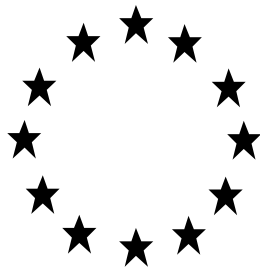
Renewal Assessment Report
under Regulation (EC) 1107/2009



Trinexapac-ethyl
Active substance data

Volume 3

Annex B.7

Residue data

Rapporteur Member State: Lithuania

Co-Rapporteur Member State: Latvia

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Versions of RAR, Vol 3-Annex B.7 (AS) Trinexapac-ethyl

Date	Reason for revision
December 2016	First draft submitted to the applicant and co-RMS
March 2017	First version submitted to EFSA and COM
December 2017	Revised version in the light of comments, reporting table, evaluation table and submission of additional data
February 2018	Revised version in light of the outcome of Expert consultation

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B.7 Residue data

Trinexapac-ethyl is the ISO common name for 4-(cyclopropyl-hydroxymethylene)-3,5-dioxocyclohexanecarboxylic acid ethyl ester (IUPAC). Trinexapac-ethyl is a plant growth regulator. It is taken up via leaves and shoots which results in morphological symptoms such as reduction of crop height or reduced elongation by inhibition of a certain step in the gibberellins biosynthesis. The main trinexapac-ethyl metabolite is trinexapac acid (CGA179500), which is stated as trinexapac acid in the entire document.

The information included in this Annex covers the compound (CGA163935), formulated as a 250 g active substance (a.s.)/L micro-emulsion formulation (ME; A8785F). Trinexapac Task Force (consisting of Syngenta Crop Protection AG, Cheminova A/S, Adama Celsius B.V. and HELM AG) submitted a dossier for the renewal of trinexapac-ethyl. The presented representative uses in Northern and Southern Europe are in the cultivation of winter wheat and winter and spring barley. In Northern and Southern Europe, trinexapac-ethyl will be applied once to wheat at a maximum of 125 g a.s./ha at BBCH 25-49. For winter and spring barley trinexapac-ethyl will be applied once at a maximum of 200 g a.s./ha and of 150 g a.s./ha, at BBCH 25-49 and 25-37 respectively.

In accordance with Commission Implementing Regulation (EU) 844/2012, the studies evaluated in this part include the relevant studies evaluated in the DAR and Addendum (2003, 2005) which are presented in more detail as well as new studies submitted by the applicants for the renewal. Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for trinexapac and to the EFSA Scientific Report for trinexapac (EFSA Scientific Report (2005) 57, 1-70), and in particular the endpoints provided in Appendix I.

Where the conclusions of the EU review had specific areas of concern on trinexapac-ethyl, new data and/or reviews and/or risk assessments have been provided. Where additional and/or new data on trinexapac-ethyl are provided, a justification has been included. Also a justification has been given if new data are required but none were provided.

A brief summary of the literature search undertaken can be found in CA B.7.8 and full details are provided in Appendix I~~I~~.

When reference is made to European evaluations (DAR, EFSA Reasoned Opinion,…) or to guidelines, full references are provided at the end of the document, before the appendices (see **Cited Documents**).

Application form for modifying MRL for rye was provided by the applicants. Although it is not a representative use, it has been agreed that the evaluation can be performed as part of the Annex I Renewal process. All information to support this MRL is extrapolated from wheat.

B.7.1 Storage stability of residues prior to analysis

The storage stability of trinexapac acid was evaluated in the DAR 2003. Studies were done not according to the current guidelines, however these studies can provide information on stability of trinexapac acid during storage. Therefore they were re-evaluated by the RMS in the current submission and reported below. ~~No~~ New studies on stability of ~~trinexapac acid and other metabolites were~~ metabolites CGA313458, CGA 113745 and cyclopropane carboxylic acid (CGA224439) were provided after commenting period and assessed for the new AIR dossier.

~~TTF informed, that storage stability studies for CGA313458, CGA 113745 and cyclopropane carboxylic acid (CGA224439) are currently on going and will be completed by April 2017.~~

B.7.1.1 Stability of residues in plants and plant products

Study 1
EU reviewed storage stability study in plants

Reference:	**Sack St. (1998)** Stability of residues of CGA 179500 (metabolite of trinexapac-ethyl, CGA 163935) in deep freeze stored analytical specimens of wheat (grain and straw) and rapeseed. Final report. (~~KCA 6.1 / 01 ;~~ KIIA 6.3.2.1 / 01)
Report No.:	105/95
Guideline:	US-EPA OPPTS 860.1380 US-EPA Pesticide assessment guideline Storage stability study, subdivision 0, Residue Chemistry.no guideline in force
GLP:	Yes
Previous evaluation:	DAR (2003)
Material and methods:	
Test Material:	Trinexapac acid (CGA 179500)
Batch No. and purity:	KGL 3552/1+2, 98% for the 0, 3, 6 and 12 month interval study; BPS 20/103, 99% for the 24 month interval study
Test system:	The storage stability of CGA 179500, the major metabolite of CGA 163935, was investigated in wheat (grain and straw) and rapeseed samples, originating from untreated control material of residue trials. Homogenised samples were fortified with 0.5 mg/kg of CGA 179500 (about 25 times the LOQ) and stored at -18°C over a period of 3, 6, 12 and 24 months. The samples were handled and stored comparable to the samples from the field trials. The homogenized and fortified substrate was stored in polyethylene containers in deep freeze rooms exactly like residue specimens. For each analysis, analytical subspecimens were weighed out and the containers put back to the freeze room immediately afterwards. Samples for storage stability tests were prepared from substrates which were taken from the remaining untreated (control) test material of current Novartis Residue Trials: Wheat grain: trial 3069/94, wheat straw: trial 3068 + 3069/94 (controls mixed), and rapeseed: trial 3067/94. These substrates were already sufficiently homogenized according to internal SOPs in course of the trials mentioned. The homogenized untreated crop materials were spiked with CGA 179500 2 ml of stock solution of CGA 179500 (200 µg/mL acetonitrile)) were diluted with methanol to a volume of 100 ml yielding a concentration of CGA 179500 in this spiking solution of 4 µg/mL. 200 g of each type of substrate were fortified with 25 ml spiking solution. Maximum evenness of

	distribution of the liquid added over the crop material was ensured by addition of the spiking solution in portions of about 2 ml using Pasteur pipette. Drops were even distributed over the whole lot of substrate. The substrate was well mixed. The next portion of the 25 ml spiking solution was added after the methanol (applied with each portion) was evaporated at room temperature. Each of the three so treated substrates was equally distributed into four plastic containers (about 50 g, each). The appropriate containers were removed from the freezer and the necessary amount of substrate was weighed out. It was removed without thawing, by drilling holes into the frozen substrate.
Test conditions:	Stored at -18^{0}C The substrate was stored under identical conditions as common residue specimens, i.e. in the same type of container (high-density polyethylene, with a screw cap) as normally used for storing residue specimens, and in the same deep freeze room, equipped with a thermostat set at a - temperature of minus 20 °C.
Analytical method:	The analytical method used (REM 137.02) comprises extraction, clean-up and HPLC quantification using UV (280 nm) detection. Extraction and clean-up was performed as outlined in the method description. Only the final determination by HPLC was different to that one described in REM 137.02: For this study a two column HPLC-system with the following conditions was used to analyse for CGA 179500: Final volume: 2 (5) ml Solvent for injection: 5 vol./95 vol. ACN/water + 0.03 M H_3P0_4 Injection volume: 100 µL (50 µL, 300 µL) Column 1: GromSil C4, 250 mm x 2 mm i.d. + guard column Eluent 1: 0.2 (0.3) mL/min 15 vol./85 vol. ACN/water + 0.03 M H_3P0_4 Column 2: Inertsil Phenyl, 250 mm x 2 mm i.d. Eluent 2: 0.2 mL/min 25 vol./75 vol. ACN/water + 0.03 M H_3P0_4 Detection: UV, 280 nm Because of the extended analytical period of two years minor adaptations of final volume, injection volume and flow rates for compensation of different sensitivity of the LC-System used could not be avoided. The less typical values are given in brackets. However, within each individual analytical sequence all analytical parameters were always kept constant. Quantitation was performed by alternate injections of cleaned up specimens and external standards. Interpolation was done by method of weighted least squares based on regression of 1st order (according to General Calculation Method REM 119.06 [2]). DILUTIONS: All specimens analysed were whole homogenized crop parts. The liquid content of the analytical subspecimens of 3 g was 0.6 ml for wheat grain and straw, and 1.5 ml for rapeseed. The extraction solvent volume added was 30 ml. This gave a final extraction volume of 30.6 ml for wheat grain and straw, and a volume of 31.5 ml for rapeseed, whereof an aliquot of 4 ml was taken for all substrates. This typically resulted in a final volume for injection of 2 ml. The typical injection volume was 100 µL. The formal specimen size injected (FSSI) was then 19.6 mg (grain and straw) or 19.0 mg (rapeseed).
Limit of quantification:	0.02 mg/kg
Method validation:	Method REM 137.02 was validated in study Forrer, 1991 for wheat grain and straw, barley grain and straw and rapeseed (please refer to Vol 3 CA B.5.1.2.1 ~~(with reference to the DAR Annex IIA.4.2.1 Method 1))~~. The validated/evaluated fortification levels were 0.04, 0.2 mg/kg. The recoveries (%, mean ~~from one sample~~ plus range) at fortification level of 0.04 mg/kg were:

Wheat grain: 94%; Wheat straw: 104%; Barley grain: 98% (98, 97, 89%); Barley straw: 102% (100, 96, 110%); Rapeseed: 105%;

The recoveries (%, mean ~~from one samples~~ plus range) at fortification level of 0.2 mg/kg were:

Wheat grain: 80%; Wheat straw: 99%; Barley grain: 95% (98, 97, 89%); Barley straw: 94% (89, 97, 96%); Rapeseed: 98%;

Additionally, the same method REM 137.02 was validated in study Sack, 1999 for wheat grain and straw and rapeseed in first laboratory and wheat grain and rapeseed in second laboratory (please refer to Vol 3 CA B.5.1.2.1).

The validated/evaluated fortification levels were 0.02, 0.2 mg/kg. The recoveries (%, mean from 5 samples plus range) at fortification level of 0.02 mg/kg were:

1 laboratory - Wheat grain: 98% (101, 97, 99, 102, 92%); Wheat straw: 104% (98, 109, 102, 110, 99%); Rapeseed: 90% (93, 75, 95, 96, 93%);

2 laboratory - Wheat grain: 78% (83, 80, 75, 76, 78%); Rapeseed: 77% (78, 76, 79, 76, 77%);

The recoveries (%, from 5 samples) at fortification level of 0.2 mg/kg were:

1 laboratory - Wheat grain: 85% (93, 91, 84, 85, 71%); Wheat straw: 89% (90, 79, 92, 93, 90%); Rapeseed: 78% (79, 81, 77, 79, 73%);

2 laboratory - Wheat grain: 72% (74, 70, 70, 73, 75%); Rapeseed: 70% (72, 71, 69, 68, 72%);

Results

The amount of residues of CGA179500 found after the different storage periods in three different matrices are summarised in Table B.7.1.1-1. The data represent the mean of 3 determinations (at t=0 months for 5 determinations). In addition to the absolute concentration (mg/kg) the relative amount (in % with t=0 months as 100%), corrected for the corresponding procedural recoveries, are given. Residues of CGA 179500 in wheat (grain and straw) and rapeseed stored under identical conditions as specimens from residue studies (i.e. at or below minus 18 °C) are considered as stable over a period of at least one years (at least 90 % of the residue can be recovered). This was measured at fortification (residue) levels of approximately 0.5 mg/kg. In the case of wheat straw the amount of CGA 179500 that could be recovered after an extended storage period of about 2 years dropped to 75 %. Therefore, for straw samples stored longer than about 1.5 years a correction factor of 1.3 for the residues found might be considered.

Table B.7.1.1 - 1. Residues of CGA 179500 in rape seed, wheat grain and straw

Crop commodity	Storage period (months)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Residue level in freezer storage stability sample (% of nominal spiking level, corrected*)	Residue level in freezer storage stability sample (% of nominal spiking level, uncorrected**)	Procedural recovery for freshly spiked control sample (%) (range plus mean)
Wheat grain Samples fortified at 0.5 mg/kg	0	0.46 0.48 0.46 0.45 0.48 (0.47)	100	93	85 85 (85)
	3	0.50 0.46 0.47 (0.48)	95	95.6	95 88 (92)
	6	0.46 0.45 0.48 (0.46)	97	92.6	87 86 (87)

	12	0.42 0.43 0.45 (0.43)	91	86.6	90 84 (87)
	24	0.36 0.40 0.43 (0.40)	90	79.4	80 82 (81)
Wheat straw Samples fortified at 0.5 mg/kg	0	0.43 0.38 0.45 0.41 0.40 (0.42)	100	83	93 93 (93)
	3	0.45 0.45 0.46 (0.45)	114	90.8	93 84 (89)
	6	0.46 0.46 0.46 (0.46)	97	92.2	106 105 (106)
	12	0.40 0.41 0.40 (0.40)	93	80.4	99 94 (97)
	24	0.32 0.30 0.32 (0.31)	75	61.8	90 94 (92)[x]
Rapeseed Samples fortified at 0.5 mg/kg	0	0.45 0.41 0.42 0.38 0.41 (0.42)	100	83	91 91 (91)
	3	0.43 0.41 0.42 (0.42)	98	84	95 93 (94)
	6	0.41 0.37 0.37 (0.38)	89	76.8	95 94 (95)
	12	0.36 0.34 0.37 (0.36)	91	71.6	88 84 (86)
	24	0.35 0.37 0.38 (0.37)	84	73	95 94 (95)

No signal (0 mm) at retention time of CGA 179500 in all controls (all < 0.02 mg/kg, with one exception [x])

*****-At each time point, the average of the concentrations found was corrected for the corresponding mean procedural recovery. The so corrected concentrations found for CGA 179500 at day 0 (day of the treatment of storage samples) were set to 100 %. Percentages of recovered CGA 179500 for the individual storage periods were calculated as ratio to these 100% values, For example

**- calculated by the RMS LT, formula "Residue level in freezer storage stability sample (mean)*100/0.5 mg/kg".

[x] corrected for signal in control (non-corrected recoveries: 108 and 110 %)

RMS comments and conclusion

Residues of trinexapac acid (CGA 179500) in ~~wheat~~ cereal grain ~~and straw (high starch content commodity)~~ as well as in rapeseed ~~(high oil content commodity)~~ can be considered as stable for at least 24 months when stored at -18°C. Residues of trinexapac acid (CGA 179500) in wheat straw can be considered as stable for at least 12 months when stored at -18°C. Some cereal samples from the residue trials were stored up to 25.5 months. As the degradation of trinexapac acid is slow (in grain, 90% (79.4 % uncorrected) of trinexapac acid was recovered after 24 months), ~~it is considered~~ the applicant consireders that there is no impact on the levels of trinexapac acid in the samples. RMS agrees with EFSA that trials not adequately supported by storage stability shall be excluded from the assessment.

Each series of analyses (sequence) was accompanied by two freshly fortified specimens to check the procedural recoveries and by the corresponding control specimen to check for background and interferences. With one exception (straw, 2 years) no signal at retention time of CGA 179500 was detected in all other control (untreated) samples.

The assessment of analytical method validation taken from DAR 2003 was inserted in RAR Vol 3 CA B.5.1.2.1 as such (also provided below *in italics*), as limitations identified at that time were not addressed during renewal,

and new methods for data generation have been recently developed in order to lower the LOQ (0.01 mg/kg), be compliant with the latest guideline (SANCO/3029/99 rev.4) and measure all compounds of interest.

The analytical method used (REM 137.02) was validated for the determination of the metabolite CGA 179500 in wheat and rapeseed. With this method it is feasible to determine CGA 179500 in wheat grain and straw with an LOQ of 0.02 mg/kg. It is noted that no confirmatory method was submitted. Although method 137.02 was also validated in wheat grain and rapeseed in another laboratory of the notifier. This is only acceptable if the independent laboratory had not been involved in any way in the method development. At this moment there is no information about this, and therefore an independent laboratory validation (ILV) still is required. Additional validation data with a confirmatory method (for instance HPLC-MS/MS) and an ILV are considered necessary to use method 137.02 for routine monitoring of the metabolite CGA 179500 in cereals.

This study was performed prior to the adoption of the OECD guideline 506 for stability of pesticide residues in stored commodities. Representative chromatograms for wheat grain and straw and rapeseeds lacked information on retention times and other analytical parameters. Only for wheat grain and straw appended chromatograms and raw data complement the original report were provided in a technical letter. Despite these minor deficiencies and method validation showing recoveries of the acceptable range (70-110%) the RMS considers the stability study as sufficient to cover wheat grain and rapeseed samples stored for up to 24 months, and wheat straw samples stored for up to 12 months ~~the proposed uses of this application~~.

The following deviations from OECD 506 (adopted 16 October 2007) were observed:

Representative chromatograms could not be sufficiently assessed for rapeseeds because of missing information on retention times and other analytical parameters.

The fortification level of 25X LOQ used in the study is remarkably higher than the suggested level of OECD 506 (10X LOQ) and the levels tested for the validation of the analytical method (1N, 2N and 10N LOQ).

Samples were homogenized before fortification and freezer storage. In residue trials provided it is not specified, if samples were homogenized before storage in freezing conditions. According to OECD 506 it is preferred that the form of the commodity in a freezer storage stability study should be, as far as possible, the same as that in the corresponding MOR studies. Since the use of a homogenate in the freezer storage stability study is likely to present a worst case versus the use of a whole commodity, it was considered acceptable.

Certificates of analysis of the test substance not provided (check of the purity).

Applicant's response to the EFSA's comment "Apart of the stability period actually demonstrated for trinexapac acid, it is noted that only a single commodity (wheat grain or rape seed) instead of the two required by OECD 506 for the high starch and high oil content commodity groups was presented. Data are not sufficient to conclude on stability for the two categories":

In the storage stability study blank matrix was fortified with a known amount of trinexapac acid and stored deep frozen. The fortification was made at a level substantially higher than the LOQ. As a known chemical at a known concentration is used for the fortifications, confirmatory techniques are not relevant. Proving the LOQ is also not relevant as the amount of chemical is substantially higher. Together with procedural recoveries at every time point, Syngenta believes that the study was valid at the time and hence the storage stability data for trinexapac acid does in principle, cover the AIR representative uses of trinexapac ethyl on wheat grain and straw.

In order to claim stability for the whole groups of high oil and high starch crops further work is required.

For product renewal further storage stability work will be carried out to cover all the crop groups to current guidelines. Work is already in progress.

RMS LT agrees with the applicant that even though the fortification level is much higher than recommended in the OECD 506 and validation of the method is not according to recent guidelines, the study could be used for the current submission with the requirement for the applicant to provide a new study (to recent guidelines) and fully covering the length of storage in field trials.

Study 2

New storage stability study of metabolite CGA224439 in processed commodities

Reference:	**Watson G. (2017)** Trinexapac-ethyl: Storage Stability of Residues of metabolite CGA224439 (CPCA) in Crop Matrices Stored Frozen for up to Twelve Months. Final report and Final report amendment 1. Syngenta File No CA876_10009 (KCA 6.1 / 01)
Report No.:	RES-00030
Guideline:	Commission of the European Communities, Storage Stability of Residue Samples; 7032/VI/95 (Appendix H, rev.5), dated 22/7/97. OECD Guidelines for the Testing of Chemicals 506. Stability of Pesticide Residues in Stored commodities. (16 October 2007). Residue Chemistry Test Guidelines OPPTS 860.1380 Storage Stability Data, EPA 712-C-95-177, August 1996. Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test Material:	Common name Cyclopropanecarboxylic acid (CGA 1224439) Code name CSAA228610, CAS Number 1759-53-1
Batch No. and purity:	STBB9094V, 99.0%; certificated 27 January 2011, expiration 28 September 2016 A0373586, 99.9%; certificated 06 June 2016, expiration 20 February 2018
Test system:	The storage stability of CGA 224439 was investigated in wheat (grain, flour, bran, bread and beer), originating from either online shop, local supermarket or local

	health food shop. The stability of CGA224439 standard solutions was also assessed (3 and 6 months storage in acetonitrile). Homogenised samples were fortified with CGA 224439 at a nominal rate of 0.1 mg/kg (10 times the LOQ). Five sub-samples were immediately taken and analysed for residues of the fortified material. The remaining samples were stored deep frozen at approximately <-18 °C for up to 12 months with duplicate fortified sub-samples being taken at intervals (1, 3, 6 and 12 months) and analysed. Sub-samples (4.0 g) of the bulk homogenised control sample were weighed into glass jars (60 mL size with screw cap) and labelled with unique sample numbers. Individual samples of bread, bran, grain, flour and beer were fortified with a known amount of standard solution containing CPCA in acetonitrile at a rate of 0.10 mg/kg. Specimens were kept deep frozen at -18°C or below. The samples remained frozen throughout the study. The zero-time samples were extracted for analysis after fortification; three replicates of CPCA were analysed. Duplicate stored samples of CPCA were taken for analysis after one, three, six and twelve months of frozen storage. Untreated samples of all matrices were weighed into glass jars (60 mL with screw cap) and stored for use as control and procedural recovery samples at each analysis interval alongside the fortified stored samples for analysis. Spare fortified samples were prepared for any potential repeat analyses. Freshly fortified samples for each matrix were prepared in duplicate at each time point and were extracted alongside the stored fortified samples and stored control sample. Duplicate control subsamples were fortified with CPCA at a fortification level of 0.10 mg/kg. The freshly fortified samples served as procedural recovery samples to evaluate method performance and to correct the stored samples for procedural recovery. All sample jars were labelled with the study number, specimen reference number and storage interval.
Test conditions:	Stored at <-18^{0}C.
Analytical method:	Crop matrix samples were analysed for CPCA using analytical method GRM020.15A Extraction for Grain, Flour, Bran and Bread: Representative amounts of crop matrix (4.0 g) were weighed into glass jars (60 mL size with screw cap). Fortification of recovery samples was performed after hydration of the crop by adding 0.01M HCl in acetonitrile/water (50/50 v/v) (4 mL) to the matrix, samples were then left for 30 minutes at room temperature. 0.01M HCL in acetonitrile/water (50/50 v/v) was added (20 mL minus the volume of solvent added for hydration) and homogenised at high speed for 2 minutes. Jars were then placed in a centrifuge at 4000 rpm for 3 minutes. The supernatant was decanted into a clean glass vial (28 mL capacity). 0.01M HCL in acetonitrile/water (50/50 v/v) (20 mL) was added to the remaining sample and shaken briefly to break up and disperse the crop pellet. Samples were then homogenised at high speed for 2 minutes. The initial supernatant was added back into the extraction vessel containing the second extract and sample matrix. The extraction vessel was capped and mixed thoroughly. The jars were centrifuged at 4000 rpm for 3 minutes. Extraction for Beer: Representative amounts of crop matrix (4.0 g = 4 mL) were weighed into glass jars (60 mL size with screw cap). Fortification of recovery samples was performed at this point. 0.01M HCL in acetonitrile/water (50/50 v/v) (40 mL) was added to the sample and shaken thoroughly to mix. Liquid-liquid Partition and Sample Dilution: An aliquot of the extract (20 mL) was transferred to a glass vial (28 mL capacity) and

the contents of a Supel QuE Citrate (EN) tube i.e. magnesium sulphate (4 g), sodium chloride (1 g), sodium citrate dibasic sesquihydrate (0.5 g) and sodium citrate tribasic dehydrate (1 g) was added. The vial was caped and shaken by hand (approx. 10 seconds) to thoroughly mix. The vials were then centrifuged at 3000 rpm for 3 minutes. An aliquot (1 mL) was transferred to a glass culture tube containing 3 mL of acetonitrile. The tube was capped and shaken to mix then centrifuged at 3000 rpm for 3 minutes.

Derivatisation:

1 mL of the diluted acetonitrile extract was transferred to a clean glass culture tube taking care not to disturb the precipitate at the bottom of the original culture tube. 50 µL of derivatising solution (25 mM mix of 2-Hydrazinoquinoline, triphenylphosphine and 2,2'-Dipyridyl disulphide in acetonitrile) was added and incubated in a driblock heater (60°C, 1 hour). Samples were removed from the heater and allowed to cool to room temperature before evaporating the extracts to dryness under a gentle stream of air (dri-block, 40°C). Once dry the extracts were immediately reconstituted with 0.5 mL of deionised water with the aid of an ultrasonic bath. Extracts were transferred to an autosampler vial and analysed via LC-MS/MS

Note: All glass jars and tubes used were lined with aluminium foil to minimise matrix interferences. The use of plastic was avoided throughout the procedure for the same reason.

The final extracts were analysed for the CPCA derivative using an HPLC (Agilent Technologies) coupled to an API 4000 tandem mass spectrometer with electrospray ionisation.

Limit of quantification: 0.01 mg/kg

Method validation: Method GRM 020.15A was validated in study Watson G., 2016 "Validation of a method for the determination of residues of CPCA in processed commodity matrices by LC-MS/MS". The LC-MS/MS method GRM020.015A has been provided in support of generation of data for registration. It uses CPCA derivatization to CPCA HQ. The method is considered to remain specific to the analyte of interest if the derivatised species is specific to that analyte.' This is important for further considerations to completely address validation of method GRM020.15A for the determination of CPCA residues in cereal processed commodity matrices of grain, flour, bran, bread and beer taken into account its acceptably demonstrated accuracy, precision and repeatability at the LOQ of 0.01 mg/kg and over concentration ranges (please refer to Vol 3 CA B.5.1.2.1).

The validated/evaluated fortification levels were 0.01, 0.1 mg/kg. The recoveries are provided below:

Matrix	m/z transition	Fortification level (mg/kg)	Recovery (%)		RSD	n
			Range	Mean		
Beer	primary 228 → 160	0.01	82-97	87	6.7	5
		0.1	88-96	92	3.1	5
	confirmatory 228 → 69	0.01	82-98	87	7.4	5
		0.1	87-95	91	2.9	5
Bread	primary 228 → 160	0.01	61-70	66	5.9	5
		0.1	71-80	76	6.0	5
	confirmatory 228 → 69	0.01	63-74	68	6.7	5
		0.1	71-81	76	5.7	5
Bran	primary 228 → 160	0.01	70-86	76	8.6	5
		0.1	77-86	81	4.4	5

	confirmatory 228 → 69	0.01	61-93	79	15.2	5
		0.1	77-86	81	4.4	5
Grain	primary 228 → 160	0.01	73-91	82	9.3	5
		0.1	71-99	88	11.5	5
	confirmatory 228 → 69	0.01	73-94	84	10.9	5
		0.1	70-98	87	12.1	5
Flour	primary 228 → 160	0.01	81-92	85	5.7	5
		0.1	79-99	89	8.4	5
	confirmatory 228 → 69	0.01	81-93	84	5.9	5
		0.1	79-98	88	8.0	5

Results

The results of the zero-time, 1 month, 3 months, 6 months and 12 months analysis in all matrices for CPCA are documented in Table B.7.1.1-2.

Residues are presented as uncorrected for mean recoveries. The mean uncorrected and corrected residues, as well as the derived mean corrected recovery (%) are also presented. The time intervals are reported in months storage times (nominal) and in days (actual) for the stored samples.

There was no significant decrease (>30% as compared to the nominal fortification value) in the observed residue levels of CGA224439 in cereal grain, flour, bran, bread and beer when stored frozen at <-18oC for a period of 12 months. At the 6 month time point for bran a decrease of 32 % as compared to the nominal fortification value was observed but at the 12 month time point a decrease of 25% (corrected) was observed which is not deemed to be significant.

The stability of CGA224439 standard solutions prepared in acetonitrile was assessed after 3 and 6 months refrigerated storage. A 0.025 µg/mL CGA224439 standard solution (intermediate standard used to prepare an LOQ equivalent derivatised calibration standard) was shown to be stable for up to 3 months when stored refrigerated (2 - 8°C) in a clear glass vial but wasn't stable for 6 months when stored refrigerated (2 - 8°C) in a clear glass. Standards are deemed to be stable if the difference between a stored and freshly prepared standard is ≤ 10%. Results are presented in Table B.7.1.1-3.

Table B.7.1.1-2. Residues of CGA 224439 in cereal processed commodities

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Mean corrected stored sample residue (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (mg/kg)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)**
Wheat grain Samples fortified at 0.10 mg/kg	0 (0)	0.08322; 0.09152; 0.08848 (0.08774)	87.7	0.09442	94	0.09365 0.09220	93	2
	1 (30)	0.09677; 0.10072 (0.09874)	98.7	0.09425	94	0.10424 0.10530	105	4
	3 (104)	0.10181; 0.10669 (0.10425)	104.3	0.09779	98	0.10902 0.10418	107	9
	6 (183)	0.09805; 0.09222 (0.09513)	95.1	0.09303	93	0.11391 0.09062	102	6
	12 (365)	0.07807; 0.08049 (0.07928)	79.3	0.07200	72	0.11200 0.10823	110	0
Flour Samples fortified at 0.10 mg/kg	0 (0)	0.11268; 0.10229; 0.09467 (0.10321)	103.2	0.09942	99	0.10284 0.10479	104	1
	1 (37)	0.07600; 0.07561 (0.07581)	75.8	0,09819	98	0.07681 0.07760	77	4
	3 (104)	0.07828; 0.07308 (0.07568)	75.7	0.09333	93	0.08282 0.07936	81	3
	6 (183)	0.08133; 0.08845 (0.08489)	84.9	0.09304	93	0.09696 0.08552	91	2
	12 (364)	0.08641; 0.07936 (0.08288)	82.9	0.09240	92	0.08465 0.09476	90	1
Bread Samples fortified	0 (0)	0.09550; 0.08740; 0.09883 (0.09391)	93.9	0.10423	104	0.08656 0.09363	90	2

Annex B.7 (AS): Residue data

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Mean corrected stored sample residue (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (mg/kg)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)**
at 0.1 mg/kg	1 (30)	0.09592; 0.11597 (0.10595)	106	0.11420	114	0.09148 0.09406	93	5
	3 (104)	0.10625; 0.09554 (0.10089)	100.9	0.10113	101	0.10342 0.09611	100	2
	6 (195)	0.07062; 0.07057 (0.07060)	70.6	0.09707	97	0.07244 0.07301	73	1
	12 (363)	0.11006; 0.10814 (0.10910)	109.1	0.09948	99	0.10222 0.11712	110	1
Beer Samples fortified at 0.1 mg/kg	0 (0)	0.09871; 0.10130; 0.09409 (0.09803)	98.0	0.10145	101	0.09581 0.09746	97	2
	1 (30)	0.09223; 0.09028 (0.09125)	91.3	0.09173	92	0.10123 0.09773	99	2
	3 (104)	0.09221; 0.08461 (0.08841)	88.4	0.10588	106	0.08600 0.08100	83	2
	6 (183)	0.09601; 0.08039 (0.08820)	88.2	0.10308	103	0.08701 0.08412	86	1
	12 (364)	0.09758; 0.09229 (0.09493)	94.9	0.09207	92	0.10404 0.10217	103	0
Bran Samples fortified at 0.1 mg/kg	0 (0)	0.06722; 0.07502; 0.07512 (0.07245)	72.5	0.08718	87	0.07517 0.09104	83	2
	1	0.09783; 0.09662	97.2	0.09431	94	0.10132	103	3

Annex B.7 (AS): Residue data

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Mean corrected stored sample residue (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (mg/kg)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)**
	(30)	(0.09722)				0.10486		
	3 (105)	0.06886; 0.07808 (0.07347)	73.5	0.07579	76	0.09274 0.10116	97	2
	6 (184) (*196)	0.07283 (*0.05626); 0.06128 (*0.05416) (0.06706 (*0.05521))	67.1 (*55.2)	0.06671 (*0.06884)	67 (*69)	0.09288 (*0.08444) 0.10816 (*0.07598)	101 (*80)	4 (*2)
	12 (364)	0.09306; 0.07669 (0.08487)	84.9	0.07505	75	0.10885 0.11732	113	1

Please note: The above table has been produced using Microsoft Excel therefore due to rounding, slight discrepancies are present when calculations are carried out using the figures displayed

* Confirmation extraction

** Extracts was proven to be stable for at least 7 days stored at 2-8°C (please refer to RAR Vol 3 CA B.5.1.2.1)

A = [Mean Procedural Recovery Sample Residue (mg/kg) / Nominal Fortification Level (mg/kg)] x 100

B = [Mean Uncorrected Stored Sample Residue (mg/kg) / Mean Procedural Recovery (%)] x 100

C = Based on nominal fortification level = [Mean Corrected Stored Sample Residue (mg/kg) / Nominal Fortification Level (mg/kg)] x 100

Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected) being below <70% are coloured in green.

Table B.7.1.1-3. Stability of CGA224439 standard solutions

Analyte	Standard Reference	Analyte Concentration** (µg/mL)	Storage Period (Months)	Difference (%)
CGA224439*	OLD1-13.9.16	0.025	3	+3.0
	NEW1-13.9.16	0.025		
CGA224439*	OLD1-1.9.16	0.025	6	-48.6
	NEW1-1.9.16	0.025		

*determined as the HQ derivative

**concentration of the CGA224439 standard in acetonitrile prior to derivatisation. The derivatised standard concentration is 0.001 µg/mL

RMS comments and conclusion

Residues of CPCA (CGA 224439) in cereal processed matrices (cereal grain, flour, bread, bran and beer) can be considered as stable for at least 12 months when stored at -18°C. Although at the 6 month time point for bran a decrease of 32.9 % as compared to the nominal fortification value was observed, additional confirmation extraction performed showed even worse results (44.8%). No explanations were provided nor in the report nor from the applicant. As at the 12 month time point recovery showed good results (decrease of only 15%), residues of CPCA (CGA 224439) were considered stable for 12 months. Samples from extraction to analysis were stored for up to 9 days, conditions during this period not stated in the report, but extracts are considered to be stable for up to 7 days stored refrigerated (2 - 8°C) (please refer to Vol 3 CA B.5.1.2.1).

CGA224439 standard solutions prepared in acetonitrile have been shown to be stable for up to 3 months when stored refrigerated (2 - 8°C) in a clear glass vial.

Samples from wheat and barley processing studies were stored up to 6 months for barley processed fractions (Watson G., 2016) and up to 7 months for wheat processed fractions (Watson G., 2016a), thus storage period in these studies is considered fully covered by storage stability data.

Study is considered suitable for evaluation.

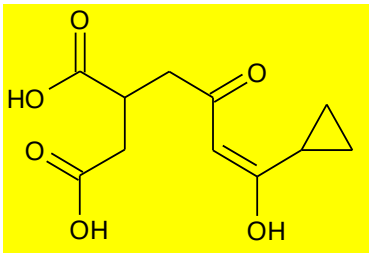
Deviations from OECD 506:

None, although it was not mentioned in the report if samples were stored in the dark and not specified cereals species form which flour, bran, bread and beer were made. Storage conditions for extracts not stated.

Study 3

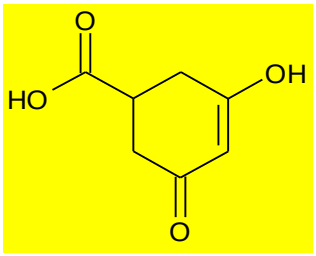
New storage stability study of metabolites CGA313458 and CGA 113745 in processed commodities

Reference:	**Langridge G. (2017)** Trinexapac-ethyl: Storage Stability of Residues of metabolites CGA113745 and CGA313458 in Crop Matrices Stored Frozen for up to Twelve Months. Final report. (KCA 6.1 / 02)
Report No.:	CEMR-7358
Guideline:	Commission of the European Communities, Storage Stability of Residue Samples; 7032/VI/95 (Appendix H, rev.5), dated 22/7/97. OECD Guidelines for the Testing of Chemicals 506. Stability of Pesticide Residues in Stored commodities. (16 October 2007). Residue Chemistry Test Guidelines OPPTS 860.1380 Storage Stability Data, EPA 712-C-95-177, August 1996. Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test Material:	IUPAC name: 2-(4-cyclopropyl-2,4-dioxo-butyl)-succinic acid Code name: CGA 313458, CAS Number: not in registry



IUPAC name: 3-hydroxy-5-oxocyclohex-3-enecarboxylic acid

Code name: CGA 113745, CAS Number: 56066-20-7



Batch No. and purity:	CGA 313458: DAH-XXXV-15, 98.2% and 96.1%; certificated 27 Mar 2014 and 27 Mar 2017, expiration 31 Mar 2016 and 31 Mar 2018 (certificates of analysis included in the report) CGA 113745: MES 420/1, 99%; certificated 20 Aug 2015, expiration 31 Aug 2017(certificate of analysis included in the report)
Test system:	The storage stability of CGA313458 and CGA113745 was investigated in brewing and baking samples (wheat grain, flour, bran, beer and bread) stored under frozen storage conditions for up to twelve months for CGA313458 and six months for CGA113745. Samples originated from either online health food supplies or organic farm. The stability of CGA313548 and CGA113475 in working and stock standard solutions stored refrigerated at between 2 - 8 °C for at least 123 and 179 days respectively was also assessed. Wheat grain and bread samples were homogenised using a robot coupe with dry ice. No preparation was required for the flour, bran and beer samples. One untreated sample was analysed at each time point to ensure that no residues of CGA313458 or CGA113745 were present above 30% of the limit of quantification. Individual stock solutions of CGA313458 and CGA113745 at 200 µg/mL were prepared in acetonitrile. Fortification standards were prepared by serial dilution of the stock solutions using ultra-pure water. Significant matrix effects (>20%) were observed during the method validation and therefore matrix-matched standards were used for sample quantification. Individual samples of each matrix were fortified with a known amount of each standard solution containing CGA313458 or CGA113745 in ultra-pure water at a rate of 0.10 mg/kg. Each sample was left to stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction or sealed and transferred to the freezer to simulate conditions under which actual samples are stored prior to their analysis. Triplicate samples of the each matrix were analysed for CGA313458 and CGA113745 at zero-days. Duplicate samples were analysed for CGA313458 at one

month, 3 months, 6 months, 8 months and 12 months. Duplicate samples were analysed for CGA113745 at one month, 3 months and 6 months.

All sample bottles were labelled with the study number, specimen reference number, matrix type and storage interval

Test conditions: Stored at <-18^{0}C.

Analytical method: Residues of **CGA313458** were analysed according to method **GRM020.013A**, "Trinexapac Ethyl – Analytical Method GRM020.013A for the Determination of the Metabolite CGA313458 in Brewing and Baking Commodities". The limit of quantification (LOQ) of the method was 0.01 mg/kg.

10 g sub samples of brewing or baking fractions were extracted by sequential homogenisation with 80/20 v/v acetonitrile/water and 50/50 v/v acetonitrile/water. An aliquot of the combined extracts equivalent to 0.2 g (2 mL) was evaporated to remove the acetonitrile. The sample was diluted with ultra-pure water and the pH adjusted to pH 7 – 9 with dilute ammonium hydroxide solution. Samples were partitioned twice with ethyl acetate to remove co-extractives then the aqueous samples were filtered through an Oasis HLB SPE cartridge. Alternatively, samples may be analysed directly from the primary extracts without any further sample clean-up where there is sufficient instrument sensitivity.

Residues of **CGA113745** were analysed according to method **GRM020.014A**, "Trinexapac Ethyl – Analytical Method GRM020.014A for the Determination of the Metabolite CGA113745 in Brewing and Baking Commodities". The limit of quantification (LOQ) of the method was 0.01 mg/kg.

For liquid commodities, 1.0 g sub samples of liquid brewing fractions were filtered through a Chromabond (EC) C18 SPE cartridge before being diluted with ultra-pure water.

For non-liquid commodities, 10 g sub samples were extracted twice by homogenisation with 0.2% ammonia in ultra-pure water. A 4 mL aliquot of the combined sample is acidified, mixed and centrifuged and 2 mL of the supernatant (equivalent to 0.2 g matrix) is taken through an Oasis WCX SPE cartridge procedure to remove endogenous co-extractives.

Final determination was carried out by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

Limit of quantification: 0.01 mg/kg

Method validation: Methods GRM 020.13A and GRM 020.14A were validated in study Langridge G., 2016 "Trinexapac-ethyl – Validation of Syngenta Methods GRM020.013A and GRM020.14A for the Determination of Residues of Trinexapac-ethyl Metabolites CGA313458 & CGA113745 in process fractions by LC-MS/MS".

Method 020.13A was developed and validated for CGA313458 determination in processed food: beer, bread, bran, wheat grain and flour. The validated fortification levels were 0.01, 0.1 mg/kg. The recoveries are provided below:

Matrix	m/z transition	Fortification level (mg/kg)	Recovery (%)		RSD	n
			Range	Mean		
Beer	primary 241.0 → 69.0	0.01	100-106	103	2.2	5
		0.1	97-105	100	3.2	5
	confirmatory 241.0 → 83.0	0.01	93-99	97	2.6	5
		0.1	96-102	99	2.8	5
Bread	primary	0.01	95-110	103	5.9	5

	241.0 → 69.0	0.1	76-82	79	2.9	5
	confirmatory 241.0 → 83.0	0.01	71-77	74	3.0	5
		0.1	72-87	79	6.9	5
Bran	primary 241.0 → 69.0	0.01	61-82	74	10.7	5
		0.1	75-107	93	13.6	5
	confirmatory 241.0 → 83.0	0.01	92-108	103	7.2	5
		0.1	69-88	75	10.5	5
Grain	primary 241.0 → 69.0	0.01	94-107	101	6.3	5
		0.1	100-109	106	3.3	5
	confirmatory 241.0 → 83.0	0.01	113-118	115	1.9	5
		0.1	100-109	104	3.4	5
Flour	primary 241.0 → 69.0	0.01	94 - 101	98	2.8	5
		0.1	78 - 83	80	2.4	5
	confirmatory 241.0 → 83.0	0.01	82 - 87	85	3.3	5
		0.1	76 - 81	79	2.4	5

Method 020.14A was developed and validated for **CGA113745** determination in processed food such as beer and bread. The applicant informed that the method needs to be further developed for bran, wheat grain and flour due to low extractability in these matrices. The validated fortification levels were 0.01, 0.1 mg/kg. The recoveries in bread and beer are provided below:

Matrix	**m/z transition**	**Fortification level (mg/kg)**	**Recovery (%)**		**RSD**	**n**
			Range	**Mean**		
Beer	primary 155.0 → 69.0	0.01	101-107	104	2.2	5
		0.1	80-84	82	1.8	5
	confirmatory 155.0 → 57.0	0.01	70-101	81	15.8	5
		0.1	76-83	80	3.3	5
Bread	primary 155.0 → 69.0	0.01	63-87	75	11.4	5
		0.1	73-88	81	7.7	5
	confirmatory 155.0 → 57.0	0.01	87-109	98	8.7	5
		0.1	86-99	92	5.5	5

Results

The results of the zero-time, 1 month, 3 months, 6 months, 8 months and 12 months analysis in all matrices for metabolite CGA 313458 are documented in Table B.7.1.1-4.

The results of the zero-time, 1 month, 3 months and 6 months analysis in all matrices for metabolite CGA 113745 are documented in Table B.7.1.1-5.

Residues are presented as uncorrected for mean recoveries. The mean uncorrected and corrected residues, as well as the derived mean corrected recovery (%) are also presented. The time intervals are reported in months storage times (nominal) and in days (actual) for the stored samples.

The residues of CGA313458 showed no significant decrease (>30% as compared to the zero-time value) in wheat grain, flour, beer and bread after storage deep frozen for up to twelve months and in bran after storage deep frozen for up to six months.

The residues of CGA113745 showed significant decrease (>30% as compared to the zero-time value) in wheat grain, flour, bran, beer and bread after storage deep frozen.

The analyses of the control samples showed that no residues of CGA313458 or CGA113745 were present above 30% of the limit of quantification.

The stability of CGA313548 and CGA113475 in working and stock standard solutions stored refrigerated at between 2 - 8 °C for at least 123 and 179 days respectively was assessed. The stored standard solutions were within ± 20 % of the freshly prepared solutions, indicating storage stability under the storage conditions used, and results are presented in table B.7.1.1-6 and B.7.1.1-7 for CGA 313458 and CGA 113745 respectively.

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Table B.7.1.1-4. Residues of CGA 313458 in cereal processed commodities

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (%)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
Wheat grain Samples fortified at 0.10 mg/kg	0 (0)	0.0865, 0.0799, 0.0772 (0.0812)	81.2	0.1027, 0.0949, 0.0917 (0.0965)	96	84, 85	84	1
	1 (28)	0.0761, 0.0691 (0.0726)	72.6	0.0963, 0.0875 (0.0919)	92	77, 81	79	0
	3 (98)	0.0913, 0.0881 (0.0897)	89.7	0.1103, 0.1065 (0.1084)	108	96, 69	83	2
	6 (182)	0.0786, 0.0701 (0.0743)	74.3	0.0783, 0.0698 (0.0741)	74	94, 107	100	1
	8 (255)	0.0502, 0.0650 (0.0576)	57.6	0.0648, 0.0839 (0.0743)	74	84, 71	77	10
	12 (372)	0.0690, 0.0713 (0.0701)	70.1	0.0893, 0.0924 (0.0908)	91	76, 79	77	1
Flour Samples fortified at 0.10 mg/kg	0 (0)	0.0992, 0.1051, 0.1028 (0.1024)	102.4	0.1084, 0.1149, 0.1122 (0.1118)	112	87, 96	92	1
	1 (32)	0.0700, 0.0808 (0.0754)	75.4	0.0915, 0.1057 (0.0986)	99	76, 77	76	0
	3 (102)	0.0735, 0.0730 (0.0733)	73.3	0.0924, 0.0918 (0.0921)	92	76, 83	80	1

Annex B.7 (AS): Residue data

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (%)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
	6 (188)	0.0657, 0.0498 (0.0577)	57.7	0.0890, 0.0675 (0.0783)	78	74, 73	74	2
	8 (264)	0.0514, 0.0575 (0.0544)	54.4	0.0721, 0.0807 (0.0764)	76	72, 70	71	2
	12 (372)	0.0783, 0.0809 (0.0796)	79.6	0.1018, 0.1052 (0.1035)	104	75, 79	77	0
Bran Samples fortified at 0.1 mg/kg	0 (0)	0.0872, 0.0866, 0.0859 (0.0866)	86.6	0.1013, 0.1006, 0.0998 (0.1006)	101	88, 84	86	1
	1 (29)	0.0785, 0.0742 (0.0763)	76.3	0.0853, 0.0805 (0.0829)	83	91, 93	92	1
	3 (101)	0.0705, 0.0729 (0.0717)	71.7	0.0862, 0.0891 (0.0877)	88	85, 78	82	1
	6 (185)	0.0682, 0.0554 (0.0618)	61.8	0.0802, 0.0652 (0.0727)	73	78, 92	85	1
	12 (380)	0.0528, 0.0539 (0.0533)	53.3	0.0671, 0.0685 (0.0678)	68	76, 81	79	0
	12 (389) (repeat)	0.0397, 0.0431 (0.0414)	41.4	0.0481, 0.0522 (0.0501)	50	83, 82	83	0
Beer Samples	0	0.0981, 0.0980, 0.0987	98.2	0.0968, 0.0967, 0.0974	97	104, 99	101	0

Annex B.7 (AS): Residue data

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (%)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
fortified at 0.1 mg/kg	(0)	(0.0982)		(0.0970)				
	1 (31)	0.0950, 0.1030 (0.0990)	99	0.0889, 0.0964 (0.0926)	92.6	109, 105	107	0
	3 (101)	0.0936, 0.0981 (0.0959)	95.9	0.0809, 0.0848 (0.0828)	82.8	118, 113	116	2
	6 (187)	0.0927, 0.0922 (0.0924)	92.4	0.0861, 0.0856 (0.0858)	85.8	107, 109	108	0
	8 (257)	0.0927, 0.0922 (0.0787)	78.7	0.0795, 0.0761 (0.0778)	77.8	101, 102	101	0
	12 (375)	0.0927, 0.0922 (0.0806)	80.6	0.0819, 0.0779 (0.0799)	79.9	100, 102	101	0
Bread Samples fortified at 0.1 mg/kg	0 (14)**	0.0956, 0.0800, 0.0841 (0.0866)	86.6	0.1325, 0.1108, 0.1165 (0.1200)	120	73, 71	72	1
	1 (29)	0.0741, 0.0812 (0.0777)	77.7	0.0895, 0.0980 (0.0938)	93.8	76, 89	83	1
	3 (101)	0.0946, 0.0958 (0.0952)	95.2	0.0858, 0.0869 (0.0864)	86.4	109, 111	110	1
	6 (186)	0.0737, 0.0777 (0.0757)	75.7	0.0947, 0.0999 (0.0973)	97.3	73, 82	78	4
	8 (255)	0.0527, 0.0591 (0.0559)	55.9	0.0705, 0.0790 (0.0747)	74.7	75, 75	75	5

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (%)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
	12 (380)	0.0716, 0.0729 (0.0722)	72.2	0.0855, 0.0871 (0.0863)	86.3	95, 73	84	0

Please note: The above table has been produced using Microsoft Excel therefore due to rounding, slight discrepancies are present when calculations are carried out using the figures displayed

* Extracts was proven to be stable for at least 6 days stored at 2-8°C (please refer to RAR Vol 3 CA B.5.1.2.1)

** Day zero analysis was repeated using a spare set already fortified

A = [Mean Procedural Recovery Sample Residue (mg/kg) / Nominal Fortification Level (mg/kg)] x 100

B = [Mean Uncorrected Stored Sample Residue (mg/kg) / Mean Procedural Recovery (%)] x 100

C = Based on nominal fortification level = [Mean Corrected Stored Sample Residue (mg/kg) / Nominal Fortification Level (mg/kg)] x 100

Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected) being below <70% are coloured in green.

Table B.7.1.1-5. Residues of CGA 113745 in cereal processed commodities

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (mg/kg)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
Wheat grain Samples fortified at 0.10 mg/kg	0 (0)	0.0978, 0.0909, 0.0832 (0.0906)	90.6	0.0911, 0.0847, 0.0775 (0.0844)	84.4	111, 104	107	0
	1 (35)	0.0191, 0.0169 (0.0180)	18.0	0.0192, 0.0170 (0.0181)	18.1	94, 105	99	1

Annex B.7 (AS): Residue data

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (mg/kg)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
	3 (106)	0.0128, 0.0121 (0.0124)	12.4	0.0164, 0.0155 (0.0160)	16.0	80, 75	78	0
	6 (177)	0.0102, 0.0121 (0.0112)	11.2	0.0136, 0.0161 (0.0149)	14.9	69, 82	75	0
Flour Samples fortified at 0.10 mg/kg	0 (0)	0.1031, 0.0742, 0.1038 (0.0937)	93.7	0.1005, 0.0723, 0.1011 (0.0913)	91.3	96, 109	103	0
	1 (35)	0.0110, 0.0227 (0.0169)	16.9	0.0115, 0.0239 (0.0177)	17.7	94, 96	95	1
	3 (106)	0.0155, 0.0174 (0.0165)	16.5	0.0181, 0.0203 (0.0192)	19.2	87, 85	86	0
	6 (177)	0.0128, 0.0112 (0.0120)	12.0	0.0159, 0.0139 (0.0149)	14.9	86, 75	80	0
Bran Samples fortified at 0.1 mg/kg	0 (0)	0.0693, 0.0685, 0.0653 (0.0677)	67.7	0.1083, 0.1071, 0.1021 (0.1059)	105.9	66, 62	64	2
	1 (39)	0.0052, 0.0051 (0.0052)	5.2	0.0075, 0.0072 (0.0074)	7.4	72, 68	70	0
	3 (106)	0.0069, 0.0061 (0.0065)	6.5	0.0087, 0.0077 (0.0082)	8.2	68, 90	79	0

Annex B.7 (AS): Residue data

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (mg/kg)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
	6 (175)	0.0000, 0.0000 (0.0000)	0.0	0.0000, 0.0000 (0.0000)	0.0	77, 77	77	1
Beer Samples fortified at 0.1 mg/kg	0 (0)	0.0913, 0.0928, 0.1001 (0.0947)	94.7	0.1048, 0.1065, 0.1149 (0.1087)	108.7	85, 89	87	0
	1 (31)	0.0223, 0.0263 (0.0243)	24.3	0.0222, 0.0262 (0.0242)	24.2	102, 99	100	0
	2 (66)	0.0243, 0.0271 (0.0257)	25.7	0.0233, 0.0259 (0.0246)	24.6	106, 103	105	0
	3 (106)	0.0170, 0.0189 (0.0180)	18.0	0.0242, 0.0270 (0.0256)	25.6	71, 70	71	0
	6 (177)	0.0133, 0.0131 (0.0132)	13.2	0.0142, 0.0141 (0.0142)	14.2	94, 92	93	1
Bread Samples fortified at 0.1 mg/kg	0 (0)	0.0995, 0.1008, 0.1069 (0.1024)	102.4	0.1055, 0.1069, 0.1134 (0.1086)	108.6	95, 95	94	1
	1 (35)	0.0220, 0.0254 (0.0237)	23.7	0.0257, 0.0297 (0.0277)	27.7	83, 88	86	1
	2 (64)	0.0160, 0.0191 (0.0176)	17.6	0.0220, 0.0261 (0.0240)	24.0	79, 67	73	1
	3 (106)	0.0221, 0.0211 (0.0216)	21.6	0.0253, 0.0242 (0.0248)	24.8	81, 93	87	4
	6	0.0173, 0.0195	18.4	0.0194, 0.0218	20.6	94, 85	89	0

Crop commodity	Storage period months (days)	Residue level in freezer storage stability sample (mg/kg) (range plus mean)	Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected)	Corrected stored sample residue (range plus mean) (mg/kg) (B)	Mean corrected stored sample recovery % of nominal (C)	Procedural recovery for freshly spiked control sample (mg/kg)	Mean procedural recovery for freshly spiked control sample (%) (A)	Extraction to analysis interval (days)*
	(176)	(0.0184)		(0.0206)				

Please note: The above table has been produced using Microsoft Excel therefore due to rounding, slight discrepancies are present when calculations are carried out using the figures displayed

* For both matrices (beer and bread) validated for the determination of CGA113745 residues, final sample extracts were shown not to be stable after storing for a period of at least 6 days (GRM020.014A). Therefore these samples should be analysed as soon as possible after extraction (please refer to RAR Vol 3 CA B.5.1.2.1)

A = [Mean Procedural Recovery Sample Residue (mg/kg) / Nominal Fortification Level (mg/kg)] x 100

B = [Mean Uncorrected Stored Sample Residue (mg/kg) / Mean Procedural Recovery (%)] x 100

C = Based on nominal fortification level = [Mean Corrected Stored Sample Residue (mg/kg) / Nominal Fortification Level (mg/kg)] x 100

Mean residue level in freezer storage stability sample (% of nominal spiking level, uncorrected) being below <70% are coloured in green.

Table B.7.1.1-6. Stability of CGA 313458 standard solutions

CGA 313458 Primary transition (m/z 241.0→69.0)				
Standard Concentration (ng/mL)	**Stored Standard Response (prepared on 5th January 2017)[1]**	**Fresh Standard Response (prepared on 8th May 2017)[2]**	**% Difference**	**Storage Period Shown**
10.0	1001289	1094983	9.4	123 days
10.0	975310	1025113	5.1	
10.0	1076152	1061363	-1.4	
Mean:			4.4	
Standard Concentration (ng/mL)	**Stored Standard Response (prepared on 5th January 2017)[3]**	**Fresh Standard Response (prepared on 8th May 2017)[4]**	**% Difference**	**Storage Period Shown**
10.0	975744	1094983	12.2	123 days
10.0	913920	1025113	12.2	

10.0	1010226	1061363	5.1	
Mean:			9.8	

1 - Prepared by serial dilution in ultra-pure water from a stored 200 µg/mL solution in acetonitrile.

2 - Prepared by serial dilution in ultra-pure water from a fresh 200 µg/mL solution in acetonitrile.

3 - Prepared by serial dilution in ultra-pure water from a stored 1.0 µg/mL solution in ultra-pure water.

4 - Prepared by serial dilution in ultra-pure water from a fresh 200 µg/mL solution in acetonitrile.

Table B.7.1.1-7. Stability of CGA 113745 standard solutions

CGA 113745 Primary transition (m/z 155.0→69.0)				
Standard Concentration (ng/mL)	**Stored Standard Response (prepared on 8th August 2016)[1]**	**Fresh Standard Response (prepared on 3rd February 2017)[2]**	**% Difference**	**Storage Period Shown**
10.0	890057	875535	-1.6	179 days
10.0	902897	900158	-0.3	
10.0	923501	910969	-1.4	
Mean:			-1.1	
Standard Concentration (ng/mL)	**Stored Standard Response (prepared on 8th August 2016)[3]**	**Fresh Standard Response (prepared on 3rd February 2017)[4]**	**% Difference**	**Storage Period Shown**
10.0	765565	875535	14.4	179 days
10.0	799124	900158	12.6	
10.0	815251	910969	11.7	
Mean:			12.9	

1 - Prepared by serial dilution in ultra-pure water from a stored 200 µg/mL solution in acetonitrile.

2 - Prepared by serial dilution in ultra-pure water from a fresh 200 µg/mL solution in acetonitrile.

3 - Prepared by serial dilution in ultra-pure water from a stored 1.0 µg/mL solution in ultra-pure water.

4 - Prepared by serial dilution in ultra-pure water from a fresh 200 µg/mL solution in acetonitrile.

Conclusion

Residues of CGA313458 have been shown to be stable in wheat grain, flour, beer and bread when stored deep frozen at <-18oC for up to twelve months and in bran when stored deep frozen at <-18oC for up to six months.

Residues of CGA113745 have been shown not to be stable in wheat grain, flour, bran, beer and bread when stored deep frozen at <-18°C.

These crop commodities are representative of those specified in EU and OECD guidelines. Analytical method GRM020.14A for CGA113745 gave poor chromatography during the processing study so development work was carried out and the chromatography was improved. The improved chromatography was consequently used in the storage stability study to analyse for CGA113745 in processed matrices. CGA113475 was shown to be unstable in the presence of crop matrices - degrading to only 20% of the initial amount over 30 days.

Thus it can be assumed that inaccurate levels of CGA113745 were found in both the pre-processed incurred grain samples and the processed commodities due to degradation in storage and poor chromatography. Therefore any data regarding residue levels of CGA113745 in the processing studies on wheat and barley should be disregarded.

RMS comments and conclusion

Residues of CGA 313458 in cereal processed matrices (cereal grain, flour, bread, bran and beer) can be considered as stable for at least 12 months for grain and beer, 6 months for bran and bread and 3 months for flour when stored at -18°C. It should be noted, that recovery in grain after 8 months storage was 57.6% and 70.1 % after 12 months. It was considered acceptable, as extract before analysis was stored for 10 days, although extracts were proven to be stable for at least 6 days stored at 2-8°C. RMS considers this was degradation during the time from extraction to analysis, but not during freezing storage, taking also into account that the conditions from extraction to analysis were not specified in the report.

Samples for metabolite CGA 313458 from wheat and barley processing studies were stored up to 8 months for barley processed fractions (Langridge G., 2016) and up to 5 months for wheat processed fractions (Watson G., 2016a), thus storage period in these studies is considered covered by storage stability data in all matrices except flour, bran and bread.

CGA 313458 standard solutions prepared in acetonitrile have been shown to be stable for up to 123 days when stored refrigerated (2 - 8°C).

Residues of CGA 113745 in cereal processed matrices (cereal grain, flour, bread, bran and beer) are not stable. Only up to 24% were recovered after 1 month of freezer storage (-18°C).

Extracts in this study were analysed 0-4 days after extraction. For both matrices (beer and bread) validated for the determination of CGA113745 residues, final sample extracts were shown not to be stable after storing for a period of at least 6 days (GRM020.014A). Therefore these samples should be analysed as soon as possible after extraction.

Samples for metabolite CGA 113745 from wheat and barley processing studies were stored up to 11.5 months for barley processed fractions (Langridge G., 2016) and up to 12 months for wheat processed fractions (Watson G., 2016a), thus storage period in these studies is not covered by storage stability data. Any data regarding residue levels of CGA113745 in the processing studies on wheat and barley should be disregarded and have been struck through.

CGA 113745 standard solutions prepared in acetonitrile have been shown to be stable for up to 179 days when stored refrigerated (2 - 8°C).

Study is considered suitable for evaluation.

Applicant's position:

Analytical method GRM020.14A for CGA113745 gave poor chromatography during the processing study so development work was carried out and the chromatography was improved. The improved chromatography was used in the storage stability study to analyse for CGA113745 in processed matrices and showed that CGA113475 was unstable in the presence of crop matrices - degrading to only 20% of the initial amount over 30 days. Thus it can be assumed that inaccurate levels of CGA113745 were found in both the pre-processed incurred grain samples and the processed commodities due to degradation in storage and poor chromatography including possible co-elution with other components.

Deviations from OECD 506:

It was not mentioned in the report if samples were stored in the dark and not specified cereals species form which flour, bran, bread and beer were made. Storage conditions for extracts not stated.

B.7.1.2 Stability of residues in animal products

Study 1

EU reviewed storage stability study in animals

Reference:	**Sack St. (2000)** Residues of CGA 179500 in milk, blood and tissues (muscle, fat, liver, and kidney) of dairy cattle resulting from feeding of CGA 179500 (metabolite of trinexapac-ethyl, CGA 163935) at three dose levels. Final report (~~KCA 6.1 / 02; KCA 6.4.2 / 01~~ KIIA 6.3.2.2 / 01 & KIIA 6.4.2 / 01)
Report No.:	330/99
Guideline:	Directive 91/414/EC, 7031/VI/95, appendix G. Directive 96/68/EC, L277
GLP:	Yes

Previous evaluation: DAR (2003)

Material and methods:

Test Material: Trinexapac acid (CGA 179500)

Batch No. and purity: BPS 520/103 99%

Test system: **Milk** of the control cow of days 0,1, 2, 3, 5, 8, 12 was used. Closed vials were thawed, then shaked. About 5 mL liquid removed from each sample and combined. Sub-samples of 2 g were weighed out and each fortified with 0.1 mL of an aqueous solution containing 0.001 mg analyte/mL and 0.5 vol.% acetonitrile to get a fortification level of 0.05 mg/L.

Tissues (round muscle, omental fat, liver, kidney) of the control cow were weighed out and fortified with a solution of 0.01 mg analyte/mL in acetonitrile+ethanol (5v+95v). The volume added to sub-samples of 10 g was 0.2 mL (0.1 mL for fat), to get a fortification level of 0.2 mg/kg (0.1 mg/kg for fat).

Blood of the control cow was fortified. Closed vials were thawed, then shaked. Sub-samples of exactly 5 g liquid were fortified with 0.1 mL of a solution of 0.005 mg analyte/mL in acetonitrile+water (2.5v+97.5v) to get a fortification level of 0.1 mg/kg.

Of each kind, ten fortified specimens were prepared and stored at or below -18^{0}C. Five sub-specimens were used for analysis, the other five served as reserve.

Test conditions: Stored at -18^{0}C

Analytical method: Analytical method REM 137.12 was used for the analysis. It was modified for the determination with LC-LC-MS/MS.

Procedural recoveries: The performance of the method was checked with each series of specimen analyses by performing procedural recovery tests.

The results of the storage stability tests were corrected for the procedural recoveries.

Limit of quantification: 0.005 mg/kg for milk

0.02 mg/kg for tissues and blood

Method validation: The performance of the method REM 137.12 was checked with each series of specimen analyses by performing procedural recovery tests. ~~The Method REM 137.12 was validated prior to sample analysis~~. The validated/evaluated fortification levels were 0.02 and 0.2 mg/kg for tissues; 0.01 and 0.1 mg/kg for blood; 0.005 and 0.05 mg/kg for milk. ~~The average recoveries (%) were:~~

~~Liver: 82%; Kidney: 95%; Muscle round: 91%; Tenderloin: 81%; Diaphragm: 81%; Fat perirenal: 89%; Fat omental: 81%; Blood: 95%; Milk: 104%;~~

~~Please refer to Vol 3 CA B.5.1.2.2 (with reference to the DAR Annex IIA.4.2.1 Method 2) for details of evaluation of analytical method REM 137.12.~~

The recoveries are provided below:

Matrix	Fortification level (mg/kg)	Recovery (%)		n
		Range	Mean	
Liver	0.02	80	80	1
	0.2	83	83	1
Kidney	0.02	102	102	1
	0.2	88	88	1

Muscle round	0.02	97	97	1
	0.2	85	85	1
Tenderloin	0.02	80	80	1
	0.2	81	81	1
Diaphragm	0.02	80	80	1
	0.2	82	82	1
Fat perirenal/omental	0.02	103/71	103/71	1/1
	0.2	75/91	75/91	1/1
Blood	0.01	93	93	1
	0.1	96	96	1
Milk	0.005	97, 88, 92, 92, 105, 105, 100, 103, 111, 120, 121, 118	104	12
	0.05	103, 103, 101, 105, 108, 103, 102, 105, 100, 102, 103	103	11

Results

Parts of each control substrate were fortified with known amounts of CGA 179500 and stored at or below minus 18°C to check the stability of the analyte in the investigated substrates. The storage period was chosen to cover the range between arrival of the specimens and analysis. The results are given in Table B.7.1.2-1. The average recovery in percent of the theoretical initial value was found to be 82 % for muscle, 89 % for milk, 85 % for kidney, 96 % for fat and 102 % for blood. The initial values were not determined. The results (% of nominal spiking level) are presented both as corrected and uncorrected for procedural recovery values. ~~were corrected for the procedural recoveries~~.

Table B.7.1.2 - 1. Residues of CGA 179500 muscle, liver, kidney, fat, milk and blood

Crop commodity	Weight fortified (g)	Storage period (months)	Residue level in freezer storage stability sample (mg/kg)*	Residue level in freezer storage stability sample (% of nominal spiking level) (range plus mean)** [range plus mean, uncorrected]***	Procedural recovery for freshly spiked control sample (%)
Muscle Samples fortified at 0.2 mg/kg	10	3 (91 d.)	0.172, 0.172, 0.154, 0.152, 0.174	86, 86, 77, 76, 87 (82) [70.5, 70.5, 63.1, 62.3, 71.3 (67)]	83, 81
Liver Samples fortified at 0.2 mg/kg	10	3 (94 d.)	0.168, 0.166, 0.169, 0.174, 0.172	84, 83, 85, 87, 86 (85) [75.6, 74.7, 76.5, 78.3, 77.4 (77)]	85, 95
Kidney Samples fortified at 0.2 mg/kg	10	3 (95 d.)	0.175, 0.171, 0.173, 0.163, 0.163	87, 86, 87, 81, 82 (85) [77, 76.1, 77, 71.7, 72.6 (75)]	89, 88

Fat omental Samples fortified at 0.1 mg/kg	10	3 (101 d.)	0.0961, 0.0956, 0.0982, 0.0932, 0.0954	96, 96, 98, 93, 95 (96) [93.6, 93.6, 95.6, 90.7, 92.6 (94)]	98, 97
Milk Samples fortified at 0.05 mg/kg	2	4 (121 d.)	~~89, 86, 90, 88, 93~~ 0.0445, 0.0430, 0.0451, 0.0440, 0.0465	89, 86, 90, 88, 93 (89) [96.6, 93.3, 97.7, 95.5, 100.9 (97)]	108, 109
Blood Samples fortified at 0.1 mg/kg	5	3 (83 d.)	102, 99, 104, 104, 101 0.102, 0.0 99, 0.104, 0.104, 0.101	102, 99, 104, 104, 101 (102) [106.1, 103, 108.2, 108.2, 105 (106)]	99, 109

* - This data was extracted by the applicant from the raw data package but was not reported

** - The results of the storage stability tests were corrected for the procedural recoveries.

*** - Recoveries were corrected, but the procedural recoveries are available and a back calculation can be performed (uncorrected recovery = corrected recovery x procedural recovery / 100). The average uncorrected recoveries are calculated by the applicant.

RMS comments and conclusion

Residues of trinexapac acid (CGA 179500) in muscle, liver, kidney, fat and blood can be considered as stable for at least 3 months and in milk for at least 4 months when stored at -18°C. Storage stability in animal matrices was tested as part of the feeding study. This study was performed prior to the adoption of the OECD guideline 506 for stability of pesticide residues in stored commodities. Ten fortified specimens were prepared for each matrix and stored at or below -18°C. Five sub-specimens were used for analysis, the other five served as reserve. Each series of analyses was accompanied by two freshly fortified specimens to check the procedural recoveries. The average recovery for muscle is below the 70 % (67 %), the range of measurements is around 70% (three values >70 and two values <70%). It is explained by the applicant that recoveries for both stored commodities and procedural recoveries are similar and both low, which suggests that there may not be a decline on storage. The corrected recovery for muscle is above 70%; this indicates that the “low” uncorrected recovery is due to the analytical method and is not a decline on storage. Despite the minor deficiencies the RMS considers the stability study as sufficient to cover the proposed uses of this application.

Method REM 137.12 was validated in study Sack, 1995a and ILV study Gasser, 2001 (Please refer to Vol 3 CA B.5.1.2.2)

Method 137.12 was validated for the determination of the metabolite CGA 179500 in animal products. The validation data also include data from an independent laboratory validation on meat and milk. No confirmatory method has been submitted for animal products. With method 137.12 it is feasible to determine the metabolite CGA 179500 in animal products with an LOQ of 0.02 mg/kg for eggs, meat and offal, and 0.01 mg/kg for milk.

~~No deviations from OECD 506 (adopted 16 October 2007) were observed.~~

Deviations from OECD 506:

Initial value at zero-time point not determined;

Fat and blood samples were fortified at 5xLOQ spiking level (instead of 10xLOQ);

Dates of fortification and analysis not provided.

Summary of storage stability data

The potential for degradation of residues during storage has been previously assessed in the framework of the peer review for trinexapac-ethyl and re-evaluated by the RMS LT for the purpose of renewal. The metabolism studies showed that trinexapac-ethyl is degraded rapidly in plants and that trinexapac acid (CGA179500) is the main metabolite. Consequently, storage stability of trinexapac acid was demonstrated for the following periods in the commodities listed in the Table B.7.1.2-2 below when frozen (approximately -18°C).

Table B.7.1.2 – 2: Summary of stability data for trinexapac acid

Commodity Category	Commodity	Maximum Storage Period (month) for which stability was demonstrated	Report Reference	EU-review reference
EU Reviewed Data				
High Oil Content	Rape seed	24	105/95	The Netherlands, 2003
High Starch Content	Wheat grain	24	105/95	The Netherlands, 2003
No group	Wheat straw	12~~24~~	105/95	The Netherlands, 2003
Animal Meat	Muscle	3[(a)]	330/99	The Netherlands, 2003
Animal Fat	Fat (omental)	3[(a)]	330/99	The Netherlands, 2003
Animal Liver	Liver	3[(a)]	330/99	The Netherlands, 2003
Animal Kidney	Kidney	3[(a)]	330/99	The Netherlands, 2003
Animal Blood	Blood	3[(a)]	330/99	The Netherlands, 2003
Milk	Milk	4[(a)]	330/99	The Netherlands, 2003

(a): storage stability in animal matrices was tested only for 3-4 months as a part of a feeding study.

Additionally, high temperature hydrolysis studies showed that metabolites CGA313458, CGA 113745 and CGA 224439 were formed during processing. Therefore storage stability studies for these metabolites covering the length of storage in processing studies were submitted by TTF. Storage stability of metabolites CGA313458, CGA 113745 and CGA 224439 was demonstrated for the following periods in the commodities listed in the Table B.7.1.2-3 below when frozen (approximately -18°C). ~~TTF informed that storage stability studies for CGA313458, CGA 113745 and cyclopropane carboxylic acid (CGA224439) are currently on-going~~.

Table B.7.1.2 – 3: Summary of stability data for metabolites CGA313458, CGA 113745 and CGA 224439 in processed cereal commodities

Commodity	Maximum Storage Period (month) for which stability was demonstrated
New Data	

Commodity	Maximum Storage Period (month) for which stability was demonstrated		
	CGA313458	CGA 113745	CGA 224439
Wheat grain	12	Not stable after 30 days	12
Flour	3	Not stable after 30 days	12
Bran	6	Not stable after 30 days	12
Bread	6	Not stable after 30 days	12
Beer	12	Not stable after 30 days	12

B.7.2 Metabolism, distribution and expression of residues

B.7.2.1 Plants

In the trinexapac-ethyl DAR 2003, the primary metabolism in plants was evaluated on cereals and grass crops (wheat, rice and grass), and pulses and oilseeds (oilseed rape). Studies were conducted using [^{14}C-cyclohexyl]-trinexapac-ethyl and were not performed according to the current guidelines (OECD 501). The existing plant metabolism studies were not performed according to the current guidelines (unextracted fraction above the trigger value of 25% of the Total Recovered Radioactivity; non identified or uncharacterized fractions above the trigger value of 10% TRR…) however many of these studies provided information on metabolism of trinexapac-ethyl in plants. These studies were not re-evaluated by the RMS LT and summaries for each study presented in the initial DAR were therefore reported hereafter. However new metabolism studies in oilseed rape and wheat were conducted for the new AIR dossier to provide further argumentation to the previous understanding of plant metabolic pathway. These studies are in line with current standards, according to GLP and follows OECD guideline 501."

The metabolism of trinexapac-ethyl was investigated for foliar applications on cereals and grass crops (wheat, rice, grass) and on pulses and oilseeds (rape) using [^{14}C-cyclohexyl]-trinexapac-ethyl. The characteristics of all these studies are summarized in Table 7.2.1-1.

Table 7.2.1-1: Summary of available metabolism studies

Group	Crop	Label Position	Application and Sampling Details				Report Reference	EU-review reference
			Method, F or G[(a)]	Rate (kg a.s./ha)	No	Sampling (DAT)		
EU Reviewed Data								
Pulses and oilseeds	Spring rape	^{14}C-cyclohexyl	Foliar, G	0.40	1	0, 14, 65	4/91 7/93	The Netherlands, 2003 Considered as supplementary

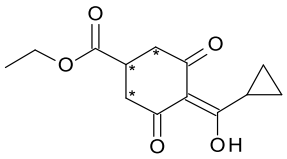
Group	Crop	Label Position	Application and Sampling Details				Report Reference	EU-review reference
			Method, F or G[(a)]	Rate (kg a.s./ha)	No	Sampling (DAT)		
Cereals and grass crops	Spring wheat	^{14}C-cyclohexyl	Foliar, G	0.15	1	0, 1, 2, 7, 14, 21	20/90	The Netherlands, 2003
	Spring wheat	^{14}C-cyclohexyl	Foliar, F	0.15	1	0, 25, 48, 71	6/93	The Netherlands, 2003 Considered as supplementary
	Paddy rice	^{14}C-cyclohexyl	Foliar, G	Scenario 1: 0.04 Scenario 2: 0.16	1	Scenario 1: Foliage 0, 7, 21 Grain, husks, straw: 82 Scenario 2: Grain, straw: 60	11/96	The Netherlands, 2003
	Grass	^{14}C-cyclohexyl	Foliar, F	0.56	1	22, 46, 102	623-00	The Netherlands, 2003
New Data								
Pulses and oilseeds	Spring oilseed rape	^{14}C-cyclohexyl	Foliar, G	0.394	1	Foliage[(b)]: 21 Whole plant[(b)]: 67-91	20120173	-
Cereals and grass crops	Spring wheat	^{14}C-cyclohexyl	Foliar, F	0.211	1	Forage: 7 Hay: 34 Grain, straw: 62	20120098	-

(a): Outdoor/field application (F) or glasshouse/protected/indoor application (G)
(b): Only seeds were analysed

Study 1

EU reviewed metabolism study in spring rape

Reference:	**Nicollier G. (1991)** Distribution and Degradation of [^{14}C-cyclohexyl]-CGA 163935 in greenhouse grown spring rape (~~KCA 6.2.1 / 01~~ KIIA 6.1.3.2 / 01)
Report No.:	90GN15BPR1 (4/91)
Project No.:	90GN15B
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residues Chemistry, Series 171-4 (a))1)&(2), U.S. Environmental Protection Agency, Washington, D.C., October 1982; Agricultural chemicals Laws and Regulations, Japan, Metabolism in Plants, Society of Agricultural Chemical Industry (1985)
GLP:	The OECD principles of Good Laboratory Practice, Paris/France 1981 The Procedure and Principles of Good Laboratory Practice in Switzerland, Federal Department of the Interior, 1986;

	U. S. Environmental Protection Agency Good Laboratory practice standards, Pesticide Programs (40 CFR Part 160)
Reference:	**Nicollier G. (1993)** Metabolism of [^{14}C-cyclohexyl]-CGA 163935 in greenhouse grown spring rape (KIIA 6.1.3.2 / 02)
Report No.:	90GN15BPR2 (7/93)
Project No.:	90GN15B
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residues Chemistry, Series 171-4 (a))1)&(2), U.S. Environmental Protection Agency, Washington, D.C., October 1982; Agricultural chemicals Laws and Regulations, Japan, Metabolism in Plants, Society of Agricultural Chemical Industry (1985)
GLP:	The OECD principles of Good Laboratory Practice, Paris/France 1981 The Procedure and Principles of Good Laboratory Practice in Switzerland, Federal Department of the Interior, 1986; U. S. Environmental Protection Agency Good Laboratory practice standards, Pesticide Programs (40 CFR Part 160)
Previous evaluation:	In DAR 2003
Material and methods:	
Test item: Position of the radiolabel (* = ^{14}C position)	[^{14}C-cyclohexyl]-trinexapac-ethyl 
Lot/Batch No.:	GAN-XVII-72
Radiochemical Purity:	95-98% (Specific activity 1.85MBq/mg (50 µg/mg).
Test concentration:	0.40 kg a.s./ha
Test system:	Spring rape (variety *Tobin Canola*) grown in eleven plastic pots (46x28x25 cm) in a greenhouse, filled with soil from Klus (Klus research Station, Switzerland). Two rows of spring rape plants (15 seeds/row) were seeded in each container. 37 days after sowing spring rape received a foliar spray treatment with 0.40 kg as/ha radio-labelled CGA 163935. Plant samples were collected at 30 minutes, 14 and 65 DAT. Whole plants were separated into green parts and flowering parts, and into stalks, pods and seeds at harvest. One soil core per container was taken and dried before analysis. ~~No indication of storage conditions and time between harvest and analysis stated in the report~~.
Stage of application:	The stem elongation stage (37 days after sowing).
No. of applications:	One foliar treatment.
Sampling time points:	Plant samples were collected at 30 minutes, 14 and 65 DAT.
Study dates:	Biological phase: September 19, 1990 – November 23, 1990 Analytical phase: September 21, 1990 – August 30, 1991 (Nicollier, 1991) April 2, 1993 (Nicollier, 1993)

Method of analysis:	For the identification and characterization of residue components, plant material was extracted with methanol/water (8:2, v/v). After the cold extraction, a hot Soxhlet extraction with methanol was performed. Thereafter, the not-extracted radioactivity was determined by combustion analysis. Extracts were partitioned with methylenechloride. Seeds were homogenized and extracted with hexane (oil fraction). The meal was extracted with methanol/water (8:2, v/v). Sugar conjugated metabolites were cleaved using cellulase enzyme digestion. Total radioactivity in plant samples was determined by combustion analysis. Distribution and translocation of radioactivity in spring rape plants was visualized by autoradiography. The residue was identified and characterized by TLC. Additional HPLC methods were used for characterisation and purification of metabolites. Structural identification of residue components was performed by MS and NMR spectroscopy. The study in spring rape combines two separate studies by the notifier (Nicollier, 1991; Nicollier, 1993). Study Nicollier 1991 was dealing with the distribution and degradation of CGA 163935 foliarly applied to spring rape plants. Study Nicollier 1993 presents data concerning the characterization and identification of metabolites as well as degradation pathways in spring rape after spray application of ^{14}C-labelled CGA 163935.
Limit of detection:	0.002 mg/kg
Method validation:	Extracted radioactivity at maturity was 72.8% in stalks, 82.7% in pods and 73.1% in meal.

Results

The distribution and identification of radioactive residues in spring rape treated with CGA 163935 in the greenhouse are summarised in table B.7.2.1-2.

Translocation of the radioactivity into untreated plant parts or new growths was demonstrated by the higher residue levels in flowers at 14 DAT (6.8 mg eq/kg) and in pods at 65 DAT (6.7 mg eq/kg). Autoradiography also revealed transfer of radioactivity in the new growth leaves and in the pods by one month after treatment. Highest residue levels are found in pods (6.7 mg eq/kg) followed by stalks (3.1 mg eq/kg) and seeds (1.4 mg eq/kg). After processing of seeds, most of the residue is found in meal (1.4 mg eq/kg) and a relatively small amount in oil (0.034 mg eq/kg). Mature plant parts do not contain detectable levels (< 0.001 mg eq/kg) of the parent compound CGA 162935. The major residue component found in rapeseed meal and pods is the metabolite CGA 179500, also present as sugar conjugates. In stalks, CGA 351210 (I4b) is the major residue component. The sum of the free and sugar conjugated CGA 179500 is accounting for 31%, 3.5%, 18% and 9.7% TRR in meal, oil, pods and stalks, respectively. The sum of the free and conjugated metabolite 4-(cyclopropyl-alpha-hydroxy-methylene)-3,5-dioxocyclohexane methanol (I4b= CGA 351210) represents 5.2%, 16%, 16% and 28% TRR in meal, oil, pods and stalks, respectively. Other minor metabolites identified in meal, pods, and stalks are CGA 313458 and CGA 312753. In addition, a minor residue faction in rapeseeds is associated with lipids.

Table B.7.2.1-2: Residues in spring rape after greenhouse application of CGA 163935 (rate 0.40 kg as/ha)

	30 min whole tops1		14 DAT green parts1		14 DAT flowering parts1		65 DAT seeds/total2		65 DAT seeds/oil2		65 DAT seeds/meal2		65 DAT pods2		65 DAT stalks2	
	mg eq/kg	% TRR	Mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
TRR	6.2	100	0.82	100	6.8	100	1.4	100	0.034	100	1.4	100	6.7	100	3.1	100
Organosoluble		95		92		100		68		100		~~40~~ 39.9		~~32~~ 31.6		19.2
Water soluble								26				26.2		46.4		~~45~~ 44.6
Identified		19		1.5		1.0		~~41~~ 37.6		~~54~~ 19.5		~~4~~ 37.8		~~193~~ 37.2		~~54~~ 44.1
CGA 163935	1.2	193	0.012	1.53	0.068	1.03							<0.001		<0.001	
CGA 179500 free								29		3.5		29.5		18		8.9
CGA 179500 conj.								1.1		-		1.1		0.4		0.8
CGA 312753								0.9		-		0.9		0.9		1.5
CGA 313458								1.1		-		1.1		1.9		4.9
I*4b4free (CGA 351210)								2.1		16.0		1.7		7.7		6.0
I4b 4conj (CGA 351210)								3.4		-		3.5		8.3		22
I8 (lipid)								1.7		69.5		-		-		-
II3b5								1.8		-		1.8		1.9		2.2
II7**										-		-		1.2		3.4
Characterised		<1		1		<1		41		2		5		7		2

	30 min whole tops1		14 DAT green parts1		14 DAT flowering parts1		65 DAT seeds/total2		65 DAT seeds/oil2		65 DAT seeds/meal2		65 DAT pods2		65 DAT stalks2	
	mg eq/kg	% TRR	Mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
I2								0.7		-		0.7		0.3		0.5
I2b								2.6		-		2.7		3.0		-
I3								0.2		0.6		0.2		0.1		-
I6								1.8		2.2		1.8		-		0.7
II1								4.5		-		4.6		3.5		2.6
II2										-		-		0.9		1.2
II4								2.8		-		2.9		1.3		2.3
II5										-				2.2		
II8								-		-		-		1.1		-
II9								-		-		-		1.7		-
Unresolved								26.5		8.2		26.9		29.0		21.0
Cold extracted		95.2		92.4		100.7		68.3		41.7		65.9		80.2		66.1
Soxhlet extracted		0.6		1.0		0.4		1.8 3.2		58.3		1.9		1.3		1.6
Microwave extraction										-		12.9		6.4		
Not extracted		3.3		10.4		0.8		14.0		-		14.3		11 10.9		27.2
Total extracted		95.8		93.4		101.1		82.0		100		80.7		81.5		67.7
Total (extracted+not extracted)		99.1		103.8		101.9		96.0		100		95.6		98.8		94.9

[1] Data from Nicollier, 1991, [2] Data from Nicollier, 1993, [3] calculated by the Rapporteur, [4] identified as 4-(cyclopropyl-alpha-hydroxy-methylene)-3,5-dioxocyclohexane methanol (CGA 351210),

[5] identified as isocitric acid or isocitric acid lactone, * TLC fraction, ** treatment with cellulase released aglycones $I_4 + I_5$

RMS comments and conclusions (Netherlands 2003)

The distribution and degradation of the parent compound CGA 163935 in spring rape was investigated in a greenhouse study after one application of the radio-labelled compound at a rate of 0.40 kg as/ha (2 x N). The parent compound CGA 163935 is mainly degraded by hydrolysis of the parent ethyl ester to the free acid CGA 179500. CGA 179500 is reduced to 4-(cyclopropyl-alpha-hydroxy-methylene)-3,5-dioxocyclohexane methanol (CGA 351210= I4b). CGA 179500 is also further oxidized by opening of the six-membered ring to yield 3-carboxy-7-cyclopropyl-5,7-diketo-heptanoic acid (CGA 313458). Cleavage of the six-membered ring followed by stepwise oxidations and decarboxylation is thought to lead to the formation of isocitric acid or isocitric acid lactone, and after dehydration, to 1,2,3-propene tricarboxylic acid (CGA 312753).

The GAP in the present study is in accordance with the current GAP that prevails in several NEU member states (0.38 vs 0.40 kg a.s./ha). Oilseed rape is not a representative use.

Guidelines and limitations

It is noted that, despite the extensive efforts for identification and characterization, several unresolved fractions in rape seeds, meal, pods and stalks represent 27%, 27%, 29% and 21% TRR, respectively. ~~No indication of storage conditions and time between harvest and analysis stated in the report.~~ ~~All residues except parent are expressed only in percentage and not accurate (mg/kg) values.~~ Despite these limitations, the study is considered acceptable for the overall evaluation.

Comments RMS LT

RMS LT agrees with the comments and limitations identified by RMS Netherlands 2003 except for final conclusion.

Additionally, all residues except parent are expressed only in percentage and not accurate (mg/kg) values.

No indication of storage conditions and exact time between harvest and analysis stated in the reports. Based on study dates presented in the reports, samples were analysed within 3 days to 11 months (Nicollier, 1991, tops, green and flowering parts) and 31 months (Nicollier, 1993, mature seeds, pods and stalks). No evidence provided proving that results were not affected by long storage period.

Purity of reference standards not provided.

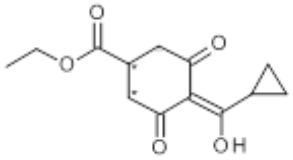
Study is considered supplementary only.

Study 2
EU reviewed metabolism study in spring wheat

Reference:	**Krauss J. H. (1990)** Uptake, Distribution and Degradation of [^{14}C-cyclohexyl]-CGA 163935 in Field grown Spring Wheat (~~KCA 6.2.1 / 02~~ KIIA 6.1.3.1 / 01)

Project No.:	89 JK 02.1
Project Report No.:	20/90
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residues Chemistry, Series 171-4 (a))1)&(2), U.S. Environmental Protection Agency, Washington, D.C., October 1982; Agricultural chemicals Laws and Regulations, Japan, Metabolism in Plants, Society of Agricultural Chemical Industry (1985)
GLP:	The OECD principles of Good Laboratory Practice, Paris/France 1981 The Procedure and Principles of Good Laboratory Practice in Switzerland, Federal Department of the Interior, 1986; U. S. Environmental Protection Agency Good Laboratory practice standards, Pesticide Programs (40 CFR Part 160)
Previous evaluation:	In DAR 2003

Material and methods:

Test item:	[^{14}C-cyclohexyl]-trinexapac-ethyl
Position of the radiolabel (* = ^{14}C position)	
Lot/Batch No.:	B-1036.1A
Radiochemical Purity:	97% (specific radioactivity 1.71MBq/mg (46.2µg/mg)
Test concentration:	0.150 kg a.s./ha
Test system:	Greenhouse experiment: Spring wheat (variety *Besso*) grown in a greenhouse in 28 small pots (6x6x5 cm, 5 plants/pot) containing sandy loam soil received a spray treatment with 0.15 kg as/ha radio-labelled CGA 163935 (0.75 x N) at 2 weeks after sowing. Plant samples were collected at time intervals of 0.5 and 4 h, and at 1, 2, 7, 14 and 21 DAT. Aerial parts of five plants of a single pot were cut off, pooled, weighted, washed 3 times in acetone/water 1:1 – sum of the washings considered as surface radioactivity. Samples were immediately frozen – homogenized with an "Omni" mixer in presence of liquid nitrogen. Roots were freed from soil, washed with acetonitrile/water 8:2, dried and treated as described for the green parts. Field experiment: A plot of 200x300 cm was sown with spring wheat (variety *Besso*) and received a spray treatment with 0.15 kg as/ha radio-labelled CGA 163935 (0.75 x N) at 6 weeks after sowing. Plants of the first interval were kept unsectioned whereas plants at ear and at milky stage were divided into ears and green parts. At maturity plants were divided into grains, husks and straw. A 1.5 m^2 control plot, situated about 50 m from the treated plot, provided plant and soil material for background analyses. For determination of the total ^{14}C residues by combustion, the plant material was homogenized in the presence of liquid nitrogen. Replicates of homogenized samples were immediately taken for combustion. The remaining samples were stored in a deep-freezer at -18 °C before extraction.
Study dates:	Biological phase: April 25, 1989 – August 18, 1989

	Analytical phase: May 23, 1989 – May 3, 1990
Stage of application:	2 weeks (greenhouse); 6 weeks (field) after sowing.
No. of applications:	One
Sampling time points:	Greenhouse experiment: Plant samples were collected at 30 minutes, 4 h, 1, 2, 7, 14 and 21 DAT. Field experiment: Plant samples were collected at 3 h, 25 and 48 DAT.
Method of analysis:	Aerial and root parts of plants were homogenized and extracted with acetonitrile: water (8:2, v/v). The parent compound was determined in aerial plant extracts by 2-dimensional (D) TLC and in root extracts by 1-D TLC. Non-extractable radioactivity of plant samples was determined by combustion analysis. The total radioactivity in plant samples was considered equal to the sum of the extractable and non-extractable radioactivity. Translocation of the compound was studied using autoradiography.
Limit of detection:	0.002 mg/kg
Method validation:	Extracted radioactivity was 74.9% in grain, 49.2% in husks and 38.1% in straw.

Results

The distribution and identification of radioactive residues in spring wheat treated with CGA 163935 in a greenhouse are summarised in table B.7.2.1-3.

In this short-term greenhouse study on spring wheat, leaf surface radioactivity of whole tops decreased rapidly within 1 day as a result of penetration and volatilization. The content of the parent compound CGA 165935 of the leaf surface also decreased rapidly within 1 day. The metabolite CGA 179500 remained a minor metabolite on the plant surface. After the first 24 hours ca. 80% of the recovered radioactivity had penetrated the plant surface, and the metabolite CGA 179500 was identified as a major metabolite in plant leaf extracts. After that time, the amount of the metabolite CGA 179500 decreased slowly. The content of the parent compound CGA 165935 in plant leaf extracts was low shortly after application and became undetectable (<0.002 mg eq/kg) 14 days after application. The apparent half-life of the parent compound in/on wheat leaves was estimated to be less than ca. 4h.

In roots, radioactivity was found to increase until 1 DAT and thereafter decreased. The parent compound CGA 165935 was identified shortly after application in the roots reached a maximum at 1 DAT and became undetectable 7 days after application.

Translocation of radioactivity from treatment plant parts into new growths could be demonstrated by autoradiography.

Results from the field experiment are integrated in study 3 Krauss J. H. (1993) and Table B.7.2.1-4.

RMS comments and conclusions (Netherlands 2003)

After foliar application of CGA 165935 at a rate of 0.15 kg as/ha to spring wheat grown in a greenhouse, the compound is initially transferred from the surface into the leaves and metabolized to CGA 179500 rapidly with a half-life of ca. 4 h. The parent compound is also taken up from soil by the roots. Translocation of residue to other plant parts is found to take place.

The GAP in the present study is in accordance with the current GAP that prevails in several NEU member states (0.125 vs 0.150 kg a.s./ha).

Guidelines and limitations

A rapid decrease of the initially applied radioactivity is noted within 4h after application according to the authors of the study. This is probably caused by volatilization during drying of the deposit. Furthermore, it is noted that a high proportion of the residue was not extracted and no efforts were made to further resolve the not extracted fraction. Within these limitations the study was considered acceptable for the overall evaluation.

Comments RMS LT

It is noted that the application rate of CGA 165935 was 20% above the intended use (cGAP) proposed for renewal. From samples to analysis samples were stored deep frozen for a maximum of 12 months, samples were stored in a deep-freezer at -18 °C before extraction, although time and conditions from extract to analysis are not stated.

Purity of the reference substances not reported.

Only the parent values are presented in both percentage and actual values.

Samples were extracted and combusted for TRR as well as analysed for parent and CGA179500 within 12 months time. Parent and CGA179500 were only reference substances in this study.

A high proportion of the residue was not extracted. After a cold extraction (with methanol/water 8:2), a hot Soxhlet extraction with methanol was performed, but still leaving unextracted 25.1%, 50.8% and 61.9% in grains, husks and straw respectively.

Study is considered suitable for evaluation.

Table B.7.2.1-3: Residues in spring wheat after greenhouse application of CGA 163935 (rate 0.15 kg as/ha)

	0.5 h whole tops/leaves		0.5 h roots		4 h whole tops/leaves		4 h roots		1 DAT whole tops/leaves		1 DAT roots		2 DAT whole tops/leaves		2 DAT roots	
	mg eq/kg	% TRR	Mg eq /kg	% TRR	mg eq /kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq /kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
TRR	5.5	100	0.15	100	2.4	100	0.77	100	1.7	100	2.1	100	1.1	100	1.2	100
LEAF SURFACE																
Organosoluble		84				43				20				20		
Identified		70				32				14				12.5		
CGA 163935	3.8	69			0.76	31			0.21	12			0.12	10.5		
CGA 179500		0.6				5.0				2.0				2.0		
PENETRATED																
Organosoluble		16		69		55		60		75		60		74		55
Identified		13		10		37		3		38		12		39		1.3
CGA 163935	0.65	12	0.015		0.27	11	0.024		0.03	1.2	0.26		0.03	2.5	0.015	
CGA 179500		0.8				26				37				36		
Not extracted[1]		0.1		31		2.0		40		4.5		40		6		45

[1] from the penetrated radioactivity

Table B.7.2.1-3: Residues in spring wheat after greenhouse application of CGA 163935 (rate 0.15 kg as/ha) (Continued)

	7 DAT whole tops/leaves		7 DAT roots		14 DAT whole tops/leaves		14 DAT roots		21 DAT whole ops/leaves		21 DAT roots	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq /kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	Mg eq /kg	% TRR
TRR	0.57	100	0.41	100	0.47	100	0.18	100	0.31	100	0.13	100
LEAF SURFACE												
Organosoluble		8.3				6.2				5.1		
Identified		3.2				1.0		4		0.9		11
CGA 163935	0.01	1.7			<0.002	0.5			<0.001	0.1		
CGA 179500		1.5				0.5				0.8		
PENETRATED												
Organosoluble		83		38		84		13		81		11
Identified		37		<0.25		35		<0.5		30		<0.5
CGA 163935	0.02	0.3	<0.001		<0.002	< 0.1	<0.001		<0.001	<0.1	<0.001	
CGA 179500		37				35				30		
Not extracted[2]		8.3		62		10		87		14		89

[1] calculated by the Rapporteur

[2] from the penetrated radioactivity

Study 3

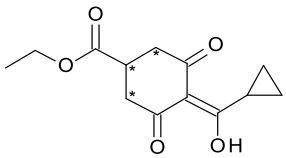
EU reviewed metabolism study in spring wheat

Reference:	**Krauss J. H. (1993)** Metabolism of [14C-cyclohexyl]-CGA 163935 in Field grown Spring Wheat (~~KCA 6.2.1 / 03~~ KIIA 6.1.3.1 / 02)
Project No.:	89 JK 02
Project Report No.:	89JK02PR2 (plant metabolism report 6/93)
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residues Chemistry, Series 171-4 (a))1)&(2), U.S. Environmental Protection Agency, Washington, D.C., October 1982; Agricultural chemicals Laws and Regulations, Japan, Metabolism in Plants, Society of Agricultural Chemical Industry (1985)
GLP:	The OECD principles of Good Laboratory Practice, Paris/France 1981 The Procedure and Principles of Good Laboratory Practice in Switzerland, Federal Department of the Interior, 1986; U. S. Environmental Protection Agency Good Laboratory practice standards, Pesticide Programs (40 CFR Part 160)
Previous evaluation:	In DAR 2003

Material and methods:

Test item: [^{14}C-cyclohexyl]-trinexapac-ethyl

Position of the radiolabel (* = ^{14}C position)



Lot/Batch No.: B-1036.1A

Radiochemical Purity: 97% (specific radioactivity 1.71MBq/mg (46.2µg/mg)

Test concentration: 0.150 kg a.s./ha

Test system: Greenhouse experiment:

Spring wheat (variety *Besso*) grown in a greenhouse in 28 small pots (6x6x5x cm, 5 plants/pot) containing sandy loam soil received a spray treatment with 0.15 kg as/ha radio-labelled CGA 163935 (0.75 x N) at 2 weeks after sowing. Plant samples were collected at time intervals of 0.5 and 4 h, and at 1, 2, 7, 14 and 21 DAT. Aerial parts of five plants of a single pot were cut off, pooled, weighted, washed 3 times in acetone/water 1:1 – sum of the washings considered as surface radioactivity. Samples were immediately frozen – homogenized with an "Omni" mixer in presence of liquid nitrogen. Roots were freed from soil, washed with acetonitrile/water 8:2, dried and treated as described for the green parts.

Field experiment:

A plot of 200x300 cm was sown with spring wheat (variety *Besso*) and received a spray treatment with 0.15 kg as/ha radio-labelled CGA 163935 (0.75 x N) at 6 weeks after sowing. For autoradiography two or three plants were collected at ear emergence and at maturity. Plants of the first interval were kept unsectioned whereas plants at ear and at milky stage were divided into ears and green parts. At maturity

	plants were divided into grains, husks and straw. A 1.5 m^2 control plot situated about 50 m from the treated plot, provided plant and soil material for background analyses. For determination of the total ^{14}C residues by combustion, the plant material was homogenized in the presence of liquid nitrogen. Replicates of homogenized samples were immediately taken for combustion. The remaining samples were stored in a deep-freezer at 18 ^0C before extraction. In order to generate a sufficient amount of selected grain metabolites, also stem injection experiments were performed (Krauss, 1993). For this purpose, spring wheat (variety Besso) was grown under greenhouse conditions. Six weeks old plants (one-node-stage) were injected with radio-labelled CGA 163935 (about 40-50 µg/plant). At maturity, i.e. 69 days after application, all injected plants were harvested and analysed. Furthermore, in vitro incubation experiments with a spring wheat homogenate were carried out. Spring wheat (variety Besso) was grown under greenhouse conditions to a height of about 20 cm. Leaves were cut, homogenised and suspended in water. After addition of radio-labelled CGA 163935, the homogenate was incubated at room temperature for 45 days under shaking. The in vitro reaction was monitored by 2D-TLC. After filtration, the water phase was partitioned with ethyl acetate and the organic phase was further purified by HPLC. Plant parts were homogenized and extracted with methanol: water (8:2, v/v). A further partitioning of acidified extracts was carried out with methylene chloride. For the stem injection and in vitro plant material, an extra partitioning step with ethyl acetate was carried out. The water phase was further subjected to XAD column chromatography and separated into a water phase and a 50% acetonitrile phase. After the "cold" extraction, a "hot" Soxhlet extraction with methanol was performed. Sugar conjugated metabolites were hydrolysed using cellulase enzyme digestion. Extracts were analysed by TLC. Analysis of residue components was performed using 1 or 2-D TLC, liquid chromatography, HPLC, and electrophoresis. Structural identification was performed with GC-MS analysis, and MS and NMR spectroscopy. The non-extractable radioactivity was determined by combustion analysis. The total radioactivity in plant samples was considered equal to the sum of the extractable and non-extractable radioactivity. Translocation of the compound was studied using autoradiography.
Study dates:	Biological phase: April 25, 1989 – August 18, 1989 Analytical phase: May 23, 1989 – May 3, 1990 (Study Krauss, 1990) February 28, 1993 (Study Krauss, 1993)
Stage of application:	2 weeks (greenhouse); 6 weeks (field) after sowing.
No. of applications:	One
Sampling time points:	Greenhouse experiment: Plant samples were collected at 30 minutes, 4 h, 1, 2, 7, 14 and 21 DAT. Field experiment: Plant samples were collected at 3 h, 25("ear emergence"), 48 ("milky stage") and 71 DAT ("maturity").
Method of analysis:	Aerial and root parts of plants were homogenized and extracted with acetonitrile: water (8:2, v/v). The parent compound was determined in aerial plant extracts by 2-dimensional (D) TLC and in root extracts by 1-D TLC. Non-extractable radioactivity of plant samples was determined by combustion analysis. The total radioactivity in plant samples was considered equal to the sum of the extractable and non-extractable radioactivity. Translocation of the compound was studied using autoradiography.
Limit of quantification:	Not stated

Method validation:	Extraction efficiency with acetonitrile:water (8:2, v/v) was 84.9, 88.6 and 88.8 % for grain, husks and straw respectively.

Results

The distribution and identification of radioactive residues in spring wheat treated with CGA 163935 in the field are summarised in table B.7.2.1-4. This study is a continuation of the previous study Krauss 1990 (report includes data and results of earlier study).

Following treatment of spring wheat in the field with CGA 165935 at a rate of 0.15 kg as/ha, the parent compound is initially found as one of the major residue components, along with the metabolite CGA 179500 in whole tops. Already at 25 DAT, the parent compound becomes undetectable in ears and leaves, while the metabolite CGA 179500 is the major component detected. The concentration of the metabolite CGA 179500 remains at about the same level (24-28% TRR) in ears and grain, but is decreasing with time in leaves. At harvest, CGA 179500 in free and conjugated form is accounting for about 35%, 27% and 21% of total residues in grains, husks and straw, respectively. The metabolite CGA 329773 is representing 11% and 3.1% of total residues in the grains and in straw, respectively. Minor metabolites identified include CGA 275537 (2.1% and 2.4% in grains and straw, respectively) and CGA 312753 (4.3% and 1.8% in husks and straw, respectively).

RMS comments and conclusions (Netherlands 2003)

The metabolic fate of CGA 163935 was studied in field grown spring wheat after foliar application of the radio-labelled substance at a rate of 0.15 mg as/ha. Also stem injection experiments and in vitro incubations were carried out to aid in the elucidation of the metabolic pathways. Based on the combined data of these experiments, the parent compound CGA 163935 is mainly degraded by hydrolysis of the ethyl ester to the free acid CGA 179500. A small amount of CGA 179500 is conjugated to sugar. Aromatization of the 6-membered ring of CGA 179500 by hydroxylation, elimination of water and keto-enol tautomerism, yields 4-cyclopropanecarbonyl-3,5-dihydroxybenzoic acid (CGA 329773). A second metabolic route of the parent compound CGA 163935 includes cleavage and oxidation of the 6-membered ring followed by stepwise oxidation and decarboxylation. Terminal metabolites of this pathway are tricarboxylic acids such as 1,2,3-propane tricarboxylic acid (CGA 275537) and a monoethyl ester derivative of 1,2,3-propene tricarboxylic acid (CGA 312753).

Guidelines and limitations

It is noted that the application rate of CGA 165935 was 25% below the intended use. A considerable fraction of the total residue remained unresolved, i.e. 32, 47 and 60% in grains, husks and straw, respectively. This is explained by the notifier as a result of the tendency of the metabolite fractions to adsorb to the matrix. The study was considered suitable for evaluation.

Table B.7.2.1-4: Residues in spring wheat after field application of CGA 163935 (rate 0.15 kg as/ha)

	0 DAT whole tops[1]		25 DAT ears[1]		25 DAT leaves[1]		48 DAT ears[1]		48 DAT leaves[1]		71 DAT grain[2]		71 DAT husks[2]		71 DAT straw[2]	
	mg eq/kg	% TRR	mg eq/ kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/ kg	% TRR	Mg eq/kg	% TRR
TRR	0.80	100	0.26	100	0.23	100	0.47	100	0.44	100	0.46[4]	100	0.44[4]	100	0.54[4]	100
Organosoluble[3]		59		38		21		41		8.1		30		16		8.3
Water soluble[3]		24		45		66		38		64		32		31		32
Identified		55		28		13		25		2.8		49		31		28
CGA 163935	ca 0.21	31	<0.001	<0.3	< 0.001	<0.3	< 0.001	< 0.3	< 0.001	< 0.3		-		-		-
CGA 179500 (total)		24		28		13		25		2.8		28		17		13
Free form												24.1		10		4.5
Conjugated (released after cellulose treatment)												2.0		-		-
Released from debris with 1M NaOH												1.7		-		-
Released from debris after Soxhlet and autoclave extraction												-		6.7		8.3
I*$_{2a}$ = CGA 312753 (total)												-		4.3		1.8
Free form												-		3.4		0.3
Released from debris after Soxhlet and autoclave extraction												-		1.0		1.5
II$_1$ = CGA 179500 sugar conjugate												6.8		10		8.0

	0 DAT whole tops[1]		25 DAT ears[1]		25 DAT leaves[1]		48 DAT ears[1]		48 DAT leaves[1]		71 DAT grain[2]		71 DAT husks[2]		71 DAT straw[2]	
	mg eq/kg	% TRR	mg eq/ kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/ kg	% TRR	Mg eq/kg	% TRR
II_2 = CGA 275537 (total)												3.1		NA		2.4
Free form												3.1		-		2.4
II_3 = CGA 329773 (total)												11		NA		3.1
Free form												10.9		-		3.1
Characterised		2.5		2.0		2.8		3.0		3.0		36		55		67
I_1*												-		6.1		0.5
I_4												0.7		0.6		2.4
II_4												1.7		NA		NA
Unresolved												32		47		60
Soxhlet extracted		2.5		2.0		2.8		3.0		3.0		1.7		1.5		3.8
Not extracted		12		13		15		18		25		15		11		11

[1] Data from Krauss 1990

[2] Data from Krauss 1993

[3] calculated by the Rapporteur

[4] total residue data from study 1B (Krauss1990)

NA = not analysed

* TLC fraction

RMS LT comments and conclusions

The GAP in the present study is in accordance with the current GAP that prevails in several NEU member states (0.125 vs 0.150 kg a.s./ha). It is noted that the application rate of CGA 165935 was 20% above the intended use proposed for renewal.

Some deviations from OECD 501were noticed.

Residue levels expressed as % TRR only. Purity of reference substances not stated. LOQ was not stated in the report, it was explained by the applicant that "LOQ is not defined in this report, but is included in the newly submitted rape report (Piskorski R. (2015a) Metabolism of [^{14}C]-trinexapac-ethyl in spring wheat".

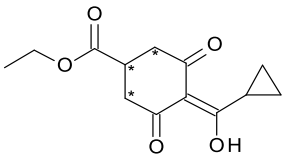
As mentioned in study 2, samples were extracted and combusted for TRR as well as analysed for parent and CGA179500 within 12 months time (Krauss, 1990. Parent and CGA179500 were only reference substances in this study). Analysis of extracts for other metabolites as well as extraction and analysis of PES performed up to 43 months from sampling. Samples were stored in a deep-freezer at -18°C before extraction, although time and conditions from extract to analysis are not stated. Assumption that results for other metabolites except CGA179500 were affected by long storage period could not be ruled out.

Study is considered supplementary.

Study 4

EU reviewed metabolism study in rice

Reference:	**Gross D. (1996)** Behaviour and Metabolism of CGA 163935 in Greenhouse Grown Paddy Rice after Application of [3,5-Cyclohexadion-1,2,6-^{14}C] Labelled Material (~~KCA 6.2.1 / 04~~ KIIA 6.1.3.3 / 01)
Project No.:	94DG53
Project Report No.:	94DG53PR1. Plant metabolism report PRM 11/96
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residues Chemistry, Series 171-4 (a))1)&(2), U.S. Environmental Protection Agency, Washington, D.C., October 1982; Directive 91/414/EEC, The Council of the European Union, Brussels; Agricultural chemicals Laws and Regulations, Japan, Metabolism in Plants, Society of Agricultural Chemical Industry (1985)
GLP:	The OECD principles of Good Laboratory Practice, Paris/France 1981 The Procedure and Principles of Good Laboratory Practice in Switzerland, Federal Department of the Interior, 1986; U.S. EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA): 40 CFR part 160; U.S. EPA Tox Substances Control Act (TSCA): 40 CFR part 792
Previous evaluation:	In DAR 2003

Material and methods:	
Test item:	[^{14}C-cyclohexyl]-trinexapac-ethyl
Position of the radiolabel (* = 14C position)	
Lot/Batch No.:	GAN-XVII-72
Radiochemical Purity:	>95% (specific radioactivity 1.85MBq/mg (50.0µg/mg)
Test concentration:	0.04 kg a.s./ha, 0.16 kg as/ha
Test system:	Rice seeds (about 1000 seeds, variety *Oryza sativa ssp. Japonica cv Koshishikari*) soaked in water for five days, were sown in a 20x30 cm seedling box. 17 days old rice was transplanted in bunches of about 4 to 5 plants into 15 rectangular polyethylene containers (47x29x25 cm). Rice plants grown in pots in a greenhouse received a foliar spray treatment with radio-labelled CGA163935 at a rate of 0.040 kg as/ha in the growth stage of stem elongation (42 days after transplantation, BBCH 37-41) or at a rate of 0.16 kg as/ha at a later growth stage (64 days after transplantation, early panicle emergence). One hour, 7and 21 days after treatment totally three bunches of rice plants were taken at any given times from different containers (1X treatment). At maturity (82 DAT) the rest of the plant material was harvested and divided into grains, husks and straw. At 4X treatment plant samples were taken only at maturity (60 DAT) and divided into grains, husks and straw.
Stage of application:	At stem elongation (42 DAT with 0.04 kg as/ha) or at early panicle emergence (64 DAT with 0.16 kg as/ha)
No. of applications:	One
Sampling time points:	Plant samples of the low dose group were taken at 1 h as well as at 7 and 21 DAT. At maturity (82 DAT) the rest of the plant material was harvested and divided into grains, husks and straw. Samples of the high dose group were only taken at maturity (60 DAT).
Method of analysis:	For analysis, all plant material was homogenized. Aliquots were taken for combustion analysis (in triplicates), and the remaining samples were kept frozen until further analysis. Homogenized plant material was repeatedly extracted with methanol/water (8:2, v/v). After cold extraction, a hot extraction with n-propanol/water (8:2, v/v) was performed. Acidic metabolites were isolated by anion exchange chromatography (DEAE Sephadex). Conjugated metabolites were cleaved using Macerozym enzyme digestion. In the extracts, the parent compound and metabolites were determined and quantified by TLC. Total radioactive residue in plant parts and residues in non-extracted plant material were determined by combustion analysis using liquid scintillation counting.
Study dates:	Biological phase: December 26, 1994 – May 15, 1995 Analytical phase: February 22, 1995 – December 22, 1995
Storage stability:	All samples used for analysis were kept frozen at -18°C. Analysis had been completed within 2.5 month after harvest, no storage stability test was performed
Limit of detection:	0.001 mg/kg
Method validation:	The extracted radioactivity with methanol/water was 16.2%, 55.0% and 56.5% in grains, husks and straw of the 0.04 kg as/ha treatment. Another 12.5%, 8.3% and

10.4% of the respective plant parts were solubilized by harsh microwave extraction.

Results

The distribution and identification of radioactive residues in rice treated with CGA 163935 in the greenhouse are summarised in tables B.7.2.1-5 (application rate of 0.04 kg/ha) and B.7.2.1-6 (application rate of 0.16 kg/ha).

The distribution of the label in rice plants was also studied by autoradiography. The autoradiogram showed even distribution of the radioactivity in the whole plant and denser labelling of new growth, indicating that the residue is translocated from the treated plant parts into new growths.

The content of the parent compound (CGA 163935) decreases rapidly in foliage and is at the 0.040 kg as/ha application rate undetectable in all plant parts at maturity. At the high application rate (0.16 kg as/ha), the parent compound is detected at low levels. In addition to the parent compound CGA 163935, 11 significant metabolite fractions are found. At maturity, the metabolite CGA 179500 is the major metabolite in grains and husks. In grains, CGA 179500 is mainly found in its free form, whereas in straw and husks the major part of it is found as conjugate with sugars and/or other plant constituents. Other minor metabolites identified are CGA 512753, CGA 275537, CGA 313458, and CGA 329773.

In straw, CGA 275537 is the major metabolite identified whereas CGA 179500 is also present at relevant levels.

RMS comments and conclusions (Netherlands 2003)

The uptake, distribution and metabolism of the parent compound CGA 163935 was studied in rice following application at a rate of 0.040 or 0.16 kg as/ha. The metabolic pathway of the parent compound (CGA 163935) in rice plants is proposed to proceed via hydrolysis of the ester bond to CGA 179500. This is followed to a small extent by aromatization of the 6-membered ring, presumably by hydroxylation followed by elimination of water and keto-enol tautomerism, to yield 4-cyclopropanecarbonyl-3,5-dihydroxybenzoic acid (CGA 329773). The 6-membered ring of CGA 179500 is also cleaved to produce 3-carboxy-7-cyclopropyl-5,7-diketoheptanoic acid (CGA 313458), in free and conjugated form. Further stepwise oxidation/decarboxylation following the cleavage of the 6-membered ring give rise to saturated and unsaturated tricarboxylated acids such as tricarballylic acid (CGA 275537) and aconitic acid (CGA 312753). Finally, CGA 179500, CGA 313458 and CGA 275537 are conjugated with sugars and/or other plant constituents.

Tricarballylic acid (CGA 275537) and aconitic acid (CGA 312753) are intermediates of the citric acid cycle (Krebs cycle). These intermediates are used for de-novo synthesis of sugars, fatty acids and certain amino acids. It is reasonable to assume that CGA 275537 and CGA 312753 are metabolized in the Krebs cycle and integrated by de-novo synthesis into the plant matrix.

Guidelines and limitations

Total extraction from 0.04 kg a.s./ha treatment was very low (28.7%, 63.3% and 66.9% for grain, husks and straw respectively). Rice is not a representative use. The study was considered acceptable for the overall evaluation.

Table B.7.2.1-5: Residues in rice after greenhouse application of CGA 163935 (rate 0.040 kg as/ha)

	1 h foliage		7 DAT foliage		21 DAT foliage		82 DAT grain		82 DAT husks		82 DAT straw	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
TRR	0.57	100	0.14	100	0.066	100	0.085	100	0.17	100	0.16	100
Organosoluble		97		89		41		10		6.1		56
Water soluble						45		6.0		49		-
Identified		86		43		36		23		50		29
CGA 163935 (parent)	0.37	66	0.008	5.5	0.001	1.6	<0.001	0.1	0.003	1.8	0.001	0.9
CGA 179500	0.10	18	0.036	26	0.017	25	0.010	12	0.015	8.9	0.008	4.9
CGA 329773							0.003[1]	2.9 [1]	0.001	0.7		
CGA 313458	0.012	2.2	0.007	5.1	0.002	2.6					0.007	4.6
CGA 275537		-	0.006	4.0	0.003	3.9	<0.001	0.5	0.005	3.2	0.031[4]	19 [4]
CGA 312753		-	0.004	2.5	0.002	2.6	0.007[2]	8.0 [2]	0.058[3]	35 [3]		
Characterised		9		49		52		16		18		45
II_1			0.025	18	0.014	22					0.048	29
II_2			0.008	6.0	0.004	5.4						
II_4			0.004	2.9	0.002	2.7			0.003			
II_6			0.009	6.5	0.005	7.1			0.007	4.4	0.004	2.4
II_7			0.003	1.9					0.002	1.3		
Unresolved	0.043	7.6	0.014	9.8	0.006	8.9	0.004	4.3	0.007	4.3	0.006	3.8

	1 h foliage		7 DAT foliage		21 DAT foliage		82 DAT grain		82 DAT husks		82 DAT straw	
	mg eq/kg	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**
Cold extracted		97.2		88.6		82.1		16.2		55.0		56.5
Microwave extract		1.2		3.7		5.5		12.5		8.3		10.4
Not extracted	0.008	1.4	0.011	8.1	0.010	16	0.061	72	0.069	41	0.065	40
Total (extracted+not extracted)		99.8		100.4		103.2		100.7		104.4		107.4

* TLC fraction,

[1] the sum of cold and microwave extracts including CGA 313458 and fractions II_7, and II_6

[2] the sum of cold and microwave extracts including CGA 313458 and fractions II_1, II_2 and II_4

[3] the sum of cold and microwave extracts including fractions II_1 and II_2

[4] the sum of cold and microwave extracts including CGA 312753 and fractions II_2 and I, I_{4a} and CGA 275537

Table B.7.2.1-6 Residues in rice after greenhouse application of CGA 163935 (rate 0.16 kg as/ha)

	60 DAT grains		60 DAT husks		60 DAT straw		60 DAT straw (not-extracted)	
	Mg eq/kg	% TRR	Mg eq/kg	% TRR	mg eq/kg	% TRR	% TRR	% fraction
TRR	1.1	100	2.2	100	1.6	100	19	100
Organosoluble								
Water soluble								
Identified		45		63		31		5
CGA 163935 (parent)(total)	<0.01	0.4*		6.2		1.4		
Free form		0.4		6.2		1.3		
Released from debris following microwave extraction		-		-		0.1		
CGA 179500 (total)		36		30		8.6		0.9
Free form		20.2		11.0		6.7		
Conjugated (released with 0.1M NaOH)		3.8		18.5		1.7		
Released from debris following digestion with 0.5 N NaOH and hydrolysis of starch at pH 1 / or microwave extraction		11.6/-		-/0.4		-/0.2		
CGA 329773 (total)		2.5		1.2		0.8		1.5
Free form		0.8		0.9		0.8		
Released from debris following digestion with 0.5 N NaOH and hydrolysis of starch at pH 1 / or microwave extraction		1.7/-		-/0.3		-		
CGA 313458 (total)		3.3		7.4		7.2		
Free form		-		0.4		4.1		
Conjugated (released with 0.1M NaOH)		0.2		6.5		2.3		
Released from debris following digestion with 0.5 N NaOH and hydrolysis of starch at pH 1 / or microwave extraction		3.1/-		-/0.5		-/0.8		
CGA 275537 (total)		3.2		17		13		3.0
Free form		0.3		-		0.8		
Conjugated (released with 0.1M NaOH)		1.6		16.8		12.2		
Released from debris following digestion with 0.5 N NaOH and hydrolysis of starch at pH 1 / or microwave extraction		1.3/-		-/0.2		-/0.3		
CGA 312753				1.1[1]				

	60 DAT grains		60 DAT husks		60 DAT straw			60 DAT straw (not-extracted)	
	Mg eq/kg	% TRR	Mg eq/kg	% TRR	mg eq/kg	% TRR		% TRR	% fraction
Characterised		36		21		49			82
II**$_1$		14 [2]		9.9[3]		31[4]			13
II_2						0.7			5.3
II_{2a}									4.4
II_{3a}				3.0[3]					
II_{3b}		1.3							
II_4						2.3			
II_6		1.6		0.4		1.4			
II_7		1.2		0.3		0.8			
II_{10a}				0.2		0.2			
II_{10b}				0.7					
Glucose		8.0							
STR-E5(E4)									36
STR-E7(E6)									7.1
STR-R6(R5)									3.6
Pectin fraction									0.5
Cellulose fraction									7.0
Lignin fraction									0.3
Unresolved		9.5		6.2		13			5.1
Cold extracted		33.9		78.0		73.2			
Microwave extract		19.6		5.3		8.2			
Not extracted		45.0		16		19			
Total (extracted + not extracted)		98.5		99.3		100.5			

* total level observed was 6.4 mg/kg of which 6.0 mg/kg is most probably an artefact

** TLC fraction,

[1] including fraction II_2

[2] consisting of CGA 179500 and CGA 275537 in various conjugated forms (esters of sugars and/or plant constituents)

[3] consisting of CGA 179500, CGA 275537 and CGA 313458 in various conjugated forms (esters of sugars and/or plant constituents)

[4] consisting of several less polar derivatives

Comments RMS LT

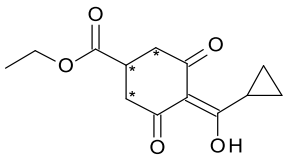
Quantitative TLC analysis of the cold and microwave extracts of all plant parts hed been completed within 2.5 months after harvest, no storage stability test was performed and is not required.

Deviations from OECD 501:

Purity of reference substances not provided.

RMS LT agrees with the conclusions made by RMS NL, study is considered suitable for evaluation.

Study 5
EU reviewed metabolism study in grass

Reference:	**Ray W. J., May-Hertl U. (2003)** [1,2,6-^{14}C]Cyclohexyl-CGA-163935: Nature of the residue in Field Grown Grass (~~KCA 6.2.1 / 05~~ KIIA 6.1.3.4 / 01)
Study No.:	623-00
Guideline:	Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the residue – Plants, Livestock
GLP:	U.S. EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA): 40 CFR part 160 with some exceptions*;
Previous evaluation:	In DAR 2003
Material and methods:	
Test item:	[^{14}C-cyclohexyl]-trinexapac-ethyl
Position of the radiolabel (* = 14C position)	
Lot/Batch No.:	BPM-XXIV-58
Radiochemical Purity:	98.1% (specific radioactivity 1.55MBq/mg (42.3µg/mg)
Test concentration:	0.50 kg as/ha (target application rate 0.56 kg as/ha)
Test system:	Tall fescue (82RH variety), grown in three field plots of 1.9 m x 3.0 m received one post foliar broadcast spray of 0.56 kg as/ha radio-labelled CGA 163935 46 days prior to swathing of the grass for harvest of mature grass seeds. Pre-forage samples were taken 22 days after application and hay samples were collected 46 days after application. Forage regrowth was collected 102 days after application. Soil samples were also taken at each of the harvest intervals. The entire plots were harvested using hand clippers. Plants were harvested, placed on plastic, and allowed to dry in the field for 13 days. The hay samples were separated into straw, seed and seed screenings.
Stage of application:	46 days prior to swathing

No. of applications: One

Sampling time points: 22 DAT - pre-forage samples; 46 DAT - hay samples; 102 DAT - forage regrowth.

Method of analysis: Subsamples of hay, straw, seed and seed screenings were homogenised, extracted with an acetonitrile-water mixture (ratio 4:1, v/v) and purified using C-18 solid phase extraction. The initial post-extracted solids of 22-day forage, straw, seed and seed screenings were refluxed with different mixtures of organic solvents (acetonitrile, n-propanol and methanol) and water (ratio 4:1, v/v). Neutral solvent extracts were hydrolysed using cellulase or a mixed cellulase, amylocellulase, β-glucosidase treatment. The extracts were analysed by HPLC-UV and 1D- and 2D-TLC. Identification was performed by co-chromatography with known standards and LC/MS/MS. Radioactivity in non-extractable solids was determined by liquid scintillation counting (LSC) after combustion; radioactivity in liquid samples and extracts was determined by LSC directly.

All samples were placed and labelled in double plastic bags, weighted and placed in frozen storage before sending to VBRC for analysis. Proven to be stable after 13 months:

Storage conditions: Samples were stored at -20°C for 13 months from sample to analysis.

Concentrated sample extracts were stored at -20°C or 4°C between chromatographic analyses.

Storage stability:

TABLE 8. COMPARISON OF INITIAL AND FINAL STABILITY EXTRACTIONS AND PROFILES

	Straw		Seed Screenings		Regrowth Forage (105 day)	
	Initial	Final	Initial	Final	Initial	Final
Date Collected	7/20/01	7/20/01	7/20/01	7/20/01	9/14/01	9/14/01
Date Extracted	11/5/01	12/10/02	11/7/01	12/10/02	11/27/01	12/10/02
Extraction Results (%TRR):						
ACN/Water Extract	70.36	63.30	45.03	44.70	55.54	46.40
PES	28.23	34.83	53.35	48.07	34.83	41.62
%TRR in Major HPLC Peaks:						
G1	15.38[a]	11.16[a]	1.98	0.50	4.40	2.22
G2			4.68	6.72	9.34	15.90
G3	4.50	4.54	2.95	3.74	2.72	3.37
G4	6.41	7.15	4.15	3.33	6.61	2.66
G5	6.34	4.86	6.61	6.81	10.23	3.74

[a] Metabolite A (G1)/CGA-275537 (G2) were not separated in this substrate.

Limit of quantification: ~~0.003~~ 0.004 mg/kg

Method validation: The extractable radioactivity (extracted with 4:1 ACN/water) for the grass samples was 75.54% (forage), 70.36% (straw), 45.62% (seeds), 45.03% (seed screenings) and 55.54 (regrowth forage).

* - NOAA and Western Regional Technical Centre weather data were not collected under GLP protocol. Irrigation amounts are approximate. Weights of samples recorded at the Western Regional Technical Centre are from a balance that was not calibrated under GLP. The soil characterization was not done under a GLP protocol. Data generated was dated and signed upon completion, but in some instances not entered into the LNB in a prompt fashion. Three reference substances used in this study were non-GLP.

Results

The distribution and identification of radioactive residues in field grown grass treated with 0.56 kg/ha CGA 163935 are summarised in table B.7.2.1-7.

The initial extraction with acetonitril:water (4:1 v/v) released 76% (22 day forage), 67% (straw), 54% (seeds), 45% (seed screenings) and 51% (~~105~~ 102 day regrowth forage) of the total radioactivity. The majority of the remaining radioactivity was released by the subsequent reflux treatments using organic solvents-water mixtures (102 day regrowth forage was not exposed to these treatments).

In none of the grass samples examined, the parent compound was detected. In the extracts of 22 day forage, straw, seeds and seed screenings, the metabolites CGA 179500 (see guidelines and limitations), CGA 275537, Metabolite A and Metabolite C were the major metabolites identified, exceeding 10% of the TRR in one or more of the samples.

Table B.7.2.1-7: Residues in field grown grass after a field application of CGA 163935 (rate 0.50 kg as/ha)

	Forage 22 day		**Straw**		**Seeds**		**Seed screenings**		**Forage 102 day regrowth**	
	mg eq/kg	**% TRR**	**Mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**
TRR	2.0	100	4.8	100	5.5	100	7.1	100	0.054	100
Water soluble[1]										
Identified	0.92	46	2.4	51	2.8	51	6.6	55	0.018	33
CGA 275537 (Total, rounded)	0.28	13.9	0.81	17.0	0.91	16.6	1.2	16.6	0.005	9.3
Free form	0.163	8.05	0.271	5.68	0.249	4.57	0.334	4.68	0.005	9.34
Released from debris following reflux / microwave extraction	0.117	5.76/-	0.538	11.27/ -	0.656	- /12.04	0.849	- /11.91		-
CGA 179500 (Total, rounded)	~~0.13~~ 0.33	~~6.1~~ 16.3	~~0.38~~ 1.07	~~7.9~~ 22.3	0.80	~~15~~ 14.7	0.91	~~13~~ 12.7	0.006	~~10~~ 10.2
Free form	0.105	5.18	0.303	6.34	0.474	8.69	0.471	6.61	0.006	10.23
Conjugated (released after enzyme treatment)		10.2		14.4		-		-		-
Released from debris following reflux / and microwave extraction	0.018	0.91/-	0.077	1.62/-	0.329	-/6.04	0.435	-/6.1		-
Metabolite A / SYN540405 (Total, rounded)	0.15	7.4	0.48	10	0.10	1.9	0.27	3.8	0.002	4.4
Free form	0.142	7.02	0.463	9.7	0.104	1.9	0.141	1.98	0.002	4.4
Released from debris following reflux / and microwave extraction	0.009	0.44/-	0.029	0.6/-		-	0.127	-/1.78		-
Metabolite B / SYN540406 (Total, rounded)	0.17	8.6	0.27	5.6	0.46	8.3	0.70	9.9	0.001	2.7
Free form	0.141	6.96	0.215	4.5	0.155	2.84	0.210	2.95	0.001	2.72
Released from debris following reflux / and microwave extraction	0.033	1.62/-	0.053	1.1/-	0.299	-/5.48	0.491	-/6.89		-
Metabolite C / NOA433257 (Total, rounded)	0.20	9.8	0.45	9.4	0.53	9.6	3.5	11.8	0.004	6.6

	Forage 22 day		Straw		Seeds		Seed screenings		Forage 102 day regrowth	
	mg eq/kg	% TRR	Mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
Free form	0.172	8.48	0.306	6.41	0.205	3.77	0.296	4.15	0.004	6.61
Released from debris following reflux / and microwave extraction	0.026	1.26/-	0.144	3.02/-	0.315	-/5.78	0.539	-/7.56		-
Characterised	0.97	49	2.4	49	2.8	52	3.7	51	0.012	25
Unknown region 1 (Total, rounded)	0.16	8.0	0.32	6.8	0.42	7.7	0.51	7.2	0.005	10
Initial extract	0.112	5.55	0.15	3.15	0.144	2.64	0.211	2.96	0.005	10.04
Released from debris following reflux / and microwave extraction	0.048	2.36/-	0.171	3.57	0.280	-/5.14	0.298	-/4.19		
Unknown region 2 (Total, rounded)	0.18	8.8	0.49	11	0.26	4.7	0.41	5.7	nd	nd
Initial extract	0.161	7.95	0.382	8.0	0.138	2.53	0.216	3.03		
Released from debris following reflux / and microwave extraction	0.017	0.83/-	0.107	2.23	0.118	-/2.16	0.189	2.65		
Unknown region 3(Total, rounded)	0.020	0.99	0.37	7.8	0.47	8.5	0.67	9.4	0.001	2.7
Initial extract		-	0.224	4.69					0.001	2.69
Released from debris following reflux / and microwave extraction	0.02	0.99/-	0.146	3.06	0.465	8.52	0.669	-/9.39		
Unknown region 4 (Total, rounded)	0.36	18	0.43	8.9	0.92	17	1.2[2]	17[2]	0.004	6.9
Initial extract	0.324	15.99	0.427	8.93	0.517	9.48	0.628	8.81	0.004	6.85
Released from debris following reflux / and microwave extraction	0.043	2.12/-			0.399[2]	-/7.32[2]	0.589	-/8.27		
Unknown region 5 (Total, rounded)	0.15	7.3	0.49	10	0.38	7.1	0.48	6.7	0.001	2.7
Initial extract	0.148	7.31	0.301	6.3	0.382	7.01	0.476	6.68	0.001	2.66
Released from debris following reflux / and microwave extraction			0.185	3.88/-						
Unknown region 6 (Total, rounded)	0.10	5.0	0.26	5.4	0.40	7.4	0.34	4.7	0.001	2.6
Initial extract	0.057	2.79	0.08	1.68	0.139	2.55	0.105	1.47	0.001	2.63
Released from debris following reflux / and microwave extraction	0.045	2.23/-	0.175	3.67	0.259	-/4.75	0.230	3.23		
Total extracted (ACN/water)	1.527	75.28	3.12	65.39	2.51	45.98	3.089	43.32	0.03	58.17
Total extracted from PES	0.376	18.52	1.625	34.03	3.120	57.23	4.418	61.96	-	-

[1] ~~Values are summations of amounts detected in the initial extracts (with acetonitril:water (4:1, v/v) and amounts detected in reflux extracts; calculated by the RMS. Initial extract + PES~~

[2] Value represents part of region 4 + 5

nd: not detectable

The levels of radioactive residues in soil samples collected after the 1st application, 45 day harvest and ~~105~~ 102 day harvest were 0.37, 0.079 and 0.083 mg eq/kg, respectively. The extractable residues represented 71% TRR, 7.7% TRR and 8.8% TRR of soil samples collected after the 1st application, 45 day harvest and 105 day harvest, respectively.

RMS comments and conclusions (Netherlands 2003)

After a single post foliar application of CGA 165935 at a rate of 0.56 kg as/ha to field grown tall fescue, CGA 179500, CGA 275537 and Metabolite C were the major metabolites identified, exceeding 10% of the TRR in one or more of the samples of 22 day forage, straw, seeds and seed screenings. In none of the grass samples examined, the parent compound was detected. The proposed metabolic pathway of CGA 165935 in grass is presented in figure B.7.2.1-1.

~~Considering that amenity grass is not intended for livestock feeding, no intake of CGA 165935 residues via amenity grass by livestock is anticipated~~.

The GAP in the present study is 2.8 times higher than the critical GAP that prevails in several NEU member states (0.20 vs 0.56 kg a.s./ha). However, grass is not a representative use.

Guidelines and limitations

The residue values of CGA 179500 in 22 day forage and straw, as they are presented in table 7 of the original study report, could not be retrieved from the raw data by the RMS. Furthermore, the identified residue components in the initial extract and in the reflux extracts were summed in table 7 of the original study report, where that was not the case for the characterised residue components. No explanation was provided for this difference. Considering that no livestock intake is anticipated and considering that this limitation is only of low value (does not alter the proposed metabolic pathway or proposed major metabolites), the study is considered acceptable.

Assessment

The evaluated study on grass metabolism indicates that trinexapac-ethyl metabolism in grass both quantitatively and qualitatively differs to some extent from metabolism in wheat, rice and rape. This does not influence the residue definition for plant products since there is no intake of grass by humans.

The metabolites found in grass forage and straw are structurally related with the parent and/or with the metabolites CGA 275537 and CGA 329773, of which toxicological data are provided. Considering this structural relationship and the toxicological data provided on parent and the metabolites CGA 275537 and CGA 329773, the metabolites detected in grass forage and straw are considered of little toxicological concern. Therefore, no additional animal metabolism studies and livestock feeding studies are considered necessary.

Comments RMS LT

The discrepancy mentioned in "guidelines and limitations" by RMS NL due to residue values of CGA 179500 in 22 day forage and straw, as they are presented in table 7 of the original study report, could not be retrieved from the raw data by the RMS, was explained by the applicant stating that additional amount of conjugated CGA 179500 were extracted after enzyme treatment, thus leading to higher total amounts (as detailed in table B.7.2.1-7).

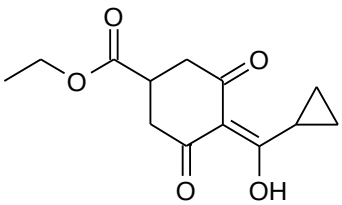
Poor extractability was observed only in seeds, sedd screenings and 102 days regrowth forage (45.98 %, 43.32 % and 58.17 % TRR extracted, respectively). Additional 57 % and 62 % TRR from seeds and seed screenings respectively was extracted from PES after reflux and microwave treatment. Only for 102 days regrowth forage samples no attempts to extract and analyse TRR in PES (34.83 % TRR) are mentioned nor the explanation provided in the report.

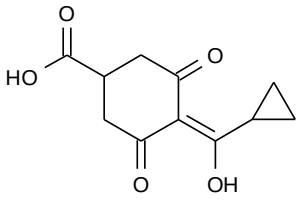
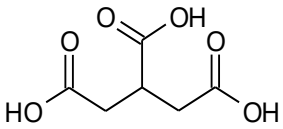
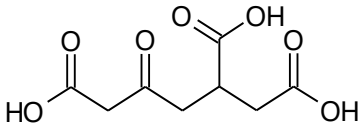
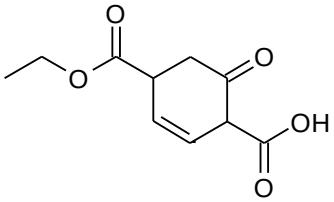
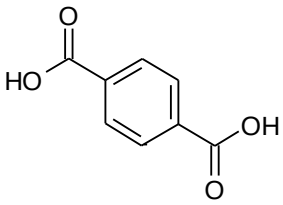
Storage stability was proven based on repeat analyses at the beginning (11/5/01-2/5/02) and the end (12/02) of analytical phase. Qualitatively, no new major metabolites were formed and no characterised metabolites were depleted, qualitatively, the metabolite profiles were unchanged after 13 months of frozen storage. Although the length of storage of extracts is not detailed in the report, results are considered not to be affected by storage.

Study report includes a statement regarding LOQ, "based on an average background of 40 dpm, a specific activity of 42.3 µCi/mg and an aliquot size of 200 mg, the limit of quantitation was calculated to be 0.004 ppm in tissue and milk samples". As this is grass metabolism study, explanation for this discrepancy would be desirable. RMS is of the opinion that might be a copy-paste mistake.

Despite minor limitations the study is considered suitable for evaluation.

Table B.7.2.1-8 List of identified compounds in field grown grass

Code	Compounds	Description
CGA 163935		Parent compound 4-(Cyclopropyl-alpha-hydroxy-methylene - 3,5-dioxocyclohexanecarboxylic acid ethyl ester

CGA 179500		4-(Cyclopropyl-alpha-hydroxy-methylene - 3,5-dioxocyclohexanecarboxylic acid
CGA 275537		1,2,3-propanetricarboxylic acid (tricarballylic acid)
Metabolite A (SYN540405)		4-oxopentane-1,2,5-tricarboxylic acid
Metabolite B (SYN540406)		4-ethoxycarbonyl-6-oxo-cyclohex-2-ene-1-carboxylic acid
Metabolite C (NOA433257)		Terephthalic acid

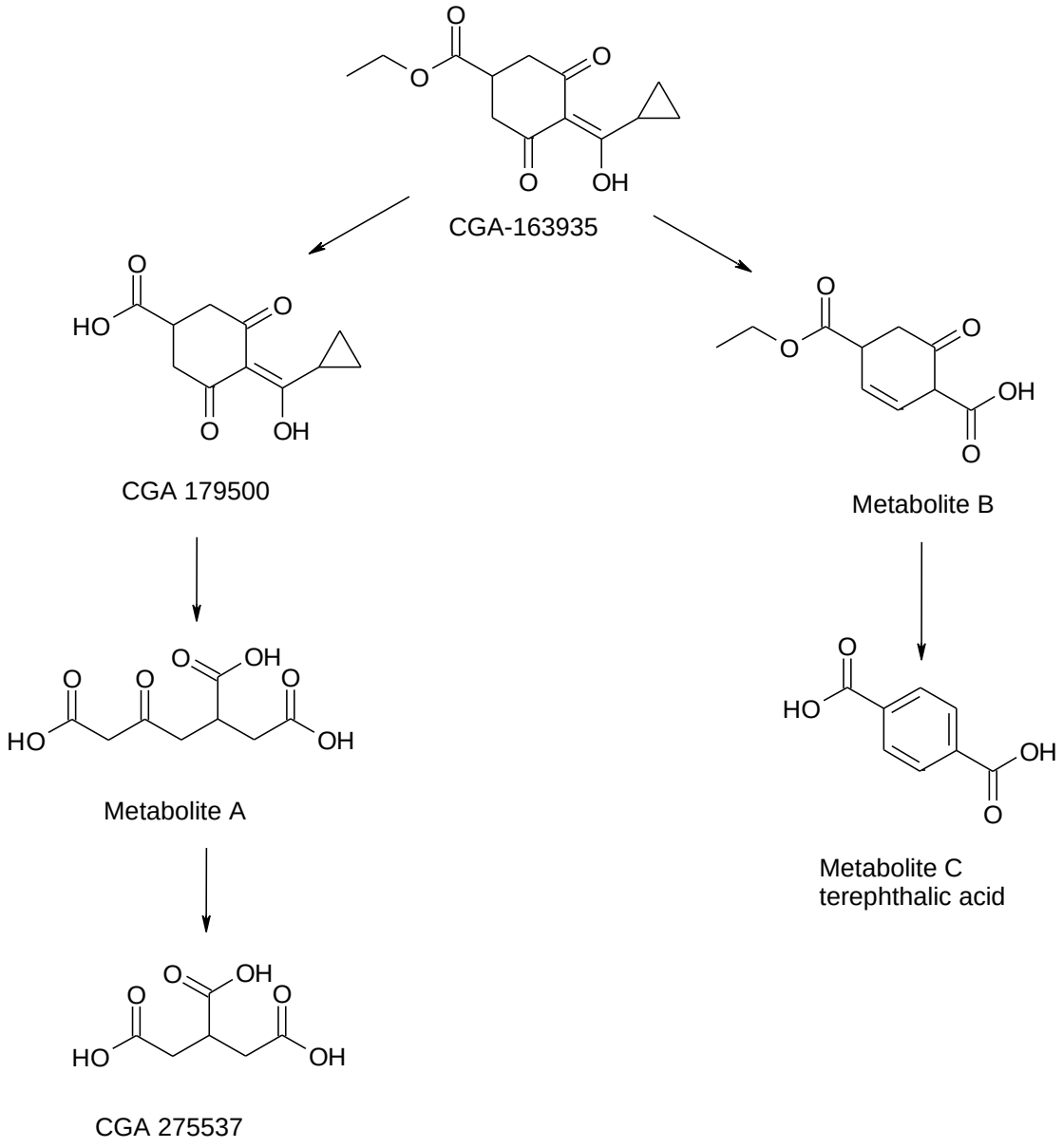


Figure B.7.2.1-1 Proposed metabolic pathway of CGA 163935 in grass

Remark RMS Netherlands, 2003

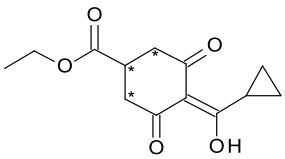
Considering that no livestock intake is anticipated (see intended uses) the results of this metabolism study (field grown grass) have not been included in the (overall) risk assessment by the RMS Netherlands.

Study 6

New metabolism study in oilseed rape

Reference:	**Piskorski R. (2015)** Trinexapac-ethyl – Metabolism of [^{14}C]Trinexapac-ethyl in Oilseed Rape. Final Report. (KCA 6.2.1 / 0~~6~~61)
Study No.:	20120173
Guideline:	OECD Guideline for the Testing of Chemicals, 501, Metabolism in Crops (January 2007). Nature of the Residue - Plants, Livestock; United States Environmental Protection Agency; Residue Chemistry Test Guidelines OPPTS 860.1300; (August 1996). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Japanese MAFF Guideline on the Application for Agricultural Chemicals Registration (12 Nohsan No. 8147, November 2000).
GLP:	OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final]. The Swiss Ordinance on Good Laboratory Practice, adopted on 18th May 2005 [OGLP, SR 813.112.1].
Previous evaluation:	Submitted for the purpose of renewal

Material and methods:

Test item:	[^{14}C]-Trinexapac-ethyl
Position of the radiolabel (* = ^{14}C position)	
Lot/Batch No.:	RDR-XV-70
Radiochemical Purity:	98.6% (specific radioactivity 2.468 MBq/mg)
Test concentration:	393.8 g a.s./ha
Test system:	Oilseed rape plants (variety *Jumbo*) were grown in total of 9 containers (40 x 60 x 40 cm). The biological phase was carried out under greenhouse conditions at the IES Ltd facilities from 17 January, 2013 (application) to 18 April, 2013 (harvest of mature plants).Containers were filled with sandy loam soil to approximately 7 cm from the top. The soil was allowed to settle for 5 days. To prevent infestation with wireworms, four days before sowing, the soil was treated with Dursban 750 WG at a rate of 3.0 g/m2. Oilseed rape seeds were sown directly into the containers at a planting density of 5 kg/ha. The radiolabelled test item, [^{14}C]-trinexapac-ethyl was formulated to a specification approximating the microemulsion MODDUS ME 250 A8587F using the blank formulation EXF228A. The oilseed rape plants were treated with the formulated test item at BBCH 55 by foliar spray application at a nominal rate of 375 g a.s./ha and a spray volume rate of 250 L/ha. The achieved application rate was 393.8 g a.s./ha. The system was maintained under controlled climatic

	conditions with the following artificial light conditions: day – 14 h; night – 10 h. Foliage samples were collected from 2 crates 21 days after application (BBCH 57-67). Fully ripe plants were collected from 6 crates between 67 and 91 days after application (BBCH 89) and left to dry in the greenhouse. At each harvest, the plant was cut above ground (no roots were harvested). Dried seeds were separated from pods manually and the pods were included in the trash sample. A total of 3 samples were collected (foliage, trash and seeds). Foliage and trash (including pods) were retained deep frozen as a contingency. Only the seeds were analysed. The soil was sandy loam and the soil characteristics were: pH (7.79), sand (54.26%), silt (33.03%), clay (12.71%), cation exchange capacity (CEC, 8.58 meq/100 g) and organic carbon (0.72%). A seed sub-sample (250 g) was homogenised using a food processor and stored at approximately -20 ^{0}C. Initial chromatographic analysis of the commodity extracts took place within 6 months after harvest. These extracts were then re-chromatographed 16 months later upon study completion. Comparison of the initial and final radiocomponent profiles obtained showed that no significant change in the profile occurred during the interim period of storage.
Stage of application:	BBCH 55
Storage:	Initial analysis of the sampled seeds combined extracts took place within 6 months after harvest. Extracts were analysed within 16 months. Samples and extracts stored at <-20°C.
No. of applications:	One foliar application
Sampling time points:	Oilseed rape plants were harvested at two growth stages: foliage at BBCH 57-67 (21 days after application) and whole plants at the crop maturity (BBCH 89), 67-91 days after application. Only the seeds harvested at maturity were analysed.
Method of analysis:	The seed sample was extracted with acetonitrile:water/hexane mixtures. Following separation of phases the residues present in the acetonitrile:water fractions were partitioned between diethyl ether:hexane and water. Polar radiocomponents present in the aqueous phases were hydrolysed with diluted sodium hydroxide. Non-polar residues present in hexane fractions (associated with endogenous oils) were saponified (base hydrolysed) and partitioned between diethyl ether and water. Residues present in partitioned/hydrolysed fractions were subject to thin layer chromatography (TLC) analysis to enable the quantification of radiocomponents and the identification/characterisation of residues by their comparison with authentic reference standards of parent and its metabolites. Results obtained by TLC were confirmed by high-performance liquid chromatography (HPLC). Non-extractable radioactive residues were characterised by sodium dodecylsulphate (SDS) extraction, mineral acid extraction under mild conditions as well as mineral acid extraction under reflux conditions followed by dabsyl chloride derivatization and glucose analysis.
Limit of quantification	0.008

Results

The total radioactive residues (TRR) present in mature seeds was 0.394 mg/kg of which 67.4% TRR was rendered solvent extractable at room temperature (see Table B.7.2.1-9).

The extracted radioactivity was analysed by chromatography. The identified components for each sample are summarised in Table B.7.2.1-10.

No residues of parent trinexapac-ethyl were detected in seeds.

The trinexapac-ethyl metabolite accounting for the highest proportion of the radioactive residue was trinexapac-acid (CGA179500) accounting for 21.8% TRR (0.086 mg/kg; found in both the free and conjugated forms of the metabolite). A much lower level of a tricarballylic acid metabolite, CGA275537, was also detected (1.0% TRR; 0004 mg/kg; found exclusively in the conjugated form).

Another major radiocomponent was also detected following saponification of radioactivity associated with hexane-extracted endogenous oils and identified as [^{14}C]-oleic acid (22.7% TRR; 0.090 mg/kg). This result demonstrates: i) a large proportion of [^{14}C]-trinexapac-ethyl derived seed residue (at least 22.7% TRR) was extensively and completely metabolised to small ^{14}C-containing moieties (i.e. the terminal products of [^{14}C]-trinexapac-ethyl metabolism) and ii) natural incorporation of these small ^{14}C moieties into the biosynthetic pool of fatty acids and also into the broader endogenous pool is a significant feature of trinexapac-ethyl metabolism in the seed.

The levels of other individual unassigned radiocomponents from extracted residues never exceeded 6.7% TRR 0.027 mg/kg).

In further characterisation of the unextracted radioactive residues with SDS, only 5.8% TRR (0.023 mg/kg) was rendered soluble indicating only a small proportion of the residue was associated with proteinaceous material. No chromatographic analysis was undertaken due to low residue levels released and the high levels of endogenous coextractives present.

Following extraction of the unextractable radioactive residues with 1 M HCl at 60 °C and 6 M HCl at 140 °C (reflux), a further 4.0% TRR (0.016 mg/kg) and 15.2% TRR (0.060 mg/kg) was rendered water soluble respectively. No chromatographic analysis was undertaken on 1M HCl extract due to low residue levels released and the high levels of endogenous coextractives present. The HCl reflux extract was subjected to treatment with dabsyl chloride to derivatise amino acids present in the sample followed by TLC co-chromatography with (i) dabsyl derivative of glutamate; and (ii) [^{14}C] glucose. It was not possible to conclude on the presence of natural incorporation into amino acids however there was some evidence of natural incorporation of ^{14}C into glucose. A further 16.2 % TRR (0.064 mg/kg) remained as unextractable residues, however based on the harsh extraction conditions employed any residue present would not be bioavailable. Also, evidence of extensive natural incorporation into oil seed indicates that the residue is due to further incorporation into natural components.

Table B.7.2.1-9: Summary of total radioactive residues and extractability in oilseed rape treated with [14C]-trinexapac-ethyl at a rate of 393.8 g a.s./ha

Crop Commodity	Extractable Radioactivity		Non-extractable Radioactivity		TRR
	%TRR	mg/kg	%TRR	mg/kg	mg/kg
Seed	67.4	0.266	32.6	0.129	0.394

Table B.7.2.1-10: Identification and characterisation of radioactive residues in oilseed rape following one application of [^{14}C]-trinexapac-ethyl at a rate of 393.8 g a.s./ha

		SEED	
TRR by summation mg/kg		0.394 [1]	
TRR by direct quantification mg/kg		0.427 [2]	
Percentage of TRR for chromatography, %		67.9	
Origin of component	**Component**	**% TRR [a]**	**Residue (mg/kg) [a]**
Chromatographed [3]	CGA179500	21.8 (2.8)	0.086 (0.011)
	CGA275537	1.0 (1.0)	0.004 (0.004)
	Oleic acid	22.7	0.090
	Unassigned [4]	15.1	0.059
	Baseline [5]	7.3	0.029
	Other fractions [6]	1.4	0.006
	Losses/gains on fractionation [7]	1.9 (Gains)	0.008 (Gains)
	Unextracted [8]	32.6	0.128
	Total	100.0	0.394

a - Values without parentheses are the sum of both the free and conjugated forms. The values within parentheses indicate the proportion of the TRR that is in the conjugated forms.

1- TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.

2- The radioactive residue determined by direct quantification employing combustion/LSC.

3- The components of the TRR that were derived from chromatographic analysis.

4- Unassigned radiocomponents which chromatographed away from origin in (i) 2D-TLC SSA

comprising at least 12 discrete components, none of which >1.7% TRR (>0.007 mg/kg) (acetonitrile:water derived fractions) or (ii) 1D-TLC SS4 comprising at least 2 components, none of which >3.6% TRR (>0.014 mg/kg) (hexane derived fractions).

5- Polar material on origin of radiochromatogram of acetonitrile:water derived fractions using 2D-TLC SSA (6.7% TRR; 0.027 mg/kg) and of hexane derived fraction using 1D-TLC SS4 (0.6% TRR; 0.002 mg/kg).

6- Extractable residues in 1 fraction produced during processing that was not analysed due to low residue levels.

7- The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

8- Radioactivity remaining in debris after extraction with aqueous acetonitrile/hexane mixtures. The nature of the residues was characterised further by reflux extraction with 6 M HCl, 1 M HCl and SDS extractions.

RMS comments and conclusion

Following foliar spray application of [^{14}C]-trinexapac-ethyl (393.8 g a.s./ha) to oilseed rape plants at the growth stage BBCH 55, oilseed rape plants were harvested at two growth stages: at BBCH 57-67 (21 days after application) and at crop maturity (BBCH 89), 67-91 days after application, yet only the seeds harvested at maturity were analysed.

All samples and extracts were stored at -20°C prior to analysis. Initial analysis of the sampled seeds combined extracts took place within 6 months after harvest. In order to demonstrate the storage stability of the seed extract during the interim period between initial and final analysis, chromatographic profiles obtained initially were compared with profiles of the same extract obtained at the completion of analysis (16 months later). Residues were not affected by storage.

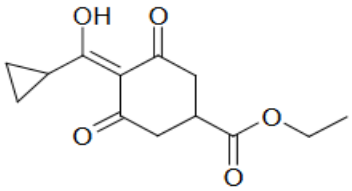
Total radioactive residues in the seed commodity were determined to be 0.394 mg/kg. Metabolism of parent trinexapac-ethyl was extensive and complete (parent was not detected); Trinexapac-acid CGA179500 was the principal metabolite identified (21.8% TRR; 0.086 mg/kg) and was detected in both free and conjugated forms; The tricarballylic acid metabolite CGA275537 was also identified but at much lower levels (1.0% TRR; 0.004 mg/kg) and was detected in the conjugated form exclusively; Natural incorporation of ^{14}C into crop endogenous constituents was observed (quantified at a level of at least 22.7% TRR; 0.090 mg/kg). The observed metabolites of trinexapac-ethyl arose via: De-esterification of the parent ethyl ester; De-esterification of the parent ethyl ester followed by the ring opening and the elimination of cyclopropylhydroxyethylene moiety with the oxidation of carbonyl groups.

The GAP in the present study is in accordance with the current GAP that prevails in several NEU member states (0.40 vs 0.393 kg a.s./ha). Oilseed rape is not a representative use.

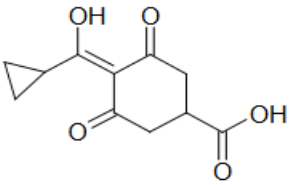
Deviations from OECD 501:

Purity of reference standards except parent was not provided in the report.

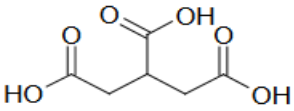
The study was well performed and reported and suitable for evaluation.



CGA163935



CGA179500



CGA275537

Small ^{14}C containing moieties

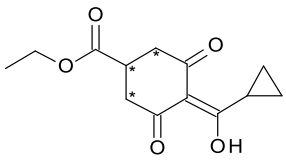
Natural incorporation into endogenous pool

Figure B.7.2.1-2: Proposed metabolic pathway of trinexapac-ethyl in oilseed rape

Study 7

New metabolism study in spring wheat

Reference:	**Piskorski R. (2015a)** Trinexapac-ethyl – Metabolism of [14C]-Trinexapac-ethyl in spring wheat (KCA 6.2.1 / 072)

Study No.:	20120098
Guideline:	OECD Guideline for the Testing of Chemicals, 501, Metabolism in Crops (January 2007) EPA Residue Chemistry Test Guideline OCSPP 860.1300, Nature of the Residue in Plants (August 1996) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market Japanese MAFF Guidelines on the Application for Agricultural Chemicals Registration Nohsan No. 8147 (November 2000)
GLP:	OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final]
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test item:	[^{14}C]-Trinexapac-ethyl ([cyclohexanedione-1,2,6-^{14}C]-CGA163965)
Position of the radiolabel (* = ^{14}C position)	
Batch No.:	TAA-I-47
Radiochemical Purity:	99.2% (specific radioactivity 2.468 MBq/mg)
Test concentration:	211g a.s./ha
Test system:	Spring wheat plants (variety *Monsoon*) were grown in a total of 10 containers (40 x 60 x 40cm). The wheat plants were treated with the formulated test item of ^{14}C trinexapac-ethyl at BBCH 37 by foliar spray application at a nominal rate of 200 g a.i./ha and the spray volume rate of 250 L/ha. The test item was applied as a formulation with a hand-held sprayer. Eight containers were treated with the test item, giving approximately 2 m^2 of the total treated plant surface. For every set of 2 crates, 15 mL of the application solution was applied as a spray foliar treatment with a hand-held sprayer. Ninth container was treated with the blank formulation only and the tenth was left untreated; both were grown for control purposes. Containers were maintained outdoors under natural conditions. Wheat plants were harvested by cutting the stems of the plants approximately 10 cm above the soil with a knife or scissors. After harvest, all samples were weighed, and hay and mature samples were dried in the greenhouse. After drying, the mature harvest was separated into straw and grain; the straw sample contained also chaff. All samples were homogenized with a food processor (forage under liquid nitrogen) and stored at approximately -20 °C prior to analysis.
Storage:	Initial analysis of the wheat sampled commodities combined extracts took place < 6 months after harvest. The original aqueous and organic phases of partitioned grain

	extract (analysed initially 5.5 months after harvest) were re-analysed 34 months after harvest, i.e. upon study completion. Comparison of initial and final radiocomponent profiles showed that no significant changes in the profiles had occurred during the interim period of storage. Stored at <-20°C.
Stage of application:	BBCH 37
No. of applications:	One foliar application
Sampling time points:	Wheat plants were collected on three occasions, as an immature crop at a forage growth stage (BBCH 43, 7 days after application), at a hay growth stage (BBCH 77, 34 days after application) and at maturity (BBCH 89; as grain and straw, 62 days after application).
Method of analysis:	Sub-samples of each wheat commodity were extracted sequentially with acetonitrile/water mixtures. Aliquots of the extracts were radioassayed by LSC. Aliquots of the post extraction solid (PES) were radioassayed by combustion analysis/LSC. The radioactive residue present in the solvent extracts was added to those of the PES in order to determine the total radioactive residue (TRR) of each commodity. Extracts containing significant quantities of radioactivity were combined and concentrated prior to TLC/HPLC analysis. Sub-samples of extracts were subject to acid and/or base hydrolysis to affect the release of metabolites from their conjugated forms. Unextracted residues in the post extraction debris from hay, straw and grain were further characterised using the clean fraction procedure to separate the residue into lignin, hemicellulose and cellulose containing fractions. Additionally, unextracted residues in the post extraction debris from grain were subject to enzyme hydrolysis to release metabolites from their conjugated forms and cleave ^{14}C starch to ^{14}C glucose. Residues present in the principal residue containing fractions derived from both pre- and post-hydrolysis procedures were subject to thin layer chromatography (TLC)/bioimage analysis for quantification and identification by co-chromatography with authentic reference standards of parent trinexapac-ethyl and its postulated metabolites. Results obtained by TLC were confirmed by High-Performance Liquid Chromatography (HPLC). Additionally, LC-MS/MS analysis was undertaken to confirm the presence of radiocomponents and to identity an additional radiocomponent for which no reference material was available.
Limit of Quantification	0.005 mg/kg for forage and grain; 0.007 mg/kg for hay and straw

Results

Radioactive residues were comparable in all commodities ranging from 1.366 mg/kg in straw up to 2.002 mg/kg in hay. Good extractability was achieved in forage, grain and hay (≥84.1% TRR) with lower extractability in straw (64.8% TRR) (see the summary table B.7.2.1-11for details).

Following chromatographic analysis of extractable radioactivity prior to and post hydrolysis of polar residues, the components identified are summarised in tables B.7.2.1-12 to B.7.2.1-15 for each experiment.

Parent (CGA163935) was detected only in forage with a low residue level of 0.3% TRR, 0.006 mg/kg.

Metabolites accounting for the highest proportion of the radioactive residue in commodities were:

•The trinexapac acid metabolite, CGA179500, accounting for 40.0% TRR in grain, 22.6% TRR in hay, 22.1% TRR in forage and 5.5% TRR in straw.

•The tricarballylic acid ethyl ester metabolite CGA300405, accounting for 20.7% TRR in forage, 9.6% TRR in straw, 8.0% TRR in hay and 0.8% TRR in grain.

•The tricarballylic acid metabolite CGA275537, accounting for 10.3% TRR in hay, 8.1% TRR in straw, 7.8% TRR in forage and 2.0% TRR in grain.

•Residues of all three of the above metabolites were found in both the free and conjugated forms in all commodities.

•A hydroxylated metabolite of trinexapac acid (SYN548584) with the position of the hydroxyl group in two possible locations on the cyclohexanedione ring. This was identified at levels of 12.1% TRR in grain, 5.1% TRR in hay, 3.3% TRR in forage and 1.9% TRR in straw. Residues in forage, hay and straw were found in the free form only but were found in both the free and conjugated forms in grain.

The aromatic diol acid metabolite CGA329773 was generally present at lower proportions of the radioactive residue in commodities accounting for 0.1 - 1.4% TRR in forage, hay and straw and not detected in grain.

Remaining unassigned organosoluble residues derived from extracted radioactivity comprised a complex mixture of radiocomponents, the collective and individual levels of which are summarised below:

•Forage: Collective unassigned residue 7.7% TRR (0.140 mg/kg), no individual radiocomponent of which >1.4% TRR (0.025 mg/kg).

•Hay: Collective unassigned residue 8.3% TRR (0.167 mg/kg), no individual radiocomponent of which >1.9% TRR (0.038 mg/kg).

•Grain: Collective unassigned residue 9.5% TRR (0.137 mg/kg), no individual radiocomponent of which >1.8% TRR (0.026 mg/kg).

•Straw: Collective unassigned residue 4.2% TRR (0.057 mg/kg), no individual radiocomponent of which >1.3% TRR (0.017 mg/kg).

Remaining unassigned aqueous soluble residues derived from extracted radioactivity were characterised as follows:

•A range of hydrolysis conditions (acid and base) were employed to release radiolabelled metabolites from their sugar/amino acid conjugates. Any radiocomponents not transformed by these hydrolysis conditions were deemed unlikely to be conjugates but free metabolites.

•TLC staining techniques indicated that major proportions of this aqueous residue were associated with endogenous components (supported by the highly polar nature of the components by TLC analysis) and is acidic in nature.

•HPLC and TLC analysis indicates the presence of citric acid and the chromatographic behaviour of the remaining radioactive components indicates they are either of similar polarity or more polar than citric acid.

•Evidence of natural incorporation from analysis of unextracted residues and the presence of citric acid and CGA275537 (tricarballylic acid, a naturally occurring compound found in grasses) suggests these polar components are likely to be part of an overall pathway to natural products.

Further characterisation of the unextracted radioactive residues using a clean fractionation technique (Moens, 2000) employed for the hay, grain and straw samples released additional small amounts of the same metabolites as observed in the extractable fractions. The largest residue identified after applying this technique was CGA300405 in all samples analysed (0.3 - 0.6% TRR; 0.004 - 0.013 mg/kg). Acid hydrolysis of a hemicellulose fraction (11.8%TRR; 0.161 mg/kg) derived from straw showed the majority of this fraction to comprise [14C]-glucose, demonstrating extensive natural incorporation into endogenous components had occurred. This result is consistent with the detection of both citric acid (a component of the citric acid cycle) and CGA275537 and is highly indicative of incorporation of small 14C containing moieties into the broader pool of natural biosynthetic products. Initial analysis of the wheat sampled commodities combined extracts took place < 6 months after harvest. The original extracts of forage and grain were re-analysed 34 months after harvest, i.e. upon study completion. Comparison of initial and final radiocomponent profiles showed that only small changes in the profiles had occurred during the interim period of storage.

Table B.7.2.1-11: Summary of total radioactive residues and extractability in wheat treated with [^{14}C]-trinexapac-ethyl at a rate of 211 g a.s./ha

Commodity	Extractable Radioactivity		Non-extractable Radioactivity		TRR
	%TRR	mg/kg	%TRR	mg/kg	mg/kg
Forage	94.8	1.708	5.1	0.092	1.801
Hay	88.8	1.778	11.2	0.224	2.002
Grain	84.1	1.215	15.9	0.230	1.444
Straw	64.8	0.886	35.2	0.481	1.366

Table B.7.2.1-12: Identification and characterisation of radioactive residues in wheat forage following one application of [^{14}C]-trinexapac-ethyl at a rate of 211 g a.s./ha

		FORAGE	
TRR by summation mg/kg		1.801 [1]	
TRR by direct quantification mg/kg		1.846 [2]	
Percentage of TRR for chromatography, %		93.9 [3]	
Origin of component	**Component**	**% TRR[a]**	**Residue (mg/kg)[a]**
Chromatographed [4]	CGA163935	0.3 (N/D)	0.006 (N/D)
	CGA179500	22.1 (1.7)	0.399 (0.030)
	CGA300405	20.7 (3.6)	0.374 (0.065)
	CGA275537	7.8 (5.0)	0.141 (0.091)
	CGA329773	0.7 (N/D)	0.012 (N/D)
	Hydroxylated CGA179500	3.3 (N/D)	0.060 (N/D)
	Unassigned in pre-hydrolysis organosoluble fraction [5]	7.7	0.140
	Unassigned in post hydrolysis fraction [6]	24.0	0.431
	Baseline components in pre- and post-hydrolysis fractions [7]	7.2	0.129
	Losses/gains on fractionation [8]	1.2 (Loss)	0.017 (Loss)
	Unextracted [9]	5.1	0.092
	Total	100.0	1.801

N/D not detected

a - Values without parentheses are the sum of both the free and conjugated forms. The values within parentheses indicate the proportion of the TRR that is in the conjugated forms.

1 - TRR determined by summation of radioactivity present in extracts and debris following solvent extraction.

2 - The radioactive residues determined by direct quantification by combustion/LSC.

3 - Percentage of TRR for chromatography.

4 - The components of the TRR derived from chromatographic analysis.

5 - Unassigned radiocomponents chromatographed by 2D-TLC comprising at least 10 discrete components, none of which

>1.4% TRR (>0.025 mg/kg).

6 - Unassigned radiocomponents chromatographed by 2D-TLC comprising at least 4 discrete components, none of which >12.7% TRR (>0.228 mg/kg). Further investigation by HPLC and 1D-TLC indicates presence of citric acid and components similar in nature or more polar than citric acid, suggesting they are likely to be part of an overall pathway to natural products.

7 - Polar material on origin of radiochromatogram using 2D-TLC. Characterisation of radioactivity using TLC staining techniques (iodine and bromocresol green) indicates residue is associated with endogenous components and acidic in nature. More forcing chromatographic conditions confirm radioactivity remains at the origin. This, alongside evidence of natural incorporation from the feed commodity debris analysis, characterises this radioactivity as similar in nature to that found in unextracted material.

8 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

9 - Radioactivity remaining in debris after extraction with acetonitrile:water.

Table B.7.2.1-13: Identification and characterisation of radioactive residues in wheat hay following one application of [^{14}C]-trinexapac-ethyl at a rate of 211 g a.s./ha

		HAY	
TRR by summation mg/kg		2.002 [1]	
TRR by direct quantification mg/kg		1.967 [2]	
Percentage of TRR for chromatography, %		88.0 [3]	
Origin of component	**Component**	**% TRR[a]**	**Residue (mg/kg)[a]**
Chromatographed [4]	CGA179500	22.6 (2.0)	0.453 (0.041)
	CGA300405	8.0 (1.4)	0.161 (0.027)
	CGA275537	10.3 (4.6)	0.206 (0.092)
	CGA329773	1.4 (N/D)	0.027 (N/D)
	Hydroxylated CGA179500	5.1 (N/D)	0.102 (N/D)
	Unassigned in pre-hydrolysis organosoluble fraction [5]	8.3	0.167
	Unassigned in post hydrolysis fraction [6]	11.1	0.222
	Baseline components in pre- and post-hydrolysis fractions [7]	21.2	0.425
	Losses/gains on fractionation [8]	0.8 (Loss)	0.015 (Loss)
	Unextracted [9]	11.2	0.224
	Total	100.0	2.002

N/D not detected

a - Values without parentheses are the sum of both the free and conjugated forms. The values within parentheses indicate the proportion of the TRR that is in the conjugated forms.

1 - TRR determined by summation of radioactivity present in extracts and debris following solvent extraction.

2 - The radioactive residues determined by direct quantification by combustion/LSC.

3 - Percentage of TRR for chromatography.

4 - The components of the TRR derived from chromatographic analysis.

5 - Unassigned radiocomponents chromatographed by 2D-TLC comprising at least 9 discrete components, none of which >1.9% TRR (>0.038 mg/kg).

6 - Unassigned radiocomponents chromatographed by 2D-TLC and comprising at least 4 discrete components, none of which >6.7% TRR (>0.135 mg/kg). Further investigation by HPLC and 1D-TLC indicates presence of citric acid and components similar in nature or more polar than citric acid, suggesting they are likely to be part of an overall pathway to natural products.

7 - Polar material on origin of radiochromatogram using 2D-TLC. Characterisation of radioactivity using TLC staining techniques (iodine and bromocresol green) indicates residue is associated with endogenous components and acidic in nature. More forcing chromatographic conditions confirm radioactivity remains at the origin and also demonstrated that no single component exceeds 10.8% TRR (0.216 mg/kg). This, alongside evidence of natural incorporation from the feed commodity debris analysis, characterises this radioactivity as similar in nature to that found in unextracted material.

8 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

9 - Radioactivity remaining in debris after extraction with acetonitrile:water. The nature of the residues was characterised further by the clean fractionation technique.

Table B.7.2.1-14: Identification and characterisation of radioactive residues in wheat grain following one application of [^{14}C]-trinexapac-ethyl at a rate of 211 g a.s./ha

		GRAIN	
TRR by summation mg/kg		1.444 [1]	
TRR by direct quantification mg/kg		1.515 [2]	
Percentage of TRR for chromatography, %		76.6 [3]	
Origin of component	**Component**	**% TRR[a]**	**Residue (mg/kg)[a]**
Chromatographed [4]	CGA179500	40.0 (12.2)	0.577 (0.176)
	CGA300405	0.8 (0.2)	0.012 (0.002)
	CGA275537	2.0 (0.3)	0.030 (0.004)
	Hydroxylated CGA179500 (SYN548584)	12.1 (0.3)	0.175 (0.004)
	Unassigned in pre-hydrolysis organosoluble [5]	9.5	0.137
	Unassigned in post hydrolysis fraction [6]	7.8	0.113
	Baseline components in post-	4.3	0.063

	hydrolysis fraction [7]		
	Losses/gains on fractionation [8]	7.6 (Loss)	0.107 (Loss)
	Unextracted [9]	15.9	0.230
	Total	100.0	1.444

a - Values without parentheses are the sum of both the free and conjugated forms. The values within parentheses indicate the proportion of the TRR that is in the conjugated forms.

1 - TRR determined by summation of radioactivity present in extracts and debris following solvent extraction.

2 - The radioactive residues determined by direct quantification by combustion/LSC.

3 - Percentage of TRR for chromatography.

4 - The components of the TRR derived from chromatographic analysis.

5 - Unassigned radiocomponents chromatographed by 2D-TLC comprising at least 9 discrete components, none of which >1.8% TRR (>0.026 mg/kg).

6 - Unassigned radiocomponents chromatographed by 2D-TLC comprising at least 8 discrete components, none of which >2.3% TRR (>0.033 mg/kg) in Hydrolysate D.

7 - Polar material on origin of radiochromatogram using 2D-TLC. Evidence of an element of natural incorporation in grain debris analysis indicates this radioactivity is likely to be similar in nature to that found in unextracted material.

8 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

9 - Radioactivity remaining in debris after extraction with acetonitrile:water. The nature of the residue was characterised further by the clean fractionation technique and enzyme hydrolysis.

Table B.7.2.1-15: Identification and characterisation of radioactive residues in wheat straw following one application of [^{14}C]-trinexapac-ethyl at a rate of 211 g a.s./ha

		STRAW	
TRR by summation mg/kg		1.366 [1]	
TRR by direct quantification mg/kg		1.378 [2]	
Percentage of TRR for chromatography, %		60.2 [3]	
Origin of component	**Component**	**% TRR[a]**	**Residue (mg/kg)[a]**
Chromatographed [4]	CGA179500	5.5 (2.0)	0.075 (0.027)
	CGA300405	9.6 (1.7)	0.131 (0.024)
	CGA275537	8.1 (2.2)	0.111 (0.030)
	CGA329773	0.1 (N/D)	0.002 (N/D)
	Hydroxylated CGA179500	1.9	0.026

	(SYN548584)	(N/D)	(N/D)
	Citric Acid	2.0	0.027
	Unassigned in pre-hydrolysis organosoluble fraction [5]	4.2	0.057
	Unassigned in post hydrolysis fraction [6]	19.9	0.272
	Baseline components in pre- and post-hydrolysis fractions [7]	8.8	0.121
	Losses/gains on fractionation [8]	4.7 (Loss)	0.063 (Loss)
	Unextracted [9]	35.2	0.481
	Total	100.0	1.366

N/D not detected

a – Values without parentheses are the sum of both the free and conjugated forms. The values within parentheses indicate the proportion of the TRR that is in the conjugated forms.

1 - TRR determined by summation of radioactivity present in extracts and debris following solvent extraction.

2 - The radioactive residues determined by direct quantification by combustion/LSC.

3 - Percentage of TRR for chromatography.

4 - The components of the TRR derived from chromatographic analysis.

5 - Unassigned radiocomponents chromatographed by 2D-TLC comprising at least 9 discrete components, none of which >1.3% TRR (>0.017 mg/kg).

6 - Unassigned radiocomponents chromatographed by 2D-TLC. Further investigation by HPLC and 1D-TLC identified citric acid and demonstrated components similar in nature or more polar than citric acid suggesting they are likely to be part of an overall pathway to natural products. Comprises at least 5 components, none of which > 5.2% TRR (>0.071 mg/kg).

7 - Polar material on origin of radiochromatogram using 2D-TLC. Characterisation of radioactivity using TLC staining techniques (iodine and bromocresol green) indicates residue is associated with endogenous components and acidic in nature. More forcing chromatographic conditions confirm radioactivity remains at the origin. This, alongside evidence of natural incorporation from the feed commodity debris analysis, characterises this radioactivity as similar in nature to that found in unextracted material.

8 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

9 - Radioactivity remaining in debris after extraction with acetonitrile:water. The nature of the residue was characterised further by the clean fractionation technique.

RMS comments and conclusion

Following a single foliar spray application of [^{14}C]-trinexapac-ethyl at 211 g a.s./ha to spring wheat plants at growth stage BBCH 37, wheat plants were harvested at three growth stages: 7 days after application (at the forage stage BBCH 43), 34 days after application(at the hay stage BBCH 77), and 62 days after application (at maturity BBCH 89).

The total radioactive residues (TRR) for harvested commodities were 1.801 mg/kg (forage), 2.002 mg/kg (hay), 1.366 mg/kg (straw) and 1.444 mg/kg (grain).

Good extractability was achieved in forage, grain and hay (≥84.1% TRR) with lower extractability in straw (64.8% TRR). Metabolism of parent trinexapac-ethyl was extensive and almost complete (parent was detected in forage only at 0.3 % TRR). The principal metabolites identified in grain were trinexapac acid CGA179500 (40.0 % of TRR) and a hydroxylated metabolite of trinexapac acid (SYN548584) (12.1 % TRR). The principal metabolites identified in forage, hay and straw were trinexapac acid (up to 22.1 % TRR), the tricarboxylic acid ethyl ester metabolite CGA300405 (up to 20.7 % TRR) and tricarballylic acid CGA275537 (up to 10.3 % TRR).

The study was well performed and reported.

The application rate in the present study was one and a half times higher than the critical GAP proposed for winter wheat in Northern and Southern Europe (0.125 vs 0.211 kg a.s./ha).

The definitive structure of the hydroxylated trinexapac acid was not confirmed in the wheat study above so additional work has been conducted to identify the position of the hydroxyl group. There are three potential hydroxylated structures, of which two were eliminated in the additional investigations. Therefore SYN548584 is the proposed structure and further information is provided below in study 8.

Study 8

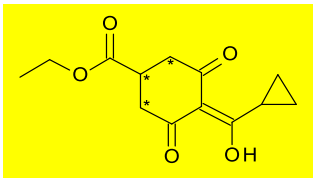
Co-chromatography of Hydroxylated Trinexapac Acid in Wheat Grain Metabolites from Wheat Study (Piskorski R. 2015a)

Reference:	**Piskorski R. (2017)** Trinexapac-ethyl –Co-chromatography of Hydroxylated Trinexapac Acid Metabolites with Wheat Grain Metabolites from Study: Metabolism of [^{14}C]-Trinexapac-ethyl in Spring Wheat (TK0070368). Final report. Syngenta File No. CGA163935_10838 (KCA 6.2.1 / 03)
Report No.:	20170023
Task No.:	TK0325771
Guideline:	OECD Guideline for the Testing of Chemicals, 501, Metabolism in Crops (January 2007) EPA Residue Chemistry Test Guideline OCSPP 860.1300, Nature of the Residue in Plants (August 1996) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market Japanese MAFF Guidelines on the Application for Agricultural Chemicals Registration (12 Nohsan No. 8147, November 24, 2000)
GLP:	Fully GLP compliant
Previous evaluation:	Submitted for the purpose of renewal

Material and methods:

Test item: [^{14}C]-Trinexapac-ethyl ([cyclohexanedione-1,2,6-^{14}C]-CGA163965) CAS No. 095266-40-3

Position of the radiolabel
(* = ^{14}C position)

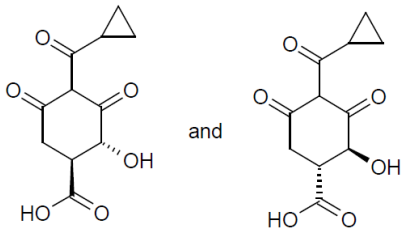
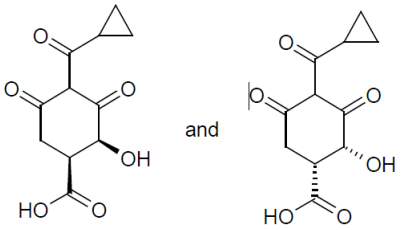


Batch No.: TAA-I-47

Radiochemical Purity: 99.2% (specific radioactivity 2.468 MBq/mg)

Test concentration: 211g a.s./ha

Standard reference compounds:

Chemical name	IES Code; R-Code	Batch Expiry date Purity Storage CoA Storage IES	Chemical structure
SYN549426	10703 R19	MES 423/1 End of September 2017 98% <10°C Approx. 4°C	
SYN549427	10702 R18	MES 424/1 End of September 2017 98% <10°C Approx. 4°C	

Test system: A wheat grain sample generated in the IES Study Number 20120098; Syngenta Task Number TK0070368 was used. The grain sample was treated with [cyclohexanedione-1,2,6-^{14}C]-CGA163935 in the scope of the crop metabolism study. The test system and test samples are repeated below for ease reference:

Spring wheat plants (variety *Monsoon*) were grown in a total of 10 containers (40 x 60 x 40cm). The wheat plants were treated with the formulated test item of ^{14}C trinexapac-ethyl at BBCH 37 by one foliar spray application at a nominal rate of 200 g a.i./ha and the spray volume rate of 250 L/ha (actual rate 211 g a.s./ha). The test item was applied as a formulation with a hand-held sprayer. Eight containers were treated with the test item, giving approximately 2 m^2 of the total treated plant surface. For every set of 2 crates, 15 mL of the application solution was applied as a spray foliar treatment with a hand-held

	sprayer. Ninth container was treated with the blank formulation only and the tenth was left untreated; both were grown for control purposes. Containers were maintained outdoors under natural conditions. Wheat plants were harvested by cutting the stems of the plants approximately 10 cm above the soil with a knife or scissors. After harvest, all samples were weighed, and hay and mature samples were dried in the greenhouse. After drying, the mature harvest was separated into straw and grain; the straw sample contained also chaff. All samples were homogenized with a food processor (forage under liquid nitrogen) and stored at approximately -20 °C prior to analysis.
Storage:	Initial analysis of the wheat sampled commodities combined extracts took place < 6 months after harvest. The original aqueous and organic phases of partitioned grain extract (analysed initially 5.5 months after harvest) were re-analysed 34 months after harvest, i.e. upon study completion. Comparison of initial and final radiocomponent profiles showed that no significant changes in the profiles had occurred during the interim period of storage. Stored at <-20°C.
Sampling time points:	Wheat plants were collected on three occasions, as an immature crop at a forage growth stage (BBCH 43, 7 days after application), at a hay growth stage (BBCH 77, 34 days after application) and at maturity (BBCH 89; as grain and straw, 62 days after application).
Method of analysis:	**Extraction and Fractionation of Residues** A sub-sample of the homogenized commodity (20 g) was extracted with solvents at a sample weight-to-volume ratio of approximately 10:1 to 5:1. The sample was extracted at room temperature four times with acetonitrile / water (4:1, v/v) and once with acetonitrile / water (1:1, v/v). The solid and liquid phases were separated by centrifugation. The radioactivity contained in the extracts was measured directly by LSC. Equal proportions of individual extracts were combined to produce an extract for LSC and TLC/HPLC analysis. Radioactivity remaining in the extracted debris was measured by LSC after combustion of appropriate aliquots. Liquid-liquid partitions were carried out between two immiscible solvents e.g. an aqueous phase and ethyl acetate. Appropriate volumes of each of the solvent were taken and carefully shaken, the phases separated and partitions were repeated as required. Radioactivity in each phase was determined by LSC. **Chromatography** HPLC employing UV and radiodetection was used to identify radiocomponents in sample extracts. TLC was also used to identify radiocomponents in sample extracts. The radioactive components were compared with standard reference compounds by co chromatography.
Limit of Quantification	0.005 mg/kg for grain;

Results

Total radioactive residues and extractability

The purpose of this study was:

• to confirm whether an unidentified metabolite in a wheat grain commodity (reported as "Hydroxylated CGA179500") from an IES Study # 20120098: Metabolism of [^{14}C]-Trinexapac-ethyl in Spring Wheat co-chromatographs with supplied reference standards.

The radioactive residues from the summation of the extractable and non-extractable radioactivity determined in this study were in good agreement with the values determined previously (see table B.7.2.1-16).

Table B.7.2.1-16: Extractability and Distribution of Radioactive Residues in Wheat

Crop Commodity	Extractable Radioactivity		Non-extractable Radioactivity		TRR [1]
	% TRR	mg/kg	% TRR	mg/kg	mg/kg
Grain – data from original metabolism study	84.1	1.215	15.9	0.230	1.444
Grain – this study	77.9	1.081	22.1	0.307	1.388

1 - mg/kg calculated directly from radioactivity extracted at room temperature, radioactivity in the debris and specific activity.

Characterisation and identification of residues

Following sequential extraction of a sub-sample of grain with acetonitrile:water mixtures, and partitioning against ethyl acetate, the radioactive residues were distributed between Aqueous fraction B (49.2% TRR; 0.683 mg/kg) and Organic fraction C (20.1% TRR; 0.279 mg/kg).

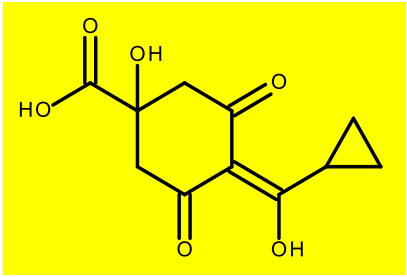
In the original metabolism study 20120098, the unidentified hydroxylated CGA179500 metabolite was found in Organic fraction C, originating from the grain. Therefore, the corresponding fraction obtained in this study was co-chromatographed with reference standards of SYN549426 and SYN549427 by HPLC and TLC. Only traces of the hydroxylated CGA179500 metabolite were found in the sample. Subsequently, to check if the metabolite remained in the aqueous phase, Aqueous fraction B was also co-chromatographed with reference standards of SYN549426 and SYN549427 by HPLC. The metabolite of interest was found in Aqueous fraction B and the sample was taken for TLC co-chromatography with reference standards of SYN549426 and SYN549427. The 2D-TLC analysis proved that the hydroxylated CGA179500 metabolite did not co-chromatograph with either SYN549426 or SYN549427.

In spite of somewhat different partitioning behaviour of the radioactive residues, the chromatographic profiles obtained in this study were comparable to those obtained in the original metabolism study, showing sufficient stability of the residues during the prolonged storage.

Conclusions

Following co-chromatography of the extractable grain residues with reference standards of SYN549426 and SYN549427, it was proven that the hydroxylated CGA179500 metabolite, proposed based on LC-MS structure elucidation in, did not correspond to the two standard references supplied.

The LC-MS/MS analysis and deuterium exchange experiments in the original metabolism study indicated two possible positions of hydroxylation, yielding either 1-hydroxy- or 2-hydroxy-metabolite. The reference standards used in this study were two diastereoisomers of the 2-hydroxy-metabolite and these structures have been ruled out by co-chromatography. Therefore, the radioactive residues identified as the hydroxylated CGA179500 metabolite can be assigned to the 1-hydroxy-CGA179500 metabolite presented below.



1-hydroxy-CGA179500

RMS comments and conclusions

RMS LT agrees with the conclusion that the reference standards used in this study (two diastereoisomers of the 2-hydroxy-metabolite) and these structures have been ruled out by co-chromatography, and therefore, the radioactive residues identified as the hydroxylated CGA179500 metabolite can be assigned to the 1-hydroxy-CGA179500 named as SYN548584.

Analysis in this study was performed 50 months after harvest (October 2012 – January 2017). Samples were stored deep frozen at -20°C, the chromatographic profiles obtained in this study were comparable to those obtained in the original metabolism study (Table B.7.2.1-16), and therefore residues are considered not to be affected by prolonged storage.

No information could be found in the report explaining the different partitioning behaviour (between organic and aqueous phases) of the radioactive residues in the original metabolism study in wheat and current study.

Study is conducted in accordance with OECD 501, reported in sufficient detail and considered suitable for evaluation.

Metabolism, distribution and expression of residue in plants - summary and conclusions

The plant metabolism of trinexapac-ethyl was carried out in four crops, representing two crop groupings – oilseeds (oilseed rape) and cereals (wheat, rice, grass). The application method was foliar for all these crops.

The representative use for trinexapac-ethyl in the EU is on barley and wheat.

All studies were performed using a cyclohexane ring radiolabelled form of trinexapac-ethyl ([^{14}C]-trinexapac-ethyl). No study was conducted using cyclopropane ring radiolabelled form of trinexapac-ethyl ([^{14}C]-trinexapac-ethyl).In one trial on spring wheat (new data), the application rate was 1.69 times higher than the critical GAP proposed for wheat in Southern and Northern Europe (0.211 *vs* 0.125 kg a.s./ha) and 1.06 times higher than the critical GAP proposed for barley in Southern and Northern Europe (0.211 vs 0.200 kg a.s./ha). ~~In grass, the application rate was 2.8 times higher than the critical GAP proposed for grass in Northern and Southern Europe (0.56 *vs* 0.2 kg a.s./ha)~~. In remaining wheat and oilseed rape trials the application rate was in line with the critical GAP proposed for wheat and oilseed rape in Southern and Northern Europe.

Trinexapac-ethyl (CGA163935) is extensively degraded in wheat, oilseed rape, rice and grass by very similar biotransformation pathways. It should be noted, that original metabolism studies (from the DAR) on oilseed rape and wheat (Nicollier, 1991 and Krauss, 1993) are considered supplementary due to deviations from OECD 501. Trinexapac-ethyl was only detected at trace levels in wheat forage and ~~in the straw and husks~~ all parts of rice. Metabolism proceeded via hydrolysis to the major metabolite trinexapac acid (CGA179500) up to 0.577 mg/kg 40 % TRR in wheat grain, followed by hydroxylation (forming hydroxylated CGA179500; 0.175 mg/kg representing 12.1 % TRR) and subsequent ring opening of the cyclohexane ring. Stepwise oxidation/decarboxylation yielded saturated and unsaturated tricarboxylated acids such as CGA275537 (tricarballylic acid; up to 0.91 mg/kg representing 17 % TRR in grass seeds), CGA312753 (aconitic acid; 0.058 mg/kg representing 35 % TRR in rice husks) and citric acid, all precursors to incorporation into the biosynthetic pool of natural products.

A secondary pathway proceeded via ring opening of the cyclohexane ring of parent leading to formation of CGA300405 (0.374 mg/kg representing 20.7 % TRR in wheat forage) and the mono ethyl esters of CGA275537 (tricarballylic acid; up to 0.206 representing 10.3 % TRR in wheat hay and 0.37 representing 17 % TRR in rice husks), CGA312753 (aconitic acid; up to 0.058 mg/kg representing 35 % TRR in rice husks). Further steps observed were aromatisation of the 6-membered ring of trinexapac acid and keto-enol tautomerism to 4-cyclopropanecarbonyl-3,5-dihydroxobenzoic acid CGA329773 (up to 0.03 representing 2.5 % TRR in rice grain and 11 % TRR in wheat grain – supplementary study) and NOA433257 (terephthalic acid; found only in grass up to 3.5 mg/kg representing 12 % TRR in seed screenings of grass) and reduction of CGA179500 to yield CGA351210 (found only in supplementary study of oilseed rape in oil, pods and stalks up to 28 % TRR).

In the new metabolism studies provided for renewal, the following metabolites – trinexapac acid (CGA179500), CGA300405, tricarballylic acid (CGA275537) and hydroxylated trinexapac acid (SYN548584) – were found in amounts more than 10 %TRR. In EU reviewed metabolism studies, the following metabolites – CGA329773, trans

aconitic acid CGA312753, metabolite A (SYN540405) and terephthalic acid NOA433257 – were found in amounts more than 10 %TRR.

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

Proposed metabolic pathway of trinexapac-ethyl in plants are presented in figure B.7.2.1-3

A list of the identified compounds in the four crop types is presented in Table B.7.2.1-16.

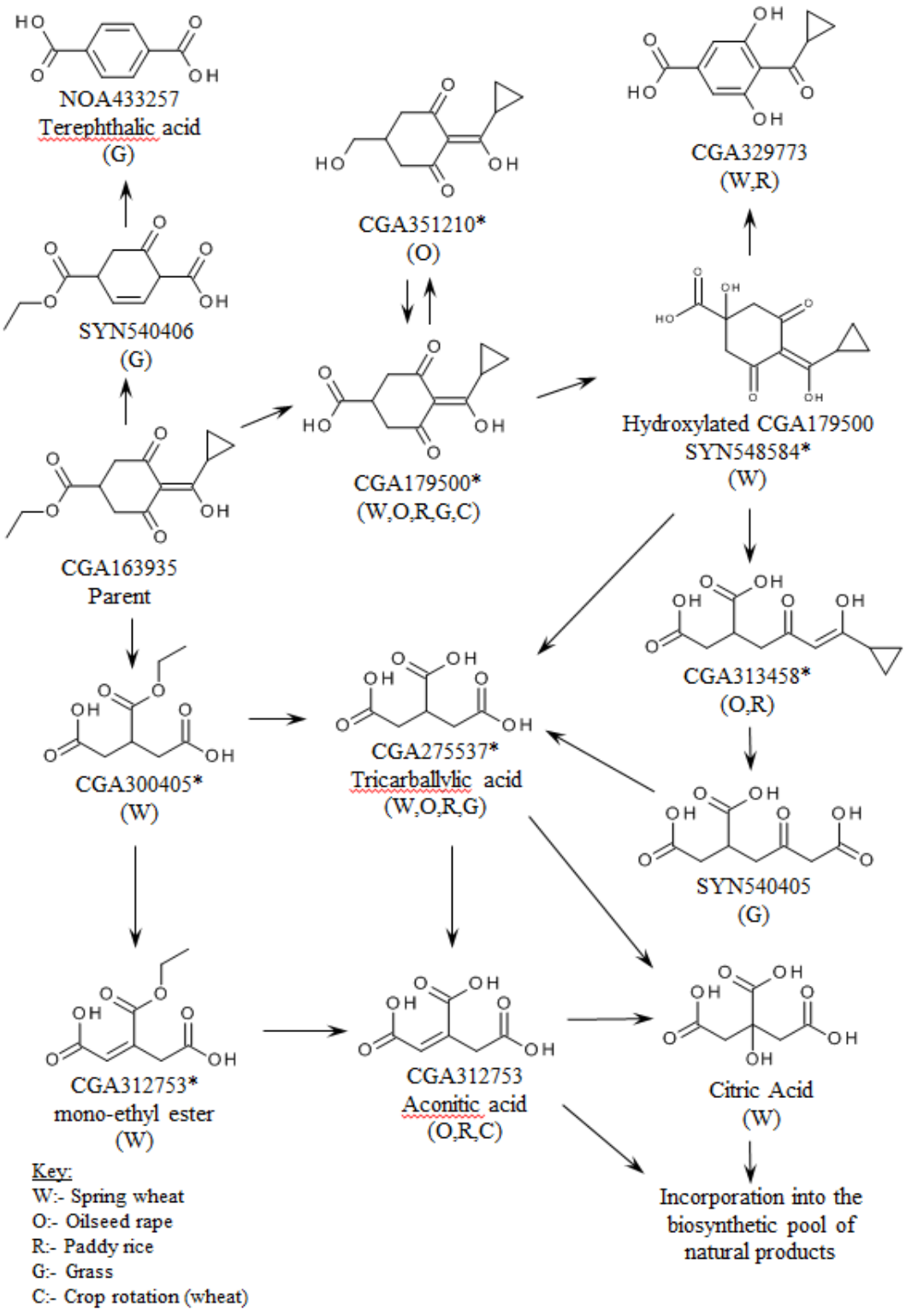


Figure B. 7.2.1-3: Proposed metabolic pathway of trinexapac-ethyl in plants

Table B.7.2.1-16: Identified compounds found in oilseed rape, wheat, rice and grass plant fractions

Designation		**Trinexapac-ethyl CGA163935**	**Trinexapac acid CGA179500#**	**CGA300405**	**Tricarballylic acid CGA275537**	**CGA329773**	**Hydroxylated CGA179500 (SYN548584)**	**Citric Acid**	**CGA313458**	**Aconitic acid CGA312753**	**Metabolite A SYN540405**	**Metabolite B SYN540406**	**Metabolite C NOA433257**	**CGA 351210#**
Chemical Name (IUPAC)		4-(cyclopropyl-hydroxymethylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester	4-(cyclopropanecarbonyl)-3,5-dioxo-cyclohexanecarboxylic acid	3-ethoxycarbonylpentanedioic acid	1,2,3-propanetricarboxylic acid	4-cyclopropanecarbonyl-3,5-dihydroxy benzoic acid	4-[cyclopropyl(hydroxy)methylene]-1-hydroxy-3,5-dioxo-cyclohexane carboxylic acid	Citric acid	3-carboxy-7-cyclopropyl-5,7-diketo-heptanoic acid	1,2,3-propene tricarboxylic acid	4-oxopentane-1,2,5-tricarboxylic acid	4-ethoxycarbonyl-6-oxo-cyclohex-2-ene-1-carboxylic acid	Terephthalic acid	2-[cyclopropyl(hydroxy)methylene]-5-(hydroxymethyl)cyclohexane-1,3-dione
New data submitted for renewal														
Presence in **oilseed rape seeds** (67-91 DAT) after one application at 0.393 kg/ha mg/kg (%TRR)***		ND	0.086 (21.8/2.8)	ND	0.004 (1.0)	ND	--	--	ND	ND	--	--	--	ND
Presence in **spring wheat** after one application at 0.211 kg/ha mg/kg (%TRR)***	Forage 7 DAT	0.006 (0.3)	0.399 (22.1/1.7)	0.374 (20.7)	0.141 (7.8)	0.012 (0.7)	0.060 (3.3)	ND	ND	ND	--	--	--	ND
	Hay 34 DAT	ND	0.453 (22.6/2.0)	0.161 (8.0)	0.206 (10.3)	0.027 (1.4)	0.102 (5.1)	ND	ND	ND	--	--	--	ND
	Grain 62 DAT	ND	0.577 (40.0/12.2)	0.012 (0.8)	0.030 (2.0)	ND	0.175 (12.1)	ND	ND	ND	--	--	--	ND
	Straw 62 DAT	ND	0.075 (5.5/2.0)	0.131 (9.6)	0.111 (8.1)	0.002 (0.1)	0.026 (1.9)	0.027 (2.0)	ND	ND	--	--	--	ND
EU reviewed data (DAR 2003)														
Presence in **rice** after one	Foliage after 1h	0.37 (66)	0.1 (18)	ND	ND	ND	--	--	0.012 (2.2)	ND	--	--	--	ND
	Foliage	0.008	0.036	ND	0.006	ND	--	--	0.007	0.004	--	--	--	ND

Designation		Trinexapac-ethyl CGA163935	Trinexapac acid CGA179500#	CGA300405	Tricarballylic acid CGA275537	CGA329773	Hydroxylated CGA179500 (SYN548584)	Citric Acid	CGA313458	Aconitic acid CGA312753	Metabolite A SYN540405	Metabolite B SYN540406	Metabolite C NOA433257	CGA 351210#
application at 0.040 [and 0.160] kg as/ha mg/kg** (%TRR)***	7 DAT	(5.5)	(26)		(4.0)				(5.1)	(2.5)				
	Foliage 21 DAT	0.001 (1.6)	0.017 (25)	ND	0.003 (3.9)	ND	--	--	0.002 (2.6)	0.002 (2.6)	--	--	--	ND
	Grain 82 [60] DAT	<0.001 (0.1) [<0.01 (0.4)]	0.01 (12) [(36)]	ND	<0.001 (0.5) [0.04 (3.2)]	0.003 (2.9) [0.03 (2.5)]	--	--	[0.04 (3.3)]	0.007 (8.0)	--	--	--	ND
	Husks 82 [60] DAT	0.003 (1.8) [(6.2)]	0.015 (8.9) [(30)]	ND	0.005 (3.2) [0.37 (17)]	0.001 (0.7) [0.03 (1.2)]	--	--	[0.16 (7.4)]	0.058 (35) [0.02 (1.1)]	--	--	--	ND
	Straw 82 [60] DAT	0.001 (0.9) [(1.4)]	0.008 (4.9) [(8.6)]	ND	0.031 (19) [0.21 (13)]	[0.01 (0.8)]	--	--	0.007 (4.6) [0.12 (7.2)]	ND	--	--	--	ND
Presence in **spring wheat** after one application at 0.150 kg as/ha mg/kg (%TRR)***	Ears/ Leaves**	ND	0.12 (25)	ND	ND	ND	--	--	ND	ND	--	--	--	--
	Roots**	0.26 (12.38)	ND	ND	ND	ND	--	--	ND	ND	--	--	--	--
	Grain	ND	0.16 (34.8/6.8) (28 free)	ND	0.014 (3.1)	0.05 (11)	--	--	ND	ND	--	--	--	--
	Husks	ND	0.12 (27/10) (17 free)	ND	ND	ND	--	--	ND	0.02 (4.3)*	--	--	--	--
	Straw	ND	0.11 (21/8) (13 free)	ND	0.01 (2.4)	0.016 (3.1)	--	--	ND	0.01 (1.8)*	--	--	--	--
Presence in **grass** after one	Forage 22 DAT	--	0.13 (6.1)	ND	0.28 (14)	ND	--	--	ND	ND	0.15 (7.4)	0.17 (8.6)	0.20 (9.8)	--
	Forage	--	0.006	ND	0.005	ND	--	--	ND	ND	0.002	0.001	0.004	--

Designation		Trinexapac-ethyl CGA163935	Trinexapac acid CGA179500#	CGA300405	Tricarballylic acid CGA275537	CGA329773	Hydroxylated CGA179500 (SYN548584)	Citric Acid	CGA313458	Aconitic acid CGA312753	Metabolite A SYN540405	Metabolite B SYN540406	Metabolite C NOA433257	CGA 351210#
application at 0.560 kg as/ha mg/kg (%TRR)***	102 DAT		(10)		(9.3)						(4.4)	(2.7)	(6.6)	
	Straw 46 DAT	--	0.38 (7.9)	ND	0.81 (16.8)	ND	--	--	ND	ND	0.48 (10)	0.27 (5.6)	0.45 (9.4)	--
	Seeds 46 DAT	--	0.80 (15)	ND	0.91 (17)	ND	--	--	ND	ND	0.10 (1.9)	0.46 (8.3)	0.53 (9.6)	--
	Seed screenings 46 DAT	--	0.91 (13)	ND	1.2 (16)	ND	--	--	ND	ND	0.27 (3.8)	0.70 (9.9)	3.5 (12)	--
Presence in **oilseed rape** after one application at 0.40 kg as/ha mg/kg (%TRR)***	Whole tops 30min	1.2 (19)	ND	--	ND	--	--	--	ND	ND	--	--	--	ND
	Green parts 14 DAT	0.012 (1.5)	ND	--	ND	--	--	--	ND	ND	--	--	--	ND
	Flowering parts 14 DAT	0.068 (1.0)	ND	--	ND	--	--	--	ND	ND	--	--	--	ND
	Seeds/ total 65 DAT	ND	0.42 (30.1/1.1)	--	ND	--	--	--	0.015 (1.1)	0.013 (0.9)	--	--	--	0.077 (5.5/3.4)
	Seeds/ oil 65 DAT	ND	0.001 (3.5)	--	ND	--	--	--	ND	ND	--	--	--	0.005 (16)
	Seeds/ meal 65 DAT	ND	0.435 (31.1/1.1)	--	ND	--	--	--	0.015 (1.1)	0.013 (0.9)	--	--	--	0.073 (5.2/3.5)
	Pods 65 DAT	ND	1.23 (18.4/0.4)	--	ND	--	--	--	0.127 (1.9)	0.06 (0.9)	--	--	--	1.07 (16/8.3)
	Stalks	ND	0.3	--	ND	--	--	--	0.152	0.047	--	--	--	0.87

Annex B.7 (AS): Residue data

Designation		Trinexapac-ethyl CGA163935	Trinexapac acid CGA179500#	CGA300405	Tricarballylic acid CGA275537	CGA329773	Hydroxylated CGA179500 (SYN548584)	Citric Acid	CGA313458	Aconitic acid CGA312753	Metabolite A SYN540405	Metabolite B SYN540406	Metabolite C NOA433257	CGA 351210#
	65 DAT		(9.7/0.8)						(4.9)	(1.5)				(28/22)

-- Metabolite was not included in the reference compounds

ND – not detected.

results presented in %free and conjugated/ %conjugated

*- ethyl ester

**- max values, 48 DAT value for wheat ears/leaves

***- Percent of the total radioactive residues

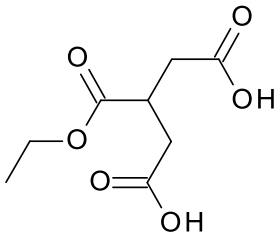
Supplementary studies are coloured in grey.

B.7.2.2 Animals

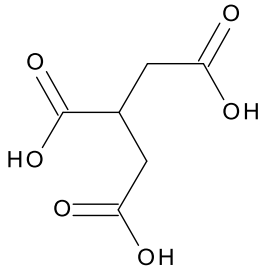
Animal metabolism studies (goat and poultry) were conducted using radiolabelled trinexapac-ethyl. As metabolites SYN548584, CGA275537, CGA300405 and CG351210 are major plant metabolites, the need for metabolism data in livestock dosed with a mixture of compounds reflecting the level of their exposure in feed items of the treated crops was addressed by the applicant and provided below *in italics*. The applicant is of the opinion, that additional metabolism studies with these metabolites are not necessary or ethical.

Position for not requiring ruminant metabolism studies dosed with CGA275537 or CGA300405

CGA300405, a tricarboxylic acid ethyl ester, and CGA275537, tricarballylic acid, were identified in crop metabolism studies. For reference, the structures are shown below:



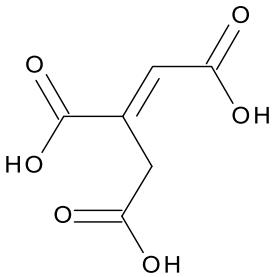
CGA300405



CGA275537

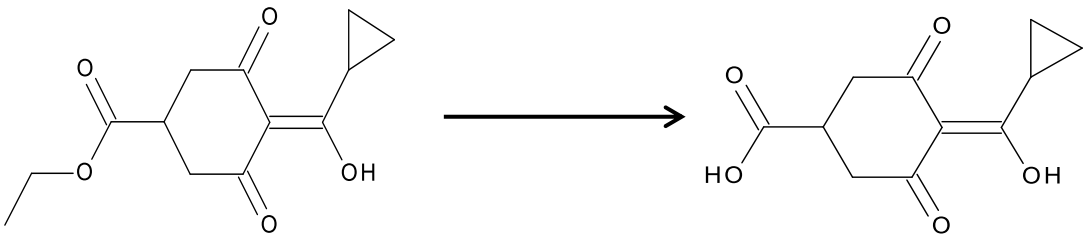
CGA300405 and CGA275537 were not identified in the livestock (goat and hen) metabolism studies and there is no evidence to suggest that CGA300405 is on the goat or hen biotransformation pathway.

The structure below, aconitic acid, is a naturally occurring component in grass forages at levels up to 5% dry weight (ref. 1-6).



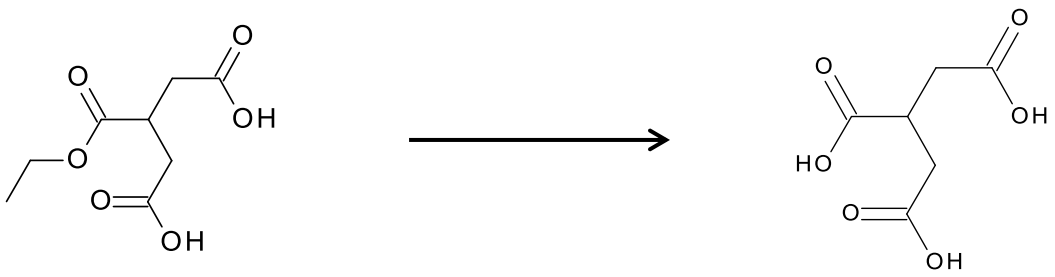
Further literature evidence (ref 7) demonstrates rapid conversion of aconitic acid in the rumen of livestock to tricarballylic acid CGA275537. Syngenta therefore concludes that a ruminant metabolism study dosed with CGA275537 is not required as the levels of tricarboxylic acid formed from endogenous aconitic acid (gram levels) will far outweigh the levels generated through the ingestion of CGA275537 derived from trinexapac-ethyl treated crops (milligram levels) in ruminants.

It is also noted that in the existing parent trinexapac-ethyl goat metabolism studies, parent is metabolised (no parent remains in any ruminant commodities) to produce trinexapac acid as shown below.



Trinexapac-ethyl Trinexapac acid

It isproposed that CGA300405 would also readily undergo ester hydrolysis to form the tricarboxylic acid (CGA275537).



CGA300405 CGA275537

Syngenta therefore concludes that a ruminant metabolism study dosed with CGA300405 is not required as the levels of tricarboxylic acid formed from endogenous aconitic acid (gram levels) will far outweigh the levels generated through the ingestion and metabolism of CGA300405 derived from trinexapac-ethyl treated crops (milligram levels) in ruminants.

References

1. *Stout, P.R., Brownell, J.R. & Bureau, R.G. (1967). Agronomy Journal **59**, 21-24*
2. *Kirkby, E.A. (1969). In Ecological Aspects of the Mineral Nutrition of Plants, pp. 215-235 [I.H. Rorison, editor]. Oxford: Blackwell Scientific Publishers*
3. *Grunes, D.L., Stout, P.R. & Brownell, J.R. (1970). Advances in Agronomy **22**, 331-374*
4. *Barta, A.L. (1973). Crop Science **13**, 113-114*
5. *Prior, R.L., Grunes, D.L., Patterson, R.P., Smith, F.W., Mayland, H.F. & Visek, W.F. (1973). Journal of Agricultural and Food Chemistry **21**, 73-77*
6. *Bohman, V.R., Horn, F.P., Stewart, B.A., Mathers, A.C. & Grunes, D.L. (1983b). Journal of Animal Science **57**, 1352-1363*
7. *Russell J.B. & Forsberg N. 1986, British Journal of Nutrition **56**, 153-162*

As SYN548584 has been found to be unstable outside the plant matrix it would be physically impossible to carry out a livestock metabolism study using this unstable metabolite.

The majority of CGA351210 is found in OSR pods and stalks. These items are not fed to animals so would not add to the dietary burden. There is a minor quantity 5.6% TRR found in the OSR seed. This adds to the dietary burden in the form of meal remaining after the oil is processed but is not considered a major increase –therefore a major vertebrate study should not be required.

RMS comments and conclusions

RMS agrees with the argumentation for not conducting animal metabolism studies with metabolites SYN548584, CGA275537, CGA300405 and CG351210 provided by the applicant.The levels of tricarballylic acid (named as tricarboxylic acid by the applicant) formed from endogenous aconitic acid (gram levels) will far outweigh the levels generated through the ingestion and metabolism of CGA300405 and CGA275537 derived from trinexapac-ethyl treated crops (milligram levels) in ruminants. Please also refer to Vol. 1 2.7.3 Definition of the residue part (3) CGA300405 and (4) tricarballylic acid (CGA275537). It should be also noted, that references reported in this position paper were not provided to RMS for re-assessment.

As SYN548584 has been found to be unstable outside the plant matrix it would be physically impossible to carry out a livestock metabolism study using this unstable metabolite.

Metabolite CGA351210 is found only in supplementary metabolism study of oilseed rape mainly in the parts not used for animal feed.

B.7.2.2.1 Poultry

The metabolism of trinexapac-ethyl in laying poultry has previously been investigated in a study that was evaluated under the framework of Directive 91/414/EEC (DAR 2003). The time of test animal sacrifice in the EU reviewed study (Cameron, 1992) was very short and the dose rate administered was unrealistically high compared to the actual animal dietary burden intakes. Therefore, another metabolism study on poultry (Powell, 2006) was conducted in order to use a dose rate and duration as recommended in OECD guidelines.

Reported metabolism studies include one study in laying hens with [^{14}C-cyclohexyl]-trinexapac-ethyl while trinexapac acid is the major residue component in livestock feed. The study is deemed relevant due to the observed rapid and near-complete metabolic transformation conversion of trinexapac-ethyl to trinexapac acid in animals. The conditions of both studies are summarised in Table 7.2.2.1-1.

Table 7.2.2.1-1: Summary of available metabolism studies in poultry

Group	Species	Label Position	No of Animals	Application Details		Sampling Details		Report Reference	EU-review reference
				Rate (mg/kg bw/d)	Duration (days)	Commodity	Time		
EU Reviewed Data									

Group	Species	Label Position	No of Animals	Application Details		Sampling Details		Report Reference	EU-review reference
				Rate (mg/kg bw/d)	Duration (days)	Commodity	Time		
Laying Poultry	Hen	^{14}C-cyclohexyl	2 4	0.4 20	4	Eggs	Daily	141798 6/93	The Netherlands, 2003 Considered as supplementary
						Excreta	Daily		
						Tissues	At sacrifice (4h)		
New Data									
Laying Poultry	Hen	^{14}C-cyclohexyl	5	0.77-0.88	10	Eggs	Daily	RJ3678B	-
						Excreta	Daily		
						Tissues	At sacrifice (22h)		

Study 1

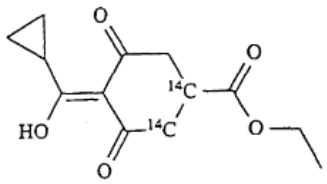
EU reviewed metabolism study in poultry

Reference:	**Cameron et al. (1992)** Distribution and excretion of [1,2-^{14}C]-cyclohexyl CGA 163935 after multiple oral administration to laying hens. (~~KCA 6.2.2 / 01~~ KIIA 6.2.2.2 / 01)
Project No:	141798
Report No.:	7478
Guideline:	US-EPA Pesticide assessment guideline subdivision O, Residue Chemistry, 1982
GLP:	Yes. In accordance with OECD principles of Good Agricultural Practice as set forth by the UK Department of Health and as accepted by the International Regulatory Authorities throughout the European Community, United States of America (FDA and EPA) and Japan (MHW, MAFF and MITI).
Previous evaluation:	In DAR 2003

Material and methods:

Test item: [1,2-^{14}C] –cyclohexyl-CGA 163935 ([1,2-^{14}C]-trinexapac-ethyl)

Position of the radiolabel



Lot/Batch No.: GAN-XVII-72

Radiochemical Purity: 99.0% Solvent system 1; 97.1% with Solvent system 2 (Specific activity: 46.3 µCi/mg (for low dose level), 7.9 µCi/mg (for high dose level)

99.3 % purity for non-radiolabelled substance

Test concentration:	0.4 mg/kg bw/day (3.8 mg/kg dietary dry matter) 20.3 mg/kg bw/day (180 mg/kg dietary dry matter)
Test system:	Two white *Leghorn* hens (1.37 – 1.51 kg bw) in one treatment group were orally dosed by hand directly into the pharynx once a day with encapsulated ^{14}C-trinexapac-ethyl at a low dose (0.4 mg/kg bw/day), another group of 4 hens were dosed with a high dose (20.3 mg/kg bw/day) for 4 days. Hens were placed in individual aluminium cages with stainless steel mesh floors and provided with feed and water *ad libitum*. The hens were housed in an isolated room with the temperature controlled at 13-23°C during the test period. The light regime (fluorescent strip lights) was 14 hours light and 10 hours dark. A seven day acclimation period proceeded the dosing period. At the end of the 4-day dosing period, all hens were sacrificed within 4 hours of the last dose. The samples were held frozen at -20°C and stored in dark until analysed.
Duration:	4 days
Sampling time points:	Eggs were collected once each day during acclimation and dosing periods, then separated into yolk and white fractions. Blood sample was taken once, prior to sacrifice. Excreta were collected cage wash performed on a daily basis in the morning. At sacrifice, samples of muscle (breast, thigh and leg), liver, skin including attached fat, peritoneal fat, kidney, gizzard contents and crop contents were taken.
Method of analysis:	Samples of the different organs, excreta, blood cells, gizzard and crop contents were combusted before analysis. Quantification of radioactivity of the samples was measured by LSC (Philips PW 4700). Samples were counted in triplicate for 5 min each. Samples for combustion were weighed into Combustocones® (Packard Instruments) and combusted using a Model 306 Tri-Carb Automatic Sample Oxidiser. The resultant $^{14}CO_2$ was absorbed in 10 ml Carbo-Sorb® and mixed automatically with 8 ml Permaflour V. Blank values for combustion samples were obtained by combusting empty Combustocones®. Blank values for liquid samples were obtained by taking 1 ml of water into 10 ml scintillation cocktail (Unisolve®, Koch-Light). For egg white and yolk samples, blank values were obtained by taking 4 ml of water into 10 ml scintillation cocktail (Unisolve®, Koch-Light).
Number of animals:	6
Method validation:	Combustion efficiency and carry-over were checked routinely several times throughout each run. Mean combustion efficiency was shown to be greater than 97 % and carry-over less than 1 % throughout the experimental period. All reported data are therefore uncorrected. The efficiency of counting for liquid and combusted samples were in the range 77-93% and 60-87%, respectively.

Results

Over the period of the experiment (76 h) excreta was the major route of elimination. For low dose scenario the mean amount excreted in excreta (88.65 %), cage wash (4.24 %), and eggs (0.01 %) accounted for 92.90 % of the

total administered dose. For high dose scenario the mean amount excreted in excreta (85.41 %), cage wash (4.36 %), and eggs (0.02 %) accounted for 89.90 % of the total administered dose. The distribution of residues of [1,2-^{14}C]-Cyclohexyl CGA 163935 in tissues and excreta are presented in table B.7.2.2.1-2.

Table B.7.2.2.1-2 Distribution of ^{14}C-residues (mean values) in tissues and excreta of laying hens following oral doses of [1,2-^{14}C]-Cyclohexyl CGA 163935 for 4 consecutive days

Matrix	0.4 mg/kg bw/d		20 mg/kg bw/d[1)]	
	mg eq/kg	% of dose	mg eq/kg	% of dose
Excreta		89		85
Eggs[2)]		0.02		0.06
Egg yolk	0.002		0.095[3)]	
Egg white	0.007		0.55	
Muscle	0.002	0.04	0.12	0.04
Liver	0.013	0.02	0.6	0.02
Kidneys	0.043	0.02	1.77	0.01
Fat	0.003	0.01	0.183	0.01
Skin	0.011	-[4)]	0.365	-[4)]
Gizzard contents	0.219	0.11	5.21	0.04
Crop content	0.959	1.08	15.78	0.37

[1)] 1 of the 4 animals of this dosing group received 2 capsules at the last time point and is not included in the assessment

[2)] Highest value measured in the samples collected daily

[3)] No eggs after the last treatment (day 4) in the high dose level group

[4)] Not calculated, because the total weight of the organ is not known

RMS comments and conclusions (Netherlands, 2003)

Absorption, distribution and excretion was determined after daily oral administration of [1, 2-^{14}C]-Cyclohexyl CGA 163935 to laying hens at two dosing levels over 4 consecutive days.

Excretion is a major elimination route for CGA 163935. The highest radioactive residues are found in crop content, gizzard content and kidneys. In eggs, total residues levels are very low and a plateau level is reached after about 50h. No accumulation of radioactivity was evident throughout the study period resulting in low tissue levels.

One of the four animals of the high dosing group received 2 capsules of the test compound at the last dosing time point (4h before termination) and was not included in the assessment. The nature of residue is described in Study 2 **Müller T. (1993)**.

RMS LT comments,~~Guidelines and limitations,~~ deviations from OECD 503 (adopted 8 January 2007):

The number of testing animals (2 and 4 for dose group) is below the recommended (10 for each dose group);

The duration of the study is shorter than recommended (4 days instead of 7).

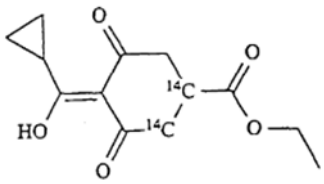
Dates of analysis for different matrices are not provided in the report (except for excreta). Samples were taken on 19 December 1989 and the experiment termination date is 3 May 1990. Time between sampling and analysis is considered to be approximately 4 months (storage stability data are not normally necessary for samples analysed within six months of collection).

Despite these deficiencies the study is considered acceptable for the overall evaluation.

Study 2
EU reviewed metabolism study in poultry

Reference:	**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 (~~KCA 6.2.2 / 02~~ KIIA 6.2.2.2 / 02)
Project No:	01TM02
Report No:	6/93
Guideline:	US-EPA Pesticide assessment guideline subdivision O, Residue Chemistry, 1982
GLP:	The OECD principles of Good Agricultural Practice (Council Decision 81/30), 1981 and OECD recommendation 83/95 concerning the "Mutual recognition of compliance with Good Agricultural Practice", 1983 The U.S. EPA Good Laboratory Practice, 40 CFR 160 (FIFRA), 1989 The U.S. EPA Good Laboratory Practice, 40 CFR 792 (TSCA), 1989 The U.S. FDA Good Laboratory Practice, 21 CFR 58 (Health and Human Services), 1987 The Japan Ministry of Agriculture, Forestry and Fisheries, NohSan, Notification No. 3850, Agricultural Production Bureau, 1984.
Previous evaluation:	In DAR 2003
Material and methods:	
Test item:	[1,2-^{14}C] –cyclohexyl-CGA 163935 / ([1,2-^{14}C]-trinexapac-ethyl)

Position of the radiolabel



Lot/Batch No.: GAN-XVII-72 radiolabelled

AMS 265-101 non-radiolabelled

Radiochemical Purity: 99.0% Solvent system 1; 97.1% with Solvent system 2 (Specific activity: 46.3 µCi/mg (for low dose level), 7.9 µCi/mg (for high dose level)

99.3 % purity for non-radiolabelled substance

Test concentration: 0.4 mg/kg bw/day (3.8 mg/kg dietary dry matter)

20.3 mg/kg bw/day (180 mg/kg dietary dry matter)

Test system: Samples taken in the disposition study in laying hens, administered with [1,2-14C]-Cyclohexyl CGA 163935 (described here as study 1, Cameron et al., 1992) were analysed in order to investigate the nature of residue of CGA 163935.

From the egg samples, equal amounts of egg white and egg yolk were pooled for both dosing groups. The animal of the high dosing group, which received accidentally a double dose at the last time point (see Guidelines and limitations), was not included in the analysis.

The samples were held frozen at -20°C and stored in dark until analysed (about 20 months).

Storage stability: Storage stability was investigated 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. Extractability and the quantitative metabolite pattern did not change.

Table 2 Storage Stability (Hen 353 LAK9, Excreta: 24 - 48 h Low Dose)

Date of Analysis	Extraction				TLC Analysis				
	% of Total Radioactivity			Exp. Recovery	% of Radioactivity Analyzed				Exp. Recovery
	Extract	Solids	Total	[%]	Fr1	Fr2	R1-R4	Total	[%]
16-Jan-90	98.0	2.0	100	106	1.2	90.3	8.4	100	95
21-Sep-90	97.2	2.8	100	102	1.3	91.3	7.4	100	94

Method of analysis: All homogenised tissue samples were extracted at least once and analysed by TLC, co-chromatograph with reference compounds. Egg white and egg yolk aliquots were extracted 3 times with acetonitrile and chromatograph using a preparative TLC. The methanol eluent of the preparative TLC spot was applied to the analytical TLC. Lean meat, liver and kidney samples were extracted 3 times with acetonitrile/water. A preparative TLC step was applied to the samples of the low dosed group before the analytical TLC. The fat and skin samples were extracted with methyl chloride/methanol, sodium phosphate buffer, hexane and again after a titration with formic acid with methyl chloride. All extracts were analysed by analytical TLC.

Number of animals: 6

Method validation: Extraction efficiency is provided in the table below:

Metabolite	Lean meat	Egg white	Egg yolk	Fat	Skin/fat	Kidneys	Liver

s	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
After low dose														
TRR	0.002	100	0.004	100	0.001	100	0.003	100	0.011	100	0.043	100	0.013	100
Extracted	0.0018	90	0.0022	55	0.00045	45	*0.002*[c]	64	*0.0033*	30	*0.039*	91	0.011	83
Not extracted	0.0002	10	0.0018	45	0.00055	55	*0.001*	*36*	*0.0077*	*70*	*0.004*	*9*	0.002	*7*
After high dose														
TRR	0.118	100	0.284	100	0.041	100	0.183	100	0.365	100	1.770	100	0.601	100
Extracted	0.107	91	0.19	68	0.020	49	0.108 c	59	0.051	14	1.58	89	0.53	88
Not extracted	0.011	9	0.009	32	0.021	51	0.075	41	0.31	86	0.20	11	0.072	12

Results

The characterisation and identification of residues of [1,2-^{14}C]-Cyclohexyl CGA 163935 in tissues of laying hens after low (0.4 mg/kg bw/d, 3.8 mg/kg feed, N=24) and high (20 mg/kg bw/d, 180 mg/kg feed, N=1176) dosing are given in tables B.7.2.2.1-3and B.7.2.2.1-4. For both dosing levels one predominant metabolite fraction was identified as CGA 179500, accounting for more than 90% of the total radioactive residue in excreta. CGA 179500 was also only identified in the different collecting intervals in excreta extracts.

Table B.7.2.2.1-3: Characterisation and identification of residues in tissues of laying hens following oral doses of 0.4 mg/kg bw/d [1,2-^{14}C]-Cyclohexyl Trinexapac-ethyl for 4 consecutive days

Metabolites	Lean meat		Egg white		Egg yolk		Fat		Skin/fat		Kidneys		Liver	
	mg /kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR
TRR[b]	0.002	100	0.004	100	0.001	100	0.003	100	0.011	100	0.043	100	0.013	100
Organo-soluble	0.0018	90	0.0022	55	0.00045	45	0.002[c]	64	0.0033	30	0.039	91	0.011	83
Water soluble														
Not extracted	0.0002	10	0.0018	45	0.00055	55	0.001	36	0.0077	70	0.004	9	0.002	7
Identified[d]	0.001	60	0.002	50	0.0004	40		60		24		84		69
CGA 163935			0.0017	43	0.00005	5								
CGA 179500	0.001	60	0.0003	7	0.00035	35	0.002	60	0.0026	24	0.036	84	0.009	69
Characterised	< 0.01 /30 [1]		0.0002 /5 [1]		0.00005 /5 [1]		< 0.01 /4 [1]		< 0.01 /6 [1]		< 0.01 /6 [1]		<0.01 /14 [1]	
Total amount per fraction (mg/kg)[a]/% TRR)[number of fractions]														

[a] ^{14}C- Cyclohexyl Trinexapac-ethyl equivalents

[b] total radioactive residue (mean values)

[c] number in italics are calculated by the Rapporteur

[d] characterisation and identification was performed on the organo-soluble fraction, which was additionally extracted.

Table B.7.2.2.1-4: Characterisation and identification of residues in tissues of laying hens following oral doses of 20.0 mg/kg bw/d [1,2-^{14}C]-Cyclohexyl Trinexapac-ethyl for 4 consecutive days

Metabolites	Lean meat		Egg white		Egg yolk		Fat		Skin/fat		Kidneys		Liver	
	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR
TRR[b]	0.118	100	0.284	100	0.041	100	0.183	100	0.365	100	1.770	100	0.601	100
Organosoluble	0.107	91	0.19	68	0.020	49	0.108[c]	59	0.051	14	1.58	89	0.53	88
Water soluble														
Not extracted	0.011	9	0.009	32	0.021	51	0.075	41	0.31	86	0.20	11	0.072	12
Identified[d]	0.058	49	0.12	44	0.016	40		44		9		53		49
CGA 163935			0.12	44	0.005	12								
CGA 179500	0.058	49			0.011	28	0.080	44	0.033	9	0.94	53	0.29	49
Characterised	0.01-0.05 /52 [1]		0.07 /24 [1]		0.04 /9 [1]		0.01-0.05 /15 [1]		0.01-0.05 /5 [1]		>0.05 /36 [1]		>0.05 /39 [1]	
Total amount per fraction (mg/kg)[a] /% TRR)[number of fractions]														

[a] ^{14}C- Cyclohexyl Trinexapac-ethyl equivalents

[b] total radioactive residue (mean values)

[c] numbers in italics are calculated by the Rapporteur

[d] characterisation and identification was performed on the organo-soluble fraction, which was additionally extracted

RMS comments and conclusions (Netherlands, 2003)

The metabolism of CGA 163935 (trinexapac-ethyl) in laying hens was studied after daily oral administration of [1,2-^{14}C]-Cyclohexyl CGA 163935 (trinexapac-ethyl) at two dosing levels over a period of 4 consecutive days. The parent compound is found in all egg samples, especially in egg white, albeit the absolute levels are very low. The metabolite CGA 179500 is present in all tissue samples analysed, except egg white after high dosing. CGA 179500 is accounting in most tissues for 60–84 % TRR and 44-53% TRR after high and low dosing, respectively.

Comments RMS LT and~~Guidelines and limitations,~~ deviations from OECD 503

Method used in the study showed poor extractability, and no information was provided if the method is suitable to determine free and conjugated forms of the metabolites. Extractability in egg white and yolk, fat and skin with attached fat was low (32-86% TRR remain unextracted). No apparent attempts for further extraction efforts to characterise/identify the non-extracted residues were mentioned in the study report, except for skin with attached fat. No additional radioactivity could be released from the non-extractable solid of the high dose pool (SFh1-1) of skin with attached fat when heated in acetonitrile/formic acid (99:1) under reflux for 2 hours.

Some major residue fractions > 0.01 mg/kg or 10% TRR ~~as well as 70-86% TRR in the not extracted fraction from skin/fat~~ were characterised but not identified – according to OECD 503 residues >10% TRR and >0.05mg/kg should be identified using all possible means. Considering the exaggerated dose levels in this study no significant residue levels are expected at the anticipated TMDI. ~~No further identification and characterisation of these fractions is considered necessary.~~

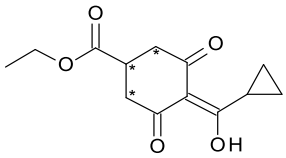
Dates of analysis for different matrices are not provided in the report. Samples were taken on 19 December 1989, sent to analytical laboratory (in frozen state) on 8, 28 August and 4 September 1990. Experimental start date is 24 September 1990 and termination only on 12 August 1991. The calculated time between sampling and analysis is considered to be approximately 20 months. Residues of CGA 179500 (metabolite of CGA 163935) is proven to be stable in animal tissues for at least 3 months (Sack St. (2000) no information is available for longer storage period), thus not covering the length of storage in the current metabolism study. Therefore the results in this study are considered not fully reliable.

The study is ~~suitable for evaluation~~ considered supplementary and can be used in the assessment only together with Powell S. (2006) as both studies show similar results.

Study 3

New metabolism study in poultry

Reference:	**Powell S. (2006).** [3,5-Cyclohexadione-1,2,6-^{14}C]-labelled Trinexapac-ethyl (CGA163935): Metabolism in Laying Hens. (KCA 6.2.2 / 0~~3~~1)

Study No:	04JH011
Report No:	RJ3678B
Syngenta file No.:	CGA163935/1048
Guideline:	Nature of the Residue - Plants, Livestock;. United States Environmental Protection Agency; Residue Chemistry Test Guidelines (OPPTS 860.1300); August 1996. Commission of the European Communities. Document 7030/VI/95 rev. 3 (22/7/97). Appendix F – Metabolism and Distribution in Domestic Animals.
GLP:	Yes. In compliance with the UK Good Laboratory Practice regulations 1999, which are in accordance with OECD principles of Good Agricultural Practice [Revised 1997].
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test item:	[3,5-Cyclohexadione-1,2,6-^{14}C]-CGA163935 ([1,2,6-^{14}C]-trinexapac-ethyl)
Position of the radiolabel (* = ^{14}C position)	
Lot/Batch No.:	BPM-XXVIII-28
Radiochemical Purity:	98.0% (Specific activity: 1.5133 MBq/mg (40.9 µCi mg^{-1})
Test concentration:	0.85 mg/kg bw/day* (8.1-10.4 mg/kg dietary dry matter)
Test system:	Five *Leghorn* hens (1.4 – 1.6 kg bw, 50 weeks age) were orally dosed by hand directly into the oesophagus once a day with encapsulated ^{14}C-trinexapac-ethyl at a dose of 8.1-10.4 mg/kg (dry weight), 1.27 mg animal/day for 10days. Hens were placed in individual metabolism cages (30 x 45 x 45 cm) and provided with feed (measured ration of protein concentrate once daily) and water *ad libitum*. The hens were housed in an isolated room with the temperature controlled at 29-33°C and humidity 36-92% during the test period. Photoperiod 24 h. A six day acclimation period proceeded the dosing period. At the end of the 10 - day dosing period, all hens were sacrificed within 22 hours of the last dose. The samples were held and transported to the laboratory frozen at ≤ -18°C.
Duration:	10 days
Sampling time points:	Eggs were collected once each day, then separated into yolk and white fractions. Blood sample was taken once, prior to sacrifice. Excreta were collected cage wash performed on a daily basis. At sacrifice, samples of muscle (breast, thigh), liver, skin including attached fat, peritoneal fat, kidney, gizzard tract and contents were taken.
Storage stability:	The composite egg white sample was extracted, fractionated and chromatographically profiled within 6 months of necropsy. Further analysis was conducted on the combined extract to characterise/identify individual components in the egg sample, and showed similar profiles, thus confirming storage stability.
Method of analysis:	Tissue samples were homogenised using standard food preparation units whilst frozen on dry ice. Radioactivity in samples was quantified by combustion and subsequent LSC analysis. Radioactivity was measured on a Packard TRI-Carb scintillation counter. Quenching was corrected by the Spectral Index of External Standard or Transformed Spectral Index of External Standard. Samples containing

	chloroform or methylene chloride were dried in a stream of nitrogen and the residues were dissolved in 2 ml tetrahydrofuran prior to mixing. Faeces and the solids after extraction were homogenised manually. The radioactivity was determined after combustion in a Packard Tri-Carb sample oxidiser. Recovery tests of the sample oxidiser were performed by combusting standards of [1-^{14}C]-n-hexane. The recoveries were always above 95% and the carryover below 0.5%. Sub-samples of egg white were homogenised in the presence of acetonitrile/water (80/20 v/v) followed by acetone using an Ultra-Turrax tissue homogeniser. Extracts containing significant quantities of radioactivity were combined and partitioned with ethyl acetate and water. The aqueous fraction was acidified to pH2 and then concentrated prior to TLC analysis. Quantitative data for fractions on TLC plates were obtained by scraping off the radioactive zones, adding about 1 ml methanol to them, followed by radiometry in scintillation mixture. Analytical TLC was performed on precoated plates of silica gel 60 F254, 0.25 mm thick. The plates were developed without chamber saturation.
Number of animals:	5
Method validation:	The efficiency of the combustor – 90.3 %, was used as a correction factor (if ≥90 %) for the combusted samples. Extractability of radioactive residues into solvent was ≥76.2% TRR (0.012 mg/kg) for egg white.

* range 0.774-0.876 mg/kg bw/day, mean calculated by RMS

Results

The radioactive residues for the edible tissues and egg yolk were found to be <0.01 mg/kg, so no further analysis was conducted. TRR in tissue and egg samples from five hens with [^{14}C]-trinexapac ethyl is summarized in table B.7.2.2.1-5. However, the initial total radioactive residues for egg whites were ≥0.01 mg/kg, so further analysis was required.

Table B.7.2.2.1-5: Summary of TRR in tissue and egg samples from five hens with [^{14}C]-Trinexapac-ethyl

Tissue	Residue (mg/kg trinexapac-ethyl equivalents)				
	Hen 1043	**Hen 1044**	**Hen 1045**	**Hen 1046**	**Hen 1047**
Liver	<0.003	0.005	0.008	0.006	<0.003
Muscle	<0.003	<0.003	<0.003	<0.003	<0.003
Skin and attached fat	<0.003	<0.003	<0.003	<0.003	<0.003
Peritoneal fat	<0.003	<0.003	<0.003	<0.003	<0.003
Egg white (maximum)	0.014	0.011	0.016	0.031	0.026
Egg yolk (maximum)	0.008	0.007	0.008	0.009	0.008

The radioactive residues in egg white are summarised in Table B.7.2.2.1-6.

Table B.7.2.2.1-6: Radioactive residues in egg white (mg/kg CGA193935 equivalents)

Collection Period (days)	Radioactive Residues (mg/kg CGA163935)					
	Hen 1043	**Hen 1044**	**Hen 1045**	**Hen 1046**	**Hen 1047**	**Mean**
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 residue for period	0.014	0.011	0.016	0.031	0.026	-

YB – yolk broke during separation from white, entire sample discarded

NS – no sample collected

Residues in egg whites have been plotted in the figure below for each individual hen. The data demonstrates that although there is some variability (mainly attributable to low residue levels), plateau is reached rapidly in egg whites in all hens, due. In addition mean residues in egg whites across all hens has been plotted to take account of biological variability and clearly demonstrates that plateau is reached rapidly.

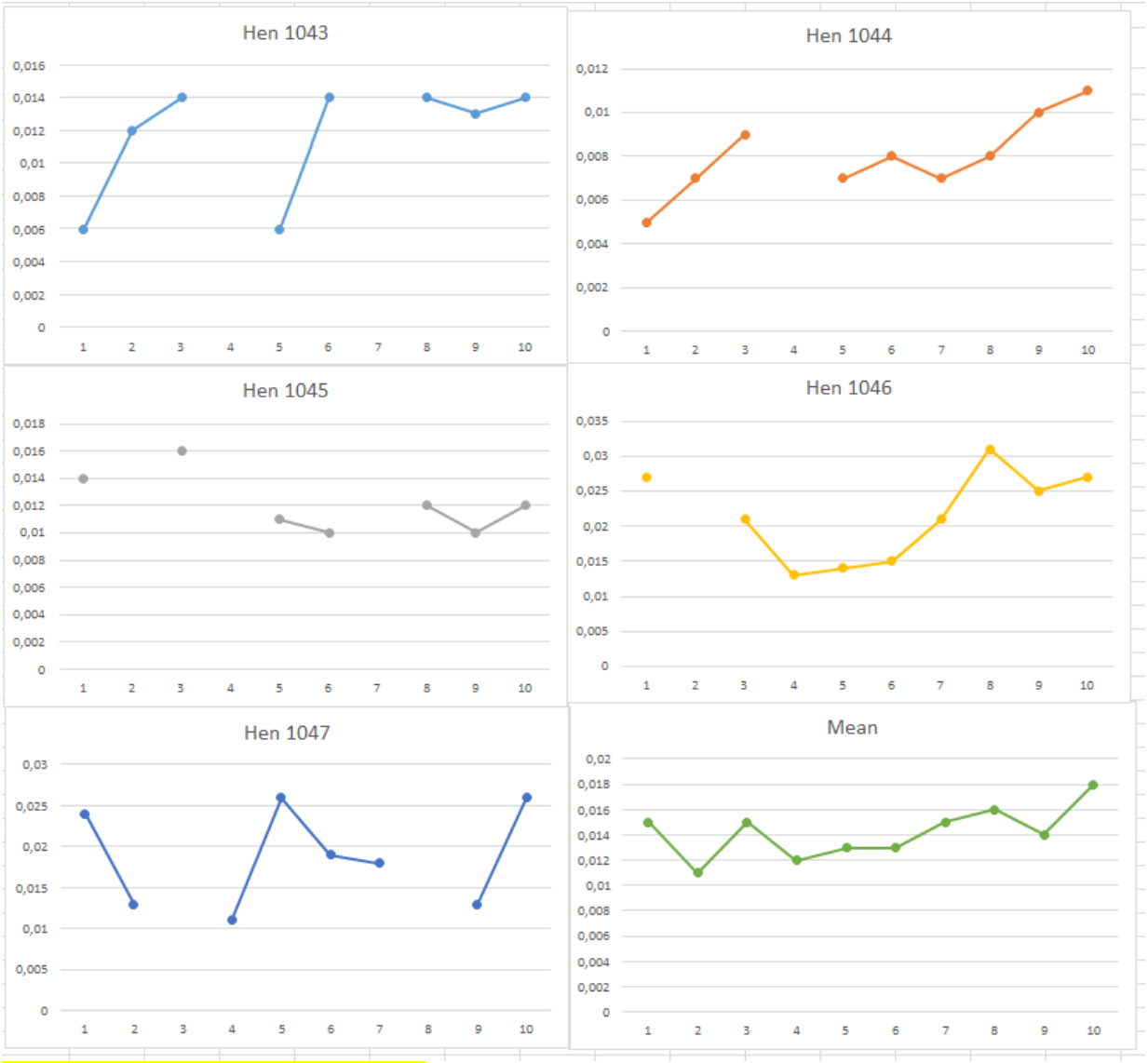


Figure 7.2.2.1-1: Residues in egg whites

Extractability of radioactive residues into solvent was ≥76.2% TRR (0.012 mg/kg) for egg white. The extracted radioactivity was analysed by chromatography. The identified components for egg white is summarised in Table B.7.2.2.1-7.

Table B.7.2.2.1-7: Summary of the characterisation and identification of components in egg white from laying hens treated with [^{14}C]-cyclohexadione CGA163935

TRR by summation mg/kg	0.017 [1]
TRR by direct quantification mg/kg	0.016 [2]
Percentage of TRR for chromatography, %	76.2

Origin of component	Component	% TRR	Residue (mg/kg)
Chromatographed [3]	CGA163935	31.0	0.005
	CGA179500	20.2	0.003
	Unassigned [4]	3.3	0.001
	Remainder [5]	6.7	0.001
	Other fractions [6]	21.6	0.004
	Losses/gains on fractionation [7]	6.6 (Loss)	0.001 (Loss)
	Unextracted [8]	10.6	0.002
	Total	100.0	0.017

1- TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.

2- The radioactive residue determined by direct quantification employing combustion/LSC.

3- The components of the TRR that were derived from chromatographic analysis.

4- Unassigned radiocomponents which chromatographed away from the origin in TLC, which contains at least 2 unknowns, none of which represents > 3.2% TRR (0.0005 mg/kg)

5- The remainder comprises diffuse areas of radioactivity within the chromatogram which cannot be assigned to discrete radioactive components

6- Extractable residues in 4 fractions that were not analysed. produced during processing that were too low for analysis. No single fraction comprised ≥ 8.2% TRR (≥0.001 mg/kg).

7- The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components..

8- Radioactivity remaining in the debris after extraction with aqueous acetonitrile and acetone. The nature of this residue was not characterised further due to radioactive residues being <0.01 mg/kg.

RMS comments and conclusions

Five hens were dosed for 10 consecutive days with ^{14}C-cyclohexadione labelled CGA163935 at a rate of 8.1 – 10.4 mg/kg in the diet, the hens were sacrificed approximately 22 hours after the final dose and necropsy of tissues of human dietary significance undertaken. Eggs were also collected during the dosing period. All tissue, eggs and excreta samples (also collected during the dosing period) were radioassayed to determine the radioactive residue (mg CGA163935 equivalents /kg sample) and the balance of dosed radioactivity recovered. Radioactivity extracted from egg white were fractionated and analysed by chromatography. The results of the analysis demonstrate that:

- [^{14}C]-trinexapac-ethyl and/or its hens biotransformation products are readily excreted as more than 87% of the dose was accounted for in the excreta.

- Total radioactive residues in egg yolk and egg white reached a maximum level of 0.009 mg/kg and 0.031 mg/kg after 8 days of dosing, respectively.
- Egg white was the only sample found to contain residues >0.01 mg/kg.
- Parent and trinexapac acid (CGA179500) were found in egg white at 0.005 mg/kg and 0.003 mg/kg respectively.

The predominant biotransformation pathway for trinexapac-ethyl in the hen is the hydrolysis of parent to the corresponding carboxylic acid, CGA179500.

It is difficult to establish a plateau from the available studies. Although max values are reached 3 to 10 days in each animal, the mean concentration curve is quite stable during the experiment. RMS agrees with the applicant that plateau is reached rapidly as quite high values is observed at 1 day in 3 of 5 hens.

Study was performed prior to adoption of OECD guidelines 503.

The following deviations from OECD 503 (adopted 8 January 2007) were observed:

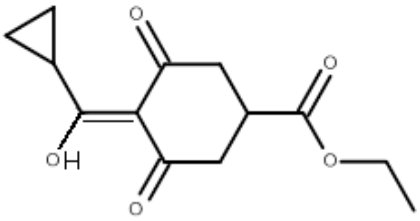
The number of testing animals (6) is slightly below the recommended (10);

The time between last dose and sacrifice is longer than recommended (22 hours instead of 6-12hours).

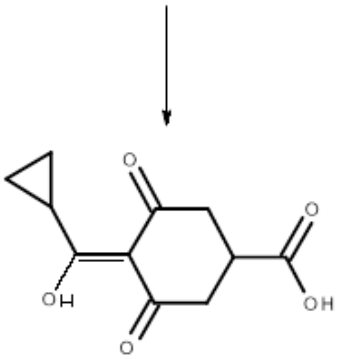
Despite these minor deficiencies the study is considered acceptable for the overall evaluation.

Proposed metabolic pathway in laying hens is shown in figure B.7.2.2.1-2.

Figure B.7.2.2.1-2: Proposed metabolic pathway for trinexapac-ethyl in laying hens



Trinexapac-ethyl



CGA179500

B.7.2.2.2 Lactating ruminants

The metabolism of trinexapac-ethyl in lactating goats was investigated in a study that was evaluated under the framework of Directive 91/414/EEC. Reported metabolism studies include two studies in lactating goat with [^{14}C-cyclohexyl]-trinexapac-ethyl while trinexapac is the major residue component in livestock feed. The study is deemed relevant due to the observed rapid and near-complete metabolic transformation conversion of trinexapac-ethyl to trinexapac acid in animals. The conditions of this study are summarised in Table B.7.2.2.2-1 and full evaluation provided below.

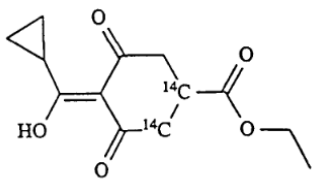
Table B.7.2.2.2-1: Summary of available metabolism studies in ruminants

Group	Species	Label Position	No of Animals	Application Details		Sampling Details		Report Reference	EU-review reference
				Rate (mg/kg bw/d)	Duration (days)	Commodity	Time		
EU Reviewed Data									
Lactating ruminants	Goat	^{14}C-cyclohexyl	1 per dose	0.2 20	4	Milk	Twice daily	141782 5/93	The Netherlands, 2003
						Urine &	Daily		

Group	Species	Label Position	No of Animals	Application Details		Sampling Details		Report Reference	EU-review reference
				Rate (mg/kg bw/d)	Duration (days)	Commodity	Time		
						faeces			Supplementary
						Tissues	At sacrifice (4h)		
			2	3	4	Milk	Twice daily	624-00	The Netherlands, 2005
						Urine & faeces	Daily		
						Tissues	At sacrifice (6h)		

Study 1

EU reviewed metabolism study in lactating goat

Reference:	**B. D. Cameron et al. (1992a)** Absorption, distribution and excretion of [1,2-^{14}C]-cyclohexyl CGA 163935 after multiple oral administration to lactating goats (~~KCA 6.2.3 / 01~~ KIIA 6.2.2.1 / 01)
Report No.:	7478
IRI project No.:	141782
Guideline:	Pesticide Assessment Guidelines Subdivision 0, Residue Chemistry, EPA, Washington, October 1982
GLP:	OECD principles of GLP as set forth by the UK Department of Health and as accepted by the International Regulatory Authorities throughout the European Community, USA (FDA and EPA) and Japan (MHW, MAFF and MITI).
Previous evaluation:	DAR 2003
Material and methods:	
Test item:	[1,2-^{14}C]-Cyclohexyl CGA 163935 (^{14}C-trinexapac-ethyl)
Position of the radiolabel	
Lot/Batch No.:	GAN-XVII-72
Radiochemical Purity:	≥98.8% (specific activity 46.34 µCi/g) Chemical purity 99.3%
Test concentration:	Low dose 7.2 mg/kg feed (equivalent to 0.2 mg/kg bw)

	High dose 694 mg/kg feed (equivalent to 19.9 mg/kg bw)
Test system:	Two lactating goats (45.0 and 43.0 kg bw) were individually housed in stainless steel metabolism cages (120 x 60 x 100 cm) with stainless steel grid floor and provided with feed and water. The room was illuminated using a 10/14, light/dark cycle with fluorescent strip lights. 7 days acclimatization period and health check was assessed prior to the first dose administration. The goats were dosed over a period of 4 consecutive days in the morning. Feed and water was provided *ad libitum*. Animals were sacrificed within 4 hours after the 4th daily dose and tissues were taken for analysis. The average feed intake for the treated goats during dosing was 1.4 kg/day. The samples were held frozen at -20°C until analysed. Storage stability was performed for urine.
Duration:	4 days
Sampling time points:	Urine and faeces were collected daily. Milk was collected twice a day, AM and PM. Blood samples were taken 0, 0.5, 1, 3, 6 and 12 h after the first dose and daily during the experiment and immediately before sacrifice. At sacrifice, samples of fat (omental, subcutaneous and renal), muscle (tenderloin, hindquarter and forequarter), kidney, liver, rumen and gall bladder contents.
Method of analysis:	Radioactivity of the samples was measured by LSC. Radioactivity in all samples was analysed using a Liquid Scintillation Analyser with automatic quench correction by the external standard channels ratio. Samples were counted in triplicate for 5 min each. Samples for combustion were weighed and combusted using a model 306 Tri-Carb Automatic Sample Oxidiser. Combustion efficiency and carry-over were checked routinely several times throughout each run. Mean combustion efficiency was shown to be greater than 97% and carry-over less than 1% throughout the experimental period. All reported data are therefore uncorrected. The efficiency of counting for liquid and combusted samples was in the range of 76-93% and 53-79% respectively.
Number of animals:	Two (one per dosing level)
Method validation	Low dose - 75 % of the total administered dose was recovered in the following samples: urine (50%), faeces (16%), cage wash (9%) and milk (0.02%). High dose – 87 % of the total administered dose was recovered in the following samples: urine (62%), faeces (19%), cage wash (6%) and milk (0.02%).

Results

The maximum concentration in blood cells and plasma after the first dose reached its maximum 1h post dosing.

The distribution of residues of [1,2-^{14}C]-Cyclohexyl CGA 163935 in tissues, milk and excreta are presented in table B.7.2.2.2-2

Table B.7.2.2.2-2: Distribution of ^{14}C-residues in tissues, milk and excreta of lactating goats following oral doses of [1,2-^{14}C]-Cyclohexyl CGA 163935 (trinexapac-ethyl) for 4 consecutive days

Matrix	0.2 mg/kg bw/d		20 mg/kg bw/d	
	mg/kg[1)]	% of dose[2)]	mg/kg[1)]	% of dose
Urine		50[3)]		62
Faeces		16		19
Milk	0.008[4)]	0.01	0.83	0.02
Muscle[4)]	0.043	2.2	2.5	1.2
Liver	0.25	0.55	12	0.27
Kidney	0.50	0.18	42	0.14
Fat[4)]	0.095	0.34	1.5	0.10
Bile	0.21	0.0	8.2	0.0
Rumen content	0.27	3.9	31	3.1

1) ^{14}C-trinexapac-ethyl equivalents

2) calculated by the notifier on the basis that total weight of muscle represents 45%, fat 6% and total blood 7% of the goat body weight

3) urine from 72-76h after 1st dosing was lost and bladder was empty post mortem

4) highest values

Tables B.7.2.2.2-3 and B.7.2.2.2-4 show the daily TRR levels in milk collected over the 4 days of the study for low and high dose respectively. The data demonstrates that plateau is reached rapidly in milk. Graphical representation of radioactive residue in milk over the dosing period for goat treated with 0.2 and 20 mg/kg bw/d [^{14}C]-CGA163935 respectively is provided in figure B.7.2.2.2-1.

Table B.7.2.2.2-3: Total radioactive residues (TRR) in milk over the dosing period from goats dosed with 0.2 mg/kg bw/d [^{14}C]-CGA163935

Day	For Individual PM and AM Collections					For 24 h Period		
	PM/AM Collection	Weight (ml)	Radioactivity (dpm/ml)	Total radioactivity (dpm)	Residue (mg/kg)	Weight (g)	Total radioactivity (dpm)	Residue (mg/kg)
1	PM Day 1	205	452	92660	0,004	585	154600	0,003
	AM Day 2	380	163	61940	0,002			
2	PM Day 2	195	671	130845	0,007	550	196165	0,003
	AM Day 3	355	184	65320	0,002			
3	PM Day 3	175	636	111300	0,006	570	192275	0,003
	AM Day 4	395	205	80975	0,002			

Table B.7.2.2.2-4: Total radioactive residues (TRR) in milk over the dosing period from goats dosed with 20 mg/kg bw/d [^{14}C]-CGA163935

Day	For Individual PM and AM Collections					For 24 h Period		
	PM/AM	Weight	Radioactivity	Total radioactivity	Residue	Weight	Total radioactivity	Residue

	Collection	(ml)	(dpm/ml)	(dpm)	(mg/kg)	(g)	(dpm)	(mg/kg)
1	PM Day 1	190	7914	1503660	0,451	530	3379100	0,363
	AM Day 2	340	5516	1875440	0,314			
2	PM Day 2	185	11663	2157655	0,664	550	3532610	0,366
	AM Day 3	365	3767	1374955	0,215			
3	PM Day 3	150	14546	2181900	0,828	520	3515750	0,385
	AM Day 4	370	3605	1333850	0,205			

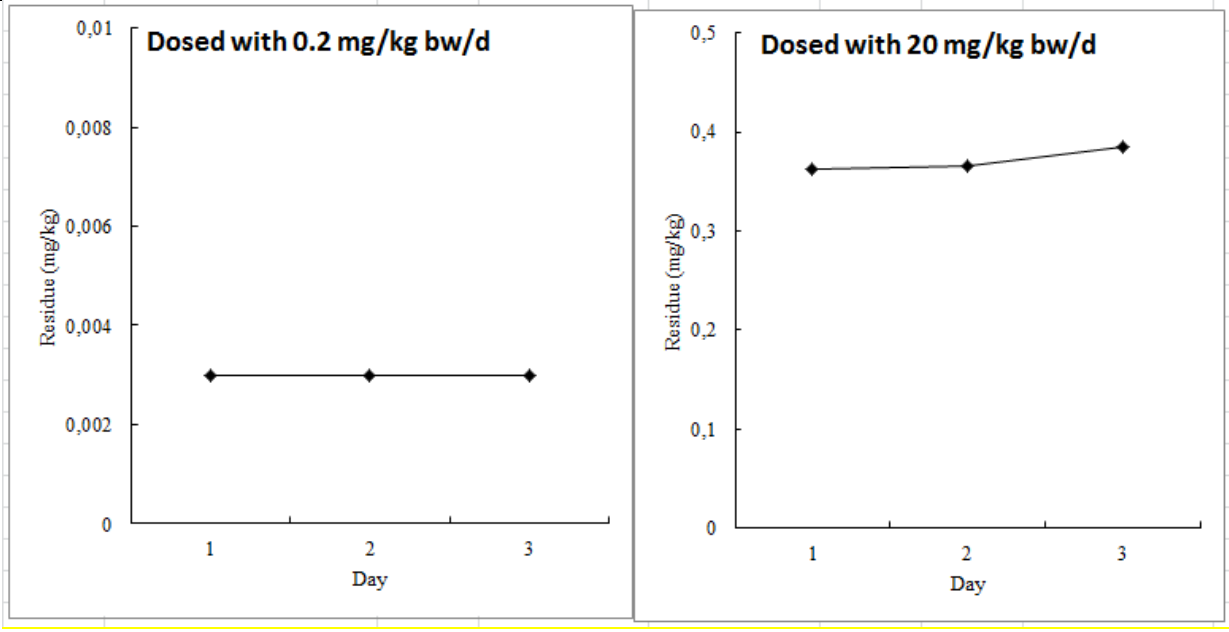


Figure B.7.2.2.2-1: Total radioactive residues (TRR) in milk over the dosing period from goats dosed with 0.2 and 20 mg/kg bw/d [^{14}C]-CGA163935

RMS comments and conclusions (The Netherlands 2003)

The uptake, distribution and elimination of [1,2-^{14}C]-cyclohexyl CGA 163935 was studied in lactating goats over a period of 4 consecutive days after application of 0.2 mg/kg bw/day (7.2 mg/kg feed) and 20 mg/kg bw/day (694 mg/kg feed). Urinary excretion is the major elimination route for CGA 163935 (trinexapac-ethyl) reaching 50 and 62% for low and high dose respectively and the remaining radioactivity was voided with faeces (16 and 19%). Only a small portion of the dose (0.02%) was eliminated with the milk at both dose levels. No accumulation of radioactivity was evident throughout the study period resulting in low tissue levels.

Highest radioactive residues are found in kidney and liver, followed by fat. In milk, low levels of residue are found which reach a plateau level within about 2-3 days.

RMS LT agrees with the above conclusions.

Testing animals were sacrificed 23 November 1989, experimental termination date is 3 May 1990, the calculated time between sample and analysis is approximately 6 months.

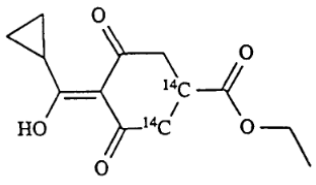
Guidelines and limitations

This study only describes the uptake, distribution and excretion of the parent compound CGA 163935 (trinexapac-ethyl) in goats. The nature of the residue is described in study 2 (Müller, 1993) below.

The study is considered suitable for evaluation.

Study 2

EU reviewed metabolism study in lactating goat

Reference:	**T. Müller (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 (~~KCA 6.2.3 / 02~~ KIIA 6.2.2.1 / 02)
Report No.:	5/93
Project No.:	01TM01
Guideline:	EPA Pesticide Assessment Guidelines, subdivision O. Residue chemistry, 1982
GLP:	OECD Good Laboratory Practice Council Decision 81/30 and Recommendation 83/95 US EPA FIFRA Good Laboratory Practice (40 CFR Part 160) US EPA TSCA Good Laboratory Practice (40 CFR Part 792) US FDA Health and Human Services Good Laboratory Practice (21 CFR Part 58) MAFF No 3850, Japan
Previous evaluation:	DAR 2003
Material and methods:	
Test item:	[1,2-^{14}C]-Cyclohexyl CGA 163935 (^{14}C-trinexapac-ethyl)
Position of the radiolabel	
Lot/Batch No.:	GAN-XVII-72 (radiolabelled) AMS 265/101 (non-radiolabelled)
Radiochemical Purity:	≥98.8% (specific activity 46.34 µCi/g) Chemical purity 99.3%
Test concentration:	Low dose 7.2 mg/kg feed (equivalent to 0.2 mg/kg bw)

	High dose 694 mg/kg feed (equivalent to 19.9 mg/kg bw)
Test system:	Samples taken in the disposition study in goats, administered with [1,2-^{14}C]-Cyclohexyl CGA 163935 (described under study 1, Cameron et al., 1992) were analysed in order to investigate the nature of residue of CGA 163935 (trinexapac-ethyl).
Method of analysis:	Milk (pool of equal amounts collected p.m. on day 1,2,3) muscle, liver and kidney samples were extracted with different acetonitrile solvent mixtures, followed by analytical TLC. Samples were analysed by TLC, co-chromatographed with reference compounds. Milk and muscle samples of the low dose were subjected to preparative TLC before the final analysis. Fat samples were subsequently partitioned with chloroform/methanol, sodium phosphate buffer, methylene chloride (after acidification), followed by analytical TLC. Samples of urine and bile fluid were analysed by TLC without pre-treatment. The data for the muscle sample represent the mean value from samples of hind-, forequarter and tenderloin muscle, and for fat the mean value from samples of omental, subcutaneous and renal fat. Radioactivity was measured on a Packard TRI-Carb scintillation counter, quenching was corrected by the SIE or TSIE method. Faeces, fat and the corresponding solids after extraction were homogenized manually. The radioactivity was determined after combustion and Carbosorb was used to trap CO2. Recovery tests of the sample oxidizer were performed by combusting standards of [1-^{14}C]-n-hexane. The recoveries were always above 95% and the carryover below 0.5%. The pattern of radioactivity on thin layer plates was detected by spark chamber radiochromatogram camera. Quantitative data for fractions on TLC plates were obtained by scraping off the radioactive zones, adding about 1 ml methanol, followed by radiometry in scintillation mixture A (Irgascint A300). Quantitative metabolite pattern were corrected for background (31 dpm). Analytical TLC was performed on precoated plates of silica gel, the following solvent systems were used: ss1 - acetonitrile/water/formic acid (90/9/1 v/v) ss2 - toluene/ethyl acetate/formic acid (50/40/10 v/v) ss3 - chloroform/1-propanol/acetic acid (80/10/10 v/v) ss4 - chloroform/ acetonitrile/formic acid (60/30/10 v/v) ss5 - dichloromethane/ethyl acetate/formic acid (70/20/10 v/v) Two-dimensional TLC was performed either in ss4 and ss5 or in ss3 and ss2 in the first and the second dimension, respectively. Preparative TLC was performed in ss1.
Number of animals:	Two
Storage stability:	Storage stability of metabolites was determined by comparison of the quantitative metabolite pattern of goat urine prior to storage (21 November 1989) and at the beginning of the experimental phase of this study (17 September 1990). The table below shows that quantitative metabolite pattern did not change.

Table 2 Storage Stability (Goat 202 LAE9, Urine: 24 - 48 h, Low Dose)

Date	December 21, 1989	September 17, 1990
Metabolite Fraction	% of Radioactivity Analyzed	% of Radioactivity Analyzed
Fr 1	0.8	0.8
Fr 2	1.3	1.1
Fr 3	94.4	95.1
R1 - R4 a)	3.5	3.0
Total	100.0	100.0
Experimental Recovery [%]	96	96

a) R1 - R4 represents areas of undefined radioactivity.

Results

The characterisation and identification of the residues of [1,2-^{14}C]-Cyclohexyl CGA 163935 present in tissues and milk are given in table B.7.2.2.2-5 for the low dosing and table B.7.2.2.2-6 for the high dosing.

Table B.7.2.2.2-5 Characterisation and identification of residues in tissues of lactating goats following oral doses of [1,2-^{14}C]-Cyclohexyl CGA 163935 (low dose level: 0.2 mg kg bw/d)

Metabolites	Milk (p.m.)		Muscle		Kidney		Liver		Fat	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	Mg eq/kg	% TRR
TRR[1]	0.006	100	0.038	100	0.50	100	0.25	100	0.045	100
Organo-soluble	0.0043[2]	71	0.036	95	0.48	96	0.22	89	0.037	82
Water soluble										
Not extracted	0.0017	29	0.002	5	0.02	4	0.03	11	0.008	18
Identified[3]		63		90		81		42		31
CGA 179500	0.004	63	0.034	90	0.40	81	0.10	42	0.014	31
Characterised Total amount per fraction (mg eq/kg)/% TRR)[number of fractions]	< 0.01 /8 [1]		< 0.01 / 5 [1]		> 0.05 / 15 [1]		> 0.05 /47 [>1]		0.01-0.05 /51 [1]	

[1] total radioactive residue

[2] numbers in italics are calculated by the Rapporteur

[3] Characterisation and identification were performed on additionally extracted organo-soluble fractions from muscle and milk.

Table B.7.2.2.2-6 Characterisation and identification of residues in tissues of lactating goats following oral doses of [1,2-^{14}C]-Cyclohexyl CGA 163935 (high dose level: 20 mg/kg bw)

Metabolites	Milk		Muscle		Kidney		Liver		Fat	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
TRR1	0.65	100	2.2	100	42	100	12	100	1.4	100
Organo-soluble	0.552	85	2.1	96	41	97	11.5 ~~12~~	96	0.97	70
Water soluble										
Not extracted	0.099	15	0.09	4	1.2	3	0.5	4	0.42	30
Identified3		76		81		82		33		67
CGA 179500	0.424	76	1.7	81	34	82	4.0	33	0.93	67
Characterised Total amount per fraction (mg eq/kg)/% TRR)[number of fractions]	0.01-0.05 /9 [1]		>0.05 /15 [1]		>0.05 /6 [1]		>0.05 /6 [>1]		0.01-0.05 /3 [1]	

[1] total radioactive residue

[2] number in italics are calculated by the Rapporteur

[3] characterisation and identification was performed on the organo-soluble fraction

[4] absolute number in table of notifier (0.492) differs from the calculated value

In urine, CGA 179500 is the major metabolite, accounting for approximately 90% of the urinary radioactivity. Minor metabolites were found, but not identified. About 12-28% of the daily dose is voided with the faeces. The predominant metabolite co-chromatographed with CGA 179500, accounting for 82-91% and 93-94% of the faecal radioactivity after low and high dosing, respectively.

RMS comments and conclusions (The Netherlands 2003)

After daily oral administration of [1,2-^{14}C]-Cyclohexyl CGA 163935 (trinexapac-ethyl) to goats at two dosing levels over 4 consecutive days, the only compound identified was CGA 179500, present in all tissue and milk samples analysed. This compound accounts for 63 – 76% TRR in milk, 81-90% TRR in muscle, 81-82% TRR in

kidneys, 33-42% TRR in liver, and 31-67% TRR in fat. Other metabolites have not been identified. The transformation of trinexapac-ethyl in the goat proceeds by the same metabolic pathway as observed in the rat.

RMS LT agrees with the above conclusions.

Guidelines and limitations

Duration of the experiment is a little bit shorter than recommended in OECD 503 (4 days instead of 5). Some residues > 0.01 mg/kg or 10% TRR were not identified, characterised only, but with regard to the exaggerated dose level this is considered acceptable.

Time from collection to sample analysis was not specified in the study report. Samples were taken at the day of sacrifice (23 November 1989) and experimental work was performed from 17 September 1990 till 15 April 1991, therefore the calculated time from sample to analysis is 10 to 17 months. No information on storage stability was presented in the report, except for urine. Residues in urine were proven to be stable for 9 months (21 December 1989 till 17 September 1990).

No information was provided if the method is suitable to determine free and conjugated forms of the metabolites.

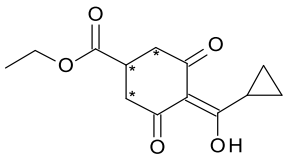
Extractability in milk (low dose) and fat (high dose) was low (29-30% TRR remain unextracted). No apparent attempts for further extraction efforts to characterise/identify the non-extracted residues were mentioned in the study report.

The study is considered supplementary ~~suitable for evaluation~~. As the results of this study are in line with study 3 results (main metabolite being CGA 179500 only), this study could also be used for the assessment.

Study 3

EU reviewed metabolism study in lactating goat

Reference:	**W. J. Ray (2002).** [1,2,6-^{14}C]-cyclohexyl-CGA-163935: Nature of the residue in lactating goats (~~KCA 6.2.3 / 03~~ KIIA 6.2.2.1 / 03)
Report No.:	624-00
Guideline:	Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue – Plants, Livestock
GLP:	EPA GLP Standards as defined by 40 CFR Part 160 with one exception – a non-GLP laboratory analysed preliminary blood samples, collected to assist in determining the health status of the test animals.
Previous evaluation:	Addendum to the DAR 2005
Material and methods:	
Test item:	[1,2,6-^{14}C]-Cyclohexyl CGA 163935 (^{14}C-trinexapac-ethyl)

Position of the radiolabel	
Lot/Batch No.:	BPM-XXIV-58
Radiochemical Purity:	97.9% (specific activity 42.3 µCi/g) Chemical purity 98.6%
Test concentration:	100 mg/kg diet as received (equivalent to 3 mg/kg bw/day)
Test system:	Two lactating goats (51.5 and 47.0 kg bw, 2 and 3 years old) were individually housed in stainless steel metabolism cages designed for the separate collection of urine and faeces. The room was illuminated using a 12-hour on/off cycle. 7 days acclimatization period and health check was assessed prior to the first dose administration. The goats were dosed over a period of 4 consecutive days in the morning, given oral doses of 150 mg of [1,2,6-^{14}C-cyclohexyl]trinexapac-ethyl contained in gelatine capsules with cellulose, equivalent to a nominal rate of 100 mg/kg diet as received (ca. 3 mg/kg bw/day, 300N TMDI for beef cattle). Animals received a measured daily quantity of a commercial goat chow plus hay and water was provided *ad libitum*. Animals were sacrificed within 6 hours after the 4th daily dose and tissues were taken for analysis. The average feed intake for the treated goats during dosing was 1.59 and 1.22 kg/day. The samples were held frozen at -20°C until analysed. All samples were extracted, profiled and all metabolites identified within 5 months of sacrifice, therefore no storage stability analyses were conducted.
Duration:	4 days
Sampling time points:	Urine and faeces were collected daily and milk twice daily. Blood samples were taken just prior to sacrifice. The following samples were collected at sacrifice (6 h after the last dose): kidneys, liver, leg and tenderloin muscle, omental and perirenal fat, bile, gastrointestinal tract (with contents). The two muscle samples were combined, as were the two fat samples.
Method of analysis:	All solid and semi-solid samples were homogenised. Radioactivity in muscle, liver, kidney, blood, GI tract and faeces was determined by combustion/LSC. Radioactivity in liquid samples was determined by LSC. Radioactivity in fat was determined by LSC after dissolving in toluene. Liver, kidney and muscle were extracted with acetonitrile/water (4/1), and fat with chloroform/methanol (4/1). Milk (day 2 pm sample) was homogenised with acetonitrile, and the solids remaining following removal of the supernatant were re-extracted with acetonitrile. Extracts of each sample were combined, concentrated, radio-assayed by LSC and profiled by C18 HPLC and silica TLC. A urine sample (24-48 hours) was filtered and profiled by C18 HPLC and silica TLC. The method of pre-treatment of the bile prior to chromatography was not reported. Metabolite identification was based on co-chromatography with unlabelled reference standards. In addition, the identity of CGA-179500 was confirmed by LC-MS/MS. Radioactivity in the post-extraction solids (PES) was quantified by combustion/LSC.
Storage stability:	Subsamples of all tissues and milk were extracted, profiled and all metabolites identified within 5 months of sacrifice. No further storage stability analyses were conducted.
Number of animals:	Two

Limit of quantification:	0.004 mg/kg
Method validation	94.5 % of the total administered dose was recovered in liver, 95% in kidney, 104% in muscle, 97.8% in fat and 90.3% in milk.

Results

Both animals were in good health throughout the study. Based on the mean weight of the animals (47.0 and 51.5 kg) and the average feed intake (1588 and 1223 g/day), the daily dose represented 95 and 125 mg/kg diet (mean 110 mg/kg diet), and 2.92 and 3.21 mg/kg bw/day (mean 3.07 mg/kg bw/day).

Total radioactivity in tissues, milk and excreta are presented in Table B.7.2.2.2-7. Results of extraction and chromatography of goat samples are shown in Table B.7.2.2.2-8.

In composite samples of milk, urine and faeces, 0.05%, 80.5% and 2.5 % of the total administered dose was recovered (total excreted 83.1%). Radioactivity in composite muscle, fat, liver and kidney represented 0.90, 0.03, 0.12 and 0.14% of the administered dose (total in tissues 1.2%), whilst blood, bile and GI tract contained 2.2, <0.01 and 3.4%, respectively. The total recovery (tissues, milk, excreta) was 89.9%.

Radioactivity extractable from tissues and milk represented 90.3-104% TRR. Parent compound was not detected in any tissue and milk. CGA-179500 was a major (>10% of TRR and/or 0.05 mg eq/kg) metabolite in milk and tissues (66.0-96.8% TRR, 0.065-5.0 mg eq./kg). CGA-113745 was a major metabolite in liver, kidney and fat (6.0-16.3% TRR, 0.012-0.35 mg eq./kg), but was not detected in muscle and milk. Two unidentified fractions G3 and G4 were found in liver and/or muscle and/or fat at 1.5-5.1% TRR (0.002-0.041 mg eq./kg), and unidentified material in the HPLC void region accounted at the most for 5.5% TRR or 0.21 mg eq./kg. PES in tissues and milk represented 4.0-9.0% TRR (0.007-0.27 mg eq./kg). The metabolite pattern in urine and bile was similar to that in tissues (only chromatograms shown).

Table B.7.2.2.2-7 Total radioactivity in goat tissues, milk and excreta after 4 doses of [1,2,6-^{14}C-cyclohexyl] trinexapac-ethyl at 150 mg/day (100 mg/kg diet)

Sample	Interval (h)	% of dose			mg/kg		
		goat 1	goat 2	composite	goat 1	goat 2	composite
milk	0-78	0.02	0.07	0.05			0.35
faeces	0-78	3.4	1.5	2.54			
urine	0-78	76.7	82.8	80.5			
total eliminated	0-78	80.1	84.4	83.1			
muscle	78	1.07	0.64	0.90	0.31	0.21	0.28
fat	78	0.05	0.02	0.03	0.15	0.065	0.11
liver	78	0.13	0.11	0.12	0.88	0.72	0.80
kidney	78	0.13	0.16	0.14	5.2	6.4	5.9

total tissue	78	1.4	0.93	1.2			
blood	78	3.10	1.28	2.19	4.9	22.3	
bile	78	0.00	0.01	<0.01	0.35	0.95	
GI tract	78	2.71	4.03	3.37	1.2	2.1	
total recovery	0-78	87.3	90.6	89.9			

Table B.7.2.2.2-8 Extraction and identification of radioactivity in composite goat milk and tissues after 4 doses of [1,2,6-^{14}C-cyclohexyl] trinexapac-ethyl at 150 mg/day (100 mg/kg diet)

	liver		kidney		muscle		fat		milk	
	%TRR	mg/kg[a]	%TRR	mg/kg[a]	%TRR	mg/kg[a]	%TRR	mg/kg[a]	%TRR	mg/kg[a]
Extractable	94.5	0.76	95.0	5.6	104	0.29	97.8	0.10	90.3	0.069
Metabolites										
CGA-113745	16.3	0.13	6.0	0.35	nd	nd	11.4	0.012	nd	nd
CGA-179500	66.0	0.53	85.3	5.0	96.8	0.27	83.9	0.089	85.3	0.065
Unknown G3	5.1	0.041	nd	nd	3.5	0.010	nd	nd	nd	nd
Unknown G4	1.5	0.012	nd	nd	nd	nd	1.5	0.002	nd	nd
Unidentified(A)	5.5	0.044	3.6	0.21	nd	nd	nd	nd	2.0	0.002
Total identified	82.3	0.66	91.3	5.4	96.8	0.27	95.3	0.10	85.3	0.065
PES	8.0	0.064	4.5	0.27	4.0	0.011	ns	ns	9.0	0.007
Total characterised	90.3	0.72	95.8	5.7	101	0.28	95.3	0.10	94.3	0.072

[a] mg/kg trinexapac-ethyl equivalents

nd = not detected (<0.004 mg/kg)

ns = no PES sample after extraction

(A) Void volume region during HPLC.

Table B.7.2.2.2-9 shows the daily TRR levels in milk collected over the 4 days of the study. The data demonstrates that plateau is reached rapidly in milk. Identification of radioactivity was performed for 2 day PM milk. Graphical representation of radioactive residue in milk over the dosing period for goat treated with 0.2 and 20 mg/kg bw/d [^{14}C]-CGA163935 is provided in figure B.7.2.2.2-2.

Table B.7.2.2.2-9: Total radioactive residues (TRR) in milk over the dosing period from goats dosed with 3 mg/kg bw/d [^{14}C]-CGA163935

Day	For Individual PM and AM Collections - Goat 995					For Individual PM and AM Collections - Goat 996				For 24 h Period		
	PM/AM Collection	Weight (g)	Radioactivity (dpm/g)	Total radioactivity (dpm)	Residue (mg/kg)	Weight (g)	Radioactivity (dpm/g)	Total radioactivity (dpm)	Residue (mg/kg)	Weight (g)	Total radioactivity (dpm)	Residue (mg/kg)
1	PM Day 1	494	6793	3355742	0,072	606	7403	4486218	0,079	1846	6173173	0,036
	AM Day 2	1067	930	992310	0,010	1525	2303	3512075	0,025			
2	PM Day	543	5540	3008220	0,059	622	8027	4992794	0,085	1834	6385274	0,037

	2											
	AM Day 3	1131	630	712530	0,007	1372	2957	4057004	0,031			
3	PM Day 3	612	4097	2507364	0,044	594	9577	5688738	0,102	1752	6221403	0,038
	AM Day 4	1149	553	635397	0,006	1149	3143	3611307	0,033			

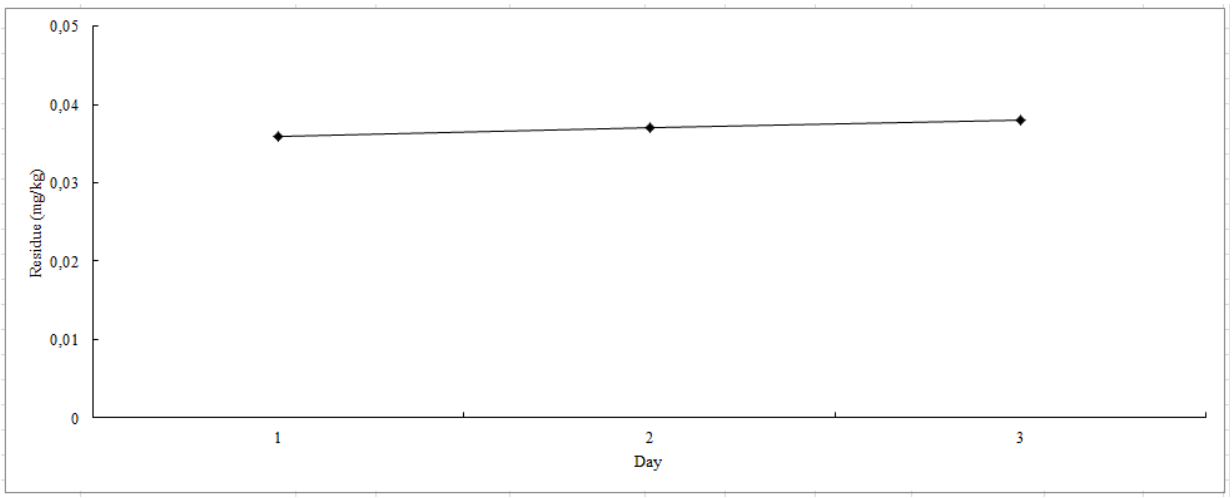


Figure B.7.2.2.2-2: Total radioactive residues (TRR) in milk over the dosing period from goats dosed with 3 mg/kg bw/d [^{14}C]-CGA163935

RMS comments and conclusions (The Netherlands 2005)

After four daily dose administrations of [1,2,6-^{14}C-cyclohexyl] trinexapac-ethyl at 150 mg/day (250N TMDI dairy cattle), equivalent to 110 mg/kg diet and 3.07 mg/kg bw/day, total RA excreted in milk, urine and faeces represented 0.05%, 80.5% and 2.5% of the administered dose, and RA in composite muscle, fat, liver and kidney was 0.90, 0.03, 0.12 and 0.14%. RA extractable from tissues and milk represented 90.3-104% TRR (PES 4.0-9.0% TRR, 0.007-0.27 mg eq./kg). Parent compound was not detected in any tissue and milk. CGA-179500 was a major metabolite in milk and tissues (66.0-96.8% TRR, 0.065-5.0 mg eq./kg). CGA-113745 was the only other major metabolite (in liver, kidney and fat: 6.0-16.3% TRR, 0.012-0.35 mg eq./kg). Unidentified fractions in tissues and milk accounted for at the most 5.5% TRR or 0.21 mg eq./kg.

RMS LT agrees with the above conclusions.

Plateau in milk is reached rapidly (during the first days of dosing).

Guidelines and limitations

The terminal residue was sufficiently characterised and relevant fractions were identified (in particular when taking into consideration the 250N dose for fractions >0.05 mg/kg). Duration of the experiment is a little bit

shorter than recommended in OECD 503 (4 days instead of 5). The study was performed in agreement with OPPTS 860.1300 and is acceptable.

Conclusion metabolism in ruminants

After oral dosing with highly exaggerated doses of trinexapac-ethyl, the highest total radioactivity residues were found in kidneys (0.50-42 mg eq/kg). Relatively low residue levels were observed in milk (0.008-0.83 mg eq/kg). Residue concentrations reached plateau levels in milk after about 2 or 3 days. Trinexapac acid was the major residue component identified in milk, meat and offal, accounting for about 66-97% TRR. In one of the goat studies, metabolite CGA113745 was also found in the liver, kidney and fat (6-16% TRR), but at low absolute levels (<0.4 mg/kg) particularly when considering the exaggerated dose rate administered to the animals, anticipated residue levels would be negligible at the estimated maximum dietary burden of pesticide residues in the diet.

The metabolism studies on lactating goats were reviewed within the framework of Directive 91/414/EEC and were considered to be acceptable; the notifier considers that no further metabolism study in ruminant is required to support trinexapac-ethyl.

B.7.2.2.3 Pigs

Metabolic pathways in ruminants and rat are considered to be comparable; a metabolism study in pig is therefore not deemed necessary.

B.7.2.2.4 Fish

No study submitted.

~~Document **SANCO/10181/2013 Rev. 2.1**, 13 May 2013 states: "*In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in the form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.*"~~

~~Currently guidance for fish metabolism and fish feeding studies has not been finalised. It was noted in Section A.24 of the summary from the SCoPAFF meeting on 24 – 25 November 2014[1], that "*the Commission working document is not yet finalised and ready to be noted as a guidance document*." Additionally, "…*the Commission emphasised that for the time being there are no agreed test guidelines and that hence the pertinent data requirements can be waived*."~~

~~Additionally, log P_{ow} is below 3 for trinexapac-ethyl.~~

~~Consequently fish metabolism studies have not been conducted.~~

Metabolism, distribution and expression of residue in livestock - summary and conclusions

The metabolism of CGA 163935 was studied in lactating goats and laying hens. In all metabolism studies ^{14}C-trinexapac-ethyl was used. However, it is noted that the metabolite CGA 179500, and also CGA 351210 (a further degradation product of CGA 179500), are the major residue components in livestock feed. As such, the livestock metabolism studies with trinexapac-ethyl might be considered less relevant in first instance. Considering the fast and extensive metabolism of trinexapac-ethyl to CGA 179500 as described below, the study results using trinexapac-ethyl are nevertheless taken into consideration.

Capsules containing the test substance were administered orally to lactating goat and laying hen with concentrations corresponding to doses of 7.2, 100 and 694 mg/kg in feed to the lactating goat (corresponding to 0.2, 3 and 19.9 mg/kg bw/day) and 4, 10 and 180 mg/kg in feed to the laying hen (corresponding to 0.4, 0.85 and 20.3 mg/kg bw/day). Trinexapac-ethyl was rapidly metabolised, with the majority of the administered radioactivity excreted in the urine and faeces (83% in goat and 89% in hen).

Taking into account all metabolism studies (fully reliable and supplementary), after oral dosing with highly exaggerated doses (17-1667N rates to goat and 24-1176N rates to poultry) of the parent compound, highest residue concentrations are found in kidneys and liver of both species. Maximum residue levels were present in the kidney and liver at 42 and 12 mg trinexapac-ethyl equivalents/kg, respectively, in lactating goat and up to 1.77 and 0.6 mg trinexapac-ethyl equivalents/kg, respectively, in laying hen. Relatively low residue levels are observed in milk (up to 0.42 mg trinexapac-ethyl equivalents /kg, 76% TRR) and eggs (up to 0.01 mg trinexapac-ethyl equivalents /kg, 28 % TRR). Residue concentrations reach plateau levels in milk after about 2 to 3 days and in eggs after about 2 to 8 days. CGA 179500 is the major residue component identified in milk, meat and offal from ruminants and poultry, accounting for about 85%, 98%, and 85% TRR, respectively. CGA 113745 was the only

[1] *http://ec.europa.eu/food/plant/standing_committees/sc_phytopharmaceuticals/docs/sum_2014112425_ppr_en.pdf*

other major metabolite (in liver, kidney and fat: 6.0-16.3 % TRR, 0.012-0.35 mg trinexapac equivalents/kg). This metabolite was found only in "new" goat metabolism study and not found in "old, supplementary" goat metabolism study probably due to its long and not supported by storage data interval between sample and analysis. Also in poultry meat, offal, and egg yolk, the metabolite CGA 179500 is a major residue component representing about 50-60% TRR, 10-80% TRR, and 28-35% TRR, respectively. The exception is egg white, in which the parent compound is dominating, although being present in very low levels (0.0017-0.12 mg trinexapac-ethyl equivalents/kg, 31-44% TRR). In general, non-identified residues exceeding levels of 0.01 mg/kg or 10% TRR were observed in animal tissues in supplementary studies. However, considering the exaggerated dose levels used in the studies, no relevant residues are expected at the TMDI and no further identification is considered necessary.

The metabolic pathway of the trinexapac-ethyl in livestock comprises of hydrolysis of the ester bond to form trinexapac acid (CGA179500). CGA113745 was the only other metabolite identified in goat tissues. The observed metabolic pathway of trinexapac-ethyl in livestock is comparable to those observed in the rat, in which trinexapac acid is the major and only residue component of significance.

Overall it is concluded that the metabolite CGA 179500 is the only residue component of significance in animal products. Excretion of the residue as CGA 179500 by both livestock species is fast and extensive. In addition, the livestock feeding studies performed with CGA 179500 indicate that at a nominal residue intake, no significant residue levels of CGA 179500 are expected. Based on these considerations, no additional livestock metabolism studies are necessary.

Since metabolism in rats and ruminants was demonstrated to be similar, the findings in ruminants can also be extrapolated to pigs.

Since currently guidance for fish metabolism and fish feeding studies has not been finalised, fish metabolism studies can be waived.

A list of the identified compounds in lactating goat dosed daily for 4 days and laying hens dosed daily for 4 and 10 days with ^{14}C – trinexapac-ethyl and in rat is presented in Table B.7.2.2-1.

Table B.7.2.2-1 List of identified compounds found in lactating goat and laying hens dosed daily for 4 days and 4 and 10 days respectively with ^{14}C-trinexapac-ethyl

	Report Reference	Designation	**Trinexapac-ethyl CGA 163935**	**Trinexapac acid CGA 179500**	**CGA 113745**
		Chemical name (IUPAC)	4-(cyclopropyl-hydroxymethylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester	4-(cyclopropanecarbonyl)-3,5-dioxo-cyclohexanecarboxylic acid	3,5-dioxocyclohexanecarboxylic acid

Presence in **goat** mg/kg[a] (%TRR)[b] Low dose/high dose[d]	141782 5/93 (considered as supplementary)	Liver	--	0.1 (42)/ 4.0 (33)	-
		Kidney	--	0.4 (81)/ 34 (82)	-
		Muscle	--	0.034 (90)/ 1.7 (81)	-
		Fat	--	0.014 (31)/ 0.93 (67)	-
		Milk	--	0.004 (63)/ 0.42 (76)	-
	624-00	Liver	-	0.53 (66.0)	0.13 (16.3)
		Kidney	-	5.0 (85.3)	0.35 (6.0)
		Muscle	-	0.27 (96.8)	-
		Fat	-	0.089 (83.9)	0.012 (11.4)
		Milk	-	0.065 (85.3)	-
Presence in **hen** mg/kg[a] (%TRR)[b] Low dose/high dose[d]	141798 6/93 (considered as supplementary)	Liver	-	0.009 (69)/ 0.29 (49)	-
		Kidney	-	0.036 (84)/ 0.94 (53)	-
		Lean meat	-	0.001 (60)/ 0.058 (49)	-
		Skin and Fat	-	0.005 (84)/ 0.11 (53)	-
		Egg white	0.0017 (43)/ 0.12 (44)	0.0003 (7)	-
		Egg yolk	0.00005 (5)/ 0.005 (12)	0.00035 (35)/ 0.011 (28)	-
	RJ3678B	Liver[c]	-	-	
		Kidney[c]	-	-	
		Muscle thigh[c]	-	-	
		Skin and Fat[c]	-	-	
		Egg white	0.005 (31)	0.003 (20)	
		Egg yolk[c]	-	-	
Presence in **rat** %TRR[e]	ABR-89119	Faeces	13, 22, 39	5, 50, 79	
		Urine		92	

a - ^{14}C- Cyclohexyl Trinexapac-ethyl equivalents

b - total radioactive residue (mean values)

c - The radioactive residues for the edible tissues and egg yolk were found to be <0.01 mg/kg, so no further analysis was conducted

d - 0.2 and 20 mg/kg bw/day for goat, and 0.4 and 20.3 mg/kg bw/day for hen

e – depending of the dose

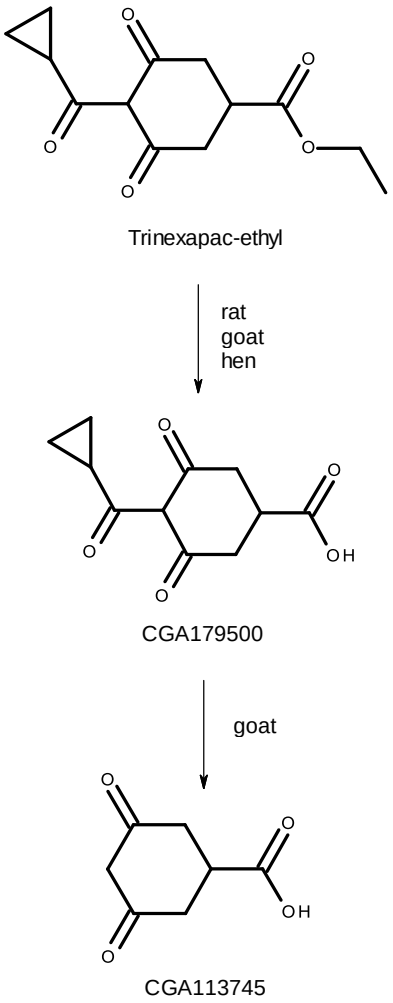


Figure B.7.2-1: Proposed metabolic pathway of trinexapac-ethyl in livestock

B.7.3 Magnitude of residue trials in plants

The representative crops in the original EU review of trinexapac-ethyl also included cereals. New trials and data are presented for these crops to replace the data originally evaluated. The new residue trials were conducted in

order to measure trinexapac acid, both free and conjugated forms since conjugates were observed in significant levels in the plant metabolism study (see Section B.7.2.1). Residue trials evaluated under Directive 91/414/EEC are not relied on in the framework of this submission because:

- they only measured the free form of trinexapac acid;
- some trials were not conducted at the proposed GAP;
- some trials were considered deficient due to the lack of raw data in the reports.

Although metabolites CGA300405 and CGA275537 are significant metabolite species observed in wheat straw (refer to Vol 3 CA B.6.2), their contribution to the animal dietary burden of pesticide residues is considered to be insignificant and they are not considered relevant for inclusion to the residue definition in plants for risk assessment (refer to Vol 3 CA B.7.4.1 and Section 2.7.3 of Volume I). Residue levels of these two metabolites have nonetheless been estimated (based on measured residue levels of free trinexapac acid) and included in calculations of residue trials results (Table B.7.3.1-3 and B.7.3.2-3).

The conversion factors – which are based on the results of metabolism-study data – used for all calculations are detailed in Appendix I.

B.7.3.1 Barley

Fifteen trials have been conducted in northern (8) and southern (7) Europe on barley at the following GAP: 1x200 g a.s./ha, with the application being made at BBCH 49. In order to provide a complete dataset for southern Europe, the residue levels from the processing study (two trials) conducted at 1×400 g a.s./ha (i.e. 2X) were adjusted to take account of the application (proportionality principle), details of those studies are presented in section B.7.5.3 Magnitude of residues in processed commodities.

As the use pattern is intended for grain production only, residue data on forage are not required. Details of the trials are summarised below. Representative GAPs on barley are presented in table B.7.3.1-1.

Table 7.3.1–1: Representative GAPs for A8587F use on barley

Crop	Outdoor/ Protected	Growth Stage	Maximum Number of Applications	Minimum Application Interval (days)	Maximum		Minimum PHI (days)
					Rate (L product/ha) [kg a.s./ha]	Water (L/ha)	
Winter barley	Outdoor	BBCH 25-49	1	not relevant	0.8 [0.2 kg a.s./ha]	100-400	not relevant
Spring barley	Outdoor	BBCH 25-37	1	not relevant	0.6 [0.15 kg a.s./ha]	100-400	not relevant

Cereals crops are treated and harvested according to growth stage and harvested at maturity. Thus a prescribed PHI is not relevant.

Studies performed in northern Europe

Study 1
Magnitude of trinexapac acid on barley

Reference:	**Andrews G. (2015)** Trinexapac-Ethyl - Residue Study on Winter Barley in northern France and Germany in 2013. Syngenta File No. A8587F_10138 (KCA 6.3.1/01)
Report No.:	TK0178789
Trial No.:	NC13039-01 NC13039-02
Guideline:	FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). European Commission Guidance Document on Residue Analytical Method, SANCO/825/00 revision 8.1 (16 Nov 2010). The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO (2002) 9
GLP:	Yes, National Good Laboratory Practice Regulations which are in accordance with OECD Principles of good Laboratory Practice (as revised in 1997) with some exceptions*
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	200.3 g a.s./ha, one application at BBCH 49 (trial 1) 195.1 g a.s./ha, one application at BBCH 47-49 (trial 2)
Sampling time points:	62 DAT (trial 1) 58 DAT (trial 2)
Method of analysis:	GRM020.05A, LOQ 0.01 mg/kg, extraction with methanol:water:pH7 phosphate buffer (30:56:14, v/v/v)

GRM020.009A, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction with acetonitrile:water (80:20, v/v)

For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)

Method validation: Procedural recovery data for trinexapac acid using analytical method GRM020.05A

Substrate (control)	Fortification level	Recovery efficiency (%)
Grain	0.01 mg/kg	97
	0.1 mg/kg	108
Mean		103
RSD (%)		-

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Substrate (control)	Fortification level	Recovery efficiency (%)
Straw	0.01 mg/kg	100
	0.01 mg/kg	100
	0.1 mg/kg	91
Mean		97
RSD (%)		5.1

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Substrate (control)	Fortification level	Recovery efficiency (%)
Grain	0.01 mg/kg	89
	0.1 mg/kg	93
Mean		91
RSD (%)		-

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Substrate (control)	Fortification level	Recovery efficiency (%)
Straw	0.05 mg/kg	83
	0.5 mg/kg	84
Mean		83
RSD (%)		-

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Two residue field trials on winter barley were conducted in northern France and Germany during 2013. Trinexapac-ethyl was applied to winter barley as A8587F, a micro-emulsion (ME) formulation containing 250 g a.i. per litre. One application was made at 200 g ai/ha for trinexapac-ethyl at growth stage BBCH 47-49. Samples were collected mechanically using a small size combine harvester, avoiding plot borders. Treated samples were collected for the determination of residues at normal commercial harvest (58 and 62 days after application). Untreated samples were collected on the same day. Additional samples of treated and untreated barley were collected for processing. Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05A to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.

Storage period: At or below -18°C for maximum of 25.4 months (NC13039-01) and 25.5 months (NC13039-02)

Extracts solutions were stored for a maximum of 16 days before analysis.

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records, including soil classification data, GPS coordinates and elevation estimate, were not generated according to GLP principles.

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05A, residues of free trinexapac acid in grain samples were 0.03 mg/kg, and in straw samples were in the range <0.01 mg/kg to 0.01 mg/kg.

Using method GRM020.009A, residues of free and conjugated trinexapac acid in grain samples were in the range 0.05 mg/kg to 0.07 mg/kg, and in straw samples were in the range <0.05 mg/kg to 0.06 mg/kg.

Details of the trials are presented in table B.7.3.1-2.

RMS comments and conclusions

In study 2013/TK0178789, two ~~acceptable~~ residue trials were conducted in accordance with the cGAP for outdoor use; two trials in northern Europe. Specimens from these trials were stored frozen for 25.4 – 25.5 months from sampling to analysis. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. Study was performed in accordance to OECD 509 and suitable for evaluation. Althought residues are considered not covered by storage stability data for both grain and straw and therefore not included in the assessment. ~~Relevant residue data for trinexapac acid (free) in grains of barley, used for MRL calculation, are:~~

~~NEU: 2 x 0.03 mg/kg~~

~~Relevant residue data for trinexapac acid (free) in straw of barley are:~~

~~NEU: <0.01; 0.01 mg/kg~~ None

~~Based on the guidance document (SANCO 7525/VI/95 – rev.10.1), residue data for barley can be extrapolated to oat when applied after the forming of edible part, and to oat, rye and wheat when applied before forming of the edible part.~~

Study 2
Magnitude of trinexapac acid on barley

Reference:	**Brown D. (2016)** Trinexapac-Ethyl - Residue Study on Barley in Northern France and the UK in 2014. Syngenta File No. A8587F_10144 (KCA 6.3.1/02)
Report No.:	36129
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.

GLP:	Yes, OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*. The national GLP requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	200.8 g a.s./ha, one application at BBCH 49 (trial 1) 199 g a.s./ha, one application at BBCH 49 (trial 2) 200 g a.s./ha, one application at BBCH 49 (trial 3) 194 g a.s./ha, one application at BBCH 49 (trial 5) 199 g a.s./ha, one application at BBCH 49 (trial 6) 193 g a.s./ha, one application at BBCH 49 (trial 7)
Sampling time points:	14 (whole plant samples), 50-74 DAT (grain, straw)
Method of analysis:	GRM020.05, LOQ 0.01 mg/kg, extraction with methanol:water:pH7 phosphate buffer. GRM020.009A, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction with acetonitrile:water (80:20, v/v) and acetonitrile:water (50:50, v/v) For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)
Method validation:	Procedural recovery data for trinexapac acid using analytical method GRM020.05

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	83
	0.01 mg/kg	82
	0.1 mg/kg	89
	0.1 mg/kg	85
	0.5 mg/kg	81
	0.5 mg/kg	92
Mean		85
RSD (%)		5
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	79
	0.01 mg/kg	76
	0.1 mg/kg	82
	0.1 mg/kg	75
	0.5 mg/kg	105
	0.5 mg/kg	110
Mean		88
RSD (%)		18
Substrate (control)	Fortification level	Recovery (%)
Straw	0.01 mg/kg	89
	0.01 mg/kg	95
	0.1 mg/kg	100
	0.1 mg/kg	101
Mean		96
RSD (%)		6

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	82
	0.01 mg/kg	75
	0.1 mg/kg	78
	0.1 mg/kg	71
	1.0 mg/kg	80
	1.0 mg/kg	75
Mean		77
RSD (%)		5
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	102
	0.01 mg/kg	99
	0.1 mg/kg	96
	0.1 mg/kg	93
	1.0 mg/kg	77
	1.0 mg/kg	68
Mean		89
RSD (%)		15
Substrate (control)	Fortification level	Recovery (%)
Straw	0.05 mg/kg	103
	0.05 mg/kg	102
	2.0 mg/kg	85
	2.0 mg/kg	81
Mean		93
RSD (%)		12

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Six residue field trials on Barley were successfully conducted in Northern France and the United Kingdom during 2014. Trinexapac-ethyl was applied to Barley as A8587F, a micro-emulsion (ME) formulation containing 250 g of trinexapac-ethyl per litre. One application, applied at growth stage 49 BBCH was made at 200 g ai/ha for trinexapac-ethyl. Treated samples were collected at 14 days after application for whole plant samples and on the first day of commercial harvest for grain and straw samples. Untreated samples were collected at 14 days after application to the treated plot for whole plant samples and on the first day of commercial harvest for grain and straw samples. Samples were taken by hand using a suitably distributive pattern. Crops were sampled using shears. For grain and straw samples the barley was threshed in the field using a minibatt. Any control samples were always taken before treated samples. Samples were analysed for trinexapac-ethyl as the analyte trinexapac acid.

Storage period: Targeting -20°C and no higher than -17.5°C for maximum of 10 months (trinexapac

	acid free) and 19 months (trinexapac acid free and conjugated) Extracts solutions were stored for a maximum of 1 day before analysis for free trinexapac acid and 8 days before analysis for trinexapac acid, free and conjugated.

* - Supplementary weather data were provided by the local meteorological office, crop maintenance records were provided by the grower. These records were not generated according to GLP principles.

Results

Residues of free trinexapac acid in barley whole plant samples taken at 14 DALA (BBCH 61 – 80) were in the range 0.03 to 0.32 mg/kg. Residues in grain samples at normal commercial harvest were in the range <0.01 to 0.36 mg/kg. Residues in straw samples taken at nominal commercial harvest were in the range <0.01 to 0.04 mg/kg.

Using method GRM020.05A no residues of free trinexapac acid were found at or above the limit of quantification (0.01 mg/kg) in any of the untreated samples, with the exception of trial 6, where a residue of 0.09 mg/kg was detected in the whole plant control and residue of 0.06 mg/kg was detected in the control grain sample.

Using method GRM020.009A residues of total free and conjugated trinexapac acid in barley whole plant samples taken at 14 DALA (BBCH 61 – 80) were in the range 0.04 to 0.30 mg/kg. Residues in grain samples at normal commercial harvest were in the range 0.01 to 0.42 mg/kg. Residues in straw samples taken at nominal commercial harvest were in the range <0.05 to 0.07 mg/kg.

No residues of total free and conjugated trinexapac acid were found at or above the limit of quantification (0.01 mg/kg) in any of the untreated samples, with the exception of trial 6, where a residue of 0.07 mg/kg was detected in the whole plant control sample and a residue of 0.11 mg/kg was detected in the grain control sample.

Details of the trials are presented in table B.7.3.1-2.

RMS comments and conclusions

In study 2016/36129, five acceptable residue trials were conducted in accordance with the cGAP for outdoor use in northern Europe. Trial sites in the UK (trial 1, 2 and 3) are more than 10 km apart, and more than 70 km apart in France (trial 5 and 7), therefore RMS considers them to be independent. For trial 6 control samples contaminated (0.09 mg/kg in whole plant; 0.06 mg/kg in grain), therefore results were not used in the assessment. Specimens from these trials were stored frozen for maximum of 10 and 19 months from sampling to analysis for trinexapac acid free and trinexapac acid free and conjugated, respectively (not assigned to exact trial). Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. Residues of trinexapac acid (free and conjugated) are not covered by storage stability data. Study was performed in accordance to OECD 509 and suitable for evaluation. Relevant residue data for trinexapac acid (free) in grains of barley, used for MRL calculation, are:

NEU: 3x<0.01; 0.04; 0.12 mg/kg.

Relevant residue data for trinexapac acid (free and conjugated) in straw of barley used in dietary burden calculation are:

NEU: ~~<0.01; 2x0.01; 0.02; 0.04 mg/kg~~ None

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for barley can be extrapolated to oat when applied after the forming of edible part, and to oat, rye and wheat when applied before forming of the edible part.

Study 3
Magnitude of trinexapac acid on barley

Reference:	**Brown D. (2016a)** Trinexapac-Ethyl - Residue Study on Barley in Belgium in 2014. Syngenta File No. A8587F_10525 (KCA 6.3.1/~~03~~05)
Report No.:	37124
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	203.0 g a.s./ha, one application at BBCH 45-49 (trial 1)
Sampling time points:	14 (whole plant samples), 64 DAT (grain, straw)
Method of analysis:	GRM020.05, LOQ 0.01 mg/kg, extraction with methanol:water: phosphate buffer. GRM020.009A, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction by sequential homogenisation with acetonitrile:water (80:20, v/v) and acetonitrile:water (50:50, v/v)

For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)

Method validation: Procedural recovery data for trinexapac acid using analytical method GRM020.05

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	80
	0.01 mg/kg	78
	1.0 mg/kg	86
	1.0 mg/kg	116
Mean		90
RSD (%)		20
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	95
	0.01 mg/kg	87
	1.0 mg/kg	98
	1.0 mg/kg	103
Mean		96
RSD (%)		7
Substrate (control)	Fortification level	Recovery (%)
Straw	0.01 mg/kg	94
	0.01 mg/kg	81
	4.0 mg/kg	66
	4.0 mg/kg	80
Mean		80
RSD (%)		14

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	92
	0.01 mg/kg	106
	1.0 mg/kg	81
	1.0 mg/kg	82
Mean		90
RSD (%)		13
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	75
	0.01 mg/kg	89
	1.0 mg/kg	75
	1.0 mg/kg	100
Mean		85
RSD (%)		14
Substrate (control)	Fortification level	Recovery (%)
Straw	0.05 mg/kg	108
	0.05 mg/kg	101
	2.0 mg/kg	81
	2.0 mg/kg	72
Mean		90
RSD (%)		19

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: One residue field trials on Barley were successfully conducted in Belgium during 2015. Trinexapac-ethyl was applied to barley as A8587F, a micro-emulsion (ME) formulation containing 250 g of trinexapac-ethyl per litre. One application, applied at

	growth stage 45-49 BBCH was made at 200 g a.i./ha for trinexapac-ethyl. Treated samples were collected at 14 days after application for whole plant samples and on the first day of commercial harvest for grain and straw samples. Untreated samples were collected at 14 days after application to the treated plot for whole plant samples and on the first day of commercial harvest for grain and straw samples. Samples were taken by hand using a suitably distributive pattern. Crops were sampled using shears. For grain and straw samples the barley was threshed in the field using a minibatt. Any control samples were always taken before treated samples. Samples were analysed for free and total (free and conjugated) trinexapac-ethyl as the analyte trinexapac acid.
Storage period:	Targeting -20°C and no higher than -12.9°C for maximum of 8 months

* - Supplementary weather data were provided by the local meteorological office, crop maintenance records were provided by the grower. These records were not generated according to GLP principles.

Results

Residues of free trinexapac acid in barley whole plant samples taken at 14 DALA (BBCH 61) were 0.27 mg/kg. Residues in grain samples at normal commercial harvest were 0.12 mg/kg. Residues in straw samples taken at nominal commercial harvest were 0.04 mg/kg.

Using method GRM020.05A no residues of free trinexapac acid were found at or above the limit of quantification (0.01 mg/kg) in any of the untreated samples.

Using method GRM020.009A residues of total free and conjugated trinexapac acid in barley whole plant samples taken at 14 DALA (BBCH 61) were 0.37 mg/kg. Residues in grain samples at normal commercial harvest were 0.26 mg/kg. Residues in straw samples taken at nominal commercial harvest were 0.09 mg/kg.

No residues of total free and conjugated trinexapac acid were found at or above the limit of quantification (0.01 mg/kg, 0.05 mg/kg for straw) in any of the untreated samples.

Details of the trial are presented in table B.7.3.1-2.

RMS comments and conclusions

In study 2016/37124, one acceptable residue trial was conducted in accordance with the cGAP for outdoor use in northern Europe. Specimens from these trials were stored frozen for maximum of 8 months from sampling to analysis. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. Results are covered by storage stability data. Study was performed in accordance to OECD 509 and suitable for evaluation.

Relevant residue data for trinexapac acid (free) in grains of barley, used for MRL calculation, are:

NEU: 0.12 mg/kg

Relevant residue data for trinexapac acid (free and conjugated) in straw of barley used in dietary burden calculation are:

NEU: ~~0.04~~ 0.09 mg/kg

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for barley can be extrapolated to oat when applied after the forming of edible part, and to oat, rye and wheat when applied before forming of the edible part.

Studies performed in southern Europe

Study 4
Magnitude of trinexapac acid on barley

Reference:	**Andrews G. (2015a)** Trinexapac-Ethyl - Residue Study on Winter Barley in Italy and Spain in 2013. Syngenta File No. A8587F_10132 (KCA 6.3.1/~~0~~403)
Report No.:	TK0178795
Trial No.:	NC13038-01 NC13038-02
Guideline:	FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). European Commission Guidance Document on Residue Analytical Method, SANCO/825/00 revision 8.1 (16 Nov 2010). The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO (2002) 9.
GLP:	Yes, National Good Laboratory Practice Regulations which are in accordance with OECD Principles of good Laboratory Practice (as revised in 1997) with some exceptions*
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	191.8 g a.s./ha, one application at BBCH 37-39 (trial NC13038-01) 208.1 g a.s./ha, one application at BBCH 39 (trial NC13038-02)
Sampling time points:	36 DAT (trial NC13038-01)

68 DAT (trial NC13038-02)

Method of analysis: GRM020.05A, LOQ 0.01 mg/kg, extraction with methanol:water:pH7 phosphate buffer (30:56:14, v/v/v)

GRM020.009A, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction with acetonitrile:water (80:20, v/v), decant supernatant and second extraction with acetonitrile:water (50:50, v/v)

For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)

Method validation: Procedural recovery data for trinexapac acid using analytical method GRM020.05A

Substrate (control)	Fortification level	Recovery
Grain	0.01 mg/kg	89
	0.01 mg/kg	92
	0.1 mg/kg	92
	0.1 mg/kg	100
	0.8 mg/kg	92
	0.8 mg/kg	96
Mean		93
RSD (%)		4.2

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Substrate (control)	Fortification level	Trinexapac
Straw	0.01 mg/kg	84
	0.1 mg/kg	71
	0.1 mg/kg	85
Mean		80
RSD (%)		9.5

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Substrate (control)	Fortification level	Trinexapac
Grain	0.01 mg/kg	77
	0.1 mg/kg	95
	1 mg/kg	73
	1 mg/kg	70
Mean		79
RSD (%)		14.1

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Substrate (control)	Fortification level	Trinexapac
Straw	0.05 mg/kg	86
	0.5 mg/kg	95
Mean		91
RSD (%)		-

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Two residue field trials on winter barley were conducted in Italy and Spain during 2013. Trinexapac-ethyl was applied to winter barley as A8587F, a micro-emulsion (ME) formulation containing 26.5% w/w of trinexapac-ethyl. One application was made at 200 g a.i./ha for trinexapac-ethyl at growth stage BBCH 37-39. In each trial, untreated and treated grain and straw samples were collected at normal commercial harvest. For trial NC13038-01 whole plants were in a mechanical thresher at the facility. In order to avoid sample contamination, the thresher was cleaned with soap and compressed air before using it. Untreated plot was threshed before the treated

	one. For trial NC13038-02 ears were separated manually from straw. Then ears were threshed with a hand thresher in order to obtain grain samples. Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05A to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.
Storage period:	At or below -18°C for maximum of 24.5 months Extract solutions were stored for a maximum of 9 days before analysis.

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records, including soil classification data, GPS coordinates and elevation estimate, were not generated according to GLP principles.

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05A, residues of free trinexapac acid in grain samples were in the range <0.01 mg/kg to 0.47 mg/kg, and in straw samples were in the range <0.01 mg/kg to 0.08 mg/kg.

Using method GRM020.009A, residues of free and conjugated trinexapac acid in grain samples were in the range <0.01 mg/kg to 0.69 mg/kg, and in straw samples were in the range <0.05 mg/kg to 0.26 mg/kg.

Details of the trials are presented in table B.7.3.1-2.

RMS comments and conclusions

In study 2015/TK0178795, two acceptable residue trials were conducted in accordance with the cGAP for outdoor use; two trials in southern Europe. Specimens from these trials were stored frozen for 4 - 24.5 months from sampling to analysis. Grain and straw samples in trial NC13038-01 were analysed after 4.5 months storage for residues of free trinexapac acid. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. RMS considers that results are covered by storage stability data for grain as well as straw sample from NC13038-01 (for free trinexapac acid). All results in straw from trial NC13038-02 and for trinexapac acid free and conjugated from trial NC13038-01 are considered not covered by storage stability data. Study was performed in accordance to OECD 509 and suitable for evaluation. Relevant residue data for trinexapac acid (free) in grains of barley, used for MRL calculation, are:

SEU: <0.01; ~~0.47~~ 0.49 mg/kg

Relevant residue data for trinexapac acid (free and conjugated) in straw of barley used in dietary burden calculation are:

SEU: ~~<0.01; 0.08 mg/kg~~ None

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for barley can be extrapolated to oat when applied after the forming of edible part, and to oat, rye and wheat when applied before forming of the edible part.

Study 5

Magnitude of trinexapac acid on barley

Reference:	**Brown D. (2016b)** Trinexapac-Ethyl - Residue Study on Barley in Southern France, Italy and Spain in 2014. Syngenta File No. A8587F_10135 (KCA 6.3.1/~~0~~504)
Report No.:	36190
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.
Deviations	Due to co- operator error, the trial 6 NCH samples (065, 067, 069 and 071), were lost and were therefore not available for analysis. However the 14 DALA samples (061 and 063) were analysed and reported.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	209 g a.s./ha, one application at BBCH 47 (trial 1) 201 g a.s./ha, one application at BBCH 45 (trial 2) 204 g a.s./ha, one application at BBCH 49 (trial 3) 200 g a.s./ha, one application at BBCH 49 (trial 4) 196 g a.s./ha, one application at BBCH 49 (trial 5) 198 g a.s./ha, one application at BBCH 47 (trial 6)
Sampling time points:	14 DAT, 48-62 DAT
Method of analysis:	GRM020.05, LOQ 0.01 mg/kg, extraction with methanol:water:pH7 phosphate buffer

GRM020.009A, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction sequential homogenisation with acetonitrile:water (80:20, v/v) acetonitrile:water (50:50, v/v)

For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)

Method validation: Procedural recovery data for trinexapac acid using analytical method GRM020.05

Matrix	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	70
	0.01 mg/kg	94
	0.1 mg/kg	62
	0.1 mg/kg	86
	0.5 mg/kg	79
	0.5 mg/kg	79
Mean		79
RSD (%)		14
Matrix	Fortification level	Recovery (%)
Grain	0.01 mg/kg	87
	0.01 mg/kg	85
	0.1 mg/kg	89
	0.1 mg/kg	85
	0.5 mg/kg	78
	0.5 mg/kg	79
Mean		84
RSD (%)		5
Matrix	Fortification level	Recovery (%)
Straw	0.01 mg/kg	79
	0.01 mg/kg	86
	0.1 mg/kg	83
	0.1 mg/kg	98
	0.5 mg/kg	97
	0.5 mg/kg	100
Mean		90
RSD (%)		10

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Matrix	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	100
	0.01 mg/kg	93
	0.1 mg/kg	99
	0.1 mg/kg	92
	1.0 mg/kg	105
	1.0 mg/kg	82
Mean		95
RSD (%)		8
Matrix	Fortification level	Recovery (%)
Grain	0.01 mg/kg	79
	0.01 mg/kg	80
	0.1 mg/kg	87
	0.1 mg/kg	94
	1.0 mg/kg	76
	1.0 mg/kg	72
Mean		81
RSD (%)		10
Matrix	Fortification level	Recovery (%)
Straw	0.05 mg/kg	105
	0.05 mg/kg	101
	2.0 mg/kg	80
	2.0 mg/kg	72
Mean		90
RSD (%)		18

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Six residue field trials on winter barley were conducted in Southern France, Italy and Spain during 2014. Trinexapac-ethyl was applied to winter barley as A8587F, a micro-emulsion (ME) formulation containing 250 g of trinexapac-ethyl per litre. One application was made at 200 g a.i./ha for trinexapac-ethyl at growth stage BBCH 39-49. Treated samples were collected 14 days after application (DAA) for whole plant samples and on the first day of commercial harvest for grain and straw samples. Untreated samples were collected 14 DAA to the treated plot for whole plant samples and on the first day of commercial harvest for grain and straw samples. In each trial, untreated and treated grain and straw samples were collected at normal commercial harvest. Crops were sampled by hands using scissors/shears/sickle. For grain and straw samples the barley was threshed at the South France base using a small plot combine Hege. Any control samples were always taken before treated samples.

Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05 to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.

Storage period: Samples were kept deep frozen targeting -20°C and no higher than -15.4°C, with a maximum of -3°C during transportation for maximum of 21 months

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles.

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05 residues of free trinexapac acid in barley whole plant samples taken at 14 DALA (BBCH 59-61) were in the range 0.02 to 0.49 mg/kg. Residues in grain samples at normal commercial harvest

(BBCH 89) were in the range 0.01 to 0.47 mg/kg. Residues in straw samples taken at nominal commercial harvest (BBCH 89) were in the range <0.01 to 0.32 mg/kg.

Using method GRM020.009A residues of total free and conjugated trinexapac acid in barley whole plant samples taken at 14 DALA (BBCH 59-61) were in the range 0.03 to 0.50 mg/kg. Residues in grain samples at normal commercial harvest (BBCH 89) were in the range 0.02 to 0.90 mg/kg. Residues in straw samples taken at nominal commercial harvest (BBCH 89) were in the range <0.05 to 0.28 mg/kg.

Details of the trials are presented in table B.7.3.1-2.

RMS comments and conclusions

In study 2016/36190, five acceptable residue trials were conducted in accordance with the cGAP for outdoor use; five trials in southern Europe. Trial sites in France (trial 1 and 2) are more than 30 km apart, trial sites in Italy (trial 3 and 4) are more than 15 km apart and more than 20 km apart in Spain (trial 5 and 6), and therefore RMS considers them to be independent. Specimens from these trials were stored frozen for maximum of 10 and 21 months from sampling to analysis for trinexapac acid (free) and trinexapac acid (free and conjugated), respectively. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. RMS considers that results are covered by storage stability data for grain. As the report does not include exact storage time for every sample, residue results for trinexapac acid free and conjugated in straw are considered not covered by storage stability data. Study was performed in accordance to OECD 509 and suitable for evaluation. Relevant residue data for trinexapac acid (free) in grains of barley, used for MRL calculation, are:

SEU: 0.01; 0.03; 0.06; 0.14; 0.47 mg/kg

Relevant residue data for trinexapac acid (free and conjugated) in straw of barley used in dietary burden calculation are:

SEU: ~~2x<0.01; 0.02; 0.13; 0.32 mg/kg~~ None

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for barley can be extrapolated to oat when applied after the forming of edible part, and to oat, rye and wheat when applied before forming of the edible part.

Study 6
Magnitude of trinexapac acid on barley

Reference:	**MacDougall J. (2016)** Trinexapac-Ethyl - Residue Processing Study on Barley in Spain and Italy in 2015. Syngenta File No. A8587F_10526 (KCA 6.3.1 / 06 & KCA 6.5.3 / ~~10~~04)

Report No.:	37194
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals No. 509, Crop Field Trials, adopted 7-Sep-2009. OECD Test Guideline 508 Magnitude of the Pesticide Residues in Processed Commodities. SANCO/825/00 rev.8.1 (16/11/2010) Guidance Document on Pesticide Residue Analytical Methods.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	403.7 g a.s./ha, one application at BBCH 49 (trial 1) 391.6 g a.s./ha, one application at BBCH 49 (trial 2)
Sampling time points:	52 DAT (trial 1), 62 DAT)trial 2)
Method of analysis:	GRM020.005, LOQ 0.01 mg/kg, extraction with methanol:water: phosphate buffer (30:56:14, v/v/v) GRM020.009A, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction sequential homogenisation with acetonitrile:water (80:20, v/v) acetonitrile:water (50:50, v/v) For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)
Method validation:	Procedural recovery data for trinexapac acid using analytical method GRM020.05

Crop	Crop Part	Fortification Level	Fortification Unit	Analyte	Percent Recovery	Mean (%)
Barley	Grain	0.01	mg/kg	Trinexapac-acid	72	96.4 (RSD=12%)
Barley	Grain	0.01	mg/kg	Trinexapac-acid	86	
Barley	Grain	0.01	mg/kg	Trinexapac-acid	103	
Barley	Grain	0.01	mg/kg	Trinexapac-acid	95	
Barley	Grain	0.01	mg/kg	Trinexapac-acid	96	
Barley	Grain	0.01	mg/kg	Trinexapac-acid	93	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	83	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	91	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	107	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	101	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	96	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	96	
Barley	Grain	2	mg/kg	Trinexapac-acid	115	
Barley	Grain	2	mg/kg	Trinexapac-acid	115	
Barley	Straw	0.01	mg/kg	Trinexapac-acid	88	84 (RSD=13%)
Barley	Straw	0.01	mg/kg	Trinexapac-acid	86	
Barley	Straw	0.1	mg/kg	Trinexapac-acid	92	
Barley	Straw	1	mg/kg	Trinexapac-acid	92	
Barley	Straw	1	mg/kg	Trinexapac-acid	82	
Barley	Straw	0.1	mg/kg	Trinexapac-acid	64	

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Crop	Crop Part	Fortification Level	Fortification Unit	Analyte	Percent Recovery	Mean (%)
Barley	Grain	0.01	mg/kg	Trinexapac-acid	64	87 (RSD=11%)
Barley	Grain	0.01	mg/kg	Trinexapac-acid	62	
Barley	Grain	0.01	mg/kg	Trinexapac-acid	80	
Barley	Grain	0.01	mg/kg	Trinexapac-acid	81	
Barley	Grain	0.01	mg/kg	Trinexapac-acid	84	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	67	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	67	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	80	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	80	
Barley	Grain	0.1	mg/kg	Trinexapac-acid	83	
Barley	Grain	2	mg/kg	Trinexapac-acid	86	
Barley	Grain	2	mg/kg	Trinexapac-acid	80	
Barley	Straw	0.05	mg/kg	Trinexapac-acid	59	81 (RSD=18%)
Barley	Straw	0.05	mg/kg	Trinexapac-acid	88	
Barley	Straw	1	mg/kg	Trinexapac-acid	87	
Barley	Straw	1	mg/kg	Trinexapac-acid	91	

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Two residue field trials on field barley were conducted in North Spain and Italy during 2015. Trinexapac-ethyl was applied to field barley as A8587F, a micro-emulsion (ME) formulation containing 250 g trinexapac-ethyl per litre. One application was made at 400 g a.i./ha. Treated and control samples were collected at normal commercial harvest (NCH) for processing and for residue analysis. Samples were shipped frozen to the analytical facility for residue analysis and at ambient temperature to the processing facility. Each field trial generated a treated and an untreated field sample of grain. The untreated and treated grain samples were put through the relevant process. The treated grain for each trial was split into 2 portions (T1 and T2) with both being taken through the procedures. Barley grain was processed into pot barley, pearl barley, flour, bran, brewing malt, malt sprouts, brewers grain (dried), brewer's yeast and beer. Relevant industrial practices and standardised procedures were applied to simulate the common processes used by industry for production of pot barley, pearl barley, flour, bran, brewing malt, malt sprouts, brewers grain (dried), brewer's yeast and beer. Crops were sampled by hand, using a suitably distributive pattern. Grain and straw samples were separated

	using a Combine Harvester/strimmer in the field. Grain and straw samples were separated using a Combine-Hege 125D. Any control samples were always taken before treated samples. Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05 to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.
Storage period:	Samples were kept deep frozen at or below -20°C with a maximum of -3°C during transportation for maximum of 8.5 months.

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles.

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05 residues of free trinexapac -acid measured in field barley grain samples taken at 52-62 days after last application (normal commercial harvest) were 0.32 and 0.11 mg/kg for Trial 1 and 2 respectively.

Residues of free trinexapac acid measured in field barley straw samples taken at 52-62 days after last application (normal commercial harvest) and analysed as per GRM020.05 were 0.09 and 0.06 mg/kg for Trial 1 and 2 respectively.

Using method GRM020.009A residues of total (free and conjugated) trinexapac acid measured in field barley grain samples taken at 52-62 days after last application (normal commercial harvest) and analysed as per GRM020.009A were 0.75 and 0.34 mg/kg for Trial 1 and 2 respectively.

Residues of total (free and conjugated) trinexapac acid measured in field barley straw samples taken at 52-62 days after last application (normal commercial harvest) and analysed as per GRM020.009A were 0.14 and 0.49 mg/kg for Trial 1 and 2 respectively.

Details of the trials are presented in table B.7.3.1-2. Results of the processing are presented in section B.7.5.3.

RMS comments and conclusions

In study 2016/37194, two residue trials using 2N dose rate were conducted in southern Europe. They were considered acceptable (in accordance with the cGAP for outdoor use) after using the proportionality approach. Recalculated results are presented below. Specimens from these trials were stored frozen for maximum of 8.5 months from sampling to analysis. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. Results are covered by storage stability data for both grain and straw. Study was performed in accordance to OECD 509 and suitable for evaluation.

Relevant residue data for trinexapac acid (free) in grains of barley, used for MRL calculation, are:

SEU: 0.06; 0.16 mg/kg

Relevant residue data for trinexapac acid (free) in straw of barley are:

SEU: ~~0.03; 0.05~~ 0.07; 0.25 mg/kg

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for barley can be extrapolated to oat when applied after the forming of edible part, and to oat, rye and wheat when applied before forming of the edible part.

Table B.7.3.1-2: Summary of supervised residue trials with barley in Northern and Southern Europe

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
NEU														
TK0178789 NC13039-01 FRANCE (Europe North) (-)	Winter barley (Etincelle)	1.01 Oct 2012 2. May 2013 3. Jul 2013	Foliar		176 L/ha	200.3 g a.s./ha (-)	15 May 2013 (-)	BBCH 49	Grain	0.03**	0.07**	62	16 Jul 2013	Method: GRM020.05A, GRM020.009A SP (max): 25.4 months (NC13039-01) 25.5 months (NC13039-02)
									Straw	<0.01**	<0.05**	62	16 Jul 2013	
TK0178789 NC13039-02 GERMANY (Europe North) (-)	Winter barley (Hobbit)	1.04 Oct 2012 2. Jun 2013 3. Jul 2013	Foliar		196 L/ha	195.1 g a.s./ha (-)	18 May 2013 (-)	BBCH 47-49	Grain	0.03**	0.05**	58	15 Jul 2013	
									Straw	0.01**	0.06**	58	15 Jul 2013	
36129 Trial 1	Winter barley	1.06 Sep 2013	Foliar		352 L/ha	200.8 g a.s./ha	05 May 2014	BBCH 49	Whole plant	0.03	0.04	14	19 May 2014	Method

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
UNITED KINGDOM (Europe North) (YO17 6QA)	(Cassia)	2. - 3. -				(-)	(-)		Grain	<0.01	0.01	71	15 Jul 2014	GRM020.05, GRM020.009A SP (max): 10 months (free) 19 months (free and conjugated) Trial 6: control samples contaminated (0.09 mg/kg in whole plant; 0.06 mg/kg in grain), results not used in the assessment Trial 1 and 2
									Straw	<0.01	<0.05**	71	15 Jul 2014	
36129 Trial 2 UNITED KINGDOM (Europe North) (YO62 7TD)	Winter barley (Saffron)	1.30 Sep 2013 2. - 3. -	Foliar		350 L/ha	199 g a.s./ha (-)	06 May 2014 (-)	BBCH 49	Whole plant	0.04	0.04	14	20 May 2014	
									Grain	<0.01	0.01	78	23 Jul 2014	
									Straw	0.01	<0.05**	78	23 Jul 2014	
36129 Trial 3 UNITED KINGDOM	Winter barley (Glacier)	1.07 Oct 2013 2. - 3. -	Foliar		350 L/ha	200 g a.s./ha (-)	08 May 2014 (-)	BBCH 49	Whole plant	0.04	0.04	14	22 May 2014	
									Grain	<0.01	0.02	74	21 Jul 2014	

Annex B.7 (AS): Residue data

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
(Europe North) (YO30 2AY)									Straw	0.01	<0.05**	74	21 Jul 2014	conducted ~ 10 km apart, considered as replicates.
36129 Trial 5 FRANCE (Europe North) (60123)	Spring barley (Sebastian)	1.12 Mar 2014 2. - 3. -	Foliar		340 L/ha	194 g a.s./ha (-)	29 May 2014 (-)	BBCH 49	Whole plant	0.20	0.16	14	12 Jun 2014	
									Grain	0.12	0.27 (0.28) §	55	23 Jul 2014	
									Straw	0.02	<0.05**	55	23 Jul 2014	
36129 Trial 6 FRANCE (Europe North) (60440)	Spring barley (Sebastian)	1.14 Mar 2014 2. - 3. -	Foliar		349 L/ha	199 g a.s./ha (-)	29 May 2014 (-)	BBCH 49	Whole plant	0.32	0.30	14	12 Jun 2014	
									Grain	0.36	0.42	50	18 Jul 2014	
									Straw	0.04	0.07**	50	18 Jul 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
36129 Trial 7 FRANCE (Europe North) (62217)	Spring barley (Beatrix)	1.11 Mar 2014 2. - 3. -	Foliar		339 L/ha	193 g a.s./ha (-)	03 Jun 2014 (-)	BBCH 49	Whole plant	0.08	0.10	14	17 Jun 2014	
									Grain	0.04	0.13	52	25 Jul 2014	
									Straw	0.04	0.07**	52	25 Jul 2014	
37124 Trial 1 BELGIUM (Europe North) (YO17 6QA)	Springbarley (Shandy)	1.16 Mar 2015 2. - 3. -	Foliar		356 L/ha	203 g a.s./ha (-)	03 Jun 2015 (-)	BBCH 45-49	Whole plant	0.27	0.37	14	17 Jun 2015	Method: GRM020.05A, GRM020.009A SP (max): 8 months
									Grain	0.12	0.26	64	06 Aug 2015	
									Straw	0.04	0.09	64	06 Aug 2015	
SEU														
TK0178795 NC13038-	Winter barley	1.19 Dec 2012	Foliar		193 L/ha	191.8 g a.s./ha	12 Jun 2013 (-)	BBCH 37-39	Grain	0.47 (0.49) §	0.69 (0.72) §	36	18 Jul 2013	Method: GRM020.05A,

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
01 ITALY (Europe South) (-)	(Margret)	2. 25 Jun to 5 Jul 2013 3. Jul 2013				(-)			Straw	0.08	0.26**	36	18 Jul 2013	GRM020.009A SP (max): 4.7 months grain and straw for trinexapac acid (free) from (NC13038-01) 24.5 months (all others)
TK0178795 NC13038-02 SPAIN (Europe South) (-)	Winter barley (Quench)	1.10 Jan 2013 2. 11 to 23 Jun 2013 3. Jul 2013	Foliar		210 L/ha	208.1 g a.s./ha (-)	21 May 2013 (-)	BBCH 39	Grain	<0.01	<0.01	68	28 Jul 2013	
									Straw	<0.01**	<0.05**	68	28 Jul 2013	
36190 Trial 1 FRANCE (Europe South) (01800)	Winter barley (Augusta)	1.13 Oct 2013 2. - 3. -	Foliar		367 L/ha	209 g a.s./ha (-)	25 Apr 2014 (-)	BBCH 47	Whole plant	0.02	0.03	14	09 May 2014	Method: GRM020.05, GRM020.009A SP (max): 10 months(free)
									Grain	0.01	0.02	59	23 Jun 2014	
									Straw	<0.01	<0.05**	59	23 Jun 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
36190 Trial 2 FRANCE (Europe South) (38790)	Winter barley (Caravan)	1.25 Oct 2013 2. - 3. -	Foliar		353 L/ha	201 g a.s./ha (-)	25 Apr 2014 (-)	BBCH 45	Whole plant	0.05	0.10	14	09 May 2014	21 months (free and conjugated) Trial 3 and 4 conducted ~15 km apart considered as replicates.
									Grain	0.03	0.06	61	25 Jun 2014	
									Straw	0.02	0.07**	61	25 Jun 2014	
36190 Trial 3 ITALY (Europe South) (27010)	Winter barley (Tatoo)	1.01 Oct 2013 2. - 3. -	Foliar		358 L/ha	204 g a.s./ha (-)	05 May 2014 (-)	BBCH 49	Whole plant	0.09	0.12	14	19 May 2014	
									Grain	0.06	0.15	62	06 Jul 2014	
									Straw	0.13	0.18**	62	06 Jul 2014	
36190 Trial 4 ITALY (Europe	Winter barley (Atomo)	1.12 Oct 2013 2. - 3. -	Foliar		351 L/ha	200 g a.s./ha (-)	05 May 2014 (-)	BBCH 49	Whole plant	0.43	0.42	14	19 May 2014	
									Grain	0.47	0.90	62	06 Jul 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
South) (26866)									Straw	0.32	0.28**	62	06 Jul 2014	
36190 Trial 5 SPAIN (Europe South) (25180)	Spring barley (Crystalia)	1.07 Feb 2014 2. - 3. -	Foliar		344 L/ha	196 g a.s./ha (-)	29 Apr 2014 (-)	BBCH 49	Whole plant	0.15	0.19	14	13 May 2014	
									Grain	0.14	0.14	48	16 Jun 2014	
									Straw	<0.01	<0.05**	48	16 Jun 2014	
36190 Trial 6 SPAIN (Europe South) (25242)	Spring barley (Explorer)	1.11 Jan 2014 2. - 3. -	Foliar		347 L/ha	198 g a.s./ha (-)	28 Apr 2014 (-)	BBCH 47	Whole plant	0.49	0.50	14	12 May 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
37194 Trial 1 SPAIN (Europe South) 25180	Spring barley (Scrabble)	1.20 Jan 2015 2. - 3. -	Foliar		203 L/ha	403.7 g a.s./ha (-)	27 Apr 2015 (-)	BBCH 49	Grain	0.32 (0.16)§	0.75 (~~0.38~~ 0.37)§	52	18 Jun 2015	Method: GRM020.005, GRM020.009A SP (max): 8.5 months
									Straw	0.09 (~~0.05~~ 0.04)§	0.14 (0.07)§	52	18 Jun 2015	
37194 Trial 2 Italy (Europe South) 26866	Winter barley (Arda)	1.10 Oct 2014 2. - 3. -	Foliar		197 L/ha	391.6 g a.s./ha (-)	30 Apr 2015 (-)	BBCH 49	Grain	0.11 (0.06)§	0.34 (0.17)§	62	01 Jul 2015	
									Straw	0.06 (0.03)§	0.49 (0.25)§	62	01 Jul 2015	

(a) According to Codex (or other e.g. EU) classification

(b) Only if relevant

(c) Year must be indicated

(d) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)

(*) Indicates sample taken prior to application

(#) Indicates corrected Residue values

(^) PHI calculated using cut date

(+) Indicates calculated Residue value

(§) indicates residue level which changed after scaling to 200 g a.s./ha application rate ~~obtained from processing studies scaled down by a factor of 2~~ (proportionality principle).

Annex B.7 (AS): Residue data

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)***		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			

(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included.

(DBA) Days Before Application

(**) residue levels not supported by storage stability data, not used in the assessment

SP (max): Maximum storage period

For risk assessment and MRL calculation/comparison purposes, individual residue-level values from the trials are summarised in Table B.7.3.1-3. Values in the grey cells are residue levels estimates calculated using trinexapac acid (free) data and conversion factors ~~(refer to Appendix I for details)~~; values may slightly vary, depending on the conversion factor used. Conversion factors were derived from wheat metabolism study. Conversion factors from free trinexapac acid to metabolite SYN 548584 are of 0.44 in grain and 0.55 in straw, to metabolite CGA 300405 are 0.03 in grain and 2.73 in straw, and to metabolite CGA 275537 are 0.07 in grain and 2.31 in straw*. Median conversion factor has been derived from free trinexapac acid to trinexapac acid (free & conjugated~~) as well as for the sum of trinexapac acid (free & conjugated) and OH-CGA179500~~.

*From wheat metabolism study: Residues of free trinexapac acid=0.401 mg/kg in grain and 0.048 mg/kg in straw.

Residues of SYN548584=0.175 mg/kg in grain and 0.026 mg/kg in straw. To convert free trinexapac residues to SYN548584 the free trinexapac value should be multiplied by 0.175/0.401 = 0.436 (0.44 to 2 decimal places)for grain and 0.026/0.048 = 0.545 (0.55 to 2 decimal places).

Residues of CGA300405=0.012 mg/kg in grain and 0.131 mg/kg in straw. To convert free trinexapac residues to CGA300405 the free trinexapac value should be multiplied by 0.012/0.401 = 0.029 (0.03 to 2 decimal places)for grain and 0.131/0.048 = 2.729 (2.73 to 2 decimal places).

Residues of CGA275537=0.03 mg/kg in grain and 0.111 mg/kg in straw. To convert free trinexapac residues to CGA275537 the free trinexapac value should be multiplied by 0.03/0.401 = 0.074 (0.07 to 2 decimal places)for grain and 0.111/0.048 = 2.312 (2.31 to 2 decimal places).

Table B.7.3.1-3: Overview of the currently available residues trials data in barley

Crop	Zone	Individual residue levels (mg trinexapac acid equivalents/kg)					
		CGA179500 (free)	**CGA179500 (free and conjugated)**	**OH-CGA179500[a] (SYN 548584)**	**CGA300405[b]**	**CGA275537[c]**	**~~Sum of CGA179500 (free & conj.) and OH-CGA179500[d]~~**
Barley grain	NEU	~~0.03~~	~~0.07~~	~~0.01~~	~~<0.01~~	~~<0.01 0~~	~~0.08~~
		~~0.03~~	~~0.05~~	~~0.01~~	~~<0.01~~	~~<0.01~~	~~0.06~~
		~~<0.01~~	~~0.01~~	~~<0.01~~	~~<0.01~~	~~<0.01~~	~~0.02~~
		<0.01	0.01	<0.01	<0.01	<0.01	~~0.02~~
		<0.01	0.02	<0.01	<0.01	<0.01	~~0.02~~
		0.12	~~0.27~~ ~~0.28[e]~~0.27	0.05	<0.01	0.01	~~0.32~~

Crop	Zone	Individual residue levels (mg trinexapac acid equivalents/kg)					
		CGA179500 (free)	CGA179500 (free and conjugated)	OH-CGA179500[a] (SYN 548584)	CGA300405[b]	CGA275537[c]	~~Sum of CGA179500 (free & conj.) and OH-CGA179500[d]~~
		0.04	0.13	0.02	<0.01	<0.01	~~0.15~~
		0.12	0.26	0.05	<0.01	0.01	~~0.31~~
STMR		~~0.03~~ 0.04	~~0.06 0.08~~ 0.13	0.01	<0.01	<0.01	~~0.07~~
HR		0.12	~~0.27 0.28~~ 0.27	0.05	<0.01	0.01	~~0.32~~
Median CF		-	~~2.2 2.6~~ 2.17	-	-	-	~~2.46~~
Calculated MRL		~~0.3~~	-	-	-	-	~~-~~
Barley grain	SEU	~~0.47~~0.49[e]	~~0.69~~0.72[e]	0.21	0.01	0.03	~~0.90~~
		<0.01	<0.01	<0.01	<0.01	<0.01	~~0.02~~
		0.01	0.02	<0.01	<0.01	<0.01	~~0.02~~
		0.03	0.06	0.01	<0.01	<0.01	~~0.07~~
		~~0.06~~	~~0.15~~	~~0.03~~	~~<0.01~~	~~<0.01~~	~~0.18~~
		0.47	0.90	0.21	0.01	0.03	~~1.11~~
		0.14	0.14	0.06	<0.01	0.01	~~0.20~~
		0.16[e]	~~0.38~~0.37[e]	0.07	<0.01	0.01	~~0.45~~
		0.06[e]	0.17[e]	0.03	<0.01	<0.01	~~0.2~~
STMR		~~0.06~~ 0.1	~~0.15~~ 0.16	0.03	<0.01	<0.01	~~0.20~~
HR		~~0.47~~0.49	0.90	0.21	0.01	0.03	~~1.11~~
Median CF		-	~~1.9 2.0~~ 1.96	-	-	-	~~2.35~~
Calculated MRL		~~0.9~~ 1.0	-	-	-	-	~~-~~
Barley straw	NEU	~~<0.01~~	~~<0.05~~	~~0.01~~	~~0.03~~	~~0.02~~	~~0.06~~
		~~0.01~~	~~0.06~~	~~0.01~~	~~0.03~~	~~0.02~~	~~0.07~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.02	~~<0.05~~	0.01	0.05	0.05	~~0.06~~
		0.04	~~0.07~~	0.02	0.11	0.09	~~0.09~~
		0.04	0.09	0.02	0.11	0.09	~~0.11~~
STMR		~~0.01~~ 0.02	~~<0.05~~ 0.09	0.01	0.03	0.02	~~0.06~~
HR		0.04	0.09	0.02	0.11	0.09	~~0.11~~
Median CF		-	~~3.44~~ 2.25[f]	-	-	-	~~4.88~~

Crop	Zone	Individual residue levels (mg trinexapac acid equivalents/kg)					
		CGA179500 (free)	**CGA179500 (free and conjugated)**	**OH-CGA179500[a] (SYN 548584)**	**CGA300405[b]**	**CGA275537[c]**	**~~Sum of CGA179500 (free & conj.) and OH-CGA179500[d]~~**
Barley straw	SEU	0.08	~~0.26~~	0.04	0.22	0.19	~~0.30~~
		~~<0.01~~	~~<0.05~~	~~0.01~~	~~0.03~~	~~0.02~~	~~0.06~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.02	~~0.07~~	0.01	0.05	0.05	~~0.08~~
		0.13	~~0.18~~	0.07	0.35	0.30	~~0.25~~
		0.32	~~0.28~~	0.18	0.87	0.74	~~0.46~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		~~0.05~~ 0.04[e]	0.07[e]	0.03	0.14	0.12	~~0.10~~
		0.03[e]	0.25[e]	0.02	0.08	0.07	~~0.27~~
STMR		~~0.03~~ 0.04	~~0.07~~ 0.16	0.02	0.08	0.07	~~0.10~~
HR		0.32	~~0.28~~ 0.25	0.18	0.87	0.74	~~0.46~~
Median CF		-	~~3.75~~ 4.87[f]	-	-	-	~~4.46~~

(a): conversion factor of 0.44 in grain and 0.55 in straw

(b): conversion factor of 0.03 in grain and 2.73 in straw

(c): conversion factor of 0.07 in grain and 2.31 in straw

~~(d): Due to the rounding, the values may differ from the result of the sum of individual values from trinexapac acid (free and conjugated) and OH-CGA179500)~~

(e): residue level which changed after scaling ~~obtained from processing studies scaled down by a factor of 2~~ (proportionality principle) are marked with (e).

(f): Calculated from one/two trial results

B.7.3.2 Wheat

Twenty trials have been conducted in northern (12) and southern (8) Europe on wheat at the following GAP: 1×125 g a.s./ha, with the application being made at BBCH 49. Twelve trials have been conducted in northern Europe because the eight residue trials conducted in 2015 were located around two main geographical points (although these latter were more than 30 km apart). Moreover, the residue levels from the processing study (two trials in southern Europe) conducted at 1×400 g a.s./ha (i.e. 3.2X) were scaled down taking account of the proportionality principle to provide a larger and statistically more robust dataset.

As the use pattern is intended for grain production only, residue data on forage are not required. Details of the trials are summarised below. Representative GAPs on wheat are presented in table B.7.3.2-1.

Table 7.3.2–1: Representative GAPs for A8587F use on wheat

Crop	Outdoor/ Protected	Growth Stage	Maximum Number of Applications	Minimum Application Interval (days)	Maximum		Minimum PHI (days)
					Rate (L product/ha) [kg a.s./ha]	Water (L/ha)	
Winter wheat	Outdoor	BBCH 25-49	1	not relevant	0.5 [0.125 kg a.s./ha]	100-400	not relevant

Cereals crops are treated and harvested according to growth stage and harvested at maturity. Thus a prescribed PHI is not relevant.

Studies performed in northern Europe

Study 1

Magnitude of trinexapac acid on wheat

Reference:	**Brown D. (2016c)** Trinexapac-Ethyl - Residue Study on Wheat in Northern France, and the UK in 2014. Syngenta File No. A8587F_10145 (KCA 6.3.2/01)
Report No.:	36094
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	125.6 g a.s./ha, one application at BBCH 49 (trial 1) 124.3 g a.s./ha, one application at BBCH 49 (trial 2) 125.4 g a.s./ha, one application at BBCH 49 (trial 3) 128.6 g a.s./ha, one application at BBCH 49 (trial 4)

120.6 g a.s./ha, one application at BBCH 49-51 (trial 5)
117.8 g a.s./ha, one application at BBCH 45-49 (trial 6)
118.7 g a.s./ha, one application at BBCH 49 (trial 7)
125.3 g a.s./ha, one application at BBCH 49 (trial 8)

Sampling time points: 14 DAT (whole plant),
63-71 DAT (grain and straw)

Method of analysis: GRM020.05, LOQ 0.01 mg/kg, extraction with methanol:water: phosphate buffer.
GRM020.009A**, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction by sequential homogenisation with acetonitrile:water (80:20, v/v) and acetonitrile:water (50:50, v/v)
For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)

Method validation: Procedural recovery data for trinexapac acid using analytical method GRM020.05

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	84
	0.01 mg/kg	83
	1.0 mg/kg	96
	1.0 mg/kg	68
Mean		83
RSD (%)		14
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	97
	0.01 mg/kg	88
	1.0 mg/kg	116
	1.0 mg/kg	117
Mean		104
RSD (%)		14
Substrate (control)	Fortification level	Recovery (%)
Straw	0.01 mg/kg	77
	0.01 mg/kg	84
	1.0 mg/kg	91
	1.0 mg/kg	94
Mean		86
RSD (%)		9

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	75
	0.01 mg/kg	88
	1.0 mg/kg	78
	1.0 mg/kg	67
Mean		77
RSD (%)		11
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	68
	0.01 mg/kg	116
	0.01 mg/kg	72
	0.01 mg/kg	79
	0.10 mg/kg	83
	0.10 mg/kg	73
	1.0 mg/kg	88
	1.0 mg/kg	75
Mean		82
RSD (%)		19
Substrate (control)	Fortification level	Recovery (%)
Straw	0.05 mg/kg	72
	0.05 mg/kg	89
	2.0 mg/kg	72
	2.0 mg/kg	67
Mean		75
RSD (%)		13

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Eight residue field trials on wheat were conducted in Northern France and the United Kingdom during 2014. Trinexapac-ethyl was applied to wheat as A8587F, a micro-emulsion (ME) formulation containing 250 g of trinexapac-ethyl per litre. One application, (applied at growth stage 45-51 BBCH was made at a target rate of 125 g a.i./ha for trinexapac-ethyl. Treated samples were collected at 14 days after application for whole plant samples and on the first day of commercial harvest for grain and straw samples. Untreated samples were collected at 14 days after application to the treated plot for whole plant samples and on the first day of commercial harvest for grain and straw samples. Crops were sampled by hands using shears. For grain and straw samples the wheat was threshed in the field using a minibatt. Any control samples were always taken before treated samples.

Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05 to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.

Storage period: Samples were kept deep frozen targeting -20°C and no higher than -17.5°C (-2°C in trial 5, but samples remained frozen) with a maximum of -5°C during transportation for maximum of 21 months.

Extract solutions were stored for a maximum of 3 days before analysis.

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles. There was a lack of positive confirmation regarding the freezer storage of some samples after preparation.

**- Protocol states that method GRM020.01A would be used for the analysis of free and conjugated trinexapac acid, after discussions with the Sponsor this was replaced with GRM020.009A, finalised as GRM020.09A

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05 residues of free trinexapac -acid measured in wheat whole plant samples taken at BBCH 61 - 65 were in the range 0.03 to 0.23 mg/kg, residues in wheat grain at BBCH 89 - 92 were in the range 0.05 to 0.37 mg/kg, residues in wheat straw at BBCH 89 - 92 were in the range <0.01 to 0.07 mg/kg.

Using method GRM020.009A residues of total (free and conjugated) trinexapac acid measured in wheat whole plant samples taken at BBCH 61 - 65 were in the range 0.04 to 0.24 mg/kg, residues in wheat grain at BBCH 89 - 92 were in the range 0.04 to 0.36 mg/kg, residues in wheat straw at BBCH 89 - 92 were <0.05 mg/kg.

Details of the trials are presented in table B.7.3.2-2.

RMS comments and conclusions

In study 2016/36094, eight acceptable residue trials were conducted in accordance with the cGAP for outdoor use in northern Europe. The nearest distance between trials in UK is 29 km, and 9 km between trials in the FR, therefore RMS consideres them to be independent. Specimens from these trials were stored frozen for 7-21 months, for maximum of 11 and 21 months from sampling to analysis for trinexapac acid free and trinexapac acid free and conjugated, respectively. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. RMS considers that results are covered by storage stability data for grain. As the report does not include exact storage time for every sample, residue results for trinexapac acid free and conjugated in straw are considered not covered by storage stability data. Study was performed in accordance to OECD 509 and suitable for evaluation. Relevant residue data for trinexapac acid (free) in grains of wheat, used for MRL calculation, are:

NEU: 0.05; 0.08; 0.09; 0.1; ~~0.11~~ 0.12; 0.22; ~~0.24~~ 0.23; ~~0.37~~ 0.39 mg/kg

Relevant residue data for trinexapac acid (free and conjugated) in straw of wheat used in dietary burden calculation are:

NEU: ~~4x<0.01; 2x0.02; 0.03; 0.07 mg/kg~~ None

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for wheat can be extrapolated to rye when applied after the forming of edible part, and to oat, rye and barley when applied before forming of the edible part.

Study 2

Magnitude of trinexapac acid on wheat

Reference:	**Brown D. (2016d)** Trinexapac-Ethyl - Residue Study on Wheat in Poland, Czech Republic, Austria and Germany in 2015. Syngenta File No. A8587F_10527 (KCA 6.3.2/~~02~~03)
Report No.:	37231
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	128.8 g a.s./ha, one application at BBCH 49 (trial 1) 125.9 g a.s./ha, one application at BBCH 49 (trial 2) 133.1 g a.s./ha, one application at BBCH 49 (trial 3) 127.3 g a.s./ha, one application at BBCH 49 (trial 4)
Sampling time points:	57-65 DAT (grain and straw)
Method of analysis:	GRM020.05, LOQ 0.01 mg/kg, extraction with methanol:water: phosphate buffer. GRM020.009A**, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction by sequential homogenisation with acetonitrile:water (80:20, v/v) and acetonitrile:water (50:50, v/v) For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)
Method validation:	Procedural recovery data for trinexapac acid using analytical method GRM020.05

Free Trinexapac Acid

Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	94
	0.01 mg/kg	95
	1.0 mg/kg	89
	1.0 mg/kg	94
Mean		93
RSD (%)		3
Substrate (control)	Fortification level	Recovery (%)
Straw	0.01 mg/kg	78
	0.01 mg/kg	98
	1.0 mg/kg	100
	1.0 mg/kg	100
Mean		94
RSD (%)		11

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Free and Conjugated Trinexapac Acid

Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	60
	0.01 mg/kg	85
	1.0 mg/kg	71
	1.0 mg/kg	77
Mean		73
RSD (%)		14
Substrate (control)	Fortification level	Recovery (%)
Straw	0.05 mg/kg	108
	0.05 mg/kg	110
	2.0 mg/kg	112
	2.0 mg/kg	110
Mean		110
RSD (%)		1

Recoveries in percent (%). The lowest fortification level is at the limit of quantification.

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Four residue field trials on wheat were conducted in Germany, Poland, Austria and the Czech Republic during 2015. Trinexapac-ethyl was applied to wheat as A8587F, a micro-emulsion (ME) formulation containing 250 g of trinexapac-ethyl per litre. One application, (applied at growth stage 49 BBCH was made at a target rate of 125 g a.i./ha for trinexapac-ethyl. Treated samples were collected on the first day of commercial harvest for grain and straw samples. Crops were sampled by hands using shears/knife or using a Wintersteiger plot combine and cleaned with a Pfeuffer Laboratory seed cleaner.

	For grain and straw samples the wheat were separated in the field using a minibatt/plot harvester. Any control samples were always taken before treated samples. Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05 to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.
Storage period:	Samples were kept deep frozen targeting -20°C with a maximum of -5°C during transportation for maximum of 7 months. Extract solutions were stored for a maximum of 1 day before analysis

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles.

**- Protocol states method GRM020.01A would be used for the analysis of free and conjugated trinexapac acid, after discussions with the Sponsor this was replaced with GRM020.009A, finalised as GRM020.09A

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05 residues of free trinexapac -acid in treated wheat grain taken on the first day of commercial harvest were found to be in the range of 0.03 to 0.07 mg/kg. Residues of free trinexapac acid in treated wheat straw taken on the first day of commercial harvest were found to be in the range of <0.01 to 0.01 mg/kg.

Using method GRM020.009A residues of total (free and conjugated) trinexapac acid in treated wheat grain taken on the first day of commercial harvest were found to be in the range of 0.01 to 0.07 mg/kg. Residues of total (free and conjugated) trinexapac acid in treated wheat straw taken on the first day of commercial harvest were found to be <0.05 mg/kg.

Details of the trials are presented in table B.7.3.2-2.

RMS comments and conclusions

In study 2016/37231, four acceptable residue trials were conducted in accordance with the cGAP for outdoor use in northern Europe. Specimens from these trials were stored frozen for maximum of 7 months from sampling to analysis. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in wheat straw for at least 12 months when stored at -18°C. Results are covered by storage stability data. Study was performed in accordance to OECD 509 and suitable for evaluation.

Relevant residue data for trinexapac acid (free) in grains of wheat used for MRL calculation are:

NEU: 0.03; 0.05; 0.06; 0.07 mg/kg

Relevant residue data for trinexapac acid (free and conjugated) in straw of wheat used in dietary burden calculation are:

NEU: ~~2x<0.01; 2x0.01~~ 4x<0.05 mg/kg

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for wheat can be extrapolated to rye when applied after the forming of edible part, and to oat, rye and barley when applied before forming of the edible part.

Studies performed in southern Europe

Study 3
Magnitude of trinexapac acid on wheat

Reference:	**Brown D. (2016e)** Trinexapac-Ethyl - Residue Study on Wheat in Southern France, Italy and Spain in 2014. Syngenta File No. A8587F_10141 (KCA 6.3.2/~~0~~302)
Report No.:	36220
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	124.4 g a.s./ha, one application at BBCH 41 (trial 1) 128.1 g a.s./ha, one application at BBCH 47-49 (trial 2) 127.5 g a.s./ha, one application at BBCH 49 (trial 3) 129.6 g a.s./ha, one application at BBCH 49 (trial 4) 125 g a.s./ha, one application at BBCH 49 (trial 5) 122.3 g a.s./ha, one application at BBCH 43 (trial 6)

125.9 g a.s./ha, one application at BBCH 49 (trial 7)
127.6 g a.s./ha, one application at BBCH 47 (trial 8)

Sampling time points: 14 DAT (whole plant)
62-80 DAT (grain and straw)

Method of analysis: GRM020.05, LOQ 0.01 mg/kg, extraction with methanol:water: phosphate buffer (30:56:14, v/v/v)
GRM020.009A**, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction by sequential homogenisation with acetonitrile:water (80:20, v/v) and acetonitrile:water (50:50, v/v)
For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)

Method validation: Procedural recovery data for trinexapac acid using analytical method GRM020.05

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	96
	0.01 mg/kg	114
	1.0 mg/kg	81
	1.0 mg/kg	97
Mean		97
RSD (%)		14
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	85
	0.01 mg/kg	74
	1.0 mg/kg	69
	1.0 mg/kg	62
Mean		73
RSD (%)		13
Substrate (control)	Fortification level	Recovery (%)
Straw	0.01 mg/kg	74
	0.01 mg/kg	93
	1.0 mg/kg	90
	1.0 mg/kg	62
Mean		80
RSD (%)		18

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Substrate (control)	Fortification level	Recovery (%)
Whole Plant	0.01 mg/kg	102
	0.01 mg/kg	91
	1.0 mg/kg	74
	1.0 mg/kg	68
Mean		84
RSD (%)		19
Substrate (control)	Fortification level	Recovery (%)
Grain	0.01 mg/kg	84
	0.01 mg/kg	73
	0.10 mg/kg	70
	0.10 mg/kg	77
	1.0 mg/kg	81
	1.0 mg/kg	86
Mean		78
RSD (%)		8
Substrate (control)	Fortification level	Recovery (%)
Straw	0.05 mg/kg	73
	0.05 mg/kg	69
	2.0 mg/kg	75
	2.0 mg/kg	69
Mean		71
RSD (%)		4

Recoveries in percent (%). The lowest fortification level is at the limit of quantification

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Eight residue field trials on wheat were conducted in Southern France, Italy and Spain during 2014. Trinexapac-ethyl was applied to wheat as A8587F, a micro-emulsion (ME) formulation containing 250 g of trinexapac-ethyl per litre. One application, (applied at growth stage 39-49 BBCH was made at a target rate of 125 g a.i./ha for trinexapac-ethyl. Treated and untreated samples were collected at 14 days after application for whole plant samples and on the first day of commercial harvest for grain and straw samples. Crops were sampled by hands using shears/scissors. For grain and straw samples the wheat were separated in the field using a hand thresher/ minibatt/plot combine Hege 125. Any control samples were always taken before treated samples. Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05 to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.

Storage period: Samples were kept deep frozen targeting -20°C and no higher than -15.4°C with a maximum of -3°C during transportation for maximum of 21months.

Extract solutions were stored for a maximum of 6 days before analysis.

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles. There was a lack of positive confirmation regarding the freezer storage of some samples after preparation.

**- Protocol states method GRM020.01A would be used for the analysis of free and conjugated trinexapac acid, after discussions with the Sponsor this was replaced with GRM020.009A, finalised as GRM020.09A

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05 residues of free trinexapac acid in wheat whole plant samples taken at BBCH 51 - 65 were in the range 0.05 to 0.20 mg/kg, residues in wheat grain at BBCH 89 were in the range 0.03 to 0.08 mg/kg and residues in wheat straw at BBCH 89 were in the range <0.01 to 0.08 mg/kg.

Using method GRM020.009A residues of total (free and conjugated) trinexapac acid in wheat whole plant samples taken at BBCH 51 - 65 were in the range 0.06 to 0.23 mg/kg, residues in wheat grain at BBCH 89 were in the range 0.03 to 0.12 mg/kg and residues in wheat straw at BBCH 89 were in the range <0.05 to 0.18 mg/kg.

Details of the trials are presented in table B.7.3.2-2.

RMS comments and conclusions

In study 2016/36220, eight acceptable residue trials were conducted in accordance with the cGAP for outdoor use in southern Europe. Specimens from these trials were stored frozen for 8-21 months, for maximum of 11 and 21 months from sampling to analysis for "trinexapac acid free" and "trinexapac acid free and conjugated", respectively. Residues of trinexapac acid (CGA 179500) in cereal grain can be considered as stable for at least 24 months and in straw for at least 12 months when stored at -18°C. RMS considers that results are covered by storage stability data for grain. As the report does not include exact storage time for every sample, residue results for trinexapac acid free and conjugated in straw are considered not covered by storage stability data. Study was performed in accordance to OECD 509 and suitable for evaluation.

Relevant residue data for trinexapac acid (free) in grains of wheat used for MRL calculation are:

SEU: 3x0.03; 2x0.05; 2x0.06; 0.08 mg/kg

Relevant residue data for trinexapac acid (free and conjugated) in straw of wheat used in dietary burden calculation are:

SEU: ~~5x<0.01; 0.01; 0.03; 0.08 mg/kg~~ None

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for wheat can be extrapolated to rye when applied after the forming of edible part, and to oat, rye and barley when applied before forming of the edible part.

Study 4
Magnitude and processing of trinexapac acid on wheat

Reference:	**MacDougall J. (2016a)** Residue Processing Study on Wheat in France and Spain in 2015. Syngenta File No. A8587F_10524 (KCA 6.3.2/04&K-CA 6.5.3/07)
Report No.:	37278
Guideline:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 544/2011 and 545/2011 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals No. 509, Crop Field Trials, adopted 7-Sep-2009. OECD Test Guideline 508 Magnitude of the Pesticide Residues in Processed Commodities. SANCO/825/00 rev.8.1 (16/11/2010) Guidance Document on Pesticide Residue Analytical Methods.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	A8587F
Lot/Batch No:	SMO3A0004
Test concentration:	250 g a.s./L
Test conditions:	400.6 g a.s./ha, one application at BBCH 49 (trial 1) 406.2 g a.s./ha, one application at BBCH 49 (trial 2)
Sampling time points:	69 DAT (trial 1), 66 DAT (trial 2)
Method of analysis:	GRM020.05, LOQ 0.01 mg/kg, extraction with methanol:water: phosphate buffer. GRM020.009A, LOQ 0.01 mg/kg (grains), LOQ 0.05 mg/kg (straw), extraction sequential homogenisation with acetonitrile:water (80:20, v/v) acetonitrile:water (50:50, v/v) For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA 4.1.2/02; KCA.4.1.2/03a and KCA.4.1.2/04a)
Method validation:	Procedural recovery data for trinexapac acid using analytical method GRM020.05

Crop	Crop Part	Fortification Level	Fortification Unit	Analyte	Percent Recovery	Mean (%)
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	81	82 (RSD=10%)
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	89	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	81	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	82	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	68	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	76	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	82	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	92	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	78	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	78	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	73	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	83	
Wheat	Grain	2	mg/kg	Trinexapac-acid	91	
Wheat	Grain	2	mg/kg	Trinexapac-acid	98	
Wheat	Straw	0.01	mg/kg	Trinexapac-acid	87	87 (RSD=4%)
Wheat	Straw	0.01	mg/kg	Trinexapac-acid	82	
Wheat	Straw	0.1	mg/kg	Trinexapac-acid	90	
Wheat	Straw	0.1	mg/kg	Trinexapac-acid	90	

Procedural recovery data for trinexapac acid using analytical method GRM020.009A

Crop	Crop Part	Fortification Level	Fortification Unit	Analyte	Percent Recovery	Mean (%)
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	88	85 (RSD=16%)
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	89	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	81	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	81	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	68	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	77	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	72	
Wheat	Grain	0.01	mg/kg	Trinexapac-acid	68	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	84	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	102	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	109	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	108	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	77	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	85	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	73	
Wheat	Grain	0.1	mg/kg	Trinexapac-acid	72	
Wheat	Grain	3.	mg/kg	Trinexapac-acid	106	
Wheat	Grain	3.	mg/kg	Trinexapac-acid	85	
Wheat	Straw	0.05	mg/kg	Trinexapac-acid	77	80 (RSD=3%)
Wheat	Straw	0.05	mg/kg	Trinexapac-acid	80	
Wheat	Straw	1	mg/kg	Trinexapac-acid	81	
Wheat	Straw	1	mg/kg	Trinexapac-acid	82	

Reference items: Trinexapac acid (CGA179500) Batch No MLA-372/1, purity 99%

Test system: Two residue field trials on field wheat were conducted in France and Spain during 2015. Trinexapac-ethyl was applied to field wheat as A8587F, a micro-emulsion (ME) formulation containing 250 g trinexapac-ethyl per litre. One application was made at 400 g a.i./ha. Treated and control samples were collected at normal commercial harvest (NCH) for processing and for residue analysis. Samples were shipped frozen to the analytical facility for residue analysis and at ambient temperature to the processing facility. Each field trial generated a treated and an untreated field sample of grain. The untreated and treated grain samples were put through the relevant process. The treated grain for each trial was split into 2 portions (A and B) with both being taken through the procedures. Wheat grain was processed into cleaned grain, waste (offal), white flour, total bran, shorts, middlings, wholemeal flour, wholemeal bread, germ, dry gluten, dry starch and gluten feed meal.

	Relevant industrial practices and standardised procedures were applied to simulate the common processes used by industry for production of cleaned grain, waste (offal), white flour, total bran, shorts, middlings, wholemeal flour, wholemeal bread, germ, dry gluten, dry starch and gluten feed meal. Crops were harvested by a combine harvester (62MB11). Any control samples were always taken before treated samples. Samples were analysed for trinexapac acid (CGA179500) using two different analytical methods: GRM020.05 to measure free trinexapac acid and GRM020.009A to measure free and conjugated trinexapac acid.
Storage period:	Samples were kept deep frozen at or below -18°C with a maximum of -14.1°C during transportation for maximum of 8 months.

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles. In addition, for trial 2, soil analysis, GPS references and elevations, photos, wind speed, pressure at application and humidity of the grain.

Results

Residues in the untreated specimens were <LOQ.

Using method GRM020.05 residues of free trinexapac -acid measured in field wheat grain samples taken at 66-69 days after last application (normal commercial harvest) were in the range 0.41 to 1.16 mg/kg.

Residues of free trinexapac -acid measured in field wheat straw samples taken at 66-69 days after last application (normal commercial harvest) and analysed as per GRM020.05 were 0.28 and 0.17 mg/kg for Trial 1 and 2 respectively.

Using method GRM020.009A residues of total (free and conjugated) trinexapac acid measured in field wheat grain samples taken at 66-69 days after last application (normal commercial harvest) were in the range 0.51 to 2.76 mg/kg.

Residues of total (free and conjugated) trinexapac acid measured in field wheat straw samples taken at 66-69 days after last application (normal commercial harvest) and analysed as per GRM020.009A were 0.10 and 0.30 mg/kg for Trial 1 and 2 respectively.

Details of the trials are presented in table B.7.3.2-2. Results of the processing are presented in section B.7.5.3.

RMS comments and conclusions

In study 2016/37278, two residue trials using 3.2N dose rate were conducted in southern Europe. They were considered acceptable (in accordance with the cGAP for outdoor use) after using the proportionality approach. Study was performed in accordance to OECD 509 and suitable for evaluation. Recalculated results are presented below.

Relevant residue data for trinexapac acid (free) in grains of barley used in MRL calculation are:

SEU: 0.15; ~~0.28~~ 0.27 mg/kg

Relevant residue data for trinexapac acid (free and conjugated) in straw of barley used in dietary burden calculation are:

SEU: ~~0.05~~ 0.03; 0.09 mg/kg

Based on the guidance document (SANCO 7525/VI/95 - rev.10.1), residue data for wheat can be extrapolated to rye when applied after the forming of edible part, and to oat, rye and barley when applied before forming of the edible part.

Table B.7.3.2-2: Summary of supervised residue trials with wheat in Northern and Southern Europe

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
NEU														
36094 Trial 1 UNITED KINGDOM (Europe North) (YO25 8JW)	Winter wheat (Cordiale)	1.15 Sep 2013 2. - 3. -	Foliar		352 L/ha	125.6 g a.s./ha (-)	26 May 2014 (-)	BBCH 49	Whole plant	0.04	0.04	14	09 Jun 2014	Method: GRM020.005, GRM020.009A SP (max): 21 months Trial 7 and 8 conducted 9 km apart considered as replicates.
									Grain	0.09	0.06	65	30 Jul 2014	
									Straw	0.02	<0.05**	65	30 Jul 2014	
36094 Trial 2 UNITED KINGDOM (Europe North) (YO17 6RY)	Winter wheat (Revelation)	1.30 Sep 2013 2. - 3. -	Foliar		348 L/ha	124.3 g a.s./ha (-)	30 May 2014 (-)	BBCH 49	Whole plant	0.07	0.13	14	13 Jun 2014	
									Grain	0.22	0.23	69	07 Aug 2014	
									Straw	0.03	<0.05**	69	07 Aug 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
36094 Trial 3 UNITED KINGDOM (Europe North) (YO30 2AY)	Winter wheat (JB Diego)	1.31 Oct 2013 2. - 3. -	Foliar		351 L/ha	125.4 g a.s./ha (-)	03 Jun 2014 (-)	BBCH 49	Whole plant	0.04	0.05	14	17 Jun 2014	
									Grain	0.05	0.04	71	13 Aug 2014	
									Straw	<0.01	<0.05**	71	13 Aug 2014	
36094 Trial 4 UNITED KINGDOM (Europe North) (YO7 2HA)	Winter wheat (Santiago)	1.16 Dec 2013 2. - 3. -	Foliar		360 L/ha	128.6 g a.s./ha (-)	17 Jun 2014 (-)	BBCH 49	Whole plant	0.23 (0.22) §	0.24 (0.23) §	14	01 Jun 2014	
									Grain	0.24 (0.23) §	0.36 (0.35) §	71	27 Aug 2014	
									Straw	0.07	<0.05**	71	27 Aug 2014	
36094 Trial 5	Winter wheat	1.25 Oct 2013	Foliar		338 L/ha	120.6 g a.s./ha	09 May 2014	BBCH 49-51	Whole plant	0.05	0.06	14	23 May 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
FRANCE (Europe North) (60440)	(Apache)	2. - 3. -				(-)	(-)		Grain	0.08	0.06	70	18 Jul 2014	
									Straw	<0.01	<0.05**	70	18 Jul 2014	
36094 Trial 6 FRANCE (Europe North) (80300)	Spring wheat (Lennox)	1.08 Apr 2014 2. - 3. -	Foliar		330 L/ha	117.8 g a.s./ha (-)	16 Jun 2014 (-)	BBCH 45-49	Whole plant	0.03	0.10 (0.11) §	14	30 Jun 2014	
									Grain	0.37 (0.39) §	0.17 (0.18) §	64	19 Aug 2014	
									Straw	0.02	<0.05**	64	19 Aug 2014	
36094 Trial 7 FRANCE (Europe North) (60490)	Winter wheat (Koreli)	1.31 Oct 2013 2. - 3. -	Foliar		333 L/ha	118.7 g a.s./ha (-)	16 May 2014 (-)	BBCH 49	Whole plant	0.10 (0.11) §	0.11 (0.12) §	14	30 May 2014	
									Grain	0.11 (0.12) §	0.10 (0.11) §	68	23 Jul 2014	
									Straw	<0.01	<0.05**	68	23 Jul 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
36094 Trial 8 FRANCE (Europe North) (60113)	Winter wheat (Pakito)	1.23 Oct 2013 2. - 3. -	Foliar		351 L/ha	125.3 g a.s./ha (-)	16 May 2014 (-)	BBCH 49	Whole plant	0.06	0.09	14	30 May 2014	
									Grain	0.10	0.08	63	18 Jul 2014	
									Straw	<0.01	<0.05**	63	18 Jul 2014	
37231 Trial 1 GERMANY (Europe North)	Winter wheat (Cubus)	1.19 Oct 2014 2. - 3. -	Foliar		361 L/ha	128.8 g a.s./ha (-)	28 May 2015 (-)	BBCH 49	Grain	0.07	0.06	57	24 Jul 2015	Method: GRM020.05, GRM020.009A SP (max): 7 months
									Straw	0.01	<0.05	57	24 Jul 2015	
37231 Trial 2 POLAND (Europe North) (47-270)	Winter wheat (Arkadia)	1.31 Oct 2014 2. - 3. -	Foliar		353 L/ha	125.9 g a.s./ha (-)	28 May 2015 (-)	BBCH 49	Grain	0.06	0.06	56	23 Jul 2015	
									Straw	<0.01	<0.05	56	23 Jul 2015	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
37231 Trial 3 AUSTRIA (Europe North) (4063)	Winter wheat (Capo)	1.31 Oct 2014 2. 5-10 Jun 2015- 3. -	Foliar		372 L/ha	133.1 g a.s./ha (-)	18 May 2015 (-)	BBCH 49	Grain	0.05	0.07	66	23 Jul 2015	
									Straw	0.01	<0.05	66	23 Jul 2015	
37231 Trial 4 CZECH REPUBLIC (Europe North) (68724)	Winter wheat (Dagmar)	1.06 Nov 2014 2. - 3. -	Foliar		356 L/ha	127.3 g a.s./ha (-)	19 May 2015 (-)	BBCH 49	Grain	0.03	0.01	65	23 Jul 2015	
									Straw	<0.01	<0.05	65	23 Jul 2015	
SEU														
36220 Trial 1 FRANCE (Europe	Winter wheat (Apache)	1.31 Oct 2013 2. - 3. -	Foliar		348 L/ha	124.2 g a.s./ha (-)	30 Apr 2014 (-)	BBCH 41	Whole plant	0.05	0.06	14	14 May 2014	Method: GRM020.05, GRM020.009A SP (max):
									Grain	0.03	0.04	80	19 Jul 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
South) (01990)									Straw	<0.01	<0.05**	80	19 Jul 2014	21 months
36220 Trial 2 FRANCE (Europe South) (38790)	Winter wheat (Solhio)	1.18 Oct 2013 2. - 3. -	Foliar		359 L/ha	128.1 g a.s./ha (-)	07 May 2014 (-)	BBCH 47-49	Whole plant	0.14	0.16	14	21 May 2014	
									Grain	0.03	0.08	80	26 Jul 2014	
									Straw	<0.01	<0.05**	80	26 Jul 2014	
36220 Trial 3 ITALY (Europe South) (26813)	Winter wheat (Bologna)	1.10 Nov 2013 2. - 3. -	Foliar		357 L/ha	127.5 g a.s./ha (-)	05 May 2014 (-)	BBCH 49	Whole plant	0.10	0.11	14	19 May 2014	
									Grain	0.05	0.08	63	07 Jul 2014	
									Straw	0.03	<0.05**	63	07 Jul 2014	
36220 Trial 4	Winter wheat	1.16 Oct 2013	Foliar		363 L/ha	129.6 g a.s./ha	06 May 2014	BBCH 49	Whole plant	0.20 (0.19) §	0.23 (0.22) §	14	20 May 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
ITALY (Europe South) (27050)	(Bologna)	2. - 3. -				(-)	(-)		Grain	0.06	0.12	62	07 Jul 2014	
									Straw	0.08	0.18**	62	07 Jul 2014	
36220 Trial 5 SPAIN (Europe South) (25670)	Winter wheat (Mecano)	1.20 Nov 2013 2. - 3. -	Foliar		350 L/ha	125 g a.s./ha (-)	25 Apr 2014 (-)	BBCH 49	Whole plant	0.06	0.07	14	09 May 2014	
									Grain	0.03	0.03	67	01 Jul 2014	
									Straw	<0.01	<0.05**	67	01 Jul 2014	
36220 Trial 6 SPAIN (Europe South) (252806)	Winter wheat (Soissons)	1.04 Nov 2013 2. - 3. -	Foliar		343 L/ha	122.3 g a.s./ha (-)	25 Apr 2014 (-)	BBCH 43	Whole plant	0.11	0.13	14	09 May 2014	
									Grain	0.06	0.11	68	02 Jul 2014	
									Straw	<0.01	0.05**	68	02 Jul 2014	

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
36220 Trial 7 SPAIN (Europe South) (29540)	Winter wheat (Antequera)	1.10 Dec 2013 2. - 3. -	Foliar		353 L/ha	125.9 g a.s./ha (-)	28 Apr 2014 (-)	BBCH 49	Whole plant	0.15	0.17	14	12 May 2014	
									Grain	0.08	0.09	64	01 Jul 2014	
									Straw	0.01	<0.05**	64	01 Jul 2014	
36220 Trial 8 SPAIN (Europe South) (29313)	Winter wheat (Garcia)	1.11 Nov 2013 2. - 3. -	Foliar		357 L/ha	127.6 g a.s./ha (-)	28 Apr 2014 (-)	BBCH 47	Whole plant	0.14	0.18	14	12 May 2014	
									Grain	0.05	0.08	64	01 Jul 2014	
									Straw	<0.01	<0.05**	64	01 Jul 2014	
37278 Trial 1	Winter wheat	1.01 Nov 2014	Foliar		227 L/ha	400.6 g a.s./ha	29 Apr 2015 (-)	BBCH 49	Grain	0.49 (0.15)§	0.62 (0.19)§	69	07 Jul 2015	Method: GRM020.05,

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			
FRANCE (Europe South) (31340)	(Ascott)	2. 13 May 2015 3. 05 Jul 2015-				(-)			Straw	0.28 (0.09)§	0.10 (0.03)§	69	07 Jul 2015	GRM020.009A SP (max): 8 months
37278 Trial 2 Spain (Europe South) (06250)	Winter wheat (Exotic)	1.03 Nov 2014 2. 05 May 2015 3. 22 Jun 2015	Foliar		254 L/ha	406.2 g a.s./ha (-)	17 Apr 2015 (-)	BBCH 49	Grain	0.88 (~~0.28~~ 0.27)§	1.40 (~~0.44~~ 0.43)§	66	22 Jun 2015	
									Straw	0.17 (0.05)§	0.30 (0.09)§	66	22 Jun 2015	

(a) According to Codex (or other e.g. EU) classification

(b) Only if relevant

(c) Year must be indicated

(d) Minimum number of days after last application (Label pre-harvest interval, PHI, underline)

(*) Indicates sample taken prior to application

(#) Indicates corrected Residue values

(^) PHI calculated using cut date

(+) Indicates calculated Residue value

(§) indicates residue level which changed after scaling to 125 g a.s./ha application rate ~~obtained from processing studies scaled down by a factor of 3.2~~ (proportionality principle).

(**) residue levels not supported by storage stability data, not used in the assessment

Annex B.7 (AS): Residue data

Report No. Trial No. Location (Region) (Postcode)	Commodity/ Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of Treatment	Application rate per treatment			Date of treatment(s) or no of treatment(s) and last date Application Interval (days) (c)	Growth Stage at Treatment	Portion Analysed	Residue found (Uncorrected) (mg/kg)		PHI (d)	Sample Date (Cut Date) (d)	Trial Details (e)
				kg a.s./hl	Water	Rate (Additive Type, Rate)				Trinexapac acid, free	Trinexapac acid, free and conjugated			

(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included.

(DBA) Days Before Application

SP (max): Maximum storage period

For risk assessment and MRL calculation/comparison purposes, individual residue-level values from the trials are summarised in Table B.7.3.2-3. Values in the grey cells are residue levels estimates calculated using trinexapac acid data and conversion factors ~~(refer to Appendix I for details)~~; values may slightly vary, depending on the conversion factor used. Conversion factors were derived from wheat metabolism study. Conversion factors from free trinexapac acid to metabolite SYN 548584 are of 0.44 in grain and 0.55 in straw, to metabolite CGA 300405 are 0.03 in grain and 2.73 in straw, and to metabolite CGA 275537 are 0.07 in grain and 2.31 in straw*. Median conversion factors have been derived from free trinexapac acid to trinexapac acid (free & conjugated) ~~as well as for the sum of trinexapac acid (free & conjugated) and OH-CGA179500~~.

*From wheat metabolism study: Residues of free trinexapac acid=0.401 mg/kg in grain and 0.048 mg/kg in straw.

Residues of SYN548584=0.175 mg/kg in grain and 0.026 mg/kg in straw. To convert free trinexapac residues to SYN548584 the free trinexapac value should be multiplied by 0.175/0.401 = 0.436 (0.44 to 2 decimal places) for grain and 0.026/0.048 = 0.545 (0.55 to 2 decimal places).

Residues of CGA300405=0.012 mg/kg in grain and 0.131 mg/kg in straw. To convert free trinexapac residues to CGA300405 the free trinexapac value should be multiplied by 0.012/0.401 = 0.029 (0.03 to 2 decimal places) for grain and 0.131/0.048 = 2.729 (2.73 to 2 decimal places).

Residues of CGA275537=0.03 mg/kg in grain and 0.111 mg/kg in straw. To convert free trinexapac residues to CGA275537 the free trinexapac value should be multiplied by 0.03/0.401 = 0.074 (0.07 to 2 decimal places) for grain and 0.111/0.048 = 2.312 (2.31 to 2 decimal places).

Table B.7.3.2-3: Overview of the currently available residues trials data in wheat

Crop	Zone	Individual residue levels (mg trinexapac acid equivalents/kg)					
		CGA179500 (free)	**CGA179500 (free and conjugated)**	**OH-CGA179500[a] (SYN 548584)**	**CGA300405[b]**	**CGA275537[c]**	**~~Sum of CGA179500 (free & conj.) and OH-CGA179500[d]~~**
Wheat grain	NEU	0.09	0.06	0.04	<0.01	0.01	~~0.10~~
		0.22	0.23	0.10	0.01	0.02	~~0.33~~
		0.05	0.04	0.02	<0.01	<0.01	~~0.06~~
		~~0.24~~ 0.23[e]	~~0.36~~ 0.35[e]	~~0.11~~ 0.10	0.01	0.02	~~0.47~~
		0.08	0.06	0.04	<0.01	0.01	~~0.10~~
		~~0.37~~ 0.39[e]	~~0.17~~ 0.18[e]	~~0.16~~ 0.17	0.01	0.03	~~0.33~~
		~~0.11~~ 0.12[e]	~~0.10~~ 0.11[e]	0.05	<0.01	0.01	~~0.15~~

Crop	Zone	Individual residue levels (mg trinexapac acid equivalents/kg)					
		CGA179500 (free)	CGA179500 (free and conjugated)	OH-CGA179500[a] (SYN 548584)	CGA300405[b]	CGA275537[c]	~~Sum of CGA179500 (free & conj.) and OH-CGA179500[d]~~
		~~0.10~~	~~0.08~~	~~0.04~~	~~<0.01~~	~~0.01~~	~~0.12~~
		0.07	0.06	0.03	<0.01	<0.01	~~0.09~~
		0.06	0.06	0.03	<0.01	<0.01	~~0.09~~
		0.05	0.07	0.02	<0.01	<0.01	~~0.09~~
		0.03	0.01	0.01	<0.01	<0.01	~~0.02~~
STMR		~~0.09~~ 0.08	~~0.07~~ 0.06	0.04	<0.01	0.01	~~0.10~~
HR		~~0.37~~ 0.39	~~0.36~~ 0.35	~~0.16~~ 0.17	0.01	0.03	~~0.47~~
Median CF		-	~~0.88~~ 0.86	-	-	-	~~1.31~~
Calculated MRL		0.6	-	-	-	-	~~-~~
Wheat grain	SEU	0.03	0.04	0.01	<0.01	<0.01	~~0.05~~
		0.03	0.08	0.01	<0.01	<0.01	~~0.09~~
		0.05	0.08	0.02	<0.01	<0.01	~~0.10~~
		0.06	0.12	0.03	<0.01	<0.01	~~0.15~~
		0.03	0.03	0.01	<0.01	<0.01	~~0.04~~
		0.06	0.11	0.03	<0.01	<0.01	~~0.14~~
		0.08	0.09	0.04	<0.01	0.01	~~0.13~~
		0.05	0.08	0.02	<0.01	<0.01	~~0.10~~
		0.15[e]	0.19[e]	0.07	<0.01	0.01	~~0.26~~
		~~0.28~~ 0.27[e]	~~0.44~~ 0.43[e]	0.12	0.01	0.02	~~0.56~~
STMR		0.06	0.09	0.03	<0.01	<0.01	~~0.12~~
HR		~~0.28~~ 0.27	~~0.44~~ 0.43	0.12	0.01	0.02	~~0.56~~
Median CF		-	1.60	-	-	-	~~2.02~~
Calculated MRL		0.4	-	-	-	-	~~-~~
Wheat straw	NEU	0.02	~~<0.05~~	0.01	0.05	0.05	~~0.06~~
		0.03	~~<0.05~~	0.02	0.08	0.07	~~0.07~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.07	~~<0.05~~	0.04	0.19	0.16	~~0.09~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.02	~~<0.05~~	0.01	0.05	0.05	~~0.06~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~

Crop	Zone	Individual residue levels (mg trinexapac acid equivalents/kg)					
		CGA179500 (free)	**CGA179500 (free and conjugated)**	**OH-CGA179500[a] (SYN 548584)**	**CGA300405[b]**	**CGA275537[c]**	**~~Sum of CGA179500 (free & conj.) and OH-CGA179500[d]~~**
		0.01	<0.05	0.01	0.03	0.02	~~0.06~~
		<0.01	<0.05	0.01	0.03	0.02	~~0.06~~
		0.01	<0.05	0.01	0.03	0.02	~~0.06~~
		<0.01	<0.05	0.01	0.03	0.02	~~0.06~~
STMR		0.01	<0.05	0.01	0.03	0.02	~~0.06~~
HR		0.07	<0.05	0.04	0.19	0.16	~~0.09~~
Median CF		-	~~3.95~~ 5	-	-	-	~~4.80~~
Wheat straw	SEU	<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.03	~~<0.05~~	0.02	0.08	0.07	~~0.07~~
		0.08	~~0.18~~	0.04	0.22	0.18	~~0.22~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		<0.01	~~0.05~~	0.01	0.03	0.02	~~0.06~~
		0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		<0.01	~~<0.05~~	0.01	0.03	0.02	~~0.06~~
		0.09[e]	0.03[e]	0.05	0.25	0.21	~~0.08~~
		0.05[e]	0.09[e]	0.03	0.14	0.12	~~0.12~~
STMR		<0.01	~~<0.05~~ 0.06[f]	0.01	0.03	0.02	~~0.06~~
HR		0.09	~~0.18~~ 0.09[f]	0.05	0.25	0.21	~~0.22~~
Median CF		-	~~3.61~~ 1.07[f]	-	-	-	~~4.44~~

(a): conversion factor of 0.44 in grain and 0.55 in straw

(b): conversion factor of 0.03 in grain and 2.73 in straw

(c): conversion factor of 0.07 in grain and 2.31 in straw

(d): due to the rounding, the values may differ from the result of the sum of individual values from trinexapac acid (free and conjugated) and OH-CGA179500~~)~~

(e): residue levels which changed after scaling ~~obtained from processing studies and scaled down by a factor of 3.2~~ (proportionality principle) are marked with (e).

(f); Calculated from two values.

B.7.3.2-4: **Overview of the available residues trials data**

Crop	Region/ Indoor (a)	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs (b)	Recommendations/comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
Representative uses						
Monitoring residue definition: ~~trinexapac acid~~ ~~Sum of~~ trinexapac ~~acid~~ and its salts, expressed as trinexapac ~~acid~~ (cereal/grass) **Risk assessment residue definition:** **~~sum of~~ trinexapac, ~~acid (~~free and conjugated~~)~~ ~~and OH-trinexapac acid, expressed as trinexapac acid~~ (cereal grain)(provisional);** **Trinexapac, free and conjugated plus CGA 300405 (cereal fodder items/grass) provisional (expressed as trinexapac or separate, pending its toxicological relevance)**						
Barley grain	NEU	Mo: ~~3~~ 2x<0.01; ~~2x0.03~~; 0.04; 2x0.12; RA: ~~3x0.02; 0.06; 0.08; 0.15; 0.31; 0.32~~ 2x0.01; 0.02; 0.13; 0.26; 0.27; ~~Scaled:~~ ~~Mo: 3x<0.01; 0.04; 2x0.12;~~ RA: 2x0.01; 0.02; 0.13; 0.26; ~~0.28~~ 0.27.	A total of ~~86~~ 5 GAP compliant acceptable trials ~~were conducted in northern EU~~ (two trials giving residues of 0.03 mg/kg not sufficiently covered by storage stability data were excluded from the calculations) ~~MRL_{OECD}: 0.23/0.3 0.27/0.3~~ Number not sufficient to derive a MRL proposal.	~~0.3*~~	~~0.32~~0.28 (~~HR_{Mo}: 0.12~~)	~~0.07~~0.08 (~~$STMR_{Mo}$: 0.03~~)
	SEU	~~Mo: <0.01; 0.01; 0.03; 0.06; 0.06 0.11; 0.14; 0.16 0.32; 0.47; 0.47.~~ ~~RA: 2x0.02; 0.07; 0.18; 2x0.20; 0.45; 0.90; 1.11. <0.01; 0.02; 0.06; 0.14; 0.15; 0.17 0.34; 0.38; 0.69; 0.75; 0.90~~ ~~Scaled:~~ Mo: <0.01; 0.01; 0.03; 0.06; ~~0.06~~; 0.14; 0.16; 0.47; 0.49. RA: <0.01; 0.02; 0.06; 0.14; ~~0.15~~; 0.17; 0.37; 0.72; 0.90	A total of ~~9~~ 6 GAP compliant plus 2 overdosed acceptable trials were conducted in southern EU. ~~2 residue levels obtained from processing studies (2N rate), these results are scaled by a factor of 2 (proportionality principle) and underlined in this table.~~ Complete dataset adjusted to 1N application rate. Scaled values are double-underlinded MRL_{OECD}: ~~0.9/0.9~~ ~~0.92/1.0~~ 0.51 (unrounded)	~~0.9~~1.0	~~1.11~~0.90 (HR_{Mo}: ~~0.47~~0.49)	~~0.20~~0.15 0.16 ($STMR_{Mo}$: ~~0.06~~ 0.10)
	~~NEU/SEU~~	~~Scaled:~~ ~~Mo: 4x<0.01; 0.01; 0.03; 0.04; 2x0.06; 2x0.12; 0.14; 0.16;~~	~~Combined datasets as being similar (Mann-Whitney U-test).~~	~~0.8~~	~~0.90~~ (~~HR_{Mo}:~~	~~0.14~~ (~~$STMR_{Mo}$:~~

Crop	Region/ Indoor (a)	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs (b)	Recommendations/comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
		~~0.47; 0.49.~~ ~~RA: <0.01; 2x0.01; 2x0.02; 0.06; 0.13; 0.14; 0.15; 0.17; 0.26; 0.28; 0.37; 0.72; 0.90.~~	~~MRL_{OECD}: 0.74/0.8~~ ~~Although values 0.47 and 0.49 mg/kg might be an outliers. MRL proposal without these values~~ ~~MRL_{OECD}: 0.28/0.3~~		~~0.49)~~	~~0.06)~~
Wheat grain	NEU	Mo: 0.03; 2x0.05; 0.06; 0.07; 0.08; 0.09; ~~0.10~~; 0.11; 0.22; 0.24; 0.37 RA: ~~0.02; 0.06; 3x0.09; 2x0.10; 0.12; 0.15; 2x0.33; 0.47~~ 0.01; 0.04; 4x0.06; 0.07; ~~0.08~~; 0.10; 0.17; 0.23; 0.36; ~~Scaled:~~ ~~Mo: 0.03; 2x0.05; 0.06; 0.07; 0.08; 0.09; 0.10; 0.12; 0.22; 0.23; 0.39~~ ~~RA: 0.01; 0.04; 4x0.06; 0.07; 0.08; 0.11; 0.18; 0.23; 0.35;~~	A total of ~~12~~ 11 Gap compliant acceptable trials ~~were conducted in northern EU~~. MRL_{OECD}: ~~0.53/0.6~~ ~~0.54/0.6~~ 0.53 (unrounded)	0.6	~~0.47~~ 0.35 0.36 (HR_{Mo}: 0.37 ~~0.39~~)	~~0.10~~ ~~0.07~~ 0.06 ($STMR_{Mo}$: ~~0.09~~ 0.08)
	SEU	~~Mo: 3x0.03; 2x0.05; 2x0.06; 0.08; 0.15 0.49; 0.28 0.88~~ ~~RA: 0.04; 0.05; 0.09; 2x0.10; 0.13; 0.14; 0.15; 0.26; 0.56~~ 0.03; 0.04; 3x0.08; 0.09; 0.11; 0.12; 0.62. 1.40; ~~Scaled:~~ Mo: 3x0.03; 2x0.05; 2x0.06; 0.08; 0.15; 0.27; RA: 0.03; 0.04; 3x0.08; 0.09; 0.11; 0.12; 0.19; 0.43;	A total of ~~10~~ 8 GAP compliant plus 2 overdosed acceptable trials ~~were conducted in southern EU~~. ~~2 residue levels obtained from processing studies (3.2N rate), these results are scaled by a factor of 3.2 (proportionality principle) and underlined in this table.~~ Complete dataset adjusted to 1N application rate. Scaled values are double-underlinded MRL_{OECD}: ~~0.39/0.4~~ ~~0.38/0.4~~ 0.39 (unrounded)	0.4	~~0.56~~ 0.43 (HR_{Mo}: ~~0.28~~ 0.27)	~~0.12~~ 0.09 ($STMR_{Mo}$: 0.06)
	NEU/SEU	~~Scaled:~~ Mo: 4x0.03; 4x0.05; 3x0.06; 0.07; 2x0.08; 0.09; ~~0.10~~; 0.12; 0.15; 0.22; 0.23; 0.27; 0.39; RA: 0.01; 0.03; 2x0.04; 4x0.06; 0.07; 4x0.08; 0.09; ~~2x~~0.11; 0.12; 0.18; 0.19;0.23; 0.35; 0.43;	Combined datasets ~~as being similar~~ (~~Mann-Whitney~~ U-test, 5%). Complete NEU and SEU datasets adjusted to 1N application rate. Scaled values are double-underlined.	0.5	0.43 (HR_{Mo}: 0.39)	0.08 ($STMR_{Mo}$: 0.07)

Crop	Region/ Indoor (a)	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs (b)	Recommendations/comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
			MRL_{OECD}: ~~0.48/0.5~~ 0.49 (unrounded) ~~Although~~ value 0.39 mg/kg detected as potential ~~might be an~~ outlier. ~~MRL proposal without this value~~ ~~MRL_{OECD}: 0.37/0.4~~			
Barley straw	NEU	Mo: ~~2x~~<0.01; ~~3~~2x~~<~~0.01; 0.02; 2x0.04 RA: ~~5x0.06; 0.07;~~ ~~0.09; 0.11~~ Trinexapac (free & conjugated): 3x<0.05; 0.07; 0.09 CGA300405: not analysed for	~~Scaling didn't change any results.~~ ~~$STMR_{RA}$ was calculated from one value.~~ Number of trials not suffient to derive input values for the dietary burden calculation. Values in straw for which storage stability was not demonstrated are underlined	No MRL calculation for feed items. -	~~0.11~~ ~~0.09~~ (~~HR_{Mo}: 0.04~~)	~~0.06~~ ~~0.09~~ (~~$STMR_{Mo}$: 0.01~~ ~~0.02~~)
	SEU	~~Mo:~~ ~~3x2x<0.01; 0.02~~; ~~0.03~~ 0.06; ~~0.05~~ ~~0.09~~; ~~0.08~~; ~~0.13; 0.32~~ ~~RA: 3x0.06; 0.08; 0.10; 0.25; 0.27; 0.30; 0.46~~ ~~0.14; 0.49~~ ~~Scaled:~~ Mo: 2x<0.01; 0.02; 0.03; 0.04; ~~0.13~~; 0.32 RA: ~~0.07; 0.25~~ Trinexapac (free & conjugated): 3x<0.05; 0.07; 0.07; 0.25; 0.26; 0.28 CGA300405: not analysed for	~~2 residue levels obtained from processing studies (2N rate), these results are scaled by a factor of 2 (proportionality principle) and underlined in this table.~~ ~~$STMR_{RA}$ was calculated from two values~~ Number of trials not suffient to derive input values for the dietary burden calculation. Values in straw for which storage stability was not demonstrated are underlined. Complete dataset adjusted to 1N application rate. Scaled values are double-underlinded		~~0.46~~ ~~0.25~~ (~~HR_{Mo}: 0.32~~)	~~0.10~~ ~~0.16~~ (~~$STMR_{Mo}$: 0.03~~)
	~~NEU/SEU~~	~~Mo: 3x<0.01; 2x0.01; 2x0.02; 2x0.04; 0.06; 0.09; 0.08; 0.13; 0.32~~ ~~RA: 0.09; 0.14; 0.49~~ ~~Scaled:~~	~~Combined datasets as being similar (Mann-Whitney U-test).~~ ~~Value of 0.32 mg/kg might be an outlier.~~		~~0.25~~ (~~HR_{Mo}: 0.32~~)	~~0.09~~ (~~$STMR_{Mo}$: 0.03~~)

Crop	Region/ Indoor (a)	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs (b)	Recommendations/comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
		~~Mo: 3x<0.01; 2x0.01; 2x0.02; 0.03; 3x0.04; 0.08; 0.13; 0.32~~ ~~RA: 0.07; 0.09; 0.25~~				
Wheat straw	NEU	Mo: ~~6~~ 5x<0.01; 2x0.01; 2x0.02; 0.03; 0.07 RA: ~~10x0.06; 0.07; 0.09~~ ~~4x<0.05~~ trinexapac (free & conj): 4 x <0.05; 7 x <0.05 CGA300405: not analysed for	~~Scaling didn't change any results.~~ ~~$STMR_{RA}$ was calculated from four values.~~ STMR/HR tentative only. Calculated only for trinexapac (free & conjugated) and including also residue levels from trials not fully covered by demonstrated storage stability (underlined)	No MRL calculation for feed items	~~0.09~~ <0.05 ~~(HR_{Mo}: 0.07)~~	~~0.06~~ <0.05 ~~($STMR_{Mo}$: 0.01)~~
	SEU	~~Mo: 5x<0.01; 0.01; 0.03~~; ~~0.05~~; ~~0.08~~; ~~0.09~~ ~~0.17; 0.28~~ ~~RA: 6x0.06; 0.07; 0.08; 0.12; 0.22~~ ~~0.10; 0.30~~. ~~Scaled:~~ Mo: 5x<0.01; 0.01; 0.03; 0.05; 0.08; 0.09 RA: ~~0.03; 0.09~~ trinexapac (free & conj):6 x <0.05; 0.05; 0.03; 0.09; 0.17 CGA300405: not analysed for	~~2 residue levels obtained from processing studies (3.2N rate), these results are scaled by a factor of 3.2 (proportionality principle) and underlined in this table.~~ ~~$STMR_{RA}$ was calculated from two values.~~ Adjusted to 1N application rate. Scaled values are double-underlinded STMR/HR tentative. Calculated only for trinexapac (free & conjugated) and including also residue levels from trials not fully covered by demonstrated storage stability (underlined)		~~0.22~~ 0.09 ~~(HR_{Mo}: 0.09)~~	0.06 ~~($STMR_{Mo}$: 0.01)~~
	NEU/SEU	~~Mo: 11x<0.01; 3x0.01; 2x0.02; 2x0.03; 0.07; 0.08; 0.17; 0.28~~ ~~RA: 4x<0.05; 0.10; 0.30~~ ~~Scaled:~~ ~~Mo: 11x<0.01; 3x0.01; 2x0.02; 2x0.03; 0.05; 0.07; 0.08; 0.09~~ ~~RA: 4x<0.05; 0.03; 0.09~~ RA: Trinexapac (free & conj): 4 x <0.05; 13 x <0.05; 0.05; 0.03; 0.09; 0.17	Combined datasets ~~as being similar~~ (~~Mann-Whitney~~ U-test, 5%) ~~according to Mo values~~. ~~No test available for RA values (since n< 3)~~ Adjusted to 1N application rate. Scaled values are double-underlinded. STMR/HR tentative. Calculated only for trinexapac (free & conjugated) and including also residue levels from trials not fully covered by demonstrated storage stability (underlined)		~~0.09~~ 0.17 ~~(HR_{Mo}: 0.09)~~	0.05 ~~($STMR_{Mo}$: 0.01)~~
MRL application						

Crop	Region/ Indoor (a)	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs (b)	Recommendations/comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
Rye grain	NEU + SEU	No data provided	Extrapolation from wheat possible. See wheat results and calculations	0.5	0.43	0.08
Rye straw	NEU + SEU	No data provided	Extrapolation from wheat possible. See wheat results and calculations	-	0.17	0.05
	~~NEU~~	~~Mo: 0.03; 2x0.05; 0.06; 0.07; 0.08; 0.09; 0.10; 0.11; 0.22; 0.24; 0.37~~ ~~RA: 0.02; 0.06; 3x0.09; 2x0.10; 0.12; 0.15; 2x0.33; 0.47~~ ~~Mo: 0.03; 2x0.05; 0.06; 0.07; 0.08; 0.09; 0.10; 0.11; 0.22; 0.24; 0.37~~ ~~RA: 0.01; 0.04; 4x0.06; 0.07; 0.08; 0.10; 0.17; 0.23; 0.36;~~ ~~Scaled:~~ ~~Mo: 0.03; 2x0.05; 0.06; 0.07; 0.08; 0.09; 0.10; 0.12; 0.22; 0.23; 0.39~~ ~~RA: 0.01; 0.04; 4x0.06; 0.07; 0.08; 0.11; 0.18; 0.23; 0.35;~~	~~No residue trials provided for rye.~~ ~~Extrapolation from wheat according to Guidelines on Comparability, extrapolation, group tolerances and data requirements for setting MRLs (Doc. SANCO 7525/VI/95 – rev.10.1, 01-12-2015) is possible.~~ ~~A total of 12 trials for wheat were conducted in northern EU.~~ ~~MRL_{OECD}: 0.53/0.6 0.54/0.6~~ ~~A total of 10 trials for wheat were conducted in southern EU. 2 residue levels obtained from processing studies (3.2N rate), these results are scaled by a factor of 3.2 (proportionality principle) and underlined in this table.~~ ~~MRL_{OECD}: 0.39/0.4 0.38/0.4~~ ~~Combined datasets as being similar (Mann-Whitney U-test).~~ ~~MRL_{OECD}: 0.48/0.5~~ ~~Although value 0.39 mg/kg might be an outlier. MRL proposal without this value MRL_{OECD}: 0.37/0.4~~	~~0.6~~	~~0.47 0.35 (HR_{Mo}: 0.37 0.39)~~	~~0.10 0.07 ($STMR_{Mo}$: 0.09)~~
	~~SEU~~	~~Mo: 3x0.03; 2x0.05; 2x0.06; 0.08; 0.15; 0.28~~ ~~RA: 0.04; 0.05; 0.09; 2x0.10; 0.13; 0.14; 0.15; 0.26; 0.56~~ ~~Mo: 3x0.03; 2x0.05; 2x0.06; 0.08; 0.49; 0.88~~ ~~RA: 0.03; 0.04; 3x0.08; 0.09; 0.11; 0.12; 0.62. 1.40;~~ ~~Scaled:~~ ~~Mo: 3x0.03; 2x0.05; 2x0.06; 0.08; 0.15; 0.27;~~ ~~RA: 0.03; 0.04; 3x0.08; 0.09; 0.11; 0.12; 0.19; 0.43;~~		~~0.4~~	~~0.56 (HR_{Mo}: 0.28)~~ ~~0.43 (HR_{Mo}: 0.27)~~	~~0.12 ($STMR_{Mo}$: 0.06)~~ ~~0.09 ($STMR_{Mo}$: 0.06)~~
	~~NEU/SEU~~	~~Scaled:~~ ~~Mo: 4x0.03; 4x0.05; 3x0.06; 0.07; 2x0.08; 0.09; 0.10; 0.12; 0.15; 0.22; 0.23; 0.27; 0.39;~~ ~~RA: 0.01; 0.03; 2x0.04; 4x0.06; 0.07; 4x0.08; 0.09; 2x0.11;~~		~~0.5~~	~~0.43 (HR_{Mo}: 0.39)~~	~~0.08 ($STMR_{Mo}$: 0.07)~~

Crop	Region/ Indoor (a)	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs (b)	Recommendations/comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg) (c)	STMR (mg/kg) (d)
		~~0.12; 0.18; 0.19;0.23; 0.35; 0.43;~~				

Summary of the data on formulation equivalence **OECD Guideline 509**

~~Representative use is early in the growing season. No further consideration required.~~

Summary of data on residues in pollen and bee products (Regulation (EU) No 283/2013, Annex Part A, point 6.10.1)

~~Not a current EU requirement as there is no agreed established guidance document.~~

~~Wheat and barley are not considered as being melliferous and are therefore not considered relevant for honey production. In addition, the crops are generally considered to be of low attractiveness to bees. Nevertheless residue study in honey is in progress and will be available on I quarter of 2018~~. Data gap. Information is requested on the potential transfer of trinexapac and its degradation products in pollen and bee products

(a): NEU or SEU for northern or southern **outdoor** trials in EU member states (**NEU+SEU** if both zones), **Indoor** for glasshouse/protected trials, **Country** or **Country/indoor** if non-EU location.

(b): Residue levels in trials conducted according to GAPs reported in ascending order (*e.g.* 3x <0.01, 0.01, 6x 0.02, 0.04, 0.08, 3x 0.10, 2x 0.15, 0.17). When residue definition for monitoring and risk assessment differs, use **Mo**/**RA** to differentiate data expressed according to residue definition for **Mo**nitoring and **R**isk **A**ssessment.

(c): **HR**, highest residue. When residue definition for monitoring and risk assessment differs, HR according to definition for monitoring reported in brackets (HR_{Mo}).

(d): **STMR**, supervised trials median residue. When residue definition for monitoring and risk assessment differs, HR according to definition for monitoring reported in brackets ($STMR_{Mo}$).

(*): High uncertainty of MRL due to small dataset.

B.7.4 Feeding studies

The results of the dietary burden calculations are reported in Volume 1 Table 2.7.5-2. Since the calculated dietary burdens for all groups of livestock (except breeding swine) were found to be above the trigger value of 0.004 mg/kg bw/d, further investigation of residues in commodities of animal origin is necessary.

B.7.4.1 Poultry

No livestock feeding studies on poultry were submitted.

~~According to the metabolism studies (see B.7.2.2.1), it is concluded that after exposure to the maximum dietary burden (about 16 750 times lower than the dose level of the metabolism studies), residue levels in poultry commodities are expected to remain below the enforcement LOQ of 0.01 mg/kg in tissues and eggs (only small amounts of trinexapac ethyl equivalents/kg were found in egg white 0.0196 mg/kg, liver 0.013 mg/kg, skin 0.011 mg/kg and kidney 0.043 mg/kg). Hence, no livestock feeding study is needed.~~

B.7.4.2 Ruminants

The transfer of residues from cattle into tissues and milk was assessed in the framework of the first Annex I inclusion. The study is presented below.

Study 1
EU reviewed feeding study with dairy cattle

Reference:	**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. (~~KCA 6.4.2 / 01 & KCA 6.1 / 02~~ KIIA 6.3.2.2 / 01 & KIIA 6.4.2 / 01)
Report No.:	330/99
Guideline:	Directive 91/414/EC, 7031/VI/95, appendix G. Directive 96/68/EC, L277
GLP:	Yes. Principles of GLP of the OECD (Paris 1981, revised in 1997); GLP Ordinance of Switzerland (Bern, 2000); EPA GLP Standards 40 CFR Part 160, USA.
Previous evaluation:	DAR 2003

Material and methods:

Test item: CGA 179500 (trinexapac acid)

Batch No: MLA-372/1 (purity 99 %)

Test concentration: 40.4 mg CGA 179500 (2 mg as/kg feed; 0.0676 mg/kg bw) for 1X-group
121.2 mg CGA 179500 (5.6 mg as/kg feed; 0.2055 mg/kg bw) for 3X-group
404.0 mg CGA 179500 (20 mg as/kg feed; 0.7051 mg/kg bw) for 10X-group

Test system: Eleven lactating cows of Holstein breed, divided into three groups with 3 cows each, and two as control were used*. One group received daily capsules containing 40 mg of CGA 179500, another group received daily capsules containing 120 mg of CGA 179500 and third group received daily capsules containing 400 mg of CGA 179500. Feeding was by treatment group, with 9 kg of dairy concentrate per cow, following each milking. Hay and water were offered ad libitum. The cows were dosed for 28-29 consecutive days and sacrificed between 20 and 24 hours after receiving the final dose.

Duration: 28-29 days

Sampling time points: Milk was collected on day 0 (pre-dose) and after 1, 2, 3, 5, 8, 12, 15, 19, 22 and 28 days. Equal amounts of morning and evening milk were combined and two aliquots of about 20 ml taken for analysis. Animals were sacrificed after 29 days (1 animal/group) or 30 days (remaining 2 animals/group) of dosing, approximately 20h after the last treatment. Samples of dairy concentrate, hay, and water consumed by cows during the study were collected on days 0 and 29. If analysis of specimens would have indicated contamination of foodstuff, these samples would have been analysed.

Method of analysis: Fat, milk and tissue samples (liver, kidney, perirenal fat, omental fat, tenderloin, round muscle and diaphragm) were analysed using method REM 137.12, modified for the measurement with LC-MS-MS (extraction with acetonitrile/water (35 vol + 65 vol), +0.2% formic acid (eluent 1) and extraction with acetonitrile/water (65 vol + 35 vol), +0.2% formic acid (eluent 2).

Storage: -18°C for maximum of 3 months from sample to analysis (muscle, liver, kidney) and 4 months for milk.

Number of animals: 11

Method validation: The performance of the method was checked with each series of specimen analyses by performing procedural recovery tests. The overall recovery of trinexapac acid (%) was:

Sample	Percent of CGA 179500 found (%)		Average (%)	LOQ (mg/kg)
	Fortification 0.02 (mg/kg)	Fortification 0.2 (mg/kg)		
Liver	80	83	82	0.02
Kidney	102	88	95	
Muscle round	97	85	91	
Tenderloin	80	81	81	
Diaphragm	80	82	81	

Fat perirenal Omental	 103 71	 75 91	 89 81	
Blood	93 (0.01 mg/kg)	96 (0.1 mg/kg)	95	0.01
Milk	0.005 mg/kg: 97, 88, 92, 92, 105, 105, 100, 103, 111, 120, 121, 118	0.05 mg/kg: 103, 103, 101, 105, 108, 103, 102, 105, 100, 102, 103	104	0.005

Storage stability: For the animal matrices muscle, liver, kidney, fat omental, milk and blood, storage stability data for CGA 179500 up to 3 months (storage at -18°C) were provided. For each matrix, 5 samples were analysed (see B.7.1.2)

* - it is stated in the report, that 3 cows each for dose groups 1X, 3X, 10X and 2 cows as control were used, although results for only one control cow were presented.

Results

The content of CGA 179500 found in representative capsules collected at the beginning (day 0) and at the end (day 29) of the administration was in the range of 87% - 100% of the nominal values. This shows sufficient stability of the test substance in capsules, individual results provided in table B.7.4.2 – 1.

No residues were found in muscle (tenderloin, round) and omental fat. One residue at 0.02 mg/kg was found in diaphragm (10X dose group). Two residues of 0.03 mg/kg were found in liver (10X dose group). Maximum residues values of about 0.03, 0.05 and 0.29 mg/kg were found in kidney of dose groups 1X, 3X and 10X, respectively. The average residues in blood were 0.02, 0.03 and 0.13 of dose groups 1X, 3X and 10X, respectively. The residues in milk and tissue samples of dairy cattle after application of different concentrations of CGA 179500 over 28 – 29 days are summarised in tables B.7.4.2-2 and B.7.4.2-3. The results are not corrected for the recovery values. In figure B.7.4.2 – 1 it is shown that plateau of residues in milk is reached in 2-3 days. Bodyweights of test animals both before and during the duration of feeding is presented in table B.7.4.2-4. The dose level in livestock expressed on a mg/kg body weight (BW) basis is calculated for the 21 February 2000 bodyweights results.

Table B.7.4.2 - 1. Measured content of CGA 179500 in representative capsules

Dose level	Animal number	Nominal value (mg)	CGA 179500 found in capsules mg*	
			Collected on day 0	Collected on day 29
control	968	0	0	0
1X	969	40	37.3 (93%)	39.5 (99%)
3X	972	120	104.3 (87%)	119.4 (100%)
10X	975	400	379.9 (95%)	399.7 (100%)

* - Percent of nominal content of CGA 179500 in parentheses.

Table B.7.4.2 - 2. Residues of trinexapac acid (CGA 179500) in ~~milk and~~ tissues from dairy cattle dosed with three concentrations of GA 179500

Residue found	Tissue sample1	0 mg as/kg feed (control)	2 mg as/kg feed (1X)	5.6 mg as/kg feed (3X)	20 mg as/kg feed (10X)
CGA 179500 (mg/kg)	Muscle	< 0.02	< 0.02	< 0.02	< 0.02
	Fat	< 0.02	< 0.02	< 0.02	0.02
	Liver	< 0.02	< 0.02	< 0.02	0.03
	Kidneys	< 0.02	0.03	0.05	0.29
	Milk	< 0.005	< 0.005	< 0.005	0.011
	Blood	< 0.01	0.02	0.04	0.17

[1] Highest observed values

Dose group	Cow No.	Residues of CGA 179500 (mg/kg)							
		Muscle tenderloin	Muscle round	Diaphragm	Liver	Kidney	Perirenal fat	Omental fat	Blood
0X	8	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.01
1X (9.7 N)*	4	<0.02	<0.02	<0.02	<0.02	0.03	<0.02	<0.02	0.014
	7	<0.02	<0.02	<0.02	<0.02	0.03	<0.02	<0.02	0.016
	10	<0.02	<0.02	<0.02	<0.02	0.03	<0.02	<0.02	0.023
	Average	<0.02	<0.02	<0.02	<0.02	0.03	<0.02	<0.02	0.018
3X (29.1)*	1	<0.02	<0.02	<0.02	<0.02	0.04	<0.02	<0.02	0.031
	9	<0.02	<0.02	<0.02	<0.02	0.05	<0.02	<0.02	0.036
	11	<0.02	<0.02	<0.02	<0.02	0.03	<0.02	<0.02	0.027
	Average	<0.02	<0.02	<0.02	<0.02	0.04	<0.02	<0.02	0.031
10X (100.7)*	2	<0.02	<0.02	<0.02	<0.02	0.08	<0.02	<0.02	0.059
	3	<0.02	<0.02	0.02	0.03	0.13	<0.02	<0.02	0.150
	5	<0.02	<0.02	<0.02	0.03	0.29	0.02	<0.02	0.167
	Average	<0.02	<0.02	0.02	0.03	0.17	0.02	<0.02	0.125

* - N rate compared to highest expected intake for dairy cattle presented in Volume 1 Table 2.7.5-2.

Table B.7.4.2 - 3. Residues of trinexapac acid (CGA 179500) in milk from dairy cattle dosed with three concentrations of GA 179500

Group/Cow No	Residues of CGA 179500 in milk (mg/kg)									
	0X/8	1X/4	1X/7	1X/10	3X/1	3X/9	3X/11	10X/2	10X/3	10X/5
D 0 (8-Mar-00)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Day 1	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	0.005
Day 2	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.006	0.006
Day 3	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	0.005
Day 5	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	0.011(1)
Day 8	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	<0.005	0.005

Day 12	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	0.005
Day 15	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.006	0.005
Day 19	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005(2)	0.006(3)
Day 22	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	0.005
Day 28	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	0.005

The results were not corrected for recovery values;

(1) – average of three analyses (0.010, 0.011, 0.011 mg/kg)

(2) – average of three analyses (<0.005 mg/kg each)

(3) – average of two analyses (0.0061 and 0.005 mg/kg)

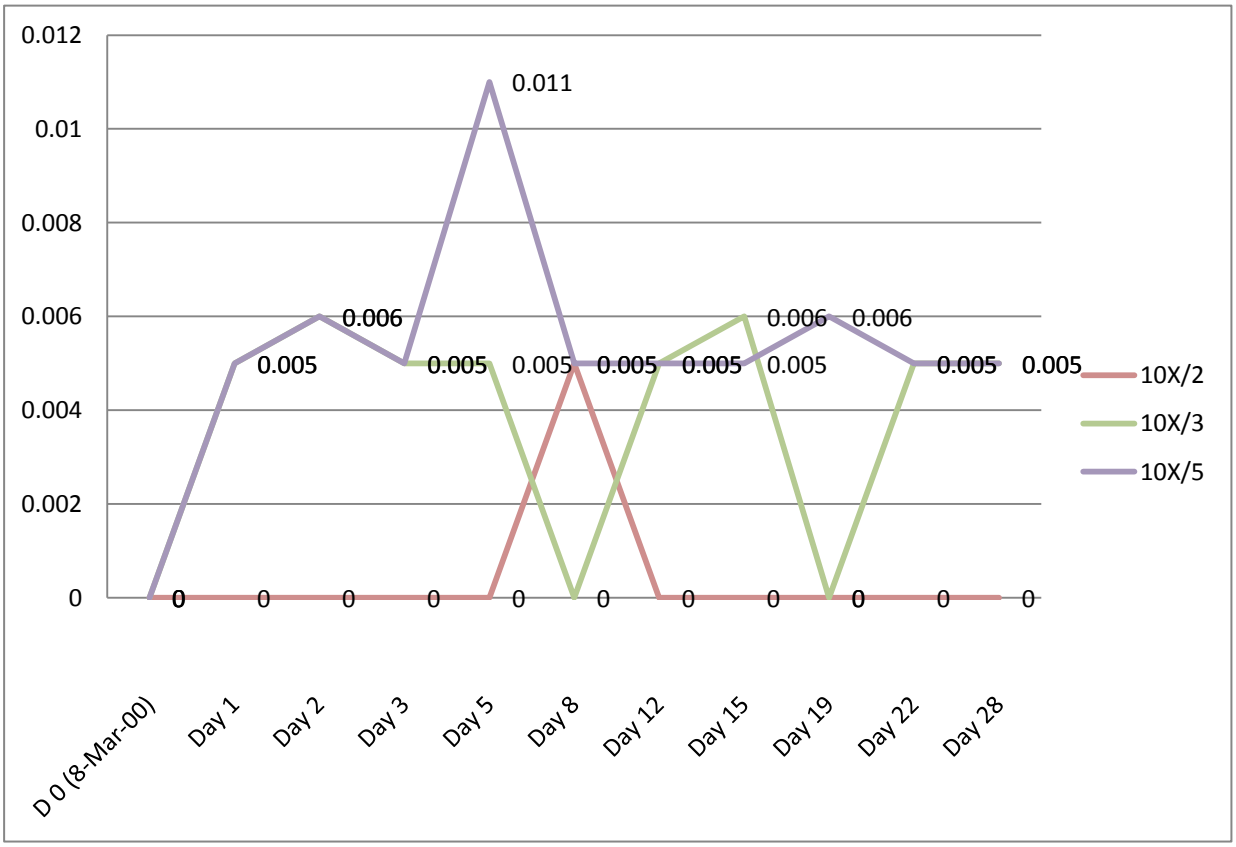


Figure B.7.4.2-1 Residues of CGA 179500 in milk (mg/kg) of 10X dose cows

Table B.7.4.2 - 4. Bodyweights of the testing animals (cows)

Cow No./ Dose group	Dates			Weight gain		Dose rate Mg/kg bw*
	21-Feb-00	8-Mar-00	5-Apr-00	From start	During dosing period	
8 / control	594	588	554	-40	-34	-

4 / 1X	588	584	612	24	28	0.068
7 / 1X	636	650	656	20	6	0.063
10 / 1X	550	560	574	24	14	0.073
1 / 3X	688	700	720	32	20	0.174
9 / 3X	558	568	564	6	-4	0.215
11 / 3X	506	518	514	8	-4	0.237
2 / 10X	588	578	588	0	10	0.680
3 / 10X	526	564	540	14	-24	0.761
5 / 10X	588	606	598	10	-8	0.680

* - Calculated for the before dose period (21-Feb-2000) body weight

RMS comments and conclusion (Netherlands 2003)

The kidney was the only tissue of all samples analysed were a clear dose dependent increase of CGA 179500 residues was found. The residues in muscle and fat were below or around the LOQ of 0.02 mg/kg. In the liver the residue level was just above the LOQ only in the highest dose group. Residues in milk samples were only found in the highest dosed group, reaching 0.011 mg/kg. No detectable residues are expected in ruminant products at a nominal intake of CGA 179500 via feed (0.30-0.40 mg/kg feed). The storage stability data provided in this study show that CGA 179500 is stable during storage at -18°C for at least three months.

RMS LT agrees with the above conclusions.

Method REM 137.12 was validated in study Sack, 1995a and ILV study Gasser, 2001 (Please refer to Vol 3 CA B.5.1.2.2)

Method 137.12 was validated for the determination of the metabolite CGA 179500 in animal products. The validation data also include data from an independent laboratory validation on meat and milk. No confirmatory method has been submitted for animal products. With method 137.12 it is feasible to determine the metabolite CGA 179500 in animal products with an LOQ of 0.02 mg/kg for eggs, meat and offal, and 0.01 mg/kg for milk.

Time from sample to analysis is covered by storage stability data.

~~No deviations from OECD guideline 505 were observed.~~ ~~Study is considered as suitable for evaluation.~~

Deviations from OECD 505:

Sampling of meat and edible tissues are not reported in sufficient detail – weight of the samples and sampling methods not provided – could not conclude if sample material/method/analytical sample preparation/weight of laboratory sample comply OECD 505.

Milk samples are a bit smaller than recommended (0.4 L instead of 0.5 L)

LOQ for the tissues are higher than recommended (0.02 instead of 0.01 mg/kg).

Despite the above deviations, RMS LT is of the opinion that study is suitable for evaluation.

According to the results of the dietary burden calculations, lambs ~~and rams/ewes~~ are the most exposed ruminants to residues of trinexapac acid, therefore the MRL calculation is based on their dietary burden calculations. Residue values have been derived using the transfer factor methodology.

The available data are considered sufficient for deriving MRLs in ruminants. These MRLs were derived in compliance with the latest recommendations on this matter (FAO, 2009; OECD, 2013) and are summarised in Table B.7.4.2-4. Significant residues in tissues and milk of ruminants are not expected and MRLs for these commodities can be established at the LOQ (0.01* mg/kg).

Table B.7.4.2-4: Overview of feeding studies

Commodity	Dietary burden		Results of the livestock feeding study						Median residue (mg/kg)[a]	Highest residue (mg/kg)[b]	Calculated MRL (mg/kg)	CF for RA[c]
	Med. (mg/kg bw/d)	Max. (mg/kg bw/d)	Dose Level (mg/kg bw/d)	No	Result for enforcement		Result for RA					
					Mean (mg/kg)	Max. (mg/kg)	Mean (mg/kg)	Max. (mg/kg)				
EU data (Report 330/99; The Netherlands, 2003)												
Residue definition for enforcement: Sum of trinexapac acid and its salts, expressed as trinexapac acid.												
Ruminant meat	~~0.009~~ 0.006	~~0.017~~ 0.010	0.068	3	<0.02	<0.02	<0.02	<0.02	<0.01	<0.01	0.01*	-
			0.21	3	<0.02	<0.02	<0.02	<0.02				
			0.71	3	<0.02	<0.02	<0.02	<0.02				
Ruminant fat			0.068	3	<0.02	<0.02	<0.02	<0.02	<0.01	<0.01	0.01*	-
			0.21	3	<0.02	<0.02	<0.02	<0.02				
			0.71	3	<0.02	0.02	<0.02	0.02				
Ruminant liver			0.068	3	<0.02	<0.02	<0.02	<0.02	<0.01	<0.01	0.01*	-
			0.21	3	<0.02	<0.02	<0.02	<0.02				
			0.71	3	0.03	0.03	0.02	0.03				
Ruminant kidney			0.068	3	0.03	0.03	0.03	0.03	<0.01	<0.01	0.01*	-
			0.21	3	0.04	0.05	0.04	0.05				
			0.71	3	0.17	0.29	0.17	0.29				

Commodity	Dietary burden		Results of the livestock feeding study						Median residue (mg/kg)[a]	Highest residue (mg/kg)[b]	Calculated MRL (mg/kg)	CF for RA[c]
	Med. (mg/kg bw/d)	Max. (mg/kg bw/d)	Dose Level (mg/kg bw/d)	No	Result for enforcement		Result for RA					
					Mean (mg/kg)	Max. (mg/kg)	Mean (mg/kg)	Max. (mg/kg)				
Milk	~~0.007~~ 0.005	~~0.012~~ 0.007	0.068	30	<0.005[d]	N/A	<0.005[d]	N/A	<0.005	<0.005	0.01*	-
			0.21	30	<0.005[d]	N/A	<0.005[d]	N/A				
			0.71	30	0.005[d]	N/A	0.005[d]	N/A				

N/A: Not applicable – only the mean values are considered for calculating MRLs in milk.
(*): Indicates that the MRL is set at the limit of analytical quantification.
(a): Median residue value according to the enforcement residue definition, derived by interpolation/extrapolation from the feeding study for the median dietary burden (FAO, 2009).
(b): Highest residue value (tissues, eggs) or mean residue value (milk) according to the enforcement residue definition, derived by interpolation/extrapolation of the maximum dietary burden between the relevant feeding groups of the study (FAO, 2009).
(c): The median conversion factor for enforcement to risk assessment.
(d): Mean residue level from day 1 until day 28 (3 cows, 10 sampling days).

B.7.4.3 Pigs

No livestock feeding studies on pigs were submitted.

B.7.4.4 Fish

No study has been submitted.

B.7.5 Effects of processing

As quantifiable residues of trinexapac acid are expected in the treated crops, a study investigating the nature of residues in processed commodities is required.

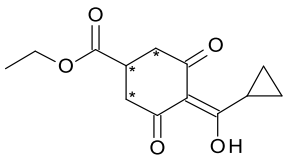
The effect of processing on the nature of trinexapac-ethyl and trinexapac acid was investigated in the framework of the peer review. Both studies were conducted simulating representative hydrolytic conditions for pasteurisation (20 minutes at 90°C, pH 4), boiling/brewing/baking (60 minutes at 100°C, pH 5) and sterilisation (20 minutes at 120°C, pH 6).

As the study with trinexapac acid was covered by data protection, two members of the Trinexapac-ethyl Task Force (Adama and Cheminova) have conducted their own high temperature hydrolysis study in order to support their own PPP. All these four studies are presented below.

B.7.5.1 Nature of residues

Study 1

EU reviewed high temperature hydrolysis study of trinexapac-ethyl

Reference:	**Cadalbert R., Buckel T. (2001)** Hydrolysis of [1,2,6-^{14}C]-Cyclohexadione labelled CGA 163935 under processing conditions. (KCIIIA 6.5.1 / 01)
Study No.:	01RC02
Guideline:	Directive 91/414/EC, Annex II: Effects on the nature of residues
GLP:	OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final]
Previous evaluation:	DAR 2003
Material and methods:	
Test Item:	[1,2,6-^{14}C]-Trinexapac-ethyl
Position of the radiolabel: (* = ^{14}C position)	
Lot/Batch No:	ILA-103.2B
Purity:	98.0 %, specific activity 2.53 MBq/mg
Test system:	3 different sterilised aqueous buffers solutions were used: pH 4 (0.01M citrate, 20 min, 90^{0}C), pH 5 (0.01M acetate, 60 min, 100^{0}C), and pH 6 (0.01M phosphate, 20 min, 120^{0}C). The range of hydrolytic conditions represented the processes of pasteurisation, baking/brewing/boiling and sterilisation. The starting concentration of the test substance was about 4.9 mg/L. Buffer solutions containing the radiolabelled test item at initial concentrations of average 4.92 mg/L were sterilized by sterile filtration and the glassware by autoclaving. After treatment samples were cooled down to ambient temperature.
Sampling time points:	At time 0 and after 20 or 60 minutes of incubation, duplicate samples per pH value were taken. All analyses were performed directly after sampling and processing, thus no storage stability test was necessary.
Method of analysis:	After determination of the pH, the samples were neutralised and total radioactivity determined by LSC. Moreover, subsamples were analysed in duplo by HPLC using UV detection and 2D-TLC. The total recovery for all samples set up ranged from 98.7 % to 99.4 % of the applied radioactivity.
Limit of quantification:	0.003 mg/L (LOD: 0.002 mg/L)
Date of experiment:	March 2001 – September 2001

Results

The results of various hydrolytic conditions on ^{14}C -CGA-163935 are summarised in table B.7.5.1-1

Table B.7.5.1-1:Radioactivity after Incubation of 14C-CGA 163935 under representative hydrolytic conditions

Process simulated	pH	Incubation		Radioactive Fractions after Incubation (% TRR)[1)]	
		Temp. (°C)	Time (min)	^{14}C-CGA 163935	Unknown
Pasteurisation	4	90	20	99	1.3
Boiling, brewing, baking	5	100	60	99	0.6
Sterilisation	6	120	20	99	0.8

[1] TRR, total radioactive residue

RMS comments and conclusions (The Netherlands 2005)

In processing procedures such as boiling, brewing, baking, sterilization and pasteurisation, CGA 163935 can be considered as hydrolytically stable with negligible degradation products.

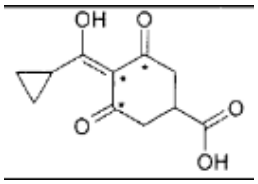
Only in the process of pasteurisation four radioactive fractions amounting to 1.3 % of total radioactivity were found. An identification of these fractions is not required since their expected concentration under field conditions is below 0.01 mg/kg, which is the limit value for further analytical studies.

Since trinexapac-ethyl is not relevant residue component in edible commodities, this study was not considered of relevance by the RMS NL and not re-evaluated by the RMS LT.

Study 2

EU reviewed high temperature hydrolysis study of trinexapac acid (Syngenta)

Reference:	**Mound E. L. (2004)** ^{14}C-Cyclohexyl Trinexapac acid (CGA 179500) aqueous hydrolysis at 90, 100 & 120^{0}C. (KCIIA 6.5.1 / 02)
Study No.:	03JH004
Guideline:	Directive 91/414/EC, 7035/VI/95, appendix E, rev. 5
GLP:	UK GLP Regulations 1999 which are in accordance with OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final]
Previous evaluation:	Addendum to the DAR 2005
Material and methods:	
Test Item:	[^{14}C]Trinexapac acid

Position of the radiolabel: (* = ^{14}C position)	
Lot/Batch No:	CDC-XI-78-1
Purity:	≥96.2%, specific activity 1750 Bq/µg
Test system:	The behaviour of [1,2,6-^{14}C] trinexapac acid (CGA 179500) was studied under conditions simulating pasteurisation, baking/brewing/boiling and sterilisation. Aliquots (37.8 µL) of a stock solution of [1,2,6-14C] trinexapac acid in acetonitrile were added to glass vials containing 4.95 mL of 0.1M buffer solution of pH 4, pH 5 and pH 6, at a concentration of 5 mg/L. The test solutions were incubated in the dark whilst stirred continuously under the following conditions: 25 minutes at pH 4 and 90°C (simulating pasteurisation); 60 minutes at pH 5 and 100°C (simulating baking, brewing and boiling); 20 minutes at pH 6 and 120°C (simulating sterilisation). Control solutions were incubated under identical conditions, but no heating was applied (ambient temperature). Duplicate samples (treated solutions) or single samples (control solutions) were taken at the end of the incubation period.
Sampling time points:	At time 0 and after 20 or 60 minutes of incubation, duplicate samples per pH value were taken.
Method of analysis:	Incubation vials were rinsed with acetonitrile and total radioactivity in combined solution and rinses was determined by LSC. Solutions were analysed by normal phase TLC with confirmation by reversed phase HPLC. Metabolite identification was based on co-chromatography with unlabelled reference standards. In addition, the identity of CGA 313458 was confirmed by NMR. The pH of the buffer solutions was determined prior to the test and found to be within 0.1 unit of target. The stock solution was analysed by LSC (homogeneity confirmed) and TLC (radiochemical purity 96.2-97.0%).
Storage stability:	TLC profiling was completed within 6 months of application but the qualitative HPLC was carried out at 7 months. 2D TLC analysis was carried out to confirm that there were no significant changes in the chromatographic profile after 7 months.
Limit of quantification:	Not stated

Results

At pH 4, 5 and 6, respectively, radioactivity recovered in control solutions at the end of incubation represented 99.1, 92.2 and 95.3% AR, of which 93.9, 83.7 and 88.6% AR was trinexapac acid (equivalent to 94.8%, 90.8% and 93.0% of the radioactivity recovered). Considering that the radiochemical purity of ^{14}C-trinexapac acid in the treatment solutions was 97.0, 96.4 and 96.2%, the degradation in the control solutions was limited.

The results for the treated solutions are summarised in Table B.7.5.1-2. At the end of incubation under the various conditions tested, trinexapac acid had degraded and represented 51-59% AR. Degradation products identified were CGA 313458 (16-21% AR) and CGA 113745 (9.6-12% AR). Unidentified fractions represented 3.4-10.2% AR (no individual compound >5.0% AR).

Table B.7.5.1-2: Recovery (range for duplicates) and identification (duplicate means) of radioactivity after incubation of [1,2,6-^{14}C] trinexapac acid under conditions simulating pasteurisation, baking/brewing/boiling and sterilisation.

	% of applied		
	pH 4 (90°C, 25* min) (pasteurisation)	**pH 5 (100°C, 60 min) (baking/brewing/ boiling)**	**pH 6 (120°C, 20 min) (sterilisation)**
RA recovered	103-106	100-104	100-104
trinexapac acid (CGA 179500)	52.5	58.5	50.9
CGA 113745	9.6	10.5	11.6
CGA 313458	19.7	16.1	21.0
others[(a)]	9.1	3.4	10.2

* Experimental time continued for further 5 minutes over 20 minute target. This was judged not to significantly alter the results and in effect gave a worst case scenario for the pH4 experiment.

(a) No individual unknown >5.0% AR.

RMS comments and conclusions (The Netherlands 2005)

At the end of incubation under the various conditions tested (25 minutes, pH 4, 90°C; 60 minutes, pH 5, 100°C; 20 minutes, pH 6, 120°C), trinexapac acid had degraded and represented 51-59% AR. Degradation products identified were CGA 313458 (16-21% AR) and CGA 113745 (9.6-12% AR). Unidentified fractions represented 3.4-10.2% AR (no individual compound >5.0% AR). Study was well performed and reported.

Deviations from OECD 507

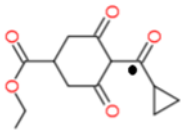
Sterility was not investigated, but degradation in the control samples was found to be limited. The pH at the end was not measured, but an influence of the addition of trinexapac acid on pH, if any, would be very limited, considering the buffer strength (0.1M) and the low concentration of the test compound (2E-05M).

LOQ not clearly stated in the report. It was explained by the applicant that this study was conducted prior to the adoption of OCED guideline 507 (2007), and was therefore conducted to meet the requirements given in EC Directive 91/414, Appendix E, 7035/VI/95, 22nd July 1997. EC directive 91/414 does not state that a LOQ should be provided.

Study 3

New high temperature hydrolysis study of trinexapac acid (Adama)

Reference: **Scullion P. (2012)**. [^{14}C] Trinexapac acid: Simulated processing – Aqueous

	hydrolysis at 90, 100 and 120°C. (KCA 6.5.1 / ~~0~~301)
Study No.:	C93481
Syngenta file No.	CGA179500_11002
Guideline:	91/414/EEC Annex II part A section 6 and Annex III part A section 8; Commission of the European Communities, Document 7035/VI//95 rev Appendix E – Processing studies.
GLP:	Swiss Ordinance relating to Good Laboratory Practice adopted May 18th, 2005 [SR 813.112.1], which is based on OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted on November 26th, 1997 by decision of the OECD Council [C 997)186/Final]. The second amendment to the report had some exceptions*.
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test Item:	[^{14}C]-Trinexapac acid which was derived by hydrolysis from [^{14}C]Trinexapac-ethyl
Position of the radiolabel: * - denotes the position of ^{14}C	
Lot/Batch No:	07BLY089 (of [^{14}C]Trinexapac-ethyl)
Radiochemical purity:	99.10% after conversion to the acid, specific activity 2.93 MBq/mg based on molecular weight of 252.3 g/mol for the unlabelled Trinexapac-ethyl
Preparation of the stock solution:	The test item was generated at the test site by hydrolysis of the [^{14}C]Trinexapac-ethyl. 17.3 mg of [^{14}C]Trinexapac-ethyl was dissolved in 20 mL water and placed in an ultrasonic bath for 5 minutes. The solution was adjusted to pH 9 by addition of 3 drops of ammonium hydroxide (25% v/v). The solution was heated for 48 hours at 40°C. The solution was used without further modification for application of the test item. Radiolabelled purity was determined to be 99.1%. The amount of [^{14}C]Trinexapac acid in the application solution was determined by liquid scintillation counting (LSC) and found to be 79.7 mg/L based on the measured radioactivity (15732800 dpm per mL) and the specific activity of 3.29 MBq/mg.
Preparation of the test solutions:	For preparation of the [^{14}C]Trinexapac acid labelled test solutions, aliquots (pH 4: 30 mL, pH 5: 40 mL and pH 6: 30 mL) of the sterilised aqueous buffer solutions at pH 4, pH 5 and pH 6 were separately added to 50 mL measuring cylinders, followed by 0.617 mL of the application solution. Additional volumes of the respective sterile buffer solutions were then added to reach a final volume of 50 mL. All treated buffer solutions were thereafter mixed and degassed in an ultrasonic bath for about 5 minutes. 15 mL aliquots of the test item application solutions were transferred into high pressure flasks (45 mL capacity) and incubated in an oil bath. The amount of test item in each buffer solution was determined by measuring triplicate samples of up to 1 mL of each buffer solution by LSC.
Test system:	Deionised water was further purified using an ELGA water purifier unit. The following buffer solutions were used (prepared in purified water): pH 4 acetate buffer: 500 mL 0.1 M acetic acid was added to 500 mL 0.1 M sodium acetate and the pH adjusted with acetic acid.

	pH 5 acetate buffer: 200 mL 0.1 M acetic acid and 500 mL 0.1 M sodium acetate were mixed and diluted to 1 L with purified water. The pH was adjusted with acetic acid. pH 6 acetate buffer: 500 mL 0.1 M sodium acetate will be adjusted to pH 6 with 0.1 M acetic acid. The buffer solutions, except pH 4 were diluted to 1 L with purified water. The final concentration of the buffer solutions was 0.05 mol/L acetate. The buffer solutions were autoclaved for 31 minutes at 121°C. All glass equipment were sterilised prior to use by rinsing with an ethanol/ water (70/30, v/v) solution. All treatments were performed on a sterile bench under laminar flow conditions. High pressure glass flasks (45 mL capacity) incubated in an oil bath.
Experimental conditions:	Buffered solutions of [^{14}C]Trinexapac acid (1 mg/L) were incubated in duplicates in high pressure glass flasks immersed in an oil bath for the specific durations and temperatures. The study was performed at pH 4, 5 and 6 at temperatures of 90°C, 100°C and 120°C, respectively. The temperatures were maintained at a constant value throughout incubation and no significant variation of the pH values was observed in the buffered solutions. [^{14}C]Trinexapac acid was tested at an initial nominal concentration of 1 mg/L. Initial measured concentrations were 1.014, 0.995 and 0.979 mg/L at pH values of 4, 5 and 6, respectively.
Sampling time points:	At time 0 and after incubation (20 or 60 minutes) the samples were taken, measured for total radioactivity and analysed for the nature of degradates. All analyses were performed within 6 months period, thus no storage stability test was necessary (main study). Samples were stored at -20°C.
Method of analysis:	The quantity of radioactivity was determined by Packard liquid scintillation counters (LSC) equipped with DPM and luminescence options. Triplicate aliquots of the samples (up to 1 mL) were measured in 10 mL of scintillation mixture. HPLC was used as the primary method to determine the amounts of test item and degradation products in the samples. Selected samples were analysed by one-dimensional TLC in order to confirm the results obtained by HPLC. TLC was performed on pre-coated silica plates (5×20 cm; layer thickness of 0.25 mm). Samples were mixed with the unlabelled test item and the mixture was applied to the plate (about 1 cm band). The unlabelled reference items were also spotted near the radioactive band. The plates were developed with chamber saturation using chloroform/methanol/formic/water (62/30/2/6; v/v/v/v) as the solvent. The unlabelled reference items were visualized by UV light at a wavelength of 254 nm. All TLC plates were submitted to the phosphor imaging technique. TLC proved to be of limited use in assigning the hydrolysis products and confirmation of the HPLC results was therefore performed by LC-MS. After separation on a reversed phase HPLC column, the eluent flow was split. About 0.41 mL/min of the eluent flow was subjected to first UV- and second ^{14}C analysis. The remaining 0.2 mL/min was split again: about 0.08 mL/min of the flow was delivered to MS and subsequent analysis and the remainder went to waste. The UV- (TSP UV 2000 operating at a wavelength of 254 nm) and ^{14}C- (Berthold LB509 with a solid scintillator flow cell) detectors operated in series. An additional study was performed in order to identify the transformation product M5. Analysis was performed by NMR
Method validation:	The mean recoveries of radioactivity for the test item were 97.9 ± 0.3% (pH 4; 90°C), 98.4 ± 0.1% (pH5; 100°C) and 98.8 ± 0.3% (pH 6; 120°C).
Limit of quantification:	0.005 mg/L (LOD: 0.003 mg/L) for HPLC

	0.6 µg/L for LSC
Date of experiment:	21.02.2011 to 23.03.2011 (main study) 28.07.2011 to 30.11.2011 (additional study)
Additional study:	Additional work was performed in order to identify the transformation product M5
Preparation of the stock solution:	A stock solution of unlabelled trinexapac acid (208026/A) was prepared by dissolving 26.32 g in 25 mL pH 5 acetate buffer.
Preparation of the test solution for NMR analysis:	The test solution for NMR analysis was prepared by adding the unlabelled trinexapac acid (208026/A) to 0.5 mL of the [^{14}C]-trinexapac acid to make a final volume of 20 mL. The solution was heated for 60 minutes in a closed vessel at approximately 100 °C. The amount of [^{14}C] was measured by LSC to be 8080000 dpm/20 mL corresponding to a new specific activity of approximately 6500 Bq/mg. Analysis by HPLC indicated M5 was formed at 3.74% of the applied radioactivity.
Method of analysis:	HPLC was used as the primary method to determine the amounts of test item and degradation products in the samples and for fractionation of M5. Detection was performed with UV detection at 275 nm. For ^{14}C detection, 1 mL of eluent was continuously mixed with 2 mL of Flo-Scint A. The LOQ of the HPLC method was 0.005 mg/L. The samples and reference compounds were analysed by LC-MS on two separate systems: a triple quadrupole MS with in-line radioactivity detector (two methods were used, LC1 and LC2) and a Bruker MaXis Q-TOF capable of high mass resolution (according to LC methods LC3 to LC8). The sample of M5 was characterised by NMR spectroscopy using a high-performance digital 600 MHz NMR spectrometer Avance III (by Bruker) equipped with a 5 mm TCI cryoprobe head (1H/13C inversely, Z gradient). All NMR spectra were recorded in water/ D_2O by using standard pulse sequences and pre-saturation (pr). For NMR measurements, the radioactive HPLC fraction (546.5 µL) was dissolved in 60.2 µL D_2O spiked with 6 µg DMSO as reference standard. The solution was then transferred into an NMR glass capillary (OD = 5 mm). The 1H NMR DMSO signals were referenced to δ = 2.613 ppm and the respective ^{13}C NMR signals to δ = 39.4 ppm. Due to the intensive water signal (about 105 times larger than the target signal), water suppression by pre-saturation was applied to all spectra recorded.

* - No claim of compliance is made for the determination of accurate masses. The accurate mass analyses were subject to multi point calibration within each sample run and each spectrum was linearly corrected based on a constant lock mass, therefore it is considered that the integrity of these results remains intact.

No claim of compliance is made for data analysis using the Bruker software package data analysis including SmartFormula. The software was, however, successfully installed by the manufacturer and its applicability was proofed with known compounds.

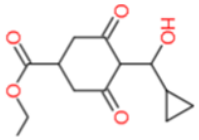
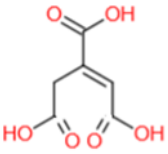
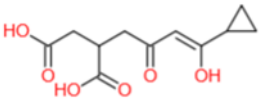
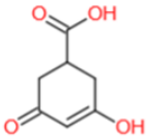
Electronic LCMS data will be stored under non-GLP conditions. Relevant, printable LCMS data used for interpretation was printed and archived as raw data under GLP.

NMR analysis was not performed under GLP and is therefore excluded from the statement of compliance. Although not being included in a national GLP compliance-monitoring program, Fraunhofer Institute for Toxicology and Experimental Medicine ITEM has been chosen as a test location, because they are recognized experts in their fields of work.

Reference items used in the study is presented in the table B.7.5.1-3below:

Table B.7.5.1-3: List of reference items used in the study.

Name	Structure

Trinexapac ethyl (R1)	
Trans-aconitic acid (R2)	
2-((Z)-4-cyclopropyl-4-hydroxy-2-oxo-but-3-enyl)-succinic acid CGA 313458 (R3A)	
3-hydroxy-5-oxo-cyclohex-3-enecarboxylic acid CGA 113745 (R3B)	

Results

Radiochemical purity of the test item

The radiochemical purity of the purified test item was determined to be 99.1% by HPLC before application. The test item proved to be stable in pH 5 and pH 6 during the application procedure since no degradation of [^{14}C]Trinexapac acid was observed in the control samples. At pH 4 some degradation was evident, with the radiolabelled purity measured as 93.95% in the control sample. This is consistent with previous studies which have shown that Trinexapac acid to be less stable at lower pH.

Experimental conditions

The temperatures were kept constant throughout the incubation period and no variation of the pH was observed in the buffer solutions.

No colonies of bacteria formed in either the test solutions at the start and end of the respective incubation periods or the negative control after 5 days of incubation at room temperature. These solutions were therefore considered to be sterile within the whole incubation period. The positive control samples were no longer sterile.

Balance of radioactivity

The mean recoveries of radioactivity for [^{14}C]Trinexapac acid were 97.9 ± 0.3% (pH 4), 98.4 ± 0.1% (pH 5) and 98.8 ± 0.3% (pH 6) (Table B.7.5.1-4).

Table B.7.5.1-4: Radioactivity balance of [^{14}C]trinexapac acid in the buffer solutions before and after incubation

Replicate	Radioactivity (% of applied)					
	pH 4 (90°C)		pH 5 (100°C)		pH 6 (120°C)	
	0 min	20 min	0 min	60 min	0 min	20 min
A	97.7	98.3	98.5	98.3	98.6	99.2
B	97.7	98.0	98.5	98.3	98.6	nr
Mean ± SD	97.9 0.3		98.4 0.1		98.8 0.3	
	Radioactivity (mg/L)					
A	0.991	0.997	0.980	0.978	0.966	0.971
B	0.991	0.994	0.980	0.978	0.966	nr
Mean ± SD	0.993 0.003		0.979 0.001		0.968 0.003	

nr no result due to vessel failure during assay

SD standard deviation

Degradation of [^{14}C]Trinexapac acid during processing

The quantitative determination of [^{14}C]Trinexapac acid was carried out based on the results of the HPLC analysis. A number of hydrolysis products were detected (Table B.7.5.1-5).

At pH 4 and 90°C (20 minutes, simulating pasteurisation) the test item decreased to 85.8% of applied radioactivity. A number of hydrolysis products were detected, with M5 amounting to 5.4% of applied radioactivity and M6 to 4.7% of applied radioactivity. M6 was shown to correspond to R3A but M5 could not be identified with the available reference items. All other detected products were below or equal to 1% of the applied radioactivity. The pH 4 control sample also showed a small amount of degradation with [^{14}C]Trinexapac acid corresponding to 91.8% of the applied radioactivity.

At pH 5 and 100°C (60 minutes, simulating baking/brewing/boiling) the test item decreased to 63.2% of applied radioactivity. Three hydrolysis products, M1, M5 and M6 were detected at levels of 1.0%, 16.3% and 17.7% of applied radioactivity, respectively. Whilst M6 was identified as R3A, M1 and M5 did not correspond to any of the available reference items.

After incubation at pH6 and 120°C (20 minutes, simulating the process of sterilisation) the test item decreased to 82.1% of applied radioactivity. Four hydrolysis products were detected with M1, M4, M5 and M6 accounting for 0.9%, 4.0%, 3.8% and 8.4% of applied radioactivity, respectively. M6 was identified as R3A but M1, M4 and M5 could not be identified with the available reference items.

Table B.7.5.1-5: Distribution of radioactivity of [14C]trinexapac acid in the buffer solutions before and after incubation at different temperatures

Pattern mean % of applied (mg/L)*	Incubation time					
	pH 4 (90°C)		pH 5 (100°C)		pH 6 (120°C)	
	0 min	20 min	0 min	60 min	0 min	20 min
Parent	91.8 (0.931)	85.8 (0.987)	98.5 (0.98)	63.2 (0.629)	98.6 (0.966)	82.1 (0.804)
M1	nd	nd	nd	1.0 (0.01)	nd	0.9 (0.008)
M2	nd	0.3 (0.003)	nd	nd	nd	nd
M3	nd	0.3 (0.003)	nd	nd	nd	nd
M4	nd	nd	nd	nd	nd	4.0 (0.04)
M5	nd	5.4 (0.055)	nd	16.3 (0.162)	nd	3.8 (0.038)
M6 (=R3A)	nd	4.7 (0.048)	nd	17.7 (0.176)	nd	8.4 (0.083)
M7	nd	1.0 (0.01)	nd	nd	nd	nd
M8	5.9 (0.06)	0.7 (0.008)	nd	nd	nd	nd

* mg in parent equivalents/Litre buffer solution

nd not detected

R3A -2-((Z)-4-cyclopropyl-4-hydroxy-2-oxo-but-3-enyl)-succinic acid CGA 313458

Assignment of the hydrolysis products using the reference compounds supplied was problematic with both HPLC and TLC analysis. In HPLC-UV, only one peak was detected for R3 although it was known to be a mix of two components, R3A and R3B. In TLC, R3 gave two major and one minor spots and therefore could not be used to assign the hydrolysed products. As TLC proved to be of limited value in confirming the HPLC results, LC-MS was used as an alternative.

M6 was identified by LC-MS as R3A and corresponded to a number of small adjacent peaks on the ^{14}C trace with retentions times between 15 minutes and 21 minutes which were therefore summed.

Reference item R3B could not be quantified by radiocounting as it would not contain the ^{14}C label. However, it is estimated to be at a very low level in the samples as there is no discrepancy in the balance of applied radioactivity before and after incubation.

A mass spectra of metabolite M5 (retention time 16.5 min by LC-MS) produced no ions which could be interpreted as a possible hydrolysis product in the mass range analysed.

Additional study

LC-MS of reference items: Available reference items R1, R2, R3A and R3B and the parent substance Trinexapac were used for the interpretation of LC-MS analyses (using methods LC1-LC3) of the radioactive sample.

Transformation products M5 and M6: Using LC3, the pH 5 60 min (conc.) sample gave accurate mass and isotopic mass separation measurements for both M6 and parent, which was in good agreement with the calculated value. No result was identified for M5. A number of different LC and MS methods were tried in order to identify M5 including:

- Use of UPLC (ultra performance liquid chromatography) columns and shorter gradients to improve peak height (LC5)
- Removal of formic acid from eluents to reduce background ions and improve negative ion sensitivity (LC4)
- Use of UPLC columns and ammonium acetate buffers (LC6)
- Use of acetonitrile rather than methanol as the organic eluent (LC7 and LC8)
- Use of higher (greater sensitivity) and lower (for thermally labile compounds) source temperatures
- Adjustment of MS parameters to increase sensitivity of lower molecular weight ions.

Various software packages were also used to try and identify M5.

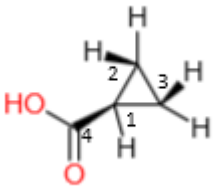
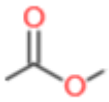
NMR: To simplify elucidation of the unknown structure M5, potential target compounds were spiked to the NMR sample. All spectra were recorded under similar conditions. The resulting NMR spectra of the mixtures were then qualitatively and quantitatively matched against the original spectra.

The NMR measurements were considerably hampered by a variety of interfering compounds including water. All chemical shifts of the identified target compound and several impurities are summarised in Table B.7.5.1-6.

Furthermore, the low concentration of the target compound was critical. Only half of the expected amount was found which had a negative impact especially for the recording of the ^{13}C NMR correlation spectra. However, the high-field shifted symmetrical protons H-2a,b and H-3a,b (both $\delta = 0.86$ ppm) suggest a cyclopropane ring as an

integral part of the unknown compound. Assignment of the attached group was a little more difficult due to many similar sized signals of the impurities.

Table B.7.5.1-6: ^{1}H and ^{13}C NMR chemical shifts and concentration assessment (based on 1H NMR data) of the main components from the radioactive HPLC cut measured in D2O using DMSO as internal standard

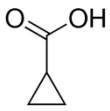
Compound	Chemical shifts (δ)		Concentration (µg/mL)
	^{1}H NMR	^{13}C NMR	
 Cyclopropane carboxylic acid	H-1 1.530 H-2a,3a 0.860 H-2b,3b 0.860	C-1nd C-29.4 C-39.4 C-4181.1*	9.2
HCOOH Formic acid	H-18.138	C-1167.1	1893.0
CH_3OH Methanol	H-13.245	C-149.8	116.0
 Methyl formiate	H-18.029 H-23.657	C-1165.2 C-252.2	87.9
CH_3CN Acetonitrile	H-11.954	C-11.5 C-2119.8	17.8
CH_3COOH Acetic acid	H-11.974	C-1- C-2160.0	1.5
CH_3COCH_3 Acetone	H-1/1' 2.155	C-131.0 C-2216.1	2.7

nd not detected

* weak signal

The use of reference substances introduced more clarity. The first assumption was the presence of cyclopropyl methyl ketone, but the NMR spectra spiked with a small amount of the respective reference (9 µg) did not confirm this. For the cyclopropane carboxylic acid reference, however, a seamless mapping of all available NMR signals was seen in the spectra. The C-H connectivity of all components was furthermore confirmed by an additionally performed HSQC (heteronuclear single quantum coherence) experiment.

Although some C atoms remained hardly detectable (C-1 and C-4 due to the very low concentration), NMR analysis showed the structure of M5 was cyclopropane carboxylic acid:



RMS comments and conclusions

The study results show that [^{14}C]-Trinexapac acid is not stable under conditions representative of pasteurisation, baking, brewing, boiling and sterilisation.

[^{14}C]Trinexapac acid was hydrolysed to 85.8% of the applied radioactivity at pH4 and 90°C (20 minutes, simulating pasteurisation). The pH 4 control sample kept at ambient temperature also showed a small amount of degradation with [^{14}C]-Trinexapac acid corresponding to 91.8% of applied radioactivity. A number of hydrolysis products were detected, with M5 amounting to 5.4% of the applied radioactivity and M6 to 4.7% of the applied radioactivity. All other detected products were below or equal to 1% of the applied radioactivity.

At pH 5 and 100°C (60 minutes, simulating baking/ brewing/ boiling) the test item was hydrolysed to 63.2% of applied radioactivity. Two main hydrolysis products M5 and M6 were detected at levels of 16.3% and 17.7% of applied radioactivity, respectively. M6 was identified as R3A (2-((Z)-4-cyclopropyl-4-hydroxy-2-oxo-but-3-enyl)-succinic acid CGA 313458), after additional study M5 was shown to be cyclopropane carboxylic acid.

At pH 6 and 120°C (20 minutes, simulating the process of sterilisation) the test item corresponded to 82.1% of applied radioactivity. Four hydrolysis products were detected, with M1, M4, M5 and M6 accounting for 0.9%, 4.0%, 3.8% and 8.4% of applied radioactivity, respectively.

Study was well performed and reported.

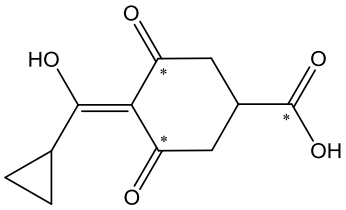
Deviations from OECD 507:

No deviations from OECD guideline 507 were observed.

Study 4
New high temperature hydrolysis study of trinexapac acid (Cheminova)

Reference:	**Flörchinger, M (2008)**. Abiotic Degradation (Hydrolysis) of [^{14}C]-Trinexapac under Typical Conditions (pH, Temperature and Time) of Processing. (KCA 6.5.1 / ~~0~~402)
Study No.:	S08-03106
Guideline:	EU 1607/VI/97 rev.2 from 10/06/1999: Guidelines for the generation of data concerning residues as provided in Annex II part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market 7035/VI/95 rev.5: Appendix E – Processing studies
GLP:	OECD Principles of Good Laboratory Practice

German principles of GLP, which are based on OECD GLP.

Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test Item:	[^{14}C]-Trinexapac acid (CAS No. 143294-89-7)
Position of the radiolabel: * - denotes the position of ^{14}C	
Lot/Batch No:	2384CJW001-3
Purity:	98.9 %, specific activity 53.90 mCi/mmol
Test system:	50 mL of citrate buffer (pH 4, and 6) or acetate buffer (pH 5) was added to the test vials followed by 10 µL of the radioactive standard (10 µCi/10 µL in acetone) and 90 µL of cold standard (2.31 g/L in acetone) to obtain a concentration of 5 mg/L Trinexapac and an overall radioactivity of 10 µCi per vial. Aliquots of each treatment buffer solution were stabilised with 1/10 volume acidified acetonitrile, the total radioactivity determined by LSC and characterised using TLC to give the pre-processing values. Duplicate preparations of each treatment buffer solution were weighed and the pH 4 solutions were heated to 90°C for 20 minutes, the pH 5 solutions were heated to 100°C for 60 minutes and the pH 6 solutions were heated to 120°C for 20 minutes, in climatic chambers. A control samples from each pH group was incubated at room temperature for the test duration. All samples were kept in the dark to avoid prospective degradation as a result of photolysis.
Sampling time points:	At time 0 and after 20 or 60 minutes of incubation, duplicate samples per pH value were taken. All analyses were performed within 6 months period, thus no storage stability test was necessary.
Method of analysis:	After equilibration of the samples at ambient temperature, the test and control samples were weighed and then stabilised by addition of 1/10 volume acidified acetonitrile before being taken for quantification by LSC and characterisation by TLC.
Method validation:	The post-hydrolysis quantification results based on the actual amount of radioactivity applied to the solutions shows recoveries ranging from 96.6 to 101.5% for the test samples and 98.5% to 103.6% for the control samples. These results indicate that there was no significant loss of radioactivity during the experimental procedures.

Sample	Radioactive Recovery (%)					
	pH4, 90°C, 20 min		pH5, 100°C, 60 min		pH6, 120°C, 20 min	
	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	Vial 2
Treated Incubate	96.6	101.5	100.0	99.3	99.7	100.1

	Incubate Control (Vial 3)	99.6	103.6	98.5

Limit of quantification: Not stated

Date of experiment: October 2008 – December 2008

Results

Aliquots of all test and control samples were analysed by TLC and it was demonstrated that the majority of the recovered radioactivity was [^{14}C]-Trinexapac. The TLC results show that no metabolites are formed during processing under conditions 1 (pH 4, 90°C, 20 min) and 3 (pH 6, 100°C, 60 min). Processing under condition 2 (pH 5, 100°C, 60 min) increased the content of the known metabolites 3-carboxyl-7-cyclopropyl-5,7-dioxoheptanoic acid (1.5%) and Cyclodion acid (1.7%). No other hydrolysis products were formed during processing.

The levels of [^{14}C]-Trinexapac acid in the test samples are summarised in table B.7.5.1-7.

Table B.7.5.1-7: Summary of levels of [^{14}C]-trinexapac acid and metabolites after processing

	Levels of [^{14}C]-Trinexapac acid and metabolites (%)					
	pH4, 90°C, 20 min		**pH5, 100°C, 60 min**		**pH6, 120°C, 20 min**	
	before	**after**	**before**	**after**	**before**	**after**
Treated sample:						
Trinexapac acid	96.1	95.2	96.3	93.1	98.9	97.7
3-carboxyl-7-cyclopropyl-5,7-dioxoheptanoic acid	2.7	2.7	2.0	3.5	1.1	2.3
Cyclodion acid CGA113745 (3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid)	1.2	2.2	1.7	3.4	-	-
Control sample:						
Trinexapac acid	96.6	96.8	96.2	97.5	99.2	98.8
3-carboxyl-7-cyclopropyl-5,7-dioxoheptanoic acid	1.9	1.6	2.5	1.8	0.8	1.2
Cyclodion acid CGA113745 (3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid)	1.5	1.6	1.4	0.7	-	-

RMS comments and conclusions

The study results show that Trinexapac acid is hydrolytically stable under conditions representative of pasteurisation, baking, brewing, boiling and sterilisation. Study is suitable for the overall assessment.

Deviations from OECD 507:

LOQ not stated in the report. It was explained by the applicant that this study was conducted to meet the requirements given in EC Directive 91/414, Appendix E, 7035/VI/95, 22nd July 1997. EC directive 91/414 does not state that a LOQ should be provided.

Summary

The effect of processing on the nature of trinexapac-ethyl and trinexapac acid was investigated in the framework of the peer review. Studies were conducted by Syngenta simulating representative hydrolytic conditions for pasteurisation (20 minutes at 90°C, pH 4), boiling/brewing/baking (60 minutes at 100°C, pH 5) and sterilisation (20 minutes at 120°C, pH 6). Two other studies were conducted by the members of the Task Force and are therefore submitted. Results of all these studies are presented in the table B.7.5.1-8.

In the studies conducted by Syngenta and Cheminova, trinexapac acid was radiolabelled in the cyclohexane ring while the Adama study has been conducted with a different radiolabelled position (cyclopropane ring).

The Syngenta and Adama studies show that trinexapac acid degrades under elevated temperatures conditions, but represents the major part of the residue (~51-86% TRR). Degradation products identified are CGA313458 (~4-21% TRR), CGA113745 (~10-12% TRR) and cyclopropane carboxylic acid (CGA224439) (~5-18% TRR), which haven't been found in the rat metabolism.

The Cheminova study shows that trinexapac acid remains stable under pasteurisation, baking/boiling/brewing and sterilisation conditions – which is different from the Syngenta and Adama studies.

It can be concluded that the nature of residues in processed commodities is different to the one in raw agricultural commodities.

Table B.7.5.1-8: Summary of high temperature hydrolysis studies

Conditions	Identified Compounds (%)	Report Reference	EU-review reference
EU Reviewed Data			
Trinexapac-ethyl			
Pasteurisation (20 min, 90°C, pH 4)	Trinexapac-ethyl (99%)	01RC02	The Netherlands, 2003
Baking, boiling, brewing (60 min, 100°C, pH 5)	Trinexapac-ethyl (99%)		
Sterilisation (20 min, 120°C, pH 6)	Trinexapac-ethyl (99%)		

Trinexapac acid			
Pasteurisation (20 min, 90°C, pH 4)	Trinexapac acid (52.5%) CGA113745 (9.6%) CGA313458 (19.7%)	RJ3480B (Syngenta)	The Netherlands, 2005
Baking, boiling, brewing (60 min, 100°C, pH 5)	Trinexapac acid (58.5%) CGA113745 (10.5%) CGA313458 (16.1%)		
Sterilisation (20 min, 120°C, pH 6)	Trinexapac acid (50.9%) CGA113745 (11.6%) CGA313458 (21.0%)		
New data			
Trinexapac acid			
Pasteurisation (20 min, 90°C, pH 4)	Trinexapac acid (85.8%) CGA313458 (4.7%) CGA224439 (5.4%)	C93481 (Adama)	-
Baking, boiling, brewing (60 min, 100°C, pH 5)	Trinexapac acid (63.2%) CGA313458 (~~3.8~~ 17.7%) CGA224439 (16.3%)		
Sterilisation (20 min, 120°C, pH 6)	Trinexapac acid (82.1%) CGA313458 (8.4%) CGA224439 (~~17.7~~ 3.8%)		
Pasteurisation (20 min, 90°C, pH 4)	Trinexapac acid (95.2%) CGA313458 (2.7%)* CGA113745 (2.2%)*	S08-03106 (Cheminova)	-
Baking, boiling, brewing (60 min, 100°C, pH 5)	Trinexapac acid (93.1%) CGA313458 (3.5%)* CGA 113745 (3.4%)*		
Sterilisation (20 min, 120°C, pH 6)	Trinexapac acid (97.7%) CGA313458 (2.3%)*		

* - these metabolites were found before and after hydrolysis in both control and treated samples at quite equal amounts and are not considered as degradation products.

B.7.5.2 Distribution of residues in peel and pulp

Not relevant based on the intended uses.

B.7.5.3 Magnitude of residues in processed commodities

As residues of trinexapac acid are expected to exceed 0.1 mg/kg in the RAC and as several degradates (>10 %TRR) were formed in the high temperature hydrolysis studies, investigation of the magnitude of residues in processed commodities has been conducted.

Processing studies of barley and wheat have been evaluated in the DAR 2003, but only trinexapac acid (free form) was measured in those studies. Eight studies (study 1 to 8) were conducted in order to investigate the influence of processing of the residue in winter and spring barley after single application of trinexapac-ethyl (CGA 163935). One study (study 13) was conducted in order to investigate the influence of processing of the residue in winter wheat after single application of trinexapac-ethyl (CGA 163935) at a rate of 0.2 kg as/ha. Those studies were ~~not~~ re-evaluated by the RMS LT and presented in combined form reflecting the style of the DAR.

Three additional studies on barley and wheat were conducted in 2006 and 2008; they measured the residue levels of trinexapac acid (free or free and conjugated) in flour and milling by-products. Details of these studies (study 9 for barley and study 14 to 15 for wheat) are summarised below.

New processing studies on barley (study 10 to 12) and wheat (study 16 to 18) have been conducted, in order to:

- mimic the representative processing conditions such as baking and brewing;
- measure trinexapac acid (free and conjugated) in raw agricultural commodities (RAC) and processed products;
- measure processing degradates CGA313458, CGA113745 and cyclopropane carboxylic acid (CPCA, also referred to as CGA224439).

The studies have been conducted at an elevated application rate (1×400 g a.s./ha; 2N for barley and 3.2N for wheat). In each study, two trials were conducted and the samples from each trial were split into two portions and taken through the processing procedures separately. Four residue values and processing factors were then derived for each processed commodity. Details of these studies are summarised below.

Barley

Study 1 to 8
EU reviewed processing studies of the residue in winter and spring barley

Reference:	**Maffezzoni M. (1999)** Residue study with CGA 163935 + Ethephon in or on winter barley in north of France. (~~KCA 6.5.3 / 01~~ KIIA 6.5.3.2 / 01)
Report No.:	9821701
Reference:	**Maffezzoni M. (1999a)** Residue study with CGA 163935 + Ethephon in or on winter barley in north of France. (~~KCA 6.5.3 / 02~~ KIIA 6.5.3.2 / 02)
Report No.:	9821702
Reference:	**Maffezzoni M. (1999b)** Residue study with CGA 163935 + Ethephon in or on spring barley in north of France. (~~KCA 6.5.3 / 03~~ KIIA 6.5.3.2 / 03)
Report No.:	9821801

Reference:	**Maffezzoni M. (1999c)** Residue study with CGA 163935 + Ethephon in or on spring barley in north of France. (~~KCA 6.5.3 / 04~~ KIIA 6.5.3.2 / 04)
Report No.:	9821802
Reference:	**Maffezzoni M. (1999d)** Residue study with CGA 163935 in or on spring barley in north of France. (~~KCA 6.5.3 / 05~~ KIIA 6.5.3.2 / 05)
Report No.:	9822002
Reference:	**Maffezzoni M. (1999e)** Residue study with CGA 163935 in or on spring barley in north of France. (~~KCA 6.5.3 / 06~~ KIIA 6.5.3.2 / 06)
Report No.:	9822001
Reference:	**Maffezzoni M. (1999f)** Residue study with CGA 163935 in or on winter barley in north of France. (~~KCA 6.5.3 / 07~~ KIIA 6.5.3.2 / 07)
Report No.:	9821902
Reference:	**Maffezzoni M. (1999g)** Residue study with CGA 163935 in or on winter barley in north of France. (~~KCA 6.5.3 / 08~~ KIIA 6.5.3.2 / 08)
Report No.:	9821901
Guideline:	Directive 91/414/EC; 7029/VI/95, appendix B FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990)
GLP:	OECD Principles of Good Laboratory Practice (GLP) in France and of the OECD, in accordance with the protocol and in compliance with sops in use at ADME Bioanalyses*.
Previous evaluation:	DAR 2003
Material and methods:	
Test material:	Trinexapac-ethyl (CGA 163935)
Lot/Batch No:	802067 (study 1 to 5)
Sampling time points:	Barley grain samples were collected 67-90 DAT, and were processed in malt, tepral wort and beer.
Test concentration:	122 g a.s./ha, one application (study 1) 127 g a.s./ha, one application (study 2) 71 g a.s./ha, one application (study 3) 774 g a.s./ha, one application (study 4) 150 g a.s./ha, one application (study 5) 147 g a.s./ha, one application (study 6) 217 g a.s./ha, one application (study 7) 198 g a.s./ha, one application (study 8)
Test system:	Eight studies were conducted in order to investigate the influence of processing of the residue in winter and spring barley after a single application of CGA 163935. Barley grain was harvested and processed to beer, malt and wort fractions. ≥50 kg of treated and untreated grain samples each were shipped to processing test facility. These were processed into:

Study	Grain end of	Malt	Tepral Wort	Beer

	steeping			
Study 1	13 kg	7 kg	771 ml	17.5 L
Study 2	13 kg	7 kg	790 ml	17 L
Study 3	13 kg	7 kg	804 ml	18.5 L
Study 4	16 kg	9 kg	799 ml	18.5 L
Study 5	13 kg	7 kg	787 ml	18.5 L
Study 6	13 kg	7 kg	791 ml	18 L
Study 7	13 kg	7 kg	785 ml	17 L
Study 8	13 kg	7 kg	765 ml	17 L

Storage conditions until shipment: At or below -18°C for specimens of analysis;
At ambient temperature for process specimens.

Method of analysis: The amount of the metabolite CGA 179500 in the different (by-)products was measured applying method AGR/MOA/TRIN-06 (based on Ciba-method REM 137.08). For detail evaluation of the analytical method, please refer to Vol 3 CA B.5.1.2.2 (KCA 4.1.2.15).

Recoveries are provided below:

Crop	Fortification level	Grain	Malt	Wort	Beer
Barley	0.02 mg/kg	80; 83; 85; 70%	95, 108; 80; 91 %	-	-
	0.20 mg/kg	81, 72; 80; 76%	94; 91; 89; 91 %	-	-
	0.01 mg/l	-	-	89; 100; 101; 93 %	80; 89; 96; 96 %
	0.10 mg/l	-	-	92; 93; 91; 90 %	89; 84; 84; 84 %

Mean procedural recovery of trinexapac acid were in the range 70% to 85% (78% mean) for grain, in the range 80% to 108% (92% mean) for malt, in the range 89% to 101% (94% mean) for wort and in the range 80% to 96% (88% mean) for beer.

Limit of quantification: 0.02 mg/kg for grain and malt
0.01 mg/kg for wort and beer

Reference items: Trinexapac acid (free) Number BPS 520/103

Purity 99%

***-** Parts not performed according to GLP: recording weather data, characterization of soil and maintenance treatments.

Results

The test material for the processing studies was grown in different trials. The characteristic features of these trials are summarised in table B.7.5.3-1.

Table B.7.5.3-1. Barley treatment data and the references of the accessory reports

Location	Variety	Application rate kg as/ha		No. of applications	DAT	Reference
North France (Le Thour)	Plaisant (Winter barley)	CGA 163935	0.12	1	90	Maffezzoni, 1999
North France (Brazey en Plaine)	Plaisant (Winter barley)	CGA 163935	0.13	1	83	Maffezzoni, 1999a
North France (St Hilaire le Petit)	Alexis (Spring barley)	CGA 163935	0.07	1	74	Maffezzoni, 1999b
North France (Esbarres)	Prisma (Spring barley)	CGA 163935	0.77	1	67	Maffezzoni, 1999c
North France (Esbarres)	Prisma (Spring barley)	CGA 163935	0.15	1	67	Maffezzoni, 1999d
North France (St Hilaire le Petit)	Alexis (Spring barley)	CGA 163935	0.15	1	74	Maffezzoni, 1999e
North France (Brazey en Plaine)	Plaisant (Winter barley)	CGA 163935	0.22	1	83	Maffezzoni, 1999f
North France (Le Thour)	Plaisant (Winter barley)	CGA 163935	0.20	1	90	Maffezzoni, 1999g

The levels and transfer factors of CGA 179500, the major metabolite of CGA 163935, in winter and spring barley grains and processed samples are presented in table B.7.5.3-2. Residues in untreated samples were in all cases below 0.02 mg/kg for grain and malt, and below 0.01 mg/kg for wort and beer.

Table B.7.5.3-2. CGA 179500 levels in processed winter and spring barley grain samples and concomitant transfer factors*

Product	Total residue CGA 179500 (mg/kg or mg/L)[1]	Transfer factor	Reference
Grain	0.06; 0.06 (0.06)	-	Maffezzoni, 1999
Malt	0.03; 0.03 (0.03)	0.5	
Wort	<0.01; <0.01 (<0.01)	<0.2	
Beer	<0.01; <0.01 (<0.01)	<0.2	

Product	Total residue CGA 179500 (mg/kg or mg/L)[1]	Transfer factor	Reference
Grain	<0.02	not applicable	Maffezzoni, 1999a
Malt	<0.02		
Wort	<0.01		
Beer	<0.01		
Grain	<0.02	not applicable	Maffezzoni, 1999b
Malt	<0.02		
Wort	<0.01		
Beer	<0.01		
Grain	0.03; 0.03 (0.03)	-	Maffezzoni, 1999c
Malt	0.02; 0.02 (0.02)	0.7	
Wort	<0.01; <0.01 (<0.01)	<0.3	
Beer	<0.01; <0.01 (<0.01)	<0.3	
Grain	0.06; 0.06 (0.06)	-	Maffezzoni, 1999d
Malt	0.04; 0.04 (0.04)	0.7	
Wort	0.01; 0.01 (0.01)	0.2	
Beer	<0.01; <0.01 (<0.01)	<0.2	
Grain	0.03; 0.03 (0.03)	-	Maffezzoni, 1999e
Malt	0.03; 0.02 (0.03)	0.8	
Wort	<0.01; <0.01 (<0.01)	<0.3	
Beer	<0.01; <0.01 (<0.01)	<0.3	
Grain	<0.02	not applicable	Maffezzoni, 1999f
Malt	<0.02		
Wort	<0.01		
Beer	<0.01		
Grain	0.08; 0.07 (0.08)	-	Maffezzoni, 1999g
Malt	0.05; 0.05 (0.05)	0.7	
Wort	0.01; 0.01 (0.01)	0.1	
Beer	<0.01; <0.01 (<0.01)	<0.1	

***** data not corrected for recovery

[1] mg/kg for grain and malt; mg/L for wort and beer/ data represent range and the mean of 2 determinations

RMS comments and conclusion (RMS Netherlands)

The processing of barley grain to beer did not result in a transfer of the metabolite CGA179500 into either wort or beer. The CGA 179500 residue levels for both processing products were around or below the LOQ (0.01 mg/L). Some transfer takes place into the malt fraction resulting in concentrations between the LOQ (0.02 mg/kg) and 0.05 mg/kg. All transfer factors were <1, indicating a reduction of residues after processing of barley grain to malt, wort and beer.

The studies are considered as suitable for evaluation.

RMS LT comments

Results from study 2, 3 and 7 (Maffezzoni, 1999a; Maffezzoni, 1999b and Maffezzoni, 1999f) were not used in the calculation of processing factors as residues in grain (RAC) were below LOQ, therefore these studies were not re-evaluated by the RMS LT.

Deviations from OECD 508:

Study 1: Residues in grain is less than recommended in OECD 508 (0.06 instead of 0.1 or 10 times the LOQ). Grains used for processing were stored at ambient temperature for 7-12 months from sample to processing, and for maximum of 3-4 months from processing till analysis – storage conditions for this period not reported.

Study 4: Residues in grain is less than recommended in OECD 508 (0.03 instead of 0.1 or 10 times the LOQ). Grains from sample to processing were stored for 6-11 months at ambient temperature, from processing till analysis – for maximum of 4-5 months, storage conditions for this period not reported.

Study 5: Residues in grain is less than recommended in OECD 508 (0.06 instead of 0.1 or 10 times the LOQ). Grains from sample to processing were stored for 6-11 months at ambient temperature, from processing till analysis – for maximum of 4-5 months, storage conditions for this period not reported.

Study 6: Residues in grain is less than recommended in OECD 508 (0.03 instead of 0.1 or 10 times the LOQ). Grains from sample to processing were stored for 6-11 months at ambient temperature, from processing till analysis – for maximum of 4-5 months, storage conditions for this period not reported.

Study 8: Residues in grain is less than recommended in OECD 508 (0.08 instead of 0.1 or 10 times the LOQ). Grains used for processing were stored at ambient temperature for 7-12 months from sample to processing, and for maximum of 3-4 months from processing till analysis – storage conditions for this period not reported.

Residue values in grain were obtained from samples stored deep frozen, no analysis of grain stored at ambient temperature and just before the processing was performed. Residue levels in grain are covered by storage stability data, whereas residue levels in processed commodities are questionable due to quite long storage at ambient temperature of grain before processing. No data showing that the storage did not affect the results of the study were available in the study reports. According to OECD 508 for pre-harvest uses, samples should be processed as soon as possible following harvest in order to keep the integrity of the RAC.

Processing factors derived from studies 1, 4, 5, 6 and 8 are considered not reliable due to unclear impact of 6-12 months storage at ambient temperature of grain prior processing and were not used in the assessment.

Study 9

New processing study of the magnitude of residue in barley

Reference:	**Mäyer T. (2010)** Trinexapac-ethyl – Magnitude of the Residues in or on Barley. Syngenta Crop Protection, Greensboro, USA. Syngenta File No. CGA163935_50026. (KCA 6.5.3 / ~~09~~01)
Report No.:	T003422 (including two trials the USA)
Guideline:	U.S. EPA OPPTS 860 Series Guidelines
GLP:	EPA Good Laboratory Practice Standards (40 CFR Part 160) with some exceptions*
Previous evaluation:	Submitted for the purpose of renewal
Reason for submission:	Measure trinexapac acid (free and conjugated) in raw agricultural commodities (RAC) and processed products
Material and methods:	
Test material:	Trinexapac-ethyl (CGA 163935)
Lot/Batch No:	ID 428456
Sampling time points:	Grain samples were collected at normal commercial harvest time (45 DAT), and were processed in pearled barley, flour and bran.
Test concentration:	0.129 g a.s./ha, one application at BBCH 32 (treatment 2 and 3) 0.644 g a.s./ha, one application 45 PHI (treatment 4)
Test system:	Two processing trials were conducted with a trinexapac-ethyl 250 EC formulation (CGA 163935) containing 0.25 kg a.s./L product. The product was applied to barley as a foliar broadcast spray with 19 - 468 L water/ha. Four application regimes were followed. Treatment 1 corresponded to the non-treated (control) plot. Treatment 2 received 0.129 kg a.s./ha at BBCH 32. Treatment 3 and 4 received 0.129 kg a.s./ha or 0.644 kg a.s./ha, respectively, 45 days prior to harvest of mature grain. Barley was processed in a manner that simulated industrial practice as closely as possible. The moisture content of whole barley was determined. All samples were oven-dried at 54 71°C to a moisture content of 11-13.5%. Samples were then cleaned by aspiration and screening. Light impurities were separated from the whole barley by aspiration. 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 Chopin mill to break the grains. Subsequently, the broken grain was fed onto sifter screens (0.14 and 0.80 mm) to obtain course bran, break flour and middlings. Course 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 sifting bran and middlings were combined as well. Because of compliance monitoring requirements and sample size, the samples were processed by batch rather than continuously, as in commercial operation.
Method of analysis:	All samples were analysed for residues of trinexapac-ethyl (CGA 163935) expressed as its plant metabolite trinexapac (CGA 179500) using analytical method GRM 020.01A. Residues of trinexapac (CGA 179500) were extracted from the sample matrices using acetonitrile/1N hydrochloric acid solution (80:20, v/v). An aliquot of the extract was passed with water through a pre-conditioned C8-SPE cartridge. The SPE cartridge was rinsed with water followed by water/acetonitrile (80:20, v/v). The analyte was eluted from the cartridge with formic acid aqueous solution/acetonitrile (80:20, v/v). Final determination was done by high performance liquid

chromatography (HPLC) with tandem triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantitation (LOQ) for trinexapac (CGA 179500) was 0.01 mg/kg for all matrices.

For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/08 and KCA.4.1.2/09).

The recoveries from barley grain, pearled barley, flour and bran fortified at the LOQ and 100-2000 multiples of the LOQ ranged from 72% to 114% for trinexapac (CGA 179500).

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥3)
Barley grain	0.01-11	16	72-111	89 ± 12.4
Pearled barley	0.01-20	2	108-114	111 ± N/A
Barley flour	0.01-20	2	102-111	106 ± N/A
Barley bran	0.01-20	2	91-100	96 ± N/A

Limit of quantification: 0.01 mg/kg

Storage conditions: Frozen at -20°C for up to 16 months from sampling to analysis.

Reference items: Trinexapac acid (CGA 179500), Lot Number GB-XLII-8B

Purity 99.9%

***** NOAA weather data was not collected according to the FIFRA-GLP requirements of 40 CFR Part 160;

Spray-mix storage stability data were not generated as required in 40 CFR Part 160;

The application of maintenance chemicals and irrigation practices did not conform to GLP requirements;

Analysis of soil characteristics did not conform to GLP requirements;

Field history from growers records were not generated not maintained under GLP;

Sample weights taken in the field were determined by non-GLP procedures.

Results

Residues in barley grain were 0.51 - 0.75 mg/kg after the low-dose treatment (application rate 0.129 kg a.s./ha), and 5.6 - 6.7 mg/kg after the high-dose treatment (application rate 0.644 kg a.s./ha). The residues of trinexapac (CGA179500) were slightly concentrated in processed fractions of pearled barley and barley bran from both treatments. Residues in barley flour were reduced as compared to the residue of the corresponding barley grain sample. Transfer factors of trinexapac (CGA179500) residues were 0.86 - 1.5 for pearled barley, 1.6 - 2.2 for barley bran, and 0.25 - 0.63 for barley flour. For details see Table A3.7-1. (Table B.7.5.3-3).

Table B.7.5.3-3. Residues of trinexapac acid (CGA 179500, free and conjugated) in barley and processed commodities

Description of specimens	Application rate [kg a.s./ha]	Residue [mg/kg]	Mean residue [mg/kg]	Transfer factor
Trial C13ND081704				
Barley grain*	0.129	0.50, 0.55, 0.48	0.51	-
	0.644	9.1, 5.2, 5.9	6.7	-
Pearled barley	0.129	0.78, 0.72	0.75	1.5
	0.644	10.1, 8.6	9.4	1.4
Barley flour	0.129	0.26, 0.31	0.29	0.57
	0.644	4.6, 3.7	4.2	0.63
Barley bran	0.129	1.0, 1.1	1.1	2.2
	0.644	10.8, 10.9	10.9	1.6
Trial C13ND081705				
Barley grain*	0.129	0.65, 0.84, 0.76	0.75	-
	0.644	5.2, 5.4, 6.1	5.6	-
Pearled barley	0.129	0.82, 0.89	0.86	1.2
	0.644	5.5, 4.1	4.8	0.86
Barley flour	0.129	0.24, 0.18	0.21	0.28
	0.644	1.4, 1.4	1.4	0.25
Barley bran	0.129	1.4, 1.3	1.4	1.9
	0.644	10.1, 9.6	9.9	1.8

* pre-processing Formulation = trinexapac-ethyl 250 EC Values are averages of multiple samples

RMS comments and conclusion

In the present study barley treated with trinexapac-ethyl (CGA 163935) at two different rates (0.129 and 0.644 kg a.s./ha) was processed into pearled barley, flour and bran. Residues of trinexapac acid in barley were not concentrated in flour (TF<1). Residues of trinexapac acid were slightly concentrated in pearled barley (TF = 0.86 - 1.5) and barley bran (TF = 1.6 - 2.2). However, the higher application rate was three times higher than the critical GAP for winter barley (200 g a.s./ha). Residue results are covered by storage stability data. The study was well performed and reported and suitable for evaluation.

Studies 10 to 12

New processing studies of the magnitude of trinexapac acid (free and total) and metabolites (CGA313458, CGA224439 and CGA113745) residue in barley

Reference: **MacDougall J. (2016)** Trinexapac-ethyl – Residue Processing Study on Barley in Spain and Italy in 2015. (Syngenta File No. A8587F_10526). (KCA 6.5.3 / ~~1~~004&

	KCA 6.3.1 / 06)
Report No.:	37194 (including two trials in SEU)
Study No.:	699779
Reference:	**Watson G. (2016)** Trinexapac-ethyl Analysis of Barley Processing Phase Specimens for CPCA from Study 699779 'Trinexapac-ethyl –Residue Processing Study on Barley in Spain and Italy in 2015' (Syngenta File No. A8587F_10526). (KCA 6.5.3 / ~~11~~05)
Report No.:	RES-00027
Study No.:	RES-00027
Reference:	**Langridge G. (2016)** Trinexapac-ethyl Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Barley Process Fractions. INTERIM REPORT submitted March 2016, (KCA 6.5.3 / 06) (Syngenta File No. CGA313458_10001) **Langridge G. (2016b)** Trinexapac-ethyl Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Barley Process Fractions. FINAL REPORT submitted January 2017. (KCA 6.5.3 / 10)
Report No.:	CEMR-7354
Study No.:	CEMR-7354
Guideline:	Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 244/2011 (283/2013) and 245/2011 (284/2013) implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals, Number 508 (2008): Magnitude of the Pesticide Residues in Processed Commodities and Number 509 (2009): Crop field trials. SANCO/825/00 rev.8.1 (16/11/2010) Guidance Document on Pesticide Residue Analytical Methods. European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.

Previous evaluation:	Submitted for the purpose of renewal
Reason for submission:	Measure trinexapac acid (free and total) and processing metabolites in raw agricultural commodities (RAC) and processed products in order to derive processing factors
Material and methods:	
Test material:	A8587F Trinexapac-ethyl
Lot/Batch No:	SMO3A0004
Sampling time points:	Grain samples were collected at normal commercial harvest time, and were processed to pot barley, pearl barley, flour, bran, brewing malt, malt sprouts, brewers grain (dried), brewer's yeast and beer
Test concentration:	403.7 g a.s./ha, one application at BBCH 49 (trial 1) 391.6 g a.s./ha, one application at BBCH 49 (trial 2)
Test system:	In study 699779 two residue field trials on field barley were conducted in North Spain and Italy during 2015. Trinexapac-ethyl was applied to field barley as A8587F, a micro-emulsion (ME) formulation containing 250 g trinexapac-ethyl per litre. Treated and control samples were collected at normal commercial harvest (NCH) for processing and for residue analysis. Samples were shipped frozen to the analytical facility for residue analysis and at ambient temperature to the processing facility. Each field trial generated a treated and an untreated field sample of grain. The untreated and treated grain samples were put through the relevant process. The treated grain for each trial was split into 2 portions (T1 and T2) with both being taken through the procedures. Barley grain was processed into pot barley, pearl barley, flour, bran, brewing malt, malt sprouts, brewers grain (dried), brewers' yeast and beer. Relevant industrial practices and standardised procedures were applied to simulate the common processes used by industry for production of pot barley, pearl barley, flour, bran, brewing malt, malt sprouts, brewers grain (dried), brewers' yeast and beer. Two analytical procedures were used to analyse the collected samples. Study RES-00027was conducted to generate results on the magnitude of residues for CPCA (CGA224439) in the barley processing specimens. Study CEMR-7354 was conducted to analyse processed fractions of barley for residues of CGA313458 and CGA113745 originally generated as part of Charles River Study Number 699779.
Processing phase:	Two follow-up procedures have been carried out on the processing of barley. Prior to each follow up processing study, barley samples collected and analysed to give a pre-processed residue value. Pot Barley and Pearl Barley processing: Before pot barley and pearl barley production, grain samples were cleaned and an optimal moisture content of barley grain of ca 14% was achieved. The samples were then hulled using a "Vertikal-Schälmaschine". Each sample was hulled until the stipulated abrasion for pot barley (20-25%) and the stipulated abrasion for pearl barley (30-35%) was reached. Flour and Bran processing During flour and bran production, grain samples were hulled using a "Vertikal-Schälmaschine". Each sample was hulled until the stipulated abrasion of 30-35% was reached. Abrasion was then sieved to bran and flour. The hulled grain was milled to flour. Afterwards the flour of the sieved abrasion and the flour of the milled hulled

grain were then mixed.

Brewing Malt and Malt Sprouts processing

Before brewing malt and malt sprouts production, grain samples were cleaned and sieved. Following sieving, a combined wet and dry steeping was conducted until a degree of steeping between 42-45% was achieved. After steeping, germination was conducted and samples were placed into a kiln for drying. Following kiln-drying the germs were removed mechanically using a trimmer and the malt sprouts were sampled. The malt was stored at room temperature until brewing to produce brewing malt. Brewing Malt samples were collected directly before brewing began.

Brewer's Grain (dried) processing

Samples of brewing malt were taken and mashed to allow enzyme degradation. The brewer's malt was milled and then mixed with brew water. Mashing was then started in a heatable tun. After mash boiling, the wort was separate from the insoluble malt components (brewer's grain). The extract remaining in the brewer's grain was then extracted by washing with hot water. The wort separation was done using a refining vat. After separation, brewer's grain was dried at 50°C until a dry matter content of <10% was reached and sampled as brewers grain dried.

Brewer's Yeast and Beer processing

During production of brewer's yeast, hop pellets were added and the separated wort boiled to deactivate the enzymes of the malt, sterilise the wort, extract and isomerise the essential components of the hops, precipitate high molecular proteins (called "Bruch") and expel unwanted aromatic substances. After boiling, the flocs (hops draff) were separated in a whirlpool causing the sludge to deposit on the bottom in the shape of a cone. An intra-plant circulation was used for cooling and ventilating. Oxygen was added to prepare the conditions for the start of fermentation. The pure culture yeast fermented sugar of the wort to alcohol and CO_2 as well as unwanted by-products (diacetyl, higher alcohols and others). Primary fermentation was carried out in bottom fermentation containers. As soon as the extract content of the fermented young beer was 2% higher than the final attenuation, storage began. Before maturation the young beer was cooled down. During the main fermentation the yeast was deposited on the tank bottom. At the beginning of maturation the young beer was stored at room temperature (warm maturation to break down the diacetyl) in casks. The young beer was then stored under pressure (approximately 0.7-1.2 bar) at ca 0-2°C (cold maturation) for 4 weeks. During this time the remaining extract was fermented. Unwanted flavour and odorous substances were decomposed or expelled. The rack beer was filtered using a special filter combination. During filtration, all organisms harming the beer (bacteria and yeast) were removed and sludge particles were separated. The final product beer was then sampled.

Method of analysis: Analytical procedure GRM020.005 was used to determine the free Trinexapac Acid residue. Residues of trinexapac acid are extracted with methanol/water/phosphate buffer solution. Extracts are centrifuged and an aliquot is acidified with 0.1M hydrochloric acid. Extracts are subjected to an Oasis HLB solid phase extraction (SPE) clean up. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA4.1.2/02). Procedural recovery data for trinexapac acid (free):

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥3)
Barley grain	0.01-2	14	72-115	96.4 ± 12

Straw	0.01-1	6	64-92	84 ± 13
Pot barley	0.01-1	6	82-100	92 ± 8
Pearled barley	0.01-1	6	91-109	101 ± 8
Bran	0.01-1	6	77-96	86 ± 7
Barley flour	0.01-2	6	50-95	81 ± 20
Brewing malt	0.01-1	6	55-100	86 ± 19
Malt sprouts	0.01-1	6	79-101	92 ± 10
Brewer grain, dried	0.01-2	6	94-103	100 ± 3
Brewer's yeast	0.01-1	6	83-96	90 ± 6
Beer	0.01-2	6	71-100	90 ± 14

Analytical procedure GRM020.009A was used to determine the total Trinexapac Acid residue (free and conjugated). Residues of trinexapac acid are extracted with an acetonitrile/water solution. Extracts are centrifuged and an aliquot is hydrolysed overnight in the presence of 1M Sodium Hydroxide. The hydrolysed extracts are portioned with ethyl acetate prior to clean up with an IST silica cartridge. Eluent from the first SPE stage are reduced to dryness prior to reconstitution with 0.1M hydrochloric acid followed by further clean up by Oasis HLB solid phase extraction (SPE). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/03a and KCA.4.1.2/04a) Procedural recovery data for total trinexapac acid:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥3)
Barley grain	0.01-2	12	62-86	87 ± 11
Straw	0.05-1	4	59-91	81 ± 18
Pot barley	0.01-1	6	71-89	76 ± 11
Pearled barley	0.01-1	6	82-90	86 ± 4
Bran	0.01-1	6	64-105	76 ± 20
Barley flour	0.01-1	6	76-90	82 ± 7
Brewing malt	0.01-1	6	72-88	83 ± 7
Malt sprouts	0.01-1	6	67-100	78 ± 15
Brewer grain, dried	0.01-2	6	56-78	72 ± 11
Brewer's yeast	0.01-1	6	82-99	93 ± 7
Beer	0.01-1	6	74-85	78 ± 5

Analytical procedure GRM020.15A was used to determine the residues of cyclopropanecarboxylic acid (CPCA or CGA224439). Residues are double extracted with an aliquot of prepared matrix with acetonitrile/water (50/50, v/v) + 0.01M HCl by maceration. Add magnesium sulphate, sodium chloride, sodium citrate dibasic

sesquihydrate and sodium citrate tribasic dehydrate to the sample extract to partition the organic and aqueous phase. Dilute an aliquot of the acetonitrile extract (x4). Derivatise an aliquot of the diluted acetonitrile extract by incubation at 60 ºC after addition of 2-Hydrazinoquinoline, triphenylphosphine and 2,2'-Dipyridyl disulphide.

Concentrate the derivatised extract to dryness and re-dissolve in deionised water Determination by HPLC-MS/MS. LOQ 0.01 mg/kg. For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/13) Procedural recovery for CPCA:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean (%)
Barley grain	0.01-0.05	2	70-99	85
Pot barley	0.01-0.05	2	98-99	99
Pearled barley	0.01-0.05	2	89-89	89
Bran	0.01-0.25	2	109*-124	117
Flour	0.01-0.05	2	66-74	70
Brewing malt	0.01-0.05	2	95-117	106
Malt sprouts	0.01-0.05	2	79-135	107
Brewers grain	0.01-0.05	2	92-97	95
Brewer's yeast	0.01-0.25	2	101-110	105
Beer	0.01-0.05	2	111-125	118
			Overall mean recovery (%)	99
			Overall RSD (%)	18.7

* - mean of two injections

Analytical procedure GRM020.013A draft was used to determine the residues of CGA313458. Residues were extracted by sequential homogenisation with 80/20 v/v acetonitrile/water and 50/50 v/v acetonitrile/water. An aliquot of the combined extracts equivalent to 0.2 g (2 mL) was evaporated to remove the acetonitrile. The sample was diluted with ultra-pure water and the pH adjusted to pH 7 –9 with dilute ammonium hydroxide solution. Samples were partitioned twice with ethyl acetate to remove co-extractives then the aqueous samples were filtered through an Oasis HLB SPE cartridge. Alternatively, samples may be analysed directly from the primary extracts without any further sample clean-up where there was sufficient instrument sensitivity. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/12). Procedural recovery for CGA313458:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean (%)	RSD (%)
Barley grain[a]	0.01-0.1	6	69-87	80	8.0
Bran	0.01-0.1	2	72-78	75	5.7
Flour[b]	0.01-0.1	2	71-71	71	0.0
Brewer's	0.01-0.1	8	73-105	87	11.9

yeast					
Beer	0.01-0.1	2	92-107	100	10.7
			Overall	83	13.0

a – Malt sprouts, Brewing malt and Brewer's grain, dried.

b – Pot barley and Pearled barley

The lowest fortification level is at the limit of quantification.

Mean RSD (%) are calculated using rounded figures.

Recoveries were not corrected for control residue.

Analytical procedure GRM020.014A draft was used to determine the residues of CGA113745. 1mL of beer samples were filtered through a Chromabond C18(EC) SPE cartridge and made up to a final volume of 10 mL with ultra-pure water. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). For the determination of CGA113745 in non-liquid brewing fractions 10 g sub samples of bread, grain, bran and flour were extracted by sequential homogenisation with 0.2% v/v ammonia in ultra-pure water. An aliquot of the combined extracts equivalent to 0.4 g (4 mL) was acidified and a 2mL aliquot was subjected to an Oasis WCX SPE clean-up. The sample was eluted with 10% acetonitrile in ultra-pure water and the acetonitrile removed by evaporation before the sample was made to 2mL with ultra-pure water. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/12). Procedural recovery for CGA113745:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean (%)	RSD (%)
Grain[a]	0.01-0.1	6	80-114	97	14.1
Bran	0.01-0.1	2	63-70	67	7.4
Flour	0.01-0.1	2	101-108	105	4.7
Yeast	0.01-0.1	6	94-107	103	5.9
Beer	0.01-0.1	2	70-72	71	2.0
			Overall	88.6	6.8

a – Malt sprouts, Brewing malt, Brewers grain (dried), Pot barley and Pearled barley

The lowest fortification level is at the limit of quantification.

Mean RSD (%) are calculated using rounded figures.

Recoveries were not corrected for control residue.

Sample storage conditions :

Specimens were stored frozen (-18 ^{0}C) for a maximum period of ~~240 days from receipt to analysis for CGA313458 and 349 days from receipt to analysis for CGA113745~~ 12 months from sample to analysis for CGA 113745;

Maximum 7 months from sample to analysis and 6 months from processing to analysis for CGA 313458 and CGA 224439;

Maximum 8.5 months from sample to analysis and 6 months from processing to analysis for CGA 179500 (trinexapac acid).

	Extract solutions were stored for a maximum of 2 days before analysis. Stability of the analytes in the specimen extracts was proven by the corresponding procedural recovery specimens, which were stored under the same conditions together with the sample extracts.
Limit of quantification:	GRM020.005 0.01 mg/kg (free trinexapac acid) GRM020.009A 0.01 mg/kg for all matrices, 0.05 mg/kg for straw (free and conjugated trinexapac acid) GRM020.15A 0.01 mg/kg GRM020.013A draft 0.01 mg/kg GRM020.014A draft 0.01 mg/kg for beer samples only
Reference items:	Trinexapac acid (CGA179500) Batch Number MLA-372/1, purity 99.0% CPCA (CGA224439) Lot Number STBB9094V, purity 99.0% CGA313458 Batch Number DAH-XXXV-15, purity 96.1% CGA113745 Batch Number MES 420/1, purity 99.0%

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles.

Results

Residues of trinexapac acid (free) in barley grain at harvest were 0.11 and 0.32 mg/kg (Table B.7.3.1-2), before processing were 0.15 to 0.27 mg/kg (Table B.7.5.3-4 to 6). Residues of trinexapac acid (free and conjugated) in barley grain at harvest were 0.34 and 0.75 mg/kg (Table B.7.3.1-2.), before processing were 1.56 to 1.90 mg/kg (Table B.7.5.3-4 to 6).

Trinexapac acid (free) residues in processed commodities were up to 0.14 in pot barley, up to 0.13 in pearled barley, up to 0.46 in bran, up to 0.25 in flour, up to 0.20 in brewing malt, up to 0.25 in malt sprouts, up to 0.10 in brewers' grain (dried), up to 0.41 in brewers' yeast and up to 0.04 mg/kg in beer (Table B.7.5.3-4 to 6).

Trinexapac acid (free and conjugated) residues in processed commodities were up to 0.45 in pot barley, up to 0.33 in pearled barley, up to 0.81 in bran, up to 0.97 in flour, up to 0.99 in brewing malt, up to 0.22 in malt sprouts, up to 0.24 in brewers' grain (dried), up to 0.42 in brewers' yeast and up to 0.11 mg/kg in beer (Table B.7.5.3-4 to 6).

Residues of metabolite CGA313458 in barley grain and processed fractions were all below LOQ (0.01 mg/kg) except for one beer sample where residue of 0.01 mg/kg was measured (Table B.7.5.3-4 to 6).

Residues of metabolite CGA224439 in barley grain at harvest were 0.03 to 0.05 mg/kg, up to 0.02 in pot barley and pearled barley, up to 0.12 in bran, up to 0.03 in flour, up to 0.01 in brewing malt, up to 0.03 in malt sprouts, up to 0.01 in brewers' grain (dried), up to 0.11 in brewers' yeast and up to 0.02 mg/kg in beer (Table B.7.5.3-4 to 6).

Residues of metabolite CGA113745 in barley grain at harvest were 0.01 to 0.03 mg/kg and found only in two bran samples at the level of 0.01 mg/kg (Table B.7.5.3-6). Although due to instability of metabolite CGA113745 and poor chromatography, all results should be disregarded and have been struck through.

Transfer factors of residues from barley grain to processed commodities are presented in tables from B.7.5.3-7 to B.7.5.3-~~11~~ 10. Transfer Factor = residue in processed product/residue in RAC.

Table B.7.5.3-4. Residues of trinexapac acid and metabolites in processed barley (pot and pearled) commodities

Sample Name	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 313458	CGA 224439	~~CGA 113745~~
Trial T1A							
1-010-4	Prior processing	Grain	0.21	1.68	<0.01	0.03	~~0.02~~
1-010-5	Prior processing	Grain	0.25	1.82	<0.01	0.03	~~0.02~~
1-010-6	Grain	Pot Barley	0.13	0.39	<0.01	0.01	~~<0.01~~
1-010-7	Grain	Pearled Barley	0.12	0.30	<0.01	<0.01	~~<0.01~~
Trial T1B							
1-010-8	Prior processing	Grain	0.22	1.91	<0.01	0.03	~~0.02~~
1-010-9	Prior processing	Grain	0.24	1.83	<0.01	0.03	~~0.02~~
1-010-10	Grain	Pot Barley	0.14	0.45	<0.01	0.01	~~<0.01~~
1-010-11	Grain	Pearled Barley	0.13	0.33	<0.01	<0.01	~~<0.01~~
Trial T2A							
2-020-46	Prior processing	Grain	0.15	1.58	<0.01	0.03	~~0.03~~
2-020-47	Prior processing	Grain	0.16	1.58	<0.01	0.04	~~0.02~~
2-020-48	Grain	Pot Barley	0.13	0.23	<0.01	0.02	~~<0.01~~
2-020-49	Grain	Pearled Barley	0.12	0.27	<0.01	0.02	~~<0.01~~
Trial T2B							
2-020-50	Prior processing	Grain	0.23	1.56	<0.01	0.04	~~0.02~~
2-020-51	Prior processing	Grain	0.25	1.63	<0.01	0.04	~~0.02~~
2-020-52	Grain	Pot Barley	0.14	0.28	<0.01	0.02	~~<0.01~~
2-020-53	Grain	Pearled Barley	0.13	0.28	<0.01	0.02	~~<0.01~~

Table B.7.5.3-5. Residues of trinexapac acid and metabolites in processed barley (bran and flour) commodities

Sample Name	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 313458	CGA 224439	~~CGA 113745~~
Trial T1A							
1-010-15	Prior processing	Grain	0.21	1.86	<0.01	0.03	~~0.02~~
1-010-16	Prior processing	Grain	0.21	1.74	<0.01	0.03	~~0.02~~
1-010-17	Grain	Bran	0.39	0.67	<0.01	0.12	~~<0.01~~
1-010-18	Grain	Flour	0.16	0.89	~~<0.01~~	0.02	~~<0.01~~
Trial T1B							
1-010-19	Prior processing	Grain	0.27	1.77	<0.01	0.04	~~0.02~~
1-010-20	Prior processing	Grain	0.26	1.87	<0.01	0.03	~~0.02~~
1-010-21	Grain	Bran	0.46	0.81	<0.01	0.12	~~<0.01~~
1-010-22	Grain	Flour	0.25	0.97	~~<0.01~~	0.02	~~<0.01~~
Trial T2A							
2-020-57	Prior processing	Grain	0.23	1.64	<0.01	0.04	~~0.03~~
2-020-58	Prior processing	Grain	0.19	1.63	<0.01	0.04	~~0.03~~
2-020-59	Grain	Bran	0.17	0.20	<0.01	0.10	~~0.01~~
2-020-60	Grain	Flour	0.17	0.58	~~<0.01~~	0.03	~~<0.01~~
Trial T2B							
2-020-61	Prior processing	Grain	0.25	1.63	<0.01	0.04	~~0.03~~
2-020-62	Prior processing	Grain	0.18	1.64	<0.01	0.04	~~0.02~~
2-020-63	Grain	Bran	0.16	0.28	<0.01	0.09	~~0.01~~
2-020-64	Grain	Flour	0.19	0.60	~~<0.01~~	0.03	~~<0.01~~

Table B.7.5.3-6. Residues of trinexapac acid and metabolites in processed barley (beer) commodities

Sample Name	Processing	Processed product	Residue levels (mg/kg)

			Trinexapac Acid (free)	Total trinexapac acid	CGA 313458	CGA 224439	~~CGA 113745~~
Trial T1A							
1-010-29	Prior processing	Grain	0.22	1.78	<0.01	0.03	~~0.01~~
1-010-30	Prior processing	Grain	0.21	1.81	<0.01	0.03	~~0.01~~
1-010-31	Grain	Brewing Malt	0.19	0.99	<0.01	<0.01	~~<0.01~~
1-010-32	Grain	Malt Sprouts	0.25	0.22	<0.01	0.03	~~<0.01~~
1-010-33	Grain	Brewers Grain, Dried	0.07	0.24	<0.01	<0.01	~~<0.01~~
1-010-34	Grain	Brewer's Yeast	0.40	0.42	<0.01	0.06	~~<0.01~~
1-010-35	Grain	Beer	0.02	0.11	<0.01	0.01	~~<0.01~~
Trial T1B							
1-010-36	Prior processing	Grain	0.19	1.90	<0.01	0.03	~~0.02~~
1-010-37	Prior processing	Grain	0.24	1.26	<0.01	0.04	~~0.03~~
1-010-38	Grain	Brewing Malt	0.11	0.99	<0.01	<0.01	~~<0.01~~
1-010-39	Grain	Malt Sprouts	0.24	0.18	<0.01	0.03	~~<0.01~~
1-010-40	Grain	Brewers Grain, Dried	0.07	0.19	<0.01	<0.01	~~<0.01~~
1-010-41	Grain	Brewer's Yeast	0.41	0.14	<0.01	0.06	~~<0.01~~
1-010-42	Grain	Beer	0.04	0.11	<0.01	0.01	~~<0.01~~
Trial T2A							
2-020-71	Prior processing	Grain	0.22	1.65	<0.01	0.05	~~0.02~~
2-020-72	Prior processing	Grain	0.24	1.62	<0.01	0.05	~~0.02~~
2-020-73	Grain	Brewing Malt	0.20	0.70	<0.01	0.01	~~<0.01~~
2-020-74	Grain	Malt Sprouts	0.16	0.11	<0.01	0.02	~~<0.01~~
2-020-75	Grain	Brewers Grain, Dried	0.10	0.16	<0.01	0.01	~~<0.01~~
2-020-76	Grain	Brewer's Yeast	0.36	0.30	<0.01	0.11	~~<0.01~~
2-020-77	Grain	Beer	0.04	0.09	<0.01	0.02	~~<0.01~~
Trial T2B							
2-020-78	Prior processing	Grain	0.26	1.58	<0.01	0.04	~~0.02~~

Sample Name	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 313458	CGA 224439	~~CGA 113745~~
2-020-79	Prior processing	Grain	0.25	1.63	<0.01	0.05	~~0.02~~
2-020-80	Grain	Brewing Malt	0.20	0.64	<0.01	0.01	~~<0.01~~
2-020-81	Grain	Malt Sprouts	0.14	0.12	<0.01	0.02	~~<0.01~~
2-020-82	Grain	Brewers Grain, Dried	0.10	0.16	<0.01	0.01	~~<0.01~~
2-020-83	Grain	Brewer's Yeast	0.39	0.27	<0.01	0.11	~~<0.01~~
2-020-84	Grain	Beer	0.03	0.08	0.01	0.02	~~<0.01~~

The median transfer factors for each commodity from the follow-up studies are calculated and presented in Tables B.7.5.3-7 to B.7.5.3-~~11~~ 10. The average residue level in the RAC has been considered for the calculations because residues were measured in duplicate.

Transfer factors for trinexapac acid and the processing metabolites were derived by calculating the ratio of residue levels of residues into processed commodities to the residue levels in the RAC.

Table B.7.5.3-7. Summary of transfer factors into processed barley products – trinexapac acid (free)

Process	Transfer factors				Median Transfer Factor*
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Pot Barley	0.57	0.61	0.81	0.58	~~0.64~~ 0.65
Pearl Barley	0.52	0.57	0.75	0.54	0.60
Bran	1.86	1.70	0.81	0.73	1.28
Flour	0.76	0.93	0.81	0.86	~~0.84~~ 0.85
Brewing Malt	0.86	0.50	0.87	0.77	0.75
Malt Sprouts	1.14	1.09	0.70	0.54	0.87
Brewers' Grain (dried)	0.32	0.32	0.43	0.38	~~0.36~~ 0.37
Brewers' Yeast	1.82	1.86	1.57	1.50	1.69
Beer	0.09	0.18	0.17	0.12	~~0.14~~ 0.15

*- Median calculated for average results of A and B replicates.

Table B.7.5.3-8. Summary of transfer factors into processed barley products – total trinexapac acid (free+conjugates)

Process	Transfer factors				Median Transfer Factor*
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Pot Barley	0.22	0.24	0.15	0.18	0.20
Pearl Barley	0.17	0.18	0.17	0.18	0.18
Bran	0.37	0.45	0.12	0.17	0.28
Flour	0.49	0.53	0.35	0.37	0.44
Brewing Malt	0.55	0.63	0.43	0.40	~~0.50~~ 0.51
Malt Sprouts	0.12	0.11	0.07	0.07	~~0.09~~ 0.10
Brewers' Grain (dried)	0.13	0.12	0.10	0.10	~~0.11~~ 0.12
Brewers' Yeast	0.23	0.09	0.18	0.17	0.17
Beer	0.06	0.07	0.05	0.05	0.06

*- Median calculated for average results of A and B replicates.

Table B.7.5.3-9. Summary of transfer factors into processed barley products – CGA224439

Process	Transfer factors				Median Transfer Factor*
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Pot Barley	0.33	0.33	0.57	0.50	0.43
Pearl Barley	n.c.	n.c.	0.57	0.50	0.54
Bran	4	3.43	2.5	2.25	3.05
Flour	0.67	0.57	0.75	0.75	0.69
Brewing Malt	n.c.	n.c.	0.20	0.22	0.21
Malt Sprouts	1	0.86	0.40	0.44	0.68
Brewers' Grain (dried)	n.c.	n.c.	0.20	0.22	0.21
Brewers' Yeast	2	1.71	2.20	2.44	2.09
Beer	0.33	0.29	0.40	0.44	0.37

n.c.: not calculated because residues were <LOQ (0.01 mg/kg)

*- Median calculated for average results of A and B replicates.

Table B.7.5.3-10. Summary of transfer factors into processed barley products – CGA313458

Process	Transfer factors				Median Transfer Factor**
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Pot Barley	n.c.	n.c.	n.c.	n.c.	-

Process	Transfer factors				Median Transfer Factor**
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Pearl Barley	n.c.	n.c.	n.c.	n.c.	-
Bran	n.c.	n.c.	n.c.	n.c.	-
Flour	n.c.	n.c.	n.c.	n.c.	-
Brewing Malt	n.c.	n.c.	n.c.	n.c.	-
Malt Sprouts	n.c.	n.c.	n.c.	n.c.	-
Brewers' Grain (dried)	n.c.	n.c.	n.c.	n.c.	-
Brewers' Yeast	n.c.	n.c.	n.c.	n.c.	-
Beer	n.c.	n.c.	n.c.	1	1*

n.c.: not calculated because residues were <LOQ (0.01 mg/kg)

***** Results from one sample

**- Median calculated for average results of A and B replicates.

Table B.7.5.3-11. Summary of transfer factors into processed barley products – CGA113745

Process	Transfer factors				Median Transfer Factor
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Pot Barley	n.c.	n.c.	n.c.	n.c.	-
Pearl Barley	n.c.	n.c.	n.c.	n.c.	-
Bran	n.c.	n.c.	0.33	0.4	0.37
Flour	n.c.	n.c.	n.c.	n.c.	-
Brewing Malt	n.c.	n.c.	n.c.	n.c.	-
Malt Sprouts	n.c.	n.c.	n.c.	n.c.	-
Brewers' Grain (dried)	n.c.	n.c.	n.c.	n.c.	-
Brewers' Yeast	n.c.	n.c.	n.c.	n.c.	-
Beer	n.c.	n.c.	n.c.	n.c.	-

n.c.: not calculated because residues were <LOQ (0.01 mg/kg)

RMS comments and conclusion

Residues of trinexapac acid (free) and CGA224439 in barley grain were concentrated in bran and brewers' yeast (TF>1). Residues of trinexapac acid (free and conjugated) and metabolite CGA313458 were not concentrated in any of the processed fractions. Metabolite CGA113745 was not concentrated in any of the processed fractions and found only in bran at level 0.01 mg/kg. CGA113745 was found to be unstable in brewing and baking samples

(wheat grain, flour, bran, beer and bread) stored under frozen storage conditions.Only 20% CGA113745 was found after 30 days and samples in this study were analysed after maximum of 12 months of storage. ~~However, the application rate was two times higher than the critical GAP (400 g a.s./ha).~~.

Analytical method GRM020.14A for CGA113745 gave poor chromatography during the processing study so development work was carried out and the chromatography was improved. The improved chromatography was used in the storage stability study to analyse for CGA113745 in processed matrices and showed that CGA113475 was unstable in the presence of crop matrices - degrading to only 20% of the initial amount over 30 days.

Thus it can be assumed that inaccurate levels of CGA113745 were found in both the pre-processed incurred grain samples and the processed commodities due to degradation in storage and poor chromatography including possible co-elution with other components. Therefore any data regarding residue levels of CGA113745 in the processing studies on wheat and barley should be disregarded and have been struck through.

Barley samples for trinexapac acid analysis from sampling to analysis were stored for up to 8.5 months. From processing till analysis – stored for up to 6 months. Trinexapac acid is stable in grain for at least 24 months. Results are covered by storage stability data.

Samples for metabolite CGA 313458 analysis from sampling to analysis were stored for up to 7 months. From processing till analysis – stored for up to 6 months for pot barley, pearl barley, flour, bran brewing sprouts, brewer‘s grain and brewing malt, and up to 5 months for brewer‘s east and beer. As the metabolite CGA 313458 was shown to be stable for only 3 months on flour, any data regarding residue levels of this metabolite in flour in the processing studies on wheat and barley should be disregarded and have been struck through. Results are covered by storage stability data, exept for flour. Residue levels of CGA 313458 in flour as well as transfer factor in to flour should be assessed further.

Barley samples for metabolite CGA 224439 analysis from sampling to analysis were stored for up to 7 months. From processing till analysis – stored for up to 6 months. CGA 224439 is stable in grain and processed products for at least 12 months. Results are covered by storage stability data.

Studies were well performed and reported and suitable for evaluation.

Wheat

Study 13

EU reviewed processing study of the magnitude of residue in wheat

Reference:	**Gasser A. (2001)** Residue study with trinexapac-ethyl (CGA 163935) in or on winter wheat in France (North). (~~KCA 6.5.3 / 13~~ KIIA 6.5.3.1 / 01)
Report No.:	3011/00

Guideline:	Procedures and principles of GLP in Switzerland and of other involved OECD countries; OECD Principles on GLP (as revised in 1997) [C(97)186/Final]; FAO Guidelines on producing pestice residues data from supervised trials (Rome, 1990); Commission of the European Communities, 7029/VI/95 (rev. 5, working document) and 7035/VI/95 (rev. 5, working document); Guidelines and Criteria for the preparation and presentation of complete dossiers and of summary dossiers for the inclusion of active substances in Annex I of Directive 91/414/EEC (Article 5.3 and 8.2), 1996.
GLP:	Swiss Ordinance relating to GLP, adopted February 2nd, 2000 [RS 813.016.5]. This ordinance is based on the OECD principles of GLP, as revised in 1997; Processing part was performed in compliance with the national GLP-regulation of the involved country, based on the aforementioned OECD principles of GLP with some exceptions*
Previous evaluation:	In DAR 2003
Material and methods:	
Test material:	Trinexapac-ethyl (CGA 163935)
Lot/Batch No:	802067/1
Sampling time points:	Grain samples were collected at normal commercial harvest time (BBCH 89, 67-68 DAT), and were processed to bran, flour, whole meal flour and whole-grain bread.
Test concentration:	0.2 g a.s./ha, one application at BBCH 51
Test system:	Winter wheat, grown in Northern France (Izy) was treated at growth stage BBCH 51 with a single application of trinexapac-ethyl (CGA 163935) at a rate of 0.2 kg as/ha. The compound was applied by foliar spraying of the formulation ME 250. Grain collected at 68 DAT, was processed into different fractions (bran, bread, middlings, epidermis, and flour). The parent compound was determined in grains only and the metabolite CGA 179500 in grain and all processed fractions The study comprises a balance study and the determination of transfer factors.
Method of analysis:	The analytical methods applied to the grain samples and all fractions obtained during processing are based on methods REM 137.01 for the parent compound and REM 137.02 for the metabolite with some modifications: clean-up steps were omitted and residue levels were quantified by LC-MS/MS. For both methods – residues extracted with methanol/phosphate buffer pH7 (30:70) and final determination by LC-MS/MS. For detail evaluation of these analytical methods please refer to Vol 3 CA B.5.1.2.1 (with reference to the DAR Annex IIA.4.2.1 Method 1 and Method 2) Procedural recovery data:

Recoveries in percent (%). The lowest fortification level is at the limit of quantitation:

Substrate (control)	Fortification Level [mg/kg]	Trinexapac-ethyl (CGA 163935)	CGA 179500
Retained grain (RAC)	0.02 / 0.20	107 / 108	93 / 99
Straight flour (for whole-meal)	0.02 / 0.20	-	84 / 98
Low grade meal	0.02 / 0.20	-	106 / 102
Dough	0.02 / 0.20	-	112 / 103
Bran (scoured total bran)	0.02 / 0.20	-	96 / 104
Offal	0.02 / 0.20	-	100 / 100
Whole-grain bread	0.01 / 0.10	-	100 / 93
Whole-meal flour	0.01 / 0.10	-	112 / 101

Limit of quantification: 0.01 mg/kg

Storage conditions: Samples stored refrigerated (at 4°C) for 3 months from sample to processing; Processed fractions stored deep frozen (<-18°C) for 7 months from processing to analysis

Reference items: Trinexapac-ethyl (CGA 163935), Batch No. AMS 265/102, purity 99.6%
Trinexapac acid (CGA 179500), Batch No. BPS 520/103, purity 99%

***** Supporting analyses were determined according to high industrial standards but not under GLP regulations. Descriptive data of the test system, the soil, maintenance and climatic conditions were not recorded under GLP.

Results

In the field grain samples collected at harvest, CGA 163935 was not detectable (<0.02 mg/kg).

Balance study

The results of the balance study are presented in table B.7.5.3-12. The balance calculations are based on the concentration of CGA 179500 measured in grain and all processed fractions.

Table B.7.5.3-12. Mass balance of CGA 179500 in wheat fractions after processing[1]

Process/Fraction	Fraction Weight (kg)	Fraction Weight (%)	Residues found (mg/kg)	Content in fraction (mg)	Residues calculated (% of the initial amount)
PROCESSING TO CLEANED GRAINS					
Grain RAC	11	100	0.28	3.0	100
Cleaned grains	10	99	0.30	3.1	103

Process/Fraction	Fraction Weight (kg)	Fraction Weight (%)	Residues found (mg/kg)	Content in fraction (mg)	Residues calculated (% of the initial amount)
Offal	0.15	1.4	0.38	0.06	1.9
Total	11	100		3.1	105
PROCESSING OF FLOUR (TYPE 550)					
Grain RAC	11	100	0.28	3.0	100
Intermediate fractions					
Coarse bran	1.7	16	1.1	1.9	64
Fine bran (middlings)	0.87	8.2	0.72	0.62	21
Straight flour	7.7	72.7	0.082	0.63	21
Low grade meal (toppings)	0.79	7.5	0.41	0.32	11
Total	11	105		3.5	116
Final (remaining) fractions – Total mass balance					
Offal	0.15	1.4	0.38	0.060	1.9
Epidermis	0.007	0.10	0.13	0.001	0.03
Remain. low grade meal (toppings)	0.46	4.4	0.41	0.19	6.3
Bran (scoured total bran)	1.8	17	1.1	2.0	67
Flour (type 550)	8.0	76	0.091	0.73	24
Total	10	99		3.0	100
PROCESSING OF WHOLE-MEAL FLOUR					
Grain RAC	11	100	0.28	3.0	100
Intermediate fractions					
Bran (total, part of whole-meal)	2.6	24	0.99	2.5	84
Straight flour (for whole-meal)	7.8	74	0.085	0.66	22
Total	10	98		3.2	106
Final (remaining) fractions – Total mass balance					
Offal	0.15	1.4	0.38	0.060	1.9
Whole-meal flour	10	98	0.29	3.0	99
Total	10	99		3.0	100
PROCESSING OF WHOLE GRAIN BREAD					
Grain RAC	13 [2]	100	0.24[2]	3.0	100
Intermediate fractions					
Dough	20[2]	161	0.18[2]	3.6	121
Final (remaining) fractions – Total mass balance					

Process/Fraction	Fraction Weight (kg)	(%)	Residues found (mg/kg)	Content in fraction (mg)	Residues calculated (% of the initial amount)
Offal	0.17 [2]	1.4	0.38	0.06	1.9
Whole-grain bread	18 [2]	142	0.15[2]	2.7	89
Total	18 2	143	-	2.7	91

[1] based on dry weight (actual fresh weights were corrected for moisture content and for total amount of the original RAC specimen used for the processing)

[2] fresh weight

Transfer factors

The levels of metabolite CGA 179500 in various processed wheat grain samples and the respective transfer factors are summarised in table B.7.5.3-13.

Table B.7.5.3-13. Levels and transfer factors of CGA 179500 in wheat grain and wheat samples after processing

Product	Total residue CGA 179500 (mg/kg)[1]	Transfer factor[1]
Grain (RAC)	0.24; 0.25; 0.24; 0.23 (0.24)	-
Bran (scoured total bran)	0.96; 1.00; 0.82; 0.92 (0.93)	4.0; 4.0; 3.4; 4.0 (3.8)
Flour (type 550)	0.078; 0.084; 0.078; 0.072 (0.078)	0.33; 0.34; 0.33; 0.31 (0.3)
Whole meal flour	0.25; 0.25; 0.22; 0.23 (0.24)	1.0; 1.0; 0.92; 1.0 (1.0)
Whole-grain bread	0.15; 0.17; 0.15; 0.14 (0.15)	0.63; 0.68; 0.63; 0.61 (0.6)

[1] range and mean values of 4 replicates

RMS comments and conclusion (RMS Netherlands, 2003)

After processing of wheat grain into different products such as cleaned grains, flour type 550, whole-meal flour, and dough or bread, most of the residue is transferred to cleaned grains, scoured total bran, whole-meal flour, and dough and bread, respectively. Concentration of the residue is found in the bran fraction, whereas reduction of the residue is observed in flour fractions. Producing whole meal flour however, does not change the concentration of residue. A reduction of residue concentration is observed after processing into flour type 550 and whole grain bread.

Guidelines and limitations:

Wheat grain could not be processed into wheat germ, however samples of the epidermis fraction, included wheat germ. The study is considered suitable for evaluation.

RMS LT comments and conclusion

Even though study was performed before OECD 508, no deviations from this guidance were observed. Transfer factors obtained as a ratio between residues in grain (after storage, before processing) and processed fraction. Residue results are covered by storage stability data. Study is suitable for evaluation.

Study 14
New processing study of the magnitude of residue in wheat

Reference:	**Mäyer T. (2010a)** Trinexapac-ethyl – Magnitude of the Residues in or on Wheat. Final report. Syngenta Crop Protection, Greensboro, USA. Syngenta File No. CGA163935_50036. (KCA 6.5.3 / ~~1~~402)
Report No.:	T003605-07 (including two trials in the USA)
Guideline:	U.S. EPA OPPTS 860 Series Guidelines
GLP:	EPA Good Laboratory Practice Standards (40 CFR Part 160) with some exceptions*
Previous evaluation:	Submitted for the purpose of renewal
Reason for submission:	Measure trinexapac acid (free and conjugated) in raw agricultural commodities (RAC) and processed products
Material and methods:	
Test material:	Trinexapac-ethyl (CGA 163935)
Lot/Batch No:	ID 428456 (A7725M)
Sampling time points:	Grain samples were collected at normal commercial harvest time.
Test concentration:	0.129 g a.s./ha, one application at BBCH 32 (treatment 2 and 3) 0.644 g a.s./ha, one application 45 PHI (treatment 4)
Test system:	Two processing trials were conducted with a trinexapac-ethyl 250 EC formulation (CGA 163935) containing 0.25 kg a.s./L product. The product was applied to wheat as a foliar broadcast spray with 19 - 468 L water/ha. Four application regimes were followed. Treatment 1 corresponded to the non-treated (control) plot. Treatment 2 received 0.129 kg a.s./ha at BBCH 32. Treatment 3 and 4 received 0.129 kg a.s./ha or 0.644 kg a.s./ha, respectively, 45 days prior to harvest of mature grain. Wheat was processed into aspirated grain fractions (AGFs), bran, flour, middlings, shorts and germ in a manner that simulates industrial practice as closely as possible. Moisture content of the wheat samples was determined and samples were dried in an oven at 43 – 57°C to a moisture content of 11-13.5%. Dried samples were placed in a dust generation room, containing a holding bin, bucket conveyors and a screw conveyor. As the samples were moved in the room for 120 minutes, aspiration was used to remove light impurities. Light impurities were classified using the following sieve size: No. 8 (2.36 mm), No 10 (2.0 mm), No 16 (1.18 mm), No 20 (0.85 mm), and No 40 (0.425 mm). After removing a portion for ash content determination, the remainder AGF was collected and placed in a freezer.

For production of further processing samples, light impurities were separated using an aspirator and foreign particles removed by screening. For production of germ samples, cleaned wheat was adjusted to 16% water content, passed through a disc mill and sieved to remove the bran from the germ fraction. The germ sample was then sifted to separate the endosperm, and aspirated again.

For milling, the cleaned wheat was adjusted to 17.5% water content. The sample was then fed through a Chopin mill to break the grains. Subsequently, the broken grain was fed onto sifter screens (0.14 and 0.80 mm) to obtain course bran, break flour and middlings. Course 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 sifting bran and middlings were combined as well.

Because of compliance monitoring requirements and sample size, the samples were processed by batch rather than continuously, as in commercial operation. From sampling to extraction, samples were stored frozen for a maximum of 17.1 months.

Method of analysis:

All samples were analysed for residues of trinexapac (CGA 179500) using analytical method GRM 020.01A. Residues of trinexapac (CGA 179500) were extracted from the sample matrices using acetonitrile/1N hydrochloric acid solution (80:20, v/v). An aliquot of the extract was passed with water through a pre-conditioned C_8-SPE cartridge. The SPE cartridge was rinsed with water followed by water/acetonitrile (80:20, v/v). The analyte was eluted from the cartridge with formic acid aqueous solution/acetonitrile (80:20, v/v). Final determination was done by high performance liquid chromatography (HPLC) with tandem triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantitation (LOQ) for trinexapac (CGA 179500) was 0.01 mg/kg for all matrices.

The recoveries from wheat grain, AGF, bran, flour, middlings, shorts and germ fortified at the LOQ and 100-2000 multiples of the LOQ ranged from 70% to 120% for trinexapac (CGA 179500). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/08 and KCA.4.1.2/09).

Concurrent procedural recoveries for CGA179500 from wheat commodities are summarized below:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥ 3)
Wheat grain	0.01-15	24	83-120	102 ± 9.8
Wheat AGF	0.01-20	2	74-86	80 ± N/A
Wheat bran	0.01-20	6	84-120	103 ± 12.6
Wheat flour	0.01-20	2	107-115	111 ± N/A
Wheat middlings	0.01-10	4	96-116	103 ± 8.7
Wheat shorts	0.01-10	4	70-117	88 ± 23.9
Wheat germ	0.01-15	4	74-114	97 ± 17.5

Method verification recoveries are presented below:

Summary of Method Verification Recoveries					
Matrix	**Analyte**	**Spike Level (ppm)**	**Sample Size (n)**	**Recoveries (%)**	**Overall Mean (%) ± std. dev.**
Wheat Grain	CGA179500	0.01 0.10	2 2	110, 112 106, 106	108 ± 3.0
Wheat AGF	CGA179500	0.01 0.10	2 2	82, 83 96, 96	89 ± 7.8
Wheat Bran	CGA179500	0.01 0.10	2 2	112, 102 114, 116	111 ± 6.2
Wheat Flour	CGA179500	0.01 0.10	2 2	84, 78 95, 111	92 ± 14
Wheat Middlings	CGA179500	0.01 0.10	2 2	76, 75 85, 83	80 ± 5.0
Wheat Shorts	CGA179500	0.01 0.10	2 2	81, 84 84, 89	84 ± 3.3
Wheat Germ	CGA179500	0.01 0.10	2 2	93, 108 94, 93	97 ± 7.3

Limit of quantification: 0.01 mg/kg

Storage conditions: Stored at <-10°C for a maximum of 17.1 months from sample to extraction.

Reference items: Trinexapac acid (CGA 179500), Lot Number GB-XLII-8B

Purity 99.9%

***** NOAA weather data was not collected according to the FIFRA-GLP requirements of 40 CFR Part 160;

Spray-mix storage stability data were not generated as required in 40 CFR Part 160;

The application of maintenance chemicals and irrigation practices did not conform to GLP requirements;

Analysis of soil characteristics did not conform to GLP requirements;

Field history from growers records were not generated nor maintained under GLP;

Sample weights taken in the field were determined by non-GLP procedures.

Results

Mean residues of trinexapac (CGA 179500) in wheat grain were 0.30 - 1.7 mg/kg after the low-dose treatment (application rate 0.129 kg a.s./ha), and 5.3 - 9.8 mg/kg after high-dose treatment (application rate 0.644 kg a.s./ha). Residues were concentrated in processed fractions of wheat bran from both treatments and of wheat middlings from one of the low-dose application trials. Transfer factors (TF) were between 1.5 and 2.2 for wheat bran, and between 0.3 and 11.7 for wheat middlings. Residues in wheat AGF, germ, shorts and flour were the same or reduced as compared to the residue of the corresponding wheat grain sample. Corresponding transfer factors were 0.2 - 0.8 for AGF, 0.9 – 1.4 for wheat germ, 0.03 - 0.6 for shorts and 0.4 - 0.5 for wheat flour. For details see table B.7.5.3-14.

Table B.7.5.3-14. Residues of trinexapac acid (CGA 179500) in wheat and processed commodities

Description of specimens	Application rate [kg a.s./ha]	Residue [mg/kg]	Mean residue [mg/kg]	Transfer factor
Trial C13ND078468				
Wheat grain*	0.129	0.328, 0.308, 0.277	0.30	-
	0.644	5.57, 4.90, 5.73	5.4	-
Wheat AGF	0.129	0.24, 0.23	0.24	0.8
	0.644	4.77, 4.03	4.4	0.8
Wheat bran	0.129	0.611, 0.707	0.66	2.2
	0.644	7.85, 9.33	8.6	1.6
Wheat flour	0.129	0.155, 0.108	0.13	0.4
	0.644	2.42, 2.88	2.7	0.5
Wheat middlings	0.129	3.67, 3.29, 3.39	3.5	11.7
	0.644	2.83, 2.45, 2.31	2.5	0.5
Wheat shorts	0.129	0.150, 0.195, 0.197	0.18	0.6
	0.644	0.124, 0.172, 0.167	0.15	0.03
Wheat germ	0.129	0.598, 0.310, 0.352	0.42	1.4
	0.644	5.88, 4.28, 5.11	5.1	0.9
Trial W01TX078473				
Wheat grain*	0.129	1.75, 1.66, 1.54	1.7	-
	0.644	10.2, 9.55, 9.50	9.8	-
Wheat AGF	0.129	0.35, 0.34	0.35	0.2
	0.644	3.50, 3.47	3.5	0.4
Wheat bran	0.129	2.28, 4.63	3.5	2.1
	0.644	15.8, 104**, 14.4, 13.8	14.7	1.5
Wheat flour	0.129	0.90, 0.582	0.74	0.4
	0.644	3.67, 3.80	3.7	0.4
Wheat middlings	0.129	0.82, 0.422, 0.429	0.56	0.3
	0.644	5.73, 5.48, 4.93	5.4	0.6
Wheat shorts	0.129	0.925, 1.15, 0.948	1.0	0.6
	0.644	5.23, 6.25, 5.61	5.7	0.6
Wheat germ	0.129	1.62, 1.52, 1.34	1.5	0.9
	0.644	9.78, 11.1, 11.6	10.8	1.1

* pre-processing

** Sample outlier not used for statistical evaluation

Formulation = trinexapac-ethyl 250 EC

Values are averages of multiple samples

RMS comments and conclusion

In the present study wheat treated with trinexapac-ethyl (CGA 163935) at two different rates (0.129 and 0.644 kg a.s./ha) was processed into aspirated grain fractions (AGF), bran, flour, middlings, shorts and germ. Trinexapac acid residues slightly concentrated in wheat bran from both the low dose and high dose application rates (TF = 1.5 - 2.2) and wheat middlings from one of the low-dose trials (TF 11.7). In all other fractions residues of trinexapac were not concentrated (similar to or less than 1 times the residue of their corresponding grain samples (TF ≤ 1). However, the higher application rate was five times higher than the critical GAP for wheat (125 g a.s./ha). Residue results are covered by storage stability data. The study was well performed and reported and suitable for evaluation.

Study 15

New processing study of the magnitude of residue in wheat

Reference:	**Ediger K. (2006)** Trinexapac-ethyl – Magnitude of the Residues in or on Wheat. Final report. Syngenta Crop Protection, Greensboro, USA. Syngenta File No. CGA163935/1053. (KCA 6.5.3 / ~~15~~03)
Report No.:	T002695-03 (including two processing trials in the USA)
Guideline:	U.S. EPA OPPTS 860 Series Guidelines
GLP:	EPA Good Laboratory Practice Standards (40 CFR Part 160) with some exceptions*
Previous evaluation:	Submitted for the purpose of renewal
Reason for submission:	Measure trinexapac acid (free) in raw agricultural commodities (RAC) and processed products in order to derive more robust processing factors
Material and methods:	
Test material:	Trinexapac-ethyl (CGA 163935)
Lot/Batch No:	FL-040485
Sampling time points:	Grain samples were collected 45 DALA.
Test concentration:	0.129 g a.s./ha (treatment 3) and 644 g a.s./ha (treatment 5)
Test system:	Two processing trials were conducted with a trinexapac-ethyl 250 EC formulation (CGA 163935) containing 0.25 kg a.s./L product. The product was applied to wheat as a foliar broadcast spray with 9 - 227 L water/ha. Five application regimes were followed. Treatment 1 corresponded to the non-treated (control) plot. Treatment 2 received 0.129 kg a.s./ha at BBCH 32. Treatments 3, 4 and 5 received 0.129, 0.385 or 0.644 kg a.s./ha, respectively, 45 days prior to harvest of mature grain. Wheat was processed into aspirated grain fractions (AGFs), bran, flour, middlings, shorts and germ in a manner that simulates industrial practice as closely as possible. Moisture content of the wheat samples was determined and samples were dried in an oven at 43 – 57°C to a moisture content of 10-13%. Dried samples were placed in a dust generation room, containing a holding bin, bucket conveyors and a screw conveyor. As the samples were moved in the room for 120 minutes, aspiration was used to

remove light impurities. Light impurities were classified using the following sieve size: No. 8 (2.36 mm), No 10 (2.0 mm), No 16 (1.18 mm), No 20 (0.85 mm), and No 40 (0.425 mm). After removing a portion for ash content determination, the remainder AGF was collected and placed in a freezer.

For production of further processing samples, light impurities were separated using an aspirator and foreign particles removed by screening. For production of germ samples, cleaned wheat was adjusted to 16% water content, passed through a disc mill and sieved to remove the bran from the germ fraction. The germ sample was then sifted to separate the endosperm, and aspirated again.

For milling, the cleaned wheat was adjusted to 17.5% water content. The sample was then fed through a Chopin mill to break the grains. Subsequently, the broken grain was fed onto sifter screens (0.14 and 0.80 mm) to obtain course bran, break flour and middlings. Course 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 sifting bran and middlings were combined as well.

Because of compliance monitoring requirements and sample size, the samples were processed by batch rather than continuously, as in commercial operation. From sampling to extraction, samples were stored frozen for a maximum of 18.2 months.

Method of analysis: All samples were analysed for residues of trinexapac (CGA 179500) using analytical method No. 110-01. Residues of trinexapac (CGA 179500) were extracted from the sample matrices using acetonitrile/sodium phosphate (pH 7). An aliquot of the extract was passed with water through a pre-conditioned C_8-SPE cartridge. The SPE cartridge was rinsed with acetonitrile/phosphoric acid aqueous solution and the analyte was eluted from the cartridge with acetonitrile/phosphoric acid (30:70 v:v). Final determination was done by high performance liquid chromatography (HPLC) with tandem triple quadrupole mass spectrometric detection (LC-MS/MS). The LOD (limit of detection), defined by the lowest standard injected, is 0.025 ng. The LOQ (limit of quantitation), defined as the lowest fortification level, is 0.05 mg/kg.

The recoveries from wheat grain, AGF, bran, flour, middlings, shorts and germ fortified at the LOQ and 100-2500 multiples of the LOQ ranged from 70% to 118% for trinexapac (CGA 179500). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/10).

Concurrent procedural recoveries for CGA179500 from wheat commodities are summarized below:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥ 3)
Wheat grain	0.05 - 15	24	72-109	89 ± 10
Wheat AGF	0.5 - 5	4	101-118	108 ± 8.4
Wheat bran	0.5 - 25	4	77-98	87 ± 9.5
Wheat flour	0.05 - 5	4	89-118	101 ± 13
Wheat middlings	0.05 - 5	4	80-87	85 ± 4.2
Wheat shorts	0.05 - 10	4	78-109	94 ± 13
Wheat germ	0.05 - 10	4	70-110	91 ± 16

Limit of quantification: 0.05 mg/kg

Storage conditions: Stored at -20°C for a maximum of 18.2 months from sample to extraction.

Reference items:	Trinexapac acid (CGA 179500), Lot Number GB-XLII-8B
Purity	99.8%

***** Ancillary data such as field history and gross sample weight determinations were not collected according to FIFRA-GLP requirements;

Tank mix data are not generated as required in 40 CFR Part 160.113(a)(1) and (3)

Results

Mean residues of trinexapac acid (CGA 179500) in wheat grain were 0.39 - 1 mg/kg after the low-dose treatment (application rate 0.129 kg a.s./ha), and 4.2 - 11 mg/kg after high-dose treatment (application rate 0.644 kg a.s./ha). Residues were concentrated in processed fractions of wheat bran from both treatments and of wheat shorts from one of the low-dose application trials. Transfer factors (TF) were between 2.1 and 2.5 for wheat bran, and between 0.45 and 1.4 for wheat shorts. Residues in wheat AGF, germ, middlings and flour were the same or reduced as compared to the residue of the corresponding wheat grain sample. Corresponding transfer factors were 0.16 - 0.35 for AGF, 0.3 – 1.1. for wheat germ, 0.4- 0.5 for middlings and 0.24 - 0.32 for wheat flour. For details see table B.7.5.3-15.

Table B.7.5.3-15. Residues of trinexapac acid (CGA 179500) in wheat and processed commodities

Description of specimens	Application rate [kg a.s./ha]	Residue [mg/kg]	Transfer factor
Trial NN-FR-04-5418/ND			
Wheat grain*	0.129	1.0	-
	0.644	11	-
Wheat AGF	0.129	0.35	0.35
	0.644	3.3	0.3
Wheat bran	0.129	2.2	2.2
	0.644	23	2.09
Wheat flour	0.129	0.32	0.32
	0.644	3.4	0.31
Wheat middlings	0.129	0.41	0.41
	0.644	4.9	0.45
Wheat shorts	0.129	0.45	0.45
	0.644	7.5	0.68
Wheat germ	0.129	0.29	0.29
	0.644	9.4	0.85
Trial SC-FR-04-5430/OK			

Description of specimens	Application rate [kg a.s./ha]	Residue [mg/kg]	Transfer factor
Wheat grain*	0.129	0.39	-
	0.644	4.2	-
Wheat AGF	0.129	0.09	0.23
	0.644	0.68	0.16
Wheat bran	0.129	0.98	2.5
	0.644	10	2.38
Wheat flour	0.129	0.12	0.31
	0.644	1.0	0.24
Wheat middlings	0.129	0.19	0.49
	0.644	1.8	0.43
Wheat shorts	0.129	0.56	1.4
	0.644	4.6	1.1
Wheat germ	0.129	0.40	1.0
	0.644	4.5	1.1

* pre-processing

Formulation = trinexapac-ethyl 250 EC

RMS comments and conclusion

In the present study wheat treated with trinexapac-ethyl (CGA 163935) at two different rates (0.129 and 0.644 kg a.s./ha) was processed into aspirated grain fractions (AGF), bran, flour, middlings, shorts and germ. Trinexapac acid residues concentrated in wheat bran from both the low dose and high dose application rates (TF = 2.09 - 2.5). There was a slight concentration found in the winter wheat shorts and germ (TF 1.4 and 1.1 respectively). In all other fractions residues of trinexapac were not concentrated (similar to or less than 1 times the residue of their corresponding grain samples (TF $\leq$ 1). However, the higher application rate was five times higher than the critical GAP for wheat (125 g a.s./ha). Residue results are covered by storage stability data. The study was well performed and reported and suitable for evaluation.

Studies 16 to 18

New processing studies of the magnitude of trinexapac acid (free and total) and metabolites (CGA313458, CGA224439 and CGA113745) residue in wheat

Reference:	**MacDougall J. (2016a)** Trinexapac-ethyl – Residue Processing Study on Wheat in France and Spain in 2015. (Syngenta File No. A8587F_10524). (KCA 6.5.3 / ~~16~~07 & KCA 6.3.2 / 04)
Report No.:	37278 (including two trials in SEU)

Study No.:	699784
Reference:	**Watson G. (2016a)** Trinexapac-ethyl Analysis of Wheat Processing Phase Specimens for CPCA from Study 699784 'Trinexapac-ethyl –Residue Processing Study on Wheat in France and Spain in 2015'. Final report. (Syngenta File No. CA876_10003). (KCA 6.5.3 / ~~17~~08)
Report No.:	RES-00028
Study No.:	RES-00028
Reference:	**Langridge G. (2016a)** Trinexapac-ethyl Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Wheat Process Fractions. INTERIM REPORT submitted March 2016. (KCA 6.5.3 / 09) (Syngenta File No. CGA313458_10002). **Langridge G. (2016c)** Trinexapac-ethyl Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Wheat Process Fractions. FINAL REPORT submitted January 2017 (KCA 6.5.3 / 11) (Syngenta File No. CGA313458_10011)
Report No.:	CEMR-7355
Study No.:	CEMR-7355
Guideline:	Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 244/2011 (283/2013) and 245/2011 (284/2013) implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals, Number 508 (2008): Magnitude of the Pesticide Residues in Processed Commodities and Number 509 (2009): Crop field trials. SANCO/825/00 rev.8.1 (16/11/2010) Guidance Document on Pesticide Residue Analytical Methods. European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007). Working document of the Commission of the European Communities, Directorate General for Agriculture, VI B II-1, Appendix E, 7035/VI/95 rev. 5 of 22.07.1997.
GLP:	Yes. OECD Principles of good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17,OECD, Paris 1998 according to country specific regulations. In addition, the field phase was performed in accordance with Consensus Document "The application of the GLP principles to field studies", ENV/JM/MONO(99)22. The analytical phase of this study was performed in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM(98)17, OECD, Paris 1998 as incorporated into the UK Statutory Instrument for GLP with some exceptions*.

Previous evaluation:	Submitted for the purpose of renewal
Reason for submission:	Measure trinexapac acid (free and total) and processing metabolites in raw agricultural commodities (RAC) and processed products in order to derive processing factors

Material and methods:

Test material:	A8587F Trinexapac-ethyl
Lot/Batch No:	SMO3A0004
Sampling time points:	Grain samples were collected at normal commercial harvest time (66-69 DALA), and were processed into cleaned grain, waste (offal), white flour, total bran, shorts, middlings, wholemeal flour, wholemeal bread, germ, dry gluten, dry starch and gluten feed meal.
Test concentration:	400.6 g a.s./ha, one application at BBCH 49 (trial 1) 406.2 g a.s./ha, one application at BBCH 49 (trial 2)
Test system:	In study 37278 two residue field trials on field wheat were conducted in South France and Spain during 2015. Trinexapac-ethyl was applied to field wheat as A8587F, a micro-emulsion (ME) formulation containing 250 g trinexapac-ethyl per litre. One application was made at a target rate of 400 g a.i./ha for trinexapac-ethyl. Treated and control samples were collected at normal commercial harvest (NCH) for processing and for residue analysis. Samples were shipped frozen to the analytical facility for residue analysis and at ambient temperature to the processing facility. Each field trial generated a treated and an untreated field sample of grain. The untreated and treated grain samples were put through the relevant process. The treated grain for each trial was split into 2 portions (T1 and T2) with both being taken through the procedures. Wheat grain was processed into cleaned grain, waste (offal), white flour, total bran, shorts, middlings, wholemeal flour, wholemeal bread, germ, dry gluten, dry starch and gluten feed meal. Relevant industrial practices and standardised procedures were applied to simulate the common processes used by industry. In study 37278 two analytical procedures were used to analyse the collected samples – GRM020.05 to determine free trinexapac acid and GRM020.009A to determine total trinexapac acid. Study RES-00028 was conducted to generate results on the magnitude of residues for CPCA (CGA224439) in the wheat processing specimens. Study CEMR-7355 was conducted to analyse processed fractions of wheat for residues of CGA313458 and CGA113745 originally generated as part of Charles River Study Number 699784. This final report contains analytical results for CGA313458 and CGA113745 in wheat grain, cleaned grain, waste (offal), white flour, total bran, shorts, wholemeal flour, wholemeal bread, dry starch and gluten feed meal.
Processing phase:	Two follow-up procedures have been carried out on the processing of wheat. Prior to each follow up processing study, wheat samples were analysed to give a pre-processed residue value. White flour processing: Wheat grain specimens were cleaned using a single grading unit and a sample of cleaned grain was taken. The water content of the wheat grains was measured and adjusted. About 5 kg of wheat grains were conditioned in a kneading machine for a minimum of 5 hours to increase the water content to approximately 17%. Samples of shorts were taken. The remaining shorts were placed through a mill consisting of reduction rolls and screened. After the reduction stage, fine bran and reduction flour

were recovered. After weighing, coarse bran and fine bran were combined to obtain total bran and milling flour and reduction flour were obtained to obtain white flour. White flour and total bran samples were taken. The fine brans were placed through sieves and screened. After division, middling samples were taken.

Wholemeal flour processing:

Wheat grain specimens were cleaned with a single grading unit and a sample of cleaned grain was taken. The water content of the wheat grains was measured and adjusted. The wheat grains were conditioned in a kneading machine for a minimum of 5 hours to increase the water content to approximately 17%. Wheat grains were placed through a mill consisting of break rolls. After the break stage, coarse bran and milling flour were recovered. Shorts were placed through a mill consisting of reduction rolls and screened. After the reduction stage, fine bran and reduction flour were recovered. Coarse bran, fine bran, milling flour and reduction flour were combined to obtain wholemeal flour. A sample of wholemeal flour was taken.

Wholemeal bread processing:

The wholemeal bread processing was made from the wholemeal flour obtained. Dry baker yeasts were rehydrated with sugar and water. The flour, the water and the yeasts preparation were put in the kneading machine. Five minutes before the end of the kneading, salt was added. The obtained dough was placed in a pan at ambient temperature for 45 minutes. The dough was divided in several little portions and were covered and kept at ambient temperature for 15 minutes. Each portion was shaped in baguette. The baguettes were covered and kept at ambient temperature for a minimum of 2 hours. The baguette was baked in an oven at 250°C for approximately 30 minutes. Wholemeal bread samples were taken.

Germ extraction processing:

Wheat grain specimens were cleaned with a single grading unit. The cleaned grains were weighed and placed in a container. The same quantity of water was added. The steeping lasted 12 h minimum. After straining, a portion of grains was set down on absorbing paper. Germs were removed from wheat grain with a cutter. A sample of germs was taken.

Gluten and starch of wheat flour separation processing:

Wheat grain specimens were cleaned with a single grading unit. The water content of the wheat grains was measured and adjusted. The wheat grains were conditioned in a kneading machine for a minimum of 5 hours to increase the water content to approximately 17%. Wheat grains were placed through a mill consisting of break rolls. After the break stage, coarse bran and milling flour were recovered. Shorts were placed through a mill consisting of reduction rolls and screened. After the reduction stage, fine bran and reduction flour were recovered. After weighing, milling flour and reduction flour were combined to obtain white flour. The gluten and starch separation processing was made with the obtained white flour. A dough was prepared and after rest, washed with water to separate starch milk and gluten. Wet gluten was dried in an oven regulated at 50 °C. Dry gluten samples were taken. After settling of starch milk in cold room, wet starch was dried in an oven regulated at 50 °C. Dry starch samples were taken. Dry gluten and dry starch were ground separately with a mill (hammer-type) and mixed (1 / 1). Gluten feed meal samples were taken.

Method of analysis: Analytical procedure GRM020.05 was used to determine the free Trinexapac Acid residue. Residues of trinexapac acid are extracted with methanol/water/phosphate buffer solution. Extracts are centrifuged and an aliquot is acidified with 0.1M hydrochloric acid. Extracts are subjected to an Oasis HLB solid phase extraction (SPE) clean up. Final determination is by high performance liquid chromatography

with triple quadrupole mass spectrometric detection (LC-MS/MS). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA 4.1.2/01; KCA4.1.2/02). Procedural recovery data for trinexapac acid (free):

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥3)
Wheat grain	0.01-2	14	73-98	82 ± 10
Straw	0.01-0.1	4	82-90	87 ± 4
Cleaned grain	0.01-2	6	93-101	97 ± 3
Waste (offal)	0.01-2	6	79-115	99 ± 12
White flour	0.01-2	6	71-90	79 ± 9
Total bran	0.01-2	6	60-90	76 ± 15
Shorts	0.01-2	6	88-102	97 ± 6
Middlings	0.01-2	6	71-99	78 ± 14
Wholemeal flour	0.01-2	6	81-85	83 ± 2
Wholemeal bread	0.01-2	6	92-107	101 ± 6
Germ	0.01-1.25	6	93-119	106 ± 10
Dry gluten	0.01-2	6	72-95	86 ± 9
Dry starch	0.01-0.1	4	87-100	93 ± 8
Gluten feed meal	0.01-2	6	75-91	83 ± 7

Analytical procedure GRM020.009A was used to determine the total Trinexapac Acid residue (free and conjugated). Residues of trinexapac acid are extracted with an acetonitrile/water solution. Extracts are centrifuged and an aliquot is hydrolysed overnight in the presence of 1M Sodium Hydroxide. The hydrolysed extracts are portioned with ethyl acetate prior to clean up with an IST silica cartridge. Eluent from the first SPE stage are reduced to dryness prior to reconstitution with 0.1M hydrochloric acid followed by further clean up by Oasis HLB solid phase extraction (SPE). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/03a and KCA.4.1.2/04a). Procedural recovery data for total trinexapac acid:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥3)
Wheat grain	0.01-3	18	68-109	85 ± 16
Straw	0.05-1	4	77-82	80 ± 3
Cleaned grain	0.01-2	6	90-102	96 ± 5
Waste (offal)	0.01-3	6	74-114	93 ± 16
White flour	0.01-2	6	88-105	95 ± 7
Total bran	0.01-3	6	83-113	97 ± 13

Shorts	0.01-2	6	90-124	106 ± 11
Middlings	0.01-2	6	82-112	97 ± 10
Wholemeal flour	0.01-2	6	84-91	88 ± 4
Wholemeal bread	0.01-2	6	75-100	85 ± 10
Germ	0.01-1.25	6	88-116	106 ± 12
Dry gluten	0.01-2	6	67-115	92 ± 20
Dry starch	0.01-2	6	76-118	98 ± 16
Gluten feed meal	0.01-2	6	73-86	78 ± 7

Analytical procedure GRM020.15A was used to determine the residues of cyclopropanecarboxylic acid (CPCA or CGA224439). Residues are double extracted with an aliquot of prepared matrix with acetonitrile/water (50/50, v/v) + 0.01M HCl by maceration. Added magnesium sulphate, sodium chloride, sodium citrate dibasic sesquihydrate and sodium citrate tribasic dehydrate to the sample extract to partition the organic and aqueous phase. Diluted an aliquot of the acetonitrile extract (x4). Derivatised an aliquot of the diluted acetonitrile extract by incubation at 60 ºC after addition of 2-Hydrazinoquinoline, triphenylphosphine and 2,2'-Dipyridyl disulphide. Concentrated the derivatised extract to dryness and re-dissolve in deionised water Determination by HPLC-MS/MS. LOQ 0.01 mg/kg. For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/13). Procedural recovery for CPCA:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean ± RSD (if n ≥3)
Wheat grain	0.01-0.1	4	90-97	94 ± 3
Waste (offal)	0.01-0.05	2	62-81	72
White flour	0.01-0.05	2	88-110	99
Total bran	0.01-0.05	2	97-106	101
Wholemeal flour	0.01-0.05	2	93-100	97
Wholemeal bread	0.01-0.05	2	100-123	112
Germ	0.01-0.05	2	72-88	80
Dry gluten	0.01-0.05	2	99-121	110
Dry starch	0.01-0.05	2	99-109	104
Gluten feed meal	0.01-0.05	2	99-102	101

Analytical procedure GRM020.013A draft was used to determine the residues of CGA313458. Residues were extracted by sequential homogenisation with 80/20 v/v acetonitrile/water and 50/50 v/v acetonitrile/water. An aliquot of the combined extracts equivalent to 0.2 g (2 mL) was evaporated to remove the acetonitrile. The

sample was diluted with ultra-pure water and the pH adjusted to pH 7 –9 with dilute ammonium hydroxide solution. Samples were partitioned twice with ethyl acetate to remove co-extractives then the aqueous samples were filtered through an Oasis HLB SPE cartridge. Alternatively, samples may be analysed directly from the primary extracts without any further sample clean-up where there was sufficient instrument sensitivity. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/12). Procedural recovery data are provided in the table below:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean (%)	RSD (%)
Wheat grain[a]	0.01-0.1	4	57-84	74	16.1
Bread[b]	0.01-0.1	2	66-74	70	8.1
Bran	0.01-0.1	2	89-90	90	0.8
Flour[c]	0.01-0.1	2	71-73	72	2.0
			Overall	75	13.5

a – Cleaned grain and Waste (offal).

b – Dry starch and Gluten feed meal.

c – Shorts.

The lowest fortification level is at the limit of quantification.

Mean RSD (%) are calculated using rounded figures.

Recoveries were not corrected for control residue.

NOTE: Due to the limited amount of the retained samples received, samples of cleaned grain and waste (offal) were analysed alongside grain procedural recoveries, samples of dry starch and gluten freed meal were analysed alongside bread procedural recoveries and samples of shorts were analysed alongside with flour procedural recoveries as these were considered similar matrices.

Analytical procedure GRM020.014A draft was used to determine the residues of CGA113745. For the determination of CGA113745 in non-liquid brewing fractions 10 g sub samples of bread, grain, bran and flour were extracted by sequential homogenisation with 0.2% v/v ammonia in ultra-pure water. An aliquot of the combined extracts equivalent to 0.4 g (4 mL) was acidified and a 2mL aliquot was subjected to an Oasis WCX SPE clean-up. The sample was eluted with 10% acetonitrile in ultra-pure water and the acetonitrile removed by evaporation before the sample was made to 2mL with ultra-pure water. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). For detail evaluation of this analytical method please refer to Vol 3 CA B.5.1.2.1 (KCA.4.1.2/12). Procedural recovery data are provided in the table below:

Substrate	Fortification level [mg/kg]	Sample size [n]	Range of recoveries [%]	Mean (%)	RSD (%)
Wheat grain[a]	0.01-0.1	4	77-93	85	7.7

Bread[b]	0.01-0.1	2	93-101	97	5.8
Bran	0.01-0.1	2	67-67	67	0.0
Flour[c]	0.01-0.1	2	83-88	86	4.1
			Overall	84	13.2

a – Cleaned grain and Waste (offal).

b – Dry starch and Gluten feed meal.

c – Shorts.

The lowest fortification level is at the limit of quantification.

Mean RSD (%) are calculated using rounded figures.

Recoveries were not corrected for control residue.

NOTE: Due to the limited amount of the retained samples received, samples of cleaned grain and waste (offal) were analysed alongside grain procedural recoveries, samples of dry starch and gluten freed meal were analysed alongside bread procedural recoveries and samples of shorts were analysed alongside with flour procedural recoveries as these were considered similar matrices.

Limit of quantification: GRM020.05 0.01 mg/kg (free trinexapac acid)

GRM020.009A 0.01 mg/kg for all matrices, 0.05 mg/kg for straw (free and conjugated trinexapac acid)

GRM020.15A 0.01 mg/kg

GRM020.013A draft 0.01 mg/kg

GRM020.014A V3 draft 0.01 mg/kg

Reference items: Trinexapac acid (CGA179500) Batch Number MLA-372/1, purity 99.0%

CPCA (CGA224439) Lot Number STBB9094V, purity 99.0%

CGA313458 Batch Number DAH-XXXV-15, purity 96.1%

CGA113745 Batch Number MES 420/1, purity 99%

Sample storage conditions : Specimens were stored frozen (<-18 ^{0}C) for a maximum period of 8 months from sample to analysis for CGA 179500 (trinexapac acid);

Maximum 7 months from sample to analysis (6 months from processing to analysis for germ only) for CGA 224439;

Maximum 7.5 months from sample to analysis (7 months from processing to analysis, 6 months for germ) for CGA 313458;

Maximum 15 months from sample/processing to analysis (12 months from receipt to analysis) for metabolite CGA 113745.

~~Specimens were stored frozen for a maximum period of 156 days from receipt to analysis for CGA313458 and 363 days from receipt to analysis for CGA113745.~~

Extract solutions were stored for a maximum of 13 days before analysis (for CGA 313458).

Stability of the analytes in the specimen extracts was proven by the corresponding procedural recovery specimens, which were stored under the same conditions together with the sample extracts.

*- Supplementary weather data were provided by the local meteorological office and crop maintenance records were provided by the grower. These records were not generated according to GLP principles. In addition, for trial 2, soil analysis, GPS references and elevations, photos, wind speed, pressure at application and humidity of the grain.

Results

Residues of trinexapac acid (free) in wheat grain at harvest were 0.49 and 0.88 mg/kg (Table B.7.3.2-2), before processing were in the range 0.41 to 1.16 mg/kg (Table B.7.5.3-16 to 19). Residues of trinexapac acid (free and conjugated) in wheat grain at harvest were 0.62 and 1.40 mg/kg (Table B.7.3.2-2), before processing were in the range 0.51 to 2.76 mg/kg (Table B.7.5.3-16 to 19).

Trinexapac acid (free) residues in processed commodities were up to 1.11 in cleaned grain, up to 1.06 in waste (offal), up to 0.54 in white flour, up to 1.04 in total bran, up to 0.88 in shorts, up to 0.48 in middlings, up to 1.06 in wholemeal flour, up to 0.88 in wholemeal bread, up to 1.13 in germ, up to 0.30 in dry gluten, up to 0.09 in dry starch and up to 0.20 mg/kg in gluten feed meal (Table B.7.5.3-16 to 19).

Trinexapac acid (free and conjugated) residues in processed commodities were up to 2.44 in cleaned grain, up to 2.48 in waste (offal), up to 0.97 in white flour, up to 2.07 in total bran, up to 1.17 in shorts, up to 1.00 in middlings, up to 1.83 in wholemeal flour, up to 1.49 in wholemeal bread, up to 0.95 in germ, up 0.52 in dry gluten, up to 0.13 in dry starch and up to 0.42 mg/kg in gluten feed meal (Table B.7.5.3-16 to 19).

Residues of metabolite CGA313458 in wheat grain and processed fractions were all below LOQ (0.01 mg/kg) except for wholemeal bread, where residue up to 0.02 mg/kg was measured (Table B.7.5.3-16 to 19). Results in flour, bran and bread samples are not covered by storage stability data, therefore should be disregarded and have been struck through.

Residues of metabolite CGA224439 in wheat grain at harvest were in the range 0.01 to 0.03 mg/kg, up to 0.03 in cleaned grain, up to 0.04 in waste (offal), up to 0.02 in white flour, up to 0.04 in total bran, up to 0.03 in shorts, up to 0.02 in middlings, up to 0.03 in wholemeal flour, up to 0.05 in wholemeal bread, up to 0.04 in germ and up to 0.08 mg/kg in dry gluten. CGA224439 was not observed in dry starch and in gluten feed meal (<0.01 mg/kg) (Table B.7.5.3-16 to 19).

Residues of metabolite CGA113745 in wheat grain and processed fractions were all below LOQ (0.01 mg/kg) (Table B.7.5.3-16 to 19). Although due to instability of metabolite CGA113745 and poor chromatography, all results should be disregarded and have been struck through.

Transfer factors of residues from wheat grain to processed commodities are presented in tables from B.7.5.3-20 to B.7.5.3-~~24~~ 23. Transfer Factor = residue in processed product/residue in RAC.

Table B.7.5.3-16 Residues of trinexapac acid and metabolites in processed wheat (white flour processing) commodities

Sample Name*	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 224439	CGA 313458	~~CGA 113745~~
Trial T1A							
CVE-15-21490 003/005	Prior processing	Wheat Grain	0.64	1.11	0.02	<0.01	~~<0.01~~
CVE-15-21490 004	Prior processing	Wheat Grain	0.53	0.95	0.02		
CVE-15-21490 011/012	After Cleaning	Cleaned Grain	1.11	1.16	0.02	<0.01	~~<0.01~~
CVE-15-21490 017/018	After Cleaning	Waste (offal)	0.31	0.97	0.03	<0.01	~~<0.01~~
CVE-15-21490 023/024	After Mixing	White Flour	0.31	0.39	0.02	~~<0.01~~	~~<0.01~~
CVE-15-21490 029/030	After Mixing	Total Bran	0.63	1.01	0.02	~~<0.01~~	~~<0.01~~
CVE-15-21490 035/036	After Milling	Shorts	0.57	0.63	0.02	<0.01	~~<0.01~~
CVE-15-21490 041/042	After Screening	Middlings	0.33	0.55	0.02	-**	~~**~~
Trial T1B							
CVE-15-21490 006/008	Prior processing	Wheat Grain	0.51	0.85	0.02	<0.01	~~<0.01~~
CVE-15-21490007	Prior processing	Wheat Grain	0.51	1.01	0.02		
CVE-15-21490 013/014	After Cleaning	Cleaned Grain	0.52	1.00	0.02	<0.01	~~<0.01~~
CVE-15-21490 019/020	After Cleaning	Waste (offal)	0.26	0.69	0.03	<0.01	~~<0.01~~
CVE-15-21490 025/026	After Mixing	White Flour	0.31	0.43	0.01	~~<0.01~~	~~<0.01~~
CVE-15-21490 031/032	After Mixing	Total Bran	0.58	0.65	0.02	~~<0.01~~	~~<0.01~~
CVE-15-21490 037/038	After Milling	Shorts	0.40	0.54	0.01	<0.01	~~<0.01~~
CVE-15-21490 043/044	After Screening	Middlings	0.29	0.54	0.01	-**	~~**~~

Sample Name*	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 224439	CGA 313458	~~CGA 113745~~
Trial T2A							
CVE-15-21490 107/109	Prior processing	Wheat Grain	0.99	2.10	0.02	<0.01	~~<0.01~~
CVE-15-21490108	Prior processing	Wheat Grain	0.90	2.22	0.03		
CVE-15-21490 115/116	After Cleaning	Cleaned Grain	1.10	2.44	0.03	<0.01	~~<0.01~~
CVE-15-21490 121/122	After Cleaning	Waste (offal)	1.02	2.47	0.03	<0.01	~~**~~
CVE-15-21490 127/128	After Mixing	White Flour	0.54	0.97	0.02	~~<0.01~~	~~<0.01~~
CVE-15-21490 133/134	After Mixing	Total Bran	1.04	2.07	0.03	~~<0.01~~	~~<0.01~~
CVE-15-21490 139/140	After Milling	Shorts	0.88	1.04	0.03	<0.01	~~<0.01~~
CVE-15-21490 145/146	After Screening	Middlings	0.47	1.00	0.02	-**	~~**~~
Trial T2B							
CVE-15-21490 110/112	Prior processing	Wheat Grain	0.86	2.46	0.03	<0.01	~~<0.01~~
CVE-15-21490111	Prior processing	Wheat Grain	1.02	2.52	0.02		
CVE-15-21490 117/118	After Cleaning	Cleaned Grain	0.99	2.34	0.03	<0.01	~~<0.01~~
CVE-15-21490 123/124	After Cleaning	Waste (offal)	1.06	2.48	0.04	<0.01	~~**~~
CVE-15-21490 129/130	After Mixing	White Flour	0.45	0.87	0.02	~~<0.01~~	~~<0.01~~
CVE-15-21490 135/136	After Mixing	Total Bran	1.01	2.00	0.04	~~<0.01~~	~~<0.01~~
CVE-15-21490 141/142	After Milling	Shorts	0.87	1.17	0.03	<0.01	~~<0.01~~
CVE-15-21490 147/148	After Screening	Middlings	0.48	0.92	0.02	-**	~~**~~

* - Sample name for free and total trinexapac acid and CGA224439 / sample name for CGA 313458 and CGA 113745

** - Not enough sample available for analysis

Table B.7.5.3-17. Residues of trinexapac acid and metabolites in processed wheat (wholemeal bread) commodities

Sample Name*	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 224439	CGA 313458	~~CGA 113745~~
Trial T1A							
CVE-15-21490 047/049	Prior processing	Wheat Grain	0.65	1.15	0.02	<0.01	~~<0.01~~
CVE-15-21490048	Prior processing	Wheat Grain	0.63	0.97	0.02		
CVE-15-21490 055/056	After Mixing	Wholemeal Flour	0.59	0.71	0.02	~~<0.01~~	~~<0.01~~
CVE-15-21490 061/062	After Baking	Wholemeal Bread	0.57	0.64	0.03	~~0.01~~	~~<0.01~~
Trial T1B							
CVE-15-21490 050/052	Prior processing	Wheat Grain	0.58	0.55	0.02	<0.01	~~<0.01~~
CVE-15-21490051	Prior processing	Wheat Grain	0.52	0.96	0.01		
CVE-15-21490 057/058	After Mixing	Wholemeal Flour	0.50	0.76	0.02	~~<0.01~~	~~<0.01~~
CVE-15-21490 063/064	After Baking	Wholemeal Bread	0.40	0.63	0.03	~~0.01~~	~~<0.01~~
Trial T2A							
CVE-15-21490 151/153	Prior processing	Wheat Grain	0.72	2.26	0.03	<0.01	~~<0.01~~
CVE-15-21490152	Prior processing	Wheat Grain	0.91	2.44	0.03		
CVE-15-21490 159/160	After Mixing	Wholemeal Flour	1.06	1.83	0.03	~~<0.01~~	~~<0.01~~
CVE-15-21490 165/166	After Baking	Wholemeal Bread	0.88	1.49	0.05	~~0.02~~	~~<0.01~~
Trial T2B							
CVE-15-21490 154/156	Prior processing	Wheat Grain	1.12	2.43	0.02	<0.01	~~<0.01~~
CVE-15-21490155	Prior processing	Wheat Grain	1.16	2.61	0.03		
CVE-15-21490 161/162	After Mixing	Wholemeal Flour	1.06	1.59	0.03	~~<0.01~~	~~<0.01~~
CVE-15-21490 167/168	After Baking	Wholemeal Bread	0.87	1.44	0.05	~~0.02~~	~~<0.01~~

* - Sample name for free and total trinexapac acid and CGA224439 / sample name for CGA 313458 and CGA 113745

Table B.7.5.3-18. Residues of trinexapac acid and metabolites in processed wheat (germ) commodities

Sample Name*	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 224439	CGA 313458	~~CGA 113745~~
Trial T1A							
CVE-15-21490 067/069	Prior processing	Wheat Grain	0.41	0.64	0.02	<0.01	~~<0.01~~
CVE-15-21490068	Prior processing	Wheat Grain	0.46	1.10	0.02		
CVE-15-21490075	After Extraction	Germ	0.42	0.34	0.03	-**	~~-**~~
Trial T1B							
CVE-15-21490 070/072	Prior processing	Wheat Grain	0.50	1.20	0.02	<0.01	~~<0.01~~
CVE-15-21490071	Prior processing	Wheat Grain	0.57	1.15	0.02		
CVE-15-21490 077/078	After Extraction	Germ	0.37	0.33	0.02	-**	~~-**~~
Trial T2A							
CVE-15-21490 171/173	Prior processing	Wheat Grain	0.91	2.44	0.03	<0.01	~~<0.01~~
CVE-15-21490172	Prior processing	Wheat Grain	0.92	2.23	0.03		
CVE-15-21490 179/180	After Extraction	Germ	1.09	0.95	0.04	-**	~~-**~~
Trial T2B							
CVE-15-21490 174/176	Prior processing	Wheat Grain	0.98	2.53	0.03	<0.01	~~<0.01~~
CVE-15-21490175	Prior processing	Wheat Grain	1.02	2.44	0.03		
CVE-15-21490 181/182	After Extraction	Germ	1.13	0.66	0.03	-**	~~-**~~

* - Sample name for free and total trinexapac acid and CGA224439 / sample name for CGA 313458 and CGA 113745

** - Not enough sample available for analysis

Table B.7.5.3-19. Residues of trinexapac acid and metabolites in processed wheat (flour separation process) commodities

Sample Name*	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 224439	CGA 313458	~~CGA 113745~~
Trial T1A							
CVE-15-21490 081/083	Prior processing	Wheat Grain	0.61	0.86	0.02	<0.01	~~<0.01~~

Sample Name*	Processing	Processed product	Residue levels (mg/kg)				
			Trinexapac Acid (free)	Total trinexapac acid	CGA 224439	CGA 313458	~~CGA 113745~~
CVE-15-21490082	Prior processing	Wheat Grain	0.55	1.16	0.02		
CVE-15-21490089/090	After Drying	Dry Gluten	0.19	0.26	0.04	-**	~~-**~~
CVE-15-21490095/096	After Drying	Dry Starch	0.05	0.11	<0.01	<0.01	~~<0.01~~
CVE-15-21490101/102	After Mixing	Gluten Feed Meal	0.13	0.19	<0.01	<0.01	~~<0.01~~
Trial T1B							
CVE-15-21490084/086	Prior processing	Wheat Grain	0.57	1.15	0.02	<0.01	~~<0.01~~
CVE-15-21490085	Prior processing	Wheat Grain	0.57	0.51	0.02		
CVE-15-21490091/092	After Drying	Dry Gluten	0.17	0.26	0.04	-**	~~-**~~
CVE-15-21490097/098	After Drying	Dry Starch	0.05	0.09	<0.01	<0.01	~~<0.01~~
CVE-15-21490103/104	After Mixing	Gluten Feed Meal	0.13	0.16	<0.01	<0.01	~~<0.01~~
Trial T2A							
CVE-15-21490185/187	Prior processing	Wheat Grain	0.89	2.76	0.03	<0.01	~~<0.01~~
CVE-15-21490186	Prior processing	Wheat Grain	0.93	2.32	0.03		
CVE-15-21490193/194	After Drying	Dry Gluten	0.25	0.52	0.04	-**	~~-**~~
CVE-15-21490199/200	After Drying	Dry Starch	0.08	0.13	<0.01	<0.01	~~<0.01~~
CVE-15-21490205/206	After Mixing	Gluten Feed Meal	0.15	0.42	<0.01	<0.01	~~<0.01~~
Trial T2B							
CVE-15-21490188/190	Prior processing	Wheat Grain	1.06	2.65	0.03	<0.01	~~<0.01~~
CVE-15-21490189	Prior processing	Wheat Grain	1.12	2.64	0.03		
CVE-15-21490195/196	After Drying	Dry Gluten	0.30	0.50	0.08	-**	~~-**~~
CVE-15-21490201/202	After Drying	Dry Starch	0.09	0.13	<0.01	<0.01	~~<0.01~~
CVE-15-21490207/208	After Mixing	Gluten Feed Meal	0.20	0.38	<0.01	<0.01	~~<0.01~~

* - Sample name for free and total trinexapac acid and CGA224439 / sample name for CGA 313458 and CGA 113745
** - Not enough sample available for analysis

The median transfer factors for each commodity from the follow-up studies are calculated and presented in Tables B.7.5.3-20 to B.7.5.3-~~24~~ 23. The average residue level in the RAC has been considered for the calculations of free and total trinexapac acid and metabolite CGA 224439 because residues were measured in duplicate.

Transfer factors for trinexapac acid and the processing metabolites were derived by calculating the ratio of residue levels of residues into processed commodities to the residue levels in the RAC.

Table B.7.5.3-20. Summary of transfer factors into processed wheat products – trinexapac acid (free)

Process	Transfer factors				Median Transfer Factor*
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Cleaned Grain	1.88	1.02	1.16	1.05	1.28
Waste (offal)	0.53	0.51	1.08	1.13	0.81
White Flour	0.53	0.61	0.57	0.48	0.55
Total Bran	1.07	1.14	1.09	1.07	1.09
Shorts	0.97	0.78	0.93	0.93	0.90
Middlings	0.56	0.57	0.49	0.51	0.53
Wholemeal Flour	0.92	0.91	1.29	0.93	1.01
Wholemeal Bread	0.89	0.73	1.07	0.76	0.86
Germ	0.95	0.69	1.18	1.13	0.99
Dry Gluten	0.33	0.30	0.27	0.28	0.30
Dry Starch	0.09	0.09	0.09	0.08	0.09
Gluten Feed Meal	0.22	0.23	0.16	0.18	0.20

*- Median calculated for average results of A and B replicates.

Table B.7.5.3-21. Summary of transfer factors into processed wheat products – total trinexapac acid (free+conjugates)

Process	Transfer factors				Median Transfer Factor*
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Cleaned Grain	1.13	1.08	1.13	0.94	~~1.10~~ 1.07
Waste (offal)	0.94	0.74	1.14	1.00	0.96
White Flour	0.38	0.46	0.45	0.35	0.41

Process	Transfer factors				Median Transfer Factor*
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Total Bran	0.98	0.70	0.96	0.80	0.86
Shorts	0.61	0.58	0.48	0.47	0.54
Middlings	0.53	0.58	0.46	0.37	0.49
Wholemeal Flour	0.67	1.00	0.78	0.63	0.77
Wholemeal Bread	0.60	0.83	0.63	0.57	0.6
Germ	0.39	0.28	0.41	0.27	0.34
Dry Gluten	0.26	0.31	0.20	0.19	0.24
Dry Starch	0.11	0.11	0.05	0.05	0.08
Gluten Feed Meal	0.19	0.19	0.17	0.14	0.17

*- Median calculated for average results of A and B replicates.

Table B.7.5.3-22. Summary of transfer factors into processed barley products – CGA224439

Process	Transfer factors				Median Transfer Factor*
	699784/1 A	699784/1 B	699784/2 A	699784/2 B	
Cleaned Grain	1	1	1.20	1.20	1.10
Waste (offal)	1.50	1.50	1.20	1.60	~~1.45~~ 1.50
White Flour	1	0.5	0.80	0.80	~~0.78~~ 0.80
Total Bran	1	1	1.20	1.60	~~1.20~~ 1.10
Shorts	1	0.50	1.20	1.20	~~0.98~~ 1.10
Middlings	1	0.50	0.80	0.80	~~0.78~~ 0.80
Wholemeal Flour	1	1.30	1	1.20	~~1.13~~ 1.10
Wholemeal Bread	1.50	2	1.67	2	~~1.79~~ 1.84
Germ	1.50	1	1.33	1	~~1.21~~ 1.17
Dry Gluten	2	2	1.33	2.67	2.00
Dry Starch	n.c.	n.c.	n.c.	n.c.	-
Gluten Feed Meal	n.c.	n.c.	n.c.	n.c.	-

n.c.: not calculated because residues were <LOQ (0.01 mg/kg)

*- Median calculated for average results of A and B replicates.

Table B.7.5.3-23. Summary of transfer factors into processed wheat products – CGA313458

Process	Transfer factors	Median Transfer

	699784/1 A	699784/1 B	699784/2 A	699784/2 B	Factor
Cleaned Grain	n.c.	n.c.	n.c.	n.c.	-
Waste (offal)	n.c.	n.c.	n.c.	n.c.	-
White Flour	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	-
Total Bran	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	-
Shorts	n.c.	n.c.	n.c.	n.c.	-
Middlings	n.c.	n.c.	n.c.	n.c.	-
Wholemeal Flour	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
Wholemeal Bread	~~1~~	~~1~~	~~2~~	~~2~~	~~1.5*~~
Germ	n.c.	n.c.	n.c.	n.c.	-
Dry Gluten	n.c.	n.c.	n.c.	n.c.	-
Dry Starch	n.c.	n.c.	n.c.	n.c.	-
Gluten Feed Meal	n.c.	n.c.	n.c.	n.c.	-

n.c.: not calculated because residues were <LOQ (0.01 mg/kg)

~~* calculated presumed that residue amount in RAC is 0.01 mg/kg (real amount <0.01 mg/kg)~~

~~Table B.7.5.3-24. Summary of transfer factors into processed wheat products – CGA113745~~

~~Process~~	~~Transfer factors~~				~~Median Transfer Factor~~
	~~699784/1 A~~	~~699784/1 B~~	~~699784/2 A~~	~~699784/2 B~~	
~~Cleaned Grain~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Waste (offal)~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~White Flour~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Total Bran~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Shorts~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Middlings~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Wholemeal Flour~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Wholemeal Bread~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Germ~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Dry Gluten~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Dry Starch~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~
~~Gluten Feed Meal~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~n.c.~~	~~-~~

~~n.c.: not calculated because residues were <LOQ (0.01 mg/kg)~~

RMS comments and conclusion

Residues of trinexapac acid (free) were concentrated in cleaned grain and total bran (TF 1.09-1.28). Residues of trinexapac acid (free and conjugated) were slightly concentrated only in cleaned grain (TF 1.1). Residues of trinexapac acid (free and total) were slightly concentrated in trial 2, but the ~~mean~~ median TF remained <1. Metabolite CGA313458 was ~~concentrated in wholemeal bread (TF 1.5), but was~~ not detected in any other fraction, although results in white and wholemeal flour, wholemeal bread and bran are not reliable. Residue of CGA224439 was concentrated in waste (offal), wholemeal bread and dry gluten (TF 1.45-2.00), and slightly concentrated in cleaned grain, total bran, wholemeal flour and germ (TF 1.10-~~1.21~~ 1.17).

Metabolite CGA113745 was not detected in any sample (<0.01 mg/kg). Although CGA113745 was found to be unstable in brewing and baking samples (wheat grain, flour, bran, beer and bread) stored under frozen storage conditions. Only 20% CGA113745 was found after 30 days and samples in this study were analysed after maximum of 15 months of storage.

Analytical method GRM020.14A for CGA113745 gave poor chromatography during the processing study so development work was carried out and the chromatography was improved. The improved chromatography was used in the storage stability study to analyse for CGA113745 in processed matrices and showed that CGA113475 was unstable in the presence of crop matrices - degrading to only 20% of the initial amount over 30 days.

Thus it can be assumed that inaccurate levels of CGA113745 were found in both the pre-processed incurred grain samples and the processed commodities due to degradation in storage and poor chromatography including possible co-elution with other components. Therefore any data regarding residue levels of CGA113745 in the processing studies on wheat and barley should be disregarded and have been struck through.

Wheat samples for trinexapac acid analysis from sampling to analysis were stored for up to 8 months. Trinexapac acid is stable in grain for at least 24 months. Results are covered by storage stability data.

Samples for metabolite CGA 313458 analysis from sampling to analysis were stored for up to 7.5 months. From processing till analysis – stored for up to 7 (6 months for germ). As the metabolite CGA 313458 was shown to be stable for only 3 months on flour, 12 months in grain and 6 months in bran and bread, any data regarding residue levels of this metabolite in flour, bran and bread in the processing studies on wheat and barley should be disregarded and have been struck through. No information regarding storage stability in remaining processed commodities were provided. Residue levels of CGA 313458 in flour, bran and bread as well as transfer factor in to flour, bran and bread should be assessed further.

Wheat samples for metabolite CGA 224439 analysis from sampling to analysis were stored for up to 7 months. From processing till analysis – stored for up to 6 -7 months. CGA 224439 is stable in grain and processed products for at least 12 months. Results are covered by storage stability data.

However, the application rate was three times higher than the critical GAP (400 g a.s./ha). Studies were well performed and reported.

Deviations:

Due to insufficient sample weights, 20 out of 96 samples for CGA113745 and 19 for CGA313458 could not be analysed. None of the germ and dry gluten samples were analysed for these metabolites.

Evaluation of processing metabolites

Residue levels of trinexapac acid (free and conjugated) ranged from 0.5–2.8 mg/kg in wheat grain and from 1.56–1.9 mg/kg in barley grain. Residue levels in processed commodities were all above the LOQ, allowing derivation of robust processing factors.

Both processing studies showed that residue levels of CGA313458 were below the LOQ (0.01 mg/kg) in virtually all matrices studied (except in one beer sample where it was found at 0.01 mg/kg and wholemeal bread where it was found at 0.01-0.02 mg/kg). Although results in barley bran, wheat bran, flour and bread samples are not covered by storage stability data. Magnitude of CGA 313458 in above mentioned processed commodities and processing factors should be further assessed. ~~Therefore no processing factor has been derived for this metabolite as is it not present in significant quantity in any of the commodities studied.~~

CPCA was recovered in low amounts in the grain (0.02-0.05 mg/kg) and in the processed commodities in the following low amounts:

- <0.01-0.03 mg/kg in all barley processed products, except bran (0.12 mg/kg) and brewers' yeast (0.11 mg/kg);
- <0.01-0.05 mg/kg in all wheat processed products, except dry gluten (0.08 mg/kg).

However, these residue levels are not significant when compared to the initial residue levels of trinexapac acid; the processing factors derived are consequently all very low (Table B.7.5.3-24).

Table B.7.5.3-24 Processing factors for cyclopropane carboxylic acid

Processed Commodity	Median PF*
Barley, pot	0.01
Barley, pearled	0.01

Barley, bran	0.06
Barley, flour	0.01
Barley, brewing malt	0.01
Barley, malt sprouts	0.02
Barley, brewers' grain	0.01
Barley, brewers' yeast	0.05
Barley, beer	0.01
Wheat, waste (offal)	0.02
Wheat, bran	0.02
Wheat, shorts	0.01
Wheat, middlings	0.01
Wheat, white flour	0.01
Wheat, wholemeal flour	0.02
Wheat, wholemeal bread	0.02
Wheat, germ	0.02
Wheat, dry gluten	0.03
Wheat, dry starch	0.01
Wheat, gluten feed meal	0.01

*Processing Factor calculated as residue of CPCA in processed product/residue of total trinexapac acid in RAC

The barley and wheat processing studies showed that residue levels of CGA113745 were below the LOQ (0.01 mg/kg) in all matrices, except bran (at 0.01 mg/kg). Although germ and dry gluten samples weight were not enough for analysis. ~~Therefore no processing factor has been derived for this metabolite~~. Nevertheless all residue results are not covered by storage stability data and the metabolite is proven to be unstable. Residue levels of this metabolite in RAC and processed commodities as well as processing factors should be further assessed.

Based on the results from the processing studies and the exposure calculations (see also Volume 1 section 2.7.9), the proposed residue definition for processed commodities for monitoring is "sum of trinexapac acid and its salts, expressed as trinexapac acid" ~~trinexapac-acid (free)~~; the proposed residue definition for risk assessment is ~~sum of~~ trinexapac acid (free and conjugated) ~~and OH-trinexapac-acid~~, expressed as trinexapac acid (see also Volume 1 Section 2.7.3). The possible inclusion of metabolite CGA 113745 in residue definition in processed commodities should be further assessed when data on magnitude in RAC and processed commodities will be available.

The median processing factors derived for monitoring and risk assessment, as well as median conversion factors are summarised in Table B.7.5.3-25. In order to derive robust processing factors, data from all available

processing studies were considered. The conversion factors were derived from the studies where trinexapac acid (free) and trinexapac acid (free and conjugated) were both measured.

The processing factors for trinexapac acid (free) and the conversion factors are derived for monitoring purposes. The risk assessment (see Volume 1 section 2.7.9) has been performed with processing factors for risk assessment.

Table B.7.5.3-25: Summary of processing studies and available processing factors

Processed Commodity	Number of Studies[a]	Median PF_{Mo} [b]	Median PF_{RA} [c]	Median CF [d]	Report References	Source
Residue definition for enforcement: ~~trinexapac acid (free)~~ sum of trinexapac acid and its salts, expressed as trinexapac acid **Residue definition for risk assessment: sum of trinexapac acid and its salts (free and conjugated) ~~and OH trinexapac acid~~, expressed as trinexapac acid**						
Barley, pot	~~4/4~~ 2/2	~~0.64~~ 0.65	0.20	~~0.31~~ 0.32	~~9821701~~ ~~9821702~~ ~~9821801~~ ~~9821802~~ ~~9822002~~ ~~9822001~~ ~~9821902~~ ~~9821901~~ T003422-07 37194	~~The Netherlands, 2003~~ New data
Barley, pearled	~~4/8~~ 2/6	0.60	~~0.70~~ 1.01	0.30		
Barley, bran	~~4/8~~ 2/6	1.28	~~1.07~~ 1.70	0.21		
Barley, flour	~~4/8~~ 2/6	~~0.84~~ 0.85	~~0.43~~ 0.44	~~0.52~~ 0.51		
Barley, brewing malt	~~9/4~~ 2/2	~~0.70~~ 0.75	~~0.50~~ 0.51	~~0.73~~ 0.69		
Barley, malt sprouts	~~4/4~~ 2/2	0.87	~~0.09~~ 0.10	0.11		
~~Barley, wort~~	~~5/0~~	~~0.22~~	~~-~~	-		
Barley, brewers' grain	~~4/4~~ 2/2	~~0.36~~ 0.37	~~0.11~~ 0.12	~~0.32~~ 0.33		
Barley, brewers' yeast	~~4/4~~ 2/2	1.69	0.17	0.10		
Barley, beer	~~9/4~~ 2/2	~~0.18~~ 0.15	0.06	~~0.44~~ 0.42		
Wheat, waste (offal)	~~4/4~~ 2/2	0.81	0.96	1.29	3011/00 T003605-07 T002695-03 37278	The Netherlands, 2003 New data
Wheat, bran	~~9/8~~ 7/6	~~1.93~~ 2.20	~~1.36~~ 1.56	0.79		
Wheat, shorts	~~8/8~~ 6/6	0.91	~~0.50~~ 0.59	0.60		
Wheat, middlings	~~8/8~~ 6/6	~~0.49~~ 0.47	~~1.87~~ 0.51	0.91		
Wheat, white flour	~~9/8~~ 7/6	~~0.42~~ 0.32	0.43	0.75		
Wheat, wholemeal flour	~~5/4~~ 3/2	~~1.01~~ 1.00	~~0.77~~ 0.78	0.78		
Wheat, wholemeal bread	~~5/4~~ 3/2	~~0.82~~ 0.81	0.66	~~0.79~~ 0.77		
Wheat, germ	~~8/8~~ 6/6	~~0.90~~ 0.93	~~0.71~~ 0.92	0.35		
Wheat, dry gluten	~~4/4~~ 2/2	0.30	~~0.24~~ 0.25	~~0.82~~ 0.81		
Wheat, dry starch	~~4/4~~ 2/2	0.09	0.08	~~0.91~~ 0.89		
Wheat, gluten feed meal	~~4/4~~ 2/2	0.20	~~0.17~~ 0.18	0.88		

Mo: monitoring; RA: risk assessment

(a): All available processing studies have been considered, i.e. even those where trinexapac acid (free) or trinexapac acid (free and conjugated) were not measured. In such cases, two numbers are displayed - e.g., 4/8 means that 4 studies measured trinexapac acid (free) and 8 studies measured total trinexapac acid (free and conjugated).

(b): The median processing factor is obtained by calculating the median of the individual processing factors of each processing study. Those processing factors are based on residue levels of trinexapac acid (free) and therefore derived for monitoring purposes.

(c): The median processing factor is obtained by calculating the median of the individual processing factors of each processing study. Those processing factors are based on residue levels of total trinexapac acid (free and conjugated) and therefore are the ones used for the risk assessment calculations.

(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors of each processing study. They are derived for monitoring purposes.

(e): Conversion factor derived based on the studies were both trinexapac acid (free) and total trinexapac acid (free and conjugated) were measured.

B.7.6 Residues in rotational crops

B.7.6.1 Metabolism in rotational crops

The metabolism of trinexapac-ethyl in rotational crops was investigated in lettuce, sugar beet, radish, winter wheat and corn using [^{14}C-cyclohexyl]-trinexapac-ethyl. One confined rotational crop study investigating the nature of residues following different plant-back intervals has been investigated during the peer review; a new study has been conducted in 2010 in order to cover a higher application rate. These studies are summarised in Table B.7.6.1-1; full details of both studies are summarised below.

Table B.7.6.1-1: Summary of metabolism studies in rotational crops

Group	Crop	Label Position	Application and Sampling Details				Report Reference	Source
			Method, F or G[(a)]	Rate (kg a.s./ha)	Sowing Interval (DAT)	Harvest Interval (DAT)		
EU Reviewed Data								
Leafy vegetables	Lettuce	^{14}C-cyclohexyl	Bare soil, F	0.15	99, 119	129, 169	23/92	The Netherlands, 2003
Root and tuber vegetables	Sugar beet				343, 407, 496	387, 515, 693		
Cereals	Winter wheat				173, 299, 343, 407	227, 479, 567, 695		
	Corn				369, 407, 496	400, 476, 654		
New data								
Leafy vegetables	Lettuce	^{14}C-cyclohexyl	Bare soil, F	0.33	30, 120, 270	Immature: 86, 183, 290 Mature: 113, 198,	1802W	-

Group	Crop	Label Position	Application and Sampling Details				Report Reference	Source
			Method, F or G[(a)]	Rate (kg a.s./ha)	Sowing Interval (DAT)	Harvest Interval (DAT)		
						309		
Root and tuber vegetables	Radish				30, 120, 309	83, 183, 350		
Cereals	Winter wheat				30, 120, 270	Forage: 83, 168, 296 Hay: 168, 209, 315 Grain, straw: 231, 251, 352		

Study 1

EU reviewed metabolism study on lettuce, sugar beet, wheat and corn after soil application of ^{14}C-trinexapac-ethyl (rotational crop)

Reference:	**Krauss J.H. (1992)** Outdoor confined accumulation study on rotational crops after bareground soil application of [^{14}C-Cyclohexyl]CGA 163935 (KCIIA 6.6.1 / 01)
Report No.:	23/92
Project No.:	89JK03
Guideline:	EPA Guideline 165-1, Confined accumulation studies on rotational crops; Agricultural chemicals laws and regulations, Japan, Metabolism in plants, Society of Agricultural Chemical Industry. (1985)
GLP:	Yes. The study was performed in compliance of the OECD principles of GLP, Paris/France 1981; The procedure and principles of GLP in Switzerland, Federal Department of the Interior, 1986; The US EPA GLP standards, Pesticide programs (40 CFR 160).
Previous evaluation:	DAR 2003
Material and methods:	
Test item:	[^{14}C-Cyclohexyl]CGA 163935 (trinexapac-ethyl)
Lot/Batch No.:	B-1036.1A
Radiochemical Purity:	98% (specific radioactivity 1.71 MBq/mg (46.2 µCi/mg)
Test concentration:	150 g a.s./ha
Test system:	The study was conducted outside at the Ciba-Geigy research farm in Klus, Switzerland in sandy loam soil. The soil characteristics were: pH (7.3), organic carbon (1.28%), sand (30.6%), silt (44.4%), clay (25.0%). The test compound [^{14}C-cyclohexyl] CGA 163935 was applied to the soil as EC formulation by spraying to a 2x2m test plot at a rate of 0.15 kg as/ha. The bareground treated plot was divided into

	four sections in which three rotational crops were subsequently planted (1 m^2 for each crop) ^{14}C-cyclohexyl labelled trinexapac-ethyl was applied at a rate of 0.15 kg a.s./ha to bare ground plots using a spraying device with four 80o T-Jet flat-fan nozzles with a flow of 0.78 l/min at ca. 3 bar overpressure. The rate applied is 25 % below the max proposed application rate for barley (proposed GAP for wheat – 125 g/ha, barley – 150-200 g/ha). Four rotational crops, lettuce (variety *Sorraya*), sugar beet (variety *KWS*), corn (variety *Blizzard*) and winter wheat (variety *Zenta*), were planted in the treated areas after 69 days (lettuce), 119 days (wheat), 299 days (sugar beets) and 338 days (corn). Plant samples were harvested at different time points after planting/seeding: lettuce at 30 and 50 days, winter wheat at 54, 180, 224 and 288 days; sugar beets at 44, 108, and 197 days, corn at 31, 69 and 158 days. Soil samples were taken 1h after application of the radio-label and after 69, 99, 119, 173, 299, 324, 369, 407, 496 days (at each sampling of plants) and were divided into a 0-5 cm, 5-10 cm, 10-20 cm and 20-30.4 cm layers. After harvest, all samples were stored at -18°C or analysed at the same day. The following plant parts were analysed: whole tops, stalks, husks, grains of winter wheat; tops, roots of sugar beets; whole tops, stalks, cobs, grains of corn, and lettuce heads.
No. of applications:	One
Method of analysis:	The fresh or dry plant parts as well as the dried soil layers were homogenized. After homogenization each sample was radioassayed by combusting three aliquots, ~1 to 2 g, in a biological Materials Oxidiser. The ^{14}C-laballed material in the samples is thus converted to $^{14}CO_2$ and absorbed in an appropriate scintillation cocktail. Only soil samples were extracted with methanol (3 times, the last time hot Soxhlet), in order to determine the not extractable radioactivity. TLC was applied to detect the parent compound. The LOQ for both methods (combustion, TLC) is 0.001 mg/kg.
Limit of quantification:	0.001 mg/kg

Results

The total radioactive residues in plant parts of different rotational crops are summarised in table B.7.6.1-2.

Table B.7.6.1-2: Total residues in rotational crops

Sample	DAT	Harvest (days after seeding)	TRR (mg eq/kg)
Lettuce heads	99	30	0.001
	119	50	0.001
Winter wheat			
Whole tops	173	54	0.001
Whole tops	299	180	<0.001
Whole tops	343	224	<0.001
Whole tops	407	288	<0.001
Stalks	407	288	0.002

Sample	DAT	Harvest (days after seeding)	TRR (mg eq/kg)
Husks	407	288	0.001
Grains	407	288	<0.001
Sugar beets			
Tops	343	44	<0.001
Roots	343	44	0.001
Tops	407	108	<0.001
Roots	407	108	<0.001
Tops	496	197	<0.001
Roots	496	197	<0.001
Corn			
Whole tops	369	31	0.001
Whole tops	407	69	<0.001
Stalks	496	158	<0.001
Cobs	496	158	<0.001
Grain	496	158	<0.001

The total residue in all sample material analysed was below or around the LOQ. The only exception is the stalks from winter wheat, which contained 0.002 mg eq/kg. With regard to these low residue levels, no further attempt was made to elucidate and characterise the nature of residue.

In soil the radioactivity in the upper layer declined from 0.256 mg eq/kg after application to 0.034 – 0.044 mg eq/kg parent equivalents at harvest of corn and sugar beets. More than 79% TRR in the upper soil layer was non-extractable at all harvests times. No attempts were made to identify or characterise the soil residue.

RMS comments and conclusion (Netherlands, 2003)

The uptake of CGA 163935 in rotational crops, as analysed in lettuce, winter wheat, sugar beets and corn after direct application of 0.15 kg as/ha radio-labelled compound to the soil, is very low (<0.01 mg/kg). The application rate of CGA 163935 was 25% below the proposed GAP for barley (150 g instead of 200 g as/ha). The study is considered suitable for evaluation.

Comments and conclusions RMS LT

RMS LT agrees with the conclusions made by RMS NL. TRR in rotational crops were at or below 0.001 mg/kg, therefore identification or characterisation is not required.

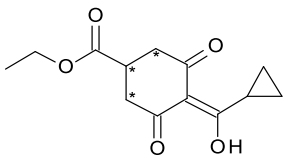
Additional deviations from OECD 502 were noticed:

Rotational intervals did not assess circumstances of crop failure or closely rotated crops (7-30 days recommended). Crops were planted 99, 173, 343 and 369 days after treatment for lettuce, wheat, sugar beet and corn respectively.

Study is considered acceptable for evaluation, even though not fully addressing the metabolism in rotational crops.

Study 2

New metabolism study on lettuce, radish and wheat after soil application of ^{14}C-trinexapac-ethyl (rotational crop)

Reference:	**Quistad G.B., Kovatchev A. (2010)** Trinexapac-Ethyl – Uptake and Metabolism in Confined Rotational Crops. PTRL-West, USA. Final report. (Syngenta File No. CGA163935_50024). (KCA 6.6.1 / 02).
Report No.:	1802W
Task No.:	T001384-08
Guideline:	Residues Chemistry Test Guidelines, OPPTS 860.1850. Confined Accumulation in Rotational Crops. United States Environmental Protection Agency, August 1996. Residues in or on Treated Products, Food and Feed; Official Journal of the European Communities; Commission Directive 96/68/EEC; October 1996. OECD Guidelines for the Testing of Chemicals, Test No. 502: Metabolism in Rotational Crops, adopted 8 January 2007 Japanese Ministry of Agriculture, Forestry and Fisheries. Guideline on the Application for Agricultural Chemicals Registration (12 Nohsan No. 8147, November 24, 2000).
GLP:	Yes. EPA GLP Standards, 40 CFR Part 160, with some exceptions*
Previous evaluation:	Submitted for the purpose of renewal
Reason for submission:	To cover a higher application rate
Material and methods:	
Test item:	[^{14}C-cyclohexanedione-1,2,6] Trinexapac-ethyl
Position of radiolabel: (*=^{14}C position)	
Lot/Batch No.:	RDR-IV-51
Radiochemical Purity:	99.3% (specific radioactivity 2.449 KBq/mg)
Test concentration:	0.350 kg a.s./ha (achieved 0.333-0.334 kg a.s./ha)
Test system:	The study was conducted outside in a field plot in Madera, California in sandy loam soil. The soil characteristics were: pH (7.4), organic carbon (1.3%), sand (81%), silt (8%), clay (11%). [^{14}C-Cyclohexanedione-1, 2, 6]-Trinexapac-ethyl radiochemical was formulated with Palisade™ EC inerts and applied as a diluted aqueous solution Three rotational crops, lettuce (variety *Salad Bowl*), radish (variety *Crimson Giant*) and wheat (variety *Certified Summit*), were planted in the treated areas after 30, 120,

	270 and 309 days. Radishes from 270 day PBI did not produce root bulbs apparently due to the summer and were replanted at 309 DAT. Rotational crops used were wheat (forage, hay, straw, grain) as the small grain or cereal, lettuce (immature and mature) as the leafy vegetable and radish (foliage and root) as the root vegetable. Rotational crops were sown in treated soil at the following plantback intervals (PBI) in days after treatment (DAT): 30, 120 and 270. In addition, radishes were also sown at 309 day PBI due since the 270 day PBI crop succumbed to the summer heat. Raw agricultural commodities (RACs) were harvested at appropriate intervals for analysis. Except for total radioactive residues (TRR) found in wheat foliage and lettuce RACs at the 30 day PBI (< 0.02 mg/kg), all other RACs throughout the study were <0.01 mg/kg by combustion.
Storage stability:	No storage stability determination was required since processing, extraction and quantitative analysis of extracts did not exceed 6 months from harvest. Comparison of the initial and final radio-component profiles showed little or no significant changes had occurred during the interim period of freezer storage
No. of applications:	One
Method of analysis:	Chopped subsamples of RACs (TRR >0.01 mg/kg) were sequentially macerated and/or mechanically shaken with initially 50-100% ACN (50% ACN in water twice, then 100% ACN once). Following centrifugation and/or vacuum filtration, supernatants were combined and residues quantified by HPLC, either directly and/or as an aqueous concentrate (ACN evaporated off). Additional characterization was attempted by TLC and/or a different HPLC system against available reference standards. For standards without UV absorbance, plates were dipped in a 10% copper sulphate solution in 10% phosphoric acid in methanol after scanning for radioactivity. Plates were then heated at 100° C for approximately 5 minutes to visualize standards. PES with TRR of >0.01 mg/kg and >10% TRR were extracted with 0.1M KOH and subsequently with 24% KOH (hemi-cellulose digestion). Since most RACs were <0.01 mg/kg (by combustion), only few RACs required extraction. However, 30 day PBI lettuce and wheat foliage RACs had TRRs >0.01 mg/kg and were extracted as described above. Initial 50-100% ACN extractability was low in all extracted RACs (<42% TRR), so the post-extracted solids (PES) were further extracted with 0.2M KOH and/or 24% KOH, as needed. For 30 day PBI lettuce RACs, aliquots of the aqueous concentrates of the combined ACN/water supernatants were each further characterized by enzymatic (Driselase/β-glucosidase) and/or mild acid (1M HCl, overnight at room temperature) treatments to determine if the polar unknowns were sugar conjugates. The aqueous hydrolysate was partitioned with ethyl acetate (EtOAc) and partitioned fractions applied to HPLC when feasible. The ACN/water supernatant(s) of 30 day PBI lettuce was also further characterized by TLC. The final PES was determined by combustion and subsequent liquid scintillation counting (LSC).
Limit of quantification:	0.001 mg/kg

*- Supporting data such as historical data, plot slope, Soil Conservation Service data; Pesticide history; Some plot observations made prior to application of the substance.

Results

Total residues determined by initial combustion and sum of fractions in rotational crop RACs are given in TableB.7.6.1-3. The highest TRRs were in 30 day PBI immature and mature lettuce and wheat forage and hay, between 0.011 and 0.017 mg/kg. All other RACs were <0.01 mg/kg by the initial combustions, not requiring extraction. For RACs > 0.01 mg/kg, Tables B.7.6.1-4 and B.7.6.1-5 provide TRRs for the initial extraction and/or subsequent PES extractions/treatments. Initial extractability with 50-100% ACN was low (<42% TRR) for these RACs. In 30 day PBI lettuce RACs, 24% KOH treatment released approximately 41-43% TRR (0.006-0.007 mg/kg) from the PES suggesting bound 14C-residues were associated with natural plant products (incorporated, entrapped and/or conjugated).

Table B.7.6.1-3. Summary of total radioactive residues in rotated crop samples grown in soil treated with [^{14}C-cyclohexanedione-1, 2, 6]-trinexapac-ethyl

Trinexapac-ethyl Treated Commodity	Plant Back Interval (Days)	Days between planting and harvest	TRR by Initial Combustion (mg/kg)	TRR by Summation of Extractable and Non-Extractable Radioactivity (mg/kg)
Wheat forage	30	53	0.010	0.010
	120	48	0.004	0.004
	270	26	0.002	NA
Wheat hay	30	138	0.009	0.011
	120	89	0.009	0.009
	270	45	0.008	NA
Wheat straw	30	201	0.003	NA
	120	131	0.005	NA
	270	82	0.004	NA
Wheat grain	30	201	0.005	NA
	120	131	0.008	0.007
	270	82	0.003	NA
Radish root	30	53	0.002	NA
	120	63	0.002	NA
	309*	41	0.001	NA
Radish foliage	30	53	0.005	NA
	120	63	0.007	NA
	309*	41	0.001	NA
Immature Lettuce	30	56	0.010	0.011
	120	63	0.004	NA
	270	20	0.007	NA

Trinexapac-ethyl Treated Commodity	Plant Back Interval (Days)	Days between planting and harvest	TRR by Initial Combustion (mg/kg)	TRR by Summation of Extractable and Non-Extractable Radioactivity (mg/kg)
Mature Lettuce	30	83	0.018	0.017
	120	78	0.004	NA
	270	39	0.001	NA

NA - not applicable (not extracted since <0.01 mg/kg).

*- Radishes from 270 day PBI did not produce root bulbs apparently due to the summer and were replanted at 309 DAT.

The components from the combined ACN/water extraction for each sample are summarized in Table B.7.6.1-4 for lettuce and table B.7.6.1-5 for wheat for extracted RACs: 30 day PBI lettuce (immature and mature), 30 day PBI wheat forage and hay and 120 day PBI wheat forage, hay and grain. Any slight discrepancies within these tables between %TRR and their corresponding mg/kg values are due to rounding. Unextractable residues (final PES) were determined by combustion.

One or more minor polar components were observed in the 30 day PBI lettuce RACs by HPLC. The same combined 50-100% ACN supernatant of the mature lettuce was also applied to two TLC solvent systems (both silica-gel). However, the polar residues remained at or near the TLC origin in both solvent systems. Further characterization of the aqueous concentrate from each combined supernatant (ACN evaporated off) included separate enzymatic and mild acid treatments for both 30 day PBI immature and mature lettuce. Subsequent EtOAc partitioning and/or chromatography of the EtOAc fraction of each lettuce hydrolysate demonstrated the hydrolysis attempts failed to release any identifiable residues.

In 30/120 day wheat forage and/or hay, two minor residues (each < 0.002 mg/kg) matched CGA179500 (free acid) and CGA312753 reference standards on both HPLC and TLC.

Figure B 7.6.1-1.provides a proposed metabolic pathway for [^{14}C-Cyclohexanedione-1, 2, 6]-Trinexapac-Ethyl in rotational crops following application to bare soil.

In this study, no individual extractable ^{14}C-residue was found to be > 0.01 mg/kg for any RAC at any PBI.

Table B.7.6.1-4: Summary of characterization and identification of residues in lettuce samples grown in soil previously treated with [^{14}C-cyclohexanedione-1, 2, 6]-trinexapac-ethyl

Plantback interval (DAT)		30		120		270	
Immature lettuce							
TRR by sum of fractions mg/kg		0.011		0.004[a]		0.007[a]	
Origin of component	Component	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR

Initial extraction[b] applied to chromatography, %TRR		0.004	36.4	NA	NA
Chromatography of Initial Extraction (ACN/water combined supernatants)	CGA163935	ND	ND		
	CGA179500	ND	ND		
	Unassigned Peaks (n =2, each ≤ 0.002 mg/kg)	0.003	27.3		
	Non-defined (each < 0.001mg/kg)	<0.001	<9.1		
PES Characterization	0.1M KOH	0.001	9.1		
	Hemi-cellulose extract[d]	0.005	45.5		
Unextractable	Final PES[a]	0.001	9.1		
Totals[e]		0.011	100.1		

Mature lettuce							
TRR by sum of fractions mg/kg		0.017		0.004		0.001	
Origin of component	Component	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Initial extraction[c] applied to chromatography, %TRR		0.007	41.2	NA		NA	
Chromatography of Initial Extraction (ACN/water combined supernatants)	Trinexapac-Ethyl	ND	ND				
	CGA179500	ND	ND				
	Unassigned Peaks[f]	0.007	41.2				
	Non-defined (each < 0.001mg/kg) [g]	<0.001	<5.9				
PES Characterization	0.1M KOH	0.001	5.9				
	Hemi-cellulose extract[d]	0.006	35.3				
Unextractable	Final PES[a]	0.003	17.6				
Totals[e]		0.017	100.0				

ND = not detected. NA = not applicable. [a]- Determined by combustion. [b]- Combined supernatants of 50-100% ACN in water extracts. Additional characterization included separate treatment of the concentrated, combined extract with: 1) 1 M HCl treatment (overnight at room temperature), and then partitioned with EtOAc (18.2% EtOAc and 81.8% aqueous) and 2) Driselase/ß-glucosidase and partitioned (after acidification) with EtOAc (EtOAc phase, 29.0%, 0.001 ppm, 9.1% TRR and aqueous phase, 71.0%, 0.003 ppm, 27.3% TRR).

[c]- Combined supernatants of 50-100% ACN in water extracts. Additional characterization included separate treatment of the concentrated, combined extract with: 1) Driselase/ß-glucosidase and partitioned (after acidification) with EtOAc (EtOAc phase 31.2%, 0.002 ppm, 12.9% TRR) and (Aqueous phase 68.8%, 0.005 ppm, 28.3% TRR); HPLC of EtOAc phase gave 62.2%, 0.001 ppm at RT 4.3 min. and 2) 1 M HCl treatment (overnight at room temperature), then partitioned with EtOAc (22.4% in EtOAc and 77.6% in aqueous phases).

[d]- 24% KOH.

[e]- Totals = ACN/water extractable residues + PES characterization + Final PES (Unextractable).

[f]- On TLC silica (chloroform:ACN:formic acid, 5:5:1), polar unknown remained at origin.

[g]- Non-defined TRR excluded from "Totals" since <0.001 mg/kg.

Table B.7.6.1-5: Summary of characterization and identification of residues in wheat samples grown in soil previously treated with [^{14}C-cyclohexanedione-1, 2, 6]-trinexapac-ethyl

Plantback interval (DAT)		**30**		**120**		**270**	
Wheat Forage							
TRR by sum of fractions mg/kg		0.010		0.004 (0.004[a])		0.002[a]	
Origin of component	Component	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Initial extraction applied to chromatography, %TRR		0.005	50.0	0.002	50.0	NA	
Chromatography of Initial Extraction (ACN/water combined supernatants)	Trinexapac-ethyl	ND	ND	ND	ND		
	CGA179500[b]	0.002	20.0	ND	ND		
	CGA312753[c]	0.001	10.0	ND	ND		
	Unassigned Peaks (n =2, each ≤ 0.002 mg/kg)	-	-	≤0.002	≤50.0		
	Non-defined (each < 0.001mg/kg)	0.002	20.0	-	-		
Unextractable	Final PES[a]	0.005	50.0	0.002	50.0		
Totals[d]		0.010	100.0	0.004	100.0		
Wheat Hay							
TRR by sum of fractions mg/kg		0.011 (0.009[a])		0.009 (0.009[a])		0.008[a]	
Origin of component	Component	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Initial extraction applied to chromatography, %TRR		0.004	36.4	0.003	33.3	NA	
Chromatography of Initial Extraction (ACN/water combined supernatants)	Trinexapac-Ethyl	ND	ND	ND	ND		
	CGA179500[b]	0.001	9.1	ND	ND		
	CGA312753[b]	0.002	18.2	0.001	11.1		
	Unassigned Peaks (n≤3, each ≤0.001 mg/kg)	≤0.001	≤9.1	≤ 0.002	≤ 22.2		
	Non-defined (each ≤0.001mg/kg) [e]	-	-	≤ 0.001	≤ 11.1		
PES Characterization	0.1M KOH	NA	NA	0.001	11.1		
	Hemi-cellulose extract[f]	NA	NA	0.004	44.4		
Unextractable	Final PES[a]	0.007	63.6	0.001	11.1		
Totals[d]		0.011	100.0	0.009	99.9		

Wheat Grain							
TRR by sum of fractions mg/kg		0.005[a]		0.007 (0.008[a])		0.003[a]	
Origin of component	Component	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Initial extraction applied to chromatography, %TRR		NA		0.001	14.3	NA	
Chromatography of Initial Extraction (ACN/water combined supernatants)	Unassigned peak(s)			0.001	14.3		
	Non-defined (each ≤0.001mg/kg) [e]			≤ 0.001[e]	≤ 14.3[e]		
Unextractable	Final PES[a]			0.006	85.7		
Totals[d]				0.007	100.0		

ND = not detected. NA = not applicable (not extracted). [a]- Determined by combustion. [b]- Confirmed by TLC. [c]- Combined supernatants of 50-100% ACN in water extracts concentrated, acidified and partitioned with EtOAc; the aqueous fraction values are reflected by the non-defined TRRs above and the EtOAc fraction containing, CGA179500 and a polar component (Rt 4.8 min, possibly matching CGA312753 reference standard), was applied to HPLC and/or TLC. [d]- Totals = ACN/water extractable residues + Final PES (Unextractable). [e]- Values excluded from "Totals" since very low. [f]- 24% KOH

RMS comments and conclusion

After one application of trinexapac-ethyl applied to bare ground at a rate of 0.3 kg a.s./ha (1.5N (300 g/ha instead of 200 g/ha) the maximum rate of the representative crops (barley), the total radioactive residues in all RACs were very low < 0.01 mg/kg, except for some 30 day PBI foliage RACs (lettuce and wheat) were slightly above 0.01 mg/kg. However, no individual extractable ^{14}C-residue was found to be > 0.01 mg/kg for any RAC at any PBI. No extractable residue match parent. These finding suggest extensive and rapid soil degradation of parent and likely mineralization to CO_2, since little ^{14}C was take-up into any rotational crop.

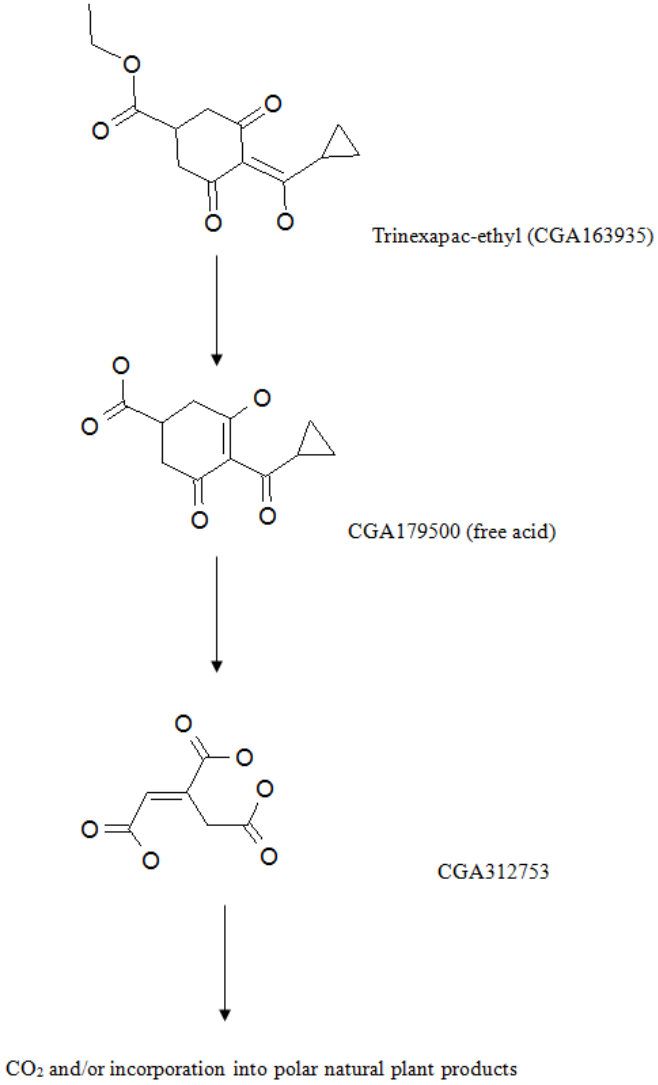


Figure B.7.6.1-1. Proposed metabolic pathway of trinexapac-ethyl in confined rotational crops.

B.7.6.2 Magnitude of residues in rotational crops

Studies on the magnitude of trinexapac-ethyl residues in rotational crops are not required. Considering that in the above rotational crop metabolism study was carried out on a bare soil with 0.75N to ~~1.75~~ 1.65N application rate, it can be concluded that trinexapac-ethyl residue levels in rotational commodities are not expected to exceed 0.01 mg/kg, provided that trinexapac-ethyl is applied in compliance with the representative GAP.

B.7.7 Other studies

No studies belonging to the category ‘other studies’ were submitted.

B.7.7.1 Effects on the residue level in pollen and bee products

No data submitted.

The applicant informed that a honey residue study is in progress and will be available during first quarter 2018.

B.7.8 References relied on

Literature search:

A brief summary of initial literature search and additional literature search including more metabolites following the REQUEST for ADDITIONAL INFORMATION from the EFSA is provided below. Full document includind criteria for relevance with which decisions to select studies in the dossier were made, search methods and results is presented in Appendix I.

RMS considers the methodology and the results his literature search to be appropriate and conducted according to EFSA Journal 2011;9(2):2092. Criteria for study relevance were developed and reported. Reasons for choosing such databases clearly stated. All studies were excluded during rapid assessment as being "obviously irrelevant records" based on titles.

Literature search report summarises the search for "scientific peer-reviewed open literature on trinexapac and its relevant metabolites dealing with metabolism and residues data which may impact health, the environment and non-target species and published within the last ten years before the date of submission of the dossier" in accordance with Article 8(5) of Regulation (EC) No. 1107/2009.

In summary, a very broad preliminary search labelled Initial Search and a Top-Up Search were conducted to identify references that included the active substance trinexapac, or its major metabolites, or representative formulations. A separate search on three additional metabolites was carried our separately from those searches and is labelled Additional Search. All searches were done in conjunction with any of the key words set out in Table 9.5-1.

The names searched for trinexapac were:

- Trinexapac ethyl, trinexapac, cimectacarb
- PRIMO MAXX, PRIMO, MODDUS
- 3-ethoxycarbonylpentanedioic acid
- 2,4-cyclopropyl-2,4-dioxo-butyl succinic acid
- Cyclopropane carboxylic acid
- 3,5-dioxocyclohexanecarboxylic acid

A succinct summary of the methodology employed in the selection of the literature to be assessed in detail or not is given below.

1) A very broad search was conducted in 16 scientific source databases for trinexapac-ethyl and its metabolites or its representative formulation.

2) Duplicates titles from between the data bases were automatically removed from the output.

3) A rapid assessment of the titles was conducted to remove any additional duplicates and any obviously irrelevant titles (where enough information was available from the title alone).

4) A further rapid assessment was conducted using summary abstracts and any clearly irrelevant titles were removed.

5) A detailed assessment of the full-text documents for the remaining titles was conducted using the criteria developed for study relevance.

6) Any relevant papers were highlighted and assessed for reliability.

A further search was made in August 2017 for the following metabolites:

CGA275537 (tricarballylic acid, CAS Number: 99-14-9, IUAPC name: 1,2,3-Propanetricarboxylic acid)
SYN548584 (hydroxylated trinexapac acid) 4-(cyclopropanecarbonyl)-1-hydroxy-3,5-dioxo-cyclohexanecarboxylic acid
CGA329773 4-(cyclopropanecarbonyl)-3,5-dihydroxy-benzoic acid
CGA351210 2-[cyclopropyl(hydroxy)methylene]-5-(hydroxymethyl)cyclohexane-1,3-dione
SYN540405 4-oxopentane-1,2,5-tricarboxylic acid
SYN540406 4-ethoxycarbonyl-6-oxo-cyclohex-2-ene-1-carboxylic acid
CGA300405 3-ethoxycarbonyl-pentanedioic acid

An overview of the results is summarised in the table below.

Data requirement(s) captured in the search	**Number (Initial Search)**	**Number (Top-Up Search)**	**Number (Additional Search)**
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	114	28	60
Number of *summary records* excluded from the search results after rapid assessment for relevance**	114	28	60
Total number of *full-text* documents assessed in detail*	0	0	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	0

Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	0

Data requirement(s) captured in the Further metabolite search Aug2017	**Number (Additional Search)**
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	139
Number of *summary records* excluded from the search results after rapid assessment for relevance**	139
Total number of *full-text* documents assessed in detail*	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

*both from bibliographic databases and other sources of peer-reviewed literature

**aligned with EFSA Journal 2011; 9(2):2092: rapid assessment means exclusion of “obviously irrelevant records” based on titles.

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EFSA (European Food Safety Authority), 2011. Risk assessment for pirimiphos-methyl residues resulting from cross-contamination. EFSA Journal 2011;9(11):2436. [48 pp.] doi:10.2903/j.efsa.2011.2436.

EFSA (European Food Safety Authority), 2012. Review of the existing maximum residue levels (MRLs) for trinexapac according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2012;10(1):2511, [38 pp.] doi:10.2903/j.efsa.2012.2511.

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FAO/WHO, 2013. Pesticide residues in food - 2013: evaluations / Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Geneva, Switzerland, 17–26 September 2013. Paper 220. Part 1, Residues, pp 1595 – 1716.

France, 2009. Additional report to the draft assessment report on the active substance prohexadione prepared by the rapporteur Member State France in consultation with Slovakia in the framework of Council Regulation (EC) No 737/2007, May 2009.

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OECD, 2007. Guideline for the testing of chemicals. 502 Metabolism in rotational crops. 18 pp.

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OECD, 2009. Guidance document on the definition of residue, Series on Pesticides No. 31, Series on Testing and Assessment No. 63. ENV/JM/MONO(2009)30, 38 pp.

OECD, 2009. Guideline for the testing of chemicals. 509 Crop field trial. 44 pp.

OECD, 2013. Guidance document on residues in livestock, Series on Pesticides No.73. ENV/JM/MONO(2013)8, 77pp.

OECD, 2016. Guidance document on crop field trials, Series on Pesticides No. 66. Series on Testing and Assessment No. 164. ENV/JM/MONO(2011)50/REV1, 43 pp.

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Reference list

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.1 ~~KCA 6.1 / 01~~ KIIA 6.3.2.1/01	Sack St.	1998	Stability of residues of CGA 179500 (metabolite of Trinexapac-ethyl, CGA 163935) in deep freeze stored analytical specimens of wheat (grain and straw) and rapeseed Novartis Crop Protection AG, Basel, Switzerland Novartis Crop Protection AG, Basel, Switzerland, 105/95 GLP not published Syngenta File No CGA163935/0562	N	N	-	SYN	~~KIIA 6.3.2.1/01~~ DAR 2003
6.1 & 6.4.2 ~~KCA 6.1 / 02 & KCA 6.4.2 / 01~~ KIIA 6.3.2.2 / 01 & KIIA 6.4.2 / 01	Sack St.	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 Novartis Crop Protection AG, Basel, Switzerland, 330/99 GLP not published Syngenta File No CGA179500/0030	N	N	-	SYN	~~KIIA 6.3.2.2 / 01 & KIIA 6.4.2 / 01~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.1 KCA 6.1 / 01	Watson G.	2017	Trinexapac-ethyl: Storage Stability of Residues of metabolite CGA224439 (CPCA) in Crop Matrices Stored Frozen for up to Twelve Months. Final Report and Final Report Amendment 1 Syngenta ResChem Analytical Limited Unit 27 Derwent Business Centre, Clarke Street, Derby, DE1 2BU, UK, RES-00030 GLP Not published Syngenta File No CA876_10009	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.1 KCA 6.1 / 02	Langridge G.	2017	Trinexapac-ethyl – Storage Stability of Residues of Metabolites CGA113745 and CGA313458 in Crop Matrices Stored Frozen for up to Twelve Months. CEM Analytical Services Ltd (CEMAS) Berkshire, UK, CEMR-7358 GLP Not published Syngenta File No. CGA113745_10003	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.2.1 ~~KCA 6.2.1 / 01~~ KIIA 6.1.3.2 / 01	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, 4-91 GLP not published Syngenta File No CGA163935/0209	N	N		SYN	~~KIIA 6.1.3.2 / 01~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.2.1 KIIA 6.1.3.2 / 02	Nicollier G.	1993	Metabolism of [14C-cyclohexyl]-CGA 163935 in greenhouse grown spring rape Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, 7-93 GLP not published	N	N		SYN	DAR 2003
6.2.1 ~~KCA 6.2.1 / 02~~ KIIA 6.1.3.1 / 01	Krauss J. H.	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, 20-90 GLP not published Syngenta File No CGA163935/0086	N	N		SYN	~~KIIA 6.1.3.1 / 01~~ DAR 2003
6.2.1 ~~KCA 6.2.1 / 03~~ KIIA 6.1.3.1 / 02	Krauss J. H.	1993	Metabolism of [^{14}C-Cyclohexyl]-CGA 163935 in Field Grown Spring Wheat Novartis Crop Protection AG, Basel, Switzerland Ciba-Geigy Ltd., Basel, Switzerland, 6/93 GLP not published Syngenta File No CGA163935/0303	N	N		SYN	~~KIIA 6.1.3.1 / 02~~ DAR 2003
6.2.1 ~~KCA 6.2.1 / 04~~ KIIA 6.1.3.3 / 01	Gross D.	1996	Behaviour and metabolism of CGA 163935 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, 11/96 GLP not published Syngenta File No CGA163935/0482	N	N		SYN	~~KIIA 6.1.3.3 / 01~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.2.1 ~~KCA 6.2.1 / 05~~ KIIA 6.1.3.4 / 01	Ray W. J., 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, 623-00 GLP not published Syngenta File No CGA163935/0862	N	N		SYN	~~KIIA 6.1.3.4 / 01~~ DAR 2003
6.2.1 KCA 6.2.1 / 0~~6~~1	Piskorski R.	2015	Trinexapac-ethyl - Metabolism of [^{14}C]-Trinexapac-ethyl in Oilseed Rape Syngenta Innovative Environmental Services, Witterswil, Switzerland, 20120173 GLP not published Syngenta File No CGA163935_10561	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-
6.2.1 KCA 6.2.1 / 0~~7~~2	Piskorski R.	2015a	Trinexapac-ethyl - Metabolism of [^{14}C]-Trinexapac-ethyl in Spring Wheat Syngenta Innovative Environmental Services, Witterswil, Switzerland, 20120098 GLP not published Syngenta File No CGA163935_10644	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.2.1 KCA 6.2.1 / 03	Piskorski R.	2017	Trinexapac-ethyl -Co-chromatography of Hydroxylated Trinexapac Acid Metabolites with Wheat Grain Metabolites from Study: Metabolism of [^{14}C]-Trinexapac-ethyl in Spring Wheat (TK0070368) Syngenta Innovative Environmental Services, Witterswil, Switzerland, 20170023 GLP not published Syngenta File No CGA163935_10838	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.2.2 ~~KCA 6.2.2 / 01~~ KIIA 6.2.2.2 / 01	Cameron B. D., et al.	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, 9128 GLP not published Syngenta File No CGA163935/0277	Y	N		SYN	~~KIIA 6.2.2.2 / 01~~ DAR 2003
6.2.2 ~~KCA 6.2.2 / 02~~ KIIA 6.2.2.2 / 02	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, 6/93 GLP not published Syngenta File No CGA163935/0306	Y	N		SYN	~~KIIA 6.2.2.2 / 02~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.2.2 KCA 6.2.2 / 0~~3~~1	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, RJ3678B 04JH011 GLP not published Syngenta File No CGA163935/1048	Y	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	-
6.2.3 ~~KCA 6.2.3 / 01~~ KIIA 6.2.2.1 / 01	Cameron B. D. et al.	1992a	Absortption, distribution and excretion of (1, 2 - ^{14}C) - cyclohexyl CGA 163935 after multiple oral administration to lactating goats. Novartis Crop Protection AG, Basel, Switzerland Inveresk Res. Int. Ltd., United Kingdom, 7478 GLP not published Syngenta File No CGA163935/0276	Y	N		SYN	~~KIIA 6.2.2.1 / 01~~ DAR 2003
6.2.3 ~~KCA 6.2.3 / 02~~ KIIA 6.2.2.1 / 02	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, 5-93 GLP not published Syngenta File No CGA163935/0305	Y	N		SYN	~~KIIA 6.2.2.1 / 02~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.2.3 ~~KCA 6.2.3 / 03~~ KIIA 6.2.2.1 / 03	Ray W. J.	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, 624-00 T000624-00 GLP not published Syngenta File No CGA163935/0944	Y	N		SYN	~~KIIA 6.2.2.1 / 03~~ Addendum to DAR 2005
6.3 KCA 6.3.1 / 01	Andrews G.	2015	Trinexapac-ethyl- Residue Study on Winter Barley in northern France and Germany in 2013 Syngenta Battelle UK Ltd, Chelmsford, Essex, UK, TK0178789 GLP not published Syngenta File No A8587F_10138	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-
6.3 KCA 6.3.1 / 02	Brown D.	2016	Trinexapac-ethyl - Residue Study on Barley in Northern France and the UK in 2014 Syngenta Charles River Laboratories, Edinburgh, United Kingdom, 36129 GLP not published Syngenta File No A8587F_10144	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.3 KCA 6.3.1 / ~~03~~05	Brown D.	2016a	Trinexapac-ethyl - Residue Study on Barley in Belgium in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland Charles River Laboratories, Edinburgh, United Kingdom, 37124 GLP not published Syngenta File No A8587F_10525	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	Trinexapac Task Force	-
6.3 KCA 6.3.1 / ~~04~~03	Andrews G.	2015a	Trinexapac-ethyl - Residue Study on Winter Barley in Italy and Spain 2013 Syngenta Battelle UK Ltd, Chelmsford, Essex, UK, TK0178795 GLP not published Syngenta File No A8587F_10132	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	-
6.3 KCA 6.3.1 / ~~05~~04	Brown D.	2016b	Trinexapac-ethyl - Residue Study on Barley in Southern France, Italy and Spain in 2014 Syngenta Charles River Laboratories, Edinburgh, United Kingdom, 36190 GLP not published Syngenta File No A8587F_10135	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.3 & 6.5.3 KCA 6.3.1 / 06 & KCA 6.5.3 / ~~10~~04	MacDougall J.	2016	Trinexapac-ethyl - Residue Processing Study on Barley in Spain and Italy in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland Charles River Laboratories, Edinburgh, United Kingdom, 37194 GLP published Syngenta File No A8587F_10526	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.3 KCA 6.3.2 / 01	Brown D.	2016c	Trinexapac-ethyl - Residue Study on Wheat in Northern France and the UK in 2014 Syngenta Charles River Laboratories, Edinburgh, United Kingdom, 36094 GLP not published Syngenta File No A8587F_10145	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-
6.3 KCA 6.3.2 / ~~02~~03	Brown D.	2016d	Trinexapac-ethyl - Residue Study on Wheat in Poland, Czech Republic, Austria and Germany in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland Charles River Laboratories, Edinburgh, United Kingdom, 37231 GLP not published Syngenta File No A8587F_10527	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.3 KCA 6.3.2 / ~~03~~02	Brown D.	2016e	Trinexapac-ethyl - Residue Study on Wheat in Southern France, Italy and Spain in 2014 Syngenta Charles River Laboratories, Edinburgh, United Kingdom, 36220 GLP not published Syngenta File No A8587F_10141	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	-
6.3 KCA 6.3.2 / 04 ~~&~~ KCA 6.5.3 / ~~16~~07	MacDougall J.	2016a	Trinexapac-ethyl - Residue Processing Study on Wheat in France and Spain in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland Charles River Laboratories, Edinburgh, United Kingdom, 37278 GLP not published Syngenta File No A8587F_10524	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	Trinexapac Task Force	-
6.4.2 & 6.1 ~~KCA 6.4.2 / 01~~ ~~&~~ ~~KCA 6.1 / 02~~ KIIA 6.3.2.2 / 01 & KIIA 6.4.2 / 01	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 Novartis Crop Protection AG, Basel, Switzerland, 330/99 GLP not published Syngenta File No CGA179500/0030	Y	N		SYN	~~KIIA 6.3.2.2 / 01 & KIIA 6.4.2 / 01~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.1 ~~KCA 6.5.1 / 01~~ KIIA 6.5.1 / 01	Cadalbert R., Buckel T.	2001	Hydrolysis of [1,2,6- ^{14}C]-Cyclohexanedione Labelled CGA 163935 under Processing Conditions Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection AG, Basel, Switzerland, 01RC02 GLP not published Syngenta File No CGA163935/0733	N	N		SYN	~~KIIA 6.5.1 / 01~~ DAR 2003
6.5.1 ~~KCA 6.5.1 / 02~~ KIIA 6.5.1 / 02	Mound E. L.	2004	[^{14}C]Cyclohexyl Trinexapac Acid (CGA179500): Aqueous Hydrolysis at 90, 100 & 120 degrees C Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, RJ3480B GLP not published Syngenta File No CGA179500/0036	N	N		SYN	~~KIIA 6.5.1 / 02~~ DAR 2003
6.5.1. KCA 6.5.1 / ~~03~~01	Scullion P.	2012	[^{14}C]Trinexapac acid: Simulated Processing - Aqueous Hydrolysis at 90, 100 and 120 °C ADAMA Celsius Property B.V., Amsterdam, Netherlands Harlan Laboratories Ltd., Itingen, Switzerland, C93481 GLP not published Syngenta File No CGA179500_11002	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Adama Celsius	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.1 KCA 6.5.1 / ~~0~~402	Florchinger M.	2008	Abiotic Degradation (Hydrolysis) of ^{14}C-Trinexapac under Typical Conditions (pH, Temperature and Time) of Processing CHEMINOVA A/S, Lemvig, Denmark Eurofins - GAB, Niefern Öschelbronn, Germany, S08-03106 GLP not published Syngenta File No CGA179500_11004	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Cheminova	-
6.5.3 ~~KCA 6.5.3 / 01~~ KIIA 6.5.3.2 / 01	Maffezzoni M.	1999	Residue Study with CGA 163935 + Ethephon in or on Winter Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9821701 GLP not published Syngenta File No CGA163935/0613	N	N		SYN	~~KIIA 6.5.3.2 / 01~~ DAR 2003
6.5.3 ~~KCA 6.5.3 / 02~~ KIIA 6.5.3.2 / 02	Maffezzoni M.	1999a	Residue Study with CGA 163935 + Ethephon in or on Winter Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9821702 GLP not published Syngenta File No CGA163935/0614	N	N		SYN	~~KIIA 6.5.3.2 / 02~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 ~~KCA 6.5.3 / 03~~ KIIA 6.5.3.2 / 03	Maffezzoni M.	1999b	Residue Study with CGA 163935 + Ethephon in or on Spring Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9821801 GLP not published Syngenta File No CGA163935/0615	N	N		SYN	~~KIIA 6.5.3.2 / 03~~ DAR 2003
6.5.3 ~~KCA 6.5.3 / 04~~ KIIA 6.5.3.2 / 04	Maffezzoni M.	1999c	Residue Study with CGA 163935 + Ethephon in or on Spring Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9821802 GLP not published Syngenta File No CGA163935/0616	N	N		SYN	~~KIIA 6.5.3.2 / 04~~ DAR 2003
6.5.3 ~~KCA 6.5.3 / 05~~ KIIA 6.5.3.2 / 05	Maffezzoni M.	1999d	Residue Study with CGA 163935 in or on Spring Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9822002 GLP not published Syngenta File No CGA163935/0617	N	N		SYN	~~KIIA 6.3.1.1 / 06 & KIIA 6.5.3.2 / 05~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 ~~KCA 6.5.3 / 06~~ KIIA 6.5.3.2 / 06	Maffezzoni M.	1999e	Residue Study with CGA 163935 in or on Spring Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9822001 GLP not published Syngenta File No CGA163935/0618	N	N		SYN	~~KIIA 6.3.1.1 / 07 &~~ ~~KIIA 6.5.3.2 / 06~~ DAR 2003
6.5.3 ~~KCA 6.5.3 / 07~~ KIIA 6.5.3.2 / 07	Maffezzoni M.	1999f	Residue Study with CGA 163935 in or on Winter Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9821902 GLP not published Syngenta File No CGA163935/0619	N	N		SYN	~~KIIA 6.3.1.1 / 08 &~~ ~~KIIA 6.5.3.2 / 07~~ DAR 2003
6.5.3 ~~KCA 6.5.3 / 08~~ KIIA 6.5.3.2 / 08	Maffezzoni M.	1999g	Residue Study with CGA 163935 in or on Winter Barley in North of France Novartis Crop Protection AG, Basel, Switzerland ADME - Bioanalyses, Aigues-Vives, France, 9821901 GLP not published Syngenta File No CGA163935/0620	N	N		SYN	~~KIIA 6.3.1.1 / 09 &~~ ~~KIIA 6.5.3.2 / 08~~ DAR 2003

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 KCA 6.5.3 / ~~09~~01	Mayer T.	2010	Trinexapac-ethyl - Magnitude of the Residues in or on Barley Syngenta Crop Protection, Inc., Greensboro, USA Syngenta Crop Protection, Inc., Greensboro, USA, ML08-1507-SYN GLP not published Syngenta File No CGA163935_50026	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-
6.5.3 KCA 6.5.3 / ~~10~~04 & KCA 6.3.1 / 06	MacDougall J.	2016	Trinexapac-ethyl - Residue Processing Study on Barley in Spain and Italy in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland Charles River Laboratories, Edinburgh, United Kingdom, 37194 GLP published Syngenta File No A8587F_10526	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 KCA 6.5.3 / ~~11~~05	Watson G.	2016	Analysis of Barley Processing Phase Specimens for CPCA from Study 699779 Trinexapac-ethyl - Residue Processing Study on Barley in Spain and Italy in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland ResChem Analytical Limited, Derby, UK, RES-00027 GLP not published Syngenta File No CA876_10004	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.5.3 KCA 6.5.3 / ~~12~~06	Langridge G.	2016	Trinexapac-ethyl - Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Barley Process Fractions Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland CEM Analytical Services Ltd (CEMAS) - Berkshire, UK, CEMR-7354-INT GLP not published Syngenta File No CGA313458_10001	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 ~~KCA 6.5.3 / 13~~ KIIA 6.5.3.1 / 01	Gasser A.	2001	Residue Study with Trinexapac-Ethyl (CGA 163935) in or on Winter Wheat in France (North) Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection AG, Basel, Switzerland, 3011/00 GLP not published Syngenta File No CGA163935/0734	N	N		SYN	~~KIIA 6.5.3.1 / 01~~ Dar 2003
6.5.3 KCA 6.5.3 / ~~1~~402	Mayer T.	2010a	Trinexapac-ethyl - Magnitude of the Residues in or on Wheat Syngenta Syngenta Crop Protection, Inc., Greensboro, USA, ML08-1504-SYN GLP not published Syngenta File No CGA163935_50036	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-
6.5.3 KCA 6.5.3 / ~~15~~03	Ediger K.	2006	Trinexapac-ethyl - Magnitude of the Residues in or on Wheat Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, Inc., Greensboro, USA, T002695-03 GLP not published Syngenta File No CGA163935/1053	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 KCA 6.5.3 / ~~16~~07 & KCA 6.3.2 / 04	MacDougall J.	2016a	Trinexapac-ethyl - Residue Processing Study on Wheat in France and Spain in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland Charles River Laboratories, Edinburgh, United Kingdom, 37278 GLP not published Syngenta File No A8587F_10524	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.5.3 KCA 6.5.3 / ~~17~~08	Watson G.	2016a	Analysis of Wheat Processing Phase Specimens for CPCA from Study 699784 Trinexapac-ethyl - Residue Processing Study on Wheat in France and Spain in 2015 Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland ResChem Analytical Limited, Derby, UK, RES-00028 GLP not published Syngenta File No CA876_10003	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 KCA 6.5.3 / ~~18~~09	Langridge G.	2016a	Trinexapac-ethyl - Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Wheat Process Fractions Syngenta, CHEMINOVA A/S, Lemvig, Denmark, ADAMA Agriculture B.V., Schaffhausen, Switzerland CEM Analytical Services Ltd (CEMAS) - Berkshire, UK, CEMR-7355-INT GLP not published Syngenta File No CGA313458_10002	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.5.3 KCA 6.5.3 / 10	Langridge G.	2016b	Trinexapac-ethyl - Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Barley Process Fractions Syngenta, ADAMA Agriculture B.V., Schaffhausen, Switzerland, CHEMINOVA A/S, Lemvig, Denmark CEM Analytical Services Ltd (CEMAS) - Berkshire, UK, CEMR-7354 GLP not published Syngenta File No CGA313458_10010	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.5.3 KCA 6.5.3 / 11	Langridge G.	2016c	Trinexapac-ethyl - Determination of Trinexapac-ethyl Metabolites CGA313458 and CGA113745 in Wheat Process Fractions Syngenta CEM Analytical Services Ltd (CEMAS) - Berkshire, UK, CEMR-7355 GLP not published Syngenta File No CGA313458_10011	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Trinexapac Task Force	-
6.6 ~~KCA 6.6.1 / 01~~ KIIA 6.6.1 / 01	Krauss J. H.	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, 23-92 GLP not published Syngenta File No CGA163935/0265	N	N		SYN	~~KIIA 6.6.1 / 01~~ DAR 2003
6.6 KCA 6.6.1 / ~~02~~01	Quistad G., Kovatchev A.	2010	^{14}C-Trinexapac-ethyl - Uptake and Metabolism in Confined Rotational Crops Syngenta Crop Protection, Inc., Greensboro, USA PTRL West, Inc., Hercules, USA, 1802W GLP not published Syngenta File No CGA163935_50024	N	Y	New data; eligible for data protection according to SANCO/12576/2012	SYN	-

Data point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	~~Data point in~~ Previous evaluation ~~(DAR)~~
6.9 KCA 6.9 / 01	Sochard B.	2015	Trinexapac - MCA S6 - risk assessment metabolites for representative uses Syngenta Not GLP not published Syngenta File No CGA163935_10675 This is CONFIDENTIAL INFORMATION	N	N	NA	SYN*	-
6.9 KCA 6.9 / 02	Sochard B.	2015a	Trinexapac - MCA S6 - risk assessment metabolites for rye Syngenta Not GLP not published Syngenta File No CGA163935_10676 This is CONFIDENTIAL INFORMATION	N	N	NA	SYN*	-

* - Data confidentiality is requested for these data. Disclosure of the information might undermine the company commercial interests by providing access to company specific know-how used to develop unique positions and approaches to risk assessment.

Appendix I Literature search

Uncorrected text (including table numbers) written by the applicant is provided below:

LITERATURE DATA

Title

This document is a Literature Review Report for trinexapac and the EU representative formulation A8587F.

Author(s) of the review

Syngenta
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Summary: A brief summary indicating the purpose of the report, the methodology employed and the results obtained

This report summarises the search for "scientific peer-reviewed open literature on trinexapac and its relevant metabolites dealing with metabolism and residues data which may impact health, the environment and non-target species and published within the last ten years before the date of submission of the dossier" in accordance with Article 8(5) of Regulation (EC) No. 1107/2009.

The search strategy is detailed in the tables below. In summary, a very broad preliminary search labelled Initial Search and a Top-Up Search were conducted to identify references that included the active substance trinexapac, or its major metabolites, or representative formulations. A separate search on three additional metabolites was carried our separately from those searches and is labelled Additional Search. All searches were done in conjunction with any of the key words set out in Table 9.5-1.

The names searched for trinexapac were:

- Trinexapac ethyl, trinexapac, cimectacarb
- PRIMO MAXX, PRIMO, MODDUS
- 3-ethoxycarbonylpentanedioic acid
- 2,4-cyclopropyl-2,4-dioxo-butyl succinic acid
- Cyclopropane carboxylic acid
- 3,5-dioxocyclohexanecarboxylic acid

A succinct summary of the methodology employed in the selection of the literature to be assessed in detail or not is given below.

1) A very broad search was conducted in 16 scientific source databases (detailed in Table 9.5-2) for trinexapac-ethyl and its metabolites or its representative formulation, using the search terms listed in Table 9.5.1.
2) Duplicates titles from between the data bases were automatically removed from the output.
3) A rapid assessment of the titles was conducted to remove any additional duplicates and any obviously irrelevant titles (where enough information was available from the title alone).
4) A further rapid assessment was conducted using summary abstracts and any clearly irrelevant titles were removed.
5) A detailed assessment of the full-text documents for the remaining titles was conducted using the criteria developed for study relevance (see Table 9.4.2-1).
6) Any relevant papers were highlighted and assessed for reliability.

A further search was made in August 2017 for the following metabolites:

CGA275537 (tricarballylic acid, CAS Number: 99-14-9, IUAPC name: 1,2,3-Propanetricarboxylic acid)
SYN548584 (hydroxylated trinexapac acid) 4-(cyclopropanecarbonyl)-1-hydroxy-3,5-dioxo-cyclohexanecarboxylic acid
CGA329773 4-(cyclopropanecarbonyl)-3,5-dihydroxy-benzoic acid
CGA351210 2-[cyclopropyl(hydroxy)methylene]-5-(hydroxymethyl)cyclohexane-1,3-dione
SYN540405 4-oxopentane-1,2,5-tricarboxylic acid
SYN540406 4-ethoxycarbonyl-6-oxo-cyclohex-2-ene-1-carboxylic acid

CGA300405 3-ethoxycarbonyl-pentanedioic acid

An overview of the results is summarised in the table below and further details are provided in Section 9.5.

Data requirement(s) captured in the search	**Number (Initial Search)**	**Number (Top-Up Search)**	**Number (Additional Search)**
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	114	28	60
Number of *summary records* excluded from the search results after rapid assessment for relevance**	114	28	60
Total number of *full-text* documents assessed in detail*	0	0	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	0

*both from bibliographic databases and other sources of peer-reviewed literature
**aligned with EFSA Journal 2011; 9(2):2092: rapid assessment means exclusion of "obviously irrelevant records" based on titles.

Data requirement(s) captured in the Further metabolite search Aug2017	**Number (Additional Search)**
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	139
Number of *summary records* excluded from the search results after rapid assessment for relevance**	139
Total number of *full-text* documents assessed in detail*	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

Protocol

Statement of the objective of the review

The review has the objective of identifying "scientific peer-reviewed open literature on trinexapac and its potentially relevant metabolites dealing with metabolism and residue studies which may impact health, the environment and non-target species and published within the last ten years before the date of submission of the dossier" in accordance with Article 8(5) of Regulation (EC) No. 1107/2009.

Criteria for relevance with which decisions to select studies in the dossier were made

Table 9.4.2-1: List of Criteria for relevance for each data requirement

Data requirements(s) (indicated by the correspondent CA data point(s))	**Criteria for relevance**
Metabolism and residues data (CA 6.1 to 6.9)	
Summary	The relevance criteria applied to determine whether a literature reference was relevant for the residues and metabolism sections of the active substance renewal process are given below. 1. Well defined test material. e.g. are purity and batch data provided? 2. Applicable test species. e.g. is the crop a representative use; were relevant animal commodities used? 3. Study conditions should not differ significantly from guidelines and recommended protocols. e.g. did the study meet the relevant guidelines? 4. Trial site/test system not previously exposed to the test material or other contaminants e.g. was the compound used previously at the trial site; was the animal feed free from the compound? 5. Sufficient experimental information is provided to substantiate and evaluate whether the study conclusions and endpoints are robust. e.g. were storage intervals recorded; are weather conditions and plot histories available? 6. Validated Analytical methodology employed. e.g. were control samples used, acceptable recoveries obtained, clear

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	example chromatograms given? 7. Study conditions do not interfere with the interpretation of the study results. e.g. starting processing material residue is robust and there is measurable residue in processed products?
8.1 Storage stability **Storage stability studies, plant and animal**	Storage Stability Studies 1. Well defined test material (including purity/content) 2. Applicable test species 3. Study conditions should not differ significantly from guidelines and recommended protocols. 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. 6. Study conditions should not interfere with the interpretation of the study results. 7. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. Notes for above criteria 1. Well defined test material (including purity/content) • e.g. was the active ingredient purity and expiry date noted? • e.g. is the source of the commodity given? 2. Applicable test species • e.g. was the test species in the same crop group as the representative use? • e.g. was the testing carried out on relevant animal commodities? 3. Study conditions should not differ significantly from recommended protocols. • e.g. were samples stored for the appropriate times and at -18°C? • e.g. was degradation on storage < 30%? • e.g. was adequate extraction efficiency demonstrated to use the method? 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. • e.g. were storage intervals appropriate? • e.g. were storage temperatures recorded? • e.g. were all components of the residue definition analysed for? 6. Study conditions should not interfere with the interpretation of the study results. • e.g. was the study conducted within a GLP facility and to the correct GLP standards? 7. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. • e.g. what methodologies were used and were the methods validated in the matrices? • e.g. were acceptable recoveries obtained? • e.g. were control samples analysed and were they 'clean'? • e.g. were representative clear chromatograms provided?
8.2 Metabolism	Primary Crop Studies Notes for criteria 1. Well defined test material (including purity/content) • e.g. if radiolabelled test item was used, was an appropriate isotope used (e.g. ^{14}C and not ^{3}H)? • e.g. if radiolabelled test item was used, was the labelling

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	position(s) appropriate to capture potential metabolites? • e.g. if radiolabelled test item was used, was the specific activity adequate to meet an LOQ of 0.01 mg/kg? • e.g. was the test material formulated? If so, was the formulation used representative of the commercial formulation? Did the formulation contain adjuvants/safener/synergist where applicable? If not formulated was a reasonable justification provided? 2. Applicable test species • N.B. any crop used for food and/or feed could be relevant from a metabolism perspective as results can be extrapolated to other crops 3. Study conditions should not differ significantly from guidelines and recommended protocols. • e.g. Does the application method reflect the intended used pattern e.g. foliar, soil, seed or post-harvest treatment • e.g. Is the GAP relevant? Correct rate, application interval, PHI, spray volume, BBCH (if applicable)? • e.g. were appropriate RACs sampled (these must cover the RACS of all crops within the test species crop group e.g. trash from OSR could act as a proxy for soybean hay)? • e.g. were samples stored deep frozen? 4. Trial site/test system not previously exposed to the test material or other contaminants. • e.g. Is plot history supplied, e.g. evidence that compound not used that year or previous year, and information on other plant protection products? 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. • Examples as in 3 above • e.g. Were metabolites identified by appropriate techniques (e.g. co-chromatography with known standards using two dissimilar chromatographic systems or by techniques capable of positive structural identification e.g. MS, NMR)? 6. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. • e.g. Were relevant control experiments carried out when harsher techniques (e.g. acid/base hydrolysis) were used to identify metabolites (i.e. to ensure metabolites identified are not merely artefacts)? • e.g. were representative clear chromatograms provided to support metabolite identification? • e.g. where sample analysis exceeded 6 months from sample collection was storage stability of samples demonstrated? 7. Study conditions should not interfere with the interpretation of the study results. • e.g. if the test item is photolabile was the study carried out outdoors? <u>Metabolism in Rotational Crops</u>

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	Notes for criteria 1. Well defined test material (including purity/content) • e.g. if radiolabelled test item was used, was an appropriate isotope used (e.g. ^{14}C and not ^{3}H)? • e.g. if radiolabelled test item was used, was the labelling position(s) appropriate to capture potential metabolites? • e.g. if radiolabelled test item was used, was the specific activity adequate to meet an LOQ of 0.01 mg/kg? • e.g. was the test material formulated? If so, was the formulation used representative of the commercial formulation? Did the formulation contain adjuvants/safener/synergist where applicable? If not formulated was a reasonable justification provided? 2. Applicable test species • N.B. relevant crop groupings are small grain, root and tuber, leafy vegetable (soybean and rice if relevant to product). Bulb vegetable (e.g. onions and garlic) should not be used. 3. Study conditions should not differ significantly from guidelines and recommended protocols. • e.g. Is it an application to bare soil? Is it sandy loam (only exception is if the compound is limited to use on a single soil type other than sandy loam)? • e.g. Is the application rate relevant? Equivalent to maximum seasonal rate on rotated crops? • e.g. Do plantbacks reflect representative rotational intervals based on expected agricultural use for the pesticide and typical rotational practices e.g. 7-30 days, 60-270 days, 270-365 days? • e.g. were appropriate RACs for human food and livestock feed sampled? • e.g. were samples stored deep frozen? 4. Trial site/test system not previously exposed to the test material or other contaminants. • e.g. Is plot history supplied, i.e. evidence that compound not used that year or previous year, and information on other plant protection products? 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. • Examples as in 3 above • e.g. Were metabolites identified by appropriate techniques (e.g. co-chromatography with known standards using two dissimilar chromatographic systems or by techniques capable of positive structural identification e.g. MS, NMR)? 6. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. • e.g. Were relevant control experiments carried out when harsher techniques (e.g. acid/base hydrolysis) were used to identify metabolites (i.e. to ensure metabolites identified are not merely artefacts)? • e.g. were representative clear chromatograms provided to support metabolite identification?

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	• e.g. where sample analysis exceeded 6 months from sample collection was storage stability of samples demonstrated? 7. Study conditions should not interfere with the interpretation of the study results. <u>Livestock Metabolism Studies</u> Notes for criteria 1. Well defined test material (including purity/content) • e.g. if radiolabelled test item was used, was an appropriate isotope used (e.g. ^{14}C and <u>not</u> ^{3}H)? • e.g. if radiolabelled test item was used, was the labelling position(s) appropriate to capture potential metabolites? • e.g. if radiolabelled test item was used, was the specific activity adequate to meet an LOQ of 0.01 mg/kg? 2. Applicable test species • Ruminant, poultry, pig, fish, any edible animal 3. Study conditions should not differ significantly from guidelines and recommended protocols. • e.g. is the dosing level extreme (i.e. had detrimental effect on animal health)? • e.g. was the application form appropriate, i.e. orally dosed capsule? • e.g. was the dosing period appropriate, i.e. plateau reached in milk or eggs, or up to 7 days in ruminant and 14 days in poultry? • e.g. were the animals healthy? • e.g. were the animals acclimatized (i.e. feeding, milk/egg production normal)? • e.g. was sacrifice time appropriate (i.e. no more than 24 hours after last dose)? • e.g. were appropriate edible tissues/milk/eggs sampled? • e.g. were samples stored deep frozen? 4. Trial site/test system not previously exposed to the test material or other contaminants. • e.g. is it clear that the animal was <u>not</u> pre-dosed • e.g. is it clear that the animal feed did <u>not</u> contain treated substance? 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. • Examples as in 3 above • e.g. Were metabolites identified by appropriate techniques (e.g. co-chromatography with known standards using two dissimilar chromatographic systems or by techniques capable of positive structural identification e.g. MS, NMR)? 6. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. • e.g. Were relevant control experiments carried out when harsher techniques (e.g. acid/base hydrolysis) were used to identify metabolites (i.e. to ensure metabolites identified are not merely artefacts)? • e.g. were representative clear chromatograms provided to support metabolite identification?

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	• e.g. where sample analysis exceeded 6 months from sample collection was storage stability of samples demonstrated? 7. Study conditions should not interfere with the interpretation of the study results.
8.3 Residue studies	Published monitoring reports were not considered relevant due to the fact that it would not be possible to determine whether or not a misuse scenario had resulted in the residue levels reported. <u>Crop Studies</u> 1. Well defined test material (including purity/content) 2. Applicable test species 3. Study conditions should not differ significantly from guidelines and recommended protocols. 4. Trial site/test system not previously exposed to the test material or other contaminants. 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. 6. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. 7. Study conditions should not interfere with the interpretation of the study results. <u>Notes for above criteria</u> 1. Well defined test material (including purity/content) • e.g. was the formulation comparable to the proposed representative formulation? 2. Applicable test species • e.g. is it a representative use crop? 3. Study conditions should not differ significantly from guidelines and recommended protocols. • e.g. Is the GAP relevant? Correct rate, application method, interval, PHI, spray volume, BBCH (if applicable), region, indoor/outdoor, control samples taken? • e.g. were weather details available? • e.g. were the control plots well separated from treated plots? • e.g. was the field phase conducted according to GLP? • e.g. were samples stored deep frozen? Were appropriate numbers of samples taken, e.g. 2kg of apples? • e.g. was appropriate sampling methodology employed? Was the sample handling traceable? 4. Trial site/test system not previously exposed to the test material or other contaminants. • e.g. Plot history supplied, e.g. evidence that compound not used that year or previous year, and information on other plant protection products (e.g. to check for common metabolites). 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. • Examples as in 3 above and also, have they proposed an endpoint, e.g. MRL, what statistical methods have they used for this? 6. Study conditions should not interfere with the interpretation of the study results. 7. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc.

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	• e.g. Was a validated method used, were acceptable recoveries obtained, were control samples analysed, were control samples 'clean', were representative clear chromatograms provided, Was the analytical phase conducted according to GLP? Were all components of the residue definition analysed for? Were samples analysed within a time period covered by storage stability data?
8.4 Livestock Feeding studies	Same criteria as for crop studies, examples could be as above with the following additions. Livestock Feeding Studies Notes 1. Well defined test material (including purity/content) 2. Applicable test species • e.g. Ruminant, poultry, pig, fish, any edible animal. 3. Study conditions should not differ significantly from recommended protocols. • e.g. is the dosing level extreme? • e.g. was the application form appropriate, e.g. capsule? • e.g. was the number of test species correct, e.g. three cows, nine hens? • e.g. was the dosing period appropriate, e.g. minimum 28 days? • e.g. were control animals included? • e.g. were the animals healthy? • e.g. were the animals acclimatized? 4. Trial site/test system not previously exposed to the test material or other contaminants. • e.g. is it clear that additional animal feed did not contain treated substance? 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. 6. Study conditions should not interfere with the interpretation of the study results. 7. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc.
8.5 Processing	High Temperature Hydrolysis Notes for criteria 1. Well defined test material (including purity/content) • e.g. if radiolabelled test item was used, was an appropriate isotope used (e.g. ^{14}C and not ^{3}H)? • e.g. if radiolabelled test item was used, was the labelling position(s) appropriate to capture potential metabolites? • e.g. if radiolabelled test item was used, was the specific activity adequate to meet an LOQ of 0.01 mg/kg? • N.B. If water solubility of test item is < 0.01 mg/L then no study is required and can be deemed non-relevant 2. Applicable test system • e.g. Was the test undertaken in a sterilised buffer medium? 3. Study conditions should not differ significantly from guidelines and recommended protocols. • e.g. Were the temperature and pH conditions applied typical of

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	processing operations carried out on commodities relevant to the test item? • e.g. were samples stored deep frozen? 4. Trial site/test system not previously exposed to the test material or other contaminants. 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. • Examples as in 3 above • e.g. Were metabolites identified by appropriate techniques (e.g. co-chromatography with known standards using two dissimilar chromatographic systems or by techniques capable of positive structural identification e.g. MS, NMR)? 6. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. • e.g. Were relevant control experiments carried out when harsher techniques (e.g. acid/base hydrolysis) were used to identify metabolites (i.e. to ensure metabolites identified are not merely artefacts)? • e.g. were representative clear chromatograms provided to support metabolite identification? • e.g. where sample analysis exceeded 6 months from sample collection was storage stability of samples demonstrated? 7. Study conditions should not interfere with the interpretation of the study results. <u>Field Studies</u> 1. Well defined test material (including purity/content) 2. Applicable test species 3. Study conditions should not differ significantly from guidelines and recommended protocols. 4. Trial site not previously exposed to the test material or other contaminants. 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. 6. Study conditions should not interfere with the interpretation of the study results. 7. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. <u>Notes for above criteria</u> 1. Well defined test material (including purity/content) • e.g. was the formulation comparable to the proposed representative formulation? 2. Applicable test species • e.g. is it a representative use crop? 3. Study conditions should not differ significantly from guidelines and recommended protocols. • NB. Processing studies can be conducted at elevated rates and shorter PHI and grown under different conditions to maximize residues. • e.g. were weather details available? • e.g. were control plots well separated from treated plots? • e.g. was the field phase conducted according to GLP? Were processed samples stored deep frozen? Were appropriate

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	numbers of samples taken, e.g. 2kg of apples? • e.g. was appropriate sampling methodology employed? • e.g. was the sample handling traceable? • e.g. was processing conducted in order to mimic industrial processing? Did the processing result in the correct process fractions as required in the guidelines. • e.g. is material balance clearly traceable? 4. Trial site not previously exposed to the test material or other contaminants. • e.g. was the plot history supplied, e.g. evidence that compound not used that year or previous year, and information on other plant protection products (e.g. to check for common metabolites)? 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. • Examples as in 3 above and also, have they proposed an endpoint, e.g. Have transfer factors been generated for the main processing products generated? • Has a clear description of the processing methodology used been provided e.g. flow diagram? 6. Study conditions should not interfere with the interpretation of the study results. • e.g. Starting processing material residue is robust and there is measurable residue in processed products? 8. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. • e.g. was a validated method used, were acceptable recoveries obtained, were control samples analysed, were control samples 'clean', were representative clear chromatograms provided? • e.g. was the analytical phase conducted according to GLP? • e.g. were all components of the residue definition analysed for? • e.g. were samples analysed within a time period covered by storage stability data?
8.6 Residues in succeeding crops	Same criteria as for crop residue studies, examples could be subtly different, e.g. acceptable PBIs, crop types, again monitoring information should not be considered relevant.
8.7 Proposed residue definition and MRLs	Not required? MRLs would only be affected if residues generated that would be covered in 8.3. Residue definition would only be affected if data generated in another section, e.g. metabolism/tox.
8.8 Proposed PHI, re-entry and withholding periods	Not required? Or could there be animal safety reports that might affect withholding periods – would these be required here, I think they would actually be better in the tox review.
8.9 Other/special studies	Not required.
8.10 Risk assessment	Not required – any adverse findings for the risk assessment will have to be due to a data point from one of the other sections, or from tox data.

* Recommended protocols under each data point include but are not limited to those listed in the Commission Communications 2013/C 95/01 and 2013/C 95/02

Search methods

Date of Initial Search	26.06.2014
Date of most recent update to search	05.05.2015
Date of Additional Search	05.05.2015
Date span of the search	10 years

Further search for a number of metabolites not covered in the Initial or additional searches mentioned above.

Date of Further Search	31st August 2017
Date span of the search	12 years

Table 9.5-1: Detailed Search Parameters for Metabolism and Residues data (CA 6.1 to 6.9)

Search Strategy	
L1	QUE (143294-89-7 OR TRINEXAPAC? OR 95266-40-3 OR CGA163935)
L2	QUE ((CGA(W)163935) OR CIMECTACARB OR (PRIMO(W)MAXX))
L3	QUE ((PRIMO OR MODDUS)(10A)(PESTICID? OR HERBICID? OR GROWTH?))
L4	QUE (TRINEXAPAC(W)(ETHYL OR ET))
L5	**QUE L1-4 TRINEXAPAC-ETHYL**
L6	QUE (26976-75-0 OR 389126-49-2 OR (CGA(W)300405) OR CGA300405)
L7	QUE (3(W)ETHOXYCARBONYLPENTANEDIOIC(W)ACID)
L8	QUE ((1(W)2(W)3(W)PROPANETRICARBOXYL?)(3A)(2(W)(METHYL OR ETHYL)))
L9	QUE (3(W)METHOXYCARBONYLPENTANEDIOIC(W)ACID)
L10	**QUE L6-9 METABOLITES**
L11	QUE L5 OR L10

Search Strategy

Plus

L1 QUE (CGA313458 OR (CGA(W)313458))
L2 QUE (2(2W)4(W)CYCLOPROPYL(W)2(W)4(W)DIOXOBUTYL(W)BUTANEDIOIC(W)ACID)
L3 QUE (2(2W)4(W)CYCLOPROPYL(W)2(W)4(W)DIOXO(W)BUTYL(W)BUTANEDIOIC(W)ACID)
L4 QUE (2(2W)4(W)CYCLOPROPYL(W)2(W)4(W)DIOXOBUTYL(W)SUCCINIC (W)ACID)
L5 QUE (2(2W)4(W)CYCLOPROPYL(W)2(W)4(W)DIOXO(W)BUTYL(W)SUCCINIC(W)ACID)
L6 QUE (56066-20-7 OR 84011-71-2 OR 42858-60-6 OR 38163-24-5)
L7 QUE (18917-09-4 OR 1759-53-1 OR (DIHYDRORESORCYLIC(W)ACID))
L8 QUE (3(W)HYDROXY(W)5(W)OXOCYCLOHEX(W)3(W)ENECARBOXYLIC(W)ACID)
L9 QUE (3(W)CYCLOHEXENE(W)1(W)CARBOXYL?)(2A)(3(W)HYDROXY(W) 5(W)OXO)
L10 QUE ((CYCLOHEXANECARBOXYLIC(W)ACID)(2A)(3(W)5(W)DIOXO))
L11 QUE (3(W)5(W)DIOXOCYCLOHEXANECARBOXYLIC(W)ACID)
L12 QUE (3(W)5(W)DIOXO(W)CYCLOHEXANECARBOXYLIC(W)ACID)
L13 QUE (3(W)5(W)DIOXO(W)CYCLOHEXANE(W)CARBOXYLIC(W)ACID)
L14 QUE (3(W)5(W)DIOXOCYCLOHEXANE(W)CARBOXYLIC(W)ACID)
L15 QUE (5(W)CARBOXY(W)1(W)3(W)CYCLOHEXANEDIONE)
L16 QUE (5(W)CARBOXY(W)1(W)3(W)CYCLOHEXANE(W)DIONE)
L17 QUE (DIHYDRO(3W)ALPHA(3W)RESORCYLIC(W)ACID)
L18 QUE ((1(W)CARBOXYCYCLOPROPYL) OR (1(W)CARBOXY(2A)CYCLOPROPYL))
L19 QUE ((CYCLOPROPANECARBOXYLIC OR (CYCLOPROPANE(W)CARBOXYLIC))(W)acid)
L20 QUE (L18 AND (1759-53-1 OR 18917-09-4 OR 38163-24-5))
L21 QUE (CARBOXYCYCLOPROPANE OR (CYCLOPROPYLCARBOXYLIC(W)ACID))
L22 QUE (CARBOXY(W)CYCLOPROPANE OR (CYCLOPROPYL(W)CARBOXYLIC(W)ACID))
L23 QUE ((DIHYDRO(W)RESORCYLIC(W)ACID) OR (CYCLOPROPIONIC(W)ACID))
L24 QUE (L1-L5 OR L6-L18 OR L20-23)

Plus

L1 QUE (METABOL? OR RESIDUE# OR TRANSFORM? OR BIOTRANSFORM?)
L2 QUE (DEGRAD? OR BIODEGRAD? OR FATE# OR MRL OR MRLS)
L3 QUE (CONJUGAT? OR EXCRET? OR ELIMINAT?)
L4 QUE (FOOD# OR FEED# OR DIET# OR DIETARY OR CONSUMER? OR HUMAN#)
L5 QUE (CONTAMINAT? OR SAFE? OR EXPOS? OR ANALY? OR ASSES?)
L6 QUE (INTAKE? OR (IN(W)TAKE?) OR SURVEY? OR RISK?)
L7 QUE (TOXIC? OR STUDY? OR STUDIES?)
L8 QUE (L4(10A)(L5 OR L6 OR L7))
L9 QUE (LIVESTOCK# OR COW# OR GOAT# OR CATTLE# OR BULLOCK#)
L10 QUE (BOVINE? OR BOVIDAE? OR BOS OR BULL# OR HEIFER? OR CAPRA#)
L11 QUE (SHEEP# OR EWE OR EWES OR RAM# OR SWINE# OR PIGLET#)
L12 QUE (PIG# OR SUIDAE? OR SUS OR OVIS OR OX OR OXEN)
L13 QUE (RUMINANT? OR HEN# OR CHICKEN# OR FOWL# OR TURKEY?)
L14 QUE (DUCK# OR GOOSE OR GEESE OR CAPON# OR POULTRY?)
L15 QUE (MEAT OR MILK OR EGG# OR TISSUE#)
L16 QUE (((BROKEN? OR BREAK?)(W)(DOWN OR UP)) OR BREAKDOWN?)
L17 QUE (BREAKSDOWN? OR UPTAKE? OR PROCESSING? OR BOUND?)
L18 QUE ((NON(W)EXTRACTAB?) OR (ROTATIONAL(3A)CROP#))
L19 QUE ((L1 OR L2 OR L3) OR L8 OR (L9 OR L10 OR L11 OR L12 OR L13 OR L14) OR (L15 OR L16 OR L17 OR L18))

Search Strategy for further metabolite search in August 2017

L1 QUE SPE=ON ABB=ON PLU=ON (**CGA(W)275537** OR **CGA275537** OR 99-14-9 OR 850848-65-6 OR 854811-52-2 OR (PROPANETRICARBOXYLIC OR TRICARBALLYLIC OR CARBOXYGLUTARIC OR CARBALLYLIC)(W)ACID)

L2 QUE SPE=ON ABB=ON PLU=ON (TRICARBOXYPROPANE OR AI(W)942(W)42301799 OR AI942(W)42301799 OR AI(W)94242301799 OR AI94242301799 OR NSC(W)2347 OR NSC2347)

L5 QUE SPE=ON ABB=ON PLU=ON (**CGA(W)300405** OR **CGA300405** OR 2109252-96-0 OR PROPANETRICARBOXYLIC(W)ACID(1W)ETHYL(W)ESTER OR PROPAN#(W)TRICARBOXYLIC(W)ACID(1W)ETHYL(W)ESTER)

L6 QUE SPE=ON ABB=ON PLU=ON (**SYN(W)548584** OR **SYN548584** OR CYCLOPROPYL(W)HYDROXY(W)METHYLEN#(2W)DIOXO(1W)HYDROXYL(W)CYCLOHEXANECARBOXYLIC(W)ACID OR CYCLOPROPYL(W)HYDROXY(W)METHYLEN#(2W)DIOXO(1W)HYDROXYL(W)CYCLOHEXAN#(W)CARBOXYLIC(W)ACID)

L7 QUE SPE=ON ABB=ON PLU=ON (CYCLOPROPANECARBONYL(1W)HYDROXY(2W)DIOXO(W)CYCLOHEXANECARBOXYLIC(W)ACID OR CYCLOPROPANECARBONYL(1W)HYDROXY(2W)DIOXO(W)CYCLOHEXAN#(W)CARBOXYLIC(W)ACID OR CYCLOPROPANECARBONYL(2W)DIHYDROXY(1W)OXO(W)CYCLOHEXA(1W)ENECARBOXYLIC(W)ACID)

L8 QUE SPE=ON ABB=ON PLU=ON (CYCLOPROPANECARBONYL(3W)TRIHYDROXY(W)CYCLOHEXA(2W)DIENECARBOXYLIC(W)ACID OR HYDROXY(2W)DIOXO(1W)CYCLOPROPANECARBONYL(W)CYCLOHEXANECARBOXYLIC(W)ACID)

L9 QUE SPE=ON ABB=ON PLU=ON (**CGA(W)329773** OR **CGA329773** OR CYCLOPROPANECARBONYL(2W)DIHYDROXY(W)BENZOIC(W)ACID OR CYCLOPROPAN#(W)CARBONYL(2W)DIHYDROXY(W)BENZOIC(W)ACID OR DIHYDROXY(1W)CYCLOPROPANECARBONYL(W)BENZOIC(W)ACID)

L10 QUE SPE=ON ABB=ON PLU=ON (**CGA(W)351210** OR **CGA351210** OR CYCLOPROPANECARBONYL(1W)HYDROXYMETHYL(W)CYCLOHEXAN#(2W)DION# OR CYCLOPROPANCARBONYL(1W)HYDROXY(1W)HYDROXYMETHYL(W)CYCLOHEX(1W)ENON#)

L11 QUE SPE=ON ABB=ON PLU=ON (CYCLOPROPANCARBONYL(2W)DIHYDROXY(1W)HYDROXYMETHYL(W)CYCLOHEXA(2W)DIEN# OR CYCLOPROPYL(W)HYDROXY(W)METHYLEN#(1W)HYDROXYMETHYL(W)CYCLOHEXA(2W)DIEN#(2W)DIOL OR CYCLOPROPYL(W)HYDROXY(W)METHYLEN#(1W)HYDROXYMETHYL(W)CYCLOHEXAN#(2W)DION#)

L12 QUE SPE=ON ABB=ON PLU=ON (**SYN(W)540405** OR **SYN540405** OR OXOPENTAN#(3W)TRICARBOXYLIC(W)ACID OR HYDROXYPENTA(1W)EN#(3W)TRICARBOXYLIC(W)ACID OR HYDROXY(W)PENT#(1W)EN#(3W)TRICARBOXYLIC(W)ACID)

L13 QUE SPE=ON ABB=ON PLU=ON (**SYN(W)540406** OR **SYN540406** OR ETHOXYCARBONYL(1W)OXO(W)CYCLOHEX(1W)EN#(1W)CARBOXYLIC(W)ACID OR ETHOXYCARBONYL(1W)HYDROXY(W)CYCLOHEXA(2W)DIEN#(1W)CARBOXYLIC(W)ACID)

L14 QUE SPE=ON ABB=ON PLU=ON (ETHOXY(W)HYDROXY(W)METHYLENE(1W)HYDROXY(W)CYCLOHEXA(2W)DIEN#(1W)CARBOXYLIC(W)ACID)

L15 QUE SPE=ON ABB=ON PLU=ON ((L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14))

Table 9.5-2: Detailed Search Parameters for Metabolism and Residues data (CA 6.1 to 6.9)

Provider	Database	Justification	Limits applied	Number*	Further Search Aug 2017
Host STN	MEDLINE	Contains information on every area of medicine providing comprehensive coverage from 1948 to present. Sources include journals and chapters in books or symposia. The database is updated 5 times each week with an annual reload and therefore stays very current in its cover.	10 years	20	27
	EMBASE	The database, covers worldwide literature in the biomedical and pharmaceutical fields, including biological science, biochemistry, human medicine, forensic science, pediatrics, pharmacy, pharmacology and drug therapy, pharmacoeconomics, psychiatry, public health, biomedical engineering and instrumentation, and environmental science. Sources include more than 4,000 journals from approximately 70 countries, monographs, conference proceedings, dissertations, and reports. The databases covers data from 1974-present and is updated daily.		1	5
	EMBAL	The database provides early access to bibliographic data and the abstracts for references that will appear in EMBASE. Bibliographic information for references is available in EMBAL for the latest 8 weeks of EMBASE data. The database covers the worldwide literature on the biomedical and pharmaceutical fields. Bibliographic information, abstracts, and author keywords are searchable. Sources include over 4,000 journals. The database covers current data and is updated daily.		0	0
	ESBIOBASE	A database providing comprehensive coverage of the entire spectrum of biological research worldwide. Coverage includes the following areas: applied microbiology, biotechnology, cancer research, cell & developmental biology, clinical chemistry, ecological & environmental sciences, endocrinology, genetics, immunology, infectious diseases, metabolism, molecular biology, neuroscience, plant and crop science, protein biochemistry, and toxicology. Records are selected from over 1,700 international scientific journals, books, and conference proceedings. The database covers the period 1994 - present and is updated weekly.		5	0
	AGRICOLA	A bibliographic database containing selected worldwide literature of agriculture and related fields. Coverage of the database includes agricultural economics and rural sociology, agricultural production, animal sciences, chemistry, entomology, food and human nutrition, forestry, natural resources, pesticides, plant science, soils and fertilizers, and water resources. Also covered are related areas such as biology and biotechnology, botany, ecology, and natural history. The database draws on bibliographies, serial articles, book chapters, monographs, computer files, serials, maps, audiovisuals, and reports. It covers the period 1970-present and is updated monthly.		3	3
	BIOSIS	A large and comprehensive worldwide life science database covers original research reports, reviews, and selected U.S. patents in biological and biomedical areas, with subject coverage ranging from aerospace biology to zoology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. It covers the period 1926 – present and is updated weekly.		14	11

Provider	Database	Justification	Limits applied	Number*	Further Search Aug 2017
	CABA	Covers worldwide literature from all areas of agriculture and related sciences including biotechnology, forestry, and veterinary medicine. Sources include journals, books, reports, published theses, conference proceedings, and patents. It covers the period 1973-present and is updated weekly.		55	5
	CAPLUS	Covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences including applied, macromolecular, organic, physical, inorganic, and analytical chemistry. Current sources include over 8,000 journals, patents, technical reports, books, conference proceedings, dissertations, product reviews, bibliographic items, book reviews, and meeting abstracts. Electronic-only journals and Web preprints are also covered. Cited references are included for journals, conference proceedings and basic patents from the U.S., EPO, WIPO, and German patent offices added to the CAS databases from 1999 to the present. Also provides early access to the bibliographic information, abstracts and CAS Registry Numbers for documents in the process of being indexed by CAS. Covers the period 1907 – present and is updated daily		87	67
	FSTA	The database provides worldwide coverage of all scientific and technological aspects of the processing and manufacture of human food products including basic food sciences, biotechnology, hygiene and toxicology, engineering, packaging, and all individual foods and food products. Sources include more than 2,200 journals, books, reviews, conference proceedings, patents, standards, and legislation. It covers the period 1969 – present and is updated weekly.		2	2
	FROSTI	The database contains citations to the worldwide literature on food science and technology including food and beverages, analytical methods, quality control, manufacturing, microbiology, food processing, health and nutrition, recipes, and additives. Sources include approximately 800 scientific and technical journals, bulletins, technical reports, conference proceedings, grey literature, and British, European (EP), U.S., Japanese, and international (PCT) patent applications. Covers the period 1972 – present and is updated twice weekly.		1	0
	GEOREF	Covers international literature on geology and geosciences. Sources include the Bibliography of North American Geology, Bibliography and Index of Geology Exclusive of North America, Geophysical Abstracts, Bibliography of Fossil Vertebrates, selected records from Geoline and from geology sections of PASCAL and state and national geological surveys. Covers the period 1669 – present and is updated twice a month.			0
	TOXCENTER	Covers the pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals. It is composed of the following subfiles: BIOSIS, CAplus, IPA and MEDLINE and sources include abstracts, books and book chapters, bulletins, conference proceedings, journal articles, letters, meetings, monographs, notes, papers, patents, presentations, research and project summaries, reviews, technical reports, theses, translations, unpublished material, web reprints. Covers the period 1907 – present and is updated weekly			38

Provider	Database	Justification	Limits applied	Number*	Further Search Aug 2017
	PQSCITECH	Is a huge resource in all areas of science and technology from engineering to lifescience. The file is a merge of 25 STN databases formerly known as CSA databases (Cambridge Scientific Abstracts): AEROSPACE, ALUMINIUM, ANTE, AQUALINE, AQUASCI, BIOENG, CERAB, CIVILENG, COMPUAB, CONFSCI, COPPERLIT, CORROSION, ELCOM, EMA, ENVIROENG, HEALSAFE, LIFESCI, LISA, MATBUS, MECHENG, METADEX, OCEAN, POLLUAB, SOLIDSTATE, and WATER. Sources are journals, patents, books, reports, and conference proceedings spanning the period 1962 – present and it is updated monthly.		1	1
	PASCAL	The database provides access to the world's scientific and technical literature including physics and chemistry, life sciences (biology, medicine, and psychology), applied sciences and technology, earth sciences, and information sciences. French and European literature is particularly well represented. Approximately 5,000 journal titles are indexed. References to theses and to conference proceedings are also included. Spans the period 1977 to present and is updated weekly			
	SCISEARCH	Is an international index to the literature covering virtually every subject area within the broad fields of science, technology, and biomedicine. SciSearch contains all the records published in Science Citation Index Expanded™ and additional records from the Current Contents series of publications. Bibliographic information and cited references from over 5,600 scientific, technical, and medical journals are contained in the database. Spans the period 1974 to present and is updated weekly.		13	5
	ANABST	Covers worldwide literature on analytical chemistry. The ANABSTR file contains bibliographic records with abstracts (since 1984) for documents reported in printed Analytical Abstracts. Sources for ANABSTR include journals, books, conference proceedings, reports, and standards. Spans the period 1980 to present and is updated weekly.			

* Total number of summary records retrieved after removing duplicates

Table 9.5-3: Detailed Search Parameters for Web searches

Website name and service publisher	URL	Justification	Search terms	Limits applied	Number*
A web search has not been conducted as the database search reported above is considered to provide an adequately comprehensive search of the quality peer reviewed literature.					

* Total number of summary records or full-text documents retrieved after removing duplicates

Table 9.5-4: Detailed Search Parameters for Journal Table of Contents

Journal name	Journal URL or publisher	Dates, volumes and issues searched	Method of searching	Search terms	Number*
A search for journal table of contents has not been conducted as the database search reported above is considered to provide an adequately comprehensive search of the quality peer reviewed literature.					

* Total number of summary records or full-text documents retrieved after removing duplicates

Table 9.5-5: Detailed Search Parameters for Reference Lists

Bibliographic details of documents whose reference lists were scanned	Number*
A search for reference lists has not been conducted as the database search reported above is considered to provide an adequately comprehensive search of the quality peer reviewed literature.	

* Total number of summary records or full-text documents retrieved after removing duplicates

Results

Table 9.6-1: Results of study selection process

Data requirement(s) captured in the search	Number (Initial Search)	Number (Top-Up Search)	Number (Additional Search)
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	114	28	60
Number of *summary records* excluded from the search results after rapid assessment for relevance**	114	28	60
Total number of *full-text* documents assessed in detail*	0	0	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	0

*both from bibliographic databases and other sources of peer-reviewed literature
**aligned with EFSA Journal 2011; 9(2):2092: rapid assessment means exclusion of "obviously irrelevant records" based on titles.

Table 9.6-2: Results of study selection process Aug 2017

Data requirement(s) captured in the Further metabolite search Aug2017	Number (Additional Search)
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	164
Number of *summary records* excluded from the search results after rapid assessment for relevance**	164
Total number of *full-text* documents assessed in detail*	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

For the initial rapid assessment the study titles and abstracts were scanned to identify studies of potential relevance to crop and livestock metabolism and/or residue studies in the context of human exposure through the diet. Studies clearly not within the remit of Regulation (EU) No. 283/2013 and regulation (EU) No. 284/2013 (such as metabolism studies in environmental compartments or microorganisms, other environmental fate studies, toxicological studies, efficacy studies, studies on plants other than crops, and mode of action studies) were eliminated.

Here is a summary of the 164 titles removed during rapid assessment of the Aug 2017metabolite search:

- 4 titles were discounted as they were duplicates
- 20 titles were removed as they related to describing the development and use of analytical methods on various commodities, but not specifically for the trinexapac metabolites in question.
- 90 titles were removed as they concerned studies on genes, biological processes, cells, bacteria and biosynthesis, so not relevant to the metabolism and residues section. A number of references for fumonosins -maize toxins were also considered not relevant.

- 16 titles were related to environmental fate, ecology, efficacy and plant disease, so not relevant to the metabolism and residues section.
- 30 titles were discounted as they related to polymers, inorganic salt complexes, catalysts and geology.
- 4 titles were relating to fumonisins in food and or feed but contained no specific information pertaining to the specific metabolites in question so were discounted

No titles were identified as potentially relevant or unclear during the rapid assessment for relevance, and therefore have not been considered further here or in MCA Section 6.