

PIRIMICARB

First draft prepared by

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Explanation.....	207
Evaluation for acceptable daily intake.....	207
Biochemical aspects	207
Absorption, distribution and excretion	207
Metabolism.....	212
Toxicological studies	218
Acute toxicity	218
Short-term studies of toxicity	220
Long-term studies of toxicity and carcinogenicity.....	237
Genotoxicity	245
Reproductive toxicity	246
Multigeneration studies	246
Developmental toxicity.....	248
Special studies.....	251
Delayed neurotoxicity.....	251
Neurotoxic potential	252
Other toxicological studies.....	254
Observations in humans	261
Comments.....	262
Toxicological evaluation.....	268
References	270
Appendix	275

Explanation

Pirimicarb is the ISO approved common name for 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate. It is a selective aphicide that is used extensively on a broad range of crops, including vegetable, cereal and orchard crops. The mode of action of pirimicarb is by inhibition of acetylcholinesterase activity.

Pirimicarb was evaluated by the JMPR in 1976, 1978 and 1982 (Annex 1, references 26, 30 and 38); an acceptable daily intake (ADI) of 0–0.02 mg/kg bw was established in 1983. Pirimicarb was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR), using new data not previously reviewed and relevant data from previous evaluations.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Groups of two male and two female rats were given either [¹⁴C]pyrimidinyl-labelled pirimicarb (radiochemical purity, >98%) or [¹⁴C]carbamoyl-labelled pirimicarb (radiochemical purity, >98%) as a single oral dose at 1 mg/kg bw. At intervals of 6 h and 24 h after

dosing, one rat of each sex from each group was killed and rapidly frozen for whole body autoradiography. The excretion of radioactivity in urine, faeces and exhaled air was monitored for 24h.

Excretion of both [^{14}C]pyrimidinyl-labelled and [^{14}C]carbamoyl-labelled pirimicarb was rapid, with >50% of the administered radiolabel being eliminated within 6 h after dosing (Table 1). In the case of [^{14}C]pyrimidinyl-labelled pirimicarb, most of the excreted radiolabel was in the urine. Urinary excretion of radioactivity over 24h accounted for 83% and 89% of the administered dose for male and female rats, respectively. Faecal excretion accounted for >6% of the administered radiolabel and about 0.1% was recovered from exhaled air. In the case of [^{14}C]carbamoyl-labelled pirimicarb, however, the principal route of elimination was via exhaled air, with >66% of the dose recovered as [^{14}C]labelled carbon dioxide over 24h for both sexes. Urinary and faecal excretion accounted for <18% and 3% of the administered dose, respectively. These findings are consistent with the extensive cleavage of the carbamate moiety and the separate fates of this and the substituted pyrimidine ring.

Whole-body autoradiography indicated that there was widespread systemic distribution of radioactivity from both labelled compounds.

Six hours after the administration of [^{14}C]pyrimidinyl-labelled pirimicarb, there was a high concentration of the radiolabel in the liver, showing a reticular pattern of distribution. High levels were also seen in the renal medulla, gastrointestinal mucosa and gastrointestinal tract contents. Moderate labelling was observed in the Harderian gland and lower levels occurred in the remaining tissues. In rats killed 24h after dosing, lower concentrations of radiolabel were observed in all tissues, the highest concentration being found in the liver.

Six hours after the administration of [^{14}C]carbamoyl-labelled pirimicarb, high levels of labelling were present in the buccal cavity, gastrointestinal tract contents, the gastric mucosa and in the liver, which showed a more homogeneous distribution of radioactivity than seen in the liver of rats given [^{14}C]pyrimidinyl-labelled pirimicarb. In the female rat, the retina and lens were also prominently labelled. After 24h, there was some labelling in the seminal vesicles.

Table 1. Recovery (%) of radiolabel over 6 h and 24 h after administration of ^{14}C -labelled pirimicarb as a single oral dose at 1 mg/kg bw

Sample	[^{14}C]pyrimidinyl-pirimicarb				[^{14}C]carbamoyl-pirimicarb			
	Male		Female		Male		Female	
	0–6 h	0–24 h	0–6 h	0–24 h	0–6 h	0–24 h	0–6 h	0–24 h
Urine	64.4	83.2	50.4	89.4	8.6	14.2	8.8	17.0
Faeces	<0.1	5.3	NS	3.3	<0.1	2.6	NS	1.1
Carbon dioxide	NA	0.1	NA	0.1	NA	66.6	NA	66.8
Cage wash	2.4	5.7	19.4	2.4	1.3	3.6	2.2	0.7
Total	66.8	94.3	69.8	95.2	9.9	87.0	11.0	85.6

From Brown (1997a)

NA, not applicable; NS, no sample

There did not appear to be any pronounced sex difference in either the excretion profiles or in tissue distribution for either [^{14}C]pyrimidinyl- or [^{14}C]carbamoyl-labelled pirimicarb (Brown, 1997a).

The tissue distribution and excretion of pirimicarb-associated radioactivity was studied in groups of five male and five female Alpk:AP_rSD rats after oral administration of [^{14}C]pyrimidinyl-labelled pirimicarb (radiochemical purity, >99%) at a dose of 1 mg/kg bw in corn oil. The rats were housed individually in metabolism cages from which urine and faeces were collected daily. The rats were killed 4 days after dosing, when representative samples of tissues were removed and analysed for residual radioactivity by liquid scintillation counting. The results are presented in Tables 2 and 3.

Pirimicarb was extensively absorbed. Over 4 days, males excreted a mean of 92% of the administered dose, with 79% being eliminated in urine and 11% in the faeces. Over the same period, females excreted >90% of the administered dose, with 82% in the urine and 7% in the faeces. The rate of excretion was rapid with >80% of the administered dose being excreted within 24 h after dosing in both sexes. Excretion was essentially complete within 4 days. No sex difference was apparent in either the routes or rates of excretion.

Table 2. Mean recovery (%) of radiolabel 4 days after administration of [^{14}C]pyrimidinyl-labelled pirimicarb at a single oral dose of 1 mg/kg bw (mean of values for five rats)

Sample	Males	Females
Urine	78.6	81.9
Faeces	11.5	6.8
Contents of the gastrointestinal tract post mortem	<0.1	<0.1
Cage wash	2.1	1.6
Tissues (including carcass)	1.8	1.9
Total	94.1	92.3

From Brown (1997b)

Table 3. Tissue concentrations of radioactivity (μg pirimicarb equivalents/g) 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 1 mg/kg (mean of values for five rats)

Tissue	Males	Females
Brain	0.002	0.003
Gastrointestinal tract	0.006	0.007
Gonads	0.007	0.015
Heart	0.014	0.014
Kidneys	0.018	0.017
Liver	0.057	0.058
Lungs	0.018	0.019
Spleen	0.009	0.011
Abdominal fat	0.004	0.003
Bone	0.016	0.010
Muscle	0.008	0.009
Blood	0.045	0.046
Plasma	0.031	0.034
Residual carcass	0.013	0.016

From Brown (1997b)

The limit of detection was 0.0004 μg pirimicarb equivalents/g of tissue for all tissues analysed

At the end of the experiment, <2% of the administered dose remained in the tissues in both sexes, the highest concentration of radioactivity being in the liver (pirimicarb equivalents, <0.06 µg/g). All other tissue concentrations were lower than that present in plasma (pirimicarb equivalents, 0.03 µg/g). Less than 0.05% of the administered dose remained in the gastrointestinal tract contents in both sexes. There was no sex difference in tissue distribution of radioactivity (Brown, 1997b).

In an experiment similar to that described above (Brown, 1997b), the tissue distribution and excretion of radioactivity was studied in rats after administration [¹⁴C]pyrimidinyl-pirimicarb as a single oral dose at 50 mg/kg bw (radiochemical purity, >98%). The results are presented in Tables 4 and 5.

Over 4 days, males eliminated a mean of 95% of the dose, of which >83% was in the urine and 10% in the faeces. Over the same period, females eliminated >93% of the dose, of which 74% was in urine and >16% was in faeces. The rate of excretion was rapid with >85% of the dose eliminated by males (79% in urine) and >76% by females (69% in urine) within 24 h of dosing. Excretion was essentially complete within 4 days, with <0.07% of the dose remaining in the gastrointestinal tract contents of both sexes. No pronounced sex difference was apparent in the rate of excretion.

Table 4. Recovery (% of administered dose) of radioactivity 4 days after administration of [¹⁴C]pyrimidinyl-pirimicarb as a single oral dose at 50 mg/kg bw (mean of values for five rats)

Sample	Males	Females
Urine	83.6	74.1
Faeces	10.1	16.6
Terminal gastrointestinal tract contents	<0.1	<0.1
Cage wash	1.3	2.0
Tissues (including carcass)	2.1	1.7
Total	97.1	94.5

From Brown (1997c)

Table 5. Mean tissue concentrations of radioactivity (µg equivalents pirimicarb/g) 4 days after administration of [¹⁴C]pyrimidinyl-pirimicarb as a single oral dose at 50 mg/kg (mean of values for five rats)

Tissue	Male	Female
Brain	0.163	0.124
Gastrointestinal tract	0.352	0.235
Gonads	0.549	0.514
Heart	1.026	0.557
Kidneys	1.272	0.740
Liver	1.914	1.638
Lungs	1.457	0.776
Spleen	0.602	0.423
Abdominal fat	0.216	0.164
Bone	0.508	0.290
Muscle	0.644	0.413
Blood	3.185	1.805
Plasma	2.354	1.130
Residual carcass	0.814	0.717

From Brown (1997c)

Limit of detection was 0.02 µg equivalent pirimicarb/g of tissue for all tissues analysed

When the rats were killed after 4 days, approximately $\leq 2\%$ of the administered dose was found in the tissues for both sexes, the highest concentration of radioactivity being found in the liver ($1.9\mu\text{g}$ and $1.6\mu\text{g}$ pirimicarb equivalents/g for males and females respectively). Concentrations in plasma were $2.4\mu\text{g}$ equivalents/g in males and $1.1\mu\text{g}$ equivalents/g in females; concentrations in all other tissues were lower. No marked sex difference was apparent in the tissue distribution of radioactivity (Brown, 1997c).

To determine whether repeated dosing affects the metabolic fate of pirimicarb, the tissue distribution and excretion of radioactivity was studied in groups of five male and five female AP₅D rats given [^{14}C]pyrimidinyl-pirimicarb (radiochemical purity, $>98\%$) as a single oral dose at 1 mg/kg bw . These rats had previously been given unlabelled pirimicarb (purity, 99.9% ; reference No. Y00032/048/001) as 14 consecutive daily oral doses at 1 mg/kg bw . In all other respects, this experiment was similar to that described in Brown, 1997b. The results in male and female rats were essentially the same (Tables 6 and 7).

Table 6. Recovery (% of administered dose) of radioactivity 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 1 mg/kg bw to rats previously given unlabelled pirimicarb as 14 consecutive daily oral doses at 1 mg/kg bw (mean of values for five rats)

Sample	Males	Females
Urine	79.4	77.9
Faeces	14.8	15.0
Terminal contents of gastrointestinal tract	<0.1	<0.1
Cage wash	2.2	3.7
Tissues (including carcass)	2.1	2.0
Total	98.4	98.7

From Brown (1997d)

Table 7. Mean tissue concentrations of radioactivity (μg pirimicarb equivalents/g) 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 1 mg/kg bw to rats previously given unlabelled pirimicarb as 14 consecutive daily oral doses at 1 mg/kg bw (mean of values for five rats)

Tissue	Male	Female
Brain	0.002	0.002
Gastrointestinal tract	0.008	0.008
Gonads	0.008	0.013
Heart	0.016	0.013
Kidneys	0.023	0.020
Liver	0.062	0.058
Lungs	0.023	0.018
Spleen	0.011	0.011
Abdominal fat	0.003	0.003
Bone	0.008	0.007
Muscle	0.011	0.010
Blood	0.055	0.047
Plasma	0.040	0.033
Residual carcass	0.017	0.017

From Brown (1997d)

The limit of detection was $0.0004\mu\text{g}$ pirimicarb equivalent/g of tissue for all tissues analysed

Over 4 days, both males and females eliminated a mean of 96% of the administered dose, with approximately 79% appearing in the urine and 15% in the faeces. The rate of excretion was rapid, with >80% of the administered dose being eliminated (71–74% in the urine) in both sexes within 24 h after dosing. Excretion was effectively complete after 4 days, with <0.1% of the administered dose being present in the gastrointestinal tract contents in both sexes.

When the rats were killed after 4 days, approximately $\leq 2\%$ of the radiolabel was found in the tissues in both sexes, with the highest concentration of radiolabel being found in the liver (0.06 μg pirimicarb equivalents/g). Concentrations in plasma were 0.04 μg equivalents/g in males and 0.03 μg equivalents/g in females; concentrations in all other tissues were lower.

A comparison of the routes and rates of excretion and the tissue distribution of radioactivity after repeated dosing in this experiment with the corresponding results from rats given ^{14}C -labelled pirimicarb as a single dose at 1 mg/kg bw (Brown, 1997b) shows no marked differences, although a greater proportion of the radiolabelled dose was eliminated in the faeces after repeated dosing. A higher recovery of the administered dose was also seen in this study. Hence, the repeated administration of pirimicarb to rats at this dose appears to have no pronounced effect on excretion or tissue distribution profiles (Brown, 1997d).

1.2 Metabolism

The biotransformation of pirimicarb was investigated in groups of male and female AP₁SD rats given [^{14}C]pyrimidinyl-pirimicarb (radiochemical purity, >98%) as a single oral dose at either 1 or 50 mg/kg bw, or as a single oral dose at 1 mg/kg bw after repeated oral doses of unlabelled pirimicarb at 1 mg/kg bw (purity, 99.9%; reference No. Y00032/048/001). An additional group of bile-duct cannulated male and female rats were given ^{14}C -labelled pirimicarb as a single oral dose at 50 mg/kg bw. The structural identification of metabolites isolated from the urine, bile and faeces was made using mass spectroscopy, proton nuclear magnetic resonance spectroscopy and co-chromatography with reference standards. Metabolites were quantified by high performance liquid chromatography.

Male and female bile-duct cannulated rats excreted a mean of 64% and 54%, respectively, of the orally administered radioactivity in the urine, and 13% and 16% in the bile within 48 h (Table 8). The mean percentage recoveries in the faeces were <1% and 4% of the administered dose in males and females, respectively. These values are taken to represent the unabsorbed dose, which thereby indicate that absorption after oral administration was extensive.

Table 8. Mean recovery (%) of administered radioactivity over 48 h in pairs of bile-duct cannulated rats given ^{14}C -labelled pirimicarb as a single oral dose at 50 mg/kg bw

Sample	Males	Females
Urine	64.0	53.8
Bile	13.2	16.5
Faeces	0.8	3.6
Total	77.9	73.9

From Gledhill (1998)

In rats, pirimicarb was extensively metabolized, giving rise to 24 metabolites, of which 17 were identified. A proposed pathway for the biotransformation of pirimicarb in rats is presented in Figure 1, with the relative proportions of metabolites shown in Tables 9 to 11. The structures and names of the rat metabolites characterized are shown in Figure 2. The major proposed route of biotransformation was via hydrolysis of the carbamate function (leading mainly to the formation of carbon dioxide), to give the 4-hydroxypyrimidine (R31805, 062/06). This was either excreted directly, or as the glucuronide conjugate, or was *N*-demethylated to produce the most abundant metabolite, the *N*-methyl hydroxypyrimidine (R34865, 062/07), which was either *O*-conjugated with glucuronide or further *N*-demethylated to the amine (R31680, 062/14). All of these metabolites were excreted predominantly in the urine, accounting for a cumulative total of 46–69% of the administered dose, irrespective of dose or sex.

The excretion of a lower proportion of the administered dose as the glucuronide conjugates of R31805 (<3% in both sexes, as CTL III and CTL XVI) in bile-duct cannulated rats than in non-cannulated rats (10% and 14% in males and females, respectively) is attributed to the reabsorption of biliary hydroxypyrimidines and their subsequent glucuronidation and elimination in the urine. After hydrolysis of the carbamate moiety, other minor hydroxylated metabolites included metabolite CTL XVII and its *N*-demethylated product (6-hydroxymethyl R34865).

Table 9. Quantification^a of metabolites in bile-duct cannulated rats given [¹⁴C]pyrimidinyl-pirimicarb orally as a single nominal dose at 50 mg/kg bw

Metabolite No.	Males				Females			
	Urine	Bile	Faeces	Total	Urine	Bile	Faeces	Total
R34865	28	2	T	30	21	0	T	21
R31680	5	4	0	9	7	0	0	7
<i>O</i> -glucuronide conjugate of R31805 (CTL III)	—	—	—	—	1	1	T	2
R31805	10	0	T	10	15	1	T	16
6-hydroxymethyl R34865	7	0	0	7	—	—	—	—
CTL VI	4	0	0	4	—	—	—	—
6-hydroxymethyl R34865 + CTL VI	—	—	—	—	5	0	0	5
CTL VII	2	0	0	2	1	0	0	1
<i>O</i> -glucuronide conjugate of R34865	—	—	—	—	0	1	0	1
CTL IX	—	—	—	—	0	7	0	7
<i>O</i> -glucuronide conjugate of hydroxylated pirimicarb (062/01) (CTL X)	0	T	0	T	—	—	—	—
<i>O</i> -glucuronide conjugate of hydroxylated pirimicarb (062/01) (CTL XI)	0	2	0	2	0	2	0	2
R35140	0	T	0	T	—	—	—	—
<i>O</i> -glucuronide conjugate of carbamate R34836	0	T	0	T	—	—	—	—
CTL XIV	0	1	0	1	—	—	—	—
CTL XV	0	2	0	2	—	—	—	—
<i>O</i> -glucuronide conjugate of R31805	1	0	0	1	2	0	0	2
Hydroxylated R31805 (CTL XVII)	—	—	—	—	0	T	0	T
Unknown 1	T	T	T, NR	T	0	0	T, NR	T
Unknown 2	0	1	NR	1	0	T	NR	T
Unknown 3	3	1	NR	4	2	1	NR	3
Unknown 4	1	0	0	1	—	—	—	—
Unknown 10	1	T	0	1	T	0	0	T
Unknown 11	—	—	—	—	1	0	0	1
Unknown 12	—	—	—	—	1	T	0	1
Unknown 11 + 12	2	T	0	2	—	—	—	—
Totals	66	14	T	80	56	13	T	73

From Gledhill (1998)

T, trace level of metabolite; NR, could not be resolved as single metabolites

^aExpressed as a percentage of the administered dose

Table 10. Quantification^a of metabolites in male rats given ¹⁴C-labelled pirimicarb as a single oral dose or as a single oral dose after 14 repeated doses of unlabelled pirimicarb

Metabolite No.	Single dose at 1 mg/kg bw			Repeated doses at 1 mg/kg bw			Single dose at 50 mg/kg bw		
	Urine	Faeces	Total	Urine	Faeces	Total	Urine	Faeces	Total
R34865	26	1	27	34	1	35	33	1	34
R31680	10	T	10	9	T	9	10	T	10
O-glucuronide conjugate of R31805 (CTL III)	2	T	2	5	T	5	3	0	3
R31805	6	T	6	8	T	8	12	1	13
6-hydroxymethyl R34865	2	T	2	6	T	6	6	0	6
CTL VI	11	T	11	4	T	4	5	0	5
CTL VII	3	0	3	5	0	5	1	0	1
O-glucuronide conjugate of R31805 (CTL XVI)	3	T	3	2	T	2	7	0	7
Unknown 1	4	NR	NR	4	2	6	1	1, NR	NR
Unknown 2	4	1, NR	9, NR	—	—	—	3	NR	5, NR
Unknown 3	1	0	1	—	—	—	—	—	—
Unknown 4	1	0	1	—	—	—	1	0	1
Unknown 5	3	0	3	—	—	—	—	—	—
Unknown 8	—	—	—	—	—	—	T	0	T
Unknown 10	0	T	T	0	T	T	2	T, NR	2
Unknown 11	—	—	—	—	—	—	0	NR	T
Unknown 12	—	—	—	—	—	—	—	—	—
Total	76	2	78	77	3	80	84	3	87

From Gledhill (1998)

T, trace level of metabolite; NR, could not be resolved as single metabolites

^aExpressed as a percentage of the administered dose

Table 11. Quantification^a of metabolites in female rats given ¹⁴C-labelled pirimicarb as a single oral dose only or as a single oral dose after 14 repeated doses of unlabelled pirimicarb

Metabolite No.	Single dose at 1 mg/kg bw			Repeated doses at 1 mg/kg bw			Single dose at 50 mg/kg bw		
	Urine	Faeces	Total	Urine	Faeces	Total	Urine	Faeces	Total
R34865	48	1	49	38	1	39	31	1	32
R31680	6	T	6	6	T	6	3	0	3
O-glucuronide conjugate of R31805 (CTL III)	8	T	8	9	1	10	4	T	4
R31805	6	T	6	4	0	4	15	1	16
6-hydroxymethyl R34865	4	T	4	3	T	3	4	0	4
CTL VI	3	T	3	4	1	5	2	0	2
CTL VII	5	0	5	3	0	3	3	0	3
O-glucuronide conjugate of R31805 (CTL XVI)	1	T	1	3	T	3	10	T	10
Hydroxylated R31805 (CTL XVII)	—	—	—	—	—	—	—	—	—
Unknown 1	1	NR	NR	2	2	4	1	NR	NR
Unknown 2	1	1, NR	3, NR	—	—	—	1	1, NR	3, NR
Unknown 3	—	—	—	—	—	—	—	—	—
Unknown 6	2	0	2	—	—	—	—	—	—
Unknown 7	1	0	1	—	—	—	—	—	—
Unknown 10–12	0	T	T	0	T	T	0	T	T
Totals	86	2	88	72	5	77	74	3	77

From Gledhill (1998)

T, trace level of metabolite; NR, could not be resolved as single metabolites

^aExpressed as a percentage of the administered dose

The carbamate function was also subject to displacement by glutathione, which produced a number of metabolites. Hydroxylation of a ring methyl group followed by displacement of the carbamate moiety by glutathione produced metabolite CTL XIV, and its *N*-demethylated equivalent, metabolite CTL XV. In female rats, the intact glutathione

From Gledhill (1998)

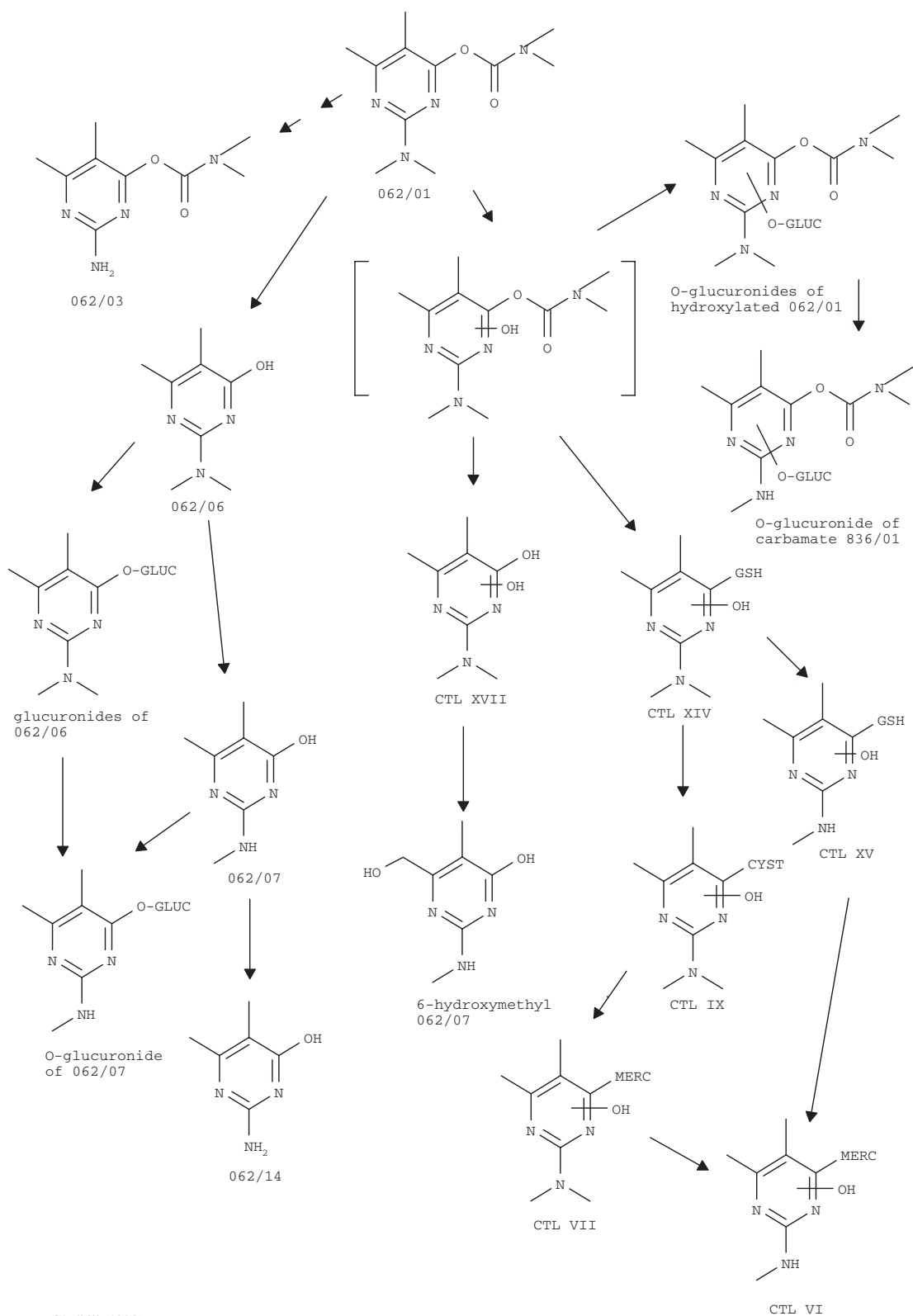


Figure 2. Structure and nomenclature of characterized metabolites of pirimicarb in the rat

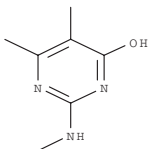
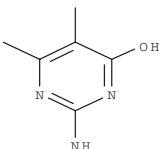
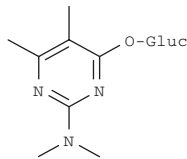
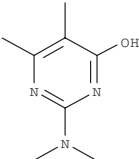
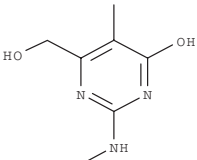
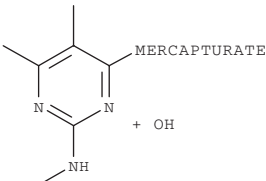
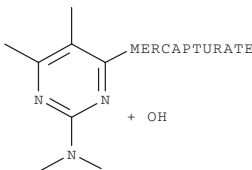
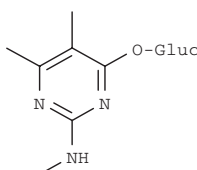
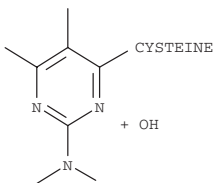
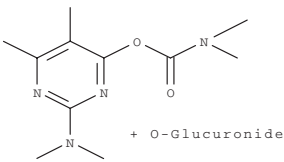
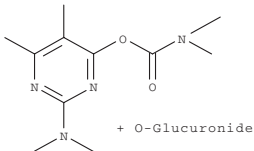
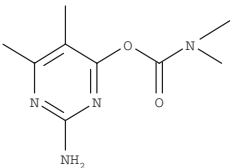
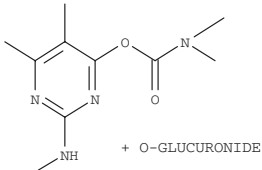
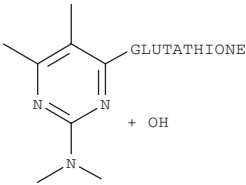
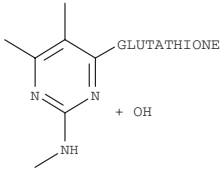
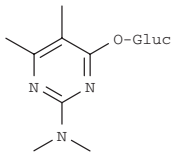
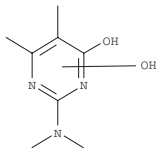
Metabolite No.	Structure and nomenclature	Metabolite No.	Structure and nomenclature
R34865 CTL I	 5,6-dimethyl-2-methylamino-4-hydroxypyrimidine	R31680 CTL II	 2-amino-5,6-dimethyl-4-hydroxypyrimidine
CTL III	 5,6-dimethyl-2-dimethylamino-4-hydroxypyrimidine glucuronide conjugate (O-glucuronide conjugate of R31805; isomer of CTL XVI)	R31805 CTL IV	 5,6-dimethyl-2-dimethylamino-4-hydroxypyrimidine
CTL V	 6-hydroxymethyl-2-methylamino-5-methylpyrimidin-4-ol (6-hydroxymethyl R34865)	CTL VI	 MERCAPTURATE + OH
CTL VII	 MERCAPTURATE + OH	CTL VIII	 5,6-dimethyl-2-methylamino-4-hydroxypyrimidine glucuronide conjugate (O-glucuronide conjugate of R34865)

Figure 2. Continued

Metabolite No.	Structure and nomenclature	Metabolite No.	Structure and nomenclature
CTL IX	 hydroxylated 4-cysteinyl- 5,6-dimethyl-2-dimethyl aminopyrimidine	CTL X	 (O-glucuronide conjugate of hydroxylated pirimicarb (062/01); isomer of CTL XI)
CTL XI	 (O-glucuronide conjugate of hydroxylated pirimicarb (062/01); isomer of CTL X)	R35140 CTL XII	 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate
CTL XIII	 (O-glucuronide conjugate of carbamate 836/01)	CTL XIV	
CTL XV		CTL XVI	 (O-glucuronide conjugate of R31805; isomer of CTL III)
CTL XVII	 (Hydroxylated R31805)		

From Gledhill (1998)

conjugate was not seen, but the corresponding cysteine conjugate (CTL IX) and mercapturate (CTL VII) were present. The *N*-demethylated mercapturate (CTL VI) was also present in the urine. There was a sex difference in the metabolic profile, with males showing a more extensive range of 4-hydroxypyrimidines than females.

While all urinary and faecal metabolites of [¹⁴C]pyrimidinyl had lost the carbamate function, the analysis of samples of bile collected soon after dosing showed the presence of small amounts of R35140 (062/03) and the O-glucuronide conjugates of hydroxylated pirimicarb (062/01) and carbamate R34836 (836/01), both of which contained the intact carbamate moiety.

There were dose-dependent differences in the relative proportions of urinary metabolites, with more of the *N*-dimethyl 4-hydroxy metabolites R31805, 6-hydroxymethyl R34865 and O-glucuronide conjugate of R31805 and less of the *N*-demethylated metabolites R34865 and R31680 being found at 50 mg/kg bw than at 1 mg/kg bw. Rats at the highest dose excreted less of the methoxy-4-mercapturates, CTL VI and CTL VII, but the males excreted a higher proportion of the dose as 6-hydroxymethyl R34865.

There were fewer differences between the sexes in rats given repeated doses of pirimicarb than in rats given a single dose at 1 mg/kg bw, but the differences were not pronounced (Gledhill, 1998).

2. Toxicological studies

2.1 Acute toxicity

The acute oral toxicity of this pesticide was evaluated in groups of five male and five female fasted Alpk:AP_rSD rats given pirimicarb (purity, 97.6%; reference No. P16) at a dose of 100, 150 or 200 mg/kg bw by gavage in corn oil. The highest dose was selected on the basis of a preliminary study. The rats were observed for 14 days after dosing. At 100 mg/kg bw, there were no deaths and signs of slight toxicity were resolved by day 2. At 150 mg/kg bw, three males and three females died and slight signs of toxicity in the survivors were resolved by day 7. At 200 mg/kg bw, four males and all five females died; signs of mild toxicity in the surviving male were resolved by day 8. All gross pathological findings at autopsy were considered to be non-specific or incidental and not of toxicological importance. The acute oral median lethal dose (LD₅₀) was calculated to be 152 mg/kg bw (95% CI, 118–197) in males and 142 mg/kg bw (95% CI, 121–166) in female rats (Lees & Connolly, 1995a).

In a study of acute percutaneous (dermal) toxicity, a group of five male and five female Alpk:AP_rSD rats was given pirimicarb (purity, 97.6%; reference No. P16) at a dose of 2000 mg/kg bw. Pirimicarb was mixed with approximately 0.5 ml of deionized water and applied at a dose of approximately 20–21 mg/cm² for males and 17–18 mg/cm² for females to the shaved intact skin, which was then covered by a gauze patch and an occlusive dressing for 24 h. The application site was then washed with water. Observations for mortality and clinical signs were made at 1 h and 4 h after application and then once daily for 14 days. Body weights were measured on days 1, 3, 5, 8 and 15. Autopsies were performed on all rats on day 15. No deaths occurred in this study and there were no significant signs of toxicity. There was never a significant loss in body weight in male rats and all males exceeded their initial body weight by day 5. Small body-weight losses were observed in female rats on day 3, but all females were gaining weight by day 8. Slight irritation of the skin

(including desquamation and small, scattered scabs) was noted in one male and two female rats. All dermal irritation had cleared by day 12. The dermal LD50 for pirimicarb was >2000 mg/kg bw in both male and female rats (Lees & Connolly, 1995b).

In a study of acute toxicity, groups of five male and five female Alpk:AP_fSD rats were given pirimicarb (purity, 97.4%; reference No. P18) by inhalation. Exposures were for 4 h to target atmospheric particulate concentrations of 400, 800 or 1200 µg/l. Analysed mean ($\pm$ standard deviation) concentrations of pirimicarb were 414 ± 47 , 747 ± 100 and 1065 ± 207 µg/l, respectively, and the corresponding mass median aerodynamic diameters ($\pm$ geometric standard deviations) of the particles were 3.05 ± 2.04 , 3.46 ± 1.89 and 3.02 ± 2.03 µm. No deaths occurred in the group with the lowest exposure. In the group with intermediate exposure, one female died, and in the group with the highest exposure, all rats either died or were killed in extremis. Clinical signs consistent with moderate toxicity and irritation to the respiratory tract were recorded in all treated rats either during or immediately after exposure. Surviving rats showed a rapid recovery from these effects. The median lethal concentration (LC50) of pirimicarb was 948 µg/l (95% CI, 746–1204) for male rats and 858 µg/l (95% CI, 703–1047) female rats (Parr-Dobrzanski, 1994).

The potential for pirimicarb (purity, 97.6%; reference No. P16) to cause acute ocular irritation was evaluated in six young male adult New Zealand White rabbits. Approximately 100 mg of pirimicarb was administered to one eye of each rabbit. The eyes were examined and assessed for irritation according to the Draize method. The rabbits were also assessed for possible initial pain response according to a six-point scale. There were no deaths in the study. Pirimicarb produced practically no initial pain (a few blinks only within 1–2 min) or slight initial pain (blinks and attempts to open the exposed eye, but the reflex closes it) in all six rabbits (class 1–2 on the six-point scale). Signs of irritation were limited to slight conjunctival redness in all rabbits and slight chemosis in one of them. These signs were no longer evident by day 2. The maximum mean total score (out of 110) was 2.3 at 2 h, reducing to 1.0 at 1 day and zero at 2 days. The final irritation assessment was that pirimicarb is practically non-irritant to the eyes of rabbits (Lees & Doyle, 1995c).

The acute dermal irritation potential of pirimicarb (purity, 97.6%; reference No. P16) was evaluated in six young male adult New Zealand White rabbits. Approximately 500 mg of pirimicarb was mixed with approximately 0.5 ml of deionized water and applied to the shaved intact skin (an area of approximately 2.5 cm $\times$ 2.5 cm), which was then covered by impermeable rubber sheeting for 4 h, after which the application site was exposed and washed with water. The Draize scale was used to assess the degree of erythema and oedema at the application sites approximately 30–60 min and 1, 2, 3, 4, 7 and 12 days after removal of the dressings. Signs of skin irritation were limited to very slight erythema (score of 1 on a scale of 0 to 4) observed in two rabbits, one for up to day 2, the other for up to day 7. The latter rabbit showed desquamation on day 7, but not on day 12. There were no signs of irritation in any of the other rabbits. The Meeting concluded that pirimicarb is practically non-irritant to the skin of rabbits (Lees & Connolly, 1995c).

The potential of pirimicarb (purity, 97.3%; reference No. RS088/E) to produce delayed contact hypersensitivity in male Porcellus:Dunkin Hartley guinea-pigs was assessed by a method based on the maximization test of Magnusson & Kligman (1970). The doses of pirimicarb used were selected on the basis of a preliminary screen. Groups of 20 test and 10 control male albino guinea-pigs were used for the main study. Two main procedures were involved: (1) the induction of an immune response; and (2) a challenge to that response.

Guinea-pigs were induced by intradermal injection of the following: (1) Freund complete adjuvant plus corn oil at the ratio of 1 : 1; (2) a 3% w/v dilution of pirimicarb in corn oil; or (3) a 3% w/v dilution of pirimicarb in a 1 : 1 preparation of Freund complete adjuvant and corn oil. Three pairs of intradermal injections (each of 0.05–0.1 ml in volume) were made, one of each pair on either side of the dorsal midline. One week later, 0.2–0.3 ml of pirimicarb as a 75% w/v preparation in corn oil was applied topically, and retained under an occlusive dressing for 48 h. Guinea-pigs in the control group were treated in the same way, but without the inclusion of pirimicarb.

For the challenge, 2 weeks after completion of the induction phase, a 75% w/v preparation of pirimicarb in corn oil (volume, 0.05–0.1 ml) was applied to the shorn left flank of the test and control animals and held under an occlusive dressing for 24 h. A 30% w/v preparation of pirimicarb in corn oil (volume, 0.05–0.1 ml) was applied to the shorn right flank of test and control animals and held under an occlusive dressing for 24 h. Skin sites were examined approximately 1 and 2 days after removal of the dressings in both induction and challenge phases, and the degree of erythema was quantified and recorded. The sensitizing potential of a 40% solution of aqueous formaldehyde was assessed in a study using a method similar to that described above and serving as a positive control.

No response was seen in any of the control animals. The guinea-pigs treated with formaldehyde showed a net percentage response calculated to be 94%. Twenty-four hours after the removal of the dressings, six of the 19 test animals (the bandage slipped from one test animal that was therefore excluded from the analysis), showed scattered mild or moderate diffuse redness in response to the challenge application with a 75% w/v dilution of pirimicarb in corn oil. The net percentage response was calculated to be 32%. After challenge with a 30% w/v preparation of pirimicarb in corn oil, scattered mild redness to intense redness and swelling was seen in nine of the 19 test animals. The net percentage response was 47%. The Meeting concluded that pirimicarb does have skin sensitizing potential under the conditions of the maximization test (Rattray & Leah, 1990).

2.2 *Short-term studies of toxicity*

There were no short-term studies of toxicity in mice and no 28-day studies in rats.

Rats

In an 8-week feeding study, groups of 20 Wistar-derived female rats of the Alderley Park strain were fed diets containing pirimicarb technical (purity, 97.7%; reference No. BX 189) at a concentration of 0, 100, 175, 250 or 750 ppm of diet, equal to 0, 12.2, 20.4, 29.2 or 84.8 mg/kg bw per day for 8 weeks. The objective of this study was to establish a definite no-effect level for the depression in growth observed in previous 90-day (Griffiths & Conning, 1968), 2-year (Clapp et al., 1972), three-generation (Fletcher & Sothern, 1971) and paired feeding (Richards et al., 1978) studies in rats. The clinical condition of the rats was monitored throughout the study, while body weights, food consumption (including food wastage) and water consumption were measured weekly. Analysis of the diets confirmed that the achieved concentration and homogeneity of pirimicarb were satisfactory. Stability of pirimicarb in diet was confirmed in an earlier study.

There was no effect on the clinical condition of the rats and variations in water consumption were considered not to be compound-related. The body-weight gain of rats at 750 ppm was significantly lower than that of controls by week 3 and remained so until week

Table 12. Mean body-weight gain (g) at selected time-points in female rats fed diets containing pirimicarb for 8 weeks

Week	Dietary concentration (ppm)				
	0 (control)	100	175	250	750
1	31.0	28.6	32.7	29.7	28.0
4	94.4	91.2	93.0	93.4	82.8**
8	147.0	141.1	146.8	144.2	132.8**

From Paul et al. (1995)

** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

Table 13. Overall mean food consumption and overall mean food wastage in female rats fed diets containing pirimicarb for 8 weeks

	Dietary concentration (ppm)				
	0 (control)	100	175	250	750
Food consumption (g)	1200	1215	1163	1172	1135**
Food wastage (g)	371.6	385.4	418.7	352.5	525.9**

From Paul et al. (1995)

** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

8 (Table 12). Overall weight gain was slightly but not statistically significantly reduced for animals at 100 or 250 ppm compared with that of controls.

In rats fed diets containing pirimicarb at 750 ppm, there was a statistically significant overall reduction in food consumption (Table 13). There were some occasional statistically significant, sporadic reductions in food consumption at 175 and 250 ppm, but overall the difference from controls was not significant. There was no effect on water consumption or food utilization at any dose, but food wastage was statistically significantly increased in animals at 750 ppm. The effect on body-weight gain could therefore be caused by either toxicity or reduced food palatability. No distinction between these possibilities could be made from this experiment. The no-observed-adverse-effect level (NOAEL) for rats in this study was 250 ppm, equal to 29 mg/kg bw per day. However, in comparison with the four earlier studies cited above, this seems to be very close to a lowest-observed-adverse-effect level (LOAEL), so the clear NOAEL is 175 ppm, equal to 20 mg/kg bw per day (Paul et al., 1995).

In an 8-week feeding study, groups of 12 Wistar-derived female rats of the Alderley Park strain were given access ad libitum to diets containing pirimicarb technical (purity, 97.7%; reference No. BX 189) at a concentration of 0, 250 or 750 ppm, equal to 0, 27.5 or 89.1 mg/kg bw per day for 8 weeks. These treated rats were paired with four groups of rats fed restricted diets, the amount given to these rats being determined by the amount eaten by rats given access to food ad libitum. Rats given diets containing pirimicarb at 250 and 750 ppm ad libitum, equal to 27.5 and 89.1 mg/kg bw per day, respectively, were paired with groups of rats given restricted access to either control diet or diets containing pirimicarb at 250 or 750 ppm, equal to 25.7 and 75.0 mg/kg bw per day, respectively.

After 8 weeks of treatment, all groups were fed control diet ad libitum for a recovery period of 8 weeks. Throughout the study, body weights and food and water consumption

Table 14. Description of a feeding study in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Study part	Feeding regime	Group	Dietary concentration (ppm)
1	Ad libitum	1	0 (control)
		2	250
		3	750
2	Restricted to the amount of food eaten by the animals in group 2 (250 ppm, ad libitum)	4	0 (control)
		5	250
3	Restricted to the amount of food eaten by the animals in group 3 (750 ppm ad libitum)	6	0 (control)
		7	750

From Richards et al. (1978)

Table 15. Mean body-weight gain (g) at selected time-points from week 0 during the treatment period and from week 8 during the recovery period in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Week	Dietary concentration (ppm)						
	Feeding ad libitum			Restricted feeding			
	0 (control)	250	750	0 (control)	250	0 (control)	750
1	37.1	35.4	31.2**	28.5	24.3**	27.2	23.0**
4	101.8	100.3	89.3**	85.0	79.3*	79.0	73.6*
8	147.0	142.0	128.5**	132.4	127.3	120.3	117.7
9	6.9	6.3	8.5	8.7	11.5	12.7	14.8
13	27.2	29.9	33.8*	32.3	36.9	36.5	42.3
16	30.8	35.4	38.8	35.8	43.1	41.3	48.5

From Richards et al. (1978)

*Statistically significant difference from appropriate control group mean, $p < 0.05$ (Student *t*-test)

**Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

were measured periodically. Analysis of the diets confirmed that the achieved concentration and stability of pirimicarb were satisfactory throughout the study.

There was no effect on the clinical condition of the rats, and variations in water consumption were considered not to be compound-related. The effects on body weight and food consumption are described below, according to the feeding regime.

Ad libitum diets: There was a slight depression in growth at 250 ppm that was more marked at 750 ppm (Table 15). This reduction in growth was completely reversible after a recovery period of 8 weeks in rats previously fed at 250 ppm ad libitum, but although those fed at 750 ppm grew better than did the controls during the recovery period, they gained less weight overall. Food consumption and food utilization showed a dose-related reduction during the treatment period (Table 16). During the recovery period, food consumption of rats at 250 ppm was only marginally higher than that of the controls, but at 750 ppm was consistently higher from week 11 onwards. Food utilization tended to be more efficient and, at 750 mg/kg, differences were statistically significant from the controls (Table 17).

Restricted diets: body-weight gain was reduced at both 250 ppm and 750 ppm compared with that in the control group (Table 15). This was essentially confined to the first week, but the resultant difference in growth was maintained during the treatment period. This depression in growth was completely reversible during the 8-week recovery period. Food consumption in control and treated animals was marginally reduced during treatment

and was similar during recovery (Table 16). Food utilization was slightly less efficient than that of controls during the treatment period and was apparently improved (not statistically significant) during recovery (Table 17).

The Meeting concluded that there was no evidence that the reduced body-weight gain was caused by unpalatability of diets containing pirimicarb or as a result of anorexia. There was evidence that the growth depression was a toxic response, as the effect was immediate in all treated groups and was accompanied by poorer food utilization. Whilst the effect was only slight at 250 ppm, it supports the trends seen in previous long-term studies. The depression was completely reversible in rats fed restricted diets and in those fed at 250 ppm *ad libitum*, and almost so in animals fed at 750 ppm *ad libitum* (Richards et al., 1978).

Groups of 25 male and 25 female Alderley Park SPF rats were either fed diets containing pirimicarb (reference No. and purity not reported) at a concentration of 0, 250 or 750 ppm, equal to 12.9 or 38.8 mg/kg bw per day in males and 15.3 or 47.1 mg/kg bw per day in females, or were given pirimicarb at a dose of 25 mg/kg bw per day orally by gavage for 12 weeks, followed by a recovery period lasting until week 16. Body weights and food consumption were measured throughout the study. Blood was taken for haematological assessment from five rats of each sex per group before exposure and from all remaining rats at the end of the study. Before exposure and periodically during the exposure period, plasma and erythrocyte cholinesterase activities were measured. At the end of the study, cholinesterase activity was measured in brain samples from designated animals, and selected organs were weighed from five rats of each sex per group. A range of tissues from all animals was examined microscopically.

Table 16. Overall mean food consumption (g) in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Weeks	Dietary concentration (ppm)						
	Ad libitum feeding			Restricted feeding			
	0 (control)	250	750	0 (control)	250	0 (control)	750
1–8	1056	1052	1003*	946	942	904	894
9–16	1097	1082	1051	1061	1072	1060	1061

From Richards et al. (1978)

* Statistically significant difference from appropriate control group mean, $p < 0.05$ (Student *t*-test)

** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

Table 17. Mean food utilization (g food/g weight gained) in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Weeks	Dietary concentration (ppm)						
	Ad libitum feeding			Restricted feeding			
	0 (control)	250	750	0 (control)	250	0 (control)	750
1–8	7.20	7.45	7.84**	7.16	7.45*	7.58	7.64
9–16	37.3	33.7	29.4*	35.9	27.5	27.1	23.4

From Richards et al. (1978)

* Statistically significant difference from appropriate control group mean, $p < 0.05$ (Student *t*-test)

** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

Analysis of the diets showed that achieved concentrations were satisfactory throughout the study. There were no adverse effects of pirimicarb on survival or evidence of compound-related clinical changes. There was no clear evidence of an effect of pirimicarb on body weight, some statistically significant differences from the controls being inconsistent and not part of a dose-response relationship. Food utilization was statistically significantly reduced compared with that of the controls in females given diets containing pirimicarb at 750 ppm during weeks 1–4.

Statistically significant differences from control values were seen in several haematological parameters in rats treated with pirimicarb. These were, however, inconsistent and there was no evidence of a dose-response relationship in any of the changes seen.

Plasma cholinesterase activity was reduced in males given pirimicarb at 25 mg/kg bw by gavage during most weeks of the dosing period, but not during the recovery period (Table 18). The differences seen in males at weeks 15 and 16 were considered to be spurious as they occurred in the recovery period after a value similar to that of controls before termination. In females, plasma cholinesterase activity was reduced during all of the dosing period. Females at the lower doses were affected, but to a lesser degree. Reductions in plasma cholinesterase activity provide evidence for the absorption of pirimicarb.

There was no effect on brain cholinesterase activity. There were, however, decreases in erythrocyte cholinesterase activity at 25 mg/kg bw in males at weeks 2 and 4 and in females at weeks 1 and 4, and at 750 mg/kg in females in weeks 8 and 10 (Table 19). Although statistically significant differences from the control values were seen at some time-points, the decreases were too small to be of toxicological significance.

There were no compound-related macro- or microscopic findings. There were no effects on organ weights (Griffiths & Conning, 1995).

It is concluded that no compound-related changes were seen in rats fed diets containing pirimicarb at 250 or 750 ppm. In contrast, plasma cholinesterase activity in rats

Table 18. Plasma cholinesterase activity (μ moles of acetic acid/ml per min) in rats receiving either diets containing pirimicarb or pirimicarb by oral gavage for 12 weeks followed by a 4-week recovery period^a

Week	Dose or dietary concentration							
	Males				Females			
	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day
Before exposure	0.57	0.59	0.64	0.56	2.32	3.03	2.86	2.43
1	0.54	0.52	0.53	0.55	3.28	2.49*	2.55*	2.38**
2	0.52	0.52	0.49	0.33**	2.48	2.32	1.99	1.63**
4	0.64	0.60	0.56	0.45**	2.90	2.40*	2.26**	1.26**
6	0.61	0.60	0.52	0.40**	3.16	2.77	2.83	2.21**
8	0.68	0.59	0.55*	0.47**	2.84	2.43	2.68	1.56**
10	0.61	0.61	0.48	0.63	3.85	3.06*	3.10*	2.14**
12	0.68	0.66	0.62	0.44**	3.56	3.13	3.08	2.29**
15	0.75	0.71	0.63*	0.71	4.13	4.15	3.68	3.85
16	0.79	0.69	0.56**	0.74	4.04	3.99	3.97	3.55

From Griffiths & Conning (1995)

^aFor animals tested during the recovery period (weeks 12 to 16), the adjusted mean values are shown for week 1 onwards)

*Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

**Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

Table 19. Erythrocyte cholinesterase activity (μ moles of acetic acid/ml per min) in rats receiving either diets containing pirimicarb or pirimicarb by oral gavage for 12 weeks followed by a 4-week recovery period^a

Week	Dose or dietary concentration							
	Males				Females			
	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day
1	1.08	1.06	1.17	0.98	1.16	1.17	1.13	0.90**
2	1.30	1.42	1.46	1.16	1.24	1.36	1.37	1.43
4	0.92	0.99	1.01	0.79*	1.41	1.24	1.26	1.08*
8	1.11	1.02	1.08	0.96	1.34	1.22	1.16*	1.20
10	1.10	1.19	1.13	1.05	1.14	1.13	0.98*	1.09

From Griffiths & Conning (1995)

^aFor animals tested during the recovery period (weeks 12 to 16), the adjusted mean values are shown

*Statistically significant difference from control group mean, $p < 0.05$ (Student t -test, two-sided)

**Statistically significant difference from control group mean, $p < 0.01$ (Student t -test, two-sided)

receiving pirimicarb at a dose of 25 mg/kg bw per day by gavage was reduced if the examination was made within 4 h of the dose being given. Since feeding with diets containing pirimicarb at 750 ppm resulted in a daily intake that was greater than that provided by administration of pirimicarb at a dose of 25 mg/kg bw per day by gavage, it is evident that the rapid absorption of pirimicarb can result in a $C_{\max}$ after dosing by gavage that is unobtainable from a diet containing pirimicarb at 750 ppm. Reductions in erythrocyte cholinesterase activity were too small to be of toxicological significance.

Groups of five male and five female rats received daily applications of pirimicarb (purity, 97.6%; reference No. P16) at a dose of 0, 40, 200, or 1000 mg/kg bw per day to the shaved, intact skin on 15 days (5 days per week) for 21 days. The test site was covered with gauze dressing followed by layers of stretch gauze and self-adhesive bandages. The exposure period was approximately 6 h/day. After treatment, the bandages were removed, and excess test substance was washed from the skin with water and mild soap. Body weight, food consumption, and clinical signs were evaluated throughout the study. Blood samples were collected before the rats were killed and subjected to examination post mortem at which selected organs were weighed and kidney, liver, treated and untreated skin, from animals in the control group and at the highest dose, were examined histopathologically.

None of the animals died before the scheduled end of the study and there were no signs of skin irritation, clinical signs of systemic toxicity or compound-related effects on body weight or food consumption. Also, there were no compound-related effects on organ weights, gross post-mortem or histopathological findings.

There were no compound-related effects on any haematological parameter.

At 1000 mg/kg bw per day, plasma alkaline phosphatase activity was reduced by about 20% in males ($p < 0.05$, Student t -test) and plasma cholesterol was about 34% higher in females ($p < 0.01$, Student t -test). There was a small but statistically significant reduction in brain cholinesterase activity at 1000 mg/kg bw per day, with no accompanying clinical signs of toxicity (Table 20). No statistically significant effects were observed at lower doses and there was no reduction of erythrocyte cholinesterase activity. There was a statistically significant reduction in plasma cholinesterase activity in males at 1000 mg/kg bw per day

Table 20. Cholinesterase activity (IU/g) in rats receiving pirimicarb by dermal application

Tissue	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	40	200	1000	0 (control)	40	200	1000
Brain	8.35	7.73	7.47	6.81**	10.54	10.33	9.35	8.17**
Erythrocyte	2216	2314	2298	2200	2390	2270	2258	2168
Plasma	557	483	459*	448**	1228	1056	941**	741**

Lees & Leah (1995)

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

and in females at 200 and 1000 mg/kg bw per day, but this was considered not to be of toxicological importance (Lees & Leah, 1995).

The NOAEL for dermal systemic toxicity was 200 mg/kg bw per day on the basis of reduced brain cholinesterase activity reduction at 1000 mg/kg bw per day.

Dogs

The report upon which this description is based contains information from two studies [referred to as part 1 (IHR241) and part 2 (IHR248)] that were combined because they were inter-related and had overlapping ranges of doses. Additional statistical analyses were carried out on both studies and included in this combined report as an addendum.

In part 1, groups of four male and four female beagle dogs were given diets delivering pirimicarb (purity, 94%; reference No. not reported) at a dose of 0, 4, 10 or 25 mg/kg bw per day for 90 days. Two males and two females from each group were then killed (one male only in the group at 25 mg/kg bw per day, as one male in this group became ill and died after only 10 weeks of treatment) and the remainder continued untreated for a further 28 days. Records were kept of their clinical condition, body weight, plasma, erythrocyte and brain cholinesterase activity, blood and urine chemistry, haematology (peripheral blood and bone marrow), terminal organ weights and histopathology.

In part 2, groups of four male and four female beagle dogs received pirimicarb at a dose of 0, 0.4, or 1.8 mg/kg bw per day for 90 days, while a fourth group received pirimicarb at 4.0 mg/kg bw per day for 180 days, the purpose being to determine a no-observed-effect level (NOEL) for the haematological changes seen in part 1. The observations and measurements made were similar to those made in part 1 but omitted the measurement of erythrocyte cholinesterase activity and the routine blood and urine chemistry.

In part 1, one male at 25 mg/kg bw per day became ill and was killed after 10 weeks of treatment, having lost 1.5 kg weight in 10 days and developed lethargy, urinary incontinence and severe anaemia. At post mortem, this dog was found to have abdominal ascites, a heavy nematode infestation of the ileum and congestion of the thymus, spleen and liver. It also had pronounced haematological abnormalities that are described below. The remaining dogs showed no clinical abnormality during the course of the experiment. There was a small compound-related reduction in body weight in males receiving pirimicarb at 25 mg/kg bw per day, with statistically significant differences from the values for controls occurring in most weeks between 5 and 14, the maximum difference being 6% ($p < 0.01$,

Student *t*-test) in week 14 (while in week 1 there had been a non-significant excess weight of almost 2%). There was no statistically significant effect on body weight among males at 10 or 4 mg/kg bw per day and no effects in females of either groups treated with pirimicarb.

During the course of the study, one male and one female at 25 mg/kg bw per day and one female at 10 mg/kg bw per day developed macrocytic anaemia. The male was killed (see above) and examination of the bone marrow post mortem showed marked erythropoietic hyperplasia with delayed maturation of the erythrocyte series and the presence of numerous megaloblasts. At this time (week 10), anaemia was not suspected in any of the other dogs. Statistical analysis of peripheral blood showed that there was an increase in mean erythrocyte diameter in both sexes at 25 mg/kg bw per day at 90 days (achieving statistical significance in females only) (Table 21). Circulating erythroblasts were markedly increased in individual dogs at 10 or 25 mg/kg bw per day, but the mean values did not achieve statistical significance. Examination of bone marrow in the recovery phase showed increases of myeloblasts and lymphocytes in males that had been receiving pirimicarb at a dose of 10 mg/kg bw per day and in the normoblast population in the males that had been receiving pirimicarb at 25 mg/kg bw per day (Table 22). In both males and females, there were increases (at all doses) in megaloblast counts both before and after recovery, but the data were erratic and showed no indication of a dose-related response. An interpretation of this observation is not evident.

There was a reduction in plasma cholinesterase activity at 25 mg/kg bw per day compared with that for controls, the maximum reductions being 30% at week 2 in male and 28% at week 1 in females (Table 23). Activity was sporadically reduced at 10 mg/kg bw per day, but there were no effects at 4 mg/kg bw per day. There was evidence of a reduction in

Table 21. Selected haematology parameters^a at day 90 in dogs fed diets containing pirimicarb ("part 1")

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
Erythrocyte diameter (µm)	6.00	6.09	6.03	6.22	6.03	6.06	6.14	6.34*
Circulating erythroblasts/500 leukocytes	0.5	2.5	3.0	16.5	0.0	1.5	42.0	27.3

From Hodge (1995a)

*Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

^aAdjusted mean values

Table 22. Bone-marrow differential cell counts^a in dogs fed diets containing pirimicarb ("part 1")

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
Myeloblasts	0.7	0.8	2.3**	1.2	1.0	1.1	1.5	0.8
Lymphocytes	1.5	2.0	6.8**	1.7	4.2	3.6	1.0*	2.1
Early normoblasts	4.6	6.3	5.8	10.5**	4.1	6.1	4.8	7.2
Megaloblasts	3.8	13.7	8.2	16.2	1.8	24.4*	10.3	15.1

From Hodge (1995a)

^aRecovery phase; adjusted mean values

*Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

**Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

Table 23. Blood plasma cholinesterase activity^a ($\mu\text{mol/ml per min}$) in dogs fed diets containing pirimicarb ("part 1")

Week	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
1	2.25	2.22	1.83**	1.61**	2.17	2.14	1.76**	1.57**
2	2.15	2.16	1.83*	1.50**	2.18	2.02	1.71*	1.82*
4	2.46	2.46	2.25	2.10	2.54	2.30	1.97*	2.11*
6	2.48	2.67	2.11	1.98*	2.59	2.32	1.96	1.98*
8	2.61	2.52	2.06	2.52	2.11	2.19	2.09	2.54
10	3.01	2.59	2.45	1.92*	2.82	2.75	1.86	1.87
12	2.78	2.74	2.29	1.82**	2.60	2.90	2.55	2.12
<i>Recovery period</i>								
1	2.92	2.94	2.83	3.69**	2.83	2.67	2.83	3.50
4	2.68	2.59	2.57	2.84	2.80	2.71	3.07	2.79

From Hodge (1995a)

^a Adjusted mean values shown for week 2 onwards.

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

Table 24. Erythrocyte cholinesterase activity^a ($\mu\text{mol/ml/min}$) in dogs fed diets containing pirimicarb ("part 1")

Week	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
6	1.73	1.70	1.58	1.44	2.06	2.03	1.86	1.80
8	1.73	1.69	1.65	1.35	1.62	1.98	2.09	1.69
10	1.91	1.72	1.69*	1.37**	2.10	1.92	1.71*	1.58*
12	1.95	1.74	1.63	1.42**	2.09	1.99	1.89	1.65**

From Hodge (1995a)

^a Adjusted mean values

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

erythrocyte cholinesterase activity in the groups receiving pirimicarb at a dose of 10 and 25 mg/kgbw per day between weeks 6 and 12 in males and at weeks 10 and 12 in females. There was no effect at 4 mg/kgbw per day (Table 24).

There appeared to have been a compensatory increase in both plasma and erythrocyte cholinesterase activity after cessation of dosing. There was no evidence for a reduction in brain cholinesterase activity.

No compound-related effects on the blood and urine chemistry were observed.

No toxicologically significant reductions or increases in organ weights were observed. An apparent reduction in adrenal weight adjusted for body weight in females at 25 mg/kgbw per day was attributable to one unusually high value among the controls.

Extra-medullary haematopoiesis was observed in the spleens and lymph nodes of dogs with severe anaemia. Treated dogs tended to show a higher incidence of focal

inflammatory lesions in the liver and reactive changes in lymph nodes, but the differences were not marked.

In part 2, there was no evidence of any compound-related effect on clinical condition or body weights. Also, there was no evidence for any adverse effect on the haematological parameters measured at 0.4 or 1.8 mg/kg bw per day. However, serum iron levels were significantly reduced in females at 4 mg/kg bw per day at 60 and 90 days (Table 25).

Changes in the bone marrow were observed in the group receiving a dose of 4.0 mg/kg bw per day at 90 and/or 180 days (Table 26). These included increases in myelocytes in males; decreases in neutrophils in males and females; and increases in megaloblasts in both sexes at all time-points. It is noted that there was a substantial difference in the values for megaloblasts in the control groups in part 1 and part 2.

There was no evidence of inhibition of either plasma or brain cholinesterase.

Table 25. Serum concentrations of iron^a (mg/100 ml) in female dogs fed diets containing pirimicarb ("part 2")

Period	Dose (mg/kg bw per day)			
	0 (control)	0.4	1.8	4.0
Before dosing	132.2	104.3	128.7	130.4
30 days	143.3	133.0	156.9	110.8
60 days	192.0	160.1	128.9	104.0*
90 days	198.3	150.5	139.8	114.0**

From Hodge (1995a)

^a Adjusted mean values shown

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

Table 26. Bone marrow differential cell counts^a in female dogs fed diets containing pirimicarb ("part 2")

Parameter/period	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	0.4	1.8	4.0	0 (control)	0.4	1.8	4.0
<i>Myelocytes</i>								
90 days	2.6	3.2	1.7	5.0**	3.8	3.5	4.3	4.7
180 days	—	—	—	4.9	—	—	—	3.2
<i>Neutrophils</i>								
90 days	18.7	16.5	16.6	12.1*	14.0	13.6	12.1	10.9
180 days	—	—	—	9.2	—	—	—	9.1
<i>Megaloblasts</i>								
30 days	0.6	2.0	1.4	3.7**	0.6	1.2	1.5	3.4**
60 days	0.9	1.8	1.7	4.2**	0.8	1.3	1.9	5.4**
90 days	0.8	1.1	3.0	6.6**	0.5	0.9	1.4	4.9**
180 days	—	—	—	7.3	—	—	—	6.4

From Hodge (1995a)

^a Adjusted mean values shown except at 180 days.

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

The only difference in organ weights compared with control values was a decrease in liver weight, adjusted for body weight, in males in all treatment groups. There was no dose–response relationship and the differences appear to result from two high control values.

The gross and microscopic findings showed no abnormalities attributable to treatment. There was evidence of splenic haematopoiesis, such as occurred in part 1, in only two dogs maintained for 180 days at 4 mg/kg bw per day. The NOAEL for pirimicarb was 1.8 mg/kg bw per day on the basis of haematological toxicity at 4.0 mg/kg bw per day (Hodge, 1995a).

Two groups of dogs were given diets containing pirimicarb (purity, 98.0%; reference No. Bx189 Dope D) as a suspension in Tween 80 for up to 16 weeks. One group of one male and one female, received pirimicarb at a dose of 2 mg/kg bw per day throughout this period. The second group of three males and three females, received pirimicarb at a dose of 25 mg/kg bw per day, increased to 50 mg/kg bw per day in two animals over weeks 5 and 6, and in all dogs of this group during weeks 8–12. A third group of one male and one female served as a control and received only Tween 80 in the diet. All groups were maintained on normal diet for 7 weeks after the dosing period. The condition and behaviour of all dogs was monitored periodically during the day. Body weights were recorded weekly and daily food intake was measured by weighing any uneaten food. Blood was taken for haematological investigations twice before the dosing period and then weekly for the remainder of the study. Bone marrow biopsies were performed on all dogs once before the dosing period and at 2-week intervals for the remainder of the study. Serological investigations were performed on all dogs at weekly intervals throughout the study. Erythrocyte and plasma cholinesterase activities were measured in all dogs on four (weekly) occasions before the dosing period, on day 1 of treatment (at 1, 2, 3, 4, 5 and 6 h) and subsequently at weekly intervals throughout the study. Clinical chemistry parameters were examined twice before the dosing period and then after 6 and 13 weeks. At the end of the study, 6 out of 10 dogs were killed and subjected to examination post mortem. Selected organs were weighed and a comprehensive range of tissues were taken and stored.

One dog was killed after 43 days of the study (28 days at 25 mg/kg bw per day, 10 days at 50 mg/kg bw per day and not dosed for 5 days) after clinical deterioration over the preceding 14 days. Autopsy revealed the probable cause of the deterioration as an intussusception of the ileum. (An intussusception is a form of intestinal obstruction caused by the prolapse of a part of the intestine into the adjoining intestinal lumen). It was not possible to establish any causal relationship between treatment and this condition. Salivation and a general loss of condition was associated with treatment in some dogs at 50 mg/kg bw per day. There were minimal changes in condition in one animal at 25 mg/kg bw per day and no changes were seen at 2 mg/kg bw per day. Body-weight gain was adversely affected in males at 25 mg/kg bw per day over the first 4 weeks of the study and in all dogs when the dose was increased to 50 mg/kg bw per day. Food intake was slightly reduced and feeding behaviour was affected at 50 mg/kg bw per day. It was not clear whether these effects were attributable to toxic effects of pirimicarb or to a decreased palatability of the diet.

Anaemia and reticulocytosis were associated with treatment at 50 mg/kg bw per day, as shown by reduced haemoglobin, erythrocyte volume fraction and erythrocyte count and an increase in the reticulocyte count of some dogs (Table 27). Responses to reduction of the dose to 25 mg/kg bw per day or to cessation of dosing varied, but generally there were returns towards, or to, control. Reticulocytes tended to increase in dogs M 693 and F 699 before they decreased once more.

Table 27. Haematology data (selected parameters) at week 12 in dogs fed diets containing pirimicarb

Dose/Dog No.	Parameter			
	Haemoglobin (g/dl)	Erythrocyte volume fraction (%)	Erythrocyte count ($10^6/\text{mm}^3$)	Reticulocyte (%)
<i>Control</i>				
M 691	13.9	45	6.89	0.6
F 696	13.9	47	6.27	0.6
<i>2 mg/kg bw per day</i>				
M 692	14.6	51	6.80	0.8
F 697	15.5	51	6.98	0.6
<i>25/50 mg/kg bw per day</i>				
M 693	D	D	D	D
M 694	3.8	15	2.16	0.4
M 695	13.1	47	5.50	2.6
F 698	13.3	43	5.76	3.8
F 699	9.7	31	4.04	0.8
F 700	12.1	37	5.02	3.2

From Fox (1998)

D, dead; F, female; M, male

At 50 mg/kg bw per day, changes in bone marrow were associated with an increase in the number of normoblasts from week 9 and a tendency towards suppression of bone marrow activity (hypoplasia). These effects were reversed when the dose was reduced to 25 mg/kg bw per day (after week 12) and all marrow samples were considered normal 1 week after cessation of treatment.

Inhibition of plasma cholinesterase activity compared with control values reached up to 85% at 25 mg/kg bw per day and >90% at 50 mg/kg bw per day. This effect was reversed within 7 days after cessation of treatment. Erythrocyte cholinesterase activity was not affected by treatment at any dose. There were no changes in other clinical pathology parameters.

Both absolute and relative spleen weights were at least fourfold greater in all treated females, while the only control female had a comparatively small spleen. Owing to the small number of dogs in the study and the lack of background data on these dogs, it was not possible to determine whether the changes were truly associated with treatment, although this would seem to be a reasonable assumption. It was considered that the changes in spleen weight were probably a reflection of the haematological changes described above. On the other hand, the splenic weight differences were also seen in the group receiving pirimicarb at 2 mg/kg bw per day, in which there were no signs of anaemia.

The results of this study demonstrate a rapid drop in haemoglobin concentration and a rise in reticulocyte numbers in two animals at 50 mg/kg bw per day. These changes, typical of haemolytic anaemia, are similar to those seen in previous studies in beagle dogs. The changes in the bone marrow appearances correlated well with the changes in peripheral blood haematology and plasma cholinesterase activity was markedly inhibited at 25 and 50 mg/kg bw per day indicating an expected biological response to treatment. The response of both dogs that received 2 mg/kg bw per day was in general similar to those seen in control dogs indicating that in this study, 2 mg/kg bw per day might be an NOAEL (Fox, 1978). Nevertheless, it could also be argued that, given the uncertainties regarding observations on the spleen, this study is unsuitable for reaching any such conclusion.

Groups of four male and four female beagle dogs were given gelatin capsules containing pirimicarb (purity, 97.5%; reference No. P22) orally at a dose of 0, 3.5, 10 or 35/25 mg/kg bw per day for a period of at least 1 year. The initial high dose of 35 mg/kg bw per day was given for 1 week, after which dosing was suspended for 2 weeks and the dose then reduced to 25 mg/kg bw per day from week 4 for the remainder of the study.

Clinical observations (including ophthalmoscopy) were made and body weights, food consumption and clinical pathology parameters, including haematology and plasma and erythrocyte cholinesterase activity, were measured throughout the study. At the end of the dosing period, the dogs were killed and subjected to a full post-mortem examination. Bone marrow smears were taken at this time. Selected organs were weighed, brain cholinesterase activity was measured and specified tissues were taken for histopathology.

Pirimicarb administered at the initial high dose of 35 mg/kg bw per day produced clear, clinical signs of toxicity (including tremors, salivation, thin appearance, sides pinched in, unsteady gait, subdued behaviour, irregular breathing and/or occasional coughing) and a significant reduction in appetite and subsequent body-weight loss during week 1. One female at 35/25 mg/kg bw per day was killed for humane reasons in week 36 after significant body-weight loss and moderately severe anaemia. Also at this dose of 35/25 mg/kg bw per day, there were clinical signs of toxicity, e.g. tremors, thin appearance, irregular breathing and coughing, a marginally increased incidence of fluid faeces and reductions in body-weight gain (46% in males, 23% in females over 52 weeks) and food consumption. The body-weight gain deficits were statistically significant over weeks 2–24 ($p < 0.01$) in males and at week 2 ($p < 0.01$) in females. The reductions in food consumption did not appear to correlate well with the reduced body-weight gain, in that they occurred with statistical significance erratically throughout the study in females, but were statistically significant in males only in week 1. Slightly reduced food consumption was also seen in several females receiving pirimicarb at a dose of 10 mg/kg bw per day. However, in the absence of any treatment-related effects on body weight at this dose, this was considered to be of no toxicological significance.

There were no treatment-related effects that were detected by ophthalmoscopy.

One bitch at 25 mg/kg bw per day developed anaemia that was first observed at week 13 and progressed until, at week 36, the dog was killed for humane reasons. Increased bone marrow cellularity and a decreased myeloid:erythroid ratio indicative of increased erythropoietic activity was also seen in this animal. No other significant haematological or bone-marrow effects were observed in dogs of any group.

Increased erythropoietic activity of the bone marrow was seen in the female dog that was killed in week 36. In those dogs that survived to the end of the study, there were no treatment-related changes in haematology or urine chemistry. At a dose of 35/25 mg/kg bw per day, there were slightly reduced plasma concentrations of albumin (in weeks 13, 26 and 52, $p < 0.05$, for males; in week 26 for females, $p < 0.01$) and total protein (in week 13, $p < 0.01$, and week 53, $p < 0.05$, for males; in week 26 for females, $p < 0.05$).

There were no changes in plasma, erythrocyte or brain cholinesterase activities that were attributable to treatment with pirimicarb in the female dosed at 35/25 mg/kg bw per day that was killed for humane reasons during week 36. There was no evidence from any of the surviving dogs of an adverse effect on plasma cholinesterase activity throughout the

study. Erythrocyte cholinesterase activity in females receiving pirimicarb at 35/25 mg/kg bw per day was statistically significantly lower (by approximately 21%) than concurrent control values in week 52, when adjusted for values obtained before dosing (Table 28). In addition, brain cholinesterase activity for females at 25 mg/kg bw per day, was statistically significantly lower (by approximately 22%) than concurrent control values in week 53. For males receiving pirimicarb at 25 mg/kg bw per day, and for males and females at 3.5 or 10 mg/kg bw per day, there were no changes in erythrocyte or brain cholinesterase activity that could be attributed to administration of pirimicarb.

There were no treatment-related changes in organ weights or macroscopic findings.

Histopathological changes consistent with an increase in erythrocyte breakdown (including increased haemosiderin pigmentation in the liver, extramedullary haematopoiesis in the spleen and bone marrow hyperplasia) were seen in the female dog that was killed in week 36. In the remaining dogs in the group treated with pirimicarb at 35/25 mg/kg bw per day and that survived to week 53, there was increased haemosiderin pigmentation of the liver in three out of four males and increased haemosiderin pigmentation of the spleen in one out of four males and one out of three females. Increased haemosiderin pigmentation of the spleen only was also apparent in one out of four females that received pirimicarb at a dose of 10 mg/kg bw per day. The NOAEL was 3.5 mg/kg bw per day on the basis of haemosiderin deposition in the liver and spleen at 35/25 mg/kg bw per day and in the spleen at 10 mg/kg bw per day (Horner, 1998).

Groups of four male and four female beagle dogs were fed pirimicarb (reference No. and purity not reported) at a measured dose of 0, 0.4, 1.8 or 4.0 mg/kg bw per day continuously for 2 years. The animals were observed daily and detailed clinical examinations were made at intervals of 3 months. Body weights were measured weekly for the first 12 weeks and then monthly throughout the remainder of the study. At 3-month intervals, blood samples were taken for haematology, cholinesterase activity and biochemistry; urine was collected for analysis; and electrocardiography readings were taken. Bone marrow samples were taken and examined at 4–6-month intervals. At the end of the exposure period, the dogs were killed and subjected to a full post-mortem examination. Selected organs were weighed and specified tissues were taken from all groups for subsequent histopathological examination. Histopathological examination of the brain and spinal cord was undertaken

Table 28. Cholinesterase activity (U/l) in dogs fed capsules containing pirimicarb for at least 1 year

Tissue	Dose (mg/kg bw per day)							
	Males				Females			
	0	3.5	10	35/25	0	3.5	10	35/25
<i>Erythrocytes</i>								
Before dosing	2823	3360	2500	2713	3010	2528	2843	2508
Week 52	3068	3850	2553	2818	3435	2728	3060	2633
Week 52, adjusted ^a	3095	3309	2921	2962	3253	2942	3015	2579**
<i>Brain</i>								
Week 52	4.82	5.00	4.84	4.48	5.68	5.52	5.11	4.43*

From Horner (1998)

^aAdjusted (analysis of covariance) for values obtained before dosing

*Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

**Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

on animals in the control group and in the group receiving pirimicarb at a dose of 4.0 mg/kg bw per day only.

All dogs survived the exposure period and maintained a satisfactory standard of clinical health throughout. There were no adverse changes in the growth rates of the dogs.

No changes were found in plasma glucose, urea, sodium or potassium concentrations, alkaline phosphatase activity, or bromosulphthalein (BSP) retention times. No changes were detected in the urine analyses. Apart from a slight increase in the erythroid:myeloid ratio in two females at 4 mg/kg bw per day, no adverse changes were seen in the bone marrow and there were no abnormalities in the haematological parameters measured. No significant differences in serum iron or folate levels were detected between any of the groups.

No adverse effects of treatment were seen at post-mortem examination and no histopathological changes attributable to treatment were identified. There was no effect on organ weights. The NOAEL was 1.8 mg/kg bw per day on the basis of slight increases in erythroid:myeloid ratio, indicative of increased erythropoiesis, in two out of four females at 4.0 mg/kg bw per day (Hodge, 1995b).

In an earlier study, pirimicarb was found to produce anaemia when fed to pure-bred dogs (Conning et al., 1968). Of the 24 dogs given this compound, three became anaemic and 14 others had detectable changes in their bone-marrow cells. Subsequent studies involving another 48 dogs reproduced some of the bone-marrow effects, but none of the dogs became anaemic. The bone-marrow effects were not progressive and the incidence was much lower than in the first study (Conning et al., 1969; Garner et al., 1972).

Initial observations on the three anaemic dogs suggested that the anaemia was of the megaloblastic type, thus implying some interference with nucleic acid synthesis. A number of drugs, particularly the anticonvulsants, are known to produce megaloblastic anaemia in man by interfering with the absorption and metabolic activities of vitamin B12 and folic acid (Kidd & Mollin, 1957; Flexner & Hartman, 1960; MacIntosh & Hutchinson, 1960; Huennekens, 1966; Reynolds et al., 1965). Overt anaemia is uncommon in human patients receiving these drugs, but about one third show changes in their bone-marrow cells that are indicative of megaloblastic erythropoiesis (Chanarin, 1969). Administration of folic acid or folinic acid at large doses of invariably corrects the anaemia and abolishes the bone-marrow irregularities (Chanarin, 1969).

Megaloblastic erythropoiesis also develops in association with a number of haematopoietic disorders, especially haemolytic anaemia (Dacie, 1962). Conversely, intramedullary haemolysis is commonly encountered in classical megaloblastosis caused by vitamin B12 or folate deficiency (Dacie, 1967a).

When the study described below (Garner et al., 1995) was begun, it was considered that the most likely explanation for the anaemia was an abnormality of erythropoiesis. Normal plasma levels of folate and vitamin B12 excluded the possibility that the absorption from the gut was impaired, but an effect at the cellular level still had to be considered. The absence of changes in leukocytes and platelets was contraindicative, and other studies with rats, guinea-pigs and ferrets had also failed to produce anaemia or bone-marrow changes (Griffiths & Conning, 1968; Clapp et al., 1972; Garner, 1972; Garner & Smith,

1972). In the study described below, the complete failure to correct the anaemia displayed by the two affected dogs with high doses of vitamin B12, B6, folic acid and folinic acid endorses the conclusion that the anaemia is not primarily megaloblastic.

The incidence of anaemia and the declining frequency of bone-marrow changes in the three reported studies in dogs raised the possibility of an idiosyncratic defect. Family studies showed that all dogs used in the first 90-day experiment were the offspring of five females, three of which were mated to the same male. The discovery of congenital factor VII deficiency (Garner et al., 1967) led to changes in the dog breeding policy and a substantial number of new breeding stock were introduced to eliminate this blood coagulation defect. Consequently, dogs used in studies after 1969 differed intrinsically from those in earlier studies. In the study described below, no evidence of a erythrocyte metabolic defect or abnormal haemoglobin structure was found in any of the four dogs used in the study or in a number of untreated stock dogs. There was, however, no doubt that the antibody detected in their sera was related to the administration of pirimicarb: it was not present before dosing; serial studies showed a dose-response relationship; and withdrawal of pirimicarb resulted in the disappearance of the antibody within 6 weeks.

Harris (1954) described a drug-induced haemolytic anaemia resulting from a erythrocyte-drug-antibody reaction in patients given the schistosomicide, Stibophen. Since then, a number of other drugs have been reported to cause haemolytic anaemia by this mechanism. All of the earlier literature relate to studies in human patients. Drug-induced autoimmune anaemia in man may be broadly classified into two types:

Type A: Haemolysis is acute and intravascular, resulting in haemoglobinaemia and haemoglobinuria. The required dose is low and there is usually some evidence of previous sensitizing exposure. Gamma-globulin cannot usually be detected on the erythrocyte surface, but components of complement can be demonstrated with suitable antisera.

Type B: Haemolysis is chronic and essentially extravascular. Haemoglobin degradation products may be present in plasma. Free haemoglobin cannot usually be detected either in plasma or urine. Large doses of the drug are required to produce an effect. Anaemia takes several weeks to develop. There is no evidence of sensitization, i.e. normal therapeutic doses of the drug can subsequently be given without adverse effect. Gamma-globulin can always be detected on the surface of circulating erythrocytes. Normal erythrocytes cannot be substituted for those of an affected person in vitro despite the presence of the drug. Antibodies are usually IgG immunoglobulins, but mixed IgM/IgG immunoglobulins are occasionally encountered.

Erythrocytes coated with IgG (auto-) antibodies are removed by the reticuloendothelial system. These antibodies may arise spontaneously or in association with diseases (systemic lupus erythematosus (SLE), lymphoma, chronic lymphocytic leukaemia) or after stimulation by a drug (e.g. α -methyldopa, l-dopa). The diagnostic laboratory hallmark is a Coombs' (antiglobulin) test, and the autoantibodies may be related to a portion of the Rhesus (Rh) locus (demonstrated for α -methyldopa; Bakemeier & Leddy, 1968). High-dose penicillin or cephalosporins may result in an antibody directed against an antibiotic-erythrocyte membrane complex; cessation of exposure to the drug results in the disappearance of accelerated destruction.

Pirimicarb-induced haemolytic anaemia could be included in the type B category, which also includes penicillin and α -methyldopa, the more common causes of this effect (Bunn & Rosse, 2001). Both require exposure at very high doses and for a protracted period to produce the effect, and both stimulate the production of IgG, but while the presence of antibody to penicillin can be demonstrated in vitro using normal cells if penicillin is in the test system (the antigen being a drug-hapten [erythrocyte] combination) the antibodies to α -methyldopa or pirimicarb cannot. Also in contrast to penicillin, the addition of α -methyldopa or pirimicarb to serum containing the specific antibody does not neutralize their effects. While dogs and humans may react differently to pirimicarb, it cannot be assumed that humans, like the rodents used in the safety evaluation of pirimicarb, will not respond with a haemolytic anaemia, given the susceptible genetic background and exposure. Therefore, the haemolytic anaemia described in a few dogs exposed to pirimicarb is presumed to be relevant.

In a study of chemically-induced autoimmune haemolytic anaemia, groups one male and one female unrelated young adult dogs were fed diets containing pirimicarb (purity, 95%; reference No. not reported) at measured doses of either 25 or 50 mg/kg bw per day. Pirimicarb was suspended in "Dispersol" OG and sprayed onto the morning food ration for each dog. Samples of blood, urine and bone marrow were obtained before exposure and at 2-weekly intervals for the first 12 weeks of the study. Thereafter, the sampling frequency was determined by the clinical and laboratory findings, or by the therapeutic regimen. Male dog No. 146 (receiving a dose of 25 mg/kg bw) and female dog No. 7 (receiving a dose of 50 mg/kg bw) were dosed continuously for at least 110 weeks.

Haematinics were administered to one dog at each dose that showed evidence of anaemia. From week 18, the male at 50 mg/kg bw and from week 24, the female at 25 mg/kg bw were treated with vitamin B12, vitamin B6, iron, pyridoxine and/or folic acid. At weeks 48 and 56 (for the male and female respectively), treatment with all haematinics and dosing with pirimicarb was stopped.

After the results of this phase of the study were obtained, a further investigation was designed to assess the possible involvement of immune mechanisms. Using a chequerboard analysis, the sera of pirimicarb-treated and untreated dogs were analysed for antibody reaction.

In an investigation carried out after their haematological recovery, the two susceptible dogs were dosed again with pirimicarb, the female at 2 mg/kg bw per day between weeks 80 and 86, and the male at 1 mg/kg bw per day between weeks 86 and 92; it was reported that this dog was subsequently dosed at 2 mg/kg bw per day.

The female at 50 mg/kg bw day and the male at 25 mg/kg per day did not become anaemic after continuous dosing for >110 weeks. The results of biochemical, haematological and clinical observations for these dogs were consistently normal.

The male at 50 mg/kg bw per day and the female at 25 mg/kg per day showed clear indications of disturbed erythropoiesis 10 weeks after the start of dosing. Peripheral blood films revealed changes in the size and shape of the erythrocytes and a few nucleated erythrocytes were regularly found. No abnormalities were seen in either the leukocytes or the platelets. When the haemoglobin levels had fallen to 50% of the value measured before exposure, the affected animals were treated with the haematinics listed above.

The two affected dogs were clinically healthy. Falling haemoglobin concentrations were accompanied by reticulocytosis and a marked erythroid hyperplasia, but with no evidence of haemorrhage. Differential cell counts showed a change in the pattern of developing erythrocytes, giving the appearance of transitional megaloblastosis.

Haemolysis was a prominent feature and failure to correct the anaemia with the various haematinics suggested this as the probable cause of the anaemia. Simultaneous withdrawal of pirimicarb and all haematinics was followed by complete haematological recovery. Biochemical tests of liver and kidney function revealed no abnormality. During the period of haematological normality, the biological half-life of the erythrocytes was determined for all the dogs used in this study. The effect of further administration of pirimicarb to the male previously dosed at 50 mg/kg bw per day was to shorten the erythrocyte half-life and produce anaemia.

Genealogical investigation of the four dogs used in this study showed that the male dosed at 50 mg/kg bw per day was the offspring of a bitch that was also the mother of an affected dog used in a previous study. The low incidence of anaemia suggested that sensitivity to pirimicarb might be genetically determined.

Washed erythrocytes from each of the two anaemic dogs were strongly agglutinated by specific anti-gamma globulin serum, while those of the other two dogs were not. Twenty untreated dogs also had negative results in this test. Free antibody was demonstrated in the sera of both anaemic dogs, but not in the sera from either the two unaffected dogs or the 25 untreated dogs.

In an analysis of sera and cells from 20 untreated dogs, no cross-reaction between them was observed. It was shown that the antibody and the antigenicity of the erythrocytes were related in time to the administration of pirimicarb. The antibody was not present in the serum samples before exposure and the erythrocytes possessed the appropriate antigen only when the dogs were receiving pirimicarb. Withdrawal of pirimicarb was followed by a marked decline in antibody titre within 6 weeks and circulating erythrocytes did not react with specific antiglobulin serum. Characterization of the antibody showed it to be an immune type, probably IgG.

The female dog previously dosed at 25 mg/kg bw per day was dosed at 2 mg/kg bw per day for 14 weeks without evidence of a haematological effect. No antibody was detectable in the serum of this animal at the end of this period.

The male dog previously dosed at 50 mg/kg bw per day was dosed at 1 mg/kg bw per day for 12 weeks without evidence of a haematological effect. The antibody had not been completely absent from the serum of this animal, but the titre did not increase, being <1 in 2. It was reported that the dose was then increased to 2 mg/kg bw per day, although the length of the dosing period was not stated. The NOAEL for anaemia in sensitive dogs was 2 mg/kg bw per day (Garner et al., 1995).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

The carcinogenic potential of pirimicarb has been evaluated in three lifetime feeding studies in mice, two in the Alderley Park Swiss-derived mouse and one in the C57 black

mouse. The earliest study (Palmer & Samuels, 1974) was reviewed by the JMPR in 1978, when a request was made to the notifier for another study of carcinogenicity to be undertaken in an appropriate species using a currently (i.e. for 1978) accepted protocol. This study suffered from the high incidence of respiratory disease. Consequently, the Palmer & Samuels (1974) study will not be reviewed here.

Groups of 60 male and 60 female Alderley Park Swiss-derived mice were fed pelleted diets containing pirimicarb (reference Nos BX189 and WED/G/9680; purities, 97.7% and 98.2%, respectively) at a concentration of 0, 0, 200, 400 or 1600 ppm for up to 96 weeks. These concentrations provided doses equivalent to 0, 0, 30, 60 or 240 mg/kg bw per day.

Clinical observations were made, body weights and food consumption were measured and at intervals urine analysis was undertaken to assess the amount of pirimicarb metabolite present. All mice, including any found dead or killed prematurely, were subjected to a full examination post mortem. At the scheduled end of the exposure period, specified tissues were taken for subsequent histopathology and bone-marrow smears were prepared and stored for possible future examination.

The analysis of pirimicarb in rodent diet presented some problems caused by the addition of water to diet before pelleting. These effects were related to a change in the degree of chemical binding of pirimicarb to dietary components and were reduced by a change in diet from expanded Porton rat diet with a vitamin E supplement (PRDE) to Porton combined diet (PCD) (same supplier). No evidence was obtained to show a facile chemical degradation of pirimicarb in diet caused by the pelleting process. Analytical methods were established to adequately monitor the pirimicarb content of diets periodically throughout the study. Availability of dietary pirimicarb was demonstrated by analysis of the hydroxypyrimidine metabolite of pirimicarb in urine (Banham et al., 1980).

The rate of mortality was increased for females at 1600 ppm during weeks 30 to 60 and shortly before termination. None of the other groups showed any differences in rates of mortality compared with the two control groups. There were no specific clinical signs attributable to treatment.

Reduced body-weight gain occurred at intervals throughout the study in male and female mice at 1600 ppm. The main effects were during the first 8 weeks and during weeks 54 to 60 ($p < 0.01$, Student *t*-test). Similar effects on body-weight gain were seen in males at 400 ppm during the first 3 weeks of the study ($p < 0.05$, Student *t*-test).

Food consumption was difficult to measure due to large amounts of wastage, which was consistently damp. These data, therefore, are not fully reliable. Irrespective of these problems, it was apparent that the mice given diets containing pirimicarb at a concentration of 1600 ppm, and to a lesser extent females at 400 ppm, were wasting more food than the others. At 1600 ppm, males had reduced food consumption throughout the study; this was also true for females after week 28. Food utilization was reduced in both sexes at 1600 ppm; this being most apparent during the first 4 weeks, but females continued to show a reduced food utilization over the first 12 weeks. The hydroxypyrimidine metabolite of pirimicarb was detected in all of the urine samples from mice receiving pirimicarb and none was detected in the controls. There was some evidence of a relationship between treatment and the amount of pirimicarb metabolite excreted in females although the correlation was not as good for males.

No treatment-related non-neoplastic effects were found. A number of different types of neoplasms were seen in all groups. In particular, five types occurred at a high frequency: lymphosarcoma, pulmonary tumours (mainly adenomas), hepatic, Harderian gland and pituitary gland tumours. Other types occurred much less frequently. Numbers of tumour-bearing animals per group were similar for males and females, but a number of differences were observed for certain tumour types, particular lymphosarcoma, pulmonary tumours and hepatic nodules. All the tumours seen are common in this strain of mouse and none was unique to mice treated with pirimicarb (Table 29).

There was no evidence of group differences in males in either the incidence or time of occurrence of lymphosarcomas. In females, the incidence of lymphosarcomas in the group receiving 1600ppm was increased and the latency decreased when compared with the group 1 controls. However, these differences were of the same magnitude when control groups 1 and 2 were compared. Therefore, little significance can be attached to the finding in the group receiving pirimicarb at 1600ppm.

There was a significant increase in the incidence of pulmonary adenomas in males and females at 1600ppm. The incidence of such tumours in mice in historical controls has varied from 0% to 28% for males and from 0% to 15.5% for females and illustrates the variability of tumour incidence in the Alderley Park mouse. The incidences of pulmonary adenomas at 1600ppm were higher than the ranges for historical controls, particularly in females. There was, however, no evidence of development of malignancy in the lungs.

There was a significant increase in liver tumours in male and female mice at 1600ppm. This finding, however, must be considered against the high and variable values for data from controls, both in the present study and in historical studies. Also, these tumours arose in the absence of any hepatotoxicity lesions in this particular study (i.e. incidences of non-neoplastic lesions in the livers of male and female mice at 1600ppm were no higher, and often lower, than in the control groups) or in any other study with pirimicarb. However, the diagnostic categories used (and which are no longer used) were type A and type B nodules. Type A nodules include both hyperplastic nodules and benign neoplasms (i.e. pre-neoplastic nodules constitute a proportion of this category), while type B nodules include

Table 29. Total numbers of selected tumours in mice fed diets containing pirimicarb for 96 weeks

Neoplasm	Dietary concentration (ppm)									
	Males					Females				
	0 (control group 1)	0 (control group 2)	200	400	1600	0 (control group 1)	0 (control group 2)	200	400	1600
<i>Liver</i>										
No. of mice examined	58	59	59	58	57	58	59	57	58	59
Type A nodule	3	9	5	9	15	1	2	3	6	4
Type B nodule	4	6	13	8	17	2	0	3	3	5
<i>Lung</i>										
No. of mice examined	59	60	59	59	58	59	59	59	59	59
Adenoma	9	8	9	8	17	9	4	9	11	18
Carcinoma	0	1	0	0	1	0	1	0	1	0
<i>Pituitary</i>										
No. of mice examined	52	46	45	40	53	51	55	50	52	41
Adenoma	4	6	1	0	0	22	16	16	16	4
Lymphosarcoma	13	15	13	13	14	11	25	18	18	24

From Sotharan et al. (1980)

tumours that show morphological characteristics of malignancy. In males at 1600 ppm, the incidence of both type A and type B nodules were clearly increased compared with that in both control groups. In addition, however, type B nodules were increased in males at 200 ppm, but not at 400 ppm. It is considered, therefore, that these liver tumours provide equivocal evidence for a carcinogenic effect of treatment in this study.

The incidence of pituitary gland adenomas was reduced in males (where, because of the lower incidences in the control group, the reduction was not significant) and in females at 1600 ppm.

The NOAEL for toxicity in mice was 200 ppm, equivalent to 30 mg/kg bw per day, on the basis of reduced body weights at 400 ppm, equivalent to 60 mg/kg bw per day. The NOAEL for carcinogenicity was 400 ppm, equivalent to 60 mg/kg bw per day, on the basis of equivocal evidence for increased incidences of malignant liver tumours in male mice and benign lung tumours in male and female mice at 1600 ppm, equivalent to 240 mg/kg bw per day (Sotheran et al., 1980).

Groups of 55 male and 55 female C57BL/10J_{CD-1} Alpk mice were fed diets containing pirimicarb (purity, 97.5%; reference No. P22) at a concentration of 0, 50, 200 or 700 ppm for at least 80 weeks. These concentrations provided doses equal to 0, 6.7, 26.6 or 93.7 mg/kg bw per day for males, respectively, and 0, 9.0, 37.1 or 130.3 mg/kg w/day for females, respectively. Clinical observations, body weights and food consumption were measured and at week 53 blood smears were taken for haematology. All mice, including any found dead or killed prematurely, were subjected to a full examination post mortem. At scheduled termination, cardiac blood samples were taken for haematology, selected organs were weighed and specified tissues taken for subsequent histopathology.

Pirimicarb had no adverse effects on survival and there was no evidence of treatment-related clinical changes. Females given diet containing pirimicarb at a concentration of 700 ppm showed a slight increase in the incidence of eye discharge when compared with that in controls; however, as the incidence was similar to that seen in male controls and treated groups and there was no adverse histopathology, it was considered that this observation was unrelated to treatment with pirimicarb. An increased incidence of subcutaneous masses seen in females at 700 ppm was unrelated to any adverse histopathological changes and was considered to be of no toxicological significance.

At 700 ppm, there was a clear reduction in body weight (by a maximum of 6% and 8% in males and females respectively, $p < 0.01$, Student *t*-test). Food consumption was sporadically reduced in males at 700 ppm, but not in females. There was reduced food utilization efficiency in both sexes at 700 ppm (males, 21%; females, 13%, $p < 0.01$, Student *t*-test) pirimicarb and in females at 200 ppm (8%, $p < 0.05$, Student *t*-test). Other small, but statistically significant, differences from control animals on body weight or food consumption e.g. in males at 50 ppm, were not considered to be toxicologically significant as no effects were apparent at 200 ppm.

At the end of the exposure period, both the erythrocyte counts and the mean cell haemoglobin concentrations were increased, while the mean cell volume and the mean cell haemoglobin were reduced in both sexes at 200 and 700 ppm. There was no evidence of anaemia, no compound-related histopathological changes in bone marrow and no evidence of increased extramedullary haemopoiesis. Decreases in mean cell haemoglobin in males

at 50 ppm were considered to be of no toxicological importance in the absence of changes in other erythrocyte parameters at this dose.

Liver weights were slightly increased in both sexes at 700 or 200 ppm. After adjustment for body weight, the increases were 6% and 9% in males and females 200 ppm, respectively, and 19% and 21% in males and females in the group receiving pirimicarb at a concentration of 700 ppm, respectively ($p < 0.01$). In the absence of any histopathological changes, these increases are considered to be of no toxicological importance. The increased incidence of eye discharge in females at 700 ppm seen at clinical observation was also seen macroscopically and there was also a decreased incidence of uterine distension at this dose.

In males, there was an increase in minimal mononuclear cell infiltration of the renal pelvis at 200 and 700 ppm; however, these increased incidences were similar to the incidences seen in all groups of females, including the controls. In females, there was a slightly increased incidence of minimal lymphoid proliferation in the lung at all doses when compared with controls and an increased incidence of pigmentation of the spleen at 700 ppm.

There was no effect of treatment with pirimicarb on the overall incidence of tumours in mice of either sex. The only neoplastic finding of significance was a small, but statistically significant increase in lung adenomas in females at 700 ppm (Table 30). The incidence, although it was low, was outside the incidence for historical controls (Table 31). A slightly higher, but non-significant incidence of lung adenoma was also observed in males. There was also a small increase the incidence of Harderian gland adenomas in males at 700 ppm and a decrease in the incidence of lymphosarcomas in males of the same group (Rattray, 1998).

It is concluded that oral administration of pirimicarb at a dose of up to 700 ppm for at least 80 weeks produced a small increase in the incidence of benign lung tumours in females, but not in males. The NOAEL for toxicity was 50 ppm, equal to 6.7 mg/kg bw per day, on the basis of slight haematological changes at 200 ppm, equal to 26.6 mg/kg bw per day.

Table 30. Selected neoplastic findings in the lungs of mice fed diets containing pirimicarb for at least 80 weeks

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	50	200	700	0	50	200	700
Adenoma	1	1	1	3	0	0	0	6
Keratinizing squamous epithelioma	0	0	0	0	0	0	0	1

From Rattray (1998)

Table 31. Historical control range of lung adenoma in studies of carcinogenicity in mice

Study duration (No.)	Dates	Range of incidence	
		Males	Females
2-year (15)	March 1984–November 1994	0/50–4/50	0/60–3/55
80-week (6)	May 1994–October 1988	1/50–4/55	0/50–2/55

From Rattray (1998)

Rats

Three studies (which pre-dated the establishment of good laboratory practice (GLP) of carcinogenicity with pirimicarb in rats, in which there were high incidences of respiratory disease, are not reviewed here because they are considered inadequate for risk assessment.

Groups of 64 male and 64 female Alpk:ApfSD rats were fed diets containing pirimicarb (purity, 97.6%; reference No. RS/88/E BXE/583) at a concentration of 0, 75, 250 or 750 ppm for up to 104 weeks. These concentrations provided doses equal to 0, 3.7, 12.3 or 37.3 mg/kg bw per day and 4.7, 15.6 or 47.4 mg/kg bw per day for males and females respectively. Twelve rats of each sex from each group were killed after 52 weeks, while the remainder continued to be exposed to pirimicarb until they were killed after 105 weeks. In addition, satellite groups of 36 male and 36 female rats were fed the same diets; 8 rats of each sex per group were killed after 26, 52 and 78 weeks and 12 rats of each sex per group were killed after 104 weeks, for determination of plasma, erythrocyte and brain cholinesterase activities.

Clinical observations (including ophthalmoscopy), body weights, food consumption, haematology and blood and urine chemistry were measured. At the scheduled times, the rats were killed and subjected to a full examination post mortem. Blood samples were taken at this time, selected organs were weighed and specified tissues were taken for subsequent histopathology.

There was no effect on the incidence of mortality in any of the treated groups and the incidence of clinical abnormalities was generally low, with no evidence for a relationship with treatment. In particular, there were no observations indicative of neurological dysfunction. There were no treatment-related ophthalmological changes.

Body-weight gain was reduced in the group receiving diet containing pirimicarb at a concentration of 750 ppm, such that final body weights were approximately 6% and 13% lower than those of the controls for males and females respectively. At 250 ppm, male body-weight gain was marginally, but statistically significantly reduced during the first 13 weeks of the study and female body-weight gain was slightly reduced throughout the study. Final body weights of the female rats at 250 ppm were approximately 6% lower than those of the controls, while values for male rats were similar to those for the controls. Although there was a marginal reduction in body-weight gain for females at 75 ppm during the second year, this was considered not to be of toxicological significance as it was small, did not form part of a dose-response relationship and values for females in the control group were atypically high during the second year. Furthermore, given the normal body weight progression in rats over a 2-year study, the changes that occur in the first year are more important than those occurring later. At 52 weeks there were statistically significant deficits in body-weight gain in both males (6%) and females (10%) at 750 ppm ($p < 0.01$, Student *t*-test), but not at any lower dose.

Food consumption was generally reduced throughout the study amongst males and females at 750 ppm and there was a marginal reduction amongst females at 250 ppm. There was no effect on males at 250 ppm or on either sex at 75 ppm. Food utilization was generally lower in the groups receiving diet containing pirimicarb at a concentration of 250 and

750 ppm, particularly during weeks 1–4, although at 250 ppm the difference from controls was small.

Haematological analysis showed slight increases in some of the erythrocyte parameters in females in the main group, particularly at week 52. At 750 ppm, these parameters were: haemoglobin concentration, erythrocyte volume fraction, mean cell haemoglobin and mean cell haemoglobin concentration. Both haemoglobin concentration and erythrocyte volume fraction were also increased in females of the main study at week 52 at 250 ppm, and at week 26 at 750 ppm. As similar changes were not seen in the larger, satellite groups of females killed at 52 weeks, and they were largely confined to data obtained for week 52, they are considered to be of no toxicological significance. In males, mean cell volume and mean cell haemoglobin were slightly higher in the group receiving pirimicarb at 750 ppm in weeks 26, 52 and 78 of the main study, but as other erythrocyte parameters were not affected these changes are considered to be incidental to treatment. Several other statistically significant changes were seen, but were all either minor and/or not consistent over a period of time and therefore considered not to be related to treatment.

There were increased levels of plasma cholesterol and triglycerides. Plasma cholesterol concentrations were statistically significantly increased in males in the main study at weeks 13, 26, 52, 78 and 104 at 750 ppm, and at weeks 13 and 26 at 250 ppm, while, in the males killed at week 52, there were no significant increases. In females in the main study, statistically significant increases were observed at weeks 13, 26, 52 and 78 at 750 ppm, at weeks 13 and 26 at 250 ppm and at week 13 at 75 ppm. This finding was supported by an increase in plasma cholesterol in females at 750 mg/kg killed at week 52. Plasma triglyceride concentrations were statistically significantly increased in males in the main study at weeks 52 and 78 in the group receiving pirimicarb at 750 ppm, a result that was supported by increases in plasma triglycerides in males at 750 and 250 ppm killed at week 52. In females in the main study, statistically significant increases were observed at weeks 13 and 26 for the group receiving pirimicarb at 750 ppm and week 26 for the group at 250 ppm, while there were no significant increases in the females killed at week 52. Thus, although there were clear indications of an effect on blood lipids, particularly on cholesterol in males, there were also inconsistencies in the observations that render interpretation difficult. Plasma alkaline phosphatase activities were statistically significantly reduced in males in the main study at weeks 52 and 78 for the group at 750 ppm and at week 13 for the group at 250 ppm, while in the males killed at week 52 the reduction for the group at 750 ppm almost reached statistical significance. In females in the main study, a statistically significant decrease was observed at week 52 for the group at 750 ppm, which was supported by a similar reduction in the females at 750 mg/kg killed at week 52.

Urinary concentrations of protein were increased in males at 750 ppm at week 51 and in all treated males at week 77, but these deviations were not consistent over time and because of the variability of individual urinary concentrations of protein in ageing rats, this is considered to be of no toxicological significance. All other statistically significant differences in urinary parameters were small, inconsistent with time and considered to be of no toxicological significance.

Plasma cholinesterase activities were lower than those of controls for females at 250 ppm (week 26) and at 250 ppm and 750 ppm (weeks 52 and 104) (Table 32). Occasional reductions were also seen in males at these doses, but were with no indication of a

Table 32. Plasma cholinesterase activity(U/l) in rats fed diets containing pirimicarb

Week	Dietary concentration (ppm)							
	Males				Females			
	0 (control)	75	250	750	0 (control)	75	250	750
26	618	599	548*	571	2014	1986	1682*	1745
52	802	777	775	747	2154	2008	1848*	1692**
78	924	848	672**	767*	1851	1891	1808	1748
104	767	952	843	842	2449	2186	2007*	1898**

From Tinston (1992)

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

dose-response relationship and were therefore considered to be of no toxicological significance. There was no effect on erythrocyte or brain cholinesterase activity, therefore the effects on plasma cholinesterase activities were considered to be evidence only of absorption of pirimicarb.

No treatment-related macroscopic changes were seen at autopsy. In rats killed at 52 weeks, liver weights, adjusted for body weight, were slightly increased in males and females at 750 ppm (12% and 11%, respectively) and in females at 250 ppm (9%), while, at the end of the study, adjusted liver weights were increased in males and females only at 750 ppm (9% and 12%, respectively). No treatment-related changes were seen in any other organ weights.

Male rats fed diet containing pirimicarb at 750 ppm showed small increases in the incidence and severity of necrosis of the brain, vacuolization of the adrenal cortex, minimal renal pelvic transitional cell hyperplasia, renal pelvic vascular ectasia, minimal hepatocellular hypertrophy and altered hepatocytes (clear cell). Males fed at 250 ppm had increased incidences of renal pelvic transitional cell hyperplasia and renal pelvic vascular ectasia. Females showed an increased incidence of slight renal pelvic transitional cell hyperplasia at 250 ppm and 750 ppm. There was also an increased severity of sciatic nerve demyelination and an increased severity and incidence of voluntary muscle degeneration. These changes in the nerve and voluntary muscle were probably related and were considered to represent an exacerbation of a spontaneous age-related change. Increased incidences of findings in a variety of other organs were considered of no toxicological significance.

The incidence of all tumours combined, both benign and malignant, was increased, but the incidence was not statistically significant. There was, however, an overall increased incidence in the number of males with malignant tumours at 250 ppm and 750 ppm, which was statistically significant (Fisher's exact test). This increase was associated with a variety of different malignancies arising in different organs, rather than a single type of tumour and may indicate an increased rate of progression to malignancy. There were also low incidences of astrocytoma of the brain in all treated groups of males and in females at 750 ppm; while these were not statistically significant and no dose-response relationship was evident, none occurred in the controls, so their significance should not be summarily dismissed. The incidences were, for the control group and groups receiving rising doses respectively, in male rats: 0/64, 3/64, 2/64, 3/64 and female rats: 0/64, 1/64, 0/64, 2/64. Data available on controls from 15 studies in this strain of rat in the performing laboratory for 1980–1987 provide a context in which to assess these astrocytomas. In male rats there were 15 astrocytomas

among 542 controls (mean, 2.76%; maximum, 4.83%). In female rats there were 8 astrocytomas among 542 controls (mean, 1.48%; maximum, 3.85%). Thus, the highest incidences in the current study (males, 3/64, 4.69%; females, 2/64, 2.13%) lie just within the upper limits of the ranges for historical controls. Nevertheless, the occurrence of relatively high incidences in the males and females of treated groups within one experiment is a matter of some concern when no astrocytomas were diagnosed in either males or females of the concurrent control groups.

The NOAEL was 75 ppm, equal to a mean dose of 3.7 mg/kg bw per day, on the basis of reductions in body weight and increases in plasma cholesterol and triglycerides at 250 ppm, equal to 12.3 mg/kg bw per day (Tinston, 1992).

Overall, the Meeting concluded that pirimicarb is unlikely to pose a carcinogenic risk to man.

2.4 Genotoxicity

Pirimicarb was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 33). There was no evidence of genotoxicity in vitro in tests for gene mutation in two studies in bacteria (*S. typhimurium* and *E. coli*) (Trueman, 1980; Callander, 1995). There was, however, a significant positive response in one study for mutations in mouse lymphoma L5178Y cells in vitro, using the currently recommended microwell version of this assay (Clay, 1996). There were increases in the numbers of both small and large mutant colonies at concentrations that permitted high levels of survival. This distinction is made because the assay is believed to be able to detect both gene mutations (manifest as large colonies) and large regions of damage, including small deletions and microscopically visible chromosomal aberrations (manifest as small colonies, because they grow more slowly).

In a single study for chromosomal aberrations in cultures of human lymphocytes taken from two donors, no significant increases in the proportion of abnormal cells were observed

Table 33. Studies of genotoxicity with pirimicarb

End-point	Test object	Dose (LED/HID)	Purity (%)	Result	Reference
<i>In vitro</i>					
Gene mutation	<i>S. typhimurium</i> strains TA100, TA1535, TA1537, TA1538 TA98 ($\pm$ S9)	2500 μ g/plate	Approx. 98	Negative ^b	Trueman (1980)
Gene mutation	<i>S. typhimurium</i> strains TA100, TA1535, TA1537, TA98; <i>E. coli</i> WP2, WP2uvrA ($\pm$ S9)	5000 μ g/plate	97.6	Negative ^b	Callander (1995)
Gene mutation	Mouse lymphoma L5178Y cells, <i>Tk</i> ⁺ locus ($\pm$ S9)	1400 μ g/ml –S9 100 μ g/ml +S9	97.5	Negative Positive	Clay (1996)
Chromosomal aberration	Human lymphocytes cells ($\pm$ S9)	500 μ g/ml	98.2	Negative	Wildgoose et al. (1987)
<i>In vivo</i>					
Unscheduled DNA synthesis in vivo/in vitro	Liver cells from male Alpk:ApfSD rats, 4 h and 12 h after dosing	200 mg/kg bw per os, single dose	98.3	Negative	Kennelly (1990)
Micronucleus formation	Bone-marrow cells from male C57BL/6JfCD-1/Alpk mice, 24 h, 48 h and 72 h after dosing	69.3 mg/kg bw, per os, single dose	97.3	Negative	Jones & Howard (1989)
Dominant lethal mutation	Male CD1 mice, 1–8 weeks after dosing	20 mg/kg bw, per os, five doses	Not reported	Negative	McGregor (1974)

LED, lowest effective dose; HID, highest ineffective dose; S9, 9000 $\times$ g supernatant from rodent liver

after incubation exposure for 3 h in an experiment conducted in the presence and absence of an exogenous activation system (Wildgoose et al., 1987).

No genotoxic activity was observed in three experiments conducted in vivo: an assay for unscheduled DNA synthesis in rat liver cells, an assay for micronucleus formation in bone marrow and an assay for dominant lethal effects in mice.

For the study of unscheduled DNA synthesis in vivo/in vitro in liver, groups of male Alpk:APfSD rats were killed either 4 h or 12 h after the administration of pirimicarb as a single oral dose at 50, 100 or 200 mg/kg bw (4 h) or 50 or 100 mg/kg bw (12 h), by gavage. A vehicle (corn oil, 10 ml/kg bw) control and a positive control (6-*p*-dimethylaminophenylazobenzthiazole, 40 mg/kg bw) group of four rats were dosed and subgroups of two rats per group were killed at 4 h and 12 h. Pirimicarb did not induce unscheduled DNA synthesis at any dose or time-point, while the positive control induced substantial DNA repair (Kennelly, 1990).

In the test for micronucleus induction in mouse bone-marrow cells, groups of 15 male and 15 female C57BL/6JfCD-1/Alpk mice were given pirimicarb (technical) at a dose of 0, 43.3 or 69.3 mg/kg bw orally by gavage on a single occasion, these doses being 50% and 80% of the median lethal dose. A group of five males and five female was given the positive control substance cyclophosphamide as an oral dose at 65 mg/kg bw. Subgroups of five males and five females per group were killed approximately 24, 48 and 72 h after treatment (except for animals in the positive control group, which were killed after 24 h). No increases in micronucleated polychromatic erythrocytes were observed in either sex in any group dosed with pirimicarb. Large increases in the incidence of micronucleus formation were observed in the positive control group (Jones & Howard, 1989).

In the dominant lethal effects experiment, groups of 15 male CD-1 mice of proven fertility were dosed with pirimicarb at a dose of 0, 10 or 20 mg/kg bw per day orally by gavage for 5 days. Positive control groups of a similar size were dosed with ethylmethanesulfonate, either intraperitoneally at a dose of 150 mg/kg bw on a single occasion, or orally at 100 mg/kg bw per day on 5 days. The males were then mated with fresh pairs of virgin females at weekly intervals for 8 weeks and the females were killed 15 or 16 days after the beginning of each mating period. They were assessed for dominant lethal effects; no effects were found in the groups treated with pirimicarb, while the positive control groups showed substantial effects in mating weeks 1 and 2, particularly in the group treated with multiple oral doses (McGregor, 1974).

Thus, the single indication of mutagenic activity observed in mouse lymphoma L5178Y cells in vitro was not supported by other assays in vitro or by results from assays in vivo. The Meeting concluded that pirimicarb is unlikely to be genotoxic.

2.5 *Reproductive toxicity*

(a) *Multigeneration studies*

Rats

Groups of 26 male and 26 female weanling Alpk:APfSD rats (F_0 generation) were fed diets containing pirimicarb (purity, 97.3%; reference No. RS/88/E) at a concentration of 0, 50, 200 or 750 ppm, equal to 0, 5.41, 21.7 or 81.8 mg/kg bw per day for males and 0, 5.64,

22.5 or 83.5 mg/kg bw per day for females, averaged over the first 10 weeks of exposure in a two-generation study of reproduction. After 10 weeks, the rats were mated and allowed to rear the ensuing F_{1a} litters to weaning. The breeding programme was repeated with the F_1 parents (selected from the F_{1a} offspring) after 10 weeks of exposure. The dietary exposures for the F_1 parental generation were equal to 0, 5.76, 23.2 or 90.1 mg/kg bw per day for males and 0, 6.04, 24.3 or 96.3 mg/kg bw per day for females, averaged over the first 10 weeks of exposure. The ensuing F_{2a} litters were reared to weaning. The diets containing pirimicarb were fed continuously throughout the study. A gross pathological examination was carried out on all parents and selected offspring. Histopathological examination was restricted to abnormalities and reproductive tract from animals suspected to be infertile.

In the F_0 generation, there were no adverse effects of pirimicarb on survival or evidence of treatment-related clinical changes. Administration of pirimicarb at 750 ppm was associated with reduced body-weight gain. Mean body weights at the end of the pre-mating period were lower than control values by 5% and 7% for F_0 males and females respectively (Table 34). As a result of the differences in starting body weights, the mean body weights of the F_1 animals at the end of the pre-mating period were lower by 12% and 11% respectively for males and females fed pirimicarb at 750 ppm compared with controls. The reduced body-weight gain in females fed at 750 ppm persisted throughout gestation and lactation in both generations, although a lesser effect was seen for the F_1 females. There were no adverse effects on body-weight gain in animals fed at 200 or 50 ppm.

Food consumption and food utilization were reduced at 750 ppm during the pre-mating period, particularly during weeks 1–4, as well as during gestation, but not during lactation. There were no adverse effects on food consumption at 200 or 50 ppm.

Table 34. Body weight (g) of rats fed diets containing pirimicarb in a multigeneration study

Generation/time	Dietary concentration (ppm)							
	Males				Females			
	0	50	200	750	0	50	200	750
<i>F₀ parents</i>								
Pre-mating:								
Initial weight	84.2	83.8	83.1	84.5	77.1	79.9	78.4	77.5
Final weight	434.5	443.6	435.0	414.9*	259.0	262.4	253.0	240.5**
Pregnancy:								
Initial weight	—	—	—	—	261.8	265.2	262.3	243.0**
Final weight	—	—	—	—	380.2	384.3	378.0	354.9**
Lactation:								
Initial weight	—	—	—	—	291.4	292.1	285.5	272.7**
Final weight	—	—	—	—	331.6	331.9	333.9	304.6**
<i>F₁ parents</i>								
Pre-mating:								
Initial weight	76.3	77.0	74.6	68.2	70.3	71.7	70.8	65.2
Final weight	440.7	442.7	437.9	386.2**	251.6	257.0	244.4	224.7**
Pregnancy:								
Initial weight	—	—	—	—	263.3	266.1	255.3	233.5**
Final weight	—	—	—	—	389.8	390.6	380.3	348.2**
Lactation:								
Initial weight	—	—	—	—	298.5	308.8	297.1	274.2**
Final weight	—	—	—	—	340.5	342.8	331.3	304.9**

From Moxon (1991)

* Statistically significant difference from control group mean, $p < 0.05$ (Student t -test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student t -test, two-sided)

There was no evidence of an adverse effect of pirimicarb on fertility or general reproductive performance at any of the doses tested. There was no effect of pirimicarb on litter size at birth or pup survival. Mean pup birth weight, per sex, was slightly lower in the group receiving pirimicarb at 750 ppm than in the controls, particularly in the F_{2a} litters. There was a subsequent effect on body weight at 750 ppm in both the F_{1a} and F_{2a} offspring, with body weights at day 29 being reduced by 7% and 8% for F_{1a} males and females and by 15% and 10% for F_{2a} males and females respectively. There was no effect on body weights of offspring at 50 or 200 ppm.

There were no compound-related macroscopic or microscopic findings in parents or offspring in either generation. There was no effect on testis weight in either generation. The NOAEL for reproductive parameters was 750 ppm, equal to 88 mg/kgbw per day (calculated from the pre-mating periods of the F₀ and F₁ parents), the highest dose tested. The NOAEL for toxicity in adult rats was 200 ppm, equal to 22.9 mg/kgbw per day (calculated from the pre-mating periods of the F₀ and F₁ parents), on the basis of reduced body-weight gain at 750 ppm, equal to 88 mg/kgbw per day. The NOAEL for offspring toxicity was 200 ppm, on the basis of reduced pup-weight gain at 750 ppm. There were no effects on reproductive indices at doses of up to 750 ppm, the highest dose tested (Moxon, 1991). The Meeting concluded that pirimicarb is not a reproductive toxicant in rats.

(b) *Developmental toxicity*

Rats

In a study of developmental toxicity, groups of 24 time-mated, female Alpk:ApfSD rats were given pirimicarb (purity, 97.3%; reference No. RS/88/E/BXE583) at a dose of 0, 0, 10, 25 or 75 mg/kgbw per day in corn oil by gavage on days 7 to 16 of gestation. The day of confirmation of mating (when spermatozoa were detected) was designated day 1 of gestation. The control group of animals received corn oil only. On day 22 of gestation, the females were killed and their uteri examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external/visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

One pregnant rat at 75 mg/kgbw per day was killed on day 7 of gestation after a dosing accident. All other rats survived for the duration of the study. Clinical observations in the dams were of low incidence or not dose-related and of the type commonly seen in the strain of rat used. Body-weight gain was lower in rats at 75 mg/kgbw per day during the pre-dosing period (25%) and there was a statistically significant decrease in body-weight gain compared with that of controls throughout the dosing period in this group (26% during days 7 to 16 of gestation). The body-weight gains in all treated groups were similar to those of rats in the control group during the post-dosing period, but overall weight gain during gestation remained statistically significantly lower for dams at 75 mg/kgbw per day. There was a statistically significant reduction in food consumption for rats receiving pirimicarb at a dose of 75 mg/kgbw per day, compared with that of controls during the dosing period (days 7 to 16 of gestation). Food consumption for all treated groups was similar to that of controls during the post-dosing period. Macroscopic changes seen post mortem were of a type and incidence commonly seen in the strain of rat used in this study and were considered not to be related to treatment.

Administration of pirimicarb at 75 mg/kgbw per day produced a significant reduction in fetal weight (8%, $p < 0.01$), with associated small reductions in litter weight and gravid

uterus weight. There was no evidence of any difference in the number or survival of the fetuses in utero at any dose, when compared with control values.

Major defects were seen in four fetuses in this study; 1, 1, 0 and 2 occurring in the groups at 0, 10, 25 and 75 mg/kg bw per day, respectively. These were: extreme dilation of the left ureter; umbilical hernia; fusion of arches of the second and third lumbar vertebrae; and a sixth digit on a hind paw. The defects were clearly unrelated and of low incidence and therefore considered to be spontaneous and not a result of treatment with pirimicarb.

The overall proportion of fetuses with minor external or visceral soft tissue defects was not statistically significantly increased by treatment with pirimicarb. Overall, there was a clear statistically significant increase in the incidence of fetuses with minor skeletal defects at 75 mg/kg bw per day. The incidences of fetuses with minor skeletal defects were: 50/265 (19.5%), 70/287 (24.5%), 65/270 (25.5%) and 92/244 (36.3%, $p < 0.01$) at 0, 10, 25 and 75 mg/kg bw per day, respectively. Almost all fetuses in all groups had some kind of skeletal variation, although the incidence was significantly higher at 75 mg/kg bw per day: 255/265 (96.5%), 276/287 (95.8%), 262/270 (97.3%) and 243/244 (99.6%, $p < 0.01$). These minor defects and variants were mainly incomplete ossification of cervical centra, sternebrae, odontoid and calcaneum, but also frequent in all groups were full ossification of the transverse processes of the fourth lumbar vertebra and bipartite fifth sternebrae.

The occurrence of an unossified centrum of the third cervical vertebra (minor defect) was statistically significantly increased at 75 mg/kg bw per day and was slightly increased at 10 mg/kg bw per day. However, the lack of effect at 25 mg/kg bw per day and the fact that 8 out of 23 affected fetuses were from one litter in the group at the lowest dose suggests that the increase seen at this dose is of no toxicological significance. The incidences in all test groups were within the ranges for historical controls. The incidence of unossified second cervical vertebrae (a variant) also became non-significant at 10 mg/kg bw per day after exclusion of this single litter.

There were statistically significant increases in the incidence of bipartite fifth sternebrae (minor defect) at 25 and 75 mg/kg bw per day. The incidence at 25 mg/kg bw per day was just outside the range for historical controls. A bipartite fifth sternebra is a slightly more retarded form of a partially ossified fifth sternebra and therefore these two findings should be considered together for interpretative purposes. Even when combined, however, there was no statistically significant differences between the control group and the group receiving pirimicarb at a dose of 25 mg/kg bw per day.

Only two external/visceral variants (slightly dilated and kinked ureter) were recorded. The incidence of the latter at 10 mg/kg bw per day was statistically significantly higher than that of the controls, but was within expected ranges when compared with historical data. In the absence of a dose-response relationship, this small increase was considered to be unrelated to treatment.

Most fetuses at all doses had one or more skeletal variants. Five types of skeletal variant showed statistically significant differences in one or more treated groups. The incidence of unossified odontoid was statistically significantly increased in all treatment groups with the highest incidence occurring at 75 mg/kg bw per day; however, as no dose-response relationship was apparent at the lower doses, the magnitude of the differences was small compared with the values for controls and the values fell within the range for recent

historical controls, the increased incidence at these doses is considered not to be of toxicological significance. The incidences were: 43/265 (16.2%), 76/287 (26.5%, $p < 0.01$), 60/270 (22.2%, $p < 0.05$) and 101/244 (41.4%, $p < 0.01$) at 0, 10, 25 and 75 mg/kg bw per day, respectively.

The incidence of unossified centrum of the second cervical vertebra was significantly increased at 75 mg/kg bw per day and was slightly increased at 10 mg/kg bw per day, but was within the range for historical controls.

There was a statistically significant increase in the incidence of fully ossified transverse processes of the fourth lumbar vertebra, unossified calcaneum and partially ossified fifth sternebra at 75 mg/kg bw per day compared with controls. The increased incidence of fully ossified transverse processes of the fourth lumbar vertebra represents a slight increase in ossification, in contrast to the other observations that are suggestive of a delay in ossification.

There was a statistically significant increase compared with control values in the degree of reduced ossification of the *manus* (scored on a six-point scale) at 75 mg/kg bw per day, but no effects were seen at lower doses: 2.92, 3.06, 2.94 and 3.14 ($p < 0.05$) at 0, 10, 25 and 75 mg/kg bw per day, respectively.

There was no evidence that pirimicarb is teratogenic to the rat at any of the doses tested in this study. Administration of pirimicarb at a dose of 75 mg/kg bw per day resulted in maternal toxicity and fetotoxicity as demonstrated by reductions in fetal weight, litter weight and gravid uterus weight. There was evidence of poorer ossification at this dose. Administration of pirimicarb at a dose of 25 mg/kg bw per day did not result in any maternal or fetal toxicity and is considered to be the no-effect level, although there were sporadic increases in some variants at this dose and at 10 mg/kg bw per day. The NOAELs in the study of developmental toxicity in rats were 25 mg/kg bw per day for the dams, on the basis of decreases in food consumption and body-weight gains at 75 mg/kg bw per day, and 25 mg/kg bw per day for the fetuses, on the basis of increased incidences of minor defects and variants at 75 mg/kg bw per day (Hodge, 1989).

The Meeting concluded that pirimicarb is neither a teratogen nor a developmental toxicant in rats.

Rabbits

In a study of developmental toxicity, groups of 20 artificially inseminated, female New Zealand White rabbits were given pirimicarb (purity, 97.3%; reference No. RS/88/E/BXE583) at a dose of 0 (19 animals), 2, 10 or 60 mg/kg bw per day by gavage in corn oil on days 7 to 19 of gestation inclusive. The day of artificial insemination was designated day 1 of gestation. On day 30 of gestation, the females were killed and their uteri examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

Two does at 60 mg/kg bw per day were killed during gestation for humane reasons. The clinical observations recorded for these rabbits before their death and the findings recorded at examination post mortem were non-specific and considered not attributable to

pirimicarb as the remaining animals in this group did not show similar signs. Administration of pirimicarb at a dose of 60 mg/kg bw per day resulted in a marked reduction in body-weight gain during the dosing period (days 7 to 19). This reduction was mainly confined to the first 3 days of dosing and there was some indication of weight recovery after the dosing period. There were no significant difference from the controls in body-weight gain at 10 mg/kg bw per day and the significant reductions that occurred at 2 mg/kg bw per day were not clearly not related to treatment. There was a reduction in food consumption in the groups receiving pirimicarb at a dose of 10 or 60 mg/kg bw per day during the dosing period. Differences in food consumption at 10 mg/kg bw per day were considered to reflect pre-dosing differences relative to the control group in which two animals had high food consumption.

No treatment-related changes were observed at any dose at examination post mortem. The incidence of cysts in the fatty tissue surrounding the ovaries (0, 3, 4 and 5, in the control group, and at 2, 10 and 60 mg/kg bw per day respectively) was within the incidence for historical controls.

There was no evidence of any adverse effects on the number, growth or survival of the fetuses in utero caused by treatment with pirimicarb at any dose. The incidences of fetuses with major defects were 5, 3, 2 and 5 in the groups receiving pirimicarb at a dose of 0, 2, 10 or 60 mg/kg bw per day respectively. The defects were of various types and occurred across all of the groups. There were a number of major defects in the treated groups that were not seen in the concurrent control group, but which have been seen previously in control or treated animals in the laboratory in which the study was performed. A gross malformation of the pinna was seen, however, in one fetus at 60 mg/kg bw per day and this had not been recorded previously in the laboratory in which the study was performed. In the absence of any dose-related trends, these isolated defects are considered to be spontaneous occurrences and not related to treatment with pirimicarb. There was no effect on the incidence of external/skeletal minor defects or variants.

The maternal NOAEL in this study of developmental toxicity in rabbits was 10 mg/kg bw per day on the basis of reductions in body-weight gain at 60 mg/kg bw per day, and the NOAEL for developmental toxicity was 60 mg/kg bw per day, the highest dose tested (Milburn et al., 1989).

The Meeting concluded that pirimicarb is not teratogenic and does not present a developmental hazard in rabbits.

2.6 *Special studies*

(a) *Delayed neurotoxicity*

No studies of delayed neurotoxicity with pirimicarb were available, it being argued that, because it is a carbamate insecticide and not an organophosphorus compound, such studies are not necessary. The insecticidal carbamates have shown no evidence of an ability to induce a delayed neurotoxic syndrome similar to that seen with some organophosphorus compounds. In a review of 17 carbamates administered as single doses at doses ranging from half to several times the LD₅₀, no behavioural evidence of neurotoxicity was observed during observation periods of 22 days, and no degeneration of the sciatic nerve was seen at autopsy (Baron, 1991).

(b) *Neurotoxic potential*

Groups of 15 male and 15 female Alpk:AP₅SD (Wistar-derived) rats were given technical-grade pirimicarb (purity, 97.6% w/w; batch No. P16) as single, oral doses at 0, 10, 40 or 110 mg/kg bw by gavage. Ten rats of each sex per group were allocated to the main study and were killed 2 weeks later. The remaining five rats of each sex per group were included as a satellite group for the purpose of measuring brain, erythrocyte and plasma cholinesterase activities, and brain neuropathy target esterase activity, on day 1.

All rats were observed before exposure and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed during week -1, and on days 1, 8 and 15. Locomotor activity was also monitored during week -1, and on days 1, 8 and 15. Body weights and food consumption were measured on occasions throughout the study. At the end of the scheduled period, five rats of each sex per group were killed and subjected to a full examination post mortem. Brain weight, length and width were recorded for these animals. Blood samples were taken and the brain was removed from selected animals for determination of brain, erythrocyte and plasma cholinesterase activities, and brain neuropathy target esterase activity. Selected nervous system tissues were removed, processed and examined microscopically.

One female that received pirimicarb at a dose of 40 mg/kg bw and two males and two females that received a dose of 110 mg/kg bw died or were killed for humane reasons after dosing on day 1. One other female that received pirimicarb at a dose of 10 mg/kg bw was killed for humane reasons on day 1; however, as the clinical signs and macroscopic findings seen were consistent with a possible dosing accident, the demise of this animal could not be attributed to administration of pirimicarb. A similar conclusion cannot be reached in the other cases.

Transient, treatment-related clinical signs were seen on day 1 for the majority of animals at 110 mg/kg bw, and for a few females at 10 or 40 mg/kg bw, with full recovery usually occurring by the following day. There was no adverse effect on body weight, although food consumption was slightly, but significantly, reduced during week 1 for males at 40 or 110 mg/kg bw.

Motor activity was significantly reduced in males at 40 and 110 mg/kg bw 6–10 min after dosing on day 1. Slightly lower landing foot splay and slightly increased time to tail flick were seen on day 1 for females at 110 mg/kg bw, but not for males. Locomotor activity was also slightly reduced for females at 40 or 110 mg/kg bw on day 1. On days 8 and 15, however, landing foot splay, time to tail flick and locomotor activity for these rats were normal.

Inhibition of brain cholinesterase activity of (method based on that of Ellman et al., 1961, but not mentioned in the report) was apparent in both sexes after dosing with pirimicarb at 110 mg/kg bw on day 1 and was considered to be indicative of neurotoxic potential (males: control, 12.62 IU/g; 110 mg/kg bw, 9.71 IU/g; $p < 0.01$; females: control, 12.04 IU/g; 110 mg/kg bw, 9.68 IU/g; $p < 0.05$), but not on day 15.

Reductions in erythrocyte (15%) and plasma (45–51%) cholinesterase activity in rats at 110 mg/kg bw, and reductions in plasma cholinesterase activity for rats at 10 or 40 mg/kg bw, were also seen on day 1, but not on day 15.

There was no evidence among rats surviving to termination of any treatment-related effects on brain weight, length or width and no macroscopic findings that could be attributable to treatment with pirimicarb. Comprehensive histopathological evaluation revealed no treatment-related changes in the central or peripheral nervous system of animals that received pirimicarb at a dose of 110mg/kg bw.

At 110mg/kg bw, there were clear cholinergic signs in some animals (whether they occurred in the same few animals was not specified), especially in females (not uncommon with carbamates and organophosphates), without a clear correlation with a toxicological significant inhibition of brain or erythrocytic acetylcholinesterase activity. It is likely that this was attributable to the usual technical problems associated with this assay in animals treated with carbamates (i.e. quick spontaneous reactivation in vivo and in vitro during preparation of the sample for the assay. It is not possible from the report to ascertain whether greater inhibition of cholinesterase activity occurred in symptomatic animals. It is difficult to discard the data obtained at 40mg/kg bw as not significant, while those (very few) at 10mg/kg bw might be considered incidental. Observations and tissue sampling were performed 3 h after dosing, which was reported to be the time of peak effect (presumably inhibition of acetylcholinesterase activity); however, with carbamates, differences in sampling and measurement time of 0.5 h can be relevant. In conclusion, the NOAEL for neurotoxic potential in rats given a single dose of pirimicarb was 10mg/kg bw. This evaluation was based on mortalities, adverse clinical signs and effects on landing foot splay, time to tail flick and locomotor activity on day 1, reductions in food consumption during week 1 and significant inhibition of brain, erythrocyte and plasma cholinesterase activities at 110 mg/kg bw and reduced food consumption, transient clinical signs, reduced motor activity and inhibition of plasma cholinesterase activity at 40mg/kg bw. It is recognized that the effect is C_{max} -dependent, and reversible (Horner, 1996a).

Groups of 12 male and 12 female Alpk:AP₁SD (Wistar-derived) rats were given diets containing technical-grade pirimicarb (purity, 97.6% w/w; batch No. P16) at a concentration of 0, 75, 250 or 1000 ppm for 90 days. These concentrations were equal to 0, 5.6, 19.2 and 77.1 mg/kg bw for males and 0, 6.6, 21.8 and 84.4 mg/kg bw for females.

All rats were observed before exposure and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed in weeks -1, 5, 9, and 14. Locomotor activity was also monitored in weeks -1, 5, 9 and 14. Body weights and food consumption were measured weekly throughout the study, and food utilization was calculated. At the end of the scheduled period, six rats of each sex per group were killed and subjected to a full examination post mortem. Brain weight, length and width were recorded and selected nervous system tissues were removed from these animals, processed and examined microscopically. Blood samples were taken from the remaining six rats of each sex per group at termination for determination of plasma and erythrocyte cholinesterase activities, and the brains of these animals were removed and submitted for determination of brain cholinesterase and neuropathy target esterase activities.

Throughout the study, there were no clinical signs that could be attributed to the administration of pirimicarb. At dietary concentrations of 250 or 1000 ppm, there were treatment-related effects on growth associated with reductions in food consumption and/or food utilization. Group mean body weight, adjusted for initial weight, for males and females at 1000 ppm was statistically significantly lower than that of concurrent controls throughout

weeks 2 to 14. At week 14, group mean adjusted body weight values for males and females at this dose were 91.5% and 92.1% of those for concurrent controls, respectively. For rats at 250 ppm, group mean body weight, adjusted for initial weight, was statistically significantly lower than that of concurrent controls throughout weeks 7 to 14 for males and during weeks 9 and 12–14 for females. At week 14, group mean adjusted body weight values for males and females at this dose were 94.1% and 95.1% that of concurrent controls, respectively.

Group mean food consumption for males and females at 1000 ppm was slightly lower than that of concurrent controls throughout the study, with these differences often attaining statistical significance for females. Group mean food utilization was statistically significantly lower than that of concurrent controls for males at 250 or 1000 ppm during weeks 1–4 and 5–8. Food utilization for females at 250 or 1000 ppm was also slightly lower than that of controls during weeks 1–4, although these differences did not attain statistical significance. Slightly reduced food utilization was also seen for males at 75 ppm during weeks 5–8. However, in the absence of any effects on food utilization during weeks 1–4, and as no effects on body weight and food consumption were apparent for these animals throughout the study, this was considered to be of no toxicological significance.

During the functional observation batteries (FOB), there were no clinical signs that could be attributed to administration of pirimicarb. In addition there were no changes in any of the quantitative or qualitative components of the FOB or effects on locomotor activity that could be attributed to administration of pirimicarb.

At autopsy, there were no treatment-related effects on brain weight, length or width, on brain, erythrocyte and plasma cholinesterase activities or on neuropathy target esterase activity. Comprehensive histopathological evaluation of the nervous system revealed no evidence of any treatment-related changes. The NOAEL was 75 ppm, equal to 5.6 mg/kg bw per day, on the basis of reductions in body weight at 250 ppm, equal to 19.2 mg/kg bw per day. The NOAEL for neurotoxic potential, after dietary administration of pirimicarb for 90 consecutive days, was 1000 ppm, equal to 77.1 mg/kg bw per day, the highest dose tested (Horner, 1996b).

(c) *Other toxicological studies*

(i) *Studies of toxicity with metabolites of pirimicarb*

Studies of toxicity have been conducted on a number of metabolites of pirimicarb from three different classes: the carbamate metabolites, the hydroxypyrimidine metabolites, and the guanidine metabolites. The International Union of Pure and Applied Chemistry (IUPAC) names, code names and occurrence of the metabolites are detailed in Appendix 1.

Acute toxicity of metabolites

The acute oral toxicities of pirimicarb hydroxypyrimidine metabolite 2-amino-5,6-dimethylpyrimidin-4-ol (R31680; 062/14) and carbamate metabolite 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140; 062/03, reference No. and purity not given) were evaluated in groups of six fasted female Alderley Park SPF Albino rats given either 2-amino-5,6-dimethylpyrimidin-4-ol (R31680) at a dose of 2500 mg/kg bw (apparently the only dose) or 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140) at a dose of 50, 64, 80, 100, 500 or 1000 mg/kg bw by gavage in Tween 80. The rats were

observed for 14 days after dosing. After dosing with 2-amino-5,6-dimethylpyrimidin-4-ol (R31680), some of the animals became subdued and showed slight urinary incontinence for a few days, but no deaths occurred. In rats dosed with 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140), toxic signs typical of cholinesterase inhibition (muscular fibrillation, salivation, urinary incontinence and chromodacryorrhoea) were observed. All surviving animals had fully recovered within 3 days. The LD₅₀ for 2-amino-5,6-dimethylpyrimidin-4-ol (R31680) was >2500 mg/kg bw, which was thus significantly less toxic than pirimicarb, while the LD₅₀ for 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140) was calculated to be 79 mg/kg bw (95% CI, 72–88) in female rats, which is comparable with the LD₅₀ of pirimicarb itself in fasted rats (Parkinson, 1974).

The acute oral toxicities of pirimicarb hydroxypyrimidine metabolites 2-dimethylamino-5,6-dimethyl pyrimidin-4-ol (R31805) and 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol (R34865; 062/07), both of which are plant and mammalian metabolites, and carbamate metabolites desmethylformamido pirimicarb (R34885; 062/04), desmethyl pirimicarb (R34836; 836/01), both of which are plant metabolites (reference No. and purity not given), were evaluated in groups of three fasted female Alderley Park SPF albino rats treated at dose ranges that varied for each substance given by gavage in propylene glycol. The rats were observed for 14 days after dosing.

The acute LD₅₀s values were: desmethylformamido pirimicarb (R34885), 50–100 mg/kg bw; desmethyl pirimicarb (R34836), 200–400 mg/kg bw; 2-dimethylamino-5,6-dimethyl pyrimidin-4-ol (R31805), 800–1600 mg/kg bw; and 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol (R34865), 2000–2500 mg/kg bw.

For desmethylformamido pirimicarb (R34885) and 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol (R34865), no specific signs of toxicity were observed, while for desmethyl pirimicarb (R34836) and 2-dimethylamino-5,6-dimethyl pyrimidin-4-ol (R31805), observations were made that were suggestive of the inhibition of cholinesterase activity. Only desmethylformamido pirimicarb (R34885) showed toxicity comparable with that of pirimicarb itself; the other three metabolites were less toxic than pirimicarb (Lefevre & Parkinson, 1989).

The acute oral toxicities of pirimicarb guanidine metabolites guanidine hydrochloride, methyl guanidine sulfate and dimethyl guanidine hydrochloride (batch Nos and purities not given) were evaluated in groups of six fasted female Alderley Park SPF albino rats treated with these substances at dose ranges that varied for each metabolite, by gavage in water. The rats were observed for 14 days after dosing. The signs of toxicity observed were subdued behaviour, piloerection and urinary incontinence. These signs were no longer evident after 5–7 days. The LD₅₀ values were: guanidine hydrochloride, 1105 mg/kg bw (95% CI, 1000–1220 mg/kg bw); methyl guanidine sulfate, 1105 mg/kg bw (95% CI, 950–1285 mg/kg bw); dimethyl guanidine hydrochloride, 1445 mg/kg bw (95% CI, 1215–1720 mg/kg bw). All three compounds were thus appreciably less toxic than pirimicarb itself (Parkinson, 1975).

Repeated doses of metabolites

The plant carbamate metabolite desmethylpirimicarb (purity, >98% w/w; R34836; batch No. R34836P4) was studied for its general toxic and haematological effects and its effects on cholinesterase inhibition in Alderley Park SPF albino rats treated with repeated

doses. The study consisted of two parts. For the toxicity and haematological investigation, groups of 20 male and 20 female rats were given desmethylpirimicarb (R34836) at a dose of 25 or 100 mg/kg bw per day by gavage. A control group of 10 males and 10 females was given the vehicle only ("Lissatan" AC). Half the animals at each doses were given 14 consecutive daily doses and the remainder were given 28 consecutive daily doses. General observations, body weight, blood and urine biochemistry and haematological parameters were measured. At the end of the study, all the rats were examined post mortem. Selected tissues were examined histopathologically.

For the assessment of cholinesterase inhibition, groups of five rats of each sex per group were given R34836 at a dose of 0, 1.5, 5, 25 or 100 mg/kg bw per day for 28 days. Blood samples were taken from all rats before dosing, 1 h after the 14th dose and 1 h after the 28th dose for measurement of erythrocyte and plasma cholinesterase activity. Brain cholinesterase activity was measured at autopsy.

In the first part of the study, one rat at 25 mg/kg was found dead after 10 doses, while none of the other rats in this group showed any clinical effects of treatment. At 100 mg/kg bw, five males and six females died during the study and there were some clinical signs of cholinesterase inhibition (described as fibrillations). The deaths occurred between two and seven doses. All the rats at 25 mg/kg bw gained weight, as did most of the surviving rats at 100 mg/kg bw.

No changes in clinical pathology were seen in rats at 25 mg/kg bw. At 100 mg/kg bw, males showed an increase in protein levels in pooled urine after the 14th and 27th doses, while there were no changes in the females. Examination of blood and bone marrow did not show any evidence of toxicity at either dose. Histological examination of the group receiving a dose at 100 mg/kg bw revealed no changes, so the group at 25 mg/kg bw was not examined.

In the segment of the study in which cholinesterase activity was analysed, no significant inhibition of erythrocyte, plasma or brain cholinesterase activity was detected in animals at 1.5 or 5 mg/kg bw. After 15 doses, there was no evidence of any inhibition of erythrocyte or plasma cholinesterase in rats at 25 mg/kg bw, but after 28 doses there was significant inhibition (55%) of plasma cholinesterase in female rats. At 100 mg/kg bw, there was significant inhibition of plasma cholinesterase in females after both 14 doses (41%) and 28 doses (63%). Brain and erythrocyte cholinesterase activity in the females and erythrocyte, plasma and brain cholinesterase activity in the males were not inhibited after either 14 or 28 doses. The NOAEL was 25 mg/kg bw on the basis of increased urinary protein at 100 mg/kg bw per day (Parkinson, 1979).

The plant carbamate metabolite desmethylformamidopirimicarb (purity, >98% w/w; R34885; 062/04, batch No. R34885P) was studied for its general toxic and haematological effects and its inhibitory effects on cholinesterase activity in Alderley Park SPF albino rats given repeated doses. The study consisted of two parts. For the toxicity and haematological investigation, groups of 10 male and 10 female rats were given desmethylformamidopirimicarb (R348385) at a dose of 12.5 or 50 mg/kg bw per day by gavage. A control group of five males and five females was given the vehicle only ("Lissatan" AC). Half the animals at each dose were given 14 consecutive daily doses and the remainder were given 28 consecutive daily doses. General observations, body weight, blood and urine

biochemistry and haematological parameters were measured. At the end of the study, all the rats were examined post mortem. Selected tissues were examined histopathologically.

For the assessment of cholinesterase inhibition, five males and five females of each sex per group were given R34885 at a dose of 0, 3 or 12.5 mg/kg bw per day for 28 days. Blood samples were taken from all rats before dosing, 1 h after the 14th dose and 1 h after the 28th dose for measurement of erythrocyte and plasma cholinesterase activity. Brain cholinesterase activity was measured at autopsy.

In the first part of the study, no clinical abnormalities were seen at either dose and all rats gained weight during the course of the study. No compound-related changes were seen in the blood or urine of any animals at either dose. Examination of blood and bone marrow did not show any evidence of toxicity at either dose. Histological examination of the group of animals at 50 mg/kg bw revealed no changes, so the group at 12.5 mg/kg bw was not examined.

In the part of the study in which cholinesterase activity was investigated, no significant inhibition of erythrocyte, plasma or brain cholinesterase activity was detected in animals at 3 mg/kg bw. At 12.5 mg/kg bw there was no evidence of any significant effect on erythrocyte or brain cholinesterase activity after 14 or 28 doses, or of any effect on plasma cholinesterase after 14 doses. There was significant inhibition (46%) of plasma cholinesterase activity in females after 28 doses, while there was no effect in males. The NOAEL was 12.5 mg/kg, the highest dose tested (Parkinson, 1978).

A group of 10 male and 10 female Alderley Park SPF albino rats was given the pirimicarb plant carbamate metabolite desmethyl pirimicarb (R34836; 836/01, batch No. and purity not given) at a dose of 100 mg/kg bw per day by gavage in propylene glycol on 5 days per week for 2 weeks. The rats were observed for signs of toxicity, and 24 h after the final dose, blood was taken for haematological analyses and for measurement of cholinesterase activity. All rats were examined post mortem and selected tissues from four males and four females were examined histopathologically.

Slight fibrillations, incontinence and salivation were apparent within 30 min of each dose, but these signs abated within 2 h and the rats appeared to have completely recovered within 24 h. Food consumption and body weight was unaffected, with the exception of one female that died because of a dosing accident.

There was no inhibition of brain or erythrocyte cholinesterase activity, although plasma cholinesterase, a less reliable indicator of anticholinesterase activity, was inhibited by approximately 25% in females.

Haematological examination (in comparison with "control" values rather than a usual concurrent control) showed that both males and females were slightly hypochromic (mean cell haemoglobin: males, 32.7% versus 30.6%; females, 33.3% versus 30.8%), and males had reticulocytosis (reticulocytes as a percentage of erythrocytes: 4.50 versus 7.20).

No gross abnormalities were detected at examination post mortem. At histopathological examination, four males and one female showed increased haemopoietic activity in the spleen, and haemopoietic cells were also seen in the thymus in one male and one female,

and a slightly reactive thymus was seen in one male and one female. An NOAEL could not be determined from this study, in which a single dose was tested (Fletcher, 1971a).

A group of 10 male and 10 female Alderley Park SPF albino rats were given the pirimicarb plant carbamate metabolite desmethylformamidopirimicarb (R34885, 062/04), batch No. and purity not given) at a dose of 25 mg/kg bw per day by gavage in propylene glycol on 5 days per week for 2 weeks. The rats were observed for signs of toxicity and, 24 h after the final dose, blood was taken for haematological analyses and for measurement of cholinesterase activity. All rats were examined post mortem and selected tissues from four males and four females were examined histopathologically.

No signs of toxicity were noted and there was no effect on food consumption or body weight. There was no inhibition of plasma, erythrocyte or brain cholinesterase activities 24 h after the final dose. However, it should be noted that as R34885 is a carbamate, peak inhibition would be expected 1–4 h after dosing, hence, a potential effect would have been missed in this study. This potential problem was addressed in a subsequent study (see Parkinson, 1978).

Haematological examination revealed slight hypochromia in both males and females (mean cell haemoglobin: males, 32.7% versus 31.2%; females, 33.3% versus 30.2%). However, comparison was with “normal” values rather than a concurrent control. There was no effect on clotting function. No gross abnormalities were seen at examination post mortem and the only significant histopathological abnormality seen was increased haemopoietic activity in the spleen in four males and one female. A NOAEL could not be determined from this study (Fletcher, 1971b).

The Fletcher (1971b) study was repeated with special reference to the haematological evaluation. Desmethylformamidopirimicarb (purity, >98%; R34885, 062/04, batch No. R34885P₅) was prepared as an aqueous suspension in 0.5% “Lissatan” AC and administered at a dose of 12.5 or 50 mg/kg bw per day by gavage to four groups of 10 male and 10 female Alderley Park SPF albino rats for either 14 or 28 days. Control groups of five males and five females were given the vehicle only for either 14 or 28 days. These rats were observed for signs of toxicity and 24 h after the final dose, blood was taken for haematological analyses. All rats were examined post mortem and selected tissues from animals in the group dosed at 50 mg/kg bw per day were examined histopathologically. No cholinesterase activities were measured in these rats. Parallel groups of five male and five female Alderley Park SPF albino rats were given R34885 at a dose of 0, 3 or 12.5 mg/kg bw per day for 28 consecutive days. These rats were bled (tail vein) twice in the 7 days before dosing began and then 1 h after each of the 14th and 28th doses for measurement of erythrocyte and plasma cholinesterase activity. Brain cholinesterase activity was also measured post mortem.

There were no effects of treatment on the clinical condition or behaviour of the rats, on body-weight gain, chemical analysis of blood, haematology or histopathology at either 12.5 or 50 mg/kg bw per day.

No significant inhibition of erythrocyte, plasma or brain cholinesterase activity was detected at 3 mg/kg bw per day. At 12.5 mg/kg bw per day, there was no evidence of any significant effect on the cholinesterase activity in erythrocytes after 14 or 28 doses, in plasma after 14 doses or in brain after 28 doses. There was, however, a significant, 46% inhibition

of plasma cholinesterase activity in female rats after 28 days, but there was no significant inhibition in male rats. The NOAEL for pirimicarb metabolite R34885 was 3 mg/kg bw per day on the basis of inhibition of plasma cholinesterase activity 1 h after dosing (Parkinson, 1978).

Pirimicarb metabolite R31805 has been tested in short-term studies of toxicity in vivo. As this metabolite is converted to R34865 in vivo, these studies on R31805 are also effectively testing R34865. Both metabolites have been tested individually, however, for acute oral toxicity and for genotoxicity.

Groups of 12 male and 12 female Alpk:AP_rSD (Wistar derived) rats were given diets containing technical-grade pirimicarb hydroxypyrimidine metabolite 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (purity, 100% w/w; R31805; 062/06, batch No. P4) at a concentration of 0, 80, 240 or 800 ppm for 90 consecutive days. These dietary concentrations were equal to received doses of 0, 6.6, 19.5 or 65.6 mg/kg bw for males and 0, 7.3, 22.0 and 73.9 mg/kg bw for females. Satellite groups of four males and four females were dosed at the same dietary concentrations for 90 days and used for measurement of cholinesterase activity.

Clinical observations, body weights and food consumption were measured for all rats throughout the study. Detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness were made and, for all main-phase rats, assessment of motor activity was performed during week 12, urine sampling during week 13 and ophthalmoscopy during weeks -1 and 13. At the scheduled end of the exposure period, all rats were killed and the main phase animals were subjected to a full examination post mortem. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no treatment-related effects on body weight or food consumption and there were no treatment-related clinical signs, ophthalmoscopic findings or effects on the FOB.

Among males at 800 ppm, mean motor activity was higher from 36–50 min and overall compared with values for concurrent controls. However, in the absence of any associated clinical signs or pathology findings, the increase in motor activity was considered to be of no toxicological significance.

Plasma cholesterol concentrations were increased in males receiving 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805) at a dietary concentration of 800 ppm and in females at all doses. Plasma total protein was increased in males at 800 ppm and triglycerides were increased in both sexes at this dose. Increases were seen in alanine aminotransferase and aspartate aminotransferase activities in females at 240 and 800 ppm. In the absence of any associated adverse pathology findings, these changes in blood chemistry parameters were considered to be of no toxicological significance.

There were no treatment-related effects on haematology or urine clinical chemistry parameters and no evidence of inhibition of cholinesterase activity.

There were no treatment-related pathology findings. After adjustment for final body weight, group mean liver weights were statistically significantly higher than those of

controls for males receiving 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805) at a dietary concentration of 800 ppm and for females at 240 ppm and 800 ppm. However, in the absence of any associated pathology findings in the liver, the increased liver weights may indicate a slight adaptive change and are considered unlikely to be of toxicological importance.

The NOAEL for 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805) was 240 ppm, equal to 19.5 mg/kg bw per day, on the basis of blood chemistry changes at 800 ppm, equal to 65.6 mg/kg bw per day (Lees, 2001).

Genotoxicity of metabolites

2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805; 062/06)

The pirimicarb metabolite 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805; 062/06) was tested for genotoxicity in a number of assays, both in vitro and in vivo (Table 34). In *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P and WP2P *uvrA* no significant response was observed (Callander, 2000a), while it did induce significant responses in L5178Y *Tk*^{+/−} cells, both in the presence of metabolic activation (S9) with treatment for 4 h and in the absence of S9 with treatment for 24 h (Clay, 2001a). No significant response was observed in a test for clastogenicity using cultured human lymphocytes treated in vitro in either the presence or absence of metabolic activation (Fox, 2001a).

No significant responses were observed in an assay for unscheduled DNA synthesis assay in vivo/in vitro in rat liver cells (Clay, 2001c) or an assay for micronucleus formation in bone marrow in mice (Clay, 2001d).

5,6-dimethyl-2-(methylamino)pyrimidin-4-ol (R34865; 062/07)

The pirimicarb metabolite 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol (R34865; 062/07) was tested for genotoxicity in a number of assays, both in vitro and in vivo (Table 35). In *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P

Table 35. Results of studies of genotoxicity with pirimicarb metabolite 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805; 062/06)

End-point	Test object	Dose (LED/HID)	Reference No./Purity (%)	Result	Reference
Gene mutation in vitro	<i>S. typhimurium</i> strains TA100, TA1535, TA1537, TA98; <i>E. coli</i> WP2, WP2 <i>uvrA</i> (±S9 mix)	5000 µg/plate	P4/100	Negative	Callander (2000a)
Gene mutation in vitro	Mouse lymphoma L5178Y cells, <i>Tk</i> ⁺ locus (±S9)	125 µg/ml −S9 (24 h)	P4/100	Positive (24 h)	Clay (2001a)
		500 µg/ml +S9 (4 h)		Positive (4 h)	
Chromosomal aberration in vitro	Human lymphocytes (±S9)	1670 µg/ml	P4/100	Negative	Fox (2001a)
Unscheduled DNA synthesis in vivo/in vitro	Male Alpk:APfSD rat liver cells 4 & 12 h after dosing	200 mg/kg bw per os × 1	P4/100	Negative	Clay (2001c)
Micronucleus formation in vivo	Male C57BL/6JfCD-1/Alpk mouse bone-marrow cells 24, 48 & 72 h after dosing	125 mg/kg bw, per os × 1	P4/100	Negative	Clay (2001d)

LED, lowest effective dose; HID, highest ineffective dose

Table 36. Results of studies of genotoxicity with pirimicarb metabolite 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol (R34865; 062/07)

End-point	Test object	Dose (LED/HID)	Reference No./purity (%)	Result	Reference
Gene mutation in vitro	<i>S. typhimurium</i> strains TA100, TA1535, TA1537, TA98; <i>E. coli</i> WP2, WP2 <i>uvrA</i> ($\pm$ S9)	5000 μ g/plate	P5/100	Negative	Callander (2000b)
Gene mutation in vitro	Mouse lymphoma L5178Y cells, <i>Tk</i> ⁺ locus ($\pm$ S9)	500 μ g/ml –S9 (24 h)	P5/100	Positive (24 h)	Clay (2001b)
		1530 μ g/ml +S9 (4 h)		Negative	
Chromosomal aberration in vitro	Human lymphocytes ($\pm$ S9)	500 μ g/ml	P5/100	Negative	Fox (2001b)

LED, lowest effective dose; HID, highest ineffective dose

and WP2P *uvrA*, no significant response was observed (Callander, 2000b), while it did induce significant responses in L5178Y *Tk*^{+/–} cell assay in the absence of metabolic activation with treatment for 24 h; no significant responses were observed in this assay in either the presence or absence of metabolic activation after treatment for 4 h (Clay, 2001b). No significant response was observed in a test for clastogenicity using cultured human lymphocytes treated in vitro in either the presence or absence of metabolic activation (Fox, 2001b).

3. Observations in humans

The symptoms of carbamate poisoning are similar to those of organophosphorus poisoning, i.e. they occur as a result of inhibition of acetyl cholinesterase activity, although they are less severe and with a much more rapid onset and shorter duration of effect. Typical features are excessive salivation and pupillary constriction in less severe cases, with muscular twitching, incontinence, convulsions and death from respiratory muscle paralysis in more severe cases (Echobichon, 2001).

In cases of inhalational or dermal overexposure, these incapacitating symptoms tend to reduce further exposure by preventing the operator from working. Recovery is rapid and complete, and there is no evidence to suggest chronic or cumulative effects. In cases of suspected poisoning, a blood sample should be taken for analysis of whole blood or erythrocyte cholinesterase activity. Up to 40% cholinesterase inhibition may occur without evidence of systemic symptoms (Lotti, 2000).

The Stewardship Department of Syngenta (formerly Zeneca/ICI Agrochemicals), which includes the occupational health function, has maintained a database of incidents involving chemical exposure of workers since 1983. At the time it was set up it was used to formally record reports of clinical conditions arising during work at the company's research station (at Jealott's Hill, Berkshire) and formulation plant (at Yalding, Kent). Information is gathered at the time of the incident and is recorded on a standard form by the occupational health nurse or the doctor. From the start of 1994, such data have been collected from all sites around the world, using a pro-forma.

Interrogation of the database revealed one report of adverse reactions from a product containing pirimicarb. This was a case of neck skin irritation in a filling and packing operator.

In a report on the medical surveillance of workers involved in production of pirimicarb formulations from 1969 to 1973 at the ICI Yalding factory, Maidstone, United Kingdom (Bagness et al., 1975), blood cholinesterase activities were routinely investigated each week for plasma and erythrocyte activities. Baseline values were established on two to four samples taken before exposure. Inhibitions of 20% were considered within normal limits, with inhibition of > 30% resulting in movement of workers.

During 1969, two of five workers exposed to pirimicarb (for 9–144 h) showed inhibition of cholinesterase activity of >30%. These two workers had processed 14 or 32 tons of Pirimor (an industrial formulation of pirimicarb), and the second, who had been exposed for approximately 144 h, reported headache and nausea. Both cases were attributed to plant failure leading to exposure to pirimicarb dust. Half suits (details not provided) were reportedly worn during charging and filling operations. A third worker was moved after having processed 16 tons of Pirimor because he was feeling unwell, but without showing cholinesterase inhibition.

During 1970, full air suits were worn during such work, with 10 workers exposed to pirimicarb for 8–25 h. No problems with illness or cholinesterase depression were encountered.

During 1971–1973, a new formulation plant designed to minimize production of airborne dust and using exhaust ventilation was used for the production of Pirimor. Depressions of, principally plasma, cholinesterase activity of >20% were detected on numerous occasions.

Workers were often moved when these depressions exceeded 30%. No cases of illness were reported and cholinesterase activity returned to normal limits within days.

In 1972–73, 150 tons of Pirimor were produced during 120, 8 h shifts. On one occasion a worker was removed with depression of plasma and erythrocyte cholinesterase activity caused by a failure of containment equipment.

During the 1973 production of the 50% WG formulation, two workers employed continuously with pirimicarb were monitored. Inhibition of cholinesterase activity (generally <20% reduction in erythrocytes and up to 69% reduction in plasma) was not prevented by the engineering, personal protection equipment (gloves, overalls, ori-nasal dust masks or filtered air hood) and hygiene systems. This failure was attributable to exposure to pirimicarb vapour from the granulation and fluidized bed drying process. When these were ventilated and personal protective hoods (supplied with fresh air) were used, no depression of cholinesterase activity or signs of illness were seen.

Comments

Kinetic studies in rats have demonstrated that pirimicarb administered orally to male and female rats is rapidly and extensively absorbed (>70% of the administered dose) and widely distributed. Radioactivity from [¹⁴C]pyrimidinyl-labelled pirimicarb was excreted predominantly in the urine, while radioactivity from [¹⁴C]carbamoyle-labelled pirimicarb was excreted predominantly in expired air. Tissue retention of radioactivity was low. There were no pronounced sex differences in the routes or rates of excretion. Pirimicarb was extensively metabolized, giving rise to 24 metabolites, 17 of which were identified. The

major metabolic pathway involves the loss of the carbamate moiety to produce a range of substituted hydroxypyrimidines, some of which are glucuronide conjugates.

The acute oral LD₅₀ for pirimicarb was 152 mg/kgbw in male rats and 142 mg/kgbw in female rats, while the acute dermal LD₅₀ of pirimicarb was >2000 mg/kg in both male and female rats. The 4 h inhalation LC₅₀ of pirimicarb in rats was 0.948 mg/l of air in males and 0.858 mg/l of air in males and females respectively. Pirimicarb is not irritating to the rabbit eye or skin. It does, however, have skin sensitizing potential under the conditions of the Magnusson & Kligman maximization test.

In a 21-day study of dermal toxicity in rats, there were no signs of irritation and no clinical signs of systemic toxicity, but there was a reduction in brain cholinesterase activity at 1000 mg/kgbw per day. The NOAEL was 200 mg/kgbw per day.

Acetylcholinesterase that has been inhibited by pirimicarb is rapidly reactivated (both in vivo and in vitro). This property hampers the reliable determination of acetylcholinesterase inhibition in erythrocytes and brain in treated animals, and special attention must be given to crucial methodological features (e.g. time between sampling and measurement, sample temperature and dilution). Consequently, the most reliable indicators of an effect are clinical signs, which usually occur when acetylcholinesterase inhibition is >50% at nerve terminals.

In experiments with multiple doses, common toxicological targets are blood and acetylcholinesterase activity.

Three dietary studies of up to 90 days in duration have been conducted in rats. In the first study (in which animals were given diets containing pirimicarb at a concentration of 250 or 750 ppm for 90 days) there were no adverse clinical, haematological or other pathological effects. A reduction in plasma cholinesterase activity was seen at 750 ppm, providing evidence for the absorption of the compound from the intestinal tract. In the second study (in which animals were given diets containing pirimicarb at a concentration of 250 or 750 ppm for 8 weeks) a clear reduction in body-weight gain was seen at 750 ppm, with a slight reduction at 250 ppm. These growth reductions were completely reversible after an 8-week recovery period. In the final study (in which animals were given diets containing pirimicarb at a concentration of 100, 175, 250 or 750 ppm for 8 weeks) there were no adverse clinical effects. A reduction in body-weight gain and food consumption was seen at 750 ppm. Owing to the effect on body-weight gain in the second study at 250 ppm, the Meeting concluded that the overall NOAEL in short-term studies in rats was 175 ppm, equivalent to 17.5 mg/kgbw per day.

Reports of three studies of 13–16 weeks in duration in dogs were available. In the first study, beagle dogs were given diets delivering pirimicarb at a dose of 0, 4, 10 or 25 mg/kg bw per day for at least 90 days. Body weight was reduced at the highest dose and plasma cholinesterase activity was reduced at 10 and 25 mg/kgbw per day. Bone-marrow changes, indicative of increased erythropoiesis, were observed in the terminal blood films in all treatment groups. Three out of 32 animals (two receiving 25 mg/kgbw per day and one receiving 10 mg/kgbw per day) developed anaemia. Two dogs of each sex per group were killed after 90 days (one male only in the group receiving 25 mg/kgbw per day, as one had been killed after 10–11 weeks) and the remainder were allowed to recover untreated for 28 days. Partial recovery from the bone-marrow changes was evident at 28 days after cessation of

treatment. A NOAEL for this study was not identified, therefore a second study with diets delivering pirimicarb at a dose of 0.4, 1.8 and 4 mg/kg bw per day for at least 90 days was conducted. An additional group received pirimicarb at 4 mg/kg bw per day for 180 days. There were no adverse clinical or pathological effects, but dogs at 4 mg/kg bw per day showed evidence of increased erythropoietic activity in the bone marrow. The NOAEL was 1.8 mg/kg bw per day. In the third study, foxhounds were given diets delivering pirimicarb at a dose of 0, 2 or 25/50 mg/kg bw per day for 16 weeks, followed by a 7-week recovery period. The dose of 25 mg/kg bw per day was increased to 50 mg/kg bw per day from week 8. Anaemia and reticulocytosis developed in dogs receiving a dose of 50 mg/kg bw per day, and bone-marrow changes, characterized by an increase in normoblasts and hypoplasia, were observed. Both the anaemia and bone-marrow changes were reversible when the dose was reduced to 25 mg/kg bw per day, or upon cessation of treatment. The NOAEL in this study in dogs was 2 mg/kg bw per day.

In addition, there were two 2-year dietary studies in dogs, and a more recent, guideline-compliant, 1-year study in which pirimicarb was administered in capsules. In the first 2-year dietary study, designed to reproduce and characterize the anaemia, two out of four unrelated beagles had an immune haemolytic anaemia when exposed to pirimicarb at a dose of 25 or 50 mg/kg bw per day for at least 3 months. Dogs at up to and including 2 mg/kg bw per day did not show such effects. The anaemia was completely reversible after withdrawal of the compound. Other dogs in the same study showed no haematological changes when exposed to pirimicarb at a dose of up to 50 mg/kg bw per day for 2 years. In the second dietary study, pirimicarb was administered at a dose of 0.4, 1.8 or 4 mg/kg bw per day for 2 years. There were no adverse changes in growth rate, blood and urine clinical chemistry, organ weights or histopathology. At 4 mg/kg bw per day, there were reductions in haemoglobin concentration and erythrocyte volume fraction in males, and a slight increase in the erythroid to myeloid ratio in two females. No adverse changes were detected in the bone marrow. None of the dogs developed overt anaemia. In the most recent study, groups of beagles were dosed orally with gelatine capsules containing pirimicarb at a dose of 0, 3.5, 10 or 25/35 mg/kg bw per day for 1 year. The highest dose of 35 mg/kg bw per day could not be sustained owing to adverse clinical signs in week 1, so from week 4 onwards, the dose was reduced to 25 mg/kg bw per day. One female dog receiving 25 mg/kg bw per day was killed humanely in week 36 after significant body-weight loss and the development of anaemia. The haematological changes in this dog were characterized by increased erythropoietic activity in the bone marrow and by histological changes consistent with increased erythrocyte breakdown. No other dog showed any treatment-related haematological changes; however, increased haemosiderin deposition was observed in the liver and spleen of dogs at 25 mg/kg bw per day. The NOAEL in this study was 3.5 mg/kg bw per day; this value is very close to the LOAEL of 4 mg/kg bw identified in three other experiments. The overall NOAEL was 2 mg/kg bw per day in dogs.

The carcinogenic potential of pirimicarb has been assessed in feeding studies of 80 and 96 weeks duration in mice and 104 weeks in rats. In both species, the highest dose tested induced moderate levels of toxicity. Two studies of carcinogenicity were conducted in Alderley Park Swiss-derived mice and one with in C57 black Alderley Park mice; however, one of the studies in Swiss-derived mice, a study that pre-dated the establishment of GLP, was not considered adequate for the assessment of the carcinogenic potential of pirimicarb, owing to a high incidence of respiratory disease. Similarly, three pre-GLP studies in rats were not adequate for carcinogenicity assessment owing to high incidences of respiratory disease.

In Alderley Park Swiss-derived mice given diets containing pirimicarb at a concentration of 0, 0, 200, 400 or 1600 ppm for up to 96 weeks, there was a significant increase in the incidence of liver tumours classified as type A (hyperplastic nodules and benign neoplasms) and type B nodules (which showed characteristics of malignancy) at the highest dose (equivalent to approximately 240 mg/kg bw per day), with no evidence of nodule induction at lower doses. The incidence of liver nodules was above the range for historical controls for the test laboratory. This finding was not confirmed in C57 black Alderley Park mice given diets containing pirimicarb at a concentration of 0, 50, 200 or 700 ppm for at least 80 weeks, where there was no evidence that liver tumours induced by pirimicarb at doses of up to 700 ppm (equal to approximately 94 and 130 mg/kg bw per day in males and females, respectively). Thus, the significant response was only seen at a very high dose and after a prolonged exposure time in a mouse strain with a high and variable background incidence of liver tumours.

Also in Alderley Park Swiss-derived mice, the incidence of pulmonary adenoma was significantly increased in both sexes at the highest dose, but with no significant response at lower doses. Data from concurrent and historical controls indicated a high and variable spontaneous background incidence of pulmonary adenomas in this strain of mouse. Given the overall variability in the incidence of pulmonary adenoma in these mice, the observation of an increased tumour incidence at the highest dose does not give cause for concern in terms of cancer risk. A small, statistically significant, increase in the incidence of pulmonary adenoma was also observed in female (but not male) C57 black Alderley Park mice at the highest dose tested, with no evidence of pulmonary adenoma induction at lower doses. In contrast to Swiss-derived mice, C57 black mice have a low spontaneous background incidence of pulmonary adenoma. Therefore, the occurrence of these tumours is considered to be treatment-related. The absence of a significant response in male mice could be a chance difference in the incidence of an uncommon tumour type. The Meeting concluded that oral administration of pirimicarb at a dose of up to 700 ppm, equal to 94 and 130 mg/kg bw per day for males and females, respectively, for at least 80 weeks produced a small increase in the incidence of benign lung tumours in females, but not in males. The NOAEL for non-neoplastic effects was 50 ppm, equal to 6.7 mg/kg bw per day, on the basis of slight haematological changes at 200 ppm, equal to 26.6 mg/kg bw per day in the 80-week study.

A 2-year study in rats showed that dietary administration of pirimicarb at 0, 75, 250 or 750 ppm resulted in reduced body-weight gains and food consumption in both sexes at 750 ppm, indicating that a maximum tolerated dose had been achieved. There was also a slight reduction in body-weight gain at 250 ppm in females. There were increases in plasma cholesterol at all observation times at 750 ppm, at weeks 13 and 26 at 250 ppm and (in females only) at week 13 at 75 ppm. Plasma concentrations of triglycerides were increased at 750 ppm in males at weeks 52 and 78, and in females at weeks 13 and 26. Males fed diets containing pirimicarb at 750 ppm showed a small increase in incidence and severity of necrosis in the brain. The significance of this is equivocal, but could not be dismissed as being incidental to treatment with pirimicarb. Females fed diets containing pirimicarb at 750 ppm showed an increased severity of sciatic nerve demyelination and an increased severity and incidence of voluntary muscle degeneration that were considered to be an exacerbation of a spontaneous age-related change. Overall, the findings in the brain, sciatic nerve and voluntary muscle were minor, confined to the highest dose and did not elicit any clinical signs of increased neurological dysfunction. Plasma cholinesterase activity was slightly reduced in females at 250 and 750 ppm, demonstrating the absorption of the test substance, but brain and erythrocyte cholinesterase activities were not affected at any dose. The

NOAEL for non-neoplastic effects was 75 ppm, equal to 3.7 mg/kg bw per day, on the basis of reductions in body weights and increases in plasma cholesterol and triglycerides at 250 ppm, equal to 12.3 mg/kg bw per day. There was an overall higher, but non-significant incidence in the number of male rats with tumours at 250 and 750 ppm. This reflected an increased incidence of males with multiple tumours at 250 ppm and of males with single tumours at 750 ppm. There were also small increased incidences of astrocytoma of the brain in all treated groups and in females at 750 ppm, but these were not statistically significant and no dose-response relationship was evident. As there was a decreased incidence of males with multiple tumours at 750 ppm and there were no consistent effects across doses, the Meeting concluded that pirimicarb did not induce a carcinogenic response in any tissue.

The Meeting concluded that pirimicarb did not demonstrate clear evidence of carcinogenic potential in mice or rats. The liver tumours were not consistently found in the two studies in mice, while the benign lung tumours were found only at the highest dose and with clear evidence for a threshold. There were no compound-related increases in any tumour type in rats.

Pirimicarb was tested for genotoxicity in an adequate range of studies, both in vitro and in vivo. The results observed were largely negative. A small increase in mutant frequency in the assay for mutation in L5178Y mammalian cells, in the presence of metabolic activation, was considered not to be a significant alert for genotoxicity. Pirimicarb has shown no evidence of genotoxic potential in several test systems in vivo.

The Meeting concluded that pirimicarb is unlikely to pose a genotoxic risk to humans.

Because the results of the studies of carcinogenicity in rodents were judged not to provide evidence of carcinogenic potential, an evaluation supported by the lack of genotoxic potential, the Meeting concluded that pirimicarb is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, the NOAEL for adult rats and for their offspring was 200 ppm, equal to 23 mg/kg bw per day in adults, on the basis of systemic toxicity in the parental rats and reduced body-weight gain in the parental rats and the offspring at a dose of 750 ppm, equal to 88 mg/kg bw per day; no other signs of reproductive toxicity were observed at this dose, the highest tested. In studies of developmental toxicity in rats, the NOAEL for fetal toxicity and maternal toxicity was 25 mg/kg bw per day on the basis of reduced fetal weight and maternal body-weight gains at 75 mg/kg bw per day. In studies of developmental toxicity in rabbits, the NOAEL for fetal and developmental toxicity was 60 mg/kg bw per day, the highest dose tested, and the NOAEL for maternal toxicity for 10 mg/kg bw per day, on the basis of reduced food consumption and body-weight gains at 60 mg/kg bw per day. The results from the two studies of developmental toxicity and the study of reproductive toxicity demonstrated that fetuses and pups were not more susceptible than adults to toxicity caused by pirimicarb.

In a study of acute neurotoxicity in rats, a single oral administration of pirimicarb at 110 mg/kg bw per day by gavage resulted in early mortalities, adverse clinical signs and reductions in brain, erythrocyte and plasma cholinesterase activities. These clinical and enzyme activity changes were transient and were not associated with histopathological changes in the nervous system. At a dose of 40 mg/kg bw, there was evidence of toxicity seen as a single mortality, transient adverse clinical signs in a few rats and reduced

motor activity on day 1. Plasma cholinesterase activity was reduced, but this observation was not accompanied by biologically significant reductions in brain or erythrocyte cholinesterase activity at this dose. The Meeting concluded that the NOAEL for acute neurotoxic potential was 10 mg/kg bw per day and this value formed the basis for the ARfD. The acute toxic effects of pirimicarb are due to inhibition of acetylcholinesterase activity at nerve terminals. Inhibition of acetylcholinesterase by carbamates (such as pirimicarb) and organophosphates involves the carbamoylation or phosphorylation of the active site on the enzyme. Plasma cholinesterase is inhibited by a similar mechanism; therefore, although this is a toxicologically irrelevant target, its inhibition serves as an indicator of exposure and a surrogate for the response of acetylcholinesterase. The degree of enzyme inhibition is dependent on the concentration of inhibitor, a property that is particularly significant for carbamates because of the short occupation half-life at the active site of the enzyme (a few minutes, both in vitro and in vivo). Consequently, plasma cholinesterase activity was inhibited after a single dose of pirimicarb at 25 mg/kg bw by gavage, but not after dietary exposure corresponding to a daily dose of about 40 mg/kg bw, when the C_{max} would have been lower.

In a 90-day study of neurotoxicity, rats fed diets containing pirimicarb at a concentration of 250 or 1000 ppm resulted in toxicity evident as reduced growth and food consumption or /utilization. There were no treatment-related effects on the functional observational battery, motor activity, cholinesterase and neurotoxic esterase activities or neuropathology. The NOAEL for neurotoxicity in this study was 1000 ppm, equal to 77 mg/kg bw per day, the highest dose tested.

Studies of toxicity have been conducted on a number of metabolites of pirimicarb: three carbamate metabolites, three hydroxypyrimidine metabolites, and three guanidine metabolites. The acute toxicities of two carbamates (the desmethyl pirimicarb and the desmethylformamido pirimicarb metabolites) were of the same order as that of pirimicarb itself, while the LD_{50} s of all the other seven metabolites were less or considerably less than that of pirimicarb itself. In addition, some of these metabolites have been subjected to testing in studies of toxicity after with repeated doses and some to assays for genotoxicity. Desmethyl pirimicarb and desmethylformamido pirimicarb had effects on cholinesterase that were similar to those caused by pirimicarb itself and are included in the residue definition, since they occur in plants. In 28- and 90-day studies of toxicity in rats, the hydroxypyrimidine metabolite, 2-dimethylamino-5,6-dimethylpyrimidin-4-ol (and, by implication, its mammalian metabolite, 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol) was of low toxicity, the NOAEL being was 240 ppm, equal to 19.5 mg/kg bw per day, on the basis of blood chemistry changes at 800 ppm, equal to 65.6 mg/kg bw per day. NOAELs could not be identified because testing was restricted to single doses in the cases of desmethyl pirimicarb (100 mg/kg bw per day for 2 weeks) and desmethylformamido pirimicarb (25 mg/kg bw per day for 2 weeks). Each of these metabolites caused slight hypochromia (reduced haemoglobin concentrations per cell). Genotoxicity tests were conducted with the hydroxypyrimidines, 2-dimethylamino-5,6-dimethylpyrimidin-4-ol and 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol. Both metabolites, like pirimicarb itself, produced some weak evidence of mutagenic effects in the assay in mouse lymphoma cells, but not in other assays. The Meeting concluded that, within the limitations of studies conducted (short-term and only in rats), desmethyl pirimicarb and desmethylformamido pirimicarb have toxicological profiles similar to that of pirimicarb itself.

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

There have been reports of production workers with inhibition of plasma and erythrocyte cholinesterase activity of sufficient severity to result in their movement to other work areas.

Toxicological evaluation

An ADI of 0–0.02 mg/kg bw was established for pirimicarb and its dimethyl carbamate metabolites on the basis of the overall NOAEL of 2 mg/kg bw per day in 90-day and 2-year studies in dogs treated by dietary administration, and with a safety factor of 100.

The Meeting established an ARfD of 0.1 mg/kg bw for pirimicarb on the basis of a NOAEL of 10 mg/kg bw in a study of acute neurotoxicity in rats. Although a reduced safety factor would be supported by the reversibility of clinical signs and the $C_{\max}$ -dependency of the effects, a safety factor of 100 was used in consideration of the steep dose–response curve (indicated by a mortality at the LOAEL) and the lack of reliable measurement of acetylcholinesterase inhibition. Haematotoxicity in dogs was also considered as a possible end-point for an ARfD; however, in one study in dogs haematological parameters were measured during treatment before the onset of anaemia, indicating that this condition did not occur after a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month and 21-month studies of toxicity and carcinogenicity ^a	Toxicity	50 ppm, equal to 6.7 mg/kg bw per day	200 ppm, equal to 27 mg/kg bw per day
		Carcinogenicity	200 ppm, equal to 37 mg/kg bw per day	700 ppm, equal to 94 mg/kg bw per day
Rat	24-month study of toxicity and carcinogenicity ^a	Toxicity	75 ppm, equal to 3.7 mg/kg bw per day	250 ppm, equal to 12.3 mg/kg bw per day
		Carcinogenicity	750 ppm, equal to 37 mg/kg bw per day ^c	—
	Two-generation study of reproductive toxicity ^a	Parental toxicity	200 ppm, equal to 22 mg/kg bw per day	750 ppm, equal to 88 mg/kg bw per day
		Offspring toxicity	200 ppm, equal to 23 mg/kg bw per day	750 ppm, equal to 88 mg/kg bw per day
	Developmental toxicity ^b	Maternal toxicity	25 mg/kg bw per day	75 mg/kg bw per day
	Single-dose neurotoxicity ^b 3-month study of neurotoxicity ^a	Embryo- and fetal toxicity	25 mg/kg bw per day	75 mg/kg bw per day
		Neurotoxicity	10 mg/kg bw	40 mg/kg bw per day
Rabbit	Developmental toxicity ^b	Neurotoxicity	1000 ppm, equal to 81 mg/kg bw per day ^c	—
		Maternal toxicity	10 mg/kg bw per day	60 mg/kg bw per day
Dog	90-day and 2-year studies of toxicity ^a	Embryo and fetal toxicity	60 mg/kg bw per day ^c	—
		Toxicity	2 mg/kg bw per day	4 mg/kg bw per day

^a Dietary administration

^b Gavage administration

^c Highest dose tested

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

*Studies that would provide information useful to the continued evaluation
of the compound*

Further observations in humans

Summary of critical end-points for pirimicarb

Absorption, distribution, excretion and metabolism in animals

Rate and extent of oral absorption	Rapid; >80% absorbed
Dermal absorption	No study of direct dermal absorption available, but brain cholinesterase activity was inhibited after application of pirimicarb to the skin of rats, indicating absorption by this route
Distribution	Distributed throughout the body; highest concentrations in liver and fat
Potential for accumulation	Low, owing to rapid excretion
Rate and extent of excretion	Rapid, >80% excretion within 24 h
Metabolism in animals	Extensive
Toxicologically significant compounds (animals, plants and environment)	Parent and the metabolites desmethyl pirimicarb and desmethylformamido pirimicarb

Acute toxicity

Rat, LD ₅₀ , oral	142 mg/kg bw
Rat, LC ₅₀ , inhalation	0.858 mg/l (4 h)
Rabbit, LD ₅₀ , dermal	>2000 mg/kg bw
Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Not irritating
Skin sensitization	Sensitizing (Magnusson and Kligman test)

Short-term studies of toxicity

Target/critical effect	Body-weight gain decrement, haemolytic anaemia or cholinesterase inhibition
Lowest relevant oral NOAEL	1.8 mg/kg bw per day: (3_month study in dogs)
Lowest relevant dermal NOAEL	2000 mg/kg bw per day (21_day study in rats)
Lowest relevant inhalation NOAEC	No data available

Genotoxicity

No genotoxic potential: negative in vivo, one study gave positive results in vitro

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Blood/anaemia, increased plasma lipids
Lowest relevant NOAEL	2 mg/kg bw per day (24-month study in dogs) 3.7 mg/kg bw per day (24_month study in rats)

Carcinogenicity

Benign lung tumours in mice induced by a non-genotoxic mode of action; a clear NOAEL was identified; therefore pirimicarb is unlikely to pose a carcinogenic risk to humans

Reproductive toxicity

Reproductive target/critical effect	Reduced parental and offspring body weight, clinical signs
Lowest relevant reproductive NOAEL	23 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; reduced fetal body weight at maternally toxic doses
Lowest relevant developmental NOAEL	25 mg/kg bw per day (rat)

Neurotoxicity/delayed neurotoxicity

Target/critical effect	Nervous system/cholinergic signs
Lowest relevant NOAEL	10 mg/kg bw

90-day neurotoxicity

Target/critical effect	Nervous system/cholinergic signs
Lowest relevant NOAEL	77 mg/kg bw per day

Other toxicological studies

Desmethyl pirimicarb and desmethylformamido pirimicarb have acetylcholinesterase-inhibiting activity in rats (no studies in dogs)

Medical data

There have been a few reports of cholinesterase inhibition in workers exposed during manufacture

Summary

	<i>Value</i>	<i>Study</i>	<i>Safety factor</i>
ADI	0–0.02 mg/kg bw	Dog; haematological changes in short- and long-term studies	100
ARfD	0.1 mg/kg bw	Rat; mortality and clinical signs of neurotoxicity in a study of acute neurotoxicity	100

References

- Bagness, J.E., Harmer, C.M. & Willis, G.A. (1975) Observations on operators during formulation. ICI Plant Protection Division. Unpublished report No. TMF 1126 C (study dates, 1969–1973).
- Bakemeier, R.F. & Leddy, J.P. (1968) Erythrocyte autoantibody associated with alpha-methyldopa: heterogeneity of structure and specificity. *Blood*, **32**, 1–14.
- Banham, P.B., Carter, A. & James, J. (1980) Pirimicarb diet analysis: analytical investigations associated with the lifetime feeding study in the mouse (PM0002). Unpublished report No. CTL/P/511 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Before guidelines and before GLP.
- Baron, R.L. (1991) Carbamate insecticides. In: Hayes, W.J. & Laws, E.R., eds, *Handbook of Pesticide Toxicology, Vol. 3. Classes of Pesticides*. New York: Academic Press, pp. 1125.
- Brown, A. (1997a) Pirimicarb: whole body autoradiography in the rat. Unpublished report No. CTL/P/5383 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Zeneca Agrochemicals. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.
- Brown, A. (1997b) Pirimicarb: excretion and tissue distribution of a single oral dose (1 mg/kg) in the rat. Unpublished Report No. CTL/P/5329 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Zeneca Agrochemicals. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987) Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.
- Brown, A. (1997c) Pirimicarb: excretion and tissue distribution of a single oral dose (50 mg/kg) in the rat. Unpublished report No. CTL/P/5381 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.
- Brown, A. (1997d) Pirimicarb: excretion and tissue distribution of a single oral dose (1 mg/kg) in the rat following repeat dosing. Unpublished Report No. CTL/P/5382 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.
- Bunn, H.F. & Rosse, W. (2001) Hemolytic anemias and acute blood loss (Chapter 108). In: Braunwald, E., Fauci, A., Kasper, K.L., Hauser, S.L., Longo, D.L. & Jameson, I.L., eds, *Harrison's Principles of Internal Medicine*, 15th Ed., New York: McGraw-Hill, Medical Publishing Division.
- Callander, R.D. (1995) Pirimicarb: an evaluation of the mutagenic potential using *S. typhimurium* and *E. coli*. Unpublished report No. CTL/P/4798 from Central Toxicology Laboratory, Zeneca. Conducted according to OECD guideline 471 and 472 (1983a, b). GLP compliant.
- Callander, R.D. (2000a) Pirimicarb metabolite 062/06 (R031805): bacterial mutation assay in *S. typhimurium* and *E. coli*. Unpublished report No. CTL/YV4619/REG/REPT from Central Toxicology Laboratory; Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 471/472 (1983). GLP compliant.
- Callander, R.D. (2000b) Pirimicarb metabolite 062/07 (R034865): bacterial mutation assay in *S. typhimurium* and *E. coli*. Unpublished report No. CTL/YV4620/REG/REPT from Central Toxicology Laboratory; Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 471/472 (1983). GLP compliant.
- Chanarin, I. (1969) *The Megaloblastic Anaemias*. Oxford & Edinburgh: Blackwell Scientific Publications, pp. 40–63, 708–714.
- Clapp, M.J., Garner, R. & Litchfield, M.H. (1972) Pirimicarb: 2-year feeding study in the rat. Unpublished report No. HO/IH/P/24 from ICI Ltd, Industrial Hygiene Research Laboratories.
- Clay, P. (1996) Pirimicarb: L5178Y TK^{+/−} mouse lymphoma mutation assay. Unpublished report No. CTL/P/5080 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 476 (1983). GLP compliant.
- Clay, P. (2001a) Pirimicarb metabolite 062/06 (R031805): L5178Y TK^{+/−} mouse lymphoma mutation assay. Unpublished report No: CTL/VV0225/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 4736 (1997). GLP compliant.

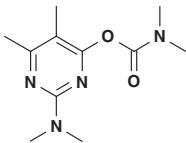
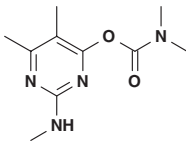
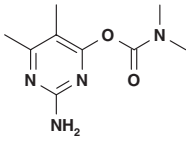
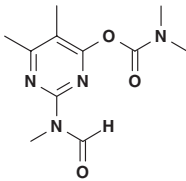
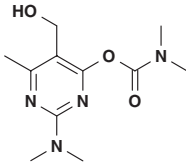
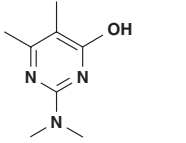
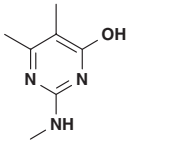
- Clay, P. (2001b) Pirimicarb metabolite 062/07 (R034865): L5178Y TK⁺ mouse lymphoma mutation assay. Unpublished report No: CTL/VV0226/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 476 (1997). GLP compliant.
- Clay, P. (2001c) Pirimicarb metabolite 062/06 (R031805): *in vivo* rat liver unscheduled DNA synthesis assay. Unpublished report No: CTL/SR1104/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 486 (1997). GLP compliant.
- Clay, P. (2001d) Pirimicarb metabolite 062/06 (R031805): mouse bone marrow micronucleus test. Unpublished report No. CTL/SM1105/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 474 (1997). GLP compliant.
- Clapp, M.J., Garner, R. & Litchfield, M.H. (1972) Pirimicarb: 2-year feeding study in the rat. Unpublished report No. HO/IH/P/24 from ICI Central Toxicology Laboratory. Submitted to WHO by ICI Ltd.
- Conning, D.M., Garner, R. & Griffiths, D. (1968) Ninety day oral toxicity of PP062—beagle dogs (Part 1). Unpublished report No. CTL/R/241 from ICI Ltd, Industrial Hygiene Research Laboratories. Submitted to WHO by ICI Ltd.
- Conning, D.M., Garner, R. & Griffiths, D. (1969) Ninety day oral toxicity of PP062—beagle dogs (Part 2). Unpublished report No. CTL/R/248 from ICI Ltd, Industrial Hygiene Research Laboratories. Submitted to WHO by ICI Ltd.
- Dacie, J.V. (1962) *The Haemolytic Anaemias, Part II. Auto-immune Haemolytic Anaemia*. London: J. & A. Churchill.
- Dacie, J.V. (1967) *The Haemolytic Anaemias, Part III. Congenital and Acquired*. London: J. & A. Churchill.
- Echobichon, D.J. (2001) Toxic effects of pesticides. In: Klaassen, C.D., ed., *Casarett and Doll's Toxicology*. 6th ed., New York, USA: Mc Graw Hill.
- Ellman, G.L., Courtney, D.K., Andres, V. & Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 88–95.
- Fletcher, K. (1971a) Pirimicarb (PP062): subacute oral toxicity of the plant metabolite, desmethylpirimicarb. Unpublished report No. HO/IH/T/842 from Industrial Hygiene Research Laboratories (now known as Central Toxicology Laboratory), Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Fletcher, K. (1971b) Pirimicarb (PP062): subacute oral toxicity of the plant metabolite, desmethylformamidopirimicarb. Unpublished report No. HO/IH/T/843 from Industrial Hygiene Research Laboratories (now known as Central Toxicology Laboratory), Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Fletcher, K. & Sotheran, J. (1971) Pirimicarb. 3-generation reproduction study in rat. Study No. CTL/R/339. Unpublished report from ICI Central Toxicology Laboratory. Submitted to WHO by ICI Ltd.
- Flexner, J.M. & Hartmann, R.C. (1960): Megaloblastic anemia associated with anticonvulsant drugs. *Am. J. Med.*, **28**, 386–396.
- Fox, T. (1978) Pirimicarb: dietary toxicity study in foxhounds. Unpublished report No. CTL/C/543 (Hazleton Report No. 1371-72/2) from Hazleton Laboratories, Europe Ltd, Harrogate, England. Submitted to WHO by Syngenta Crop Protection AG. Conducted before the guidelines and GLP.
- Fox, V. (2001a) Pirimicarb metabolite 062/06 (R031805): *in vitro* cytogenetic assay in human lymphocytes. Unpublished report No. CTL/SV1030/REG/REPT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 473 (1997). GLP compliant.
- Fox, V. (2001b) Pirimicarb metabolite 062/07 (R34865): *in vitro* cytogenetic assay in human lymphocytes. Unpublished report No. CTL/SV1031/REG/REPT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 473 (1997). GLP compliant.
- Garner, R. (1972) Pirimicarb anaemia (a chemically-induced autoimmune haemolytic anaemia). Unpublished report No CTL/P/61 from ICI Ltd, Industrial Hygiene Research Laboratories.
- Garner, R., Conning, D.M. & Hermoso-Perez, C. (1967) Factor VII deficiency in beagle dog plasma and its use in the assay of the human factor VII. *Nature (Lond.)* **216**, 1130–1132.
- Garner, R. & Smith, •• (1972) Pirimicarb: 2-year feeding study in the rat. Unpublished report No. CTL/P/24 from ICI Ltd, Industrial Hygiene Research Laboratories.
- Garner, R., Phillips, C.E. & Slack, P. (Report revised in 1995 but originally issued in 1972) First revision to pirimicarb (PP062): pirimicarb anaemia (a chemically-induced autoimmune haemolytic anaemia). Unpublished report No. CTL/P/61 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. No guidelines applicable. Conducted before GLP.

- Gledhill, A.J. (1998) Pirimicarb: biotransformation in the rat. Unpublished report No. CTL/P/5530 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.
- Griffiths, D. & Conning, D.M. (Report revised in 1995 but originally issued in 1968) First revision to ninety-day oral toxicity of PP062—albino rats. Unpublished report No. CTL/R/237 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conduct consistent with 87/302/EEC B.26 and OECD 408 (1981), but before GLP.
- Harris, J.W. (1954) Studies on the mechanism of a drug-induced haemolytic anemia. *J. Lab. Clin. Med.*, **44**, 809–810 (Abstract 73).
- Hodge, M.C.E. (1989) Pirimicarb: teratogenicity study in the rat. Unpublished report No. CTL/P/2745 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 414 (1981).
- Hodge, M.C.E. (1995a) Subchronic studies with pirimicarb (PP062) in the beagle dog: Ninety-day oral toxicity of PP062—beagle dogs (Part 1) and oral toxicity of PP062—beagle dogs (Part 2). Unpublished report No. CTL/P/4593 incorporating CTL/R/241 and CTL/R/248 (previously IHR/241 and IHR/248) from Central Toxicology Laboratory (formerly known as Industrial Hygiene Research Laboratories), Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Hodge, M.C.E. (1995b) First revision to pirimicarb (PP062): 2-year feeding study in beagle dogs'. Unpublished report No. CTL/R/337 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 452 (1981), 67/548/EEC B.30, US EPA Pesticide Guidelines Subdivision F, reference No. 83-1 (1994).
- Horner, S.A. (1996a) Pirimicarb: acute neurotoxicity study in rats. Unpublished report No. CTL/P/5232 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to FIFRA § 82–88.
- Horner, S.A. (1996b) Pirimicarb: subchronic neurotoxicity study in rats. Unpublished report No. CTL/P/5233 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to FIFRA § 81–87.
- Horner, S.A. (1998) Pirimicarb: 1-year oral toxicity study in dogs. Unpublished report No. CTL/P/5690 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 452 (1981), 87/302/EEC OJ L133 (1988), US EPA Pesticide Guidelines Subdivision F, reference No. 83-1 (1994). GLP compliant.
- Huennekens, F.M. (1966) Biochemical functions and interrelationships of folic acid and vitamin B12. *Prog. Hematol.*, **5**, 83–104.
- Jones, K. & Howard, C.A. (1989) Pirimicarb (technical): an evaluation in the mouse micronucleus test. Unpublished report No. CTL/P/2641 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 474 (1983). GLP compliant.
- Kennelly, J.C. (1990) Pirimicarb: assessment for the induction of unscheduled DNA synthesis in rat hepatocytes *in vivo*. Unpublished report No. CTL/P/2824 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 486 (1983). GLP compliant.
- Kidd, P. & Mollin, D.L. (1957) Megaloblastic anemia and vitamin-B12 deficiency after anticonvulsant therapy: report of two cases, *BMJ*, **ii**, 974–976.
- Lees, D. (2001) Pirimicarb metabolite 062/06 (R031805): 90-day dietary toxicity study in rats. Unpublished report No. CTL/ PR1184 from Syngenta UK. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 408 (1981), 87/302/EEC B.26, US EPA OPPTS 870.3100. GLP compliant.
- Lees, D. & Connolly, H.J. (1995a) Pirimicarb: acute oral toxicity to the rat. Unpublished report No. CTL/P/4802 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.
- Lees, D. & Connolly, H.J. (1995b) Pirimicarb: acute dermal toxicity to the rat. Unpublished report No. CTL/P/4855 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.
- Lees, D. & Connolly, H.J. (1995c) Pirimicarb: skin irritation to the rabbit. Unpublished report No. CTL/P/4858 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.

- Lees, D. & Doyle, C.E. (1995) Pirimicarb: eye irritation to the rabbit. Unpublished report No. CTL/P/4706 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.
- Lees, D. & Leah, A.M. (1995) Pirimicarb: 21-day dermal toxicity to the rat. Unpublished report No. CTL/P/4805 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 410 (1981), 92/69/EEC B.9. GLP compliant.
- Lefevre, V.K. & Parkinson, G.R. (1974) Pirimicarb (PP062; 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate): acute oral toxicities of plant and mammalian metabolites. Unpublished report No. CTL/P/101B from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted before GLP.
- Lotti, M. (2000) Organophosphorus compounds. In: Spencer, P.S. & Schaumburg, H.H., eds, *Experimental and clinical neurotoxicology*, 2nd Ed., New York, USA: Oxford University Press.
- McGregor, D.B. (1974) Dominant lethal study in mice of ICI PP062. Zeneca unpublished report No. CTL/C/256 from Inveresk Research International. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 478 (1983). Conducted before GLP.
- MacIntosh, P.C. & Hutchinson, J.L. (1960) Megaloblastic anaemia due to anticonvulsant therapy: report of a case responding to vitamin B12. *Can. Med. Ass. J.*, **82**, 365–368.
- Magnusson, B. & Kligman, A.M. (1970) *Allergic contact dermatitis in the guinea pig. Identification of contact allergens*. Springfield, Illinois: Charles C. Thomas.
- Milburn, G.M. (1989) Pirimicarb: teratogenicity study in the rabbit. Unpublished report No. CTL/P/2680 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD 414 (1981).
- Moxon, M.E. (1991) Pirimicarb: multigeneration study in the rat. Unpublished report No. CTL/P/2940 Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 416 (1983). GLP compliant.
- Palmer, S.M. & Samuels, D.M. (1974) Pirimicarb: 80-week carcinogenic study in mice. CTL Study No. HO/CTL/P121/B. Submitted to WHO by ICI.
- Parkinson, G.R. (1974) Pirimicarb metabolites (R31680 and R35140): acute oral toxicity. Unpublished report No. HO/CTL/P/144 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Parkinson, G.R. (1975) Oral toxicities of three guanidine compounds. Unpublished Report No. CTL/P/160 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Parkinson, G.R. (1978) Pirimicarb metabolite R34885: subacute oral toxicity to rats. Unpublished report No. CTL/P/402 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Parkinson, G.R. (Report revised in 1979 but originally issued in 1978) Pirimicarb metabolite R34836: subacute oral toxicity to rats. Unpublished report No. CTL/P/401 (revised) from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Parr-Dobrzanski, R.J. (1994) Pirimicarb: 4-hour acute inhalation toxicity study in the rat. Unpublished report No. CTL/P/4522 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.
- Paul, D., Richards, D., Banham, P.B. & Weight, T.M. (Original report date: 1978; revised by Hodge, M.C.E., 1995) First revision to pirimicarb: growth study to determine a no-effect level in the female rat. Unpublished report No. CTL/P/408 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Rattray, N.J. (1998) Pirimicarb: 80-week carcinogenicity study in mice. Unpublished report No. CTL/P/5839 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 451 (1983). GLP compliant.
- Rattray, N. & Leah, A.M. (1990) Pirimicarb: skin sensitisation to the guinea pig. Unpublished report No. CTL/P/3087 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.
- Reynolds, E.H., Hallpike, J.F., Phillips, B.M. & Matthews, D.M. (1965) Reversible absorptive defects in anticonvulsant megaloblastic anaemia. *J. Clin. Path.*, **18**, 593–598.
- Richards, D., Banham, P.B. & Weight, T.M. (1978) Pirimicarb: paired feeding study in the female rat. Unpublished report No. CTL/P/407 Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.
- Sotheran, M.F., Banham, P.B., Jackson, D.G., Taylor, K., Weight, T.M. & Woollen, B.H. (1980) Pirimicarb: lifetime feeding study in the mouse. Unpublished report No. CTL/P/0491 from Central Toxicology

- Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted before OECD guidelines and GLP.
- Tinston, D.J. (1992) Pirimicarb: Two-year feeding study in rats. Unpublished report No. CTL/P/3040 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 453 (1983). GLP compliant.
- Trueman, R.W. (1980) An examination of pirimicarb for potential mutagenicity using the Salmonella/microsome reverse mutation assay. Unpublished report No. CTL/P/540 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted to a protocol that was consistent with the OECD guideline 471 (1983). GLP compliant.
- Wildgoose, J., Howard, C.A., Richardson, C.R. & Randall, V. (1987) Pirimicarb: a cytogenetic study in human lymphocytes *in vitro*. Unpublished report No. CTL/P/1655 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 473 (1983). GLP compliant.

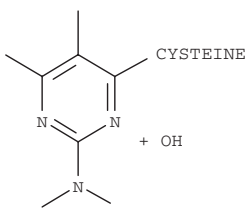
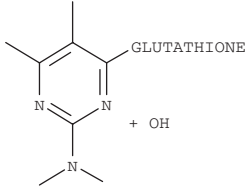
APPENDIX

Compound name	IUPAC name	Structure	Study in which metabolite was identified
Pirimicarb 062/01 R32062 I X (as O-Glucuronide)	2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate		Parent active ingredient Primary crops—lettuce, potato, apple, wheat Crop rotation Rat biotransformation
836/01 Desmethyl pirimicarb R34836 III XIII (as O-Glucuronide)	5,6-dimethyl-2-(methylamino)pyrimidin-4-yl dimethylcarbamate		Primary crops—lettuce, potato, apple, wheat Crop rotation Soil aerobic degradation Soil anaerobic degradation Soil surface photolysis Field soil dissipation Aqueous photolysis Water sediment Rat biotransformation
062/03 R35140 IV XII	2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate		Primary crops—lettuce Crop rotation Field soil dissipation Aqueous photolysis Rat biotransformation
062/04 Desmethylformamido pirimicarb R34885 II	5,6-dimethyl-2-(methylformamido)pyrimidin-4-yl dimethylcarbamate		Primary crops—lettuce, potato, apple, wheat Crop rotation Soil aerobic degradation Soil anaerobic degradation Soil surface photolysis Field soil dissipation Aqueous photolysis Water sediment
062/05 R238359	2-dimethylamino-5-hydroxymethyl-6-methylpyrimidin-4-yl dimethylcarbamate		Tentative—primary crop—apple
062/06 R31805 V III (rat as O-glucuronide conjugate) IV (rat as free metabolite)	2-dimethylamino-5,6-dimethylpyrimidin-4-ol		Primary crops—lettuce, potato, apple, wheat Crop rotation Livestock—hen, goat Soil aerobic degradation Soil anaerobic degradation Field soil dissipation Aqueous photolysis Rat biotransformation
062/07 R34865 VI VIII (as O-glucuronide conjugate)	5,6-dimethyl-2-(methylamino)pyrimidin-4-ol		Primary crops—lettuce, apple Crop rotation Livestock—hen, goat Soil aerobic degradation Soil anaerobic degradation Field soil dissipation

Compound name	IUPAC name	Structure	Study in which metabolite was identified
062/08 R238177	2-dimethylamino-6-hydroxymethyl-5-methylpyrimidin-4-yl dimethylcarbamate		Primary crops—wheat, lettuce
062/09 Guanidine R12378 X	Guanidine		Crop rotation Tentative—wheat primary crop
062/10 1,1-Dimethylguanidine R16210 VIII	1,1-Dimethylguanidine		Primary crops—potato, apple, wheat Soil surface photolysis Aqueous photolysis
062/11 Methylguanidine R16192 IX	1-Methylguanidine		Primary crops—potato, wheat Soil surface photolysis
062/12 R7272	1,1-Dimethylurea		Not detected
062/13 R57659	Methylurea		Not detected
062/14 R31680 VII II	2-Amino-5,6-dimethylpyrimidin-4-ol		Tentative—lettuce primary crop Livestock—hen, goat Field soil dissipation Rat biotransformation
062/15 R404094	2-Dimethylamino-5-hydroxymethyl-6-methylpyrimidin-4-ol		Tentative—lettuce primary crop Tentative—apple primary crop
062/16 R404137	2-Dimethylamino-6-hydroxymethyl-5-methylpyrimidin-4-ol		Tentative—apple primary crop
062/17 R407392	2-(N-methylformamido)-5,6-dimethylpyrimidin-4-ol		Tentative—lettuce primary crop
062/18 R406405	5-Hydroxymethyl-6-methyl-2-(methylamino)pyrimidin-4-ol		Tentative—lettuce primary crop Tentative—milk from goat study

Compound name	IUPAC name	Structure	Study in which metabolite was identified
062/19 R407135	2-Amino-5-hydroxymethyl-6-methylpyrimidin-4-ol		Not detected
062/20 R409239	2-Amino-6-hydroxymethyl-5-methylpyrimidin-4-ol		Not detected
062/21 R409238 V (rat)	6-Hydroxymethyl-5-methyl-2-(methylamino)pyrimidin-4-ol		Tentative—lettuce crop metabolism Rat biotransformation
062/22 R409464	2-Dimethylamino-5,6-dimethyl-4-(β-D-glucos-6-yl)pyrimidine		Not detected
062/23 R4715	2-Amino-4-hydroxy-6-methylpyrimidin-5-carboxylic acid		Not detected
062/24 R26021	β-d-Glucosylurea		Not detected
062/25 R59480	2-Amino-6-hydroxypyrimidin-4-carboxylic acid		Not detected
062/26 R99366	5,6-Dimethylpyrimidin-2,4-diol		Not detected
062/27 R16229	1-Acetylguanidine		Not detected
062/28 R32379	1-Acetyl-3,3-dimethylguanidine		Soil surface photolysis

Compound name	IUPAC name	Structure	Study in which metabolite was identified
062/29 R411934	2,3-Diacetyl-1,1-dimethylguanidine		Soil surface photolysis
062/30 R411893	3,3-Dimethyl-1-(2-oxopropionyl)guanidine		Not detected
062/31 R413303	(<i>r</i>)-3-Aza-5-glutamino-4-oxo-6-(2-dimethylamino-5,6-ylthio)hexanoic acid		Not detected
062/32 R35251	2-Dimethylamino-4-hydroxy-6-methylpyrimidin-5-al		Not detected
062/33 R414656	2-Dimethylamino-6-hydroxy-5-methylpyrimidin-4-al		Not detected
062/34 R414657	2-Dimethylamino-6-hydroxy-5-methylpyrimidin-4-carboxylic acid		Not detected
Urea R1498	Urea		Primary crop—apple
Metabolite A			Crop rotation
VI			Rat biotransformation

Compound name	IUPAC name	Structure	Study in which metabolite was identified
IX	4-Cysteiny1-hydroxymethyl-2-dimethylmethylamino-pyrimidine		Rat biotransformation
XIV			Rat biotransformation