5. EVALUATION OF DATA FOR ACCEPTABLE DAILY INTAKE AND ACUTE DIETARY INTAKE FOR HUMANS, MAXIMUM RESIDUE LEVELS AND SUPERVISED TRIAL MEDIAN RESIDUE VALUES

5.1 AMINOPYRALID (220)

Toxicology

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 CCPR. All pivotal studies with aminopyralid and aminopyralid TIPA were certified as complying with GLP.

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 pre-treatment 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 non-labelled 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 pre-treatment 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 pre-treated with repeated doses of unlabelled aminopyralid, the absorption of aminopyralid (based on lower T_{max}, higher area under the curve for concentration–time [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 pre-treated 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 pre-treatment in non-pregnant and early-stage pregnant rabbits and single dose after pre-treatment in late-stage pregnant rabbits). Based on 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 LD50s are both > 5000 mg/kg bw, and by inhalation, the LC50 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. Based on 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 gastrointestinal 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 *Salmonella 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 foetal 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 unco-ordination 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 unco-ordination 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 unco-ordination was found. However, at doses at which unco-ordination 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 unco-ordination 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 foetuses, 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.

A toxicological monograph was prepared.

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; foetal 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

RESIDUE AND ANALYTICAL ASPECTS

Aminopyralid was evaluated for residues and toxicology by the 2006 JMPR and a discussion of residue aspects included in the report of the 2006 JMPR. As the toxicological evaluation was not completed in 2006 a dietary risk assessment could not be conducted and recommendations for maximum residue levels were not made. The current Meeting has completed the evaluation of the toxicological data and, based on the evaluation of the 2006 JMPR, recommendations are now made for maximum residue levels in various commodities.

Recommendations

On the basis of the data from supervised trials and farm animal feeding studies reported by the 2006 JMPR, the Meeting concluded that the residue levels as listed in Annex 1 are appropriate for establishing maximum residue limits and for IEDI assessment.

Definition of the residue for plants and animals (for compliance with MRLs and estimation of dietary intake): aminopyralid and its conjugates that can be hydrolysed, expressed as aminopyralid.

DIETARY RISK ASSESSMENT

Long-term intake

The evaluation of aminopyralid resulted in recommendations for MRLs and STMR values for raw and processed commodities. The International Estimated Daily Intakes (IEDI) of aminopyralid in the 13 GEMS/Food cluster diets, based on estimated STMRs were all < 1% of the maximum ADI of 0.9 mg/kg bw. The Meeting concluded that the long-term intake of residues of aminopyralid from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The 2007 JMPR decided that an ARfD is unnecessary. The Meeting therefore concluded that the short-term intake of aminopyralid residues is unlikely to present a public health concern.