

AMINOCYCLOPYRACHLOR

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Explanation.....	3
Evaluation for acceptable daily intake.....	4
1. Biochemical aspects	4
1.1 Absorption, distribution and excretion.....	4
1.2 Biotransformation	11
2. Toxicological studies.....	13
2.1 Acute toxicity.....	13
2.2 Short-term studies of toxicity.....	13
2.3 Long-term studies of toxicity and carcinogenicity.....	18
2.4 Genotoxicity	20
2.5 Reproductive and developmental toxicity.....	21
(a) Single or multigeneration studies.....	21
(b) Developmental toxicity	27
2.6 Special studies	28
(a) Neurotoxicity	28
(b) Immunotoxicity	28
(c) Study on the photolytic degradate, cyclopropane carboxylic acid (CPCA)	29
3. Observations in humans	34
Comments.....	34
Toxicological evaluation	37
References	40

Explanation

Aminocyclopyrachlor is the International Organization for Standardization (ISO)–approved common name for 6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service (CAS) number 858956-08-8. Aminocyclopyrachlor belongs to the pyrimidine carboxylic acid chemical family and is an auxin-mimicking herbicide used for selective control of weeds, invasive species and brush in pasture. The methyl ester (CAS No. 858954-83-3) is also used as a herbicide, and information on this compound was also assessed.

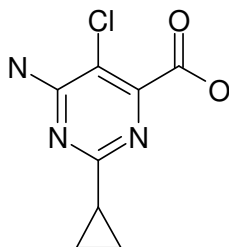
A complete toxicological data package was provided on aminocyclopyrachlor. A subset of toxicological studies was provided on aminocyclopyrachlor-methyl based on the weight of evidence from pharmacokinetic and metabolism studies and the results of bridging studies, which indicated the toxicological equivalence of the two compounds. For comparative purposes, doses of aminocyclopyrachlor-methyl are expressed as aminocyclopyrachlor acid equivalents (ae), calculated using the respective relative molecular masses (227.65 versus 213.62). The chemical structure of aminocyclopyrachlor is given in Fig. 1.

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

All studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and complied, where appropriate, with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the

European Union or United States Environmental Protection Agency. Minor deviations from these protocols were not considered to affect the integrity of the studies.

Fig. 1. Chemical structure of aminocyclopyrachlor (DPX-MAT28)

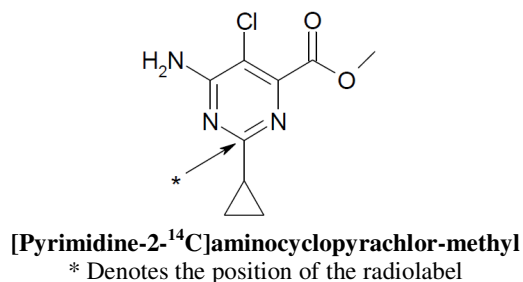
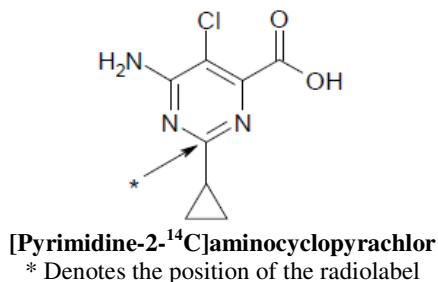


Evaluation for acceptable daily intake

1. Biochemical aspects

Absorption, distribution, metabolism and excretion studies were conducted in rats using [pyrimidine-2-¹⁴C]aminocyclopyrachlor or [pyrimidine-2-¹⁴C]aminocyclopyrachlor-methyl (Fig. 2).

Fig. 2. Chemical structures of [pyrimidine-2-¹⁴C]aminocyclopyrachlor and [pyrimidine-2-¹⁴C]aminocyclopyrachlor-methyl



1.1 Absorption, distribution and excretion

Rats

Himmelstein (2010a) undertook pilot mass balance and definitive pharmacokinetic and metabolism studies in CrI:CD[®](SD) rats dosed with [pyrimidine-2-¹⁴C]aminocyclopyrachlor (99.5% radiochemical purity) in 0.5% (weight per volume [w/v]) methyl cellulose. In the pilot mass balance study, one male and one female rat each received a single gavage dose of ¹⁴C-labelled aminocyclopyrachlor at 25 mg/kg body weight (bw). At 24-hour intervals, exhaled air (up to 48 hours after dosing), urine and faeces (up to 168 hours after dosing) were collected. Rats were killed at 168 hours, and tissues were sampled. Radioactivity was quantified in plasma, excreta and tissues by liquid scintillation counting (LSC). Total recovery of radioactivity was 69.9% and 107.9% of the administered radioactive dose in the male and female, respectively. In the absence of any detectable radioactivity in exhaled air, no further collection of exhaled air was performed after 48 hours. The majority of radioactivity was recovered equally in urine and faeces within the first 24 hours after dosing (male rat: totals of 36% and 32% of the administered dose in urine and faeces, respectively, up to 168 hours after dosing; female rat: totals of 56% and 52% of the administered dose in urine and faeces, respectively, up to 168 hours after dosing). No radioactivity was detected in

tissues. A low level of radioactivity was detected in the carcass of the male rat (0.039% of the administered dose); no radioactivity was detected in the carcass of the female rat.

In the definitive pharmacokinetic study, rats (four of each sex per dose) received a single gavage dose of ^{14}C -labelled aminocyclopyrachlor at 25 or 500 mg/kg bw. Blood was sampled at various times up to 30 hours after dosing for the analysis of radioactivity in plasma and red blood cells by LSC. Two additional rats (one of each sex) received a single gavage dose of 500 mg/kg bw, and plasma was collected at 2 hours to assist with the development of the analytical method used in the metabolism study. Pharmacokinetic parameters are summarized in Table 1. Radioactivity was rapidly absorbed, with peak plasma concentrations reached at 0.4–1.0 hour after dosing and peak concentrations in red blood cells reached at 0.3–1.0 hour after dosing. The ratio of the maximum concentrations of radioactivity ($C_{\max}$) in red blood cells and plasma ranged from 0.33 to 0.48, indicating limited potential for uptake and binding to red blood cells. Mean plasma elimination half-lives were consistent in males and females (5.6 and 5.7 hours, respectively) and between the low and high doses. There was a proportional increase in the $C_{\max}$ and area under the concentration–time curve ($\text{AUC}_{0-\infty}$) from the low to the high dose, with the latter suggesting linear first-order kinetic processes for uptake and elimination (Himmelstein, 2010a).

Table 1. Mean pharmacokinetic parameters in plasma and red blood cells of rats following a single oral dose of ^{14}C -labelled aminocyclopyrachlor at 25 or 500 mg/kg bw

Parameter	25 mg/kg bw dose				500 mg/kg bw dose			
	Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma								
$C_{\max}$ ($\mu\text{g eq/g}$)	3.8	0.9	5.0	1.2	57.3	14.2	61.6	13.0
$T_{\max}$ (h)	0.5	0.4	0.4	0.1	0.6	0.3	1.0	0.7
$t_{1/2}$ (h)	5.6	0.5	5.7	0.4	5.6	0.3	5.7	0.7
$\text{AUC}_{0-\infty}$ ($\mu\text{g eq}\cdot\text{h/g}$)	7.0	1.4	9.0	1.9	150.8	28.7	168.4	26.2
Red blood cells ^a								
$C_{\max}$ ($\mu\text{g eq/g}$)	1.3	0.3	2.0	0.7	27.2	6.2	28.7	4.7
$T_{\max}$ (h)	0.5	0.4	0.3	0.1	0.6	0.3	1.0	0.7

$\text{AUC}_{0-\infty}$: area under the concentration–time curve from time 0 to infinity; bw: body weight; $C_{\max}$: peak concentration; eq: equivalent; SD: standard deviation; $t_{1/2}$: half-life; $T_{\max}$: time to reach $C_{\max}$

^a Only $C_{\max}$ and $T_{\max}$ are presented. Time course data were insufficient to calculate other kinetic parameters.

Source: Himmelstein (2010a)

[Pyrimidine-2- ^{14}C]aminocyclopyrachlor-methyl (99.4% radiochemical purity) in 0.5% (w/v) methyl cellulose was administered as a single gavage dose to CrI:CD[®](SD) rats under the same experimental conditions used in the preceding study by Himmelstein (2010a). In the pilot mass balance study, total recovery of radioactivity was 89.2% and 82.6% of the administered dose in the male and female, respectively. In the absence of any detectable radioactivity in exhaled air, no further collection of exhaled air was performed after 48 hours. In both rats, the majority of radioactivity (79%) was recovered in urine within 24 hours of dosing. The cumulative amount of radioactivity in urine and faeces at 168 hours after dosing was 80.38% and 6.67%, respectively, in the male and 79.27% and 3.06%, respectively, in the female. The higher proportion of radioactivity excreted in urine suggests a higher level of gastrointestinal absorption than for ^{14}C -labelled aminocyclopyrachlor. At 168 hours after dosing, most tissues contained no detectable radioactivity, with the exception of the gastrointestinal tract (0.009% of the administered dose) and carcass (0.144% of the administered dose). Pharmacokinetic parameters are summarized in Table 2. At the same nominal dose, a single gavage dose of ^{14}C -labelled aminocyclopyrachlor-methyl resulted in a more rapid uptake of radioactivity than for ^{14}C -labelled aminocyclopyrachlor (up to approximately 2-fold), with $C_{\max}$ and $\text{AUC}_{0-\infty}$ values also noticeably higher (2- to 5-fold and 1.4- to 2.85-fold,

respectively). Plasma elimination half-lives were approximately twice those of ^{14}C -labelled aminocyclopyrachlor. Peak concentrations of radioactivity in red blood cells were 36–49% of those observed in plasma, indicating very limited potential for binding to red blood cells (Himmelstein 2010b).

Table 2. Mean pharmacokinetic parameters in plasma and red blood cells of rats following a single oral dose of ^{14}C -labelled aminocyclopyrachlor-methyl at 25 or 500 mg/kg bw

Parameter	25 mg/kg bw dose				500 mg/kg bw dose			
	Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma								
$C_{\max}$ ($\mu\text{g eq/g}$)	20.0	7.4	16.7	0.8	126.0	14.9	113.9	27.6
$T_{\max}$ (h)	0.3	0.0	0.3	0.0	0.4	0.1	0.5	0.0
$t_{1/2}$ (h)	13.3	0.6	10.9	1.2	8.7	4.1	11.6	4.1
$\text{AUC}_{0-\infty}$ ($\mu\text{g eq}\cdot\text{h/g}$)	14.8	1.4	14.5	1.5	255.8	59.8	241.5	47.8
Red blood cells ^a								
$C_{\max}$ ($\mu\text{g eq/g}$)	7.2	2.8	8.2	1.8	54.7	5.1	48.1	10.1
$T_{\max}$ (h)	0.3	0.1	0.3	0.0	0.4	0.1	0.6	0.3

$\text{AUC}_{0-\infty}$: area under the concentration–time curve from time 0 to infinity; bw: body weight; $C_{\max}$: peak concentration; eq: equivalent; SD: standard deviation; $t_{1/2}$: half-life; $T_{\max}$: time to reach $C_{\max}$

^a Only $C_{\max}$ and $T_{\max}$ are presented. Time course data were insufficient to calculate other kinetic parameters.

Source: Himmelstein (2010b)

In a series of experiments, Himmelstein (2010c) examined the absorption, distribution, metabolism and elimination of [pyrimidine-2- ^{14}C]aminocyclopyrachlor (99.5% radiochemical purity) in 0.5% (w/v) methyl cellulose in CrI:CD[®](SD) rats. Radioactivity was quantified by LSC, with metabolites analysed by high-performance liquid chromatography (HPLC) and liquid chromatography with mass spectrometry (LC-MS).

In a mass balance study, rats were administered a single gavage dose of ^{14}C -labelled aminocyclopyrachlor at 0 mg/kg bw (one of each sex), 25 mg/kg bw (four of each sex) or 500 mg/kg bw (four of each sex). Exhaled air was collected to 48 hours after dosing, whereas urine and faeces were collected to 72 hours after dosing. Rats were killed at 72 hours, and tissues were sampled. Radioactivity was quantified in excreta, tissues and carcass, whereas metabolites were analysed in urine and faeces. In a repeated-dose experiment, also designed to analyse the excretion of radioactivity and the formation of metabolites, 24 male and six female rats were administered ^{14}C -labelled aminocyclopyrachlor at 25 mg/kg bw per day for 14 days. Urine and faeces were collected daily from each rat. Three males were killed on days 2, 6, 10, 14 (six rats) and 15 for the analysis of radioactivity in whole blood, plasma, red blood cells, liver, kidney, fat and muscle. Three males were also killed on days 13 and 16, with three females killed on days 13 and 16 for a more comprehensive analysis of radioactivity in tissues and the carcass.

The mass balance of radioactivity is summarized in Table 3. No radioactivity was detected in exhaled air. Recovery of radioactivity was greater than 97%, with the majority detected in urine and faeces, in approximately equal proportions, at the low single dose. At the high single dose, a higher proportion of radioactivity was detected in faeces, suggesting a reduction in gastrointestinal absorption. The majority of radioactivity was excreted within 24 hours of a single oral dose. Other than the skin, kidneys, gastrointestinal tract and its contents, which contained relatively low levels of radioactivity, there was no detectable radioactivity in any other sampled tissues (blood, bone

marrow, brain, fat, heart, lungs, spleen, liver, pituitary, thyroid, thymus, ovaries, testes, pancreas, adrenals, uterus, muscle, bladder and bone) 72 hours after a single 25 or 500 mg/kg bw oral dose of ^{14}C -labelled aminocyclopyrachlor (Table 4). In the repeated-dose experiment, the pattern of tissue distribution of radioactivity was consistent with that occurring after a single dose, with tissue radioactivity rapidly increasing to apparent steady state by day 3. Table 5 summarizes the concentrations of radioactivity in various tissues at 6 and 72 hours after the final repeated dose. Radioactivity was rapidly eliminated from tissues following the cessation of dosing, with no indication of any accumulation.

Table 3. Cumulative mass balance in rats following oral dosing with ^{14}C -labelled aminocyclopyrachlor

Sample	Mean % of administered radioactivity					
	25 mg/kg bw (single dose)		500 mg/kg bw (single dose)		25 mg/kg bw (repeated dose)	
	Males	Females	Males	Females	Males	Females
Urine						
6 h	35.75	45.12	22.14	31.93	—	—
12 h	40.78	50.29	33.73	36.20	—	—
24 h	46.08	55.08	38.08	41.59	38.65	49.06
48 h	47.64	56.21	39.86	43.70	38.45	51.51
72 h	47.74	56.51	40.04	44.08	39.53	51.79
16 days	—	—	—	—	38.85	55.26
Faeces						
6 h	0.18	0.49	0.38	0	—	—
12 h	24.16	23.17	18.58	5.37	—	—
24 h	44.02	38.12	47.73	40.43	42.28	44.44
48 h	47.69	39.29	54.22	50.23	42.68	48.96
72 h	47.70	39.48	54.76	51.08	40.45	50.94
16 days	—	—	—	—	36.57	57.25
Cage wash	2.43 ^a	2.87 ^a	1.84 ^a	2.08 ^a	2.62 ^b	4.04 ^b
Feed residue	0.328 ^a	0.316 ^a	0.648 ^a	0.243 ^a	0.253 ^b	0.146 ^b
Tissues + carcass	0.064 ^a	0.222 ^a	0.085 ^a	0.184 ^a	0.030 0 ^b	0.026 3 ^b
Recovery	98.5 ^a	99.4 ^a	97.4 ^a	97.7 ^a	97.01 ^b	98.03 ^b

bw: body weight

^a Cumulative radioactivity to 72 hours.

^b Cumulative radioactivity to 16 days.

Source: Himmelstein (2010c)

The excretion of radioactivity was analysed in groups of bile duct-cannulated rats that were administered a single gavage dose of ^{14}C -labelled aminocyclopyrachlor at 0 mg/kg bw (one of each sex), 25 mg/kg bw (four of each sex) or 500 mg/kg bw (four of each sex). Urine, faeces and bile were collected at 0–6, 6–12, 12–24 and 24–48 hours after dosing. Rats were killed at 48 hours, and radioactivity was analysed in blood, urine, faeces, bile, the gastrointestinal tract and its contents, tissues, carcass and cage wash. At both doses, the majority of absorbed radioactivity was excreted in the urine (22.2–34.5%), with a relatively small proportion recovered in bile (0.13–0.25%). Based on

the concentration of radioactivity in urine, bile and carcass (not including the gastrointestinal tract and its contents), the estimated extent of gastrointestinal absorption was 22.4–34.9%. This estimate of gastrointestinal absorption is lower than an estimate based on the level of radioactivity in urine, tissues and carcass in the mass balance experiment conducted in non-bile duct–cannulated rats (39.9–56.2%) at the same doses. It is noted that in the bile duct–cannulated rats, the majority of radioactivity was eliminated unabsorbed in the faeces (57.9–68.5% of the administered dose), which suggested that under the experimental conditions, gastrointestinal absorption was reduced. The author proposed that this was due to the combination of the intake of the drinking solution (pH 6.3) containing dextrose (276 mmol/L), potassium chloride (6.7 mmol/L) and sodium chloride (154 mmol/L) to help maintain hydration and bile flow and the aminocyclopyrachlor (acid dissociation constant [pK_a] 4.65) dosing preparation (pH 7). On this basis, the estimate of gastrointestinal absorption from the mass balance experiment (accounting for 37–57% of the administered dose by 72 hours) is considered a more reliable estimate of the absorbed dose.

Table 4. Mean radioactivity in selected tissues 72 hours after a single oral dose of ^{14}C -labelled aminocyclopyrachlor

Tissue	Mean radioactivity (% of the administered dose)			
	Male		Female	
	25 mg/kg bw	500 mg/kg bw	25 mg/kg bw	500 mg/kg bw
Carcass ^a	0.067	0.058	0.200	0.101
Skin	0.043	< LOD	< LOD	< LOD
Kidney	0.000	0.000 2	< LOD	0.000 3
Gastrointestinal tract	0.001	0.001 6	0.001	0.002 9
Gastrointestinal tract contents	0.013	0.040	0.022	0.080
Total	0.034	0.042	0.023	0.083

bw: body weight; LOD: limit of detection

^a Percentage of dose recovered in carcass is not included in the total.

Source: Himmelstein (2010c)

The tissue distribution of radioactivity was further analysed in groups of eight rats of each sex that were administered a single gavage dose of ^{14}C -labelled aminocyclopyrachlor at 25 or 500 mg/kg bw. At 1 and 6 hours after dosing (the approximate time to $C_{\max}$ [$T_{\max}$] and $T_{\max} + 5$ hours, respectively), four rats of each sex per group were killed, and radioactivity was analysed in various tissues. Results are summarized in Table 6. The majority of radioactivity was associated with the gastrointestinal tract contents. Other than the gastrointestinal tract and its contents, the concentration of radioactivity in all sampled tissues was low, with the highest concentrations detected in the skin, muscle, whole blood, kidney and liver. There was no indication of accumulation in any tissue. A comparison of the level of radioactivity at 6 hours after dosing in this study with that at 72 hours in the mass balance study (Table 3) indicated relatively rapid elimination of radioactivity from tissues.

Himmelstein (2010d) examined the absorption, distribution, metabolism and elimination of a single gavage dose of 25 mg/kg bw of [pyrimidine-2- ^{14}C]aminocyclopyrachlor-methyl (99.2% radiochemical purity) in 0.5% (w/v) methyl cellulose when administered to CrI:CD[®](SD) rats. Radioactivity was quantified by LSC, with metabolites analysed by HPLC and LC-MS.

In a mass balance and tissue distribution experiment, four rats of each sex were dosed with ^{14}C -labelled aminocyclopyrachlor-methyl, with a separate male and female serving as controls. Urine and faeces were collected predosing and at 0–6, 6–12, 12–24, 24–48 and 48–72 hours. Exhaled volatiles and carbon dioxide were collected predosing and at 0–6, 6–12, 12–24 and 24–48 hours. Rats were killed 72 hours after dosing, and various tissues were collected for the analysis of

radioactivity. There were no signs of toxicity. The mean recovery of radioactivity was at least 98%, with the majority detected in urine and relatively little detected in faeces and exhaled air (Table 7). The majority of radioactivity was excreted in urine within 24 hours of dosing. At 72 hours after dosing, relatively low levels of radioactivity were detected in the majority of sampled tissues, with the highest levels detected in the carcass, skin, fat and gastrointestinal tract contents (Table 8).

Table 5. Mean radioactivity in selected tissues 6 and 72 hours after the final repeated oral dose of ¹⁴C-labelled aminocyclopyrachlor

Tissue	Mean radioactivity (% of the administered dose)			
	Male		Female	
	6 h after last dose	72 h after last dose	6 h after last dose	72 h after last dose
Carcass ^a	0.137 74	0.026 29	0.133 74	0.022 98
Skin	0.013 69	0.002 70	0.010 21	0.002 39
Whole blood	0.008 06	0.000 20	0.004 32	0.000 20
Bone marrow	NA	0.001 03	NA	NA
Brain	0.000 09	0.000 02	0.000 12	0.000 03
Fat	0.005 90	0.002 45	0.003 03	0.002 21
Heart	0.000 16	0.000 02	0.000 12	NA
Lungs	0.000 30	0.000 03	0.000 34	0.000 03
Spleen	0.000 10	0.000 01	0.000 05	NA
Liver	0.010 36	0.000 31	0.002 80	0.000 20
Kidney	0.005 32	0.000 07	0.003 29	0.000 12
Gastrointestinal tract	0.219 29	0.000 22	0.164 95	0.000 18
Gastrointestinal tract contents	7.142 52	0.002 61	7.105 25	0.002 55
Pituitary	< LOD	0.000 01	< LOD	< LOD
Thyroid	< LOD	0.000 01	< LOD	NA
Thymus	0.000 06	0.000 02	0.000 06	NA
Ovaries	–	–	< LOD	< LOD
Testes	0.001 12	0.000 04	–	–
Pancreas	0.000 30	0.000 02	0.000 13	< LOD
Adrenals	0.000 03	0.000 01	< LOD	< LOD
Uterus	–	–	0.000 09	< LOD
Muscle	0.034 61	0.002 02	0.008 54	< LOD
Bladder	0.003 36	0.000 01	0.000 48	< LOD
Bone	0.001 26	0.000 34	0.000 68	< LOD
Total	7.446	0.010	7.304	0.008

LOD: limit of detection; NA: not available

^a Percentage of dose recovered in carcass is not included in the total.

Source: Himmelstein (2010c)

Table 6. Mean radioactivity in selected tissues 1 and 6 hours after a single oral dose of ¹⁴C-labelled aminocyclopyrachlor

Tissue	Mean radioactivity (% of the administered dose)							
	Male				Female			
	25 mg/kg bw		500 mg/kg bw		25 mg/kg bw		500 mg/kg bw	
	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h
Carcass ^a	3.458	5.167	4.154	0.276	3.187	0.354	2.462	0.427
Skin	1.306	0.073	0.780	0.057	1.368	0.060	0.695	0.047
Whole blood	1.020	0.039	0.587	0.029	0.954	0.034	0.492	0.029
Bone marrow	0.072	0.053	0.042	0.007	0.076	0.011	0.039	0.005
Brain	0.004	0.000 8	0.002 4	0.000 7	0.005	0.001	0.002 7	0.000 8
Fat	0.238	0.025	0.375	0.023	0.210	0.019	0.106	0.017
Heart	0.026	0.001 2	0.015	0.000 9	0.021	0.001	0.010 8	0.000 9
Lungs	0.036	0.001 9	0.024	0.001 6	0.038	0.002	0.023 2	0.001 5
Spleen	0.006	0.000 6	0.038	0.000 4	0.006	0.001	0.003 1	0.000 3
Liver	0.750	0.038	0.392	0.030	0.517	0.023	0.264	0.018
Kidney	0.894	0.025	0.562	0.030	1.072	0.051	0.431	0.024
Gastrointestinal tract	13.083	1.363	11.913	1.753	9.544	1.428	10.847	3.928
Gastrointestinal tract contents	58.039	46.766	63.535	67.032	51.504	41.749	64.058	56.035
Pituitary	0.000 4	< LOD	0.002	< LOD	0.001	< LOD	0.000 3	< LOD
Thyroid	0.000 6	< LOD	0.002	0.000 3	0.001	< LOD	0.001 0	< LOD
Thymus	0.006	0.000 4	0.004	0.004	0.007	0	0.003 9	0.003
Ovaries	—	—	—	—	0.006	0	0.002 9	0.003
Testes	0.025	0.003 1	0.031	0.002	—	—	—	—
Pancreas	0.023	0.001 4	0.038	0.003 4	0.020	0.001	0.018 7	0.000 7
Adrenals	0.001	0.000 1	0.019	0.000 1	0.005	0	0.001 1	0.000 1
Uterus	—	—	—	—	0.018	0.008	0.018 2	0.001
Muscle	1.189	0.098	2.008	0.057	0.993	0.067	0.385	0.052
Bladder	0.235	0.030	0.126	0.003	0.124	0.004	0.138	0.003
Bone	0.088	0.008	0.072	0.018	0.104	0.007	0.049	0.006
Total	77.043	48.500	80.567	69.042	66.594	43.453	77.591	60.168

bw: body weight; LOD: limit of detection

^a Percentage of dose recovered in carcass is not included in the total.

Source: Himmelstein (2010c)

The excretion of radioactivity was analysed in a group of bile duct-cannulated rats (four of each sex). Urine, faeces and bile were collected at 0–6, 6–12, 12–24 and 24–48 hours after dosing. Rats were killed 48 hours after dosing. Mean total recovery of radioactivity was 91.7% in males and 93.2% in females. The majority of the radioactivity was recovered in the urine (87.2% and 87.1% in

males and females, respectively), with only minor amounts recovered in the carcass plus gastrointestinal tract (0.13% and 0.26% in males and females, respectively) and bile (0.60% and 0.46% in males and females, respectively). By summation, the mean percentage of absorbed radioactivity was 87.9% and 87.8% in male and female rats, respectively. Comparison of the estimated absorption from the bile duct–cannulated rats was consistent with the cumulative excretion in urine of non-bile duct–cannulated animals from the mass balance experiment (Himmelstein, 2010d).

Table 7. Cumulative mass balance in rats following a single oral dose of 25 mg/kg bw of ^{14}C -labelled aminocyclopyrachlor-methyl

Sample	Mean % of administered radioactivity			
	Males		Females	
	Mean	SD	Mean	SD
Urine				
6 h	85.87	5.42	79.52	7.34
12 h	90.49	1.72	81.68	7.11
24 h	91.53	1.97	83.44	6.31
48 h	92.30	2.09	84.96	5.25
72 h	92.37	2.07	85.06	5.18
Faeces				
6 h	0.03	0.05	0.07	0.15
12 h	2.41	1.17	2.54	0.75
24 h	4.25	0.79	3.91	0.52
48 h	4.54	0.81	4.33	0.55
72 h	4.59	0.81	4.44	0.55
Exhaled air ^a	0.015	0.002	0.016	0.002
Cage wash ^b	1.30	0.45	5.04	4.07
Feed residue ^b	0.36	0.39	3.35	3.73
Tissues + carcass ^b	0.11	0.03	0.11	0.04
Recovery ^b	98.7	1.7	98.0	1.4

bw: body weight; SD: standard deviation

^a Radioactivity recovered in the carbon dioxide trap at 0–6 hours after dosing only – no radioactivity was detected in volatile organic or water traps.

^b Cumulative radioactivity to 72 hours.

Source: Himmelstein (2010d)

1.2 Biotransformation

In the pilot metabolism study by Himmelstein (2010a) described in section 1.1 above, rats received a single gavage dose of ^{14}C -labelled aminocyclopyrachlor at 0 mg/kg bw (one of each sex) or 500 mg/kg bw (three of each sex) and were killed 30 minutes later for the analysis of plasma metabolites by HPLC and LC-MS. Urine and faeces collected until 24 hours after dosing in the pilot mass balance study were also analysed for metabolites. Aminocyclopyrachlor was the only compound detected in plasma, urine and faeces.

Table 8. Mean radioactivity in selected tissues 72 hours after a single oral dose of 25 mg/kg bw of ¹⁴C-labelled aminocyclopyrachlor-methyl

Tissue	Mean radioactivity (% of administered dose)	
	Males	Females
Carcass ^a	0.088 5	0.080 5
Skin	0.021 0	0.017 1
Whole blood	0.002 6	0.002 5
Bone marrow ^b	< LOD	< LOD
Brain	0.000 3	0.000 5
Fat	0.014 9	0.015 3
Heart	0.000 2	0.000 3
Lungs	0.000 4	0.000 5
Spleen	< LOD	< LOD
Liver	0.003 8	0.003 5
Kidney	0.000 8	0.001 2
Gastrointestinal tract	0.001 9	0.002 1
Gastrointestinal tract contents	0.013 1	0.016 6
Pituitary	< LOD	< LOD
Thyroid	< LOD	< LOD
Thymus	0.000 2	< LOD
Ovaries	—	< LOD
Testes	0.000 4	—
Pancreas	0.000 2	0.000 2
Adrenals	NA	< LOD
Uterus	—	< LOD
Muscle	0.015 0	0.014 7
Bladder	0.000 3	< LOD
Bone	0.002 3	0.002 7
Total	0.076 5	0.071 5

LOD: limit of detection; NA: not available

^a Percentage of dose recovered in carcass is not included in the total.

Source: Himmelstein (2010d)

In the study in rats by Himmelstein (2010b) described in section 1.1 above, the only metabolite detected in plasma, urine and faeces was the free acid form of aminocyclopyrachlor.

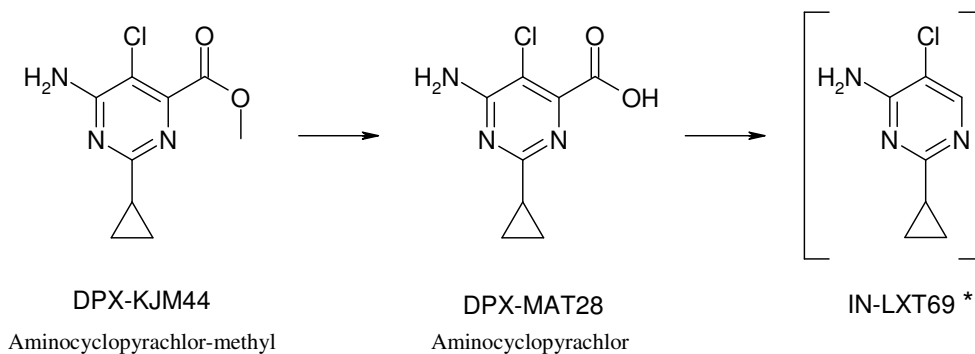
In the study in rats by Himmelstein (2010c) described in section 1.1 above, metabolites were analysed in urine, faeces and bile collected at various times up to 288 hours after the commencement of dosing. Only parent aminocyclopyrachlor was detected. On this basis, aminocyclopyrachlor is not metabolized to any great extent in rats.

In the study in rats by Himmelstein (2010d) described in section 1.1 above, the analysis of urine, faeces and bile for potential metabolites of aminocyclopyrachlor-methyl detected

aminocyclopyrachlor, the ester cleavage product of aminocyclopyrachlor-methyl, as the predominant metabolite. A very low level of aminocyclopyrachlor-methyl was quantifiable in urine (0.10% and 0.08% of the administered dose in males and females, respectively) and bile (0.003% of the administered dose in males) shortly (0–6 hours) after dosing, but at no other times. No additional metabolism of aminocyclopyrachlor-methyl was evident under the conditions of the current study.

The proposed metabolic pathway of aminocyclopyrachlor-methyl in rats is shown in Fig. 3, noting that low levels of the compound designated IN-LXT69 were detected in 90-day rat (Mawn, 2010a; Anand, 2011c) and dog (Mawn, 2008b; Luckett, 2011) studies only.

Fig. 3. Proposed metabolic pathway of aminocyclopyrachlor-methyl in rats



* Small amounts detected in rats and dogs, only following repeated dietary exposure.

2. Toxicological studies

2.1 Acute toxicity

The results of tests of the acute toxicity of aminocyclopyrachlor and aminocyclopyrachlor-methyl, including skin and eye irritation and skin sensitization studies, are summarized in Table 9. There were no deaths in any of these studies. In the oral dosing study with aminocyclopyrachlor, one female rat had diarrhoea the day after dosing (Carpenter, 2011a), but otherwise no clinical signs were observed in any of the studies. There was no effect on body weight, and there were no gross lesions.

2.2 Short-term studies of toxicity

Mice

In a 90-day toxicity study, aminocyclopyrachlor (90.5% purity) was admixed in the diet and fed ad libitum to groups of 15 CrI:CD1(ICR) mice of each sex at a dietary concentration of 0, 300, 1000, 3000 or 7000 parts per million (ppm). The mean intakes of aminocyclopyrachlor were 0, 47, 154, 459 and 1088 mg/kg bw per day for males and 0, 61, 230, 649 and 1629 mg/kg bw per day for females, respectively. Deaths, clinical signs, body weight and feed consumption were recorded throughout the dosing period. Ophthalmoscopy was performed prior to the commencement of dosing and at the end of the study. Five mice of each sex per group were killed on day 60, and blood was collected for the analysis of aminocyclopyrachlor metabolites. In the remaining mice, blood was sampled at the end of the exposure period for the analysis of standard haematological parameters and total protein. Survivors were killed after approximately 90 days and necropsied, organs were weighed and histopathology was performed.

Plasma metabolite analysis revealed only parent aminocyclopyrachlor, with metabolite IN-LXT69 below the limit of quantification (LOQ) of 10 ng/mL. The mean plasma concentration of

aminocyclopyrachlor was slightly higher in males than in females at all dose levels, with the highest concentrations reaching 2484 and 2313 ng/mL at 7000 ppm in males and females, respectively.

Table 9. Results of studies of the acute toxicity of aminocyclopyrachlor and aminocyclopyrachlor-methyl

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw) or LC ₅₀ (mg/L)	Reference
Aminocyclopyrachlor							
Rat	CrI:CD(SD)	F	Oral	92.2	Deionized water	> 5 000	Carpenter (2011a)
Rat	CrI:CD(SD)	M + F	Dermal	92.2	Deionized water	> 5 000	Carpenter (2011b)
Rat	CrI:CD(SD)	M + F	Inhalation MMAD = 3.2–3.7 µm	90.5	Air	> 5.4	Anand (2011a)
Rabbit	NZW	M	Skin irritation	90.5	Deionized water	Not irritating	Carpenter (2011c)
Rabbit	NZW	M	Eye irritation	90.5	None	Not irritating	Carpenter (2011d)
Mouse	CBA/JHsd	F	LLNA	90.5	DMF	Not sensitizing	Carpenter (2011e)
Aminocyclopyrachlor-methyl							
Rat	CrI:CD(SD)	F	Oral	96.9	0.5% (w/v) aqueous methyl cellulose	> 5 000	Carpenter (2007a)
Rat	CrI:CD(SD)	M + F	Dermal	96.9	Deionized water	> 5 000	Carpenter (2007b)
Rabbit	NZW	M	Skin irritation	96.9	Deionized water	Not irritating	Carpenter (2007c)
Rabbit	NZW	M	Eye irritation	96.9	None	Not irritating	Carpenter (2007d)
Mouse	CBA/JHsd	F	LLNA	96.9	DMSO	Not sensitizing	Carpenter (2011e)

DMF: *N,N*-dimethylformamide; DMSO: dimethyl sulfoxide; F: female; LC₅₀: median lethal concentration; LD₅₀: median lethal dose; LLNA: local lymph node assay; M: male; MMAD: mass median aerodynamic diameter; NZW: New Zealand White; w/v: weight per volume

There was no treatment-related effect on any recorded or analysed parameter. The no-observed-adverse-effect level (NOAEL) was 7000 ppm (equal to 1088 mg/kg bw per day for males and 1629 mg/kg bw per day for females), the highest dietary concentration tested (Mawn, 2008a; Anand, 2011b).

Rats

Aminocyclopyrachlor (90.5% purity) was admixed in the diet and fed ad libitum to groups of CrI:CD(SD) rats (15 of each sex per group) at a dietary concentration of 0, 600, 2000, 6000 or 18 000 ppm for 94 or 95 days (males) or 96 or 97 days (females). The mean intakes of

aminocyclopyrachlor were 0, 35, 114, 349 and 1045 mg/kg bw per day for males and 0, 45, 146, 448 and 1425 mg/kg bw per day for females at 0, 600, 2000, 6000 and 18 000 ppm, respectively. Observations for mortalities and clinical signs were made daily. Body weight and feed consumption were recorded weekly. Ophthalmoscopy was performed prior to the commencement of dosing and at the end of the study. Five rats of each sex per group were terminated on day 56, and blood was collected for the analysis of aminocyclopyrachlor metabolites. A functional observational battery and motor activity assessment were performed prior to the commencement of dosing and during weeks 4, 8 and 13. Blood and urine were sampled at the end of the exposure period for the analysis of haematological, clinical chemistry or urine analysis parameters. Survivors were killed after approximately 90 days and necropsied, organs were weighed and histopathology was performed. The latter also included an analysis of the brain, central nerves and peripheral nerves.

Parent aminocyclopyrachlor was the most abundant analyte detected in plasma, reaching 10 268 ng/mL in males and 13 428 ng/mL in females (LOQ = 10 ng/mL) at the highest dietary concentration of 18 000 ppm. Relatively low concentrations of the metabolite IN-LXT69 were detected at the same dietary concentration (92 ng/mL in males and 80 ng/mL in females; LOQ = 10 ng/mL), but not at lower doses.

There were no deaths and no treatment-related clinical signs. At 18 000 ppm, mean body weight of males was up to 9% lower ($P < 0.05$) than the control values from day 28 to day 91 of treatment. In females, mean body weight was up to 8% ($P < 0.05$) lower than the control values at days 56, 70 and 77. At this same dietary concentration, mean body weight gain from day 0 to day 91 was significantly lower ($P < 0.05$) than the control values (15% and 18% lower than the control values in males and females, respectively). Whereas mean feed consumption was generally comparable across all groups, overall (i.e. days 0–91) mean feed conversion efficiency was 11% and 17% lower ($P < 0.05$) than the control values in high-dose males and females, respectively. There was no treatment-related effect on body weight parameters at lower doses. There were no treatment-related ophthalmological abnormalities and no effects on the functional observational battery or motor activity assessment. Haematological, clinical chemistry and urine analysis parameters were unremarkable. There were no treatment-related macroscopic, organ weight or histopathological findings.

The NOAEL was 6000 ppm (equal to 349 and 448 mg/kg bw per day for males and females, respectively), based on reduced body weight, body weight gain and feed conversion efficiency at 18 000 ppm (equal to 1045 and 1425 mg/kg bw per day for males and females, respectively) (Mawn, 2010a; Anand, 2011c).

Aminocyclopyrachlor-methyl (90.5% purity) was admixed in the diet and fed ad libitum to groups of CrI:CD(SD) rats (10 of each sex per group) at a dietary concentration of 0, 600, 2000, 6000 or 18 000 ppm for 94 or 95 days (males) or 95 or 96 days (females). The mean intakes of aminocyclopyrachlor-methyl were 0, 35, 117, 347 and 1022 mg/kg bw per day for males and 0, 43, 133, 405 and 1219 mg/kg bw per day for females at 0, 600, 2000, 6000 and 18 000 ppm, respectively. These doses are equal to 0, 33, 110, 326 and 961 mg ae/kg bw per day for males and 0, 40, 125, 381 and 1146 mg ae/kg bw per day for females, respectively.

Observations for mortalities and clinical signs were made daily. Body weight and feed consumption were recorded weekly. Ophthalmoscopy was performed prior to the commencement of dosing and at the end of the study. Blood was collected from five rats of each sex per group on days 60 and 87 for the analysis of aminocyclopyrachlor metabolites. A functional observational battery and motor activity assessment were performed prior to the commencement of dosing and during weeks 4, 8 and 13. Blood and urine were sampled at the end of the exposure period for the analysis of haematological, clinical chemistry or urine analysis parameters. Survivors were killed after approximately 90 days (days 94–95 and 95–96 for males and females, respectively) and necropsied, organs were weighed and histopathology was performed. The latter also included an analysis of the brain, central nerves and peripheral nerves.

Metabolite analysis revealed no parent compound above the LOQ of 10 ng/mL. Aminocyclopyrachlor was the main plasma metabolite, reaching maximum concentrations of 20 660 and 29 784 ng/mL at 18 000 ppm in males and females, respectively (LOQ = 10 ng/mL). Plasma concentrations of the metabolite IN-LXT69 at this same dose were 439 and 415 ng/mL in males and females, respectively (LOQ = 10 ng/mL).

There were no deaths and no treatment-related clinical signs. At 18 000 ppm, mean body weight was 5–9% lower than the control value in males and 5–11% lower than the control value in females, the difference being statistically significant ($P < 0.05$) in females from days 42 to 84 except day 63. Also at 18 000 ppm, overall body weight gain was 14% and 24% lower ($P < 0.05$) than the control values in males and females, respectively. Concomitant with the reduction in body weight gain was a reduction in feed consumption, which was 9% and 11% lower than the control values ($P < 0.05$) in males and females, respectively, over days 1–91 of exposure. At 18 000 ppm, overall feed conversion efficiency was also lower than the control values (5% lower in males and 15% lower in females), but not statistically significantly so. There was no treatment-related effect on body weight parameters at lower doses.

There were no treatment-related ophthalmological abnormalities and no effects on the functional observational battery or motor activity assessment. Haematological, clinical chemistry and urine analysis parameters were unremarkable. There were no treatment-related macroscopic, organ weight or histopathological findings.

The NOAEL was 6000 ppm (equal to 326 and 381 mg ae/kg bw per day for males and females, respectively), based on reduced body weight, body weight gain and feed consumption at 18 000 ppm (equal to 961 and 1146 mg ae/kg bw per day for males and females, respectively) (Mawn, 2010b; Anand, 2011d).

Dogs

Aminocyclopyrachlor (90.5% purity) was admixed in the diet at a concentration of 0, 250, 1250, 5000 or 15 000 ppm, and 400 g food was offered daily to groups of Beagle dogs (four of each sex) for 90 days. The mean intakes of aminocyclopyrachlor were 0, 6.5, 33, 126 and 426 mg/kg bw per day for males and 0, 7.0, 38, 124 and 388 mg/kg bw per day for females at 0, 250, 1250, 5000 and 15 000 ppm, respectively. Dogs were observed twice daily for mortality and clinical signs, with a more detailed clinical examination performed weekly. A neurobehavioural assessment was performed weekly, with observations covering changes in activity, gait, posture, strength and response to handling, as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation). Body weight was recorded weekly, and feed consumption was recorded daily. Ophthalmoscopy was performed prior to the commencement of dosing and at scheduled termination. Blood and urine were sampled prior to the commencement of dosing and during weeks 4, 8 and 12 for the analysis of haematological, clinical chemistry or urine analysis parameters. Additional blood samples were collected during week 9 for the analysis of aminocyclopyrachlor metabolites. All dogs were killed at the end of the study and necropsied, and their organs were weighed. Tissues were collected and examined histopathologically. Samples of liver were analysed for cytochrome P450 enzymes.

The main plasma analyte was aminocyclopyrachlor, with higher concentrations present in female dogs compared with male dogs at the same dietary concentration of 15 000 ppm (82 575 ng/mL versus 63 000 ng/mL, respectively; LOQ = 10 ng/mL). IN-LXT69 was detected in male and female dogs only at 15 000 ppm at concentrations marginally above the LOQ of 10 ng/mL (i.e. 12 ng/mL).

There were no deaths and no treatment-related clinical signs or neurobehavioural findings. Body weight and feed consumption were unaffected by treatment. Ophthalmoscopy was unremarkable. There was no treatment-related effect on haematological, clinical chemistry or urine analysis parameters. There were no macroscopic or microscopic findings that were attributable to treatment. Total hepatic cytochrome P450 levels were unaffected by treatment. However, concentrations of cytochrome P450 enzymes CYP1A1 and CYP2B1/2 were increased 5- and 2-fold,

respectively, in male dogs at 5000 and 15 000 ppm (Table 10). In females, a 2-fold increase in the concentration of CYP2B1/2 occurred at 5000 ppm, with a 2-fold increase in CYP2E1 at 1250 ppm (Table 10).

Table 10. Liver enzyme analysis in dogs exposed to aminocyclopyrachlor via the diet for 90 days

Cytochrome	Mean ($\pm$ SD) content (net intensity) (nmol/mg microsomal protein)				
	0 ppm	250 ppm	1 250 ppm	5 000 ppm	15 000 ppm
Males					
Total P450 ^a	0.407 $\pm$ 0.103	0.409 $\pm$ 0.115	0.367 $\pm$ 0.047	0.320 $\pm$ 0.046	0.405 $\pm$ 0.163
CYP1A1	173 $\pm$ 78	255 $\pm$ 212	Not detected	869 $\pm$ 279*	899 $\pm$ 502
CYP1A2	Not detected	Not detected	Not detected	Not detected	Not detected
CYP2B1/2	6 466 $\pm$ 195	8 097 $\pm$ 303	6 673 $\pm$ 195	14 537 $\pm$ 687*	12 333 $\pm$ 300
CYP2E1	774 $\pm$ 526	890 $\pm$ 300	709 $\pm$ 215	1121 $\pm$ 0	487 $\pm$ 5
CYP3A2	14 061 $\pm$ 7 742	8 054 $\pm$ 4 453	10 164 $\pm$ 4 440	8 635 $\pm$ 4 759	10 505 $\pm$ 6 058
CYP4A1/2/3	110 960 $\pm$ 5 614	109 584 $\pm$ 8 517	100 190 $\pm$ 2 079	102 521 $\pm$ 6 806	99 175 $\pm$ 4 382*
Females					
Total P450 ^a	0.421 $\pm$ 0.060	0.396 $\pm$ 0.078	0.378 $\pm$ 0.042	0.394 $\pm$ 0.049	0.415 $\pm$ 0.054
CYP1A1	781 $\pm$ 204	835 $\pm$ 407	1 086 $\pm$ 327	979 $\pm$ 270*	1 033 $\pm$ 176
CYP1A2	Not detected	Not detected	Not detected	Not detected	Not detected
CYP2B1/2	13 086 $\pm$ 651	7 477 $\pm$ 314	6 963 $\pm$ 610	25 237 $\pm$ 583*	17 513 $\pm$ 865
CYP2E1	481 $\pm$ 120	693 $\pm$ 92	900 $\pm$ 194*	594 $\pm$ 300	523 $\pm$ 211
CYP3A2	19 631 $\pm$ 9 223	19 028 $\pm$ 12 474	20 628 $\pm$ 5 434	23 226 $\pm$ 3 427	22 411 $\pm$ 2 447
CYP4A1/2/3	10 4017 $\pm$ 6 971	111 144 $\pm$ 1 494	117 163 $\pm$ 5 892*	113 233 $\pm$ 5 705	120 065 $\pm$ 1 655*

ppm: parts per million; SD: standard deviation; *: $P < 0.05$ (statistically significant results are in boldface type)

Source: Mawn (2008b); Luckett (2011)

The NOAEL was 15 000 ppm (equal to 426 mg/kg bw per day for males and 388 mg/kg bw per day for females), the highest dietary concentration tested (Mawn, 2008b; Luckett, 2011).

Aminocyclopyrachlor (90.5% purity) was admixed in the diet at a concentration of 0, 1250, 5000, 15 000 or 30 000 ppm, and 400 g of food was offered daily to groups of Beagle dogs (four of each sex) for 1 year. Mean daily intakes were 0, 38, 178, 465 and 1077 mg/kg bw per day for males and 0, 47, 175, 542 and 1073 mg/kg bw per day for females at 0, 1250, 5000, 15 000 and 30 000 ppm, respectively. Dogs were observed twice daily for mortalities and clinical signs. Body weight was recorded weekly. Feed consumption was recorded daily. Ophthalmoscopy was performed prior to the commencement of dosing and prior to termination. Blood and urine were sampled prior to the commencement of dosing and on days 90, 188 and 366 for the analysis of haematological, clinical chemistry or urine analysis parameters. Following termination, dogs were necropsied and their organs weighed. Tissues were collected from the high-dose and control groups for histopathological examination, with potential target tissues examined at lower doses as necessary.

There were no deaths and no treatment-related clinical signs. Body weight and feed consumption were unaffected by treatment. Ophthalmoscopy was unremarkable. There was no

treatment-related effect on any haematological, clinical chemistry or urine analysis parameters. There were no treatment-related macroscopic abnormalities or effects on organ weights. Histopathological examination revealed an increase in hepatic vacuolation, consistent with glycogen accumulation, in treated females (0/4, 1/4, 0/4, 1/4 and 2/4 dogs at 0, 1250, 5000, 15 000 and 30 000 ppm, respectively). The vacuolation was graded as minimal (Grade 1) with the exception of the vacuolation in one high-dose female, which was graded as slight (Grade 2). In the absence of similar findings in males, changes in clinical chemistry parameters indicative of liver toxicity or a dose-related increase in severity, this finding was not considered adverse.

The NOAEL was 30 000 ppm (equal to 1077 and 1073 mg/kg bw per day for males and females, respectively), the highest dietary concentration tested (Han, 2010).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Aminocyclopyrachlor (90.5% purity) was admixed in the diet at a concentration of 0, 300, 1000, 3000 or 7000 ppm and fed ad libitum to groups of 60 male and 60 female Crlj:CD1 (ICR) mice for 18 months. The mean intakes of aminocyclopyrachlor were 0, 39, 133, 393 and 876 mg/kg bw per day for males and 0, 50, 171, 527 and 1190 mg/kg bw per day for females at 0, 300, 1000, 3000 and 7000 ppm, respectively. Observations for mortalities and clinical signs were made at least once daily. Body weight and feed consumption were recorded weekly to week 13 and every 2 weeks thereafter. Ophthalmological examinations were made prior to the commencement of dosing and prior to scheduled termination. Blood was sampled at 12 and 18 months for haematological analysis. Following termination, mice were necropsied, organs weighed and tissues examined histopathologically.

There was no treatment-related effect on any parameter, and there were no neoplastic or non-neoplastic changes at any dose. On this basis, the NOAEL for chronic toxicity and carcinogenicity was 7000 ppm (equal to 876 mg/kg bw per day for males and 1190 mg/kg bw per day for females), the highest dietary concentration tested (Huh, 2010).

Rats

Aminocyclopyrachlor (90.5% purity) was admixed in the diet at a concentration of 0, 600, 2000, 6000 or 18 000 ppm and fed ad libitum to groups of Crl:CD(SD) rats (80 of each sex per group) for 2 years. Mean daily intakes of aminocyclopyrachlor were 0, 27, 97, 279 and 892 mg/kg bw per day for males and 0, 29, 100, 309 and 957 mg/kg bw per day for females at 0, 600, 2000, 6000 and 18 000 ppm, respectively. Satellite groups of 10 rats of each sex per group were killed after 1 year. Rats were observed at least twice daily for mortalities and clinical signs. Body weight and feed consumption were recorded weekly to week 13 and every 2 weeks thereafter. Ophthalmoscopy was performed prior to the commencement of dosing and after 1 (satellite groups) and 2 years. Blood and urine were collected at approximately 12, 18 and 24 months for the analysis of haematological, clinical chemistry or urine analysis parameters. For the satellite groups, blood and urine were collected at 26 weeks and 12 months. Following scheduled termination after 1 or 2 years, rats were necropsied and their organs weighed. Histopathology was performed on the standard range of tissues.

There were no treatment-related deaths or clinical signs. At the highest dose, mean body weight and body weight gain were significantly lower ($P < 0.01$ or 0.05) than the control values in both sexes (Table 11). Also at the highest dose, feed consumption (females) and feed conversion efficiency (both sexes) were significantly lower than the control values ($P < 0.01$ or 0.05) (Table 11). There were no treatment-related ophthalmological abnormalities or haematology, clinical chemistry, urine analysis, organ weight or macroscopic findings.

Microscopic examination of rats killed after 2 years of exposure revealed an increased incidence of brain tumours in high-dose males (Table 12); no brain tumours or preneoplastic lesions were observed in satellite groups of rats killed after 1 year. Statistical analysis using the Cochran-

Armitage trend test determined that this increase was significant ($P < 0.05$); however, analyses using the Peto and Fisher exact tests detected no significance. A single undifferentiated glioma was observed in one high-dose male, and an oligodendroglioma was observed in one 6000 ppm male. Two granular cell tumours were also observed in 18 000 ppm males. The incidence of combined glial tumours was significantly increased ($P < 0.05$) in high-dose males using either the Cochran-Armitage trend test or Peto analysis, but not by the Fisher exact test. In females, no significant difference in the incidence of brain tumours was found.

Table 11. Effect of 2 years of dietary exposure to aminocyclopyrachlor on body weight and feed parameters in rats

Parameter	Mean value (% of control)				
	0 ppm	600 ppm	2 000 ppm	6 000 ppm	18 000 ppm
Mean body weight (g)					
Males					
Day 0	216.3	216.2 (100%)	216.3 (100%)	210.4** (97%)	214.2 (99%)
Day 91	565.6	571.3 (101%)	567.3 (100%)	555.0 (98%)	536.0** (95%)
Day 357	791.8	796.1 (101%)	805.2 (102%)	803.1 (101%)	737.3** (93%)
Day 721	877.6	879.3 (100%)	856.2 (98%)	840.9 (96%)	788.5 (90%)
Females					
Day 0	158.7	158.6 (100%)	157.1 (99%)	155.0* (98%)	154.2** (97%)
Day 91	298.8	302.4 (101%)	303.6 (102%)	300.3 (101%)	286.6** (96%)
Day 357	426.5	427.0 (100%)	431.6 (101%)	424.8 (100%)	394.0* (92%)
Day 721	535.2	538.7 (101%)	533.3 (100%)	518.8 (97%)	487.3 (91%)
Mean body weight gain (g)					
Males					
Days 0–91	349.2	355.1 (102%)	351.0 (101%)	344.6 (99%)	321.8** (92%)
Days 0–357	576.1	579.9 (101%)	589.2 (102%)	592.9 (103%)	523.3** (91%)
Days 0–721	664.4	664.9 (100%)	641.3 (97%)	633.0 (95%)	577.0 (87%)
Females					
Days 0–91	140.2	143.8 (103%)	146.5 (104%)	145.3 (104%)	132.4* (94%)
Days 0–357	267.9	268.6 (100%)	274.3 (102%)	269.8 (101%)	239.7* (89%)
Days 0–721	379.5	382.3 (101%)	374.9 (99%)	360.2 (95%)	332.7 (88%)
Feed consumption (g)					
Males					
Days 0–91	24.3	24.6 (101%)	24.6 (101%)	24.3 (100%)	23.8 (98%)
Days 0–357	24.7	24.7 (100%)	25.0 (101%)	24.5 (99%)	24.3 (98%)
Days 0–721	24.6	24.6 (100%)	25.3 (103%)	23.8 (103%)	24.3 (99%)
Females					
Days 0–91	16.5	16.4 (99%)	16.5 (100%)	16.7 (101%)	16.1* (98%)
Days 0–357	16.4	16.2 (99%)	16.3 (99%)	16.5 (101%)	16.2 (99%)
Days 0–721	16.7	16.8 (101%)	16.9 (101%)	17.1 (102%)	16.9 (101%)

Table 11 (continued)

Parameter	Mean value (% of control)				
	0 ppm	600 ppm	2 000 ppm	6 000 ppm	18 000 ppm
Feed conversion efficiency (g body weight gain/g feed consumed)					
Males					
Days 0–91	15.7	15.9 (101%)	15.7 (100%)	15.6 (99%)	14.8** (94%)
Days 0–357	6.6	6.6 (100%)	6.6 (100%)	6.8 (103%)	6.0** (91%)
Days 0–721	3.8	3.7 (97%)	3.5 (92%)	3.7 (97%)	3.3 (87%)
Females					
Days 0–91	9.3	9.6 (103%)	9.7 (104%)	9.6 (103%)	9.1 (98%)
Days 0–357	4.6	4.6 (100%)	4.7 (102%)	4.6 (100%)	4.2* (91%)
Days 0–721	3.1	3.1 (100%)	3.1 (100%)	2.9 (94%)	2.7 (87%)

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (statistically significant results are in boldface type)

Source: Moon (2010)

Age- and sex-matched historical control data from four other studies ($n = 267$) conducted by the performing laboratory between 2006 and 2009 indicated that the mean incidence of astrocytomas was 1.1% (range 0–1.5%), and the mean incidence of total glial neoplasms was 3.0% (range 0–4.4%). The incidence of these neoplasms in the current study was marginally outside of this range. A comparison with a more extensive historical control database from Charles River Laboratories, which supplied the rats for the study, indicated that the incidence of astrocytoma was at the historical control maximum. An analysis of published historical control data by the study author indicated that the control maximum for the incidence of astrocytoma was up to 11%.

The increased incidence of brain tumours in high-dose males was not considered treatment related on the basis of the following considerations:

- Microscopic analysis of brains from both males and females detected no preneoplastic changes, including glial cell proliferation.
- There was no decrease in the latency of brain tumour development in either sex.
- The higher survival to termination in high-dose males (36/70 versus 27/70 in the controls) increases the probability of the development of age-related neoplasms.
- A similar pattern of increase did not occur in females.
- The incidence of astrocytoma was well within the published historical control range.
- A pathology working group convened by the sponsor concluded that the slight difference in the incidence of astrocytomas in high-dose males was not treatment related (Hardisty, 2010).
- Aminocyclopyrachlor was negative in the standard battery of genotoxicity tests.

The NOAEL for chronic toxicity was 6000 ppm (equal to 279 mg/kg bw per day for males and 309 mg/kg bw per day for females), based on reduced body weight, body weight gain, feed consumption and feed conversion efficiency at 18 000 ppm (equal to 892 mg/kg bw per day for males and 957 mg/kg bw per day for females). The NOAEL for carcinogenicity was 18 000 ppm (equal to 892 mg/kg bw per day for males and 957 mg/kg bw per day for females), the highest dietary concentration tested (Moon, 2010).

2.4 Genotoxicity

The results of genotoxicity studies on aminocyclopyrachlor or aminocyclopyrachlor-methyl are summarized Table 13.

Table 12. Incidence of brain tumours in rats after 2 years of dietary exposure to aminocyclopyrachlor

Parameter	Number of rats with finding (% incidence)					CRL historical control incidence	
	0 ppm	600 ppm	2 000 ppm	6 000 ppm	18 000 ppm	Mean	Range
Males							
<i>N</i>	70	70	70	70	69	2 146	–
Astrocytoma ^M	0	0	0	1	3* (4.35%)	1.21%	0–4.29%
Glioma, undifferentiated ^M	0	0	0	0	1 (1.45%)	0.14%	0–1.92%
Oligodendroglioma ^M	0	0	0	1	0	0.14%	0–2%
Granular cell tumour ^B	0	0	0	0	2 (2.90%)	0.37%	0–2%
Combined glial tumours	0	0	0	2	4* (5.8%)	–	–
Females							
<i>N</i>	70	70	70	70	70	2 344	–
Astrocytoma ^M	0	2 (2.9%)	1 (1.4%)	0	2 (2.8%)	0.38%	0–2.31%
Glioma, undifferentiated ^M	0	0	1 ^a (1.4%)	0	0	0.04%	0–1.43%
Oligodendroglioma ^M	0	0	0	0	1	0.21%	0–2.00%
Granular cell tumour ^B	0	0	0	1	0	0.21%	0–2.00%
Combined glial tumours	0	2 (2.9%)	2 (2.9%)	0	2 (2.9%)	–	–

B: benign; CRL: Charles River Laboratories; M: malignant; ppm: parts per million; *: $P < 0.05$ (statistically significant results are in boldface type)

^a Alternatively diagnosed as a pineal carcinoma (non-glial tumour).

Source: Moon (2010)

2.5 Reproductive and developmental toxicity

(a) Single or multigeneration studies

Rats

In a one-generation study, aminocyclopyrachlor-methyl (99.7% purity) was admixed in the diet at a concentration of 0, 600, 5000 or 17 000 ppm and fed ad libitum to CrI:CD[®](SD)IGS BR rats (10 rats of each sex per concentration) through premating, mating, gestation and lactation. Observations for mortalities and clinical signs were made daily, with a more detailed clinical assessment undertaken weekly. Body weight and feed consumption were recorded weekly during the premating period and for dams on days 0, 7, 14 and 21 of gestation and lactation. A functional observational battery and a motor activity assessment were undertaken in F₀ rats prior to the commencement of dosing and at day 27 (males) or 28 (female) of the premating exposure period. Blood was sampled from five F₀ rats of each sex per group on day 29 for the analysis of

haematological and clinical chemistry parameters, with blood also sampled on days 60 (males) and 74–90 (females) for the analysis of coagulation parameters. Following weaning, F₁ body weights and feed consumption were recorded weekly until sexual maturity. Litter evaluations were made on postnatal days 0, 4, 7, 14 and 21. The time of vaginal patency or preputial separation was recorded. Following scheduled termination, all parental rats were necropsied, and the following organs were weighed: testes, ovaries (with oviducts), pituitary gland, epididymides, uterus (with cervix), adrenal glands, prostate, liver and seminal vesicles (with coagulating glands and their fluids). These organs, in addition to the thyroid, vagina, pancreas and any gross lesions, were examined histopathologically in five rats of each sex in the control and high-dose groups. In one male and one female F₁ weanling from each litter, the pancreas, thyroid and any gross lesions were examined histopathologically.

Table 13. Results of genotoxicity assays on aminocyclopyrachlor or aminocyclopyrachlor-methyl

End-point	Test object	Concentration or dose and vehicle	Purity (%)	Results	Reference
Aminocyclopyrachlor					
Gene mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535 and TA1537 <i>Escherichia coli</i> WP2 uvrA	1.5–5 000 µg/plate (±S9) DMSO vehicle	90.5	Negative	Wagner & VanDyke (2011)
Gene mutation	CHO cells	750–2 150 µg/mL (±S9) DMSO vehicle	90.5	Negative	Clarke (2011)
Cytogenetic test	Human lymphocytes	267–2 136 µg/mL (±S9) DMSO vehicle	90.5	Negative	Gudi & Rao (2011)
In vivo micronucleus	CrI:CD1(ICR) mice (bone marrow)	500, 1 000 or 2 000 mg/kg bw	90.5	Negative	Krsmanovic & Huston (2007)
Aminocyclopyrachlor-methyl					
Gene mutation	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537 <i>E. coli</i> WP2 uvrA	100–5 000 µg/plate (±S9) DMSO vehicle	95.3	Negative	Ford (2005)

bw: body weight; CHO: Chinese hamster ovary; DMSO: dimethyl sulfoxide; S9: 9000 × g supernatant fraction from rat liver homogenate

The mean dietary intakes of aminocyclopyrachlor-methyl are summarized in Table 14.

There were no treatment-related deaths or clinical signs. Mean body weight, body weight gain, feed consumption and feed conversion efficiency were unaffected by treatment of F₀ parental males during premating exposure. In F₀ females, there were no significant intergroup differences in mean body weight during premating. Mean body weight gain from days 0 to 28 of premating exposure was 35% lower than the control value in high-dose F₀ females, but this difference was not statistically significant. Feed consumption by high-dose F₀ females was significantly lower ($P < 0.05$) than the control values on premating days 0–7 (12% lower), 7–14 (15% lower) and 0–28 (11% lower), but there was no difference in feed conversion efficiency. During gestation, there were no significant intergroup differences in mean body weight, body weight gain, feed consumption or feed conversion efficiency in F₀ dams. During lactation, mean body weight was unaffected by treatment in F₀ dams, whereas there was a significant increase ($P < 0.05$) in mean body weight gain in high-

dose dams (26.4 versus 0.7 g in the controls). There were no significant differences in feed consumption or feed conversion efficiency during lactation.

Table 14. Mean intakes of aminocyclopyrachlor-methyl in a one-generation reproduction study in rats

Group	Mean intake (mg/kg bw per day) ^a		
	600 ppm	5 000 ppm	17 000 ppm
Males			
F ₀ pre mating	38 (36)	304 (285)	1 059 (994)
F ₁ (weaning to termination)	60 (56)	491 (461)	1 697 (1 592)
Females			
F ₀ pre mating	44 (41)	352 (330)	1 190 (1 118)
F ₀ gestation	52 (49)	346 (325)	1 199 (1 125)
F ₀ lactation	77 (72)	681 (629)	2 340 (2 196)
F ₁ (weaning to termination)	63 (59)	518 (486)	1 787 (1 677)

bw: body weight; F₀: parental generation; F₁: first filial generation; ppm: parts per million

^a Doses are expressed as aminocyclopyrachlor-methyl, with the equivalent dose of aminocyclopyrachlor acid equivalents contained in parentheses.

Source: Lewis (2006)

There were no treatment-related effects observed during the functional observational battery in F₀ parental rats, and there was no treatment-related effect on motor activity. Haematological and clinical chemistry parameters were unaffected by treatment.

There was no treatment-related effect on any reproduction or litter parameter or developmental landmarks. At the highest dose, mean pup weight was lower than the control values on days 7, 14 and 21 of lactation (6%, 9% and 17% lower than the control values, respectively), with the reductions on days 14 and 21 being statistically significant ($P < 0.05$).

In F₁ male weanlings at the highest dose, mean body weight was significantly lower ($P < 0.05$) than the control values on post-weaning days 0, 7, 14 and 21 (up to 17% lower than the control values), whereas there was no significant difference in body weight gain. Mean feed consumption was significantly lower ($P < 0.05$) than the control values at the highest dose on post-weaning days 0–7, 14–21, 21–28 and 0–39 (up to 17% lower than the control values), but there were no differences in feed conversion efficiency. Feed consumption was also significantly lower ($P < 0.05$) than the control values at 5000 ppm on post-weaning days 0–7 and 14–21; however, in the absence of any corresponding effects on body weight gain, these differences were not considered adverse. There were no intergroup differences in feed conversion efficiency in F₁ male weanlings.

In high-dose F₁ female weanlings, mean body weight was significantly lower ($P < 0.05$) than the control values on post-weaning days 0 and 7 (15% and 11% lower, respectively), whereas there was no significant difference in body weight gain. At the highest dose, overall feed consumption (postnatal days 0–39) was significantly lower ($P < 0.05$) than the control value (8% lower), but there was no effect on feed conversion efficiency.

There were no treatment-related organ weight, macroscopic or histopathological findings.

The NOAEL for reproductive toxicity was 17 000 ppm (equal to 994 mg ae/kg bw per day for males and 1118 mg ae/kg bw per day for females), the highest dietary concentration tested. The

NOAEL for both parental toxicity and offspring toxicity was 5000 ppm (equal to 285 mg ae/kg bw per day for males and 330 mg ae/kg bw per day for females) for reduced body weight or pup weight, body weight gain and feed consumption at 17 000 ppm (equal to 994 mg ae/kg bw per day for males and 1118 mg ae/kg bw per day for females) (Lewis, 2006).

Aminocyclopyrachlor (90.5%) was admixed in the diet at a concentration of 0, 500, 1500, 5000 or 17 000 ppm and fed ad libitum to two parental generations of CrI:CD(SD) rats (28 of each sex per group) and their offspring. Observations for mortalities and clinical signs were made daily. Body weight and feed consumption were recorded weekly during the premating period and for dams on days 0, 1, 14 and 21 of gestation and lactation. Litter evaluations were made on postnatal days 0, 4, 7, 14 and 21. Standard reproduction parameters were calculated. Sperm parameters for all F₀ and F₁ parental males were evaluated. Samples were collected from all F₀ and F₁ females from 3 weeks prior to mating until the end of the cohabitation period for the evaluation of the estrous cycle. A quantitative evaluation of primordial and growing follicles was conducted on 10 lactating F₁ control and high-dose females. Following scheduled termination, all parental rats were necropsied, and the following organs were weighed: testes, ovaries (with oviducts), liver, right cauda epididymides, uterus (with cervix), kidneys, spleen, seminal vesicles (with coagulating glands), brain, prostate, pituitary, adrenal glands and thyroid. These same organs were also examined histopathologically.

The mean intakes of aminocyclopyrachlor by both parental generations of rats are summarized in Table 15.

Table 15. Mean intakes of aminocyclopyrachlor in a two-generation study of reproduction in rats

Group	Mean intake (mg/kg bw per day)			
	500 ppm	1 500 ppm	5 000 ppm	17 000 ppm
Males				
F ₀ premating	30	92	299	1 048
F ₁ premating	42	126	426	1 522
Females				
F ₀ premating	36	110	367	1 243
F ₀ gestation	33	100	331	1 158
F ₀ lactation	59	175	599	2 215
F ₁ premating	46	141	465	1 666
F ₁ gestation	32	102	336	1 192
F ₁ lactation	64	189	651	2 243

bw: body weight; F₀: parental generation; F₁: first filial generation; ppm: parts per million

Source: Lewis (2011a)

There were no treatment-related deaths or clinical signs.

In F₀ males, mean body weight and body weight gain were significantly lower ($P < 0.05$) than the control values at 5000 and 17 000 ppm. The magnitude of the body weight reduction ranged from 6% to 7% at 5000 ppm and from 6% to 9% at 17 000 ppm and was evident from week 5. At 5000 ppm, cumulative body weight gain to day 70 was 14% lower than the control value. At 17 000 ppm, cumulative body weight gain to days 70 and 119 was 17% and 13% lower than the control values, respectively. Similar differences in body weight parameters occurred in F₁ males. During premating, mean weekly body weight was significantly lower ($P < 0.05$) than the control values at 5000 ppm (from week 9) and 17 000 ppm (every week), ranging from 7% to 9% and from 12% to

15%, respectively. Mean cumulative body weight gain to day 70 or 105 was 7% or 9% lower, respectively, at 5000 ppm and 13% or 15% lower, respectively, at 17 000 ppm. A similar decrease in body weight and body weight gain did not occur in females at 5000 ppm. As the weight of evidence from all other studies on aminocyclopyrachlor or aminocyclopyrachlor-methyl did not indicate any sex differences, the significant changes in body weight and body weight gain seen in males at 5000 ppm in this study were not considered treatment related.

In F_0 females, mean body weight and body weight gain were significantly lower ($P < 0.05$) than the control value only at 17 000 ppm. During premating, mean body weight was 6–7% lower than the control values over the last 3 weeks of exposure, with cumulative body weight gain (days 0–70) 16% lower than the control value. During gestation, mean body weights were up to 8% lower than the control values on days 7, 14 and 21, with cumulative body weight gain (days 0–21 of gestation) 12% lower than the control value. During the lactation period, mean body weights were 5–9% lower than the control values, with only the difference on days 0 and 7 being statistically significant ($P < 0.05$); overall body weight gain during lactation was comparable to the control value. At 17 000 ppm in F_1 females, mean body weight was up to 14% lower than the control values ($P < 0.05$) on days 0, 7 and 14 and thereafter recovered to be within 7% of the control value by the end of the exposure period. Cumulative body weight gain was within 5% of the control value and not statistically significantly different. At 5000 ppm in F_1 maternal rats, mean body weight was up to 6% lower than the control value, but differences were not statistically significant. There was no treatment-related effect on mean body weight or body weight gain during gestation and lactation in F_1 dams.

During the premating period, feed consumption was unaffected by treatment in F_0 males. At 17 000 ppm, feed conversion efficiency was significantly lower ($P < 0.05$) than the control values throughout the exposure period (up to 24% lower than the control value). Statistically significant differences in feed conversion efficiency occurred inconsistently at lower doses and were considered incidental findings. In F_1 males at 17 000 ppm, mean feed consumption was significantly lower than the control values ($P < 0.05$) during the premating exposure period (5–12% lower), and mean feed conversion efficiency was also significantly ($P < 0.05$) reduced (9–25% lower).

In F_0 females, there was no treatment-related effect on feed consumption during premating, gestation or lactation. At 17 000 ppm, mean feed conversion efficiency over days 0–70 of the premating phase was 12% lower ($P < 0.05$) than the control values, 26% lower ($P < 0.05$) during the first week of gestation and unaffected during lactation. There was no treatment-related effect on feed consumption or feed conversion efficiency in F_1 females during premating, gestation or lactation.

There was no treatment-related effect on sperm parameters, estrous cycling or reproduction parameters. There were no treatment-related clinical signs in pups and no effects on litter parameters. At 17 000 ppm, mean pup weight was significantly lower ($P < 0.05$) than the control values on postnatal days 0, 4, 14 and 21 in the F_1 generation (7–15% lower than the control values) and on postnatal day 21 in the F_2 generation (8% lower than the control value). At 5000 ppm in the F_1 generation, mean pup weight was also significantly lower ($P < 0.05$) than the control value (7% lower). Despite these lower body weights, body weight gain of pups was comparable among the treatment and control groups.

There was no treatment-related effect on developmental landmarks. The slight delay in preputial separation in F_1 males at 17 000 ppm (44.0 versus 42.9 days in the control) was not statistically significant and was within the performing laboratory's historical control range (39.9–45.0 days) and therefore was considered secondary to the lower body weight at weaning.

There were no treatment-related gross abnormalities. Organ weight findings are summarized in Table 16. Mean absolute and relative adrenal weights were significantly higher ($P < 0.05$) than the control weights in F_0 males at 5000 and 17 000 ppm; in the absence of any accompanying histopathological findings, similar effects in females or similar effects in F_1 males, these differences were not considered treatment related. At 17 000 ppm, significantly reduced ($P < 0.05$) absolute and relative spleen weights were determined in F_1 and F_2 weanlings and F_1 males; in the absence of any

accompanying histopathology, these reductions in spleen weight were considered a secondary effect of the lower body weight.

Table 16. Mean organ weights in F_0 and F_1 parental rats exposed to aminocyclopyrachlor

Organ	Mean organ weights (g or %)									
	0 ppm		500 ppm		1 500 ppm		5 000 ppm		17 000 ppm	
	M	F	M	F	M	F	M	F	M	F
F_0 adults										
Adrenals										
Absolute (g)	0.051	0.078	0.054	0.072	0.057	0.071	0.057*	0.073	0.058*	0.075
Relative (%)	0.009	0.023	0.009	0.021	0.010	0.021	0.010*	0.022	0.010*	0.023
F_1 adults										
Liver										
Absolute (g)	21.95	14.60	21.07	14.05	21.67	14.49	20.10	14.57	18.26*	14.78
Relative (%)	3.403	4.216	3.418	4.065	3.478	4.202	3.409	4.300	3.357	4.404
Spleen										
Absolute (g)	0.979	0.621	0.908	0.606	0.957	0.647	0.900	0.646	0.825*	0.617
Relative (%)	0.152	0.180	0.148	0.176	0.155	0.188	0.153	0.191	0.152	0.185
F_1 weanlings										
Spleen										
Absolute (g)	0.266	0.264	0.254	0.243	0.238	0.247	0.232	0.232	0.199*	0.182*
Relative (%)	0.448	0.471	0.448	0.443	0.421	0.450	0.433	0.444	0.391*	0.382*
F_2 weanlings										
Spleen										
Absolute (g)	0.289	0.267	0.253	0.258	0.275	0.272	0.256	0.261	0.243*	0.226
Relative (%)	0.467	0.459	0.420	0.443	0.450	0.469	0.432	0.469	0.432	0.424

F: female; M: male; ppm: parts per million; *: $P < 0.05$ (statistically significant results are in boldface type)

Source: Lewis (2011a)

At 17 000 ppm in F_1 females, the incidence of slight thyroid follicular cell hypertrophy was increased compared with the controls (8/28 versus 2/28, respectively). There were no other treatment-related histopathological findings, including effects on the spleen.

The NOAEL for reproductive toxicity was 17 000 ppm (equal to 1048 mg/kg bw per day), the highest dietary concentration tested. The NOAEL for parental toxicity was 5000 ppm (equal to

299 mg/kg bw per day), based on reduced body weight, body weight gain and feed conversion efficiency in males at 17 000 ppm (equal to 1048 mg/kg bw per day). The NOAEL for offspring toxicity was 5000 ppm (equal to 299 mg/kg bw per day), based on reduced pup weights at 17 000 ppm (equal to 1048 mg/kg bw per day) (Lewis, 2011a).

(b) *Developmental toxicity*

Rats

Aminocyclopyrachlor (90.5% purity) in 0.5% (w/v) methyl cellulose was administered by gavage to groups of 25 pregnant CrI:CD(SD) rats at a dose of 0, 30, 100, 300 or 1000 mg/kg bw per day from day 6 to day 20 of gestation. Dams were observed daily for clinical signs of toxicity, with body weight and feed consumption recorded throughout this period. On day 21 of gestation, surviving dams were killed, and the following parameters were recorded or calculated: pregnancy rate, total resorptions, mean number of implantations, live fetuses, dead fetuses, corpora lutea counts and pup sex ratio. Fetuses were examined for external, visceral and skeletal abnormalities.

There was no treatment-related effect on any parameter. On this basis, the NOAEL for maternal toxicity and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Lewis, 2011b).

Rabbits

Aminocyclopyrachlor (90.5% purity) in 0.5% (w/v) methyl cellulose was administered by gavage to groups of 22 pregnant New Zealand White rabbits at a dose of 0, 100, 300, 500 or 1000 mg/kg bw per day from day 7 to day 28 of gestation. Dams were observed daily throughout gestation for clinical signs of toxicity, with body weight and feed consumption recorded throughout this period. On day 29 of gestation, surviving dams were killed, and the following parameters were recorded or calculated: pregnancy rate, total resorptions, mean number of implantations, live fetuses, dead fetuses, corpora lutea counts and pup sex ratio. Fetuses were examined for external, visceral and skeletal abnormalities.

At 1000 mg/kg bw per day, one dam was found dead on day 13 of gestation, with two dams aborting their litters on day 20 or day 26 of gestation. Clinical signs observed in the decedent included increased respiration at the 1 hour post-dosing observation (gestation day 7) and decreased defecation daily from gestation days 9 to 13. This dam lost 394 g of body weight from the commencement of dosing and consumed less than 14 g feed per day for 4 days prior to death. A postmortem examination could not establish the cause of death. A second dam also died at the highest dose, but this was attributable to an intubation error. Clinical signs observed in the two dams that aborted their litters included decreased defecation several days prior to abortion and red material in the cage pan or anogenital area on the day of abortion. Additionally, both dams lost 636–643 g body weight (approximately 17–19%) from the first day of dosing to the day of abortion. Reduced feed consumption was noted from gestation day 13 or 17 (≤ 17 g/day). These abortions may be considered a secondary effect of body weight loss or reduced feed consumption. There were no treatment-related deaths or clinical signs at lower doses.

There were no significant intergroup differences in mean body weight. Although mean body weight gain was lower than the control values at 500 and 1000 mg/kg bw per day, only mean body weight gain of high-dose dams over gestation days 14–17 was significantly lower ($P < 0.01$) than the control value (a mean loss of 9 g versus a gain of 70 g in the controls). Overall body weight gain during the treatment period (days 7–29 of gestation) was 273, 218, 292, 173 and 166 g at 0, 100, 300, 500 and 1000 mg/kg bw per day, respectively, with the decreases at 500 and 1000 mg/kg bw per day (37% and 40% lower than the control values, respectively) not being statistically significant. At 1000 mg/kg bw per day, mean feed consumption was significantly lower ($P < 0.05$) than the control values on gestation days 14–15 and 15–16 (31% lower), with feed consumption over days 14–17 of gestation also being significantly lower ($P < 0.05$; 27%) than the control value. Although feed consumption was lower than the control value at 500 mg/kg bw per day, the difference was not statistically significant.

There were no treatment-related macroscopic findings. In the high-dose dam that was found dead on gestation day 13, red material was noted around the nasal area and accessory spleen, and a white precipitate was observed in the abdominal cavity adjacent to the right kidney; nine normally developing implantations were noted in utero. Necropsy confirmed that an intubation error was the cause of the second death at 1000 mg/kg bw per day. In the two 1000 mg/kg bw per day dams that aborted their litters, one had one early resorption and red material around the urogenital area, dark red contents in the vagina and nine early resorptions in utero. The second dam had one late resorption, red material around the urogenital area, cystic oviducts, thick white contents in the stomach and nine late resorptions (mummified) in utero.

There was no treatment-related effect on litter parameters or the incidence of external, visceral or skeletal abnormalities.

The NOAEL for maternal toxicity was 500 mg/kg bw per day, based on deaths, clinical signs, reduced body weight gain, reduced feed consumption and abortions at 1000 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Edwards, 2011).

2.6 *Special studies*

(a) *Neurotoxicity*

In an acute neurotoxicity study, groups of 10 Crl:CD(SD) rats of each sex received a single gavage dose of aminocyclopyrachlor (purity 90.5%) in 0.5% (w/v) aqueous methyl cellulose at 0, 200, 1000 or 2000 mg/kg bw. Observations were made twice daily for deaths and clinical signs. Body weights were recorded pretreatment and daily following dosing. A functional observational battery and a motor activity assessment were performed pretreatment, at 2 hours after dosing (i.e. the approximate time to peak effect) and at days 8 and 15. On day 15, all survivors were killed, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Five rats of each sex per group were killed, their brain weights were recorded and a neurohistopathological examination was performed.

There were no deaths and no treatment-related clinical signs. Mean body weight and body weight gain were unaffected by treatment. At 2000 mg/kg bw, mean feed consumption was significantly lower ($P < 0.01$ or 0.05) than the control values in both sexes 1–2 days after dosing (17% lower in males and 15% lower in females). In the absence of any other treatment-related effects or evidence of systemic toxicity or neurotoxicity, this effect on feed consumption was not considered adverse. The functional observational battery and motor activity assessment were unremarkable. There were no treatment-related macroscopic findings or neurohistopathological abnormalities.

The NOAEL was 2000 mg/kg bw per day, the highest dose tested (Barnett, 2011).

(b) *Immunotoxicity*

Mice

In a study designed to evaluate immunotoxic potential, aminocyclopyrachlor (90.5% purity) was admixed in the diet at a concentration of 0, 300, 3000 or 7000 ppm and fed ad libitum to groups of 10 male Crl:CD1(ICR) mice for 28 days. The mean intakes of aminocyclopyrachlor were 0, 45, 425 and 1056 mg/kg bw per day at 0, 300, 3000 and 7000 ppm, respectively. A positive control group of five male mice was administered an intraperitoneal injection of cyclophosphamide monohydrate (25 mg/kg bw) on days 23–28. Deaths, clinical signs, body weight and feed consumption were recorded throughout the dosing period. Prior to termination, the immune system was stimulated by injecting sheep red blood cells (sRBCs) on day 23, with blood samples collected from each mouse on day 28. Serum samples were analysed for sRBC-specific immunoglobulin M (IgM) antibodies to provide a quantitative assessment of humoral immune response. Mice were killed on day 28 and necropsied, and selected organs were weighed (brain, spleen and thymus).

There was no treatment-related effect on any recorded or analysed parameter. The NOAEL was 7000 ppm (equal to 1056 mg/kg bw per day), the highest dietary concentration tested (Hoban, 2011a).

Rats

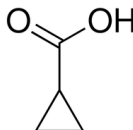
In a study designed similarly to the study conducted by Hoban (2011a) in mice, aminocyclopyrachlor (90.5% purity) was admixed in the diet at a concentration of 0, 600, 6000 or 18 000 ppm and fed ad libitum to groups of 10 male Crl:CD(SD) rats for 28 days. The mean intakes of aminocyclopyrachlor were 0, 42, 407 and 1277 mg/kg bw per day at 0, 600, 6000 and 18 000 ppm, respectively.

There was no treatment-related effect on any recorded or analysed parameter, including immune function. The NOAEL was 18 000 ppm (equal to 1277 mg/kg bw per day), the highest dietary concentration tested (Hoban, 2011b).

(c) Study on the photolytic degradate, cyclopropane carboxylic acid (CPCA)

Cyclopropane carboxylic acid (CPCA) (CAS No. 1759-53-1) is an environmental degradate of aminocyclopyrachlor (Fig. 4), which forms only as an aqueous photolytic metabolite. Low concentrations were detected as a possible extraction artefact in a grass metabolism study. However, dietary exposure to CPCA from rotational crops and animal products is highly unlikely, as CPCA was not detected in aerobic or anaerobic soil metabolism studies, nor was it detected in a goat metabolism study.

Fig. 4. Chemical structure of cyclopropane carboxylic acid (IN-V0977)



In a 90-day study, CPCA (99.7% purity) in deionized water was administered by gavage to Crl:CD(SD) rats (10 of each sex per group) at a dose of 0, 2, 10, 30 or 60 mg/kg bw per day. Observations for mortalities and clinical signs were made daily, with a more detailed clinical assessment undertaken weekly. Body weight and feed consumption were recorded weekly. Ophthalmological and neurobehavioural assessments (consisting of a modified functional observational battery and a motor activity assessment) were performed prior to the start of dosing and near the end of the exposure period. Blood and urine were sampled at the end of the exposure period for the analysis of haematological and clinical chemistry or urine analysis parameters. At the end of the exposure period, survivors were killed (day 92 or 93 in males; day 93 or 94 in females) and necropsied, and their organs were weighed. Histopathology was performed on the standard range of tissues.

There were no treatment-related deaths or clinical signs. There were no adverse effects on body weight, body weight gain, feed consumption or feed conversion efficiency. Although mean body weight at the end of the exposure period and overall body weight gain were higher than the control values in both sexes, these differences were not statistically significant. This increase was coincident with increased feed consumption, which was also not statistically significant. There were no treatment-related ophthalmological or neurobehavioural findings.

Selected haematological, clinical chemistry, urine analysis, organ weight, macroscopic and histopathological findings are summarized in Table 17. Mean neutrophil and monocyte counts were significantly increased ($P < 0.05$) in high-dose males (1.6- and 2.25-fold higher than the control values, respectively), with mean monocyte counts also significantly increased ($P < 0.05$) in high-dose females (2-fold higher than the control value). There were a number of perturbations in clinical chemistry parameters that collectively indicated hepatotoxicity or altered hepatic function, including

Table 17. Effects of exposure to CPCA in a 90-day gavage study in rats^a

Parameter	Males					Females				
	0 mg/kg bw per day	2 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	60 mg/kg bw per day	0 mg/kg bw per day	2 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	60 mg/kg bw per day
Haematology										
Neutrophils ($\times 10^3/\mu\text{L}$)	1.51	1.07	1.07	1.32	2.46*	0.70	0.68	0.78	0.61	0.62
Monocytes ($\times 10^3/\mu\text{L}$)	0.20	0.16	0.15	0.22	0.45*	0.11	0.10	0.11	0.17	0.22*
Clinical chemistry										
AST (U/L)	101	120	99	132	298*	109	115	108	207*	150*
ALT (U/L)	35	34	34	39	62*	44	37	43	47	38
Total bile acids ($\mu\text{mol/L}$)	9.1	11.4	9.6	9.3	22.1	10.1	9.3	8.6	19.0	24.9*
Total protein ($\mu\text{mol/L}$)	7.3	7.0	7.1	6.8*	6.7*	7.6	7.5	7.7	7.6	7.5
Globulin (g/dL)	3.5	3.3	3.3	3.1*	3.0*	3.2	3.3	3.2	3.2	3.2
Triglycerides (mg/dL)	62	60	65	64	121*	62	56	62	53	64
BUN (mg/dL)	14	14	14	12	15	17	17	18	22*	24*
Creatinine (mg/dL)	0.41	0.38	0.36*	0.32*	0.32*	0.47	0.50	0.45	0.46	0.49
Inorganic phosphorus (mg/dL)	6.8	6.7	6.3	6.3	6.5	4.7	5.2	5.3	5.7*	6.5*
Urine analysis										
pH	7.6	7.3	7.2	7.3	6.8**	6.8	6.8	6.9	6.4*	6.2**

Parameter	Males					Females				
	0 mg/kg bw per day	2 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	60 mg/kg bw per day	0 mg/kg bw per day	2 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	60 mg/kg bw per day
Organ weights										
Liver										
Absolute (g)	14.368	14.087	15.339	15.162	17.458*	7.004	7.222	7.733	9.182*	9.950*
Relative (%)	2.596	2.468	2.613	2.713	2.996*	2.457	2.499	2.595	3.104*	3.262*
Kidney										
Absolute (g)	3.386	3.575	3.773	3.688	4.104*	1.713	1.854	1.822	2.048*	2.089*
Relative (%)	0.612	0.627	0.644	0.663	0.708*	0.602	0.641	0.611	0.693*	0.689*
Macroscopic findings (number of rats/10)										
Liver: discoloration, pale	0	0	0	0	1	0	0	0	6	8
Histopathology (number of rats/10)										
Heart: vacuolation of myocardium										
Total	0	0	0	0	8	0	0	0	9	10
Minimal	0	0	0	0	3	0	0	0	4	1
Mild	0	0	0	0	5	0	0	0	2	7
Moderate	0	0	0	0	0	0	0	0	3	2
Heart: cardiomyopathy										
Total	4	4	2	7	10	1	1	2	9	10
Minimal	2	2	1	3	0	1	1	2	6	2
Mild	2	2	1	3	4	0	0	0	3	7
Moderate	0	0	0	1	6	0	0	0	0	1
Liver: fatty change, periportal										
Total	3	1	1	2	10	0	0	0	8	9

Table 17 (continued)

Parameter	Males					Females				
	0 mg/kg bw per day	2 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	60 mg/kg bw per day	0 mg/kg bw per day	2 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	60 mg/kg bw per day
Minimal	2	1	1	1	8	0	0	0	3	3
Mild	1	0	0	1	2	0	0	0	4	5
Moderate	0	0	0	0	0	0	0	0	1	1
Liver: infiltrate, mononuclear cell										
Total	8	7	3	5	6	1	1	1	6	8
Minimal	8	7	3	5	6	1	1	1	6	6
Mild	0	0	0	0	0	0	0	0	0	2
Thymus: necrosis, lymphoid										
Total	0	0	0	0	5	0	2	1	6	5
Minimal	0	0	0	0	5	0	2	0	6	5
Severe	0	0	0	0	0	0	0	1	0	0
Pancreas: zymogen, decreased										
Total	0	0	0	3	5	0	1	2	10	10
Minimal	0	0	0	2	4	0	1	2	4	4
Mild	0	0	0	1	1	0	0	0	6	6

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; U: units; *: $P < 0.05$; **: $P < 0.01$ (statistically significant results are in boldface type)

^a All results expressed as the mean.

Source: Carpenter (2012)

increased aspartate aminotransferase (AST) (60 mg/kg bw per day males and 30 and 60 mg/kg bw per day females), increased alanine aminotransferase (ALT) (60 mg/kg bw per day males), increased total bile acids (60 mg/kg bw per day females), increased triglycerides (60 mg/kg bw per day males) and decreased globulins and total protein (30 and 60 mg/kg bw per day males). Blood urea nitrogen (BUN) and inorganic phosphorus levels were increased in females at 30 and 60 mg/kg bw per day, concomitant with an increase in kidney weight. However, in the absence of any effect on creatinine or histopathological changes in the kidneys, these increases in BUN and inorganic phosphorus levels were considered to be of uncertain toxicological significance. Urinary pH was significantly reduced ($P < 0.01$ or 0.05) at 30 and 60 mg/kg bw per day in males and at 60 mg/kg bw per day in females, which was attributable to the renal excretion of the acidic test material.

Mean liver weight and relative liver weight were increased in males at 60 mg/kg bw per day (22% and 15% higher than the control values, respectively) and in females at 30 and 60 mg/kg bw per day (31% and 42% higher than the control values, respectively, at 30 mg/kg bw per day; 32% and 42% higher than the control values, respectively, at 60 mg/kg bw per day). These increases in liver weight correlated with pale discoloration observed macroscopically and histopathological abnormalities, including minimal to mild periportal fatty change and minimal to moderate mononuclear infiltrates within the liver. The periportal fatty change was characterized by numerous small clear vacuoles within the cytoplasm of the periportal hepatocytes, with the distribution of the affected lobules locally extensive or diffuse and with females more severely affected than males. The mononuclear cell infiltrates comprised focal to multifocal clusters of mononuclear inflammatory cells.

Mean kidney weight and relative kidney weight were also increased in both sexes at these same doses (21% and 16% higher than the control values, respectively, in males at 60 mg/kg bw per day; 20% and 22% higher than the control values, respectively, in females at 30 mg/kg bw per day; 22% and 14% higher than the control values, respectively, in females at 60 mg/kg bw per day). There were no treatment-related macroscopic or histopathological kidney abnormalities, and therefore these increases in weight are unlikely to be toxicologically significant.

At 60 mg/kg bw per day in males and at 30 and 60 mg/kg bw per day in females, histopathological findings in the heart included minimal to moderate myocardial vacuolation, which consisted of numerous small clear vacuoles within the sarcoplasm of cardiac myocytes. The distribution of the affected myocytes was described as being locally extensive or diffuse. At these same doses, the incidence and severity of cardiomyopathy were also increased; this consisted of one or more areas of myocyte degeneration/necrosis, with a mononuclear inflammatory cell infiltrate. Other treatment-related histopathological findings occurring at these doses included minimal lymphoid necrosis in the thymus (characterized by the presence of a small number of scattered lymphocytes with pyknotic nuclei) and a minimal to mild decrease in zymogen in the pancreas (consisting of reduced eosinophilic staining zymogen within the apical cytoplasm of pancreatic acinar cells).

The NOAEL was 10 mg/kg bw per day, based on increased AST (females only), increased total bile acids (females only), decreased globulin with an associated decrease in total protein (males only) and adverse microscopic findings in the heart, liver (females only) and thymus (females only) at 30 mg/kg bw per day (Carpenter, 2012).

As this NOAEL is lower than that for aminocyclopyrachlor (349 mg/kg bw per day) following 90 days of dietary exposure, and as CPCA was not detected in rat metabolism studies, its toxicological relevance was evaluated using JMPR's metabolite assessment scheme, included in the guidance document for WHO monographers.¹ This scheme uses the threshold of toxicological concern (TTC) and read-across approaches as tools. In the absence of dietary intake data, a read-across approach was taken. CPCA was classified as a Cramer class II substance and had no structural alerts for deoxyribonucleic acid (DNA) or protein binding, carcinogenicity or mutagenicity. Using the

¹ http://www.who.int/entity/foodsafety/areas_work/chemical-risks/jmpr_Guidance_Document_FINAL.pdf

JMPR metabolite decision-tree, CPCA was determined to be a substance that would not be expected to be a safety concern.

3. Observations in humans

There were no reports submitted on the health of workers involved in the manufacture or use of aminocyclopyrachlor or aminocyclopyrachlor-methyl. No cases of human poisoning have been reported.

Comments

Biochemical aspects

In studies conducted using [^{14}C]aminocyclopyrachlor, maximum concentrations of radioactivity in plasma (C_{max}) were reached at 0.4–1 hour after a single gavage dose of 25 or 500 mg/kg bw. Gastrointestinal absorption was estimated to be 37–57%. The plasma elimination half-life was approximately 5.5 hours. More than 90% of the administered dose was excreted within 24 hours of dosing, with equal proportions recovered in urine and faeces at the low dose and a higher proportion of radioactivity detected in faeces at the high dose, suggesting a reduction in gastrointestinal absorption at the high dose. There was no evidence of tissue accumulation. Aminocyclopyrachlor was not metabolized to any great extent and was the only compound identified in plasma, urine and faeces. Low concentrations of an additional plasma metabolite, IN-LXT69, were detected only in 90-day rat and dog studies.

In studies conducted on [^{14}C]aminocyclopyrachlor-methyl using the same nominal doses of 25 or 500 mg/kg bw, radioactivity was more rapidly absorbed than for [^{14}C]aminocyclopyrachlor (up to approximately 2-fold), with C_{max} and $\text{AUC}_{0-\infty}$ values also higher (2- to 5-fold and 1.4- to 2.85-fold, respectively). Gastrointestinal absorption was estimated to be 87%. The plasma half-life values were approximately twice those of [^{14}C]aminocyclopyrachlor. The majority of the radioactivity was excreted in urine within 24 hours of dosing. There was no evidence of tissue accumulation. The main metabolite detected in plasma, urine and faeces was the free acid form of aminocyclopyrachlor. Low concentrations of aminocyclopyrachlor-methyl were detected in urine and bile shortly after dosing, but at no other times. Low concentrations of IN-LXT69 were detected in a 90-day rat study at the highest dietary concentration tested.

Toxicological data

The Meeting noted that the kinetic differences between aminocyclopyrachlor-methyl and aminocyclopyrachlor do not appear to translate to any discernible difference in toxicity.

The oral and dermal LD_{50} values in rats for both aminocyclopyrachlor and aminocyclopyrachlor-methyl were greater than 5000 mg/kg bw. In rats, the LC_{50} for aminocyclopyrachlor was greater than 5.4 mg/L. Aminocyclopyrachlor and aminocyclopyrachlor-methyl were not skin or eye irritants in rabbits, nor were they skin sensitizers in mice (local lymph node assay).

In repeated-dose toxicity studies in rats and dogs, the main adverse effects were confined to reduced body weight, body weight gain and feed consumption. No toxicity was observed in mice up to the highest tested dietary concentration.

In a 28-day study in male mice, which tested dietary concentrations of 0, 300, 3000 and 7000 ppm aminocyclopyrachlor (equal to 0, 45, 425 and 1056 mg/kg bw per day, respectively), the NOAEL was 7000 ppm (equal to 1056 mg/kg bw per day), the highest dietary concentration tested.

In a 90-day toxicity study in mice, which tested dietary aminocyclopyrachlor concentrations of 0, 300, 1000, 3000 and 7000 ppm (equal to 0, 47, 154, 459 and 1088 mg/kg bw per day for males and 0, 61, 230, 649 and 1629 mg/kg bw per day for females, respectively), the NOAEL was 7000 ppm (equal to 1088 mg/kg bw per day), the highest dietary concentration tested.

In a 28-day study in male rats, which tested dietary aminocyclopyrachlor concentrations of 0, 600, 6000 and 18 000 ppm (equal to 0, 42, 407 and 1277 mg/kg bw per day, respectively), the NOAEL was 18 000 ppm (equal to 1277 mg/kg bw per day), the highest dietary concentration tested.

In a 3-month toxicity study in rats, which tested dietary aminocyclopyrachlor concentrations of 0, 600, 2000, 6000 and 18 000 ppm (equal to 0, 35, 114, 349 and 1045 mg/kg bw per day for males and 0, 45, 146, 448 and 1425 mg/kg bw per day for females, respectively), the NOAEL was 6000 ppm (equal to 349 mg/kg bw per day), based on reduced body weight, body weight gain and feed conversion efficiency at 18 000 ppm (equal to 1045 mg/kg bw per day).

In another 3-month toxicity study in rats, which tested dietary aminocyclopyrachlor-methyl concentrations of 0, 600, 2000, 6000 and 18 000 ppm (equal to 0, 33, 110, 326 and 961 mg ae/kg bw per day for males and 0, 40, 125, 381 and 1146 mg ae/kg bw per day for females, respectively), the NOAEL was 6000 ppm (equal to 326 mg ae/kg bw per day), based on reduced body weight, body weight gain and feed consumption at 18 000 ppm (equal to 961 mg ae/kg bw per day).

In a 90-day toxicity study in dogs, which tested dietary aminocyclopyrachlor concentrations of 0, 250, 1250, 5000 and 15 000 ppm (equal to 0, 6.5, 33, 126 and 426 mg/kg bw per day for males and 0, 7.0, 38, 124 and 388 mg/kg bw per day for females, respectively), the NOAEL was 15 000 ppm (equal to 388 mg/kg bw per day), the highest dietary concentration tested.

In a 52-week toxicity study in dogs, which tested dietary aminocyclopyrachlor concentrations of 0, 1250, 5000, 15 000 and 30 000 ppm (equal to 0, 38, 178, 465 and 1077 mg/kg bw per day for males and 0, 47, 175, 542 and 1073 mg/kg bw per day for females, respectively), the NOAEL was 30 000 ppm (equal to 1073 mg/kg bw per day), the highest dietary concentration tested.

The overall NOAEL in dogs from the 90-day and 52-week studies was 1073 mg/kg bw per day.

In an 18-month toxicity and carcinogenicity study in mice, which tested dietary aminocyclopyrachlor concentrations of 0, 300, 1000, 3000 and 7000 ppm (equal to 0, 39, 133, 393 and 876 mg/kg bw per day for males and 0, 50, 171, 527 and 1190 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 7000 ppm (equal to 876 mg/kg bw per day), the highest dietary concentration tested. No carcinogenicity was observed in this study.

In a 2-year study in rats, which tested dietary aminocyclopyrachlor concentrations of 0, 600, 2000, 6000 and 18 000 ppm (equal to 0, 27, 97, 279 and 892 mg/kg bw per day for males and 0, 29, 100, 309 and 957 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 6000 ppm (equal to 279 mg/kg bw per day), based on reduced body weight, body weight gain, feed consumption and feed conversion efficiency at 18 000 ppm (equal to 892 mg/kg bw per day). No carcinogenicity was observed in this study.

The Meeting concluded that aminocyclopyrachlor is not carcinogenic in mice or rats.

Aminocyclopyrachlor and aminocyclopyrachlor-methyl were tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that aminocyclopyrachlor is unlikely to be genotoxic.

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

In a one-generation reproductive toxicity study, which tested dietary aminocyclopyrachlor-methyl concentrations of 0, 600, 5000 and 17 000 ppm (equal to 0, 36, 285 and 994 mg ae/kg bw per day for males and 0, 41, 330 and 1118 mg ae/kg bw per day for females, respectively), the NOAEL for reproductive toxicity was 17 000 ppm (equal to 994 mg ae/kg bw per day), the highest dietary concentration tested. The NOAEL for both parental toxicity and offspring toxicity was 5000 ppm (equal to 285 mg ae/kg bw per day), for reduced body weight or pup weight, body weight gain and feed consumption at 17 000 ppm (equal to 994 mg ae/kg bw per day).

In a two-generation reproductive toxicity study in rats, which tested dietary aminocyclopyrachlor concentrations of 0, 500, 1500, 5000 and 17 000 ppm (equal to 0, 30, 92, 299

and 1048 mg/kg bw per day for males and 0, 36, 110, 367 and 1243 mg/kg bw per day for females, respectively), the NOAEL for reproductive toxicity was 17 000 ppm (equal to 1048 mg/kg bw per day), the highest dietary concentration tested. The NOAEL for parental toxicity was 5000 ppm (equal to 299 mg/kg bw per day), based on reduced body weight, body weight gain and feed conversion efficiency in males at 17 000 ppm (equal to 1048 mg/kg bw per day). The NOAEL for offspring toxicity was 5000 ppm (equal to 299 mg/kg bw per day), based on reduced pup weight at 17 000 ppm (equal to 1048 mg/kg bw per day).

In a developmental toxicity study in rats, which tested aminocyclopyrachlor doses of 0, 30, 100, 300 and 1000 mg/kg bw per day, the NOAEL for both maternal toxicity and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, which tested aminocyclopyrachlor doses of 0, 100, 300, 500 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity was 500 mg/kg bw per day, based on deaths, clinical signs, reduced body weight gain, reduced feed consumption and abortions at 1000 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

The Meeting concluded that aminocyclopyrachlor is not teratogenic.

In an acute neurotoxicity study in rats, which tested aminocyclopyrachlor doses of 0, 200, 1000 and 2000 mg/kg bw, the NOAEL was 2000 mg/kg bw, the highest dose tested.

The Meeting concluded that aminocyclopyrachlor is not neurotoxic.

No effects on the immune system were noted in 28-day studies in mice and rats at aminocyclopyrachlor doses up to 7000 ppm (equal to 1056 mg/kg bw per day) in mice and 18 000 ppm (equal to 1277 mg/kg bw per day) in rats.

The Meeting concluded that aminocyclopyrachlor is not immunotoxic.

Toxicological data on metabolites and/or degradates

The Meeting noted the formation of a photolytic degradate of aminocyclopyrachlor, CPCA, which was not detected in rat metabolism studies. Low concentrations of this compound were detected as a possible extraction artefact in a grass metabolism study. Dietary exposure to CPCA from rotational crops and animal products is unlikely, as CPCA was not detected in aerobic or anaerobic soil metabolism studies, nor was it detected in a goat metabolism study.

In a 90-day toxicity study in rats, which tested CPCA doses of 0, 2, 10, 30 and 60 mg/kg bw per day administered by gavage, the NOAEL was 10 mg/kg bw per day, based on increased AST (females only), increased total bile acids (females only), decreased globulin with an associated decrease in total protein (males only) and adverse microscopic findings in the heart, liver (females only) and thymus (females only) at 30 mg/kg bw per day.

As CPCA appeared to be more toxic than aminocyclopyrachlor in rats following repeated oral dosing, and as it was not detected in rat metabolism studies, its toxicological relevance was assessed using JMPR's metabolite assessment scheme included in the guidance document for WHO monographers. On the basis of this assessment, the Meeting concluded that CPCA is unlikely to be a safety concern, even if not an artefact of extraction.

Human data

No information was provided on the health of workers involved in the manufacture or use of aminocyclopyrachlor or aminocyclopyrachlor-methyl. No information on accidental or intentional poisoning in humans is available.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–3 mg/kg bw per day, expressed as aminocyclopyrachlor acid equivalents, based on a NOAEL of 279 mg/kg bw per day for reduced body weight, body weight gain, feed consumption and feed conversion efficiency at 892 mg/kg bw per day in a 2-year study of toxicity in rats, with the application of a 100-fold safety factor. The ADI is supported by the NOAELs of 299 mg/kg bw per day and 285 mg ae/kg bw per day from the reproductive toxicity studies in rats conducted on aminocyclopyrachlor and aminocyclopyrachlor-methyl, respectively. The ADI is established for the sum of aminocyclopyrachlor and its methyl ester, expressed as acid equivalents.

The Meeting concluded that it is not necessary to establish an acute reference dose (ARfD) for aminocyclopyrachlor in view of its low acute oral toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment based on studies conducted on aminocyclopyrachlor and aminocyclopyrachlor-methyl

Species	Study	Effect	NOAEL	LOAEL
Aminocyclopyrachlor				
Mouse	Eighteen-month study of toxicity and carcinogenicity ^a	Toxicity	7 000 ppm, equal to 876 mg/kg bw per day ^b	–
		Carcinogenicity	7 000 ppm, equal to 876 mg/kg bw per day ^b	–
Rat	Ninety-day study of toxicity ^a	Toxicity	6 000 ppm, equal to 349 mg/kg bw per day	18 000 ppm, equal to 1 045 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity ^a	Toxicity	6 000 ppm, equal to 279 mg/kg bw per day	18 000, equal to 892 mg/kg bw per day
		Carcinogenicity	18 000 ppm, equal to 892 mg/kg bw per day ^b	–
	Two-generation study of reproductive toxicity ^a	Reproductive toxicity	17 000 ppm, equal to 1 048 mg/kg bw per day ^b	–
		Parental toxicity	5 000 ppm, equal to 299 mg/kg bw per day	17 000 ppm, equal to 1 048 mg/kg bw per day
		Offspring toxicity	5 000 ppm, equal to 299 mg/kg bw per day	17 000 ppm, equal to 1 048 mg/kg bw per day
	Developmental toxicity study ^c	Maternal toxicity	1 000 mg/kg bw per day ^b	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day ^b	–
Rabbit	Developmental toxicity study ^c	Maternal toxicity	500 mg/kg bw per day	1 000 mg/kg bw per day
		Embryo and fetal toxicity	1 000 mg/kg bw per day ^b	–

Species	Study	Effect	NOAEL	LOAEL
Dog	Ninety-day and 1-year studies of toxicity ^{a,d}	Toxicity	30 000 ppm, equal to 1 073 mg/kg bw per day ^b	–
Aminocyclopyrachlor-methyl				
Rat	Ninety-day study of toxicity ^{a,e}	Toxicity	6 000 ppm, equal to 326 mg ae/kg bw per day	18 000 ppm, equal to 961 mg ae/kg bw per day
	One-generation study of reproductive toxicity ^{a,e}	Reproductive toxicity	17 000 ppm, equal to 994 mg ae/kg bw per day ^b	–
		Parental toxicity	5 000 ppm, equal to 285 mg ae/kg bw per day	17 000 ppm, equal to 994 mg ae/kg bw per day
		Offspring toxicity	5 000 ppm, equal to 285 mg ae/kg bw per day	17 000 ppm, equal to 994 mg ae/kg bw per day

^a Dietary administration.

^b Highest dose tested.

^c Gavage administration.

^d Two studies combined.

^e Conducted on aminocyclopyrachlor-methyl; doses expressed as aminocyclopyrachlor acid equivalents (ae).

Estimate of acceptable daily intake (ADI)

0–3 mg/kg bw

Estimate of acute reference dose (ARfD)

Unnecessary

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

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

Critical end-points for setting guidance values for exposure to aminocyclopyrachlor and aminocyclopyrachlor-methyl

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption	Rapid; 37–57% aminocyclopyrachlor; 87% aminocyclopyrachlor-methyl
Distribution	Rapid tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Limited; hydrolysis of aminocyclopyrachlor-methyl to aminocyclopyrachlor
Toxicologically significant compounds in animals and plants	Aminocyclopyrachlor

<i>Acute toxicity</i>	
Rat, LD ₅₀ , oral	> 5 000 mg/kg bw
Rat, LD ₅₀ , dermal	> 5 000 mg/kg bw
Rat, LC ₅₀ , inhalation	> 5.4 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
<i>Short-term studies of toxicity</i>	
Target/critical effect	Reduced body weight, body weight gain and feed consumption
Lowest relevant oral NOAEL	349 mg/kg bw per day (rat) (aminocyclopyrachlor) 326 mg ae/kg bw per day (rat) (aminocyclopyrachlor-methyl)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
<i>Long-term studies of toxicity and carcinogenicity</i>	
Target/critical effect	Reduced body weight, body weight gain, feed consumption and feed conversion efficiency
Lowest relevant NOAEL	279 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
<i>Genotoxicity</i>	
	Unlikely to be genotoxic
<i>Reproductive toxicity</i>	
Target/critical effect	No evidence of reproductive toxicity (rat)
Lowest relevant parental NOAEL	299 mg/kg bw per day (two-generation study with aminocyclopyrachlor)
Lowest relevant offspring NOAEL	299 mg/kg bw per day (two-generation study with aminocyclopyrachlor)
Lowest relevant reproductive NOAEL	1 048 mg/kg bw per day, highest dose tested (two-generation study with aminocyclopyrachlor)
<i>Developmental toxicity</i>	
Target/critical effect	No evidence of developmental toxicity (rat and rabbit)
Lowest relevant maternal NOAEL	500 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	1 000 mg/kg bw per day, highest dose tested (rat and rabbit)
<i>Neurotoxicity</i>	
Acute neurotoxicity NOAEL	2 000 mg/kg bw per day, highest dose tested (rat)
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data
<i>Other toxicological studies</i>	
Immunotoxicity NOAEL	1 056 mg/kg bw per day, highest dose tested (mouse)

Medical data

No data

Summary

	Value	Study	Safety factor
ADI	0–3 mg/kg bw	Two-year study of toxicity (rat)	100
ARfD	Unnecessary	–	–

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