AMINOPYRALID

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Explanation

Aminopyralid is the International Organization for Standardization (ISO) approved name for 4-amino-3,6-dichloropyridine-2-carboxylic acid (Chemical Abstracts Service, CAS No. 150114-71-9). It is a post-emergent auxin-type herbicide for the control of a wide variety of broadleaf weed species. Most of the toxicological studies evaluated were performed with the aminopyralid acid. However, some studies were performed with the commercially available aqueous solution of aminopyralid triisopropylammonium (TIPA) salt, called GF-871, since products are also marketed in this form. Throughout the current evaluation, aminopyralid acid is termed aminopyralid and its TIPA salt is termed aminopyralid TIPA. GF-871 is a 41.3–41.9% aqueous solution of aminopyralid TIPA corresponding to approximately 21.7% aminopyralid. If not otherwise stated, doses are given as aminopyralid equivalents.

Aminopyralid has not been evaluated previously by the Meeting. The evaluation of aminopyralid was scheduled for the 2006 JMPR but, owing to incomplete submission of data, was postponed to the present Meeting. Aminopyralid was reviewed at the request of the Codex Committee on Pesticide Residues (CCPR). All pivotal studies with aminopyralid and aminopyralid TIPA were certified as complying with good laboratory practice (GLP).

Figure 1. Chemical structure of aminopyralid

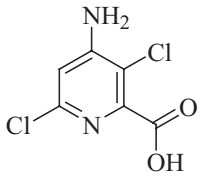
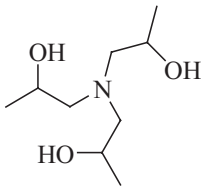


Figure 2. Chemical structure of TIPA



TIPA, triisopropylammonium

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

Rat

The absorption, distribution, metabolism and excretion of aminopyralid were studied in groups of four male Fischer 344 rats given single and multiple oral daily doses of [^{14}C]aminopyralid (purity, 99.5%; radiochemical purity, 98.6%; specific activity, 1.014 GBq/mmol; batch No. F380-135a) by gavage. Animals received either a single higher dose of 1000 mg/kg bw or a lower dose of 50 mg/kg bw. An additional group of four male rats was given non-radiolabelled test material as a daily dose at at 50 mg/kg bw per day (days 1 to 14) and radiolabelled test material as a single dose (day 15) at 50 mg/kg bw per day. Aminopyralid was administered in 0.5% methylcellulose in distilled water. The radiolabelled preparations also contained 5.3% ethanol (v/v) (equivalent to approximately 300 mg/kg bw for in groups receiving higher and lower doses). The study complied with GLP.

Throughout the study, the animals looked healthy and showed no changes in appearance or behaviour. During the 7-day collection period, the percentages of radioactivity excreted in the groups receiving a single lower dose or a single higher dose were comparable, most of the excretion being completed by 24 h. Renal and faecal excretion were slightly greater than 40%, with a tendency to

higher renal excretion in the group receiving a single lower dose. In the group receiving multiple lower doses, excretion via the urine amounted to approximately 60% and via faeces was nearly 30%. More details are presented in Table 1. Biliary excretion of aminopyralid was assumed to be very low, on the basis of studies with the closely-related substances triclopyr, picloram, fluroxypyr and 2,4-D. Therefore, the company did not perform experiments to analyse the biliary excretion of aminopyralid and the percentage of absorbed material was calculated as the sum of the renally excreted and residual substance in the tissues and the carcass. On this basis, the percentage of absorbed material was estimated to be 42%, 50% and 59% for the groups receiving the single higher dose, the single lower dose and multiple lower doses, respectively.

The urinary elimination was biphasic, with elimination half-lives $t_{1/2\alpha}$ and $t_{1/2\beta}$ of 3–4 h and 10–12 h, respectively (see Table 2). For both half-lives, there were no significant differences between the three dosing groups, 74.0–93.1% of the administered dose being excreted within the first 24 h after dosing.

As can be seen from Table 3, after a depletion period of 168 h, virtually all of the examined tissues had radioactivity levels of less than 0.01% of the administered dose. Only in the skin of the rats in the group receiving a single higher dose were notable levels detected, but with high interindividual variability (1–66 µg eq/g).

Pooled samples of urine from 0–6 h and 6–12 h, together contained 78–90% of the total radioactivity recovered in the urine and in all dose groups unchanged aminopyralid comprised more than 96% of the radiolabel present. The remainder of the radiolabel in the urine, approximately 4%,

Table 1. Recovery of radioactivity over 7 days in rats given radiolabelled aminopyralid by gavage

Sample	Recovery (% of administered dose)		
	1000 mg/kg bw (single dose)	50 mg/kg bw (single dose)	50 mg/kg bw per day (rpeat dose)
Urine	41.3 ± 8.6	49.8 ± 4.8	58.65 ± 5.5
Faeces	43.4 ± 7.7	43.1 ± 5.8	33.0 ± 4.6
Volatiles	0.01 ± 0.00	NA	NA
Cage wash	9.7 ± 3.1	3.0 ± 0.9	3.4 ± 0.7
Tissues/carcass	0.73 ± 0.72	0.07 ± 0.04	0.09 ± 0.02
Total recovery	95.2 ± 10.5	96.0 ± 1.7	95.0 ± 1.8
Absorbed material	42	50	59

From Liu (2004)
NA, not applicable, no collection performed.

Table 2. Average urinary elimination half-lives in rats given radiolabelled aminopyralid by gavage

Urinary elimination half-life (h)	Dose		
	1000 mg/kg bw (single dose)	50 mg/kg bw (single dose)	50 mg/kg bw per day (repeat dose)
t1/2α	3.8 ± 0.5	2.9 ± 1.2	3.3 ± 0.7
t1/2β	10.9 ± 2.7	10.2 ± 3.3	12.3 ± 3.7

From Liu (2004)

Table 3. Tissue distribution of radiolabel at 168 h after dosing in rats given radiolabelled aminopyralid by gavage

Tissue	Dose					
	1000 mg/kg bw (single dose)		50 mg/kg bw (single dose)		50 mg/kg bw per day (repeat dose)	
	% of dose	µg Eq/g	% of dose	µg Eq/g	% of dose	µg Eq/g
Whole blood	NA	0.608	NA	0.026	NA	0.029
Kidneys	< 0.01	0.541	< 0.01	0.020	< 0.01	0.021
Liver	< 0.01	0.607	< 0.01	0.024	< 0.01	0.027
Perirenal fat	NA	0.072	NA	0.004[a]	NA	0.004[a]
Gastrointestinal tract and contents	0.03	2.56	< 0.01	0.019	< 0.01	0.025
Skin	0.45	27.0	0.03	0.074	0.05	0.148
Spleen	< 0.01	0.609	< 0.01	0.017	< 0.01	0.015[a]
Remaining carcass	0.26	4.45	0.04	0.030	0.05	0.032

From Liu (2004)
NA, not applicable.
[a]Average of 50% of the values for the limit of detection within the group.

comprised three unknown components, which were also found in the dosing formulation and hence were not further characterized. In pooled samples of faeces from all dose groups at 0–24 h and a pooled sample from the group receiving the single higher dose at 24–48 h, only unchanged parent compound was identified. These results suggest that aminopyralid is not metabolized (Liu, 2004).

To compare the patterns of absorption, distribution, metabolism and excretion of aminopyralid and aminopyralid TIPA, two groups of four male Fischer 344 rats received single equimolar doses of either [^{14}C]aminopyralid (batch No. DE3-E1004-77, INV1893) at 50 mg/kg bw or [^{14}C]aminopyralid TIPA at 96 mg/kg bw by oral gavage. [^{14}C]Aminopyralid TIPA was prepared using [^{14}C]aminopyralid (batch No. DE3-E1004-77, INV1893) by adding appropriate amounts of TIPA.

The test material was 94.5% chemically and 98.25% radiochemically pure active ingredient. Both test materials had a specific activity of 1.058 GBq/mmol and were administered in 0.5% methylcellulose in distilled water. Dosing preparations containing aminopyralid (acid form) appeared to be much more like a suspension than did the TIPA salt, which was readily soluble. Aminopyralid was re-suspended before gavage by shaking by hand. Rats were fitted with indwelling jugular vein cannulae and blood samples (14) were taken at intervals until 120 h after dosing. The study complied with GLP.

Throughout the study, the animals looked healthy and showed no changes in appearance or behaviour. Calculated as the sum of renal excretion and remaining radioactivity in tissues and carcass, 46% and 43% of aminopyralid in the acid form and as the TIPA salt were absorbed, respectively. Faecal excretion amounted to about 50% for both compounds. Except for one male with 0.02% of the administered dose in the skin, no radioactivity at above the limit of quantitation of ≤ 0.01% of administered dose (LOQ) was recovered in the tissues of rats treated with aminopyralid. In rats treated with aminopyralid TIPA, traces of radioactivity at slightly greater than the LOQ were identified in the kidney and the spleen. Plasma peak concentrations were achieved at 0.25 h after dosing resulting in 26 and 16 µg acid equivalents/g plasma in rats treated with aminopyralid or aminopyralid TIPA, respectively. For both compounds, urinary excretion was nearly completed within the first 24 h after dosing and the biphasic elimination parameters were comparable.

Table 4. Recovery of radioactivity over 5 days in rats given radiolabelled aminopyralid or aminopyralid TIPA by gavage

Sample	Recovery (% of administered dose)	
	Aminopyralid	Aminopyralid TIPA
Urine	46.3 ± 8.0	42.5 ± 8.8
Faeces	50.6 ± 4.6	54.3 ± 5.6
Cage wash	0.2 ± 0.1	0.2 ± 0.1
Tissues/carcass	0.01 ± 0.01	0.0 ± 0.0
Total recovery	97.1 ± 3.5	97.0 ± 3.1
Absorbed material	46	43

From Domoradzki et al. (2004)
TIPA, triisopropylammonium

Table 5. Average plasma and urinary elimination half-lives in rats given radiolabelled aminopyralid or aminopyralid TIPA by gavage

Half-life (h)	Aminopyralid	Aminopyralid TIPA
Plasma		
$t_{1/2\alpha}$	0.34 ± 0.08	0.51 ± 0.21
$t_{1/2\beta}$	8.8 ± 2.3	13.0 ± 7.5
Urine		
$t_{1/2\alpha}$	2.8 ± 1.1	2.5 ± 0.8
$t_{1/2\beta}$	7.8 ± 2.2	10.7 ± 1.7

From Domoradzki et al. (2004)
TIPA, triisopropylammonium

In pooled samples of urine and faeces of animals treated with aminopyralid, only one labelled peak was found, corresponding to unchanged aminopyralid. On analysis of pooled samples of urine and faeces from rats in the group treated with aminopyralid TIPA, the result was essentially the same but a minor labelled peak was observed, but not further characterized because it accounted for only 0.34% of the administered dose (Domoradzki et al., 2004).

Rabbit

The absorption, distribution, metabolism and excretion of aminopyralid were studied in three groups of three female New Zealand White (NZW) rabbits given single and multiple oral daily doses by gavage. Three non-pregnant animals received a single dose of radiolabelled test material at 371 mg/kg bw, three pregnant animals received a single dose of radiolabelled aminopyralid at 362 mg/kg bw on day 7 of gestation and another three pregnant animals received repeated daily doses of nonlabelled aminopyralid at 279 mg/kg bw from day 7 of gestation until day 21 of gestation, followed by a single dose of radiolabelled aminopyralid at 279 mg/kg bw on day 22 of gestation. The variation between doses administered was the consequence of inadvertently using different dose preparations, but this was judged not to affect the interpretation of the results. For blood sampling, vascular access ports were placed into the jugular vein 2 weeks before mating. All animals were killed 72 h after dosing. Additionally, plasma from rabbits that were not pregnant, from pregnant animals on day 7 of gestation and on day 22 of gestation and for comparison purposes from non-pregnant female Fischer 344 rats was used for studies of plasma protein binding with radiolabelled aminopyralid. The study complied with GLP.

The test material was 94.5% chemically and 98.25% radiochemically pure active ingredient and had a specific activity of 1.058 GBq/mmol (batch No. of labelled compound, TSN 102298, DE3-E1004-77, INV1893).

Renal excretion was the major route of elimination in all three groups. In the group of pregnant rabbits that were pre-treated with nonlabelled aminopyralid, faecal excretion was substantially less, while renal excretion was minimally increased when compared with that in the other groups. The only radioactive peak in pooled samples of urine and faeces was identified as unchanged aminopyralid.

Other than the gastrointestinal tract, which showed some radioactivity at termination, the spleen in the group of pregnant rabbits that were pre-treated with nonlabelled aminopyralid was the only organ showing some radioactivity.

In all dosed groups, radioactivity was detected in the plasma within 10 min after dosing and T_{max} was achieved within 1 h. The plasma elimination half-life was lowest in the group of pretreated pregnant rabbits (4.1 h) and highest in the group of non-pregnant rabbits (7.2 h). There was increased early renal excretion, shorter T_{max} and higher plasma area under the curve of concentration–time (AUC) in pregnant rabbits given repeated doses, indicating more rapid and extensive absorption in these animals when compared with non-pregnant rabbit or pregnant rabbits given a single dose on day 7 of gestation.

Table 6. Recovery of radioactivity in rabbits given radiolabelled aminopyralid by gavage

Sample	Recovery (% of radiolabelled dose)		
	Not pregnant	Pregnant	
		Treated on day 7 of gestation	Treated on days 7–22 of gestation
Expired air	NC	NC	NC
Tissues	0.15 ± 0.05	0.11 ± 0.10	0.11 ± 0.04
Carcass	NA	NA	NA
Cage wash	1.19 ± 1.35	0.36 ± 0.42	0.52 ± 0.45
Urine:			
0–12 h	39.0 ± 11.5	47.5 ± 11.8	61.5 ± 5.7
12–24 h	33.5 ± 7.5	29.5 ± 3.9	21.7 ± 3.3
24–48 h	3.86 ± 0.71	5.33 ± 3.96	1.88 ± 0.26
24–72 h	0.38 ± 0.28	0.23 ± 0.03	0.67 ± 0.23
0–72 h	76.6 ± 7.5	82.5 ± 7.6	85.7 ± 2.5
Faeces:			
0–24 h	19.6 ± 5.5	15.3 ± 6.1	7.1 ± 4.0
24–48 h	0.53 ± 0.42	0.64 ± 0.83	0.33 ± 0.30
48–72 h	0.06 ± 0.01	0.09 ± 0.03	0.08 ± 0.02
0–72 h	20.3 ± 5.8	16.1 ± 6.8	7.54 ± 4.26
Total	98.2 ± 2.9	99.1 ± 2.9	93.9 ± 4.4
Urinary half-life (h)	6.47 ± 0.98	5.96 ± 0.41	6.77 ± 0.40

From Hansen et al. (2005)
NA, not analysed; NC, Not collected

Table 7. Pharmacokinetic parameters in rabbits given radiolabelled aminopyralid by gavage

Plasma parameter	Not pregnant	Pregnant, , treated on day 7 of gestation	Pregnant, treated on days 7–22 of gestation
C_{max} (µg/g plasma)	50 ± 16	50 ± 15	71 ± 5
T_{max} (h)	1.3 ± 0.6	1.6 ± 0.1	0.5 ± 0.4
$t_{½}$ (h)	7.2 ± 2.6	4.4 ± 1.6	4.1 ± 0.7
K_{el} (h^{-1})	0.106 ± 0.045	0.180 ± 0.085	0.171 ± 0.033
AUC (µg•h/ml)	334 ± 13	265 ± 39	441 ± 55

From Hansen et al. (2005)
AUC, area under the curve of concentration–time.

Table 8. Plasma protein binding of radiolabelled aminopyralid in rabbits treated by gavage

Concentration of radiolabelled aminopyralid	Plasma protein binding (%)			
	Rat	Not pregnant	Pregnant, treated on day 7 of gestation	Pregnant, treated on days 7–22 of gestation
10 µg/ml	72.3	68.2	65.7	58.1
33 µg/ml	70.3	67.5	62.7	53.4
154 µg/ml	55.5	53.4	47.3	43.1

From Hansen et al. (2005)

Plasma protein binding of aminopyralid was moderate, about 40–70%, and was greater in rats than in rabbits. In rabbits, it was slightly lower in pregnant animals on day 22 of gestation after pretreatment.

In summary, the pharmacokinetic and plasma-protein-binding studies indicated a somewhat higher bioavailability in late-stage pregnant rabbits than in non-pregnant or early-stage pregnant rabbits, although the difference in terms of unbound compound was not more than twofold (Hansen et al., 2005).

1.2 Effects on enzymes and other biochemical parameters

No information was available.

2. Toxicological studies

2.1 Acute toxicity

(a) Oral administration

Groups of five overnight-fasted male and female Fischer 344 rats received aminopyralid (purity, 94.5%; batch No. TSN102319/F0031-143) at a dose of 5000 mg/kg bw by gavage as two doses at 2500 mg/kg administered with an interval of 1 h, as a 50% mixture in 0.5% aqueous methylcellulose. The rats were observed for mortality, clinical signs and body-weight development during the 14 days after dosing and were then necropsied. The study complied with GLP.

One male rat died on test day 3, its appearance before death being consistent with a moribund condition. Clinical observations in the surviving rats revealed a high incidence of various combinations of perineal, perioral and perinasal soiling, watery faeces and a lower incidence of periocular soiling, decreases in muscle tone, resistance to removal, extensor-thrust, and reactivity to handling were observed. All surviving animals recovered completely by the time of study termination. Four rats had transient body-weight loss during the first week of the study, but all rats gained weight over the duration of the study. On necropsy, the male that died had treatment-related gross findings consisting of haemolysis, gas in the gastrointestinal tract and perineal soiling. Surviving animals had no treatment-related gross pathological changes.

The acute oral median lethal dose (LD_{50}) for aminopyralid was > 5000 mg/kg bw (Brooks & Yano, 2001b).

Groups of five overnight fasted male and female Fischer 344 rats received a single dose of undiluted GF-871 (41.9% aminopyralid TIPA; batch No. TSN103622/E-1175-52) at a dose of 5000 mg/kg bw (equivalent to a dose of aminopyralid of 1085 mg/kg bw) by gavage. The rats were observed for mortality, clinical signs and body-weight development during the next 14 days and were then necropsied. The study complied with GLP.

All animals survived the 14-day observation period and gained weight. Clinical observations consisted of bilateral cloudy eyes in all animals on day 1, lacrimation, watery or soft faeces and soiling of the periocular and/or perineal regions. All clinical signs resolved by test day 4. No treatment-related pathological changes were observed.

The acute oral LD_{50} for aminopyralid TIPA was > 5000 mg/kg bw, equivalent to 1085 mg/kg bw expressed as aminopyralid (Wilson et al., 2002b).

(b) Dermal application

Five male and five female Fischer 344 rats were treated by dermal patch application for 24 h with aminopyralid (purity, 94.5%; batch No. TSN102319/F0031-143) at a dose of 5000 mg/kg bw, moistened with 0.5 ml of 0.5% aqueous methylcellulose. The rats were observed for mortality, clinical signs and body-weight development during the next 14 days and were then necropsied. The study complied with GLP.

All animals survived the 14-day observation period. Clinical observations consisted of perioral soiling in one male and two females on test day 1 or 2 and periocular soiling in one male on test day 1. All animals lost weight by test day 2, but gained weight over the remainder of the study. There were no gross pathological observations.

The acute dermal LD_{50} of aminopyralid was > 5000 mg/kg bw (Brooks & Yano, 2001a).

Five male and five female Fischer 344 rats were treated by dermal patch application for 24 h with GF-871 (41.9% aminopyralid TIPA; batch No. TSN103622/E-1175-52) at a dose of 5000 mg/kg bw (equivalent to aminopyralid at a dose of 1085 mg/kg bw). The rats were observed for mortality, clinical signs and body-weight development during the next 14 days and were then necropsied. The study complied with GLP.

All animals survived the 14-day observation period. After a body-weight loss on test day 2 in all animals, all animals gained weight over the remainder of the study. Clinical observations consisted of perineal soiling in one male and reddening of the skin on the test site in two males. There were no gross pathological observations.

The acute dermal LD_{50} of aminopyralid TIPA was > 5000 mg/kg, equivalent to 1085 mg/kg bw expressed as aminopyralid (Wilson et al., 2002a).

(c) Exposure by inhalation

Five male and five female Fischer 344 rats were exposed by nose-only inhalation for 4 h to a dust aerosol containing aminopyralid (purity, 94.5%; batch No. TSN102319/F0031-143) at 5.5 mg/l and with a mass median aerodynamic diameter (MMAD) of 2.5 µm (geometric standard deviation, ± 2.45). The rats were observed for mortality, clinical signs and body-weight development during the 14 days after exposure and were then necropsied. The study complied with GLP.

Immediately after exposure, gasping was observed in one male and bilateral ptosis in four males and five females. There were no other toxicologically significant clinical observations immediately after exposure. During the 14-day observation period, toxicologically significant clinical observations were limited to one female during the first week. This animal was noted with dried red material around the nose, ptosis or complete closure of the eyes and/or yellow material on the urogenital area. All animals were considered normal by day 7. Initially, a minimal body-weight decrease was observed for both sexes (9 out of 10 animals), but this effect was no longer seen on day 3 and in all animals no relevant findings were recorded on terminal necropsy.

The acute median lethal concentration (LC_{50}) after exposure to aminopyralid by inhalation was > 5.5 mg/l (Kiplinger, 2001).

Five male and five female Fischer 344 rats were exposed by nose-only inhalation for 4 h to a dust aerosol containing GF-871 (41.9% aminopyralid TIPA, equivalent to aminopyralid at 1.26 mg/l, E-1175-52) at 5.79 mg/l and with a MMAD of 2.9 µm (geometric standard deviation, ± 1.67). The rats were observed for mortality, clinical signs and body-weight development during the 14 days after exposure and were then necropsied. The study complied with GLP.

All animals survived the treatment and post-exposure period and the only treatment-related signs were extensive soiling and body-weight loss on day 2. Thereafter, all animals gained weight and no treatment-related observations were made upon necropsy.

The acute LC_{50} on inhalation exposure to GF-871 was > 5.79 mg/l, equivalent to aminopyralid at 1.26 mg/l (Landry & Krieger, 2002).

(d) Dermal and ocular irritation

One male and two female NZW rabbits were exposed dermally on the intact skin for 4 h to 500 mg of aminopyralid (purity, 94.5%; batch No. F0031-143) moistened with 0.3 ml of 0.5% aqueous methylcellulose. The study complied with GLP.

Over the 3-day study period, no signs of dermal irritation were observed and the animals gained weight normally (Brooks, 2001a).

One male and two female NZW rabbits were exposed dermally on the intact skin for 4 h to 0.5 ml of undiluted GF-871 (41.9% aminopyralid TIPA, equivalent to approximately 108.5 mg of aminopyralid, batch No. TSN103622/ E-1175-52). The study complied with GLP.

The female rabbits showed very slight erythema within the first 48 h and 72 h after unwrapping, respectively. These signs resolved within 7 days. All animals gained weight normally (Brooks & Radtke, 2002a).

Two male and one female NZW rabbits were treated with 100 mg of undiluted aminopyralid (purity, 94.5%; batch No. F0031-143) in powder form in the conjunctival sac of the right eye. Animals were observed for 35 days for ocular and body-weight changes. The study complied with GLP.

One hour after dosing, all rabbits had slight conjunctival redness, moderate chemosis, and moderate discharge. Twenty-four hours after dosing, all rabbits had moderate to marked conjunctival redness, moderate chemosis, moderate to marked discharge, and slight corneal opacity. The two male rabbits also

had slight irritation of the iris 24 h after dosing. Forty-eight hours after dosing, all rabbits had moderate to marked conjunctival redness, slight to moderate chemosis, moderate to marked discharge, slight corneal opacity, and slight irritation of the iris. The ocular irritation slowly diminished over time. Corneal vascularization was noted in all three animals starting on day 15 and continuing for one rabbit until day 36. Two rabbits had ocular irritation that continued to study termination. All rabbits gained body weight normally throughout the study. The Meeting concluded that aminopyralid is irritating to the eyes (Brooks, 2001b).

One male and two female NZW rabbits were treated with 0.1 ml of undiluted GF-871 (41.9% aminopyralid TIPA, equivalent to approximately 21.7 mg of aminopyralid; batch No. TSN013622/E-1175-52) in the conjunctival sac of the right eye. Animals were observed for 3 days for ocular and body-weight changes. The study complied with GLP.

One hour after dosing, the male and one of the two females showed slight conjunctival redness, which resolved by test day 2. There were no other signs of ocular irritation. The Meeting concuded that GF-871 causes slight initial irritation to the eyes (Brooks & Radtke, 2002b).

(e) Dermal sensitization

Ten male and ten female Hartley-derived guinea-pigs were used to evaluate the dermal sensitization potential of aminopyralid according to the Magnusson & Kligman method. The study complied with GLP. Aminopyralid (batch No. TSN102319/F0031-143) was administered intradermally at a dose of 5% w/v, followed 1 week later by a topical dose at 100% w/v. Two weeks later, the animals were challenged with 100% aminopyralid, applied topically. Aminopyralid was not a contact sensitizer, since there was no dermal reaction in any of the test animals, but clear reactions with the positive-control compounds 1-chloro-2,4-dinitrobenzene (DNCB) and α-hexylcinnamaldehyde (HCA) (Wilson, 2001).

Ten male and ten female Hartley-derived guinea-pigs were used to evaluate the dermal sensitization potential of GF-871 (41.9% aminopyralid TIPA; batch No. E-1175-52) according to the Magnusson and Kligman method. The study complied with GLP. GF-871 was administered intradermally at a dose of 1% w/v, followed 1 week later by a topical dose of 100% w/v. Two weeks later, the animals were challenged with GF-871 at 100%, applied topically.

GF-871 was not a contact sensitizer, since there was no dermal reaction in any of the test animals, but clear reactions with the positive-control compounds DNCB and HCA (Brooks & Wilson, 2002).

2.2 Short-term studies of toxicity

Mouse

In a 4-week feeding study, groups of five male and five female CD-1 mice were fed diets containing aminopyralid (purity, 95.4%; batch No. F-0031-125, TSN102095) to supply doses of 0, 10, 100, 500 or 1000 mg/kg bw per day. Animals were examined twice per day for general health, moribundity and mortality, and weekly detailed clinical examinations were performed. Feed consumption and body-weight development were recorded regularly. At the end of the study, haematological investigations and clinical chemistry including coagulation time and urine analysis parameters were analysed, organ weights recorded and gross pathology and histopathology performed. The study complied with GLP.

Male and female mice at the highest dose had statistically significantly lower white blood cell counts compared with the concurrent controls, but values were within the range for historical controls in females and slightly less than the lower bound in males. There were no histological changes in the bone marrow and no evidence of leukocyte migration from the blood. Two males showed hepatocyte hypertrophy and decreased hepatocellular glycogen. There was no change in liver weight. The Meeting considered that these effects were not of toxicological significance. The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg bw per day, the highest dose tested (Yano & Dryzga, 2000).

Table 9. Acute toxicity of aminopyralid

Test article	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
Aminopyralid	Rat	F344	M & F	Dermal	> 5000	—	94.5	Brooks & Yano (2001a)
Aminopyralid TIPA	Rat	F344	M & F	Dermal	> 5000, equivalent to > 1085 aminopyralid	—	Not reported	Wilson et al. (2002a)
Aminopyralid	Rat	F344	M & F	Oral	> 5000	—	94.5	Brooks & Yano (2001b)
Aminopyralid TIPA	Rat	F344	M & F	Oral	> 5000, equivalent to > 1085 aminopyralid	—	21.8% aminopyralid equivalents	Wilson et al. (2002b)
Aminopyralid	Rat	F344	M & F	Inhalation	—	> 5.5	94.5	Kiplinger (2001)
Aminopyralid TIPA	Rat	F344	M & F	Inhalation	—	> 5.79, equivalent to > 1.26 aminopyralid	21.8% aminopyralid equivalents	Landry & Krieger (2002)
Aminopyralid	Rabbit	NZW	M & F	Dermal irritation	No irritation	—	94.5	Brooks, (2001a)
Aminopyralid TIPA	Rabbit	NZW	M & F	Dermal irritation	Slight irritation	—	21.8% aminopyralid equivalents	Brooks & Radtke (2002a)
Aminopyralid	Rabbit	NZW	M & F	Ocular irritation	Irritant	—	94.5	Brooks, (2001b)
Aminopyralid TIPA	Rabbit	NZW	M & F	Ocular irritation	Slightly irritant	—	21.8% aminopyralid equivalents	Brooks & Radtke (2002b)
Aminopyralid	Guinea-pig	Hartley-derived albinos	M & F	Dermal sensitization	No sensitization	—	94.5	Wilson, (2001)
Aminopyralid TIPA	Guinea-pig	Hartley-derived albinos	M & F	Dermal sensitization	No sensitization	—	21.8% aminopyralid equivalents	Brooks & Wilson (2002)

F344, Fischer 344; F, female; NZW, M, male; New Zealand White.

In a 13-week feeding study, groups of 10 male and 10 female CD-1 mice were fed diets containing aminopyralid (purity, 94.5%; batch No. F0031-143, TSN102319) to supply doses of 0, 10, 100, 500 or 1000 mg/kg bw per day. Daily cage-side observations, weekly detailed clinical observations, ophthalmological examinations, body weights, feed consumption, haematology and clinical chemistry were evaluated, a gross necropsy was conducted with extensive histopathological examination of tissues and organ weights were recorded. The study complied with GLP.

The only statistically significant finding was a slight increase in plasma sodium concentrations in groups of males at 100, 500 or 1000 mg/kg bw per day (79–181 mmol/l vs 175 mmol/l in males in the control group). The plasma sodium concentrations of the controls in this study were greater than the range for historical controls. The Meeting considered that the changes in plasma sodium concentration were not of toxicological significance, since the effect was minimal and in its severity was

not dose-dependant. Sodium was also slightly but significantly increased in females of the group at 500 mg/kg bw per day only. No other treatment-related changes were identified in any of the parameters evaluated. There were no changes in leukocyte number or in hepatic histology.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Stebbins et al., 2001).

Rat

In a 4-week feeding study, groups of five male and five female Fischer 344 rats were fed diets containing aminopyralid (purity, 95.4%; batch No. F-0031-125, TSN 102095) to supply doses of 0, 10, 100, 500 or 1000 mg/kg bw per day. Animals were examined twice per day for general health, moribundity and mortality, and weekly detailed clinical examinations were performed. Feed consumption and body-weight development were recorded regularly. At the end of the study, haematological and clinical chemistry (including coagulation time) and urine analysis parameters were analysed, organ weights recorded and gross pathology and histopathology performed. The study complied with GLP.

The increases in prothrombin time (15.3 s in the males at the highest dose vs 13.1 s in males in the control group) and of plasma urea nitrogen (16 mg/dl in females at the highest dose vs 14 mg/dl in the females in the control group) in one sex at the highest dose were statistically significant, but fell well within ranges for historical controls. Three males and two females at 500 mg/kg bw and all rats at the highest dose showed enlarged caeca without any histological findings. Caecal enlargement is often an adaptive physiological response. Although treatment-related effects were identified at the highest dose and to a minor degree also at 500 mg/kg bw per day, the Meeting considered these to be not adverse.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Stebbins & Day, 2000).

In a 4-week study of dermal toxicity, groups of 10 male and 10 female Fischer 344 rats received aminopyralid (purity, 94.5%; batch No. F-0031-143, TSN102319) at a dose of 0, 100, 500, or 1000 mg/kg bw per day for 6 h/day on 28 consecutive days. The test substance was applied as a suspension in 0.5% aqueous methylcellulose to a shaved area of at least 10% of the total body surface by semi-occlusion with a gauze dressing, non-absorbent cotton. Approximately 6 h after application, the exposure site was wiped with a water-dampened towel to remove any residual test material. Daily cage-side observations, weekly dermal and detailed clinical observations and ophthalmological examinations were performed, body weights and feed consumption recorded, haematology, clinical chemistry and urine analysis parameters analysed, and a gross necropsy with extensive histopathological examination of tissues and organ weights was conducted. The study complied with GLP.

Males at the lowest and the intermediate doses had statistically significant weight increases of the full but not of the empty caeca. The effect was not dose-dependent and there was no significant change in the group at the highest dose or in any of the groups of females. The effect was therefore judged by the Meeting not to be compound-related. In males at 500 and 1000 mg/kg bw per day, very slight to slight hyperplasia of the epidermis at the test sites was observed. There were no signs of any systemic effects attributable to dermal exposure.

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested (Stebbins et al., 2002).

In a 13-week feeding study, groups of 10 male and 10 female Fischer 344 rats were fed diets containing aminopyralid (purity, 94.5%; batch No. F0031-143, TSN102319) to supply doses of 0, 10, 100, 500 or 1000 mg/kg bw per day. Additionally, recovery groups of 10 male and 10 female rats were fed diets containing aminopyralid at 0 or 1000 mg/kg bw per day for 13 weeks and were given control feed for an additional 4 weeks to evaluate the reversibility of any effects induced during the 13 weeks of treatment with aminopyralid. Daily cage-side observations, weekly detailed clinical and ophthalmologic examinations were performed, body weights and feed consumption recorded, haematology, clinical chemistry and urine analysis parameters analysed, and a gross necropsy with extensive histopathological examination of tissues and organ weights was conducted. The study complied with GLP.

The data from urine analysis revealed some treatment-related changes. These included decreases in pH at 500 mg/kg bw per day and above, most likely to be a consequence of the urinary excretion of the test substance, which is an acid, and decreases of protein and ketone concentrations in the urine of males and females at the highest dose. A complete reversal of these effects was observed in the recovery group. There were no histopathological correlates of these changes. In male and female rats, absolute and relative weights of full and empty caeca were statistically significantly increased in the groups at 500 and the 1000 mg/kg bw per day; for the empty caeca of females this was only significant at the highest dose. These weight increases essentially returned to normal within the 4-week recovery period. The only treatment-related histopathological alteration was a very slight and diffuse hyperplasia of the mucosal epithelium of the caecum and the ileum in all males of the group at the highest dose. This effect was reversible in the recovery group. These changes are fully consistent with a physiological adaptation of the caecum. In males, slight but statistically significant decreases in thymus weights were identified in the groups at 10, 500 and the 1000 mg/kg bw per day, but a clear dose–response relationship was not evident. These changes were within the range for historical controls. There were no significant changes in prothrombin time or plasma urea nitrogen in any group.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Dryzga & Stebbins, 2001).

In a 13-week feeding study, groups of 10 male and 10 female Fischer 344 rats were fed diets containing GF-871 (41.3% aminopyralid TIPA; batch No. 173-162-1A, TSN104110) to supply doses of 0, 465, 1211 or 2421 mg/kg bw per day, or 0, 101, 263 or 525 mg/kg bw per day expressed as aminopyralid. Daily cage-side observations, weekly detailed clinical and ophthalmological examinations were performed, body weights and feed consumption recorded, haematology, clinical chemistry and urine analysis parameters analysed, and a gross necropsy with extensive histopathological examination of tissues and organ weights was conducted. The study complied with GLP.

Treatment-related changes were confined to urine parameters and caecal weights. In the group at the highest dose, slightly higher urine volumes in both sexes and slightly reduced urine specific gravity in females were recorded. Absolute and relative weights of the full caeca were statistically significantly increased in both sexes at the intermediate and the highest dose, while the weights of the empty caeca were only increased in females at the highest dose. These changes were not considered to be toxicologically significant.

The NOAEL was 2421 mg/kg bw per day, or 525 mg/kg bw per day expressed as aminopyralid, the highest dose tested (Stebbins & Dryzga, 2004).

Dog

In a 4-week feeding study, groups of two male and two female beagle dogs were fed diets containing aminopyralid (purity, 95.4%; batch No. F0031-125, TSN102095) at a concentration of (0, 0.15, 0.45, or 1.5%, equal to 0, 62, 193 and 543 mg/kg bw per day. Animals were examined twice per day for general health, moribundity and mortality, and weekly detailed clinical examinations were performed. Feed consumption and body-weight development were recorded regularly. Before treatment and in the final week of treatment, detailed ophthalmic examinations were performed. At the beginning and the end of the study, haematological and clinical chemistry (including coagulation time) and urine analysis parameters were analysed, while after termination, organ weights were recorded and gross pathology and histopathology performed. The study complied with GLP.

Feed intake showed inconsistent reduction in males at the highest dose, but there was no obvious treatment-related effect. Slight changes in some erythrocyte parameters apparent in females on a groupwise comparison were not evident when the comparison was made with pretreatment values. There were no treatment-related effects identified in this study.

The NOAEL was 1.5% aminopyralid in the feed, equal to 543 mg/kg bw per day, the highest dose tested (Stebbins & Baker, 2000).

In a 13-week feeding study, groups of four male and four female beagle dogs were fed diets containing aminopyralid (purity, 94.5%; batch No. F-0031-143, TSN102319) at a concentration of 0, 0.15, 0.75, or 3.0%, equal to 0, 52.7, 232, 929 mg/kg bw per day. Animals were examined twice per day for general health, moribundity and mortality, and weekly detailed clinical examinations were performed. Feed consumption and body-weight development were recorded regularly. Before treatment and in the final week of treatment, detailed ophthalmic examinations were performed. At the beginning, in the middle and at the end of the study, haematological and clinical chemistry (including coagulation time) and urine analysis parameters were analysed, at the end of the study, organ weights were recorded and gross pathology and histopathology performed. The study complied with GLP.

A statistically significant slight increase in absolute and relative liver weights in males and females in the group at the highest dose was observed. The toxicological significance of this effect is questionable since the effect was marginal, within or near the range for historical controls and a histopathological correlate was not identified. All males and females at the highest dose showed slight and diffuse hyperplasia and hypertrophy of the mucosal epithelium of the stomach. The mucosal hyperplasia was characterized by increased numbers of mucous cells and chief cells in the fundus of the stomach. Mucous cell hyperplasia was also noted in the pylorus of the stomach. Hypertrophy of mucous cells, characterized by increased cytoplasmic volume, was most prominent in mucous cells of the pylorus. There was no accompanying degeneration, necrosis or inflammation of the mucosa of the stomach.

The NOAEL was 0.75% aminopyralid in the feed, equal to 232 mg/kg bw per day, on the basis of histological changes in the stomach at the next higher dose (Stebbins & Baker, 2002).

In a 1-year feeding study, groups of four male and four female beagle dogs were fed diets containing aminopyralid (purity, 94.5%; batch No. F-0031-143, TSN102319) at a concentration of 0, 0.03, 0.3, or 3.0%, equal to 0, 9.2 (females), 93.2 (females), 967 (males) mg/kg bw per day, the lowest dose relative to body weight was attained in males of the group at 3.0%, in the other two treated groups this was in the females). Animals were examined twice per day for general health, moribundity and mortality, and weekly detailed clinical examinations were performed. Feed consumption and body-weight development were recorded regularly. Before treatment and before termination, detailed ophthalmic examinations were performed. At the beginning, at weeks 14 (both sexes) and 26 (males)/27 (females) and at termination, haematological and clinical chemistry (including coagulation time) and urine analysis parameters were analysed, and at the end of the study, organ weights were recorded and gross pathology and histopathology performed. The study complied with GLP.

All observed effects were restricted to the groups at the highest dose. At the end of the study, females had a 9% lower body weight. Both sexes showed statistically significant increased relative liver weights (males, 21.6%; and females, 10.6%) and in two males and two females centrilobular to midzonal hepatocyte hypertrophy was found. In two females the stomach mucosa was diffusely thickened and in all animals at the highest dose mucosal hyperplasia and hypertrophy, very slight or slight chronic inflammation and slight lymphoid hyperplasia were observed.

The NOAEL was 0.3%, equal to 93.2 mg/kg bw per day, on the basis of histopathological changes in the gastric mucosa at the highest dose of 3.0% (Stebbins & Day, 2003b).

2.3 Long-term studies of toxicity and carcinogenicity

Mouse

In an 18-month study of toxicity and carcinogenicity, groups of 50 male and 50 female CD-1 mice were fed diets containing aminopyralid (purity, 94.5%; batch No. F0031-143, TSN102319) adjusted to provide doses of 0, 50, 250, or 1000 mg/kg bw per day. Monthly in the first year, then at month 17 and at study termination, animals were examined for clinical signs and from month 6 to

18, monthly for palpable masses. Body weight and feed consumption were recorded monthly, clinical chemistry and haematology parameters at month 12 and at final termination. At study termination, ophthalmological examinations and histopathology on tissues were performed, and organ weights were recorded. The study complied with GLP.

No treatment-related effects were observed in males at any dose. All females receiving aminopyralid showed increased cumulative mortality (Table 11), which was most pronounced in the group at the highest dose (42%) and statistically significant in this group and and the group at the lowest dose (34%). The increased mortality in the group at the lowest dose was not considered to be compound-related as there was no increase in any toxicological correlate, and there was no relationship with dose when considered with values for the group at the intermediate dose (30%). The mean cumulative mortality in females of six groups of historical controls in studies performed by the company was 23% (range, 18–32%). The most common cause of pre-term death in the group at the highest dose was nephropathy. There were 11 female mice with early death or moribund status with nephropathy at 1000 mg/kg bw per day, compared with 4, 2, and 4 mice at 0, 50, or 250 mg/kg bw per day, respectively. However, the overall incidence of nephropathy in females at the highest dose was not increased, nor was its severity. Nevertheless, it is possible that the increased mortality in females at the highest dose was due to exacerbation of the effects of age-related nephropathy in these animals. An increased incidence of bilateral pale kidneys, reduced amount of body fat, haemolysed blood in the gastronintestinal tract, atelectasis of the lung and perineal soiling were found (Table 10). These effects most likely reflect the moribund status of the animals. There were no changes in incidences of tumours, which would be indicative of a carcinogenic potential for aminopyralid.

The NOAEL was 250 mg/kg bw per day on the basis of the increased mortality in females at 1000 mg/kg bw per day (Stebbins & Day, 2003a).

Table 10. Number of females for which pathological observations were made among mice fed diets containing aminopyralid for 18 months

Observation	Dose (mg/kg bw per day)			
	0	50	250	1000
Bilateral pale kidneys	5	6	7	13
Decreased amount of fat	4	5	8	9
Haemolysed blood in gastrointestinal tract	1	5	1	10
Atelectasis of the lung	0	0	2	4
Perineal soiling	2	5	3	8

From Stebbins & Day (2003a)

Table 11. Cumulative mortality in females at study termination among mice fed diets containing aminopyralid for 18 months

Dose (mg/kg bw per day)	Cumulative mortality (%)
0	16
50	34
250	30
1000	42
Mean for historical controls (range)	23 (18–32)

From Stebbins & Day (2003a)

Rat

In a 24-month study of toxicity and carcinogenicity, groups of 65 male and 65 female Fischer 344 rats were fed diets containing aminopyralid (purity, 94.5%; batch No. F0031-143, TSN102319) adjusted to provide aminopyralid at a dose of 0, 5, 50, 500, or 1000 mg/kg bw per day. From each group, five males and five females were pre-selected for evaluation of long-term toxicity, five males and five females for long-term neurotoxicity and five males and five females for evaluation of long-term toxicity and neurotoxicity after 12 months. The remaining 50 animals were treated for another 12 months. The following parameters were measured in the long-term toxicity and/or carcinogenicity groups: daily cage-side observations, monthly detailed clinical observations for the first 12 months then at 15, 18, 21, and 24 months, monthly palpable tumour observations on all rats in the study of carcinogenicity for months 12–24, ophthalmic examinations, body weights, feed consumption, organ weights and gross necropsy with extensive histopathological examination of tissues. Haematology, clinical chemistry, and urine analysis data were collected from the groups after 3, 6, 12, 18, and 24 months of dosing. The study complied with GLP.

The long-term neurotoxicity part of this study is described in section 2.6.

No clinical signs of toxicity were observed and mortality at the end of the study was comparable in all groups. Nevertheless, there seemed to be an effect of treatment on the time to death in males at the highest dose, but it did not attain statistical significance. Body weights in males were statistically significantly reduced at 500 and the 1000 mg/kg bw per day and in the females at 1000 mg/kg bw per day (Table 12). In both sexes, the body-weight reduction developed in the first weeks and remained throughout the study more or less at the same level. Minimally increased feed intake and consequently reduced feed efficiency was observed in males at 1000 mg/kg bw per day. Slightly reduced but not statistically significant body-weight decrements were also observed in males at 5 and 50 mg/kg bw per day, but were not considered to be treatment-related. The males and females at 500 and 1000 mg/kg bw per day at the 12-month interim necropsy and at the end of the study had statistically significantly increased absolute and relative weights of the full and the empty caeca (Table 13). Histopathologically, the incidence of very slight hyperplasia of the caecal mucosa was increased in males and females of the group at the highest dose, being statistically significant only in males. In all groups, no treatment-related effects on haematological parameters were observed and in clinical chemistry, only females at the highest dose showed slightly increased aspartate aminotransferase (AST) activities in the first year of treatment. Urine analysis provided a consistent pattern of treatment-related changes for males and females at 500 and 1000 mg/kg bw per day. The effects included increased urine volumes, decreased specific gravity and protein and ketone content and reduced pH values. As there was no renal histopathological correlate, these changes were most likely to be adaptive effects to the high concentrations of aminopyralid in the diet. The caeca of rats at 500 or 1000 mg/kg bw per day were enlarged owing to the presence of increased amounts of semi-solid contents of similar consistency to that normally found in rats. As only the volume but not the consistency and appearance of the faeces was changed, it is postulated that there was increased colonic water resorption with compensatory renal excretion of the additional water, which led to the somewhat increased urine volume and decreased specific gravity. Decreased urine pH was attributed to renal excretion of aminopyralid, which is an acid.

There were no treatment-related increases in tumour incidences. In females at the highest dose, a decrease in the incidence of pituitary adenomas was observed.

The NOAEL was 500 mg/kg bw per day on the basis of slight but statistically significant body-weight decreases in males at 1000 mg/kg bw per day. Aminopyralid was not carcinogenic to rats under the conditions of this study (Johnson & Dryzga, 2003).

Table 12. Body weights of rats fed diets containing aminopyralid in a 24-month feeding study

Test day	Body weight (g)				
	Dose (mg/kg bw per day)				
	0	5	50	500	1000
Males					
1	150.5	147.8	147.9	149.5	151.3
29	255.0	252.1	251.9	253.9	252.5
91	334.3	330.1	328.1	328.9	325.9
120	353.1	346.4	342.3*	344.8*	339.7*
176	385.1	379.5	375.8*	374.9*	370.1*
260	418.8	413.1	410.1	407.3*	401.1*
364	444.1	436.6	433.9	430.1*	421.4*
540	466.4	454.1	457.4	444.3*	436.2*
729	431.4	420.3	421.8	409.1	412.9
Females					
1	113.7	113.6	113.7	113.1	112.8
29	154.3	156.2	155.0	153.9	151.4
91	182.5	182.8	183.3	182.4	178.0*
120	188.5	188.4	187.7	187.2	183.7*
176	200.5	201.0	200.6	199.1	196.3*
260	214.0	216.4	217.1	214.4	210.8
364	229.1	233.1	231.7	231.1	224.9
540	275.4	278.2	279.7	273.9	264.2
729	294.4	294.0	305.1	289.4	283.5

From Johnson & Dryzga (2003)
* Statistically different from mean for control group according to Dunnett test, alpha = 0.05.

Table 13. Weight of caecum of rats fed diets containing aminopyralid in a 24-month feeding study

Caecal weights	Dose (mg/kg bw per day)				
	0	5	50	500	1000
Males					
Full caecum (g)	5.21	4.98	5.52	9.05§	15.5§
Relative weight (g/100g)	1.27	1.25	1.39	2.33§	4.00§
Empty caecum (g)	2.40	2.38	2.34	3.12§	3.57§
Relative weight (g/100g)	0.584	0.592	0.586	0.807§	0.925§
Females					
Full caecum (g)	4.00	4.17	4.21	5.72§	10.4§
Relative weight (g/100g)	1.44	1.52	1.47	2.12§	3.94§
Empty caecum (g)	1.86	1.89	1.96	2.14*	2.59*
Relative weight (g/100g)	0.671	0.687	0.685	0.795§	0.980

From Johnson & Dryzga (2003)
* Statistically different from mean for control group according to Dunnett test, alpha = 0.05.
** Statistically different from mean for control group according to Wilcoxon test, alpha = 0.05.

2.4 Genotoxicity

Aminopyralid (batch No. F0031-143, TSN102319) and aminopyralid TIPA (batch No. 173-162-1A, TSN104110) were tested for genotoxicity in assays for reverse mutation in *S. typhimurium* and *E. coli*, an assay for HGPRT forward mutation in Chinese hamster ovary cells (CHO), an assay for chromosomal aberration in rat lymphocytes in vitro and an assay for micronucleus formation in mouse bone marrow in vivo. All four studies complied with GLP.

The results of all assays except that for chromosomal aberration in rat lymphocytes were clearly negative (Table 14). Chromosomal aberrations were only found after 24 h of treatment (but not after 4 h) in the absence of metabolic activation (S9) at concentrations that clearly reduced mitotic indices, indicative of a cytotoxic potential for aminopyralid (Table 15).

Table 14. Results of studies of genotoxicity with aminopyralid and aminopyralid TIPA

Test article	End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro						
Aminopyralid	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A	100–5000 µg/plate ± S9, in DMSO	94.5	Negative	Mecchi (2001)
Aminopyralid TIPA	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A	33.3–5000 µg/plate (7.3–1090 µg/plate aminopyralid equivalents) in DMSO ± S9, in DMSO	21.8% aminopyralid equivalents	Negative	Charles & Mecchi (2004)
Aminopyralid	Forward mutation	*Hgprt* locus in CHO cells	31.25–2070 µg/ml ± S9, in DMSO	94.5	Negative	Linscombe et al. (2001)
Aminopyralid TIPA	Forward mutation	*Hgprt* locus in CHO cells	250–4000 µg/ml (54.5–872 µg/ml aminopyralid equivalents) ± S9, in DMSO	21.8% aminopyralid equivalents	Negative	Schisler et al. (2004)
Aminopyralid	Chromosomal aberrations	Rat lymphocytes	32.3–2070 µg/ml ± S9, in DMSO	94.5	Negative +S9 Positive −S9 (see Table 15)	Linscombe et al. (2002)
Aminopyralid TIPA	Chromosomal aberrations	Rat lymphocytes	1000–4000 µg/ml (218–872 µg/ml aminopyralid equivalents) ± S9, in DMSO	21.8% aminopyralid equivalents	Negative	Linscombe et al. (2004)
In vivo						
Aminopyralid	Micronucleus formation	Male CD-1 mice	2 × 500–2000 mg/kg bw on two consecutive days	94.5	Negative	Spencer & Gorski (2002)
Aminopyralid TIPA	Micronucleus formation	Male CD-1 mice	2 × 500–2000 (109–436 aminopyralid equivalents) mg/kg bw on two consecutive days	21.8% aminopyralid equivalents	Negative	Spencer et al. (2004)

CHO, Chinese hamster ovary cells; S9, 9000 × *g* supernatant from rat livers; TIPA, triisopropylammonium

Table 15. Results of assays for chromosomal aberration with aminopyralid in rat lymphocytes

Concentration (μg/ml)	−S9		+S9	
	Effect (%)[a]	RMI (%)	Effect (%)[a]	RMI (%)
4-h treatment				
0	1.0	100	0.5	100
64.7	—	114.5	0.5	82.1
517.5	1.5	113.3	—	69.0
1035	1.0	92.8	0.5	72.6
2070	1.5	63.9	1.0	63.1
24-h treatment				
0	0.5	100	0.5	100
62.5	—	—	2.0	95.0
125	1.0	97.7	—	81.8
750	1.0	60.2	—	—
1000	4.5 [b]	50.4	1.0	81.0
1400	5.0 [b]	33.1	—	—
1700	7.5 [b]	27.8	—	—
2070	—	18.0	2.5	84.3
24-h treatment				
0	2.5	100	—	—
1000	4.5	47.8	—	—
1400	7.0 [b]	37.7	—	—
1700	7.5 [b]	29.0	—	—

From Linscombe et al. (2002)
RMI, relative mitotic index; S9, 9000 × *g* supernatant from rat livers.
[a] Cells with aberrations excluding gaps.
[b] Significantly different (alpha = 0.05) from negative controls.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rat

Groups of 30 male and 30 female CD rats were fed diets containing aminopyralid (purity, 94.5%; batch No. F-0031-143, TSN102319) and supplying a dose of 0, 50, 250 or 1000 mg/kg bw per day for approximately 10 weeks before breeding, and continuing during breeding (2 weeks), gestation (3 weeks) and lactation (3 weeks) for each of two generations to evaluate the potential for reproductive toxicity and effects on neonatal growth and survival. A comprehensive evaluation of male and female reproductive systems was conducted, including an evaluation of estrous cyclicity, gonadal function, mating performance, conception, gestation, parturition, lactation and weaning, as well as survival, growth and development of the offspring. In-life observations, body weights, feed consumption and litter data were recorded. In addition, a gross necropsy of the P_1 and P_2 adults was conducted with extensive histopathological examination of reproductive organs and other tissues. The study complied with GLP.

In P_1 and P_2 male and female rats at 250 and the 1000 mg/kg bw per day, the absolute and relative weights of full end empty caeca were increased; this effect did not attain statistical significance in females at 250 mg/kg bw per day. There were no histopathological correlates of these effects. This

change was considered to be a physiological adaptation and not of toxicological significance. There were no other treatment-related findings in either the parameters for general health or reproductive performance.

The NOAELs for general toxicity and for reproductive toxicity were both 1000 mg/kg bw per day, the highest dose tested (Marty et al., 2003).

(b) Developmental toxicity

Rat

Groups of 25 time-mated female CD rats received aminopyralid (purity, 94.5%; batch No. F-0031-143, TSN102319) at a dose of 0, 100, 300, or 1000 mg/kg bw per day as a suspension in 0.5% aqueous Methocel (A4M® methylcellulose) in a volume of 4 ml/kg, by oral gavage, starting on day 6 of gestation and continiuning until day 20. Clinical examinations were conducted throughout the study, and body weights and feed consumption were recorded regularly. On day 21, dams were terminated and the fetuses delivered by caesarian section. The weights of gravid uteri, the numbers of corpora lutea, implantation sites, viable fetuses and resorptions were recorded. Fetuses were sexed and body weights measured and they were subjected to external, visceral, craniofacial and skeletal examinations. The study complied with GLP.

In all the parameters examined in this study, no treatment-related findings were found. Under the conditions of this study, aminopyralid did not show evidence of any maternal or embryo/fetal toxicity or teratogenicity.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Carney & Tornesi, 2001).

Groups of 25 time-mated female CD rats received GF-871 (41.3% aminopyralid TIPA) at a dose of 0, 484, 1211 or 2421 mg/kg bw per day in a volume of 4 ml/kg, by oral gavage, starting on day 6 of gestation and continuing until day 19. This dosing regime provided aminopyralid at a dose of 0, 105, 263 or 525 mg/kg bw per day. Clinical examinations were conducted throughout the study, and body weights and feed consumption were recorded regularly. On day 21, dams were terminated and the fetuses delivered by caesarian section. The weights of gravid uteri, the numbers of corpora lutea, implantation sites, viable fetuses and resorptions were recorded. Fetuses were sexed and body weights measured and they were subjected to external, visceral, craniofacial and skeletal examinations. The study complied with GLP.

No treatment-related effects on dams or fetuses were observed even at the highest dose tested.

The NOAEL for GF-871 was 2421 mg/kg bw per day, or 525 mg/kg bw per day as aminopyralid equivalents, the highest dose tested (Zablotny & Thorsrud, 2004).

Rabbit

Groups of 26 time-mated female NZW rabbits received aminopyralid (purity, 94.5%; batch No. F-0031-143, TSN102319) at a dose of 0, 25, 100, or 250 mg/kg bw per day by oral gavage suspensions in 0.5% aqueous Methocel (A4M® methylcellulose) in a volume of 4 ml/kg, starting on day 7 of gestation and continuing until day 27 (phase I). Since there was no evidence of maternal toxicity at the highest dose, in a second part of this study with the same design, mated rabbits were given aminopyralid in suspension at a dose of 0, 500, or 750 mg/kg bw per day (phase II). On day 28, dams were terminated and the fetuses were delivered by caesarian section. The weights of gravid uteri, the numbers of corpora lutea, implantation sites, viable fetuses and resorptions were recorded. Fetuses were sexed and body weights measured and they were subjected to external, visceral, craniofacial and skeletal examinations. The study complied with GLP.

Mean body-weight gain was significantly reduced at 500 and 750 mg/kg bw per day (−11.8 g and −70.0 g vs 25 g in the rabbits in the control group) on days 7−10 of gestation. Thereafter, the reduced body-weight gain remained significant for rabbits at the highest dose only. Intermittently

in both groups, feed consumption was reduced. In the group at the highest dose, higher incidences of decreased amounts of faeces and of uncoordinated gait shortly after dosing were observed. In one rabbit, the observation was made on the first day of treatment (Table 16).Two animals showed the same effect on the second day of treatment. Uncoordinated gait was also observed in rabbits at 500 mg/kg bw per day, but it was much less pronounced and was first observed on the second day of treatment. In both groups, these effects were seen in 23 out of 26 animals and lasted for only 2 h at a time and were completely resolved thereafter. Also, the degree of severity of uncoordinated gait did not increase with time. Two rabbits at the highest dose were killed after being found to be in a moribund condition on day 17 of gestation; these rabbits showed uncoordinated gait, decreased activity, perineal urine soiling and decreased amounts of faeces. At gross necropsy, examination of these animals showed that both rabbits were pregnant and had pale kidneys, watery and dark caecal contents, and erosions/ulcers in the glandular mucosa of the stomach. The moribund condition of these rabbits was considered to be treatment-related. Another dam at 750 mg/kg bw per day and one dam at 500 mg/kg bw per day died during the treatment period, with signs indicative of complications arising from incorrect gavage dosing. At termination, one dam at 500 mg/kg bw per day had ulcers/erosions in the glandular mucosa of the stomach. Owing to the severity of the clinical signs, all rabbits at the highest dose were terminated before day 20 of gestation and were not available for the evaluation of reproductive and fetal parameters.

Weights of gravid uteri, the numbers of corpora lutea, implantation rates, numbers of viable fetuses and resorptions were not impaired by treatment at any dose. Additionally, no treatment-related changes in the incidences of malformations, numbers of malformed fetuses or numbers of affected litters were observed.

The NOAEL for maternal toxicity was 250 mg/kg bw per day and the NOAEL for developmental toxicity was 500 mg/kg bw per day, the highest dose evaluated (Marty et al., 2002).

Groups of 26 time-mated female NZW rabbits received GF-871 (41.3% aminopyralid TIPA; batch No. 173-162-1A, TSN104110) at a dose of 0, 484, 1211 or 2421 mg/kg bw per day in a volume of 4 ml/kg bw by oral gavage on days 7 to 27 of gestation. This regimen supplied aminopyralid at doses of 0, 105, 263 or 525 mg/kg bw per day.

On day 28 of gestation, dams were terminated and the fetuses delivered by caesarian section. The weights of gravid uteri, the numbers of corpora lutea, implantation sites, viable fetuses and resorptions were recorded. Fetuses were sexed and body weights measured and they were subjected to external, visceral, craniofacial and skeletal examinations. The study complied with GLP.

Three rabbits at the highest dose and one at the intermediate dose were killed between days 14 and 18 owing to severe body-weight loss and poor general condition. In all treated groups, a dose-related reduction in feed consumption was observed, resulting in a statistically significant reduction in body-weight gain only in rabbits at the highest dose. Of rabbits at the highest dose, 19 out of 26 showed uncoordinated gait on several days, usually starting and resolving within 1–2 h after dosing. Six rabbits in this group also had repetitive chewing behaviour. Two animals in the group at the intermediate dose and one at the lowest dose also showed one incident of uncoordinated gait. Uncoordinated gait was not apparent until after dosing for a number of days (6 days) (Table 16).

No organ-weight or pathological changes were observed in dams and reproductive parameters were not impaired in any treated group. In the group at the highest dose, fetal body weights were decreased for females (10.5%), males (7.4%), and both sexes combined (9.8%). The fetal weights were also reduced when compared with those in historical control groups. The incidence, the nature and the lack of a consistent pattern of fetal malformations, found in all groups, do not imply any relationship with treatment.

Therefore, the NOAEL for maternal toxicity with GF-871 was less than 484 mg/kg bw per day, or 105 mg/kg bw per day expressed as aminopyralid equivalents, on the basis of uncoordinated gait

in one rabbit in the group at the lowest dose. The NOAEL for developmental toxicity with GF-871 was 1211 mg/kg bw per day, or 263 mg/kg bw per day expressed as aminopyralid equivalents, on the basis of fetal body-weight reduction in the group at the highest dose (Carney & Tornesi, 2004a).

In a supplemental study, groups of 26 time-mated female NZW rabbits were given GF-871 (41.3% aminopyralid TIPA; batch No. 173-162-1A, TSN104110) at a dose of 0, 121 or 363 mg/kg bw per day in a volume of 4 ml/kg bw by oral gavage, from day 7 to day 27 of gestation. This study was designed to identify a NOAEL for maternal toxicity, which was 0, 26 or 78 mg/kg bw per day expressed as aminopyralid.

On day 28 of gestation, dams were terminated and the fetuses were delivered by caesarian section. In-life, body-weight changes, feed consumption and clinical observations were recorded. On necropsy, gross pathology in dams was performed and the pregnancy status was examined. The study complied with GLP.

The only findings were restricted to one rabbit at the lowest dose and three rabbits at the highest dose, which showed uncoordination at one time-point. In the rabbit at the lowest dose, the effect occurred at day 10 of gestation, equivalent to treatment day 4, but this was not compound-related, as the rabbit was suffering from a fatal dosing injury. In the rabbits at the highest dose, the effect occurred on days 14, 25 and 26, equivalent to treatment days 8, 19 and 20, respectively (Table 16). Concomitantly, two of the affected rabbits at the highest dose also showed repetitive chewing behaviour. No other investigated parameters in this or the group at 121 mg/kg bw showed any treatment-related changes.

The NOAEL for maternal toxicity with GF-871 was 121 mg/kg bw per day, or26 mg/kg bw per day expressed as aminopyralid equivalents (Carney & Tornesi, 2004b).

2.6 Special studies: neurotoxicity

Rat

In a study of acute neurotoxicity, 10 male and 10 female Fischer 344 rats were given aminopyralid (purity, 94.5%; batch No. TSN102319/F0031-143) as a single dose at 0, 500, 1000 or 2000 mg/kg bw as a suspension in aqueous 0.5% Methocel® (methylcellulose) by oral gavage in a volume of 10 ml/kg bw.

Daily cage-side observations were performed on all rats and clinical examinations on test days 2, 3 and 4. Before dosing and on days 8 and 15 after dosing, body weights were recorded and the rats were tested for motor activity and subjected to a functional observational battery (FOB). At study termination after 2 weeks, five males and females per dose were killed for evaluation of pathological and histopathological changes in central and peripheral nervous tissues. The remaining five males and females per dose were also killed and a standard set of tissues was preserved. The study complied with GLP.

Clinical signs were restricted to increased faecal soiling in males at the highest dose and urine soiling in females at the highest dose. These findings resolved within the study period and whether this soiling was indicative of a neurotoxic potential at the highest dose is not clear.

There were no other signs of neurotoxicity or systemic toxicity.

The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested, and the NOAEL for general toxicity was 1000 mg/kg bw (Marable et al., 2001).

In a 12-month dietary study of neurotoxicity, which was part of the 24-month study of toxicity and carcinogenicity, groups of 10 male and 10 female Fischer 344 rats were fed diets containing aminopyralid (purity, 94.5%; batch No. F0031-143, TSN102319) at concentrations adjusted to provide doses of 0, 5, 50, 500, or 1000 mg/kg bw per day. The study design and parameters investigated were the same as in the main 24-month study. Five males and females per group were pre-selected for neurotoxicity evaluation and five males and females per group to evaluate both toxicity and

neurotoxicity after 12 months. Before dosing and at 1, 3, 6, 9 and 12 months, a FOB and an evaluation of motor activity were performed for these rats. At termination, five males and females per group (and all rats in the control group and the group at the highest dose) were subjected to neuropathological evaluation. The study complied with GLP.

In males at the highest dose, a treatment-related increase in defaecation level was observed. This effect might be related to the caecal enlargement observed in the main study at 500 and 1000 mg/kg bw per day in both sexes and which was considered to represent physiological adaptation. There were no other treatment-related changes indicating a neurotoxic potential or systemic toxicity.

The NOAEL for neurotoxicity and for general toxicity was 1000 mg/kg bw per day, the highest dose tested (Maurissen et al., 2003).

Rabbit

In a study in three non-pregnant female NZW rabbits, aminopyralid (purity not stated) was administered at a dose of 500 mg/kg bw by oral gavage in 0.5% Methocel® for 22 days (phase I) and then at 1000 mg/kg bw for another 20 days (phase II). No control group was included in this study as it was intended to look for predefined uncoordination that would not occur in rabbits in the control group. Behavioural observations (open field and sensorimotor coordination) were performed daily throughout the study (rabbits were observed in open field for 15 min after dosing and then subsequently in their home cages at unscheduled times throughout the day), weekly body-weight changes were recorded and daily clinical cage-side observations were made. In each of the two phases of the study, clinical chemistry parameters were investigated and at the end of the study all rabbits were necropsied, with special focus on the gastrointestinal tract and the brain.

During phase I, neither behavioural nor obvious clinical chemistry changes were observed. In phase II, one rabbit showed clear signs of uncoordinated gait on several days starting typically in the first 2 h after dosing. The first observation was made on day 2 of treatment. A second rabbit had similar signs on day 19, while the third rabbit was free of behavioural signs for the whole study. The results of clinical chemistry were not notable. On necropsy, all rabbits showed adherent mucus in the linings of the stomach and the animal with the repeated behavioural signs was free of further histological changes. The other two rabbits had ascites in the abdominal cavity and one showed decreased adipose tissue (Marable & Day, 2004).

Two groups of 10 time-mated NZW rabbits were given GF-871 (41.3% aminopyralid TIPA; batch No. 173-162-IA/TSN104110) at a dose of 0 or 800 mg/kg bw (173.6 mg/kg bw expressed as aminopyralid equivalents) by gavage on day 7 of gestation until day 27 of gestation to evaluate the potential for neuropathological changes. Daily body weights, feed consumption and clinical observations (approximately 1 h after dosing) were recorded (including observations of incoordination) and on day 28 of gestation, an extensive investigation on neurological tissues was performed: brain—cerebrum, thalamus/hypothalamus, midbrain, pons, cerebellum, medulla; pituitary gland, trigeminal ganglia with nerve; spinal cord—cervical swelling (C3–C6), lumbar swelling (L1–L4); dorsal root ganglia (cervical and lumbar); dorsal and ventral roots (cervical and lumbar); peripheral nerves—proximal sciatic, proximal and distal tibial, sural; eyes with optic nerve, skeletal muscle (anterior tibial and gastrocnemius). The study complied with GLP.

One rabbit in the control group was killed after having aborted and one rabbit in the treatment group died accidentally during clinical examination. After an initial body-weight loss, all animals gained weight and at the end no statistically significant difference from the control group was evident.

Three rabbits in the treatment group showed slight to moderate/severe signs of uncoordinated gait at isolated, infrequent time-points, one of these rabbits died accidentally (see above). The first day of this observation was recorded on day 7 (slight), 9 (slight) or 14 (moderate/severe) (this was the animal that died) of gestation in these three rabbits, equivalent to treatment days 1, 3 and 9,

respectively (Table 16). Gross pathology and histopathology revealed no treatment-related changes in the central or peripheral nervous system. However, one treated rabbit showed acute degeneration of several muscle fibers. This finding was not accompanied by inflammation or kidney changes (indicative of chronic lesion and high release of myoglobin, respectively).

In summary, no neuropathological potential of GF-871 was evident from this study (Yano & Zablotny, 2005).

Table 16. Day on which first observation was recorded of uncoordination in pregnant rabbits treated with aminopyralid or aminopyralid TIPA[a]

Aminopyralid								Aminopyralid TIPA (GF-871)							
Marty et al. (2002)				Carney & Tornesi (2004b)				Carney & Tornesi (2004a)				Yano & Zablotny (2005)			
Dose as aminopyralid equivalents (mg/kg bw)															
500		750		26		78		105		263		525		173.6	
GD	TD	GD	TD	GD	TD	GD	TD	GD	TD	GD	TD	GD	TD	GD	TD
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	2	7	1	—	—	—	—	—	—	—	—	—	—	—	—
9	3	8	2	—	—	—	—	—	—	—	—	—	—	—	—
10	4	8	2	—	—	—	—	—	—	—	—	—	—	—	—
10	4	9	3	—	—	—	—	—	—	—	—	—	—	7	1
11	5	9	3	—	—	—	—	—	—	—	—	12	6	9	3
11	5	9	3	—	—	—	—	—	—	—	—	13	7	14	9
11	5	9	3	—	—	—	—	—	—	—	—	15	9		
12	6	10	4	—	—	—	—	—	—	—	—	18	12		
12	6	10	4	—	—	—	—	—	—	—	—	18	12		
13	7	10	4	—	—	—	—	—	—	—	—	19	13		
14	8	11	5	—	—	—	—	—	—	—	—	19	13		
15	9	13	7	—	—	—	—	—	—	—	—	20	14		
17	11	13	7	—	—	—	—	—	—	—	—	20	14		
17	11	14	8	—	—	—	—	—	—	—	—	20	14		
19	13	14	8	—	—	—	—	—	—	—	—	22	16		
20	14	14	8	—	—	—	—	—	—	—	—	23	17		
21	15	14	8	—	—	—	—	—	—	—	—	24	18		
22	16	15	9	—	—	—	—	—	—	—	—	24	18		
24	18	15	9	—	—	—	—	—	—	—	—	25	19		
25	19	15	9	—	—	—	—	—	—	—	—	25	19		
26	20	16	10	—	—	14	8	—	—	—	—	25	19		
27	21	18	12	—	—	25	19	—	—	16	10	26	20		
27	21	19	13	10[c]	4[c]	26	20	16	10	18	12	26	20		

GD, day of gestation; TD, treatment day; TIPA, triisopropylammonium.

[a] For each animal in the respective studies, the first day (day of gestation and treatment day) on which uncoordination was observed is given (day 7 of gestation is equivalent to day 1 of treatment)

[b] 26 animals were used in (Marty et al., 2002; Carney & Tornesi, 2004a; Carney & Tornesi, 2004b) and 10 animals in (Yano & Zablotny, 2005)

[c] Not treatment-related, fatal dosing injury.

The same five time-mated NZW rabbits received GF-871 via gavage according to the following dosing regimen: 500 mg/kg bw per day (equivalent to 108.5 mg/kg bw per day expressed as aminopyralid equivalents) from day 7 to day 15 of gestation; 1000 mg/kg bw per day (equivalent to 217.0 mg/kg bw per day expressed as aminopyralid equivalents) from day 16 to day 23 of gestation; or two doses at 1000 mg/kg bw given at an interval of 1 h on day 23 of gestation, in order to make a detailed evaluation of uncoordination in pregnant rabbits. Two additional rabbits served as controls. Rabbits were monitored daily for clinical signs of toxicity, body weight and feed consumption. In addition, expanded clinical observations were performed after dosing on day 23 of gestationby an independent neurotoxicology consultant (Dr J. Ross, Ross Toxicology Services, LLC).

For this study, no detailed results were available but only the following summary.

No clinical signs were noted in the treated rabbits during the first phase of dosing at 500 mg/kg bw per day. However, after increasing the dose to 1000 mg/kg bw per day on day 16 of gestation, uncoordination was noted at least once in all five rabbits, with these signs first appearing as early as day 18 and as late as day 23 of gestation. At the expanded clinical observations on day 23 of gestation, five out of five dosed rabbits exhibited abnormal behaviour, characterized by uncoordination. Hindlimbs and forelimbs were affected. Affected animals were easily displaced in the lateral direction by pushing with the hands. There were no consistent changes in muscle strength as evaluated by observing external ear position, head position and extensor thrust reflexes. There were no autonomic or somatic signs of convulsive activity, such as urination, defaecation, or paroxysmal movements, no signs of myoclonus or tremors. There were no apparent changes in level of consciousness as evaluated by observations of the rabbits' attention to external stimuli in the environment and no apparent change in vestibular function as evaluated by observing head position and spontaneous nystagmus. Optokinetic nystagmus was normal. The most severely affected rabbits had a slowed righting reaction, but this appeared to be secondary to uncoordination, and not due to either weakness or depression of the central nervous system. The primary neurological change was uncoordination of all four limbs (Carney, 2004).

3. Observations in humans

No information was available.

Comments

Biochemical aspects

In a study of the absorption, distribution, metabolism and excretion of radiolabelled aminopyralid administered by oral gavage, male rats were given single doses at 50 or 1000 mg/kg bw, or a single dose at 50 mg/kg bw after 14 days pretreatment with unlabelled aminopyralid at a dose of 50 mg/kg bw per day. Male rats also received radiolabelled aminopyralid TIPA salt as a single oral gavage dose at 96 mg/kg bw (equal to aminopyralid at 50 mg/kg bw). The pharmacokinetic behaviour of aminopyralid and its TIPA salt was very similar. Of the administered dose, 42–59% was absorbed and rapidly excreted in the urine, most within the first 24 h. Excretion was biphasic, with half-lives of 3.0–3.8 h and 10.2–12.3 h. Biliary excretion was assumed to be negligible. After a depletion period of 168 h, virtually all tissue samples showed concentrations of radiolabel of less than 0.01% of the administered dose. Aminopyralid was not metabolized in rats; more than 95% of the radiolabel was accounted for. On the basis of lack of coordination in gait after exposure to aminopyralid and aminopyralid TIPA in rabbits but not in rats, an extensive study of the absorption, distribution, metabolism and excretion of aminopyralid in rabbits was performed. A group of non-pregnant rabbits received radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw. A group of pregnant rabbits ("late-stage") received nonlabelled aminopyralid on days 7–21 of gestation and

then radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw on day 22 of gestation. An additional group of pregnant rabbits ("early-stage") received radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw on day 7 of gestation, without pretreatment with unlabelled aminopyralid. Using plasma from animals in these three groups and from a group of non-pregnant rats given radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw, a study of plasma-protein binding was performed. In late-stage pregnant rabbits pretreated with repeated doses of unlabelled aminopyralid, the absorption of aminopyralid (on the basis of lower Tmax, higher AUC and increased renal excretion) was somewhat more rapid and greater than in non-pregnant and early-stage pregnant rabbits. Plasma-protein binding was lower (43–58%) in late-stage pregnant rabbits pretreated with repeated doses of unlabelled aminopyralid than in non-pregnant and early-stage pregnant rabbits (47–68%). The difference in bioavailability (expressed as unbound compound) of aminopyralid was at most twofold. However, interpretation of these results remains ambiguous because of the different dosing regimens used (single dose without pretreatment in non-pregnant and early-stage pregnant rabbits and single dose after pretreatment in late-stage pregnant rabbits). On the basis of renal excretion of radiolabel, absorption of aminopyralid in rabbits was close to 80% or greater, being 20–40% higher than in rats.

Toxicological data

Aminopyralid and aminopyralid TIPA have low acute toxicity in rats when administered orally, dermally or by inhalation. The oral and the dermal LD_{50}s are both > 5000 mg/kg bw, and by inhalation, the LC_{50} is > 5.5 mg/l, the highest dose tested. Aminopyralid is clearly irritating to the eye, while aminopyralid TIPA is only slightly irritating. Aminopyralid is not a dermal irritant, while aminopyralid TIPA is a slightly irritant. In guinea-pigs, aminopyralid and aminopyralid TIPA produced no signs of skin-sensitizing potential, as tested by the Magnusson & Kligman method.

In short-term feeding studies in mice, rats and dogs and in a study of dermal exposure studying rats, animals received aminopyralid at doses of up to 1000 mg/kg bw per day. Body weight was reduced only in female dogs receiving aminopyralid at 967 mg/kg bw per day, the highest dose tested in a 1-year study. Males and females at this dose also showed a slight increase in relative liver weights accompanied by hepatocyte hypertrophy in two out of four animals per sex. In male and female rats at doses of 500 mg/kg bw per day and greater, reversibly increased absolute and relative weights of full and empty caeca were observed and slight mucosal hyperplasia of the caecum and the ileum was found in males at the highest dose. These changes were considered to be a consequence of physiological adaptation. Mucosal hyperplasia of the stomach was observed in all dogs at 967 mg/kg bw per day. Treatment-related clinical chemistry changes were restricted to the urine of rats at 500 mg/kg bw per day and greater, where decreased pH values and decreased concentrations of protein and ketone were found. Changes in the pH of the urine were most likely due to urinary excretion of the unchanged, acid parent compound. Generally, aminopyralid was well tolerated by mice, rats and dogs in short-term studies. The NOAEL for aminopyralid in mice was 1000 mg/kg bw per day (the highest dose tested), 1000 mg/kg bw per day in rats (the highest dose tested) and 93.2 mg/kg bw per day in dogs, on the basis of histopathological changes in the gastric mucosa at the highest dose tested. In a 13-week feeding study in rats with aminopyralid TIPA, the same effects on caecal weights and urine chemistry were observed as with aminopyralid. On the basis of the lack of other effects, the NOAEL for aminopyralid TIPA in rats was 2421 mg/kg bw per day as GF-871, equal to aminopyralid at 525 mg/kg bw per day, the highest dose tested.

In an 18-month study in mice and a 24-month feeding study in rats, diets adjusted to provide aminopyralid at maximal doses of 1000 mg/kg bw per day did not induce any increases in the incidence of neoplastic findings.

Mortality was increased in all groups of female mice receiving aminopyralid, but appeared to be compound-related only in animals at the highest dose. Animals that died showed an increased incidence of age-related nephropathy. Although the overall incidence of nephropathy was not increased in this or any treated group, exacerbation of the effects of age-related nephropathy in these animals may have been responsible for the increase in mortality. Other treatment-related signs of toxicity in this group e.g. pale kidneys, reduced body fat, haemolysed blood in the gatrointestinal tract, atelectasis of the lung and perineal soiling, were most likely a reflection of the moribund state of the animals. The NOAEL was 250 mg/kg bw per day on the basis of increased mortality in females at 1000 mg/kg bw per day. Although the final cumulative mortality in rats was comparable at all doses, the onset of mortality in males at 1000 mg/kg bw per day appeared earlier. As statistical significance and clear treatment-related causes for death were lacking, the Meeting did not consider this finding as being related to treatment.

Slightly reduced body-weight gain was observed in male rats at 500 mg/kg bw per day and above. Increased absolute and relative weights of full and empty caeca were observed in males and females at 500 mg/kg bw per day and above, accompanied by very slight mucosal hyperplasia of the caeca, which was statistically significant only in males at the highest dose. This finding is common to many substances and, due to the lack of further histological changes, was not considered to be an adverse finding. In males and females at 500 and 1000 mg/kg bw per day, increased urine volumes, decreased specific gravity and protein and ketone contents and reduced pH values were observed without any renal histopathological correlate. These changes were not considered to be toxicologically significant, the change in pH most likely reflecting urinary excretion of the acidic parent compound. The NOAEL in this feeding study in rats was 500 mg/kg bw per day on the basis of slight but statistically significant body-weight decreases in males at 1000 mg/kg bw per day.

Aminopyralid was not carcinogenic in mice or rats.

Aminopyralid and its TIPA salt were tested for genotoxicity in an adequate range of tests, including an assay for reverse mutation in *S. typhimurium* and *E. coli*, an assay for forward mutation in *Hgprt* with Chinese hamster ovary cells, an assay for chromosomal aberration in rat lymphocytes in vitro and an assay for micronucleus formation in mouse bone marrow in vivo. In the assay for chromosomal aberration in rat lymphocytes in vitro, an increased frequency of chromosomal aberration was seen only after 24 h of treatment at clearly cytotoxic concentrations of aminopyralid and in the absence of metabolic activation. The results of all other assays showed no evidence for genotoxicity.

The Meeting concluded that aminopyralid and aminopyralid TIPA are unlikely to be genotoxic.

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

In a two-generation study of reproduction in rats given diets containing aminopyralid, only increases in the weight of full and empty caeca in parental animals treated with aminopyralid were observed and no changes in reproductive parameters were observed. The caecal changes were not considered to be toxicologically significant. The NOAEL for general toxicity and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested. In pregnant rats and rabbits treated with aminopyralid by gavage, even the highest doses tested, 1000 and 500 mg/kg bw per day, respectively, did not induce any treatment-related malformations in the offspring. When rats and rabbits were treated with aminopyralid TIPA, no treatment-related malformations occurred, but fetal body-weight reduction was observed in only in rabbits at high doses (aminopyralid equivalents, 526 mg/kg bw per day).

In rabbits treated with aminopyralid at doses of 500 mg/kg bw per day and greater, body-weight gain was reduced, and uncoordinated gait was evident immediately after dosing, lasting for approximately 2 h. Neither the severity nor the duration of uncoordinated gait increased as the study progressed. Additionally, two rabbits treated with aminopyralid at the highest dose were killed in a

moribund condition on day 17 of gestation, being found to have pale kidneys, watery and dark caecal contents and erosions/ulcers in the glandular mucosa of the stomach. Owing to severe clinical signs observed at 750 mg/kg bw per day, all remaining animals in this study were killed at day 20 of gestation and were not available for evaluation of reproductive performance. Therefore, the NOAEL for maternal toxicity was 250 mg/kg bw per day. In pregnant rabbits treated with aminopyralid TIPA, maternal toxicity was evident as uncoordinated gait at a dose of 78 mg/kg bw per day as aminopyralid equivalents and greater. At higher doses, the clinical effects observed were similar to those seen with aminopyralid. The NOAEL for maternal toxicity was 26 mg/kg bw per day as aminopyralid equivalents.

In a study of acute toxicity and a 1-year study of neurotoxicity in rats treated with aminopyralid, no signs of behavioural changes and no histopathological findings suggesting neurotoxic potential were observed. Faecal and urine soiling at 2000 mg/kg bw in the study of acute toxicity were most likely due to general toxicity rather than indicative of specific neurotoxicity. The NOAELs for neurotoxicity in these two studies were 2000 and 1000 mg/kg bw per day as aminopyralid, respectively, the highest doses tested.

To obtain further insight into the possible mode of action leading to uncoordinated gait in rabbits, pregnant and non-pregnant animals were treated with aminopyralid and aminopyralid TIPA. Again, uncoordinated gait was observed but without any histopathological correlate in the central or peripheral nervous system. Additionally, no changes in consciousness, muscle strength or autonomic and somatic control were observed. For the acute dietary risk assessment, the occurrence of uncoordination after one or two doses in pregnant rabbits treated with aminopyralid may be the only relevant end-point. The NOAEL for aminopyralid for this effect was 250 mg/kg bw per day. In one study with aminopyralid TIPA, slight uncoordination was seen in one animal after 1 day of treatment at a dose of 173.6 mg/kg bw per day as aminopyralid equivalents. In another two studies of developmental toxicity in rabbits receiving aminopyralid TIPA, a dose-related increase in the incidence of uncoordination was found. However, at doses at which uncoordination was observed, i.e. 78, 105, 263 and 525 mg/kg bw per day as aminopyralid equivalents, the effect occurred only after at least six exposures. The NOAEL was 26 mg/kg bw per day as aminopyralid equivalents. The Meeting concluded that the weight of evidence indicated that the NOAEL for acute uncoordination was 250 mg/kg bw as aminopyralid. Effects observed in short-term studies in dogs were not considered relevant for establishing an acute reference dose.

The Meeting concluded that the existing data were adequate to characterize the potential hazard to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.9 mg/kg bw based on a NOAEL of 93.2 mg/kg bw per day identified on the basis of histological changes in the gastric mucosa at higher doses in a 1-year study in dogs, and a safety factor of 100.

The Meeting concluded that it was not necessary to establish an ARfD for aminopyralid. The only end-point that might be suitable as a basis for establishing an ARfD for aminopyralid was uncoordinated gait in the studies of developmental toxicity in rabbits. Although this effect was observed after repeated exposure at 78 mg/kg bw with a NOAEL of 26 mg/kg bw, it was observed after one or two exposures only at higher doses, with a NOAEL of 250 mg/kg bw. As this effect was dependent on C_{max} and in view of the kinetics of aminopyralid and the dynamics of this response, the Meeting considered it appropriate to adjust the safety factor. Applying a safety factor of 25 to the NOAEL of 250 mg/kg bw would result in a putative ARfD of 10 mg/kg bw which is greater than the JMPR-recommended cut-off value for an ARfD of 5 mg/kg bw.

Levels relevant to risk assessment

Species	Test material	Study	Effect	NOAEL[b]	LOAEL[b]
Mouse	Aminopyralid	Eighty-week study of toxicity and carcinogenicity[a]	Toxicity	250 mg/kg bw per day	1000 mg/kg bw per day
			Carcinogenicity	1000 mg/kg bw per day[c]	—
Rat	Aminopyralid	Two-year study of toxicity and carcinogenicity[a]	Toxicity	500 mg/kg bw per day	1000 mg/kg bw per day
			Carcinogenicity	1000 mg/kg bw per day[c]	—
	Aminopyralid	Two-generation study of reproductive toxicity[a]	Parental toxicity/	1000 mg/kg bw per day[c]	—
			Offspring toxicity	1000 mg/kg bw per day[c]	—
	Aminopyralid	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day[c]	—
			Embryo/ fetotoxicity	1000 mg/kg bw per day[c]	—
	Aminopyralid TIPA	Developmental toxicity[d]	Maternal toxicity	525 mg/kg bw per day[c]	—
			Embryo/ fetotoxicity	525 mg/kg bw per day[c]	—
	Aminopyralid	Long-term study of neurotoxicity	Neurotoxicity	1000 mg/kg bw per day[c]	—
Rabbit	Aminopyralid	Developmental toxicity[d]	Maternal toxicity	250 mg/kg bw per day	500 mg/kg bw per day
			Embryo/ fetotoxicity	500 mg/kg bw per day[c]	—
	Aminopyralid TIPA	Developmental toxicity[d]	Maternal toxicity	26 mg/kg bw per day	78 mg/kg bw per day
			Embryo/ fetotoxicity	263 mg/kg bw per day	526 mg/kg bw per day
Dog	Aminopyralid	One-year study of toxicity[a]	Toxicity	93.2 mg/kg bw per day	967 mg/kg bw per day

TIPA, triisopropylammonium.
[a] Dietary administration.
[b] Values for aminopyralid TIPA are given as aminopyralid equivalents.
[c] Highest dose tested.
[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.9 mg/kg bw

Estimate of acute reference dose

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 exposures

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 42–59%
Dermal absorption	No data
Distribution	Extensive
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid
Metabolism in animals	Minimal. No metabolites identified.
Toxicologically significant compounds in animals, plants and the environment	Aminopyralid
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.5 mg/l
Rabbit, dermal irritation	Aminopyralid is not an irritant, aminopyralid TIPA is a slight irritant.
Rabbit, ocular irritation	Aminopyralid is an irritant, aminopyralid TIPA is not an irritant.
Skin sensitization (test method used)	Not a sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Histopathological changes in stomach
Lowest relevant oral NOAEL	93.2 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day, the highest dose tested (4-week study in rats)
Lowest relevant inhalation NOAEC	No studies available
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increased mortality in female mice
Lowest relevant NOAEL	250 mg/kg bw per day (18-month study in mice)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects in rats
Lowest relevant reproductive NOAEL	1000 mg/kg bw per day, the highest dose tested
Developmental target/critical effect	No developmental effects in rats and rabbits with aminopyralid; fetal body-weight changes with aminopyralid TIPA.
Lowest relevant developmental NOAEL	263 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	
	No neurotoxic effects in rats with aminopyralid and aminopyralid TIPA; uncoordinated gait in pregnant rabbits, with both aminopyralid and aminopyralid TIPA.
Lowest relevant NOAEL	26 mg/kg bw per day (repeated doses), 250 mg/kg bw (single dose)
Medical data	
	No data available

Summary

	Value	**Study**	**Safety factor**
ADI	0–0.9 mg/kg bw	Dog, 1-year study	100
ARfD	Unnecessary	—	—

TIPA, triisopropylammonium

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