

CHLORFENAPYR

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Explanation

Chlorfenapyr is the International Organization for Standardization (ISO)–approved name for 4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-1H-pyrrole-3-carbonitrile (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 122453-73-0). Chlorfenapyr is a contact and stomach insecticide that acts, following metabolic activation, as an uncoupler of oxidative phosphorylation in mitochondria. It has limited systemic activity.

Chlorfenapyr has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies with chlorfenapyr were certified to be compliant with good laboratory practice (GLP), unless otherwise specified.

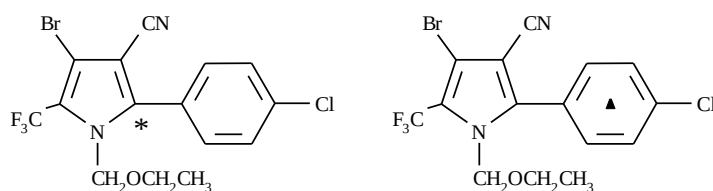
Evaluation for acceptable daily intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of chlorfenapyr were investigated in rats. Two of the studies (Okada, 1994a,b) were not certified as being compliant with GLP.

The structural formulae of [2-pyrrole-¹⁴C]chlorfenapyr and [phenyl(U)-¹⁴C]chlorfenapyr are given in Figure 1.

Figure 1. Structural formulae of radioactively labelled chlorfenapyr used in toxicokinetic studies



* denotes position of ¹⁴C or ¹³C at the 2-position of the pyrrole ring [2-pyrrole-¹⁴C]

▲ uniformly labelled ¹⁴C phenyl ring [phenyl(U)-¹⁴C]

1.1 Absorption, distribution and excretion

Groups of five male and five female Sprague-Dawley rats were treated with the radiolabelled compounds as follows:

- SOLD: single oral administration of low-dose labelled compound;
- SOHD: single oral administration of high-dose labelled compound;
- MOLD: daily oral administration of low-dose non-radioactive compound for 14 days, followed by single oral administration of low-dose labelled compound;
- Control: single oral administration of dosing vehicle.

The control group comprised three animals of each sex. The radiolabelled test substance was suspended in aqueous (0.5% weight per weight [w/w]) sodium salt of carboxymethylcellulose and administered orally by gavage.

The actual oral dose rate for each treatment group is shown in Table 1.

Table 1. Doses used in toxicokinetics study

Treatment	Dose (mg/kg bw)	
	[2-pyrrole- ¹⁴ C]	[phenyl(U)- ¹⁴ C]
SOLD	19.3	20.0
SOHD	208.3	205.1
MOLD	19.2	21.7
Control	—	—

From Mallipudi (1994)

bw, body weight; MOLD, multiple oral low dose; SOHD, single oral high dose; SOLD, single oral low dose

All urine, faeces and cage rinses were collected at the following time intervals: 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96, 96–120, 120–144 and 144–168 hours post-dosing. Selected tissue samples were collected at termination. Specific radioactivity was determined by high-performance liquid chromatography (HPLC) of the analyte and standard chlorfenapyr. Total radioactive residue (TRR) in the blood, tissues/organs and faeces or post-extraction residuum was determined by combustion followed by liquid scintillation counting. Radioactivity in the urine, as well as in liquid chromatographic column eluates, was measured by liquid scintillation counting.

The absorbed chlorfenapyr-related residue was distributed throughout the body and detected at concentrations ranging from 0.02 to 24.3 µg equivalents (Eq) chlorfenapyr per gram tissue in all tissue and organ matrices of all treatment groups. The mean residual percentage of administered radioactivity in blood, carcasses and tissues at 7 days post-dosing ranged from less than 0.01% to 3.37% (Table 2). The concentration of radioactive residue was 0.58–7.75 µg Eq chlorfenapyr per gram tissue in blood, 1.0–24.3 µg Eq chlorfenapyr per gram tissue in fat, 0.09–1.05 µg Eq chlorfenapyr per gram tissue in muscle, 0.34–3.10 µg Eq chlorfenapyr per gram tissue in kidneys and 0.90–6.69 µg Eq chlorfenapyr per gram tissue in liver. Brain showed the lowest concentration of radioactivity (0.02–0.19 µg Eq chlorfenapyr per gram tissue) among all tissues evaluated. Fat showed the highest concentration of radioactivity (1.0–24.3 µg Eq chlorfenapyr per gram tissue). The animal carcasses showed 0.23–3.08 µg Eq of radioactive residues per gram tissue (0.58–3.37% of the administered dose). In general, the highest concentration level in each tissue/organ was obtained from the high-dose groups. Sex-related differences were noted in residue levels for all dose groups. Concentrations were higher by 2- to 3-fold in female rats than in male rats in blood and most tissues, but were the same in liver.

A pilot metabolism study showed only trace amounts of radiocarbon (< 0.01% of the dose) in volatile organic compounds and no radioactivity in the expired carbon dioxide over a 7-day period. During the first 24 hours following administration of radiolabelled chlorfenapyr, approximately 70% of the dose was excreted (66% in faeces and 4% in urine). Within 48 hours after dosing, approximately 88% of the dose had been excreted (82% in faeces and 6% in urine). The administered oral dose was eliminated over a 7-day period mainly via faeces (80–106%) and to a much lesser extent via urine (5.3–11.2%), regardless of the treatment regimen or position of ¹⁴C label.

The excretion of chlorfenapyr-related radioactivity in the rat is summarized in Table 3 (urine) and Table 4 (faeces).

In summary, there was no evidence of elimination of chlorfenapyr-related radioactivity via respiration. The principal route of elimination of orally administered chlorfenapyr was via faeces. There are no substantial ¹⁴C label-related differences in the absorption, distribution or elimination of radioactivity in the rat. In general, the higher concentrations of radiocarbon in the tissues and organs were obtained with the high-dose treatment. Blood and tissue radiocarbon concentrations appeared higher in female rats than in male rats (Mallipudi, 1994).

In another study on the disposition of chlorfenapyr, groups of four male and four female Sprague-Dawley rats were treated as follows:

- low dose: 1.85 MBq/2 mg at a dosing volume of 4 ml/kg body weight (bw), single oral administration;
- high dose: 1.85 MBq/20 mg at a dosing volume of 4 ml/kg bw, single oral administration.

The radiolabelled test substance was administered orally by gavage as an aqueous solution in 1% sodium carboxymethylcellulose plus 1% Tween 80. Blood was sampled at 15 and 30 minutes and 1, 2, 4, 8, 12, 24, 48, 72, 120 and 168 hours post-dosing. Urine was collected 6 and 12 hours post-dosing. Urine and faeces were collected at 24, 48, 72, 120 and 168 hours post-dosing. Organs and tissues from four animals of each sex were collected at 1, 8, 24 and 168 hours post-dosing. In bile duct-cannulated animals, bile was collected at 3, 6, 12 and 24 hours; urine was collected at 6, 12 and 24 hours; and faeces was collected at 24 hours post-dosing.

Table 2. Mean radioactivity detected in blood carcass and tissue samples 7 days following dosing with ^{14}C -labelled chlorfenapyr

Matrix	% of radioactive dose in matrix					
	SOLD		MOLD		SOHD	
	M	F	M	F	M	F
[2-pyrrole-^{14}C]						
Blood	0.08	0.21	0.10	0.32	0.04	0.10
Bone (femur)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Brain	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Carcass	1.58	3.06	1.50	3.02	0.58	1.51
Fat (body)	0.14	0.26	0.07	0.14	0.03	0.17
Heart	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Kidneys	0.02	0.03	0.02	0.03	< 0.01	< 0.01
Liver	0.48	0.40	0.36	0.38	0.15	0.17
Lungs	< 0.01	0.03	0.01	0.03	< 0.01	< 0.01
Muscle (thigh)	< 0.01	0.02	< 0.01	0.03	< 0.01	< 0.01
Ovaries	NA	< 0.01	NA	< 0.01	NA	< 0.01
Skin (shaved)	0.03	0.05	0.03	0.07	0.01	0.04
Spleen	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Testes	0.02	NA	0.02	NA	< 0.01	NA
[phenyl(U)-^{14}C]						
Blood	0.07	0.22	0.06	0.22	0.04	0.08
Bone (femur)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Brain	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Carcass	1.41	3.37	1.12	2.63	0.76	1.38
Fat (body)	0.16	0.35	0.07	0.20	0.03	0.18
Heart	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Kidneys	0.02	0.03	0.01	0.03	< 0.01	< 0.01
Liver	0.37	0.38	0.28	0.31	0.17	0.15
Lungs	< 0.01	0.02	< 0.01	0.02	< 0.01	< 0.01
Muscle (thigh)	< 0.01	0.03	< 0.01	0.02	< 0.01	< 0.01
Ovaries	NA	< 0.01	NA	< 0.01	NA	< 0.01
Skin (shaved)	0.02	0.06	0.02	0.05	0.01	0.03
Spleen	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Testes	0.01	NA	< 0.01	NA	< 0.01	NA

From Mallipudi (1994)

F, female; M, male; MOLD, multiple oral low dose; NA, not applicable; SOHD, single oral high dose; SOLD, single oral low dose

After pyrrole- ^{14}C -labelled chlorfenapyr was administered orally, ^{14}C began to be detected in the blood of the systemic circulation as early as 15 minutes, the first measurement interval; levels increased slowly, with a peak concentration in plasma ($C_{\max}$) occurring at 8–12 hours. Based on the results, it was inferred that absorption of chlorfenapyr from the digestive tract was relatively slow. Concentrations of radiolabel declined slowly and monophasically, decreasing to about 7–13% of $C_{\max}$ by 168 hours, with ^{14}C -labelled chlorfenapyr gradually translocating from the systemic circulation to various organs and tissues. The plasma elimination half-life of radiolabel was approximately 56 hours.

Table 3. Cumulative radioactivity detected in urine following dosing with ¹⁴C-labelled chlorfenapyr

Collection interval (h)	% of radioactive dose in urine ^a					
	SOLD		MOLD		SOHD	
	M	F	M	F	M	F
[2-pyrrole-¹⁴C]						
0-4	0.2	0.2	0.9	1.0	0.1	< 0.1
0-8	1.3	1.3	3.0	2.9	0.2	0.8
0-24	6.3	4.5	7.1	7.4	1.7	2.1
0-48	8.8	6.4	9.1	9.7	3.9	4.4
0-72	9.7	7.3	9.8	10.3	4.4	4.9
0-168	11.2	8.5	11.1	11.2	5.3	6.0
[phenyl(U)-¹⁴C]						
0-4	0.8	0.9	1.2	1.3	0.7	0.7
0-8	2.3	2.1	2.9	3.1	1.0	1.3
0-24	6.4	4.7	6.6	6.9	3.2	4.2
0-48	8.4	6.4	7.8	8.6	6.1	5.7
0-72	9.2	7.0	8.4	9.1	6.7	6.1
0-168	10.4	8.1	9.4	10.2	7.6	6.8

From Mallipudi (1994)

F, female; M, male; MOLD, multiple oral low dose; SOHD, single oral high dose; SOLD, single oral low dose

^a Includes cage rinses from 0- to 144-hour samples plus cage wash and cage wipe from 144- to 168-hour samples.**Table 4. Cumulative radioactivity detected in faeces following dosing with ¹⁴C-labelled chlorfenapyr**

Collection interval (h)	% of radioactive dose in faeces					
	SOLD		MOLD		SOHD	
	M	F	M	F	M	F
[2-pyrrole-¹⁴C]						
0-4	NS	NS	NS	ND	< 0.1	NS
0-8	NS	NS	< 0.1	NS	< 0.1	1.7
0-24	66.7	57.1	77.1	63.2	65.5	57.7
0-48	84.5	74.7	90.9	89.6	83.0	78.6
0-72	89.0	82.4	94.9	96.8	85.4	84.0
0-168	93.7	92.0	99.4	106.0	87.8	88.5
[phenyl(U)-¹⁴C]						
0-4	NS	NS	NS	ND	< 0.1	NS
0-8	NS	NS	NS	NS	< 0.1	4.4
0-24	62.3	57.4	79.2	68.0	60.1	72.6
0-48	76.5	70.4	85.7	80.3	74.9	91.4
0-72	81.0	76.2	88.1	86.0	78.1	94.7
0-168	86.1	83.9	91.4	92.7	80.1	97.5

From Mallipudi (1994)

F, female; M, male; MOLD, multiple oral low dose; ND, not detectable; NS, no sample; SOHD, single oral high dose; SOLD, single oral low dose

Maximum concentrations of chlorfenapyr in the liver, adrenals and fat were higher than those detected in plasma, with most tissues showing maximum levels between 1 and 8 hours. The per cent tissue distribution was elevated in tissues such as fat, muscle, skin and liver. Carbon-14 distributed among tissues did not show any tendency to remain there, and concentrations declined rapidly, with tissue concentrations decreasing to less than 10% of the maximum by 168 hours.

The radioactivity found in selected tissues of rats following administration of the low and high doses is summarized in Tables 5 and 6, respectively. Blood, plasma, fat, muscle and skin weights were assumed to be 7%, 4%, 5%, 40% and 22% of body weight, respectively.

The administered oral dose was almost completely excreted into the urine and faeces over a 168-hour period. Residues in tissues and carcass at 168 hours represented 2–5% of the oral dose. The main excretion route was faecal (75–85%), which was 5–10 times the excretion via the urinary route (8–16%). In bile duct-cannulated animals within the first 24 hours, between 2% and 19% of the ^{14}C was excreted into the faeces, between 4% and 6% into the urine and between 17% and 30% into the bile (Table 7). Cumulative excretion of ^{14}C into the bile was 3–7 times higher than that into the urine, suggesting that biliary excretion was the main route of ^{14}C excretion after absorption from the digestive tract. Faecal excretion thus included both the unabsorbed ^{14}C portion of the oral dose and biliary ^{14}C . It is likely that a portion of biliary ^{14}C entered the enterohepatic circulation.

Table 5. Summary of the radioactivity found in selected tissues of rats administered ^{14}C -labelled chlorfenapyr at 2 mg/kg bw

Tissue	Tissue radioactivity (% of dose)							
	Males ($n = 4$ at each time point)				Females ($n = 4$ at each time point)			
	1 h	8 h	24 h	168 h	1 h	8 h	24 h	168 h
Blood	2.03	4.18	2.79	0.49	2.34	3.68	3.10	0.54
Plasma	1.89	4.25	2.74	0.47	2.15	3.68	3.04	0.51
Thyroid	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lung	0.12	0.13	0.07	0.01	0.16	0.14	0.10	0.02
Liver	5.66	3.19	2.07	0.58	7.13	3.29	2.35	0.54
Adrenal	0.02	0.01	< 0.01	< 0.01	0.04	0.01	0.01 ^a	< 0.01
Kidney	0.40	0.39	0.25	0.05	0.47	0.40	0.29	0.04
Spleen	0.03	0.02	0.01	< 0.01	0.04	0.03	0.02	< 0.01
Pancreas	0.08	0.06	0.03	0.01 ^a	0.15	0.12	0.06	0.02 ^b
Fat	5.01	16.4	11.7	1.05	5.12	14.1	19.3	1.81
Brown fat	0.07	0.15	0.05	0.01 ^b	0.11	0.26	0.11	0.01 ^a
Muscle	4.29	2.90	1.62	0.28	6.67	3.71	2.40	0.30
Skin	3.53	7.44	3.48	0.56	4.89	9.42	6.30	0.80
Prostate gland/uterus	0.02	0.02	0.02	< 0.01	0.03	0.04	0.04	0.01
Stomach ^c	36.1	0.26	0.07	0.02	22.5	0.52	0.13	0.02
Small intestine ^c	40.5	8.77	2.22	0.14	37.8	7.63	2.36	0.20
Caecum ^c	0.10	27.7	6.98	0.16	2.57	40.7	7.30	0.27
Large intestine ^c	0.11	17.5	4.25	0.13	0.24	8.84	3.89	0.28

From Okada (1994a)

^a Mean of three animals, when tissue content was < 0.01% in all other animals of the group.

^b Mean of two animals, when tissue content was < 0.01% in all other animals of the group.

^c Including contents.

Table 6. Summary of the radioactivity found in selected tissues of rats administered ^{14}C -labelled chlorfenapyr at 20 mg/kg bw

Tissue	Tissue radioactivity (% of dose)							
	Males ($n = 4$ at each time point)				Females ($n = 4$ at each time point)			
	1 h	8 h	24 h	168 h	1 h	8 h	24 h	168 h
Blood	0.95	2.12	2.06	0.33	0.89	3.37	3.02	0.33
Plasma	0.90	2.14	2.01	0.30	0.82	3.39	3.15	0.30
Thyroid	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lung	0.08	0.07	0.05	0.01	0.08	0.13	0.10	0.01
Liver	3.98	2.00	1.65	0.39	4.02	3.61	1.92	0.37
Adrenal	0.02	0.01 ^a	< 0.01	< 0.01	0.02	0.02	0.01 ^b	< 0.01
Kidney	0.27	0.17	0.13	0.03	0.23	0.40	0.25	0.03
Spleen	0.02	0.02	0.01	< 0.01	0.02	0.02	0.02	< 0.01
Pancreas	0.07	0.06	0.03	< 0.01	0.09	0.16	0.07	< 0.01
Fat	3.97	15.6	8.54	0.21	2.62	14.2	15.4	0.67
Brown fat	0.05	0.20	0.03	< 0.01	0.06	0.33	0.14	< 0.01
Muscle	3.39	2.04	1.24	0.17	3.57	4.03	2.73	0.19
Skin	2.83	6.79	2.68	0.31	2.84	12.4	8.59	0.42
Stomach ^c	30.7	0.27	0.19 ^b	0.02 ^a	41.4	2.03	0.10	0.01 ^b
Small intestine ^c	62.3	5.01	1.07	0.08	55.2	9.36	2.89	0.12
Caecum ^c	0.06	43.2	1.77	0.10	0.05	35.1	10.2	0.16
Large intestine ^c	0.06	13.6	2.66	0.08	0.07	14.1	3.53	0.17

From Okada (1994a)

^a Mean of two animals, when tissue content was < 0.01% in all other animals of the group.

^b Mean of three animals, when tissue content was < 0.01% in all other animals of the group.

^c Including contents.

Table 7. Summary of the cumulative radioactivity found in bile, urine and faeces (bile duct-cannulated rats)

Sampling time (h)	Cumulative excreted radioactivity (% of dose)					
	2 mg/kg bw			20 mg/kg bw		
	Bile	Urine	Faeces	Bile	Urine	Faeces
Males						
0–3	4.1 ± 4.81	—	—	1.8 ± 1.54	—	—
0–6	9.5 ± 7.34	0.9 ± 0.62	—	4.8 ± 2.81	0.6 ± 0.32	—
0–12	18.3 ± 8.24	1.5 ± 0.59	—	10.7 ± 6.80	1.1 ± 0.56	—
0–24	30.1 ± 11.3	4.0 ± 1.25	9.7 ± 9.58	17.4 ± 9.14	5.5 ± 3.42	18.8 ± 15.5
Females						
0–3	4.7 ± 3.07	—	—	1.4 ± 0.60	—	—
0–6	11.5 ± 4.22	0.8 ± 0.59	—	4.7 ± 0.94	0.8 ± 0.47	—
0–12	19.6 ± 7.73	2.0 ± 1.23	—	9.8 ± 2.61	1.8 ± 0.77	—
0–24	24.1 ± 8.03	4.8 ± 2.29	2.3 ± 2.40	19.9 ± 7.35	4.4 ± 1.70	10.8 ± 6.31

From Okada (1994a)

—, not assayed

In summary, the absorption of orally administered chlorfenapyr was relatively slow, and translocation from the systemic circulation to tissues was gradual. More than 90% of the administered dose of chlorfenapyr was excreted into the faeces and urine by 168 hours. The principal route of elimination was via faeces, whereas the urinary route was minor. Faecal excretion included both the unabsorbed portion of the oral dose and the biliary fraction. Biliary excretion represented the main route of excretion after absorption from the digestive tract. It is also likely that a portion of the biliary excreta entered the enterohepatic circulation. No sex-related differences were observed in the described findings (Okada, 1994a).

In another study, groups of four male and four female Sprague-Dawley rats were treated as follows:

- low dose: 1.85 MBq/2 mg at a dosing volume of 4 ml/kg bw, single oral administration;
- high dose: 1.85 MBq/20 mg at a dosing volume of 4 ml/kg bw, single oral administration.

The radiolabelled test substance was administered orally by gavage as an aqueous solution in 1% sodium carboxymethylcellulose plus 1% Tween 80. Urine was sampled at 12, 24, 48 and 72 hours post-dosing. Faeces was collected at 24, 48 and 72 hours post-dosing. In bile duct-cannulated animals, bile was collected at 3, 6, 12 and 24 hours; urine was collected at 6, 12 and 24 hours; and faeces was collected at 24 hours post-dosing.

Almost all ^{14}C detected in faeces was unchanged chlorfenapyr. There was no unchanged chlorfenapyr in bile. This finding indicated that the unchanged chlorfenapyr in the faeces did not come from the bile, but consisted of chlorfenapyr that was not absorbed from the gastrointestinal tract and was directly excreted into the faeces. It follows, therefore, that the per cent absorption of chlorfenapyr from the gastrointestinal tract can be obtained by subtracting the percentage of faecal excretion of chlorfenapyr from the administered dose:

- male rats (2 mg/kg bw): $100\% - 17.0\% = 83.0\%$
- female rats (2 mg/kg bw): $100\% - 23.1\% = 76.9\%$
- male rats (20 mg/kg bw): $100\% - 35.2\% = 64.8\%$
- female rats (20 mg/kg bw): $100\% - 33.0\% = 67.0\%$

In summary, the per cent absorption of chlorfenapyr from the gastrointestinal tract was approximately 80% and 65% in the 2 mg/kg bw and 20 mg/kg bw groups, respectively, with an apparent decrease in absorption with increasing dose. Chlorfenapyr was absorbed unchanged; there was no evidence that degradation occurred in the digestive tract (Okada, 1994b).

1.2 Biotransformation

In the Mallipudi (1994) study described in section 1.1, the urinary residue consisted of M-4 along with sulfate and amino acid or peptide conjugates of M-4 and some minor unknown polar metabolites; the parent compound and its *N*-dealkylated product (M-8) were not detected in the urine (Table 8).

Faecal radiocarbons were composed mainly of unchanged chlorfenapyr (M-9), which accounted for 38–72% of the administered dose 48 hours after dosing. There were several identified minor metabolites (Table 9), each of which accounted for less than 5% of the dose over the same time period. The metabolites included the *N*-dealkylated product (M-8), M-4 (a 4-hydroxy-5-carboxypyrrole derivative of M-8), M-5 (a 4-oxo-5-hydroxypyrrole derivative of M-8), M-7A-RAT (a carboxymethylmethoxy derivative of M-9), M-6 (a desbromo derivative of M-7A-RAT) plus many minor polar unknowns.

Table 8. Summary of radioactive residue components in rat urine

Group/component	Residue in urine (% of dose)											
	[2-Pyrrole- ¹⁴ C]						[Phenyl(U)- ¹⁴ C]					
	0–12 h		12–24 h		24–48 h		0–12 h		12–24 h		24–48 h	
	M	F	M	F	M	F	M	F	M	F	M	F
SOLD												
<i>Total</i>	3.3	2.4	3.0	2.1	2.5	1.9	4.1	3.1	2.3	1.6	2.0	1.7
M-1	0.09	0.05	0.10	0.05	0.05	0.04	0.37	0.30	0.17	0.11	0.07	0.08
U-2	0.21	0.09	0.18	0.06	0.07	0.06	0.17	0.10	0.08	0.04	0.09	0.05
M-1A	0.35	0.15	0.10	0.04	0.24	0.17	0.12	0.11	0.10	0.03	0.08	0.05
M-2	0.72	0.26	0.68	0.29	0.40	0.22	1.36	0.65	0.62	0.27	0.32	0.32
M-3	0.39	0.25	0.47	0.20	0.53	0.23	0.31	0.29	0.20	0.14	0.51	0.21
M-4	0.45	0.27	0.29	0.21	0.24	0.21	0.72	0.53	0.26	0.20	0.18	0.17
M-5	0.54	0.86	0.77	0.90	0.76	0.68	0.65	0.79	0.64	0.62	0.54	0.56
M-6	0.09	0.07	0.12	0.08	0.08	0.07	0.05	0.06	0.05	0.02	0.06	0.02
Unknowns	0.45	0.29	0.25	0.24	0.15	0.18	0.25	0.20	0.13	0.12	0.11	0.13
MOLD												
<i>Total</i>	5.0	4.7	2.1	2.7	2.0	2.3	5.0	4.8	1.6	2.1	1.2	1.7
M-1	0.06	0.07	0.03	0.07	0.02	0.09	0.04	0.05	0.01	0.02	0.01	0.02
U-2	0.24	0.15	0.09	0.05	0.05	0.03	0.31	0.23	0.11	0.15	0.02	0.08
M-1A	0.36	0.39	0.15	0.25	0.19	0.23	0.92	0.60	0.18	0.24	0.04	0.18
M-2	0.77	0.37	0.30	0.28	0.27	0.24	0.75	0.57	0.37	0.24	0.13	0.26
M-3	0.66	0.50	0.34	0.31	0.38	0.26	0.20	0.24	0.08	0.15	0.27	0.10
M-4	1.06	0.82	0.37	0.33	0.44	0.29	0.93	0.85	0.16	0.20	0.08	0.14
M-5	1.03	1.59	0.58	0.96	0.43	0.80	1.20	1.56	0.51	0.79	0.53	0.65
M-6	0.17	0.16	0.07	0.11	0.06	0.13	0.17	0.19	0.06	0.08	0.04	0.08
Unknowns	0.59	0.51	0.15	0.28	0.14	0.21	0.44	0.46	0.10	0.21	0.08	0.18
SOHD												
<i>Total</i>	0.5	1.4	1.2	0.7	1.6	1.5	1.5	3.2	1.7	1.0	2.9	1.5
M-1	0.01	0.02	0.01	0.01	0.12	0.11	0.22	0.62	0.19	0.10	0.16	0.11
U-2	0.02	0.04	0.05	0.29	0.06	0.03	—	—	—	—	—	—
M-1A	0.02	0.02	0.19	0.08	0.14	0.10	0.07	0.09	0.09	0.05	0.15	0.09
M-2	0.13	0.32	0.14	0.07	0.40	0.23	0.48	0.93	0.50	0.18	0.86	0.24
M-3	0.05	0.11	0.10	0.05	0.19	0.08	0.09	0.15	0.17	0.06	0.29	0.11
M-4	0.07	0.26	0.18	0.14	0.09	0.18	0.32	0.72	0.18	0.12	0.25	0.13
M-5	0.09	0.32	0.28	0.19	0.42	0.55	0.15	0.39	0.32	0.33	0.62	0.55
M-6	0.02	0.05	0.06	0.04	0.02	0.05	0.02	0.05	0.04	0.02	0.04	0.03
Unknowns	0.06	0.22	0.17	0.09	0.13	0.17	0.13	0.19	0.19	0.10	0.47	0.21

From Mallipudi (1994)

—, not detected; F, female; M, male

Table 9. Summary of radioactive residue components in rat faeces

Group/ component	Residue in faeces (% of dose)											
	[2-Pyrrole- ¹⁴ C]						[Phenyl(U)- ¹⁴ C]					
	0–12 h		12–24 h		24–48 h		0–12 h		12–24 h		24–48 h	
	M	F	M	F	M	F	M	F	M	F	M	F
SOLD												
<i>Total</i>	4.0	9.9	62.7	47.2	17.8	17.6	11.8	14.1	50.5	43.3	14.2	13.0
U-1 (polar)	0.02	0.02	3.92	1.94	1.85	0.34	0.10	0.04	3.38	0.77	2.88	1.46
M-1	< 0.01	< 0.01	—	—	0.11	0.13	—	—	—	0.16	0.31	0.43
U-2	< 0.01	< 0.01	0.58	0.27	0.32	0.71	0.08	—	0.64	—	0.43	0.96
M-1A	0.02	0.06	0.27	0.60	0.34	1.03	0.08	0.04	0.81	0.39	0.54	0.53
M-2	0.03	0.05	1.93	0.94	1.28	1.04	0.11	0.08	1.69	0.99	0.81	0.92
M-3	0.08	0.17	3.80	1.41	1.15	0.85	0.16	0.22	1.34	1.47	0.75	0.83
M-4	0.06	0.09	2.65	1.67	1.74	1.34	0.17	0.28	1.74	1.22	0.64	0.85
M-5	0.08	0.16	2.62	2.00	1.76	1.65	0.29	0.37	1.70	0.93	0.70	0.80
M-6	0.04	0.10	1.39	1.01	0.87	0.80	0.17	0.11	1.28	0.53	0.51	0.62
M-7	0.05	0.12	2.57	1.60	1.52	2.11	0.15	0.18	1.35	1.29	1.03	1.54
M-7A	0.02	0.05	0.80	0.57	0.39	0.45	0.11	0.07	0.66	0.65	0.24	0.31
M-8	0.06	0.15	0.87	0.71	0.23	0.25	0.15	0.24	0.58	0.51	0.14	0.13
M-9 (parent)	3.32	8.58	35.29	29.97	1.53	3.61	9.60	12.44	29.76	32.43	1.50	1.43
MOLD												
<i>Total</i>	50.5	30.3	26.6	32.9	13.8	26.4	56.7	25.1	21.8	42.9	6.5	12.3
U-1 (polar)	0.53	0.57	0.81	1.29	1.64	3.34	0.88	0.26	0.74	0.93	1.54	1.33
M-1	—	—	0.03	0.19	0.08	0.05	0.11	0.14	0.09	0.26	0.03	0.03
U-2	0.39	0.22	0.52	0.54	0.13	0.50	0.06	0.18	0.50	0.92	0.08	0.25
M-1A	0.26	0.11	0.88	0.85	0.50	0.83	0.45	0.19	0.76	0.51	0.17	0.43
M-2	1.00	0.24	1.43	1.52	1.29	1.16	0.63	0.33	0.51	1.55	0.37	0.63
M-3	1.87	0.35	1.40	1.05	0.76	1.46	1.99	1.02	1.56	2.35	0.50	0.63
M-4	1.67	1.03	2.33	2.50	1.57	2.92	1.68	0.89	1.41	2.80	0.48	1.18
M-5	1.84	0.76	2.23	2.30	1.09	2.15	1.14	0.50	1.28	2.15	0.47	1.44
M-6	1.22	0.42	0.99	1.81	0.55	1.30	0.97	0.43	0.81	1.32	0.37	1.01
M-7	1.59	0.84	1.99	2.31	1.38	2.04	0.73	0.39	0.84	1.93	0.40	1.03
M-7A	0.73	0.33	0.61	0.69	0.09	0.15	0.75	—	0.48	0.50	0.15	0.21
M-8	0.92	0.60	0.61	0.62	0.30	0.55	0.79	0.30	0.30	0.72	0.17	0.18
M-9 (parent)	33.68	22.70	8.67	13.08	1.15	2.71	43.57	19.13	9.01	22.25	0.36	1.10
SOHD												
<i>Total</i>	26.3	28.2	39.2	40.5	17.5	20.9	24.5	51.5	35.6	21.1	14.8	18.8
U-1 (polar)	0.12	0.03	0.22	0.15	0.66	0.51	0.07	0.02	0.92	0.31	1.41	0.87
M-1	—	< 0.01	—	0.03	0.23	0.06	< 0.01	0.01	0.04	0.02	0.08	0.05
U-2	< 0.01	< 0.01	0.21	0.12	0.12	0.44	0.01	0.06	0.07	0.12	0.09	0.14
M-1A	0.01	< 0.01	0.11	0.11	0.52	0.33	0.08	0.02	0.17	0.15	0.37	0.33
M-2	0.02	0.01	0.43	0.37	0.88	0.76	0.12	0.20	0.43	0.17	1.17	1.19
M-3	0.17	0.09	0.86	0.57	1.46	1.52	0.16	0.25	0.70	0.31	1.15	1.32
M-4	0.03	0.05	1.05	0.42	1.51	1.40	0.13	0.12	0.42	0.23	1.12	1.09

Group/ component	Residue in faeces (% of dose)											
	[2-Pyrrole- ¹⁴ C]						[Phenyl(U)- ¹⁴ C]					
	0–12 h		12–24 h		24–48 h		0–12 h		12–24 h		24–48 h	
	M	F	M	F	M	F	M	F	M	F	M	F
M-5	0.13	0.04	0.99	0.30	1.43	2.39	0.19	0.14	0.50	0.25	1.17	1.26
M-6	0.11	0.03	0.57	0.25	0.63	1.03	0.14	0.26	0.33	0.18	0.69	0.58
M-7	0.09	0.07	2.02	0.36	1.66	1.95	0.19	0.21	0.57	0.30	1.51	1.47
M-7A	0.08	0.06	0.35	0.12	0.35	0.53	0.19	0.26	0.54	0.10	0.34	0.34
M-8	0.35	0.39	0.63	0.52	0.19	0.26	0.36	0.71	0.45	0.31	0.19	0.20
M-9 (parent)	24.25	26.86	34.24	35.58	3.47	5.87	22.0	47.34	28.92	17.94	2.27	7.15

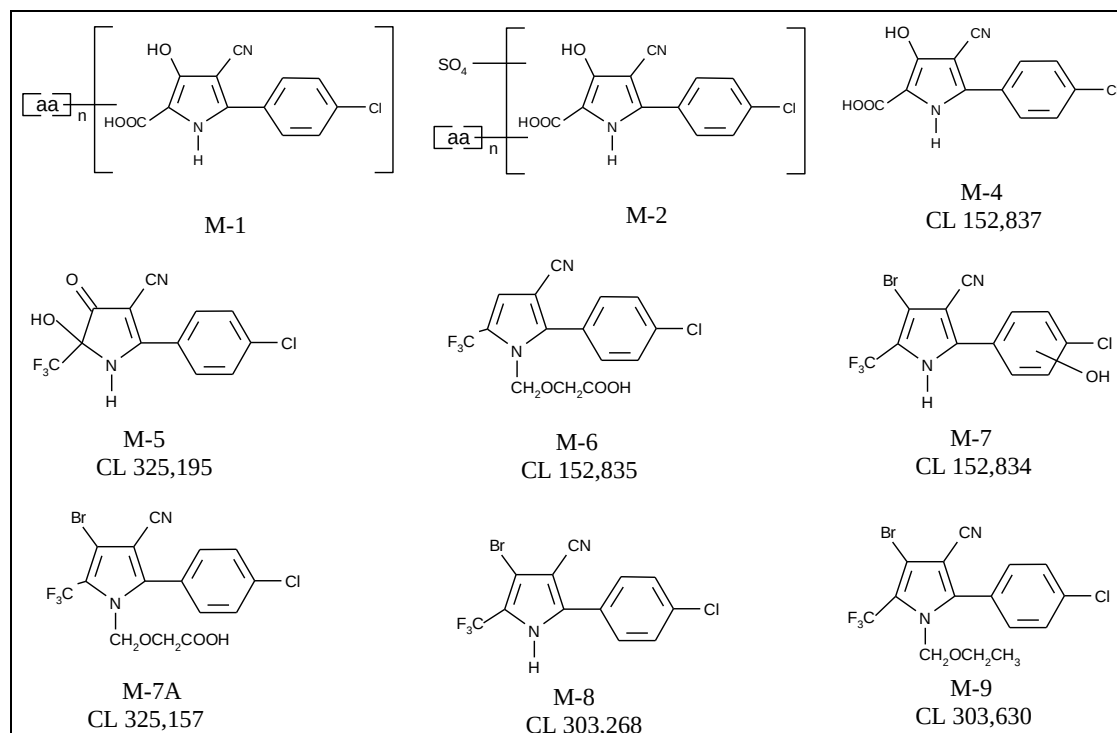
From Mallipudi (1994)

—, not detected; F, female; M, male

Analysis of tissue extracts showed that fat contained mainly unchanged chlorfenapyr (65.8–93.9% of the TRR; 4.37–22.82 µg Eq chlorfenapyr per gram tissue), whereas muscle, kidney and liver also contained more polar metabolites, which included M-8 (0.6–1.3% of the TRR), M-7, M-5, M-4, M-7A-RAT and M-6. Chlorfenapyr (M-9) and M-8 constituted 1.0–5.8% (0.03–0.18 µg Eq/g tissue) and 1.9–5.3% (0.03–0.15 µg Eq/g tissue) of the kidney TRR. Chlorfenapyr (M-9) and M-8 constituted 2.9–8.3% (0.15–0.51 µg Eq/g tissue) and 1.6–5.2% (0.09–0.32 µg Eq/g tissue) of the liver TRR. The parent chlorfenapyr and metabolites M-8, M-5 and M-4 constituted 6.5–30.9% (0.04–0.27 ppm), 0.4–1.9% (< 0.01–0.01 µg Eq/g tissue), 4.6–16.1% (0.02–0.16 µg Eq/g tissue) and 3.6–20.6% (0.02–0.21 µg Eq/g tissue) of the TRR in the muscle, respectively.

The chemical structures of the identified metabolites of chlorfenapyr in the rat are illustrated in Figure 2.

Figure 2. Chemical structures of identified metabolites of chlorfenapyr (CL 303,630) in the rat



In summary, orally administered chlorfenapyr excreted via the faeces consisted mainly of the unchanged compound plus minor amounts of *N*-dealkylated, debrominated and hydroxylated oxidation products. The absorbed residue was metabolized via *N*-dealkylation, dehalogenation, hydroxylation and conjugation. The unchanged compound and its less polar metabolites were found in tissues such as fat and liver, whereas more polar metabolites and conjugates were present in the urine and in the highly perfused tissues, such as kidney and liver. The bond between the phenyl and pyrrole rings appears to remain intact (Mallipudi, 1994).

In the study by Okada (1994a) described in section 1.1, 11 components were detected in urine, which accounted for more than 0.1% of the dose individually, of which U-9 was a major metabolite detected at the highest level in all the groups. It was followed by U-3, U-4, U-7 and U-8, which showed relatively high levels.

In faeces, as in urine, continuous elution was observed, including minor peaks; there were 25 components individually containing more than 0.1% of the dose. The peak showing the highest level was F-25, which proved to be unchanged chlorfenapyr. Other than this, F-6, F-9, F-12 and F-20 showed relatively high levels. Metabolites such as F-20 showing relatively longer retention times on HPLC columns were non-polar ones, which were not present in the urine.

Also in bile, a number of peaks were present, but polar metabolites eluting at the retention time of 10–20 minutes accounted for a far higher proportion than in the urine and faeces. The metabolites eluted in this range were mixtures with complex composition and showed subtle differences in elution pattern with different samples (they were likely to be conjugates of 2-(4-chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-2-pyrrolidine-3-carbonitrile, or PY-4-CO-5-OH). Other than these polar metabolites, components similar to urinary and faecal metabolites, such as B-9, B-11 and B-17, were detected.

Contents of total metabolites in the urine are shown in Table 10. After a peak (U-1) eluting at the solvent front, there was continuous elution of ^{14}C , with minor peaks, suggesting that chlorfenapyr undergoes complex metabolism.

Table 10. Summary of the total urinary metabolites collected through 72 hours, determined using HPLC with refractive index detector

Metabolite peak	Identification	Contents (% of dose)			
		2 mg/kg bw		20 mg/kg bw	
		Males	Females	Males	Females
U-1	—	0.2	0.1	0.1	—
U-2	Conjugated metabolite ^a	1.2	0.5	0.7	0.5
U-3	Conjugated metabolite ^a	1.4	0.9	1.3	0.8
U-4	Conjugated metabolite ^a	1.8	0.8	1.7	0.6
U-5	—	0.9	0.5	0.4	0.4
U-6	—	0.6	—	0.1	—
U-7 (F-9/B-9)	PY-4-CO-5-COOH-5-H	1.1	0.8	0.8	0.6
U-8	—	1.0	0.9	0.8	0.8
U-9 (F-12/B-11)	PY-4-CO-5-OH	2.7	2.8	2.3	2.7
U-10	PY-4-CO-5-H	0.1	—	0.1	—
U-11	PY-4-OH	0.6	0.3	0.4	0.3
Procedural loss	[SepPak [®]]	1.4	0.8	0.9	0.7
Total		13.0	8.4	9.6	7.4

From Okada (1994b)

—, not detected (< 0.1% of dose)

^a Conjugates of PY-4-CO-5-OH and its further metabolized compounds.

Table 11 shows the contents of total metabolites in the faecal samples. As in the urine, there was continuous elution of minor peaks, with 25 individual components, each representing more than 0.1% of the dose. The highest concentration was peak F-25, which corresponded to unchanged chlorfenapyr. F-6, F-9, F-12 and F-20 were non-polar metabolites not present in the urine.

Table 11. Summary of the total faecal metabolites collected through 72 hours, determined using HPLC with refractive index detector

Metabolite peak	Identification	Contents (% of dose)			
		2 mg/kg bw		20 mg/kg bw	
		Males	Females	Males	Females
F-1	—	0.7	0.6	0.7	0.2
F-2	Conjugated metabolite ^a	0.8	1.2	0.7	0.9
F-3	Conjugated metabolite ^a	2.2	1.2	1.6	1.1
F-4	Conjugated metabolite ^a	1.0	0.7	0.3	0.7
F-5	Conjugated metabolite ^a	1.3	1.4	1.1	1.5
F-6	Conjugated metabolite ^a	8.5	7.3	6.3	4.3
F-7	—	1.9	1.3	—	1.5
F-8	—	2.1	1.2	0.7	0.9
F-9 (U-7/B-9)	PY-4-CO-5-COOH-5-H	1.4	2.5	2.2	2.4
F-10	—	—	—	0.1	0.4
F-11	—	—	—	0.1	—
F-12 (U-9/B-11)	PY-4-CO-5-OH	3.8	3.1	2.8	2.5
F-13	—	—	0.3	0.2	0.2
F-14	—	—	0.5	0.3	0.2
F-15	—	1.4	1.2	1.1	1.5
F-16	—	—	0.2	—	—
F-17	—	—	0.2	—	0.2
F-18	—	—	0.1	—	—
F-19	—	—	0.1	0.6	0.2
F-20 (B-17)	M- ω -COOH	1.6	1.4	0.9	1.1
F-21	—	—	0.1	—	—
F-22	PY	0.3	0.6	0.3	0.3
F-23	M-4-H	0.5	0.4	0.7	0.6
F-24	—	—	—	0.1	—
F-25	Chlorfenapyr	17.0	23.1	35.2	33.0
Procedural loss	[SepPak [®]]	4.2	5.2	6.3	6.5
Unextracted ¹⁴ C	—	19.0	16.5	16.9	17.0
Total		67.7	70.4	79.2	77.2

From Okada (1994b)

—, not detected (< 0.1% of dose)

^a Conjugates of PY-4-CO-5-OH and its further metabolized compounds.

Bile samples contained polar metabolites (B-2 through B-6), which accounted for higher proportions than metabolites eluted at similar retention times in the urine or faeces (Table 12).

Table 12. Summary of the total biliary metabolites collected through 24 hours, determined using HPLC with refractive index detection

Metabolite peak	Identification	Contents (% of dose)			
		2 mg/kg bw		20 mg/kg bw	
		Males	Females	Males	Females
B-1	—	0.7	0.6	0.4	0.3
B-2	Conjugated metabolite ^a	4.3	3.4	2.8	3.3
B-3	Conjugated metabolite ^a	5.2	5.0	3.2	4.0
B-4	Conjugated metabolite ^a	2.3	1.2	1.1	1.0
B-5	Conjugated metabolite ^a	5.8	4.7	3.3	3.6
B-6	Conjugated metabolite ^a	3.1	2.6	1.9	2.1
B-7	—	0.5	—	—	—
B-8	—	0.3	0.3	0.2	0.2
B-9 (U-7/F-9)	PY-4-CO-5-COOH-5-H	1.2	0.8	0.7	0.7
B-10	—	—	0.2	—	—
B-11 (U-9/F-12)	PY-4-CO-5-OH	1.5	1.4	0.8	1.3
B-12	—	0.2	0.1	0.2	0.1
B-13	PY-4-CO-5-H	0.5	0.4	0.6	0.4
B-14	—	0.4	0.6	0.4	0.4
B-15	—	0.1	—	—	—
B-16	—	—	—	—	—
B-17 (F-20)	M- ω -COOH	0.2	—	0.1	0.1
Procedural loss	[SepPak [®]]	3.8	2.8	1.7	2.4
Total		30.1	24.1	17.4	19.9

From Okada (1994b)

—, not detected (< 0.1% of dose)

^a Conjugates of PY-4-CO-5-OH and its further metabolized compounds.

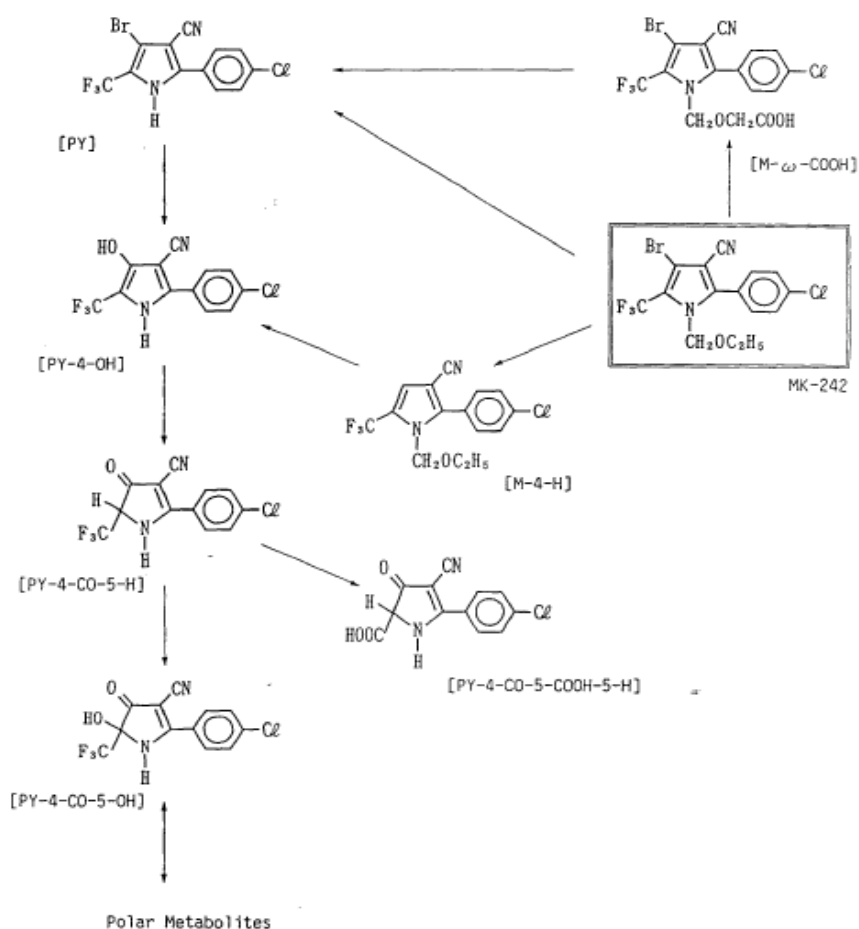
In summary, the major metabolic pathway of absorbed chlorfenapyr was the formation of PY-4-CO-5-OH by *N*-dealkylation, followed by transformation at position 4 of the pyrrole ring. Other metabolites retaining the *N*-alkyl group included M-4-H and M- ω -COOH. All the metabolites that either were successfully identified or had their structure estimated retained both the pyrrole and phenyl rings, which suggested that the bond between the two rings was resistant to cleavage during the metabolic transformation of chlorfenapyr. There was evidence of reabsorption of biliary metabolites (Okada, 1994b), supporting the existence of enterohepatic circulation of chlorfenapyr, previously suggested in a separate report (refer to Okada, 1994a).

The proposed metabolic pathway of chlorfenapyr in rats (Okada, 1994b) is illustrated in Figure 3.

2. Toxicological studies

2.1 Acute toxicity

Acute toxicity studies on chlorfenapyr are summarized in Table 13.

Figure 3. Proposed metabolic pathway of chlorfenapyr in rats**Table 13. Summary of acute toxicity, including irritancy and skin sensitization, of chlorfenapyr**

Type of test	Result	Purity; batch no.	Reference
Mouse, oral	LD ₅₀ = 45 mg/kg bw (males) LD ₅₀ = 78 mg/kg bw (females)	94.5%; AC 7504-59A	Bradley (1994a)
Rat, oral	LD ₅₀ = 441 mg/kg bw (males) LD ₅₀ = 1152 mg/kg bw (females)	94.5%; AC 7504-59A	Lowe (1993)
Rabbit, dermal	LD ₅₀ > 2000 mg/kg bw	94.5%; AC 7504-59A	Fischer (1992)
Rat, 4 h inhalation	LC ₅₀ = 0.83 mg/l (males)	94.5%; AC 7504-59A	Hoffman (1993)
Rabbit, skin irritation	Not irritating	94.5%; AC 7504-59A	Bradley (1993a)
Rabbit, eye irritation	Not irritating	94.5%; AC 7504-59A	Bradley (1993b)
Guinea-pig, skin sensitization (Magnusson & Kligman)	Not sensitizing	94.5%; AC 7504-59A	Otake (1995)

LC₅₀, median lethal concentration; LD₅₀, median lethal dose

*(a) Lethal doses**Oral administration*

In a test for acute oral toxicity, five mice (CRL:CD[®]BR) of each sex per dose received chlorfenapyr (BAS 306 I) (lot no. AC 7504-59A; purity 94.5%) in 0.5% carboxymethylcellulose and tap water by oral gavage at a dose level of 35, 70 or 140 mg/kg bw (dose volume 25 ml/kg bw) following a preliminary range-finding study. The study was conducted in compliance with United States Environmental Protection Agency (USEPA) test guideline OPPTS 870.1100_EPA 81.1. Overt signs of toxicity were observed at the 140 mg/kg bw dose level and were limited to decreased activity during the first 2 hours following dosing. Mortality occurred in both sexes at all levels of dosing and generally occurred during the first 24 hours following dosing. No gross pathological changes in surviving mice could be attributed to ingestion of the test material. Gross pathological changes in decedents were limited to bright red lungs in one mouse at 140 mg/kg bw. There were no consistent necropsy findings associated with active ingredient administration.

The median lethal dose (LD₅₀) was 45 mg/kg bw for males and 78 mg/kg bw for females (Bradley, 1994a).

The acute oral LD₅₀ of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was evaluated by oral gavage in 0.5% weight per volume (w/v) carboxymethylcellulose and tap water in five male rats (CRL:CD(SD)[®]BR) per dose group at a dose level of 156.25, 312.5, 625, 1250 or 2500 mg/kg bw and five female rats (CRL:CD(SD)[®]BR) per dose group at a dose level of 625, 1250 or 2500 mg/kg bw. In both cases, chlorfenapyr was administered at a constant dose volume of 10 ml/kg bw. The study was conducted partially in compliance with USEPA test guideline OPPTS 870.1100_EPA 81.1. Mortalities were observed in a dose-dependent manner in both sexes, starting from a dose level of 312.5 mg/kg bw in males and from a dose level of 625 mg/kg bw in females.

Signs of toxicity included hyperthermia and prostration in both sexes at dose levels of 625 mg/kg bw and above and prostration and salivation in males at dose levels of 312.5 mg/kg bw and above. Signs of hyperthermia included warm to the touch and an elevation of rectal body temperature, which was observed between 15 minutes and 24 hours after dosing. The majority of clinical signs were resolved in surviving animals by 24 hours after treatment. Gross pathological findings, such as muscle tetany, abdominal muscle striation, and congested, pale and mottled liver and kidney, were observed in both sexes at dose levels of 625 mg/kg bw and above and in males also at a dose level of 312.5 mg/kg bw.

The LD₅₀ of chlorfenapyr was calculated to be 441 and 1152 mg/kg bw for males and females, respectively (Lowe, 1993).

Dermal application

In a test for acute dermal toxicity, five male and five female rabbits (New Zealand White strain) were exposed to chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) moistened with tap water at a dose level of 2000 mg/kg bw by dermal occlusive application to intact skin for a 24-hour period. The application site encompassed an area equivalent to approximately 10% of the body surface area. The protocol was in compliance with USEPA test guideline OPPTS 870.1200_EPA 81.1. One female died on day 2 of the study. Prior to death, no overt signs of toxicity were observed. At necropsy, the animal exhibited pale kidneys, a pale spleen, mottled lungs and evidence of haemorrhage externally in the anogenital region. This haemorrhage was possibly caused by the occlusive wrapping during the 24-hour exposure period. The death did not appear to be treatment related. No overt signs of toxicity were observed. Body weights and body weight gains were generally unaffected in surviving animals. No treatment-related gross lesions were observed in surviving animals at termination of the 14-day observation period. The dermal LD₅₀ in male and female rabbits was greater than 2000 mg/kg bw (Fischer, 1992).

Exposure by inhalation

In a whole-body inhalation study, five rats (Sprague-Dawley CDR) of each sex per dose group were exposed to a mean analytical concentration of 0.34, 0.71, 1.8 or 2.7 mg/l (by gravimetry) of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) (dust) for 4 hours. An additional five rats of each sex served as controls. The study protocol was partially in compliance with test method B.2 of Directive 92/69/EEC and Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 403, Acute Inhalation Toxicity (1981). The mean analytical concentrations along with the mass median aerodynamic diameter (MMAD) and the corresponding geometric standard deviation (GSD) are summarized in Table 14.

Table 14. Acute inhalation toxicity of chlorfenapyr in rat: particle size data

Group	Nominal concentration (mg/l)	Analytical concentration (mg/l)	Mortality			MMAD (µm)	GSD
			Male	Female	Total		
I	—	0	0/5	0/5	0/10	—	—
II	17	1.8	4/5	1/5	5/10	7.3	2.0
III	5.8	0.71	2/5	0/5	2/10	7.0	1.9
IV	3.9	0.34	1/5	0/5	1/10	8.1	2.3
V	80	2.7	5/5	1/5	6/10	5.9	1.9

From Hoffman (1993)

Fourteen animals died before the end of the study. All remaining animals were examined post mortem for the presence of grossly visible abnormalities. Among toxicological findings, only discoloration of the lungs and tan/brown skin discoloration were believed to be treatment related. The median lethal concentration (LC_{50}), based on the analytical concentration, was calculated to be 1.9 mg/l for the combined sexes, 0.83 mg/l for the males and greater than 2.7 mg/l for the females.

The study (Hoffman, 1993) has the following weak points:

- The differences between the nominal and analytical exposure concentrations were attributed to impaction or sedimentation of the dust on the surface of the exposure chamber. In this context, although a whole-body exposure is foreseen by the guideline used, no precaution was taken to prevent licking of the test substance from the skin.
- The mean MMAD was calculated to be 7.1 µm with a GSD of 2.
- The results demonstrate that approximately 32% of the particles were less than 5 µm in size and 71% of the particles were less than or equal to 10 µm in size.

(b) Dermal irritation

The skin irritation potential of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was tested in three male albino rabbits (New Zealand White strain). The study was conducted according to test method B.4 of Directive 92/69/EEC and OECD Test Guideline No. 404, Acute Dermal Irritation/Corrosion (1992). The animals were exposed to 0.5 g chlorfenapyr moistened with tap water, applied to a 6.5 cm² gauze pad and applied to trunk skin under an occlusive cover for 4 hours. A barely perceptible erythema was observed in two of the three test animals at the 1-hour observation. At the 24-hour observation, all signs of skin irritation had resolved. No further signs of irritation were observed at 24, 48 or 72 hours after removal of the test material. Chlorfenapyr was not irritating to rabbit skin (Bradley, 1993a).

(c) *Ocular irritation*

The eye irritation potential of chlorfenapyr was investigated in three male rabbits (New Zealand White albino) by instillation of 0.1 g into the conjunctival sac of the left eye. Eyes were examined at -6, 1, 24, 48 and 72 hours and at 4 days. The study was conducted in accordance with test method B.5 of Directive 92/69/EEC and OECD Test Guideline No. 405, Acute Eye Irritation/Corrosion (1987). Eye irritation at 1 hour after treatment was characterized by slight redness of the conjunctiva, slight chemosis and slight discharge in all three animals. At 24 hours post-dosing, eye irritation was characterized by a diffuse area of corneal opacity (1/3), slight iritis (1/3), slight (2/3) to moderate (1/3) conjunctival redness and slight (1/3) to moderate (1/3) chemosis. At 48 hours post-dosing, corneal opacities were unchanged, the iritis had resolved and conjunctival irritation was limited to slight redness (3/3). At 72 hours post-dosing, eye irritation was limited to slight redness of the conjunctiva in one animal, while all signs of irritation had resolved in the remaining test animals. All signs of irritation had resolved in the remaining animal by 4 days post-dosing. Chlorfenapyr is considered to be practically non-irritating to the eye in this test (Bradley, 1993b).

(d) *Dermal sensitization*

The skin sensitization potential of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was investigated using topical and intradermal (maximization) methods in groups of 40 (control and test) female Hartley guinea-pigs. The protocol was partly in compliance with test method B.6 of Directive 92/69/EEC and OECD Test Guideline No. 406, Skin Sensitisation (1992). Chlorfenapyr was dissolved in olive oil. Test concentrations were selected on the basis of preliminary testing and were 2% for intradermal injection, 10% by occlusive application over 48 hours for dermal induction (seen to be moderately irritating) and 0.4% for application for 24 hours for challenge (highest non-irritant concentration). No dermal reaction was reported in the test substance groups. Therefore, chlorfenapyr was concluded not to be sensitizing in this study (Otake, 1995).

2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

Chlorfenapyr technical (lot no. AC6943-61A; purity 98.4%) was fed to groups of five male and five female CD-1 albino mice for 28 days at a dietary concentration of 0 (control), 160, 240, 320, 480 or 640 ppm (equal to 0, 30.1, 43.6, 62.3, 100.5 and 143.6 mg/kg bw per day for males and 0, 33.7, 57.8, 71.1 and 122.6 mg/kg bw per day for females, respectively; dose not calculated for 640 ppm females, as it caused death in all females by day 5). The protocol was partially in compliance with test method B.7 of Directive 92/69/EEC and OECD Test Guideline No. 407, Repeated Dose 28-Day Oral Toxicity Study in Rodents (1981). Deviation from the protocol was due to the absence of blood biochemistry analysis.

Mortality (Table 15) occurred in male mice at the 640 ppm (4/5), 480 ppm (1/5) and 240 ppm (1/5) dietary levels and in female mice at the 640 ppm (5/5), 480 ppm (3/5) and 320 ppm (1/5) levels. All mortality occurred during the first 7 days of the study. Signs of toxicity were observed in both sexes at the 640 and 480 ppm levels and in females at the 320 ppm level. Signs of toxicity observed included ataxia, depression, stiff gait and body drop.

Dose-related decreases in body weight gain (Table 15) were noted at all dietary levels, although these were not statistically significant. Body weight gains were reduced in the surviving male at 640 ppm (41%), in surviving males and females at 480 ppm and 320 ppm (combined average of 62% and 27%, respectively) and in males at 240 ppm (30%). Weight gains for both sexes were slightly reduced at 160 ppm (average of 21%).

Statistically significant haematological changes (Table 15) observed at termination consisted of decreased lymphocyte counts and increased eosinophil counts in surviving females at 480 ppm when compared with controls. The total white blood cell count for females at 480 ppm was

Table 15. Oral 28-day toxicity of chlorfenapyr in mouse

	0 ppm		160 ppm		240 ppm		320 ppm		480 ppm		640 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F ^a
Mortality	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	1/5	3/5	4/5	5/5
Body weight (g)	36.6	28.8	35.8	27.2	34.6	28.8	34.8	26.9	32.4*	25.7	33.5	—
(week 4)	(—)	(—)	(↓2%) ^b	(↓5.6%)	(↓5.5%)	(0%)	(↓5%)	(↓6.6%)	(↓11.5%*)	(↓10.8%)	(↓9.5%)	(—)
Body weight total	7.6	4.6	6.4	3.4	5.3	4.6	5.8	3.2	3.8	1.2	4.4	—
gain (g) ^c	(—)	(—)	(↓15.8%) ^b	(↓26%)	(↓30%)	(0%)	(↓23.7%)	(↓30%)	(↓50%)	(↓74%)	(↓41%)	(—)
Organ weights												
Absolute liver weight (g)	1.4	1.2	1.6	1.19	1.6	1.4*	1.6	1.3	1.7	1.4	2	—
Relative liver weight (% change relative to control)	—	—	↑9.6%	↓4.1%	↑13%	↑8.6%	↑14%	↑9%	↑30%*	↑23%*	↑47%	
Haematology												
Lymphocytes (%)	66.8	81	67.8	78	62.5	79.8	61.2	77.8	72.8	63.5*	84	—
Eosinophils (%)	—	—	0	0.2	0.3	0.8	0	0.8	0	6*	0	—

From Fischer (1991b)

* $P < 0.05$ (Williams' test)^a No parameters except mortality could be assessed in females at 640 ppm.^b Per cent change relative to controls.^c Body weight gain in grams during weeks 1–4.

comparable to that of controls. It should be noted that these results are based on only two surviving females (out of five) in the 480 ppm group. The increase in eosinophil counts was not considered biologically significant because eosinophil counts for males at 480 and 640 ppm (the highest concentration tested) were comparable to those of controls. Similarly, lymphocyte counts for males at 480 ppm were comparable to those of controls, and a slight, but not statistically significant, increase in lymphocyte counts was observed for males at 640 ppm. As changes in lymphocyte counts noted for females in the 480 ppm group (highest concentration at study termination) were based on observations in only two animals and were not observed in males at a higher concentration (640 ppm), they were not considered to be biologically significant.

Increased absolute and relative liver weights (Table 15) were observed in both sexes at dose levels of 240 ppm and higher and in males also at the 160 ppm level, although they were statistically significant only at 480 ppm. It was noted that the relative liver weight in one of the control males was somewhat low. These increases were accompanied by hepatocellular hypertrophy, which was observed microscopically at all treatment levels.

In conclusion, the no-observed-adverse-effect level (NOAEL) for chlorfenapyr in the mouse was 160 ppm (equal to 30.1 mg/kg bw per day in males and 33.7 mg/kg bw per day in females), based on decreased body weight gain, mortality and increased relative liver weight seen at 240 ppm (equal to 43.6 mg/kg bw per day in males and 57.8 mg/kg bw per day in females) (Fischer, 1991b).

Chlorfenapyr (lot no. AC 7171-141A; purity 93.6%) was fed to five groups of 20 male and 20 female albino CD-1 mice for 13 weeks at a dietary level of 0 (control), 40, 80, 160 or 320 ppm (equal to 0, 7.1, 14.8, 27.6 and 62.6 mg/kg bw per day for males and 0, 9.2, 19.3, 40.0 and 78.0 mg/kg bw per day for females, respectively).

Two mice at the 320 ppm level died during the study (Table 16). These deaths were attributed to ingestion of chlorfenapyr. Clinical signs of toxicity, consisting of diuresis and tremors, were observed in one male at the 320 ppm level from day 14 through day 19 of the study. No other signs of toxicity could be attributed to the test material at any other dietary level during the study period. Feed consumption at all dietary levels was comparable to that of controls for most of the measurement intervals. Treatment-related reductions in body weight gain were noted for both sexes at the 320 ppm level and for females at the 160 ppm level when compared with control values.

Haematological changes noted at the 320 ppm level included statistically significant increases in haematocrit and erythrocyte counts in males and a statistically significant increase in white blood cell counts in females. Changes in clinical chemistry parameters noted at the 320 ppm level included increased sodium and decreased albumin in males and increased potassium in females. Dose-dependent, statistically significant increases in liver to body weight ratios were observed in both sexes at the 320 ppm level and in males at the 160 ppm level. Spleen to body weight ratios were also significantly increased in male mice at the 320 and 160 ppm dietary levels. The percentage change was very similar at the two dose levels.

No treatment-related macroscopic changes were noted at termination. Microscopic evaluation revealed treatment-related hypertrophy of liver parenchymal cells in male mice at the 320 ppm (19/20 males; 10/20 females), 160 ppm (13/20 males; 4/20 females), 80 ppm (6/20 males; 0/20 females) and 40 ppm (1/20 males; 0/20 females) dietary levels, compared with controls (0/20 males; 0/20 females). There was no evidence of progressive degenerative change, toxic necrosis or proliferative change. Male and female mice at the 320 ppm level exhibited spongiform (encephalo)myelopathies in the brain (19/20 males; 19/20 females) and spinal cord (18/20 males; 19/20 females). One male at the 160 ppm level also exhibited myelopathy in the spinal cord.

Table 16. Oral 90-day toxicity of chlorfenapyr in mouse

	0 ppm		40 ppm		80 ppm		160 ppm		320 ppm	
	M	F	M	F	M	F	M	F	M	F
Mortality	0/20	1/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20	1/20
Body weight (g) (week 13)	41	34.1	39.9	34.3	39.9	33.6	40.6	32.5	38*	31.6*
	(—)	(—)	(↓2.7%) ^a	(↑0.6%)	(↓2.7%)	(↓1.5%)	(↓1.0%)	(↓4.7%)	(↓7.3%*)	(↓7.3%*)
Body weight total gain (g) ^b	11.8	9.8	10.4	9.7	10.2	9.6	11.9	8.1	8.7*	7*
	(—)	(—)	(↓11.9%) ^a	(↓1.0%)	(↓1.9%)	(↓2.0%)	(↑0.8%)	(↓17.3%)	(↓26.3%*)	(↓28.6%*)
Organ weights										
Absolute liver (g)	2.46	2.04	2.54	2.17	2.46	2	2.64	2	2.63	2.21
Relative liver (% change relative to control)	—	—	↑5.0%	↑6.2%	↑2.0%	↑3.6%	↑10.3%*	↑5.7%	↑13.3%*	↑18.0%*
Absolute spleen (g)	0.12	0.16	0.13	0.16	0.14	0.15	0.15*	0.15	0.15	0.16
Relative spleen (% change relative to control)	—	—	↑6.5%	↑4.4%	↑12.9%	0%	↑25.2%*	↑2.2%	↑25.5%*	↑15.6%
Blood chemistry										
Sodium (week 13, % change relative to control)	—	—	↑1.0%	↑0.2%	↓0.1%	↓0.6%	↑0.5%	↑1.9%	↑1.4%*	↑4.1%
Potassium (week 13, % change relative to control)	—	—	↑4.9%	−2.5%	0%	−2.5%	0%	↑11.3%	^c	↑32.5%*
Total protein (week 13, % change relative to control)	—	—	↑1.9%	↑5.6%	↑1.9%	0%	↑3.8%	↑3.7%	↑1.9%	↑9.3%*
Albumin (week 13, % change relative to control)	—	—	0%	0%	↓4.0%	↓6.9%	↓8.0%	↓6.9%*	↓12.0%*	↓6.9%
Haematology										
Haematocrit (% change relative to control)	—	—	↑2.6%	↓2.4%	↑1.9%	↑3.6%	↑1.0%	↓2.6%	↑17.9%*	↑8.4%
RBC (% change relative to control)	—	—	↑2.5%	↓6.1%	↑1.3%	↓3.7%	↑2.5%	↓6.1%	↑11.4%*	↑1.2%
Lymphocyte (% change relative to control)	—	—	↑2.6%	↓1.1%	↑14.0%*	↓2.2%	↑12.1%*	↓4.7%	↑10.5%	↓3.0%
WBC (% change relative to control)	—	—	↑85.0%	↓12.9%	↑85.0%	↑9.7%	↑25.0%	↓25.8%	↑60.0%	↑109.7%*
Neutrophile (% change relative to control)	—	—	↓5.3%	↑5.3%	↓31.2%*	↑8.4%	↓27.9%*	↑21.1%	↓22.6%	↑6.3%
Histopathology (n = 20)										
Myelopathies of the brain	0	0	0	0	0	0	0	0	19	19
Myelopathies of the spinal cord	0	0	0	0	0	0	1	0	18	19

From Fischer (1994)

F, female; M, male; RBC, red blood cells; WBC, white blood cells

* $P < 0.05$ (Williams' test)^a Per cent change relative to controls.^b Body weight gain in grams during weeks 1–13.^c No evaluation done, sample exhibited haemolysis.

The NOAEL for chlorfenapyr in the mouse was 80 ppm (equal to 14.8 mg/kg bw per day in males and 19.3 mg/kg bw per day in females), based on increased relative spleen weight and myelopathy in brain and spinal cord in male mice seen at 160 ppm (equal to 27.6 mg/kg bw per day). Hepatic hypertrophy observed in males in the absence of any other histopathological correlate was not considered adverse (Fischer, 1994).

Rats

Chlorfenapyr technical (lot no. AC 6943-61A; purity 98.4%) was fed to six groups of five male and five female Sprague-Dawley albino rats for 28 days at a dietary concentration of 0 (control), 600, 900, 1200, 1600 or 2000 ppm (equal to 0, 68.3, 106.3, 134.2, 176.8 and 243.0 mg/kg bw per day in males and 0, 74.8, 110.5, 147.2, 192.7 and 247.9 mg/kg bw per day in females, respectively). The selection of dose levels was based on a preliminary study. The protocol was in compliance with test method B.7 of Directive 92/69/EEC and OECD Test Guideline No. 407, Repeated Dose 28-Day Oral Toxicity Study in Rodents (1981).

Mortality (Table 17) occurred in males at the 2000 ppm (2/5) and 1600 ppm (1/5) dietary levels. All females survived until study termination. No overt signs of toxicity that could be attributed to administration of the test material were observed in the surviving animals during the study.

Dose-related, statistically significant decreases in feed consumption and body weight gain were noted at 1200–2000 ppm. Reductions in body weight gain occurred in both males and females and averaged 42% at 2000 ppm, 28% at 1600 ppm, 16% at 1200 ppm, 12.5% at 900 ppm and 6% at 600 ppm. Liver weights (absolute and relative to body weight) were increased in all dose groups and were dose dependent. Relative kidney weight was dose dependently increased in males from 900 ppm.

Haematological parameters were unaffected by treatment with chlorfenapyr. Values for alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), blood urea nitrogen and total protein were somewhat increased over control values in both male and female rats at the 2000, 1600 and 1200 ppm dietary levels.

Hepatocellular hypertrophy was observed in all surviving animals (3/3 males and 5/5 females) at 2000 ppm and in 75% (3/4) of surviving males and 40% (2/5) of surviving females at 1600 ppm.

In conclusion, the NOAEL for chlorfenapyr in rats was 600 ppm (equal to 68.3 mg/kg bw per day in males and 74.8 mg/kg bw per day in females), based on a relative liver weight increase (> 20% of control in both sexes) and ALT increase at 900 ppm (equal to 106.3 mg/kg bw per day in males and 110.5 mg/kg bw per day in females) (Fischer, 1991a).

Chlorfenapyr technical (lot no. AC 7171-141A; purity 93.6%) was fed to six groups of 20 male and 20 female albino rats for 13 weeks at a dietary concentration of 0 (control), 150, 300, 600, 900 or 1200 ppm (equal to 0, 10.9, 22.0, 44.9, 69.5 and 92.2 mg/kg bw per day in males and 0, 12.5, 26.1, 51.8, 75.4 and 102.8 mg/kg bw per day in females). The protocol was in compliance with test method B.7 of Directive 92/69/EEC and OECD Test Guideline No. 408, Repeated Dose 90-Day Oral Toxicity Study in Rodents (1981).

No treatment-related mortality was noted at any dose level (Table 18). Low incidences of clinical signs, including ataxia (3/20), decreased activity (3/20), dark material around nose (4/20) and anorexia (5/20), were observed in a few males of the 1200 ppm group. An increased incidence of brown material around the nose was also observed in males at 1200 ppm (9/20).

Body weights and body weight gains were lower than control values for both sexes at 1200 and 900 ppm and for males only at 600 ppm. Total body weight gains were decreased in males and females by 37% and 24%, respectively, at 1200 ppm, by 25% and 21%, respectively, at 900 ppm and by 14% and 8%, respectively, at 600 ppm.

Table 17. Oral 28-day toxicity of chlorfenapyr in rat

	0 ppm		600 ppm		900 ppm		1200 ppm		1600 ppm		2000 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
Mortality	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	2/5	0/5
Body weight (g)	312	228	304	217	280	215	277	205*	248*	189*	219*	174*
(week 4)	(—)	(—)	(↓2.6%) ^a	(↓4.9%)	(↓10.3%)	(↓5.7%)	(↓11.3%)	(↓10%*)	(↓20.6%*)	(↓17.1%*)	(↓30%*)	(↓23.7%*)
Body weight total	212	145	204	134	179.4	131.8	176.8	122.4*	149*	107*	113.7*	92.2*
gain (g) ^b	(—)	(—)	(↓3.8%) ^a	(↓7.6%)	(↓15%)	(↓10%)	(↓17%)	(↓15.6%)	(↓30%*)	(↓26%*)	(↓46%*)	(↓37%*)
Organ weights												
Absolute liver (g)	10.2	7.4	10.6	8.7	11	9*	11.5	9.3*	12.5*	9.9*	11.8	10.2*
Relative liver (%)	3.6	3.6	3.9	4.4*	4.4*	4.6*	4.6*	5*	5.6*	5.7*	6.1*	6.5*
Absolute kidney (g)	2.4	1.8	2.1	1.7	2	1.7	2	1.6*	1.6*	1.5*	1.6*	1.4*
Relative kidney (%)	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.7	0.9	0.8	0.9
Blood chemistry												
BUN (% change relative to control)	—	—	↑13.3%	↑26.8%	↑17.3%	↑19.7%	↑28.0%	↑35%*	↑48%*	↑42%*	↑71%*	↑56%*
Albumin (% change relative to control)	—	—	↓7.3%	↓5.1%	↓12.2%	↓7.7%	↓12%*	↓5.1%	↓15%*	↓10%*	↓17%*	↓10%*
Total protein (% change relative to control)	—	—	↓2.7%	↑6.0%	↓4.1%	↑6.0%	↓1.4%	↑9%*	↑4.1%	↑9%*	↓4.1%	↑10%*
ALT (% change relative to control)	—	—	↓10.3%	↑22.2%	↑6.1%	↑67.0%	↑25.7%	↑36.2%	↑43%*	↑79%*	↑71%*	↑98%*
GGT (U/l)	0	0.6	0	0.8	0.2	1	0.6	1.4	2.8*	2	4.7*	5.4*

From Fischer (1991a)

ALT, alanine aminotransferase; BUN, blood urea nitrogen; F, female; GGT, gamma-glutamyl transpeptidase; M, male; U, units; * $P < 0.05$ (Williams' test)^a Per cent change relative to controls.^b Body weight gain in grams during weeks 1–4.

Table 18. Oral 90-day toxicity of chlorfenapyr in rat

	0 ppm		150 ppm		300 ppm		600 ppm		900 ppm		1200 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
Mortality	0/20	1/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20	0/20	0/20
Body weight (g) (week 13)	531	313.4	502	324.2	501	311.5	478.3*	299.8	441.2*	274.4*	390.7*	270.3*
	(—)	(—)	(↓5.5%) ^a	(↓3.4%)	(↓5.6%)	(↓0.7%)	(↓10%*)	(↓4.4%)	(↓17%*)	(↓12.5%*)	(↓26.5%*)	(↓13.8%*)
Body weight total gain (g) ^b	376.2	188.9	347.1	192.4	347.7	184.1	323.4*	174.5	283.6*	148.5*	237.1*	143.9*
	(—)	(—)	(↓8%)* ^a	(↑2%)	(↓8%)	(↓3%)	(↓14%*)	(↓8%)	(↓25%*)	(↓21%*)	(↓37%*)	(↓24%*)
Organ weights												
Absolute liver (g)	13.8	8.19	14.2	8.51	14.9	8.74	15	9.58	16*	9.7	14.9	10.31
Relative liver (% change relative to control)	—	—	↑7.2%	0%	↑13%*	↑6.7%	↑19.5%*	↑21.7%*	↑38%*	↑33%*	↑45%*	↑42.3%*
Absolute kidney (g)	3.31	2.26	3.46	2.28	3.34	2.23	3.21	2.24	2.99*	2*	2.69*	1.99*
Relative kidney (% change relative to control)	—	—	↑9%	↓1.3%	↑6%	0%	↑6%	↑3.8%	↑7.5%	↑1.2%	↑10.6%	↑1.2%
Absolute spleen (g)	0.8	0.58	0.8	0.59	0.83	0.61	0.83	0.61	0.97*	0.68*	0.95*	0.71*
Relative spleen (% change relative to control)	—	—	0%	↓6%	↑6.2%	↑4.4%	↑12.5%	↑9.9%	↑43%*	↑30%*	↑62.5%	↑40%*
Blood chemistry												
BUN												
- week 6 (% change relative to control)	—	—	↑5.6%	↑3.5%	↑19.7%	↑7.9%	↑18.3%	↑24.4%	↑24.6%	↑17.2%	↑57.7%*	↑37.4%*
- week 13 (% change relative to control)	—	—	↓6.5%	↓4.5%	↑2.3%	↓10%	↑4.6%	↓3.2%	↑8.7%	↓5.1%	↑30%*	↑5.7%
Total protein												
- week 13 (% change relative to control)	—	—	↑4%	↑3.8%	↑4%	0%	↑4%	0%	↑6.6%*	↑7.7%*	↑1.3%	↑7.7%*
Albumin												
- week 6 (% change relative to control)	—	—	0%	↓5.5%	0%	↓8.2%	↓2.8%	↓5.5%	↓2.8%	↓2.8%	↓5.6%*	↓5.5%

	0 ppm		150 ppm		300 ppm		600 ppm		900 ppm		1200 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
- week 13 (% change relative to control)	—	—	0%	0%	↑2.4%	↓5%	↓2.5%	↓5%	↓2.5%	↓2.4%	↓10%*	↓5%
ALP												
- week 6 (% change relative to control)	—	—	↓18.2%	↓5.9%	↓5%	↑25%	↑5%	↑18.4%	↑44%*	↑14.4%	↑21%	↑44.6%
- week 13 (% change relative to control)	—	—	↓3%	↓7%	↑14%	↑35%	↑30%	↑23%	↑111%*	↑72%*	↑104%*	↑102%*
Haematology												
Haematocrit (% change relative to control)	—	—	↑1%	↑0.2%	↓3%	↑0.5%	↓2.4%	↓6%	↓4.5%	↓7.3%*	↓7.5%*	↓9%*
Haemoglobin (% change relative to control)	—	—	1.3	↓0.6	↓3.8	↓1.3	↓3.8	↓6.9*	↓5.7	↓9.4*	↓7.0*	↓8.8*
RBC (% change relative to control)	—	—	↑2.4%	0%	↓3.6%	0%	↓2.4%	↓6.2%	↓3.6%	↓8.6%*	↓10.8%*	↓8.6%*
Platelets (% change relative to control)	—	—	↑5.8%	↓2.8%	↑4.1%	↑9.4%	↑11.3%	↑12.1%	↑21.1%*	↑14.1%	↑20.0%*	↑13.8%*
Histopathology (n = 20)												
Vacuolation of brain and spinal cord	0	0	0	0	0	0	1	0	2	0	2	0
Vacuolation of sciatic nerve	0	0	0	0	0	0	0	0	0	0	1	0
Vacuolation of optic nerve	0	0	0	0	0	0	0	0	0	0	1	0

From Fischer (1993)

ALP, alkaline aminotransferase; BUN, blood urea nitrogen; F, female; M, male; RBC, red blood cells; * $P < 0.05$ (Williams' test)

^a Per cent change relative to controls.

^b Body weight gain in grams during weeks 1–13.

Red blood cell parameters (haematocrit, erythrocyte count and haemoglobin) were reduced for both sexes at 1200 ppm and for females at 900 ppm; in females, a significant reduction of haemoglobin was also evident at the 600 ppm dose level. Haematocrit and erythrocyte count were reduced in this group, but not significantly. Platelet counts were elevated in both sexes at 1200 and 900 ppm, but the changes were statistically significant only in males. Serum alkaline phosphatase level tended to be higher after 6 weeks and was clearly elevated in both sexes at 1200 and 900 ppm after 13 weeks of treatment. GGT level in both sexes and ALT level in males were elevated at 1200 ppm after 6 weeks, but not 13 weeks. Blood urea nitrogen was elevated at 1200 ppm in both sexes after 6 weeks, but only in males after 13 weeks. Also after 13 weeks, total serum protein was elevated in females at 1200 ppm and in both sexes at 900 ppm. Albumin was reduced after 6 and 13 weeks in males at 1200 ppm.

Increased absolute and relative liver and spleen weights (correlated with decreased red cell parameters) were observed at termination in the 1200 and 900 ppm groups. Other organ weight changes at these treatment levels were secondary to body weight decreases.

Hepatic parenchymal cell hypertrophy was observed in one male at 1200 ppm and one male at 900 ppm. Spongiform myelopathy (vacuolation) of the brain and spinal cord was seen in two males at 1200 ppm, two males at 900 ppm and one male at 600 ppm. In the two males at 1200 ppm with myelopathies of the brain and spinal cord, one also exhibited this lesion in the sciatic nerve and the other in the optic nerve. Testicular atrophy was observed in two males at 1200 ppm and three males at 900 ppm.

The NOAEL for chlorfenapyr in the rat was 300 ppm (equal to 22.0 mg/kg bw per day in males and 26.1 mg/kg bw per day in females), based on increases in relative liver weight, alkaline phosphatase and blood urea nitrogen and, in females, changes in red cell parameters seen at 600 ppm (equal to 44.9 mg/kg bw per day in males and 51.8 mg/kg bw per day in females) (Fischer, 1993).

Dogs

Chlorfenapyr technical (lot no. AC 7504-59A; purity 95.4%) was fed to four groups of four male and four female purebred Beagle dogs for 90 days at a dietary concentration of 0 (control), 60, 120 or 300 ppm. These concentrations were based on a range-finding study in which dogs exhibited body weight losses at 300 ppm and above and reductions in feed consumption within short feeding intervals (less than 2 weeks) at 400 ppm and above. A stability study conducted at room temperature on 60 and 300 ppm mock batches of chlorfenapyr in Certified Canine Diet Meal 5007 demonstrated that these batches were stable for a period of 14 days. Diets were assayed weekly for the first 4 weeks and monthly thereafter. The batches resulted in mean nominal percentages of 95.4%, 95.1% and 97.2% for the three treatment groups, respectively. Dietary concentrations were 60, 120 and 300 ppm; due to poor feed consumption (and subsequent body weight losses and/or emaciated appearance in a few dogs) in the high-dose group, the dose level was decreased on test day 15 to 240 ppm and on test day 26 to 200 ppm. Substance intake was as follows: 2.1, 3.9, 4.4, 6.0 and 7.3 mg/kg bw per day for males and 2.2, 4.5, 6.0, 5.8 and 7.1 mg/kg bw per day for females.

Reduction of the dietary concentration of the high-dose group to 200 ppm was well tolerated, with feed consumption and body weight gain paralleling that of the control group. There were no effects on feed consumption or body weight gain in the 120 or 60 ppm groups (Table 19).

No treatment-related changes in ophthalmology, haematology, clinical chemistry, urine analysis, organ weight or pathology were evident at any dose level.

Based on these results, the NOAEL was judged to be 120 ppm (equal to 3.9 mg/kg bw per day in males and 4.5 mg/kg bw per day in females), based on decreased body gain at 200 ppm (equal to 7.3 mg/kg bw per day in males and 7.1 mg/kg bw per day in females) (Kelly, 1993).

Table 19. Oral 90-day toxicity of chlorfenapyr in dog: mean body weight changes in selected weeks

Week	Body weight change (kg)							
	0 ppm		60 ppm		120 ppm		200 ppm ^a	
	M	F	M	F	M	F	M	F
1	0.10	0.00	0.50	0.40	0.10	0.40	-1.10	-0.1
2	0.00	0.00	0.40	0.20	0.10	0.10	-1.60	-1.00
4	0.40	0.70	0.40	0.60	0.30	0.50	-1.3**	-0.9**
5	0.80	0.80	0.90	0.80	0.70	0.70	-0.1*	-0.6*
13	1.10	1.10	1.10	1.80	1.80	0.90	0.40	0.00

From Kelly (1993)

F, female; M, male; * $P < 0.05$; ** $P < 0.01$ ^a Dose level was decreased on test day 15 from 300 ppm to 240 ppm and then decreased again on test day 26 from 240 to 200 ppm.

Chlorfenapyr (lot no. AC 7504-59A; purity 95.4%) was fed to four groups of male and female Beagle dogs for 1 year at a dietary concentration of 0 (control), 60, 120 or 240 ppm. The control, 60 ppm and 120 ppm groups consisted of five male and five female dogs, whereas the 240 ppm group consisted of six animals of each sex. Dietary batches of chlorfenapyr at concentrations of 60 and 240 ppm were found to be stable over a period of 14 days at room temperature or frozen. Substance intake was 0, 2.1, 4.0 and 8.7 mg/kg bw per day for males and 0, 2.3, 4.5 and 10.1 mg/kg bw per day for females at 0, 60, 120 and 240 ppm, respectively.

No mortality occurred during the study. One male dog in the 240 ppm group exhibited excessive salivation during weeks 1 through 4 of the study. No other clinical signs of toxicity were noted. Body weights (Table 20) and body weight gains (Table 21) for animals in the 240 ppm group were lower than concurrent control values during the study. Body weight gains following 52 weeks of treatment for males and females in the control group were 1.6 kg and 1.5 kg, respectively, whereas weight gain for both sexes in the 240 ppm group was only 0.5 kg.

Table 20. Oral 1-year toxicity of chlorfenapyr in dog: body weight in selected weeks

Week	Body weight (% of control)							
	0 ppm		60 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
26	—	—	↑4%	↓7%	↑6%	↓6%	↓1%	↓17%
52	—	—	↑4%	↓6%	↑7%	↓8%	↑10%	↓17%

From Kelly (1994)

F, female; M, male

Table 21. Oral 1-year toxicity of chlorfenapyr in dog: body weight changes in selected weeks

Week	Body weight change (% of control)							
	0 ppm		60 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
26	—	—	↑62%	↓36%	↑54%	↓29%	0%	↓91%
52	—	—	↓19%	↓13%	↑53%	↓31%	↓69%	↓67%

From Kelly (1994)

F, female; M, male

Feed consumption and feed efficiency values of treated animals were, in general, comparable to control values. Ophthalmoscopic examinations, haematology, clinical chemistry, urine analysis and organ weight data did not reveal any indications of adverse treatment-related effects. No treatment-related macroscopic changes were observed at study termination.

For microscopic findings, the lymphoid cell population (namely, the severity rating, or the number and size of lymphoid follicles) in the stomach of several dogs in the 120 and 240 ppm groups was slightly increased compared with concurrent controls (Table 22). However, the variable severity of the lymphoid cell population may represent individual animal variation of the lymphofollicular tissue. The latter tissue has been associated with infection of one or more types of spiral-shaped bacteria (i.e. *Helicobacter felis* and/or *Gastrospirillum hominis*), believed to be part of the natural gastric flora in dogs. The lymphoid cell population in the stomach of several dogs was considered to be within normal physiological range, and the gastric lymphoid cell findings were not considered to represent a toxicologically significant effect of the compound.

Table 22. Oral 1-year toxicity of chlorfenapyr in dog: histopathological findings in the stomach

	Incidence of finding							
	0 ppm		60 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
<i>Number of animals in the dose group</i>	5	5	5	5	5	5	6	6
Increased lymphocytes/plasma cells, lamina propria								
- minimal	1	1	—	2	1	2	2	2
- mild	1	—	1	—	3	—	3	2
- moderate	—	—	2	—	—	—	1	—
Hypertrophy/hyperplasia of lymphoid follicles								
- minimal	3	3	—	—	—	1	—	—
- mild	2	2	3	5	2	3	1	1
- moderate	—	—	2	—	3	1	5	5
Blind reading (1)								
- minimal	4	3	1	—	—	1	—	—
- mild	—	—	1	3	1	—	1	1
- moderate	1	2	2	2	2	2	2	3
- marked	—	—	1	—	2	2	3	2
Blind reading (2)								
- minimal	3	—	1	—	1	—	—	—
- mild	2	—	3	2	—	2	1	—
- moderate	—	1	1	2	3	2	2	1
- marked	5	—	—	1	1	2	3	4

From Kelly (1994)

F, female; M, male

The NOAEL for dietary administration of chlorfenapyr to purebred Beagle dogs was 120 ppm (equal to 4.0 mg/kg bw per day for males and 4.5 mg/kg bw per day for females), based on reduced body weight and body weight gain at 240 ppm (equal to 8.7 mg/kg bw per day for males and 10.1 mg/kg bw per day for females) (Kelly, 1994).

(b) Dermal application

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was administered dermally to four groups of six male and six female New Zealand White rabbits at a dose level of 0 (control), 100, 400 or 1000 mg/kg bw per day. Animals were treated for a 6-hour period, 5 days/week, for 4 weeks.

No treatment-related mortalities or clinical signs were observed in any dose group. All animals were free of significant dermal effects and ocular changes throughout the study. Mean body weight and feed consumption values of all test animals were comparable to control values.

A very slight decrease in mean erythrocyte count was observed in females in the 1000 mg/kg bw per day dose group. Dose-related elevations in mean serum cholesterol values were evident in the 400 and 1000 mg/kg bw per day dose groups. Other treatment-related changes in clinical chemistry values were elevations in the mean serum ALT for females in the 1000 mg/kg bw per day dose group (Table 23). Urine analysis values for all treated groups were comparable to control data.

Increased absolute and relative liver weights, relative to control values, were noted for animals in the 400 and 1000 mg/kg bw per day dose groups (Table 24). Cytoplasmic vacuolation of the liver was exhibited by several animals in the 400 and 1000 mg/kg bw per day dose groups. The vacuoles were of different sizes, the affected hepatocytes did not exhibit a consistent lobular pattern and no hepatocellular necrosis was associated with this morphological change.

Table 23. Dermal 28-day toxicity of chlorfenapyr in rabbit: haematology and clinical chemistry findings

	0 mg/kg bw per day		100 mg/kg bw per day		400 mg/kg bw per day		1000 mg/kg bw per day	
	M	F	M	F	M	F	M	F
RBC (million/ μ l)	5.9	5.89	6.2	5.76	6.08	5.57	5.62	5.37*
Hb (g/dl)	12.6	11.9	12.8	12.1	12.5	11.6	11.7	11.4
ALT (IU/l)	43	39	54	61	65	57	46	77*
Cholesterol (mg/dl)	58	67	59	66	93*	115*	108**	131**

From Blaszcak (1993)

ALT, alanine aminotransferase; F, female; Hb, haemoglobin; IU, international units; M, male; RBC, red blood cells; * $P < 0.05$; ** $P < 0.01$

Table 24. Dermal 28-day toxicity of chlorfenapyr in rabbit: liver gross pathology and histopathology

	0 mg/kg bw per day		100 mg/kg bw per day		400 mg/kg bw per day		1000 mg/kg bw per day	
	M	F	M	F	M	F	M	F
Absolute liver weight (g)	53.59	58.26	55.45	57.78	61.82	67.77*	72.29**	78.17**
Relative liver weight (%)	2.28	2.51	2.38	2.46	2.80**	2.94**	3.05**	3.38**
Cytoplasmic vacuolation of hepatocytes	0/6	0/6	0/6	0/6	1/6	3/6	4/6	4/6

From Blaszcak (1993)

F, female; M, male; * $P < 0.05$; ** $P < 0.01$

In conclusion, the NOAEL for chlorfenapyr was 100 mg/kg bw per day, based on haematological findings and a liver weight increase together with ALT increase and cytoplasmic vacuolation in hepatocytes seen at 400 mg/kg bw per day (Blaszczak, 1993).

(c) *Exposure by inhalation*

Fifteen male and 15 female Wistar rats per test group were exposed (nose only) to dynamic inhalation containing dust aerosol atmospheres (Table 25) of chlorfenapyr (batch no. 2181H88HV; purity 97.8%) for 6 hours per working day, 5 days/week, for approximately 90 days (65 exposures). From the 15 animals of each sex per group, 10 were designated as main group animals and 5 as recovery group animals. The main group animals were terminated 1 day after the exposure period; the recovery group animals were terminated after a recovery period of 28 days. The doses were 0 mg/m³ (control air) (group 0 dose / 01 recovery), 5 mg/m³ (group 1 dose / 11 recovery), 20 mg/m³ (group 2 dose / 21 recovery), 40 mg/m³ (group 4 dose / 41 recovery) and 80 mg/m³ (group 3 dose / 31 recovery).

Table 25. Inhalation 90-day toxicity of chlorfenapyr in rat: particle size data

Target concentration (mg/m ³ solids)	Measured concentration, mean ± SD (mg/m ³ solids)	Particle size distribution (MMAD), mean ± SD (µm)
Air control	n.d.	n.d.
5	5.1 ± 0.6	1.9 ± 0.2
20	20 ± 3	2.8 ± 0.1
80	79 ± 7	n.d.
40	41 ± 5	2.1 ± 0.3

From Ma-Hock et al. (2005)

MMAD, mass median aerodynamic diameter; n.d., not determined; SD, standard deviation

Inhalation of a chlorfenapyr dust aerosol led to the premature death of male animals at a concentration of 80 mg/m³ within the first 3 exposure days (7/15 male animals: 4/10 in the main group and 3/5 in the recovery group). Because of this high lethality, surviving animals of this group were terminated prematurely. Substitute groups were exposed to 40 mg/m³. However, one male animal was found dead after each of study day 35 and study day 80.

In addition to lethality, a slightly increased respiration rate was observed in animals exposed to 40 mg/m³. This finding is considered to be substance related. Alopecia or injuries that were observed in individual animals were considered to be incidental.

There were no statistically significant changes in the mean body weight (Table 26) or mean body weight gain (Table 27) in animals exposed to 5 or 20 mg/m³. Statistically significant differences were noted only in the substitute animals (groups 4 and 41). These changes in body weight and body weight gain were also considered to be incidental, as they were not concentration or time related.

Table 26. Inhalation 90-day toxicity of chlorfenapyr in rat: statistically significant changes in mean body weights

Test group	Sex	Test concentration (mg/m ³)	Study days	Change in mean body weight
4	Male	40	–4, 0 and 7	Decreased
4	Female	40	14	Increased
41	Male	40	–4 to 119	Decreased

From Ma-Hock et al. (2005)

Table 27. Inhalation 90-day toxicity of chlorfenapyr in rat: statistically significant changes in mean body weight gain

Test group	Sex	Test concentration (mg/m ³)	Study days	Change in mean body weight gain
4	Female	40	7, 14	Increased
41	Male	40	21, 28, 119	Decreased

From Ma-Hock et al. (2005)

A functional observational battery was carried out on assigned animals before exposure, during exposure (on study day 84), at the end of exposure (on study day 92) and at the end of the recovery period (study day 119). The functional observational battery started with passive observations, without disturbing the animals, followed by removal from their cage and open-field observations. Thereafter, sensorimotor tests and reflex tests were conducted. No substance-related effects were observed in home cage observations. The grip strength (forelimbs) was statistically significantly increased in males of the 40 mg/m³ group at the end of recovery. This change was considered to be incidental and not of biological relevance. Motor activity was measured on the same day and with the same animals on which the functional observational battery was performed. The measurement was performed in the dark using the Multi-Varimex System with four infrared beams per cage. The numbers of beam interrupts were counted over 12 intervals, each lasting 5 minutes. Measurements ended exactly 60 minutes thereafter. There were some variations of motor activity (mostly increases and some decreases) seen at some intervals in both sexes. These changes were considered to be incidental, because there was no concentration–response relationship and the finding was not consistently observed among intervals.

Regarding clinical pathology findings, inhalation of the test compound at 40 mg/m³ caused slight increases in white blood cells (Table 28) and lymphocytes at the end of the exposure period. These findings were assessed as being treatment related. At the end of the recovery period, leukocytes and lymphocytes were decreased when compared with the respective control values. The prolonged prothrombin times in the animals of both sexes exposed to 40 mg/m³ are also considered treatment related and are indicative of slight disturbance of coagulation (Table 29).

Table 28. Inhalation 90-day toxicity of chlorfenapyr in rat: white blood cell counts

Day	White blood cells (billions/l)							
	0 mg/m ³		5 mg/m ³		20 mg/m ³		40 mg/m ³	
	M	F	M	F	M	F	M	F
93	4.62	2.76	4.69	3.52	5.21	3.22	5.92*	3.96*
120	6.11	4.88	4.56*	3.86	6.25	3.7	3.97*	2.59**

From Ma-Hock et al. (2005)

F, female; M, male; * $P < 0.05$; ** $P < 0.01$

Table 29. Inhalation 90-day toxicity of chlorfenapyr in rat: clotting analyses

Day	Clotting (HQT, s)							
	0 mg/m ³		5 mg/m ³		20 mg/m ³		40 mg/m ³	
	M	F	M	F	M	F	M	F
93	29.5	26.9	30.4	26.7	30.6	27.3	32.9**	28.7*
120	28.7	26.6	30.2	26.4	29.5	26.5	29.7	27.1

From Ma-Hock et al. (2005)

F, female; HQT, Hepato-Quick test; M, male; * $P < 0.05$; ** $P < 0.01$

Regarding pathology, the two animals dosed with 40 mg/m³ that did not survive to termination of the study were examined by histopathology and showed autolytic changes to a different degree and marked pulmonary congestion in both cases, with one animal showing additional macroscopic lung changes (all lung lobes with diffuse discoloration, dark red). This is considered to be related to the mode of death due to asphyxiation and/or agonal respiratory distress. Considering the lethality that occurred at 80 mg/m³, the two deaths at 40 mg/m³ are considered to be substance related.

There were no major organ weight changes in either sex in the treated groups, and those observed were not dose related and exhibited no macroscopic or microscopic pathology. All further macroscopic or histopathological findings were regarded to be incidental in origin and not related to treatment.

No treatment-related effects were observed in animals exposed to the 5 or 20 mg/m³ concentrations of the test compound. Thus, the no-observed-adverse-effect concentration (NOAEC) was 20 mg/m³, based on lethality, haematological alterations and prolonged prothrombin times seen at 40 mg/m³ (Ma-Hock et al., 2005).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed to four groups of 65 male and 65 female CD-1 albino mice for 18 months at a dietary concentration of 0 (control), 20, 120 or 240 ppm. The compound was found to be stable in the diet after 7 and 14 days of storage in the animal room. Aliquots of diet mixes at 20 and 240 ppm were submitted to the laboratory for assessment of homogeneity; results demonstrated acceptable homogeneity. Achieved test article intakes were 0, 2.8, 16.6 and 34.5 mg/kg bw per day in males and 0, 3.7, 21.9 and 44.5 mg/kg bw per day in females, respectively. From weeks 16 to 80, the achieved intakes of test article were calculated on a weekly basis whenever feed consumption and body weights were recorded (i.e. every 2 or 4 weeks).

A statistically significant increase in mortality rate was noted for females in the 240 ppm group when compared with controls. At 18 months, survival rates for females in the control, 20 ppm, 120 ppm and 240 ppm groups were 80%, 71%, 73% and 60%, respectively (Table 30). As the severity and nature of treatment-related histopathological findings (vacuolation of the brain and dermatitis) seen in high-dose females were comparable to those seen in high-dose males, these changes were not considered to be indicative of the cause of death.

There were no neoplastic or non-neoplastic findings that could be attributed to the increase in mortality in high-dose females, and the survival rate for females in the high-dose group was not remarkably different from survival rates in historical control data from the same laboratory. A relationship between higher mortality and treatment with chlorfenapyr was considered unlikely.

Treatment with chlorfenapyr was associated with a reduced growth rate and a slightly lower feed intake in males and females receiving 240 ppm and in females receiving 120 ppm. Body weight gains over the 80-week treatment period were reduced by 30% and 14% for males and females in the 240 ppm group, respectively, and by 14% for females in the 120 ppm group. Body weight gain in selected weeks is shown in Table 31. A slightly reduced feed intake was also seen in males receiving 120 ppm.

Treatment with chlorfenapyr for 80 weeks did not produce any adverse effects on clinical signs, haematology parameters or organ weights. A slight increase in the incidence of skin ulceration and scabs was noted at necropsy for animals at the 240 ppm level. This finding was microscopically presented as dermatitis.

Table 30. Carcinogenicity study in mouse: survival rate

Week(s)	Males												Females											
	0 ppm			20 ppm			120 ppm			240 ppm			0 ppm			20 ppm			120 ppm			240 ppm		
	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%
0	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
0–4	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
5–8	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
9–12	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
13–16	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
17–20	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
21–24	0	55	100	1	54	98	0	55	100	0	55	100	0	55	100	0	55	100	1	54	98	0	55	100
25–28	0	55	100	0	54	98	3	52	95	1	54	98	0	55	100	1	54	98	0	54	98	0	55	100
29–32	0	55	100	0	54	98	0	52	95	1	53	98	1	54	98	2	52	95	1	53	96	2	53	96
33–36	0	55	100	0	54	98	0	52	95	0	53	98	0	54	98	1	51	93	2	51	93	0	53	96
37–40	0	55	100	1	53	98	0	52	95	0	53	98	0	54	98	1	50	91	1	50	91	1	52	96
41–44	0	55	100	0	53	98	0	52	95	2	51	93	0	54	98	0	50	91	2	48	87	0	52	95
45–48	2	53	96	1	52	95	0	52	95	0	51	93	1	53	98	0	50	91	1	47	85	0	52	95
49–52	0	53	96	0	52	95	1	51	93	1	50	91	2	51	93	1	49	89	0	47	85	0	52	95
53–56	0	53	96	4	48	87	0	51	93	0	50	91	0	51	93	1	48	87	0	47	85	2	50	91
57–60	1	52	95	0	48	87	0	51	93	0	50	91	1	50	91	1	47	85	0	47	85	2	48	87
61–64	3	49	89	1	47	85	2	49	89	2	48	87	2	48	87	2	45	82	1	46	84	3	45	82
65–68	2	47	85	1	46	84	1	48	87	2	46	84	0	48	87	1	44	80	1	45	82	3	42	76
69–72	3	44	80	2	44	80	2	46	84	1	45	82	1	47	85	1	43	78	1	44	80	5	37	67
73–76	0	44	80	2	42	76	2	44	80	0	45	82	2	45	82	0	43	78	2	42	76	1	36	65
77–80	3	41	75	0	42	76	5	39	71	2	43	78	1	44	80	4	39	71	2	40	73	2	34	62
Termination	0	41	75	1	41	75	0	39	71	0	43	78	0	44	80	0	39	71	0	40	73	1	33	60

From Bernier (1994)

%, percentage of the original group size; D, number of animals dying during the period; S, number of animals surviving at the end of the period

Table 31. Carcinogenicity study in mouse: body weight gain in selected weeks

Week	Body weight gain (g)							
	0 ppm		20 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
0	3.18	1.69	2.59	1.29	2.89	1.28	2.29 B	1.53
10	0.62	0.88	0.51	0.88 D	0.55	0.22 E	0.54	0.40 B
20	0.65	0.63	0.54	0.32	0.42	0.21	0.22 E	0.21
30	0.79	1.35	0.49	0.95	0.22 C	0.56 E	0.48 b	0.61 E
42	0.99	0.68	1.07	0.13	0.36 C	0.53	0.30 C	0.21
50	0.52	-0.2	0.38	0.60 F	0.14 B	-0.12	0.24 a	0.16
62	0.42	0.8	0.64	0.42	0.56	0.66	0.39	0.52
70	-0.17	0.34	-0.01	0.26	-0.19	-0.01	-0.33	0.38
80	-0.49	-0.36	-0.25	0.32	-0.29	0.08	0.05	-0.15

From Bernier (1994)

a = $P < 0.05$ (Wilcoxon's test); b = $P < 0.01$ (Wilcoxon's test); B = $P < 0.01$ (Dunnett's); C = $P < 0.001$ (Dunnett's); D = $P < 0.05$; E = $P < 0.01$; F = $P < 0.001$ (t-test on adjusted means, not shown, using week 0 data as covariates)

Non-neoplastic changes associated with the test article consisted of vacuolation of the white matter of the brain. This was noted following 52 weeks of treatment in males and females receiving 240 ppm and in females receiving 120 ppm. Following 80 weeks of treatment, the incidence of vacuolation for males receiving 120 or 240 ppm was 9/39 (23%) and 33/43 (77%), respectively, and the incidence for females in these two groups was 23/40 (58%) and 33/33 (100%), respectively. Vacuolation was also noted in pre-terminal males and females receiving 240 ppm and in males receiving 120 ppm. Vacuolation was seen microscopically in spinal cord and optic nerve sections in males and females receiving 240 ppm following 80 weeks of treatment and in pre-terminal males at 240 ppm. Vacuolation of the spinal cord was also noted in pre-terminal females at 240 ppm (Table 32).

Table 32. Carcinogenicity study in mouse: selected non-neoplastic findings (n = 65 mice of each sex per group)

		0 ppm		20 ppm		120 ppm		240 ppm	
		M	F	M	F	M	F	M	F
Brain	Basophilic bodies	6	4	7	0	0	2	1	1
	Vacuolation	4	10	3	5	14	28	49	58
Liver	Focal hyperplasia	4	1	5	0	2	0	2	0
	Hypertrophy, centrilobular	0	0	1	0	4	0	4	1
	Hypertrophy, diffuse	1	0	3	0	2	0	4	0
	Inflammation, focal	19	2	16	0	10	3	7	6
Optic nerve	Vacuolation	0	0	0	0	0	1	12	14
Spinal cord lumbar	Vacuolation	0	0	0	0	2	0	11	3
Spinal cord cervical	Vacuolation	0	1	0	0	2	0	20	23
Spinal cord thoracic	Vacuolation	0	2	1	0	2	1	17	16
Skin	Dermatitis	9	3	12	1	11	6	21	9

From Bernier (1994)

F, female; M, male

The incidence of tumours was generally low, and statistical analysis of the distribution of these lesions across control and treated groups showed no evidence that their presence in any organ or tissue could be related to treatment (Table 33).

Table 33. Carcinogenicity study in mouse: selected neoplastic findings (n = 65 mice of each sex per group)

	0 ppm		20 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
Hepatocellular adenoma	8	0	10	1	4	1	2	0
Hepatocellular carcinoma	1	0	3	0	0	0	0	0
Alveolar/bronchiolar adenoma	16	7	12	7	13	8	10	9
Alveolar/bronchiolar carcinoma	2	1	1	2	3	0	4	1
Interstitial cell adenoma (testis)	1	NA	0	NA	1	NA	0	NA
Myeloid leukaemia	3	0	0	0	0	0	0	0
Adenocarcinoma (mammary gland)	NA	0	NA	2	NA	1	NA	1
Leiomyoma (uterus)	NA	5	NA	0	NA	1	NA	0
Adenocarcinoma (uterus)	NA	0	NA	0	NA	1	NA	0

From Bernier (1994)

F, female; M, male; NA, not applicable

No compound-related effects on any of the parameters evaluated were evident at the 20 ppm dietary level.

Based on the results of this study, the NOAEL for chronic toxic effects following 80 weeks of dietary administration of chlorfenapyr to mice was considered to be 20 ppm (equal to 2.8 mg/kg bw per day for males and 3.7 mg/kg bw per day for females), based on body weight effects and vacuolation of the white matter of the brain at 120 ppm (equal to 16.6 mg/kg bw per day for males and 21.9 mg/kg bw per day for females).

Treatment with chlorfenapyr at dietary levels of up to 240 ppm for 80 weeks did not produce any evidence of a carcinogenic effect. The NOAEL for oncogenic effects through 80 weeks of treatment was 240 ppm, the highest dose tested. Based on feed consumption data, this represents a daily intake of approximately 34.5 mg/kg bw per day for males and 44.5 mg/kg bw per day for females (Bernier, 1994).

Rats

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed to four groups of 65 male and 65 female Sprague-Dawley rats for 24 months at a dietary level of 0 (control), 60, 300 or 600 ppm. Achieved test article intakes were 0, 2.9, 15.0 and 30.8 mg/kg bw per day for males and 0, 3.6, 18.6 and 37.0 mg/kg bw per day for females, respectively. Formulations were stable for 14 days at room temperature and 166 days at freezer temperature.

An interim termination was conducted on 10 rats of each sex per group after 12 months of treatment. Survival was similar to or higher than that in controls and significantly increased for females at the 600 ppm level. At 24 months, survival rates were 51%, 55%, 55% and 59% for males and 36%, 35%, 44% and 58% for females in the control, 60 ppm, 300 ppm and 600 ppm groups, respectively (Table 34).

Table 34. Carcinogenicity study in rat: survival rate and historical control data

Week	Survival rate (%)									
	0 ppm		60 ppm		300 ppm		600 ppm		Historical control ^a	
	M	F	M	F	M	F	M	F	M	F
0	100	100	100	100	100	100	100	100	100	100%
52	95	98	100	100	100	97	95	100	96.88%	98.11%
104	51	36	55	35	55	44	59	58	35–67.35%	34.83–58.62%
105	59	36	55	33	54	44	55	55	—	—

From Trutter (1994)

^a Eight studies with males and nine with females conducted between April 1984 and April 1991.

There was no evidence of a treatment-related effect from clinical observations, ophthalmoscopic findings, urine analysis results or gross pathology in any of the three test groups. Dose-related decreases in mean body weight and body weight gain were seen in the 300 and 600 ppm groups. Statistically significant decreases in body weights were noted in the 300 ppm dose group at weeks 8, 14 and 23 through 92 for males and at weeks 4, 6, 7, 10, 15 through 25, 31 through 70, 92, 100 and 105 for females. For the 600 ppm level, body weights were reduced for the majority of the treatment period. Statistically significant reductions in body weight gain were noted for both sexes at the 300 and 600 ppm levels through week 52. Body weight gains for the first 52 weeks of the study were reduced by 6.4% and 12.1% for males and by 10.5% and 21.7% for females in the 300 and 600 ppm groups, respectively. Body weight gains were also reduced through week 104 at these two levels as compared with controls; the differences were statistically significant for females. Body weight gains over the 104-week treatment period were reduced by 9.1% and 9.8% for males and by 26.4% and 29.0% for females in the 300 and 600 ppm groups, respectively (Table 35).

Table 35. Carcinogenicity study in rat: body weight gain

Week(s)	Body weight gain (g)							
	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
0	51	28	60	26	62	29	67	27
1–52	487	258	478 (↓2%)	245 (↓5%)	458* (↓6%*)	231* (↓10%*)	428* (↓12%*)	202* (↓22%*)
1–104	419	295	410 (↓2%)	298 (↑1%)	381 (↓9%)	218* (↓26%*)	378 (↓10%)	210* (↓29%*)

From Trutter (1994)

F, female; M, male; * $P < 0.05$

Total mean feed consumption for week 1 through week 52 and week 1 through week 104 and total mean feed efficiency for week 1 through week 4 were significantly reduced at the 600 ppm dietary level.

Compound-related haematology findings included statistically significant decreases in erythrocyte count, haemoglobin and haematocrit values at weeks 13, 26 and 52 for males at the 600 ppm level. Erythrocyte count and haematocrit values were significantly decreased at week 13 only for females at the 600 ppm level. Reticulocyte counts were significantly increased for males at the 600 ppm level. Statistical analysis of serum chemistry data showed significant increases in mean globulin values at weeks 13 and 26 for females in the 300 ppm group, at weeks 26 and 53 for males in the 600 ppm group and at weeks 13, 26, 53 and 78 for females in the 600 ppm group. Significant decreases in albumin to globulin ratios were also noted for males in the 600 ppm groups at weeks 26 and 78. Mean

total cholesterol values were significantly increased for females in the 300 and 600 ppm groups (Table 36).

Table 36. Carcinogenicity study in rat: haematology and serum chemistry

	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
Week 52								
RBC (millions/ μ l)	8.5	7.72	8.65	7.27	8.53	7.2	7.85*	7.55
Haemoglobin (g/dl)	15.5	15.1	15.7	14.8	15.8	14.1	14.1*	14.7
Haematocrit (%)	44.2	41.7	44.4	40.2	48.1	39.2	39.6*	40.6
Reticulocytes (%)	1.8	1.5	1.4	4.6	1.6	6.7	3*	1.7
Week 26								
Albumin to globulin ratio	2.08	2.92	2.02	3.04	1.90	2.29*	1.59*	2.05*
Week 78								
Cholesterol (mg/dl)	124	105	81*	120	103	145*	140	142*
Albumin to globulin ratio	1.63	2.05	1.45	1.99	1.39*	1.87	1.34*	1.55*

From Trutter (1994)

F, female; M, male; RBC, red blood cells; * $P < 0.05$

Statistical evaluation of organ weight measurements showed treatment-related increases in the liver to terminal body weight ratios in the mid-dose and high-dose males and females at 12 months and in the high-dose males and females at 24 months (Table 37). Histopathological evaluations showed compound-related alterations in the livers of the animals of the 300 and 600 ppm groups. The microscopic changes consisted of hepatocellular enlargement, which was usually of slight severity. This enlargement was observed as a centrilobular to midzonal change in animals from the mid-dose and high-dose groups terminated after 52 weeks of treatment and also occurred as a diffuse change in occasional animals from these two groups at termination (104 weeks). The hepatocellular enlargement occurred with greatest incidence in severity in the high-dose group and correlated with the increased liver to body weight ratios noted in rats of both sexes of that group (Table 38).

Statistical analysis of tumour data showed no differences that were attributable to compound administration.

Table 37. Carcinogenicity study in rat: liver weights (12 and 24 months)

	0 ppm		60 ppm		300 ppm		600ppm	
	M	F	M	F	M	F	M	F
12 months								
Liver to body weight (%)	2.342	2.630	2.544	2.703	2.672*	3.082*	2.888*	3.282*
Absolute liver weight (g)	15.96	10.16	17.09	10.53	17.28	11.01	18.02	11.43
24 months								
Liver to body weight (%)	2.547	2.814	2.670	2.820	2.937	3.093	3.301*	3.558*
Absolute liver weight (g)	16.26	12.65	16.80	12.67	16.99	11.41	18.19	12.78

From Trutter (1994)

* $P < 0.05$

Table 38. Carcinogenicity study in rat: liver histopathological findings (24 months) (n = 65 rats of each sex per group)

	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
Diffuse hepatocellular enlargement	1	3	0	1	4	5	23	13
Centrilobular hepatocellular enlargement	2	3	1	0	13	13	24	41

From Trutter (1994)

F, female; M, male

No compound-related effects on any of the parameters evaluated were evident at the 60 ppm dietary level. Thus, the NOAEL for chronic toxic effects through 24 months of treatment with chlorfenapyr was 60 ppm (equal to 2.9 mg/kg bw per day for males and 3.6 mg/kg bw per day for females), based on reduced body weight and body weight gain and increased liver weight associated with hepatocellular enlargement at 300 ppm (equal to 15.0 mg/kg bw per day for males and 18.6 mg/kg bw per day for females). Chlorfenapyr was not carcinogenic in rats when tested at doses up to 600 ppm (equal to 30.8 mg/kg bw per day for males and 37.0 mg/kg bw per day for females) (Trutter, 1994).

2.4 Genotoxicity

A summary of genotoxicity studies is given in Table 39, and details about the studies are given below. Results from an acceptable range of in vitro and in vivo genotoxicity studies showed no indication of genotoxic potential:

- Chlorfenapyr does not induce gene mutations, as it did not induce either base pair substitution or frame shift mutation in any of the tester bacterial strains.
- Chlorfenapyr was found not to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells in vitro (with or without metabolic activation).
- Chlorfenapyr showed no evidence of clastogenic or polyploidy inducing activity.
- Chlorfenapyr did not induce mutations at the HGPRT locus in CHO cells.
- Chlorfenapyr did not cause chromosomal damage in vivo.
- Chlorfenapyr did not induce unscheduled deoxyribonucleic acid (DNA) synthesis (UDS), indicating no induction of DNA damage in cultured rat hepatocytes.

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in standard plate incorporation assays with six bacterial tester strains. A dose range-finding assay conducted at dose levels of 100–5000 µg/plate showed excessive toxicity at all doses. Therefore, the doses tested in the mutation assay were 1, 5, 10, 25 and 50 µg/plate with and without metabolic activation by hepatic S9 preparation from Aroclor 1254-treated rats with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2 *uvrA*–, along with concurrent vehicle and positive controls in two independent trials. Because of excessive toxicity at 50 µg/plate in TA1537 and TA1538, chlorfenapyr technical was retested at 0.5, 1, 5, 10, 15, 20 and 25 µg/plate in two independent trials.

Results of original and confirmation assays showed that no dose-related increases in revertant frequency or positive responses were found in the presence or absence of metabolic activation in any trial. Chlorfenapyr does not induce gene mutations, as it did not induce either base pair substitution or frame shift mutation in any of the tester bacterial strains (Mulligan, 1994a).

Table 39. Summary of genotoxicity studies of chlorfenapyr**(a) In vitro tests**

Test system/method guideline	Organism/strain(s)	Levels tested	Result	Remarks	Reference
Bacterial reverse gene mutation	<i>Salmonella typhimurium</i> and <i>Escherichia coli</i>	1–50 µg/plate	No gene mutation was induced with or without metabolic activation	Bacteriotoxicity at 100 µg/plate	Mulligan (1994a)
Chromosomal aberration	CHO cell culture	1.8–225 mg/l	No chromosomal aberration was induced with or without metabolic activation	With S9 mix: 14.1 mg/l reduced the mitotic index to 39% of the control Without S9 mix: 225 mg/l reduced the mitotic index to 32% of the control	Sharma (1994a)
	Chinese hamster lung cells	1.8–225 µg/plate	No evidence of clastogenic or polyploidy inducing activity with or without metabolic activation		Adams 1994
UDS	Primary rat hepatocytes	0.05–0.3 mg/l	No UDS was induced with or without metabolic activation	Cytotoxicity at 0.5 mg/l	San (1993)
HGPRT	CHO cell culture	5–500 mg/l (250 mg/l without S9 mix)	No mutations at the HGPRT locus in CHO cells were induced with or without metabolic activation	With S9 mix: 500 mg/l caused cytotoxicity with about 50% survival Without S9 mix: 500 mg/l caused cytotoxicity with about 30% survival	Sharma (1994b)

Table 39 (continued)*(b) In vivo tests*

Test system/ method guideline	Species/sex/number per group	Frequency of application	Sampling	Dose level	Results	Reference
Mouse micronucleus test	Mice/5 of each sex per group	Single oral gavage treatment	24, 48, 72 h post- dosing	Males: 7.5, 15, 30 mg/kg bw Females: 5, 10, 20 mg/kg bw	No effect on the number of micronucleated polychromatic erythrocytes in the bone marrow at any dose level at any time for males or females	Sharma (1994c)

CHO, Chinese hamster ovary; DNA, deoxyribonucleic acid; S9, 9000 × g supernatant fraction of rat liver homogenate; UDS, unscheduled DNA synthesis

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in the in vitro chromosomal aberration test in Chinese hamster ovary (CHO) cells in both the presence and absence of an exogenous metabolic activation system, comprising hepatic S9 preparation from Aroclor 1254-treated rats. Based on dose range-finding results (cytotoxicity to monolayers and greater than 50% reduction in mitotic indices at higher dose levels), doses tested in the definitive assay were 6.25, 12.5, 25 and 50 µg/ml with S9 metabolic activation and 12.5, 25, 50 and 100 µg/ml without activation. Limited by toxicity in the definitive test, the doses selected and evaluated included 6.25, 12.5 and 25 µg/ml with S9 metabolic activation and 25, 50 and 100 µg/ml without activation, along with concurrent untreated vehicle and positive controls. Cells were harvested 12, 24 and 48 hours after treatment. Positive controls were included in the 24-hour harvest only. Data on the frequencies of structural chromosomal aberrations and polyploid cells were collected and analysed statistically (Table 40).

The only positive result was a significant increase in the polyploid index in CHO cells at only one concentration at the 24-hour harvest and in the presence of S9 metabolic activation. Values were in the biologically acceptable range, the linear trend in the dose-response relationship was negative and this increase was not observed at 48 hours. The activity observed does not appear to have any biological significance.

Chlorfenapyr was found to be negative in this assay and thus is not considered to be a clastogenic agent (Sharma, 1994a).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 93.8%) was tested in vitro in Chinese hamster lung cells to determine whether it would cause chromosomal aberrations or polyploidy at a range of dose levels, along with concurrent controls, at three treatment periods without metabolic activation and one treatment period with metabolic activation by hepatic S9 preparation from Aroclor 1254-treated rats. Mitotic indices were calculated for cultures treated with the various concentrations of chlorfenapyr and the solvent control. High doses evaluated for chromosomal aberration analysis were selected based on 50% or greater reduction in mitotic indices in each treatment group as follows:

- 6-hour treatment with cell harvest 18 hours later at doses of 3.5, 7.0 and 14.1 µg/ml with S9 activation (set 1);
- 6-hour treatment with cell harvest 18 hours later at doses of 3.5, 7.0, 14.1 and 225 µg/ml without S9 activation (set 2);

Table 40. Chromosomal aberrations in CHO cells with chlorfenapyr technical, polyploid index, 12-, 24- and 48-hour harvest

Treatment	Flask no.	No. of cells scored	No. of polyploid cells	Polyploid index (%)	Group average (%)
12 h harvest					
With S9					
Untreated	1	100	3	3.0	2.5
	2	100	2	2.0	
Vehicle	3	100	1	1.0	1.0
	4	100	1	1.0	
25 µg/ml	5	100	1	1.0	1.0
	6	100	1	1.0	
12.5 µg/ml	7	100	0	0.0	0.5
	10	100	1	1.0	
6.25 µg/ml	11	100	0	0.0	0.0
	12	100	0	0.0	
Without S9					
Untreated	13	100	0	0.0	0.5
	14	100	1	1.0	
Vehicle	15	100	1	1.0	1.0
	16	100	1	1.0	
100 µg/ml	17	100	0	0.0	0.5
	18	100	1	1.0	
50 µg/ml	19	100	0	0.0	0.5
	20	100	1	1.0	
25 µg/ml	21	100	2	2.0	1.5
	22	100	1	1.0	
24 h harvest					
With S9					
Untreated	25	100	0	0.0	5.0
	26	100	10	10.0	
Vehicle	27	100	2	2.0	2.5
	28	100	3	3.0	
25 µg/ml	31	100	2	2.0	2.0
	32	100	2	2.0	
12.5 µg/ml	33	100	0	0.0	0.5
	34	100	1	1.0	
6.25 µg/ml	35	100	12	12.0	11.0
	36	100	10	10.0	
CP 25 µg/ml	37	100	0	0.0	0.5
	38	100	1	1.0	
Without S9					
Untreated	39	100	0	0.0	0.5
	40	100	1	1.0	

Table 40 (continued)

Treatment	Flask no.	No. of cells scored	No. of polyploid cells	Polyploid index (%)	Group average (%)
Vehicle	41	100	2	2.0	1.0
	42	100	0	0.0	
100 µg/ml	43	100	1	1.0	1.0
	44	100	1	1.0	
50 µg/ml	45	100	1	1.0	1.0
	46	100	1	1.0	
25 µg/ml	47	100	0	0.0	1.0
	48	100	2	2.0	
MMC 1 µg/ml	51	100	2	2.0	1.5
	52	100	1	1.0	
48 h harvest					
With S9					
Untreated	53	100	0	0.0	0.5
	54	100	1	1.0	
Vehicle	55	100	0	0.0	0.5
	56	100	1	1.0	
25 µg/ml	59	100	5	5.0	5.0
	60	100	5	5.0	
12.5 µg/ml	61	100	3	3.0	4.5
	62	100	6	6.0	
6.25 µg/ml	63	100	7	7.0	5.5
	64	100	4	4.0	
Without S9					
Untreated	65	100	0	0.0	0.5
	66	100	1	1.0	
Vehicle	67	100	0	0.0	0.0
	68	100	0	0.0	
100 µg/ml	69	100	2	2.0	1.5
	70	100	1	1.0	
50 µg/ml	71	100	1	1.0	0.5
	72	100	0	0.0	
25 µg/ml	73	100	2	2.0	2.0
	74	100	2	2.0	

From Sharma (1994a)

CP, cyclophosphamide; MMC, mitomycin-C; S9, 9000 × g supernatant fraction of rat liver homogenate

- 24-hour treatment with cell harvest at the end of the treatment period at doses of 1.8, 3.5 and 14.1 µg/ml without S9 activation (set 3);
- 48-hour treatment with harvest at the end of the treatment period at doses of 1.8, 3.5 and 7.0 µg/ml without S9 activation (set 4).

All positive control compounds caused large, statistically significant increases in the frequency of either aberrant cells or polyploid cells when compared with the solvent control.

Chlorfenapyr technical caused no statistically significant increases in the proportion of aberrant cells or polyploid cells in either the presence or absence of S9 mix at any treatment time.

It was concluded that chlorfenapyr technical showed no evidence of clastogenic or polyploidy inducing activity in this in vitro test system (Adams, 1994).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in the in vitro UDS assay in cultured rat hepatocyte cells. The concentrations tested were 0.05, 0.075, 0.1, 0.125, 0.15 and 0.3 µg/ml. Because of excessive toxicity at 0.3 µg/ml, the highest dose evaluated was 0.15 µg/ml. It should be emphasized that an intermediate concentration between 0.3 and 0.15 µg/ml should have been tested to confirm the results.

No significant increase in the incorporation of tritiated thymidine into nuclear DNA of the cultured cells was found at any dose level. Therefore, chlorfenapyr technical did not induce UDS, indicating no induction of DNA damage in cultured rat hepatocytes (San, 1993).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested for inducing mutations at the HGPRT locus in CHO cells in vitro. The dose selection was based on a dose range-finding assay performed at dose levels of 10, 100, 500, 1250, 2500 and 3000 µg/ml with and without metabolic activation by hepatic S9 preparation from Aroclor 1254-treated rats. There was massive precipitation from 500 to 3000 µg/ml in the test system. In addition, the monolayers exhibited excessive toxicity at dose levels of 500 µg/ml and higher. Therefore, the mutation assay was initiated with 500 µg/ml as the high dose with S9 metabolic activation. Because of the excessive toxicity at 500 µg/ml, the doses evaluated in the mutation assay were 5, 10, 50, 100 and 250 µg/ml. The assay without metabolic activation was then conducted with test dose levels of 2.5, 5, 25, 50, 100 and 250 µg/ml.

The top dose evaluated (250 µg/ml) with and without metabolic activation demonstrated toxicity and precipitation in the test system, meeting the criteria for a maximum dose level. Statistical analysis of data from both original and confirmation assays showed that mutation frequencies at any dose were not significantly greater than concurrent control values. In addition, they were found to be within the range of background mutation frequencies observed for untreated or vehicle controls in 22 other studies performed under the same conditions. Chlorfenapyr technical therefore did not induce mutations at the HGPRT locus in CHO cells (Sharma, 1994b).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in groups of 15 male and 15 female CD-1 albino mice. Animals received a single dose by gavage at a dose level of 0 (corn oil: vehicle), 7.5, 15 or 30 mg/kg bw for males and 0, 5, 10 or 20 mg/kg bw for females. Dose levels were selected based on mortality in range-finding studies. In that experiment, there was mortality in all dose groups and no demonstration of cytotoxicity to the target organ (bone marrow cells). Therefore, in order to achieve the maximum tolerated dose, an LD₅₀ was calculated from the mortality data. The top doses tested in the micronucleus assay represent 60% of the LD₅₀ values for male and female mice. Furthermore, toxicokinetics studies showed that bone marrow is reached by the active substance and that dose levels were high enough to produce clinical effects.

Five animals of each sex per group were killed at 24, 48 and 72 hours following dosing for the collection of bone marrow cells. Five males dosed at 30 mg/kg bw died within 72 hours of dosing. Clinical signs of toxicity (slight diarrhoea) were observed in females dosed at 10 and 20 mg/kg bw, but no female mortality occurred at any dose level. A combination of deaths in males at the high dose and the selection of 60% of the LD₅₀ values for males and females demonstrate that testing was performed at or above the maximum tolerated dose, thus satisfying the USEPA's criteria of dose selection in this assay. There was no effect on the number of micronucleated polychromatic erythrocytes in the bone marrow at any dose level at any time interval for males or females.

Therefore, chlorfenapyr did not cause chromosomal damage in vivo in this test system (Sharma, 1994c).

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed daily to four groups of Sprague-Dawley rats at a dietary concentration of 0 (control), 60, 300 or 600 ppm. Each parental generation consisted of 30 male and 30 female rats per group. Both parental generations were treated during a pre-mating period of 10–11 weeks, and treatment continued during both a 20-day mating period and a post-mating period. Mated females continued to be treated during the ensuing gestation, lactation and post-weaning periods until termination. F₁ and F₂ litters were culled to eight pups per litter on postnatal day 4. Through both parental generations, mean test substance intake values of chlorfenapyr administered during the pre-mating treatment periods, as derived from feed consumption data and using the nominal concentrations, approximated 5, 22 and 44 mg/kg bw per day at 60, 300 and 600 ppm, respectively. Similar test substance intake values were seen during the gestation periods; however, during the lactation periods, mean test substance intake values increased to approximately 9, 42 and 82 mg/kg bw per day for the 60, 300 and 600 ppm dietary levels, respectively. Pretest analyses of mock batches of diets at the low and high concentrations (60 and 600 ppm, respectively) confirmed that the mixing procedure developed and used for this study produced diets that were stable for at least 21 days at ambient storage.

No treatment-related mortality occurred. Parental toxicity was noted at the 300 and 600 ppm dietary levels. Reduced mean body weight gains during the mating and post-mating periods were noted for P₁ and F₁ males and females in the 600 ppm dose group. Body weight and body weight gain for the F₁ parental females in the 300 and 600 ppm dose groups were reduced ($\geq 10\%$) during gestation, as was body weight during lactation ($\geq 10\%$) (Table 41). There was no evidence of any parental toxicity at the 60 ppm dietary level.

Table 41. Multigeneration study in rat: parental body weight and body weight gain

	0 ppm		60 ppm		300 ppm		600 ppm	
	P ₁ adults	F ₁ adults	P ₁ adults	F ₁ adults	P ₁ adults	F ₁ adults	P ₁ adults	F ₁ adults
Body weight gain, males (% change relative to control)	—	—	↓5.9a	↑9.9b	↑2.5a	↓3.7b	↓25.3a**	↓17.8b*
Body weight, females (% change relative to control)								
- weeks 1–10 ^{pm}	—	—	0	↓3.8	↓3	↓9.4**	↓3.9	↓13**
- days 0, 6, 13, 20 ^{ge}	—	—	↑0.5	↓7.3*	↓2	↓9.6**	↓4	↓13.1**
- day 21 ^{la}	—	—	↓2	↓7.6**	↓2.8	↓10.7**	↓7**	↓14.2**
Body weight gain, females (% change relative to control), days 0, 6, 13, 20 ^{ge}	—	—	↑5.8	↓10.7	↑2	↓12.8	↓2	↓13.8*

From Schroeder (1994)

a, weeks 11–16; b, weeks 32–43; ge, gestation; la, lactation; pm, pre-mating; * $P < 0.05$; ** $P < 0.01$ (parametric data: Anova+Dunnett's; non-parametric data: Kruskal-Wallis + Dunn's rank sum)

Reproductive performance was not affected at any dietary level of chlorfenapyr. No adverse effects at the 60, 300 or 600 ppm dietary levels were evident from reproductive indices, gestation indices or parturition data during either litter interval (Table 42).

Table 42. Multigeneration study in rat: reproductive indices

		0 ppm		60 ppm		300 ppm		600 ppm	
		M	F	M	F	M	F	M	F
Mating (% mated/total)	P ₁	93.3	100	100	100	86.7	96.7	96.7	100
	F ₁	82.8	90	96.7	100	90	100	93.3	100
Pregnancy (% pregnant/mated)	P ₁	—	93.3	—	93.3	—	96.6	—	93.3
	F ₁	—	85.2	—	86.7	—	96.7	—	96.7
Fertility (% impregnating/mated)	P ₁	92.8	—	93.3	—	100	—	93.1	—
	F ₁	83.3	—	89.7	—	96.3	—	96.4	—

From Schroeder (1994)

F, female; M, male

Statistically significant reductions, relative to control data, in mean pup weight (postnatal days 0 through 21) were noted for the F₁ and F₂ litters in the 300 and 600 ppm dose groups (Table 43). Pup body weights from the day of birth to weaning for F₁ and F₂ litters in the 60 ppm dose group were comparable to control data.

Table 43. Multigeneration study in rat: pup body weight

		Pup body weight (g)			
		0 ppm	60 ppm	300 ppm	600 ppm
F ₁		54.1	52.9	48.9**	47.4**
F ₂		51.9	50.2	48.4*	44.8**

From Schroeder (1994)

* $P < 0.05$; ** $P < 0.01$

In the F₂ high-dose group, the mean pup viability index was lower than that of the controls, and this difference was statistically significant and considered indicative of a treatment-related response. F₂ pup live birth index was unaffected by the treatment, and pup weaning index for the high-dose group over the F₂ litter interval was 100%. Thus, no adverse effect of treatment with chlorfenapyr at a dietary level up to and including 300 ppm was evident from pup survival indices during lactation for either F₁ or F₂ litters. At the 600 ppm level, the only effect of treatment was reduced pup survival over the days 0–4 interval for the F₂ litters (Table 44).

No adverse effects of treatment with chlorfenapyr were apparent from the macroscopic postmortem evaluations of the parental animals or pups, and no adverse effect of treatment at the 600 ppm dietary level was observed on microscopic evaluations of reproductive tissues, pituitary glands or macroscopic lesions.

The NOAEL for both parental and developmental toxicity was 60 ppm (equal to 5 mg/kg bw per day), based on decreased body weight at 300 ppm (equal to 22 mg/kg bw per day for parental animals) and on decreased body weight in pups at 300 ppm (equal to 22 mg/kg bw per day). The NOAEL for reproductive toxicity was 600 ppm (equal to 44 mg/kg bw per day), the highest dose tested (Schroeder, 1994).

Table 44. Multigeneration study in rat: pup index

		0 ppm	60 ppm	300 ppm	600 ppm
Pup live birth index	F ₁	97.4	99.3	97.5	93.1
	F ₂	98.5	97.6	98.5	96.2
Pup viability index	F ₁	98.8	98.5	95.3	97
	F ₂	96.2	93.5	93.5	86.5*
Pup weaning index	F ₁	99.5	99.6	97.7	98.1
	F ₂	97.8	98.5	98.7	100

From Schroeder (1994)

* $P < 0.05$ (parametric data: Anova+Dunnett's; non-parametric data: Kruskal-Wallis + Dunn's rank sum)

(b) *Developmental toxicity*

Rats

Four groups of 25 presumed pregnant Sprague-Dawley rats were used in this study. Females were presumed pregnant on the basis of observations of sperm in smears of vaginal contents and/or copulatory plugs. Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was administered by gavage in an aqueous suspension with 0.5% carboxymethylcellulose at a dose level of 0 (control), 25, 75 or 225 mg/kg bw per day on day 6 through day 15 of gestation. These dose levels were chosen on the basis of results from a pilot study in which 160 mg/kg bw per day caused slightly reduced body weight gain and feed consumption in pregnant females and 270 mg/kg bw per day for 7 days caused body weight loss, reduced feed consumption and increased liver to body weight ratios in non-pregnant females.

There was no mortality or signs of toxicity in the definitive study. A dose level of 225 mg/kg bw per day resulted in significantly reduced maternal body weight gains, feed consumption and water consumption during intervals of the treatment period (Table 45). These parameters were also reduced during some intervals of the treatment period at 75 mg/kg bw per day, but the reductions were less severe than those at the higher dose level, and only those for feed consumption reached statistical significance. Maternal body weight gain, feed consumption and water consumption were not reduced at 25 mg/kg bw per day.

Ovarian, uterine and fetal observations were unaffected at all dose levels. No external, soft tissue or skeletal malformations or variations were attributable to treatment (Table 46).

Based on these results, the NOAEL for maternal toxicity was 75 mg/kg bw per day, based on decreased body weight gain at 225 mg/kg bw per day. The NOAEL for developmental toxicity was 225 mg/kg bw per day, the highest dose tested. Chlorfenapyr is neither a developmental toxicant nor a teratogenic agent in the rat (Martin, 1993).

Rabbits

Four groups of 20 artificially inseminated female New Zealand White rabbits were used in this study. Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was administered by gavage in an aqueous suspension with 0.5% carboxymethylcellulose at a dose level of 0 (control), 5, 15 or 30 mg/kg bw per day on day 7 through day 19 of gestation. Rabbits were terminated on day 29 of gestation. These dose levels were selected on the basis of the results of a range-finding study in which mortality occurred at 50 mg/kg bw per day and reductions in maternal body weight, body weight gain and feed consumption occurred at 25 mg/kg bw per day.

Table 45. Developmental toxicity study in rat: maternal data

Maternal parameter	0 mg/kg bw per day	25 mg/kg bw per day	75 mg/kg bw per day	225 mg/kg bw per day
Mortality	0	0	0	0
Clinical signs	—	—	—	Localized alopecia
Feed consumption				
- g/day	21.6	20.9	20.6*	19.9**
- g/kg bw per day	69.4	68.5	67.2	65.9**
Body weight (% change relative to control)				
- day 20	—	↓2.7%	↓1.3%	↓2.4%
- day 20 ^a	—	↓3%	↓1.8%	↓2.8%
Body weight gain (% change relative to control)				
- day 20	—	↓6%	↓3.5%	↓6.4%
- day 20 ^a	—	↓8.4%	↓6.5%	↓10%
Gravid uterine weight (% change relative to control)	—	0%	↑4.3%	↑2.4%

From Martin (1993)

* $P < 0.05$; ** $P < 0.01$ ^a Corrected body weight = gestation body weight minus total litter weight.**Table 46. Developmental toxicity study in rat: caesarean section data**

	0 mg/kg bw per day	25 mg/kg bw per day	75 mg/kg bw per day	225 mg/kg bw per day
No. of rats tested	25	25	25	25
No. of pregnant rats	22	25	24	25
Mean no. of corpora lutea	16.0	15.8	16.2	15.7
Mean no. of implantations	14.3	13.8	14.7	14.2
No. of litters with ≥ 1 live fetuses	22	25	24	25
Mean no. of resorptions, early/late	1.0/0.0	0.9/0.0	1.3/0.1	0.6/0.0
Mean no. of live fetuses	13.3	12.9	13.3	13.5
Live male fetuses (%)	52.9	50.3	52.6	50.1
Mean fetal weight (g), males/females	3.30/3.18	3.37/3.24	3.38/3.26	3.30/3.13
Litters/fetuses with any alteration	8/18	8/10	7/10	11/22

From Martin (1993)

There was no mortality or clinical signs of toxicity in the definitive study. No doe aborted or delivered prematurely during the study. Maternal body weight gain and feed consumption were reduced during the treatment period at 15 and 30 mg/kg bw per day, although the effects were not statistically significant and lacked a clear dose–response relationship (Table 47).

There was no evidence of maternal toxicity at 5 mg/kg bw per day. Ovarian, uterine and fetal observations were unaffected at all dose levels. No external, soft tissue or skeletal malformations or variations of fetuses were attributable to treatment (Table 48).

Table 47. Developmental study in rabbit: maternal body weight gain

Maternal parameter	0 mg/kg bw per day	5 mg/kg bw per day	15 mg/kg bw per day	30 mg/kg bw per day
Mortality	0	0	0	0
Body weight (% change relative to control)				
- day 29	—	0%	↓3.18%	↓1%
- day 29 ^a	—	↑1.4%	↓3.3%	↑1.1%
Body weight gain (% change relative to control)				
- day 29	—	↓5.7%	↓26.5%	↓25.6%
- day 29 ^a	—	↑100%	↓275%	↓22%
Gravid uterine weight (% change relative to control)	—	↓9.4%	↓3.4%	↓17.4%

From Hoberman (1993)

^a Corrected body weight = gestation body weight minus total litter weight.

Table 48. Developmental study in rabbit: caesarean section data

	0 mg/kg bw per day	5 mg/kg bw per day	15 mg/kg bw per day	30 mg/kg bw per day
No. of rabbits tested	19	20	20	20
No. pregnant	18	19	16	17
Mean no. of corpora lutea	10.8	10.2	10.6	10.1
Mean no. of implantations	8.3	7.5	8.1	7.4
No. of litters with ≥ 1 live fetuses	17	18	16	16
Mean no. of resorptions, early/late	0.3/0.1	0.3/0.1	0.1/0.1	0.7/0.2
Mean no. of live fetuses	8.4	7.6	7.9	6.9
Live male fetuses (%)	53.7	50.2	50.2	52.2
Mean fetal weight (g), males/females	44.30/43.80	44.90/44.86	43.68/43.49	45.60/45.32
Litters/fetuses with any alteration	15/42	15/39	16/39	13/33

From Hoberman (1993)

Based on these results, the NOAEL for maternal toxicity was 5 mg/kg bw per day, based on decreased body weight gain at 15 mg/kg bw per day. The NOAEL for developmental toxicity was 30 mg/kg bw per day, the highest dose tested. Chlorfenapyr is neither a developmental toxicant nor a teratogenic agent in the rabbit (Hoberman, 1993).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was administered orally, via gastric intubation, as a single dose to four groups of 10 male and 10 female Sprague-Dawley CD[®] rats at a dose level of 0 (control), 45, 90 or 180 mg/kg bw. These dose levels were chosen on the basis of results from a pilot study in which mortality occurred at 250 mg/kg bw for both sexes. The next

highest dose in the pilot study was 125 mg/kg bw, which produced no mortality but showed decreased activity and rapid breathing, indicating toxicity. All animals were observed for 2 weeks following treatment.

Treatment-related mortality occurred in the 180 mg/kg bw dose group. Two males and two females in this group were found dead on the day of treatment. Treatment with chlorfenapyr at dose levels up to 180 mg/kg bw did not affect body weights or feed consumption during the study.

At study day 1, there was a statistically significantly greater decrease in activity in the mid-dose group than in the control group. Because this response was not dose dependent, this decrease in the mid-dose group is considered to be incidental and not related to treatment.

Observations made during the functional observational battery evaluations on day 1 indicated that administration of chlorfenapyr at a dose level of 180 mg/kg bw resulted in changes in gait, locomotion and arousal. These changes were considered responses to the test material, as this dose was lethal in 20% of the animals. No functional observational battery findings were observed at lower (non-lethal) doses, 90 and 45 mg/kg bw (Table 49).

Table 49. Acute neurotoxicity study in rat

	0 mg/kg bw		45 mg/kg bw		90 mg/kg bw		180 mg/kg bw	
	M	F	M	F	M	F	M	F
No. of rats	10	10	10	10	10	10	10	10
Mortality	0	0	0	0	0	0	2	2
Body weight (g)	302.4	222.4	314.6	210.6	307.6	213.9	290.1	223.5
Motor activity								
<i>Movements: mean change score^a</i>								
- day 1	-45.6	-60.3	-39	-50.6	-61	-60.8	-55.2	-60.4
		-52.9		-44.8		-60.9**		-57.8
- day 8	11.9	-23.4	31.4	-19.1	4.2	-7.4	14.3	-4.1
		-5.7		6.2		-1.6		5.1
- day 15	-1.8	-9.8	23.6	-23.7	22.4	4.3	29.4	1.2
		-5.8		-0.1		-13.4		-15.1
Functional observation battery								
<i>Open-field observations (day 1)</i>								
Tremors	0	0	0	0	0	0	0	0
Locomotion impaired (slightly)	1	0	0	0	1	0	5	3
Gait incoordination (body drags)	0	0	0	0	0	0	3	0
Arousal (low)	1	0	0	1	2	0	5	3
<i>Reflex/physiological/manipulative observations</i>								
Touch response: no reaction	1	0	0	0	0	0	0	0
Aerial righting response: slightly uncoordinated	0	0	2	0	1	0	2	0

From Ponnock (1996)

F, female; M, male; ** $P < 0.01$

^a Change score: algebraic difference between the post-treatment movement score and the corresponding scores at pretreatment.

Cage-side observations made following the functional observational battery evaluations also indicated that 3 of 20 animals in the 180 mg/kg bw dose group and 2 of 20 animals in the 90 mg/kg

bw dose group were lethargic on the day of treatment. These observations were not noted on the following day or at 7 or 14 days following treatment (Table 50).

Table 50. Acute neurotoxicity study in rat: cage-side observations

	0 mg/kg bw		45 mg/kg bw		90 mg/kg bw		180 mg/kg bw	
	M	F	M	F	M	F	M	F
No. of rats	10	10	10	10	10	10	10	10
Cage-side observations								
- day 1	—	—	—	—	2 lethargic	—	2 lethargic, 2 found dead	1 lethargic, 2 found dead
- day 15	1 (↓ feed consumption, ↓ faecal volume)	—	1 (↓ feed consumption, ↓ faecal volume) ^a	1 (↓ feed consumption, ↓ faecal volume) ^a	—	—	—	—

From Ponnock (1996)

F, female; M, male

^a Water valve not functioning properly.

No macroscopic or microscopic compound-related changes were observed. All areas of the brain, spinal cord and peripheral nerves from treated animals were similar to those of controls.

Based on the increased mortality noted in the 180 mg/kg bw group and clinical signs of toxicity observed in the 180 and 90 mg/kg bw groups, the NOAEL for an acute administration of chlorfenapyr was 45 mg/kg bw. Chlorfenapyr was not considered to be an acute neurotoxicant (Ponnock, 1996).

Subchronic neurotoxicity

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed to four groups of 25 male and 25 female Sprague-Dawley rats for 52 weeks at a dietary concentration of 0 (control), 60, 300 or 600 ppm. Achieved test article intakes were 0, 2.6, 13.6 and 28.2 mg/kg bw per day for males and 0, 3.4, 18.0 and 37.4 mg/kg bw per day for females, respectively. The test substance was stable in rodent feed over a 14-day period. Following 13 weeks of treatment, five rats of each sex per group were anaesthetized and then perfused in situ with formalin. After 52 weeks of treatment, 10 rats of each sex in the control and 600 ppm groups and 5 rats of each sex from the 60 and 300 ppm groups were selected for perfusion. The remaining animals were fed control diet for a 16-week recovery period. At the end of the recovery period, all surviving male rats from the control and 600 ppm groups, along with 5 and 10 randomly selected male rats from the 60 and 300 ppm groups, respectively, and 5 female rats per group were selected for perfusion. All surviving animals not selected for perfusion were terminated by carbon dioxide asphyxiation.

No treatment-related mortality occurred, and the test substance had no biologically significant effects on the parameters of the functional observational battery (Table 51), body temperature or clinical observations. Motor activity (Table 51) was not affected by treatment with chlorfenapyr. There were no necropsy observations attributable to the test substance, and alterations observed in the microscopic examination (excluding neurohistological examination, see below) of tissues from female rats were considered unrelated to chlorfenapyr.

Body weights, body weight gains and feed efficiency (Table 51) were statistically significantly reduced for animals in the 300 and 600 ppm groups either at intervals during treatment or at the end of the treatment period. These findings were more prevalent in males than in females.

During the 16-week recovery period, overall body weight gains for animals in the 300 and 600 ppm groups were higher than during the treatment period. Relative mean feed consumption values (g/kg bw per day) were reduced for animals in the 600 ppm groups during the first 2 weeks of the treatment period and tended to be increased at other intervals during the exposure period. For the entire treatment period (day 1 through day 365), relative feed consumption values were increased for both sexes in the 600 ppm group and for males in the 300 ppm group. During the recovery period, little increase in relative feed consumption values was noted for the 300 and 600 ppm groups when compared with control values.

Table 51. Subchronic neurotoxicity study in rat

	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
Number of rats	25	25	25	25	25	25	25	25
Mortality	1	3	3	1	0	0	0	1
Body weight (% change relative to control), week 52	—	—	↓0.7	↑2.5	↓8.5	↓9.7	↓13.7*	↓6.9
Body weight (% change relative to control), recovery	—	—	↓3.5	↑2.4	↓6.5	↓3.11	↓3	↑0.1
Body weight gain (% change relative to control), week 52	—	—	↓0.8	↑6	↓12	↓16.6	↓21.3**	↓11.9
Feed consumption (% change relative to control), week 52	—	—	↑2.1	↓1	↑6.2**	↑3.8	↑9.7**	↑7**
Feed consumption (% change relative to control), recovery	—	—	↓1	↓3.2	↑1	↑7.8	↑2	↑6.8
Motor activity (week 52)								
Number of movements	289.6	414.7	374.6	414.3	349.4	405.8	328.0	367.3
Time spent in movement (s)	381.3	634.4	570.3	576.1	510.1	617.6	489.8	494.8
Functional observation battery								
Number of rats	19	17	17	18	20	20	20	18
<i>Open-field observations (week 52)</i>								
Tremors	0	1	0	0	0	0	0	0
Vocalizations during handling	0	0	1	1	0	0	1	0
Gait normal	19	17	17	18	20	20	20	18
Arousal normal	19	17	17	18	20	20	20	18

From Foss (1994)

F, female; M, male; * $P < 0.05$; ** $P < 0.01$

Neurohistological examination of males in the 600 ppm group terminated after 13 weeks of exposure revealed swelling of the myelin sheath in the spinal roots. A more generalized myelinopathic process, consisting of vacuolar myelinopathy, vacuolation and/or myelin sheath swelling of the brain and spinal cord, was present in male rats in the 300 and 600 ppm groups terminated after 52 weeks of exposure. This process was not associated with any evidence of myelin or axon degeneration and was not evident in the male rats terminated after the recovery period (Table 52).

No adverse effects of treatment were noted at the 60 ppm dietary level. Thus, the NOAEL for a dietary exposure to chlorfenapyr was 60 ppm (equal to 2.6 mg/kg bw per day for males and 3.4 mg/kg bw per day for females), based on body weight data and neurohistopathological findings at 300 ppm (equal to 13.6 mg/kg bw per day for males and 18.0 mg/kg bw per day for females) (Foss, 1994).

Table 52. Subchronic neurotoxicity study in rat: selected neurohistological findings

	Incidence of finding							
	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
<i>Number of rats</i>	10	10	5	5	5	5	10	10
Vacuolar myelinopathy								
Globus pallidus	0	0	0	0	0	0	4*	0
Hippocampus	0	0	0	0	0	0	6**	0
Fimbria	0	0	0	0	1	0	7**	0
Cerebellar white matter	0	0	0	0	3*	0	5*	0
Pyramids	0	0	0	0	1	0	8**	0
Stria medullaris	0	0	0	0	1	0	6**	0
Anterior commissure	0	0	0	0	1	0	7**	0
External capsule	0	0	0	0	1	0	6**	0
Internal capsule	0	0	0	0	1	0	8**	0
Corpus callosum	0	0	0	0	1	0	7**	0
Fornix	0	0	0	0	1	0	4*	0
Cerebral peduncle	0	0	0	0	2	0	8**	0
Olfactory bulb	0	0	0	0	1	0	5*	0
Olfactory tract	0	0	0	0	0	0	5*	0
Optic nerve/chiasm	0	0	0	0	1	0	7*	0
Spinal cord, cervical	0	0	0	0	1	0	7**	0
Spinal nerve roots	1	0	0	0	4*	0	9**	0
Sciatic nerve	0	0	0	0	0	0	4*	0

From Foss (1994)

F, female; M, male; * $P < 0.05$; ** $P < 0.01$ *Developmental neurotoxicity*

In a developmental neurotoxicity study, chlorfenapyr was administered by gavage as an aqueous suspension in 0.5% carboxymethylcellulose to 40 female Wistar rats at 0 (control), 5, 10 or 15 mg/kg bw per day from gestation day 6 (GD 6) through lactation day 10 (LD 10). A functional observational battery was performed on 10 dams per group on GD 7, GD 14, LD 7 and LD 14. On postnatal day 4 (PND 4), litters were culled to yield four males and four females (as closely as possible). The test material was administered by gavage to pups from PND 11 through PND 21.

Offspring were allocated for detailed clinical observations (functional observational battery) and assessment of motor activity, auditory startle reflex habituation, learning and memory (water maze testing) and neuropathy at termination (PND 62). On PND 22, the whole brain was collected from 10 pups of each sex per group for histopathological examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No deaths or clinical signs of toxicity were reported in dams during gestation or lactation. Body weight and feed consumption were not affected by treatment. Gross necropsy of dams was unremarkable, and brain weight and morphometry were similar between treated and control groups.

Pregnancy rate, gestation length, the mean number of delivered pups per dam and percentage of liveborn and stillborn pups were not affected by treatment. Pup survival in the mid-dose group (10

mg/kg bw per day) and high-dose group (15 mg/kg bw per day) was decreased, as evidenced by an increase in the number of pups cannibalized (7 and 13, respectively, versus 1 each in the control group and low-dose group) and found dead (6 and 12, respectively, versus 4 and 3 in the control group and low-dose group, respectively). Most deaths occurred during LD 1 through LD 4 (Table 53).

Table 53. Developmental neurotoxicity study in rat: maternal and litter data

Parameter	0 mg/kg bw per day	5 mg/kg bw per day	10 mg/kg bw per day	15 mg/kg bw per day
Maternal data				
Mortality	11	19	16	15
Feed consumption (g/animal per day), gestation	18.4	18.6	19	19
Feed consumption (g/animal per day), lactation	45.1	45.5	46.1	46.1
Body weight gain (g), GDs 0–20	111.4	108.6	113.6	113.1
Body weight gain (g), LDs 0–21	35.5	30.2	36	34.7
Litter data				
Total number of litters	37	36	38	39
Pups died (%)	1.3	1	1.9	3.4
Pups cannibalized (%)	0.3	0.3	2.2*	3.7**

From Schneider et al. (2006)

* $P < 0.05$; ** $P < 0.01$

Among surviving pups, no treatment-related effects on sex ratio, clinical signs, preweaning and post-weaning body weight, functional observational battery observations or learning and memory in offspring were observed. There was a statistically significant increase in the mean latency of the acoustic startle response in male pups on PND 24 (+30%), but this was not apparent at PND 60. The mean day of achieving sexual maturation in treated animals was comparable to that in the control group. A dose-related decrease in accumulated total movement distance on PND 13 was observed in treated females; the decrease (53% of the control value) was significant in the high-dose group (15 mg/kg bw per day). It was noted that the value in this group (2273 ± 1560 cm) was comparable to the historical control range (1839–2899 cm) and that the mean concurrent control value (4321 ± 2144 cm) was above the historical control range.

Brain weight and morphometric measurements of the cerebrum and cerebellum were comparable between treated and control groups on PND 22, PND 62 and PND 111. On microscopic examination at PND 22, treatment-related minimal to moderate vacuolation of the white matter was observed in several areas of the brain, including the frontal lobe, parietal lobe, midbrain, pons, cerebellum and medulla oblongata, in up to 4 out of 10 animals of each sex in the high-dose group (15 mg/kg bw per day) compared with none in the control group. As in adults, the vacuolation was reversible upon withdrawal of treatment (from PND 22 to PND 62). The low-dose group (5 mg/kg bw per day) and mid-dose group (10 mg/kg bw per day) were examined, and no lesions were observed. No treatment-related microscopic findings were observed at the PND 62 necropsy (Table 54).

For the high-dose (15 mg/kg bw per day) female pups, although there was a slightly decreased mean size of left and right brain hippocampus (approximately 5% below control) on PND 62, this change may be due to a residual effect of the vacuolation (oedema) observed on PND 22. Furthermore, there was no decrease in mean size of either side of the brain hippocampus for males or females at the high dose (15 mg/kg bw per day) on PND 111.

Table 54. Developmental neurotoxicity study in rat: neurohistopathological findings*(a) Dams*

	Histopathological finding	0 mg/kg bw per day	15 mg/kg bw per day
<i>Number of animals in the dose group</i>		10	10
Frontal lobe		0	0
Parietal lobe		0	0
Midbrain		0	0
Pons		0	0
Cerebellum		0	0
Medulla oblongata		0	0
Cervical cord		0	0
Gasserian ganglia		0	0
Cervical ganglia		0	0
Sciatic nerve	Axonal degeneration	0	1
Tibial nerve		0	0
Gastrocnemius		0	0
Optic nerve		0	0
Pituitary gland	Cyst	0	1

(b) Pups

		Incidence of finding (PND 22 / PND 62)							
		0 mg/kg bw per day		5 mg/kg bw per day		10 mg/kg bw per day		15 mg/kg bw per day	
		M	F	M	F	M	F	M	F
<i>Number of animals in the dose group</i>		10	10	10	10	10	10	10	10
Frontal lobe	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	4/0	4/0
Parietal lobe	Vacuolation white matter	0/0	0/0	0/—	0/0	0/—	0/0	2/0	4/0
Midbrain	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	0/0	1/0
Pons	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	1/0	2/0
Cerebellum	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	1/0	3/0
Medulla oblongata	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	0/0	1/0
Cervical cord		0/0	0/0	—	—	—	—	0/0	0/0
Gasserian ganglia		0/0	0/0	—	—	—	—	0/0	0/0
Cervical ganglia		0/0	0/0	—	—	—	—	0/0	0/0
Sciatic nerve	Axonal degeneration	0/0	0/0	—	—	—	—	0/1	0/1
Proximal tibial nerve	Axonal degeneration	0/1	0/0	—	—	—	—	0/0	0/0
Distal tibial nerve	Axonal degeneration	0/2	0/0	—	—	—	—	0/0	0/0
Gastrocnemius	Myofibrillar degeneration	0/1	0/0	—	—	—	—	0/0	0/0

Histopathological finding	Incidence of finding (PND 22 / PND 62)							
	0 mg/kg bw per day		5 mg/kg bw per day		10 mg/kg bw per day		15 mg/kg bw per day	
	M	F	M	F	M	F	M	F
Optic nerve	0/0	0/0	—	—	—	—	0/0	0/0
Pituitary gland	0/0	0/0	—	—	—	—	0/0	0/0

From Schneider et al. (2006)

—, not assessed; F, female; M, male; PND, postnatal day

Based on the results and findings noted above, the critical effect of multifocal vacuolation (minimal to moderate severity) of the white matter of the brain observed in rat offspring resulted in an offspring NOAEL of 10 mg/kg bw per day and an offspring lowest-observed-adverse-effect level (LOAEL) of 15 mg/kg bw per day. The maternal NOAEL was 15 mg/kg bw per day, the highest dose tested (Schneider et al., 2006).

(b) Pharmacological studies

Studies on the pharmacological action of chlorfenapyr were performed in mice, rats and rabbits to evaluate effects on the central nervous system, respiratory and cardiovascular systems, autonomic nervous system, gastrointestinal system, skeletal muscle and blood coagulation.

The effects of chlorfenapyr on general behaviour were evaluated following oral administration to three male animals per dose group as follows: vehicle control; 0.3, 1, 3, 10, 30 and 100 mg/kg bw in mice; 3, 10, 30, 100, 300 and 1000 mg/kg bw in rats.

At a dose of 3 mg/kg bw in mice, one of three animals was affected for any behavioural element: depression of grooming behaviour and reactivity, decrease of spontaneous motor activity and abnormal gait. At a dose of 10 mg/kg bw, gait was normal, whereas the frequency of prone position and slight diarrhoea increased. All these changes disappeared within 6 hours. Doses of 30 and 100 mg/kg bw induced the same behaviour changes mentioned above (except diarrhoea) to a moderate degree, with dilation of the pupil and salivation as well. At a dose of 30 mg/kg bw, one of the three mice died, and at 100 mg/kg bw, all three mice died after clonic convulsions. Oral administration of chlorfenapyr to three males per dose group (vehicle control, 1, 3, 10 mg/kg bw) had no effect on hexobarbital sleeping time in mice.

In rats, a dose of 30 mg/kg bw induced a slight decrease of spontaneous motor activity and frequency of prone position; a dose of 100 mg/kg bw and above also induced depression of grooming behaviour and reactivity, abnormal limb position and gait, and salivation and an increase in body temperature. All of the rats died after clonic convulsions within 6 hours after administration of 100 mg/kg bw and within 4 hours at 300 and 1000 mg/kg bw. Oral administration of chlorfenapyr to three male rats per dose group (vehicle control, 3, 10, 30 mg/kg bw) had no effect on rectal temperature in the low- and mid-dose groups. At 30 mg/kg bw, rectal temperature was elevated from 1 to 5 hours, with a peak increase of 1.5 °C at 3 hours. Rectal temperature had returned to normal by 7 hours.

In rabbits, oral administration of chlorfenapyr to three males per dose group (doses: 3, 10 and 30 mg/kg bw) showed no effects on the spontaneous electroencephalogram. In anaesthetized rabbits, chlorfenapyr administered intraduodenally to three males per dose group (doses: 3, 10 and 30 mg/kg bw) had no effect on respiration, blood pressure, heart rate or the electrocardiogram over the 4-hour observation period.

Effects on the autonomic nervous system were evaluated in six male rats per dose group by oral administration of chlorfenapyr at doses of 3, 10 and 30 mg/kg bw. No effect on pupil size was observed.

Chlorfenapyr administered orally (doses: 1, 3 and 10 mg/kg bw) to eight fasted male mice per dose group had no effect on the intestinal transport of a charcoal meal.

Effects on the skeletal muscle were evaluated in eight male mice per dose group by oral administration of chlorfenapyr (doses: 1, 3 and 10 mg/kg bw) using a horizontal wire traction test. No changes in muscle contractility were observed.

A study to evaluate effects on blood coagulation was performed in six male rats per dose group. Three hours after oral administration of chlorfenapyr (doses: 3, 10 and 30 mg/kg bw), blood samples were collected to measure the prothrombin time (PT) and the activated partial thromboplastin time (APTT). A dose of 30 mg/kg bw significantly prolonged the PT, but this change was not considered to be of consequence, because the extent to which PT was prolonged, less than 2 seconds, is considered to be biologically insignificant.

In conclusion, chlorfenapyr had no biologically important effects on the respiratory or cardiovascular system, autonomic nervous system, skeletal muscle system, gastrointestinal system or blood coagulation. Some pharmacological effects were observed on the central nervous system, such as changes in general behaviour and an increase in body temperature. The minimum doses to produce changes in general behaviour were 3 mg/kg bw in mice (Table 55) and 30 mg/kg bw in rats (Table 56). Effects in mice at 3 mg/kg bw were marginal and related to one animal only. Convulsions due to stimulation of the central nervous system were thought to be the cause of death observed in rats and mice after acute intoxication. No changes in the electroencephalogram were observed at non-lethal doses in rabbits. No-effect doses (NOAELs) were 3 mg/kg bw in mice, 10 mg/kg bw in rats and 30 mg/kg bw in rabbits (Horii, 1994).

(c) Toxicity of metabolites

Acute oral studies on metabolites

The acute oral toxicity of metabolites of chlorfenapyr is summarized in Table 57.

AC 312,094 (residue present in fish). In an acute oral toxicity study, five rats (Charles River Crl:CD(SD)BR) of each sex per dose received AC 312,094 (pyrrole-3-carbonitrile, 2-(*p*-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)) (lot no. AC 8698-67A; purity 96.3%) dispersed in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at a dose level of 5000 mg/kg bw. The protocol was in compliance with USEPA test guideline 81-1.

Deaths occurred in a male on day 4 (1/5 males) and in a female on day 6 (1/5 females). Clinical signs were limited to decreased activity in males only; the animals returned to normal by 24 hours post-dosing. Body weight in surviving animals was unaffected by administration of the test metabolite. There were no gross pathological changes in decedents or surviving animals. The LD₅₀ was greater than 5000 mg/kg bw (Bradley, 1994b).

AC 303,268 (residue present in rat, hen and goat). In an acute oral toxicity study, five rats (Charles River Crl:CD(SR)BR) of each sex per dose received AC 303,268 (pyrrole-3-carbonitrile, 4-bromo-2-(*p*-chlorophenyl)-5-(trifluoromethyl)) (lot no. AC 8697-44B; purity 100.3%) dispersed in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at initial dose levels of 250 and 125 mg/kg bw. Because of excessive mortality, dose levels of 62.5, 31.25, 15.6 and 7.8 mg/kg bw were tested. An additional dose level of 23.4 mg/kg bw was tested in order to calculate an LD₅₀. The protocol was in compliance with USEPA test guideline 81-1. Deaths occurred in both sexes by 8 hours post-dosing at 31.25 mg/kg bw and above.

Prostration with hind legs extended was observed in one surviving animal at doses of 31.25, 62.5 and 125 mg/kg bw, but returned to normal within 24 hours post-dosing. Other clinical signs were limited to testes protruding, writhing and tonus at necropsy in decedents. There were no relevant findings on body weight. Gross pathological changes in decedents consisted of dark or mottled livers and pronounced muscle striations. The LD₅₀ was 27 mg/kg bw in males and 29 mg/kg bw in females (Bradley, 1994c).

Table 55. Effect of chlorfenapyr on general behaviour in male mice^a

	Incidence of finding ^b																															
	3 mg/kg bw								10 mg/kg bw								30 mg/kg bw								100 mg/kg bw							
	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h				
Depression of grooming	—	1/3	—	—	—	—	—	1/3	3/3	3/3	—	—	—	—	3/3	3/3	3/3	3/3	1/2	1/2	—	3/3	1/1	—	—	—	—	—				
Depression of reactivity	—	—	1/3	1/3	—	—	—	—	—	3/3	3/3	—	—	—	—	—	3/3	3/3	1/2	1/2	—	1/3	1/1	—	—	—	—	—				
Decrease of SMA	—	1/3	—	—	—	—	—	1/3	3/3	3/3	3/3	—	—	—	3/3	3/3	3/3	3/3	1/2	1/2	1/2	3/3	1/1	—	—	—	—	—				
Abnormal body position	—	—	—	—	—	—	—	—	—	1/3	1/3	—	—	—	3/3	3/3	3/3	2/3	1/2	1/2	1/2	3/3	1/1	—	—	—	—	—				
Abnormal gait	—	—	—	1/3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—	—	—				
Increase of pupil size	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	1/1	—	—	—	—	—				
Salivation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	1/1	—	—	—	—	—				
Diarrhoea	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				
Death	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	2/3	3/3	—	—	—	—				

From Horii (1994)

SMA, spontaneous motor activity

^a No effects observed at 0.3 and 1 mg/kg bw.^b Number of animals showing abnormal clinical sign/number of animals used.

Table 56. Effect of chlorfenapyr on general behaviour in male rats^a

Clinical signs	Incidence of finding ^b																											
	30 mg/kg bw								100 mg/kg bw								300 mg/kg bw								1000 mg/kg bw			
	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h
Depression of grooming	—	—	—	—	—	—	—	—	—	3/3	—	—	—	—	—	2/3	3/3	—	—	—	—	2/3	3/3	3/3	—	—	—	—
Depression of reactivity	—	—	—	—	—	—	—	—	—	3/3	1/1	—	—	—	—	2/3	3/3	—	—	—	—	2/3	2/3	3/3	—	—	—	—
Decrease of SMA	—	—	2/3	3/3	—	—	—	—	—	3/3	1/1	—	—	—	1/3	2/3	3/3	—	—	—	—	2/3	3/3	3/3	—	—	—	—
Convulsions	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—
Abnormal body position	—	—	2/3	3/3	—	—	—	—	—	3/3	1/1	—	—	—	—	2/3	3/3	—	—	—	—	2/3	3/3	3/3	—	—	—	—
Abnormal limb position	—	—	—	—	—	—	—	—	—	—	1/1	—	—	—	—	—	3/3	—	—	—	—	—	—	3/3	—	—	—	—
Abnormal gait	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	—	—	—	—	—	—	3/3	—	—	—	—
Decrease of limb tone	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	—	—	—	—	—
Salivation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—	—	—	2/3	—	—	—	—
Increase of body temperature	—	—	—	—	—	—	—	—	—	2/3	1/1	—	—	—	—	2/3	3/3	—	—	—	—	—	3/3	3/3	—	—	—	—
Diarrhoea	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	1/3	1/3	—	—	—	—
Death	—	—	—	—	—	—	—	—	—	—	2/3	3/3	—	—	—	—	—	3/3	—	—	—	—	—	—	3/3	—	—	—

From Horii (1994)

SMA, spontaneous motor activity

^a No effects observed at 3 or 10 mg/kg bw.^b Number of animals showing abnormal clinical sign/number of animals used.

Table 57. Summary of oral acute toxicity of chlorfenapyr metabolites in the rat

Metabolite	LD ₅₀ (mg/kg bw)		Purity (%)	Lot no.	Reference
	Males	Females			
AC 312,094	> 5000	> 5000	96.3	AC 8698-67A	Bradley (1994b)
AC 303,268	27	29	100.3	AC 8697-44B	Bradley (1994c)
AC 322,250	> 5000	2500	89.0	AC 9014-97A	Bradley (1994d)
AC 325,195	776	1367	97.0	AC 9014-93B	Bradley (1994e)

AC 322,250 (residue present in goat). In an acute oral toxicity study, five rats (Charles River CRL:CD®BR) of each sex per dose received AC 322,250 (pyrrole-2-carboxylic acid, 3-bromo-5-(*p*-chlorophenyl)-4-cyano-) (lot no. AC 9014-97A; purity 89.0%) dispersed in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at a dose level of 5000 mg/kg bw. Because of high mortality of female rats, two additional dose groups of 2500 and 1250 mg/kg bw were added, with five female rats per group. The protocol was in compliance with USEPA test guideline 81-1. Deaths occurred at the 5000 and 2500 mg/kg bw dose levels by 5 days post-dosing. All females at the 1250 mg/kg bw dose level survived the 14 days post-dosing (5000 mg/kg bw: 1/5 males, 4/5 females; 2500 mg/kg bw: 4/5 males; 1250 mg/kg bw: 0/5 females). Clinical signs were limited to animals at the 5000 mg/kg bw dose level and consisted of decreased activity, diarrhoea, diuresis and ptosis. Surviving animals returned to normal between 24 and 48 hours post-dosing. There were no gross pathological changes in surviving animals that could be attributed to test material administration. Necropsy findings included a dark spleen, a test material-filled stomach and a gas-filled stomach in decedents. There were no relevant findings on body weight. The LD₅₀ was greater than 5000 mg/kg bw in males and 2500 mg/kg bw in females (Bradley, 1994d).

AC 325,195 (residue present in rat, hen and goat). In an acute oral toxicity study, five rats (Charles River CRL:CD®BR) of each sex per dose received AC 325,195 (2-pyrrolidine-3-carbonitrile, 2-(*p*-chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-) (lot no. AC 9014-93B; purity 97.0%) in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at dose levels of 312.5 mg/kg bw (males only), 625, 1250, 2500 and 5000 (both sexes). The protocol was in compliance with USEPA test guideline 81-1. Deaths generally occurred in both sexes from 2 to 24 hours post-dosing. Females at the 625 mg/kg bw and males at the 312.5 mg/kg bw dose level survived the 14-day study period (312.5 mg/kg bw: 0/5 males; 625 mg/kg bw: 2/5 males, 0/5 females; 1250 mg/kg bw: 4/5 males, 3/5 females; 2500 mg/kg bw: 5/5 males, 4/5 females; 5000 mg/kg bw: 5/5 males, 5/5 females). Major clinical signs included salivation, decreased activity, hyperthermia, chromodacryorrhoea, dyspnoea, ptosis, brown material around the nose, red material in urine, dehydration and prostration in decedents. Clinical signs in surviving animals were limited to ptosis, diuresis and brown material around the nose. There were no clinical signs observed in females at the 625 mg/kg bw dose level or in males at the 312.5 mg/kg bw dose level. Gross pathological changes observed in decedents consisted of white foci on the liver and spleen and discoloration of the spleen. There were no relevant findings on body weight. Treatment-related lesions in the liver and spleen were observed at gross necropsy for three animals that were found dead at the 1250 and 2500 mg/kg bw dose levels. Microscopic evaluation of tissues of these animals showed areas of hepatocellular necrosis and/or infarction and diffuse haemorrhage. There were no gross pathological changes in surviving animals that could be attributed to ingestion of the test material. The LD₅₀ was 776 mg/kg bw in males and 1367 mg/kg bw in females (Bradley, 1994e).

Genotoxicity studies on metabolites

Based on a dose range-finding test showing a limit of solubility (evidence of precipitate at the highest dose) in the test system, AC 312,094 was evaluated for its ability to produce mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and in *E. coli* strain WP2 *uvrA*⁻ at concentrations of 25, 50, 100, 250, 500 and 1000 µg/plate with metabolic activation by hepatic S9 preparation from Aroclor 1254-treated rats and 5, 10, 25, 50, 100 and 250 µg/plate without metabolic

activation, along with concurrent negative and positive controls. Results obtained in these assays indicated that the test material was not mutagenic, in that it did not induce either base pair substitution or frame shift mutations in any of the tester strains of bacteria with or without metabolic activation (Mulligan, 1994b).

Based on dose range-finding studies, AC 303,268 was tested in *E. coli* tester strain WP2 *uvrA*⁻ at concentrations of 10, 25, 50, 100 and 250 µg/plate (limited by solubility; evidence of precipitate in the test system at the highest dose) and 0.05, 0.10, 0.25, 0.5, 1.0 and 5.0 µg/plate (limited by toxicity) in the remaining tester strains (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538) with and without metabolic activation by hepatic S9 preparation from Aroclor 1254-treated rats, along with concurrent negative and positive controls. Results obtained in these assays indicated that the test material was not mutagenic, in that it did not induce either base pair substitution or frame shift mutations in any of the tester strains of bacteria with or without metabolic activation (Mulligan, 1994c).

Based on a dose range-finding test, AC 322,250 was tested at concentrations of 250, 500, 1000, 2500 and 5000 µg/plate in *E. coli* strain WP2 *uvrA*⁻ with and without metabolic activation by hepatic S9 preparation from Aroclor 1254-treated rats. The remaining strains (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538) were tested at concentrations of 100, 250, 500, 1000 and 2500 µg/plate with S9 metabolic activation and at 50, 100, 250, 500 and 1000 µg/plate without S9 metabolic activation, along with concurrent negative and positive controls. The highest doses in the test system with the *Salmonella* strains were limited by toxicity. Results obtained in these assays indicated that AC 322,250 was not mutagenic, as it did not induce either base pair substitution or frame shift mutations in any of the tester strains of bacteria with or without metabolic activation (Mulligan, 1994d).

Dermal absorption study

The absorption, distribution and excretion of radioactivity were studied in male rats following a single dermal administration of ¹⁴C-labelled BAS 306 I (chlorfenapyr) in the formulation concentrate BAS 306 02 I or a 1/100 (w/w) aqueous dilution of the formulation concentrate, representing an application dilution. The nominal dose levels were 2.4 or 0.0217 mg/cm² (corresponding to approximately 24 or 0.217 mg/animal and approximately 90 or 0.9 mg/kg bw). Groups of four animals each were exposed according to the following regimen:

exposure duration (hours):	8	8	8
skin wash after (hours):	8	8 + 24	8 + 120
sacrifice time (hours):	8	24	120

The following specimens/tissues were collected for total radioactivity: urine, faeces, blood cells, plasma, carcass, skin at the application site and skin surrounding application site. For balance estimates, the cage wash and skin washes, as well as the protective cover, were also checked for radioactivity.

Mean recoveries of radioactivity from all dose groups were in the range of 91.67–104.94% of the total radioactivity administered. The largest proportion of radioactivity was recovered from the skin wash. At the high dose (the formulation concentrate), a relative absorption of about 0.32% was observed after an 8-hour exposure period. At sacrifice after 24 and 120 hours, the relative absorptions were 0.66% and 1.45% of the dose, respectively. At the low dose (representing typical application dilution), a relative absorption of about 3.34% was observed after an 8-hour exposure period. At sacrifice after 24 and 120 hours, the relative absorptions were 7.80% and 14.26%, respectively.

The dermal penetration rate of the diluted product in rats was approximately 15% of the applied dose at 120 hours after an 8-hour exposure period (Fabian & Leibold, 2005).

3. Observations in humans

Chlorfenapyr technical has been produced since 1994 at one location in the USA. During this period, there have been no unusual or abnormal health effects observed among the personnel working in the plant. BASF Corporation policy mandates periodic medical monitoring of all production personnel, typically either annually or biannually. There are no medical tests specific to the handling of, or exposure to, chlorfenapyr.

Comments

Biochemical aspects

In two metabolism studies, one of which was not certified to be compliant with GLP, chlorfenapyr labelled with ^{14}C in either the pyrrole or the phenyl ring was administered by oral gavage to intact and bile duct-cannulated rats. The radiolabel was relatively slowly absorbed, the extent varying from 80% at 2 mg/kg bw to 65% at 20 mg/kg bw. The maximum concentration of radiolabel in plasma was achieved after about 8–12 hours, was dose proportional (at 2–20 mg/kg bw) and did not differ between males and females. Absorbed radiolabel was slowly distributed throughout the body, with concentrations in fat, liver and adrenals being greater than those in plasma. In general, tissue radiolabel concentration increased with dose. Blood and tissue concentrations of radiolabel were 2- to 3-fold higher in female rats than in male rats. Excretion was relatively rapid, mainly via the faeces, ranging from 80% to 106% of the administered dose in 7 days. There was little or no potential for accumulation, with 70% of the dose excreted in 24 hours and approximately 90% within 48 hours. The elimination half-life for plasma radiolabel was approximately 56 hours. Most of the chlorfenapyr in faeces was present as the unchanged compound, comprising material that was not absorbed together with material excreted via the bile, which was the main route of elimination. Faeces also contained minor amounts of *N*-dealkylated, debrominated and hydroxylated oxidation products of chlorfenapyr. Excretion via the urine was minor, representing only 5–11% of the administered dose over 7 days. There was no elimination of chlorfenapyr-related radioactivity via respiration.

The major routes of metabolism are *N*-dealkylation, dehalogenation, hydroxylation and conjugation, but not with sulfate or glucuronide. There is no cleavage of the bond between the pyrrole and phenyl rings of chlorfenapyr during its biotransformation.

Toxicological data

Chlorfenapyr technical is moderately toxic via the oral route, with LD_{50} s of 441 mg/kg bw in rats and 45 mg/kg bw in mice, and via the inhalation route, with an LC_{50} of 0.83 mg/l in rats. Chlorfenapyr was of low toxicity after dermal exposure in rabbits ($\text{LD}_{50} > 2000$ mg/kg bw). It is not irritating to the skin or eye of rabbits and is not a dermal sensitizer in the guinea-pig maximization test.

Following repeated administration of chlorfenapyr to mice, rats and dogs, decreased feed consumption and body weight gains were observed in all three species. Increased liver weights, associated with hepatocellular hypertrophy, and vacuolation in the brain and spinal cord were also noted in rats and mice.

In a 28-day study in mice, the NOAEL was 160 ppm (equal to 30.1 mg/kg bw per day), based on decreased body weight gain, mortality and increased relative liver weight at 240 ppm (equal to 43.6 mg/kg bw per day). In a 90-day study in mice, the NOAEL was 80 ppm (equal to 14.8 mg/kg bw per day), based on increased relative spleen weight and myelopathy in brain and spinal cord in males at 160 ppm (equal to 27.6 mg/kg bw per day).

In a 28-day study in rats, the NOAEL was 600 ppm (equal to 68.3 mg/kg bw per day), based on increases in relative liver weights and ALT activity at 900 ppm (equal to 106.3 mg/kg bw per day). In a 90-day study in rats, the NOAEL was 300 ppm (equal to 22 mg/kg bw per day), based on increases in relative liver weight, alkaline phosphatase activity and blood urea nitrogen and, in females, changes in red cell parameters (haemoglobin) at 600 ppm (equal to 44.9 mg/kg bw per day).

Vacuolation of the brain and spinal cord was seen at higher doses (900 ppm, equal to 69.5 mg/kg bw per day, and above).

In a 90-day dietary study in dogs, the NOAEL was 120 ppm (equal to 3.9 mg/kg bw per day), based on decreased body weight gain at 200 ppm (equal to 7.1 mg/kg bw per day). In a 1-year dietary study in dogs, the NOAEL was 120 ppm (equal to 4.0 mg/kg bw per day), based on reduced body weight and body weight gain at 240 ppm (equal to 8.7 mg/kg bw per day). The overall NOAEL for these two studies in the dog was 4 mg/kg bw per day.

Long-term studies of toxicity and carcinogenicity were performed in mice and rats, with similar NOAELs in the two species. In an 18-month dietary study in mice, the NOAEL for non-neoplastic effects was 20 ppm (equal to 2.8 mg/kg bw per day), based on decreases in body weight gain and vacuolation of the white matter of the brain at 120 ppm (equal to 16.6 mg/kg bw per day). No evidence of carcinogenicity was found.

In a 24-month dietary study in rats, the NOAEL for non-neoplastic effects was 60 ppm (equal to 2.9 mg/kg bw per day), based on reduced body weight and body weight gain and increased liver weight associated with hepatocellular hypertrophy at 300 ppm (equal to 15 mg/kg bw per day). No evidence of carcinogenicity was found.

The Meeting concluded that chlorfenapyr was not carcinogenic in rats and mice.

The potential genotoxicity of chlorfenapyr was tested in an adequate range of in vitro and in vivo studies. Chlorfenapyr showed no evidence of genotoxicity.

The Meeting concluded that chlorfenapyr was unlikely to be genotoxic in vivo.

On the basis of the lack of genotoxicity and the absence of carcinogenicity in the rat and the mouse, the Meeting concluded that chlorfenapyr is unlikely to be carcinogenic in humans.

In a two-generation reproductive toxicity study in rats, the NOAEL for effects on fertility was 600 ppm (equal to 44 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 60 ppm (equal to 5 mg/kg bw per day), based on decreased body weight and body weight gain at 300 ppm (equal to 22 mg/kg bw per day). The NOAEL for offspring toxicity was 60 ppm (equal to 5 mg/kg bw per day), based on decreased body weight of pups at 300 ppm (equal to 22 mg/kg bw per day).

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 75 mg/kg bw per day, based on decreased body weight at 225 mg/kg bw per day. The NOAEL for developmental toxicity was 225 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 5 mg/kg bw per day, based on decreased body weight gain at 15 mg/kg bw per day. The NOAEL for developmental toxicity was 30 mg/kg bw per day, the highest dose tested.

The Meeting concluded that chlorfenapyr was not teratogenic.

In an acute neurotoxicity study in rats, the NOAEL for systemic toxicity was 45 mg/kg bw, based on clinical signs of toxicity (2 lethargic animals out of 20) at 90 mg/kg bw and above. Lethality (20%) was observed at 180 mg/kg bw. There was no evidence for neuropathological effects or neurotoxicity up to the highest dose tested (180 mg/kg bw).

In a 1-year neurotoxicity study in rats, the NOAEL for neurotoxicity was 60 ppm (equal to 2.6 mg/kg bw per day), based on vacuolar myelinopathy, vacuolation and/or myelin sheath swelling of the brain and spinal cord in males at 300 ppm (equal to 13.6 mg/kg bw per day). There was no change in motor activity or other behavioural activity. The effects observed were reversible within 16 weeks.

In a developmental neurotoxicity study in rats, the NOAEL for maternal toxicity was 15 mg/kg bw per day, the highest dose tested. The NOAEL for developmental neurotoxicity was 10 mg/kg bw per day, based on an increased incidence of multifocal vacuolation (minimal to moderate severity) of the white matter of the brain on postnatal day 22 at 15 mg/kg bw per day. This effect

appears to be reversible (i.e. 38 days after end of treatment), as no adverse effects on either behaviour or neuropathology were evident in rats on PND 60.

Single-dose studies on the pharmacological action of chlorfenapyr were performed in mice, rats and rabbits to evaluate effects on the central nervous system, autonomic nervous system, respiratory and cardiovascular systems, gastrointestinal system, skeletal muscle and blood coagulation. The only relevant pharmacological effects were observed on the central nervous system, such as changes in general behaviour and an increase in body temperature. Convulsions due to stimulation of the central nervous system were thought to be the cause of death observed in rats and mice after acute intoxication. No changes in the electroencephalogram were observed at non-lethal doses in rabbits. NOAELs were 3 mg/kg bw in mice, 10 mg/kg bw in rats and 30 mg/kg bw (the highest dose tested) in rabbits, based on depression of grooming behaviour and reactivity, a decrease in spontaneous motor activity and prone position in mice and rats.

The acute oral toxicity of four chlorfenapyr animal metabolites (AC 312,094, AC 303,268, AC 322,250 and AC 325,195) was tested in Sprague-Dawley rats, and its genotoxicity was tested in microbial test systems.

AC 312,094 was of low acute oral toxicity in rats ($LD_{50} > 5000$ mg/kg bw) and showed no mutagenic potential in microbial test systems.

AC 303,268 was of high acute oral toxicity in rats ($LD_{50} = 27$ mg/kg bw). This metabolite showed no mutagenic potential in microbial test systems. It is present at significant levels in livestock.

AC 322,250 was of slight acute oral toxicity in rats ($LD_{50} = 2500$ mg/kg bw) and showed no mutagenic potential in microbial test systems.

AC 325,195 was of moderate acute oral toxicity in rats ($LD_{50} = 776$ mg/kg bw) and showed no mutagenic potential in microbial test systems.

There were no reports of adverse health effects of chlorfenapyr in manufacturing plant personnel.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for chlorfenapyr of 0–0.03 mg/kg bw, based on a NOAEL of 2.8 mg/kg bw per day for decreases in body weight gain and vacuolation of the white matter of the brain at 16.6 mg/kg bw per day in an 18-month mouse study and a NOAEL of 2.9 mg/kg bw per day for reduced body weight and body weight gain and increased liver weight associated with hepatocellular enlargement at 15 mg/kg bw per day in a 2-year rat study. This was supported by a NOAEL of 2.6 mg/kg bw per day for reversible vacuolar myelinopathy, vacuolation and/or myelin sheath swelling of the brain and spinal cord in males at 13.6 mg/kg bw per day in a 1-year study of neurotoxicity in rats. A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) for chlorfenapyr of 0.03 mg/kg bw, based on the NOAEL of 3 mg/kg bw for depression of grooming and reactivity and decreased spontaneous motor activity observed at 10 mg/kg bw in a pharmacological study in mice. A 100-fold safety factor was applied.

Based on available information, it was not possible for the Meeting to determine whether the ADI and ARfD would also cover the metabolite AC 303,268.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity ^a	Toxicity	20 ppm, equal to 2.8 mg/kg bw per day	120 ppm, equal to 16.6 mg/kg bw per day
		Carcinogenicity	240 ppm, equal to 34.5 mg/kg bw per day ^b	—
	Pharmacological study ^c	Toxicity	3 mg/kg bw	10 mg/kg bw
Rat	Two-year study of toxicity and carcinogenicity ^a	Toxicity	60 ppm, equal to 2.9 mg/kg bw per day	300 ppm, equal to 15 mg/kg bw per day
		Carcinogenicity	600 ppm, equal to 30.8 mg/kg bw per day ^b	—
	Two-generation study of reproductive toxicity ^a	Reproductive toxicity	600 ppm, equal to 44 mg/kg bw per day ^b	—
		Parental toxicity	60 ppm, equal to 5 mg/kg bw per day	300 ppm, equal to 22 mg/kg bw per day
		Offspring toxicity	60 ppm, equal to 5 mg/kg bw per day	300 ppm, equal to 22 mg/kg bw per day
	Developmental toxicity study ^c	Maternal toxicity	75 mg/kg bw per day	225 mg/kg bw per day
		Embryo and fetal toxicity	225 mg/kg bw per day ^b	—
	Acute neurotoxicity study ^c	Toxicity	45 mg/kg bw	90 mg/kg bw
		Neurotoxicity	180 mg/kg bw ^b	—
	One-year neurotoxicity study ^a	Neurotoxicity	60 ppm, equal to 2.6 mg/kg bw per day	300 ppm, equal to 13.6 mg/kg bw per day
	Developmental neurotoxicity study ^c	Maternal toxicity	15 mg/kg bw per day ^b	—
		Offspring neurotoxicity	10 mg/kg bw per day	15 mg/kg bw per day
Rabbit	Developmental toxicity study ^c	Maternal toxicity	5 mg/kg bw per day	15 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day ^b	—
Dog	Thirteen-week and 1-year studies of toxicity ^{a,d}	Toxicity	120 ppm, equal to 4 mg/kg bw per day	240 ppm, equal to 8.7 mg/kg bw per day

^a Dietary administration.^b Highest dose tested.^c Gavage administration.^d Two or more studies combined.***Estimate of acceptable daily intake for humans***

0–0.03 mg/kg bw

Estimate of acute reference dose

0.03 mg/kg bw

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

Additional studies on the toxicity of AC 303,268 to enable adequate characterization of the dietary risk from this metabolite. The Meeting was aware that additional studies on the compound have been performed but did not have access to sufficiently detailed reports to enable their evaluation.

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

Critical end-points for setting guidance values for exposure to chlorfenapyr*Absorption, distribution, excretion and metabolism in mammals*

Rate and extent of oral absorption	Slow, approximately 80% and 65% in the low-dose and high-dose groups, respectively
Dermal absorption	No data
Distribution	Extensive
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid and almost complete in 168 h in urine and faeces
Metabolism in animals	Absorbed dose metabolized by <i>N</i> -dealkylation, debromination, ring hydroxylation and conjugation
Toxicologically significant compounds in animals, plants and the environment	Chlorfenapyr, AC 303,268

Acute toxicity

Rat, LD ₅₀ , oral	441 mg/kg bw
Rabbit, LD ₅₀ , dermal	> 2000 mg/kg bw
Rat, LC ₅₀ , inhalation	0.83 mg/l (4 h aerosol, whole-body exposure)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Dermal sensitization	Not sensitizing (Magnusson & Kligman)

Short-term studies of toxicity

Target/critical effect	Decreased body weight and weight gain, increased liver weights, vacuolation of the white matter (rat and mouse)
Lowest relevant oral NOAEL	4 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	20 mg/m ³ (rat)

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Reduced growth rate and feed intake, vacuolation of the white matter (mice), haematological changes (rat)
Lowest relevant NOAEL	2.8 mg/kg bw per day (mouse carcinogenicity study)
Carcinogenicity	Not carcinogenic

Genotoxicity

Not genotoxic

<i>Reproductive toxicity</i>	
Reproduction target/critical effect	Reductions in pup body weights at parentally toxic doses
Lowest relevant parental NOAEL	5 mg/kg bw per day
Lowest relevant reproductive NOAEL	44 mg/kg bw per day (highest dose tested)
Lowest relevant offspring NOAEL	5 mg/kg bw per day
<i>Developmental toxicity</i>	
Developmental target/critical effect	Not teratogenic, no developmental toxicity
Lowest relevant maternal NOAEL	5 mg/kg bw per day (rabbit)
Lowest relevant developmental NOAEL	30 mg/kg bw per day (highest dose tested) (rabbit)
<i>Neurotoxicity</i>	
Acute neurotoxicity target/critical effect	Not acutely neurotoxic
One-year neurotoxicity target/critical effect	Vacuolation of the white matter (reversible) (rat)
Lowest relevant NOAEL	2.6 mg/kg bw per day (rat)
Neurodevelopmental toxicity target/critical effect	Vacuolation of white matter of the brain (reversible) (rat)
Lowest relevant NOAEL	10 mg/kg bw per day
<i>Other toxicological studies</i>	
Acute toxicity of metabolites	AC 312,094: rat LD ₅₀ > 5000 mg/kg bw AC 303,268: rat LD ₅₀ = 27 mg/kg bw AC 322,250: rat LD ₅₀ = 2500 mg/kg bw AC 325,195: rat LD ₅₀ = 776 mg/kg bw
Genotoxicity of metabolites	Not genotoxic
<i>Medical data</i>	
No data available	

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Eighteen-month (mouse) and 2-year (rat) studies of toxicity; 1-year neurotoxicity study (rat)	100
ARfD	0.03 mg/kg bw	Pharmacological study (mouse)	100

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