TRINEXAPAC-ETHYL (271)

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EXPLANATION

At the Forty-fourth Session of the CCPR (2012), trinexapac-ethyl was scheduled for evaluation as a new compound by 2013 JMPR.

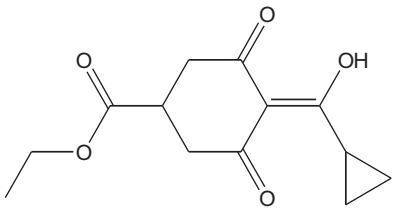
Trinexapac-ethyl is a synthetic plant growth regulator that is derived from cyclohexanecarboxylate. It is applied as a foliar spray, post-emergence and approved for use on cereal crops such as barley, durum wheat, oats, rye, triticale and wheat, oilseed rape and sugarcane as well as grassland, amenity turf and managed turf.

The manufacturer supplied information on identity, metabolism, methods of residue analysis, freezer storage stability, registered use patterns, supervised residue trials, fate of residues in processing and farm animal feeding studies.

SPECIFICATIONS

Specifications for trinexapac-ethyl have not been developed by FAO.

IDENTITY

ISO common name:	Trinexapac-ethyl
IUPAC name:	4-(cyclopropyl-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester
Chemical Abstract name:	4-(cyclopropylhydroxymethylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester
CAS Index Name: (alternative)	
CAS No.:	95266-40-3
CIPAC No.:	Not allocated
Manufacturer's experimental name:	CGA 163935
Molecular Formula:	$C_{13}H_{16}O_5$
Structural Formula:	
Molecular Weight:	252.3 g/mol

PHYSICAL AND CHEMICAL PROPERTIES

Pure Active Ingredient (except as noted)

Physical-Chemical Property	Results	Test material purity and specification	Reference
Trinexapac-ethyl			

Physical-Chemical Property	Results	Test material purity and specification	Reference
Melting point	36.1–36.6 °C.	AMS 265/102, purity 99.6%	Das 1998, CGA163935/0537
Boiling point	Trinexapac-ethyl has no boiling point at atmospheric pressure. Trinexapac-ethyl decomposed at a temperature of approximately 310 °C.	AMS 265/102, purity 99.6%	Das 2000, CGA163935/0631
Thermal stability	Trinexapac-ethyl shows no thermal effect between room temperature and 150 °C.	P.912005, TGAI	Schürch 1993, CGA163935/0299
Relative Density	Active substance, pure: 1.31 g/mL at 22 °C	AMS 265/102, purity 99.6%	Füldner 2000, CGA163935/0651
Vapour pressure	Vapour pressure curve ^{10}Log P (Pa) = 16.244 – 5637.9 × 1/T (K) extrapolated from vapour pressure curve obtained between 38 °C and 170.2 °C. Vapour pressure = 2.16×10^{-3} Pa at 25 °C	AMS 265/101, purity 99.3%	Rordorf 1990, CGA163935/0241
Henry's law constant	Henry's law constants at 25 °C: 5.4×10^{-4} Pa × m^3 × mol^{-1}. A vapour pressure of 2.16×10^{-3} Pa and a water solubility of 1.1 g/L (measured) were used to calculate the Henry's Law constant.		Burkhard 1995, CGA163935/0432
Description of the physical state and colour, purity of the ai. and of technical grade	Active substance, pure: white odourless powder Active substance as manufactured: red-brown solidified melt with weak odour	AMS 265/102, purity 99.6% P.306042, TGAI purity 96.8%	Das 2000a, CGA163935/0629 Das 2000b, CGA163935/0628
UV absorption	Solution: Neutral; Acidic; Basic Wavelength [nm]: 240.2; 277.4; 240.0; 280.4; 270.8 Molar Extinction Coefficient [L/mol × cm]: 9335; 13976; 11712; 12368; 21320	AMS 265/102, 99.6%	Roth 1997, CGA163935/0504
Solubility of purified active substance in water	pH 4.9 2.8 g/L at 25 °C pH 5.5 10.2 mg/L at 25 °C pH 8.2 21.1 mg/L at 25 °C pH 3.5 1.1 g/L at 25 °C (distilled water)	AMS 265/101, purity 99.3% AMS 265/102, purity 99.6%	Rodler 1990, CGA163935/0238 Stulz 1991 CGA163935/0351

Physical-Chemical Property	Results	Test material purity and specification	Reference
Solubility in organic solvents	[g/L at 25 °C] acetone > 500 dichloromethane > 500 ethyl acetate > 500 hexane 45 methanol > 500 octanol 420 toluene > 500	P.306042, TGAI 96.0%	Stulz 1998, CGA163935/0561
n-Octanol/ water partition coefficient	log K_{ow} at 25 °C pH 5 log K_{ow} 1.5 pH 6.9 log K_{ow} –0.29 pH 8.9 log K_{ow} –2.1	AMS 265/102, purity 99.6%	Kettner 1999, CGA163935/0621
Hydrolysis rate at pH 5, 7 and 9 under sterile and dark conditions	pH 5 $t_{0.5}$ 485–562 days pH 7 $t_{0.5}$ 828–908 days pH 9 $t_{0.5}$ 8.1 days It is considered that trinexapac-ethyl is hydrolysed to the free acid in basic solutions. Therefore hydrolysis may be a major degradation pathway in the environment in basic media with substantially slower degradation in neutral and acid environments.	PAIRA (pure active ingredient radiocarbon labelled [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl), radiochemical purity 99.3%	Spare 1989, CGA163935/0021 Spare 1990, CGA163935/0022 Spare 1992, CGA163935/0274
Direct phototransformation in sterile water using artificial light	Trinexapac-ethyl was significantly degraded in irradiated samples (pH 7.0, 20 °C, Xenon arc light) reaching 13% of the applied dose after 15 days of irradiation. Assuming a pseudo-first order kinetic, a half-life of 6.5 days was calculated, equivalent to a half-life of 21.3 equivalent days of midday summer sunlight. There was negligible degradation of the test compound in dark control vessels (< 4% in 15 days). $\Phi = 1.86 \times 10^{-3}$ at pH 7.0	Radiolabelled: [Hydroxymethylene-^{14}C] trinexapac-ethyl, radiochemical purity > 98% Non-radiolabelled: AMS 265/102, purity 99.6%	Millais 2001, CGA163935/0724
Quantum yield	The quantum yield of direct photolysis was found to be: $\Phi = 1.00 \times 10^{-3}$ at pH 7 (anionic form) $\Phi = 8.32 \times 10^{-3}$ at pH 2.3 (undissociated)	AMS 265/102, purity 99.6%	Zetzsch 1994 CGA163935/0480

Physical-Chemical Property	Results	Test material purity and specification	Reference
Dissociation in water of purified active substance	pKa = 4.57 at 20 °C	AMS 265/101, purity 99.3%	Jäkel 1990, CGA163935/0535 Burkhard 1999, CGA163935/0566
Surface tension	55.5 mN/m at 20 °C	P.306042, 96.8%	Martin 2000, CGA163935/0630
Trinexapac acid			
Hydrolysis rate at pH 4, 5, 7 and 9 under sterile and dark conditions	pH 4 $t_{0.5}$ 79.1 days at 20°C pH 5 $t_{0.5}$ 74.4 days at 20°C pH 4 $t_{0.5}$ 3.4 days at 44°C pH 5 $t_{0.5}$ 3.2 days at 44°C pH 4 $t_{0.5}$ 40 days at 25°C* pH 5 $t_{0.5}$ 37 days at 25°C* *Extrapolated values using the Arrhenius equation Trinexapac acid was found to be stable in buffer solutions at pH 7 and 9. No degradation was observed after 5 days of incubation at 50 °C in the dark.	ILA-47.2A, radiochemical purity > 99.4%	Mamouni 2006, CGA179500/0034

Formulations

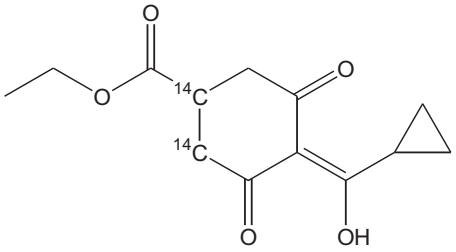
Formulation type	Active substance and content
EC (Emulsifiable Concentrate)	Trinexapac-ethyl 250 g/L
EC	Trinexapac-ethyl 249 g/L
EC	Trinexapac-ethyl 120 g/L
ME (Micro-emulsion)	Trinexapac-ethyl 250 g/L

METABOLISM AND ENVIRONMENTAL FATE

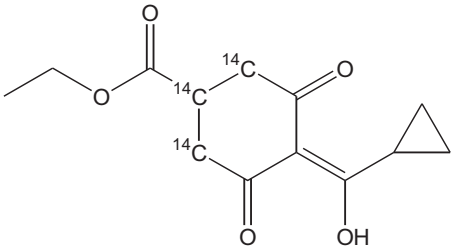
General

The studies for plant metabolism, animal metabolism and confined rotational crops were conducted with the test materials shown below, with the label position/s indicated in the following structural formulae:

[1, 2-^{14}C-cyclohexyl] trinexapac-ethyl



[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl



[Hydroxymethylene ^{14}C] trinexapac-acid

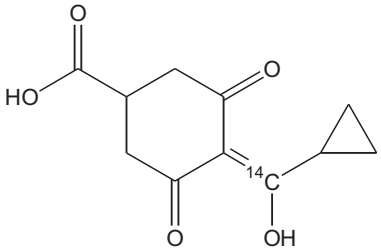
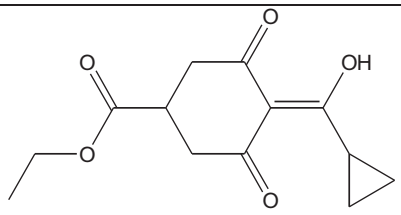
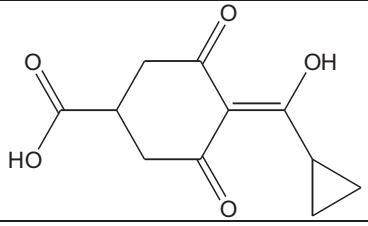
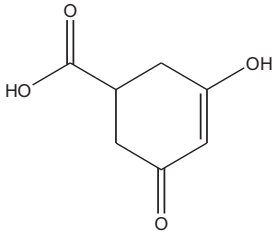
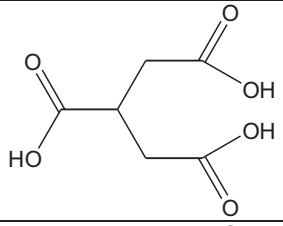
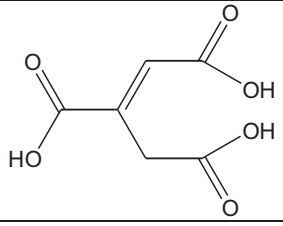
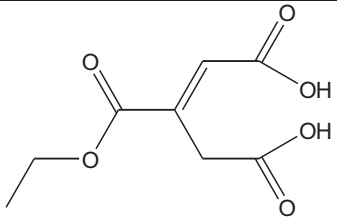
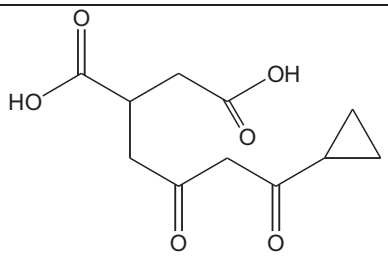
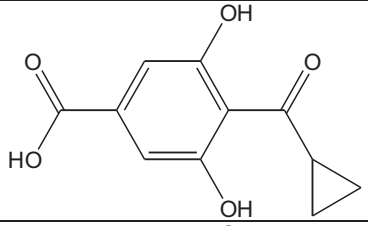
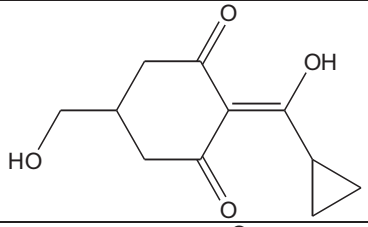
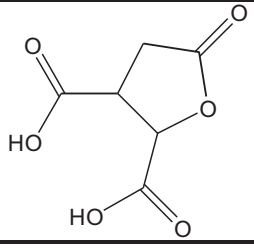


Table 1 summarises the names, codes, and structures of the parent and principle metabolites found in plant, livestock, rat and rotational crop studies.

Table 1 Trinexapac-ethyl and metabolites/degradates found in metabolism and environmental fate studies

Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
Active substance: Trinexapac-ethyl CGA 163935		4-(cyclopropyl-hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid ethyl ester	Wheat, Rapeseed, Rice, Hen (egg only)
CGA 179500 Trinexapac acid		4-(cyclopropyl-hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid	Wheat, Rapeseed, Rice, Grass, Rotational wheat, Goat, Hen
CGA 113745		3-hydroxy,5-oxocyclohexanecaboxylic acid	Goat

Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
CGA 275537 Tricarballylic acid		3-carboxy-pentanedioic acid	Wheat, Rice, Grass
CGA 312753 Trans aconitic acid		(E)-3-carboxy-pent-2-enedioic acid	Rapeseed, Rice, Rotational wheat
CGA 312753 mono-ethyl ester		(E)-3-ethoxycarbonylpent-2-enedioic acid	Wheat
CGA 313458		2-(4-cyclopropyl-4-hydroxy-2-oxo-but-3-enyl)-succinic acid	Rapeseed, Rice
CGA 329773		4-cyclopropanecarbonyl-3, 5 dihydroxy-benzoic acid	Wheat, Rice
CGA 351210		2-(cyclopropyl-hydroxymethylene)-5-hydroxymethylcyclohexane-1,3-dione	Rapeseed
– Isocitric acid lactone		5-oxo-tetrahydro-furan-2, 3 dicarboxylic acid	Rapeseed (tentative identification)

Animal metabolism

Metabolism in rats

Evaluation of the metabolism studies in rats was carried out by the WHO Core Assessment Group.

Trinexapac-ethyl undergoes limited metabolism in the rat, involving predominantly ester hydrolysis of trinexapac-ethyl to trinexapac acid. The predominant urinary metabolite was trinexapac acid (up to 100% of total urinary radioactivity), with low levels of a conjugated derivative of trinexapac acid detected only in the urine of bile-duct cannulated rats (6.3% of the administered dose). In faeces, the parent compound accounted for 5–22% of total faecal radioactivity (1–2.5% of the administered dose), with the balance comprising trinexapac acid. Bile contained mainly a conjugated derivative of trinexapac acid (2.9% of the administered dose), with low levels of the parent compound also detected (0.2% of the administered dose).

Metabolism in lactating goats

Two studies were conducted on lactating goats using [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl.

[1, 2-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered by capsule to two lactating goats (British Saanen, 43.0–47.5 kg bw during acclimatisation and 42.0–45.5 kg bw at sacrifice) at a level of either 7.2 ppm or 694 ppm in the feed for 4 consecutive days (Cameron *et al.* 1992, CGA163935/0276).

Actual doses were 7.17 or 694.0 ppm in feed based on feed consumption of 1.39 or 1.30 kg dry matter/ day or 0.20 or 19.9 mg/kg bw/day respectively. Urine, faeces and blood were collected throughout the study. Milk was sampled twice daily, in the morning and afternoon. The mean milk yield during the dosing period was 536 or 586 mL/day. Animals were sacrificed approximately 4 hours after the last dose. Samples of liver, kidney, muscle (tenderloin, hindquarter and forequarter), fat (omental, subcutaneous and renal) and rumen contents were collected.

The low dose goat eliminated 0.02%, 16.3% and 50.0% via milk, faeces and urine of the total dose applied respectively, while the corresponding values of the high dosed goat were 0.02%, 19.0% and 62.2% at 76 hours in the study.

The tissue residues of the low dosed goat were 0.035–0.043 mg/kg trinexapac-ethyl equivalents in muscles, 0.017–0.094 mg/kg trinexapac-ethyl equivalents in fat, 0.25 mg/kg equiv. in liver and 0.50 mg/kg equiv. in kidney. In the high dosed goat the residues were correspondingly higher *i.e.* 1.90–2.49 mg/kg trinexapac-ethyl equivalents in muscles, 1.20–1.55 mg/kg equiv. in fat, 12.1 mg/kg equiv. in liver and 41.9 mg/kg equiv. in kidney. The residue in the milk of the low dosed goat reached a plateau on the second day of dosing, with 0.002 mg/kg trinexapac-ethyl equivalents (am) and 0.007 mg/kg equiv. (pm). In the milk of the high dosed goat the plateau was reached on the third day when the total of am and pm is considered.

Residue levels in the corresponding tissues of the high dose goat were generally proportional but by a factor of 48 (liver)–84 (kidney) rather than the feed concentration factor of 96. An exception was subcutaneous fat, in which high dose residues were approximately 13 times the low dose residues, although it was postulated that the low dose subcutaneous fat result was an outlier.

Table 2 Recovery of radioactivity from goats dosed with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl at 7.2 ppm (low dose) and 694 ppm (high dose) in the feed for 4 consecutive days

Sample	Interval (hours)	Low dose		High dose	
		mg/kg trinexapac-ethyl equiv.	% total dose	mg/kg trinexapac-ethyl equiv.	% total dose
Total Faeces	0–76		16.3		19.0
Total Urine	0–76		50.0		62.2
Cage Wash	0–76		8.69		5.90
Total Milk	0–76		0.02		0.02
Total Eliminated	0–76		75.0		87.1

Sample	Interval (hours)	Low dose		High dose	
		mg/kg trinexapac-ethyl equiv.	% total dose	mg/kg trinexapac-ethyl equiv.	% total dose
Hindquarter muscle	76	0.035		1.90	
Forequarter muscle	76	0.043		2.49	
Tenderloin	76	0.035		2.15	
Total Muscle	76		2.17[a]		1.20[a]
Omental fat	76	0.024		1.55	
Subcutaneous Fat	76	0.094		1.20	
Renal fat	76	0.017		1.41	
Total Fat	76		0.34[a]		0.10[a]
Kidney	76	0.50	0.18	41.9	0.14
Liver	76	0.25	0.55	12.1	0.27
Total Tissue	76		3.24		1.71
Blood cells	76	0.29	1.20	17.9	0.59
Plasma	76	0.79	3.78	44.1	2.35
Bile	76	0.21	0.0	8.17	0.0
Rumen	76	0.27	3.88	31.4	3.12
Total Recovery	0–76		87.1		94.9

[a] Value calculated using mean data from the three regions taken

Table 3 Radioactivity in the milk from goats dosed with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl at 7.2 ppm (low dose) and 694 ppm (high dose) in the feed for 4 consecutive days

Study day	Low dose				High dose			
	(mg/kg trinexapac-ethyl equiv.)		% Daily Dose		(mg/kg trinexapac-ethyl equiv.)		% Daily Dose	
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
1	NA	0.004	NA	0.01	NA	0.45	NA	0.01
2	0.002	0.007	0.01	0.01	0.31	0.66	0.01	0.01
3	0.002	0.006	0.01	0.01	0.22	0.83	0.01	0.01
4	0.002	0.008[a]	0.01	0.01[a]	0.11	0.80[a]	0.01	0.01[a]

[a] Milk collected pre-sacrifice (*i.e.* 4 hours after administration of the fourth daily dose)

NA = Not Applicable

In a second study (Müller 1993, CGA163935/0305) the nature of the metabolites of trinexapac-ethyl from the previously described goat metabolism study was determined.

Radioactivity in faeces was almost completely extractable with acidified methanol/water (1:1 v/v). The extractability of the muscle, liver and kidney samples was high (> 89% of the total radioactive residues). Extraction from these matrices was by using acetonitrile/water for the low dosed goat and liver of the high dosed goat, or acetonitrile and acetonitrile/water (1:1 v/v) for kidney and muscle in the high dosed goat. In milk 53–85% of the radioactivity was recovered in the supernatants after precipitation of the proteins and lipids with acetonitrile.

In the fat pools the majority of the radioactivity (70–82%) was extracted with chloroform/ methanol (4:1 v/v for the low dose pool and 2:1 v/v for the high dose pool). In order to separate acidic from neutral compounds the extract was then partitioned with basic phosphate buffer and re-transferred into methylene chloride after acidification. Approximately 39% of the radioactivity remained in the organic layer in the low dosed pool, whereas in the high dosed pool the radioactivity of the extract was completely transferred into the basic aqueous layer and re-transferred in the methylene chloride phase for TLC analysis. It was assumed that the poor extractability from the chloroform phase of the low dose goat was due to the low residual radioactivity in a complex matrix, rather than due to differences in the nature of the metabolites present.

Trinexapac acid was the main component of TRR in urine, faeces and bile fluid of both low and high dose goats (81.6–96.1% of extractable radioactivity). Residues in muscle and kidney were also mostly trinexapac acid (80.7–89.6% TRR). In milk, trinexapac acid accounted for 45.5–76.0 % TRR. In liver and fat trinexapac acid was 30.8–67.3% TRR although for fat this represented 87.3–96.1% of the extractable radioactivity. For liver this represented only 34.1–47.2% of the extractable radioactivity, although other distinct metabolites observed in liver did not co-chromatograph with any of the reference compounds.

Table 4 Distribution of metabolites in goat tissues and milk after multiple applications of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl in the feed for 4 consecutive days

Dose Level (ppm in the feed)	Tissues	% Extracted	Quantitation (TLC)			
			Trinexapac acid			Total residues (mg/kg)
			% of radioactivity analysed	% of TRR	mg/kg	
Low (7.2)	Muscles [a]	95.4	94.0	89.6	0.034	0.038
	Fat [b]	82.1	87.3	30.8	0.014	0.045
	Liver	88.8	47.2	41.9	0.11	0.25
	Kidney	96.4	83.8	80.8	0.40	0.50
	Milk pm [c]	71.1	88.7	63.1	0.004	0.006
	Milk am [d]	52.8	86.1	45.5	0.001	0.002
High (694)	Muscles [a]	95.9	84.1	80.7	1.76	2.18
	Fat [b]	70.0	96.1	67.3	0.93	1.39
	Liver	95.8	34.1	32.7	3.96	12.1
	Kidney	97.1	84.2	81.7	34.2	41.9
	Milk pm [c]	84.6	89.8	76.0	0.49	0.65

[a] Equal amounts of fore-, hindquarter and tenderloin muscle

[b] Equal amounts of omental, subcutaneous and renal fat

[c] Pool of equal amounts collected pm on days 1, 2 and 4

[d] Pool of equal amounts collected am on days 2 and 4

ND = Not Determined

The residual radioactivity present in the rumen content 4 hours after the last dose was readily extractable (98 and 99% for the low and high dose goats respectively). The extracts contained only trinexapac acid, indicating rapid hydrolysis of trinexapac-ethyl to the corresponding carboxylic acid, under conditions present in the rumen.

In another study [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered by gelatin capsule to two lactating goats (*Capra hircus*, 47.0 and 51.5 kg bw) at a level of 95 and 125 ppm in the feed for 4 consecutive days based on feed consumption of 1.50 kg feed/ day (Ray 2002, CGA163935/0944).

Urine and faeces were collected daily and blood samples just prior to sacrifice. Milk was sampled twice daily. Animals were sacrificed approximately 6 hours after the last dose. Samples of liver, kidney, muscle (leg and tenderloin), fat (omental and perirenal), bile and gastrointestinal contents were collected. Muscle and fat samples were combined.

Approximately 90% of the total administered radioactivity was recovered; 2.54% in the faeces, 80.5% in urine, < 0.01% in the bile, 2.19% in the blood, 1.19% in the combined tissues, 3.37% in the GI tract and 0.05% in milk. The ^{14}C residue levels in composite tissues ranged from 0.106 mg/kg equiv. in the fat to 5.903 mg/kg equiv. in the kidney. The ^{14}C residue levels in composite milk ranged from 0.018 mg/kg equiv. (7–24 hour, day 1 am) to 0.078 mg/kg equiv. (0–7 hour, day 1 pm).

The extractability of the muscle, liver and kidney samples was high (> 95% of the TRRs). Extraction from these matrices was by using acetonitrile/water. The extractability of the fat samples using chloroform/methanol was 98% of the TRR. In milk 90% of the radioactivity was recovered in the supernatants after precipitation of the proteins and lipids with acetonitrile.

Parent compound was not detected in any tissue or milk. After isolation and analysis by co-chromatography and mass spectrometry, two tissue and milk metabolites were identified. Residues of trinexapac acid accounted for 66.0–96.8% TRR in milk and tissues (Table 5). CGA 113745 (formed from further hydrolysis of trinexapac acid) was a major metabolite in liver, kidney and fat (6.0–16.3% TRR) but was not detected in muscle and milk. Post-extraction solids in tissues and milk represented 4.0–9.0% TRR.

Table 5 Quantitation of metabolites in tissues and milk after multiple applications of [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl in the feed

	Liver		Kidney		Muscle		Fat		Milk (Day 2 pm)	
	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR
	0.802	100	5.903	100	0.275	100	0.106	100	0.076	100
Extractable Metabolites	0.758	94.5	5.61	95.0	0.285	103.6	0.104	97.8	0.069	90.3
CGA113745	0.131	16.3	0.354	6.0	ND	ND	0.012	11.4	ND	ND
CGA179500	0.529	66.0	5.04	85.3	0.266	96.8	0.089	83.9	0.065	85.3
Unknown G3	0.041	5.1	ND	ND	0.010	3.5	ND	ND	ND	ND
Unknown G4	0.012	1.5	ND	ND	ND	ND	0.002	1.5	ND	ND
Unidentified	0.044	5.5	0.212	3.6	ND	ND	ND	ND	0.002	2.0
Total Identified	0.660	82.3	5.39	91.3	0.266	96.8	0.101	95.3	0.065	85.3
PES	0.064	8.0	0.266	4.5	0.011	4.0	NS	NS	0.007	9.0
Total Characterised	0.724	90.3	5.66	95.8	0.277	100.8	0.101	95.3	0.072	94.3

ND = Not detected

NS = No PES sample after extraction

Metabolism in Poultry

Two studies were conducted in laying hens using [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl.

In the first study [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered daily to laying hens (White Leghorn, 1.26–1.45 kg bw prior to the start of acclimatisation until the last day of acclimatisation and 1.30–1.51 kg bw at sacrifice) using gelatine capsules at a low dose level (two hens, 0.4 mg/kg body weight equivalent to 3.8 ppm in feed) or a high dose level (four hens, 20.3 mg/kg body weight equivalent to 180 ppm in feed) for 4 consecutive days (Cameron *et al.* 1992, CGA163935/0277).

Excreta, blood and cage washes were collected throughout the study. Eggs were collected throughout the day and in the morning before subsequent administration. Egg yield for the low dose hens was 71–86% for the acclimatisation period (100% = 1 egg per hen per day) and 25–75% for the dose period, while for the high dose hens the egg yield was 86–100% for the acclimatisation period and 50–75% for the dose period. Animals were sacrificed approximately 4 hours after the last dose. Samples of lean meat (mixture of leg, thigh and breast), skin (including attached fat), peritoneal fat, liver and kidney and gizzard and crop contents were collected.

Over the period of the experiment (76 hours), 85–89% (high and low doses respectively) of the total administered dose was eliminated in excreta. Transfer of radioactivity into eggs accounted for only 0.01% and 0.02% of the total administered dose for the low and high dosed hens, respectively. A plateau was reached on Day 2 of the dosing period. Concentrations of radioactivity in egg yolk were generally less than in egg whites. Radioactivity did not accumulate in eggs with time over the period of the experiment. Concentrations of radioactivity in egg white and egg yolk in the low-dosed hens did not exceed 0.007 and 0.002 mg/kg trinexapac-ethyl equivalents, respectively. In the high dosed hens the corresponding values were 0.33 and 0.055 mg/kg trinexapac-ethyl equivalents.

Mean radioactive residues in tissues of the low-dosed hens were 0.002 mg/kg trinexapac-ethyl equivalents in lean meat (0.12 mg/kg equiv. high dose), 0.011 mg/kg equiv. in skin (including attached fat) (0.37 mg/kg equiv.), 0.003 mg/kg equiv. in peritoneal fat (0.18 mg/kg equiv.), 0.013 mg/kg equiv. in liver (0.60 mg/kg equiv.) and 0.043 mg/kg in kidney (1.77 mg/kg equiv.).

Residue levels in tissues of the high dose hens compared with levels observed in the low dose hens were greater by a factor of 33–61, which is comparable with the feed concentration factor of 47.

Table 6 Cumulative excretion of radioactivity following four consecutive daily doses of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl at an average of 3.8 ppm (low dose) and 180 ppm (high dose) in the feed

Interval (hours)	Sample	Low dose [a]	High dose [b]
		% total administered dose	% total administered dose
0–24	Excreta	24.0	20.9
	Eggs	0.01	0.01
	Cage wash	0.16	0.26
	Total excreted	24.2	21.4
0–48	Excreta	47.9	42.3
	Eggs	0.01	0.02
	Cage wash	0.97	1.04
	Total excreted	48.9	43.4
0–72	Excreta	72.2	63.0
	Eggs	0.01	0.02
	Cage wash	1.73	2.46
	Total excreted	74.0	65.5
0–76	Excreta	88.7	85.4
	Eggs	0.01	0.02
	Cage wash	4.24	4.36
	Total excreted	92.9	89.9
Gizzard contents		0.11	0.06
Crop contents		1.08	1.08
Tissues (all)		0.11	0.15
Total calculated recovery		94.2	91.1

[a] mean of two animals

[b] mean of four animals

Table 7 Radioactivity in eggs from hens dosed with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl at 3.8 ppm (low dose) and 180 ppm (high dose) in the feed for 4 consecutive days

Interval (hours)	Low dose (mg/kg equiv.) [a]		High dose (mg/kg equiv.) [b]	
	Yolk	White	Yolk	White
0-24	< 0.001	0.005	0.020	0.22
0-48	0.001	0.007	0.048	0.33
0-72	0.001	0.004	0.055	0.31
0-76	0.002	0.001	-	-

[a] mean of two animals

[b] mean of four animals

Table 8 Distribution of the radioactivity in the tissues of laying hens from hens dosed with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl at 3.8 ppm (low dose) and 180 ppm (high dose) in the feed for 4 consecutive days

Sample	Low dose (mg/kg equiv.) [a]		High dose (mg/kg equiv.) [b]	
	mg/kg trinexapac-ethyl equiv.	% total dose	mg/kg trinexapac-ethyl equiv.	% total dose
Lean meat	0.002	0.04	0.12	0.06
Skin (incl. attached fat)	0.011	NC	0.37	NC
Peritoneal fat	0.003	0.01	0.18	0.01
Liver	0.013	0.02	0.60	0.03
Kidney	0.043	0.02	1.77	0.02

Sample	Low dose (mg/kg equiv.) [a]		High dose (mg/kg equiv.) [b]	
	mg/kg trinexapac-ethyl equiv.	% total dose	mg/kg trinexapac-ethyl equiv.	% total dose
Blood cells	0.006	0.01	0.28	0.01
Plasma	0.014	0.02	0.75	0.03
Total Tissue and Blood	NA	0.11	NA	0.15
Gizzard content	0.22	0.11	5.21	0.06
Crop content	0.96	1.08	15.8	1.08
Total	NA	1.30	NA	1.29

[a] mean of two animals

[b] mean of four animals

NC = Not calculable as total weight of skin (including attached fat) is not known

NA = Not applicable

In the second study (Müller 1993a, CGA163935/0306) the nature of the metabolites of trinexapac-ethyl from the previously described hen metabolism study was determined.

A high proportion (> 97%) of the radioactivity in the excreta of low and high dosed hens was extractable using acetonitrile and acetonitrile/water (1:1 v/v), more than 90% of which was trinexapac acid. All other metabolites contributed to less than 10% of the total radioactivity.

The extractability of the radioactivity in lean meat, liver and kidney samples of low and high-dose hens was high (≥ 83% of the total radioactive residues using acetonitrile/water (1:1 v/v)).

In the fat pools the majority of the radioactivity (59–64%) was extracted with chloroform/ methanol (4:1 v/v). In order to separate acidic from neutral compounds the extract was then partitioned with basic phosphate buffer and re-transferred into methylene chloride after acidification. Approximately 11% of the radioactivity remained in the organic layer in the high dosed pool whereas in the low dosed pool the radioactivity of the extract was completely transferred into the basic aqueous layer and re-transferred in the methylene chloride phase for TLC analysis.

Radioactive residues in muscle (lean meat), liver, kidney and fat samples of low and high-dosed hens were predominantly trinexapac acid (44–84% of the total residues or 54–92% of the extractable radioactivity).

In skin including attached fat the extractability (chloroform/ methanol (4:1 v/v)) was only 14–30%. No additional radioactivity could be released from the non-extractable solid of the high-dose pool when heated in acetonitrile/ formic acid (99:1 v/v) under reflux for 2 hours. The extract was then partitioned with basic phosphate buffer and re-transferred into methylene chloride after acidification. In skin with attached fat, trinexapac acid was also the major metabolite accounting for 64–80% of the extractable radioactivity. However as the extractability was rather low, the contribution to the total radioactivity was only 9–24%.

The extractability (acetonitrile) in eggs from hens of both feed levels was 55–68% of the total radioactivity in the egg white and 45–49% in the yolk. In the yolk the major metabolite was again trinexapac acid amounting to 57–76% of the extractable radioactivity, while parent trinexapac-ethyl was present at only 10–25%. In egg white the major residue was parent accounting for 64–78% of the extractable TRR. Trinexapac acid was either not detected (high dose) or accounted for only 13% of the extractable TRR (low dose).

The gizzard contents of the low and high-dose hens, which were extracted with acetonitrile, contained unchanged parent compound (43–65%) and trinexapac acid (25–39%) indicating that hydrolysis already occurs partially in the digestive tract.

Table 9 Distribution of the radioactivity in the tissues of laying hens from hens after multiple applications with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl in the feed for 4 consecutive days

Dose Level	Tissues	% Extracted	Quantitation	
			Trinexapac acid	Other metabolites

(ppm in the feed)			% of total residues	mg/kg trinexapac-ethyl equiv.	(mg/kg)
Low (3.8)	Lean meat	90.3	59.5	0.001	0.001
	Fat	63.9	53.5	0.002	0.001
	Skin/ fat	29.9	24.0	0.003	0.008
	Liver	83.4	69.0	0.009	0.004
	Kidney	91.1	84.2	0.036	0.007
High (180)	Lean meat	90.9	49.1	0.058	0.060
	Fat	59.4	43.7	0.080	0.103
	Skin/ fat	14.1	9.0	0.033	0.332
	Liver	88.4	48.7	0.293	0.308
	Kidney	89.1	53.0	0.938	0.832

Table 10 Quantitative distribution of metabolites and parent in eggs and gizzard content after multiple applications of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl in the feed of hens for 4 consecutive days

Dose Level (ppm in the feed)	Tissues	% Extracted	Trinexapac acid		Trinexapac-ethyl	
			mg/kg trinexapac-ethyl equiv.	% of extracted	mg/kg trinexapac-ethyl equiv.	% of extracted
Low (3.8)	Egg white	55.1	0.0003	13.5	0.0017	77.8
	Egg yolk	45.4	0.0003	76.1	< 0.0001	10.2
	Gizzard content	96.5	–	38.8	–	ca. 43
High (180)	Egg white	68.3	–	ND	0.124	63.9
	Egg yolk	49.4	0.011	57.1	0.005	25.2
	Gizzard content	96.7	–	25.6	–	65.0

ND = Not Detectable

In another hen metabolism study [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered daily to five laying hens (White Leghorn, 50 weeks at acclimatisation, 1.45–1.61 kg bw at the start of acclimatisation and 1.47–1.62 kg bw at sacrifice) by gelatine capsule at 10 ppm (based on mean feed consumption of 0.125 kg/day, 1.27 mg/animal/day) in feed for 10 consecutive days (Powell 2006, CGA163935/1048).

Actual doses corresponded to 8.1–10.4 ppm feed, based on the quantity of diet consumed by each hen. Eggs were collected at 24 hour intervals throughout the morning before subsequent administration. Faeces samples were collected daily. Animals were sacrificed approximately 22 hours after the last dose. Samples of fat (skin and attached fat and peritoneal), muscle (breast and thigh), kidney, liver and gastrointestinal tract and contents were collected.

Between 87.5% and 90.6% of the administered dose was recovered in the excreta (mean 89.1%), indicating that the radioactivity was rapidly excreted. The total radioactive recoveries for the five hens ranged from 89.0–91.3% (mean 90.4%).

Table 11 Distribution of radioactivity following multiple doses of [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl in the feed

Sample	Hen 1043	Hen 1044	Hen 1045	Hen 1046	Hen 1047	Mean
	% total administered dose	% total administered dose	% total administered dose	% total administered dose	% total administered dose	% total administered dose
Egg white	0.04	0.02	0.02	0.06	0.04	0.036
Egg Yolk	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Muscle	0.2	< 0.1	< 0.1	0.1	< 0.1	< 0.12
Liver						
Skin with attached fat						
Peritoneal fat pad						
Whole blood						

Sample	Hen 1043	Hen 1044	Hen 1045	Hen 1046	Hen 1047	Mean
	% total administered dose	% total administered dose	% total administered dose	% total administered dose	% total administered dose	% total administered dose
GI tract						
Excreta	88.1	87.5	89.4	89.7	90.6	89.1
Cage wash	2.3	1.5	0.8	0.9	0.6	1.22
Total	90.6	89.0	90.3	90.7	91.3	90.4

Residue levels in the various tissue samples were all < 0.01 mg/kg equiv., indicating very little retention of the compound. Overall retention of the radioactivity in the tissues was < 0.2% of the administered dose.

Table 12 Total radioactive residues in tissue samples (expressed as trinexapac-ethyl equivalents) following multiple doses of [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl in the feed

Matrix	Hen 1043	Hen 1044	Hen 1045	Hen 1046	Hen 1047	Mean
	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.
Muscle	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Liver	< 0.003	0.005	0.008	0.006	< 0.003	0.005
Skin with attached fat	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Peritoneal fat pad	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003

Total radioactive residues (TRRs), expressed as mg/kg trinexapac-ethyl equivalents in egg yolk were very low, ranging from < 0.003–0.009 mg/kg equiv. (mean values < 0.003–0.008 mg/kg equiv.). Egg white residues ranged from 0.005–0.031 mg/kg equiv. (mean values 0.011–0.018 mg/kg equiv.). The maximum residue levels of 0.009 and 0.031 mg/kg equiv. were reached by Day 8 of the dosing period (Table 13). Overall retention of the radioactivity in eggs was < 0.1% of the administered dose.

Table 13 Total radioactive residues in eggs (expressed as trinexapac-ethyl equivalents) following multiple doses of [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl in the feed

Matrix	Hen 1043	Hen 1044	Hen 1045	Hen 1046	Hen 1047	Mean
	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.	mg/kg equiv.
Egg white						
1	0.006	0.005	0.014	0.027	0.024	0.015
2	0.012	0.007	YB	NS	0.013	0.011
3	0.014	0.009	0.016	0.021	NS	0.015
4	NS	NS	NS	0.013	0.011	0.012
5	0.006	0.007	0.011	0.014	0.026	0.013
6	0.014	0.008	0.010	0.015	0.019	0.013
7	NS	0.007	NS	0.021	0.018	0.015
8	0.014	0.008	0.012	0.031	NS	0.016
9	0.013	0.010	0.010	0.025	0.013	0.014
10	0.014	0.011	0.012	0.027	0.026	0.018
Maximum	0.014	0.011	0.016	0.031	0.026	
Egg yolk						
1	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
2	< 0.003	0.003	YB	NS	< 0.003	< 0.003
3	0.004	< 0.003	0.004	0.003	NS	0.003
4	NS	NS	NS	0.004	0.003	0.004
5	0.006	0.005	0.005	0.007	0.006	0.006
6	0.007	0.006	0.006	0.008	0.007	0.007
7	NS	0.007	NS	0.008	0.008	0.008
8	0.007	0.007	0.007	0.009	NS	0.008
9	0.007	0.007	0.008	0.009	0.008	0.008
10	0.008	0.007	0.007	0.008	0.007	0.007
Maximum	0.008	0.007	0.008	0.009	0.008	

YB = Yolk broke during separation from white, sample discarded

NS = No sample collected

TRR values for edible tissues and egg yolks were below 0.01 mg/kg trinexapac-ethyl equivalents, so no further analysis was undertaken.

A composite egg white sample (two hens, days 6–10 inclusive) was extracted with acetonitrile/ water (80:20 v/v) and acetone. After removal of acetonitrile and acidification with concentrated HCl, a liquid-liquid partition with ethyl acetate separated free organosoluble metabolites from polar residues. The major metabolites identified in the organosoluble residue were parent trinexapac-ethyl and trinexapac acid which accounted for 31.0% TRR (0.005 mg/kg equiv.) and 20.2% TRR (0.003 mg/kg equiv.) respectively (Table 14). The extract also contained at least two other components, none of which was > 0.001 mg/kg. No further analytical work was carried out on the solid debris or the aqueous fractions.

Table 14 Characterisation of ^{14}C-residues from egg whites after multiple applications with [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl in the feed

Components	Egg White	
	mg/kg equiv. trinexapac-ethyl	% TRR
Total Radioactive Residue	0.016	100.0
Identified		
Parent	0.005	31.0
Trinexapac acid	0.003	20.2
Total identified	0.008	51.2
PES	0.002	10.6
Unanalysed Aqueous Fractions	0.003	18.3
Other unanalysed fraction	0.001	3.3
Chromatographed samples	–	–
Unknown components	0.001	3.3
Remainder	0.001	6.7
Losses during work-up	0.001	6.6
Grand total	0.017[a]	100.0

[a] Slight discrepancy due to rounding of the calculated residues

Summary of animal metabolism

Animal metabolism studies were carried out with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl (goat and hens) or [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl (goats, hens and rats). No accumulation of residues was observed in any organ, tissue or animal commodity.

In the goat [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl study, parent was hydrolysed to a significant extent prior to absorption. The acid was then efficiently absorbed and excreted via urine and faeces. Of the total dose 66–81% was eliminated in milk, faeces and urine. Trinexapac acid was the main component of urine and faeces (81–96%) and accounted for 81–90% of the total residues in muscle and kidney, 46–76% of the TRRs in milk, while accounting for lower proportions in liver and fat (31–67% of the TRRs), although for fat this represented 87–96% of the extractable radioactivity. In liver this represented only 34–47% of the extractable radioactivity, although other distinct metabolites observed in liver did not co-chromatograph with any of the reference compounds.

In another study in which [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered by gelatin capsule to two lactating goats, approximately 83% was eliminated in the milk, faeces and urine. Parent compound was not detected in any tissue or milk. Residues of trinexapac acid accounted for 66.0–96.8% TRR in milk and tissues. CGA-113745 (formed from further hydrolysis of trinexapac acid) was a major metabolite in liver, kidney and fat (6.0–16.3% TRR), but was not detected in muscle and milk. The metabolite pattern in urine and bile was similar to that of tissues.

In the hen parent trinexapac-ethyl was hydrolysed to the acid in the gizzard prior to absorption. The gizzard of the low and high-dose hens contained unchanged parent compound (43–65% of extractables) and trinexapac acid (25–39% of the extractables). The acid was efficiently absorbed and excreted. Over the period of the experiment, 85–89% of the total administered dose was eliminated in excreta. A high proportion (> 97%) of the radioactivity in the excreta of low and high dosed hens was extractable, more than 90% of which was trinexapac acid. All other metabolites contributed to less than 10% of the total radioactivity. Residues in muscle (lean meat), liver, kidney and fat samples of low and high-dosed hens were predominantly trinexapac acid (44–84% of the total residues or 54–92% of the extracted radioactivity). In the skin (with subcutaneous fat) only 14–30% of the total radioactivity was extractable. Trinexapac acid was the major metabolite in the extracts (64–80% of extracted radioactivity). The major metabolite found in egg yolks was trinexapac acid (57–76% of extracted radioactivity) while parent was present at 10–25%. In egg whites the major residue was parent accounting for 64–78% of extracted radioactivity, with acid only present at up to 13%. In another study the major metabolites identified in the organosoluble residue from egg whites were parent trinexapac-ethyl and trinexapac acid which accounted for 31.0% TRR and 20.2% TRR respectively.

In the rat, residues of trinexapac-ethyl were eliminated almost completely in the urine in the form of trinexapac acid (up to 100% of total urinary radioactivity), with low levels of a conjugated derivative of trinexapac acid detected in the urine of bile-duct cannulated rats (6.3% of the administered dose). In faeces, the parent compound accounted for 5–22% of total faecal radioactivity (1–2.5% of the administered dose), with the balance comprising trinexapac acid. Bile contained mainly a conjugated derivative of trinexapac acid (2.9% of the administered dose), with low levels of the parent compound also detected (0.2% of the administered dose).

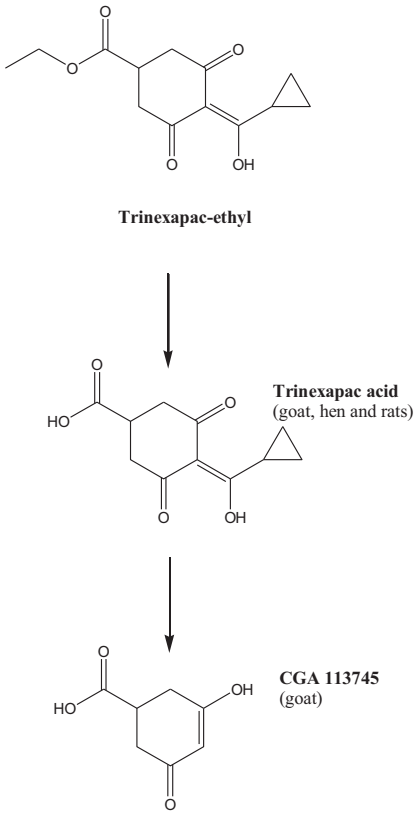


Figure 1 Proposed metabolic pathway for trinexapac-ethyl in animals

Plant metabolism

The metabolism of trinexapac-ethyl in spring wheat, spring rape, paddy rice and grass was investigated. The radiolabelled material was either [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl or [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl.

Rice

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was applied by foliar methods to paddy rice (Koshihikari) in water at rates of 40 and 160 g ai/ha (Gross 1996, CGA163935/0482). Applications were made at the lower rate 42 days after transplantation (stem elongation and stem node formation BBCH 37–41), or at the higher rate 64 days after transplantation (early panicle emergence). For the low application rate samples of rice plants were taken one hour as well as 7 and 21 days (full panicle emergence) after treatment. At maturity (82 days after treatment) the rest of the plant material was harvested and divided into grains, husks and straw.

The total radioactive residues (TRRs) are summarized below in Table 15. Total residues at harvest were 0.085 mg/kg trinexapac-ethyl equivalents in grain, 0.168 mg/kg equiv. in husks and 0.161 mg/kg equiv. in straw, indicating translocation of radioactivity from treated plants (foliage) into new growths. At maturity the parent content was less than 0.003 mg/kg in all plant parts. Non-extractable residues at maturity accounted for 40.5%, 41.1% and 72.0% of the total radioactivity in straw, husks and grain respectively.

Table 15 Distribution of radioactivity in various plant parts of rice, paddy water and soil after foliar application of [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl at 40 g ai/ha

Interval after application	Matrix	TRRs		Parent (mg/kg trinexapac-ethyl equiv.)	Extracted radioactivity (%)[b]		Non-extracted (%)[b]	Total (%)[b]
		(mg/kg trinexapac-ethyl equiv.)	(%) [a]		Cold extract [c]	Microwave[d]		
1 hour	Foliage	0.565	100	0.373	97.2	1.2	1.4	99.8
	Paddy water	0.020		NA				
7 days (Flowering)	Foliage	0.138	100	0.008	88.6	3.7	8.1	100.4
	Paddy water	0.002		NA				
21 days	Foliage	0.066	100	0.001	82.1	5.5	15.7	103.2
	Paddy water	< 0.001		NA				
82 days	Straw	0.161	100	0.002	56.5	10.4	40.5	107.4
(Maturity)	Husks	0.168	100	0.002	55.0	8.3	41.1	104.4
	Grain	0.085	100	< 0.001	16.2	12.5	72.0	100.7
	Soil	0.014		< 0.001	NA	5.1	96.2	101.3

[a] % of radioactivity found in plant part

[b] % of radioactivity found in plant part and determined by combustion

[c] Using methanol/ water (8:2 v/v)

[d] Using n-propanol/ water (8:2 v/v)

NA = not analysed

In the case of the 160 g ai/ha treatment plant samples were taken only at maturity (60 days after treatment) and divided into grains, husks and straw. The extractability of the residues from harvested material treated with 160 g ai/ha is summarized in Table 16.

Table 16 Distribution of radioactivity in various plant parts of rice after foliar application of [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl at 160 g ai/ha

Interval after application	Matrix	TRRs		Parent (mg/kg trinexapac-ethyl equiv.)	Extracted radioactivity (%)[b]		Non-extracted (%) [b]	Total (%) [b]
		(mg/kg trinexapac-ethyl equiv.)	(%)[a]		Cold extract [c]	Microwave[d]		
60 days	Straw	1.584	100	0.011	73.2	8.2	19.1	100.5
(Maturity)	Husks	2.223	100	0.078	78.0	5.3	16.0	99.3
	Grain	1.067	100	0.004	33.9	19.6	45.0	98.5

[a] % of radioactivity found in plant part

[b] % of radioactivity found in plant part and determined by combustion

[c] Using methanol/ water (8:2 v/v)

[d] Using n-propanol/ water (8:2 v/v)

In foliage unchanged parent compound decreased from 66.0% of the TRR at one hour after application, to 5.5% TRR after 7 days which indicated rapid degradation of the molecule. In addition to parent, up to ten metabolite fractions were observed, the major component being trinexapac acid (17.8–26.1% of TRR). Other metabolites observed included CGA 313458 (2.2–5.1% of the TRR), CGA 275537 or tricarballylic acid (0–4.0% of the TRR) and CGA 312753 or trans aconitic acid (0–2.6% of the TRR).

Table 17 Summary of identified components in rice foliage after treatment with [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl (40 g ai/ha treatment)

Metabolite	Rice matrix					
	Foliage E_1 [a] (1 hr after application)		Foliage E_1 [a] (7 days after application)		Foliage E_1 [a] (21 days after application)	
	(mg/kg trinexapac-ethyl equiv.)	% TRR	(mg/kg trinexapac-ethyl equiv.)	% TRR	(mg/kg trinexapac-ethyl equiv.)	% TRR
	0.565	100	0.138	100	0.066	100
II_1			0.025	18.3	0.014	21.8
II_2			0.008	6.0	0.004	5.4
II_3 CGA312753 (Aconitic acid)			0.004	2.5	0.002	2.6
II_4			0.004	2.9	0.002	2.7
II_5 CGA 275537 (Tricarballylic acid)			0.006	4.0	0.003	3.9
II_6			0.009	6.5	0.005	7.1
II_7			0.003	1.9		
II_8 CGA 313458	0.012	2.2	0.007	5.1	0.002	2.6
II_9 CGA 329773						
II_{10} Trinexapac acid	0.101	17.8	0.036	26.1	0.017	25.5
II_{11} Trinexapac-ethyl	0.373	66.0	0.008	5.5	0.001	1.6
Unresolved	0.043	7.6	0.014	9.8	0.006	8.9
Non-extractables	0.008	1.4	0.011	8.1	0.010	15.7
Total		95.0		96.7		97.8

[a] E_1 = cold extract

Extractability of residues in grain was low. Only 16.2% of the radioactivity could be extracted with methanol/ water and another 12.5% were extracted with aqueous propanol under harsh microwave conditions. The extractable radioactivity consisted mainly of trinexapac acid with 11.6% of the TRR. Absolute amounts were low, all fractions being significantly below 0.01 mg/kg, except trinexapac acid with exactly 0.01 mg/kg (Table 18).

Table 18 Summary of identified components in rice grain, husks and straw 82 days after treatment with [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl (40 g ai/ha treatment)

Metabolite	Rice matrix												Soil	
	Grain				Husks				Straw					
	E_1[a]	E_2[b]	$\Sigma tr_1 + E_2$		E_1[a]	E_2[b]	$\Sigma E_1 + E_2$		E_1[a]	E_2[b]	$\Sigma E_1 + E_2$		E_1	
	% TRR	% TRR	mg/kg equiv.	% TRR	% TRR	% TRR	mg/kg equiv.	% TRR	% TRR	% TRR	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR
			0.085	100			0.168	100			0.161	100	0.014	100
II_1	0.7	6.5	0.007	8.0	30.0	4.5	0.058	34.5	24.9	4.6	0.048	29.5	–	–
II_2	0.4								16.4	3.0	0.031	19.4	–	–
II_3 CGA312753	0.4												–	–
II_4	–				–	–	0.003	–					–	–
II_5 CGA 275537 (Tricarballylic acid)	0.5		< 0.001	0.5	3.2	–	0.005	3.2					–	–
II_6	1.3	1.2	0.003	2.9	3.9	0.5	0.007	4.4	2.0	0.4	0.004	2.4	–	–
II_7	–				1.3	–	0.002	1.3	–	–	–	–	–	–
II_8 CGA 313458	–				–	–			3.9	0.7	0.007	4.6	–	–
II_9 CGA 329773	0.4				0.7	–	0.001	0.7	–	–	–	–		0.6
II_{10} Trinexapac acid	10.0	1.6	0.010	11.6	8.3	0.6	0.015	8.9	4.2	0.7	0.008	4.9		0.7
II_{11} Trinexapac-ethyl	0.1		< 0.001	0.1	1.8	–	0.003	1.8	0.9	–	0.001	0.9		0.5
Unresolved	3.8		0.004	5.6	9.5	0.5	0.007	10.0	5.2	1.0	0.006	6.2		1.0
Non-extractables	–		0.061	72.0	–	–	0.069	41.1	–	–	0.065	40.5		96.2
Total				99.4				100.2				106.0		101.3

[a] E_1 = cold extract

[b] E_2 = microwave extract

Due to the low residues and high % of non-extractable radioactivity in grain, the matrices of the 160 g ai/ha experiment were used for further characterisation. As also observed for the 40 g ai/ha treatment, trinexapac acid was the major component. Together with the microwave fraction this metabolite accounted for 28.1% of the total residues in grain. Other metabolites observed included parent trinexapac-ethyl (0.7% of the TRR), CGA 329773 (0.7% of the TRR), CGA 313458 (1.6% of the TRR) and CGA 275537 or tricarballylic acid (2.1% of the TRR) (Table 19). Partition of the aqueous residues of E_1 with diethyl ether at pH 4 gave 73% of the radioactivity in the organic phase and 27% in the water phase. The organic phase consisted mainly of trinexapac acid and the water phase the polar fractions II_1, II_2 and II_{3a}. Further enzymatic and mild chemical treatment (0.1 N NaOH) showed that fractions II_1 and II_2 in grain consisted mainly of trinexapac acid in various conjugated forms, probably as esters of sugars and/ or plant constituents.

To characterise the methanol/water insoluble radioactivity in grain the residue R_1 (before microwave extraction) was analysed by digestion with 0.5 N NaOH followed by hydrolysis of the starch at pH 1. 70.1% of the radioactivity was eluted as a weakly acidic fraction. This radioactivity showed a similar TLC pattern to that already observed for the extractable radioactivity with fraction II_1-II_2 and II_{10} as major spots. Formation of [^{14}C]-osazone (8% of TRR) from the neutral water eluate indicated incorporation of carbon fragments into the sugar pool of the plant (Table 20).

In husks, extractable radioactivity of 160 g ai/ha treatment consisted mainly of II_1 (20.9% of the TRR), II_2 (8.5% of the TRR), trinexapac acid (13.8% TRR), tricarballylic acid (8.2% TRR) and trinexapac-ethyl (6.1% TRR) (Table 19). Partition of the aqueous residues with diethyl ether at pH 4 showed 26% of the radioactivity in the organic phase and 74% in the water phase. The organic phase consisted mainly of trinexapac acid and parent whereas the water phase consisted mainly of the polar fractions II_1, II_2 and II_{3a} as well as tricarballylic acid. The polar fractions II_1 through II_{3a} consisted mainly of acid and hydrolysis product of trinexapac acid, tricarballylic acid and CGA 313458 in various conjugated forms, most probably as esters of sugars and/or plant constituents.

In straw from the 160 g ai/ha treatment besides the dominant fractions II_1 (21.2% of the TRR) and II_2 (17.0% of the TRR), tricarballylic acid (10.7% of the TRR) was a major metabolite followed by trinexapac acid (6.9% TRR) and CGA 313458 (7.8% of the TRR) (Table 19).

Partition of the aqueous residues of E_1 of 160 g ai/ha treatment with diethyl ether at pH 4 gave 22% of the radioactivity in the organic phase and 78% in the water phase. The organic phase consisted mainly of trinexapac acid, II_8 = CGA313458, II_9 = CGA329773 and parent whereas the water phase consisted mainly of the polar fractions II_1, II_2, and II_8 as well as tricarballylic acid. Chemical hydrolysis of the water phase (0.1 N NaOH) cleaved metabolite fraction II_2 (17.0% TRR) completely in favour of tricarballylic acid and trinexapac acid proving that II_2 in straw consisted of conjugate esters of the tricarballylic acid and trinexapac acid. Fraction II_1 was not altered by acid or base treatment. To characterise the non-extractable radioactivity in straw of 160 g ai/ha treatment after cold methanol and microwave extraction the residue R_2 was analysed. After boiling in water, 35% of the residual radioactivity was extracted, containing mainly water-soluble polysaccharides of the cell wall material. The remaining insoluble part was solubilised with boiling 2 N NaOH. Using this procedure another 49.5% was released from the non-extractable radioactivity containing mainly hemicellulose and lignin of the cell wall material. Quantitation of the fractions originating from the non-extractable radioactivity in straw is given in Table 20.

Table 19 Summary of identified components in rice grain, husks and straw 60 days after treatment with [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl (160 g ai/ha treatment)

Metabolite	Rice matrix											
	Grain				Husks				Straw			
	E_1 [a]	E_2 [b]	$\Sigma tr_1 + E_2$		E_1 [a]	E_2 [b]	$\Sigma E_1 + E_2$		E_1 [a]	E_2 [b]	$\Sigma E_1 + E_2$	
	% TRR	% TRR	mg/kg equiv.	% TRR	% TRR	% TRR	mg/kg equiv.	% TRR	% TRR	% TRR	mg/kg equiv.	% TRR
			1.067	100			2.223	100			1.584	100
II_1	2.6	2.6	0.055	5.2	20.2	0.7	0.465	20.9	18.4	2.8	0.336	21.2
II_2	0.9	–	0.010	0.9	7.4	1.1	0.189	8.5	16.5	0.5	0.269	17.0
II_{3a}	1.3	0.5	0.019	1.8	5.1	–	0.113	5.1	–	–	–	–
II_{3b}	–	–	–	–	3.4	–	0.076	3.4	–	–	–	–
II_4	–	–	–	–	–	–		–	2.1	–	0.033	2.1
II_5 CGA 275537 (Tricarballylic acid)	1.9	0.2	0.022	2.1	8.0	0.2	0.182	8.2	10.4	0.3	0.169	10.7
II_6	0.8	–	0.009	0.8	2.7	–	0.060	2.7	0.6	–	0.010	0.6
II_7	0.3	1.0	0.014	1.3	1.3	0.3	0.036	1.6	–	–	–	–
II_8 CGA 313458	–	1.6	0.017	1.6	1.4	0.5	0.042	1.9	7.0	0.8	0.124	7.8
II_9 CGA 329773	–	0.7	0.007	0.7	0.8	0.3	0.024	1.1	0.8	–	0.013	0.8
II_{10} Trinexapac acid	23.0	5.1	0.30	28.1	13.4	0.4	0.307	13.8	6.7	0.2	0.109	6.9
II_{10a}	–	1.4	0.015	1.4	–	0.2	0.004	0.2	–	–	–	–
II_{10b}	–	1.7	0.018	1.7	–	0.7	0.016	0.7	–	–	–	–
II_{11} Trinexapac-ethyl	0.4	0.3	0.007	0.7	6.1	–	0.136	6.1	1.3	0.1	0.022	1.4
Unresolved	2.7	3.9	0.070	6.6	7.5	0.9	0.187	8.4	7.5	2.1	0.152	9.6
Non-extractables			0.48	45.0			0.356	16.0			0.303	19.1
Total				97.9				98.6				97.2

[a] E_1 = cold extract

[b] E_2 = microwave extract

Table 20 Summary of identified components in rice grain, husks and straw after solvent partition, 0.1 N NaOH treatment of cold extract E_1 and non-extractable analysis (160 g ai/ha treatment with [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl)

Metabolite Fractions	Rice matrix											
	Grain				Husks				Straw			
	E_1 Org. phase	E_1 Water Phase [a]	NE Non-extr. [b]	Σ	E_1 Org. phase	E_1 Water Phase [a]	NE Non-extr.	ΣE N_1 Org. phase	E_1 Org Phase	E_1 Water Phase [a]	ΣE Non extr. [b]	Σ
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
II_1		0.9	13.2	14.1		9.2	0.7	9.9		28.0	2.5	31.3
II_2							1.1	1.1	0.2		1.0	1.2
II_3 CGA312753 (Aconitic acid)												
II_{2a}											0.8	0.8
II_{3a}		1.3		1.3		3.0		3.0				
II_4										2.3		2.3
II_5 CGA 275537 (Tricarballylic acid)	0.3	1.6	1.3	3.2		16.8	0.2	17.0	0.8	12.2	0.6	13.6
II_6	0.3	0.4	0.9	1.6	0.4			0.4	0.6	0.8		1.4
II_7			1.2	1.2			0.3	0.3		0.8		0.8
II_8 CGA 313458		0.2	3.1	3.3	0.4	6.5	0.5	7.4	4.1	2.3		6.4
II_9 CGA 329773	0.8		1.7	2.5	0.9		0.3	1.2	0.8		0.3	1.1
II_{10} Trinexapac acid	20.2	3.8	11.6	35.6	11.0	18.5	0.4	29.9	6.7	1.7	0.2	8.6
II_{10a}							0.2	0.2				
II_{10b}							0.7	0.7				
II_{11} Trinexapac-ethyl	0.4		6.0	6.4	6.2			6.2	1.3			1.3
STR6[c]											8.9	8.9
Pectine Lignin Cellulose[d]											1.6	1.6
^{14}C-glucose			8.0	8.0								
Unresolved	2.2	1.0	6.3	9.5	1.5	3.8	0.9	6.2	1.6	8.9	1.0	11.5
Characterised				77.2				72.9				79.3
Identified				59.0				60.3				41.5
Non-extractables								16.0				
Total				86.7				99.5				90.8

[a] After 0.1 N NaOH treatment

[b] After basic/acid treatment of R_1 (grain) and R_2 (straw)

[c] Fractions STR-E_5(E_4) 6.8%, STR-E_7(E_6) 1.4% and STR-R_6(R_5) 0.7%

[d] Pectine 0.1%, Cellulose 1.4% and Lignin 0.1%

E_1= cold extract

NA = not analysed

The proposed metabolic pathways of trinexapac-ethyl in rice are mainly by hydrolysis of the ester bond to give trinexapac acid, aromatisation of the C_6 ring of trinexapac acid presumably by hydroxylation followed by elimination of water and keto-enol tautomerisation, yielding 4-cyclopropanecarbonyl-3,5-dihydroxy-benzoic acid (CGA 329773), cleavage of the C_6 ring of trinexapac acid to 2-(4-cyclopropyl-4-hydroxy-2-oxo-but-3-enyl)-succinic acid (CGA 313458) and stepwise oxidation/decarboxylation after cleavage of the C_6 ring of trinexapac acid yielding saturated and unsaturated tricarboxylated acids such as 3-carboxy-pentanedioic acid (tricarballylic acid = CGA 275537) and 3-carboxy-pent-2-enedioic acid (trans aconitic acid = CGA 312753) and conjugation of trinexapac acid, CGA 313458 and CGA 275537 with sugars and/ or other plant constituents.

Spring wheat

[1, 2-^{14}C-cyclohexyl] trinexapac-ethyl formulated as an emulsion concentrate was applied to spring wheat (Besso) by foliar spraying at a rate of 150 g ai/ha (Krauss 1990, CGA163935/0086). Applications were made to two week old plants (greenhouse experiment) and six week old plants (one-node stage, field experiment). In the greenhouse experiment samples were taken at seven time intervals from 0.5 hours, 4 hours, 1, 2, 7, 14 and 21 days. Plants were separated into leaves and roots. In the field experiment samples were taken at 0 days (tops), 25-days (ears and leaves at ear emergence), 48 days (ears and leaves at milky stage) and 71 days (grain, husks, straw at maturity). Soil samples were also taken.

Incorporation of radioactivity into the greenhouse grown plants is summarized below. After rapid initial dissipation of radioactivity from the leaf surface during the first day, subsequent decrease was slow for the next 20 days.

Table 21 Distribution of radioactivity in wheat whole tops and roots after foliar application of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl (greenhouse short term experiment)

Interval	Plant part	TRRs		Parent (mg/kg trinexapac-ethyl equiv.)	Recovered (%) [b]	Surface (%) [a]	Penetrated		Total (%) [c]
		(mg/kg trinexapac-ethyl equiv.)	% [a]				Cold ext. (%)	NE (%) [a]	
30 minutes	Whole tops	5.476	60.8	4.421	60.8	83.6	16.3	0.1	100.0
	Roots	0.153	0.2	0.015	0.2		68.5	31.5	100.0
	Plant Total		61.0		61.0				
	Soil Whole Pot	0.066	39.0	0.013	39.0		31.7	68.3	100.0
	Soil Total		39.0		39.0				
	Total		100.0		100.0				
4 hours	Whole tops	2.446	44.2	1.028	28.5	43.3	54.7	2.0	100.0
	Roots	0.756	1.7	0.024	1.1		59.9	40.1	100.0
	Plant Total		45.9		29.6				
	Soil Whole Pot	0.057	54.1	0.004	34.9		18.4	81.6	100.0
	Soil Total		54.1		34.9				
	Total		100.0		64.5				
1 day	Whole tops	1.721	33.3	0.226	22.3	20.1	75.5	4.5	100.1
	Roots	2.080	5.3	0.256	3.5		60.4	39.6	100.0
	Plant Total		39.2		25.8				
	Soil Whole Pot	0.066	60.8	< 0.001	40.1		12.7	87.3	100.0
	Soil Total		60.8		40.1				
	Total		100.0		65.9				
2 days	Whole tops	1.118	28.5	0.145	18.1	20.3	74.1	5.6	
	Roots	1.163	3.9	0.015	2.5		54.9	45.1	
	Plant Total		32.4		20.6				
	Soil Whole Pot	0.069	67.6	< 0.001	43.0		13.8	86.2	100.0
	Soil Total		67.6		43.0				
	Total		100.0		63.6				
7 days	Whole tops	0.587	28.7	0.012	14.5	8.3	83.4	8.3	100.0
	Roots	0.411	3.6	< 0.001	1.8		38.1	61.9	100.0
	Plant Total		32.2		16.3				
	Soil Whole Pot	0.057	67.8		34.3		15.3	84.7	100.0
	Soil Total		67.8		34.3				
	Total		100.0		50.6				
14 days	Whole tops	0.466	47.1	0.002	25.1	6.2	83.6	10.3	100.1
	Roots	0.121	4.1	< 0.001	2.2		12.7	87.4	100.1
	Plant Total		51.2		27.3				
	Soil Whole Pot	0.044	48.8		26.0		13.5	86.6	100.1

Interval	Plant part	TRRs		Parent (mg/kg trinexapac-ethyl equiv.)	Recovered (%) [b]	Surface (%) [a]	Penetrated		Total (%) [c]
		(mg/kg trinexapac-ethyl equiv.)	% [a]				Cold ext. (%)	NE (%) [a]	
	Soil Total		48.8		26.0				
	Total		100.0		53.3				
21 days	Whole tops	0.305	39.2	< 0.001	19.6	5.1	81.1	13.8	100.0
	Roots	0.127	4.4	< 0.001	2.2		11.1	88.9	100.0
	Plant Total		43.6		21.8				
	Soil Whole Pot	0.049	56.4	< 0.001	28.2		12.7	87.3	100.0
	Soil Total		56.4		28.2				
	Total		100.0		50.0				

[a] % of radioactivity found in sub-balanced plant parts/soil system; non-extractables (NE) is residual radioactivity determined after cold solvent extraction

[b] % of radioactivity recovered at interval 1

[c] % of radioactivity found in plant parts/ soil, determined by the sum of extractable and non-extractable radioactivity

The characterisation of the leaf rinse radioactivity is shown in the following table. Once penetrated, trinexapac-ethyl was hydrolysed to trinexapac acid. Parent concentration dropped to less than 2% of TRR 7 days after application, whereas the acid metabolite accounted for 30–40% of TRR in the leaf extract after 1 day and the following twenty days. After the first 24 hours approximately 80% of the recovered radioactivity had penetrated the plant surface. Translocation of radioactivity from treated plant parts into newly grown parts could be demonstrated by autoradiography.

Table 22 Residues of parent and trinexapac acid in wheat whole tops after foliar application of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl (greenhouse short term experiment)

Interval	Parent trinexapac-ethyl		Trinexapac acid	
	Surface (%) [a]	Plant extract (%) [a]	Surface (%) [a]	Plant extract (%) [a]
30 minutes	68.8	11.9	0.6	0.8
4 hours	31.4	10.6	5.0	26.0
1 day	11.9	1.2	2.0	37.0
2 days	10.5	2.5	2.0	35.6
7 days	1.7	0.3	1.5	37.2
14 days	0.5	< LD [b]	0.5	34.7
21 days	0.1	< LD [b]	0.8	29.8

[a] In % of radioactivity recovered at each interval

[b] LD = Limit of Detection (< 0.1% of radioactivity)

The incorporation of the radioactivity into the field grown spring wheat plants is summarized in the following table. Translocation of radioactivity from aerial plant parts to ears was significant. Total radioactive residues in the ears increased from 0.256 mg/kg at ear emergence (25 days after application) to approximately 0.47 mg/kg at milky stage and maturity. This behaviour of the TRR was confirmed by autoradiography. The TRR in grain, husks and straw at maturity was 0.462, 0.440 and 0.542 mg/kg respectively. Parent was always less than the limit of detection (0.001 mg/kg).

Table 23 Distribution of radioactivity in wheat whole tops, ears, grain, husks and straw after foliar application of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl (field long term experiment)

Interval	Plant Parts/ Soil Layers	TRRs		Parent (mg/kg trinexapac-ethyl equiv.)	Extracted radioactivity (%) [b]		Non-extracted (%)[b]	Total (%) [b]
		(mg/kg trinexapac-ethyl equiv.)	%[a]		Cold extraction [d]	Soxhlet [e]		
0 days	Whole tops	0.801	100	0.209	84.1	2.5	12.2	98.8
	Soil							
	0–5 cm	0.274	82.5	0.002	14.5	0.9	82.6	98.0
	5–10 cm	0.044	16.1	< 0.001	12.9	0.8	85.8	99.5
	10–20 cm	< 0.001	1.1	NA [c]	NA [c]	NA [c]	NA [c]	
	20–30 cm	< 0.001	0.3	NA [c]	NA [c]	NA [c]	NA [c]	
	Soil Total	0.034	100.0					
25 days	Ears	0.256	100	< 0.001	84.3	2.0	12.5	98.8
(Ear emergence)	Leaves	0.225	100	< 0.001	87.2	2.8	14.7	104.7
48 days	Ears	0.473	100	< 0.001	83.2	3.0	18.3	104.5
(Milky stage)	Leaves	0.438	100	< 0.001	73.5	3.0	25.3	101.8
71 days	Grain	0.462	100	< 0.001	68.4	1.7	25.1	95.2
	Husks	0.440	100	< 0.001	46.6	1.5	50.8	98.9
	Straw	0.542	100	< 0.001	41.0	4.1	61.9	107.0
	Soil							
	0–5 cm	0.071	81.0	< 0.001	6.3	0.6	92.2	99.1
	5–10 cm	0.008	11.7	NA [c]	NA [c]	NA [c]	NA [c]	
	10–20 cm	0.001	4.1	NA [c]	NA [c]	NA [c]	NA [c]	
	20–30 cm	< 0.001	3.3	NA [c]	NA [c]	NA [c]	NA [c]	
	Soil Total	0.011	100.1					

[a] In % of the radioactivity found in the sub-balanced plant parts/ soil layers

[b] In % of radioactivity found in the plant part/ soil layers and determined by combustion

[c] NA = not analysed

[d] Using methanol/ water (8:2 v/v)

[e] Using methanol

Characterisation of the TRR in the growing plants and at maturity is shown in the following table. The portion of the organosoluble radioactivity decreased from approximately 70% in the fresh green plant parts to 20–40% in straw and grain of the extractable radioactivity. The non-extractable residues increased from a few percent of the TRR in the fresh green plant parts to 25–50% of the TRR in grain and husks and 62% of the TRR in the straw at maturity.

Apart from the samples taken shortly after application, no parent compound could be detected in any plant part at later intervals. However the trinexapac acid was present in varying amounts in all plant parts. In the 25 and 48 day ears its relative amount was higher (28 and 25% of TRR respectively) than in the leaves (13 and 3% of TRR respectively). Likewise its content in grain (24% of TRR) was higher than in husks (10.0% of TRR) or in straw (4.5% of TRR).

Table 24 Extractability and residues of parent and trinexapac acid in whole tops, ears, grain, husks, straw and soil after foliar application of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl (field long term experiment)

Interval	Matrix	Organic phase (%) [a]	Water phase (%) [a]	Parent (%) [b]	Trinexapac acid (%) [b]
Application	Whole tops	69.9	28.8	31.0	24.1
0 days	Soil (0–5 cm)			4.3	7.4
	Soil (5–10 cm)			2.9	6.1
25 days	Ears	45.0	52.8	< LD [c]	28.0

Interval	Matrix	Organic phase (%) [a]	Water phase (%) [a]	Parent (%) [b]	Trinexapac acid (%) [b]
(Ear emergence)	Leaves	23.6	75.6	< LD [c]	13.3
48 days	Ears	49.2	45.8	< LD [c]	24.8
(Milky stage)	Leaves	11.0	87.2	< LD [c]	2.8
71 days	Grain	39.1	60.8	< LD [c]	24.2
(Maturity)	Husks	33.8	61.2	< LD [c]	10.0
	Straw	20.9	75.4	< LD [c]	4.5
	Soil (0–5 cm)			< LD [c]	~0.3

[a] In % of the extracted radioactivity

[b] in % of the total radioactivity found in plant parts and soil layers and determined by combustion

[c] LD = Limit of Detection (< 0.3% of radioactivity)

Eight significant metabolite fractions were characterised originating from the extracts of the field samples (see Table 25). The major metabolite fraction I_5 was identified as trinexapac acid.

The metabolite pattern in leaves, ears and husks was qualitatively similar, showing the presence of the metabolite fractions I_1, I_2, I_4 and I_5. In grain, metabolites I_1 and I_2 were not found. The water soluble metabolite fractions II_1 and II_3 were cellulase resistant. The upper soil layer at plant maturity contained trinexapac acid at 0.3% of the total radioactivity. Non-extractable radioactivity ranged from 26–92% of total residues under the extraction conditions used.

Table 25 Quantification of metabolite fractions in plant parts at maturity (field long term experiment)

Metabolite	Wheat matrix			Soil (0–5 cm) (%)
	Grain (%) [a]	Husks (%) [a]	Straw (%) [a]	
II_1	6.8	10.0	8.0	4.4
II_2	3.1	NA	2.4	NA
II_3	10.9	NA	3.1	NA
II_4	1.7	NA	NA	NA
I_1	–	6.1	~0.5	NA
I_2	–	3.3	~0.2	NA
I_4	~0.7	~0.6	~0.1	~0.4
I_5 Trinexapac acid	24.2	10.0	4.5	~0.3
Unresolved and soxhlet	25.4	14.9	21.4	1.2
Soxhlet	1.7	1.5	3.8	0.6
Non-extractable radioactivity	26.4	51.4	57.9	92.2
Total	100.9	97.8	101.9	99.1

[a] In % of the radioactivity totally found in plant parts

NA = Not analysed

A stem-injection experiment was conducted on six week old plants grown under greenhouse conditions and an incubation experiment (45 days at room temperature) of a cell suspension of treated leaves. The injection occurred with approximately 40–50 µg of [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl per plant. All injected plants were harvested at maturity, 69 days after treatment (Krauss 1993, CGA 163935/0303).

In addition to the analytical methods used in the previous report silicagel column chromatography run was applied as flash chromatography under pressure, for fractionation of samples the column was eluted with a step gradient using ethylene chloride/ methanol mixtures and for isolation, purification and analysis different HPLC systems were applied. For the analysis of the non-extractable radioactivity in grain, husks and straw, various procedures were used. After extraction at high temperature and purification by partitioning and filtration, the water phase was dialysed in membrane bags. Further techniques for the characterisation and identification of the metabolites were high voltage electrophoresis and chemical derivatisation (methylation and acetylation).

Trinexapac acid was the dominant metabolite observed in grain (27.9% of the TRR). Other important metabolites observed included II_4, CGA 275537 (II_2) and II_3 which after methylation was identified as 4-cyclopropanecarbonyl-3, 5 dihydroxy-benzoic acid (CGA 329773). Approximately 85% of the TRR was characterised in grain and 51% could be identified.

The major metabolite observed in husks was trinexapac acid (16.7% of the TRR). Other metabolites observed included CGA 275537 (II_2). Approximately 86% of the TRR was characterised in husks and 37% could be identified.

In straw, the major fraction observed in the methylene chloride extract was trinexapac acid. Metabolite fraction I_2 which contained the mono ethyl ester of the aconitic acid was also present in the organic phase. After purification of the fraction which was generated from the water phase by partition with ethyl acetate, three major fractions were obtained which corresponded to the metabolite fractions II_3, trinexapac acid and II_4. Heating a suspension of non-extractables in a propanol/water mixture released a further 47% of TRR. From these fractions the metabolite fractions I_2, I_4 and trinexapac acid could be identified. Approximately 94% of the TRR was characterised in straw and 31% could be identified. The major metabolite observed in straw was trinexapac acid (12.8% of the TRR).

Only 6.9% of the total residues in the 0–5 cm soil layer could be extracted by methanol/water extraction followed by soxhlet extraction as previously reported (Table 25), with trinexapac acid only approximately 0.3% of the total residues. Analysis of the non-extractable radioactivity was performed by microwave extraction. The major part of the soil non-extractables (55% of the total residues) was released and partitioned with ethyl acetate. Trinexapac (2.2% of the total residues) and some minor fractions were in the combined organic phase.

Table 26 Summary of identified components in spring wheat matrices at maturity after treatment with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl

Metabolite	Wheat matrix					
	Grain		Husks		Straw	
	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]
II_1 [b]	0.031	6.8 [d]	0.044	10.0	0.043	8.0
II_2 CGA275537	0.014	3.1		NA [c]	0.013	2.4
II_3 CGA329773	0.050	10.9		NA [c]	0.017	3.1
II_4	0.0078	1.7		NA [c]		NA [c]
I_1		NA [c]	0.027	6.1	0.0027	0.5
I_{2a} CGA 312753 mono-ethyl ester		NA [c]	0.019	4.3	0.0097	1.8
I_4 [e]	0.0032	0.7	0.0026	0.6	0.013	2.4
I_5 Trinexapac acid	0.129	27.9	0.073	16.7	0.069	12.8
Unresolved and soxhlet		33.6		48.7		63.4
Non-extractables [f]	0.070	15.1	0.050	11.4	0.061	11.2
Characterised		84.7		86.4		94.4
Identified		51.1		37.7		31.0
Total	0.462 [g]	99.8 [h]	0.440 [g]	97.8 [h]	0.542 [g]	105.6 [h]

[a] In % of the total radioactivity found in plant parts

[b] Radioactivity remaining at the start in TLC analysis

[c] NA = Not Analysed

[d] Includes sugar conjugate of trinexapac acid

[e] Acidic compound

[f] Obtained after alkaline hydrolysis

[g] TRR

[h] Sum of characterised and non-extractables

The proposed transformation of trinexapac-ethyl in spring wheat is mainly by hydrolysis of the ester bond to give trinexapac acid. Conjugation of trinexapac acid with sugar occurs to a small extent in grain. Aromatisation of the C_6 ring of trinexapac acid presumably by hydroxylation followed by elimination of water and keto-enol tautomerisation, yielding CGA 329773 also occurs. Cleavage of

the C_6 ring of trinexapac-ethyl was postulated as an alternative pathway. Stepwise oxidation/decarboxylation after cleavage of the 6-membered ring yielded saturated and unsaturated tricarboxylic acids such as tricarballylic acid (CGA 275537) and the mono ethyl ester of aconitic acid (CGA 312753).

Rape

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was applied to spring rape (*Tobin Canola*) under greenhouse conditions in plastic containers filled with soil (Nicollier 1991, CGA163935/0209). One treatment was made to 37 day old spring rapeseed (stem elongation) at an application rate of 400 g ai/ha. Plants were sampled at 30 minutes (whole plants), 14 days (green and flowering parts) and 65 days (maturity - stalks, pods and seeds) after application. Soil samples were also taken.

The TRRs are summarized in Table 27. TRRs of 5.74 mg/kg trinexapac-ethyl equivalents, 1.43 mg/kg equiv. and 3.14 mg/kg equiv were found in the pods, seeds and stalks respectively. No parent compound was detected (< 0.002 mg/kg) in any plant part at harvest. After processing into oil and meal only 2.4% of the radioactivity was present in the oil (TRRs 0.11 and 2.11 mg/kg equiv. respectively).

Residues in soil at maturity reached 0.064 mg/kg for the 0–5 cm layer, 0.012 mg/kg for the 5–10 cm layer and 0.012 mg/kg for the 10–20 cm layer. Parent compound was below the limit of detection (0.002 mg/kg). Non-extractable residues accounted for 93.2, 97.3 and 99.0% of the total radioactivity in the 0–5cm, 5–10 cm and 10–20 cm soil layers respectively.

Table 27 Distribution of radioactivity in various plant parts of rapeseed after foliar application of [^{14}C] trinexapac-ethyl

Interval	Matrix	TRRs		Parent (mg/kg equiv.)	Extracted radioactivity (%)[b]		Non-extracted (%) [b]	Total (%) [b]
		(mg/kg equiv.)	% [a]		Cold extraction [c]	Soxhlet [d]		
30 minutes (Stem elongation)	Whole tops	6.24	100	1.18	95.2	0.6	3.3	99.1
	Soil							
	0–5 cm	0.030	100	< 0.002	9.0	1.3	93.0	103.3
	Soil Total		100					
14 (Flowering)	Green parts	0.82	100	0.012	92.4	1.0	10.4	103.8
	Flowering parts	6.79	100	0.068	100.7	0.4	0.8	101.9
	Soil							
	0–5 cm	0.063	73.7	< 0.002	10.2	0.4	92.9	103.5
	5–10 cm	0.009	10.1	NA	NA	NA	NA	
	10–20 cm	0.009	16.3	NA	NA	NA	NA	
	Soil Total	0.025	100.0					
65 (Maturity)	Stalks	3.14	100	< 0.002	66.1	1.6	27.2	94.9
	Pods	5.74	100	< 0.002	80.2	1.3	17.3	98.8
	Seeds	1.43	100	< 0.002	NA	NA	NA	
	Meal	2.11	97.6	< 0.002	67.5	1.9	27.6	
	Oil	0.11	2.4	< 0.002	100	–	–	
	Soil							
	0–5 cm	0.064	66.3	< 0.002	8.1	0.3	93.2	101.6
	5–10 cm	0.012	13.4	NA	5.9	0.4	97.3	103.6
	10–20 cm	0.012	20.3	NA	7.1	0.4	99.0	106.5
	Soil Total		100.0					

[a] In % of the radioactivity found in the sub-balanced plant parts/ soil layers

[b] In % of radioactivity found in the plant part/ soil layers and determined by combustion

[c] Using methanol/ water (8:2 v/v)

[d] Using methanol

NA = Not Analysed

Partitioning behaviour (pH 4) of the extractable residues in pods and stalks showed that the major part of the radioactivity was located in the water phase (57.9% in pods and 67.4 % in stalks) with 39.4% (pods) and 29.0% (stalks) located in the organic phase.

At harvest no parent compound was detected in pods, seeds and stalks. Approximately 16 metabolite fractions were found in plant, seeds processed parts and soil extracts. The major metabolite observed was trinexapac acid representing 26.9, 16.2 and 8.9% of the total radioactivity in meal, pods and stalks respectively (Table 28). Aqueous fractions obtained after partition were treated with cellulase which released aglycones indicating the presence of sugar conjugates. The major metabolite fractions that were released as aglycones were II_7 and II_1–II_2. The aglycones released were chromatographed and revealed the presence of trinexapac acid (I_5) and I_4. Non-extractable radioactivity accounted for 93.2% of the radioactivity in the 0–5 cm soil extract, with one minor metabolite, I_7, being observed only in soil.

The metabolite pattern observed in spring rape compared with that obtained in spring wheat, revealed the presence of crop specific metabolite fractions.

Table 28 Summary of identified components in plants parts, processed fractions and soil at harvest after treatment with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl (400 g ai/ha treatment)

Metabolite	Rapeseed matrices			Soil (0–5 cm)
	Seeds—Meal	Pods	Stalks	% TRR [a]
	% TRR [a]	% TRR [a]	% TRR [a]	
II_1	–	3.5	2.6	4.8
II_2	–	0.9	1.2	
II_3		1.9	2.2	
II_4		1.3	2.3	
II_5		2.2		
II_6	19.0	0.9	1.5	
II_7 [b]		11.0	11.8	ND
II_8		1.1		
II_9		1.7		
II_{10}	–	1.9	4.9	
I_2	6.3	3.0	0.5	
I_3	–	–	–	
I_4	2.6	7.7	6.0	0.2
I_5 Trinexapac acid	26.9	16.2	8.9	0.4
I_6	–	0.4	0.7	0.4
I_7	–	–	–	0.3
Unresolved	12.6	24.3	21.0	2.0
Sub-total	67.4	78.0	63.7	8.1
Soxhlet	1.9	1.3	1.6	0.3
Non-extractables	27.6	17.3	27.2	93.2
Total	96.5	98.8	92.5	101.6

[a] % of radioactivity found in plant parts, processed parts and in soil

[b] Treatment with cellulase released aglycones I_4 and I_5 (trinexapac acid)

ND = Not Detected

In a subsequent study (Nicollier 1993, CGA163935/0308) with application of the radio-labelled trinexapac-ethyl and sampling of specimens as described above, additional analytical techniques were employed.

Metabolites identified are summarized below in Table 29. Forty to 46% of the TRR was identified in stalks, pods, seed meal and whole seeds while 89% of the TRR was identified in oil. In all plant parts 65–100% TRR was characterised.

In stalks organo-soluble radioactivity contained CGA 351210 and trinexapac acid while water soluble radioactivity (from a water/ methanol gradient) yielded six fractions which included trans aconitic acid (CGA 312753) and CGA 313458. Metabolite II_{3b} was tentatively identified as isocitric acid lactone while metabolite II_9 was postulated to be CGA 329773. The major fraction of the water

phase was enzymatically hydrolysed with cellulase. Two aglycones were released, one co-chromatographed with metabolite I_{4b} (CGA 351210) and the second one with metabolite I_5 (trinexapac acid).

In pods organo-soluble radioactivity again contained CGA 351210 and trinexapac acid while water soluble radioactivity (from a water/ methanol gradient) yielded four fractions which included trans aconitic acid (CGA 312753). After enzymatic hydrolysis with cellulase two aglycones, metabolites I_{4b} (CGA 351210) and I_5 (trinexapac acid) were released.

In seeds residues in oil were extracted with hexane. The two major metabolite fractions co-chromatographed were metabolite I_{4b} (CGA 35120) and metabolite I_5 (trinexapac acid). Of the oil radioactivity, 69.5% (or 1.7% of the TRR in the seed) remained attached to the lipid fraction. The meal fraction was extracted with 80% methanol. The major fractions again were metabolites I_{4b} (CGA 351210) and I_5 (trinexapac acid). Water soluble radioactivity (from a water/ methanol gradient) yielded four fractions which included trans aconitic acid (CGA 312753). After enzymatic hydrolysis with cellulase, metabolites I_{4b} (CGA 351210) and trinexapac acid were released. About 48% of the non-extractable radioactivity in meal could be solved in alkaline propanol after heating in a microwave. The major fraction of this organic phase was again trinexapac acid.

Table 29 Summary of identified components in plants parts and processed fractions at harvest after treatment with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl (400 g ai/ha treatment)

Metabolite	Rapeseed Matrices										Soil (0–5 cm)
	Seeds						Pods		Stalks		
	Meal		Oil		Whole						
	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	% TRR [a]
II_1	0.097	4.6			0.064	4.5	0.201	3.5	0.082	2.6	8.2
II_2		ND			–	ND	0.051	0.9	0.038	1.2	
II_{3b} [c]	0.038	1.8			0.026	1.8	0.109	1.9	0.069	2.2	
II_4	0.061	2.9			0.040	2.8	0.075	1.3	0.072	2.3	
II_5							0.126	2.2			
II_6 CGA312753	0.019	0.9			0.013	0.9	0.051	0.9	0.047	1.5	
II_7 [b]			–	ND			0.069	1.2	0.107	3.4	
II_8		ND			–	ND	0.063	1.1	–	ND	
II_9		ND			–	ND	0.097	1.7	–	ND	
II_{10} CGA313458	0.023	1.1			0.016	1.1	0.109	1.9	0.154	4.9	
I_2	0.015	0.7			0.010	0.7	0.017	0.3	0.016	0.5	
I_{2b}	0.057	2.7			0.037	2.6	0.172	3.0	–	ND	
I_3	0.004	0.2	0.0007	0.6	0.003	0.2	0.006	0.1	–	ND	
I_{4b} free 351210	0.036	1.7	0.0184	16.0	0.030	2.1	0.442	7.7	0.188	6.0	0.2
I_{4b} conjugated 351210	0.074	3.5	–	ND	0.048	3.4	0.476	8.3	0.681	21.7	ND
I_5 free trinexapac acid	0.624	29.5	0.004	3.5	0.415	29.0	1.039	18.1	0.279	8.9	10.2
I_{5b} conjugated trinexapac acid	0.023	1.1	–	ND	0.016	1.1	0.023	0.4	0.025	0.8	ND
I_6	0.038	1.8	0.0025	2.2	0.026	1.8	–	ND	0.022	0.7	0.4
I_7		ND		ND		ND				ND	5.7
I_8		ND	0.0799	69.5	0.024	1.7			–	ND	ND
Unresolved + Soxhlet	0.609	28.8	0.0094	8.2	0.405	28.3	1.740	30.3	0.710	22.6	21.4
Non-	0.302	14.3	–	–	0.200	14.0	0.626	10.9	0.854	27.2	55.2

Metabolite	Rapeseed Matrices										Soil (0–5 cm)
	Seeds						Pods		Stalks		
	Meal		Oil		Whole						
	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	mg/kg equiv.	% TRR [a]	% TRR [a]
extractables [d]											
Characterised		81.3		100		82.0		84.8		65.3	
Identified		39.6		89.0		41.1		39.9		46.0	
Total	2.115	95.6	0.115	100	1.430	96.0	5.742	95.7	3.141	92.5	101.6

[a] % of radioactivity found in plant parts, processed parts and in soil

[b] Treatment with cellulase released aglycones I_4 and I_5

[c] Tentatively identified as isocitric acid lactone

[d] For meal, pods and soil after hydrolysis of the non-extractables

ND = Not detected

The proposed transformation of trinexapac-ethyl in rape seed is mainly by hydrolysis of the ester bond to give trinexapac acid. The sum of free and sugar conjugated trinexapac acid accounted for 30.6%, 3.5%, 18.5% and 9.7% of total radioactivity in meal, oil, pods and stalks respectively. Reduction of trinexapac acid to 2-(cyclopropyl-hydroxymethylene)-5-hydroxymethylcyclohexane-1, 3-dione (CGA 351210) and subsequent conjugation with sugar occurs. Additional oxidation of trinexapac acid gives 2-(4-cyclopropyl-4-hydroxy-2-oxo-but-3-enyl)-succinic acid (CGA 313458) while cleavage of the C_6 ring of trinexapac-ethyl followed by stepwise oxidation/decarboxylation after cleavage of the 6-membered ring yielded isocitric acid and dehydration to trans aconitic acid (CGA 312753).

The observed metabolic pathway for rapeseed is comparable with those of wheat, grass and rice with the exception of one rape specific metabolite (CGA 351210) formed by the reduction of trinexapac acid.

Grass

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was applied by foliar methods to tall fescue grass at rates of 560.4 g ai/ha (Ray and May-Hertl 2003, CGA163935/0862). One foliar broadcast spray was made 46 days prior to swathing the grass for harvest of mature grass seed. Pre-forage samples were taken 22-days after application and hay samples were collected 46 days after application. The hay samples were dried and separated into straw, seed and seed screenings. Forage regrowth was collected 105 days after application. Soil samples were also taken at each of the harvest intervals listed above.

The TRRs and extractable and non-extractable radioactivity are summarized in Table 30. TRRs of 5.45 mg/kg trinexapac-ethyl equivalents, 7.13 mg/kg equiv. and 4.78 mg/kg equiv. were found in seeds, seed screenings and straw respectively. TRRs of 2.03 mg/kg equiv. and 0.054 mg/kg equiv. were observed for the 22 and 105 day forage samples respectively. Radioactivity extracted from 22 day forage, straw, seeds and seed screenings using acetonitrile/ water (4:1 v/v) or from 105 day regrowth forage using chloroform/methanol (4:1 v/v), ranged from 45% (seed screenings and seeds) to 75.5% (22 day forage).

Table 30 Total radioactive residues in various parts of grass after foliar application of [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl

Days After Treatment	Matrix	TRRs (mg/kg equiv.)	Extracted radioactivity (%) [a]	PES radioactivity (%) [b]	Recovery (%)
After 1st application	Soil	0.374	70.7	28.2	99.0
45	Soil	0.079	7.72	67.9	75.7
105	Soil	0.083	8.78	62.4	71.2

Days After Treatment	Matrix	TRRs (mg/kg equiv.)	Extracted radioactivity (%) [a]	PES radioactivity (%) [b]	Recovery (%)
22	Forage	2.03	75.5	20.2	95.8
46	Seeds	5.45	45.6	53.6	99.2
Swathing of mature grass	Seed screenings	7.13	45.0	53.4	98.4
seeds	Straw	4.78	70.4	28.2	98.6
105	Forage	0.054	55.5	34.8	90.4

[a] % of radioactivity found in plant part

[b] % of radioactivity found in plant part and determined by combustion

In-depth isolation procedures of extractable and non-extractable material, followed by analysis of purified metabolites enabled identification of five metabolites which are summarized below in Table 31. Degradation of parent compound was rapid as none was detected in any plant part at harvest. Eleven metabolite fractions were found, of which five major metabolites were present in all plant samples. In the various plant matrices, the two main metabolites observed were trinexapac acid representing 10.2–22.4% of the TRR and CGA 275537 (tricarballylic acid) representing 9.3–17.0% of the TRR. Radioactive residues left after solvent extraction (PES) ranged from 10.2–34.8% TRR.

Table 31 Summary of identified components in grass after treatment with [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl

Metabolite	Grass matrix									
	Forage (22 day)		Straw		Seeds		Seed Screenings		Regrowth Forage	
	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR	mg/kg equiv.	% TRR
	2.03	100	4.78	100	5.45	100	7.13	100	0.054	100
Metabolite A	0.15	7.46	0.49	10.3	0.10	1.90	0.27	3.76	0.002	4.40
CGA 275537	0.28	13.8	0.81	17.0	0.91	16.6	1.18	16.6	0.005	9.34
Metabolite B	0.17	8.58	0.27	5.60	0.45	8.32	0.70	9.84	0.001	2.72
Metabolite C (terephthalic acid)	0.20	9.74	0.45	9.43	0.52	9.55	0.84	11.7	0.004	6.61
Trinexapac acid	0.33	16.3	1.07	22.4	0.80	14.7	0.91	12.7	0.006	10.2
Unknown Region 1	0.11	5.55	0.15	3.15	0.14	2.65	0.22	3.01	0.005	10.0
Unknown Region 2	0.16	7.95	0.38	8.00	0.14	2.53	0.22	3.08	NA	NA
Unknown Region 3	NA	NA	0.22	4.69	NA	NA	NA	NA	0.001	2.69
Unknown Region 4	0.32	16.0	0.43	8.93	0.52	9.50	0.64	8.95	0.004	6.85
Unknown Region 5	0.15	7.31	0.30	6.30	0.38	7.01	0.48	6.78	0.001	2.66
Unknown Region 6	0.057	2.79	0.080	1.68	0.14	2.55	0.11	1.49	0.001	2.63
Identified	1.13	55.9	3.09	64.6	2.79	51.1	3.89	54.6	0.018	33.3
PES [a]	0.21	10.2	0.51	10.6	1.32	24.2	1.36	19.1	0.019	34.8
Characterised	1.34	66.1	3.60	75.3	4.11	75.3	5.26	73.7	0.037	68.1

Metabolite % TRRs and mg/kg are a summation of the individual values from their identification in the initial extracts, enzyme treatments plus the PES extracts (unknown regions not included in this summation).

[a] PES values obtained by subtracting total metabolites identified from the original PES values.

NA = Not Applicable

The proposed transformation of trinexapac-ethyl in grass was mainly determined by exhaustive analyses of the straw and 22 day forage samples. The major biotransformation is by hydrolysis of the ester bond to give trinexapac acid. Trinexapac acid undergoes hydrolytic ring opening and a Baeyer-Villiger like oxidation of the cyclopropylketone moiety to yield open chain metabolite A. Subsequent retro Claisen condensation provides tricarballylic acid (CGA 275537). An observed minor pathway starting from trinexapac-ethyl involves reductive elimination of one ring carbonyl group and Baeyer-Villiger like oxidation of the cyclopropyl-ketone moiety to yield metabolite B. An additional reductive elimination followed by aromatisation provides metabolite C (terephthalic acid).

Summary of plant metabolism

Rice, wheat, rape and grass (foliar treatment) metabolism studies were carried out with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl or [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl. Metabolic pathways in rice, wheat, rape and grass were comparable in all four crops.

Between 85–94%, 23–79%, 65–100% and 66–75% of TRR was characterised in the various plant parts of wheat, rice, oilseed rape and grass respectively depending on the plant part and the sampling interval after application. The portion of identified metabolites was 31–51%, 10.4–60.3%, 39.6–89.0% and 33.3–64.6% of TRR in wheat, rice, oilseed rape and grass, respectively.

Seven metabolites were identified. Trinexapac acid was the major metabolite which was present in all species and all plants parts at harvest. Parent compound was completely degraded and was not detectable (< 0.001/< 0.002 mg/kg) in any plant part of wheat, rapeseed and grass at harvest time. In rice only traces (0.001 and 0.003 mg/kg) were observed in straw and husks respectively. Further degradation of trinexapac acid proceeded *via* stepwise oxidation/ decarboxylation after cleavage of the 6-membered ring yielding saturated and unsaturated tricarboxylated acids such as tricarballylic acid (CGA 275537) and trans aconitic acid (CGA 312753), the latter compound an intermediate in the citric acid cycle (Krebs cycle). From the intermediates of the citric acid cycle and their breakdown products, sugars, fatty acids and certain amino acids were formed by de-novo synthesis. Consequently high non-extractable residues are formed (for example 72.0% in rice grains). High unresolvable radioactivity in TLC-analysis after extraction may also be in part attributed to radio-labelled matrix products or breakdown products thereof. In wheat, the ring cleavage occurred also on the parent molecule yielding the mono-ethyl ester of the aconitic acid. Further minor steps were aromatisation of the 6 membered ring of trinexapac acid and keto-enol tautomerism to 4-cyclopropanecarbonyl-3,5-dihydroxobenzoic acid (CGA 329773) and in rape reduction of trinexapac acid to 2-(cyclopropylhydroxy-methylene)-5-hydroxymethyl-cyclohexane-1,3-dione (CGA 351210) and subsequent conjugation with sugar.

Sugar conjugates of trinexapac acid were hydrolysed in some crop parts to yield free acid. Trinexapac acid sugar conjugates accounted for an increase in trinexapac acid of 11.6% TRR in rice grain and 10% and 14% TRR in grass forage and straw, respectively. Levels of sugar conjugates of trinexapac acid were not significant in oilseed rape, rice straw or wheat grain and were not investigated in wheat straw or husk.

Although not all metabolites were found in every plant species, all observed degradation and transformation steps (oxidation, decarboxylation, ring cleavage and conjugation) occurred in all crops. Therefore, the metabolic profiles revealed comparable pathways in all crops.

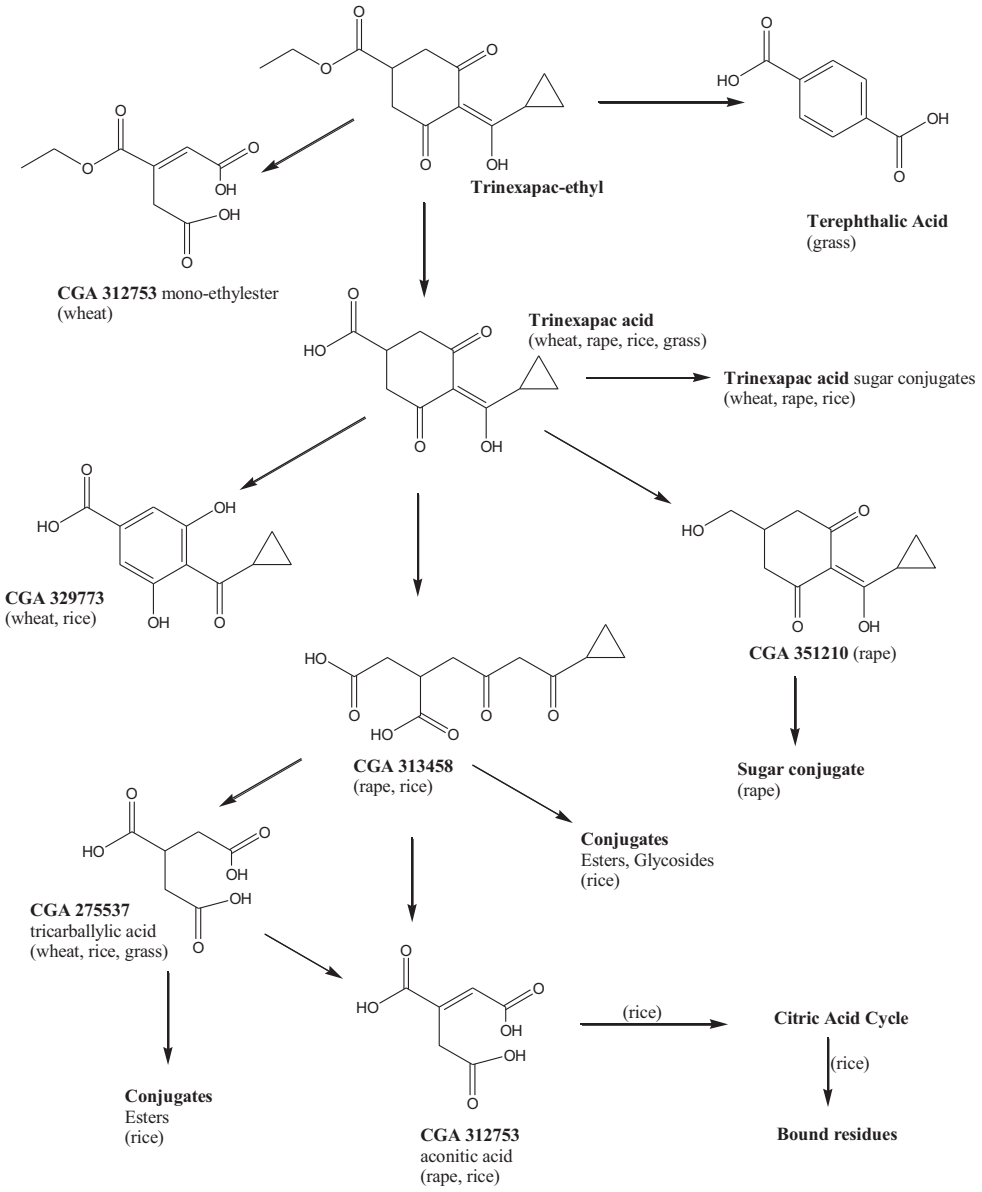


Figure 2 Proposed metabolic pathways for trinexapac-ethyl in plants

Confined Rotational Crop Studies

The metabolism of trinexapac-ethyl was investigated in three representative succeeding crops—lettuce (representative of leafy vegetables), radish (root and tuber vegetables) and wheat (cereals) (Quistad and Kovatchev 2010, CGA163935_50024). The study was performed with the test substance, [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl, applied to bare sandy loam soil, at a rate of 350 g ai/ha, followed by soil aging intervals of 30, 120 and 270 days. Crops were planted at each of the above intervals after application, to determine whether trinexapac-ethyl residues or degradates appear in follow crops. In addition radishes were sown at 309 days after soil treatment because the 270 plant back interval (PBI) crop did not produce root bulbs, due to the high summer temperatures during the cultivation interval.

Table 32 Schedule for planting/ seeding and harvest

Representative Crop Type	Crop	Planting/ Sowing [Days after Treatment]	Harvest [Days after Treatment]	Plant parts
		30	86	Heads (immature)
			113	Heads (mature)
Leafy Vegetable	Lettuce	120	183	Heads (immature)
			198	Heads (mature)
		270	290	Heads (immature)
			309	Heads (mature)
		30	83	Foliage, Roots
Root Vegetable	Radish	120	183	Foliage, Roots
		309	350	Foliage, Roots
			83	Forage
		30	168	Hay
			231	Straw, Grain
			168	Forage
Cereal	Wheat	120	209	Hay
			251	Straw, Grain
			296	Forage
		270	315	Hay
			352	Straw, Grain

Lettuce heads were harvested at immaturity (20–63 days after transplantation) and maturity (39–83 days after transplantation). Radish foliage and roots were harvested at maturity (41–63 days after sowing). Wheat was sampled as forage (26–53 days after sowing), as hay (45–138 days after sowing) and as straw and grain at maturity (82–201 days after sowing).

Total radioactive residues detected in rotational crops following soil application of [^{14}C] cyclohexyl trinexapac-ethyl were found to be:

- 0.011 mg/kg trinexapac-ethyl equivalents in lettuce head samples at immaturity (86 days after application) and 0.017 mg/kg equiv. in lettuce heads sampled at 100% maturity (113 days after application); for lettuce planted at 30 days PBI; 0.004 mg/kg equiv. and 0.007 mg/kg equiv. in immature lettuce heads of plants with 120 and 270 days PBI and 0.004 mg/kg equiv. and 0.001 mg/kg equiv. in mature lettuce heads of plants with 120 and 270 days PBI
- 0.005 mg/kg equiv., 0.007 mg/kg equiv. and 0.001 mg/kg equiv. for radish foliage of radish sown at 30, 120 and 309 days PBI respectively; 0.002 mg/kg equiv., 0.002 mg/kg equiv. and 0.001 mg/kg equiv. for radish roots sown at 30, 120 and 309 days PBI respectively
- 0.010 mg/kg equiv. in wheat forage sampled at 83 days after application and 0.011 mg/kg equiv. in wheat hay sampled 168 days after application, for plants with 30 days PBI; 0.004 mg/kg equiv. and 0.002 mg/kg equiv. for wheat forage of plants with 120 and 270 days PBI; 0.009 mg/kg equiv. and 0.008 mg/kg equiv. for wheat hay of plants with 120 and 270 days PBI, 0.003 mg/kg equiv., 0.004 mg/kg equiv. and 0.004 mg/kg equiv. for wheat straw samples and 0.005 mg/kg equiv., 0.008 mg/kg equiv. and 0.003 mg/kg equiv. for wheat grain samples of plants with 30, 120 and 270 days PBI respectively.

RACs were initially extracted with acetonitrile: water (1:1, 2×) followed by acetonitrile (1×). The combined acetonitrile: water extract supernatants were analysed by HPLC and/ or TLC. Post extraction solids were further extracted with other solvents if their total radioactive residues (TRR) were > 10%. The TRR of the final PES was determined by combustion.

The TRRs detected in rotational crops following soil application of [^{14}C] cyclohexyl trinexapac-ethyl at 350 g ai/ha are shown in Table 33.

Table 33 TRR values determined for trinexapac-ethyl confined accumulation in rotational crops

Crop	RAC	30 Days After Treatment (mg/kg trinexapac-ethyl equiv.)	120 Days After Treatment (mg/kg trinexapac-ethyl equiv.)	270 Days After Treatment (mg/kg trinexapac-ethyl equiv.)
Lettuce	Immature	0.011 [a]	0.004	0.007
	Mature	0.017 [a]	0.004	0.001
Radish [b]	Foliage	0.005	0.007	0.001
	Roots	0.002	0.002	0.001
	Forage	0.010 [a]	0.004	0.002
Wheat	Hay	0.011 [a]	0.009	0.008
	Straw	0.003	0.004	0.004
	Grain	0.005	0.008	0.003

[a] Sum of fractions (combined acetonitrile/water extracts + further PES extracts + PES). Other TRR values were determined by combustion

[b] 270 day PBI radish did not produce a root bulb, so re-sown at 309 days after treatment within the same plot

Radish RACs were not extracted with acetonitrile for analysis of residue distribution and extractable radioactivity because the TRRs were < 0.01 mg/kg. For lettuce only RACs with TRR ≥ 0.010 mg/kg were extracted (RACs with 30 days PBI). For wheat, only forage and hay specimens from plants of 30 and 120 days PBI were extracted. The extractability of radioactivity in lettuce and wheat RACs with acetonitrile was generally low at < 50% TRR. Residues of parent trinexapac-ethyl were not detectable by HPLC-UV (LOQ = 0.001 mg/kg). Residues of trinexapac acid from hydrolysis of parent and CGA 312753, formed by cleavage of the cyclohexanedione ring of trinexapac acid, were only detectable in wheat hay and forage in the range of 0.001–0.002 mg/kg.

The distribution of residual trinexapac-ethyl and extractable radioactivity in rotational crops (lettuce and wheat) is shown in Table 34.

Table 34 Summary of identified components in rotational lettuce and wheat following cultivation in soil treated with [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl after plant back intervals of 30, 120 and 270 days.

Metabolite	Crop parts											
	Immature lettuce heads		Mature lettuce heads		Wheat Forage		Wheat Hay		Wheat Straw		Wheat Grain	
	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]
Plant back interval: 30 DAT												
TRR	0.011		0.017		0.010		0.011		NA		NA	
Parent	ND		ND		ND		ND		NA		NA	
Trinexapac acid	ND		ND		0.002	20.0	0.001	9.1	NA		NA	
CGA 312753	ND		ND		ND		0.002	18.2	NA		NA	
Extractable radioactivity	0.004	36.4	0.007	41.2	0.005	50.0	0.004	36.4		NA		NA
Plant back interval: 120 DAT												
TRR	NA		NA		0.004		0.009		NA		0.007	
Parent	NA		NA		ND		ND		NA		NA	
Trinexapac acid	NA		NA		ND		ND		NA		NA	
CGA 312753	NA		NA		ND		0.001	11.1	NA		NA	
Extractable radioactivity		NA		NA		50.0	0.003	33.3		NA	0.001	14.3
Plant back interval: 270 DAT												
TRR	NA		NA		NA		NA		NA		NA	
Parent	NA		NA		NA		NA		NA		NA	
Trinexapac acid	NA		NA		NA		NA		NA		NA	
CGA 312753	NA		NA		NA		NA		NA		NA	

Metabolite	Crop parts											
	Immature lettuce heads		Mature lettuce heads		Wheat Forage		Wheat Hay		Wheat Straw		Wheat Grain	
	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]	mg/kg [a]	% TRR [b]
Extractable radioactivity		NA		NA		NA		NA		NA		NA

[a] Trinexapac-ethyl equivalents

[b] In % of radioactivity found in the plant part, determined by combustion

ND = Not detectable

NA = Not Applicable (RACS were not extracted due to low TRR detected by combustion)

The uptake of residues by the rotational crops lettuce, radish and wheat planted or sown after several intervals after application of trinexapac-ethyl to bare ground was very low. No accumulation was observed. Residues of trinexapac-ethyl in the rotational crop RACs were below the LOQ (< 0.001 mg/kg). The residues of trinexapac acid and CGA 312753 were very close to or below the LOQ (< 0.001–0.002 mg/kg). The very limited uptake of radioactive material in succeeding crops clearly indicates the lack of systemic behaviour of trinexapac-ethyl. It was concluded that residues in rotational crops are negligible.

Field Accumulation in Rotational Crops

Field rotational crop studies for trinexapac-ethyl were conducted in both the USA and Switzerland.

USA Study—application to wheat (rotational crops—wheat, radish, spinach)

In a study conducted in the USA in the 2004–2005 growing season, a 250 g/L EC formulation of trinexapac-ethyl was applied once as a broadcast spray to wheat at 203 g ai/ha (Ediger 2006b, CGA163935/1054). Radish, wheat and spinach were planted after 3 different plant back intervals (14, 30 and 45 days).

Table 35 Schedule for planting/ seeding and harvest

Representative Crop Type	Crop [Varieties]	Planting/ Sowing [Days after Treatment]	Harvest [Days after Treatment]	Plant parts
	Spinach (Tyee, Melody)	14	60–73	Leaves
Leafy Vegetable		30	75–90	Leaves
		45	91–107	Leaves
		14	55–75	Tops
			55–75	Roots
Root Vegetable	Radish (Champion, Cherry Belle)	30	73–90	Tops
			73–90	Roots
		45	79–106	Tops
			79–106	Roots
			73–75	Forage (fall)
			235–274	Forage (spring)
		14	266–287	Hay
			300–310	Straw
			300–310	Grain
			90	Forage (fall)
			250–274	Forage (spring)
Cereal	Spring and Winter Wheat	30	281–287	Hay
	(Barrie, Caledonia, Kaskaskia)		310–315	Straw
			310–315	Grain
			106–107	Forage (fall)
			266–303	Forage (spring)
		45	297–345	Hay
			331–368	Straw

Representative Crop Type	Crop [Varieties]	Planting/ Sowing [Days after Treatment]	Harvest [Days after Treatment]	Plant parts
			331–368	Grain

Spinach leaves were harvested 45–62 days after sowing. Radish tops and roots were harvested 34–61 days after sowing. Wheat was sampled as fall forage (59–62 days after sowing), spring forage (220–260 days after sowing), hay (253–300 days after sowing), straw (280–323 days after sowing) and grain (280–323 days after sowing).

Samples were stored frozen for a maximum of 23 months. Trinexapac residues in rotational crop samples were quantitated as trinexapac acid by LC/MS/MS (Method 110-01). The LOQ is 0.05 mg/kg for trinexapac acid. Concurrent method recovery data were collected in rotational crop matrices at spiking levels of 0.05 and 0.50 mg/kg and were acceptable.

The maximum residues observed in specimens from treated crops were all < 0.05 mg/kg except for one Day-14 PBI wheat straw sample at 0.07 mg/kg.

Table 36 Residues of trinexapac acid in rotational crops (spinach, radish and wheat) following application of trinexapac-ethyl to the target crop (wheat) at 203 g ai/ha

Crop	Matrix	Days After Treatment	Trinexapac Acid (mg/kg)		
			Single Values	Mean	RSD (%)
Plant back interval: 14 DAT					
Spinach	Leaves	60–73	< 0.05 (×4)	< 0.05	NA
Radish	Tops	55–75	< 0.05 (×4)	< 0.05	NA
	Roots	55–75	< 0.05 (×4)	< 0.05	NA
	Forage (fall)	73–75	< 0.05 (×4)	< 0.05	NA
	Forage (spring)	235–274	< 0.05 (×4)	< 0.05	NA
Wheat	Hay	266–287	< 0.05 (×4)	< 0.05	NA
	Straw	300–310	< 0.05 (×3), 0.07	0.06 [a]	18 [a]
	Grain	300–310	< 0.05 (×4)	< 0.05	NA
Plant back interval: 30 DAT					
Spinach	Leaves	75–90	< 0.05 (×4)	< 0.05	NA
Radish	Tops	73–90	< 0.05 (×4)	< 0.05	NA
	Roots	73–90	< 0.05 (×4)	< 0.05	NA
	Forage (fall)	90	< 0.05 (×4)	< 0.05	NA
	Forage (spring)	250–274	< 0.05 (×4)	< 0.05	NA
Wheat	Hay	281–287	< 0.05 (×4)	< 0.05	NA
	Straw	310–315	< 0.05 (×4)	< 0.05	NA
	Grain	310–315	< 0.05 (×4)	< 0.05	NA
Plant back interval: 45 DAT					
Spinach	Leaves	91–107	< 0.05 (×4)	< 0.05	NA
Radish	Tops	79–106	< 0.05 (×4)	< 0.05	NA
	Roots	79–106	< 0.05 (×4)	< 0.05	NA
	Forage (fall)	106–107	< 0.05 (×4)	< 0.05	NA
	Forage (spring)	266–303	< 0.05 (×4)	< 0.05	NA
Wheat	Hay	297–345	< 0.05 (×4)	< 0.05	NA
	Straw	331–368	< 0.05 (×4)	< 0.05	NA
	Grain	331–368	< 0.05 (×4)	< 0.05	NA

[a] For statistical calculations 0.05 was substituted for < 0.05

DAT = Days After Treatment

NA = Not Applicable

Two control straw samples had residues of 0.05 (confirmed as 0.06) and 0.07 (confirmed as 0.07) mg/kg

The results showed that rotational crops sown 14, 30 and 45 days after application of trinexapac-ethyl to the target crop, are very unlikely to contain residues of trinexapac-ethyl as its main metabolite trinexapac acid, above the LOQ of 0.05 mg/kg.

European study—application to bare soil (rotational crops—lettuce, winter wheat, sugar beet and corn)

A trial was conducted in Switzerland during 1989 to investigate residues of trinexapac-ethyl in succeeding crops, grown in soil previously treated with [^{14}C] cyclohexyl trinexapac-ethyl (Krauss 1992, CGA163935/0265). A 250 g/L EC formulation of trinexapac-ethyl was applied once to bare soil at a rate of 150 g ai/ha. Treated plots (1 m^2 each) were planted after four different time intervals (69, 119, 299 and 338 days after treatment). The crops investigated were lettuce, winter wheat, sugar beet and maize (corn).

The schedule for seeding/planting and harvesting is shown in Table 37.

Table 37 Schedule for planting/ seeding and harvest

Representative Crop Type	Crop [Varieties]	Planting/ Sowing [Days after Treatment]	Harvest [Days after Treatment]	Plant parts
Leafy Vegetable	Lettuce	69	99	Heads (50% Maturity)
	(Sorraya)		119	Heads (Maturity)
			343	Tops, Roots
Root Vegetable	Sugar Beet	299	407	Tops, Roots
	(KWS)		496	Tops, Roots
			173	Whole tops (fall, cutting) (25% maturity)
	Winter Wheat	119	299	Whole tops (50% maturity)
	(Zenta)		343	Whole tops (75% maturity)
Cereal			407	Stalks, husks, grain (100% maturity)
			369	Whole tops (25% maturity)
	Maize (Blizzard)	338	407	Whole tops (50% maturity)
			496	Stalks, husks, grain (100% maturity)

Plant samples and soil cores (0–5 cm, 5–10 cm, 10–20 cm and 20–30 cm layers) were collected. Lettuce was harvested at one-half maturity (30 days after transplantation) and maturity (50 days after transplantation). Winter wheat was sampled as a fall cutting, one quarter maturity (54 days after sowing), one half maturity (180 days after sowing), three quarters maturity (224 days after sowing), and at maturity (288 days after sowing). The sugar beets and maize were harvested at one-quarter maturity (44 and 31 days after sowing respectively), one-half maturity (108 and 69 days after sowing, respectively) and at maturity (197 and 159 days after sowing respectively).

The following tables show the distribution of radioactivity in rotational crops (lettuce, winter wheat, sugar beet and maize) and the distribution of residue trinexapac-ethyl in soil.

Table 38 Distribution of radioactivity and residual trinexapac-ethyl in rotational lettuce and soil following application of [^{14}C] cyclohexyl trinexapac-ethyl to bareground

Days After Treatment / Growth Stage of Rotational Crops	Plant Parts / Soil Layers	Total Residues		Parent (mg/kg) [a]	Extractable radioactivity (%)		Non Extractable (%) [c]	Total (%) [c]
		(mg/kg) [a]	(%) [b]		Cold [c]	Soxhlet [c]		
70 days (planting)	Soil							
	0–5 cm	0.071	85.6	NA	14.9	1.8	82.3	99.0
	5–10 cm	0.010	10.6	NA	NA	NA	NA	
	10–20 cm	0.001	2.8	NA	NA	NA	NA	
	20–30 cm	< 0.001	1.0	NA	NA	NA	NA	
	Soil Total	0.012	100.0					
99 days Lettuce 50% Maturity	Heads	0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.044	95.4	NA	10.4	1.4	87.4	99.3
	5–10 cm	0.002	2.7	NA	NA	NA	NA	
	10–20 cm	< 0.001	1.9	NA	NA	NA	NA	

Days After Treatment / Growth Stage of Rotational Crops	Plant Parts / Soil Layers	Total Residues		Parent (mg/kg) [a]	Extractable radioactivity (%)		Non Extractable (%) [c]	Total (%) [c]
		(mg/kg) [a]	(%) [b]		Cold [c]	Soxhlet [c]		
	20–30 cm	NA	NA	NA	NA	NA	NA	
	Soil Total	0.012	100.0					
119 days Lettuce 100% Maturity	Heads	0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.080	77.9	NA	10.3	1.6	87.0	98.9
	5–10 cm	0.018	17.9	NA	9.2	1.6	88.0	98.8
	10–20 cm	0.001	3.8	NA	NA	NA	NA	
	20–30 cm	< 0.001	0.4	NA	NA	NA	NA	
	Soil Total	0.015	100.0					

[a] in trinexapac-ethyl equivalents; limit of detection for combustion = 0.001 mg/kg

[b] in % of radioactivity found in the sub-balanced plant parts/ total soil system

[c] in % of radioactivity found in the plant part/ soil layer, determined by combustion

NA = Not Analysed

Table 39 Distribution of radioactivity and residual trinexapac-ethyl in rotational winter wheat and soil following application of [^{14}C] cyclohexyl trinexapac-ethyl to bare ground

Days After Treatment / Growth Stage of Rotational Crops	Plant Parts / Soil Layers	Total Residues		Parent (mg/kg) [a]	Extractable radioactivity (%)		Non Extractable (%)[c]	Total (%) [c]
		(mg/kg) [a]	(%) [b]		Cold [c]	Soxhlet [c]		
119 days (planting)	Soil							
	0–5 cm	0.072	77.0	NA	12.0	1.7	86.5	100.2
	5–10 cm	0.019	18.0	NA	9.5	1.7	86.9	98.1
	10–20 cm	0.002	4.5	NA	NA	NA	NA	
	20–30 cm	< 0.001	0.6	NA	NA	NA	NA	
	Soil Total	0.016	100.0					
173 days Fall Cutting	Whole Tops	0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.034	82.2	NA	10.8	1.4	84.0	96.2
	5–10 cm	0.004	10.0	NA	NA	NA	NA	
	10–20 cm	0.001	7.0	NA	NA	NA	NA	
	20–30 cm	< 0.001	0.9	NA	NA	NA	NA	
	Soil Total	0.007	100.0					
299 days Winter Wheat 50% Maturity	Whole Tops	< 0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.045	72.9	NA	10.1	2.5	84.6	97.2
	5–10 cm	0.008	12.7	NA	NA	NA	NA	
	10–20 cm	0.002	8.4	NA	NA	NA	NA	
	20–30 cm	0.001	6.0	NA	NA	NA	NA	
	Soil Total	0.009	100.0					
407 days Winter Wheat 75% Maturity	Whole Tops	< 0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.052	74.2	NA	10.1	1.9	78.9	90.9
	5–10 cm	0.012	17.1	NA	8.4	2.5	86.8	97.4
	10–20 cm	0.002	8.2	NA	NA	NA	NA	
	20–30 cm	< 0.001	0.5	NA	NA	NA	NA	
	Soil Total	0.010	100.0					
343 days Winter Wheat 100% Maturity	Stalks	0.002	100	NA	NA	NA	NA	
	Husks	0.001	100	NA	NA	NA	NA	
	Grain	< 0.001	100	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.028	74.4	NA	7.8	2.0	92.5	102.3
	5–10 cm	0.006	16.5	NA	NA	NA	NA	
	10–20 cm	0.001	6.4	NA	NA	NA	NA	
	20–30 cm	< 0.001	2.8	NA	NA	NA	NA	

Days After Treatment / Growth Stage of Rotational Crops	Plant Parts / Soil Layers	Total Residues		Parent (mg/kg) [a]	Extractable radioactivity (%)		Non Extractable (%)[c]	Total (%) [c]
		(mg/kg) [a]	(%) [b]		Cold [c]	Soxhlet [c]		
	Soil Total	0.005	100.0					

[a] in trinexapac-ethyl equivalents; limit of detection for combustion = 0.001 mg/kg

[b] in % of radioactivity found in the sub-balanced plant parts/ total soil system

[c] in % of radioactivity found in the plant part/ soil layer, determined by combustion

NA = Not analysed

Table 40 Distribution of radioactivity and residual trinexapac-ethyl in rotational sugar beet and soil following application of [^{14}C] cyclohexyl trinexapac-ethyl to bare ground

Days After Treatment / Growth Stage of Rotational Crops	Plant Parts / Soil Layers	Total Residues		Parent (mg/kg) [a]	Extractable radioactivity (%)		Non Extractable (%) [c]	Total (%) [c]
		(mg/kg) [a]	(%) [b]		Cold [c]	Soxhlet [c]		
299 days (planting)	Soil							
	0–5 cm	0.076	73.6	NA	9.6	2.0	86.6	98.2
	5–10 cm	0.018	17.9	NA	8.6	2.1	86.4	97.1
	10–20 cm	0.003	8.0	NA	NA	NA	NA	
	20–30 cm	< 0.001	0.4	NA	NA	NA	NA	
	Soil Total	0.014	100.0					
343 days Sugar Beet 25% Maturity	Tops	< 0.001	100.0	NA	NA	NA	NA	
	Roots	0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.063	69.2	NA	8.1	2.1	85.8	96.0
	5–10 cm	0.014	17.1	NA	10.1	1.8	86.7	98.6
	10–20 cm	0.004	10.2	NA	NA	NA	NA	
	20–30 cm	0.001	3.5	NA	NA	NA	NA	
	Soil Total	0.014	100.0					
407 days Sugar Beet 50% Maturity	Tops	< 0.001	100.0	NA	NA	NA	NA	
	Roots	< 0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.042	46.0	NA	7.3	2.0	93.1	102.4
	5–10 cm	0.019	23.8	NA	6.8	1.3	89.0	97.1
	10–20 cm	0.010	27.2	NA	NA	NA	NA	
	20–30 cm	0.001	3.0	NA	NA	NA	NA	
	Soil Total	0.013	100.0					
496 days Sugar Beet 100% Maturity	Tops	< 0.001	100	NA	NA	NA	NA	
	Roots	< 0.001	100	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.044	65.8	NA	7.5	2.4	95.9	105.8
	5–10 cm	0.017	24.0	NA	7.4	1.7	91.5	100.6
	10–20 cm	0.003	8.6	NA	NA	NA	NA	
	20–30 cm	< 0.001	1.5	NA	NA	NA	NA	
	Soil Total	0.010	100.0					

[a] in trinexapac-ethyl equivalents; limit of detection for combustion = 0.001 mg/kg

[b] in % of radioactivity found in the sub-balanced plant parts/ total soil system

[c] in % of radioactivity found in the plant part/ soil layer, determined by combustion

NA = Not analysed

Table 41 Distribution of radioactivity and residual trinexapac-ethyl in rotational maize and soil following application of [^{14}C] cyclohexyl trinexapac-ethyl to bareground

Days After Treatment / Growth Stage of Rotational Crops	Plant Parts / Soil Layers	Total Residues		Parent (mg/kg)[a]	Extractable radioactivity (%)		Non Extractable (%) [c]	Total (%) [c]
		(mg/kg) [a]	(%) [b]		Cold [c]	Soxhlet [c]		
338 days (planting)	Soil							
	0–5 cm	0.065	72.2	NA	9.8	1.9	87.6	99.3
	5–10 cm	0.019	21.8	NA	7.7	1.7	84.9	94.3
	10–20 cm	0.002	5.1	NA	NA	NA	NA	
	20–30 cm	< 0.001	0.9	NA	NA	NA	NA	
	Soil Total	0.012	100.0					
369 days Maize 25% Maturity	Whole Tops	0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.051	73.5	NA	8.1	2.0	88.7	98.8
	5–10 cm	0.013	20.6	NA	6.7	1.4	90.0	98.1
	10–20 cm	0.002	5.4	NA	NA	NA	NA	
	20–30 cm	0.001	0.6	NA	NA	NA	NA	
	Soil Total	0.009	100.0					
407 days Maize 50% Maturity	Whole Tops	< 0.001	100.0	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.047	52.4	NA	9.3	2.5	87.7	99.5
	5–10 cm	0.018	39.6	NA	7.5	1.2	92.6	101.3
	10–20 cm	0.002	7.1	NA	NA	NA	NA	
	20–30 cm	< 0.001	0.9	NA	NA	NA	NA	
	Soil Total	0.011	100.0					
496 days Maize 100% Maturity	Stalks	< 0.001	100	NA	NA	NA	NA	
	Cobs	< 0.001	100	NA	NA	NA	NA	
	Grain	< 0.001	100	NA	NA	NA	NA	
	Soil							
	0–5 cm	0.034	55.3	NA	7.1	2.2	98.7	108.0
	5–10 cm	0.015	26.9	NA	5.4	1.5	95.2	102.1
	10–20 cm	0.004	16.0	NA	NA	NA	NA	
	20–30 cm	< 0.001	1.9	NA	NA	NA	NA	
	Soil Total	0.009	100.0					

[a] in trinexapac-ethyl equivalents; limit of detection for combustion = 0.001 mg/kg

[b] in % of radioactivity found in the sub-balanced plant parts/ total soil system

[c] in % of radioactivity found in the plant part/ soil layer, determined by combustion

NA = Not analysed

Total radioactive residues in rotational crops following soil application of [^{14}C] cyclohexyl were found to be:

- 0.001 mg/kg in lettuce head samples at 50% maturity (99 days after application) and 100% maturity (119 days after application)
- 0.001 mg/kg in winter wheat whole top samples at fall cutting (173 days after application), < 0.001 mg/kg in whole tops at 50% maturity (299 days after application) and 75% maturity (343 days after application). Harvest stalks, husks and grain taken 407 days after application contained 0.002 mg/kg, 0.001 mg/kg and < 0.001 mg/kg respectively
- 0.001 mg/kg in sugar beet root samples at 25% maturity (343 days after application), < 0.001 mg/kg in sugar beet tops at 25% maturity and tops and roots taken at 50% maturity (407 days after application). and at maturity (496 days after application)

- 0.001 mg/kg in maize whole tops samples at 25% maturity (369 days after application) and < 0.001 mg/kg in whole tops taken at 50% maturity (407 days after application). Harvest stalks, cobs, and grain taken 496 days after application contained < 0.001 mg/kg.

The uptake of residues by the rotational crops lettuce, winter wheat, sugar beet and maize planted or sown after several intervals after application of trinexapac-ethyl to bareground was therefore very low (< 0.001–0.002 mg/kg). No accumulation was observed. Due to the residues in the rotational crop RACs being < 0.01 mg/kg, no characterisation was possible. The very limited uptake of radioactive material in succeeding crops clearly indicates the lack of systemic behavior of trinexapac-ethyl. It was concluded that the residues situation in rotational crops is negligible.

Greater than 90% of the TRR after application of trinexapac-ethyl to bare ground remained in the top 10 cm of soil during the entire cultivation of the succeeding crops. In soil significant residues (TRR) were only observed in the top soil layer (0–5cm) in the range of 0.065–0.076 mg/kg at planting or sowing and at 0.028–0.080 mg/kg at harvest. In layers below, the TRR did not exceed 0.019 mg/kg and was in most cases < 0.010 mg/kg. Extractables in the top soil layer amounted to 7.1–14.9% of TRR after cold extraction using 50% aqueous methanol. An additional 1.4–2.5% of TRR was extractable after a hot Soxhlet extraction with methanol. 78.9–98.7% of TRR in the top soil layer were non-extractables.

The confined and field rotational crop studies suggest that residues of trinexapac-ethyl are unlikely to occur in succeeding crops.

RESIDUE ANALYSIS

Analytical methods

Details of analytical methods including validation data were supplied for the determination of trinexapac acid or trinexapac-ethyl in plant and animal matrices, soil, water and air and are considered satisfactory. A summary of all analytical methods for plants and animals is given in Table 42.

Table 42 Summary of analytical methods developed for plant and animal matrices

Matrix	Analyte	Method No.	Detection system	LOQ	Reference
Plant	Trinexapac acid	110-01	HPLC-MS	Field grown grass (forage, straw, hay, seed) LOQ = 0.05 mg/kg	Lin 2002, CGA163935/0962 Cobin and Pyles 2002, CGA163935/0961 (ILV for 110-01)
Plant	Trinexapac acid (free and conjugated)	GRM020.01A	HPLC-MS/MS	Field grown grass (forage, straw, seed), wheat (grain, forage, straw) LOQ = 0.01 mg/kg	Lin 2008, CGA179500_50010 Thomas 2010, CGA179500_50000 (ILV for GRM020.01A)
Plant	Trinexapac acid	REM 137.02	HPLC-UV	Wheat (grain, straw), barley (grain, straw), rape seed LOQ = 0.02 mg/kg	Forrer 1991, CGA163935/0082 Sack 1999, CGA163935/0588 (Validation for REM 137.02)
Plant	Trinexapac acid	REM 137.11	HPLC-UV	Rape seed oil LOQ = 0.02 mg/kg	Sack 1995, CGA163935/0417
Plant	Trinexapac acid	REM 137.13	HPLC-MS/MS	Beans, apple, potato, wheat grain, oilseed rape LOQ = 0.01 mg/kg	Campbell and Crook 2004, CGA163935/0990 Benazeraf 2004,

Matrix	Analyte	Method No.	Detection system	LOQ	Reference
				LOQ = 0.02 mg/kg (beans)	CGA163935/0988 (ILV for REM 137.13)
Plant	Trinexapac-ethyl	REM 137.01	HPLC-UV	Wheat (grain, straw), barley (grain, straw) LOQ = 0.04 mg/kg	Forrer 1989, CGA163935/0033
Plant	Trinexapac-ethyl	REM 137.05	HPLC-UV	Rape seed LOQ = 0.02 mg/kg	Forrer 1991a, CGA163935/0142
Animal	Trinexapac acid	REM 137.12	HPLC-UV	LOQ = 0.02 mg/kg LOQ = 0.01 mg/kg (milk) LOQ = 0.005 mg/kg (milk – modified method using HPLC-MS/MS in dairy cattle feeding study)	Sack 1995, CGA163935/0440 Gasser 2004, CGA163935/0740 (ILV for REM 137.12)
Animal	Trinexapac acid	REM 137.14	HPLC-MS/MS	LOQ = 0.01 mg/kg LOQ = 0.005 mg/kg (milk)	Kwiatkowski 2004, CGA179500/0039 Benazeraf 2004, CGA163935/0989 (ILV for REM 137.14)

ILV = Independent Laboratory Validation

Plant commodities

Method 110-01

Method 110-01 for the determination of trinexapac as trinexapac acid in plant matrices by means of high performance liquid chromatography with mass spectrometry (HPLC/MS) was reported by Lin (2002, CGA163935/0962). The method includes a reflux extraction procedure which is buffered to pH 7 and therefore does not hydrolyse sugar conjugates. Residues are quantified against a trinexapac acid external standard to give residues of "free" trinexapac acid.

Samples are extracted by refluxing for 1 hour with acetonitrile/ sodium phosphate buffer (70:30, v/v) at pH 7. The sample is passed through a pre-conditioned C_{18} SPE cartridge for purification and evaporated to an aqueous solution (rotary evaporator). The aqueous solution is acidified and is then passed through a second SPE cartridge (C_8). The cartridge is rinsed with acetonitrile/ 0.05% H_3PO_4 aqueous solution (10:90, v/v) and the analyte is eluted from the column with acetonitrile/ 0.05% H_3PO_4 aqueous solution (30:70, v/v). The eluate is evaporated again to an aqueous solution, acidified and is liquid/liquid partitioned twice against a methyl tertiary butyl ether (MTBE)/hexane (63:35, v/v) with the addition of salt. The organic layers are collected and evaporated to dryness and re-dissolved in ethyl acetate. The ethyl acetate sample is passed through a pre-conditioned silica SPE column and rinsed with methanol/ ethyl acetate (15:85, v/v). The analyte is eluted from the cartridge with methanol/acetonitrile/ ethyl acetate (40:30:30, v/v/v). The eluate is evaporated to near dryness and dissolved in acetonitrile/water (10:90, v/v). The sample is injected onto a reverse phase C_8 column HPLC/MS/MS system for analysis of trinexapac acid using 0.1% acetic acid/acetonitrile (60:40, v/v) as the mobile phase. Detection in the positive ionisation mode was used (mass monitored 225).

Samples of grass (forage, hay/ straw, seed and seed screenings were spiked with trinexapac acid at levels of 0.05, 1 and 5 mg/kg and then extracted and analysed using Method 110-01. Recoveries were found to be within acceptable ranges. In all matrices tested, the individual recovery values were in the acceptable range of 70–120%. The relative standard deviations (RSD, %) for all commodities and all spiking levels were at or below 20%. The mean recovery data are summarized in Table 43. The LOQ was reported as 0.05 mg/kg in all matrices.

Table 43 Method recoveries for method 110-01: trinexapac acid in field grown grass matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
	0.05	3	72–107	87	20
Forage	1	1	75	–	–
	5	3	76–94	86	11
	0.05	3	72–99	86	16
Hay/straw	1	1	86	–	–
	5	3	90–94	92	2
	0.05	2	84, 95	90	–
Seed	1	1	98	–	–
	5	3	77–92	87	10
	0.05	3	72–87	80	10
Seed screenings	1	1	104	–	–
	5	3	75–102	92	16

The extraction efficiency of Method 110-01 was tested. Grass matrices (forage, straw, seed and seed screenings) samples from the metabolism study were analysed in triplicate.

Table 44 Extractability of residues of [^{14}C] trinexapac in grass matrices according to Method 110-01

Matrix	TRR (mg/kg) [a] Determined in Study 623-00	LSC Values (mg/kg) [b] Determined in this Study	Method 110-01 Extraction Efficiency [c]	Trinexapac acid residues (mg/kg) [d] Determined by Method 110-01	Accountability (%) [e]
Forage	2.03	1.6	80%	0.12 (4.8%)	5.9%
Straw	4.78	3.6	75%	0.75 (4.3%)	15.7%
Seed	5.45	3.2	58%	0.36 (38.9%)	6.6%
Seed screenings	7.13	4.2	59%	0.61 (16.6)	8.6%

[a] TRRs were determined by combustion analyses of radiolabelled samples in Study 623-00 (Ray and May-Hertl 2003, CGA163935/0862)

[b] Liquid scintillation counting (LSC) of the radiolabelled extracts in this study (averaged from triplicate samples)

[c] Extractability or extraction efficiency = (LSC results ÷ TRR values)× 100%

[d] Residues concentrations averaged from triplicate analysis (RSD)

[e] Accountability = (Residues found by this method ÷TRR)× 100%

It was found that the extraction efficiencies determined by Method 110-01 ranged from 58–80% for various grass matrices. Concentrations of trinexapac acid were determined by the method. Accountability ranged from 5.9% in forage to 15.7% in straw.

An independent laboratory validation report for Method 110-01 for the determination of residues of trinexapac acid (Cobin and Pyles 2002, CGA163935/0961) in plants (grass forage, hay, straw and seed) has been submitted. Minor modifications, principally concerning the silica SPE column, were suggested. Grass forage, straw and seed screenings were successfully validated during the first trial and grass hay during the second trial.

Table 45 Recovery results of method 110-01: trinexapac in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Grass Forage	0.05	2	70, 70	70	–
	0.5	2	74, 83	79	–
Grass Hay	0.05	2	42, 49 (Trial 1) 85, 90 (Trial 2)	46 88	– –
	0.5	2	88, 92 (Trial 1) 96, 96 (Trial 2)	90 96	– –
Grass Straw	0.05	2	99, 109	104	–

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
	0.5	1	102	–	–
Grass Seed Screenings	0.05	2	105, 113	109	–
	0.5	2	99, 104	102	–

Note: For hay the method was changed from trial 1 to trial 2 to improve recoveries

Concurrent recovery results obtained during the residues and processing studies are tabulated below.

Table 46 Concurrent recovery results for method 110-01: trinexapac in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid				Reference
		No.	Range [%]	Mean [%]	RSD [%]	
	0.05	4	70–86	79	9	Ediger 2006,
Spring Wheat Forage	0.50	3	75–85	81	7	451534
	5	1	111	111	–	
	0.05	2	70, 85	78	–	
Spring Wheat Hay	0.5	2	85, 120	103	–	
	5	3	104	104	–	
	0.05	3	70–92	82	14	
Spring Wheat Straw	0.5	1	82	82	–	
	2	1	80	80	–	
	5	1	95	95	–	
	0.05	4	88–94	90	5	
Spring Wheat Grain	0.5	4	72–109	96	17	
	10	1	96	96	–	
	15	1	80	80	–	
Spring Wheat Aspirated	0.5	1	101	101	–	
Grain Fractions	5.0	1	112	112	–	
Spring Wheat Bran	0.5	1	77	77	–	
	25	1	81	81	–	
Spring Wheat Flour	0.05	1	89	89	–	
	5	1	103	103	–	
Spring Wheat Middlings	0.05	1	80	80	–	
	5	1	82	82	–	
Spring Wheat Shorts	0.05	1	94	94	–	
	10	1	78	78	–	
Spring Wheat Germ	0.50	1	70	70	–	
	10	1	94	94	–	
Winter Wheat Forage	0.1	6	70–73	71	2	
	0.5	8	73–120	90	17	
Winter Wheat Hay	0.2	1	69	69	–	
	0.5	9	64–105	85	16	
Winter Wheat Straw	0.1	3	70–88	78	12	
	0.5	8	71–104	89	13	
	1	1	80	80	–	
Winter Wheat Grain	0.05	1	86	86	–	
	0.1	6	72–92	83	8	
	0.5	7	76–99	90	10	
Winter Wheat Aspirated	0.05	1	118	118	–	
Grain Fractions	0.5	1	101	101	–	
Winter Wheat Bran	0.05	1	91	91	–	
	0.5	1	98	98	–	
Winter Wheat Flour	0.05	1	92	92	–	
	0.5	1	118	118	–	
Winter Wheat Middlings	0.05	1	89	89	–	
	0.5	1	87	87	–	
Winter Wheat Shorts	0.05	1	109	109	–	

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid				Reference
		No.	Range [%]	Mean [%]	RSD [%]	
	0.5	1	96	96	–	
Winter Wheat Germ	0.05	1	110	110	–	
	0.5	1	89	89	–	
Stripped Cane	0.05	4	85–104	94	9	Ediger 2006a,
	0.50	4	80–112	95	14	451598
	0.05	4	82–111	95	16	
Stripped Matured Cane	0.50	4	97–107	102	4	
	2.0	2	94, 99	97	–	
	0.05	1	94	94	–	
Stripped Cane (before	0.5	1	100	100	–	
processing)	1.0	2	114, 124	119	–	
Molasses	0.05	1	87	87	–	
	2.0	2	81, 90	86	–	
Refined Sugar	0.05	1	92	92	–	
Spinach Leaves	0.05	2	71, 78	75	–	Ediger 2006b,
	0.5	2	87, 89	88	–	CGA163935/1054
Radish Tops	0.05	2	60, 107	84	–	
	0.5	2	80, 94	87	–	
Radish Roots	0.05	2	88, 91	90	–	
	0.5	2	89, 100	95	–	
Wheat Forage	0.05	4	74, 76, 79, 104	83	17	
	0.5	4	85, 88, 92, 100	91	7	
Wheat Hay	0.05	2	67, 70	69	–	
	0.5	2	79, 111	95	–	
Wheat Straw	0.05	2	79, 120	100	–	
	0.5	2	102, 117	110	–	
Wheat Grain	0.05	2	111, 111	111	–	
	0.5	2	91, 112	102	–	

Method GRM020.01A

A validated method (GRM020.01A) has been reported for the determination of trinexapac acid in plant matrices using HPLC-MS/MS (Lin 2008, CGA179500_50010). It is a modified version of Method 110-01. This method includes a strong acid hydrolysis procedure to release "conjugated" residues of trinexapac acid from plant matrices. Residues are quantitated against a trinexapac acid external standard to give the sum of "free" and "conjugated" trinexapac acid. Other modifications included reducing the steps for sample clean-up procedures and using HPLC/MS/MS for final determination.

Samples are refluxed for 5 hours with acetonitrile/ 1 N hydrochloric acid (80:20, v/v). An aliquot of the extract supernatant is mixed with water and passed through a C_8 SPE cartridge (pre-conditioned with acetonitrile then 0.5% formic acid). The C_8 SPE cartridge is rinsed with water then water/ acetonitrile (80:20, v/v). The analyte is eluted from the cartridge with formic acid aqueous solution/ acetonitrile (80:20, v/v). The eluent is injected onto a reverse phase ODS-2 column HPLC-MS/MS system for analysis of trinexapac acid. MS/MS detection in the negative ionization mode is used to monitor the ion transition 223→135 (quantitation) for trinexapac. The 223→179 transition can be used for the confirmatory analysis of trinexapac residues.

Samples of field grown grass commodities (forage, straw, seed screenings and seed) were spiked with trinexapac acid at levels of 0.01–1 mg/kg. Samples were extracted and analysed with Method GRM020.01A (HPLC-MS/MS). In the matrices tested the mean recoveries were within 70–120% (Table 47). The relative standard deviations (RSD, %) for all commodities and all spiking levels were below 20%. The LOQ was defined by the lowest spiking level successfully tested, which was 0.01 mg/kg for trinexapac acid in all matrices.

Table 47 Method recoveries for GRM020.01A: trinexapac acid in grass matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Forage	0.01	5	95–101	99	2
	0.1	5	85–105	97	8
	0.2	1	100	–	–
Straw	0.01	5	93–97	94	2
	0.02	5	95–100	98	2
	0.2	5	86–91	89	2
	1	1	107	–	–
Seed screenings	0.01	5	91–99	96	3
	0.1	5	97 (×5)	97	0
	0.2	1	100	–	–
Seed	0.01	5	95–97	96	1
	0.1	5	84–100	92	7

The extraction efficiency (radio-validation) of Method GRM020.01A was tested. Grass matrices (forage, straw and seed screenings) samples from the metabolism study were analysed in triplicate.

Table 48 Extractability of residues of [^{14}C] trinexapac in grass matrices according to Method GRM020.01A

Matrix	TRR (mg/kg) [a] Determined in Study 623-00	LSC Values (mg/kg) [b] Determined in this Study	Method GRM020.01A Extraction Efficiency [c]	Trinexapac acid residues (mg/kg) [d] Determined in Study 623-00	Trinexapac acid residues (mg/kg) [e] Determined by GRM020.01A
Forage	2.03	2.28	112%	0.329	0.40
Straw	4.78	4.85	101%	1.068	1.3
Seed screenings	7.13	6.37	89%	0.906	2.0

[a] TRRs were determined by combustion analyses of radiolabelled samples in Study 623-00 (Ray and May-Hertl 2003, CGA163935/0862)

[b] Liquid scintillation counting (LSC) of the radiolabelled extracts in this study

[c] Extractability or extraction efficiency = (LSC results ÷ TRR values)× 100%

[d] Data reported in Study 623-00 (Ref.)

[e] Averaged from triplicate analysis of radiolabelled samples (duplicate for seed screenings) by Analytical Method GRM020.01A LC-MS/MS analysis

It was found that the extraction efficiency and residue concentrations determined by Method GRM020.01A were in general agreement with those reported in the metabolism study. The radio-validation data confirms that the method is capable of accurately quantifying incurred residues of trinexapac acid as the sum of free and conjugated residues.

An independent laboratory validation report for Method GRM020.01A for the determination of residues of trinexapac acid in plants (wheat grain, forage and straw) has been submitted (Thomas 2010, CGA179500_50000).

For trinexapac, the transition 223→135 was used for quantitation, and 223→179 was used for confirmation. Recoveries at fortification levels of 0.01 (LOQ) and 0.1 mg/kg were provided for the quantitation transition and were found to be acceptable (Table 49). All individual recoveries were between 70–120% with a relative standard deviation < 20% for each fortification level / matrix combination. The LOQ was defined by the lowest spiking level successfully tested, which was 0.01 mg/kg for trinexapac in all matrices.

Table 49 Recovery results of method GRM020.01A: trinexapac acid in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Wheat Grain	0.01	5	86–109	95	10
	0.10	5	95–106	100	4
Wheat Forage	0.01	5	79–98	90	8
	0.10	5	85–97	91	5
Wheat Straw	0.01	5	94–108	100	5
	0.10	5	98–105	102	3

The method GRM020.01A was successfully independently validated for measurement of residues of trinexapac acid in wheat forage, straw and grain.

Method and concurrent recovery results obtained for Method GRM020.01A during the residues and processing studies are tabulated in the following tables.

Table 50 Method recoveries for method GRM020.01A: trinexapac acid in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid				Reference
		No.	Range [%]	Mean [%]	RSD [%]	
Wheat Grain	0.01	2	110, 112	111	–	Mäyer 2010,
	0.10	2	106, 106	106	–	CGA163935_50036
Wheat AGFs	0.01	2	82, 83	83	–	
	0.10	2	96, 96	96	–	
Wheat Bran	0.01	2	112, 102	107	–	
	0.10	2	114, 116	115	–	
Wheat Flour	0.01	2	78, 84	81	–	
	0.10	2	95, 111	103	–	
Wheat Middlings	0.01	2	75, 76	76	–	
	0.10	2	83, 85	84	–	
Wheat Shorts	0.01	2	81, 84	83	–	
	0.10	2	84, 89	87	–	
Wheat Germ	0.01	2	93, 108	101	–	
	0.10	2	93, 94	94	–	
Sugarcane	0.01	2	76, 70	73	–	Mäyer 2010b,
	0.10	2	80, 76	78	–	CGA163935_50038
Molasses	0.01	2	101, 89	95	–	
	0.10	2	94, 87	91	–	
Refined Sugar	0.01	2	85, 78	82	–	
	0.10	2	82, 71	77	–	
	2.5	2	71, 79	75	–	Simmons 2010,
Poultry feed	10.0	2	70, 75	38	–	CGA179500_50008
	41.0	2	79, 90	85	–	

Table 51 Concurrent recovery results of method GRM020.01A: trinexapac acid in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid				Reference
		No.	Range [%]	Mean [%]	RSD [%]	
	0.01	5	72–109	96	16	Mäyer 2010a,
Barley Hay	0.10	4	100–110	105	4	CGA163935_50026
	1.0	1	107	107	–	
	7.0	1	98	98	–	
	0.01	5	72–112	95	15	
Barley Straw	0.10	4	81–110	95	13	
	1.0	1	80	80	–	
	0.01	8	82–111	95	12	
Barley Grain	0.10	7	72–92	82	8	
	11.0	1	94	94	–	

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid				Reference
		No.	Range [%]	Mean [%]	RSD [%]	
Pearled Barley	0.01	1	108	108	–	
	20.0	1	114	114	–	
Barley Flour	0.01	1	102	102	–	
	20.0	1	111	111	–	
Barley Bran	0.01	1	91	91	–	
	20.0	1	100	100	–	
Wheat Forage	0.01	9	72–120	93	18	Mäyer 2010,
	0.10	8	84–105	96	7	CGA163935_50036
	10	1	94	94	–	
Wheat Hay	0.01	9	71–118	94	18	
	0.10	8	90–107	100	6	
	11	1	106	106	–	
	0.01	9	71–112	88	19	
Wheat Straw	0.10	8	89–107	96	6	
	1.0	1	111	111	–	
	0.01	12	83–120	101	12	
Wheat Grain	0.10	11	88–113	103	7	
	15	1	113	113	–	
Wheat AGFs	0.01	1	74	74	–	
	20	1	86	86	–	
	0.01	3	94–120	108	12	
Wheat Bran	0.10	2	106, 107	107	–	
	20	1	84	84	–	
Wheat Flour	0.01	1	107	107	–	
	20	1	115	115	–	
	0.01	2	99, 100	100	–	
Wheat Middlings	0.10	1	96	96	–	
	10	1	116	116	–	
	0.01	2	70, 117	94	–	
Wheat Shorts	0.10	1	78	78	–	
	10	1	86	86	–	
	0.01	2	74, 114	94	–	
Wheat Germ	0.10	1	100	100	–	
	15	1	99	99	–	
	0.01	6	71–100	83	14	Mäyer 2010b,
Sugarcane	0.10	5	71–92	84	10	CGA163935_50038
	10.0	1	71	71	–	
Molasses	0.01	1	85	85	–	
	20.0	1	75	75	–	
Refined Sugar	0.01	1	71	71	–	
	20.0	1	71	71	–	
Wheat Germ	0.1	8	79–109	99	11	Brown 2011,
Wheat Bran	0.1	8	85–103	93	8	CGA179500_50012
Wheat Flour	0.1	8	70–107	87	17	
	2.5	3	76, 98, 101	92	15	Simmons 2010,
Poultry feed	10.0	2	70, 81	76	–	CGA179500_50008
	41.0	1	105	–	–	

Method REM 137.02

A validated method (REM 137.02) has been reported for the determination of trinexapac acid in plant matrices using HPLC-UV (Forrer 1991, CGA163935/0082).

In the case of wheat and barley, trinexapac acid is extracted by shaking a homogenised subsample with a pH 7 methanol/aqueous phosphate buffer (30:70, v/v) to analyse for free trinexapac acid. In the case of rapeseed, trinexapac acid is extracted by shaking a homogenised subsample with a pH 7 acetonitrile/aqueous phosphate buffer (80:20, v/v) to analyse for free trinexapac acid. An extract aliquot is transferred to a pre-conditioned (methanol/water 50:50, v/v) solid phase extraction cartridge

(SAX). After washing with methanol/water (30:70, v/v) and water, trinexapac acid is eluted with sulphuric acid (0.1 N) and partitioned into dichloromethane. The organic phase is evaporated to dryness. Additional clean-up and final determination are achieved using a three-column HPLC switching system with UV-detection. The LOQ of this method was given as 0.02 mg/kg trinexapac acid. Recovery data are summarized in Table 52.

Table 52 Method recoveries for method REM 137.02: trinexapac acid in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Wheat Grain	0.04	1	94	–	–
	0.2	1	80	–	–
Wheat Straw	0.04	1	104	–	–
	0.2	1	99	–	–
Barley Grain	0.04	3	96–101	98	3
	0.2	3	89–98	95	5
Barley Straw	0.04	3	96–110	102	7
	0.2	3	89–97	94	5
Rape Seed	0.04	1	105	–	–
	0.2	1	98	–	–

In a validation study for Method 137.02 (Sack 1999, CGA163935/0588) for the final determination the HPLC system was changed from the original 3-column LC system to the 2-column system which follows the same reversed phase principles as the 3-column system. The LOQ was defined by the lowest spiking level successfully tested, which was 0.02 mg/kg for trinexapac acid in all matrices.

Samples of wheat and rape seed matrices were spiked with trinexapac acid at levels of 0.02–0.2 mg/kg. Samples were extracted and analysed with Method REM 137.02 (HPLC-UV). In the matrices tested mean recoveries were within 70–120%. The recovery data are summarized in Table 53. Relative standard deviations (RSD, %) for all commodities and all spiking levels were below 20%. Reproducibility was demonstrated by the successful validation with wheat grain and rapeseed carried out in a second laboratory, fulfilling the requirement for an enforcement method.

Table 53 Method recoveries for method REM 137.02: trinexapac acid in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Wheat Grain	0.02	5	92–102	98	4
	0.20	5	71–93	85	10
Wheat Straw	0.02	5	98–110	104	5
	0.20	5	79–93	89	6
Rape Seed	0.02	5	75–96	90	10
	0.20	5	73–81	78	4
Wheat Grain [a]	0.02	5	75–83	78	4
	0.20	5	70–75	72	3
Rape Seed [a]	0.02	5	76–79	77	2
	0.20	5	68–72	70	3

[a] Results from the second laboratory

Method REM 137.11

Details of a method (REM 137.11) for the determination of trinexapac acid in rape seed oil using HPLC-UV have been reported in the rape seed processing studies (*e.g.* Sack 1995, CGA163935/0417).

Trinexapac acid is extracted from rape seed oil four times by vigorously shaking with methanol. The combined methanol phases are diluted with pH 7 phosphate buffer. This aqueous

sample is pre-cleaned using a C18 SPE column. After acidification of the sample passed through the first column, trinexapac acid is extracted by means of a second C18 SPE column. Final determination is performed using a two column HPLC with UV-detection. Samples of rape seed oil were spiked with trinexapac acid at levels of 0.02 and 0.1 mg/kg (recoveries 88% and 87% respectively).

Method REM 137.13

A validated method (REM 137.13) has been reported for the determination of free trinexapac acid in plant matrices using HPLC-MS/MS (Campbell and Crook 2004, CGA163935/0990). The method was developed from Method 137.02 with the replacement of the HPLC-UV two column switching method by solid phase extraction (SPE) followed by single column HPLC-MS/MS.

Samples are extracted by homogenisation with pH 7 methanol/ water/ phosphate buffer (30:56:14, v/v/v) to analyse for free trinexapac acid. Extracts are centrifuged and aliquots are subsequently diluted with ultra-pure water. A SPE procedure is then carried out to facilitate sample clean up. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

Samples of broad bean seed, broad bean whole plant and broad bean remainder were spiked with trinexapac acid at levels of 0.02–4 mg/kg (whole plant), 0.02–0.2 mg/kg (remainder) and 0.02–10.0 mg/kg (seed) (Table 54). Samples were extracted and analysed using Method REM 137.13. In the matrices tested the mean recoveries were within 70–120%. The relative standard deviations (RSD, %) for all commodities and all spiking levels were ≤ 20%. MS/MS detection in the negative ionisation mode is used to monitor the ion transition 223→83 for trinexapac.

Table 54 Method recoveries for method REM 137.13: trinexapac acid in bean matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Broad Bean (Whole Plant)	0.02	5	91–104	97	5
	4	5	93–102	96	4
Broad Bean (Remainder)	0.02	5	64–96	78	20
	0.2	5	69–111	91	17
Broad Bean (Seed)	0.02	5	75–98	89	10
	10.0	5	87–96	91	4

An independent laboratory validation report for Method REM 137.13 for the determination of residues of trinexapac acid (Benazeraf 2004, CGA163935/0988) in plants has been submitted.

Samples of apple, potato, wheat grain and oilseed rape were spiked with trinexapac acid at levels of 0.01–0.1 mg/kg (apple), 0.01–0.1 mg/kg (potato), 0.01–0.3 (wheat grain) and 0.01–1 mg/kg (oilseed rape). Samples were extracted and analysed using Method REM 137.13. In the matrices tested the mean recoveries were within 70–120% and the relative standard deviations (RSD, %) for all commodities and all spiking levels were ≤ 20% (Table 55).

Table 55 Recovery results of method REM 137.13: trinexapac acid in plant matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Apple	0.01	5	63–100	84	18
	0.1	5	72–98	90	12
Potato	0.01	5	97–111	103	6
	0.1	5	88–104	98	7
Wheat Grain	0.01	5	72–96	84	12
	0.3	5	79–103	92	9
Oilseed Rape	0.01	5	74–90	85	8
	1	5	79–87	83	5

The LOQ was defined by the lowest spiking level successfully tested, which was 0.01 mg/kg each for trinexapac in all matrices.

Method REM 137.01

Method REM 137.01 has been reported for the determination of trinexapac-ethyl in wheat and barley grains and straw using HPLC-UV (Forrer 1989, CGA163935/0033).

Samples are extracted by homogenisation with pH 7 methanol/ aqueous phosphate buffer (30:70, v/v) to analyse for trinexapac-ethyl. Extracts are acidified and the compound re-extracted into dichloromethane. A SPE procedure is then carried out to facilitate sample clean up. Final determination is by a three column high performance liquid chromatography switching system with UV-detection.

Samples of wheat grain and straw were spiked with trinexapac-ethyl at levels of 0.04 and 0.2 mg/kg (Table 56). Samples were extracted and analysed using Method REM 137.01. In the matrices tested the mean recoveries were within 70–120%. The relative standard deviations (RSD, %) for all commodities and all spiking levels were ≤ 21%. Supplementary data (wheat and barley grain and straw) for confirmation of the validity of Method REM 137.01, using a simplified chromatographic system with only the first two columns, was presented in the same study. Method REM 137.01 was also validated in this study for quantitation of residues of trinexapac-ethyl in soil.

Table 56 Method recoveries for method REM 137.01: trinexapac-ethyl in cereal matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac-ethyl			
		No.	Range [%]	Mean [%]	RSD [%]
Wheat grain	0.04	6	60–111	87	21
	0.2	9	71–104	87	12
Wheat straw	0.04	2	100, 103	102	–
	0.2	7	99–106	103	3
Wheat grain [a]	0.04	5	70–104	89	16
	0.2	5	83–99	93	7
Wheat straw [a]	0.04	5	73–105	92	13
	0.2	5	82–99	93	8
Barley grain [a]	0.04	4	72–99	87	13
	0.2	4	77–99	89	11
Barley straw [a]	0.04	4	87–104	98	8
	0.2	4	86–101	94	7

[a] Supplementary data supplied with Forrer 1989, CGA163935/0033 using a simplified chromatographic system

Method REM 137.05

A validated method (REM 137.05) has been reported for the determination of trinexapac-ethyl in rape seed using HPLC-MS/MS (Forrer 1991a, CGA163935/0142).

Trinexapac-ethyl is extracted by shaking a homogenised sub-sample with a pH 7 mixture of acetonitrile and aqueous phosphate buffer. An aliquot of the extract is washed with hexane, acidified and the compound is re-extracted into dichloromethane or into tert-butyl methyl ether/ethyl acetate. Additional clean-up and final determination are achieved by a three column HPLC switching system with UV-detection. Samples of rape seed were spiked with trinexapac-ethyl at levels of 0.04 – 0.2 mg/kg. Samples were extracted and analysed with REM 137.05 (HPLC-UV). Mean recoveries were within 70–120% (Table 57). The relative standard deviations (RSD, %) for all commodities and all spiking levels were below 20%. The limit of quantitation for trinexapac-ethyl residues in crop commodities using Method REM 137.05 was established at 0.02 mg/kg.

Table 57 Method recoveries for method REM 137.05: trinexapac-ethyl in rape seed

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Rape Seed	0.04	2	80, 91	86	-
	0.2	7	78-103	91	10
Rape Seed [a]	0.04	6	72-84	78	6
	0.2	6	62-82	75	10

[a] Recoveries were generated with Method REM 137.01 using a simplified chromatographic system. Instead of the three column HPLC (as described for Method REM 137.05) the first two columns only were used.

Animal commodities

Method REM 137.12

Method REM 137.12 was developed for the determination of trinexapac acid in animal matrices (Sack 1995, CGA163935/0440). This HPLC-UV method was superseded by Method REM 137.14 (LC-MS/MS).

Method REM 137.12 involves the extraction of trinexapac acid from tissues (meat, liver or kidney) using a mixture of water/ methanol/ sodium hydroxide by shaking at room temperature. The extract is centrifuged and an aliquot is acidified. This aliquot is cleaned-up by means of solid phase extraction (SPE) using a C18 column followed by a second clean-up step on a strong anion exchange (SAX) SPE column. For milk, sub-specimens are diluted with water. After addition of sodium hydroxide an aliquot is taken and passed through a C18 SPE column. The entire pre-cleaned aliquot passed through the column is collected and acidified with phosphoric acid. Trinexapac acid is then extracted on a second C18 SPE column. For eggs, a homogenised sub-specimen (liquid part of egg only) is suspended in a mixture of water and methanol and dissolved by addition of sodium hydroxide. An aliquot of this clear solution is cleaned up/ extracted according to the same principles as applied for milk. The final extracts in all cases are analysed using HPLC-UV.

Samples of bovine meat, liver and kidney, milk and eggs were spiked with trinexapac acid at levels of 0.02–0.20 mg/kg (tissues), 0.01–0.10 mg/kg (milk) and 0.02–0.20 mg/kg (eggs). Samples were extracted and analysed using Method REM 137.12. In all the matrices tested the mean recoveries were within 70–120%. The mean recovery data are summarized in Table 58. The relative standard deviations (RSD, %) for all commodities and all spiking levels were < 20%.

The LOQ was defined by the lowest spiking level successfully tested, which was 0.01 mg/kg in milk and 0.02 mg/kg each in animal tissues and eggs.

Table 58 Recovery results of method REM 137.12: trinexapac acid in animal matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Meat	0.02	8	78–88	84	5
	0.20	8	86–95	92	3
Liver	0.02	4	86–94	89	4
	0.20	4	70–86	79	9
Kidney	0.02	4	77–85	82	5
	0.20	4	78–81	79	2
Milk	0.01	8	93–99	95	2
	0.10	8	92–101	97	3
Eggs	0.02	8	78–86	82	4
	0.20	8	78–84	81	2

An independent laboratory validation report for Method REM 137.12 for the determination of residues of trinexapac acid in animal matrices (milk and meat) has been submitted (Gasser 2001, CGA163935/0740).

Samples of meat and milk were spiked with trinexapac acid at levels of 0.01–0.10 mg/kg (milk) and 0.02–0.20 mg/kg (meat). Samples were extracted and analysed using Method REM 137.12. In both matrices tested the mean recoveries were within 70–120% and the relative standard deviations (RSD, %) for both commodities and both spiking levels were ≤ 20% (Table 59). The LOQ was defined by the lowest spiking level successfully tested, which was 0.01 mg/kg in milk and 0.02 mg/kg in meat.

Table 59 Recovery results of method REM 137.12: trinexapac acid in animal matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Milk	0.01	5	92–96	94	2
	0.10	5	87–93	91	3
Meat	0.02	5	81–90	87	4
	0.20	5	85–89	86	2

Concurrent recovery results obtained for Method REM 137.12 during the dairy cattle feeding study (Sack 2000, CGA179500/0030) are tabulated in Table 60. The method was modified to allow determination using HPLC-MS/MS.

Table 60 Mean concurrent recovery data for Method 137.12 in dairy cattle feeding study

Matrix	Spiking level (mg/kg)	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Liver	0.02	1	80	–	–
	0.2	1	83	–	–
Kidney	0.02	1	102	–	–
	0.2	1	88	–	–
Muscle round	0.02	1	97	–	–
	0.2	1	85	–	–
Tenderloin	0.02	1	80	–	–
	0.2	1	81	–	–
Diaphragm	0.02	1	80	–	–
	0.2	1	82	–	–
Fat, perirenal	0.02	1	103	–	–
	0.2	1	75	–	–
Fat, omental	0.02	1	71	–	–
	0.2	1	91	–	–
Blood	0.01	1	93	–	–
	0.1	1	96	–	–
Milk	0.005	12	88–121	104	11
	0.1	11	100–108	103	2

Method REM 137.14

Method REM 137.14 was developed for the determination of trinexapac acid in animal matrices using quantification of residues by LC-MS/MS (Kwiatkowski 2004, CGA179500/0039). It supersedes the previously developed HPLC-UV Method REM 137.12.

Animal tissue and egg matrices are extracted by shaking with water/ methanol (80:20, v/v) containing 0.33% 1M sodium hydroxide solution. Milk is extracted by shaking with water/ methanol (98:2, v/v) containing 0.2% 1M sodium hydroxide solution. Extracts are centrifuged and aliquots are diluted with ultra-pure water. After a solid phase extraction procedure is carried out, final determination is by high performance liquid chromatography with triple quadrupole mass

spectrometric detection (LC-MS/MS). MS/MS detection in the negative ionisation mode is used to monitor ion transitions 223.0→83.0 and 223.0→178.6 (confirmatory transition) for trinexapac acid.

Samples of bovine muscle, kidney, liver, fat and eggs were spiked with trinexapac acid at levels of 0.01 and 0.10 mg/kg. Samples of milk were spiked with trinexapac acid at levels of 0.005 and 0.05 mg/kg. Samples were extracted and analysed with Method REM 137.14. In all matrices tested, the mean recovery values were between 70% and 120% and the relative standard deviations (% RSD) for all commodities and all spiking levels for five tests at each spiking level were ≤ 20%. Mean recovery data for the quantitation transition are given in Table 61.

The LOQ was defined by the lowest spiking level successfully tested which was 0.01 mg/kg for egg, muscle, kidney, liver and fat, and 0.005 mg/kg for milk.

Table 61 Recovery results of method REM 137.14: trinexapac acid in animal matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Muscle (cow)	0.01	5	62–110	95	20
	0.1	5	63–92	81	15
Kidney (cow)	0.01	5	88–114	99	10
	0.1	5	77–96	91	9
Liver (cow)	0.01	5	69–95	86	12
	0.1	5	86–97	92	5
Fat (cow)	0.01	5	94–109	104	6
	0.1	5	93–102	96	4
Milk (cow)	0.005	5	88–114	106	10
	0.05	5	89–99	94	5
Eggs	0.01	5	67–119	96	20
	0.1	5	79–97	90	8

An independent laboratory validation report for Method REM 137.14 has been submitted (Benazeraf 2004, CGA163935/0989).

Samples of bovine muscle were spiked with trinexapac acid at levels of 0.01 and 0.10 mg/kg and samples of milk were spiked with trinexapac acid at levels of 0.005 and 0.05 mg/kg. Samples were extracted and analysed with Method REM 137.14 (HPLC-MS/MS). In all matrices tested, the mean recovery values were between 70% and 120% and the relative standard deviations (% RSD) for all commodities and all spiking levels for five tests at each spiking level were < 20%. Mean recovery data for the quantitation transition are given in Table 62.

Table 62 Recovery results of method REM 137.14: trinexapac acid in animal matrices

Matrix	Spiking level [mg/kg]	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Muscle	0.01	5	93–108	101	5
	0.10	5	71–108	90	17
Milk	0.005	5	91–113	99	9
	0.05	5	77–119	96	17

Method REM 137.14 was successfully independently validated for the analysis of residues of trinexapac acid at an LOQ of 0.01 mg/kg in bovine muscle and 0.005 mg/kg in bovine milk.

Method and concurrent recovery results obtained for Method REM 137.14 during the poultry feeding study (Simmons 2010, CGA179500_50008) are tabulated in Tables 63 and 64.

Table 63 Mean method validation data for Method REM 137.14 (eggs and tissues) from the laying hen feeding study

Matrix	Spiking level (mg/kg)	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Chicken Egg	0.01	3	74–92	81	12
	0.10	3	94–103	97	5
Chicken Fat	0.01	3	86–89	89	6
	0.10	3	75–101	88	15
Chicken Liver	0.01	3	86–109	98	12
	0.10	3	93–98	95	3
Chicken Kidney	0.01	3	100–116	105	9
	0.10	3	95–100	97	3
Chicken Muscle	0.01	3	89–109	102	11
	0.10	3	98–105	102	3

Table 64 Mean concurrent recovery data for Method REM 137.14 (eggs and tissues) from laying hen feeding study

Matrix	Spiking level (mg/kg)	Recoveries for trinexapac acid			
		No.	Range [%]	Mean [%]	RSD [%]
Chicken Egg	0.01	6	70–109	80	19
	0.10	6	79–95	86	8
Chicken Fat	0.01	2	92, 103	98	–
	0.10	2	99, 100	100	–
Chicken Liver	0.01	2	91, 99	95	–
	0.10	2	86, 89	88	–
Chicken Kidney	0.01	2	93, 114	104	–
	0.10	1	100	–	–
	1.0	1	91	–	–
Chicken Muscle	0.01	1	92	–	–
	0.10	1	96	–	–

Stability of pesticide residues in stored analytical samples

Plant Matrices

A freezer storage stability study was carried out on wheat grain, straw and rapeseed fortified with trinexapac acid at approximately 0.5 mg/kg and stored at ≤ -18°C (Sack 1998, CGA163935/0562). Samples were analysed using Method REM 137.02 with minor modifications concerning final determination by HPLC. Concurrent recoveries were determined together with the analytical samples. The results are summarized below in Table 65 and indicate that trinexapac acid is stable in frozen storage for at least 24 months in wheat grain and rapeseed although in the case of wheat straw, the amount of trinexapac acid that could be recovered after storage for approximately 2 years dropped to an average of 69% (75% if corrected for average concurrent recovery).

Table 65 Storage stability of trinexapac acid in plant matrices

Matrix [a]	Days Stored	Stability Recoveries [%]	Concurrent Recoveries [%]
	0	(set at) 100	85, 85
	96	85, 85, 92	88, 95
Wheat Grain	180	82, 84, 88	86, 87
	383	77, 79, 82	84, 90
	742	65, 73, 79	80, 82
	0	(set at) 100	93, 93
	93	101, 102, 103	84, 93
Wheat Straw	183	102, 104, 104	105, 106

Matrix [a]	Days Stored	Stability Recoveries [%]	Concurrent Recoveries [%]
	365	89, 89, 92	94, 99
	709	65, 71, 72	90, 94
	0	(set at) 100	91, 91
	96	89, 93, 94	93, 95
Rapeseed	167	82, 82, 89	94, 95
	376	75, 79, 81	84, 88
	720	77, 81, 82	94, 95

[a] Average fortification levels 0.55 mg/kg wheat grain and 0.45 mg/kg wheat straw and rapeseed

A freezer storage stability study was carried out on wheat processed fractions (germ, bran and flour) fortified with trinexapac acid at 0.1 mg/kg and stored at -20 ± 5 °C (Brown 2011, CGA179500_50012).

Samples were analysed using Method GRM020.01A with minor method modifications. Recoveries were run concurrently with the analytical samples. The results are summarized below in Table 66 and indicate that trinexapac acid is stable in frozen storage for at least 12 months in wheat processed fractions.

Table 66 Storage stability of trinexapac acid in plant matrices

Matrix	Days Stored	Concurrent Recoveries [%]		Stability Recoveries [%]	
		Found	Average	Found	Average
	0	103, 102	102	109, 100	104
Wheat Germ	101	105, 101	103	104, 105	104
	271	79, 84	82	83, 84	84
	381	106, 109	108	104, 113	108
	0	87, 95	91	95, 75	85
Wheat Bran	99	100, 103	102	80, 93	86
	269	85, 86	86	85, 76	80
	379	97, 88	92	104, 98	101
	0	107, 104	106	110, 105	108
Wheat Flour	102	73, 91	82	79, 81	80
	272	70, 72	71	69, 67	68
	382	87, 95	91	84, 93	88

In the [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl grass metabolism study the storage stability was determined by comparison of the metabolite profiles of extracts of straw, seed screenings and 105 day regrowth forage at the beginning of the analytical phase and at the end of the study, approximately thirteen months later. The qualitative and quantitative metabolite profiles did not change throughout this period. It was concluded that the results of the study were not considered to be affected by the length and conditions of storage.

Animal Matrices

The stability of trinexapac acid in extracts of animal matrices was tested at the same time as the dairy cattle and laying hen feeding studies (Sack 2000, CGA179500/0030 and Simmons 2010, CGA179500_50008 respectively).

In the dairy cattle feeding study, no significant degradation of residues of trinexapac acid was observed in frozen samples of muscle (average recovery 82% corrected for concurrent recoveries), milk (89%), liver (85%), kidney (85%), fat (96%) and blood (102%) stored at ≤ -18 °C over the storage period from the arrival of specimens until analysis (83-121 days).

Table 67 Storage stability of trinexapac acid in dairy cattle matrices

Matrix	Spiking Level [mg/kg]	Days Stored	Concurrent Recoveries [%]	Recoveries [%]	
				Found [a]	Average [a]
Muscle	0.20	91	83, 81	86, 86, 77, 76, 87	82
Liver	0.20	94	85, 96	84, 83, 85, 87, 86	85
Kidney	0.20	95	89, 88	87, 86, 87, 81, 82	85
Omental Fat	0.10	101	98, 97	96, 96, 98, 93, 95	96
Milk	0.05	121	108, 109	89, 86, 90, 88, 93	89
Blood	0.10	83	99, 109	102, 99, 104, 104, 101	102

[a] Recoveries found (and averages) are corrected for concurrent recoveries

In the laying hen feeding study, no significant degradation of residues of trinexapac acid was observed in frozen samples of eggs, chicken fat, chicken liver, chicken kidney and chicken muscle (average recoveries > 70%) stored at ≤ -18 °C over the storage period from the sample collection until analysis (31–82 days).

Table 68 Storage stability of trinexapac acid in poultry matrices

Matrix	Spiking Level [mg/kg]	Days Stored	Concurrent Recoveries [%]	Stability Recoveries [%]	
				Found	Average
Eggs	0.1	82	85, 84	73, 87	80
Fat	0.1	56	100, 104	93, 99	96
Liver	0.1	57	85, 85	83, 84	84
Kidney	0.1	54	94, 88	92, 97	95
Muscle	0.1	31	100, 101	97, 104	101

The stability of trinexapac acid in extracts of animal matrices was tested with the validation of Method REM 137.14 (Kwiatkowski 2004, CGA179500/0039). The stability of trinexapac acid in extracts of eggs (7 days storage), muscle (8 days storage), kidney (4 days storage), liver (5 days storage) and fat (3 days storage) was assessed by retaining water: methanol (80:20 v/v) containing 0.33% 1M sodium hydroxide and for milk (6 days storage) in water: methanol (98:2 v/v) containing 0.2% 1M sodium hydroxide extraction solutions at < 7 °C. Overall mean recoveries for trinexapac acid between 70–120% with an overall RSD of ≤ 20% for each matrix tested, indicated that the compound is stable when stored in the extract solution for the period indicated.

The stability of trinexapac acid in the final solution of acetonitrile: water (30:70 v/v) for all matrices was also assessed. Milk, eggs, muscle, kidney, liver and fat aliquots in the final solution were retained, stored at < 7 °C and re-analysed after 2 days (kidney and muscle), 3 days (eggs and fat), 4 days (liver) and 5 days (milk) following the original analysis and quantified against freshly calibrated solutions. The compound was shown to be stable when stored in the final solutions for those periods.

In the [1,2-^{14}C-cyclohexyl] trinexapac-ethyl hen metabolism study the storage stability was determined by comparison of the quantitative metabolite pattern of hen excreta at the beginning of the storage period, with that obtained from the identical sample at the beginning of the analytical work approximately 2 weeks later. The extractability and quantitative metabolite patterns did not change throughout this period. It was concluded that the results of the study were not considered to be affected by the length and conditions of storage. In the hen metabolism study using [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl, the extract of the egg white sample (taken as a representative of all the commodities) was analysed by TLC twice, approximately 6 months apart. The generated profiles were similar and therefore it was concluded that storage stability was confirmed.

In the [1,2-^{14}C-cyclohexyl] trinexapac-ethyl goat metabolism study the storage stability was determined by comparison of the quantitative metabolite pattern of goat urine prior to storage and at the beginning of the experimental phase of the study, approximately nine months later. The quantitative metabolite patterns did not change throughout this period. It was concluded that the results of the study were not considered to be affected by the length and conditions of storage.

USE PATTERNS

Information on registered uses made available to this meeting is shown in Table 69.

Table 69 Registered uses of trinexapac-ethyl on cereal grains, grasses for sugar or syrup production and sugarcane

Crop	Country	Formulation		Application				PHI [days]
		g ai/L	Type	Method	Timing	Rate, g ai/ha	Season Max. (no.) or [g ai/ha/season]	
Cereal Grains								
Barley, summer	Belgium	250	EC	Foliar	BBCH 29–32	100–150	(1)	–
Barley, spring	France	250	EC	Foliar	BBCH 25–37	150	(1)	–
Barley, spring	Germany	250	EC	Foliar	BBCH 31–37	150	(1)	–
Barley, winter	Belgium	250	EC	Foliar	BBCH 31–32	125–200	(1)	–
Barley, winter	France	250	EC	Foliar	BBCH 25–39	200	(1)	–
Barley, winter (multi-row varieties)	Germany	250	EC	Foliar	BBCH 31–49	200	(1)	–
Barley, winter (two-row varieties)	Germany	250	EC	Foliar	BBCH 31–49	150	(1)	–
Barley, winter and spring	USA	249	EC	Foliar	Feekes 5–8	90–123	(1)	45
Barley, winter and spring	USA	249	EC	Foliar	Feekes 6–7 and Feekes 8	45	(2)	45
Barley, winter and spring	USA	120	EC	Foliar	Feekes 5–8	101–123	(1)	45
Barley, winter and spring	USA	120	EC	Foliar	Feekes 6–7 and Feekes 8	56	(2)	45
Grasses (grown for seed)	France	250	EC	Foliar	BBCH 31–51	200	(1)	–
Grasses (grown for seed)	USA	249	EC	Foliar	Feekes 5–8	101–560	(1)	35[a,b]
Grasses (grown for seed)	USA	249	EC	Foliar	Feekes 5–8 and 7–10 days later	45–269	(2)	35[a,b]
Grasses (grown for seed)	USA	120	EC	Foliar	Feekes 5–8	101–560	(1)	35[a,b]
Grasses (grown for seed)	USA	120	EC	Foliar	Feekes 5–8 and 7–10 days later	56–280	(2)	35[a,b]
Oat	Belgium	250	EC	Foliar	BBCH 30–31	100	(1)	–
Oat	Germany	250	EC	Foliar	BBCH 31–37	150	(1)	–
Oat	USA	249	EC	Foliar	Feekes 5–8	90–123	(1)	45
Oat	USA	249	EC	Foliar	Feekes 4–5 and Feekes 7	45	(2)	45
Oat	USA	120	EC	Foliar	Feekes 5–8	101–123	(1)	45
Oat	USA	120	EC	Foliar	Feekes 4–5 and Feekes 7	56	(2)	45
Rye	Belgium	250	EC	Foliar	BBCH 31–32	100–125	(1)	–
Rye, winter	Germany	250	EC	Foliar	BBCH 31–39	150	(1)	–
Rye, winter	Germany	250	EC	Foliar	BBCH 39–49	75	(1)	–
Ryegrass (Italian—grass seed cultivation)	Belgium	250	EC	Foliar	BBCH 31–49	100–200	(1)	–
Ryegrass (perennial—grass seed cultivation)	Belgium	250	EC	Foliar	BBCH 31–49	100–200	(1)	–
Spelt	Belgium	250	EC	Foliar	BBCH 31–32	100–125	(1)	–
Triticale	Belgium	250	EC	Foliar	BBCH 31–32	100–125	(1)	–
Triticale	Germany	250	EC	Foliar	BBCH 31–49	150	(1)	–
Triticale	Germany	250	EC	Foliar	BBCH 39–49	75	(1)	–
Triticale	USA	249	EC	Foliar	Feekes 5–8	90–123	(1)	45
Triticale	USA	249	EC	Foliar	Feekes 4–5 and Feekes 7	45	(2)	45
Triticale	USA	120	EC	Foliar	Feekes 5–8	101–123	(1)	45
Triticale	USA	120	EC	Foliar	Feekes 4–5 and Feekes 7	56	(2)	45
Wheat, summer	Belgium	250	EC	Foliar	BBCH 30–31	100	(1)	–
Wheat, winter	Belgium	250	EC	Foliar	BBCH 31–32	100–125	(1)	–

Crop	Country	Formulation		Application				PHI [days]
		g ai/L	Type	Method	Timing	Rate, g ai/ha	Season Max. (no.) or [g ai/ha/season]	
Wheat, winter	France	250	EC	Foliar	BBCH 25–39	125	(1)	–
Wheat, winter	Germany	250	EC	Foliar	BBCH 31–49	100	(1)	–
Wheat, winter, spring, durum	USA	249	EC	Foliar	Feekes 5–8	90–123	(1)	45
Wheat, winter, spring, durum	USA	249	EC	Foliar	Feekes 4–5 and Feekes 7	45	(2)	45
Wheat, winter, spring, durum	USA	120	EC	Foliar	Feekes 5–8	101–123	(1)	45
Wheat, winter, spring, durum	USA	120	EC	Foliar	Feekes 4–5 and Feekes 7	56	(2)	45
Grasses for sugar or syrup production								
Sugarcane (for ripening)	USA	249	EC	Foliar	28–60 days before harvest	202–347	(1)	28
Sugarcane (for internode shortening for seed piece production)	USA	249	EC	Foliar	When 6 fully developed full size leaves have formed then when 6 more fully developed full size leaves have formed (minimum of 2 applications)	78–224	[347 g/ha/ crop/season]	28
Sugarcane (for ripening)	USA	120	EC	Foliar	28–60 days before harvest	202–347	(1)	28
Sugarcane (for internode shortening for seed piece production)	USA	120	EC	Foliar	When 6 fully developed full size leaves have formed then when 6 more fully developed full size leaves have formed (minimum of 2 applications)	67–224	[347 g/ha/ crop/season]	–
Oilseeds								
Oilseed rape	Belgium	250	EC	Foliar	BBCH 55–57	300	(1)	–
Oilseed rape, winter	Germany	250	EC	Foliar	BBCH 39–55	375	(1)	–

[a] Do not graze or feed forage 49 days after the last application

[b] Other crops may be planted 30 days after the last application

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on supervised trials for the uses of trinexapac on cereals (barley and wheat), oilseeds (oilseed rape) and grasses for sugar and syrup production (sugarcane).

Trials were well documented with laboratory and field reports. The former included method validation including recoveries with spiking at residue levels similar to those occurring in samples from the supervised trials. Dates of analyses or duration of sample storage were also provided. Trials were carried out in appropriately sized plots and sample sizes were within acceptable weights. Applications were generally made using backpack sprayers although occasionally tractor mounted sprayers were used. Samples were collected and stored frozen immediately or soon after sampling. Although trials included control plots, no control data are recorded in the Tables because no residues in control samples exceeded the LOQ. Residues are unadjusted for recoveries.

Residues, application rates and spray concentrations have generally been rounded to two significant figures. Residues from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels and dietary intake assessment. If a higher residue level was observed at a longer PHI than the GAP, the higher value has been used in MRL setting and dietary intake assessment. When residues were not detected they are shown as below the LOQ (*e.g.* < 0.01 mg/kg) and the LOQ value was utilised for maximum residue level estimation and dietary intake assessment.

For replicate samples (from the same plot), the mean value was used for maximum residue level estimation and dietary intake assessment. For two or more analyses of the same sample, the mean value was used for maximum residue level estimation and dietary intake assessment, with the individual results given in brackets. For multiple trials on a crop from the same location, the result from the trial yielding the highest residue was utilised for maximum residue level estimation and dietary intake assessment. In this case the trials are separated by a dotted line.

In some studies (barley, wheat, sugarcane) residues have been determined as total trinexapac (includes free trinexapac acid and conjugates of trinexapac acid) while in other studies (wheat, sugarcane and rape seed) residues have been determined as trinexapac (free trinexapac acid only). In both cases trinexapac means trinexapac acid. Residues of trinexapac-ethyl were considered to be 0 mg/kg in rape seed. Residues according to maximum GAP have been underlined.

Group	Commodity	Country	Table No.
GC Cereal Grains	Barley	USA	70
	Wheat	USA	71
	Wheat	USA	72
GS Grasses for sugar or syrup production	Sugarcane	USA	73
	Sugarcane	USA	74
SO Oilseeds	Rape seed	Germany	75
Animal Feeds	Barley hay and straw	USA	76
	Wheat forage	USA	77
	Wheat hay and straw	USA	78
	Wheat forage	USA	79
	Wheat hay and straw	USA	80
	Rape seed forage and straw	Germany	81

The results of these supervised trials are shown in the following tables:

Cereal Grains

Supervised trials were carried out on barley (12 trials—Table 70) in the USA during the 2008 and 2009 growing seasons (Mäyer 2010a, CGA163935_50026). A foliar application of a 250 g/L EC formulation was made at 127–134 g ai/ha at Feekes Growth Stage 7 (BBCH 32) or at 45 days before harvest, or (for generating processing samples) at 129 and 644 or 648 g ai/ha at 45 days before harvest. Applications were made to plots using spray volumes of 68–288 L/ha with ground equipment, except for one trial which simulated aerial application (Trial C08WI081703). Additional samples were collected at one site (Trial C30IA081702–Bagley, Iowa) to examine the residue decline. Residues of total trinexapac were quantitated as trinexapac acid by LC/MS/MS method GRM020.01A. Acceptable concurrent recovery data were obtained.

Residues in barley hay and straw are shown in Table 76. Processed fraction samples for analysis were generated from trials C13ND081704 and C13ND081705 (Table 82).

Table 70 Residues from the foliar application of trinexapac-ethyl to barley in the USA

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean [a]	
GAP, USA (barley)	1	123				45			
USA	1	132	288	BBCH 59/	Grain	45	1.3	1.2	Mäyer 2010a
(New Tripoli,				Feekes 10.5			1.1		CGA163935_50026
Pennsylvania)									E04PA081701
2009									
(Nomini)									
USA	1	132	68	BBCH 49	Grain	24	1.0		Mäyer 2010a
(Bagley, Iowa)					Grain	31	1.3		CGA163935_50026
2008					Grain	38	0.73		C30IA081702
(Robust)					Grain	45	0.90	0.95	
							1.0		
					Grain	52	1.0		
USA	1	133	19	BBCH 33	Grain	45	0.07	0.08	Mäyer 2010a
(Fitchburg, Wisconsin)							0.09		CGA163935_50026
2008									C08WI081703
(Kewaunee)									
USA	1	129	187	BBCH 51	Grain	45	0.65	0.76	Mäyer 2010a
(Northwood, North							0.86		CGA163935_50026
Dakota)									C13ND081704
2008									
(Tradition)									
USA	1	129	141	BBCH 73	Grain	45	0.75	0.83	Mäyer 2010a
(Carrington, North							0.90		CGA163935_50026
Dakota)									C13ND081705
2008									
(Tradition)									
USA	1	128	187	BBCH 73	Grain	45	0.53	0.53	Mäyer 2010a
(New Rockford, North							0.52		CGA163935_50026
Dakota)									C13ND081706
2008									
(Tradition)									
USA	1	134	146	BBCH 59	Grain	45	0.51	0.52	Mäyer 2010a
(Eldridge, North							0.53		CGA163935_50026
Dakota)									C12ND081707
2008									
(Tradition)									
USA	1	127	138	BBCH 59	Grain	45	0.56	0.50	Mäyer 2010a
(Adrian, North Dakota)							0.44		CGA163935_50026
2008									C12ND081708
(Drummond)									
USA	1	131	273	BBCH 45	Grain	45	0.05* (0.05,	0.03	Mäyer 2010a
(Monte Vista,							0.04, 0.05)		CGA163935_50026

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean [a]	
Colorado)							0.01*		E13CO081709
2008									
(C-69)									
USA	1	128	282	BBCH 45	Grain	45	0.57	0.60	Mäyer 2010a
(Madera, California)							0.62		CGA163935_50026
2008									W29CA081710
(Recleaned whole									
barley)									
USA	1	130	194	BBCH 84	Grain	45	0.72	0.72	Mäyer 2010a
(Hermiston, Oregon)							0.71		CGA163935_50026
2008									W21OR081711
(Radiant)									
USA	1	130	97	BBCH 65	Grain	45	0.52	0.44	Mäyer 2010a
(Rupert, Idaho)							0.35		CGA163935_50026
2008									W15DO081712
(IDA Gold II)									

[a]: residue values considered for estimation of STMR values

*Mean value of replicates

LOQ = 0.01 mg/kg

Supervised trials were carried out on wheat in the USA during the 2008 growing seasons (Mäyer 2010, CGA163935_50036) and the 2004 and 2005 growing seasons (Ediger 2006, 451534). The Mäyer study quantified total trinexapac residues.

Wheat (Total residues of trinexapac acid)

Supervised trials were carried out on wheat (20 trials—Table 71) in the USA during the 2008 growing season (Mäyer 2010, CGA163935_50026). A foliar application of a 250 g/L EC formulation was made at 127–134 g ai/ha at approximately Feekes Growth Stage 7 or at 45 days before harvest, or (for generating processing samples) at 129 or 130 and 646 or 649 g ai/ha at 45 days before harvest. Applications were made to plots using spray volumes of 97–252 L/ha with ground equipment, except for two trials which simulated aerial application (Trials C19KS078462 and C16SD078471). At three sites additional samples were collected to examine the residue decline. Residues of total trinexapac were quantitated as trinexapac acid by LC/MS/MS method GRM020.01A. Acceptable concurrent recovery data were obtained.

Residues in wheat forage are shown in Table 77 and in hay and straw in Table 78. Processed fraction samples for analysis were generated from trials C13ND078468 and W01TX078473 (Table 83).

Table 71 Residues from the foliar application of trinexapac-ethyl to wheat in the USA

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac [a] (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA (wheat)	1	123				45			
USA	1	130	120	BBCH 71	Grain	31	0.33		Mäyer 2010
(Suffolk, Virginia)					Grain	38	0.34		CGA163935_50036
2008					Grain	45	0.32	0.32	E07VA078460

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac [a] (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
(Winter wheat—R9184)							0.32		
					Grain	52	0.24		
USA	1	129	135	BBCH 65	Grain	45	4.03	3.32	Mäyer 2010
(Proctor, Arkansas)							2.61		CGA163935_50036
2008									C24AR078461
(Wheat—DK7710)									
USA	1	128	19	Boot	Grain	45	1.03	1.01	Mäyer 2010
(Minneapolis, Kansas)							0.98		CGA163935_50036
2008									C19KS078462
(Hard White Wheat –									
Danby)									
USA	1	129	188	BBCH 67	Grain	45	0.47	0.47	Mäyer 2010
(Northwood, North							0.47		CGA163935_50036
Dakota)									C13ND078463
2008									
(HRSW—Kelby)									
USA	1	134	183	BBCH 51	Grain	45	0.81	0.77	Mäyer 2010
(Perley, Minnesota)							0.73		CGA163935_50036
2008									C12MN078464
(Spring Wheat—Alsen)									
USA	1	130	158	Boot	Grain	45	0.30	0.27	Mäyer 2010
(St Joseph, Missouri)							0.24		CGA163935_50036
2008									C19MO078465
(Hard Red Winter									
Wheat—Kansas 2137)									
USA	1	132	134	BBCH 55	Grain	45	0.90	0.99	Mäyer 2010
(Kirklin, Indiana)							1.08		CGA163935_50036
2008									C05IN078466
(Wheat—Pioneer									
25R78)									
USA	1	129	241	BBCH 53	Grain	45	0.07	0.07	Mäyer 2010
(Madill, Oklahoma)							0.07		CGA163935_50036
2008									W01OK078467
(Winter Wheat—Jagger)									
USA	1	129	188	BBCH 65	Grain	45	0.31	0.31	Mäyer 2010
(Carrington, North							0.31		CGA163935_50036
Dakota)									C13ND078468
2008									
(Durum—Divide)									
USA	1	129	187	BBCH 65	Grain	31	0.69		Mäyer 2010
(Carrington, North					Grain	38	0.58		CGA163935_50036
Dakota)					Grain	45	0.76	0.82	C13ND078469

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac [a] (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2008							0.87		
(SWSW—WPB Nick)					Grain	52	0.61		
USA	1	130	144	BBCH 41	Grain	45	0.48	0.53	Mäyer 2010
(Velva, North Dakota)							0.57		CGA163935_50036
2008									C14ND078470
(Hard White Spring									
Wheat—Agawana)									
USA	1	130	19	BBCH 45	Grain	45	1.64	1.64	Mäyer 2010
(Lake Andes, South							1.64		CGA163935_50036
Dakota)									C16SD078471
2008									
(Spring Wheat—Briggs)									
USA	1	134	192	BBCH 51	Grain	31	0.49	0.42	Mäyer 2010
(Grand Island,							0.35		CGA163935_50036
Nebraska)					Grain	38	0.84		C17NE078472
2008					Grain	45	1.00 [b] (1.12, 0.89, 0.98)	0.78	
(Hard Red Winter Wheat—Com. Wesley)							0.56 [b] (0.23, 0.77, 0.67)		
					Grain	52	0.70		
USA	1	130	252	BBCH 52	Grain	45	0.10	0.10	Mäyer 2010
(Groom, Texas)							0.10		CGA163935_50036
2008									W01TX078473
(Winter Wheat—Cutter)									
USA	1	127	237	BBCH 52	Grain	45	0.41	0.40	Mäyer 2010
(Groom, Texas)							0.39		CGA163935_50036
2008									W01TX078476
(Winter Wheat—TAM									
212)									
USA	1	128	239	BBCH 51	Grain	45	0.33	0.32	Mäyer 2010
(Groom, Texas)							0.30		CGA163935_50036
2008									W01TX078477
(Winter Wheat—									
Jagalene)									
USA	1	131	240	BBCH 52	Grain	45	0.14	0.15	Mäyer 2010
(Goodwell, Oklahoma)							0.15		CGA163935_50036
2008									W01OK078474
(Winter Wheat—									
Ogallalla)									
USA	1	134	126	BBCH 60	Grain	45	1.10	1.14	Mäyer 2010
(Frederick, Oklahoma)							1.18		CGA163935_50036

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac [a] (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2008									C29OK078475
(Winter Wheat—Custer)									
USA	1	130	241	BBCH 51	Grain	45	0.44	0.45	Mäyer 2010
(Wellington, Texas)							0.45		CGA163935_50036
2008									W01TX078478
(Winter Wheat—TAM									
200)									
USA	1	127	97	BBCH 65	Grain	45	0.87	0.85	Mäyer 2010
(Rupert, Idaho)							0.82		CGA163935_50036
2008									W15ID078479
(Winter Wheat—									
Westbred 528)									

[a] residue values considered for estimation of STMR values

[b] Mean of three readings

[a] Mean of three readings

LOQ = 0.01 mg/kg

Wheat (Free trinexapac acid)

Supervised trials were carried out on wheat (20 trials—Table 72) in the USA during the 2004 and 2005 growing seasons (Ediger 2006, 451534). A foliar application of a 250 g/L EC formulation was made at 123–130 g ai/ha (and at 384 or 391 and 643 g ai/ha at two sites) at approximately Feekes Growth Stage 7 or at 45 days before harvest, or (for generating processing samples) at 127 or 130 and 643 g ai/ha at 45 days before harvest. Applications were made to plots using spray volumes of 92–187 L/ha with ground equipment, except for three trials which simulated aerial application (Trials 4A-FR-04-5421, NN-FR-04-5424 and NM-FR-04-5429). At three sites additional samples were collected to examine the residue decline. Residues of trinexapac were quantitated as trinexapac acid using method 110-01. Acceptable concurrent recovery data were obtained.

Residues in wheat forage are shown in Table 79 and in hay and straw in Table 80. Processed fraction samples for analysis were generated from trials NN-FR-04-5418 and SC-FR-04-5430 (Table 84).

Table 72 Residues from the foliar application of trinexapac-ethyl to wheat in the USA

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA (wheat)	1	123				45			
USA	1	129	147	BBCH 43	Grain	50	0.50	0.57	Ediger 2006
(Pikeville, North							0.64		451534
Carolina)									SJ-FR-04-5415
2005									
(Winter Wheat—Coker									
9184)									
USA	1	129	142	BBCH 55	Grain	45	1.0	0.98	Ediger 2006
(Lehi, Arkansas)							0.96		451534

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2005									SE-FR-04-5416
(Winter Wheat—									
DK9410)									
USA	1	130	138	Feekes 8	Grain	45	0.52	0.49	Ediger 2006
(Abilene, Kansas)							0.46		451534
2005									ND-FR-04-5417
(Winter Wheat—									
Burdett)									
USA	1	127	92	BBCH 61	Grain	48	0.88	0.88	Ediger 2006
(Northwood, North							0.75		451534
Dakota)							1.0		NN-FR-04-5418
2004	1	384	93	BBCH 61	Grain	48	4.3	4.5	
(Spring Wheat— Briggs)							4.6		
	1	643	94	BBCH 61	Grain	48	10	10.3	
							10 11		
USA	1	128	187	BBCH 65	Grain	44	0.97	0.99	Ediger 2006
(Campbell, Minnesota)				Full			1.0		451534
2004				flowering					NF-FR-04-5419
(Spring Wheat—Oxen)									
USA	1	130	151	BBCH 61	Grain	47	1.3	1.35	Ediger 2006
(Kirksville, Missouri)							1.4		451534
2005									ND-FR-04-5420
(Winter Wheat— Ernie)									
USA	1	127	31	BBCH 55	Grain	46	0.73	0.77	Ediger 2006
(Champaign, Illinois)							0.81		451534
2005									4A-FR-04-5421
(Winter Wheat—									
Kaskaskia)									
USA	1	130	129	BBCH 71	Grain	42	0.99	1.05	Ediger 2006
(Hinton, Oklahoma)							1.1		451534
2005					Grain	47	0.93	0.96	SC-FR-04-5422
(Winter Wheat—							0.98		
Jagalene)					Grain	51	0.83	0.80	
							0.77		
USA	1	128	187	BBCH 33	Grain	40	0.06	0.06	Ediger 2006
(New Rockford, North							< 0.05		451534
Dakota)					Grain	50	0.09	0.10	NN-FR-04-5423
2004							0.10		
(Winter Wheat—					Grain	56	0.07	0.08	
Lebsock)							0.09		
USA	1	128	19	Feekes 10.5	Grain	42	1.5	1.95	Ediger 2006
(Froid, Montana)							2.4		451534
2004									NN-FR-04-5424
(Spring Wheat—									
Challis)									

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
USA	1	129	93	Feekes 10.5	Grain	42	1.2	1.2	Ediger 2006
(Froid, Montana)							1.2		451534
2004									NN-FR-04-5425
(Spring Wheat—									
Pristine)									
USA	1	129	178	BBCH 65	Grain	45	0.44	0.46	Ediger 2006
(Trail City, South							0.48		451534
Dakota)									NF-FR-04-5426
2004									
(Spring Wheat—Freyr)									
USA	1	129	187	BBCH 61	Grain	44	0.52	0.55	Ediger 2006
(Grand Island,							0.57		451534
Nebraska)									NB-FR-04-5427
2005									
(Winter Wheat—									
Arrowsmith HW)									
USA	1	125	130	Flag leaf	Grain	31	0.90	0.88	Ediger 2006
(Larned, Kansas)							0.86		451534
2005					Grain	38	0.73	0.72	NM-FR-04-5428
(Winter Wheat— Jagger)							0.71		
					Grain	45	0.90	0.91	
							0.91		
					Grain	52	0.51	0.55	
							0.59		
USA	1	129	21	Flag leaf	Grain	45	0.77	0.77	Ediger 2006
(Larned, Kansas)							0.77		451534
2005									NM-FR-04-5429
(Winter Wheat— Jagger)									
USA	1	130	144	BBCH 45	Grain	45	0.29	0.34	Ediger 2006
(Goodwell, Oklahoma)							0.33		451534
2005							0.39		SC-FR-04-5430
(Winter Wheat— Clark)	1	391	145	BBCH 61	Grain	45	2.1	1.7	
							1.2		
	1	643	143	BBCH 61	Grain	45	5.8	4.9	
							4.6 4.2		
USA	1	123	136	BBCH 67	Grain	44	0.32	0.35	Ediger 2006
(Levelland, Texas)							0.37		451534
2005									SC-FR-04-5431
(Winter Wheat—TAM									
200)									
USA	1	130	126	Feekes 10.5	Grain	45	0.55	0.55	Ediger 2006
(Ault, Colorado)							0.54		451534
2005									NM-FR-04-5432
(Winter Wheat—									

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
Yuma)									
USA	1	127	140	BBCH 65	Grain	44	0.36	0.32	Ediger 2006
(Clovis, New Mexico)							0.27		451534
2005									SC-FR-04-5433
(Winter Wheat—TAM									
110)									
USA	1	129	139	BBCH 61	Grain	44	0.22	0.25	Ediger 2006
(Ephrata, Washington)							0.27		451534
2004									WF-FR-04-5435
(Winter Wheat—									
Stephens)									

LOQ = 0.05 mg/kg

Feekes 8 = Flag leaf (last leaf) visible but still rolled up, ear beginning to swell

Feekes 10.5 = Head emergence complete

Grasses for sugar or syrup production

Supervised trials were carried out on sugarcane in the USA during the 2008 and 2004 growing seasons (Mäyer 2010b, CGA163935_50038 and Ediger 2006a, 451598 respectively). The Mäyer study quantified total trinexapac residues.

Sugarcane (Total residues of trinexapac acid)

Supervised trials were carried out on sugarcane (eight trials—Table 73) in the USA during the 2008 growing season (Mäyer 2010b, CGA163935_50038). A foliar application of a 250 g/L EC formulation was made at 350–382 g ai/ha at 28 days before harvest, or (for generating processing samples) at 361 or 382 and 1779 or 1856 g ai/ha at 28 days before harvest. Applications were made to plots using spray volumes of 94–982 L/ha with ground equipment, except for one trial which simulated aerial application (Trial E19H1078497). At two sites (E19FL078491 and E18LA078494) additional samples were collected to examine the residue decline. Residues of total trinexapac were quantitated as trinexapac acid by LC/MS/MS method GRM020.01A. Acceptable concurrent recovery data were obtained.

Processed fraction samples for analysis were generated from trials E19FL078492 and E18LA078495 (Table 85).

Table 73 Residues from the foliar application of trinexapac-ethyl to sugar cane in the USA

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA	1	347				28			
USA	1	367	982	BBCH 49	Sugar cane	28	< 0.01	< 0.01	Mäyer 2010
(Bradenton, Florida)							< 0.01		CGA163935_50038
2008									E16FL078490
(CP88-1762)									
USA	1	356	94	BBCH 91	Sugar cane	0	< 0.01		Mäyer 2010
(Belle Glade, Florida)						7	0.02		CGA163935_50038

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2008						14	0.07		E19FL078491
(CP96-1252)						28	0.04	0.04	
							0.04		
						35	0.03		
USA	1	382	105	BBCH 91	Sugar cane	28	0.06	0.06	Mäyer 2010
(Clewiston, Florida)							0.05		CGA163935_50038
2008									E19FL078492
(CL95-0776)									
USA	1	358	221	BBCH 70	Sugar cane	28	0.24	0.22	Mäyer 2010
(Washington, Louisiana)							0.20		CGA163935_50038
2008									E18LA078493
(85-384)									
USA	1	364	225	BBCH 70	Sugar cane	0	0.36		Mäyer 2010
(Washington, Louisiana)						7	0.34		CGA163935_50038
2008						14	0.29		E18LA078494
(85-384)						28	0.27	0.30	
							0.33		
						35	0.23		
USA	1	361	223	BBCH 70	Sugar cane	28	0.40	0.42	Mäyer, 2010
(Washington, Louisiana)							0.43		CGA163935_50038
2008									E18LA078495
(85-384)									
USA	1	350	234	BBCH 39	Sugar cane	28	0.05	0.08	Mäyer 2010
(Raymondsville, Texas)							0.10		CGA163935_50038
2008									W08TX078496
(1210)									
USA	1	358	19	BBCH 95	Sugar cane	28	0.13	0.17	Mäyer 2010
(Puunene, Hawaii)							0.21		CGA163935_50038
2008									E19H1078497
(H78-7750)									

LOQ = 0.01 mg/kg

Sugarcane (Free trinexapac acid)

Supervised trials were carried out on sugarcane (eight trials—Table 74) in the USA during the 2004 growing season (Ediger 2006a, 451598). A foliar application of a 250 g/L EC formulation was made at 345–365 g ai/ha (and at 1764 g ai/ha at one site) at 28 days before harvest of mature cane, or (for generating processing samples) at 345 and 1739 g ai/ha at 28 days before harvest of mature cane. Applications were made to plots using spray volumes of 102–948 L/ha with ground equipment, except

for one trial which simulated aerial application (Trial SD-FR-04-5505). At two sites additional samples were collected to examine the residue decline. Residues of trinexapac were quantitated as trinexapac acid using method 110-01. Acceptable concurrent recovery data were obtained.

Processed fraction samples for analysis were generated from trial VN-FR-04-5501 (Table 86).

Table 74 Residues from the foliar application of trinexapac-ethyl to sugar cane in the USA

Country Location Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA	1	347				28			
USA	1	347	278	28 days to	Sugar cane	0	0.05	0.08	Ediger 2006a
(South Bay, Florida)				harvest			0.10		451598
2004						14	0.11	0.11	VN-FR-04-5500
(CP89-2143)							0.11		
						21	0.13	0.10	
							0.07		
						28	0.09	0.09	
							0.09		
						35	0.07	0.07	
							0.06		
USA	1	345	276	28 days to	Sugar cane	28	0.06	0.06	Ediger 2006a
(South Bay, Florida)				harvest			< 0.05		451598
2004									VN-FR-04-5501
(CP89-2143)									
USA	1	350	948	Mature	Sugar cane	28	0.06	0.06	Ediger 2006a
(Pahokee, Florida)				cane			< 0.05		451598
2004									VN-FR-04-5502
(CP88-1762)									
USA	1	365	102	BBCH 39	Sugar cane	0	0.09	0.07	Ediger 2006a
(Cheneyville, Louisiana)							< 0.05		451598
2004						14	0.22	0.16	SD-FR-04-5503
(LCP85-384)							0.09		
						21	0.50	0.48	
							0.46		
						28	0.11	0.14	
							0.17		
						35	0.20	0.23	
							0.25		
USA	1	365	152	28 days to	Sugar cane	28	< 0.05	< 0.05	Ediger 2006a
(Washington, Louisiana)				harvest			< 0.05		451598
2004	1	1764	147	28 days to	Sugar cane	28	1.7	0.93	SD-FR-04-5504
(LCP85-384)				harvest			0.16		
USA	1	359	21	BBCH 39	Sugar cane	28	0.17	0.25	Ediger 2006a

Country Location Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
(Bunkie, Louisiana)							0.32		451598
2004									SD-FR-04-5505
(LCP85-384)									
USA	1	356	191	BBCH 39	Sugar cane	28	0.26	0.22	Ediger 2006a
(Raymondville, Texas)							0.18		451598
2004									SA-FR-04-5506
(3388)									
USA	1	351	398	BBCH 49	Sugar cane	28	< 0.05	0.12	Ediger 2006a
(Makaweli, Hawaii)							0.19		451598
2004									WD-FR-04-5507
(77-4643)									

LOQ = 0.05 mg/kg

Oilseeds

Supervised trials were carried out on rape seed (18 trials—Table 75) in Germany during the 1991, 1992 and 1999 growing seasons (various studies). One foliar application of a 250 g/L EC formulation was made at 375–448 g ai/ha. Applications were made to plots using spray volumes of 218–400 L/ha with ground equipment. Oilseed rape samples were collected at maturity, 68–113 days after application. In four trials whole plant samples were sampled immediately after the last application (0 DALA), pods with seeds and rest of plant were collected at 49–68 DALA and straw at 93–113 DALA respectively.

Residues in rape seed were quantitated as trinexapac acid using HPLC-UV method REM 137.02 (LOQ = 0.02 mg/kg). In four trials (gr 02499, gr 02299, gr 02599 and gr 02199) method REM 137.02 with modifications was used, which determines trinexapac by HPLC-MS/MS with an LOQ of 0.02 mg/kg for seeds and 0.04 mg/kg for whole plant, remainder and straw. In addition residues of trinexapac-ethyl were quantitated in rape seed in some trials using modified Method REM 137.05. Residues of trinexapac-ethyl were quantitated in rape seed whole plant, pods with seeds, remaining plant part and straw in some trials using Method REM 137.01.

Residues in rape seed whole plant, pods with seeds, remaining plant part and straw are shown in Table 81.

Table 75 Residues from the foliar application of trinexapac-ethyl to winter rape in Germany

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)	Trinexapac-ethyl (mg/kg)	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage					
GAP, Germany	1	375		BBCH 39–55					
Germany	1	400	400	BBCH 55	Seed	96	0.04	< 0.02	Hauck 1993j
(Bötersen)									CGA163935/0316
1991									gr 20291
(Lirabon)									
Germany	1	400	400	BBCH 53–55	Seed	102	0.13	0.06[a]	Hauck 1993b
(Lorsch)									CGA163935/0317

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)	Trinexapac-ethyl (mg/kg)	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage					
1991									gr 40591
(Ceres)									
Germany	1	400	400	BBCH 55	Seed	103	0.15	< 0.02	Hauck 1993k
(Achern-Gamshurst)									CGA163935/0318
1991									gr 60491
(Libravo)									
Germany	1	448	224	BBCH 55	Seed	68	1.0	< 0.02	Hauck 1993h
(Burgwedel-Thönse)									CGA163935/0329
1992									920EK101P
(Accord)									
Germany	1	436	218	BBCH 55	Seed	77	0.31	< 0.02	Hauck 1993i
(Braunschweig-Hondelage)									CGA163935/0330
1992									920EK201P
(Lirajet 00)									
Germany	1	400	400	BBCH 55	Seed	72	0.64	< 0.02	Hauck 1993a
(Goch)									CGA163935/0331
1992									AGR/RP-W92/CGD 01
(Lirajet)									
Germany	1	400	400	BBCH 53–55	Seed	83	0.15	< 0.02	Hauck 1993c
(Zülpich-Oberelvenich)									CGA163935/0319
1992									gr 10492
(Lirajet)									
Germany	1	400	400	BBCH 51–53	Seed	71	0.26	< 0.02	Hauck 1993
(Gnarrenburg)									CGA163935/0322
1992									gr 21192
(Lirajet)									
Germany	1	400	400	BBCH 53–55	Seed	90	0.29	< 0.02	Hauck 1993d
(Rohlstorf)									CGA163935/0323
1992									gr 30392
(Ceres)									
Germany	1	400	400	BBCH 53–55	Seed	82	0.24	< 0.02	Hauck 1993e
(Ursleben)									CGA163935/0325
1992									gr 60592
(Lirajet)									
Germany	1	400	300	BBCH 53–55	Seed	80	0.90	< 0.02	Hauck 1993g
(Cunnersdorf)									CGA163935/0328
1992									RF 04/92
(Madora)									
Germany	1	400	300	BBCH 53–55	Seed	74	0.64	< 0.02	Hauck 1993f
(Egeln)									CGA163935/0327

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)	Trinexapac-ethyl (mg/kg)	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage					
1992									RF 04/92
(Liberator)									
Germany	1	375	400	BBCH 39–51	Seed	108	0.10	–	Smith 2000
(Rohlsdorf)									CGA163935/0636
1999									Trial 1
(Lirajet)									
Germany	1	375	400	BBCH 33–51	Seed	113	0.10	–	Smith 2000a
(Ivenrode)									CGA163935/0637
1999									Trial 1
(Laser)									
Germany	1	375	400	BBCH 51–53	Seed	93	0.16	–	Smith 2000b
(See)									CGA163935/0638
1999									Trial 1
(Express)									
Germany	1	375	400	BBCH 50–51	Seed	104	0.24	–	Smith 2000c
(Kandel)									CGA163935/0639
1999									Trial 1
(Mohikan)									

LOQ = 0.02 mg/kg (trinexapac acid and trinexapac-ethyl)

[a] The trinexapac-ethyl residues result for Lorsch is considered to be anomalous.

Animal Feeds

Note: Animal feed residues are expressed on a wet weight or 'as received' basis.

Table 76 Residues from the foliar application of trinexapac-ethyl to barley in the USA

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA (barley, oat)	1	123				45			
USA	1	131	189	Feekes 7	Hay	30	0.23	0.25	Mäyer 2010a
(New Tripoli,							0.26		CGA163935_50026
Pennsylvania)	1	132	288	BBCH 59/	Straw	45	0.29	0.24	E04PA081701
2009				Feekes 10.5			0.19		
(Nomini)									
USA	1	134	121	BBCH 32	Hay	0	6.7		Mäyer 2010a
(Bagley, Iowa)					Hay	10	0.70		CGA163935_50026
2008					Hay	20	0.45		C30IA081702
(Robust)					Hay	30	0.54	0.48	
							0.41		
					Hay	37	0.32		
	1	132	68	BBCH 49	Straw	24	0.28		

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
					Straw	31	0.21		
					Straw	38	0.14		
					Straw	45	0.08	0.14	
							0.20		
					Straw	52	0.13		
USA	1	133	20	BBCH 33	Hay	30	0.34	0.40	Mäyer 2010a
(Fitchburg, Wisconsin)							0.46		CGA163935_50026
2008	1	133	19	BBCH 33	Straw	45	0.01	0.02	C08WI081703
(Kewaunee)							0.02		
USA	1	129	189	BBCH 32	Hay	30	0.13	0.13	Mäyer 2010a
(Northwood, North							0.13		CGA163935_50026
Dakota)	1	129	187	BBCH 51	Straw	45	0.08	0.08	C13ND081704
2008							0.07		
(Tradition)									
USA	1	129	187	BBCH 32	Hay	30	0.04	0.05	Mäyer 2010a
(Carrington, North							0.05		CGA163935_50026
Dakota)	1	129	141	BBCH 73	Straw	45	0.12	0.12	C13ND081705
2008							0.12		
(Tradition)									
USA	1	128	187	BBCH 32	Hay	30	0.03	0.03	Mäyer 2010a
(New Rockford, North							0.03		CGA163935_50026
Dakota)	1	128	187	BBCH 73	Straw	45	0.05	0.07	C13ND081706
2008							0.08		
(Tradition)									
USA	1	129	140	BBCH 32	Hay	30	0.10	0.10	Mäyer 2010a
(Eldridge, North							0.10		CGA163935_50026
Dakota)	1	134	146	BBCH 59	Straw	45	0.08	0.09	C12ND081707
2008							0.09		
(Tradition)									
USA	1	128	139	BBCH 32	Hay	30	0.13	0.15	Mäyer 2010a
(Adrian, North Dakota)							0.16		CGA163935_50026
2008	1	127	138	BBCH 59	Straw	45	0.11	0.11	C12ND081708
(Drummond)							0.10		
USA	1	129	271	BBCH 41	Hay	30	< 0.01	≤ 0.01	Mäyer 2010a
(Monte Vista,							< 0.01		CGA163935_50026
Colorado)	1	131	273	BBCH 45	Straw	45	< 0.01	≤ 0.01	E13CO081709
2008							< 0.01		
(C-69)									
USA	1	132	193	BBCH 32	Hay	30	0.34	0.33	Mäyer 2010a
(Madera, California)							0.31		CGA163935_50026
2008	1	128	282	BBCH 45	Straw	45	0.20	0.20	W29CA081710
(Re-cleaned whole							0.19		
barley)									
USA	1	129	192	BBCH 32	Hay	30	0.20	0.18	Mäyer 2010a

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
(Hermiston, Oregon)							0.16		CGA163935_50026
2008	1	130	194	BBCH 84	Straw	45	0.09	0.08	W21OR081711
(Radiant)							0.06		
USA	1	127	97	BBCH 32	Hay	30	0.14	0.17	Mäyer 2010a
(Rupert, Idaho)							0.19		CGA163935_50026
2008	1	130	97	BBCH 65	Straw	45	0.16	0.17	W15ID081712
(IDA Gold II)							0.17		

LOQ = 0.01 mg/kg

Feekes 7 = Second node detectable

Table 77 Residues from the foliar application of trinexapac-ethyl to wheat in the USA

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA (wheat)	1	123				45			
USA	1	132	114	BBCH 32	Forage	0	2.08		Mäyer 2010
(Suffolk, Virginia)					Forage	7	0.16		CGA163935_50036
2008					Forage	14	0.19		E07VA078460
(Winter wheat—R9184)					Forage	30	0.05	0.06	
							0.07		
					Forage	37	0.12		
USA	1	129	135	BBCH 32	Forage	30	0.18	0.23	Mäyer 2010
(Proctor, Arkansas)							0.27		CGA163935_50036
2008									C24AR078461
(Wheat—DK7710)									
USA	1	130	19	BBCH 32	Forage	30	0.36	0.33	Mäyer 2010
(Minneapolis, Kansas)							0.30		CGA163935_50036
2008									C19KS078462
(Hard White Wheat –									
Danby)									
USA	1	130	188	BBCH 32	Forage	30	0.06	0.06	Mäyer 2010
(Northwood, North							0.06		CGA163935_50036
Dakota)									C13ND078463
2008									
(HRSW—Kelby)									
USA	1	131	142	BBCH 33	Forage	30	0.07	0.07	Mäyer 2010
(Perley, Minnesota)							0.06		CGA163935_50036
2008									C12MN078464
(Spring Wheat—Alsen)									
USA	1	127	155	Feekes 6–7	Forage	30	0.11	0.10	Mäyer 2010
(St Joseph, Missouri)							0.08		CGA163935_50036
2008									C19MO078465

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
(Hard Red Winter									
Wheat—Kansas 2137)									
USA	1	129	130	BBCH 32	Forage	30	0.23	0.22	Mäyer 2010
(Kirklin, Indiana)							0.20		CGA163935_50036
2008									C05IN078466
(Wheat—Pioneer									
25R78)									
USA	1	129	84	BBCH 32	Forage	30	0.09	0.10	Mäyer 2010
(Madill, Oklahoma)							0.11		CGA163935_50036
2008									W01OK078467
(Winter Wheat—Jagger)									
USA	1	130	189	BBCH 32	Forage	30	0.03	0.03	Mäyer 2010
(Carrington, North							0.02		CGA163935_50036
Dakota)									C13ND078468
2008									
(Durum—Divide)									
USA	1	129	187	BBCH 32	Forage	0	4.33		Mäyer 2010
(Carrington, North					Forage	7	0.12		CGA163935_50036
Dakota)					Forage	14	0.07		C13ND078469
2008					Forage	30	0.04	0.04	
(SWSW—WPB Nick)							0.04		
					Forage	37	0.04		
USA	1	131	140	BBCH 31	Forage	30	0.01	0.02	Mäyer 2010
(Velva, North Dakota)							0.02		CGA163935_50036
2008									C14ND078470
(Hard White Spring									
Wheat—Agawana)									
USA	1	129	20	BBCH 32	Forage	30	0.90	0.94	Mäyer 2010
(Lake Andes, South							0.98		CGA163935_50036
Dakota)									C16SD078471
2008									
(Spring Wheat—Briggs)									
USA	1	134	192	BBCH 32	Forage	0	5.57		Mäyer 2010
(Grand Island,					Forage	7	0.15		CGA163935_50036
Nebraska)					Forage	14	0.13		C17NE078472
2008					Forage	30	< 0.01	0.04	
(Hard Red Winter Wheat							0.07		
—Com. Wesley)					Forage	37	0.08		
USA	1	129	84	Feekes 7	Forage	30	0.17	0.17	Mäyer 2010
(Groom, Texas)							0.16		CGA163935_50036
2008									W01TX078473
(Winter Wheat—									

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
Cutter)									
USA	1	129	84	Feekes 7	Forage	30	0.01	0.01	Mäyer 2010
(Groom, Texas)							< 0.01		CGA163935_50036
2008									W01TX078476
(Winter Wheat—TAM									
212)									
USA	1	129	84	Feekes 7	Forage	30	< 0.01	0.02	Mäyer 2010
(Groom, Texas)							0.03		CGA163935_50036
2008									W01TX078477
(Winter Wheat—									
Jagalene)									
USA	1	130	84	BBCH 32	Forage	30	0.20	0.17	Mäyer 2010
(Goodwell, Oklahoma)							0.14		CGA163935_50036
2008									W01OK078474
(Winter Wheat—									
Ogallalla)									
USA	1	130	122	BBCH 32	Forage	30	0.40	0.38	Mäyer 2010
(Frederick, Oklahoma)							0.36		CGA163935_50036
2008									C29OK078475
(Winter Wheat—Custer)									
USA	1	129	84	Feekes 7	Forage	30	0.04	0.08	Mäyer 2010
(Wellington, Texas)							0.11		CGA163935_50036
2008									W01TX078478
(Winter Wheat—TAM									
200)									
USA	1	130	93	BBCH 32	Forage	30	0.08	0.09	Mäyer 2010
(Rupert, Idaho)							0.09		CGA163935_50036
2008									W15ID078479
(Winter Wheat—									
Westbred 528)									

LOQ = 0.01 mg/kg

Feekes 6 = First node detectable

Feekes 7 = Second node detectable

Table 78 Residues from the foliar application of trinexapac-ethyl to wheat in the USA

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA (wheat)	1	123				45			
USA	1	132	114	BBCH 32	Hay	0	2.47		Mäyer 2010
(Suffolk, Virginia)					Hay	7	0.54		CGA163935_50036

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2008					Hay	14	0.27		E07VA078460
(Winter wheat—R9184)					Hay	30	0.17	0.17	
							0.17		
					Hay	37	0.15		
	1	130	120	BBCH 71	Straw	31	< 0.01		
					Straw	38	0.07		
					Straw	45	0.08	0.07	
							0.06		
					Straw	52	0.11		
USA	1	129	135	BBCH 32	Hay	30	0.54	0.50	Mäyer 2010
(Proctor, Arkansas)							0.45		CGA163935_50036
2008	1	129	135	BBCH 65	Straw	45	0.31	0.20	C24AR078461
(Wheat—DK7710)							0.08		
USA	1	130	19	BBCH 32	Hay	30	0.55	0.59	Mäyer 2010
(Minneapolis, Kansas)							0.62		CGA163935_50036
2008	1	128	19	Boot	Straw	45	0.22	0.23	C19KS078462
(Hard White Wheat –							0.24		
Danby)									
USA	1	130	188	BBCH 32	Hay	30	0.11	0.11	Mäyer 2010
(Northwood, North							0.10		CGA163935_50036
Dakota)	1	129	188	BBCH 67	Straw	45	0.06	0.06	C13ND078463
2008							0.06		
(HRSW—Kelby)									
USA	1	131	142	BBCH 33	Hay	30	0.08	0.11	Mäyer 2010
(Perley, Minnesota)							0.14		CGA163935_50036
2008	1	134	183	BBCH 51	Straw	45	0.08	0.09	C12MN078464
(Spring Wheat—Alsen)							0.10		
USA	1	127	155	Feekes 6-7	Hay	30	0.17	0.19	Mäyer 2010
(St Joseph, Missouri)							0.20		CGA163935_50036
2008	1	130	158	Boot	Straw	45	0.03	0.03	C19MO078465
(Hard Red Winter							0.02		
Wheat—Kansas 2137)									
USA	1	129	130	BBCH 32	Hay	30	0.31	0.31	Mäyer 2010
(Kirklin, Indiana)							0.30		CGA163935_50036
2008	1	132	134	BBCH 55	Straw	45	0.19	0.17	C05IN078466
(Wheat—Pioneer							0.15		
25R78)									
USA	1	129	84	BBCH 32	Hay	30	0.02	0.03	Mäyer 2010
(Madill, Oklahoma)							0.03		CGA163935_50036
2008	1	129	241	BBCH 53	Straw	45	< 0.01	0.01	W01OK078467
(Winter Wheat—Jagger)							0.01		
USA	1	130	189	BBCH 32	Hay	30	0.04	0.04	Mäyer 2010

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
(Carrington, North							0.03		CGA163935_50036
Dakota)	1	129	188	BBCH 65	Straw	45	0.10	0.11	C13ND078468
2008							0.11		
(Durum—Divide)									
USA	1	129	187	BBCH 32	Hay	0	1.31		Mäyer 2010
(Carrington, North					Hay	7	1.33		CGA163935_50036
Dakota)					Hay	14	0.19		C13ND078469
2008					Hay	30	0.09	0.08	
(SWSW—WPB Nick)							0.07		
					Hay	37	0.09		
	1	129	187	BBCH 65	Straw	31	0.25		
					Straw	38	0.16		
					Straw	45	0.47	0.46	
							0.44		
					Straw	52	0.08		
USA	1	131	140	BBCH 31	Hay	30	0.05	0.06	Mäyer 2010
(Velva, North Dakota)							0.06		CGA163935_50036
2008	1	130	144	BBCH 41	Straw	45	0.14	0.15	C14ND078470
(Hard White Spring							0.16		
Wheat—Agawana)									
USA	1	129	20	BBCH 32	Hay	30	1.26	1.18	Mäyer 2010
(Lake Andes, South							1.09		CGA163935_50036
Dakota)	1	130	19	BBCH 45	Straw	45	0.64	0.60	C16SD078471
2008							0.56		
(Spring Wheat—Briggs)									
USA	1	134	192	BBCH 32	Hay	0	10.8		Mäyer 2010
(Grand Island,					Hay	7	0.46		CGA163935_50036
Nebraska)					Hay	14	0.42		C17NE078472
2008					Hay	30	0.14	0.08	
(Hard Red Winter Wheat							< 0.01		
—Com. Wesley)					Hay	37	0.14		
	1	134	192	BBCH 51	Straw	31	0.16		
					Straw	38	0.40		
					Straw	45	0.55	0.59	
							0.62		
					Straw	52	0.12		
USA	1	129	84	Feekes 7	Hay	30	0.31	0.30	Mäyer 2010
(Groom, Texas)							0.28		CGA163935_50036
2008	1	130	252	BBCH 52	Straw	45	0.16	0.15	W01TX078473
(Winter Wheat—Cutter)							0.13		
USA	1	129	84	Feekes 7	Hay	30	0.15	0.11	Mäyer 2010
(Groom, Texas)							0.07		CGA163935_50036

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2008	1	127	237	BBCH 52	Straw	45	0.10	0.09	W01TX078476
(Winter Wheat—TAM							0.07		
212)									
USA	1	129	84	Feekes 7	Hay	30	0.19	0.21	Mäyer 2010
(Groom, Texas)							0.22		CGA163935_50036
2008	1	128	239	BBCH 51	Straw	45	0.08	0.09	W01TX078477
(Winter Wheat—							0.10		
Jagalene)									
USA	1	130	84	BBCH 32	Hay	30	0.41		Mäyer 2010
(Goodwell, Oklahoma)	1	131	240	BBCH 52	Straw	45	0.04	0.04	CGA163935_50036
2008							0.04		W01OK078474
(Winter Wheat—									
Ogallalla)									
USA	1	130	122	BBCH 32	Hay	30	0.83	0.78	Mäyer 2010
(Frederick, Oklahoma)							0.72		CGA163935_50036
2008	1	134	126	BBCH 60	Straw	45	0.38	0.28	C29OK078475
(Winter Wheat—Custer)							0.18		
USA	1	129	84	Feekes 7	Hay	30	0.03	0.04	Mäyer 2010
(Wellington, Texas)							0.04		CGA163935_50036
2008	1	130	241	BBCH 51	Straw	45	0.03	0.04	W01TX078478
(Winter Wheat—TAM							0.04		
200)									
USA	1	130	93	BBCH 32	Hay	30	0.24	0.24	Mäyer 2010
(Rupert, Idaho)							0.24		CGA163935_50036
2008	1	127	97	BBCH 65	Straw	45	0.39	0.33	W15ID078479
(Winter Wheat—							0.26		
Westbred 528)									

LOQ = 0.01 mg/kg

Feekes 6 = First node detectable

Feekes 7 = Second node detectable

Table 79 Residues in wheat forage from the foliar application of trinexapac-ethyl to wheat in the USA

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA (wheat)	1	123				45			
USA	1	130	156	BBCH 23	Forage	26	< 0.05	< 0.05	Ediger 2006
(Pikeville, North				(Feekes 5–7)			< 0.05		451534
Carolina)									SJ-FR-04-5415
2005									
(Winter Wheat—									

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
Coker									
9184)									
USA	1	129	90	Feekes 7	Forage	30	0.07	0.09	Ediger 2006
(Lehi, Arkansas)							0.10		451534
2005									SE-FR-04-5416
(Winter Wheat—									
DK9410)									
USA	1	128	140	Feekes 8	Forage	31	0.07	0.07	Ediger 2006
(Abilene, Kansas)							0.07		451534
2005									ND-FR-04-5417
(Winter Wheat—									
Burdett)									
USA	1	128	232	BBCH 32	Forage	30	< 0.05	< 0.05	Ediger 2006
(Northwood, North							< 0.05		451534
Dakota)									NN-FR-04-5418
2004									
(Spring Wheat— Briggs)									
USA	1	129	187	Feekes 7	Forage	30	< 0.05	0.05	Ediger 2006
(Campbell, Minnesota)							0.05		451534
2004									NF-FR-04-5419
(Spring Wheat— Oxen)									
USA	1	131	141	Feekes 5–7	Forage	27	< 0.05	< 0.05	Ediger 2006
(Kirksville, Missouri)							< 0.05		451534
2005									ND-FR-04-5420
(Winter Wheat— Ernie)									
USA	1	129	39	BBCH 32	Forage	34	0.06	0.06	Ediger 2006
(Champaign, Illinois)							0.06		451534
2005									4A-FR-04-5421
(Winter Wheat—									
Kaskaskia)									
USA	1	129	128	BBCH 32	Forage	0	1.3	1.3	Ediger 2006
(Hinton, Oklahoma)							1.3		451534
2005					Forage	7	0.52	0.52	SC-FR-04-5422
(Winter Wheat—							0.52		
Jagalene)					Forage	14	0.39	0.43	
							0.46		
					Forage	31	0.13	0.11	
							0.08		
					Forage	38	< 0.05	< 0.05	
							< 0.05		
USA	1	129	189	Feekes 7	Forage	1	0.79	0.89	Ediger 2006
(New Rockford, North							0.98		451534
Dakota)					Forage	7	0.17	0.16	NN-FR-04-5423

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2004							0.15		
(Winter Wheat—					Forage	14	0.09	0.09	
Lebsock)							0.08		
					Forage	30	< 0.05	< 0.05	
							< 0.05		
					Forage	37	< 0.05	< 0.05	
							< 0.05		
USA	1	127	19	Feekes 6	Forage	33	0.13	0.12	Ediger 2006
(Froid, Montana)							0.10		451534
2004									NN-FR-04-5424
(Spring Wheat—									
Challis)									
USA	1	134	98	Feekes 6	Forage	33	< 0.05	< 0.05	Ediger 2006
(Froid, Montana)							< 0.05		451534
2004									NN-FR-04-5425
(Spring Wheat—									
Pristine)									
USA	1	128	172	BBCH 32	Forage	30	0.10	0.11	Ediger 2006
(Trail City, South							0.12		451534
Dakota)									NF-FR-04-5426
2004									
(Spring Wheat— Freyr)									
USA	1	129	186	BBCH 32	Forage	29	0.09	0.09	Ediger 2006
(Grand Island,							0.08		451534
Nebraska)									NB-FR-04-5427
2005									
(Winter Wheat—									
Arrowsmith HW)									
USA	1	123	129	Feekes 7	Forage	0	1.0	1.4	Ediger 2006
(Larned, Kansas)							1.7		451534
2005					Forage	7	0.06	0.07	NM-FR-04-5428
(Winter Wheat— Jagger)							0.08		
					Forage	14	< 0.05	< 0.05	
							< 0.05		
					Forage	30	< 0.05	< 0.05	
							< 0.05		
					Forage	37	< 0.05	< 0.05	
							< 0.05		
USA	1	129	20	Feekes 7	Forage	30	< 0.05	< 0.05	Ediger 2006
(Larned, Kansas)							< 0.05		451534
2005									NM-FR-04-5429
(Winter Wheat— Jagger)									
USA	1	128	144	BBCH 29	Forage	30	< 0.05	< 0.05	Ediger 2006
(Goodwell, Oklahoma)							< 0.05		451534

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2005									SC-FR-04-5430
(Winter Wheat—Clark)									
USA	1	128	142	BBCH 32	Forage	30	0.08	0.08	Ediger 2006
(Levelland, Texas)							0.07		451534
2005									SC-FR-04-5431
(Winter Wheat—TAM									
200)									
USA	1	128	127	Feekes 7	Forage	30	0.34	0.31	Ediger 2006
(Ault, Colorado)							0.28		451534
2005									NM-FR-04-5432
(Winter Wheat—Yuma)									
USA	1	130	139	BBCH 32	Forage	30	0.14	0.15	Ediger 2006
(Clovis, New Mexico)							0.16		451534
2005									SC-FR-04-5433
(Winter Wheat—TAM									
110)									
USA	1	129	144	BBCH 32	Forage	29	< 0.05	< 0.05	Ediger 2006
(Ephrata, Washington)							< 0.05		451534
2004									WF-FR-04-5435
(Winter Wheat—									
Stephens)									

LOQ = 0.05 mg/kg

Feekes 5 = Pseudo stem (formed by sheaths of leaves) strongly erected

Feekes 6 = First node detectable

Feekes 7 = Second node detectable

Feekes 8 = Flag leaf (last leaf) visible but still rolled up, ear beginning to swell

Table 80 Residues from the foliar application of trinexapac-ethyl to wheat in the USA

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
GAP, USA (wheat)	1	123				45			
USA	1	130	156	BBCH 23	Hay	26	0.06	0.07	Ediger 2006
(Pikeville, North				(Feekes 5–7)			0.07		451534
Carolina)	1	129	147	BBCH 43	Straw	50	0.14	0.12	SJ-FR-04-5415
2005							0.10		
(Winter Wheat—Coker									
9184)									
USA	1	129	90	Feekes 7	Hay	30	0.19	0.15	Ediger 2006
(Lehi, Arkansas)							0.10		451534

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2005	1	129	142	BBCH 55	Straw	45	< 0.05	0.06	SE-FR-04-5416
(Winter Wheat—							0.06		
DK9410)									
USA	1	128	140	Feekes 8	Hay	31	0.15	0.13	Ediger 2006
(Abilene, Kansas)							0.10		451534
2005	1	130	138	Feekes 10.5.2	Straw	45	< 0.05	< 0.05	ND-FR-04-5417
(Winter Wheat—							< 0.05		
Burdett)									
USA	1	128	232	BBCH 32	Hay	30	< 0.05	< 0.05	Ediger 2006
(Northwood, North							< 0.05		451534
Dakota)	1	127	92	BBCH 61	Straw	48	< 0.05	0.06	NN-FR-04-5418
2004							0.06		
(Spring Wheat— Briggs)	1	384	93	BBCH 61	Straw	48	0.26	0.21	
							0.16		
	1	643	94	BBCH 61	Straw	48	0.51	0.63	
							0.74		
USA	1	129	187	Feekes 7	Hay	30	0.06	0.08	Ediger 2006
(Campbell, Minnesota)							0.10		451534
2004	1	128	187	BBCH 65	Straw	44	0.11	0.11	NF-FR-04-5419
(Spring Wheat— Oxen)				Full			0.11		
				Flowering					
USA	1	131	141	Feekes 5–7	Hay	27	0.06	0.06	Ediger 2006
(Kirksville, Missouri)							0.06		451534
2005	1	130	151	BBCH 61	Straw	47	< 0.05	< 0.05	ND-FR-04-5420
(Winter Wheat— Ernie)							< 0.05		
USA	1	129	39	BBCH 32	Hay	34	0.09	0.09	Ediger 2006
(Champaign, Illinois)							0.09		451534
2005	1	127	31	BBCH 55	Straw	46	0.10	0.10	4A-FR-04-5421
(Winter Wheat—							0.09		
Kaskaskia)									
USA	1	129	128	BBCH 32	Hay	0	0.34	0.97	Ediger 2006
(Hinton, Oklahoma)							1.6		451534
2005					Hay	7	0.67	0.63	SC-FR-04-5422
(Winter Wheat—							0.58		
Jagalene)					Hay	14	0.46	0.46	
							0.46		
					Hay	31	0.20	0.19	
							0.18		
					Hay	38	0.13	0.17	
							0.20		
	1	130	129	BBCH 71	Straw	42	0.07	0.08	
							0.09		
					Straw	47	0.05	0.06	

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
							0.06		
					Straw	51	0.05	0.07	
							0.08		
USA	1	129	189	Feekes 7	Hay	1	1.0	1.1	Ediger 2006
(New Rockford, North							1.2		451534
Dakota)					Hay	7	0.19	0.19	NN-FR-04-5423
2004							0.19		
(Winter Wheat—					Hay	14	0.09	0.10	
Lebsock)							0.10		
					Hay	30	< 0.05	< 0.05	
							< 0.05		
					Hay	37	< 0.05	< 0.05	
							< 0.05		
	1	128	187	BBCH 33	Straw	40	< 0.05	< 0.05	
							< 0.05		
					Straw	50	< 0.05	< 0.05	
							< 0.05		
					Straw	56	< 0.05	< 0.05	
							< 0.05		
USA	1	127	19	Feekes 6	Hay	33	0.17	0.18	Ediger 2006
(Froid, Montana)							0.18		451534
2004	1	128	19	Feekes 10.5	Straw	42	0.08	0.08	NN-FR-04-5424
(Spring Wheat—							0.07		
Challis)									
USA	1	134	98	Feekes 6	Hay	33	< 0.05	< 0.05	Ediger 2006
(Froid, Montana)							< 0.05		451534
2004	1	129	93	Feekes 10.5	Straw	42	0.06	0.07	NN-FR-04-5425
(Spring Wheat—							0.07		
Pristine)									
USA	1	128	172	BBCH 32	Hay	30	0.11	0.11	Ediger 2006
(Trail City, South							0.11		451534
Dakota)	1	129	178	BBCH 65	Straw	45	< 0.05	< 0.05	NF-FR-04-5426
2004							< 0.05		
(Spring Wheat— Freyr)									
USA	1	129	186	BBCH 32	Hay	29	0.08	0.08	Ediger 2006
(Grand Island,							0.08		451534
Nebraska)	1	129	187	BBCH 61	Straw	44	< 0.05	< 0.05	NB-FR-04-5427
2005							< 0.05		
(Winter Wheat—									
Arrowsmith HW)									
USA	1	123	129	Feekes 7	Hay	0	2.6	2.3	Ediger 2006
(Larned, Kansas)							2.0		451534
2005					Hay	7	0.08	0.08	NM-FR-04-5428
(Winter Wheat— Jagger)							0.08		
					Hay	14	0.05	0.06	

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
							0.06		
					Hay	30	0.05	0.05	
							0.05		
					Hay	37	< 0.05	< 0.05	
							< 0.05		
	1	125	130	Flag leaf	Straw	31	0.05	0.05	
							< 0.05		
					Straw	38	< 0.05	< 0.05	
							< 0.05		
					Straw	45	< 0.05	< 0.05	
							< 0.05		
					Straw	52	< 0.05	< 0.05	
							< 0.05		
USA	1	129	20	Feekes 7	Hay	30	< 0.05	< 0.05	Ediger 2006
(Larned, Kansas)							< 0.05		451534
2005	1	129	21	Flag leaf	Straw	45	< 0.05	< 0.05	NM-FR-04-5429
(Winter Wheat—Jagger)							< 0.05		
USA	1	128	144	BBCH 29	Hay	30	< 0.05	< 0.05	Ediger 2006
(Goodwell, Oklahoma)							< 0.05		451534
2005	1	130	144	BBCH 45	Straw	45	0.08	0.07	SC-FR-04-5430
(Winter Wheat—Clark)							0.05		
	1	391	145	BBCH 45	Straw	45	0.12	0.09	
							0.06		
	1	643	143	BBCH 45	Straw	45	0.15	0.16	
							0.16		
USA	1	128	142	BBCH 32	Hay	30	0.11	0.11	Ediger 2006
(Levelland, Texas)							0.10		451534
2005	1	123	136	BBCH 67	Straw	44	< 0.05	< 0.05	SC-FR-04-5431
(Winter Wheat—TAM							< 0.05		
200)									
USA	1	128	127	Feekes 7	Hay	30	0.90	0.75	Ediger 2006
(Ault, Colorado)							0.60		451534
2005	1	130	126	Feekes 10.5	Straw	45	< 0.05	< 0.05	NM-FR-04-5432
(Winter Wheat—Yuma)							< 0.05		
USA	1	130	139	BBCH 32	Hay	30	0.19	0.19	Ediger 2006
(Clovis, New Mexico)							0.19		451534
2005	1	127	140	BBCH 65	Straw	44	< 0.05	< 0.05	SC-FR-04-5433
(Winter Wheat—TAM							< 0.05		
110)									
USA	1	129	144	BBCH 32	Hay	29	0.07	0.07	Ediger 2006
(Ephrata, Washington)							0.06		451534

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)		Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean	
2004	1	129	139	BBCH 61	Straw	44	< 0.05	< 0.05	WF-FR-04-5435
(Winter Wheat—							< 0.05		
Stephens)									

LOQ = 0.05 mg/kg

Feekes 5 = Pseudo stem (formed by sheaths of leaves) strongly erected

Feekes 6 = First node detectable

Feekes 7 = Second node detectable

Feekes 8 = Flag leaf (last leaf) visible but still rolled up, ear beginning to swell

Table 81 Residues from the foliar application of trinexapac-ethyl to winter rape in Germany

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)	Trinexapac-ethyl (mg/kg)	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage					
GAP, Germany (oilseed rape, winter)	1	375							
Germany	1	375	400	BBCH 39–51	Whole plant	0	4.5	3.7	Smith 2000
(Rohlsdorf)					Pods with seeds	56	0.28	–	CGA163935/0636
1999					Remaining Plant	56	< 0.04	–	Trial 1
(Lirajet)					type				
					Straw	108	0.11	–	
Germany	1	375	400	BBCH 33–51	Whole plant	0	2.2	3.3	Smith 2000a
(Ivenrode)					Pods with seeds	68	0.22	–	CGA163935/0637
1999					Remaining Plant	68	< 0.04	–	Trial 1
(Laser)					type				
					Straw	113	0.07	–	
Germany	1	375	400	BBCH 51–53	Whole plant	0	3.4	1.4	Smith 2000b
(See)					Pods with seeds	49	0.43	–	CGA163935/0638
1999					Remaining Plant	49	< 0.04	–	Trial 1
(Express)					type				
					Straw	93	0.07	–	
Germany	1	375	400	BBCH 50–51	Whole plant	0	2.4	2.7	Smith 2000c
(Kandel)					Pods with seeds	57	0.33	–	CGA163935/0639
1999					Remaining Plant	57	0.04	–	Trial 1
(Mohikan)					type				
					Straw	104	0.05	–	

LOQ = 0.04 mg/kg (trinexapac acid in whole plant, remainder and straw and trinexapac-ethyl in whole plant)

LOQ = 0.02 mg/kg (trinexapac acid in pods with seeds)

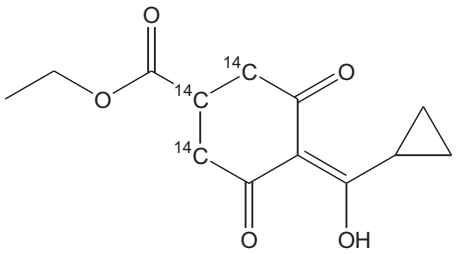
FATE OF RESIDUES IN STORAGE AND PROCESSING

Residues after processing

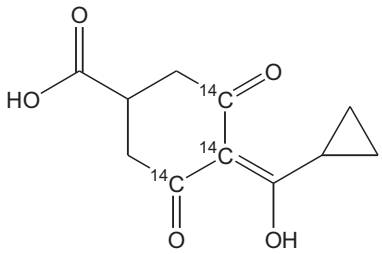
Processing studies are necessary according to the uses and the residues of trinexapac-ethyl on raw agricultural commodities. The fate of trinexapac-ethyl residues during processing of raw agricultural commodities was investigated in a number of major registered crops (barley, wheat, sugarcane and oilseed rape).

The high temperature hydrolysis of residues of trinexapac-ethyl and trinexapac acid under varying conditions were studied (Cadalbert and Buckel 2001, CGA163935/0733 and Mound and Clark 2004, CGA179500/0036 respectively). The labelled compounds used are depicted below.

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl



^{14}C-cyclohexyl labelled trinexapac-acid



Trinexapac-ethyl

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was added to aqueous buffer solutions and subjected to hydrolysis at pH 4, 5 and 6 at high temperature at concentrations of 5 mg/L (0.01 M citrate, acetate and phosphate buffers respectively). The conditions were selected to simulate hydrolysis under processing conditions and included:

- The effect of pasteurisation (pH 4 and pasteurised at 90 °C for 20 minutes)
- The effect of baking, boiling and brewing (pH 5 and baked at 100 °C for 60 minutes)
- The effect of sterilisation (pH 6 and autoclaving at 120 °C for 20 minutes).

The pH ranged from 4.22 (beginning of the trial), 4.30 (end of the trial) for the pH 4 samples, 5.04 (beginning of the trial), 5.07 (end of the trial) for the pH 5 samples and from 6.15 (beginning of the trial), 6.25 (end of the trial) for the pH 6 samples. No major loss of radioactivity material occurred. For all three processing conditions, 99% (mean) of the applied radioactivity remained after the test. Subsamples of the test solutions were analysed by HPLC and TLC. The amount of radioactive material in the samples at the end of the test mainly corresponded to parent compound (93–94% of the applied radioactivity in all samples). Minor compounds were present up to 3.3% of the applied radioactivity, however as they were present at the start of the incubation, these were considered to be impurities.

In summary, the data show that trinexapac-ethyl was not degraded during the simulation of pasteurisation (pH 4, 90 °C, 20 minutes), baking, boiling and brewing (pH 5, 100 °C, 60 minutes) and sterilisation (pH 6, 120 °C, 20 minutes).

Trinexapac acid

^{14}C-cyclohexyl labelled trinexapac acid was added to aqueous acetate buffer solutions and subjected to hydrolysis at pH 4, 5 and 6 at high temperature, at concentrations ranging from 4.30–4.87 mg/L.

The conditions were the same as described above except the pH 4 experiment, simulating the effects of pasteurisation, was run for 25 minutes.

The pH of the buffer solutions prior to the experiment were 4.01, 5.06 and 6.08. No major loss of radioactivity material occurred. For the pH 4 at 90 °C experiment, 104% (mean) of the applied radioactivity remained after the test while for the other two processing conditions, 102% (mean) of the applied radioactivity remained after the test.

The major component of the radioactive material in the samples at the end of the hydrolysis reactions corresponded to unchanged trinexapac acid; at pH 4 and 90 °C the mean value was 52.5%, at pH 5 and 100 °C the mean value was 58.5% and at pH 6 and 120 °C the mean value was 50.9%. The hydrolysis products were similar at each pH. At least two major and several minor degradates were observed. The two major components were identified as CGA313458 (at pH 4 and 90 °C the mean value was 19.7%, at pH 5 and 100 °C the mean value was 16.1% and at pH 6 and 120 °C the mean value was 21.0%) and CGA113745 (at pH 4 and 90 °C the mean value was 9.6%, at pH 5 and 100 °C the mean value was 10.5% and at pH 6 and 120 °C the mean value was 11.6%). The other degradates, comprising more than one component, represented < 11.6% in any of the hydrolysates with no individual unknown being more than 5%. Characterisation was by TLC co-chromatography and where possible by HPLC confirmation. Identification of CGA313458 was confirmed by NMR.

The proposed hydrolysis of trinexapac acid under processing conditions is as follows:

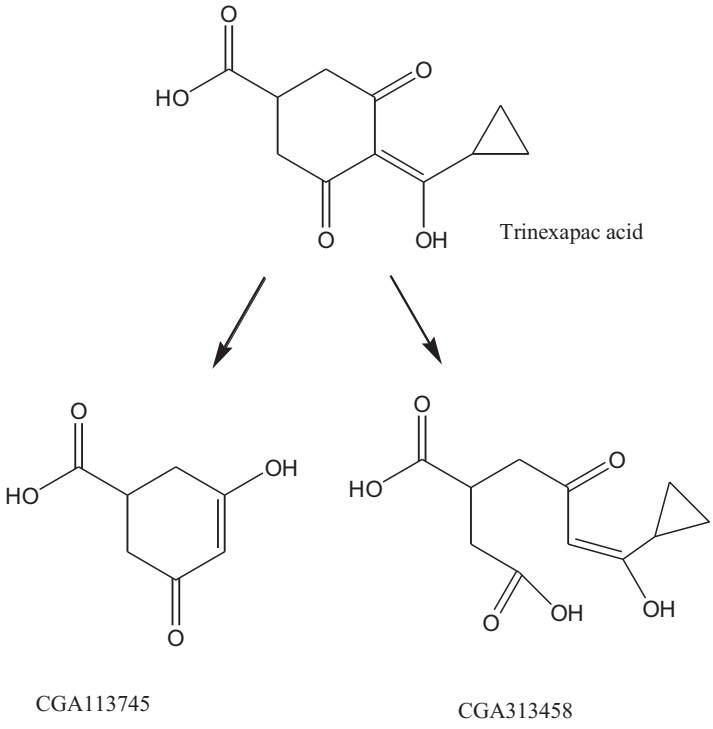


Figure 3 Hydrolysis of trinexapac acid under processing conditions

In summary, the data show that trinexapac acid undergoes limited degradation during the simulation of pasteurisation (pH 4, 90 °C, 25 minutes), during the simulation of baking, boiling and brewing (pH 5, 100 °C, 60 minutes) and during sterilisation (pH 6, 120 °C, 20 minutes).

Barley

The effect of processing (laboratory scale) on residues of trinexapac-ethyl in barley was investigated in two trials conducted in the USA in the 2008 growing season (Mayer 2010a, CGA163935_50026). Barley with incurred residues was obtained where plants were sprayed with a foliar spray at 0.129 and 648 k g ai/ha at BBCH 51 and at 0.129 and 0.644 k g ai/ha at BBCH 73. Applications were made in 141–188 L/ha using ground equipment. Barley bulk RAC samples were harvested 45 days after application.

Bulk barley grain samples were processed into processed barley commodity samples according to simulated commercial procedures into the following samples: pearled barley, flour and bran.

Barley was removed from storage and a representative RAC sample taken. Barley was oven-dried at 54–71 °C to a moisture content of 11–13.5%, then samples were cleaned by aspiration and screening. Light impurities were separated from the whole barley by aspiration. Large and small foreign particles were then separated from the barley. The cleaned barley was hulled, resulting in the fractions blocked (pearled) barley and husk. A sub-fraction of the pearled barley was then fed through a mill to break the grains. The broken grain was fed onto the sifter screens (0.14 and 0.80 mm) to obtain coarse bran, break flour and middlings. Coarse bran was sifted further to produce bran and shorts. Middlings were separated in a reduction mill to reduction flour and shorts. Break flour was combined with the reduction flour to produce barley flour. Shorts obtained after shifting bran and middlings were also combined.

Residues of total trinexapac in barley RAC and processed commodities were quantitated as trinexapac acid by LC/MS/MS using Method GRM 020.01A. Acceptable concurrent recovery data were obtained for all barley commodities.

Table 82 Residues of trinexapac in barley and processed commodities

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean		
GAP, USA (barley)	1	123				45				
USA	1	129	187	BBCH 51	Grain	45	0.50, 0.55, 0.48	0.51	–	Mäyer 2010a
(Northwood, North					Pearled		0.78, 0.72	0.75	1.5	CGA16393-5_50026
Dakota)					barley					C13ND081704p
2008					Flour		0.26, 0.31	0.29	0.57	
(Tradition)					Bran		0.95, 1.1	1.0	2.0	
	1	648	188	BBCH 51	Grain	45	9.1, 5.2, 5.9	6.7	–	
					Pearled		10.1, 8.6	9.4	1.4	
					barley					
					Flour		4.6, 3.7	4.2	0.63	
					Bran		10.8, 10.9	10.9	1.6	
USA	1	129	141	BBCH 73	Grain	45	0.65, 0.84, 0.76	0.75	–	Mäyer 2010a
(Carrington, North					Pearled		0.82, 0.89	0.86	1.2	CGA16393-5_50026
Dakota)					barley					C13ND081705p
2008					Flour		0.24, 0.18	0.21	0.28	
(Tradition)					Bran		1.4, 1.3	1.4	1.9	
	1	644	141	BBCH 73	Grain	45	5.2, 5.4, 6.1	5.6	–	

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean		
					Pearled		5.5, 4.1	4.8	0.86	
					barley					
					Flour		1.4, 1.4	1.4	0.25	
					Bran		10.1, 9.6	9.9	1.8	

Wheat

The effect of processing (laboratory scale) on residues of trinexapac-ethyl in wheat was investigated in two trials conducted in the USA in the 2008 growing season (Mäyer 2010, CGA163935_50036). Wheat with incurred residues was obtained where plants were sprayed with a foliar spray at 0.129 and 646 k g ai/ha at BBCH 65 (durum wheat) and at 0.130 and 0.649 k g ai/ha at BBCH 52 (winter wheat). Applications were made in 188–252 L/ha using ground equipment. Wheat bulk RAC samples were harvested 45 days after application.

Bulk wheat grain samples were processed according to simulated commercial procedures into the following samples: aspirated grain fractions, bran, flour, middlings, shorts and germ.

Drying and aspiration was used to generate aspirated grain fractions. Light impurities were separated by aspiration and screened, removing foreign particles (screenings) from the wheat. For wheat germ recovery, the cleaned wheat was moisture adjusted to 16%, passed through a disc mill, and sieved to remove bran from the germ fraction. The germ (with endosperm) was then sifted to separate the germ from the endosperm and was aspirated again.

The moisture content of the cleaned wheat grain was adjusted to 17.5%. The sample was fed through a mill to break the grains. The broken grain was fed onto the sifter screens (0.14 and 0.80 mm) to obtain coarse bran, break flour and middlings. Coarse bran was sifted further to produce bran and shorts. Middlings were separated in a reduction mill to reduction flour and shorts. Break flour was combined with the reduction flour to produce wheat flour. Shorts obtained after shifting bran and middlings were also combined.

Residues of total trinexapac in wheat RAC and processed commodities were quantitated as trinexapac acid by LC/MS/MS using Method GRM 020.01A. Acceptable concurrent recovery data were obtained for all wheat commodities.

Table 83 Residues of trinexapac in wheat and processed commodities

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean		
GAP, USA (wheat)	1	123				45				
USA	1	129	188	BBCH 65	Grain	45	0.33, 0.31, 0.28	0.30	–	Mäyer 2010
(Carrington, North					Wheat		0.24, 0.23	0.24	0.8	CGA163935_50036
Dakota)					AGF					C13ND078468
2008					Bran		0.61, 0.71	0.66	2.2	
(Durum–Divide)					Flour		0.16, 0.11	0.13	0.4	
					Middlings		3.67, 3.29, 3.39	3.5	11.7	
					Shorts		0.15, 0.20, 0.20	0.18	0.6	
					Germ		0.60, 0.31, 0.35	0.42	1.4	

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean		
	1	646	188	BBCH 65	Grain	45	5.57, 4.90, 5.73	5.4	–	
					Wheat		4.77, 4.03	4.4	0.8	
					AGF					
					Bran		7.85, 9.33	8.6	1.6	
					Flour		2.42, 2.88	2.7	0.5	
					Middlings		2.83, 2.45, 2.31	2.5	0.5	
					Shorts		0.12, 0.17, 0.17	0.15	0.03	
					Germ		5.88, 4.28, 5.11	5.1	0.9	
USA	1	130	252	BBCH 52	Grain	45	1.75, 1.66, 1.54	1.7	–	Mäyer 2010
(Groom, Texas)					Wheat		0.35, 0.34	0.35	0.2	CGA163935_50036
2008					AGF					W01TX078473
(Winter Wheat—					Bran		2.28, 4.63	3.5	2.1	
Cutter)					Flour		0.90, 0.58	0.74	0.4	
					Middlings		0.82, 0.42, 0.43	0.56	0.3	
					Shorts		0.93, 1.15, 0.95	1.0	0.6	
					Germ		1.62, 1.52, 1.34	1.5	0.9	
	1	649	251	BBCH 52	Grain	45	10.2, 9.55, 9.50	9.8	–	
					Wheat		3.50, 3.47	3.5	0.4	
					AGF					
					Bran		15.8, 104 [a], 14.4, 13.8	14.7	1.5	
					Flour		3.67, 3.80	3.7	0.4	
					Middlings		5.73, 5.48, 4.93	5.4	0.6	
					Shorts		5.23, 6.25, 5.61	5.7	0.6	
					Germ		9.78, 11.1, 11.6	10.8	1.1	

[a] Outlier, not used in calculations

In another study the effect of processing (laboratory scale) on residues of trinexapac-ethyl in wheat was investigated in two trials conducted in the USA in the 2004 or 2005 growing seasons (Ediger 2006, 451534). Wheat with incurred residues was obtained where plants were sprayed with a foliar spray at 0.127 and 0.643 k g ai/ha at BBCH 61 or 0.130 and 0.643 k g ai/ha at BBCH 45. Applications were made in 92–144 L/ha using ground equipment.

Bulk wheat grain samples were processed according to simulated commercial procedures into the following samples: aspirated grain fractions, bran, flour, middlings, shorts and germ (as described for Mäyer 2010, CGA163935_50036).

Residues of trinexapac in wheat RAC and processed commodities were quantitated as trinexapac acid using Method 110.01. Acceptable concurrent recovery data were obtained for all wheat commodities.

Table 84 Residues of trinexapac in wheat and processed commodities

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)	Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage					
GAP, USA (wheat)	1	123				45			
USA	1	127	92	BBCH 61	Grain	48	1.0		Ediger 2006
(Northwood, North					Wheat		0.35	0.35	451534
Dakota)					AGF				NN-FR-04-5418
2004					Bran		2.2	2.2	
(Spring Wheat—					Flour		0.32	0.32	
Briggs)					Middlings		0.41	0.41	
					Shorts		0.45	0.45	
					Germ		0.29	0.29	
	1	643	94	BBCH 61	Grain	48	11		
					Wheat		3.3	0.30	
					AGF				
					Bran		23	2.1	
					Flour		3.4	0.31	
					Middlings		4.9	0.45	
					Shorts		7.5	0.68	
					Germ		9.4	0.85	
USA	1	130	144	BBCH 45	Grain	45	0.39		Ediger 2006
(Goodwell,					Wheat		0.09	0.23	451534
Oklahoma)					AGF				
2005					Bran		0.98	2.5	SC-FR-04-5430
(Winter Wheat—					Flour		0.12	0.31	
Clark)					Middlings		0.19	0.49	
					Shorts		0.56	1.4	
					Germ		0.40	1.0	
	1	643	143	BBCH 45	Grain	45	4.20		
					Wheat		0.68	0.16	
					AGF				
					Bran		10	2.4	
					Flour		1.0	0.24	
					Middlings		1.8	0.43	
					Shorts		4.6	1.1	
					Germ		4.5	1.1	

Sugarcane

The effect of processing (laboratory scale) on residues of trinexapac-ethyl in sugarcane was investigated in two trials conducted in the USA in the 2008 growing season (Mäyer 2010b, CGA163935_50038). Sugarcane with incurred residues was obtained where plants were sprayed with one foliar spray at 0.382 and 1.856 kg ai/ha at BBCH 91 and 0.361 and 1.779 k ai/ha at BBCH 70.

Application was made 28 days prior to the harvest of the mature cane. Applications were made in 102–223 L water /ha.

Bulk sugarcane samples were processed according to simulated commercial procedures into blackstrap molasses and refined sugar.

The sugarcane was cleaned by spraying the cane. Juice was pressed out using a cane crusher repeatedly until the majority of the juice was yielded. Juice of the first pressing was screened (sieve) to remove large particles prior to pH adjustment to 7.2–7.6 with a 20% w/w calcium-oxide solution. After pH adjustment, the juice was boiled (97–100 °C) for 3 minutes. The heated juice was then immediately centrifuged to separate mud and thin juice. Thin juice was evaporated under vacuum (rotary evaporator) until the juice contained 50–60% solids (thick juice) with the temperature maintained below 70 °C. After evaporation the thick juice was filtered using cotton. During laboratory evaporation a known amount of thick juice was evaporated to 70–80% solids and a small amount of sugar was added to "seed" crystallisation of sugar granules. The remaining thick juice was slowly added and continuously evaporated, and the solution was allowed to cool. Crystallised raw sugar and molasses were separated by centrifugation. Final black strap molasses was 60–65% solids. After molasses was removed, the remaining sugar was refined.

Equal parts of raw sugar and water were combined and mixed to dissolve the sugar. The pH of the mixture was adjusted to 5.2–5.8. After resting, mixing was resumed and the pH adjusted to 7.0–7.4. After heating to approximately 59–61 °C, filter aid was added and the solution was vacuum filtered. Upon obtaining a non-cloudy filtrate, the solution was evaporated and crystallised. Refined sugar was dried at 38–54 °C until the moisture content was approximately 3.0%.

Residues of total trinexapac in sugarcane RAC and processed commodities were quantitated as trinexapac acid by LC/MS/MS using Method GRM 020.01A. Acceptable concurrent recovery data were obtained for all sugarcane commodities.

Table 85 Residues of trinexapac in sugarcane and processed commodities

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean		
GAP, USA (sugarcane)	1	347				28				
USA	1	382	105	BBCH 91	Sugarcane	28	0.04, 0.08, 0.05	0.06	–	Mäyer 2010b
(Clewiston, Florida)					Molasses		0.32, 0.32	0.32	5.3	CGA16393-5_50038
2008					Refined		< 0.01, < 0.01	< 0.01	0.2	E19FL078492p
(CL95-0776)					sugar					
	1	1856	102	BBCH 91	Sugarcane	28	0.38, 0.23 0.24	0.28	–	
					Molasses		1.7, 1.8	1.8	6.4	
					Refined		< 0.01, < 0.01	< 0.01	0.03	
					sugar					
USA	1	361	223	BBCH 70	Sugarcane	28	0.31, 0.21, 0.11	0.21	–	Mäyer 2010b
(Washington,					Molasses		1.6, 1.3	1.5	7.1	CGA16393-5_50038
Louisiana)					Refined		0.02, 0.03	0.03	0.1	E18LA078495p
2008					sugar					
(85–384)	1	1779	220	BBCH 70	Sugarcane	28	2.2, 3.0, 2.2	2.5	–	
					Molasses		12.6, 9.2	10.9	4.4	
					Refined		0.22, 0.24	0.23	0.1	

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)		Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage			Individual	Mean		
					sugar					

In another study the effect of processing (laboratory scale) on residues of trinexapac-ethyl in sugarcane was investigated in one trial conducted in the USA in the 2004 growing season (Ediger 2006a, 451598). Sugarcane with incurred residues was obtained where plants were sprayed with a foliar spray at 0.345 or 1739 kg ai/ha at 28 days prior to harvest of the mature cane. Applications were made in 276–278 L/ha using ground equipment.

Bulk sugarcane samples were processed according to simulated commercial procedures into molasses and refined sugar (as described for Mäyer 2010b, CGA163935_50038, with very minor modifications).

Residues of trinexapac in sugarcane RAC and processed commodities were quantitated as trinexapac acid using Method 110.01. Acceptable concurrent recovery data were obtained for all sugarcane commodities.

Table 86 Residues of trinexapac in sugarcane and processed commodities

Country Year (Variety)	Application				Matrix	PHI days	Trinexapac (mg/kg)	Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage					
GAP, USA (sugarcane)	1	347				28			
USA	1	345	276	28 days to	Sugarcane	28	0.06		Ediger 2006a
(South Bay, Florida)				harvest	Molasses		< 0.05	0.8	451598
2004					Refined		< 0.05	0.8	VN-FR-04-5501
(CP89-2143)					sugar				
	1	1739	278	28 days to	Sugarcane	28	0.51		
				harvest	Molasses		1.4	2.7	
					Refined		< 0.05	0.1	
					sugar				

LOQ = 0.05 mg/kg

Rape seed

The effect of processing (laboratory scale) on residues of trinexapac-ethyl in rape seed was investigated in two trials carried out in Germany during the 1994 growing season (Sack 1995a, CGA163935/0416 and Sack 1995, CGA163935/0417). Rapeseed with incurred residues were obtained where plants were sprayed a foliar spray at 0.375 kg ai/ha. Application was made 84–85 days prior to the harvest of the mature rape seeds. Applications were made in 300 L water /ha.

Bulk rape seed samples were processed into processed rape seed commodity samples (crude oil, press cake and refined oil) according to simulated commercial procedures.

The harvested rape seed samples were processed into crude oil and press cake by cold pressing or hot extraction simulating industrial practice as closely as possible. Crude oil was processed further into refined oil.

Residues of trinexapac acid in rapeseed RAC and press cake were quantitated by LC/MS/MS using modified Method REM 137.02, while Method REM 137.11 was used for the quantification of

trinexapac acid residues in crude and refined oil. Acceptable concurrent recovery data for rape seed commodities were obtained for each analyte.

Table 87 Residues of trinexapac in rapeseed and processed commodities

Country Year (Variety)	Application				Matrix	PHI days	Total Trinexapac (mg/kg)	Processing Factor	Author, Study No., Trial No.
	No	g ai/ha	Water (L/ha)	Growth Stage					
GAP, Germany (oilseed rape, winter)	1	375							
Germany	1	375	300	BBCH 55	Rapeseed	85	0.64	–	Sack 1995a
(Kötschau) 1994					Crude oil (cold pressing)		< 0.02	0.03	CGA163935/0416 FR 31/94/40
(Lirajet)					Press cake (cold pressing)		0.56	0.9	
					Crude oil (hot extraction)		< 0.02	0.03	
					Press cake (hot extraction)		0.64	1.0	
					Refined oil		< 0.02	0.03	
Germany	1	375	300	BBCH 55–57	Rapeseed	84	0.33	–	Sack 1995
(Motterwitz) 1994					Crude oil (cold pressing)		< 0.02	0.06	CGA163935/0417 FR 31/94/70
(Lirajet)					Press cake (cold pressing)		0.49	1.5	
					Crude oil (hot extraction)		< 0.02	0.06	
					Press cake (hot extraction)		0.44	1.3	
					Refined oil		< 0.02	0.06	

LOQ = 0.02 mg/kg

The results of the processing factors are summarized in the table below:

Table 88 Summary of processing factors for trinexapac residues

Raw Agricultural Commodity (RAC)	Residues Determined	Processed Commodity	Application rate (g ai/ha)	Calculated Processing factors	PF Mean (*median*)
Barley	Total trinexapac	Pearled barley	129 644–648	1.5, 1.2 1.4, 0.86	1.2
		Flour	129 644–648	0.57, 0.28 0.63, 0.25	0.43
		Bran	129 644–648	2.2, 1.9 1.6, 1.8	1.9
Wheat	Total trinexapac	Aspirated grain fractions	129–130 646–649	0.8, 0.2 0.8, 0.4	0.55
		Bran	129–130 646–649	2.2, 2.1 1.6, 1.5	1.9
		Flour	129–130 646–649	0.4, 0.4 0.5, 0.4	0.43
		Middlings	129–130 646–649	11.7, 0.3 0.5, 0.6	(*0.55*)
		Shorts	129–130 646–649	0.6, 0.6 0.03, 0.6	0.46
		Germ	129–130	1.4, 0.9	1.1

Raw Agricultural Commodity (RAC)	Residues Determined	Processed Commodity	Application rate (g ai/ha)	Calculated Processing factors	PF Mean (*median*)
			646–649	0.9, 1.1	
Wheat	Trinexapac	Aspirated grain fractions	127–130 643	0.35, 0.23 0.30, 0.16	0.26
		Bran	127–130 643	2.2, 2.5 2.1, 2.4	2.3
		Flour	127–130 643	0.32, 0.31 0.31, 0.24	0.30
		Middlings	127–130 643	0.41, 0.49 0.45, 0.43	0.46
		Shorts	127–130 643	0.45, 1.4 0.68, 1.1	0.91
		Germ	127–130 643	0.29, 1.0 0.85, 1.1	0.81
Sugarcane	Total trinexapac	Molasses	361–382 1779–1856	5.3, 7.1 6.4, 4.4	5.8
		Refined sugar	361–382 1779–1856	0.2, 0.1 0.2, 0.1	0.15
Sugarcane	Trinexapac	Molasses	345 1739	0.8 2.7	1.8
		Refined sugar	345 1739	0.8 0.1	0.45
Rapeseed	Trinexapac	Crude oil (cold pressed)	375	0.03, 0.06	0.05
		Press cake (cold pressed)	375	0.9, 1.5	1.2
		Crude oil (hot extraction)	375	0.03, 0.06	0.05
		Press cake (hot extraction)	375	1.0, 1.3	1.2
		Refined oil	375	0.03, 0.06	0.05

RESIDUES IN ANIMAL COMMODITIES

Dairy cattle transfer study (Sack 2000, CGA179500/0030)

A feeding study with trinexapac acid was conducted in lactating cows (ten *Bos taurus Holstein* dairy cattle ranging from 2–6 years of age divided into three dose groups of three cows with one control animal, 514–720 kg during dosing).

The animals received nominal doses of trinexapac acid equivalent to 0, 2 (40 mg trinexapac acid, 1×), 6 (120 mg trinexapac acid, 3×) and 20 ppm in the feed (400 mg trinexapac acid, 10×) respectively (actual doses 40.4, 121.2 and 404.0 mg respectively). The doses were administered orally *via* gelatine capsules, for a period of 29–30 consecutive days.

Milk samples from each animal were collected twice daily, at approximately 6 am and pm, and combined as one pooled sample. Milk specimens were collected on day 0 (pre-dose) and days 1, 2, 3, 5, 8, 12, 15, 19, 22 and 28 of the feeding period. The cows were sacrificed within 20–24 hours after the final dosing (one cow from each treatment group on day 29 and two cows from each group, except the control group, on day 30). Besides blood, the following tissues were collected; liver, kidney, round muscle, tenderloin muscle, diaphragm, perirenal and omental fat.

Milk, tissues and blood samples were analysed for residues of trinexapac acid using Method REM 137.12.

The residues of trinexapac acid found in milk during the treatment periods are summarized in Table 89.

Table 89 Residues of trinexapac acid in milk

Study day	Group mean (and maximum individual) residues of trinexapac acid (mg/kg)			
	0 ppm	2 ppm	6 ppm	20 ppm
0	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	< 0.005 (< 0.005)
1	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.005 (0.005)
2	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.006 (0.006)
3	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.0 05 (0.005)
5	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.007 (0.011[a])
8	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.005 (0.005)
12	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.005 (0.005)
15	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.005 (0.006)
19	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.005 (0.006[b])
22	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.005 (0.005)
28	< 0.005	< 0.005 (< 0.005)	< 0.005 (< 0.005)	0.005 (0.005)

< 0.005 denotes less than the LOQ

[a] Average of three analyses (0.010, 0.011, 0.011 mg/kg)

[b] Average of two analyses (0.0061 and 0.005 mg/kg)

No quantifiable residues of trinexapac acid were found in the milk of the 0, 2 and 6 ppm dose groups. Residues in the 20 ppm dose group ranged from < 0.005–0.011 mg/kg. Residues reached a plateau after one day.

Results of the tissue and blood analysis are summarized in Table 90.

Table 90 Residues of trinexapac acid in tissues

Treatment Group	Group mean (and maximum individual) residues in tissue for trinexapac acid (mg/kg)							
	Muscle Tenderloin	Muscle Round	Diaphragm	Liver	Kidney	Perirenal Fat	Omental Fat	Blood
0 ppm	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.010
2 ppm	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	0.03 (0.03)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	0.018 (0.023)
6 ppm	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	0.04 (0.05)	< 0.02 (< 0.02)	< 0.02 (< 0.02)	0.031 (0.036)
20 ppm	< 0.02 (< 0.02)	< 0.02 (< 0.02)	< 0.02 (0.02)	0.03 (0.03)	0.17 (0.29)	< 0.02 (0.02)	< 0.02 (0.02)	0.13 (0.17)

< 0.02 denotes less than the LOQ

No quantifiable residues of trinexapac acid were found in the tissues of the control cow. In the lowest and mid-range dose groups (2 and 6 ppm), no quantifiable residues were detected in muscle, liver and fat, although residues slightly above LOQ were observed in kidney (means 0.03 and 0.04 mg/kg respectively).

In the highest dose group (20 ppm), quantifiable residues were detected in one diaphragm sample (0.02 mg/kg), two liver samples (both 0.03 mg/kg) and one perirenal fat sample (0.02 mg/kg). Quantifiable residues were observed in all kidney samples (mean 0.17 mg/kg).

Quantifiable residues were observed in all blood samples from all dose groups (means 0.018, 0.031 and 0.13 mg/kg respectively).

Laying hen transfer study (Simmons 2010, CGA179500_50008)

A feeding study with trinexapac acid was conducted in laying hens (forty *Hyline Browns*, approximately 37 weeks old upon arrival, divided into 3 dose groups and a control group each of ten hens, 1.80–1.92 kg from 7 days before dosing until the end of dosing). The target doses of trinexapac acid were equivalent to 3.3, 9.9 and 33.0 ppm in the feed. Mean doses in the feed were determined to be 3.7, 10 and 37 ppm.

The doses were administered orally *via* gelatine capsules, for a period of 28 days. Ten animals were allocated per dose group divided into three subgroups of 3–4 birds. Eggs from each hen were collected on day 0 (pre-dose) and days 1, 3, 7, 10, 14, 17, 21, 24 and 28 of the feeding period. Egg production by group was at or above 89% with two exceptions (the control group on week 4 at 74% and the high dose group on week 4 at 75%). The animals were sacrificed within 6 hours after the last administration of the treated feed. Tissues collected were skin plus attached fat and peritoneal fat, liver, kidney and breast and thigh muscle.

Liver, kidney, breast and thigh muscle, skin with attached fat and peritoneal fat and eggs were analysed for residues of trinexapac acid using Method REM 137.14 with some modifications.

Residues of trinexapac acid found in eggs during the treatment period are summarized in Table 91. Residues in control samples were all < LOQ (< 0.01 mg/kg).

Table 91 Residues of trinexapac acid in eggs

Study day	Group mean (and maximum individual) residues of trinexapac acid (mg/kg)			
	0 ppm	3.7 ppm	10 ppm	37 ppm
0	< 0.01	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
1	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
3	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
7	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
10	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
14	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
17	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
21	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
24	–	NA	< 0.01 (< 0.01)	< 0.01 (< 0.01)
28	–	NA	< 0.01 (< 0.01)	0.013 (0.013)

< 0.01 denotes less than the LOQ

NA = Samples were not analysed, because residues of higher dose groups are < LOQ

No quantifiable residues were detected in any dose group apart from in the 37 ppm dose group at day 28 (mean residues in groups A (three hens), B (three hens) and C (four hens) were all 0.013 mg/kg.

Residues of trinexapac acid in tissues are summarized below in Table 92. Residues in control samples were all < LOQ.

Table 92 Residues of trinexapac acid in tissues

Treatment Group	Group mean (and maximum individual) residues in tissue for trinexapac acid (mg/kg)			
	Fat	Liver	Kidney	Muscle
0 ppm	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
3.7 ppm	NA	NA	0.06 (0.08)	NA
10 ppm	< 0.01 (< 0.01)	< 0.01 (< 0.01)	0.04 (0.05)	NA
37 ppm	0.03 (0.03)	0.02 (0.02)	0.45 (0.54)	< 0.01 (< 0.01)

< 0.01 denotes less than the LOQ

NA = Samples were not analysed, because residues of higher dose groups are < LOQ

No residues of trinexapac acid above LOQ (0.01 mg/kg) were detected in any muscle (breast and thigh muscle) sample from any treatment group. No residues of trinexapac acid above LOQ (0.01 mg/kg) were detected in any liver sample from any treatment group apart from the highest dose group (mean 0.02 mg/kg). No residues of trinexapac acid above LOQ (0.01 mg/kg) were detected in any fat sample from any treatment group apart from the highest dose group (mean 0.03 mg/kg). Mean residues of trinexapac acid in kidney were 0.06, 0.04 and 0.45 mg/kg in the lowest to highest dose groups respectively.

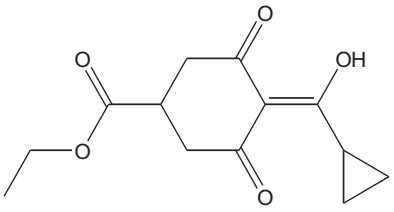
APPRAISAL

At the Forty-fourth Session of the CCPR (2012), trinexapac-ethyl was scheduled for evaluation as a new compound by 2013 JMPR.

Trinexapac-ethyl is a synthetic plant growth regulator that is derived from cyclohexanecarboxylate. It is applied as a foliar spray, post-emergence and is approved for use on cereal crops such as barley, durum wheat, oats, rye, triticale and wheat, oilseed rape and sugarcane as well as on grassland pastures.

The manufacturer supplied information on identity, metabolism, methods of residue analysis, freezer storage stability, registered use patterns, supervised residue trials, fates of residues in processing and farm animal feeding studies.

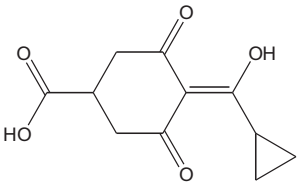
The IUPAC name is 4-(cyclopropyl-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester.



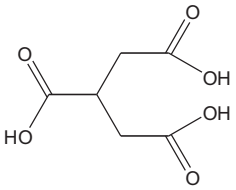
Trinexapac-ethyl

The 2013 JMPR established an ADI for trinexapac-ethyl of 0–0.3 mg/kg bw trinexapac acid equivalents.

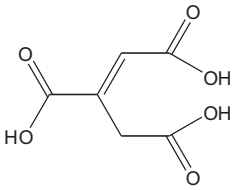
The structures of the key metabolites discussed are shown below:



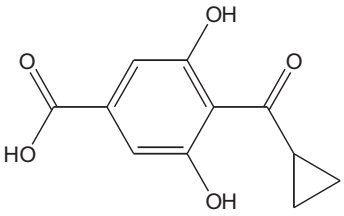
CGA 179500
Trinexapac acid



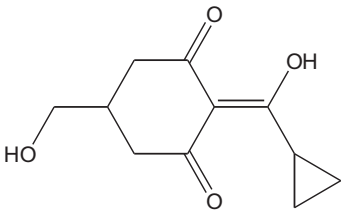
CGA 275537
Tricarballylic acid



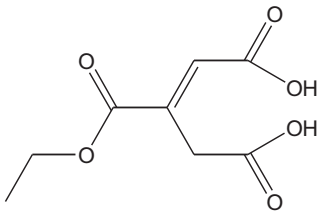
CGA 312753
Trans aconitic acid



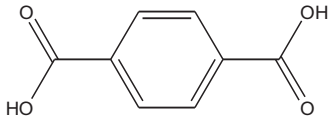
CGA 329773



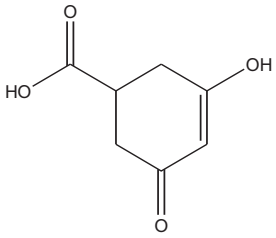
CGA 351210



CGA 312753 mono-ethyl ester



Terephthalic acid



CGA113745

Animal metabolism

The Meeting received animal metabolism studies with trinexapac-ethyl in rats, hens and goats.

Rats

Evaluation of the metabolism studies in rats was carried out by the WHO Core Assessment Group.

Trinexapac-ethyl undergoes limited metabolism in the rat, involving predominantly ester hydrolysis of trinexapac-ethyl to trinexapac acid. The predominant urinary metabolite was trinexapac acid (up to 100% of total urinary radioactivity), with low levels of a conjugated derivative of trinexapac acid detected only in the urine of bile-duct cannulated rats (6.3% of the administered dose). In faeces, the parent compound accounted for 5–22% of total faecal radioactivity (1–2.5% of the administered dose), with the balance comprising trinexapac acid. Bile contained mainly a conjugated derivative of trinexapac acid (2.9% of the administered dose), with low levels of the parent compound also detected (0.2% of the administered dose).

Goats

[1, 2-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered via capsule to lactating goats at 7.2 ppm or 694 ppm in the feed (or 0.20 or 19.9 mg/kg bw/day respectively) for 4 consecutive days. Milk was sampled twice daily, in the morning and afternoon. Animals were sacrificed approximately 4 hours after the last dose.

The low dose goat eliminated 16% and 50% via faeces and urine of the total administered dose respectively at 76 hours in the study, while the corresponding values of the high dosed goat were 19% and 62%. In milk, 0.02% of the administered dose was recovered at both dose levels. A plateau was reached after 2 days (low dose-0.002 mg/kg equiv (am) and 0.007 mg/kg equiv. (pm)) or 3 days (high dose-0.22 mg/kg equiv (am) and 0.83 mg/kg equiv. (pm)) after administration.

The tissue residues of the low dosed goat were 0.035–0.043 mg/kg equiv. in muscles, 0.017–0.094 mg/kg equiv. in fat, 0.25 mg/kg equiv. in liver and 0.50 mg/kg equiv. in kidney. In the high dosed goat the residues were correspondingly higher, i.e., 1.90–2.49 mg/kg equiv. in muscles, 1.20–1.55 mg/kg equiv. in fat, 12.1 mg/kg equiv. in liver and 41.9 mg/kg equiv. in kidney.

Residues in muscle and kidney were mostly trinexapac acid (81–90% TRR). In milk, trinexapac acid accounted for 46–76% TRR. In liver and fat trinexapac acid was 31–67% TRR, although for fat this represented 87–96% of the extracted radioactivity. For liver this represented only 34–47% of the extracted radioactivity.

In another study [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered by gelatine capsule to two lactating goats at 100 ppm in the feed (or 3.1 mg/kg bw/day) for 4 consecutive days. Milk was sampled twice daily. Animals were sacrificed approximately 6 hours after the last dose.

The goats had eliminated 3% and 81% via faeces and urine of the total administered dose respectively at 78 hours in the study while 0.05% of the administered dose was recovered in milk. Residues in the Day 2 pm sample of milk were 0.076 mg/kg equiv.

The tissue residues were 0.28 mg/kg equiv. in muscles, 0.11 mg/kg equiv. in fat, 0.80 mg/kg equiv. in liver and 5.90 mg/kg equiv. in kidney.

No parent was observed in tissues or milk. Residues in liver, kidney, muscle and fat were mostly trinexapac acid (CGA179500) (66–97% TRR). In milk, trinexapac acid accounted for 85% TRR. In liver, kidney and fat the metabolite CGA113745 accounted for 6–16% TRR. This metabolite was not detected in either muscle or milk.

Hens

[1, 2-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered daily to laying hens using gelatine capsules at a low dose level (2 hens, 0.4 mg/kg body weight equivalent to 3.8 ppm in feed) or a high dose level (4 hens, 20.3 mg/kg body weight equivalent to 180 ppm in feed) for 4 consecutive days. Eggs were collected throughout the day and in the morning before subsequent administration. Animals were sacrificed approximately 4 hours after the last dose.

Over the period of the experiment (76 hours), 85–89% (high and low doses respectively) of the total administered dose was eliminated in excreta. Transfer of radioactivity into eggs accounted for only 0.01% and 0.02% of the total administered dose for the low and high dosed hens, respectively. A plateau was reached at Day 2 of the dosing period. Concentrations of radioactivity in egg yolk were generally less than in egg whites. Concentrations of radioactivity in egg white and egg yolk in the low-dosed hens did not exceed 0.007 and 0.002 mg/kg equiv., respectively. In the high dosed hens the corresponding values were 0.33 and 0.055 mg/kg equiv.

Mean radioactive residues in tissues of the low-dosed hens were 0.002 mg/kg equiv. in lean meat (0.12 mg/kg equiv. high dose), 0.011 mg/kg equiv. in skin (including attached fat) (0.37 mg/kg equiv.), 0.003 mg/kg equiv. in peritoneal fat (0.18 mg/kg equiv.), 0.013 mg/kg equiv. in liver (0.60 mg/kg equiv.) and 0.043 mg/kg in kidney (1.77 mg/kg equiv.).

Radioactive residues in muscle (lean meat), liver, kidney and fat samples of low and high-dosed hens were predominantly trinexapac acid (44–84% TRR). In skin including attached fat in which the extractability was low, trinexapac acid was again the major metabolite accounting for 64–80% of the extracted radioactivity.

In the yolk the major metabolite was trinexapac acid accounting for 57–76% of the extracted radioactivity (0.0003–0.011 mg/kg equiv.), while parent trinexapac-ethyl was present at only 10–25% (< 0.0001–0.005 mg/kg equiv.). In egg white the major residue was parent accounting for 64–78% of the extractable TRR (0.0017–0.124 mg/kg equiv.). Trinexapac acid was either not detected (high dose) or accounted for only 13% (0.0003 mg/kg equiv.) of the extractable TRR (low dose).

In another hen metabolism study [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was orally administered daily to 5 laying hens by gelatine capsule at 8.1–10.4 ppm in feed for 10 consecutive days. Eggs were collected at 24 hour intervals throughout the morning before subsequent administration. Animals were sacrificed approximately 22 hours after the last dose.

Of the administered dose 89% was recovered in the excreta. Residue levels in the various tissue samples were all < 0.01 mg/kg equiv. Residue levels in egg yolk were very low, ranging from < 0.003–0.009 mg/kg equiv. Egg white residues ranged from 0.005–0.031 mg/kg equiv. The maximum residue levels of 0.009 and 0.031 mg/kg equiv. were reached by Day 8 of the dosing period. Overall retention of the radioactivity in eggs was < 0.1% of the administered dose.

TRR values for edible tissues and egg yolks were below 0.01 mg/kg equiv., so no further analysis was undertaken. The major metabolites identified in the organosoluble residue of the composite egg white sample were parent trinexapac-ethyl and trinexapac acid which accounted for 31% TRR and 20% TRR respectively.

Summary of animal metabolism

The metabolic pathways of trinexapac-ethyl observed in rats, goats and poultry are similar. Animal metabolism studies showed that parent was rapidly absorbed and almost completely hydrolysed to

trinexapac acid before excretion. No accumulation of residues was observed in any organ, tissue or animal commodity.

Plant metabolism

Paddy rice, spring wheat, spring rape and grass (foliar treatment) metabolism studies were carried out with [1, 2-^{14}C-cyclohexyl] trinexapac-ethyl or [1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl.

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was applied by foliar methods to rice at rates of 40 and 160 g ai/ha. TRRs in samples taken at maturity (60 days after treatment at 160 g ai/ha) were 1.58 mg/kg equiv. for straw, 2.22 mg/kg equiv. for husks and 1.07 mg/kg equiv. for grain. Trinexapac acid was the only identified compound present in rice grain at greater than 10% TRR (36% TRR) and was the major identified component of rice husks (30% TRR). It was present in rice straw at 9% TRR. Tricarballylic acid (CGA 275537) was present at 14% TRR in straw. Some parent compound was observed in grain, husks and straw (all < 10% TRR). Metabolite CGA 329773 was a minor metabolite in grain, husks and straw (< 3% TRR).

[1, 2-^{14}C-cyclohexyl] trinexapac-ethyl was applied to spring wheat by foliar spraying at a rate of 150 g ai/ha. Trinexapac acid was the only identified component in wheat grain (24% TRR) and was the only identified component of both wheat husks and wheat straw observed at > 10% of the TRR (15 and 21% TRR respectively).

A stem-injection experiment was conducted on six week old wheat plants grown under greenhouse conditions. Trinexapac acid was the major identified component of wheat grain (27.9% TRR, 0.129 mg/kg equiv.) and was the only identified component of both wheat husks and wheat straw observed at > 10% of the TRR (17% (0.073 mg/kg equiv.) and 13% TRR (0.069 mg/kg equiv.) respectively). Metabolites CGA329773 and CGA275537 were present in grain at 11% (0.050 mg/kg equiv.) and 3% TRR (0.014 mg/kg equiv.) respectively and in straw at 3 and 2% TRR respectively.

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was applied to spring rape under greenhouse conditions in plastic containers filled with soil. Trinexapac acid (free and conjugated) was the major metabolite observed in rape meal (31% TRR, 0.647 mg/kg equiv.), rape whole seeds (30% TRR, 0.431 mg/kg equiv.), pods (19% TRR, 1.06 mg/kg equiv.) and stalks (10% TRR, 0.304 mg/kg equiv.) and was also observed in oil (4% TRR, 0.004 mg/kg equiv.). Metabolite CGA 351210 was observed in oil at 16% TRR but at only 0.02 mg/kg equiv. This metabolite, only observed in rape, accounted for 16–28% TRR in pods and stalks (0.918 mg/kg equiv. and 0.869 mg/kg equiv. respectively).

[1, 2, 6-^{14}C-cyclohexyl] trinexapac-ethyl was applied by foliar methods to tall fescue grass at 560 g ai/ha. TRRs of 5.45 mg/kg equiv., 7.13 mg/kg equiv. and 4.78 mg/kg equiv. were observed for seeds, seed screenings and for straw respectively. TRRs of 2.03 mg/kg equiv. and 0.054 mg/kg equiv. were observed for 22 day forage and 105 day forage respectively. In grass, trinexapac acid was the major identified component of 22 day forage (16% TRR), straw (22% TRR) and regrowth forage (10% TRR) and was also a major component of seeds (15% TRR) and seed screenings (13% TRR). Also observed in all matrices were CGA 275537 (9-17% TRR) and terephthalic acid (7–12% TRR) the latter metabolite only observed in grass.

Summary of plant metabolism

Parent compound was completely degraded and was not detectable (< 0.001/< 0.002 mg/kg) in any plant part of wheat, rapeseed and grass at harvest time. In rice only traces (0.001 and 0.003 mg/kg) were observed in straw and husks respectively.

Trinexapac acid was therefore the major metabolite which was present in all species and all plant parts at harvest. Sugar conjugates of trinexapac acid were hydrolysed in some crop parts to yield free acid which, in some cases, significantly increased the levels of trinexapac acid recovered. Trinexapac acid sugar conjugates accounted for an increase in trinexapac acid of 11.6% TRR in rice grain and 10% and 14% TRR in grass forage and straw, respectively.

Rotational Crops

Studies of residues in confined rotational crops have been submitted in which soil was treated at 350 g ai/ha, followed by soil aging at 30, 120 and 270 days. Representative succeeding crops of lettuce (leafy vegetable), radish (root vegetable) and wheat (cereal grain) were planted at the above intervals to determine whether trinexapac-ethyl residues or degradates appear in follow crops.

The uptake of residues by the rotational crops lettuce, radish and wheat planted or sown after several intervals after application of trinexapac-ethyl to bare ground was very low. No accumulation was observed. Residues of trinexapac-ethyl in the rotational crop RACs were below the LOQ (< 0.001 mg/kg). The residues of trinexapac acid and CGA 312753 (trans aconitic acid) were very close to or below the LOQ (< 0.001–0.002 mg/kg). The very limited uptake of radioactive material in succeeding crops clearly indicates the lack of systemic behaviour of trinexapac-ethyl. It was concluded that residues in rotational crops are negligible.

The Meeting received information on field rotational crop studies for trinexapac-ethyl which were conducted in both the USA and Switzerland.

In a study conducted in the USA in the 2004–2005 growing season, a 250 g/L EC formulation of trinexapac-ethyl was applied once as a broadcast spray to wheat at 203 g ai/ha. Radish, wheat and spinach were planted after three different plant back intervals (14, 30 and 45 days). The results showed that rotational crops sown 14, 30 and 45 days after application of trinexapac-ethyl to the target crop, are very unlikely to contain residues of trinexapac-ethyl as its main metabolite trinexapac acid, above the LOQ of 0.05 mg/kg.

A trial was conducted in Switzerland during 1989 to investigate residues of trinexapac-ethyl in succeeding crops, grown in soil previously treated with [14C]cyclohexyl trinexapac-ethyl. A 250 g/L EC formulation of trinexapac-ethyl was applied once to bare soil at a rate of 150 g ai/ha. Treated plots were planted after four different time intervals (69, 119, 299 and 338 days after treatment). The crops investigated were lettuce, winter wheat, sugar beet and maize (corn).

The uptake of residues by the rotational crops lettuce, winter wheat, sugar beet and maize planted or sown after several intervals after application of trinexapac-ethyl to bare ground was therefore very low. No accumulation was observed. Residues of trinexapac-ethyl in the rotational crop RACs were below the LOQ (< 0.001 mg/kg) so no characterization was possible. The very limited uptake of radioactive material in succeeding crops clearly indicates the lack of systemic behaviour of trinexapac-ethyl. It was concluded that the residues situation in rotational crops is negligible.

The confined and field rotational crop studies suggest that residues of trinexapac acid are unlikely to occur in succeeding crops.

Methods of analysis

The Meeting received information on analytical methods suitable for the determination of residues of trinexapac acid in plant matrices and animal matrices. The methodology involves quantification of free trinexapac acid (plant and animal matrices) or quantification of both free and conjugated trinexapac acid with the inclusion of an acid hydrolysis step (plant matrices). The methods used are based on HPLC-MS/MS (LOQs of 0.01 or 0.05 mg/kg for trinexapac acid in plant matrices and 0.02 mg/kg for animal matrices (0.01 mg/kg for milk)) and HPLC-UV (LOQs of 0.02 mg/kg for trinexapac acid in plant matrices or 0.01 mg/kg in animal matrices (0.005 mg/kg for milk)).

Details of HPLC-UV methods for determining residues of parent trinexapac-ethyl have also been submitted (LOQs of 0.02 or 0.04 mg/kg).

Stability of pesticide residues in stored analytical samples

The Meeting received information on the freezer storage stability of trinexapac acid in plant and animal commodities.

Freezer storage stability studies indicate that trinexapac acid is stable for at least 24 months in wheat grain and rapeseed and up to 24 months in wheat straw and for at least 12 months in wheat

processed fractions. The stability of trinexapac acid in extracts of animal matrices was tested in the dairy cattle and laying hen feeding studies. No significant degradation of residues of trinexapac acid was observed in cattle matrices (83–121 days) or in poultry matrices (31–82 days).

The periods of storage stability studies cover the sample storage intervals of residue trials.

Definition of the residue

Animals

In the goat metabolism studies, no parent was observed in tissues and milk. Trinexapac acid accounted for 81–97% of the total residues in muscle and kidney, 31–84% of the TRRs in liver and fat and 46–85% of the TRRs in milk. In one study the metabolite CGA 113745 accounted for 6–16% TRR in liver, kidney and fat, but was not detected in either muscle or milk.

In the hen metabolism studies residues in muscle (lean meat), liver, kidney and fat samples of low and high-dosed hens were dominated by trinexapac acid (44–84% of TRR). Only in the skin (with subcutaneous fat) was the major portion of the total radioactivity (70–86%) not extractable. However trinexapac acid was the dominant metabolite in the extract (64–80% of the extractable radioactivity). The major metabolites identified in the organosoluble residue of egg whites were parent trinexapac-ethyl and trinexapac acid which accounted for 31% TRR and 20% TRR respectively.

The observed metabolic pathway of trinexapac-ethyl in livestock is comparable to that observed for the rat in which trinexapac acid is the major and only residue component of significance.

The ratio of trinexapac acid residues in muscle and fat observed in the livestock metabolism and feeding studies support the conclusion that trinexapac is not fat soluble. There is no evidence to suggest that there is significant potential for bioaccumulation in fat tissues.

It is considered that a residue definition of "Trinexapac (acid)" is appropriate for commodities of animal origin for compliance with MRLs (enforcement) and for risk assessment.

Plants

In the rice study trinexapac acid was the major identified component in rice grain (36% TRR) and rice husks (30% TRR). In the spring wheat study, trinexapac acid was the major identified component of wheat grain (28% TRR) and was the only identified component of both wheat husks and wheat straw observed at > 10% of the TRR. It was the major metabolite observed in rape meal, rape whole seeds and pods (19–31% TRR) and was also observed in oil and stalks (4–10% TRR). In grass, trinexapac acid was the major identified component of 22 day forage, straw, straw and regrowth forage (10–22% TRR) and was also a major component of seed screenings and seeds (13–15% TRR). Sugar conjugates of trinexapac acid were hydrolysed in some crop parts to yield free acid.

Trinexapac acid was present in all species and all plants parts at harvest and was generally the main component of the TRR. A definition of trinexapac acid is therefore considered suitable as the residue definition for compliance. Since conjugates of trinexapac acid were sometimes significant in edible commodities and are supposed to be of similar toxicity, the Meeting decided to include conjugates of trinexapac acid in the residue definition for risk to the acid assessment. A suitable method is available for determining residues of free and conjugated trinexapac acid.

It is therefore considered that a residue definition of "Trinexapac (acid)" is appropriate for plant commodities for compliance with MRLs (enforcement). It is considered that a residue definition of "Trinexapac and its conjugates, expressed as trinexapac acid" is appropriate for plant commodities for risk assessment.

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

Definition of the residue (for estimation of dietary intake for plant commodities): Trinexapac and its conjugates, expressed as trinexapac acid

The residue is considered not fat soluble.

Results of supervised residue trials on crops

Supervised trials were available for the use of trinexapac on barley, wheat, oilseed rape and sugarcane.

In some studies (barley, wheat and sugarcane) residues have been determined as total trinexapac (includes free trinexapac acid and conjugates of trinexapac acid) while in other studies (wheat, sugarcane and rape seed) residues have been determined as trinexapac (free trinexapac acid only). In both cases trinexapac means trinexapac acid. For dietary intake assessment (risk assessment) residues expressed as total trinexapac acid, where available, have been considered. For maximum residue level estimation (compliance) residues expressed as trinexapac acid, where available, have been considered.

Product labels were available from Belgium, France, Germany and the United States of America.

Cereals - wheat (free trinexapac acid)

Residue trials were conducted in wheat in the USA according to the critical GAP in the USA (1 application at 0.123 kg ai/ha, 45-day PHI).

For the estimation of maximum residue levels the ranked order of residues of trinexapac acid in wheat grain from supervised trials according to the GAP in the USA was 0.10, 0.25, 0.32, 0.34, 0.35, 0.46, 0.49, 0.55, 0.55, 0.57, 0.77, 0.88, 0.91, 0.98, 0.99, 1.05, 1.35 and 1.95 mg/kg.

The Meeting estimated a maximum residue level for trinexapac acid in wheat of 3 mg/kg.

The Meeting recognized that wheat (spring wheat, winter wheat and durum wheat) and triticale, barley and oats have similar GAPs and normally show comparable residues after early treatment. As application was before flowering, the Meeting decided to extrapolate the MRL estimated for wheat grain to barley, oats and triticale.

Cereals - barley

Residue trials were conducted in barley in the USA according to the GAP in the USA (1 application at 0.123 kg ai/ha, 45-day PHI).

For dietary intake purposes the ranked order of total residues of trinexapac acid in barley grain from supervised trials according to the GAP in the USA was 0.03, 0.08, 0.44, 0.50, 0.52, 0.53, 0.60, 0.72, 0.76, 0.83, 1.0 and 1.2 mg/kg.

Cereals - wheat (total residues of trinexapac acid)

Residue trials were conducted in wheat in the USA according to the GAP in the USA (1 application at 0.123 kg ai/ha, 45-day PHI).

For dietary intake purposes the ranked order of total residues of trinexapac acid in wheat grain from supervised trials according to the GAP in the USA was 0.07, 0.15, 0.27, 0.31, 0.32, 0.40, 0.45, 0.47, 0.53, 0.77, 0.78, 0.82, 0.85, 0.99, 1.01, 1.14, 1.64 and 3.32 mg/kg.

The Meeting noted that the USA GAP is the same for barley and wheat. The Meeting noted that the populations of residues data for barley and wheat matching USA GAP resulted in similar distributions of residues for barley and wheat (e.g. medians do not differ by more than 5×). Given the similarity of the datasets (confirmed by the Mann-Whitney U test), the Meeting decided to combine the datasets for barley and wheat to give a larger dataset for estimation of dietary parameters for the purposes of determining an STMR residue level for barley grain and wheat grain.

For dietary intake purposes residues were 0.03, 0.07, 0.08, 0.15, 0.27, 0.31, 0.32, 0.40, 0.44, 0.45, 0.47, 0.50, 0.52, 0.53, 0.53, 0.60, 0.72, 0.76, 0.77, 0.78, 0.82, 0.83, 0.85, 0.99, 1.0, 1.01, 1.14, 1.2, 1.64 and 3.32 mg/kg.

The Meeting estimated an STMR of 0.57 mg/kg.

The Meeting recognized that wheat (spring wheat, winter wheat and durum wheat), triticale, barley and oats have similar GAPs and normally show comparable residues after early treatment. As application was before flowering, the Meeting decided to extrapolate the STMR estimated for barley grain and wheat grain to oats and triticale.

Grasses for sugar or syrup production - sugarcane (free trinexapac acid)

Residue trials were conducted in sugarcane in the USA according to the critical GAP in the USA (1 application at 0.347 kg ai/ha, 28-day PHI).

For the estimation of maximum residue levels the ranked order of residues of trinexapac acid in sugar cane from supervised trials according to the GAP in the USA was < 0.05, 0.06, 0.09, 0.12, 0.22, 0.23 and 0.25 mg/kg.

The Meeting estimated a maximum residue level for trinexapac acid in sugarcane of 0.5 mg/kg.

Grasses for sugar or syrup production—sugarcane (total residues of trinexapac acid)

Residue trials were conducted in sugarcane in the USA according to the critical GAP in the USA (1 application at 0.347 kg ai/ha, 28-day PHI).

For dietary intake purposes the ranked order of total residues of trinexapac acid in sugar cane from supervised trials according to the GAP in the USA was < 0.01, 0.04, 0.06, 0.08, 0.17 and 0.42 mg/kg.

The Meeting estimated an STMR value for trinexapac acid in sugarcane of 0.07 mg/kg.

Oilseeds - Rape

As conjugates of trinexapac acid were not significant in the submitted rape metabolism studies, residue levels from the submitted rape seed studies, which were determined as trinexapac only, have been considered suitable for estimation of a maximum residue level and also for estimation of dietary intake parameters.

Residue trials were conducted in winter rape (canola) in Germany according to the critical GAP in Germany for winter rape (1 application at 0.375 kg ai/ha).

For the estimation of maximum residue levels and for dietary purposes the ranked order of residues of trinexapac acid in winter rape seed from supervised trials according to GAP was 0.04, 0.10, 0.10, 0.13, 0.15, 0.15, 0.16, 0.24, 0.24, 0.26, 0.29, 0.31, 0.64, 0.64, 0.90 and 1.0 mg/kg.

The Meeting estimated maximum residue level and STMR values for trinexapac acid in rape seed of 1.5 and 0.24 mg/kg respectively.

Animal feeds

The Meeting received supervised trials data for barley hay and straw, wheat forage, hay and straw and rape seed forage.

Moisture content percentages for animal feeds have not been determined. The values from the FAO Manual on the Submission and Evaluation of Pesticides Residues Data for the feeds, have been used to convert wet weight or ‘as received’ residues values to dry weight residues values.

Forage - Wheat (residues of free and conjugated trinexapac acid)

Wheat forage was collected in the trials carried out in the USA according to GAP in the USA (1 application at 0.123 kg ai/ha).

For the calculation of the livestock animal dietary burden the ranked order of total residues in wheat forage samples collected 30 days after application (wet weight) at the GAP application rate was

0.02, 0.03, 0.04, 0.06, 0.07, 0.08, 0.08, 0.09, 0.10, 0.10, 0.12, 0.17, 0.17, 0.22, 0.23, 0.33, 0.38 and 0.94 mg/kg.

For the calculation of the livestock animal dietary burden the ranked order of total residues in wheat forage samples collected 30 days after application (dry weight) at the GAP application rate was 0.08, 0.12, 0.16, 0.24, 0.28, 0.32, 0.32, 0.36, 0.40, 0.40, 0.48, 0.68, 0.68, 0.88, 0.92, 1.32, 1.52 and 3.76 mg/kg.

The Meeting estimated median and highest residue values for trinexapac acid in wheat forage (dry weight) of 0.40 and 3.76 mg/kg respectively.

The Meeting recognized that wheat (spring wheat, winter wheat and durum wheat) and triticale, barley and oats have similar GAPs and normally show comparable residues after early treatment. The Meeting decided to extrapolate the dietary parameters estimated for wheat forage to barley, oats and triticale.

Hay and Straw - Barley and Wheat

Data for wheat hay and straw were collected in the trials carried out in the USA which approximate USA GAP (1 application at 0.123 kg ai/ha).

For the estimation of maximum residue levels the ranked order of residues in wheat hay samples collected 30 days after application (wet weight) at the GAP application rate was < 0.05, < 0.05, < 0.05, 0.05, 0.06, 0.07, 0.07, 0.08, 0.08, 0.09, 0.11, 0.11, 0.13, 0.15, 0.18, 0.19, 0.19 and 0.75 mg/kg.

The ranked order of residues in wheat hay samples converted to dry weight basis at the GAP application rate was < 0.06, < 0.06, < 0.06, 0.06, 0.07, 0.08, 0.08, 0.09, 0.09, 0.10, 0.13, 0.13, 0.15, 0.17, 0.20, 0.22, 0.22 and 0.85 mg/kg.

For the estimation of maximum residue levels the ranked order of residues in wheat straw samples collected 45 days after application (wet weight) at the GAP application rate was < 0.05, < 0.05, < 0.05, < 0.05, < 0.05, < 0.05, < 0.05, < 0.05, < 0.05, < 0.05, 0.06, 0.06, 0.07, 0.07, 0.08, 0.10, 0.11 and 0.12 mg/kg.

The ranked order of residues in wheat straw samples (dry weight) at the GAP application rate was < 0.06, < 0.06, < 0.06, < 0.06, < 0.06, < 0.06, < 0.06, < 0.06, < 0.06, < 0.06, 0.07, 0.07, 0.08, 0.08, 0.09, 0.11, 0.13 and 0.14 mg/kg.

The Meeting used the wheat hay data for the free trinexapac acid to estimate a maximum residue level in wheat straw and fodder (dry) of 0.9 mg/kg. The Meeting recognized that wheat (spring wheat, winter wheat and durum wheat), triticale, barley and oats have similar GAPs and normally show comparable residues after early treatment. It was therefore decided to apply the maximum residue level recommended for trinexapac acid on wheat straw and fodder (dry) to barley, oats and triticale.

For the calculation of the livestock animal dietary burden the ranked order of total residues in barley hay samples collected 30 days after application (wet weight) at the GAP application rate was < 0.01, 0.03, 0.05, 0.10, 0.13, 0.15, 0.17, 0.18, 0.25, 0.33, 0.40 and 0.48 mg/kg.

The ranked order of total residues in barley hay samples converted to a dry weight basis at the GAP application rate was < 0.011, 0.03, 0.06, 0.11, 0.15, 0.17, 0.19, 0.20, 0.28, 0.38, 0.45 and 0.55 mg/kg.

For the calculation of the livestock animal dietary burden the ranked order of total residues in barley straw samples collected 45 days after application (wet weight) at the GAP application rate was < 0.01, 0.02, 0.07, 0.08, 0.08, 0.09, 0.11, 0.12, 0.14, 0.17, 0.20 and 0.24 mg/kg.

The ranked order of total residues in barley straw samples converted to a dry weight basis at the GAP application rate was 0.01, 0.02, 0.08, 0.09, 0.09, 0.10, 0.12, 0.13, 0.16, 0.19, 0.22 and 0.27 mg/kg.

For the calculation of the livestock animal dietary burden the ranked order of total residues in wheat hay samples collected 30 days after application (wet weight) at the GAP application rate was 0.03, 0.04, 0.04, 0.06, 0.09, 0.11, 0.11, 0.14, 0.17, 0.19, 0.24, 0.30, 0.31, 0.41, 0.50, 0.59, 0.78 and 1.18 mg/kg.

The ranked order of total residues in wheat hay samples collected 30 days after application (dry weight) at the GAP application rate was 0.03, 0.05, 0.05, 0.07, 0.10, 0.13, 0.13, 0.16, 0.19, 0.22, 0.27, 0.34, 0.35, 0.47, 0.57, 0.67, 0.89 and 1.34 mg/kg.

For the calculation of the livestock animal dietary burden the ranked order of total residues in wheat straw samples collected 45 days after application (wet weight) at the GAP application rate was 0.01, 0.03, 0.04, 0.04, 0.06, 0.09, 0.11, 0.11, 0.15, 0.15, 0.17, 0.20, 0.23, 0.28, 0.33, 0.46, 0.59 and 0.60 mg/kg.

The ranked order of total residues in wheat straw samples collected 45 days after application (dry weight) at the GAP application rate was 0.01, 0.03, 0.05, 0.05, 0.07, 0.10, 0.13, 0.13, 0.17, 0.17, 0.19, 0.23, 0.26, 0.32, 0.38, 0.52, 0.67 and 0.68 mg/kg.

The Meeting noted that the USA GAP is the same for barley and wheat. The Meeting also noted that the populations of residues data for barley and wheat hay matching USA GAP gave higher residues than the straw and resulted in similar distributions of residues (e.g., medians do not differ by more than 5×). Given the similarity of the datasets (confirmed by the Mann-Whitney U test), the Meeting decided to combine the datasets for barley and wheat hay (dry weight) to give a larger dataset for estimation of dietary parameters for the purposes of determining median and highest residue levels for barley straw and fodder (dry) and wheat straw and fodder (dry).

For the calculation of the livestock animal dietary burden the ranked order of total residues of trinexapac acid in barley and wheat hay (dry weight) collected 30 days after application (dry weight) from supervised trials according to the GAP in the USA was < 0.011, 0.03, 0.03, 0.05, 0.05, 0.06, 0.07, 0.10, 0.11, 0.13, 0.13, 0.15, 0.16, 0.17, 0.19, 0.19, 0.20, 0.22, 0.27, 0.28, 0.34, 0.35, 0.38, 0.45, 0.47, 0.55, 0.57, 0.67, 0.89 and 1.34 mg/kg.

The Meeting used the barley and wheat hay data to estimate median and highest residue values for trinexapac acid in barley straw and fodder (dry) and wheat straw and fodder (dry) of 0.19 and 1.34 mg/kg respectively, based on the combined dataset for barley and wheat hay.

The Meeting recognized that wheat (spring wheat, winter wheat and durum wheat) and barley have similar GAPs to oats and triticale and normally show comparable residues after early treatment. The Meeting decided to extrapolate the dietary parameters estimated for wheat and barley to oats and triticale.

Rape seed forage

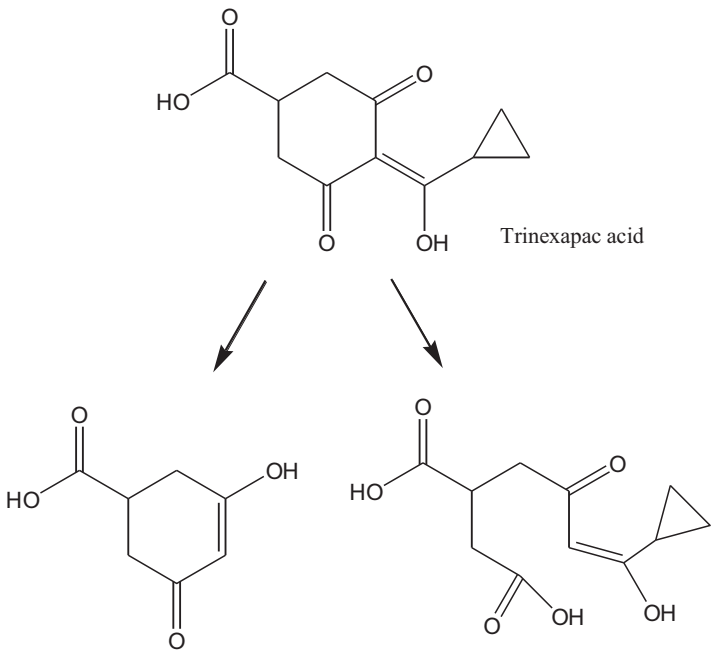
In four trials conducted according to GAP in Germany, residues in rape remaining plant type (forage) at PHIs from 49–68 days were < 0.04, < 0.04, < 0.04 and 0.04 mg/kg.

The Meeting agreed that four trials according to GAP were not sufficient to estimate a highest residue for trinexapac in rape forage.

Fate of residues during processing

High temperature hydrolysis of residues of trinexapac-ethyl and trinexapac acid under varying conditions have been reported. The [^{14}C]-labelled compounds were dissolved in aqueous buffer; at pH 4 and heated for 20 or 25 minutes at 90 ºC to simulate pasteurisation, at pH 5 and refluxed at 100 ºC for 60 minutes to simulate baking, brewing and boiling and at pH 6 at about 120 ºC in an autoclave for 20 minutes to simulate sterilisation. Trinexapac-ethyl was not degraded. Trinexapac acid underwent limited degradation and was the major component of the radioactive material at the end of the hydrolysis reactions (51–59%). It degraded into the compounds CGA113745 (10–12%) and CGA313458 (16–21%).

CGA113745 was observed as a goat metabolite in one metabolism study, while CGA313458 was observed as a rapeseed and rice metabolite. Based on a structural assessment of CGA 113745 and CGA 313458 and the estimated levels of chronic dietary intake, the Meeting concluded that these metabolites are unlikely to pose a dietary risk.



Hydrolysis of trinexapac acid under processing conditions

The Meeting also received processing studies for barley, wheat, sugarcane and rape seed. The table below summarizes STMR-P values calculated on the determined processing factors. In addition the following maximum residue levels were estimated.

Barley

Based on the total trinexapac processing factor of 1.9 for barley bran (in the absence of a trinexapac processing factor) and the barley grain MRL of 3 mg/kg, the calculated expected highest residues in barley bran are 5.7 mg/kg. The Meeting estimated an MRL for trinexapac in barley bran of 6 mg/kg.

Wheat

Based on the trinexapac processing factor of 2.3 for wheat bran and the wheat grain MRL of 3 mg/kg the calculated expected highest residues in wheat bran are 6.9 mg/kg. The Meeting estimated an MRL for trinexapac in wheat bran of 8 mg/kg.

The processing factors derived from the processing studies and the resulting recommendations for STMR-Ps (and maximum residue levels) are summarized in the table below.

Processing Factors from the Processing of Raw Agricultural Commodities (RACs) with Field-Incurred Residues from Foliar Treatment with Trinexapac-ethyl

RAC	Processed Commodity	Best Estimate Processing Factor	RAC MRL	RAC STMR	Processed Commodity STMR-P
Barley	Pearled barley	1.2	3	0.57	0.68
	Bran	1.9			1.08
	Flour	0.43			0.25
Wheat	Aspirated Grain Fractions	0.55	3	0.57	0.31

RAC	Processed Commodity	Best Estimate Processing Factor	RAC MRL	RAC STMR	Processed Commodity STMR-P
	Bran	1.9			1.08
	Flour	0.43			0.25
	Middlings	0.55			0.31
	Shorts	0.46			0.26
	Germ	1.1			0.63
Sugarcane	Molasses	5.8	0.5	0.07	0.40
	Refined sugar	0.15			0.01
Rapeseed	Press cake	1.2	1.5	0.24	0.32
	Refined oil	0.05			0.01

Except for the rapeseed study (in which processing factors were determined based on residues of trinexapac), processed commodity STMR-Ps were calculated on the basis of the total trinexapac acid processing factors.

Residues in animal commodities

Estimated maximum and mean dietary burdens of farm animals

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: wheat, barley, oat and triticale grain, straw, forage, hay and silage, wheat milled by-products (bran), wheat aspirated grain fractions, barley bran fractions, sugarcane molasses and bagasse and rape forage and rape seed meal.

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

	US-Canada		EU		Australia		Japan	
	max	mean	max	mean	max	mean	max	mean
Beef cattle	0.98	0.87	1.76	0.82	3.76 [a]	0.88	0.97	0.97 [c]
Dairy cattle	1.76	0.77	1.76	0.76	3.76 [b]	0.86 [d]	1.00	0.83

[a] Highest maximum beef or dairy cattle dietary burden suitable for HR and MRL estimates for mammalian meat

[b] Highest maximum dairy cattle dietary burden suitable for HR and MRL estimates for mammalian milk

[c] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat

[d] Highest mean dairy cattle dietary burden suitable for STMR estimates for mammalian milk

Potential poultry feed items include: wheat, barley, oat and triticale grain, straw, forage, hay and silage, wheat milled by-products (bran), wheat aspirated grain fractions, barley bran fractions, sugarcane molasses and bagasse and rape forage and rape seed meal.

Summary of poultry dietary burden for trinexapac (ppm of dry matter diet)

	US-Canada		EU		Australia		Japan	
	max	mean	max	mean	max	mean	max	mean
Poultry Broiler	0.94	0.94	0.70	0.70	0.34	0.34	0.13	0.13
Poultry Layer	0.94	0.94 [b]	1.08 [a]	0.76	0.34	0.34	0.37	0.37

[a] Highest maximum poultry dietary burden suitable for HR and MRL estimates for poultry meat and eggs

[b] Highest maximum poultry dietary burden suitable for STMR estimates for poultry meat and eggs

Farm Animal Dietary Burden

The Meeting received a lactating dairy cow feeding study which provided information on residues of trinexapac acid arising in tissues and milk when dairy cows were dosed for 29–30 days, at target feeding levels equivalent to 0, 2, 6 and 20 ppm trinexapac acid in the diet.

No quantifiable residues of trinexapac acid were observed in milk at the 0, 2 and 6 ppm dose groups. Residues in the 20 ppm dose group ranged from < 0.005–0.011 mg/kg. Residues reached a plateau after one day.

No quantifiable residues of trinexapac acid were observed in the tissues of the control cow. In the lowest and mid-range dose groups (2 and 6 ppm), no quantifiable residues were detected in muscle, liver and fat, although residues slightly above LOQ (0.02 mg/kg) were observed in kidney (highest (mean in brackets) were 0.03 (0.03) and 0.05 (0.04) mg/kg respectively). No quantifiable residues were detected in muscle in the 20 ppm dose group while the highest residues (mean in brackets) observed in liver, kidney and fat in the 20 ppm dose group were 0.03 (0.03), 0.29 (0.17) and 0.02 (< 0.02) mg/kg respectively.

The Meeting also received information on the residue levels arising in tissues and eggs when laying hens were dosed with trinexapac acid for 28 days, at feeding levels equivalent to 3.7, 10 and 37 ppm in the diet.

No quantifiable residues of trinexapac acid were observed in eggs at any feeding level except in the 37 ppm dose group at 28 days (highest and mean residues were 0.01 mg/kg).

No residues of trinexapac acid above LOQ (0.01 mg/kg) were detected in any muscle (breast and thigh muscle) sample from any treatment group. No residues of trinexapac acid above LOQ were detected in any liver sample from any treatment group apart from the highest dose group (highest and mean residues were 0.02 mg/kg). No residues of trinexapac acid above LOQ were detected in any fat sample from any treatment group apart from the highest dose group (highest and mean residues were 0.03 mg/kg). Highest (mean) residues of trinexapac acid in kidney were 0.08 (0.06), 0.05 (0.04) and 0.54 (0.45) mg/kg in the lowest to highest dose groups respectively.

Animal commodity maximum residue levels

Cattle- STMR, HR and MRLs

For highest residue level estimation, the high residues in the cattle tissues were calculated by interpolating the maximum dietary burden for beef cattle (3.76 ppm) between the relevant feeding levels (2 and 6 ppm) in the dairy cow feeding study and using the highest tissue concentrations from individual animals within those feeding groups. For highest residue level estimation, the high residues in the cattle milk were calculated by interpolating the maximum dietary burden for dairy cattle (3.76 ppm) with the lowest feeding level (2 and 6 ppm) in the dairy cow feeding study and using the highest mean milk concentrations from those feeding groups.

The STMR values for the tissues were calculated by extrapolating the mean dietary burden for beef cattle (0.97 ppm) with the 2 ppm feeding level from the dairy cow feeding study and using the mean tissue concentrations from that feeding group. The STMR values for the milk were calculated by extrapolating the mean dietary burden for dairy cattle (0.86 ppm) with the 2 ppm feeding level from the dairy cow feeding study and using the mean milk concentrations from that feeding group.

Trinexapac Feeding Study	Feed Level (ppm) for milk residues	Residues (mg/kg) in milk	Feed Level (ppm) for tissue residues	Residues (mg/kg)			
				Muscle	Liver	Kidney	Fat
HR Determination (beef or dairy cattle)							
Feeding Study	2	< 0.005	2	< 0.02	< 0.02	0.03	< 0.02
	6	< 0.005	6	< 0.02	< 0.02	0.05	< 0.02
Dietary burden and estimate of highest	3.76	< 0.005	3.76	< 0.02	< 0.02	0.04	< 0.02

residue							
STMR Determination (beef or dairy cattle)							
Feeding Study	2	< 0.005	2	< 0.02	< 0.02	0.03	< 0.02
Dietary burden and estimate of highest residue	0.86	< 0.0022	0.97	< 0.010	< 0.010	0.015	< 0.010

The Meeting estimated the following STMR values: milk 0 mg/kg; muscle 0 mg/kg; edible offal (based on kidney) 0.015 mg/kg and fat 0 mg/kg.

The Meeting estimated the following maximum residue levels: milk 0.005(*) mg/kg; meat (mammalian except marine mammals) 0.01(*) mg/kg, edible offal (based on kidney) 0.1 mg/kg and mammalian fats (except milk fats) 0.01(*) mg/kg.

Poultry - STMR, HR and MRLs

For highest residue level estimation, the high residues in the hen tissues and eggs were calculated by extrapolating the maximum dietary burden (1.08 ppm) with the lowest feeding level (3.7 ppm) in the laying hen feeding study and using the highest tissue concentrations from individual animals within that feeding group and using the highest mean egg concentration from that feeding group.

The STMR values for the tissues and eggs were calculated by extrapolating the mean dietary burden (0.94 ppm) with the lowest feeding level (3.7 ppm) from the poultry feeding study and using the mean tissue and egg concentrations from that feeding group.

Trinexapac Feeding Study	Feed Level (ppm) for egg residues	Residues (mg/kg) in egg	Feed Level (ppm) for tissue residues	Residues (mg/kg)			
				Muscle	Liver	Kidney	Fat
HR Determination (poultry broiler or layer)							
Feeding Study	3.7	< 0.01	3.7	< 0.01	< 0.01	0.08	< 0.01
Dietary burden and estimate of highest residue	1.08	< 0.003	1.08	< 0.003	< 0.003	0.023	< 0.003
STMR Determination (poultry broiler or layer)							
Feeding Study	3.7	< 0.01	3.7	< 0.01	< 0.01	0.06	< 0.01
Dietary burden and estimate of highest residue	0.94	< 0.0025	0.94	< 0.0025	< 0.0025	0.015	< 0.0025

The Meeting estimated the following STMR values: egg 0 mg/kg; muscle 0 mg/kg; edible offal (based on kidney) 0.015 mg/kg and fat 0 mg/kg.

The Meeting estimated the following maximum residue levels: eggs 0.01(*) mg/kg; poultry meat 0.01(*) mg/kg, poultry edible offal (based on kidney) 0.05 mg/kg and poultry fats 0.01(*) mg/kg.

RECOMMENDATIONS

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

Definition of the residue (for estimation of dietary intake for plant commodities): *Trinexapac and its conjugates, expressed as trinexapac acid.*

The Meeting estimated the maximum residue levels and STMR values shown below.

Commodity		MRL, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
CCN	Name			
GC 0640	Barley	3	0.57	
	Barley bran	6	1.08	
AS 0640	Barley straw and fodder, dry (dry weight)	0.9	0.19	1.34
MO 0105	Edible offal (mammalian)	0.1	0.015	
PE 0112	Eggs	0.01 (*)	0	
MF 0100	Mammalian fats (except milk fats)	0.01 (*)	0	
MM 0095	Meat (from mammals other than marine mammals)	0.01 (*)	0	
ML 0106	Milks	0.005 (*)	0	
GC 0647	Oats	3	0.57	
AS 0647	Oat straw and fodder, dry (dry weight)	0.9	0.19	1.34
PF 0111	Poultry fats	0.01 (*)	0	
PM 0110	Poultry meat	0.01 (*)	0	
PO 0111	Poultry, edible offal of	0.05	0.015	
SO 0495	Rape seed	1.5	0.24	
GS 0659	Sugar cane	0.5	0.07	
GC 0653	Triticale	3	0.57	
AS 0653	Triticale straw and fodder, dry (dry weight)	0.9	0.19	1.34
GC 0654	Wheat	3	0.57	
CM 0654	Wheat bran	8	1.08	
AS 0654	Wheat straw and fodder, dry (dry weight)	0.9	0.19	1.34
	Barley flour		0.25	
	Barley forage (dry weight)		0.40	3.76
AF 0647	Oat forage (green) (dry weight)		0.40	3.76
	Pearled barley		0.68	
	Rape seed refined oil		0.01	
DM 0659	Sugar cane molasses		0.40	
	Triticale forage (dry weight)		0.40	3.76

Commodity		MRL, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
CCN	Name			
	Wheat aspirated grain fractions		0.31	
CF 1211	Wheat flour		0.25	
	Wheat forage (dry weight)		0.40	3.76
CF 1210	Wheat germ		0.63	

DIETARY RISK ASSESSMENT

Long-term intake

The evaluation of trinexapac has resulted in recommendations for MRLs and STMRs for raw and processed commodities. Consumption data were available for 36 food commodities and were used in the dietary intake calculation. The results are shown in Annex 3. The International Estimated Daily Intakes for the 13 GEMS/Food regional diets, based on estimated STMRs were in the range 0–1% of the maximum ADI of 0.3 mg/kg bw (Annex 3).

The Meeting concluded that the long-term intake of residues of trinexapac from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-term Intake (IESTI) for trinexapac was not calculated, as it was not considered necessary to establish an ARfD.

The Meeting concluded that the short-term intake of residues of trinexapac from uses that have been considered by the JMPR is unlikely to present a public health concern.

REFERENCES

Code	Authors	Year	Title, Institute, Report reference
CGA163935/0609	Angly, H	1999	Oxidizing properties (liquids). Novartis Crop Protection AG, Basel, Switzerland. Institute of Safety and Security, Basel, Switzerland. Test Report 1999.4073.OPL. Syngenta File No CGA163935/0609. Unpublished.
CGA163935/0647	Angly, H	2000	Flammability of solids. Novartis Crop Protection AG, Basel, Switzerland. Institute of Safety and Security, Basel, Switzerland. Test Report 2000.4007.FLS. Syngenta File No CGA163935/0647. Unpublished.
CGA163935/0988	Benazeraf, L	2004	Independent laboratory validation of residue method REM 137.13 for the determination of trinexapac acid (CGA179500) in oilseed rape, potato, apple and cereal grain. Syngenta Crop Protection AG, Basel, Switzerland. ADME—Bioanalyses, Vergeze, France. Study Number SYN/TRIN/04091. Syngenta File No CGA163935/0988. Unpublished.
CGA163935/0989	Benazeraf, L	2004a	Independent laboratory validation of residue method REM 137.14 for the determination of trinexapac acid (CGA179500) in bovine muscle and bovine milk. Syngenta Crop Protection AG, Basel, Switzerland. ADME—Bioanalyses, Vergeze, France. Study Number SYN/TRIN/04092. Syngenta File No CGA163935/0989. Unpublished.
CGA163935/0413	Bissig, R	1995	Absorption and distribution kinetics, and biliary excretion of [1,2,6-^{14}C]cyclohexyl CGA 163935 in the rat. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland.015AM01 & 015AM02. Syngenta File No CGA163935/0413. Unpublished.

Code	Authors	Year	Title, Institute, Report reference
CGA163935/0654	Bourry, R	2000	Validation of method REM 137.09.Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland, 308/00. Syngenta File No CGA163935/0654. Unpublished.
CGA179500_50012	Brown, S	2011	Stability of trinexapac-ethyl as CGA179500 in wheat processed fractions under freezer storage conditions. Syngenta Crop Protection, Greensboro, USA. Morse Laboratories, LLC, Sacramento, USA. Report Number ML-10-1605-SYN. Syngenta File No CGA179500_50012. Unpublished.
CGA163935/0432	Burkhard, N	1995	Henry's Law constant. Novartis Crop Protection AG, Basel, Switzerland. Syngenta File No CGA163935/0432. Unpublished.
CGA163935/0566	Burkhard, N	1999	CGA 163935—Dissociation constant. Novartis Crop Protection AG, Basel, Switzerland. Syngenta File No CGA163935/0566. Unpublished.
CGA163935/0733	Cadalbert, R and Buckel, T	2001	Hydrolysis of [1,2,6- ^{14}C]-cyclohexanedione labelled CGA 163935 under processing conditions. Syngenta Crop Protection AG, Basel, Switzerland. Study Number 01RC02. Syngenta File No CGA163935/0733. Unpublished.
CGA163935/0277	Cameron, B, Clydesdale, K, Somers, K and Speirs, G	1992	Distribution and excretion of (1,2 - ^{14}C) cyclohexyl CGA 163935 after multiple oral administration to laying hens. Novartis Crop Protection AG, Basel, Switzerland. Inveresk Res. Int. Ltd., United Kingdom. IRI Report No. 9128. Syngenta File No CGA163935/0277. Unpublished.
CGA163935/0276	Cameron, B, Clydesdale, K, Somers, K and Speirs, G	1992a	Absorption, distribution and excretion of (1, 2 - ^{14}C) cyclohexyl CGA163935 after multiple oral administration to lactating goats. Novartis Crop Protection AG, Basel, Switzerland. Inveresk Res. Int. Ltd., United Kingdom. IRI Report No. 7478. Syngenta File No CGA163935/0276. Unpublished.
CGA163935/0990	Campbell, A and Crook, S	2004	Residue analytical method For the determination of residues of trinexapac acid (CGA179500) in crop samples. Final determination by LC-MS/MS. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta, Jealott's Hill, United Kingdom. REM 137.13. Syngenta File No CGA163935/0990. Unpublished.
CGA163935/0057	Capps, TM	1990	[Cyclohexyl-^{14}C]-CGA 163935 FIFRA Guideline rat metabolism study: metabolite characterization and identification. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, USA. ABR-89119. Syngenta File No CGA163935/0057. Unpublished.
CGA163935/0961	Cobin, J and Pyles, S	2002	Independent laboratory validation of Method 110-01, "Analytical method for the determination of residues of CGA-163935 as its plant metabolite CGA-179500 in crops by high performance, liquid chromatography with mass spectrometry detection including validation data". Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Crop Protection, Inc., Greensboro, USA. Syngenta Number 114-01. Syngenta File No CGA163935/0961. Unpublished.
CGA163935/0537	Das, R	1998	Report on melting point/ melting range. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland. EZA Study Number 60073. Syngenta File No CGA163935/0537. Unpublished.
CGA163935/0631	Das, R	2000	Boiling point/ boiling range of CGA163935. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland. Study Number 77861. Syngenta File No CGA163935/0631. Unpublished.
CGA163935/0629	Das, R	2000a	General physico-chemical properties of CGA 163935. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland. Study Number 77864. Syngenta File No CGA163935/0629. Unpublished.
CGA163935/0628	Das, R	2000b	General physico-chemical properties of CGA 163935 tech. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland, Study Number 77865. Syngenta File No CGA163935/0628. Unpublished.
451534	Ediger, K	2006	Trinexapac-ethyl—Magnitude of the residues in or on wheat. Syngenta Crop Protection, Inc., Greensboro, USA Report Number T002695-03. Syngenta File No 451534. Unpublished.
451598	Ediger, K	2006a	Trinexapac-ethyl—Magnitude of the residues in or on sugarcane and processed commodities Final report. Syngenta Crop Protection, Inc., Greensboro, USA. Report Number T003484-03. Syngenta File No 451598. Unpublished.

Code	Authors	Year	Title, Institute, Report reference
CGA163935/1054	Ediger, K	2006b	Trinexapac-ethyl—Field accumulation in rotational crops (14-, 30- and 45-Day PBI). Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Crop Protection, Inc., Greensboro, USA. Report Number T002700-03. Syngenta File No CGA163935/1054. Unpublished.
CGA163935/0033	Forrer, K	1989	CGA 163935, Liquid chromatographic determination of residues of parent compound, plant materials, soil. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. REM-137-01. Syngenta File No CGA163935/0033. Unpublished.
CGA163935/0082	Forrer, K	1991	CGA 163935 Determination of residues of the metabolite CGA179500 by liquid chromatography. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. REM-137-02. Syngenta File No CGA163935/0082. Unpublished.
CGA163935/0142	Forrer, K	1991a	CGA 163935, Liquid chromatographic determinations of residues of parent compound, rape. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. REM-137-05. Syngenta File No CGA163935/0142. Unpublished.
CGA163935/0651	Füldner, HH	2000	Density of solids. Novartis Crop Protection AG, Basel, Switzerland. Solvias AG, Basel, Switzerland. Study No. PP-99/77P.DES. Syngenta File No CGA163935/0651. Unpublished.
CGA163935/0740	Gasser, A	2001	Independent laboratory validation (ILV) of Method REM 137.12:Validation by analysis of milk and meat specimens fortified with CGA 179500 and determination of recoveries. Syngenta Crop Protection AG, Basel, Switzerland. Report on Study 312/01. Syngenta File No CGA163935/0740. Unpublished.
CGA163935/0482	Gross, D	1996	Behaviour and metabolism of CGA163935 in greenhouse grown paddy rice after application of [3,5-cyclohexadion-1, 2, 6-^{14}C] labelled material. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Plant Metabolism Report PMR 11/96. Syngenta File No CGA163935/0482. Unpublished.
CGA163935/0322	Hauck, M	1993	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3091/92. Syngenta File No CGA163935/0322. Unpublished.
CGA163935/0331	Hauck, M	1993a	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3108/92. Syngenta File No CGA163935/0331. Unpublished.
CGA163935/0317	Hauck, M	1993b	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3219/91. Syngenta File No CGA163935/0317. Unpublished.
CGA163935/0319	Hauck, M	1993c	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3086/92. Syngenta File No CGA163935/0319. Unpublished.
CGA163935/0323	Hauck, M	1993d	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3092/92. Syngenta File No CGA163935/0323. Unpublished.
CGA163935/0325	Hauck, M	1993e	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3098/92. Syngenta File No CGA163935/0325. Unpublished.
CGA163935/0327	Hauck, M	1993f	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3104/92. Syngenta File No CGA163935/0327. Unpublished.
CGA163935/0328	Hauck, M	1993g	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3105/92. Syngenta File No CGA163935/0328. Unpublished.
CGA163935/0329	Hauck, M	1993h	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3106/92. Syngenta File No CGA163935/0329. Unpublished.

Code	Authors	Year	Title, Institute, Report reference
CGA163935/0330	Hauck, M	1993i	Determination of residues of parent compound CGA163935 and metabolite CGA179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3107/92. Syngenta File No CGA163935/0330. Unpublished.
CGA163935/0316	Hauck, M	1993j	Determination of residues of parent compound CGA163935 and metabolite CGA179500–field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3216/91. Syngenta File No CGA163935/0316. Unpublished.
CGA163935/0318	Hauck, M	1993k	Determination of residues of parent compound CGA 163935 and metabolite CGA 179500—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Analytical Report 3221/91. Syngenta File No CGA163935/0318. Unpublished.
CGA163935/0535	Jäkel, K	1990	Report on dissociation constant in water. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Anal. Test. No. EAN-127306. Syngenta File No CGA163935/0535. Unpublished.
CGA163935/0621	Kettner, R	1999	Octanol / water partition coefficient of CGA 163935. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland. Study Number 77863. Syngenta File No CGA163935/0621. Unpublished.
CGA163935/0086	Krauss, J	1990	Uptake, distribution and degradation of [^{14}C] cyclohexyl]-CGA 163935 in field grown spring wheat. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Project Report 20/90. Syngenta File No CGA163935/0086. Unpublished.
CGA163935/0265	Krauss, J	1992	Outdoor confined accumulation study on rotational crops after bareground soil application of [^{14}C] cyclohexyl]- CGA 163935. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Metabolism Report 23/92. Syngenta File No CGA163935/0265. Unpublished.
CGA163935/0303	Krauss, J	1993	Metabolism of [^{14}C] cyclohexyl]-CGA163935 in field grown spring wheat. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Metabolism Report 6/93. Syngenta File No CGA163935/0303. Unpublished.
CGA179500/0039	Kwiatkowski, A	2004	Trinexapac acid (CGA179500): Validation of a residue analytical method REM 137.14 for the determination of residues in animal products (milk, eggs, muscle, kidney, fat and liver). Syngenta Crop Protection AG, Basel, Switzerland. Syngenta—Jealott's Hill, Bracknell, United Kingdom. Report Series RJ3570B. Study Number 04-S623. Syngenta File No CGA179500/0039. Unpublished.
CGA163935/0962	Lin, K	2002	Analytical method for the determination of residues of CGA-163935 as its plant metabolite CGA-179500 in crops by high performance liquid chromatography with mass spectrometry detection including validation data. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Crop Protection, Inc., Greensboro, USA. Syngenta Number 110-01. Syngenta File No CGA163935/0962. Unpublished.
CGA179500_50010	Lin, K	2008	Validation of analytical method GRM020.01A for the determination of residues of trinexapac-ethyl as CGA179500 in crops by LC-MS/MS Final report. Syngenta Crop Protection, Inc., Greensboro, USA. Report Number T002856-07. Syngenta File No CGA179500_50010. Unpublished.
CGA163935/0728	Loffler, A	2001	Dermal absorption of [3,5-cyclohexadione-1,2,6-^{14}C] CGA 163935 formulated as EC 250 (A-7725M) in the rat. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Crop Protection AG, Basel, Switzerland, 015AM03. Syngenta File No CGA163935/0728. Unpublished.
CGA179500/0034	Mamouni, A	2006	[Hydroxymethylene-^{14}C]-CGA 179500 (trinexapac-acid): hydrolysis at four different pH values. Syngenta Crop Protection AG, Basel, Switzerland. RCC Ltd., Itingen, Switzerland. RCC Study Number 770670. Syngenta File No CGA179500/0034. Unpublished.
CGA163935/0630	Martin, N	2000	Surface tension. Novartis Crop Protection AG, Basel, Switzerland. Solvias AG, Basel, Switzerland. Study No. PP-99/78T.SUR. Syngenta File No CGA163935/0630. Unpublished.
CGA163935_50036	Mayer, T	2010	Trinexapac-ethyl—Magnitude of the residues in or on wheat Final report. Syngenta Crop Protection, Inc., Greensboro, USA. Report Number T003605-07. Syngenta File No CGA163935_50036. Unpublished.

Code	Authors	Year	Title, Institute, Report reference
CGA163935_50026	Mayer, T	2010a	Trinexapac-ethyl—Magnitude of the residues in or on barley Final report. Syngenta Crop Protection, Inc., Greensboro, USA. Report Number T008422-07. Syngenta File No CGA163935_50026. Unpublished.
CGA163935_50038	Mayer, T	2010b	Trinexapac-ethyl—Magnitude of the residues in or on sugarcane. Syngenta Crop Protection, Inc., Greensboro, USA. Report Number T003606-07. Syngenta File No CGA163935_50038. Unpublished.
CGA163935/0724	Millais, A	2001	[Hydroxymethylene-^{14}C]-CGA 163935 Aqueous photolysis. Syngenta Crop Protection AG, Basel, Switzerland. Huntingdon Research Centre, Cambridgeshire, United Kingdom. NVR 059/012189. Syngenta File No CGA163935/0724. Unpublished.
CGA179500/0036	Mound, E and Clark, T	2004	[^{14}C] Cyclohexyl Trinexapac Acid (CGA179500): Aqueous hydrolysis at 90, 100 & 120 °C. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta—Jealott's Hill, Bracknell, United Kingdom. Report Series RJ3480B. Syngenta File No CGA179500/0036. Unpublished.
CGA163935/0306	Müller, T	1993	The nature of metabolites in eggs, tissues, and excreta of laying hen after multiple oral administration of [1,2-^{14}C] cyclohexyl CGA 163935. Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland. Project Report 6/93. Syngenta File No CGA163935/0306. Unpublished.
CGA163935/0305	Müller, T	1993a	The nature of the metabolites in milk, tissues, and excreta of lactating goat after multiple oral administration of [1,2-^{14}C] cyclohexyl CGA 163935. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Project Report 5/93. Syngenta File No CGA163935/0305. Unpublished.
CGA163935/0209	Nicollier, G	1991	Distribution and degradation of [^{14}C-cyclohexyl]-CGA 163935 in greenhouse grown spring rape. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Metabolism Report 4/91. Syngenta File No CGA163935/0209. Unpublished.
CGA163935/0308	Nicollier, G	1993	Metabolism of [^{14}C] cyclohexyl]-CGA 163935 in greenhouse grown spring rape. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Metabolism Report 7/93. Syngenta File No CGA163935/0308. Unpublished.
CGA163935/1048	Powell, S	2006	[3,5-Cyclohexadione-1, 2, 6-^{14}C]-labelled trinexapac-ethyl (CGA163935)—Metabolism in laying hens. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta–Jealott's Hill International, Bracknell, Berkshire, United Kingdom. Report Series RJ3678B. Study Number 04JH011. Syngenta File No CGA163935/1048. Unpublished.
CGA163935_50024	Quistad, G and Kovatchev, A	2010	[^{14}C] Trinexapac-ethyl—Uptake and metabolism in confined rotational crops Final report. Syngenta Crop Protection, Inc., Greensboro, USA PTRL West, Inc., Hercules, USA. Report Number 1802W. Syngenta File No CGA163935_50024. Unpublished.
CGA163935/0944	Ray, W	2002	[1,2,6-^{14}C] Cyclohexyl-CGA-163935: Nature of the residue in lactating goats. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Crop Protection, Inc., Greensboro, USA. Syngenta Number 624-00. Unpublished.
CGA163935/0862	Ray, W and May-Hertl, U	2003	[1,2,6-^{14}C] Cyclohexyl-CGA-163935: Nature of the residue in field grown grass. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Crop Protection, Inc., Greensboro, USA. Syngenta Number 623-00. Syngenta File No CGA163935/0862. Unpublished.
CGA163935/0238	Rodler, M	1990	Report on water solubility. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Münchwilen AG, Münchwilen, Switzerland. EZA Project Number EA-127307. Syngenta File No CGA163935/0238. Unpublished.
CGA163935/0701	Roper, CS	2001	The in vitro percutaneous absorption of [3,5-cyclohexadione-1,2,6-^{14}C]-CGA163935 formulated as EC 250 (A-7725M) through rat and human skin Report Amendment 1. Syngenta Crop Protection AG, Basel, Switzerland. Inveresk Research International, Tranent, United Kingdom, Report Nr. 19227 / Project Nr. 169640. Syngenta File No CGA163935/0701. Unpublished.
CGA163935/0241	Rordorf, B	1990	Report on vapour pressure curve. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Anal. Test No. 127 307. Syngenta File No CGA163935/0241. Unpublished.

Code	Authors	Year	Title, Institute, Report reference
CGA163935/0504	Roth, M	1997	Report on spectra. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland. EZA Project Number 50598. Syngenta File No CGA163935/0504. Unpublished.
CGA163935/0417	Sack, S	1995	Determination of residues of the metabolite CGA 179500 in rapeseed, crude oil, refined oil and presscake—field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Report 3067/94. Syngenta File No CGA163935/0417. Unpublished.
CGA163935/0416	Sack, S	1995a	Determination of residues of the metabolite CGA 179500 in rapeseed, crude oil, refined oil and presscake–field trial. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Report 3066/94. Syngenta File No CGA163935/0416. Unpublished.
CGA179500/0030	Sack, S	2000	Residues of CGA 179500 in milk, blood and tissues (muscle, fat, liver, kidney) of dairy cattle resulting from feeding of CGA 179500 (metabolite of trinexapac-ethyl, CGA 163935) at three dose levels. Novartis Crop Protection AG, Basel, Switzerland. Report Number 330/99. Syngenta File No CGA179500/0030. Unpublished.
CGA163935/0440	Sack, S	1995	CGA 163935 (trinexapac-ethyl): Determination of the metabolite CGA 179500 by liquid chromatography, animal produce (tissue, milk, eggs). Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. REM 137.12. Syngenta File No CGA163935/0440. Unpublished.
CGA163935/0407	Sack, S	1995a	Trinexapac-ethyl (CGA 163935), Determination of the metabolite CGA 179500 by liquid chromatography, Water, Residue Method Validated. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland, REM 137.09. Syngenta File No CGA163935/0407. Unpublished.
CGA163935/0562	Sack, S	1998	Stability of residues of CGA 179500 (metabolite of trinexapac-ethyl, CGA163935) in deep freeze stored analytical specimens of wheat (grain and straw) and rapeseed. Novartis Crop Protection AG, Basel, Switzerland. Study Number 105/95. Syngenta File No CGA163935/0562. Unpublished.
CGA163935/0588	Sack, S	1999	Validation of method REM 137.02 (validation by analysis of fortified specimens and determination of recoveries). Novartis Crop Protection AG, Basel, Switzerland. Report on Study 304/99. Syngenta File No CGA163935/0588. Unpublished.
CGA163935/0244	Schürch, H	1992	Report on auto-flammability of solids. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Test No. PP 92/6T.AFG. Syngenta File No CGA163935/0244. Unpublished.
CGA163935/0242	Schürch, H	1992a	Report on determination of flashpoint. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Test No. PP 92/6T.FLP. Syngenta File No CGA163935/0242. Unpublished.
CGA163935/0243	Schürch, H	1992b	Report on explosive properties. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Test No. PP 92/6T.EXP. Syngenta File No CGA163935/0243. Unpublished.
CGA163935/0299	Schürch, H	1993	Report on thermal stability and stability in air. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Test No. PP-93/4T.TSA. Syngenta File No CGA163935/0299. Unpublished.
CGA179500_50008	Simmons, D	2010	CGA179500—Magnitude of residues in meat and eggs resulting from the feeding of three levels to poultry Final report. Syngenta Crop Protection, Inc., Greensboro, USA. Report Number T009354-07. Syngenta File No CGA179500_50008. Unpublished.
CGA163935/0636	Smith, J	2000	Determination of residues of CGA 163935 and the metabolite CGA 179500 in winter oilseed rape. Novartis Crop Protection AG, Basel, Switzerland. Novartis Agro GmbH, Frankfurt, Germany. Study Report gr 02199. Syngenta File No CGA163935/0636. Unpublished.
CGA163935/0637	Smith, J	2000a	Determination of residues of CGA 163935 and the metabolite CGA 179500 in winter oilseed rape. Novartis Crop Protection AG, Basel, Switzerland. Novartis Agro GmbH, Frankfurt, Germany. Study Report gr 02299. Syngenta File No CGA163935/0637. Unpublished.

Code	Authors	Year	Title, Institute, Report reference
CGA163935/0638	Smith, J	2000b	Determination of residues of CGA 163935 and the metabolite CGA 179500 in winter oilseed rape. Novartis Crop Protection AG, Basel, Switzerland. Novartis Agro GmbH, Frankfurt, Germany. Study Report gr 02499. Syngenta File No CGA163935/0638. Unpublished.
CGA163935/0639	Smith, J	2000c	Determination of residues of CGA 163935 and the metabolite CGA 179500 in winter oilseed rape. Novartis Crop Protection AG, Basel, Switzerland. Novartis Agro GmbH, Frankfurt, Germany. Study Report gr 02599. Syngenta File No CGA163935/0639. Unpublished.
CGA163935/0021	Spare, WC	1989	Identification of hydrolysis product of CGA-163935. Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Corp., Greensboro, USA. Report No. ABR-89047. Syngenta File No CGA163935/0021. Unpublished.
CGA163935/0022	Spare, WC	1990	^{14}C-CGA 163935, Hydrolysis of ^{14}C-CGA 163935. Novartis Crop Protection AG, Basel, Switzerland. Agrisearch Inc., Frederick, Maryland, USA. Agrisearch Project No. 12152. Syngenta File No CGA163935/0022. Unpublished.
CGA163935/0274	Spare, WC	1992	Hydrolysis of ^{14}C-CGA 163935 at pH 5 and 7. Novartis Crop Protection AG, Basel, Switzerland. Agrisearch Inc., Thurmont, USA. Agrisearch Project No. 12180. Syngenta File No CGA163935/0274. Unpublished.
CGA163935/0567	Stamm, E	1999	Atmospheric oxidation of trinexapac-ethyl CGA 163935 by hydroxyl radicals; rate estimation. Novartis Crop Protection AG, Basel, Switzerland. Project 99SM47. Syngenta File No CGA163935/0567. Unpublished.
CGA163935/0351	Stulz, J	1993	Report on water solubility. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Münchwilen AG, Münchwilen, Switzerland. EZA Project Number15626. Syngenta File No CGA163935/0351. Unpublished.
CGA163935/0561	Stulz, J	1998	Report on solubility in organic solvents. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Münchwilen AG, Münchwilen, Switzerland. EZA Study Number 68509. Syngenta File No CGA163935/0561. Unpublished.
CGA179500_50000	Thomas, C	2010	Independent laboratory validation (ILV) of Syngenta analytical method GRM020.01A Analytical method for the determination of residues of trinexapac-ethyl as CGA179500 in crops by LC-MS/MS. Syngenta Crop Protection, Inc., Greensboro, USA. North Coast Laboratories, Arcata CA, USA. Study Number 110.036. Syngenta File No CGA179500_50000. Unpublished.
CGA163935/0333	Tribolet, R	1993	Sampling of air and determination of residues of parent compound by high performance liquid chromatography. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. REM 137.07. Syngenta File No CGA163935/0333. Unpublished.
CGA163935/0500	Tribolet, R	1996	Validation of Method REM 137.07 in Air; Validation by analysis of fortified specimens and evaluation of recoveries. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Report on Study 151/96. Syngenta File No CGA163935/0500. Unpublished.
CGA163935/0480	Zetzsch, C.	1994	Phototransformation of CGA 163935 in water by direct irradiation. Novartis Crop Protection AG, Basel, Switzerland. ITA Frauenhofer-Inst., Hannover, Germany. Study No. PC94-2. Syngenta File No CGA163935/0480. Unpublished.