+15%	+19%	+33%
Liver,										
Absolute	—	—	—	—	—	—	—	—	+15%	+29%
Relative								+12%	+15%	+37%
Kidney, relative	—	—	—	—	—	—	—	—	—	+12.5%
Macroscopic findings										
Heart, hard myocardium	—	—	—	—	—	—	—	—	3/4	2/4
Microscopic findings										
Heart										
Severe chronic myocarditis right atrium	—	—	—	—	—	—	—	—	3/4	2/4
Foci cartilaginous metaplasia in muscle	—	—	—	—	—	—	—	—	1/4	—

Kidney, focal chronic epithelial regeneration of renal tubules	—	—	—	—	—	—	—	—	2/4	2/4
Bone marrow, hypercellularity	—	—	—	—	—	—	—	—	4/4	2/4

From Altmann (1997)

F, female; M, male.

Macroscopic findings were limited to hard myocardium observed in three out of four males and in two out of four females at 3500 ppm (Table 24). Microscopic findings revealed severe chronic myocarditis in the right atrium of the heart in three out of four males and in two out of four females at 350 ppm. One male exhibited foci of cartilaginous metaplasia in the affected heart muscle. Focal chronic epithelial regeneration of the renal tubules was observed in the kidneys in two out of four males and two out of four females at 3500 ppm. Hypercellularity of the bone marrow was recognized in all males and in two out of four females at 3500 ppm.

Administration of cyromazine for 1 year to dogs produced effects at doses ≥ 200 ppm in males and ≥ 800 ppm in females. Target organs of toxicity were the heart, the kidney and the haematopoietic system on the basis of histopathological findings, organ-weight changes and/or haematology values. The NOAEL was 200 ppm, equal to 5.7 mg/kg bw per day, in males on the basis of haematological effects observed at the next higher dose and 800 ppm, equal to 25 mg/kg bw per day, in females on the basis of haematological effects and histopathological changes in heart and kidney observed at 3500 ppm. In the absence of biochemical or histopathological findings, the small increases in relative liver and heart weights observed in females at 800 ppm were not considered to be adverse (Altmann, 1997).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study that complied with the principles of GLP (with QA certificate provided), groups of 68 male and 68 female Charles River CD-1 mice were fed diets containing cyromazine (purity, 95.3–95.5%) at a concentration of 0, 50, 1000 or 3000 ppm for up to 2 years. The doses were equal to a mean daily intake of 0, 6.5, 126 and 384 mg/kg bw per day in males and 0, 8.2, 164 and 476 mg/kg bw per day in females. Eight male and eight female mice were assigned from each group for interim clinical pathology investigations after 52 weeks of treatment. The remaining mice were maintained on the study for 2 years. Samples of diet of all concentrations (including controls) were taken at intervals throughout the study and analysed for achieved concentration, stability and homogeneity. The mice were observed daily for appearance, behaviour, overt signs of toxicity, moribundity and mortality; detailed observations were recorded weekly. Individual body weights were obtained weekly during the initial 13 weeks of study and every 2 weeks thereafter. Individual food consumption was measured for 10 mice of each sex per group weekly for the first 13 weeks and every 2 weeks thereafter; test article consumptions were calculated using the body weight and food consumption measurements. Haematological tests were performed on eight randomly selected mice of each sex per group at 12 and 24 months. At the end of the scheduled period, the animals were killed and subjected to a full postmortem examination. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination. The study was performed before OECD TG 451 (1981) had been enacted but complied to a great extent with these requirements.

Analysis of the diets showed that the stability of cyromazine in diets was satisfactory. Mean concentrations of cyromazine found in all diets analysed were 97 ± 31%, 102 ± 14% and 99 ± 9% of target concentrations at 50, 1000 and 3000 ppm, respectively. The homogeneity of cyromazine in the diet was improved in the second half of the study by a modification of the method by which the diet was prepared.

There was no clear dose-related effect on survival (62%, 63%, 55% and 55% in males and 52%, 47%, 57% and 40% in females from the control group, and at the lowest, intermediate and highest dose, respectively, at 104 weeks). Although the lowest rate of survival was in females at 3000 ppm, survival was within the expected range (values for historical controls for survival at 24 months were: males, 44.2%; females, 44.6%). There were no treatment-related differences noted in clinical condition between control and treated mice. Statistically significantly lower body weight and body-weight gains were noted for males at 3000 ppm throughout the study and for males at 1000 ppm from weeks 12 to 87. At week 104, the decreases in body-weight gain compared with controls were 12% and 18% at 1000 and 3000 ppm, respectively. Females at these doses showed occasional statistically significant differences in body weight and body-weight gain compared with controls; however, the differences were considered not to be biologically significant. At week 104, only slightly lower mean body weight was observed in treated animals compared with controls (0%, 2.4% and 7.1% in males and 2.9%, 5.7% and 5.7% in females). Food consumption was slightly reduced for males at 1000 and 3000 ppm throughout the study and was reduced in females at 3000 ppm, particularly in the first year of the study. There were no treatment-related effects on haematological parameters.

No treatment-related macroscopic changes were noted at the 12-month interim examinations. At study termination, there was a slightly increased incidence of masses in the livers of treated males compared with controls. There was no treatment-related effect on organ weights. A slight increase in relative liver weight in males at the highest dose was related to the reduced body weight in these animals.

Microscopic examination revealed a variety of neoplastic and non-neoplastic lesions. Except for hepatocellular neoplasms in male mice, the incidence and distribution of these lesions were similar to those of spontaneous lesions in CD-1 mice of a similar age. A slight increase in the frequency of hepatocellular neoplasms (adenomas and carcinomas) was noted in treated males (incidences of 18.3%, 28.8%, 32.1% and 26.3% in the control group and at the lowest, intermediate and highest dose, respectively) but, since there was no dose–response relationship, no increase in non-neoplastic proliferative lesions and no similar effect in females, it was not considered to be treatment-related (Table 25). Pulmonary metastasis was observed in one male mouse at 1000 ppm that had hepatocellular carcinoma. In females at the highest dose, the incidence of mammary gland adenocarcinomas was higher than in the other groups (4%, 8.3%, 5.7% and 16% in the control group and at the lowest, intermediate and highest dose, respectively). Data for historical controls indicated an expected range of 0–5.0% (mean, 1.3%). A variety of other neoplasms were observed in male and female mice in the control group and in mice receiving cyromazine. The incidence and distribution of these neoplasms were approximately equal in the control group and in groups receiving cyromazine. The most frequent of these were alveolar/bronchiolar adenomas and carcinomas of the lung, adenomas and carcinomas of the Harderian glands and malignant lymphoma involving primarily the thymus, spleen and lymph nodes.

Table 25. Incidence of hepatocellular neoplasms in CD-1 mice given diets containing cyromazine for 104 weeks

Finding	Dietary concentration (ppm)							
	0[a]		50		1000		3000	
	M	F	M	F	M	F	M	F
No. of animals examined	60	56	59	57	53	57	57	57
Liver								
Hepatocellular adenoma	5	0	9	2	11	1	5	3
Hepatocellular carcinoma	6	0	8	0	6	0	9	0
Hepatocellular adenoma and carcinoma	0	0	0	0	0	0	1	0
Mammary gland								
Adenocarcinoma[b]	—	2/2	—	7/4	—	3/3	—	9/8

From Blair (1982a)

F, female; M, male.

[a] Historical control data were obtained from 19 studies conducted at IRDC between 1976 and 1978 in 1490 males and 1490 females. Hepatocellular adenomas, mean: males, 2.3%; females, 0.9%; range, males, 0–26.7%; females, 0–6.0%. Hepatocellular carcinomas, mean: males, 8.4%; females, 1.0%; range, males, 1.0–26.0%; females, 0–10.0%. Mammary gland adenocarcinomas, females: mean, 1.3%; range, 0–5.0%.

[b] No. of lesions/No. of animals with lesions.

Generalized amyloidosis was observed in male and female mice in all test groups. The tissues most frequently involved were the small intestine, kidney, liver, adrenals, thyroid, heart and ovary. In the small intestine, amyloid was generally deposited in the lamina propria. In the adrenal gland, amyloid deposition was located at the corticomedullary junction. In the kidney and liver, amyloid deposition occurred in the renal glomerulus and around hepatic veins. Amyloid deposits in the thyroid occurred in the perifollicular tissue, while the deposits were scattered in the heart and ovary. Several male and female mice had chronic inflammatory lesions in the lungs. The severity of the lesion varied but was generally mild. These lungs had patchy areas with thickened alveolar walls lined by low cuboidal epithelium. This adenomatous change was frequently accompanied by intra-alveolar accumulation of alveolar macrophages and peribronchiolar and perivascular mononuclear cell infiltrates. The lesions resembled those associated with resolving pulmonary infections with Sendi virus in mice. Inflammatory lesions in the kidney were observed in mice of both sexes. Interstitial mononuclear infiltrates were observed in the renal cortex and a few mice had proteinaceous tubular casts, regenerative tubular epithelium and tubular cysts. Cystic endometrial hyperplasia was frequently seen in female mice in the control group and in groups receiving cyromazine and was characterized by proliferation of the epithelial lining of the uterus with cystic dilatation of endometrial glands. Parovarian cysts and cystic follicles were commonly observed in the ovaries of female mice in all groups. Inflammatory, degenerative and non-neoplastic proliferative lesions were less frequently observed in other tissues. Extramedullary hematopoiesis and brown granular pigment resembling haemosiderin were present in the spleen of most of the mice. These are common findings in the spleen of mice. Nematode parasites were present in the large intestines of a few animals. The haematopoietic activity of the bone marrow, as judged by overall cellularity, was within the normal range and no differences were noted among treatment groups. Other lesions were few in number and scattered among tissues in male and female mice in the control group and in groups receiving cyromazine.

In conclusion, dietary administration of cyromazine to CD-1 mice for up to 24 months resulted in a toxicologically relevant decrease in body-weight gain in males at 3000 ppm. The lower body weights observed in males at 1000 ppm and in females at 1000 and 3000 ppm were not

considered to be biologically significant owing to the very small differences between the means. Cyromazine did not produce inflammatory, degenerative or neoplastic lesions. A slightly higher number of hepatocellular neoplasms were observed in males receiving cyromazine, but were not considered to be treatment-related owing to the absence of non-neoplastic proliferative lesions, the lack of a dose-related response and the absence of similar changes in females. In females at the highest dose, the incidence of mammary gland adenocarcinomas was higher than in the other groups, and was above the upper limit of the range for historical controls. The NOAEL was 1000 ppm in males, equal to 126 mg/kg bw per day, on the basis of changes in body weight (Blair, 1982a).

Rats

In a study that complied with the principles of GLP (with QA certificate provided), groups of 60 male and 60 female Charles River CD rats were fed diets containing cyromazine (purity, 95.3–95.5%) at a concentration of 0, 30, 300 or 3000 ppm for up to 2 years. An additional 10 males and 10 females were initiated concurrently in the control group and in the group at 3000 ppm to allow for interim sacrifices; five rats of each sex were killed after 1 year and another five rats of each sex were fed control diet for a 4-week recovery period before termination after 1 year of dosing. The doses were equal to a mean daily intake of 0, 1.5, 15 and 156 mg/kg bw per day in males and 0, 1.8, 19 and 210 mg/kg bw per day in females. Samples of diet at all dietary concentrations (including controls) were taken at intervals throughout the study and analysed for achieved concentration, stability and homogeneity. The mice were observed daily for appearance, behaviour, overt signs of toxicity, moribundity and mortality; detailed observations were recorded weekly. Individual body weights were obtained weekly during the initial 13 weeks of the study and every other week thereafter. Individual food consumption was measured for 10 randomly selected rats of each sex per group weekly for the first 13 weeks and every other week thereafter; test article consumptions were calculated using the body weight and food consumption measurements. Haematological and clinical biochemistry (blood and urine) tests were performed on eight randomly selected rats of each sex per group before study initiation and at 6, 12, 18 and 24 months. At the end of the scheduled period the animals were killed and subjected to a full postmortem examination. Terminal samples of blood and bone marrow were taken, selected organs were weighed and specified tissues were taken for subsequent histopathology examination. The study was performed before OECD TG 453 (1981) had been enacted but complied to a great extent with these requiremets. The following deviations were noted: albumin was not measured, adrenals and ovaries were not weighed and ophthalmoscopy was not performed.

Analysis of the diets showed that the stability of cyromazine in diet was satisfactory. Diet analysis at the highest dose was satisfactory. At the lowest dose, higher variation in the content of cyromazine was observed in the period up to week 59. From week 60, the procedure was changed and concentrations were satisfactory for all doses. The mean concentrations of cyromazine found in all analysed diets were 96%, 101% and 99% of nominal concentrations at 30, 300 and 3000 ppm, respectively, indicating that animals had been adequately exposed.

There were no treatment-related differences noted in clinical condition, behaviour or survival between rats in the control group and rats treated with cyromazine. At termination of the study, survival was 60–67% in males and 53–70% in females. There was a decrease in mean body weight in both sexes at 3000 ppm (20.3% in males and 28.3% in females at week 104) compared with controls. Statistically significantly lower body-weight gain (22% and 33% in males and females, respectively at week 103) and food consumption (13.2% and 9.3% in males and females, respectively) were noted in both sexes at 3000 ppm. A statistically significant decrease in body-weight gain of 6–11% was also observed during weeks 12–55 in females at 300 ppm. Occasionally lower food consumption was observed in both sexes at 300 ppm. During the last 10 weeks of the study, the body weights of

females at the intermediate dose were comparable to those of the control group. The recovery animals of both sexes gained weight over the 4-week recovery period. Weight gains of 4–6% were noted for the control group, while rats at the highest dose in the recovery group exhibited weight gains of 11–19%.

There were no treatment-related differences between rats in the control group and rats treated with cyromazine noted in the clinical pathology parameters. There were no treatment-related differences noted in macroscopic pathology between rats in the control group and rats treated with cyromazine. Reductions in organ weights (liver, kidney, heart and brain) in rats at the highest dose were associated with reduced body weights in this group.

Tumours of the pituitary gland (adenoma and adenocarcinoma) in males and females, and mammary gland tumours in females, were seen in all groups including the controls (Table 26). There was a slightly higher incidence of testis interstitial cell tumours in males at the highest dose (6 out of 57 compared with 1 out of 60, 2 out of 59 and 1 out of 58 in the control group and at the lowest and intermediate dose, respectively) and mammary gland adenocarcinomas in females at the highest dose (9 out of 59 compared with 3 out of 53, 2 out of 58, and 1 out of 58 in the control group and at the lowest and intermediate dose, respectively). Both these values for incidence, as well as that for the incidence of pituitary gland adenomas and carcinomas, were within the range for historical controls in the laboratory. Other tumours occurred much less frequently and their incidence was not treatment-related.

Table 26. Incidence of selected tumours in rats fed diets containing cyromazine for 2 years

Tumour	Dietary concentration (ppm)							
	Males				Females			
	0[a]	30	300	3000	0[a]	30	300	3000
Pituitary								
No. of animals examined	60	56	57	57	55	59	56	58
Adenoma	26	19	19	17	29	31	38	42
Adenocarcinoma	7	12	8	5	15	11	7	11
Mammary gland								
No. of animals examined	56	46	43	55	53	58	58	59
Adenoma	0	0	0	1	3	8	6	8
Adenocarcinoma	0	0	2	0	3	2	1	9
Testis	60	59	58	57	—	—	—	—
Interstitial cell tumours	1	2	1	6	—	—	—	—

From Blair (1982b)

[a] Data on historical controls (16 2-year studies conducted at IRDC between 1975 and 1979, data from 1010 males and 1071 females). Pituitary adenoma: mean, 28.5%, range, 8.3–68.0% in males; and mean, 52.5%, range, 18.3–75.0% in females. Pituitary carcinoma: mean, 2.2%, range, 0–10.0% in males; and mean, 5.0%, range, 0–24.0% in females. Mammary gland adenomas: mean, 0.3%, range: 0–3.3% in males; and mean, 4.9%, range, 0–21.7% in females. Mammary gland carcinomas: mean, 0.3%, range, 0–1.7% in males; and mean, 9.5%, range, 1.5–21.4% in females. Interstitial cell tumours of testis: mean, 7.7%, range, 0–22.0%.

Non-neoplastic findings, such as inflammatory, degenerative, developmental and hyperplastic changes were mainly seen in the kidney, lung and liver (Table 27). In the kidney, there was evidence of chronic progressive nephropathy, commonly seen in older rats. Females fed diets containing cyromazine at 3000 ppm had a slightly higher incidence of pelvic epithelial hyperplasia (a common accompaniment of chronic nephropathy) compared with other groups. However, the incidence of chronic nephropathy was lower in the group at the highest dose than in other groups. Therefore, the

observed incidence probably related to variability in degree of change as a result of ageing and was not treatment-related. Chronic respiratory disease, characterized by bronchiectasis, suppurative bronchitis and cellular infiltrates or proliferations, was evident in the lung, with a slightly higher incidence in males at 3000 ppm compared with controls. However, this finding is common and the incidence was variable, therefore it may not have been a result of treatment with cyromazine. Microscopic changes in the liver were of a type and incidence common in older rats of this strain. All the neoplastic and non-neoplastic changes observed were considered to be spontaneous lesions, with no direct evidence of a treatment-related incidence and within the normal range of variations.

Table 27. Incidence of selected microscopic findings in rats fed diets containing cyromazine for 2 years

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	30	300	3000	0	30	300	3000
Kidney								
Chronic nephropathy	41	43	40	22	14	14	11	2
Epithelial hyperplasia	1	3	0	2	1	9	6	15
Lung								
Hyperplasia	21	13	18	19	11	8	9	11
Suppurative bronchitis	16	11	13	23	9	12	6	10
Bronchiectasis	7	4	6	18	2	5	4	10
Liver								
Pericholangitis	8	11	3	6	7	12	9	3
Vacuolation	0	0	0	0	1	1	0	0
Focal vacuolation:	6	2	2	2	1	1	0	0
Hepatocytomegaly	0	0	0	0	2	5	5	3
Focal hepatocytomegaly	1	2	2	2	1	5	3	5

From Blair (1982b)

In conclusion, dietary administration of cyromazine for up to 2 years resulted in decreased body-weight gain, lower mean body weight and food consumption in male and female rats at 3000 ppm. On the basis of these effects, the NOAEL was 300 ppm, equal to 15 mg/kg bw per day in males and 19 mg/kg bw per day in females. In females, a higher incidence (but within the range for historical controls) of mammary gland tumours was observed at 3000 ppm (Blair, 1982b).

2.4 Genotoxicity

The mutagenic/genotoxic potential of technical cyromazine was investigated in a battery of tests in vitro and in vivo (Table 28). All the results were negative, with the exception of an inconclusive spot test in mice. The Meeting considered that cyromazine is not genotoxic.

Table 28. Results of studies of genotoxicity with cyromazine

End-point	Test object	Concentration[o]	Purity (%)	Results	GLP or QA	Reference
In vitro						
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	20–5000 µg/plate, in DMSO	97.5	Negative[a,b]	GLP & QA	Deparade (1988)
Reverse mutation	*S. typhimurium* TA1538 & E. coli WP2*uvrA*	20–5000 µg/plate, in DMSO	97.5	Negative[a,c]	GLP & QA	Deparade (1990)
Gene mutation, mitotic gene conversion & mitotic recombination	*Saccharomyces cerevisiae D7*	375–3000 µg/ml, in DMSO	98.9	Negative[a,d]	GLP & QA	Hool (1984)
Gene mutation	Mouse lymphoma cells, L5178Y, $Tk^{+/-}$	50–500 µg/ml, in culture medium, 4-h exposure	96.2	Negative[a,e]	GLP & QA	Beilstein (1985)
Gene mutation	Chinese hamster, V79 cells, *Hprt* locus	25–1000 µg/ml, −S9, in ethanol, 21-h exposure 100–4000 µg/ml, +S9, in ethanol, 5-h exposure	98.9	Negative[a,f]	GLP & QA	Dollenmeier (1986)
Chromosomal aberration	Human peripheral blood lymphocytes	62.5, 125, 250, 500 and 1000 µg/ml in DMSO, ± S9, 3-h treatment, harvesting 46 h after the end of treatment	96.2	Negative[a,g]	GLP & QA	Strasser (1985)
Unscheduled DNA synthesis	Rat (F344) primary hepatocytes	$1–10^{-4}$ mg/ml in DMSO, 18-h exposure	NR	Negative[h]	QA	Tong (1982)
Unscheduled DNA synthesis	Mouse (male CD-1) primary hepatocytes	$1–10^{-4}$ mg/ml in DMSO, 18-h exposure	NR	Negative[i]	QA	Tong (1983)
In vivo						
Nucleus anomalies[j] (BM cells)	Chinese hamster (three male + three female/group)	Two oral doses at 2000, 4000 and 8000 mg/kg bw (24 h apart) in CMC	98.9	Negative[k]	—	Hool (1980)
Micronucleus test (BM cells)	Mouse (Tif:MAGf SPF, NMRI derived) (five male + five female/group)	Single oral doses of 360 and 1080 mg/kg bw in CMC-Sampling time: 24, 48 and 72 h after treatment	96.3	Negative[l]	GLP & QA	Strasser (1987)
Spot test	Mouse (males: T-stock, females: C57Bl/6)	Single intraperitoneal. doses at 150, 300 and 600 mg/kg bw in sesame oil	96.2	Inconclusive[m]	GLP & QA	Strasser (1986)
Dominant lethal	Mouse (Tif:MAGf SPF, NMRI derived)	Single oral doses at 226 or 678 mg/kg bw in PEG 400	98.9	Negative[n]	—	Hool (1981)

CMC, carboxymethyl cellulose; DMSO, dimethyl sulfoxide; GLP, good laboratory practice; NR, not reported; QA, quality assurance; S9, S9, 9000 × *g* supernatant from livers of male rats.

[a] With and without metabolic activation.

[b] Cyromazine was 264assayed twice using the standard plate incorporation protocol over a dose range of 20-5000 µg/plate, ±S9 prepared from Aroclor-induced male RAI rats. The experimental protocol essentially complied with OECD TG 471 (1997).

[c] Cyromazine was assayed twice using the standard plate incorporation protocol over a dose range of 20-5000 µg/plate, ±S9 prepared from Aroclor-induced male RAI rats. The experimental protocol essentially complied with OECD TG 471 (1997).

[d] Cyromazine was tested ±S9. Two independent repeat tests were performed. Positive controls: –S9, 4-nitroquinoline-*N*-oxide; +S9, cyclophosphamide. The highest concentration of test substance was chosen on the basis of the solubility of the test substance and caused no decrease in survival of the yeast cells. Experimental protocol essentially in complied with OECD TG 480 and TG 481 (1986).

[e] Cyromazine was tested ±S9. Two independent repeat tests were performed. Positive controls: –S9, ethylmethane sulfonate; +S9, dimethylnitrosamine. Cyromazine produced no cytotoxicity at the doses tested. The experimental protocol was essentially in compliance with OECD TG 476 (1997).

[f] Cyromazine was tested ±S9. Two independent repeat tests were performed. Positive controls: −S9 mix, ethylmethane sulfonate; +S9, dimethylnitrosamine. In a preliminary assay, toxicity was observed −S9 but not +S9. Therefore, 4000 µg/ml was chosen as the highest concentration for the main assay + S9 and 2000 µg/ml −S9. The experimental protocol was essentially in compliance with OECD TG 476 (1997).

[g] Cyromazine was tested ±S9. Positive controls: −S9, mitomycin C; +S9, cyclophosphamide. Concentration-related reductions in mitotic activity were observed in cultures in a preliminary assay for cytotoxicity. The highest concentration chosen for the main assay was 1000 µg/ml (mitotic index, about 68% of control values in preliminary tests for cytotoxicity test). The experimental protocol was not totally in compliance with OECD TG 473 (1997) as a repeat test was not performed and treatment −S9 should have been for a cell cycle.

[h] Positive control was 7,12-dimethylbenzanthracene and negative control was anthracene. Unscheduled DNA synthesis (UDS) was measured by autoradiography. The highest dose tested was limited by the solubility of the test material. Cytotoxicity was observed in one of the cultures at the highest concentration (1 mg/ml) tested. The test protocol was not in compliance with OECD TG 482 (1986) as only a single test was performed and less than 50 cells per culture were evaluated for UDS. The study was performed before the GLP certification of laboratories, but was conducted according to the principles and practices of GLP.

[i] Positive control was benzo(a)pyrene and negative control was pyrene. UDS was measured by autoradiography. Three independent repeat assays were performed. No cytotoxicity was observed in any of the tests at the highest concentration (1 mg/ml) tested. The highest concentration was determined by the solubility of the test substance. The test protocol was not in compliance with OECD TG 482 (1986), as only 40 cells per culture were evaluated for UDS instead of 50. The study was performed before the GLP certification of laboratories, but was conducted according to the principles and practices of GLP.

[j] Single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leukopoietic cells and polyploid cells.

[k] Cyclophosphamide was used as positive control. Two females died after the first application of vehicle control. In the group receiving the highest dose, two females died after the second dose. The study was performed before OECD TG 474 was enacted. Less than 1000 polychromatic erythrocytes (PCE) were analysed for the presence of micronuclei. Exact number of erythrocytes was not stated in the report. Only three animals per sex were analysed.

[l] Cyclophosphamide was used as positive control. The highest dose was selected on the basis of a tolerability test showing that no deaths occurred in a group of four animals. In the test for mutagenicity, one male at the highest dose died within 72 h. The experimental protocol was not totally in compliance with OECD TG 474 (1997) as only two doses were used. Only 1000 polychromatic erythrocytes (PCE) were analysed for the presence of micronuclei.

[m] Each group consisted of 48 males and 96 females. Each untreated male was placed in a cage with two untreated females. After successful mating, females were removed from the mating cages before allocation to treatment groups. Groups of 71 presumed pregnant animals received a single intraperitoneal injection of cyromazine at the appropriate dose on day 10 after conception. Examination of the offspring for spots was started at age 12–14 days and was performed twice per week between weeks 3 and 5 after birth. The incidence of recessive spots (RS) and white mid-ventral spots (WMVS) was recorded. Total numbers of offspring examined for each group were 354, 354, 357 and 93 for the control group and the group at the lowest intermediate and highest dose, respectively. In a preliminary test, 600 mg/kg bw was found to be the highest dose not causing deaths in groups of non-pregnant female mice. At 600 mg/kg bw, a decrease in the average litter size was observed (4.33 compared with 6.61, 7.52 and 6.88 in the vehicle control group and at the lowest and intermediate dose, respectively). Survival up to the start of spot observation was reduced at the two highest doses. There was a statistically significant increase in the incidence of RS at doses of 300 (1.12%) and 600 mg/kg bw (2.15%) when compared with the concurrent control group (0%). However, owing to the low incidences of RS and the marked reduction in litter size in the group at the highest dose, it was necessary to compare the groups at the two higher doses with the cumulative negative controls using the Fischer exact test. In this test, a dose at 300 mg/kg bw

or 600 mg/kg bw did not differ significantly from the data for historical controls (RS, 0.75%) derived from experiments with the appropriate vehicle (sesame oil) alone ($p = 0.514$ and $p = 0.179$ for the groups at 300 and 600 mg/kg bw). By contrast, cumulative positive control experiments with ethylmethanesulfonate (100 mg/kg bw) yielded an average RS frequency of 1.41%. This was significantly different (Fisher exact test for RS, $p = 0.013$) from the data for historical controls (RS, 0.26%) derived from experiments with the appropriate vehicle (physiological saline or Hank BSS) alone. It was therefore concluded by the performing laboratory that the observed increase was not indicative of a mutagenic effect. The Meeting considered the study inconclusive as interpretation of the results was confounded by reduced reproductive performance of the group at the highest dose, resulting in a much smaller number of observations. The study protocol complied with OECD TG 484 (1986).

[n] Groups of 20 fertile males were treated with cyromazine. Approximately 8 h after dosing, each of the males was placed in a cage with two untreated females. After 1 week, the females were removed and replaced by another two females. This procedure was continued for 6 consecutive weeks in order to examine the effect of treatment on all stages of the spermatogenic cycle. The females were examined daily for the presence of a vaginal plug which would indicate successful mating. The day that a vaginal plug was observed was designated as day 0 of gestation. The uteri of the females were examined for live implantations, early deaths and late deaths 14 days after they were first housed with the males. Three males died within 24 h after treatment with the compound at the highest dose. The data on mating ratio, on the number of implantations and embryonic deaths are comparable for all groups. A study with a positive control, thiotepa, was carried out in 1976. This was administered in a single intraperitoneal dose at at doses of 3.65 or 11 mg/kg to male mice. These males were then housed with untreated females for 1 week. At the end of the week the females were replaced by new ones. This procedure was continued for 8 consecutive weeks. A cytotoxic and dominant lethal effect was observed in the progeny of male mice treated with thiotepa. The study complied to a great deal with OECD TG 478 (1984), except that there were only two treated groups plus controls and a positive-control study, carried out within 3 years of the main study, was reported in a supplement to the main report. This study was performed before GLP guidelines were enacted and therefore not performed under formal quality assurance, but a compliance statement was provided. Preimplantation losses could not be accurately calculated as the number of corpora lutea was not recorded.

[o] Vehicle was used as negative control. Positive control substances were used in all assays and gave the expected results.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a study that complied with the principles of GLP (with QA certificate provided), groups of 15 male and 30 female (F_0 parents) weanling Sprague-Dawley COBS CD rats were fed diet containing cyromazine (purity, 95.3%) at a concentration of 0, 30, 1000 or 3000 ppm (initially 4000 ppm, reduced to 3000 ppm after 4 weeks of feeding to the F_0 parents). The doses were equal to a mean daily intake of 0, 2.0, 64 and 228 mg/kg bw per day in F_0 males and 0, 2.3, 77 and 259 mg/kg bw per day in F_0 females, and 0, 1.6, 51 and 169 mg/kg bw per day in F_1 males and 0, 1.9, 66 and 202 mg/kg bw per day in F_1 females. After 100 days, the animals were mated and allowed to rear the ensuing F_1 litters to weaning. From these weanlings, the F_1 parents were selected and after a 120 day pre-mating period were mated to produce the F_2 litters. In each generation, on day 4 of lactation, the litters were reduced to 10 animals (where possible, of equal sex ratio). Test diets were fed continuously throughout the study. Body weights, food consumption (measured only during pre-mating periods), fertility and the general appearance, behaviour and survival of all parental rats were observed and recorded during the study. Female parental rats were also observed for duration of gestation and any difficulties during parturition. The litter parameters evaluated and recorded included pup viability on day 0 of lactation, mean litter size, pup body weights and survival during lactation and the general appearance and behaviour of the pups. Complete gross and histopathological postmortem examinations were conducted on all parental rats (F_0 and F_1 generations), on five F_1 weanlings of each sex per group not selected as members of the F_1 generation and on five F_2 weanlings of each sex per group. The remaining F_1 and F_2 weanlings were given gross internal examinations only if appearing abnormal on external examination. Those appearing normal were discarded. Samples of diet at all concetrations (including controls) were taken at intervals throughout the study and analysed for achieved concentration. Stability and homogeneity were measured.

The study was performed before OECD TG 416 (1983) was enacted and did not comply entirely with these requirements. Among points on which it failed to comply were: females were housed with males for up to three 10-day periods, food consumption was measured during the pre-mating periods only, F_1 offspring not selected for mating were as old as 30 days at postmortem examination, cervix, vagina and seminal vesicles of all animals selected for histopathology were not listed in the report as being examined, individual weights of the pups at termination have not been reported. The Meeting considered that these deviations did not compromise the scientific validity of the study.

In the early part of the study, analytical results suggestive of non-homogeneity of the diet containing cyromazine at 30 ppm were noted; results ranged from 11% of the target (average of the duplicate assays at study week 23) to 133% (study week 15). Prepared diets containing cyromazine at 1000 and 3000 ppm contained 92–143% and 72–125% of the desired concentrations, respectively. The mean concentrations found from study weeks 1–25 were 78 ± 29%, 109 ± 12% and 104 ± 9.1% of the target levels in the groups receiving cyromazine at 30, 1000 and 3000 ppm, respectively. In diets prepared for study weeks 26 to termination, concentrations of cyromazine ranged from (mean ± SD) 47% to 155% (91 ± 23%), 83% to 122% (101 ± 9.6%) and 76% to 116% (97 ± 9.6%) of the target levels in the groups at 30, 1000 and 3000 ppm; respectively. Nevertheless, although there were notable variations in concentrations in diet at 30 ppm in the first part of the study, the overall exposure was considered to be acceptable. The overall range and mean of doses received during the pre-mating period is reported in Table 29.

Table 29. Overall range and mean of dose received during the pre-mating period in a two-generation reproduction study in rats fed diets containing cyromazine

Generation/sex	Dietary concentration (ppm)					
	30		1000		3000	
	Dose received (mg/kg bw per day)					
	Range	Mean	Range	Mean	Range	Mean
F_0						
Males	3.09–1.39	1.97	101–43.7	64.1	368–157	228
Females	3.28–1.87	2.34	108–61.9	77.2	398–192	259
F_1						
Males	2.00–1.28	1.55	66.8–43.0	51.3	278–137	169
Females	2.37–1.66	1.94	81.2–57.2	66.3	246–169	202

From Blair (1981a)

One female F_1 parental rat died but the death was considered to be incidental to treatment with cyromazine. Survival was 100% in the remaining animals in both generations. The clinical condition of parental animals was not affected by treatment with cyromazine; however, there was some evidence of a viral infection affecting a small number of rats (sialodacryoadenitis virus, SDV). Dose-related and statistically significant decreases in parental mean body weight were observed in males at 1000 and 3000 ppm and females in the F_0 generation and in F_1 females as well as in F_1 males at 3000 ppm. In the F_0 generation, mean body weights were reduced by 7.5% and 17.5% respectively, for males, and 8.6% and 17.0% respectively, for females, compared with controls. In the F_1 males at 3000 ppm, mean body weights were reduced by approximately 14.5% compared with controls. Food consumption nearly paralleled the body-weight effect with slight decreases in the group at 1000 ppm group and moderate decreases in males and females of both generations at 3000 ppm.

The reproductive performance of rats treated with cyromazine at all doses was comparable with that of controls for both matings. Male fertility in the group at 3000 ppm of the F_0 generation was reduced and may have been compound-related (Table 30). Male and female fertility in the group at 30 ppm of the F_1 generation was reduced when compared with the control groups, but these observations were not consistent across generations and there was no evidence of a dose–response relationship; therefore it was not considered to be treatment-related. There were no remarkable differences in the process of parturition or the duration of gestation between females receiving cyromazine and those in the control group for either generation.

Table 30. Fertility index in a two-generation study of reproduction in rats fed diets containing cyromazine

Generation	Fertility index (%)							
	Dietary concentration (ppm)							
	Males				Females			
	0	30	1000	3000	0	30	1000	3000
F_0	86.7	86.7	92.9	66.7	80.0	90.0	90.0	76.7
F_1	86.7	66.7	100.0	93.3	86.7	76.7	86.7	90.0

From Blair (1981a)

There was no evidence of any effect of treatment on macroscopic changes on F_0 parents, F_1 adults or weanlings or F_2 pups. Occasional statistically significant differences between control and treated groups were noted in relative organ weights. However, these changes were considered to be

either sporadic or as a consequence of reduced body weights at 3000 ppm, and of no toxicological significance. There were no treatment-related microscopic findings.

Pup viability at birth and mean litter size in the treated groups of the F_1 litters were similar to control. In the F_2 litters, pup viability at birth and mean litter size were reduced in the group at 3000 ppm (decrease of 5% and 7.9%, respectively); subsequent survival was not affected. Mean pup body weight in both generations was reduced at 3000 ppm.

Dietary administration of cyromazine to rats for two generations resulted in decreased parental body weights and food consumption at doses of 1000 and 3000 ppm and decreased pup body weights at 3000 ppm. The effects at 1000 ppm were not considered to be biologically significant. Male fertility was reduced in the F_0 generation at 3000 ppm; however, reproductive performance was not affected. A decrease in pup viability at birth and mean litter size was also observed in F_2 litters at 3000 ppm. The NOAEL for parental toxicity, reproduction and offspring toxicity was 1000 ppm, equal to 64 mg/kg bw per day in males and 51 mg/kg bw per day in females (Blair, 1981a).

(b) Developmental toxicity

Rats

In a study that complied with the principles of GLP (with QA certificate provided), groups of 20 pregnant Charles River COBS®CD® rats were given cyromazine (purity, 96.3%) at a dose of 0, 100, 300 or 600 mg/kg bw per day, by gavage in 1% (w/v) aqueous CMC, on days 6–19 (inclusive) of gestation. The day that evidence of mating was detected was designated as day 0 of gestation. Before treatment, the dams were observed daily for mortality and overt changes in appearance and behaviour. They were observed daily for mortality and clinical signs of toxicity on days 6–20 of gestation. Individual maternal body weights were recorded on days 0, 6, 9, 12, 16 and 20 of gestation. On day 20 of gestation, the rats were killed and their uteri weighed and examined for live fetuses and intrauterine deaths. The abdominal cavities and organs of the dams were examined for grossly evident morphological changes. The fetuses were weighed, examined for external abnormalities and sexed. Approximately one third of the fetuses were examined for visceral abnormalities by razor-blade sectioning and the remaining two thirds were stained for skeletal examination. The study was performed before OECD TG 414 (1981) had been enacted and did not comply entirely with these requirements, e.g. food consumption was not recorded and visceral examination was carried out on one third of all fetuses. The Meeting considered that these deviations did not compromise the scientific validity of the study.

Clinical signs seen in the dams at 600 mg/kg bw per day included increased activity during the first portion of the treatment period, red nasal discharge, clear oral discharge and inactivity beginning mid-way through the treatment period. Red nasal discharge was also seen in the group at 300 mg/kg bw per day. Maternal body-weight gain was moderately reduced in the group at 600 mg/kg bw per day (days 6–20 of gestation, 69.3%; and days 0–20 of gestation, 73.5% of controls, respectively) and slightly reduced in the group at 300 mg/kg bw per day (days 6–20 of gestation, 82.5%; and days 0–20 of gestation, 85.7% of controls, respectively). A mean maternal body-weight loss occurred in both groups during the first 3 days of treatment.

There were no biologically meaningful or statistically significant differences in the mean numbers of viable fetuses, early or late resorptions, total implantations or numbers of corpora lutea. There were no biologically meaningful or statistically significant differences in the fetal sex distribution or the number of litters with malformations in any of the treatment groups. A slight decrease in mean fetal body weight in the group at 300 mg/kg bw per day (3%) and a statistically significant decrease in the group at 600 mg/kg bw per day (6%) was noted. A definite increase in fetuses with reduced ossification was noted at 600 mg/kg bw per day (Table 31). Increases occurred

in the number of litters and fetuses with developmental variations in all treated groups. Slight increases were noted in reduced ossification of the skull in all groups and in unossified sternebrae (No. 5 and/or No. 6 and other sternebrae) in the groups at 100 and 300 mg/kg bw per day. These very slight increases in the group at 100 mg/kg bw per day may have been due to random occurrence as there were no other signs of fetal toxicity. A definite increase was noted in unossified sternebrae in the group at 600 mg/kg bw per day.

Table 31. Incidence of skeletal variations of fetuses in a study of developmental toxicity in rats given cyromazine by gavage

Finding	Dose (mg/kg bw per day)			
	0 (control)[a]	100	300	600
No. of. litters examined	23	22	25	24
No. of fetuses examined skeletally	224	217	238	224
Skull, reduced ossification (No. of litters affected)	0 (0)	2 (1)	1 (1)	2 (2)
Hyoid unossified (No. of litters affected)	5 (4)	3 (3)	3 (2)	0 (0)
Vertebrae, reduced ossification (No. of litters affected)	1 (1)	2 (2)	1 (1)	1 (1)
Pubis unossified (No. of litters affected)	0 (0)	0 (0)	1 (1)	0 (0)
Metetarsals unossified (No. of litters affected)	0 (0)	0 (0)	1 (1)	0 (0)
Sternebrae No. 5 and/or No. 6 unossified (No. of litters affected)	48 (17)	63 (19)	79 (22)	171 (23)
Other sternebrae unossified (No. of litters affected)	2 (2)	5 (4)	5 (4)	24 (11)

From Rodwell (1979)

[a] IRDC reported incidence of sternebrae No. 5 and/or No. 6 unossified among historical controls: 12.5% of the fetuses in 45.7% of the litters.

Cyromazine was not teratogenic in rats given doses of up to 600 mg/kg bw per day by gavage. Signs of maternal toxicity (clinical signs of toxicity and decreased body-weight gain) were observed at 300 and 600 mg/kg bw per day and fetal toxicity (decreased body weight and reduced ossification) was observed in the group at the highest dose. The NOAEL for maternal toxicity was 100 mg/kg bw per day and the NOAEL for developmental toxicity was 300 mg/kg bw per day (Rodwell, 1979).

Rabbits

Groups of 16 artificially inseminated Dutch Belted rabbits were given cyromazine (purity, 96.3%) as a single daily dose at 0, 25, 50 or 75 (experiment 1) and 0, 10, 30 or 60 mg/kg bw per day (experiment 2) a constant volume of 1 ml/kg (in 1% (w/v) in aqueous CMC) orally by gavage on days 6–27 (inclusive) of gestation. The day of artificial insemination was designated day 0 of gestation. Before treatment, the females were observed daily for mortality and overt changes in appearance and behaviour. The females were observed daily for mortality and clinical signs of toxicity on days 6–28 of gestation. Dams not surviving to the scheduled sacrifice were necropsied and fetuses from these dams were examined externally and preserved in 10% neutral buffered formalin. Any female showing signs of abortion was sacrificed and examined for grossly evident morphological changes. Intact fetuses were examined externally and preserved in 10% neutral buffered formalin. Individual maternal body weights were recorded on days 0, 6, 12, 18, 24 and 28 of gestation. On day 28 of gestation, all surviving females were killed and the uterus was excised and weighed. The number and location of viable and non-viable fetuses, early and late resorptions, number of total implantations and corpora lutea were recorded. The dams were examined macroscopically and maternal tissues were preserved for microscopic examination only as deemed necessary by the gross findings. All

fetuses were removed, weighed and examined for external malformations. Each fetus was dissected, internally sexed and examined for visceral abnormalities and then processed for subsequent skeletal examination. The study was conducted according to the principles and practices of GLP (with QA certificate) and was performed before OECD TG 414 (1981) had been enacted but complied to a great extent, except that food consumption was not measured.

Four, two, two and one animals died in the groups at 75, 60, 50 and 25 mg/kg bw per day, respectively. There were no mortalities in the groups at 10 or 30 mg/kg bw per day or in the controls. Nine females aborted (before death or where sacrificed) between day 20 and day 27 of gestation (two each in the groups at 30, 50 and 60 mg/kg bw per day and three in the group at 75 mg/kg bw per day). In experiment 2, a reduction in the amount of faeces was seen in the treated groups at various intervals during the treatment period. This finding occurred for a longer duration with increasing dose. Macroscopic examination of the dams post mortem revealed heart failure as a cause of death for two females, one each in the groups at 25 and 75 mg/kg bw per day; lung congestion and oedema were cited as possible causes of death for two females in the group at 60 mg/kg bw per day and pneumonitis-pleuritis was determined a cause of death for one dam at 75 mg/kg bw per day. There were no treatment-related trends in necropsy findings in either experiment when compared with those for the respective control groups.

In experiment 1, mean maternal body-weight losses were observed primarily during days 6 to 12 of gestation at 25 mg/kg bw per day (–101 g) and days 6 to 18 at 50 (–261 g) and 75 (–399 g) mg/kg bw per day when compared with the control groups. This resulted in mean maternal body-weight losses over the entire treatment period in these three groups. In experiment 2, there were moderate body-weight losses during days 6 to 18 of gestation in the groups at 30 (–133 g) and 60 (–255 g) mg/kg bw per day, resulting in a slight decrease in mean maternal body-weight gain at 30 mg/kg bw per day and a slight loss in mean maternal body weight at 60 mg/kg bw per day, for the entire study period. There was no effect on body-weight gain at 10 mg/kg bw per day.

In experiment 1, the pregnancy rates in all groups, including controls, were considerably reduced (12, 9, 10 and 10 females in the groups at 0, 25, 50 and 75 mg/kg bw per day, respectively) when compared with values for historical controls (82%) for this strain of rabbit (which may have resulted from a technician error when inseminating).

In experiment 1, there were no effects on the mean number of corpora lutea or mean fetal body weight in the groups at 25, 50 or 75 mg/kg bw per day or on mean postimplantation loss and fetal sex distribution in the groups at 25 or 50 mg/kg bw per day. A slight decrease in the mean number of total implantations (19.4%) and an increase in mean postimplantation loss (400%) with a corresponding and statistically significant decrease in the mean number of viable fetuses (50.8%) was observed at 75 mg/kg bw per day. Malformations were not observed in the 11 litters examined in the control group or in the eight litters examined in the group at 50 mg/kg bw per day. The only malformations observed were fused sternebrae in one fetus from one litter (of eight litters examined) in the group at 25 mg/kg bw per day and fetal anasarca in two fetuses from one litter (of four litters examined) in the group at 75 mg/kg bw per day.

In experiment 2, there were no biologically meaningful or statistically significant differences in the mean numbers of corpora lutea, total implantations, postimplantation loss, viable fetuses or the total sex distribution in any of the treated groups when compared with the control group. Although the mean fetal body weight was slightly reduced in all groups, this finding was considered to be unrelated to treatment since there was both a wide deviation within each group and a slight increase in the number of viable fetuses in the treated groups. Although there were no malformations observed in the control group in this experiment, the genetic and developmental variations seen in the treated groups were comparable to those cited for historical controls. In only one out of 11 recently documented studies were no malformations observed in the controls. Anomalies were observed in all the treated groups (5.3%, 6.7% and 12.1% of fetuses and 33.3%, 30.8% and 40.0% of litters affected at 10,

30 and 60 mg/kg bw per day, respectively). This finding was statistically significant ($p < 0.05$) in the group at the highest dose. However, most of these anomalies occurred only once and had been observed in historical controls. The number of fetuses with anomalies at incidences that exceeded values for historical controls included the following: two with carpal flexure at 10 mg/kg bw per day, one with fused sternebrae and one with malformed sternebrae at 30 mg/kg bw per day and one with internal hydrocephaly/dome-shaped head, one with skull anomalies (malformed nasals, premaxillae and jugals), one forked thoracic rib and three with fused sternebrae at 60 mg/kg bw per day (Table 32). A slight increase in the number of fetuses and litters with 27 presacral vertebrae and a 13th full rib was noted in the treated groups compared with the control group.

Table 32. Incidence of malformations and variations of fetuses (litters) in a study of developmental toxicity in rabbits given cyromazine by gavage—experiment 2

Finding	No. of fetuses affected (No. of litters affected)				
	Dose (mg/kg per day)				
	0 (control)	10	30	60	Historical controls
Total No. examined	77 (14)	95 (15)	90 (13)	58 (10)	951 (149)
External malformations					
Carpal flexure	0 (0)	2 (2)	1 (1)	0 (0)	4 (4)
Abdominal closure defects—omphalocele	0 (0)	0 (0)	0 (0)	2 (1)	2 (2)
Soft tissue malformations					
Internal hydrocephaly/ dome-shaped head	0 (0)	1 (1)	0 (0)	1 (1)	3 (3)
Skeletal malformations					
Scoliosis with/without rib anomaly	0 (0)	1 (1)	0 (0)	1 (1)	7 (7)
Fused sternebrae	0 (0)	1 (1)	1 (1)	3 (3)	1 (1)
Malformed sternebrae	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)
Variations[a]					
Twenty-seven presacral vertebrae	5.2 (14.3)	8.4 (26.7)	10.0 (38.5)	20.7 (50.0)	68 (36)[b]
Thirteenth full rib	7.8 (21.4)	20.0 (46.7)	21.1 (69.2)	32.8 (70.0)	97 (44)[c]

From Blair (1981b)

[a] Percentage affected: fetuses (litters).

[b] Range, 1.8–14.1 (13.3–44.4).

[c] Range, 1.1–32.7 (7.7–70.0).

In conclusion, oral administration of cyromazine to pregnant Dutch Belted rabbits results in maternal toxicity at doses ≥ 25 mg/kg bw per day as shown by maternal deaths, abortions and decrease in body weight. In the first experiment, embryotoxicity was observed at 75 mg/kg bw per day (decreased total implantations, increased postimplantation losses and decrease in the number of viable fetuses), but no teratogenic effect was observed. In the second experiment, an increased incidence of malformations at above the range for historical controls were seen; however, their biological significance is uncertain. Factors relating their occurrence to treatment included the fact that a majority involved the sternum, they occurred in all treatment groups and their frequency of occurrence at 30 and 60 mg/kg bw per day exceeded values for historical controls. However, they occurred generally as single incidences.

The NOAEL for maternal toxicity was less than 25 mg/kg bw per day in the first experiment (the lowest dose tested) and 10 mg/kg bw per day in the second experiment. The NOAEL for developmental toxicity was 50 mg/kg bw per day in the first experiment on the basis of the decrease in viable fetuses and increase in postimplantation loss at 75 mg/kg bw per day, and 30 mg/kg bw per day in the second experiment on the basis of an increase in postimplantation loss and increased incidences of 27 presacral vertebrae at 60 mg/kg bw per day (Blair, 1981b).

In a second study, conducted in the same strain of rabbit and at the same laboratory, an enteric disease precluded a reliable evaluation of the effects observed. Groups of 18 inseminated rabbits were given cyromazine (purity not reported) at a dose of 0 (vehicle control, 1% CMC), 5, 10, 30 or 60 mg/kg bw per day by gavage on days 7–19 (inclusive) of gestation.

Cyromazine did not elicit developmental toxicity in this study at up to the highest dose tested, 60 mg/kg bw per day. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of body-weight loss at ≥ 30 mg/kg bw per day during the treatment period. A slight reduction in food consumption was observed at 60 mg/kg bw per day only (Schardein, 1985).

In a third study, groups of 18 artificially inseminated New Zealand White rabbits (BUK(CRL) NZW FBR) were given cyromazine (purity, 95.2%) at a dose of 0 (environmental control, undosed), 0 (vehicle control, 0.5% aqueous CMC), 5, 10, 30 or 60 mg/kg bw per day by gavage on days 7–19 (inclusive). Throughout gestation, all females were observed twice per day for signs of toxicity. Individual maternal body weights were recorded on days 0, 7, 10, 14, 20, 24 and 29 of gestation and food consumption was recorded daily from days 0–29 of gestation. On day 29 of gestation, all surviving females were sacrificed and uteri examined for live fetuses and intrauterine deaths. All fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies and developmental variations. The study was conducted according to the principles and practices of GLP (with QA certificate) and complied with OECD TG 414 (1981).

Analyses of the dosing preparations confirmed that the achieved concentrations, homogeneity and stability of cyromazine in the vehicle were satisfactory.

There were no treatment-related deaths during the study. Four females aborted and were sacrificed, one in the untreated control group, one in the group at 30 mg/kg bw per day and two in the group at 60 mg/kg bw per day (one on day 21 that may have been treatment-related). Decreased urination and defecation were noted in the groups at 30 and 60 mg/kg bw per day. At 60 mg/kg bw per day, severe maternal body-weight losses (−226 g on days 7–10, −113 g on days 7–20) and reduced food consumption (44% and 55% of vehicle control for days 7–10 and days 7–20, respectively) were seen, particularly over the first few days of the study. Mean body-weight gains and food consumption were significantly increased after the treatment period (days 20–29 of gestation: +306 g compared with +10 g in the vehicle control group and 169 g compared with 102 g in the vehicle control group, respectively). At 30 mg/kg bw per day, decreased body-weight gains during days 7–10, 10–14 and 7–20 of gestation were observed (17.5%, 87% and 69% of values for the vehicle control, respectively) as well as statistically significant decreases in food consumption during the first 3 days of treatment (82% of vehicle control). Treatment-related decreases in the mean net body-weight gain were observed in the groups at 30 and 60 mg/kg bw per day when compared with controls (days 0–29 of gestation, decrease of 6% and 14%, respectively). Mean gravid uterine weights and net maternal body weights were not affected by treatment at any dose. There were no pathological changes among the dams at the terminal necropsy which could be considered treatment-related. The pregnancy rate of the group at 10 mg/kg bw per day was somewhat lower than normally expected for this species (seven successful pregnancies out of 18 females inseminated). This was considered to be a random occurrence.

There were no treatment-related differences in the mean numbers of viable and dead fetuses, implantation sites and corpora lutea in the treated groups when compared with the vehicle and untreated

control groups. No significant differences in fetal sex ratios or fetal weights were observed in any of the treated groups. There was an increase (not statistically significant) in the mean number of early resorptions in the two groups at higher doses (mean, 1.2 and 1.7, respectively, compared with 0.8 and 0.9 in the controls) and the mean number of late resorptions in the group at the highest dose (mean, 0.6 compared with 0.1 and 0.0 in the controls). Mean postimplantation loss was increased in the group at 60 mg/kg bw per day compared with the controls (mean, 2.4 compared with 0.9 in the controls), which exceeded the incidence among historical controls (Buckshire: mean, 0.8; range, 0.7–0.9).

An increased number of fetuses with external malformations were seen in the group at 60 mg/kg bw per day compared with the control groups (Table 33). Four fetuses from one litter had open eyelid and three fetuses from another litter had shortened tails. The majority of the external malformations in the group at 30 mg/kg bw per day were observed in a fetus with multiple anomalies (spina bifida, exencephaly, cyclopia and umbilical hernia). The finding of cyclopia and related head malformations (exencephaly) in single fetuses in the groups at 10 and 30 mg/kg bw per day was not observed in the group at 60 mg/kg bw per day and was possibly of genetic origin as both fetuses were sired by the same male. Skeletal examination revealed there was a slight increase in the number of fetuses with vertebral anomalies (with associated rib anomaly) when compared with the control groups and data for historical controls. All visceral anomalies in the group at the highest dose occurred in single instances. In the group at 30 mg/kg bw per day, the percentage of visceral malformations was slightly increased in comparison with that in both control groups. In this group there were three fetuses with diaphragmatic hernia (in two litters, not observed in the group at the highest dose), two fetuses with hydrocephaly (incidence among historical controls, Buckshire: range for fetuses, 0.0–1.2; range for litters, 0.0–7.7) and one fetus with a kidney with an associated ureter anomaly. There was a statistically significant increase at 60 mg/kg bw per day in the incidence of 13th rudimentary ribs; however, the percentage of fetuses and litters affected was similar in frequency to the range for historical controls.

Table 33. Incidence of malformations of fetuses in a study of developmental toxicity in rabbits given cyromazine by gavage

Finding	No. of fetuses affected (No. of litters affected)					
	Dose (mg/kg per day)					
	0[a]		5	10	30	60
	0.5% CMC		Not treated			
Total No. examined	58 (12)	53 (13)	62 (12)	45° (7)	71 (10)	54 (11)
No. with external malformations	0 (0)	0 (0)	1 (1)	1 (1)	2 (2)	10 (5*)
Cyclopia with multiple head anomalies	0 (0)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)
No. with soft tissue malformations	1 (1)	0 (0)	1 (1)	2 (2)	6 (4)	3 (3)
No. with skeletal malformations	6 (6)	2 (2)	4 (2)	2 (1)	5 (5)	11 (6)
Vertebral anomaly with or without associated rib anomaly	4 (4)	1 (1)	3 (1)	1 (1)	3 (3)	7 (4)
Total number with malformations	7 (6)	2 (2)	5 (3)	3 (2)	10 (6)	15 (6)
Variations						
Thirteenth rudimentary ribs	2 (2)	4 (3)	5 (3)	3 (2)	7 (4)	10 (7*)

From Nemec (1985)

CMC, carboxymethyl cellulose

[a] Historical incidence of thirteenth rudimentary rib(s): 17/258 fetuses (range: 0.0–14.1%), 12/42 litters (range: 0.0–57.1%); and of vertebral anomalies: 8/258 fetuses (range: 0.0–4.7%), 7/42 litters (0.0–28.6%) (Buckshire)

[*] Statistically significant difference from control group mean, $p < 0.05$ (Fisher exact test); decreased number due to a decreased pregnancy rate attributed to random occurrence.

In New Zealand White rabbits, cyromazine produced severe maternal toxicity when administered orally at a dose of at 60 mg/kg bw per day during gestation, as shown by clinical signs of toxicity, one abortion, severe body-weight loss and decreased food consumption. Slight maternal toxicity (decreased body-weight gain and feed consumption) was also observed at 30 mg/kg bw per day. As a result of the severe maternal toxicity, postimplantation losses and resorptions increased at 60 mg/kg bw per day. At this dose, the number of fetuses (litters) with external malformations (not considered to be treatment-related), and the number of fetuses with vertebral anomalies were also increased. The NOAEL for maternal toxicity was 30 mg/kg bw per day considering that body-weight loss was marginal at that dose. The NOAEL for developmental toxicity was also 30 mg/kg bw per day (Nemec, 1985).

A study was undertaken in order to investigate the suspected deficiency in the male that sired two fetuses with a rare malformation, cyclopia, in two groups in the previous study. A total of four male New Zealand White (BUK:(CRL)NZWfBR) rabbits were used for artificial insemination, and sperm morphology and semen quality were evaluated. The male that had been used in the previous study of teratology was included to investigate whether this male would again produce fetuses showing cyclopia and/or related head effects. Three groups of at least 56 female rabbits were inseminated. Females in the 'sham' control group were 'sham' gavaged once daily from days 7 to 19 of gestation (a stainless-steel dosing cannula was used, but no material was administered). The females in the other two groups were not dosed. Clinical observations, body weights and food consumption were recorded throughout the study. On day 29 of gestation, all dams were killed and uterine contents examined. All fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies and developmental variations. The study was conducted according to the principles and practices of GLP (with QA certificate).

Sixteen females died during the experimental period; 9, 5 and 2 from control group 1 (group size, 56), control group 2 (group size, 59) and the 'sham' control group (group size, 56), respectively. The cause of death for most of these animals was attributed to a suspected bacterial infection. There were no differences in clinical observations between the groups. A total of 43, 48 and 45 gravid females were available for examination at study termination from the control group 1, control group 2 and 'sham' control groups, respectively. There were no apparent differences between the groups in maternal body-weight gain or food consumption. There were no group differences in reproductive parameters.

Although cyclopia per se was not observed in this study, a number of rare and severe defects of the head and related structures occurred (Table 34). These included hydrocephaly, acrania, cleft palate (all observed in different fetuses) and one fetus with multiple head anomalies from male No. 2749. Defects of the head (hydrocephaly, acephaly and macroglossia) also occurred in fetuses sired by other males. In addition, other malformations observed in this study had been seen in the previous study of teratology. These included spina bifida, fused or severely malaligned sternebrae, hydrocephaly, short tail, heart and/or great vessel anomalies, vertebral defects, rib anomalies, mid-line closure defects, kidney anomalies and diaphragmatic hernia.

Table 34. Incidence of malformations in control groups of (BUK:(CRL)NZWfBR) New Zealand White rabbits

Type of malformation	No. of fetuses affected (No. of litters affected)[a]		
	Control group 1	Control group 2	Sham control group
Total No. examined	182 (33)	233 (39)	186 (37)
External malformations	1 (1)	6 (6)	7 (5)
Conjoined twins	0 (0)	1 (1)	0 (0)
Acephaly	0 (0)	1 (1)	0 (0)

Spina bifida	1 (1)	1 (1)	2 (2)
Short tail	0 (0)	1 (1)	1 (1)
Brachydactyly	0 (0)	0 (0)	1 (1)
Carpal and/or tarsal flexure	0 (0)	1 (1)	3 (3)
Gastroschisis	0 (0)	0 (0)	1 (1)
Acrania	0 (0)	0 (0)	1 (1)
Head anomaly*	0 (0)	0 (0)	1 (1)
Thoraco-gastroschisis	0 (0)	0 (0)	1 (1)
Cleft palate	0 (0)	0 (0)	1 (1)
Macroglossia	0 (0)	1 (1)	0 (0)
Soft tissue malformations	4 (4)	3 (3)	3 (3)
Retroesophageal aortic arch	0 (0)	1 (1)	0 (0)
Malpositioned kidneys	0 (0)	0 (0)	1 (1)
Heart and/or great vessel anomaly	1 (1)	0 (0)	0 (0)
Hydrocephaly	2 (2)	1 (1)	1 (1)
Diaphragmatic hernia	0 (0)	1 (1)	0 (0)
Interventricular septa defect	0 (0)	0 (0)	1 (1)
Kidney and/or ureter absent	0 (0)	1 (1)	0 (0)
Iris bombe	1 (1)	0 (0)	0 (0)
Skeletal malformations	10 (9)	10 (10)	11 (9)
Extra site of ossification anterior to sternebra No. 1	0 (0)	3 (3)	1 (1)
Sternebrae fused	2 (2)	2 (2)	5 (5)
Vertebral centra anomaly	2 (2)	2 (2)	1 (1)
Vertebral anomaly with or without associated rib anomaly	3 (2)	3 (3)	4 (3)
Rib anomaly	2 (2)	0 (0)	0 (0)
Sternebra(e) mal-aligned (severe)	2 (2)	1 (1)	1 (1)
Skull anomaly	0 (0)	1 (1)	0 (0)
Bent limb bone(s)	0 (0)	1 (1)	0 (0)
Total No. with malformations	15 (13)	15 (15)	16 (12)
Variations			
Thurteenth rudimentary ribs	30 (17)	27 (15)	21 (16)

[a] The number of litters affected is given in parentheses.

From Nemec (1986b)

In conclusion, the incidence of spontaneous fetal malformations in the BUK:(CRL)NZWfBR closed colony was found to be higher (6.4–8.6% for fetuses and 32.4–39.3% for litters) than that of the two other colonies of New Zealand White rabbits that had been used in the laboratory (Hazleton Research animals: 3.9% for fetuses and 21.5% for litters; Langshaw farms: 2.8% for fetuses and 15.6% for litters). The male from the previous study sired a number of fetuses with malformations in this study, including some with severe head anomalies. These results indicate that the malformations observed in the treated groups of the previous cyromazine study (Nemec, 1985) were consistent with those seen in this colony of rabbits (BUK:(CRL)NZWfBR). The effects of mild stress by sham gavage dosing on the incidence of malformations in this study were equivocal. Although an increase in the number of visceral malformations was associated with females that were sham gavaged, there were no remarkable overall differences observed between test groups with reference to the number of fetuses

and litters with malformations. The types of developmental variations were similar to those observed in the historical controls for the laboratory and in the study of Nemec, 1985 (Nemec, 1986b).

In a fourth study, groups of 74 artificially inseminated New Zealand White (Hra:(NZW)SPF) rabbits were given cyromazine (purity, 95.2%) at a dose of 0 (vehicle control, 0.5% aqueous CMC), 5, 10 or 30 mg/kg bw per day by gavage on days 7–19 (inclusive) of gestation. Throughout gestation, all females were observed twice per day for toxicity. Individual maternal body weights were recorded on days 0, 7, 10, 14, 20, 24 and 29 of gestation and food consumption was recorded daily from days 0 to 29 of gestation. On day 29 of gestation, 25 females with viable fetuses from each group were killed for teratological investigation. The uteri were examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination. The remaining females were allowed to litter naturally. On day 4 of lactation, six young per litter were randomly selected and retained for an assessment of postpartum survival and growth up to lactation day 28. Remaining young were killed after weighing on day 4. On day 28 of lactation, offspring were killed, sexed and necropsied. A gross postmortem examination was performed on all surviving F_0 maternal females. The study was conducted according to the principles and practices of GLP (with QA certificate) and complied with OECD TG 414 (1981).

Analyses of the dosing preparations confirmed that the achieved concentration, homogeneity and stability of the test substance in vehicle were satisfactory.

There was no evidence of any effect of cyromazine in the animals at 5 or 10 mg/kg bw per day, on clinical findings, survival, body-weight gain during gestation or lactation, food consumption or maternal performance. At 30 mg/kg bw per day, there was a marked body-weight loss in dams from days 7–20 of gestation (−115 g). After the dosing period, body-weight gain was statistically increased compared with that in the control group (+206 g and +70 g, respectively). Body-weight gain during lactation in these females was generally comparable to that of controls. Food consumption in the group at 30 mg/kg bw per day was affected in a corresponding manner (days 10–14, 67% and days 14–20, 65% of values for controls).

The mean numbers of viable and nonviable fetuses, early and late resorption sites, postimplantation loss, implantation sites, corpora lutea and mean fetal weights were similar in treated and control groups.

There were no biologically meaningful or statistically significant differences in the number or percentage of fetuses with malformations or developmental variations in any of the cyromazine-treated groups compared with controls. Postnatally, there were no treatment-related alterations in duration of gestation, parturition, live birth and survival indices, sex ratio, live litter size, offspring body weight or condition throughout lactation.

In conclusion, cyromazine produced maternal toxicity (body-weight loss and decreased food consumption) at 30 mg/kg bw per day when administered orally to New Zealand White rabbits during the period of major organogenesis. The NOAEL for maternal toxicity was 10 mg/kg bw per day. Cyromazine did not induce teratogenic or fetotoxic effects at doses up to 30 mg/kg bw per day, the highest dose tested in this study (Nemec, 1986a).

2.6 Special studies: neurotoxicity

Cyromazine belongs to the chemical class of s-triazines; it acts as an insect growth regulator and is not suspected to act upon the nervous system. Except for some unspecific symptoms at doses at or above the LD_{50} observed in studies of acute toxicity , the acute, short- and long-term studies reported previously revealed neither clinical signs nor any biochemical or histopathological change that might point to a neurotoxic potential of cyromazine. The conduct of special studies in the field of neurotoxicity was therefore not necessary.

3. Studies on metabolites

There were no metabolites found to a significant amount in plants and/or soil that were not seen in animals. In soil, considering the degradation and mineralization, it was concluded that the risk of cyromazine or one of its metabolites being translocated into non-target areas, including groundwater, was low. The predicted environmental concentration (PEC) at 1 m soil depth, after use of products that contain cyromazine under realistic worst-case scenarios was less than 0.00 µg/l. If melamine occurs as a metabolite in groundwater, it is also predicted not to exceed 0.001 µg/l for most European soil and climate scenarios under common conditions of agricultural use.

Melamine is a metabolite in rats (about 7% in urine and faeces), monkeys, goats and hens. It is an important chemical commodity used for the production of melamine–formaldehyde resins. The toxicity of melamine has been extensively investigated and reported in the literature. Reviews had been released by ECETOC (1983) and IARC (1986 & 1999). Melamine has no insecticidal activity in comparison with the parent cyromazine (Rindlisbacher, 2002). In rats and humans, melamine is a metabolite of the anti-neoplastic agent hexamethylmelamine (Worzalla et al., 1974). The carcinogenic potency of melamine had been investigated in the National Toxicology Program (1983). The results are discussed below.

3.1 Absorption, distribution, excretion and metabolism

After administration of melamine as a single oral dose at 250 mg/kg bw in rats or dogs, 50% and 61.3%, respectively, of unmodified melamine was excreted in the urine in 6 h (Lipschitz & Stokey, 1945). Nearly 20% of the melamine excreted by the rats was recovered as the crystalline dimelamine-monophosphate.

In adult male Fischer 344/N rats given [^{14}C]melamine as a single oral dose at 0.38 mg, melamine was rapidly excreted (90% of the administered dose within 24 h in the urine). Negligible radioactivity was detected in exhaled air and faeces. Melamine was distributed in the body water. Kidney and bladder showed higher concentrations of radioactivity than did plasma. Virtually no residual radioactivity was observed in the tissues at 24 h after dosing or later. The plasma half-life was 2.7 h, which is in good agreement with the urinary excretion half-life of 3.0 h. Radioactivity in plasma and urine co-chromatographed with unchanged melamine, indicating that melamine is not metabolized in the rat (Mast et al., 1983).

3.2 Toxicological studies

(a) Acute toxicity

Melamine has very low acute toxicity. The oral LD_{50} values for melamine given in corn oil by gavage were reported to be 3161 and 3828 mg/kg bw in male and female Fischer 344/N rats, and 3296 and 7014 mg/kg bw for male and female B6C3F_1 mice (National Toxicology Program, 1983). White crystals were found in the stomach of three out of five male and four out of five female rats at 10 000 mg/kg bw, four out of five males and five out of five females at 6810 mg/kg bw, one out of five males and two out of five females at 3160 mg/kg bw and one out of five males at 2150 mg/kg bw. In mice, no compound-related toxic effects were observed at necropsy. In another study in mice, the oral LD_{50} was 4500 mg/kg bw. Reported signs of toxicity were lachrimation, dyspnoea, intermittent tremors and coma preceding death. Vasodilatation in the tail and ears and paralysis of the forequarters were also observed (ECETOC, 1983). Melamine applied as a paste to the skin of rabbits at 1000 mg/kg bw did not induce toxicity or local irritation (ECETOC, 1983). Introduction

of melamine powder into the rabbit eye caused mild transient irritation (ECETOC, 1983). There was no sensitization in guinea-pigs (ECETOC, 1983).

(b) Short-term studies of toxicity

Mice

Groups of five male and five female B6C3F$_1$ mice were fed diets containing melamine (purity, 97%) at a concentration of 5000, 10 000, 15 000, 20 000, or 30 000 ppm for 14 days. All animals survived to the end of the study. Treatment seemed to have no effect on body weight. A hard crystalline solid was found in the urinary bladder of all male mice and in two out of five female mice at 30 000 ppm. No other compound-related effects were observed at necropsy (National Toxicology Program, 1983).

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing melamine at a concentration of 0, 6000, 9000, 12 000, 15 000, or 18 000 ppm (equivalent to 0, 900, 1350, 1800, 2250 and 2700 mg/kg bw per day) for 13 weeks. One female at 9000 ppm died. Body-weight gain was depressed in all treated groups. The incidence of mice with bladder calculi was dose-related and was greater in males than in females. Bladder calculi were found at dietary concentrations of 12 000 ppm and greater (6 out of 10, 9 out of 10 and 7 out of 10 in males, and 1 out of 10, 3 out of 10 and 7 out of 10 in females, at 12 000, 15 000 and 18 000 ppm, respectively). Ulceration of the urinary bladder epithelium was also dose-related and was observed at dietary concentrations of 9000 ppm and greater. Bladder ulcers were multifocal or associated with inflammation (cystitis). The results were considered not to provide evidence for an association between ulceration and bladder calculi in either sex. Since the body weight effects at 6000 ppm (equivalent to 900 mg/kg bw per day) were of questionable biological significance, this dose could be considered as a NOAEL (National Toxicology Program, 1983).

Rats

In rats treated with melamine, no histological effects were seen, except for crystalline deposits in the renal tubules found after five successive intraperitoneal doses at 500 mg/kg bw per day. No symptoms were observed except for moderate transient weight loss (ECETOC, 1983).

Groups of five male and five female Fischer 344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 5000, 10 000, 15 000, 20 000, or 30 000 ppm for 14 days. Body-weight gain was reduced at dietary concentrations of 15 000 ppm and greater. A hard crystalline solid was found in the urinary bladder of most males at ≥ 10 000 ppm and in four out of five females at ≥ 20 000 ppm. The kidneys of two out of five males at 30 000 ppm were pale and pitted. The details given of the study were limited, but the highest NOAEL appeared to be 5000 ppm (National Toxicology Program, 1983).

Three 13-week studies were performed in rats.

In the first study, groups of 12 male and 12 female F344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 0, 6000, 9000, 12 000, 15 000, or 18 000 ppm, equivalent to 0, 600, 900, 1200, 1500 and 1800 mg/kg bw per day. One male at 18 000 ppm and two males at 6000 ppm died. Body-weight gain at a dietary concentration of 12 000 ppm and greater was depressed and food consumption was reduced at 18 000 ppm. Calculi were found to occur in the urinary bladder

of most male rats in a dose-related manner and in the bladder of some females at ≥ 15 000 ppm. Histopathological evaluation was performed on 10 animals of each sex from the control group and groups at, 6000 and 18 000 ppm. Diffuse epithelial hyperplasia of the urinary bladder was found in 8 out of 10 males and 2 out of 10 females at 18 000 ppm, while in rats at 6000 ppm, focal epithelial hyperplasia was only found in 1 out of 10 males and in none of the females. No other compound-related histopathological effects were observed (National Toxicology Program, 1983).

In the second 13-week study, groups of male and female F344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 0, 750, 1500, 3000, 6000, or 12 000 ppm, equivalent to 0, 75, 150, 300, 600 and 1200 mg/kg bw per day. Body-weight gain was depressed in males only at 6000 and 12 000 ppm. Urinary bladder calculi were not observed in treated or control females, but the incidence among male rats increased in a dose-related manner (1 out of 10, 2 out of 10, 5 out of 10, 7 out of 10, 9 out of 10 and 9 out of 9 in the controls and at 750, 1500, 3000, 6000 and 12 000 ppm, respectively). Hyperplasia of the transitional epithelium of the bladder was observed to occur in a dose-dependent manner for male rats at ≥ 3000 ppm (1 out of 10, 3 out of 10 and 9 out of 9in the groups at 3000, 6000 and 12 000 ppm, respectively). There was no evidence of hyperplasia of the bladder epithelium in female rats. Dose-related calcareous deposits were observed in the straight segments of the proximal tubules in females. Microscopic evaluation of the urine did not provide evidence of melamine crystalluria. The NOAEL for hyperplasia was 1500 ppm, equivalent to 150 mg/kg bw per day (National Toxicology Program, 1983).

In the third 13-week study, F344/N rats were fed diets containing melamine (purity, 97%) at a concentration of 0, 10 000 or 18 000 ppm in the presence and absence of 1% ammonium chloride in the drinking-water to see if this treatment might affect the incidence of calculus formation in the urinary tract. Ammonium chloride had no effect on calculus formation (National Toxicology Program, 1983).

Rabbits and dogs

In rabbits and dogs fed with melamine at a dose of 126 mg/kg bw per day for 1 to 4 weeks, no effects were found, either macro- or microscopically (ECETOC, 1983).

(c) Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female B6C3F_1 mice were fed diets containing melamine (purity, 97%) at a concentration of 0, 2250 or 4500 ppm (equivalent to 0, 338 or 675 mg/kg bw per day) for 103 weeks, followed by a basal diet for 2 weeks before sacrifice. All animals were observed twice daily for morbidity or mortality. Clinical signs were recorded monthly. Body weight and feed consumption by cage (five animals per cage) were recorded once per week for the first 13 weeks, monthly until week 91 and then every 2 weeks. Necropsies were performed on all animals found dead and those killed at the end of the study. Animals were examined for gross and histopathological abnormalities.

Mean body weights of male mice at the highest dose were slightly lower than those of controls after week 50 of the study (decrease in body-weight gain, 21%). Survival at termination of the study was: 39 out of 49 (80%), 36 out of 50 (72%) and 28 out of 50 (56%) in males in the control group and at the lower and higher dose, respectively and 37 out of 50 (74%), 43 out of 50 (86%) and 41 out of 50 (82%) in females in the control group and at the lower and higher dose, respectively. The

reduction in the survival of males at the highest dose was statistically significant ($p = 0.013$). No treatment-related increase in the incidence of tumours was observed. In male mice, treatment-related increases were observed in the incidence of urinary bladder calculi (2 out of 45, 40 out of 47 and 41 out of 44 in the control group and at the lower and higher dose, respectively), in the incidence of acute and chronic inflammation of the urinary bladder (0 out of 45, 25 out of 47 and 24 out of 44 in mice in the control group and at the lower and higher dose, respectively) and in the incidence of epithelial hyperplasia of the bladder (1 out of 45, 11 out of 47 and 13 out of 44 in mice in the control group and at the lower and higher dose, resectively). Urinary bladder calculi, acute and chronic inflammation of the urinary bladder and epithelial hyperplasia were seen in 4 out of 50 females at the highest dose (National Toxicology Program, 1983).

Rats

Groups of 50 male and 50 female Fischer 344/N rats were fed diets containing melamine at a concentration of 0, 2250 or 4500 ppm (males) or 0, 4500 or 9000 ppm (females) for 103 weeks, followed by a basal diet for 2 weeks before sacrifice. Doses were equivalent to 0, 113 or 225 in males and 0, 225 or 450 mg/kg bw per day in females. All animals were observed twice daily for morbidity or mortality. Clinical signs were recorded monthly. Body weight and feed consumption by cage (five animals per cage) were recorded once per week for the first 13 weeks, monthly until week 91 and then every 2 weeks. Necropsies were performed on all animals found dead and those killed at the end of the study. Animals were examined for gross and histopathological abnormalities.

Survival was significantly reduced in males at the highest dose ($p = 0.03$) from week 101. Survival rates at termination of the study were: 30 out of 49 (61%), 30 out of 50 (60%) and 19 out of 50 (38%) in males in the control group and at the lowest and highest dose, respectively and 34 out of 50 (68%), 30 out of 50 (60%) and 27 out of 50 (54%) in females in the control group and at the lower and higher dose, respectively. The incidence of transitional-cell carcinomas of the urinary bladder in males was 0 out of 45, 0 out of 50 and 8 out of 49 (trend $p \leq 0.002$) in the control group and the lower and higher dose, respectively. The incidence in the group at the highest dose was significantly higher ($p \leq 0.016$) than in the controls. There was also a dose-related incidence of bladder calculi in males (0 out of 45, 1 out of 50, and 10 out of 49 in the control group and at the lower and higher dose, respectively). In a separate study, X-ray microscopic analysis of two urinary bladder calculi obtained from male F344/N rats fed diets containing melamine at 16 000 or 19 000 ppm indicated that the principal component of the calculi was melamine. Of 49 males at the highest dose, seven had transitional-cell carcinomas and bladder calculi, one had a carcinoma without calculi and three had calculi without carcinoma (one of these rats had a papilloma and one had epithelial hyperplasia). There was therefore a statistically significant ($p < 0.001$) correlation between the presence of bladder calculi and bladder tumours. Females had no bladder calculi and one female in each of the groups at the lowest and highest dose had a papilloma of the bladder. A statistically significant increase ($p \leq 0.01$) in the incidence of chronic inflammation of the kidney was observed in females (4 out of 49, 17 out of 49 and 41 out of 47 in the control group and at the lower and higher dose, respectively). The dose–response relationship and intensity of the increased interstitial lymphoplasmocytic infiltrates and cortical fibrosis clearly set these changes apart from the minor inflammatory component that may accompany the progressive nephropathy normally encountered in ageing F344/N rats. Chronic inflammation of the kidney was not significant in male rats receiving cyromazine (National Toxicology Program, 1983).

Groups of 20 male F344 rats were fed diets containing melamine (purity, > 99%) at a concentration of 0.3%, 1% or 3% (equivalent to 150, 500 and 1500 mg/kg bw per day) for a total of 36 weeks followed by a 4-week recovery period. Ten animals per group underwent exploratory laparotomy at the end of week 36 and all animals were killed at week 40. Bladder weight in rats

receiving 3% melamine in the diet was threefold that in controls. The incidences of papillary or nodular hyperplasia were 0%, 5%, 30% and 63% in the control group and at the lowest, intermediate and highest dose, respectively; incidences of papillomatosis were 0%, 0%, 25% and 89% and those of calculi were 0%, 0%, 70% and 100% at 36 weeks and 0%, 20%, 45% and 42% at 40 weeks. Carcinomas of the urinary bladder were observed in 0 out of 20, 1 out of 20 and 15 out of 19 rats at the lowest, intermediate and highest doses and papillomas in 0 out of 20, 1 out of 20 and 12 out of 19 rats, respectively. One carcinoma and three papillomas of the ureter were also induced in 19 rats at the highest dose. The correlation between calculus formation at week 36 and tumour incidence at week 40 was highly significant ($p = 0.0065$) (Okumura et al., 1992; IARC, 1999).

Groups of 20 male F344/DuCrj rats were fed diets containing melamine (purity, 99.94%) at a concentration of 1% or 3% with or without 5% or 10% aqueous sodium chloride solution (NaCl) for a total of 36 weeks and were killed at week 40. Water intake, as a surrogate for urinary output, was increased in groups exposed to 3% melamine, with or without 5% or 10% NaCl, in groups exposed to 1% melamine with 5% or 10% NaCl and in a group exposed to 10% NaCl only; water intake was not increased in animals exposed to 1% melamine only. The incidences of calculi and papillomatosis were 30% and 75% with 3% melamine, 75% and 85% with 3% melamine plus 5% NaCl, 30% and 10% with 3% melamine plus 10% NaCl, 37% and 47% with 1% melamine, 11% and 11% with 1% melamine plus 5% NaCl, 5% and 0% with 1% melamine plus 10% NaCl. No calculi or papillomatosis were reported in controls or with 10% NaCl alone.

Urinary bladder carcinomas were observed in 4 out of 19, 18 out of 20 and 18 out of 20 rats given 1% melamine alone, 3% melamine alone or 3% melamine plus 5% NaCl, respectively. No carcinomas were observed in the groups receiving 3% melamine plus 10% NaCl or 1% melamine plus 5% or 10% NaCl. The incidences of papillomas were similarly decreased by NaCl. In contrast to the incidence of 10 out of 20 in the group given 3% melamine alone, 5 out of 20 and 3 out of 20 rats receiving 3% melamine plus 5% NaCl or 10% NaCl respectively, developed papillomas. Papillomas developed in 8 out of 19 rats receiving 1% melamine alone.

Therefore, the addition of NaCl to 1% melamine decreased the incidences of calculi and papillomatosis, in parallel with a decrease in the incidence of neoplasia. With 3% melamine, NaCl did not affect the induction of calculi or papillomatosis but decreased the incidence of neoplasia. Thus, with the lower concentration of melamine, NaCl appeared to increase urinary output and decrease the incidences of hyperplasia, calculus formation and neoplasia. Chemical analysis of the calculi showed that they contained approximately equal amounts of melamine and uric acid on a molar basis, which together accounted for 61–81% of the weight (Ogasawara et al., 1995; IARC, 1999).

On the basis of these studies, it has been concluded that bladder tumours are associated with administration of high doses of melamine, and that the tumours are related to precipitation of urinary melamine with the formation of melamine/uric acid containing urinary-tract calculi, producing urothelial toxicity and consequent regeneration of the bladder epithelium and ultimately the formation of tumours (IARC, 1999; Meek et al., 2003). Although the correlation between calculi, ulceration, hyperplasia and formation of bladder tumours has not been 100%, explanations on the basis of studies with other chemicals such as uracil have been advanced to explain the discrepancies (Clayson et al., 1995, Fukushima et al., 1992). Ulcerations secondary to calculi formation occur relatively rapidly and are repaired, even with continued presence of the calculus. It is thus not unusual to see extensive proliferation of the bladder epithelium in the presence of calculi at later time-points, such as those seen in the experiments with melamine, without an associated ulceration or intense inflammatory response. Chronic inflammation is frequently present, however. Similarly, correlation between the presence of calculi and tumours at later time-points is not 100%. This has been explained by the loss of calculi during the experiment, either by dissolution or, more likely, spontaneous evacuation from the urinary tract (Clayson et al., 1995; IARC, 1999b).

There is significantly less risk in humans for developing bladder cancer from calculi than in rodents, most likely owing primarily to the usually short time that calculi are present in humans due to anatomic and obstructive issues (IARC, 1999b).

(d) Genotoxicity

The genotoxic potential of melamine was assessed in several tests in vitro and in vivo (Table 35). With the exception of prophage induction, all the tests gave negative results. is the Meeting considered that melamine is not genotoxic.

Table 35. Results of studies of genotoxicity with melamine, a metabolite of cyromazine

End-point	Test object	Concentration	Results	Reference
Prophage induction	*E. coli* WP2s (λ)	78 µg/well	Positive[a]	Rossman et al. (1991)
Reverse mutation	*S. typhimurium his* G46, TA1530, TA1531, TA1532 and TA1534	Not stated	Negative[c]	Seiler (1973)
	S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100 *E. coli* WP2 *uvrA*	≤ 500 µg/plate	Negative[a]	Jagannath & Brusick (1977)
	S. typhimurium TA1535, TA1538, TA98 and TA100	Not stated	Negative	Lusby et al. (1979)
	S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100	≤ 5000 µg/plate, plate incorporation assay	Negative[a]	Mast et al. (1982a)
	S. typhimurium TA1535, TA1537, TA98 and TA100	≤ 5550 µg/plate, pre-incubation assay	Negative[a,b]	Haworth et al. (1983) National Toxicology Program (1983)
Gene conversion	*S. cerevisiae* D4	Not stated	Negative[a]	Jagannath & Brusick (1977)
Forward mutation	Chinese hamster ovary (CHO), *Hprt* locus	0.6–1 mg/ml	Negative[a]	Mast et al. (1982a) National Toxicology Program (1983)
Gene mutation	Mouse lymphoma L5178Y, *Tk* locus	≤ 160 µg/ml	Negative[a]	McGregor et al. (1988)
Chromosomal aberration	CHO	Not stated	Negative	National Toxicology Program (1983)
Sister chromatid exchange	CHO	≤ 1 mg/ml	Negative[a]	Mast et al. (1982a)
Unscheduled DNA synthesis	Primary rat hepatocytes	≤ 6 mg/ml	Negative	Mirsalis et al. (1983); National Toxicology Program (1983); ECETOC (1983)
Sex-linked recessive lethal mutations	*Drosophila melanogaster*	1% feed	Negative	Röhrborn (1962)
Micronucleus test (BM cells)	Mice	Single oral dose at 1000 mg/kg bw or 2 × 1000 mg/kg bw (24 h apart)	Negative	Mast et al. (1982b) National Toxicology Program (1983)

S9, 9000 × *g* supernatant from livers of Aroclor-induced rats or hamsters.

[a] With and without metabolic activation.

[b] S9 from the liver of Aroclor-induced rats or hamsters, liquid pre-incubation assay.

[c] Without S9.

(e) Reproductive toxicity

No toxic effect or gross malformation was found in fetuses of pregnant rats injected intraperitoneally with melamine at a dose of 70 mg/kg bw on days 5 and 6, 8 and 9 or 12 and 13 of gestation (Thiersch, 1957). This study was considered to be inadequate for an evaluation of prenatal toxicity owing to incomplete reporting of experimental methods and results of fetal examinations (IARC, 1999).

(f) Special studies

A study of skin initiation/promotion to investigate the initiation activity of melamine was performed in CD-1 mice. At a single topical dose of 1 μmol (approx. 6 mg/kg bw) melamine (unknown purity) in 0.2 ml acetone followed by twice-weekly applications of 10 nmol of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 ml acetone for 31 weeks, there was no increase in the incidence of papillomas in comparison to controls (acetone + TPA) (19% compared with 14% in controls) (Perrella & Boutwell, 1983; IARC, 1986).

4. Observations in humans

Routine medical examinations, including anamnesis, physical examination and comprehensive analysis of blood and urine from employees who had handled cyromazine in different manufacturing or formulating plants had revealed no adverse effects on health and no complaints had been reported (SYNGENTA, medical data from manufacturing plants, Monthey, Switzerland, and Grimsby, UK, 1994).

No cases of poisoning of workers involved in the production and formulation or field use of cyromazine had been reported to the company or in the open literature. No epidemiological study was available.

Comments

Biochemical aspects

Toxicokinetic studies in rats given ^{14}C-labelled cyromazine as single or repeated oral doses showed that the active substance is rapidly and almost completely absorbed from the gastrointestinal tract and distributed to all organs and tissues. The substance was rapidly excreted, with an initial rapid phase of 2–12 h followed by a slower phase. More than 97% of the administered dose was excreted within 24 h, almost exclusively in the urine.

Cyromazine was incompletely metabolized, essentially by methylation, hydroxylation or *N*-dealkylation. The major component present was cyromazine, which accounted for 71–72% of the radiolabel; a further 7% was attributable to melamine, 8–11% to hydroxy-cyromazine and methyl-cyromazine. Only 6% of [U-^{14}C triazine]metabolites in the urine and 13% in the faeces remained uncharacterized and comprised minor metabolites.

In monkeys (*Macaca fasicicula*), ^{14}C-labelled cyromazine was also rapidly and extensively absorbed and rapidly excreted, predominantly in the urine. Cyromazine accounted for approximately 95% of urinary radioactivity, with the remainder being attributable to melamine.

Toxicological data

Cyromazine has low acute toxicity in rats when administered orally (LD_{50} = 3387 mg/kg bw), dermally (LD_{50} > 3170 mg/kg bw) or by inhalation (4-h LC_{50} > 3.6 mg/l, the highest achievable concentration). Signs of intoxication were sedation, dyspnoea, curved position and ruffled fur after oral or dermal administration. Animals recovered from systemic symptoms within 9–12 days. After inhalation, a decrease in activity, piloerection and nasal discharge were observed; these clinical signs were no longer seen on day 2 after exposure. Cyromazine is not an irritant to the skin and eyes of rabbits. In a maximization test in guinea-pigs, cyromazine did not show any sensitizing potential.

The toxicity of cyromazine administered orally was investigated in short-term dietary studies: 90-day studies in rats and dogs and a 1-year study in dogs. The main effects were changes in body weight in rats and dogs, and haematological changes in dogs.

The NOAEL in a 90-day study in rats was 3000 ppm, equal to 232 mg/kg bw per day, the highest dose tested. Small changes in body weight were not considered to be toxicologically relevant.

In a 90-day study in dogs, cyromazine induced some reduction in body-weight gain in both sexes at 3000 ppm and in females at 1000 ppm. Food consumption was decreased at 3000 ppm. Decreased erythrocyte values (total erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) were observed in males and females at 3000 ppm and 300 ppm, respectively. The NOAEL was 300 ppm (equal to 12 mg/kg bw per day).

In a 1-year dietary study in dogs, the NOAEL was 200 ppm, equal to 5.7 mg/kg bw per day, on the basis of haematological effects observed at 800 ppm in males.

A NOAEC of 0.058 mg/l, equivalent to 9.3 mg/kg bw per day, was identified on the basis of clinical signs in a 28-day (4 h per day) study in rats treated by inhalation. Haematology was reversibly affected in males at 0.706 mg/l. In rabbits, dermal exposure to cyromazine at doses of up to 2000 mg/kg bw per day for 21 days (6 h per day) produced no systemic adverse effects and no observable skin irritation.

Long-term dietary studies of toxicity and carcinogenicity were carried out in mice and rats. Body-weight changes were the critical effects observed in these studies.

The NOAEL in a 2-year study in mice given diets containing cyromazine was 1000 ppm (equal to 126 mg/kg bw per day) on the basis of changes in body weight in males at 3000 ppm. Small and occasional decreases in body weight and food consumption observed at 1000 ppm were not considered toxicologically relevant.

In rats, dietary administration of cyromazine for 2 years resulted in a decrease in mean body weight, body-weight gain and food consumption in males and females at 3000 ppm. The NOAEL for these effects was 300 ppm, equal to 15 mg/kg bw per day.

Non-statistically significant increases in the incidence of mammary gland tumours were observed in female mice (above the highest value in the range for historical controls) and rats (within the range for historical controls) at 3000 ppm.

Cyromazine gave consistently negative results in a comprehensive range of studies of genotoxicity in vitro and in vivo, with the exception of an inconclusive spot test in mice. The Meeting concluded that cyromazine is unlikely to be genotoxic.

In view of the absence of genotoxicity and the equivocal response at the highest dose in the studies of carcinogenicity in mice and rats, the Meeting concluded that cyromazine is unlikely to pose a carcinogenic risk to humans at exposure levels relevant to residues on food.

The reproductive toxicity of cyromazine was examined in a two-generation study in rats, and in studies of developmental toxicity in rats and rabbits.

Dietary administration of cyromazine to rats for two generations resulted in decreased parental body weights and food consumption at doses of 3000 ppm, and decreased pup body weights at 3000 ppm. Male fertility was reduced in the F_0 generation at 3000 ppm. A decrease in pup viability at birth and in mean litter size was observed in F_2 litters at 3000 ppm. The NOAEL for parental toxicity, reproductive and offspring toxicity was 1000 ppm (equal to 51 mg/kg bw per day).

Cyromazine was not teratogenic to rats when administered at a dose of up to 600 mg/kg bw per day. Signs of maternal toxicity were observed at 300 and 600 mg/kg bw per day and fetal toxicity was observed at the highest dose. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of clinical signs of toxicity and decreased body-weight gain. The NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of a decrease in body weight and reduced ossification at the next highest dose.

In three studies of developmental toxicity in rabbits, the administration of cyromazine by gavage at a dose of 25 mg/kg bw per day or greater resulted in deaths, clinical signs of toxicity (decreased urination and defecation), decrease in body-weight gain, body-weight loss and decrease in food consumption in the dams. Body-weight loss was usually observed within the first few days after dosing and it was not always possible to determine whether this loss was accompanied by a reduction in food intake. The animals rapidly recovered weight after dosing stopped. The overall NOAEL for maternal toxicity was 10 mg/kg bw per day. Cyromazine did not induce teratogenic or fetotoxic effects in rabbits. Increased numbers of abortions, post-implantation losses, resorptions and vertebral variations as well as decreased numbers of viable fetuses were observed only at maternally toxic doses (60 mg/kg bw per day or greater). The overall NOAEL for developmental toxicity was 30 mg/kg bw per day.

No specific studies of neurotoxicity with cyromazine were available; however, no evidence of neurotoxicity was apparent in the available studies of toxicity.

No adverse effects were reported in personnel involved in the production and formulation of cyromazine, or in the use of this product in the field.

The metabolites found in plants, goats, hens and rats are melamine and 1-methylcyromazine; neither contain new functional groups or structural alerts. Melamine has been investigated for its toxicological properties and results were reported in the published literature. The main toxic effects of dietary exposure to melamine in rats and mice were calculi formation (constituted by melamine and uric acid), inflammatory reactions and hyperplasia in the urinary bladder. The NOAEL for urinary bladder calculi formation and hyperplasia was 1500 ppm (equivalent to 150 mg/kg bw per day) in a 90-day study in rats. Induction of carcinomas of the urinary bladder occurred in male rats fed diets containing melamine at 4500 ppm (equivalent to 225 mg/kg bw per day) for 103 weeks, but not in female rats or in male or female mice. Melamine is not genotoxic in vitro or in vivo. Although bladder tumours related to calculi formation are not considered to be species-specific, they are related to the administration of high doses (IARC, 1999b). Bladder tumours were related to precipitation of urinary melamine with the formation of melamine/uric acid-containing urinary-tract calculi, producing urothelial toxicity and consequent regeneration of the bladder epithelium and ultimately formation of tumours. The non-DNA-reactive mechanism by which melamine produced urinary bladder tumours in male rats occurred only under conditions in which calculi were produced. The risk of developing bladder cancer from calculi is significantly lower in humans than in rodents, most probably because of the usually short time that calculi are present in humans, owing to anatomic and obstructive issues. These bladder tumours are thus an effect that occurs at high doses, having a threshold that is well above the expected human exposure through residues.

The Meeting concluded that the existing database was adequate to characterize the potential hazard of cyromazine and its metabolites to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.06 mg/kg bw based on a NOAEL of 5.7 mg/kg bw per day for haematological effects detected at 23 mg/kg bw per day in males in a 1-year study of toxicity in dogs, and using a safety factor of 100.

The Meeting established an ARfD of 0.1 mg/kg bw based on a NOAEL of 10 mg/kg bw per day for body-weight loss and decrease in food consumption observed soon after dosing at 25 mg/kg bw per day or greater in dams in studies of developmental toxicity in rabbits treated by gavage and with a safety factor of 100. The reason for these effects was unknown and there is a rapid recovery on cessation of administration. Therefore, this ARfD may be conservative.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	1000 ppm, equal to 126 mg/kg bw per day	3000 ppm, equal to 384 mg/kg bw per day
		Carcinogenicity	1000 ppm, equal to 164 mg/kg bw per day	3000 ppm, equal to 476 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 15 mg/kg bw per day	3000 ppm, equal to 156 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 156 mg/kg bw per day[c]	—
	Multigeneration reproductive toxicity[a]	Parental	1000 ppm, equal to 51 mg/kg bw per day	3000 ppm, equal to 169 mg/kg bw per day
		Offspring toxicity	1000 ppm, equal to 51 mg/kg bw per day	3000 ppm, equal to 169 mg/kg bw per day
		Reproductive toxicity	1000 ppm, equal to 51 mg/kg bw per day	3000 ppm, equal to 169 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Developmental toxicity	300 mg/kg bw per day	600 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	25 mg/kg bw per day
		Developmental toxicity	30 mg/kg bw per day	60 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	200 ppm, equal to 5.7 mg/kg bw per day	800 ppm, equal to 23 mg/kg bw per day

[a] Dietary administration

[b] Gavage administration

[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.06 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 exposures

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 94–97% based on urinary excretion
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid and extensive (> 97% within 24 h, mainly via urine)
Metabolism in animals	Incomplete metabolism, essentially by methylation, hydroxylation or *N*-dealkylation
Toxicologically significant compounds	Parent compound and melamine
Acute toxicity	
Rat, LD_{50}, oral	3387 mg/kg bw
Rat, LD_{50}, dermal	> 3170 mg/kg bw
Rat, LC_{50}, inhalation	> 3.6 mg/l of air (4-h, nose only, aerosol)
Rabbit, skin irritation	Not irritating (24 h)
Rabbit, eye irritation	Not irritating
Guinea-pig, skin sensitization (test method used)	Not sensitizing (Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Decreased body-weight gain (rats and dogs), haematopoietic system (dogs)
Lowest relevant oral NOAEL	232 mg/kg bw per day (90-day study in rats) 5.7 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	2000 mg/kg bw per day, highest dose tested (3-week study in rabbits)
Lowest relevant inhalation NOAEC	0.058 mg/l air (28-day study in rats)
Genotoxicity	
	Not genotoxic in vitro and in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Decreased body-weight gain (mice and rats)
Lowest relevant NOAEL	15 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Non-statistically significant increase in incidence of mammary gland tumours in female mice (higher than historical control range) and rats at 3000 ppm
Reproductive toxicity	
Reproduction target/critical effect	Reduction in male fertility, decreased pup viability at birth and mean litter size at parentally toxic levels
Lowest relevant reproductive NOAEL	Parents: 51 mg/kg bw per day (rats) Reproductive and offspring toxicity: 51 mg/kg bw per day (rats)
Developmental target/critical effect	Embryotoxicity (rabbits) and fetotoxicity (rats) at maternally toxic doses
Lowest relevant developmental NOAEL	Maternal: 10 mg/kg bw per day (rabbits) Developmental: 30 mg/kg bw per day (rabbits)
Neurotoxicity/delayed neurotoxicity	
	No specific study; no findings in other studies

Other toxicological studies	
Toxicity of metabolites:Melamine	Oral LD_{50} in rat: 3161 mg/kg bw (males)
	Main toxic effects of dietary administration to rats and mice: calculi formation, inflammatory reactions and hyperplasia in the urinary bladder
	LOAEL calculus formation: about 150 mg/kg bw per day in rats (90 days)
	NOAEL hyperplasia: 150 mg/kg bw per day in rats (90 days) Induction of carcinomas of the urinary bladder in male rats at 225 mg/kg bw per day (2 years)
	Not carcinogenic in female rats or in mice of both sexesNot genotoxic in vitro and in vivo
	Non-genotoxic mode of action
Medical data	
	No adverse effects on health in manufacturing personnel

Summary

	Value	Study	Safety factor
ADI	0–0.06 mg/kg bw	Dog, 1-year study of toxicity	100
ARfD	0.1 mg/kg bw	Rabbit, developmental toxicity, (maternal toxicity)	100

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