5. EVALUATION OF DATA FOR ACCEPTABLE DAILY INTAKE AND ACUTE REFERENCE DOSE FOR HUMANS, MAXIMUM RESIDUE LEVELS AND SUPERVISED TRIALS MEDIAN RESIDUE VALUES

5.1 AMINOCYCLOPYRACHLOR (272)

TOXICOLOGY

Aminocyclopyrachlor is the International Organization for Standardization (ISO)–approved common name for 6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid (International Union of Pure and Applied Chemistry [IUPAC]), with Chemical Abstracts Service (CAS) number 58956-08-8. Aminocyclopyrachlor belongs to the pyrimidine carboxylic acid chemical family and is an auxin-mimicking herbicide used for selective control of weeds, invasive species and brush in pasture. The methyl ester (CAS No. 858954-83-3) is also used as a herbicide, and information on this compound was also assessed.

Aminocyclopyrachlor has not previously been evaluated by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with good laboratory practice (GLP).

Biochemical aspects

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

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

Toxicological data

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

The oral and dermal median lethal dose (LD_{50}) values in rats for both aminocyclopyrachlor and aminocyclopyrachlor-methyl were greater than 5000 mg/kg bw. In rats, the median lethal concentration (LC_{50}) for aminocyclopyrachlor was greater than 5.4 mg/L. Aminocyclopyrachlor and

aminocyclopyrachlor-methyl were not skin or eye irritants in rabbits, nor were they skin sensitizers in mice (local lymph node assay).

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

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

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

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

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

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

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

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

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

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

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

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

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

The Meeting concluded that aminocyclopyrachlor is unlikely to be genotoxic.

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

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

In a two-generation reproductive toxicity study in rats, which tested dietary aminocyclopyrachlor concentrations of 0, 500, 1500, 5000 and 17 000 ppm (equal to 0, 30, 92, 299 and 1048 mg/kg bw per day for males and 0, 36, 110, 367 and 1243 mg/kg bw per day for females, respectively), the NOAEL for reproductive toxicity was 17 000 ppm (equal to 1048 mg/kg bw per day), the highest dietary concentration tested. The NOAEL for parental toxicity was 5000 ppm (equal to 299 mg/kg bw per day), based on reduced body weight, body weight gain and feed conversion efficiency in males at 17 000 ppm (equal to 1048 mg/kg bw per day). The NOAEL for offspring toxicity was 5000 ppm (equal to 299 mg/kg bw per day), based on reduced pup weight at 17 000 ppm (equal to 1048 mg/kg bw per day).

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

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

The Meeting concluded that aminocyclopyrachlor is not teratogenic.

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

The Meeting concluded that aminocyclopyrachlor is not neurotoxic.

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

The Meeting concluded that aminocyclopyrachlor is not immunotoxic.

Toxicological data on metabolites and/or degradates

The Meeting noted the formation of a photolytic degradate of aminocyclopyrachlor, cyclopropane carboxylic acid (CPCA) (CAS No. 1759-53-1), which was not detected in rat metabolism studies. Low concentrations of this compound were detected as a possible extraction artefact in a grass metabolism study. Dietary exposure to CPCA from rotational crops and animal products is unlikely, as CPCA was not detected in aerobic or anaerobic soil metabolism studies, nor was it detected in a goat metabolism study.

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

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

Human data

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

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

Toxicological evaluation

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

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

A toxicological monograph was prepared.

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

Species	Study	Effect	NOAEL	LOAEL
Aminocyclopyrachlor				
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	7 000 ppm, equal to 876 mg/kg bw per day[b]	–
		Carcinogenicity	7 000 ppm, equal to 876 mg/kg bw per day[b]	–
Rat	Ninety-day study of toxicity[a]	Toxicity	6 000 ppm, equal to 349 mg/kg bw per day	18 000 ppm, equal to 1 045 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	6 000 ppm, equal to 279 mg/kg bw per day	18 000, equal to 892 mg/kg bw per day
		Carcinogenicity	18 000 ppm, equal to 892 mg/kg bw per day[b]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	17 000 ppm, equal to 1 048 mg/kg bw per day[b]	–
		Parental toxicity	5 000 ppm, equal to 299 mg/kg bw per day	17 000 ppm, equal to 1 048 mg/kg bw per day
		Offspring toxicity	5 000 ppm, equal to 299 mg/kg bw per day	17 000 ppm, equal to 1 048 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	1 000 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	500 mg/kg bw per day	1 000 mg/kg bw per day
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Dog	Ninety-day and 1-year studies of toxicity[a,d]	Toxicity	30 000 ppm, equal to 1 073 mg/kg bw per day[b]	–
Aminocyclopyrachlor-methyl				
Rat	Ninety-day study of toxicity[a,e]	Toxicity	6 000 ppm, equal to 326 mg ae/kg bw per day	18 000 ppm, equal to 961 mg ae/kg bw per day
	One-generation study of reproductive toxicity[a,e]	Reproductive toxicity	17 000 ppm, equal to 994 mg ae/kg bw per day[b]	–
		Parental toxicity	5 000 ppm, equal to 285 mg ae/kg bw per day	17 000 ppm, equal to 994 mg ae/kg bw per day
		Offspring toxicity	5 000 ppm, equal to 285 mg ae/kg bw per day	17 000 ppm, equal to 994 mg ae/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two studies combined.
[e] Conducted on aminocyclopyrachlor-methyl; doses expressed as aminocyclopyrachlor acid equivalents (ae).

Estimate of acceptable daily intake (ADI)

0–3 mg/kg bw

Estimate of acute reference dose (ARfD)

Unnecessary

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; 37–57% aminocyclopyrachlor; 87% aminocyclopyrachlor-methyl
Distribution	Rapid tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Limited; hydrolysis of aminocyclopyrachlor-methyl to aminocyclopyrachlor
Toxicologically significant compounds in animals and plants	Aminocyclopyrachlor
Acute toxicity	
Rat, LD_{50}, oral	> 5 000 mg/kg bw
Rat, LD_{50}, dermal	> 5 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight, body weight gain and feed consumption
Lowest relevant oral NOAEL	349 mg/kg bw per day (rat) (aminocyclopyrachlor)
	326 mg ae/kg bw per day (rat) (aminocyclopyrachlor-methyl)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight, body weight gain, feed consumption and feed conversion efficiency
Lowest relevant NOAEL	279 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	
Target/critical effect	No evidence of reproductive toxicity (rat)
Lowest relevant parental NOAEL	299 mg/kg bw per day (two-generation study with aminocyclopyrachlor)

Lowest relevant offspring NOAEL	299 mg/kg bw per day (two-generation study with aminocyclopyrachlor)
Lowest relevant reproductive NOAEL	1 048 mg/kg bw per day, highest dose tested (two-generation study with aminocyclopyrachlor)
Developmental toxicity	
Target/critical effect	No evidence of developmental toxicity (rat and rabbit)
Lowest relevant maternal NOAEL	500 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	1 000 mg/kg bw per day, highest dose tested (rat and rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw per day, highest dose tested (rat)
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity NOAEL	1 056 mg/kg bw per day, highest dose tested (mouse)
Medical data	
	No data

Summary

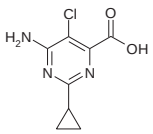
	Value	Study	Safety factor
ADI	0–3 mg/kg bw	Two-year study of toxicity (rat)	100
ARfD	Unnecessary	—	—

RESIDUE AND ANALYTICAL ASPECTS

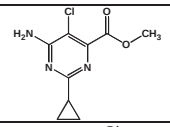
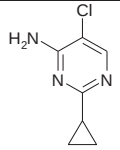
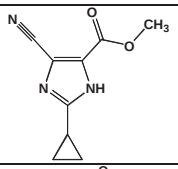
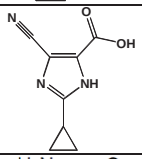
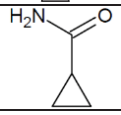
Aminocyclopyrachlor is a new pyrimidine carboxylic acid herbicide used for the control of broadleaf weeds and woody vegetation. Aminocyclopyrachlor mimics the naturally occurring phytohormone indole acetic acid (auxin) disrupting plant growth. At the Forty-fifth Session of the CCPR (2013), it was scheduled for evaluation as a new compound by the 2014 JMPR.

The Meeting received information on the metabolism of aminocyclopyrachlor and also its methyl ester (aminocyclopyrachlor-methyl, DPX-KJM44) in lactating goats and grass, methods of residue analysis, freezer storage stability, GAP information, supervised residue trials, and a cattle feeding study.

Aminocyclopyrachlor is 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid (IUPAC).



Metabolites referred to in the appraisal were addressed by their codes:

DPX-KJM44	(methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid)	
IN-LXT69	(5-chloro-2-cyclopropyl-pyrimidin-4-ylamine)	
IN-QGC48	(5-cyano-2-cyclopropyl-3H-imidazole-4-carboxylic acid methyl ester)	
IN-QFH57	(5-cyano-2-cyclopropyl-3H-imidazole-4-carboxylic acid)	
IN-Q3007	(cyclopropane carboxamide)	

The methyl ester of aminocyclopyrachlor (DPX-KJM44) was initially explored as a potential herbicide before finally settling on aminocyclopyrachlor (free acid) as the compound to be commercialised. As DPX-KJM44 is rapidly converted to aminocyclopyrachlor in both animals and plants, studies on DPX-KJM44 were submitted to the Meeting to support the evaluation of aminocyclopyrachlor.

Animal metabolism

The current meeting evaluated laboratory animal (rat) metabolism studies of orally administered aminocyclopyrachlor-methyl (DPX-KJM44) and reported that the ester is rapidly converted to the free acid (aminocyclopyrachlor). Therefore, studies with DPX-KJM44 also serve to demonstrate the metabolism of aminocyclopyrachlor.

A lactating goat was orally dosed twice daily for five consecutive days with [pyrimidine-2-^{14}C]DPX-KJM44 at a dose equivalent to 97 ppm in the feed. Approximately 85% of the administered dose was recovered in the excreta (20% faeces, 54% urine) or gastrointestinal tract (8%). The radioactivity in the tissues ranged from 0.01 mg DPX-KJM44 equivalents/kg in fat to 1.67 mg DPX-KJM44 equivalents/kg in kidney. TRR values in milk reached 0.031 mg DPX-KJM44 equivalents/kg after five days of dosing.

With the exception of one fat sample, greater than 80% of the TRR was extracted with the solvent systems used (acetone for milk and acetonitrile/water for tissues).

No intact DPX-KJM44 was detected in tissues or milk. The major component of the ^{14}C residues were aminocyclopyrachlor (kidney 55% TRR, liver 66% TRR, muscle 43% TRR, fat 47–84% TRR, milk 16% TRR). A number of minor metabolites were unable to be identified but were generally only present at low levels (< 0.01 mg DPX-KJM44 equivalents/kg).

In summary, the metabolism of DPX-KJM44 and aminocyclopyrachlor in goats is similar to metabolism in laboratory animals in the respect that the ester is de-esterified with little further breakdown of the acid (aminocyclopyrachlor).

Plant metabolism

A study on the metabolic fate of DPX-KJM44 in grass was made available to the meeting and a number of studies were located in the literature where aminocyclopyrachlor or DPX-KJM44 were sprayed onto grass and also a variety of weed species. While the literature studies were not conducted according to the protocols developed for submission of data to regulatory authorities, they were used to contribute to the weight of evidence regarding metabolism of aminocyclopyrachlor in plants. As with animal systems, following treatment with DPX-KJM44, rapid degradation occurs with the formation of aminocyclopyrachlor, thus studies with DPX-KJM44 also provide evidence on the metabolism of aminocyclopyrachlor in plants.

Grass

The metabolic fate of [pyrimidine-2-^{14}C]DPX-KJM44 in mixed grass (30 cm high) was examined following a single foliar application at 373 g ai/ha. Absorption following spraying was rapid with only 13% of the TRR in leaves recovered in surface washes from samples collected on the day of application. DPX-KJM44 was rapidly degraded, representing 25% TRR on the day of application, 14% TRR after three days and less than 9% TRR thereafter. Aminocyclopyrachlor was the major component of the ^{14}C residue comprising 64% TRR at day 0, declining to 33% TRR at 60 days after application. Minor metabolites were IN-LXT69 (4–6% TRR), IN-QGC48 (0–4% TRR), IN-QFH57 (0–2%TRR) and IN-Q3007 (0–1% TRR). Combined, the minor metabolites accounted for no more than 6.1% TRR in individual grass samples.

In a study of [^{14}C]-aminocyclopyrachlor metabolism in tall fescue grass sprayed once at 79 g ai/ha with aminocyclopyrachlor with the addition of 0.25% non-ionic surfactant, absorption from a single leaf treated with [^{14}C]-aminocyclopyrachlor was rapid with only 37% TRR recovered in surface washes 1 day after application. At eight days after application, aminocyclopyrachlor was the only compound detected in solvent extracts of plant material.

The metabolism of DPX-KJM44 and aminocyclopyrachlor was also studied in a range of weeds (black nightshade, Canada thistle, field bindweed, large crabgrass, prickly lettuce, rush skeleton weed, yellow star thistle). In those cases where DPX-KJM44 was applied, there was rapid hydrolysis to form aminocyclopyrachlor which was translocated in the plant. There was no further transformation of aminocyclopyrachlor within the three to eight day duration of the studies.

The metabolism of aminocyclopyrachlor by plants is well understood. Following application to grass (and weeds) the major residue component consists of parent aminocyclopyrachlor.

Environmental fate

The Meeting received information on the soil aerobic metabolism, soil photolysis, aqueous hydrolysis and aqueous photolysis properties of [^{14}C]-aminocyclopyrachlor and [^{14}C]-DPX-KJM44. Studies were also received on the behaviour of [^{14}C]- DPX-KJM44 in a rotational crop situation.

In soil incubation studies under aerobic conditions in the dark at 20 °C, ^{14}C-DPX-KJM44 degraded to form aminocyclopyrachlor with a DT_{50} of 0.1 days. Subsequently aminocyclopyrachlor degraded with a DT_{50} of 275 days. In studies following the aerobic degradation of ^{14}C-aminocyclopyrachlor applied to soil, the DT_{50} for degradation ranged from 120–433 days the sandy loam, clay loam and silty clay soils studied. IN-LXT69 accounted for 4.0–6.4% of the applied radioactivity (AR) at day 0 declining to 0.2–0.4% AR by day 120 of the study. Further analysis of the unextracted portion of ^{14}C demonstrated incorporation into humin (13–20% AR), fulvic acid (11% AR) and humic acid (0.3% AR) fractions present in the soil.

In four field dissipation studies where DPX-KJM44 was applied to bare soil at two sites and to grass plots at two sites, the soil DT_{50} values were 0.4–1.6 days for DPX-KJM44 and 55 to 163 days for aminocyclopyrachlor. The DT_{50} values for grass foliage were 0.4 days for DPX-KJM44 and 4.8–8.9 days for aminocyclopyrachlor.

In a soil photolysis study with application of ^{14}C-aminocyclopyrachlor on the surface of a silt loam soil the estimated DT_{50} was 61 days suggesting photolysis will contribute to soil degradation.

Aminocyclopyrachlor was stable to hydrolysis in aqueous solutions at pH 4, 7 and 9 suggesting hydrolysis plays a negligible role in its degradation. A study on the aqueous photolysis of aminocyclopyrachlor showed it is degraded on irradiation. Aminocyclopyrachlor accounted for 28% AR after 360 hours continuous irradiation. Photodegradates formed at levels above 5% AR were IN-QFH57 (14% AR), IN-LXT69 (16% AR), IN-YY905 (8% AR), IN-Q3007 (7% AR) and IN-V0977 (12% AR).

In a confined rotational crop study with cabbage, turnip and maize, a plot of sandy loam soil was treated with [^{14}C]- DPX-KJM44 at the equivalent of 75 g ai/ha with some plots treated at 369 g ai/ha and crops sown 30, 60, 120 and 300 days after soil application for cabbage and turnip and 15, 120 and 300 days after application for maize. TRR in cabbage ranged from < LOD to 0.023 mg eq./kg with 60 to 83% of the ^{14}C accounted for by aminocyclopyrachlor. For turnips, negligible ^{14}C residues were detected in roots (maximum 0.004 mg eq./kg) while ^{14}C residues in tops ranged from 0.003 to 0.011 mg eq./kg. In the two samples with sufficient residues for identification, the major components of the ^{14}C residue in tops were aminocyclopyrachlor (41–59% TRR) and DPX-KJM44 (0–17% TRR). Radioactive residues in maize ranged from 0.011 to 0.246 mg equiv/kg for forage, 0.023–0.262 mg eq./kg for stover and 0.012–0.085 mg eq./kg for maize grain. In all cases aminocyclopyrachlor was the major component of the ^{14}C residue (46–71% TRR) with DPX-KJM44 present at ≤ 10% TRR together with small amounts of IN-LXT69 (< 2%TRR). Residues of aminocyclopyrachlor present in soil are able to be taken up the rotational crops.

In summary, aminocyclopyrachlor residues in soil may contribute to residues observed in rotational crops. A field crop rotation study is desirable.

Methods of Analysis

The Meeting received description and validation data for analytical methods suitable for residue analysis of DPX-KJM4, aminocyclopyrachlor and related metabolites IN-LXT69, IN-QFH57 and IN-QGC48 in grass and DPX-KJM4, aminocyclopyrachlor and IN-LXT69 in animal commodities.

Grass samples are homogenised with 0.15 M ammonium acetate (aq) and acetonitrile, extracted with acetonitrile/0.15 M ammonium acetate (aq) 70/30 and the extracts acidified with dilute HCL. Extracts are cleaned up using SPE cartridges before analysis by LC-MS/MS. The LOQs for all analytes are 0.01 mg/kg.

Animal commodities are analysed using a different procedure. Milk samples are extracted using acetonitrile/0.1% aqueous formic acid (90:10, v/v) with analysis by LC-MS/MS. Tissue samples are homogenised and extracted with acetonitrile/0.1% aqueous formic acid with analysis by LC-MS/MS. In the case of muscle the samples are analysed following a clean-up step using solid phase extraction. The LOQs for all analytes are 0.01 mg/kg.

QuEChERS and the US FDA pesticide multiresidue methods are not suitable for analysis of aminocyclopyrachlor.

In conclusion, suitably validated methods are available for the analysis of aminocyclopyrachlor and selected metabolites in animal and plant matrices although currently there are no multi-residue methods available for aminocyclopyrachlor.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of DPX-KJM-44, aminocyclopyrachlor, IN-LXT69, IN-QFH57 and IN-QGC48 in grass and hay stored frozen. The compounds were all stable in grass and hay for the duration of the stability studies; 500 days for DPX-KJM-44, aminocyclopyrachlor, IN-LXT69 and 400 days for IN-QFH57 and IN-QGC48.

In animal matrices fortified separately with DPX-KJM44, aminocyclopyrachlor and IN-LXT69, residues were stable in milk, muscle, fat and hens eggs for at least 133 days. Aminocyclopyrachlor and IN LXT69 were stable in liver and kidney for at least 147 and 88 days, respectively. DPX-KJM44 was not stable in liver and kidney, being converted to aminocyclopyrachlor either during storage or subsequent analysis.

The periods of demonstrated stability cover the frozen storage intervals used in the residue studies.

Definition of the residue

Livestock may be exposed to residues present in feeds. In a lactating goat metabolism study with DPX-KJM44, the ester was rapidly converted to aminocyclopyrachlor which was the major component of the residue in all tissues and milk (kidney 55% TRR, liver 66% TRR, muscle 43% TRR, fat 47–84% TRR, milk 16% TRR) with no individual metabolite of aminocyclopyrachlor was identified as present at levels above 0.01 mg/kg.

Residues of aminocyclopyrachlor were higher in muscle than fat in the metabolism study while in the livestock feeding study they were much higher in fat compared to muscle (2.4 to 9.0×). Levels of aminocyclopyrachlor were higher in skim milk compared to cream. The log K_{ow} for aminocyclopyrachlor is -2.48 (pH 7) suggesting the compound is not fat soluble. Taken as a whole, the Meeting considered that residues of aminocyclopyrachlor are not fat soluble.

Following foliar application of aminocyclopyrachlor (and also DPX-KJM44) to grass, the major component of the residue is aminocyclopyrachlor (33–68% TRR). All components formed from aminocyclopyrachlor were minor (<6.1% TRR).

Based on the above the Meeting decided the residue definition for compliance with MRLs and estimation of dietary intake should be as follows:

Definition of the residue for compliance with MRL and estimation of dietary intake (for animal and plant commodities): *aminocyclopyrachlor.*

The residue is not fat soluble.

Results of supervised residue trials on crops

Grass

The Meeting received supervised residue trial data for aminocyclopyrachlor on grass (including hay). GAP in the Canada is one application at up to 264 g ai/ha with a PHI of 0 days. In trials approximating critical GAP in Canada residues in grass forage were (n=6): 13, 18, 18, 22, 25, 39 mg/kg (on an as received basis) and 58, 60, 72, 77, 79 and 81 mg/kg when corrected for reported moisture contents.

The Meeting estimated a highest and median residue of 81 and 74.5 mg/kg for grass (dry weight basis).

Residues in grass hay from field trials performed in Canada and the USA approximating GAP in Canada were (n=6): 30, 35, 40, 46, 46, 48 mg/kg (on an as received basis) and 40, 45, 46, 49, 60 and 60 mg/kg when corrected for reported moisture contents.

The Meeting estimated a maximum residue limit and median and high residues of 150, 47.5 and 60 mg/kg for grass hay.

Rotational crop residues

Soil residues of aminocyclopyrachlor are moderately persistent. The use-pattern (Canadian GAP) does not specify plant-back intervals for follow-crops. In the confined rotational crop study, where DPX-KJM44 was applied to soil at the equivalent of 70 to 346 g aminocyclopyrachlor/ha, residues of aminocyclopyrachlor in cabbage and turnip from crops planted 300 days after application to soil were below practical LOQs of 0.01 mg/kg. Residues of aminocyclopyrachlor were above 0.01 mg/kg in maize commodities (forage, stover, grain). The Meeting considered the available information on residues in rotational crops to be inadequate for the purposes of estimating maximum residue levels and STMR values to cover potential residues in such crops. The Meeting also noted that the livestock dietary burden is dominated by residues in grass and hay (100% of the diet for Australia) and that any contribution from potential residues in feeds in follow crops is minor. To enable completion of the risk assessment, the Meeting noted that residues in follow crops are unlikely to average more than 0.01 mg/kg and agreed that a value of 0.01 mg/kg could be used in estimates of consumer exposure for commodities from non-permanent crops.

Residues in animal commodities

Farm animal feeding studies

The Meeting received information on the residue levels in tissues and milk of dairy cows dosed with DPX-KJM44 at the equivalent of 73, 160, 454 and 1594 ppm in the feed for 28 consecutive days.

Residues of DPX-KJM44 and IN-LXT69 in tissues and milk and aminocyclopyrachlor in muscle were < 0.01 mg/kg at all sampling intervals and doses.

Aminocyclopyrachlor residues in milk were < 0.01 mg/kg for the 73 ppm dose group and most of the samples for the 160 ppm dose group. The maximum daily mean residues were 0.024 mg/kg for the 454 ppm dose group and 0.077 mg/kg for the 1594 ppm dose group.

In kidney mean aminocyclopyrachlor residues were 0.12, 0.31, 0.34 and 0.98 mg/kg for the 73, 160, 454 and 1594 ppm dose groups respectively. Mean residues liver residues were 0.039, 0.042, 0.049 and 0.096 mg/kg and mean fat residues were < 0.01, 0.015, 0.062 and 0.46 mg/kg for the 73, 160, 454 and 1594 ppm dose groups respectively.

Animal commodity maximum residue levels

Dietary burden calculations for beef cattle and dairy cattle and poultry are provided below. The dietary burdens were estimated using the OECD diets listed in Appendix IX of the 2009 edition of the FAO Manual.

Potential cattle feed items include: grass and grass hay.

Summary of livestock dietary burden (ppm of dry matter diet)

	US-Canada		EU		Australia		Japan	
	max	mean	Max	mean	Max	Mean	Max	Mean
Beef cattle	9	7.2	41.5	37.2	81	74.5	21.2	20.4
Dairy cattle	36.4	33.5	48.6	44.7	81[a]	74.5[b]	44.3	35.6
Broilers	0	0	0	0	0	0	0	0
Layers	0	0	8.1[c]	7.4[d]	0	0	0	0

[a] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat and milk
[b] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat and milk
[c] Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs
[d] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs

Animal commodity maximum residue levels

A lactating dairy cow feeding study was made available to the Meeting. A review of the laboratory animal and lactating goat metabolism studies showed that DPX-KJM44 is rapidly converted to aminocyclopyrachlor and significant differences are not expected in residues arising from dosing with DPX-KJM44 or aminocyclopyrachlor. The meeting decided the DPX-KJM44 feeding study could be used to estimate aminocyclopyrachlor residues in meat, edible offal and milk and agreed that in estimating residues levels, the feed levels should be expressed in terms of aminocyclopyrachlor acid equivalents.

The calculations used to estimate highest total residues for use in estimating maximum residue levels, STMR and HR values are shown below.

	Feed level (ppm) for milk residues	Residues (mg/kg) in milk	Feed level (ppm) for tissue residues	Residues (mg/kg) in			
				Muscle	Liver	Kidney	Fat
MRL beef or dairy cattle							
Feeding study [a]	68.5 (73)[c]	< 0.01	68.5 (73)[c]	< 0.01	0.064	0.17	0.015
	150 (160)[c]	0.012	150 (160)[c]	< 0.01	0.082	0.4	0.04
Dietary burden and high residue	81	0.01	81	< 0.01	0.067	0.21	0.019
STMR beef or dairy cattle							
Feeding study [b]	68.5 (73)[c]	< 0.01	68.5 (73)[c]	< 0.01	0.039	0.12	0.01
	150 (160)[c]	< 0.01	150 (160)[c]	< 0.01	0.042	0.31	0.015
Dietary burden and residue estimate	74.5	< 0.01	74.5	< 0.01	0.039	0.13	0.01

[a] highest residues for tissues and mean residues for milk

[b] mean residues for tissues and mean residues for milk

[c] feeding level expressed as acid equivalents = aminocyclopyrachlor, figure in brackets is feed level expressed in terms of DPX-KJM44

The Meeting estimated the following STMR values: milk 0.01 mg/kg; muscle 0.01 mg/kg; 0.039 mg/kg for liver and 0.13 mg/kg for kidney and fat 0.01 mg/kg.

The Meeting estimated the following maximum residue levels: milk 0.02 mg/kg; meat (mammalian except marine mammals) 0.01 mg/kg, fat 0.03 mg/kg and edible offal 0.3 mg/kg.

No information on residues of aminocyclopyrachlor in poultry were available to the meeting, therefore no maximum residue levels can be estimated for poultry commodities. However, Europe was the only region for which the poultry dietary burden was greater than zero. As aminocyclopyrachlor is not approved for use in Europe, the meeting considered there is no likelihood of residues in poultry commodities.

RECOMMENDATIONS

On the basis of the data obtained from supervised residue trials the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Definition of the residue (for compliance with MRL and estimation of dietary intake) for animal and plant commodities: *Aminocyclopyrachlor*

The residue is not fat soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The WHO Panel of the 2014 JMPR established an Acceptable Daily Intake (ADI) of 0-3 mg/kg bw for aminocyclopyrachlor.

The evaluation of aminocyclopyrachlor resulted in recommendations for MRLs and STMR values for raw and processed commodities. Where data on consumption were available for the listed food commodities, dietary intakes were calculated for the 17 GEMS/Food Consumption Cluster Diets.

The IEDIs in the seventeen Cluster Diets, based on the estimated STMRs were 0% of the maximum ADI (3 mg/kg bw). The Meeting concluded that the long-term intake of residues of aminocyclopyrachlor from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

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