AMINOCYCLOPYRACHLOR (272)

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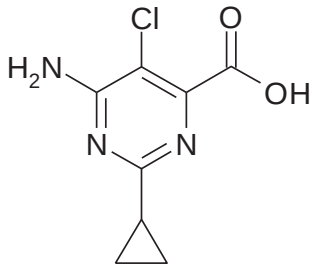
EXPLANATION

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 45th Session of the CCPR (2013), it was scheduled for the evaluation as a new compound by 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.

IDENTITY

Common name	Aminocyclopyrachlor
Chemical name	
IUPAC:	6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
CAS:	6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
Manufacturer's code numbers:	DPX-MAT28
CAS number:	858956-08-8
CIPAC Code:	
Molecular formula:	$C_8H_8ClN_3O_2$
Molecular mass:	213.62 g/mole
Structural formula:	

Formulations	Active ingredient content
SG	50% aminocyclopyrachlor
WG	39.5% aminocyclopyrachlor, 12.6% metsulfuron methyl
WG	35.3% aminocyclopyrachlor, 17.6% metsulfuron methyl

PHYSICAL AND CHEMICAL PROPERTIES

Specifications

Specifications for aminocyclopyrachlor have not been developed by FAO.

Physical and chemical properties (pure aminocyclopyrachlor 99.7%)

Property	Results (method)	Reference
Appearance	Pure aminocyclopyrachlor is a white solid with a mild fruity odour	Shalini 2007 23196
Melting point	140.5 ± 0.1 °C	Shalini 2007 23196

Property	Results (method)	Reference
Relative density	1.4732 ± 0.0777	Shalini 2007 23196
pH	3.34 ± 0.01 for a 1% aqueous suspension	Moorthy 2011 22543 rev 1
Vapour pressure	6.9215×10^{-3} kPa (5.192×10^{-8} mm Hg) at 20 °C	Moorthy 2007a 22537
Henry's Law constant ($Pa/m^3/mol$ at 20 °C)	5.24×10^{-7} (Milli-RO-water) 4.71×10^{-7} at pH 4 3.51×10^{-7} at pH 7 3.82×10^{-7} at pH 9	Hirata 2008 22545
Solubility in water at 20 °C	2.81 ± 0.1 g/L Milli-Q® water3.13 ± 0.26 g/L pH 4 4.20 ±0.14 g/L pH 7 3.87 ±0.17 g/L pH 9	Moorthy 2007b 22541
Solubility in organic solvents (at 20 °C) (g/L)	Methanol 36.747 ± 0.281 Ethyl acetate 2.008 ± 0.002 n-octanol 1.945 ± 0.027 acetone 0.960 ± 0.007 acetonitrile 0.651 ± 0.003 o-xylene 0.005 ± 0.000 n-hexane $(9.7 \pm 2.1) \times 10^{-6}$ dichloromethane 0.235 ± 0.003	Anand 2007 22542
Partition coefficient n-octanol/water (20 ± 1 °C)	log K_{ow} −1.12 ± 0.04 Milli-Q® water log K_{ow} −1.01 ± 0.01 pH 4 log K_{ow} −2.48 ± 0.02 pH 7	Manjunatha 2007 22544
Hydrolysis	Stable at pH 4,7 and 9 and at 25 °C	Manjunatha 2008 22116
Photolysis	Half-life: 7.3 days at pH 4, 25 °C and 1.3 days in sterile natural spring water	Lowrie 2008 22117 rev 1
Quantum yield	$\Phi = 2.5521 \times 10^{-4}$ molecules degraded/photon	Lowrie 2008 22117 rev. 1
Dissociation constant	pK_a 4.65 ± 0.04 at 20 ± 1 °C	Anand 2011a 22555 rev 1
Stability	Aminocyclopyrachlor was tested for stability at normal (ambient) and elevated (54 ± 2 °C) temperatures and in the presence of metal and metal ions for a period of 14 days. The results of the test show that the aminocyclopyrachlor is stable to normal and elevated temperatures and metal and metal ions	Yogeesha 2011 22539 rev 1

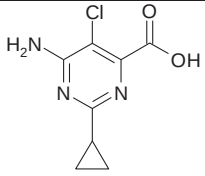
Technical grade material (aminocyclopyrachlor 92.2%)

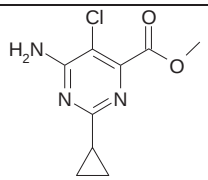
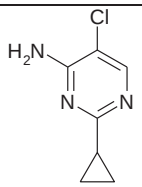
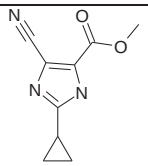
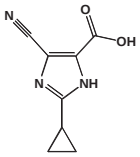
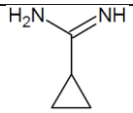
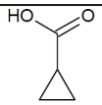
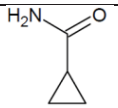
Property	Results (method)	Reference
Melting point	138.9 ± 0.1 °C	Anand 2011b 22551 rev 1
Density at 20 ºC	1.3476 ± 0.0813 g/mL	Anand 2011b 22551 rev 1

METABOLISM AND ENVIRONMENTAL FATE

Metabolites are given various abbreviations and code numbers in the studies. Structures and abbreviations and codes are shown below.

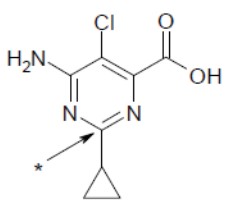
Degradation compounds from metabolism of aminocyclopyrachlor in plants, animals, soil, or water

Compound Name	Structure	Found in:
Aminocyclopyrachlor 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid CAS Number: 858956-08-8 DPX-MAT28 MW 213.62		grass, goat, rotational crops, soil

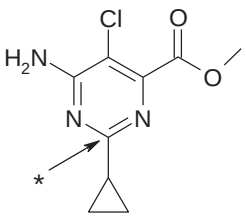
Compound Name	Structure	Found in:
Aminocyclopyrachlor-methyl Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylate CAS Number: 858954-83-3 DPX-KJM44, DPX-MAT28 methyl ester MW 227.65		
5-Chloro-2-cyclopropyl-pyrimidin-4-ylamine IN-LXT69 MW 169.61		Grass, goat, photolysis, rotational crops, soil
5-Cyano-2-cyclopropyl-3H-imidazole-4-carboxylic acid methyl ester IN-QGC48 MW 191.19		grass
5-Cyano-2-cyclopropyl-3H-imidazole-4-carboxylic acid IN-QFH57 MW 177.16		Photolysis, grass Structural formulae changed
Cyclopropane carbamidine IN-YY905		Photolysis
Cyclopropane carboxylic acid CAS NO.: 1759-53-1 IN-V0977		Photolysis, grass
Cyclopropane carboxamide CAS No.: 6228-73-5 IN-Q3007		Photolysis, grass

Animal metabolism

The Meeting received studies on the metabolism of DPX-KJM44 (aminocyclopyrachlor-methyl) in rats and lactating goats. The metabolism of aminocyclopyrachlor in animals was investigated using [^{14}C]-DPX-KJM44 while in plants studies were available for metabolism of [^{14}C]aminocyclopyrachlor and [^{14}C]-DPX-KJM44. The structural formula and the positions of the ^{14}C label are shown below. The studies on rats were evaluated by the WHO Core Assessment Group.



[pyrimidine-2-^{14}C]aminocyclopyrachlor



[pyrimidine-2-^{14}C]DPX-KJM44

Figure 1 Label positions of aminocyclopyrachlor and DPX-KJM44: marked as *

The identification of residue components in the animal and plant metabolism studies was achieved using authentic standards of the compounds involved.

Laboratory animal (rat) studies

Metabolism of aminocyclopyrachlor-methyl (DPX-KJM44) in rats was evaluated by the WHO Core Assessment Group of the 2014 JMPR. The metabolic conversion of DPX-KJM44 to aminocyclopyrachlor by plasma esterases is rapid (*in vitro* plasma $t_{1/2}$ approximately 5 minutes). As a result, administration of DPX-KJM44 to animal models will result in systemic exposures to primarily aminocyclopyrachlor. Both aminocyclopyrachlor and DPX-KJM44 have comparable pharmacokinetic profiles. Each is rapidly absorbed, and rapidly and essentially completely eliminated. For both substances the terminal compound in laboratory animals is aminocyclopyrachlor.

Lactating goat

Melville and Vance (2009 22837) studied the metabolism of DPX-KMJ44 in a lactating goat (British Saanen×, 51.3 kg bw; 2.0 kg milk/d) that was dosed orally via gelatin capsule twice daily with ring labelled [^{14}C] DPX-KMJ44 for 5 consecutive days at the equivalent of 97 ppm in the feed (feed consumption 1.45–1.73 kg DM/d). Doses were administered following milking (ca. 8:00 and 20:00 hours). Milk was collected twice daily and urine and faeces were collected daily. The goat was sacrificed approximately 6 h after the administration of the final dose and tissues taken post-mortem for quantification and analysis of radioactivity.

Composite liver, kidney, muscle, and fat samples were ground to a powder in dry ice and initially extracted with buffered acetonitrile:water (9:1, v/v). The extracted liver and kidney samples were digested with pepsin and protease to liberate additional ^{14}C-residues. Milk samples were initially partitioned with hexane and subsequently extracted with acetone. Aliquots of the extracts were analysed using liquid scintillation counting (LSC) while the post-extraction solids were dried under nitrogen, and triplicate portions were analysed using combustion and LSC.

Radioactive residues in the various extracts from each matrix were analysed by HPLC, using a C_{18} column with a mobile phase gradient of 10 mM ammonium formate and methanol. Radioactive residues were quantified by LSC analysis of the collected HPLC fractions. Radiolabelled components identified by HPLC and/or TLC co-chromatography with authentic unlabelled reference standards were later confirmed in selected samples using mass spectrometry.

All samples were extracted and analysed within 8 months of frozen storage. Reanalysis of liver and kidney extracts, stored for 11 months, showed no significant changes in the metabolite profile indicating metabolites in the extracts were stable to frozen storage.

The total recovery of dosed radioactivity was 85% with the majority of the radioactivity excreted in urine (54%) and faeces (20%). Approximately 8% remained in the contents of the gastrointestinal tract and a further 3.1% was found in the cage wash.

TRR in milk samples increased during the dosing period and reached a maximum of 0.031 mg equiv/kg in the milk collected at Day 5 of the study. Acetone extractions recovered *ca.* 80.5% of the total milk [^{14}C] residues. Aminocyclopyrachlor accounted for 15.9% TRR (0.004 mg equiv/kg). Other milk components accounting for 0.002 to 0.007 mg equiv/kg were not identified. Composite sample of milk from days 1 to 5 was separated into skim milk and cream with the majority of ^{14}C located in the cream fraction (0.016 mg equiv/kg skim milk, 0.12 mg equiv/kg cream). Residues in skim milk and cream were too low for identification.

Radioactive residues in tissues ranged from 0.01 mg equiv/kg in the omental fat to 1.67 mg equiv/kg in the kidneys. Good extractability was achieved for the milk and tissue samples with greater than 80% TRR recovered in solvent extracts (except omental fat = 47.4%). Residues remaining after solvent extraction of liver and kidney were subject to sequential treatment with pepsin and protease enzymes liberating an additional 1 to 11.6% of the TRR.

The identified metabolites in the tissue and milk samples are summarised in Table 1.

Table 1 Identification of radioactivity in tissue and milk of goats dosed orally with DPX-KJM44

Matrix	Milk [a]	Liver	Kidney	Muscle	Renal Fat	Omental fat	SC fat
TRR (mg equiv/kg)	0.023	0.30	1.67	0.042	0.016	0.010	0.026
			%TRR				
Solvent extracted	80.5	86.7	98.3	87.5	83.8	47.4	80.7
Aminocyclopyrachlor	15.9	55.6 [b]	55.3 [c]	43.3	83.8	47.4	80.7
unknowns	64.6 [d]	ND	ND	32.7 [e]	ND	–	–
Pepsin/protease digest	NC	11.6	1.0	NC	NC	NC	NC
Aminocyclopyrachlor		10.1	NA				
Unextracted	19.5	1.7	0.7	12.5	16.2	52.6	19.3
Losses during processing	–	29.3 [f]	43.0 [f]	11.5	–	–	–

[a] composite day 1–5 d sample
[b] Total characterised as aminocyclopyrachlor by TLC (concentrated extract prior to SPE clean-up) was 87.5% TRR (0.26 mg equiv/kg); confirming aminocyclopyrachlor as the only component present
[c] Total characterised as aminocyclopyrachlor by TLC (concentrated extract prior to SPE clean-up) was 93.2% TRR (1.56 mg equiv/kg); confirming aminocyclopyrachlor as the only component present
[d] Three components ranging from 0.002–0.007 mg equiv/kg
[e] Two components ranging from 0.006–0.008 mg equiv/kg
[f] Concentrated muscle and liver solvent extracts required SPE clean-up prior to HPLC analysis; efforts to improve low recoveries following SPE clean-up were unsuccessful.
ND = Not detected
NC = Not conducted
NA = Not analysed

Solvent extraction of liver recovered 86.7% TRR (0.259 mg equiv/kg). The concentrated solvent extract was subjected to SPE clean-up which resulted in losses such that the concentrated cleaned extract accounted for 57.4% TRR. Pepsin and protease digestion of the liver PES liberated a further 11.6% TRR (0.034 mg equiv/kg). Unextracted liver residues were 1.7%TRR (0.006 mg equiv/kg).

Aminocyclopyrachlor was the major extractable component in liver accounting for 55.6% TRR (0.167 mg equiv/kg) in the SPE cleaned solvent extract by HPLC. Aminocyclopyrachlor was the only component observed by TLC analysis of the concentrated extract prior to SPE clean-up accounting for 87.5% TRR (0.262 mg equiv/kg). A single radiolabelled component was detected in the pepsin digest which co-chromatographed with aminocyclopyrachlor and accounted for 10.1% TRR (0.030 mg equiv/kg).

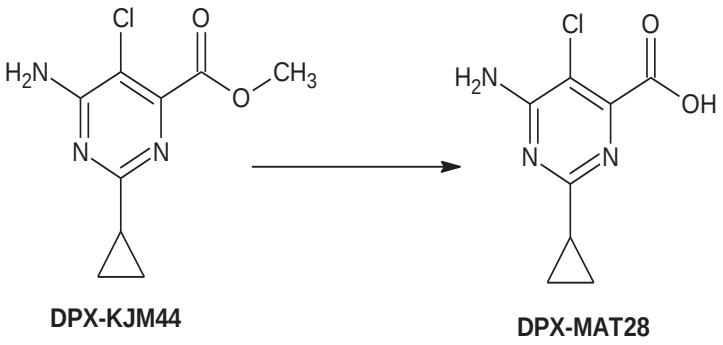
Total liver residues characterised as aminocyclopyrachlor by a combination of TLC (concentrated extract prior to SPE clean-up) and HPLC of the pepsin digest was 97.6% TRR (0.292 mg equiv/kg).

Solvent extraction of kidney recovered 98.3% TRR (1.64 mg equiv/kg). The concentrated solvent extract was subjected to SPE clean-up which resulted in losses such that the concentrated cleaned extract accounted for 55.3% TRR. Pepsin and protease digestion of the kidney PES only liberated an additional 1.0% TRR (0.017 mg equiv/kg) and were not analysed further. Unextracted kidney residues were 0.7% TRR (0.011 mg equiv/kg). Aminocyclopyrachlor was the major extractable component in kidney accounting for 55.3% TRR (0.925 mg equiv/kg) in the SPE cleaned solvent extract by HPLC. Aminocyclopyrachlor was the only component observed by TLC analysis of the concentrated extract prior to SPE clean-up accounting for 93.2% TRR (1.560 mg equiv/kg). Total kidney residues characterised as aminocyclopyrachlor by TLC (concentrated extract prior to SPE clean-up) was 93.2% TRR (1.56 mg equiv/kg).

Solvent extraction of muscle recovered 87.5% TRR (0.037 mg equiv/kg). The HPLC profile of the concentrated muscle extract showed that aminocyclopyrachlor was the major component accounting for 43.3% TRR (0.018 mg equiv/kg). Two minor components accounted for 14.4% TRR (0.006 mg equiv/kg) and 18.3% TRR (0.008 mg equiv/kg) were not identified. Unextracted muscle residues were 12.5% TRR (0.005 mg equiv/kg).

Solvent extraction of the three fat samples ranged from 47.4–83.8% TRR with lower extractability in omental fat. The HPLC profile of the renal, omental, and subcutaneous fat extracts showed that aminocyclopyrachlor was the major extractable component accounting for 83.8% TRR (0.013 mg equiv/kg), 47.4% TRR (0.005 mg equiv/kg), and 80.7% TRR (0.021 mg equiv/kg), respectively. Unextracted fat residues were ≤ 0.005 mg equiv/kg.

In summary, DPX-KJM44 was rapidly metabolised and eliminated as aminocyclopyrachlor, primarily in the excreta (74% of the administered total dose). Identified residues in tissues were exclusively aminocyclopyrachlor. A proposed metabolic pathway for DPX-KMJ44 in the lactating goat is presented in Figure 2.



DPX-KJM44 **DPX-MAT28**

Figure 2 Metabolism of DPX-KJM44 and aminocyclopyrachlor (DPX-MAT28) in lactating goats

Plant metabolism

The Meeting received plant metabolism studies with DPX-KJM44 on grass, aminocyclopyrachlor on grass and DPX-KJM44 and aminocyclopyrachlor on a variety of weeds. When applied to plants, the methyl esters of aminocyclopyrachlor (DPX-KJM44) is quickly broken down to release free acid which is readily translocated throughout the plants.

Grass

Lowrie (2009, 22393) studied the metabolism of [^{14}C]-DPX-KMJ44 in grass (Amenity mix: 30% Disco perennial ryegrass, 30% Franklin strong creeping red fescue, and 40% Vienna perennial ryegrass) grown under field conditions. A single application at a nominal rate of 373 g DPX-KMJ44/ha (spray volume 1000 L/ha) of a WP formulation was made to a 1 m^2 plot of grass growing in a sandy loam soil (67.6% sand, 15.1% silt, 17.4% clay; pH 6.5; 5.8% organic matter; 17.5 cmol/kg CEC). At the time of application the grass was approximately 30 cm in height. The grass plot was enclosed during the experiment Grass samples were collected at 0, 3, 7, 14, 30 and 60 days after application and were separately homogenised and radioactivity determined by combustion/LSC. The identity of DPX-KJM44, aminocyclopyrachlor, and the various grass metabolites were investigated by chromatographic (HPLC) comparison of the extracted residues with authentic reference standards of DPX-KJM44, aminocyclopyrachlor, IN YY905, IN V0977, IN Q3007, IN LXT69, IN QFH57, and IN QGC48. All samples were stored frozen (ca. –20 °C). Analyses of ^{14}C residues were completed within 4 months.

The grass foliage collected at each sampling point was gently washed with acetonitrile to remove the surface residues. The rinsed tissues were homogenised and portions of plant tissue were extracted twice with acetonitrile:0.2% formic acid in water (80:20, v/v; ca 150 mL) then twice with acetonitrile:0.2% formic acid in water (1:1, v/v; ca 150 mL). Levels of radioactivity in the wash and extract samples were determined by liquid scintillation counting (LSC). The unextracted radioactivity in the post extraction solids (PES) was determined by combustion analysis followed by LSC. Total

radioactive residues in each sample were determined by summing radioactivity in the surface wash, extracts and post extraction solids.

Unextracted ^{14}C residues in PES of tissues harvested 0, 7, and 60 DAT were subject to enzymatic and chemical hydrolysis. Post-extraction solids were incubated twice with a 2% solution of α-amylase in 0.2 M sodium phosphate buffer (pH 7 at 50 °C for 72 hours). After the second incubation with α-amylase, the remaining pellet was incubated with a mixture of 0.5% amyloglucosidase and 2% cellulase in 0.2 M sodium acetate buffer (pH 5 at 50 °C for 48 hours). Following each incubation the solution was separated from the pellet by centrifugation followed by decantation. The pellet remaining following enzyme hydrolysis was sequentially incubated with sodium hydroxide (0.1 N, 60 °C, 6 hours) and hydrochloric acid (1 M, 60 °C, 6 hours). The hydrolysates were separated from the remaining solids on each occasion by centrifugation and decanting.

Surface wash, solvent extracts, enzyme digests and hydrolysates were analysed using reversed phase HPLC. Radioactivity in collected fractions was quantified by LSC. LC-MS was conducted to confirm the presence of DPX-KMJ44, aminocyclopyrachlor and related metabolites.

Table 2 Characterisation of ^{14}C residues in grass following application of DPX-KJM44

				%TRR		
Days after	TRR (mg equiv	Surface wash and extracted				
application	DPX-KJM44/kg)	Surface wash	Extract 1	Extract 2	Total	Unextracted
0	15.6	13.0	76.6	8.6	98.2	1.8
3	15.4	10.3	76.2	8.3	94.8	5.2
7	12.0	5.8	75.8	10.4	92.0	7.9
14	5.9	3.1	70.3	12.0	85.4	14.6
30	4.1	4.5	57.2	11.3	73.0	27.0
60	2.4	1.2	35.4	11.6	48.2	51.7

Table 3 Identification of ^{14}C residues in grass following application of DPX-KJM44

Days after application	0	3	7	14	30	60
TRR (mg equiv DPX-KJM44/kg)	15.6	15.4	12.0	5.9	4.1	2.4
%TRR						
Extracted	99.4 [a]	94.8	95.3 [a]	85.4	73.0	76.7 [a]
IN-Q3007	< 0.1	< 0.1	0.1	0.2	0.2	1.3
IN-V0977	ND	ND	ND	ND	ND	0.5
Aminocyclopyrachlor	64.2	67.7	64.4	50.5	53.9	32.9
IN-QFH57	< 0.1	0.5	0.6	1.0	2.0	1.9
IN-LXT69	4.9	4.2	4.8	6.1	4.7	5.6
IN-QGC48	< 0.1	1.1	0.5	ND	4.1	0.6
DPX-KJM44	24.7	13.7	5.9	8.6	6.5	8.6
Unextracted	0.6	5.2	4.7	14.6	27.0	23.4
Total identified/ characterised	93.8	87.5	77.1	67.4	72.7	56.2

[a] Figures differ from Table 2 as it also includes material released from enzyme digestion and acid/base hydrolysis of post-extraction solids

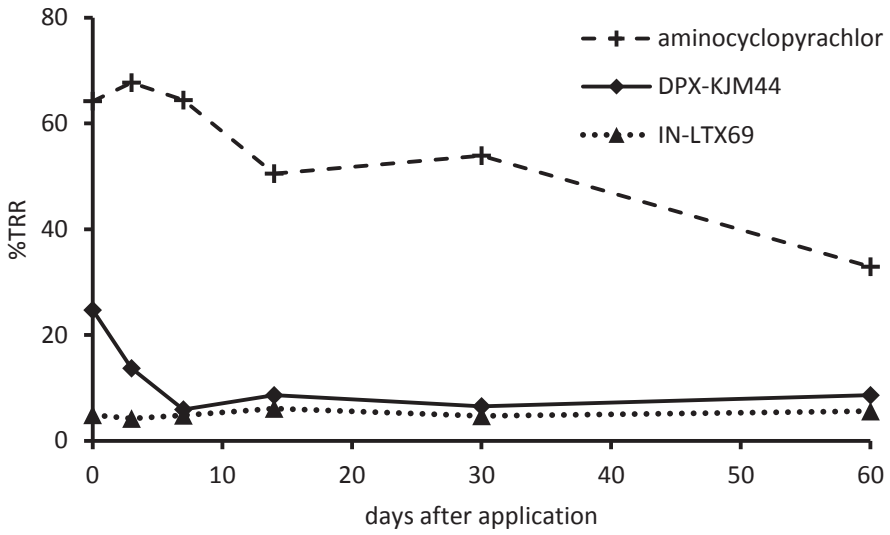


Figure 3 Plot of DPX-KJM44, aminocyclopyrachlor and IN-LTX69 residues in grass following foliar application of DPX-KJM44

Total surface wash and tissue extracted residues in grass ranged from 15.3 to 1.18 mg equiv/kg (98.2 to 48.3% TRR) at 0 DAT to 60 DAT, respectively. The unextracted residue accounted for 0.28 and 1.27 mg equiv/kg (1.8 and 51.7% TRR, respectively) at 0 DAT and 60 DAT, respectively. Only a very small amount of the TRR was released from PES by enzyme and chemical hydrolysis (1.2%) at 0 days after application; 28.5% of the TRR was released from PES by enzyme and chemical hydrolysis at 60 days after application.

TRR in grass harvested immediately following application was 15.6 mg equiv/kg. Acetonitrile surface washes removed 13.0% TRR (2.0 mg equiv/kg). Solvent extracted residues accounted for 85.2% TRR (13.3 mg equiv/kg). Unextracted residues were further investigated by enzyme 1 (amylase), enzyme 2 (a mixture of amyloglucosidase and cellulose), base and acid hydrolysis, resulting in the liberation of 0.7% TRR (0.11 mg equiv/kg), 0.3% TRR (0.041 mg equiv/kg), 0.1% TRR (0.016 mg equiv/kg), and 0.1% TRR (0.013 mg equiv/kg), respectively. Unextracted residues remaining following enzyme/chemical hydrolysis of PES accounted for 0.6% TRR (0.102 mg equiv/kg).

The identification of extracted residues was confirmed by LC-MS including several minor components including IN Q3007, IN V0977, IN QFH57, IN LXT69, and IN QGC48. Aminocyclopyrachlor (32.9 to 64.2%TRR) was the principal extracted residue in the grass samples with DPX-KJM44 (5.9 to 24.7%TRR) present at lower levels. IN-LXT69, IN V0977, IN Q3007, IN QFH57, and IN QGC48 were identified as minor grass components that individually accounted for no greater than 6.1% TRR (0.14 to 0.77 mg equiv/kg; Table 3).

Lewis *et al.* (2013) studied the absorption and translocation of ^{14}C-aminocyclopyrachlor in tall fescue (*Lolium arundinaceum* (Schreb.) SJ Darbyshire, *cv*. Confederate) following application to a tiller leaf. Plants were grown to the 5 to 7 leaf stage (single tiller) and the third leaf from the crown was covered with aluminium foil and the plants sprayed with commercially formulated aminocyclopyrachlor at 79 g ai/ha with the addition of 0.25% non-ionic surfactant (NIS). The foil was removed and five 1 µL droplets of ^{14}C-aminocyclopyrachlor and 0.25% NIS were applied to the previously covered leaf. Plants were harvested 3, 12, 24, 48, 96 and 192 hours after application and separated into ^{14}C-treated leaf, remaining above ground foliage, crown and roots. The treated leaf was washed with 50% v/v methanol:water to remove surface residues. Similarly, roots were washed with methanol:water to remove root exudates.

Absorption of ^{14}C- aminocyclopyrachlor was high accounting for 63% (100%-leaf wash %) of the applied ^{14}C by 24 hours after application (Table 4). Translocation of absorbed ^{14}C was observed as evidenced by ^{14}C accumulation in the untreated foliage and in the roots. Accumulation of ^{14}C-radioactivity in other foliage ranged from 25 to 41% of the absorbed ^{14}C and for roots from 22 to 27% at 24 to 72 hours after treatment. Metabolism was not observed with 100% of ^{14}C in solvent extracts present as aminocyclopyrachlor at 192 hours after application.

Table 4 Distribution of ^{14}C following foliar application of ^{14}C-aminocyclopyrachlor to tall fescue

Hours after				%applied ^{14}C			
application	Leaf wash	^{14}C-treated leaf	Other foliage	Crown	Roots	Root exudates	Total recovery
3	49	25	5	4	0	3	86
12	38	35	11	4	0	1	89
24	37	41	14	3	0	0	95
48	32	35	21	2	1	8	99
96	29	30	34	2	1	1	97
192	26	26	25	1	2	3	83

Following application to grass, DPX-KMJ44 was rapidly metabolised (demethylated) to form aminocyclopyrachlor. Aminocyclopyrachlor was the principal radiolabelled component found in grass samples collected immediately after treatment and up to 60 days after treatment. Aminocyclopyrachlor was decarboxylated to a lesser extent to IN-LXT69. Other minor routes of metabolism included photo-induced simultaneous elimination of hydrogen chloride and pyrimidine ring contraction yielding the imidazole-nitriles, IN-QGC48 and IN-QFH57 (from DPX-KJM44 and aminocyclopyrachlor, respectively), and pyrimidine ring opening with subsequent oxidation providing the amide IN-Q3007. Formation of the carboxylate, IN V0977 could occur through photolysis and is a minor pathway or it could be an artefact of the extraction process as hydrolysis experiments on IN-Q3007 under alkaline and acidic conditions showed that IN V0977 is produced under conditions comparable to exhaustive extraction procedures employed in the grass metabolism study (Brown 2011 31316).

The proposed metabolic pathway for DPX-KMJ44 and aminocyclopyrachlor in grass is provided in Figure 4.

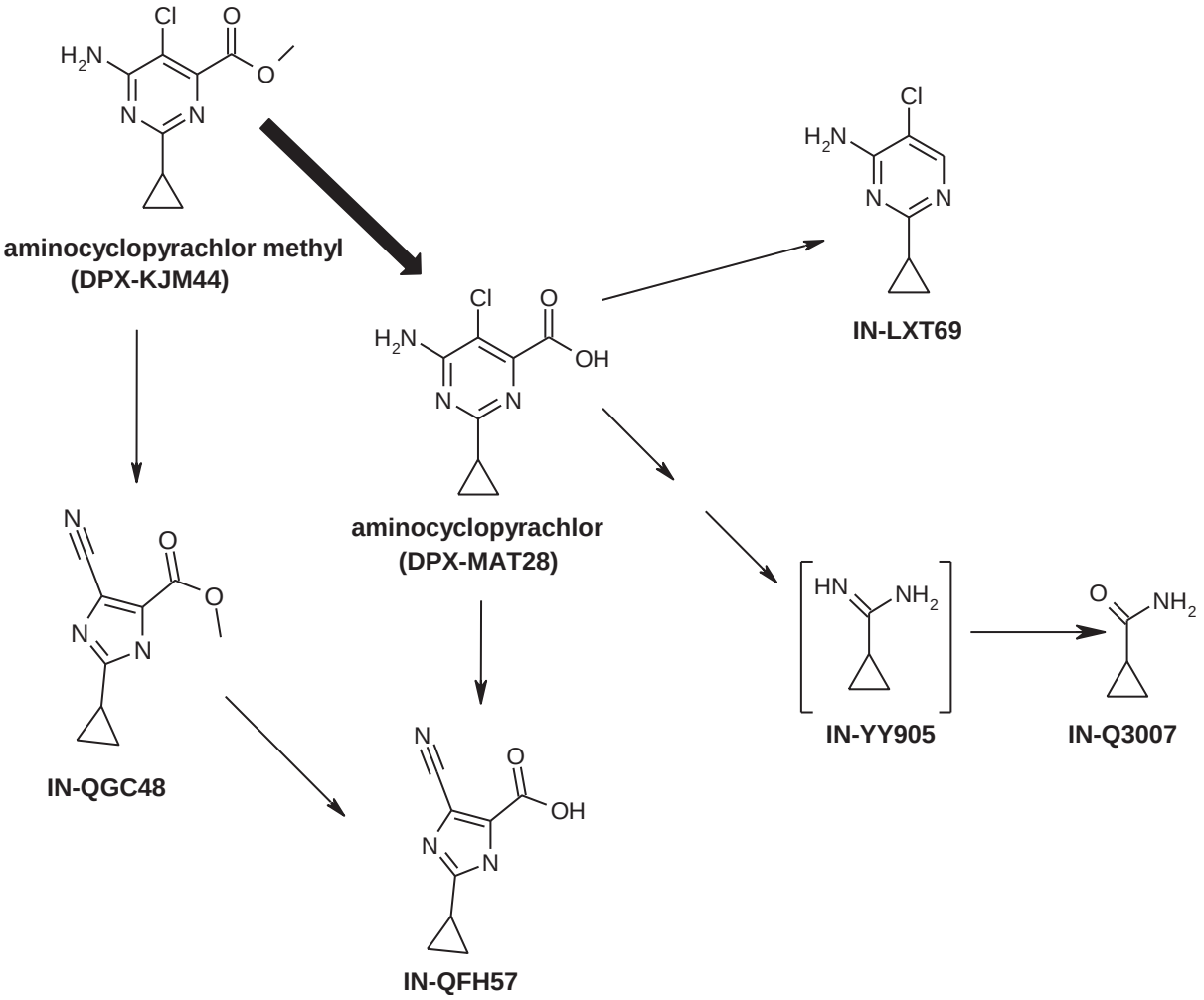


Figure 4 Metabolic pathway of DPX-KJM44 and aminocyclopyrachlor following foliar application to grass

Studies on weeds

A number of literature studies have investigated the absorption, translocation and metabolism of aminocyclopyrachlor and DPX-KJM44 in various weed species.

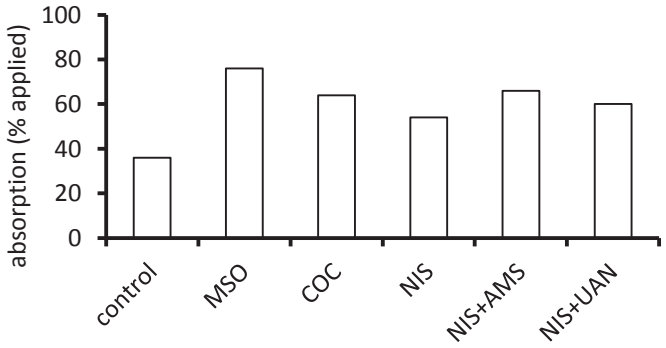
Bukun *et al.* (2010) studied absorption and translocation of both aminocyclopyrachlor and DPX-KJM44 in Canada thistle (*Cirsium arvense*). Plants at the five- to six-rosette stage were selected and the youngest fully expanded leaf on each plant was covered with aluminium foil. The plants were sprayed with formulations containing aminocyclopyrachlor or DPX-KJM44 at the equivalent of 0.14 kg ai/ha together with 1% v/v methylated seed oil (MSO). Following spray application the protected leaves were exposed and treated with formulations containing ^{14}C-herbicide. Plants were harvested at 24, 48, 96 and 192 hours after application and separated into ^{14}C-treated leaf, and above and below ground tissue. In studies on metabolism, plants were harvested at 0, 2, 6 and 24 hours after application with residues analysed by HPLC.

Metabolism of ^{14}C- DPX-KJM44 was exclusively to the free acid (aminocyclopyrachlor). By 2 hours after application, 65% of the ^{14}C in plant extracts was aminocyclopyrachlor and by 6 hours after treatment 82% of the absorbed DPX-KJM44 had been metabolised to aminocyclopyrachlor.

The effect of adjuvants on absorption was studied in a separate experiment where [^{14}C]aminocyclopyrachlor or ^{14}C- DPX-KJM44 were formulated in treatment solutions containing methylated seed oil (MSO), crop oil concentrate (COC), non-ionic surfactant (NIS), NIS plus ammonium sulphate (AMS) or NIS plus urea ammonium nitrate (UAN). Solutions were 1% MSO (v/v), 1% COC (v/v), 0.25% NIS (v/v), 0.25% NIS (v/v) plus 0.2% AMS (w/v) and 0.25% NIS (v/v) plus 1% UAN (v/v) combined with formulated product to provide a concentration when applied to leaves that was equivalent to application at 0.14 kg ai/ha. Leaves were harvested 96 hours after application and surface residues measured by washing the leaves with 90% water, 10% methanol and 0.25% NIS. Quantitation of radioactivity was by liquid scintillation spectroscopy. The fraction absorbed was determined as the difference between applied radioactivity and radioactivity in the surface wash divided by applied radioactivity.

Absorption of both DPX-KJM44 and aminocyclopyrachlor was maximised in formulations containing methylated seed oil (MSO). While absorption of DPX-KJM44 by leaves was greater than for aminocyclopyrachlor, once absorbed DPX-KJM44 is rapidly metabolised to aminocyclopyrachlor and translocation from the application site to other parts of the plant was almost identical for the two formulations.

A aminocyclopyrachlor



B DPX-KJM44

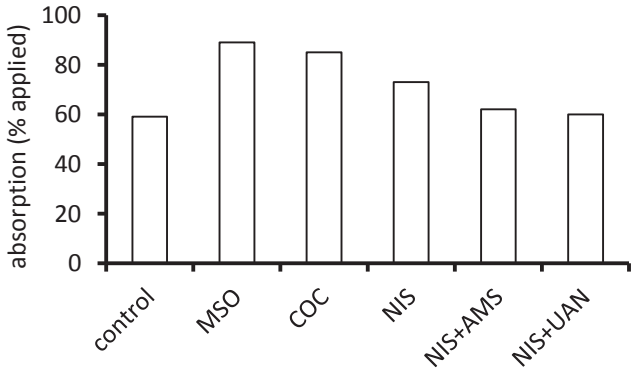


Figure 5 Effect of adjuvants on absorption of *A* aminocyclopyrachlor and *B* DPX-KJM44. MSO = methylated seed oil, COC = crop oil concentrate, NIS = non-ionic surfactant, NIS+AMS = NIS + ammonium sulphate, NIS+UAN = NIS + urea ammonium nitrate

Lindenmayer (2012) studied the absorption, translocation and metabolism of aminocyclopyrachlor in field bindweed (*Convolulus arvensis*). Field bindweed plants were grown from seed, pruned to maintain a single shoot and prevent flowering. When plants reached 12–15 leaf

stage, and vined to a height of 30 cm on a wooden stake, a single leaf midway up the stem was covered by aluminium foil and the plant sprayed with aminocyclopyrachlor (with 1% v/v MSO) at the equivalent of 0.14 kg ai/ha and a spray volume equivalent to 187 L/ha. Following the spray application, the aluminium foil was removed and the protected leaves were treated with a solution of [^{14}C]aminocyclopyrachlor plus 1% MSO v/v. Plants were harvested at 0, 6, 12, 24, 48, 96 and 192 hours after application and separated into ^{14}C-treated leaves, above-ground tissues, below ground tissue.

To determine absorption, treated leaves were washed with 90% water, 10% methanol and 0.25% NIS and the wash solution analysed by liquid scintillation spectroscopy. Radioactivity in distant plant parts was extracted by homogenising with 90% methanol, centrifuging and decanting the extract for LSC. Samples of the extracts were also subject to HPLC to determine metabolites.

Absorption of ^{14}C was rapid, reaching a maximum of 48% of the applied radioactivity by 48 hours after application. Translocation to above and below ground plant parts increased with time after application and reached 14% of the applied radioactivity by 192 hours after application. No soluble metabolites of aminocyclopyrachlor were detected in the plant extracts suggesting limited or no metabolism of aminocyclopyrachlor within 192 hours of application.

The results for Canada thistle and field bindweed described above are similar to those reported by Bell *et al.* (2011) who studied the absorption, translocation and metabolism of DPX-KJM44 and aminocyclopyrachlor on yellow star thistle (*Centaurea solstitialis L.*), rush skeleton weed (*Chondrilla juncea L.*) and prickly lettuce (*Lactuca serriola L.*). Following application of DPX-KJM44 there was rapid formation of the free acid (aminocyclopyrachlor) with a half-life of 3.5 hours and no subsequent metabolism in yellow starthistle, rush skeleton weed and prickly lettuce. Proportional herbicide movement between species was similar, with the majority translocating to developing shoots. However, in rush skeleton weed, early translocation was directed to root tissue. Highly sensitive species such as prickly lettuce absorb and translocate less material than relatively less sensitive species such as rush skeleton weed. De-esterification of DPX-KJM44 appeared to delay translocation of the resulting aminocyclopyrachlor in yellow star thistle and rush skeleton weed.

Vargas *et al.* (2014) studied the absorption, translocation, and metabolism of ^{14}C-DPX-KJM44 in large crabgrass (*Digitaria sanguinalis (L.) Scop*) and black nightshade (*Solanum nigrum L)* applied with and without diflufenzopyr (35 g ai/ha). Diflufenzopyr had minimal effects on translocation of radioactivity in either species. Accumulation of radioactivity in above ground parts of black nightshade was greater than, or equal to, that in large crabgrass by 72 h after treatment. In both species, metabolism of ^{14}C-DPX-KJM44 was rapid such that 60 to 78% of the extracted radioactivity was present as aminocyclopyrachlor at 8 hours after application.

Lym (2014) studied the absorption and translocation of aminocyclopyrachlor in leafy spurge (*Euphorbia esula*) and yellow toadflax (*Linaria vulgaris*). Application of [^{14}C]aminocyclopyrachlor was made approximately 8 weeks after planting and when plants were 10–12 cm tall. The application was to a single leaf midway on the stem of each plant. Samples were collected at 24, 48, 96 and 192 hours after application with plants separated into treated leaf, leaves above and leaves below the treated leaf as well as roots. In both weed species absorption was rapid averaging 63 and 71% of the applied ^{14}C in yellow toadflax and leafy spurge respectively at 24 hours after application. By 96 hours after application, 28 and 16.5% of the applied ^{14}C for yellow toadflax and leafy spurge respectively had translocated to other above ground plant parts. Relatively low levels of ^{14}C translocated to the roots of both weeds (< 12% applied ^{14}C).

In summary, DPX-KJM44 and aminocyclopyrachlor are rapidly absorbed and translocated by plants. Absorption is improved by the addition of spray adjuvants such as MSO or NIS. Following administration of DPX-KJM44 to plants, DPX-KJM44 is rapidly metabolised to aminocyclopyrachlor which is the major component of the residue. Following administration of aminocyclopyrachlor to plants, aminocyclopyrachlor is the major component of the residue.

ENVIRONMENTAL FATE

The FAO Manual on the Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed (2009) explains the data requirements for studies of environmental fate. The focus should be on those aspects that are most relevant to MRL setting. For aminocyclopyrachlor, supervised residue trials data are available for grass. Aerobic degradation in soil is relevant, as well as the normal requirements for hydrolysis, photolysis and rotational crop studies.

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

Aminocyclopyrachlor residues are persistent in soils. Aminocyclopyrachlor residues in soils resulting from recommended uses could contribute to the residues in succeeding crops.

Aerobic degradation in soil

The rate and route of degradation of [^{14}C]- DPX-KJM44 was investigated in a sandy loam soil (Sassafras, Maryland USA, pH 5.4, 2.1% OM) under aerobic conditions by Hirata *et al.* (2011, 22435). [^{14}C]-DPX-KJM44 was applied at a nominal rate equivalent to a single application of 0.336 kg ai/ha. The soil samples were incubated under aerobic conditions in the laboratory and maintained under moist, dark conditions at 20 ± 2 °C for up to 360 days. Samples were extracted twice with 0.2% formic acid in water:acetonitrile (20:80 v:v). For samples from day 40 onwards, an additional extraction was conducted using 0.15M ammonium acetate:acetonitrile (30:70 v:v). This extraction step was repeated to demonstrate exhaustive extraction.

The mean total recoveries of radioactivity for the 0 to 360 DAT soil samples were between 91.8 and 93.7% of applied radioactivity (AR). By 360 days, 20.4 to 25.8% of the AR was mineralised to $^{14}CO_2$ with 46.4–46.6% AR extracted with the solvent systems used and 21.4 to 25.0% remaining unextracted.

DPX-KJM44 was rapidly degraded, accounting for less than 1% AR by Day 3 as it was converted to aminocyclopyrachlor which accounted for 91.0–91.4% AR. By day 360, aminocyclopyrachlor accounted for 42.8-43.1% AR. The only other product observed was IN-LXT69 which reached a maximum between days seven and 122 accounting for 2.8–3.1% AR and declining to 2.2–2.4% AR by day 360.

The rate of degradation was estimated using single first-order (SFO) kinetics. The DT_{50} and DT_{90} values obtained are presented in Table 5.

Table 5 Summary of DT_{50} for DPX-KJM44 and aminocyclopyrachlor in Sassafras sandy loam (20 °C in the dark)

Soil: Sassafras sandy loam	DT_{50} (days)	DT_{90} (days)	r^2
DPX-KJM44	0.1	0.2	0.9999
aminocyclopyrachlor	275	912	0.9492

The proposed degradation pathway of DPX-KJM44 in soil is presented in Figure 6 and involves the rapid de-methylation of DPX-KJM44 to form aminocyclopyrachlor which then undergoes de-carboxylation to form IN-LXT69. Both $^{14}CO_2$ and bound residue are significant degradation products of DPX-KJM44 in soil.

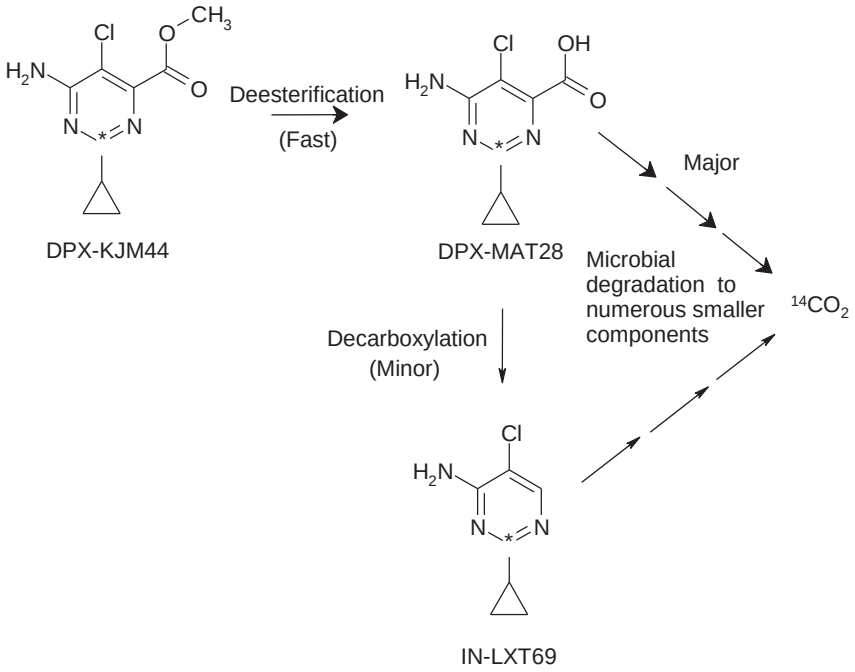


Figure 6 Proposed degradation pathway for DPX-KJM44 in soil under aerobic conditions

Manjunatha (2010, 22119) studied the degradation of aminocyclopyrachlor in three soils maintained under aerobic conditions in the dark at a nominal temperature of 20 ± 2 °C for 120 days. The moisture content of the soils was adjusted to between 40 and 60% maximum water holding capacity and as close as possible to 75% water holding capacity at 1.033 MPa. Relevant properties of the soils used are presented in Table 6.

Table 6 Properties of soils used to study aerobic degradation of aminocyclopyrachlor

Soil Name	Texture	Origin	pH		% Organic Matter	Soil biomass µg/g	
			Water	0.01 M $CaCl_2$	Walkley-Black	initial	final
Nambsheim	Sandy Loam	Europe	8.09	7.48	2.2	1394	4796
Tama	Silty Clay	USA	6.45	6.13	4.6	650	4234
Drummer	Clay Loam	USA	6.40	5.79	6.5	284	4181

[Pyrimidine-2-^{14}C] aminocyclopyrachlor was applied at a nominal rate of 0.448 mg/kg dry soil. Samples were analysed after 0, 7, 15, 30, 60, 90 and 120 days incubation. Residues in samples, other than day 0, were sequentially extracted using: (1) phosphate buffer (pH 7.4); (2) and (3) acetonitrile: pH 7.4 phosphate buffer 70:30 v/v; 4 acetonitrile: The extraction for the day 0 Drummer soil sample omitted the acetonitrile:ammonium acetate extraction. The extraction for the day 0 Nambsheim and Tama samples utilised: 1 2× acetonitrile: 0.2% (v/v) formic acid 80:20 v/v; 2 acetonitrile: 0.15 M ammonium acetate 70:30 v/v; 3 acetonitrile: pH 7.4 phosphate buffer 70:30 v/v followed by: 4 acetonitrile. Analysis of residues was by LSC and LC-MS/MS.

Extracted radioactivity recovered from samples ranged from *ca* 98 to 110% AR at day 0 decreasing to *ca* 49–81% AR after 120 days. Unextracted residues accounted for *ca* 0.6–6.9% AR at Day 0, increasing to a maximums *ca* 13–43% AR after 120 days while radioactivity associated with volatile traps reached 0.1–0.6% AR at Day 120. Following extraction, the organic matter from the Tama and Drummer soils of day 120 were fractionated into humin, fulvic acid, and humic acid. The mean radioactivity in the unextracted residue associated with humin, fulvic acid and humic acid were 13–20%, 11% and 0.3% AR, respectively.

The overall material balance ranged between *ca* 90 and 111% AR.

The amount of aminocyclopyrachlor in the solvent extracts declined from 105, 93 and 94% AR at zero time to 76, 42 and 45% AR after 120 days in the Nambsheim, Tama and Drummer soils, respectively. IN-LXT69 accounted for 4.0–6.4% AR at Day 0 declining thereafter to 0.2–0.4% AR at Day 120.

The dissipation half-lives were calculated using nonlinear first-order regression and are summarised in Table 7.

Table 7 DT_{50} and DT_{90} times for aminocyclopyrachlor in three soils (SFO kinetics)

Soil	k (day^{-1})	DT_{50} (days)	DT_{90} (days)	r^2
Nambsheim sandy loam	0.0016	433	1439	0.448
Tama silty clay	0.0058	120	397	0.964
Drummer clay loam	0.0055	126	419	0.938

Conklin and Lym (2013) studied the effect of temperature and moisture on the soil half-life of aminocyclopyrachlor on four soils from the Northern Great Plains. Relevant properties of the soils used are presented in the table below.

Table 8 Properties of Northern Great Plains soils used to study the degradation of aminocyclopyrachlor

Location	Soil	% by weight					
		Sand	Silt	Clay	Organic Matter	FCWC [a]	pH
Fargo	Fargo	5	45	50	7.0	55	7.2
Jamestown	Svea-Barnes	37	42	21	6.4	51	5.7
Medora	Glendive-Havre	5	35	60	1.2	38	8.1
Walcott	Lamoure	86	9	5	2.6	49	7.8

[a] Field capacity gravimetric water content

Ten mL of a 36 µg/kg aminocyclopyrachlor solution was mixed thoroughly with 500 g air-dry soil. The treated soil was maintained in the dark at different soil moisture contents and temperatures for 8 weeks. At the end of this period, the soil was frozen to reduce or eliminate microbial activity until soybean bioassay for aminocyclopyrachlor residues. DT_{50} values for the decline of aminocyclopyrachlor were calculated based on the soybean bioassay results (Table 9).

The rate of aminocyclopyrachlor dissipation generally increased as soil moisture content or temperature increased however, there were also effects that may be due to organic matter and clay content. The DT_{50} times for aminocyclopyrachlor are not easily predicted by a single soil characteristic.

Table 9 Effect of moisture content and temperature on DT_{50} values (days) for four soils after treatment with aminocyclopyrachlor at 39 ug/kg.

Moisture content	Temperature		Soil		
(FCWC [a])	(°C)	Fargo	Glendive-Havre	Lamoure	Svea-Barnes
22.5%	16	> 50	20	44	7
45%	16	17	54	30	3
90%	16	5	19	21	11
45%	8	37	> 112	> 80	> 88
45%	16	18	54	52	22
45%	24	11	51	13	13

[a] Field capacity gravimetric water content

The soil dissipation of aminocyclopyrachlor occurs at a slow rate. Aminocyclopyrachlor is persistent in the environment.

Field dissipation

In a series of studies, the degradation of DPX-KJM44 was studied following a single application of a wettable powder formulation to bare soil (Shepard 2009 22528, 2010a 22527) or to turf grass (Shepard 2012 22529, 2010b 22526). Treated plot areas ranged from 279 to 372 m^2. In each experiment, five replicate soil core samples at each sampling time were collected from each of three treated plots. Soil samples were collected at various intervals after depending on the site and up to 540 days after treatment. Samples were collected from depths of 0–5, 5–15, 15–30, 30–50, 50–70, and 70–90 cm. Where available, grass samples were taken from a 30 × 30 cm area by cutting the grass as close as possible to the soil surface. Soil and grass samples were extracted and analysed for residues of DPX-KJM44 and its degradation products aminocyclopyrachlor and IN-LXT69 by LC-MS/MS. The LOQ and LOD for DPX-KJM44 aminocyclopyrachlor and IN-LXT69 were 0.001 mg/kg and 0.0003 mg/kg respectively. Table 10 contains a summary of the experimental conditions.

Table 10 Experimental conditions for soil field dissipation studies on DPX-KJM44

	Location	Application rate g as/ha (g ai/ha)	Spray volume (L/ha)	Soil type	Soil pH [a]	Soil OC% [b]
Bare soil	Ontario	336 (315)	980	Brant silt loam	6.0–8.4	0.23–1.0
	California	336 (315)	890	Tagus silt loam	8.4–8.7	0.1–0.4
Turf	Georgia	168 (158)	944	Tifton sandy loam	6.3–6.4	0.7–0.9
	Ontario	336 (316)	980	Brant silt-loam	6.4–7.1	1.1–2.6

[a] Range of soil pH measured in water for soil fraction collected from 0–90 cm depth
[b] Range of soil organic carbon measured in soil fraction collected from 0–90 cm depth

Analysis of the total residue (DPX-KJM44 + aminocyclopyrachlor + IN-LXT69) in soil indicated that DPX-KJM44 was rapidly hydrolysed to aminocyclopyrachlor and quickly declined to soil concentrations that were below the limit of detection. Within 14 days after application, aminocyclopyrachlor was the major residue present. The degradation product IN-LXT69 generally showed concentrations < LOD throughout the study. Figure 7 summarises visually the degradation of DPX-KJM44 and aminocyclopyrachlor. Total residues, calculated as the sum of DPX-KJM44, aminocyclopyrachlor and IN-LXT69, are almost entirely due to aminocyclopyrachlor.

Air sampling did not recover significant quantities of DPX-KJM44 or aminocyclopyrachlor in volatile traps suggesting loss through volatilisation is not a significant dissipation mechanism.

The DPX-KJM44 concentrations in soil in units of % mass of applied parent equivalents were used to calculate the DT_{50} and DT_{90} values tabulated below. DPX-KJM44 is rapidly degraded to aminocyclopyrachlor with DT_{50} values of 0.7 to 1.6 days for the four sites studied. In contrast the DT_{50} values for aminocyclopyrachlor are much longer ranging from 55 to 163.6 days. Meaningful kinetic information could not be generated for IN-LXT69.

The dissipation of DPX-KJM44 in established grass foliage was investigated at two sites with DT_{50} values for DPX-KJM44 of 0.4 days while the DT_{50} values for aminocyclopyrachlor were 4.8 to 8.9 days (Figure 8, Table 11).

The modelling results are summarized in Table 11.

Table 11 DT_{50} and DT_{90} values for DPX KJM44 and aminocyclopyrachlor in four soils

Site	Matrix	Compound	Model	DT_{50} (days)	DT_{90} (days)	r^2
Ontario	Soil	DPX-KJM44	SFO	1.6	5.2	0.9408
		aminocyclopyrachlor	SFO	79.9	265	0.7721
California	Soil	DPX-KJM44	SFO	0.7	2.4	0.9707
		aminocyclopyrachlor	SFO	163.6	543.5	0.8048
Sycamore, Georgia	Foliage	DPX KJM44	SFO	0.4	1.4	0.8537

Site	Matrix	Compound	Model	DT_{50} (days)	DT_{90} (days)	r^2
		aminocyclopyrachlor	SFO	4.8	16.1	
	Soil	DPX-KJM44	SFO	0.8	2.7	
		aminocyclopyrachlor	SFO	55	183	
Ontario	Foliage	DPX KJM44	SFO	0.4	1.4	0.983
		aminocyclopyrachlor	DFOP	8.9	30.2	0.972
	Soil	DPX-KJM44	DFOP	0.8	3.4	0.951
		aminocyclopyrachlor	DFOP	141.4	644	0.754

SFO = Simple first-order model
DFOP = Double first-order parallel model

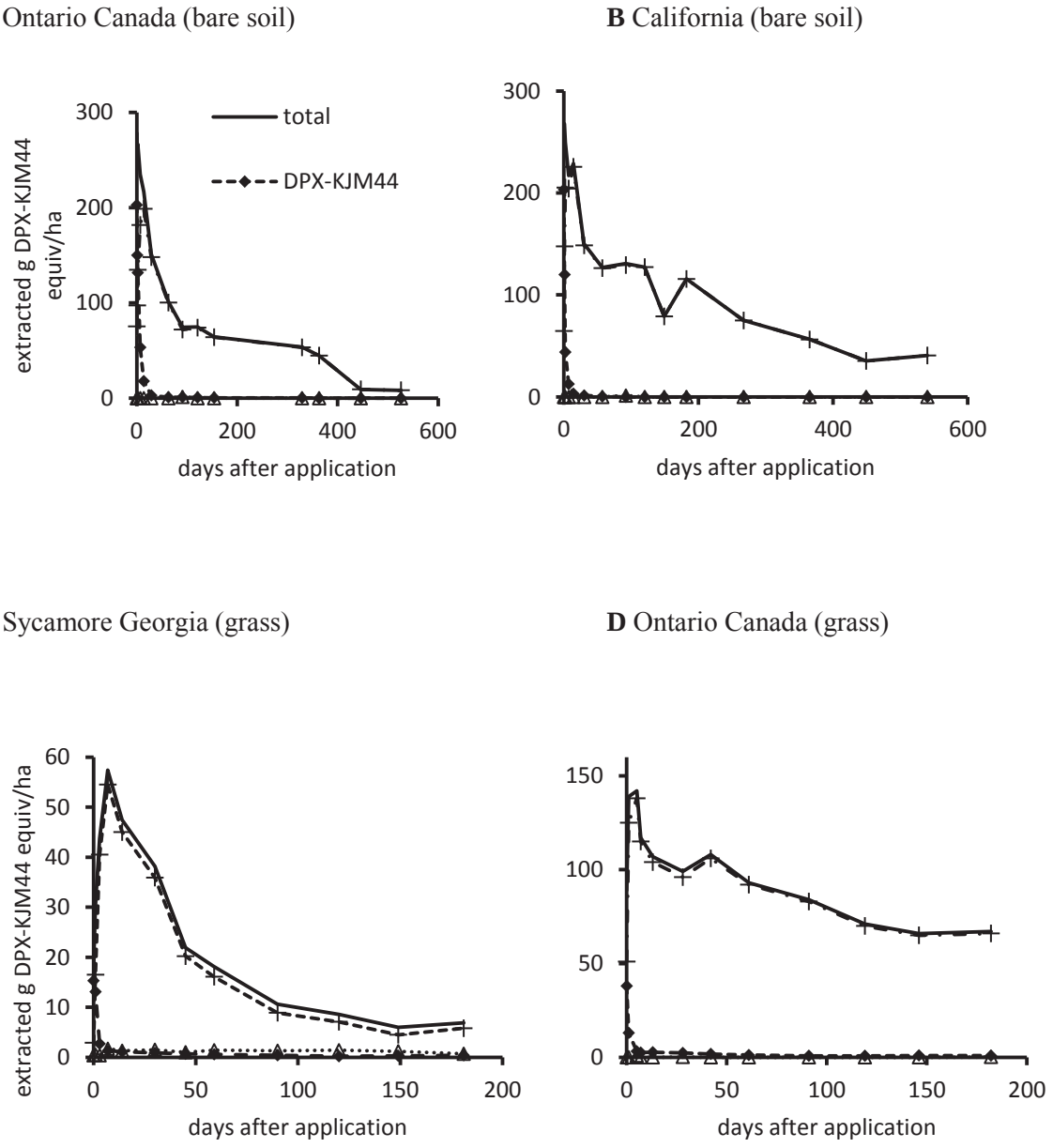


Figure 7 Evolution of residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 in soil following application of DPX-KJM44 to bare soil (A, B) and to plots of grass (C, D)

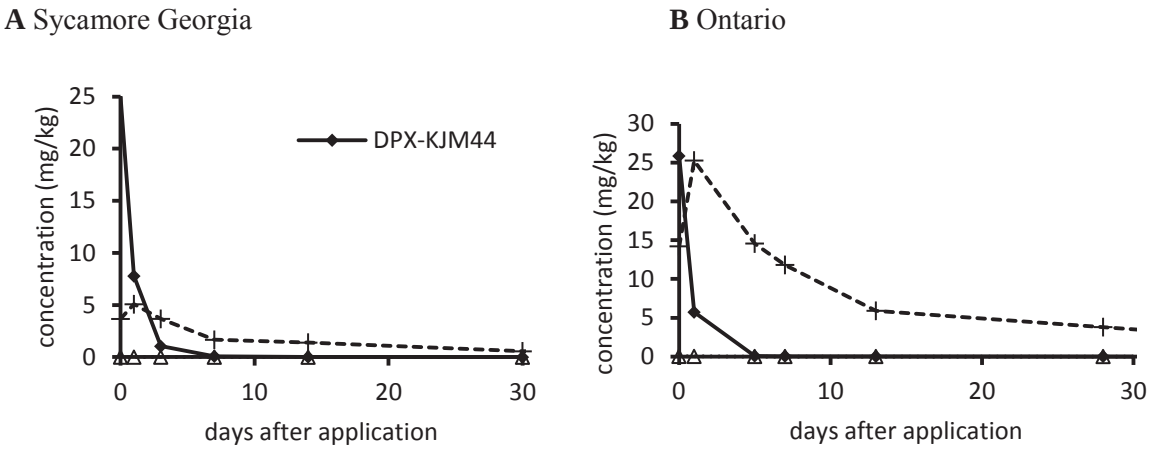


Figure 8 Evolution of residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 following application of DPX-KJM44 to plots of grass, residues in grass

Soil photolysis

Wardrope (2007 22118) studied the soil photolysis of aminocyclopyrachlor. [Pyrimidine-^{14}C]aminocyclopyrachlor was applied by pipette to thinly-layered soil (*ca* 2 mm thick) to achieve a nominal concentration of 448 µg ae/kg soil (448 ppm), equivalent to a field use rate of 336 g ai/ha soil, and irradiated under artificial irradiation (Xenon light) equipped with filters to eliminate wavelengths of < 290 nm. $^{14}CO_2$ and ^{14}C non-specific organic volatiles were collected. Samples were taken for analysis at 0, 1, 3, 5, 7, 11, and 15 days of irradiation. The soil samples were extracted twice with acetonitrile: 0.2% formic acid (8:2 v/v) followed by an extraction with acetonitrile: 0.15 M ammonium acetate (7:3 v/v). Identification of the transformation products was performed by HPLC and LC-MS analysis using authentic reference standards.

The material balance ranged from 96 to 102% and 95 to 102% of the applied radioactivity (AR) in the irradiated and dark (non-irradiated) samples, respectively. The total extracted radioactivity for irradiated samples decreased during the irradiation period, from a mean of 97% AR at Day 0 to a mean of 83% AR after 15 days. Unextracted ^{14}C-residues increased from < 4% to a maximum mean value of 17% AR after 15 days. Throughout the experimental phase, evolved $^{14}CO_2$ and ^{14}C-organic volatile radioactivity were below the limit of quantification. In the non-irradiated (dark control) samples the total extracted radioactivity remained relatively constant at all time points, with means of 97% AR at Day 1 and 92% AR at Day 15. Unextracted ^{14}C-residues increased from *ca* 2 to 5.1% AR after 15 days. Throughout the experimental phase, evolved $^{14}CO_2$ and ^{14}C-organic volatile radioactivity were below the limit of quantification.

In the irradiated samples, aminocyclopyrachlor decreased from a mean value of 94% AR at day 0, to a mean of 74% AR after 15 days. The only significant transformation product was identified as IN-LXT69, with a maximum mean concentration of 4.9% AR observed at Day 7. Multiple (a total of fourteen but up to six for any one sample) minor transformation products were detected, accounting for a combined mean maximum value of 8.7% AR at Day 11. No individual unidentified component accounted for greater than 5% AR at any time point. In the non-irradiated samples, the parent compound decreased from a mean of 94% AR at day 1 to 87% AR after 15 days. One major transformation product was identified as IN-LXT69, with a mean maximum value of 5.2% AR observed at Day 11. Eight minor transformation products were detected, none of which accounted for greater than 5% AR with a majority less than 1% AR at any time point.

To calculate the rate of degradation of aminocyclopyrachlor due to photolysis only, the first-order rate of degradation constant in the non-irradiated samples was subtracted from the first-order rate of degradation constant in the irradiated samples. The resulting corrected DT_{50} and DT_{90} values

were 61 and 204 days, respectively. These estimated DT_{50} and DT_{90} values are extrapolated beyond the limits of the observed data, 15 days.

Soil photolysis is expected to be a significant route of aminocyclopyrachlor degradation when compared to aerobic soil degradation.

Hydrolysis

Manjunatha (2008, 22116) studied the hydrolytic stability of aminocyclopyrachlor at 50 ± 0.5 °C for 5 days in dark, sterile, aqueous buffered solutions at pH 4, pH 7and pH 9. Aqueous solutions of aminocyclopyrachlor showed less than 10% degradation at the end of the 5 day study period. Aminocyclopyrachlor is considered stable to hydrolysis at pH 4, 7 and 9 ($t_{½}$ at 25 °C > 1 year).

Hydrolysis of aminocyclopyrachlor is not expected to be a significant process under environmental conditions.

Aqueous Photolysis

Lowrie (2008, 22117) studied the photolysis of aminocyclopyrachlor in sterile natural water. Solutions containing 2 µg [^{14}C]aminocyclopyrachlor/mL were continuously irradiated using light from a xenon arc lamp filtered to give a spectral distribution close to that of natural sunlight. The samples were maintained at 20 °C and were irradiated for periods up to 360 h.

The mass balance (mean across all samples) was 99% of the AR. In the pH 4 buffer system, aminocyclopyrachlor was relatively rapidly degraded accounting for 28.1% AR after 360 hours of continuous irradiation. A number of photo-degradates were formed; IN-QFH57 (13.8% AR) and IN-LXT69 (16.1% AR), IN-YY905 (8.0% AR), IN-Q3007 (6.8% AR) and IN-V0977 (12.4% AR) together with several minor components that individually accounted for < 10% AR. No significant degradation was apparent in the dark controls indicating that the degradation in irradiated samples was the result of photodegradation only.

Degradation was also rapid in the irradiated natural water test system. Aminocyclopyrachlor accounted for 5.6% AR after 144 hours of continuous irradiation and was not detected at subsequent sampling times. IN-QFH57 was the major degradation product accounting for 29.7% AR at 360 hours with other significant degradates IN-YY905, IN-Q3007 and IN-V0977 accounting for 11.7, 24.4 and 14.6% AR at 360 hours. Two other components that each accounted for more than 10% AR could not be identified though from their properties are thought to be small aliphatic molecules. Other components individually accounted for less than 10% AR.

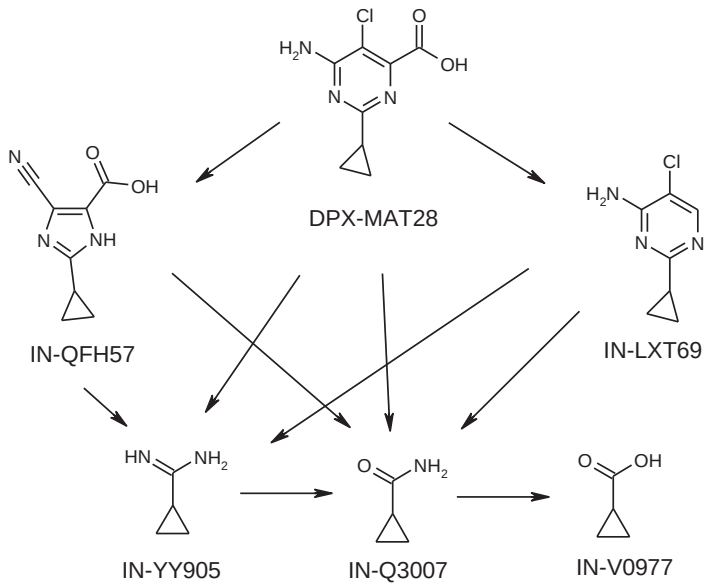


Figure 9 Proposed photodegradation pathway for aminocyclopyrachlor in pH 4 buffer and natural water.

Calculated degradation rate constants (half-lives) for aminocyclopyrachlor under constant irradiation are 7.3 days and 24.2 days for the DT_{50} and DT_{90} values in pH 4 buffer and 1.3 and 4.5 days for the DT_{50} and DT_{90} values in natural water.

Aminocyclopyrachlor is rapidly degraded by photolysis in both pH 4 sterile buffer and natural water solutions. Aminocyclopyrachlor is photodegraded through at least three separate pathways: decarboxylation to form IN-LXT69, photo-induced simultaneous elimination of hydrogen chloride and pyrimidine ring contraction to form the imidazole-nitrile IN-QHF57 and pyrimidine ring destruction to form the cyclopropyl amidine, IN-YY905 with subsequent hydrolysis to IN-Q3007 and IN-Y0977. Unidentified products are likely to be other low molecular weight compounds.

Photolysis of aminocyclopyrachlor could potentially be a significant route of degradation under environmental conditions.

Confined rotational crop studies

A confined rotational crop study was conducted on a sandy loam soil (65.9% sand, 18.7% silt, 15.4% clay; pH 6.3; 3.2% organic carbon; 15.4 meq/100 g CEC) treated with [^{14}C]-DPX-KJM44 at either 75 or 369 g ai/ha (MacDonald and Hall 2009 22559). The lower application rate was used for rotations of 15, 30, 60, 120, and 300 days while the high application rate was used with a 300 day rotation only. Seeds of cabbage and turnip were sown into the soil at 30, 60, 120 and 300 (both treatment rates) days after application (DAA). Seeds of maize (field corn) were sown into the soil at 15, 120 and 300 (both treatment rates) DAA. The trials were conducted under greenhouse conditions. The growth stages for harvest of immature crop fractions were maize forage (whole aerial portion, collected at late dough/early dent, BBCH 85), ca 50% expected mature head size (BBCH 45) for cabbage and ca 50% expected tuber diameter (BBCH 45) for turnip. Tissues were homogenised and, if necessary, extracted sequentially with acetonitrile:water (9:1, v/v) and acetonitrile:water (7:3 v/v, twice). For select samples, the post extraction solids (PES) were further extracted sequentially with enzymes (α-amylase, amyloglucosidase/cellulase combination), alkali (0.1 N NaOH at 60 °C) and acid (1 N HCl at 60 °C). The total radioactive residues (TRR) were expressed as mg equiv DPX-KJM44/kg.

Radioactive residues detected in immature and mature cabbage ranged from 0.005 to 0.027 mg equiv/kg (at both treatment rates). The majority of the ^{14}C was recovered in solvent extracts with unextracted residues amounting to no more than 0.005 mg equiv/kg. Aminocyclopyrachlor accounted for 60–61%TRR (0.008 0.014 mg equiv/kg) in mature cabbage from plots treated 30 and

60 days before sowing (low application rate) and for 83% TRR (0.010 mg equiv/kg) from plots treated 300 days before sowing (high application rate). DPX-KJM44 was only observed at trace levels (0.001 mg/kg) in mature cabbage. Unidentified residues accounted for ≤ 0.004 mg equiv/kg.

TRR in immature turnip tops at the 75 g ai/ha soil treatment rate were ≤ 0.029 mg equiv/kg while TRRs in immature turnip tubers were ≤ 0.010 mg equiv/kg. The TRR in immature turnip tops at the 369 g ai/ha soil treatment rate were 0.085 mg equiv/kg with no residues detected in the immature tubers. TRR in turnips grown from seeds sown 30, 60, 120 and 300 DAA at the lower treatment rate were ≤ 0.011 mg equiv/kg in mature turnip tops and ≤ 0.003 mg equiv/kg in the tubers. TRR in mature turnip tops and tubers from the high rate were each 0.004 mg equiv/kg.

Aminocyclopyrachlor accounted for 40 to 59% TRR (0.004–0.007 mg equiv/kg) of the ^{14}C present in mature turnip tops from crops sown 60 and 120 DAA. DPX-KJM44 (17% TRR, 0.002 mg equiv /kg) was only detected in the mature tops from the 60 DAA planting interval. Unidentified components were individually present at < 0.01 mg equiv/kg.

TRR in immature maize (forage) from seeds sown at 15, 120, 300 (75 g ai/ha) and 300 (369 g ai/ha) DAA were 0.091, 0.011, 0.028 and 0.246 mg equiv/kg, respectively; the corresponding values for stover were 0.149, 0.023, 0.058 and 0.262 mg equiv/kg, for cobs were 0.024, 0.003, ND and 0.012 mg equiv/kg, and for grain were 0.067, 0.012, 0.018 and 0.085 mg equiv/kg. The majority of the radioactivity from all maize tissues was able to be extracted with the solvent systems used (61–99% TRR). TRR in post-extraction solids did not exceed 0.009 mg equiv/kg.

Aminocyclopyrachlor was the principal component (63–70% TRR) in forage from the 15 and 120 DAA plots. DPX-KJM44 was only observed in the 15 DAA forage (10% TRR; 0.009 mg/kg) and not in forage of crops sown at later intervals after soil application. Unidentified components were also present accounting for ≤ 0.01 mg equiv/kg in total.

Aminocyclopyrachlor was also the major ^{14}C component in forage from the 300 DAA plots (low and high treatments groups) comprising 68–71% TRR. A trace amount of DPX-KJM44 (0.7% TRR; 0.002 mg/kg) was detected in forage from the high soil treatment rate. A component with similar chromatographic properties to IN LXT69 was detected at ≤ 0.01 mg equiv/kg at both soil treatment rates. Multiple (5–26) unidentified and apolar components totalling 5–21% TRR (≤ 0.05 mg equiv/kg) with individual components ≤ 0.01 mg equiv/kg were also observed in the various forage samples at both soil treatment rates. Chromatographic analysis of enzyme digests of the post extraction solids from the high soil treatment rate demonstrated the presence of aminocyclopyrachlor (11% TRR, 0.026 mg equiv/kg).

Aminocyclopyrachlor was the major component detected in all stover samples ranging from 0.014 to 0.151 mg equiv/kg. DPX-KJM44 accounted for 0.013 mg/kg in the stover sample from the 15 DAA plot, and ≤ 0.002 mg/kg in all other stover samples. A late eluting apolar unknown accounted for 2–7% TRR (≤ 0.018 mg equiv/kg) whereas a component with similar chromatographic properties to IN-LXT69 was detected at ≤ 0.001 mg equiv/kg. Multiple (12 to 27) unidentified components totalled 6–25% TRR (0.001–0.064 mg equiv/kg), with individual components ≤ 0.012 mg equiv/kg, were also observed in the various stover samples.

In grain, aminocyclopyrachlor accounted for 71–72% TRR (0.01–0.05 mg equiv/kg) in samples from the 15 and 120 DAA plots. DPX KJM44 was observed at a trace level (< 0.01 mg/kg) in the 15 DAA plot grain. Aminocyclopyrachlor accounted for 72–76% TRR in the grain from maize sown at 300 DAA at the low and high treatment rates. DPX-KJM44 (2.5% TRR) was detected in the low treatment rate sample only. Unidentified components totalled 2.2–4.2% TRR with no single component comprising greater than 0.001 mg equiv/kg were also detected.

Table 12 Identification of ^{14}C residues in rotational crops following application of ^{14}C-DPX-KJM44 to soil

	Application	Sowing	TRR			Extracted	(%TRR)			PES
RAC	rate g ai/ha	interval (DAA)	mg equiv/kg	Total	KJM44	ACP	LXT69	Apolar unknown	Un-identified	(%TRR)
Cabbage	75	30	0.023	82.4	3.3	60.0			19.1 [a]	17.6

RAC	Application rate g ai/ha	Sowing interval (DAA)	TRR mg equiv/kg	Total	KJM44	Extracted ACP	(%TRR) LXT69	Apolar unknown	Un-identified	PES (%TRR)
		60	0.013	72.4	4.6	60.6			7.2 [b]	27.6
		120	ND							
		300	ND							
	369	300	0.012	83.0	ND	83.0			ND	17.0
Turnip	75	30	0.006							
tops		60	0.010	100	17.1	40.5		ND	42.4 [c]	ND
		120	0.011	87.7	ND	59.3		23.3	5.1 [d]	12.3
		300	0.003							
	369	300	0.004							
Turnip	75	30	ND							
roots		60	ND							
		120	ND							
		300	0.003							
	369	300	0.004							
Maize	75	15	0.091	95.3	10.0	62.7	ND	4.0	1.9 [e]	4.6
forage		120	0.011	74.9	ND	69.7	ND	5.2	ND	25.1
		300	0.028	82.9	ND	68.2	1.5	4.9	8.3 [f]	17.1
	369	300	0.246	98.7	0.7	71.4	1.9	5.0	16.3 [g]	1.4
Maize	75	15	0.149	96.7	9.1	71.9	0.2	5.1	5.6 [h]	3.3
stover		120	0.023	60.9	ND	58.8	ND	2.1	ND	39.1
		300	0.058	95.2	1.2	45.9	ND	6.5	19.6 [i]	4.7
	369	300	0.262	96.8	0.9	58.0	ND	6.9	25.3 [j]	3.3
Maize	75	15	0.067	98.1	4.5	72.3		2.6	ND	1.9
grain		120	0.012	70.9	ND	70.9		ND	ND	29.1
		300	0.018	78.2	2.5	71.5		ND	4.2 [k]	21.8
	369	300	0.085	98.9	ND	76.0		1.0	1.2 [l]	1.1

[a] Two components, no single component greater than 17.5% TRR, 0.004 mg equiv/kg
[b] Three components, no single component greater than 3.4% TRR, 0.001 mg equiv/kg
[c] Three components, no single component greater than 16.4%TRR, 0.002 mg equiv/kg
[d] One single component
[e] Five components, no single component greater than 0.5%TRR, < 0.001 mg equiv/kg
[f] Eight components, no single component greater than 2.0%TRR, 0.001 mg equiv/kg
[g] 25 components, no single component greater than 1.4%TRR, 0.003 mg equiv/kg
[h] 12 components, no single component greater than 1.5% TRR, 0.002 mg equiv/kg
[i] 25 components, no single component greater than 3.0% TRR, 0.002 mg equiv/kg
[j] 27 components, no single component greater than 4.7% TRR, 0.012 mg equiv/kg
[k] Three components, no single component greater than 1.7%TRR, < 0.001 mg equiv/kg
[l] Three components, no single component greater than 0.5%TRR, < 0.001 mg equiv/kg
ACP = aminocyclopyrachlor

The proposed metabolic pathway for DPX-KJM44 in confined rotational crops is provided in Figure 10. The pathway involves de-esterification of DPX-KJM44 to form aminocyclopyrachlor. Aminocyclopyrachlor undergoes decarboxylation (to a much lesser extent) to form IN LXT69.

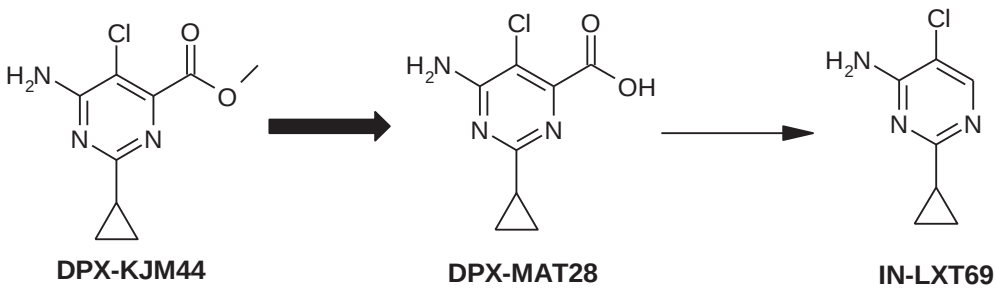


Figure 10 Proposed metabolic pathway for [^{14}C]DPX-KJM44 in confined rotational crops

Field crop rotational studies

No field crop rotational studies were made available to the meeting. From the confined rotational crop study, low levels of residues are expected in rotational crops.

METHODS OF RESIDUE ANALYSIS

Analytical methods

The Meeting received descriptions and validation data for analytical methods for residues of aminocyclopyrachlor in animal and plant matrices. The methods are suitable for analysis of DPX-KJM44, aminocyclopyrachlor and metabolites in plant matrices as well as for DPX-KJM44, aminocyclopyrachlor and IN-LXT69 in animal commodities.

A brief description of the methods used in the residue trials is given in Table 13.

Table 13 Summary of major analytical methods used for the determination of aminocyclopyrachlor and metabolites in various matrices

Method/ reference	Matrix	Extraction	Clean-up	Detection, LOQ
22582	Plant commodities	0.15 M ammonium acetate/acetonitrile. Combined extracts are diluted with 0.1 N HCl and water	aminocyclopyrachlor , DPX-KJM44, IN-LXT69, IN-QGC48: Extract aliquots concentrated under a stream of N_2 at 40 °C, diluted with 0.5% formic acid, passed through SAX SPE columns and loaded onto an Oasis® MCX SPE cartridge, eluted with 75 mM ammonium hydroxide in methanol into tubes containing 0.2% formic acid. Eluates are concentrated under stream of N_2 at 40 °C, diluted with 0.01% formic acid and filtered through 0.45 µm PTFE filter ready for analysis IN-QFH57: Extract aliquots concentrated under a stream of N_2 at 40 °C, diluted with 0.01% formic acid, loaded onto an SAX SPE cartridge, eluted with 1% formic acid in methanol into tubes containing 0.2% formic acid. Eluates are concentrated under stream of N_2 at 40 °C, diluted with 0.01% formic acid and filtered through 0.2 µm PTFE filter ready for analysis	LC-MS/MS LOQ 0.01 mg/kg
25836	Animal commodities	Milk: acetonitrile/0.1% formic acid (aq) (90:10, v/v). An aliquot is diluted with 0.01% formic acid (aq) for analysis. Tissues: acetonitrile/0.1% formic acid (aq) 90:10, 70:30 then 50:50 v/v. Extraction is on ice. Kidney, liver, fat:	Muscle: An aliquot of extract is diluted with 0.01% formic acid (aq) and passed through a MCX SPE cartridge.	LC-MS/MS LOQ 0.01 mg/kg

Plant materials

Residue Analytical Method 22582 (Penz and Nanita 2008 22582)

After grinding the samples in dry ice to a powder, the samples should be returned to the freezer until ready for analysis. The analytes were extracted sequentially from a 10 g sample by (1) soaking in 35 mL of 0.15 M ammonium acetate (aq), addition of 80 mL of acetonitrile and homogenization; and

(2) homogenization in 100-120 mL of acetonitrile/0.15 M ammonium acetate (aq) 70/30. Extracts were combined and diluted with 0.1 N HCl and water.

For analysis of DPX-KJM44, aminocyclopyrachlor, IN-LXT69, and IN-QGC48, 1–2 mL of extract aliquots were concentrated to 0.5 mL (stream of N_2, 40 °C) and diluted with 0.5% formic acid (aq). The extracts were filtered using SAX SPE cartridges and loaded onto Oasis® MCX SPE cartridges. The analytes were eluted with 15–20 mL of 75 mM ammonium hydroxide in methanol into tubes containing 1 mL of 0.2% formic acid (aq). The eluates were concentrated to a volume of 1–2 mL (stream of N_2, 40 °C), diluted with 0.01% formic acid (aq), filtered through a 0.45 μm PTFE filter and analysed by LC-MS/MS.

For analysis of IN-QFH57, 1 mL of extract aliquots were concentrated to a volume of 0.5 mL (stream of N_2, 40 °C), diluted with 0.01% formic acid (aq) and loaded onto SAX SPE cartridges. IN-QFH57 was eluted with 1% formic acid in methanol into tubes containing 0.5 mL of 0.2% formic acid (aq). The eluates were concentrated to a volume of 0.5 mL (stream of N_2, 40 °C), diluted with 0.01% formic acid (aq), filtered through a 0.2 μm PTFE filter and analysed by LC-MS/MS.

Transitions monitored for quantitation were m/z 214 → 68 for aminocyclopyrachlor (confirmation m/z 214 → 101), 228 → 68 for DPX-KJM44 (confirmation m/z 228 → 101), 170 → 76 for IN-LXT69 (confirmation m/z 170 → 103), 192→178 for IN-QGC48 (confirmation 192→132) and 176→132 for IN-QFH57 (confirmation 176→105).

Good linearity was observed for the calibration curves of DPX-KJM44, aminocyclopyrachlor, IN-LXT69, IN-QGC8 and IN-QFH57 solvent standards at 0.02–2.0 ng/mL, i.e. each had correlation coefficient, r, ≥ 0.99. Inferences were not observed for DPX-KJM44, aminocyclopyrachlor, IN-LXT69, IN-QFH57, and IN-QGC48 at their respective chromatographic retention times in the samples tested.

The LOQ determined in this method for all analytes was 0.01 mg/kg for all matrices tested. In addition, at this fortification level, the analyte peak consistently represents a signal-to-noise ratio of approximately 5–20 to 1 for the least responsive analyte.

Table 14 Aminocyclopyrachlor and metabolite recovery data obtained during validation of 22582 (recovery ± RSD)

Matrix	Level (mg/kg)	N	DPX-KJM44	Aminocyclopyrachlor	IN-QFH57	IN-QGC48	IN-LXT69	Reference
grass	0.01	5	82 ± 3	80 ± 8	91 ± 7	79 ± 14	83 ± 10	22582
forage	0.1	5	83 ± 1	79 ± 2	88 ± 5	82 ± 1	89 ± 4	Supplement No. 1,
grass	0.01	5	85 ± 6	77 ± 7	80 ± 13	84 ± 17	89 ± 4	Revision No. 2
hay	0.1	5	84 ± 3	71 ± 4	93 ± 4	87 ± 2	88 ± 2	
lettuce	0.01	5	90 ± 5	87 ± 6	75 ± 7	86 ± 10	88 ± 3	22582
	0.1	5	86 ± 3	86 ± 2	80 ± 6	93 ± 4	89 ± 4	Supplement No. 2
oranges	0.01	5	94 ± 3	94 ± 5	77 ± 6	86 ± 9	92 ± 5	
	0.1	5	93 ± 2	89 ± 3	85 ± 4	98 ± 3	93 ± 1	
sweet	0.01	5	90 ± 3	88 ± 7	87 ± 9	80 ± 11	91 ± 6	
corn	0.1	5	87 ± 8	89 ± 3	88 ± 11	90 ± 9	92 ± 7	

Rodgers (2010 30574) conducted an independent laboratory validation of the method. Two slightly different versions were studied: one, following purification, based on strong anion exchange (SAX) filtration and the other using SAX and mixed cation exchange (MCX) solid phase extraction (SPE). Additionally, the effect of replacing the 0.5% formic acid flushing solvent for the SAX cartridge with 0.1 N HCl was investigated. The simple SAX filtration method (no MCX step) combined with 0.1 N HCl flushing solvent gave satisfactory performance (Table 15).

Boughton (2011 31151) conducted an independent laboratory validation of method 22582 in grass and representative oily (corn), watery (lettuce) and acidic (oranges) crops. Acceptable recoveries were obtained for all analytes in all matrices except for IN-QFH57 in oranges.

Table 15 Aminocyclopyrachlor and metabolite recovery data obtained during independent laboratory validations of 22582

Matrix	Level (mg/kg)	N	DPX-KJM44	Aminocyclopyrachlor	IN-QGC48	IN-LXT69	IN-QFH57
Grass hay	0.01	5	69 ± 4	77 ± 5	71 ± 7	74 ± 5	–
(SAX + 0.5% formic acid)	0.1	5	75 ± 8	74 ± 8 [a]	78 ± 9	80 ± 7	–
Grass hay	0.01	5	83 ± 4	105 ± 6	87 ± 2	101 ± 17	–
(SAX + 0.1N HCl)	0.1	5	85 ± 4	108 ± 4	92 ± 3	77 ± 5	–
Lettuce	0.01	5	79 ± 4	94 ± 10	86 ± 8	85 ± 5	95 ± 5
	0.1	5	78 ± 3	84 ± 3	89 ± 2	82 ± 5	103 ± 2
Orange	0.01	5	88 ± 2	94 ± 6	95 ± 3	91 ± 5	171 ± 4
	0.1	5	88 ± 3	90 ± 3	85 ± 2	90 ± 4	171 ± 3
Corn	0.01	5	75 ± 2	77 ± 6	76 ± 10	70 ± 2	81 ± 2.5
	0.1	5	75 ± 2	76 ± 2	80 ± 1	74 ± 3	89 ± 1

[a] n=4

Nanita *et al.* (2013) reported the results of an inter-laboratory study comprising five participating laboratories in the USA that was used to evaluate the method reproducibility and ruggedness. Method validation sets for the matrix white pine were analysed at the five laboratories. One laboratory also analysed grass forage and hay samples. The results are shown in Table 16.

Table 16 Summary of aminocyclopyrachlor recoveries during method validation and concurrent fortifications for routine sample analysis at five laboratories in the USA

Laboratory	Matrix	N	%Recovery (mean ± SD) [a]
A	White pine	6	111 ± 10
B	Conifers (Norway spruce 16, white pine 7, white spruce 4)	27	100 ± 15
C	White pine	6	101 ± 7
D	White pine	10	88 ± 14
E	White pine	9	102 ± 13
A	Grass forage	10	103 ± 13
A	Grass hay	10	107 ± 5

[a] Fortification levels for aminocyclopyrachlor included 0.01, 0.1 and 1 mg/kg.

It was reported that acceptable analyte calibration curves were routinely obtained at each laboratory ($r > 0.99$, RSD response factors < 20%) and when combined with the satisfactory mean recoveries and standard deviations it is concluded the method is suitably validated for use as a single analyte method in regulatory laboratories.

RESIDUES IN FOOD OF ANIMAL ORIGIN

Residue Analytical Method 25836

The extraction procedure for milk involves extraction twice using acetonitrile/0.1% aqueous formic acid (90:10, v/v). The extracts are combined and an aliquot diluted with 0.01% aqueous formic acid prior to analysis. For tissue samples, after grinding the samples in dry ice to a powder, the samples should be returned to the freezer until ready for analysis. Muscle, liver, kidney and fat are extracted sequentially with acetonitrile/0.1% aqueous formic acid 90:10, 70:30 then 50:50 v/v (liver, kidney and muscle are extracted on ice). An aliquot of the extract of fat, liver or kidney is analysed following a dilution step with 0.01% aqueous formic acid. An aliquot from the extract of muscle is analysed following a clean-up step using mixed mode cation exchange solid phase extraction.

Aminocyclopyrachlor, DPX-KJM44, and IN-LXT69 are quantified by liquid chromatography with tandem mass spectrometry employing turbo ion spray ionisation in positive mode. Transitions monitored for quantitation were m/z 214 → 68 for aminocyclopyrachlor (confirmation m/z 214 →

101), 228 → 68 for DPX-KJM44 (confirmation m/z 228 → 101) and 170→ 76 for IN-LXT69 (confirmation m/z 170→ 103). The method LOQs were 0.01 mg/kg for each analyte.

Good linearity was observed for the calibration curves of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 solvent standards at 0.02–2.0 ng/mL, i.e. each had correlation coefficient, r, ≥ 0.99. Inferences were not observed for DPX-KJM44, aminocyclopyrachlor and IN-LXT69 at their respective chromatographic retention times in the samples tested.

As the solvent extraction procedure is close to that used in the metabolism study for which greater than 80% of the TRR were recovered from tissues and milk, the extraction procedure is considered suitable.

Recovery data reported by Pentz and Nanita (2009 25836) and also by Ward and Spence (2010 27162 rev 1) are shown in Table 17.

Table 17 DPX-KJM44, aminocyclopyrachlor and metabolite recovery data obtained during validation of the method 25836 (recovery ± RSD)

Matrix	Level (mg/kg)	N	DPX-KJM44	Aminocyclopyrachlor	IN-LXT69	Reference
muscle	0.01	5	94 ± 1	93 ± 7	74 ± 4	25836
	0.1	5	93 ± 4	97 ± 5	78 ± 3	
liver	0.01	5	81 ± 9	91 ± 4	83 ± 5	
	0.1	5	79 ± 14	89 ± 9	84 ± 13	
kidney	0.01	5	89 ± 3	99 ± 9	87 ± 14	
	0.1	5	83 ± 14	102 ± 8	91 ± 10	
fat	0.01	5	94 ± 5	87 ± 4	79 ± 3	
	0.1	5	96 ± 6	103 ± 5	87 ± 4	
milk	0.01	5	90 ± 12	95 ± 5	90 ± 10	
	0.1	5	94 ± 16	101 ± 5	97 ± 14	
Skim milk	0.01	5	106 ± 2	96 ± 3	100 ± 4	
	0.1	5	103 ± 1	101 ± 1	101 ± 2	
Heavy cream	0.01	5	97 ± 2	91 ± 2	95 ± 5	
	0.1	5	93 ± 3	100 ± 5	102 ± 1	
eggs	0.01	5	102 ± 6	94 ± 9	95 ± 5	
	0.1	5	101 ± 6	93 ± 8	99 ± 7	
fish	0.01	5	83 ± 5	84 ± 8	74 ± 6	
	0.1	5	83 ± 8	81 ± 12	78 ± 6	
muscle	0.01	3	77 ± 6	76 ± 4	73 ± 5	27162
	0.1	3	80 ± 5	75 ± 3	92 ± 10	revision no. 1
liver	0.01	3	91 ± 1	95 ± 6	91 ± 5	
	0.1	3	84 ± 4	97 ± 4	94 ± 2	
kidney	0.01	3	95 ± 7	111 ± 8	89 ± 8	
	0.1	3	100 ± 2	98 ± 5	103 ± 4	
fat	0.01	3	100 ± 4	107 ± 17	101 ± 4	
	0.1	3	100± 2	95 ± 4	97 ± 2	
milk	0.01	3	104 ± 1	89 ± 5	103 ± 2	
	0.1	3	94 ± 3	78 ± 1	93 ± 3	

In a study describing the independent laboratory validation of the method (Sheehan 2010 28151), acceptable recoveries were obtained with fortified samples though it is noted that analysis of DPX-KJM44 requires special care to ensure the compound does not hydrolyse during the analytical procedure. Initially DPX-KJM44 was hydrolysed during analysis of liver samples fortified with a mixture of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 leading to low recoveries for DPX-KJM44 and high recoveries for aminocyclopyrachlor. Subsequent minor modifications such as smaller sample sizes lead to satisfactory recoveries being obtained (liver 2). Special care should be taken to keep liver and kidney samples chilled on ice where practical during the extraction procedure.

Table 18 DPX-KJM44, aminocyclopyrachlor and metabolite recovery data obtained during validation of the method and independent laboratory validation of 25836 (recovery ± RSD)

Matrix	Level (mg/kg)	N	DPX-KJM44	Aminocyclopyrachlor	IN-LXT69	Reference
liver 1	0.01	5	31 ± 25 [a]	168 ± 20	106 ± 5	28151
	0.1	5	39 ± 31 [a]	148 ± 7	102 ± 2.8	independent
	0.01	5	33 ± 8 [b]			laboratory
	0.1	5	43 ± 13 [b]			validation
liver 2	0.01	5	83 ± 5 [a]	93 ± 19	–	
	0.1	5	80 ± 6 [a]	86 ± 9	–	
	0.01	5	97 ± 6 [b]	–	–	
	0.1	5	92 ± 5 [b]	–	–	
milk	0.01	5	107 ± 2 [a]	97 ± 11	107 ± 7	
	0.1	5	108 ± 1 [a]	101 ± 3	107 ± 2	
	0.01	5	107 ± 4 [b]			
	0.1	5	110 ± 1 [b]			
egg	0.01	5	99 ± 2 [a]	98 ± 8	101 ± 5	
	0.1	5	100 ± 1 [a]	92 ± 3	96 ± 2	
	0.01	5	99 ± 4 [b]			
	0.1	5	101 ± 3 [b]			

[a] Gradient elution
[b] Isocratic elution

Applicability of multiresidue methods

Aminocyclopyrachlor is not suitable for inclusion in QuEChERS and US FDA pesticide multi-residue methods (Nanita *et al.* 2013).

STABILITY OF RESIDUES IN STORED ANALYTICAL SAMPLES

The freezer storage stability of aminocyclopyrachlor in homogenised plant, animal tissues, milk and egg samples fortified with PDX KJM44, aminocyclopyrachlor or metabolites was studied. Except for liver and kidney, residues were stable for the duration of the storage studies.

Stability of residues in plant products

Vogl (2009b 24605) studied the freezer storage stability of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 in samples of grass forage and hay spiked separately with the compounds at a nominal rate of 0.5 mg/kg. Samples were analysed using procedures based on method 22582. The data indicate residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 are stable at approximately -20 °C for at least 500 days in grass forage and grass hay.

Table 19 Storage stability results for samples spiked with aminocyclopyrachlor, DPX-KJM44 and IN-LXT69

	Storage	aminocyclopyrachlor		DPX KJM44		IN-LXT69	
Commodity	Period (days)	(mg/kg)	Procedural Recovery (%)	(mg/kg)	Procedural Recovery (%)	(mg/kg)	Procedural Recovery (%)
Grass forage	0	0.46/ 0.47	–	0.45/ 0.46	–	0.44/ 0.46	–
	7	0.46/ 0.45	93/ 92	0.44/ 0.42	96/ 93	0.49/ 0.46	100/ 97
	14	0.40/ 0.41	86/ 91	0.40/ 0.41	85/ 89	0.43/ 0.39	87/ 87
	30	0.41/ 0.43	87/ 83	0.41/ 0.40	84/ 82	0.38/ 0.37	69/ 70
	60	0.37/ 0.40	75/ 76	0.36/ 0.36	75/ 80	0.40/ 0.40	81/ 83
	90	0.45/ 0.46	86/ 79	0.45/ 0.45	87/ 81	0.49/ 0.45	85/ 80
	150	0.41/ 0.41	79/ 89	0.41/ 0.37	82/ 90	0.41/ 0.39	82/ 89
	210	0.38/ 0.39	76/ 77	0.39/ 0.38	81/ 80	0.40/ 0.41	80/ 81
	300	0.39/ 0.42	79/ 79	0.41/ 0.38	82/ 80	0.40/ 0.45	89/ 89
	360	0.44/ 0.44	89/ 91	0.45/ 0.42	94/ 94	0.41/ 0.46	97/ 91
	500	0.41/ 0.43	88/ 87	0.44/ 0.42	90/ 92	0.44/ 0.45	93/ 93

	Storage	aminocyclopyrachlor		DPX KJM44		IN-LXT69	
Commodity	Period (days)	(mg/kg)	Procedural Recovery (%)	(mg/kg)	Procedural Recovery (%)	(mg/kg)	Procedural Recovery (%)
Grass hay	0	0.85/ 0.83	85/ 83	0.87/ 0.84	87/ 84	0.88/ 0.81	88/ 81
	7	0.78/ 0.79	81/ 81	0.89/ 0.85	85/ 82	0.79/ 0.82	85/ 85
	14	0.80/ 0.76	82/ 75	0.84/ 0.86	84/ 80	0.86/ 0.90	85/ 81
	30	0.82/ 0.84	83/ 80	0.90/ 0.93	88/ 85	0.78/ 0.82	80/ 75
	60	0.84/ 0.76	75/ 77	0.85/ 0.81	81/ 81	0.91/ 0.82	85/ 85
	90	0.90/ 0.93	92/ 89	0.95/ 0.96	99/ 98	0.93/ 0.950	96/ 92
	150	0.73/ 0.77	74/ 77	0.87/ 0.82	80/ 85	0.84/ 0.86	67/ 74
	210	0.84/ 0.81	79/ 80	0.85/ 0.86	86/ 85	0.90/ 0.91	85/ 88
	300	0.74/ 0.77	79/ 77	0.81/ 0.87	83/ 80	0.80/ 0.80	80/ 73
	360	0.88/ 0.95	95/ 97	0.89/ 0.92	97/ 95	0.91/ 0.86	89/ 90
	500	0.83/ 0.87	98/ 87	0.91/ 0.93	99/ 92	0.89/ 0.89	97/ 91

In another study, separate grass forage and grass hay control sample replicates were individually fortified with IN-QFH57 or IN-QGC48 (Vogl 2010 27890). Grass samples were fortified with the respective analyte at 0.30 mg/kg for forage and hay, and stored at approximately -20 °C.

The data indicate residues of IN-QFH57 and IN-QGC48 are stable at approximately -20 °C for at least 400 days in grass forage and grass hay.

Table 20 Storage stability results for samples spiked with aminocyclopyrachlor at 0.2–0.3 mg/kg

	Storage	IN-QFH57		IN-QGC48	
Commodity	Period (days)	(mg/kg)	Procedural Recovery (%)	(mg/kg)	Procedural Recovery (%)
Grass forage	0	0.28/ 0.27	–	0.33/ 0.33	–
	7	0.20/ 0.19	86/ 65	0.33/ 0.32	105/ 97
	14	0.23/ 0.23	91/ 90	0.31/ 0.36	119/ 118
	30	0.22/ 0.22	88/ 86	0.30/ 0.30	102/ 109
	60	0.21/ 0.21	89/ 87	0.27/ 0.27	101/ 100
	90	0.23/ 0.23	91/ 87	0.27/ 0.27	106/ 97
	150	0.23/ 0.22	86/ 77	0.28/ 0.28	117/ 109
	210	0.24/ 0.23	87/ 79	0.29/ 0.31	107/ 109
	300	0.21/ 0.20	73/ 75	0.28/ 0.28	99/ 102
	360	0.23/ 0.22	80/ 79	0.27/ 0.28	98/ 99
	400	0.25/ 0.26	101/ 94	0.29/ 0.27	105/ 91
Grass hay	0	0.35/ 0.35	–	0.31/ 0.32	–
	7	0.28/ 0.28	99/ 97	0.27/ 0.28	96/ 99
	14	0.26/ 0.26	82/ 86	0.30/ 0.30	100/ 100
	30	0.33/ 0.33	107/ 109	0.26/ 0.25	83/ 80
	60	0.25/ 0.25	94/ 81	0.23/ 0.24	76/ 79
	90	0.22/ 0.21	71/ 73	0.28/ 0.31	96/ 98
	150	0.25/ 0.28	93/ 85	0.28/ 0.29	85/ 81
	210	0.26/ 0.25	86/ 85	0.24/ 0.24	79/ 79
	300	0.30/ 0.29	114/ 113	0.27/ 0.26	92/ 98
	360	0.28/ 0.28	103/ 104	0.33/ 0.33	105/ 109
	400	0.26/ 0.26	93/ 95	0.29/ 0.31	106/ 107

Animal matrices

Aliquots of homogenized bovine muscle, liver, kidney, fat, milk and hens eggs were fortified with DPX-KJM44, aminocyclopyrachlor or IN-LXT69 at 0.2 mg/kg (Roberts and Ward 2010 26273). All samples were stored at approximately -20 °C prior to analysis after approximate intervals of 1 week, 2 weeks, 1, 2, 3 and 5 months, with the exception of kidney which was not analysed at 5 months. In addition, extracts of liver and kidney were stored at approximately -20 °C for ca. 7 and 14 days.

The freezer stability of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 was demonstrated 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. However, DPX-KJM44 was stable in liver and kidney extracts stored at *ca*. -20 °C for at least 14 days.

Table 21 Storage stability results for egg, milk and tissue samples spiked with DPX-KJM44, aminocyclopyrachlor or IN-LXT69

	Storage	DPX-KJM44	Procedural	aminocyclopyrachlor	Procedural	IN-LXT69	Procedural
Matrix	Interval (days)	(mg/kg)	Recovery (%)	(mg/kg)	Recovery (%)	(mg/kg)	Recovery (%)
Milk	0	0.22/ 0.22	98/ 101	0.18/ 0.18	87/ 94	0.22/ 0.21	99/ 106
	10	0.22/ 0.22	106/ 112	0.19/ 0.18	91/ 98	0.21/ 0.22	102/ 111
	19	0.21/ 0.21	105/ 106	0.18/ 0.18	98/ 95	0.20/ 0.21	108/ 103
	31	0.20/ 0.19	101	0.18/ 0.16	96	0.24/ 0.20	101
	59	0.19/ 0.19	96/ 95	0.19/ 0.19	97/ 106	0.21/ 0.21	106/ 107
	94	0.20/ 0.21	103/ 104	0.19/ 0.19	91/ 91	0.19/ 0.19	96/ 99
	146	0.19/ 0.20	111/ 108	0.20/ 0.18	100/ 101	0.20/ 0.20	101/ 98
muscle	0	0.23/ 0.24	111/ 125	0.16/ 0.15	71/ 82	0.17/ 0.16	84/ 86
	7	0.18/ 0.17	89/ 91	0.15/ 0.16	82/ 84	0.14/ 0.16	78/ 73
	13	0.17/ 0.18	97/ 94	0.17/ 0.19	89/ 92	0.17/ 0.17	83/ 90
	39	0.18/ 0.18	97/ 102	0.20/ 0.26	104/ 105	0.10/ 0.18	87/ 99
	55	0.15/ 0.16	86/ 90	0.12/ 0.17	103/ 100	0.16/ 0.14	88/ 91
	83	0.14/ 0.14	80/ 76	0.15/ 0.16	80/ 80	0.15/ 0.15	77/ 68
	138 [a]	0.02/ 0.02	9/ 9	0.18/ 0.18	100/ 98	0.04/ 0.04	19/ 20
liver	0	0.17/ 0.18	93/ 91	0.18/ 0.18	97/ 99	0.19/ 0.19	93/ 93
	9	0.09/ 0.09	90/ 91	0.17/ 0.16	88/ 90	0.17/ 0.17	91/ 89
	16	0.17/ 0.15	92/ 83	0.17/ 0.14	103/ 107	0.19/ 0.18	98/ 96
	28	0.15/ 0.18	95/ 92	0.18/ 0.20	119/ 113	0.19/ 0.19	99/ 101
	57	0.18/ 0.19	97/ 96	0.20/ 0.20	133/ 130	0.16/ 0.17	106/ 104
	91	0.18/ 0.17	92/ 92	0.19/ 0.19	101/ 95	0.13/ 0.16	77/ 82
	147	0.18/ 0.17	93/ 90	0.19/ 0.21	95/ 107	0.21/ 0.21	101/ 105
kidney	0	0.15/ 0.15	90/ 84	0.18/ 0.17	92/ 92	0.17/ 0.17	92/ 89
	8 [b]	0.11/ 0.11	96/ 94	0.18/ 0.18	92/ 90	0.18/ 0.17	85/ 85
	14 [c]	0.11/ 0.13	99/ 97	0.20/ 0.19	96/ 100	0.22/ 0.21	97/ 96
	26 [d]	0.11/ 0.13	102/ 99	0.21/ 0.22	108/ 108	0.18/ 0.17	102/ 93
	60	0.18/ 0.18	102/ 103	0.20/ 0.21	115/ 114	0.16/ 0.16	87/ 92
	88	0.16/ 0.17	92/ 93	0.20/ 0.22	93/ 94	0.15/ 0.15	75/ 76
Fat [f]	0	0.25/ 0.19	92/ 91	0.18/ 0.17	81/ 83	0.22/ 0.22	95/ 104
	12	0.20/ 0.20	104/ 97	0.18/ 0.18	96/ 91	0.19/ 0.20	97/ 92
	19	0.18/ 0.18	92/ 86	0.18/ 0.19	102/ 90	0.19/ 0.18	100/ 92
	46	0.20/ 0.21	102/ 104	0.20/ 0.21	113/ 116	0.22/ 0.22	111/ 118
	60	0.21/ 0.20	108/ 105	0.17/ 0.18	101/ 102	0.19/ 0.19	104/ 104
	95	0.20/ 0.20	97/ 97	0.18/ 0.15	98/ 97	0.18/ 0.17	97/ 93
	147	0.21/ 0.21	102/ 96	0.18/ 0.19	103/ 97	0.17/ 0.18	94/ 88
egg	0	0.20/ 0.19	100/ 96	0.18/ 0.17	89/ 94	0.19/ 0.19	97/ 100
	6	0.21/ 0.28	104/ 105	0.18/ 0.18	95/ 95	0.20/ 0.18	96/ 96
	18	0.19/ 0.20	101/ 103	0.18/ 0.16	100/ 101	0.17/ 0.20	102/ 107
	33	0.19/ 0.20	108/ 108	0.20/ 0.18	107/ 106	0.21/ 0.21	112/ 119
	46	0.19/ 0.19	104/ 103	0.19/ 0.18	100/ 100	0.20/ 0.20	108/ 110
	81	0.19/ 0.18	99/ 99	0.17/ 0.18	88/ 89	0.18/ 0.18	94/ 91
	133 [e]	0.16/ 0.13	89/ 87	0.19/ 0.18	101/ 99	0.21/ 0.19	100/ 99

[a] DPX-KJM44 muscle 138 d also contained 0.012 and 0.01 mg/kg aminocyclopyrachlor. No evidence of significant hydrolysis. Possible incorrect fortification. In addition, the procedural recoveries for DPX-KJM44 and IN-LXT69 were very low indicating an issue with the fortification of the muscle samples. Data for muscle day 138 should be discarded.

[b] DPX-KJM44 kidney 8 d, also contained 0.068 and 0.074 mg/kg aminocyclopyrachlor indicating significant hydrolysis of DPX-KJM44 either during storage or during sample analysis

[c] DPX-KJM44 kidney 14 d, also contained 0.070 and 0.060 mg/kg aminocyclopyrachlor indicating significant hydrolysis of DPX-KJM44 either during storage or during sample analysis

[d] DPX-KJM44 kidney 26 d, also contained 0.088 and 0.086 mg/kg aminocyclopyrachlor indicating significant hydrolysis of DPX-KJM44 either during storage or during sample analysis

[e] DPX-KJM44 egg 133 d, also contained 0.026 and 0.02 mg/kg aminocyclopyrachlor indicating significant hydrolysis of DPX-KJM44 either during storage or during sample analysis

[f] storage intervals are for aminocyclopyrachlor and IN-LXT69. Storage intervals for DPX-KJM44 were 0, 6, 13, 40, 54, 89 and 141 days.

USE PATTERNS

Aminocyclopyrachlor is a broad-spectrum herbicide that is rapidly absorbed by leaves and roots and translocated to the meristematic regions of the plant, where it is thought to act as an auxin mimic. Aminocyclopyrachlor is active on most broadleaf weeds and brush species with selectivity to most grasses and is used for the control of a range of broadleaf weeds in pasture, rangeland and industrial non-crop situations.

Table 22 Selected registered uses of aminocyclopyrachlor

Crop	Country	Formulation a		Application				PHI
		g ai/L or g ai/kg	type	Method	Rate g ai/ha	Water L/ha	No	(days)
Pasture, rangeland, industrial non-crop, industrial grassed	Canada	50%	WG	Broadcast	70 (max 70/season)	200 grd 30–50 air	1	0 b, c
Rangeland, non-crop	Canada	39.5% + 12.6% metsulfuron-methyl	WG	Broadcast	264 (max 264/season)	≤ 2000 grd 30–50 air	1	0 b, c
Pasture, rangeland, industrial non-crop	Canada	35.3% + 17.6% metsulfuron-methyl	WG	Broadcast	60	200 grd 30–50 air	1	0 b, c

[a] Product labels require the use of adjuvants: non-ionic surfactant (NIS) at 0.25% v/v or Merge Adjuvant at 1% v/v or crop oil concentrate at 1% v/v.
[b] There are no grazing or haying restrictions for non-lactating or lactating animals (including cattle, horses, sheep and goats). Grazing animals do not have to be moved off pasture or rangeland before, during or after applying aminocyclopyrachlor.
[c] If range or pasture or non-crop sites treated with aminocyclopyrachlor are to be converted to a food, feed, or fibre agricultural crop, or to a horticultural crop, a field bioassay should be completed before planting the desired crop.
grd = For application using ground based equipment
air = For application using aircraft

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

Aminocyclopyrachlor is a non-selective herbicide that is highly active against growing weeds. The Meeting received information on supervised field trials for aminocyclopyrachlor on the following crops or crop groups:

Commodity	Table No.
Grass forage	Tables 24, 25
Grass hay	Tables 26, 27

Trials were generally well documented with laboratory and field reports. Laboratory reports included method validation with procedural recoveries from spiking at residue levels similar to those occurring in samples from the supervised trials. Dates of analyses or duration of residue sample storage were also provided. Although trials included control plots, no control data are recorded in the tables except where residues in control samples exceeded the LOQ. Control samples are indicated in the summary tables with a "c". Unless stated otherwise, residue data are recorded unadjusted for recovery.

The application rates for DPX-KJM44 and aminocyclopyrachlor are expressed in terms of acid equivalents (i.e., aminocyclopyrachlor).

Residues and application rates have generally been rounded to two significant figures or, for residues near the LOQ, to one significant figure. Residue values from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels. Those results included in the evaluation are underlined.

Conditions of the supervised residue trials were generally well reported in detailed field reports. Trial designs used non-replicated plots. Field reports provided data on the sprayers used, plot size, field sample size and sampling date.

Table 23 Summary of sprayers, plot sizes and field sample sizes in the supervised trials (Shepard 2010c 24323)

Location	Year	Sprayer	Plot size	Sample size	Sample to analysis interval (days)
New Glasgow, PEI Canada	2008	CO_2 hand sprayer	20×4 m	Grass 1.1–1.2 kg hay 0.62–0.75 kg	336–376
Paris, ON, Canada	2008	CO_2 backpack sprayer	20×3 m	Grass 1.3–1.4 kg hay 0.48–0.60 kg	358–400
Elora, ON, Canada	2008	Tractor mounted boom	30×8 m 30×5 m	Grass 1.0–1.2 kg Hay 2.1–2.4 kg	142–249
Tipton, MO, USA	2008	Tractor mounted boom	9.1×15.2 m	Grass 1.5–1.9 kg Hay 0.7–0.8 kg	286–371
Springfield, NE, USA	2008	CO_2 backpack sprayer	6.1×15.2 m	Grass 1 kg Hay 0.5 kg	290–372
East Bernard, TX, USA	2008	CO_2 backpack sprayer	11.0×45.7 m	Grass 1.0–1.2 kg Hay 0.5–0.8 kg	273–401
Hinton, OK, USA	2008	CO_2 backpack sprayer	7.3×12.2 m	Grass 2.2 kg Hay 1.1 kg	247–331
Frederick, SD, USA	2008	CO_2 backpack sprayer	6.1×18.3 m	Grass 1.0–1.2 kg Hay 0.5–0.7 kg	274–365
Jamestown, ND, USA	2008	CO_2 backpack sprayer	6.1×12.2 m	Grass 1.0 kg Hay 0.5 kg	287–379
Uvalde, TX, USA	2008	CO_2 backpack sprayer	6.1×21.3 m	Grass 1.5–3.2 kg Hay 1–2 kg	295–380
Lamed, KS, USA	2008	CO_2 backpack sprayer	6.1×15.2 m 5.5×15.2 m	Grass 1.0–1.4 kg Hay 0.5–0.6 kg	282–344
Saskatoon, SK, Canada	2008	Side mounted tractor boom	4×25 m	Grass 1.2–1.6 kg Hay 1.1–1.3 kg	297–360
Hepburn, SK, Canada	2008	Tractor mounted boom	8×50 m	Grass 1.1–2.1 kg Hay 0.8–1.4 kg	257–397
Innisfail, AB, Canada	2008	Side mounted tractor boom	4×25 m	Grass 0.7–0.8 kg Hay 0.5–0.7 kg	278
Innisfail, AB, Canada	2008	Side mounted tractor boom	4×25 m	Grass 1.3–1.8 kg Hay 0.6 kg	273–377
Minto, MB, Canada	2008	Side mounted tractor boom	8×29 m	Grass 1.1 kg Hay 0.7–0.8 kg	183–300
St Andrew, MB, Canada	2008	Tractor mounted boom	3×20 m	Grass 1.0 kg Hay 0.3–0.5 kg	272–365
Arborg, MB, Canada	2008	ATV offset mounted boom	3×20 m	Grass 1.1–1.2 kg Hay 0.12–0.54 kg	268–360
Germansville, PA, USA	2008	CO_2 backpack sprayer	6.1×9.1 m	Grass 1.0–1.5 kg Hay 0.6–0.8 kg	38–97
Germansville, PA, USA	2008	CO_2 backpack sprayer	6.1×9.1 m	Grass 1.0–1.5 kg Hay 0.5–0.9 kg	38–55
Frenchtown, NJ, USA	2008	CO_2 backpack sprayer	6.1×7.6 m	Grass 1.3 kg Hay 0.5	37–65
Richland, IA, USA	2008	CO_2 backpack sprayer	15.2×12.2 m	Grass 1.0–1.2 kg Hay 0.7–0.9 kg	40–44

Where duplicate field samples from an un-replicated plot were taken at each sampling time and were analysed separately, the mean of the two analytical results was taken as the best estimate of the residues in the plot and only the means are recorded in the tables. Similarly where samples were collected from replicate plots the mean result is reported (see general consideration JMPR 2010).

Animal feeds

Grass

Twenty-two supervised residue trials with aminocyclopyrachlor or DPX-KJM44 on grass pastures were conducted in Canada and the USA in 2008 and 2009 (Shepard 2010c 24323). In these trials aminocyclopyrachlor (SL formulation) or DPX-KJM44 (WG formulations) were applied once at rates of 303–335 g ai/ha to pasture. All applications included an adjuvant at the recommended rate. Two replicate samples were collected from each treated plot. Samples of grass and hay were stored for a maximum of 401 days. Residues of aminocyclopyrachlor, DPX-KJM44, IN-LXT69, IN-QFH57 and IN-QGC48 samples were determined using method 22528. Mean concurrent recoveries for the analytes in samples fortified at 0.01 to 120 mg/kg ranged from 83 ± 11% to 95 ± 9% per analyte/matrix combination (n = 32 to 39).

Table 24 Application of DPX-KJM44 to grass: Residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 (mg/kg, mean of two replicate samples) in grass forage (rates expressed in terms of aminocyclopyrachlor equivalents)

Location, year, variety	Form	g ai/ha [e]	l/ha	GS	DAT (days) [a]	DPX-KJM44	Aminocyclopyrachlor	IN-LXT69	Moisture (%)
New Glasgow, PEI, Canada, 2008, Timothy	WG	335	209	BBCH 41	0	14 [b]	1.1 [b]	0.007 [b]	75.4
Paris, ON, Canada, 2008, Bromegrass	WG	315	200	boot	0	10	0.77	0.004	77.6
Elora, ON, Canada,	WG	316	200	BBCH 39–41	0	34	1.5	0.018	60.4
2008, Bromegrass breeder-seed EXP	WG	308	9	BBCH 39–41	0	9.9	0.75	0.007	
Tipton, MO, USA, 2008, fescue	WG	317	258	Stem elongation	0	8.8	1.0	0.005	70.6
Springfield, NE, USA, 2008, fescue K-31	WG	311	130	23 cm	0	29	5.6	0.017	77.1
East Bernard, TX,	WG	308	136	boot	–0	< 0.01	0.01	< 0.01	
USA, 2008, coastal					0	24	4.3	0.013	69.9
Bermuda grass					3	4.6	8.9	0.004	
					7	1.8	7.7	< 0.01	
					14	0.099	5.6	0.004	58.0
					21	0.05	3.6	0.004	
Hinton, OK, USA, 2008, Bermuda grass	WG	310	131	boot	0	16	1.9	0.01	69.3
Frederick, SD, USA, 2008, bluegrass/ smooth bromegrass	WG	317	94	85–90% pre-boot	0	29	4.0	0.018	58.0
Jamestown, ND, USA, 2008, bromegrass	WG	309	140	BBCH 37	0	14	3.1	0.01	70.5
Uvalde, TX, USA, 2008, Bermuda grass	WG	317	188	BBCH 45	0	29	3.2	0.017	53.1
Larned, KS, USA,	WG	317	187	BBCH 45	0	26	1.9	0.02	65.3
2008, Bermuda	WG	303	9	BBCH 45	0	11	0.53	0.006	

Location, year, variety	Form	g ai/ha [e]	l/ha	GS	DAT (days) [a]	DPX-KJM44	Aminocyclopyrachlor	IN-LXT69	Moisture (%)
grass									
Saskatoon, SK, Canada, 2008, bromegrass	WG	314	200	BBCH 32–37	0	27	2.3	0.012	69.6
Hepburn, SK,	WG	307	195	BBCH 32—37	0	< 0.01	< 0.01	< 0.01	
Canada, 2008,					0	13	2.9	0.007	77.2
meadow					3	3.9	2.8	< 0.01	
bromegrass					7	0.4	2.7	< 0.01	
					14	0.15	1.6	< 0.01	73
					21	0.027	0.92	< 0.01	
Innisfail, AB, Canada, 2008, Timothy climax	WG	319	200	BBCH 33–34	0 [c]	18	0.94	0.009	74.2
Innisfail, AB, Canada, 2008, Timothy drummond	WG	326	205	BBCH 32–34	0 [c]	18	1.8	0.01	74.8
Minto, MB, Canada, 2008, meadow bromegrass 52%, fleet and crested wheatgrass 48% Fairway	WG	310	158	6 leaves, 2 tillers	0	26	2.2	0.011	78.0
St. Andrew, MB, Canada, 2008, meadow fescue	WG	318	280	BBCH 39–40	0	13	2.0	0.008	76.6
Arborg, MB,	WG	317	281	Early boot	0	14	1.1	0.007	80.9
Canada, 2008, Barrelex tall fescue	WG	314	9	Early boot	0	13	0.71	0.006	
Germansville, PA, USA, 2008, fescue triplet mix bravo, Biltmore, Laramie	WG	309	234	Pre-boot	0 [d]	38	5.6	0.02	75.6
Germansville, PA, USA, 2008, bluegrass-unique midnight Blacksburg	WG	321	234	Pre-boot	0 [d]	47	6.6	0.028	72.2
Frenchtown, NJ, USA, 2008, tall fescue Rebel	WG	325	257	Pre-head 6–8 in	0	37	13	0.02	77.8
Richland, IA, USA, 2008, Kentucky blue grass	WG	315	181	Pre-boot	0	20	2.3	0.011	78.3

[a] DAT = days after last application (days between last application and sampling)

[b] Comparison of residues support the conclusion that control and replicate A samples were inadvertently switched. The values reported have been corrected to account for this.

[c] plot locations are the same with spray applications within one week for the two plots

[d] plot locations are the same with spray applications within one week for the two plots

[e] Adjuvants added to spray solutions are listed in order of trials in the table: 1. Agral 90 0.05% and soluble urea 2.5%; 2. Agral 90 0.05% and 28-0-0 1.0%; 3. Agral 90 0.2%, UAN 2.5%; 4. 80-20 0.25% and 28% N 1 qt/acre; 5. MSO 10% and UAN 2.5%; 6. Induce 0.25%, 32-0-0 0.25%; 7. Baron 0.9%, AMS 2.0%; 8. Preference 0.25%, N-Pa-K28 3.7%; 9. Activator 90 0.25%, 28-0-0 0.25%; 10. Dyne-Amic 0.23%, 32% liquid nitrogen 2.2%; 11. Spreader 90 0.25%, 32%UAN 0.125%; 12 Ag-Surf 0.2%, 28-0-0 0.25%; 13 Ag-Surf 0.2%, 28-0-0 0.25%; 14 Ag-Surf 0.2%, 28-0-0 0.25%; 15 Ag-Surf 0.2%, 28-0-0 0.25%; 16 Ag-Surf 0.2%, 28-0-0 0.25%; 17. Merge 0.5%, 28-0-0 0.25%; 18. Merge 0.5%, 28-0-0 0.25%; 19. Induce 0.25%, UAN 2.5%; 20. Induce 0.25%, UAN 2.5%; 21. Induce 0.25%, urea 2.5%; 22 Preference 0.25%, UAN 1 qt/ac.

Table 25 Application of aminocyclopyrachlor to grass: Residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 (mg/kg, mean of two replicate samples) in grass forage

Location, year, variety	Form	g ai/ha [c]	L/ha	GS	DAT (days) [a]	Aminocyclopyrachlor	IN-LXT69	DPX-KJM44 [b]	Moisture (%)
Paris, ON, Canada 2008, Bromegrass	SL	308	200	Boot	0	13	< 0.01	0.027	77.6
Hinton, OK, USA, 2008, Bermuda grass	SL	311	131	Boot	0	22	0.019	0.028	69.3
Frederick, SD, USA, 2008, bluegrass/ smooth bromegrass	SL	317	94	85–90% pre-boot	0	25	0.02	0.026	58.0
Uvalde, TX, USA, 2008, Bermuda grass	SL	319	190	BBCH 45	0	39	0.035	0.14	53.1
Hepburn, SK,	SL	310	197	BBCH 32–37	-0	< 0.01	< 0.01	< 0.01	
Canada, 2008,					0	18	0.004	0.016	77.2
meadow					3	9.0	0.008	0.016	
bromegrass					7	4.7	0.004	0.034	
					14	2.6	< 0.01	0.026	73
					21	1.2	< 0.01	0.024	
St. Andrew, MB, Canada, 2008, meadow fescue	SL	317	280	BBCH 39–40	0	18	0.006	0.062	76.6

[a] DAT = days after last application (days between last application and sampling)
[b] Trials are conducted in association with trials using DPX-KJM44. Residues of DPX-KJM44 are thought to arise from movement of DPX-KJM44 vapour from DPX-KJM44 treated plots to aminocyclopyrachlor treated plots.
[c] Adjuvants added to spray solutions are listed in order of trials in the table: 1. Agral 90 0.05% and 28-0-0 1.0%; 2. Baron 0.9%, AMS 2.0%; 3. Preference 0.25%, N-Pa-K28 3.7%; 4. Dyne-Amic 0.23%, 32% liquid nitrogen 2.2%; 5 Ag-Surf 0.2%, 28-0-0 0.25%; 6. Merge 0.5%, 28-0-0 0.25%;.

No residues of IN-QGC48 were detected while only occasional trace (< LOQ) residues of IN-QFH57 were detected.

Table 26 Application of DPX-KJM44 to grass: Residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 (mg/kg, mean of two replicate samples) in grass hay (rates expressed in terms of aminocyclopyrachlor equivalents)

Location, year, variety	Form	g ai/ha [e]	L/ha	GS	DALA (days) [a]	DPX-KJM44	Aminocyclopyrachlor	IN-LXT-69	Moisture (%)
New Glasgow, PEI, Canada, 2008, Timothy	WG	335	209	BBCH 41	0 + 8	29	15	0.018	37.9
Paris, ON, Canada, 2008, Bromegrass	WG	315	200	boot	0 + 10	30	3.9	0.012	34.6
Elora, ON, Canada,	WG	316	200	BBCH 39–41	0 + 18	34	4.5	0.018	19.2
2008, Bromegrass breeder-seed EXP	WG	308	9	BBCH 39–41	0 + 18	23	2.4	0.014	
Tipton, MO, USA, 2008, fescue	WG	317	258	Stem elongation	0 + 2	25	2.6	0.014	25.2
Springfield, NE, USA, 2008, fescue K-31	WG	311	130	23 cm	0 + 4	41	35	0.037	20.2

Location, year, variety	Form	g ai/ha [e]	L/ha	GS	DALA (days) [a]	DPX-KJM44	Aminocyclopyrachlor	IN-LXT-69	Moisture (%)
East Bernard, TX,	WG	308	136	boot	–0 + 4	0.042	0.013	< 0.01	
USA, 2008, coastal					0 + 4	46	16	0.029	19.0
Bermuda grass					3 + 5	9.2	20	0.01	
					7 + 4	2.8	13	0.006	
					14 + 4	0.21	9.1	0.01	15.2
					21 + 3	0.13	6.4	0.006	
Hinton, OK, USA, 2008, Bermuda grass	WG	310	131	Boot	0 + 3	31	7.1	0.022	23.4
Frederick, SD, USA, 2008, bluegrass/ smooth bromegrass	WG	317	94	85–90% pre-boot	0 + 3	38 [b]	12[B]	0.03[B]	12.8
Jamestown, ND, USA, 2008, bromegrass	WG	309	140	BBCH 37	0 + 2	21	9.2	0.015	28.3
Uvalde, TX, USA, 2008, Bermuda grass	WG	317	188	BBCH 45	0 + 2	45	6.2	0.028	6.6
Larned, KS, USA,	WG	317	187	BBCH 45	0 + 1	46	4.1	0.028	15.0
2008, Bermuda grass	WG	303	9	BBCH 45	0 + 1	19	1.1	0.012	
Saskatoon, SK, Canada, 2008, bromegrass	WG	314	200	BBCH 32–37	0 + 0	33	17	0.025	13.6
Hepburn, SK,	WG	307	195	BBCH 32–37	-0 + 11	0.005	0.007	< 0.01	
Canada, 2008,					0 + 25	14	22	0.021	11.8
meadow					3 + 22	2.5	15	0.008	
bromegrass					7 + 18	0.57	9.7	0.005	
					14 + 28	0.031	4.9	< 0.01	10.4
					21 + 21	0.038	3.1	< 0.01	
Innisfail, AB, Canada, 2008, Timothy climax	WG	319	200	BBCH 33–34	0 + 7 [c]	41	16	0.03	25.3
Innisfail, AB, Canada, 2008, Timothy drummond	WG	326	205	BBCH 32–34	0 + 9 [c]	34	24	0.024	21.7
Minto, MB, Canada, 2008, meadow bromegrass 52%, fleet and crested wheatgrass 48% Fairway	WG	310	158	6 leaves, 2 tillers	0 + 7	68	11	0.028	23.0
St. Andrew, MB, Canada, 2008, meadow fescue	WG	318	280	BBCH 39–40	0 + 20	14	22	0.016	18.1
Arborg, MB,	WG	317	281	Early boot	0 + 14	15	27	0.014	26.7
Canada, 2008, Barrelex tall fescue	WG	314	9	Early boot	0 + 14	27	12	0.016	
Germansville, PA, USA, 2009, fescue triplet mix bravo, Biltmore, Laramie	WG	309	234	Pre-boot	0 + 2 [d]	102	26	0.075	17.9
Germansville, PA, USA, 2009,	WG	321	234	Pre-boot	0 + 2 [d]	71	24	0.053	10.4

Location, year, variety	Form	g ai/ha [e]	L/ha	GS	DALA (days) [a]	DPX-KJM44	Aminocyclopyrachlor	IN-LXT-69	Moisture (%)
bluegrass-unique midnight Blacksburg									
Frenchtown, NJ, USA, 2008, tall fescue Rebel	WG	325	257	Pre-head 6–8 in	0 + 3	36	79	0.031	28.0
Richland, IA, USA, 2008, Kentucky blue grass	WG	315	181	Pre-boot	0 + 8	28	17	0.02	27.0

[a] DAT = days after last application (days between last application and sampling) + interval between cutting and sampling (windrowing time)
[b] Comparison of residues support the conclusion that control and replicate A samples were inadvertently switched. The values reported have been corrected to account for this.
[c] plot locations are the same with spray applications within one week for the two plots
[d] plot locations are the same with spray applications within one week for the two plots
[e] Adjuvants added to spray solutions are listed in order of trials in the table: 1. Agral 90 0.05% and soluble urea 2.5%; 2. Agral 90 0.05% and 28-0-0 1.0%; 3. Agral 90 0.2%, UAN 2.5%; 4. 80-20 0.25% and 28% N 1 qt/acre; 5. MSO 10% and UAN 2.5%; 6. Induce 0.25%, 32-0-0 0.25%; 7. Baron 0.9%, AMS 2.0%; 8. Preference 0.25%, N-Pa-K28 3.7%; 9. Activator 90 0.25%, 28-0-0 0.25%; 10. Dyne-Amic 0.23%, 32% liquid nitrogen 2.2%; 11. Spreader 90 0.25%, 32%UAN 0.125%; 12 Ag-Surf 0.2%, 28-0-0 0.25%; 13 Ag-Surf 0.2%, 28-0-0 0.25%; 14 Ag-Surf 0.2%, 28-0-0 0.25%; 15 Ag-Surf 0.2%, 28-0-0 0.25%; 16 Ag-Surf 0.2%, 28-0-0 0.25%; 17. Merge 0.5%, 28-0-0 0.25%; 18. Merge 0.5%, 28-0-0 0.25%; 19. Induce 0.25%, UAN 2.5%; 20. Induce 0.25%, UAN 2.5%; 21. Induce 0.25%, urea 2.5%; Preference 0.25%, UAN 1 qt/ac.

Table 27 Application of aminocyclopyrachlor to grass: Residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 (mg/kg, mean of two replicate samples) in grass hay (rates expressed in terms of aminocyclopyrachlor equivalents)

Location, year, variety	Form	g ai/ha [c]	L/ha	GS	DAT (days) [a]	Aminocyclopyrachlor	IN-LXT-69	DPX-KJM44 [b]	Moisture (%)
Paris, ON, Canada, 2008, Bromegrass	SL	308	200	Boot	0 + 10	30	0.011	0.2	34.6
Hinton, OK, USA, 2008, Bermuda grass	SL	311	131	Boot	0 + 3	46	0.08	0.11	23.4
Frederick, SD, USA, 2008,bluegrass/ smooth bromegrass	SL	317	94	85–90% pre-boot	0 + 3	35	0.05	0.084	12.8
Uvalde, TX, USA, 2008, Bermuda grass	SL	319	190	BBCH 45	0 + 2	46	0.11	0.22	6.6
Hepburn, SK, Canada, 2008, meadow bromegrass	SL	310	197	BBCH 32–37	–0 + 11	< 0.01	< 0.01	< 0.01	
					0 + 25	40	0.03	0.08	11.8
					3 + 22	25	0.021	0.081	
					7 + 18	12	0.012	0.082	
					14 + 28	5.9	0.005	0.035	10.4
					21 + 21	3.7	< 0.01	0.045	
St. Andrew, MB, Canada, 2008, meadow fescue	SL	317	280	BBCH 39–40	0 + 20	48	0.015	0.72	18.1

[a] DAT = days after last application (days between last application and sampling) + interval between cutting and sampling (windrowing time)
[b] Trials are conducted in association with trials using DPX-KJM44. Residues of DPX-KJM44 are thought to arise from movement of DPX-KJM44 vapour from DPX-KJM44 treated plots to aminocyclopyrachlor treated plots.
[c] Adjuvants added to spray solutions are listed in order of trials in the table: 1. Agral 90 0.05% and 28-0-0 1.0%; 2. Baron 0.9%, AMS 2.0%; 3. Preference 0.25%, N-Pa-K28 3.7%; 4. Dyne-Amic 0.23%, 32% liquid nitrogen 2.2%; 5 Ag-Surf 0.2%, 28-0-0 0.25%; 6. Merge 0.5%, 28-0-0 0.25%;.

No residues of IN-QGC48 were detected while only occasional trace (< LOQ) residues of IN-QFH57 were detected.

PRIMARY FEED COMMODITIES OF PLANT ORIGIN

Livestock feeding studies

Dairy cow feeding study

The transfer of aminocyclopyrachlor-methyl (DPX-KJM44) residues from feed to tissues and milk of dairy cows was studied by Roberts and Ward (2010, 26273).

DPX KJM44 was administered orally to four groups of three Holstein/Friesian cattle (4–9 yrs old; 504–704 kg bw) by gelatine capsule (with one control cow fed capsules only) for 28 days. Mean daily feed consumption during the exposure period was 14.3–16.4 kg DM (hay, *ad libitum* and 8 kg/day protein concentrate). Mean daily milk yield during the exposure period was 8.1 to 29 kg/cow/day. Based on mean daily feed consumption, the exposure was equivalent to 73, 160, 454 and 1594 ppm in the feed (1.9, 3.8, 11.4, and 37.9 mg/kg bw/day). Milk was collected twice daily (pm sampling pooled with am sampling the next day) at 11 intervals through the 28 days of dosing. Selected samples were analysed for residues of DPX-KJM44 and aminocyclopyrachlor and a metabolite, IN-LXT69. Cream and skim milk were produced from selected milk samples taken at two intervals after milk residue plateau and these samples were analysed for DPX-KJM44, aminocyclopyrachlor and IN-LXT69. Muscle, liver, kidney and fat samples were collected at sacrifice 22–24 hours after the last dose; 14–16 days in the case of the depuration animals. Due to potential instability of DPX-KJM44 in frozen storage, liver and kidney samples were extracted shortly after sacrifice. Milk, skim milk and cream samples were all analysed within approximately 2 months of collection. The maximum frozen storage intervals were 65 days for milk, 30 days for skim milk and 59 days for cream. The maximum storage intervals for tissues were 44 and 36 days for muscle and fat, respectively. Liver and kidney samples were extracted on the day of collection with the exception of selected repeat samples.

Table 28 Concurrent recovery results for DPX-KJM44, aminocyclopyrachlor and IN-LXT69 for the dairy cow feeding study

			DPX-KJM44		aminocyclopyrachlor		IN-LXT69	
	Fort level		Recoveries (%)		Recoveries (%)		Recoveries (%)	
Matrix	(mg/kg)	N	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Milk	0.01	31–36	70–116	92 ± 11	59–115	91 ± 15	63–126	100 ± 12
	0.10	31–37	86–124	108 ± 9	65–118	94 ± 10	62–113	102 ± 12
Muscle	0.01	3–4	74–82	77 ± 4	75–85	80 ± 5	64–82	75 ± 8
	0.10	3–4	80–92	85 ± 5	74–90	83 ± 9	75–82	79 ± 3
Liver	0.01	8–11	61–96	77 ± 11	88–126	107 ± 12	94–116	107 ± 8
	0.10	8–11	82–111	90 ± 8	65–121	102 ± 18	92–120	103 ± 9
Kidney [b]	0.01	8–10	85–124	93 ± 7 [a]	102–242	109 ± 7 [a]	90–110	101 ± 8
	0.10	8–10	79–113	93 ± 13	87–107	99 ± 6	92–110	100 ± 7
Fat [b]	0.01	3–4	84–93	87 ± 4	68–89	77 ± 11	91–102	95 ± 5
	0.10	4	97–102	99 ± 2	86–100	93 ± 6	81–105	95 ± 10

[a] Outliers (recovery values > 160%) omitted from calculations

[b] Note: There were 2 additional aminocyclopyrachlor kidney and fat fortifications, each, at 2 mg/kg (96 and 99% and 97 and 104% recoveries for kidney and fat, respectively).

Average aminocyclopyrachlor milk residues at each sampling interval are presented below for the two dose rates that routinely had milk residues above the LOQ.

Table 29 Daily average residues of aminocyclopyrachlor in milk of cows dosed with DPX-KJM44.

DPX-KJM44 dose	Average residue (mg/kg) in milk at various sampling intervals (days)									
	1	3	5	7	10	14	17	21	24	28
454 ppm	0.014	0.017	0.021	0.024	0.019	0.018	0.020	0.021	0.022	0.022
1594 ppm	0.038	0.050	0.055	0.053	0.050	0.053	0.061	0.059	0.071	0.077

These data suggest that residues of aminocyclopyrachlor detected in whole milk reached a plateau by 5 to 7 days of dosing.

The maximum individual milk residue for all groups at each dose level over the 28 days of dosing is summarized below (Table 30).

Table 30 Maximum residues of aminocyclopyrachlor in milk of individual cows dosed with DPX-KJM44

Analyte	Maximum individual cow daily residue in milk in mg/kg			
	73 ppm	160 ppm	454 ppm	1594 ppm
DPX-KJM44	< 0.01	< 0.01	< 0.01	< 0.01
aminocyclopyrachlor	< 0.01	0.012	0.026	0.098
IN-LXT69	< 0.01	< 0.01	< 0.01	< 0.01

The average cream and skim milk residues are presented below.

Table 31 Partitioning of residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 in skim milk and cream

Analyte	Average residue in cream and skim milk (mg/kg)				
	Matrix	73 ppm	160 ppm	454 ppm	1594 ppm
Milk day 14					
DPX-KJM44	Cream	ND	ND	ND	ND
	Skim milk	ND	ND	ND	ND
aminocyclopyrachlor	Cream	< 0.01	< 0.01	0.011	0.033
	Skim milk	< 0.01	< 0.01	0.018	0.065
IN-LXT69	Cream	ND	< 0.01	< 0.01	< 0.01
	Skim milk	ND	ND	ND	< 0.01
Milk day 21					
DPX-KJM44	Cream	ND	ND	< 0.01	< 0.01
	Skim milk	ND	ND	ND	ND
aminocyclopyrachlor	Cream	ND	< 0.01	0.012	0.033
	Skim milk	< 0.01	< 0.01	0.019	0.063
IN-LXT69	Cream	ND	< 0.01	< 0.01	< 0.01
	Skim milk	ND	ND	ND	< 0.01

ND = Not detected

Table 32 Residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 in tissues of cows dosed with DPX-KJM44

Dose (ppm)		residue in tissues in mg/kg (average)			
	Analyte	Muscle	Fat	Liver	Kidney
73	DPX-KJM44	ND ND ND (ND)	ND < 0.01 ND (< 0.01)	0.014 < 0.01 < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)
	aminocyclopyrachlor	< 0.01 ND ND (< 0.01)	0.015 ND < 0.01 (< 0.01)	0.028 0.082 < 0.01 (0.039)	0.092 0.11 0.17 (0.12)
	IN-LXT69	ND ND ND (ND)	ND ND ND (ND)	< 0.01 < 0.01 ND (< 0.01)	ND ND ND (ND)
160	DPX-KJM44	ND < 0.01 ND (< 0.01)	ND ND < 0.01 (< 0.01)	< 0.01 ND < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)
	aminocyclopyrachlor	< 0.01 < 0.01 0.012 (< 0.01)	< 0.01 < 0.01 0.040 (0.015)	0.064 0.02 0.042 (0.042)	0.40 0.29 0.23 (0.31)
	IN-LXT69	ND ND ND (ND)	ND ND ND (ND)	ND < 0.01 ND (< 0.01)	ND ND ND (ND)

454	DPX-KJM44	ND < 0.01 < 0.01 (< 0.01)	ND ND ND (ND)	ND ND ND (ND)	< 0.01 < 0.01 < 0.01 (< 0.01)
	aminocyclopyrachlor	< 0.01 < 0.01 < 0.01 (< 0.01)	0.040 0.029 0.12 (0.062)	0.075 0.025 0.046 (0.049)	0.54 0.20 0.28 (0.34)
	IN-LXT69	< 0.01 < 0.01 < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)
1594	DPX-KJM44	ND ND ND (ND)	ND ND ND (ND)	ND ND ND (ND)	< 0.01 < 0.01 < 0.01 (< 0.01)
	aminocyclopyrachlor	0.021 0.10 0.030 (0.051)	0.025 0.61 0.74 (0.46)	0.091 0.088 0.11 (0.096)	0.68 0.85 1.4 (0.98)
	IN-LXT69	< 0.01 < 0.01 < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)	< 0.01 < 0.01 < 0.01 (< 0.01)
1594	DPX-KJM44	ND	ND	ND	< 0.01
(–14 days)	aminocyclopyrachlor	0.019	0.030	< 0.01	< 0.01
	IN-LXT69	ND	ND	ND	ND
1594	DPX-KJM44	ND	ND	ND	ND
(–16 days)	aminocyclopyrachlor	ND	0.056	ND	< 0.01
	IN-LXT69	ND	ND	ND	ND

ND = Not detected.

Aminocyclopyrachlor was the only analyte with residues greater than the LOQ (0.01 mg/kg). In tissue samples obtained within approximately 24 hrs of dose completion, quantifiable aminocyclopyrachlor residues levels were highest in kidney followed by fat, liver and muscle. Where quantifiable, residue levels in fat, liver and kidney were dose dependent.

Following cessation of dosing and where present, residues in milk and tissues rapidly declined. The average residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 in milk obtained 1 day post last dose were < 0.01, 0.013 mg/kg and not detected, respectively. These residues declined to non-detectable in milk obtained 7 days post last dose.

Three days after withdrawal of dosing, residues of DPX-KJM44, aminocyclopyrachlor and IN-LXT69 declined to not detected, < 0.01 mg/kg and not detected in cream and not detected, < 0.01 mg/kg and not detected in skim milk, respectively. All residues were not detected 10 days post last dose.

Following a 15 day withdrawal period, all residues of DPX-KJM44 in tissues were not detected with the exception of kidney (< 0.01 mg/kg). Residues of aminocyclopyrachlor were < 0.01, 0.056, < 0.01 and 0.019 mg/kg in kidney, fat, liver and muscle, respectively. No residues of IN-LXT69 were detected.

Following a 17 day withdrawal period, all residues were not detected with the exception of aminocyclopyrachlor in kidney (< 0.01 mg/kg) and fat (0.03 mg/kg)

NATIONAL RESIDUE DEFINITIONS

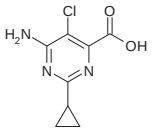
Canada: aminocyclopyrachlor

APPRAISAL

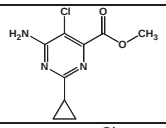
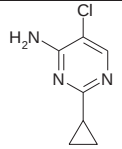
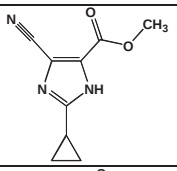
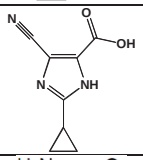
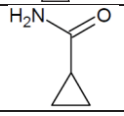
Aminocyclopyrachlor is a new pyrimidine carboxylic acid herbicide used for the control of broadleaf weeds and woody vegetation. Aminocyclopyrachlor mimics the naturally occurring phytohormone indole acetic acid (auxin) disrupting plant growth. At the Forty-fifth Session of the CCPR (2013), it was scheduled 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-2-14C]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 14C 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.

Commodity		Recommended MRL (mg/kg)		STMR or STMR-P (mg/kg)	HR, HR-P, highest residue (mg/kg)
CCN	Name	New	Previous		
MO 0105	Edible offal (mammalian)	0.3		0.039Liver 0.13Kidney	
MF 0100	Mammalian fats	0.03		0.01	
	Grass forage			74.5 dw	81 dw
AS 0162	Hay and fodder (dry) of grass	150			
MM 0095	Meat (from mammals other than marine mammals)	0.01		0.01	
ML 0106	Milks	0.02		0.01	

dw = dry weight basis

Table of recommendations animal feeds.

Commodity		Recommended MRL (mg/kg)		STMR or STMR-P (mg/kg)	HR, HR-P, highest residue (mg/kg)
CCN	Name	New	Previous		
	Grass forage			74.5 dw	81 dw

dw = dry weight basis

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.

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22551	Anand	2011b	DPX-MAT28: DPX-MAT28: Laboratory study of physicochemical properties for a. color b. odor c. physical state d. melting point e. boiling point/decomposition f. relative density g. bulk density. Advinus Therapeutics Private Limited. DuPont Report Number: DuPont-22551, Revision No. 1. Unpublished
22555	Anand	2011a	DPX-MAT28: Laboratory study of dissociation constants in water. Advinus Therapeutics Limited. DuPont Report Number DuPont-22555, Revision No. 1. Unpublished
	Bell *et al*	2011	Uptake, translocation and metabolism of aminocyclopyrachlor in prickly lettuce, rush skeletonweed and yellow starthistle. *Pest. Manag. Sci.,* 67: 1338–1348. doi: 10.1002/ps.2197
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	Bukun, B, Lindenmayer, RB, Nissen, SJ, Westra, P, Shaner, DL and Brunk,	2010	Absorption and Translocation of Aminocyclopyrachlor and Aminocyclopyrachlor-Methyl Ester in Canada Thistle (*Cirsium arvense*). *Weed Science* 58: 96-102.

Code	Author	Year	Title, Institute, Report reference
	G		
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