Document Title

Amendment of Summary of the residues in or on treated products, food and feed for

Thiacloprid

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013

Document MCA

Section 6: Residues in or on treated products, food and feed

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

Date

2016-10-04

Author(s)

[redacted]

Bayer CropScience

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2014-09-16	Original submission	M-496661-01-1
2016-10-04	Update of section Effect on the residue level in pollen and bee products (p. 72ff) to included summary of the study M-510422-01-1 and updated conclusions for section CA 6.3.2 Magnitude of residue trials in corn	M-496661-02-1

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report.

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CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

CA 6.1 Storage stability of residues

In the baseline dossier studies have been described which demonstrated that residues of thiacloprid remain stable in three crop types, i.e. apple fruit, tomato fruit and melon peel for at least 18 months at -18°C.

In 2005 a study was conducted in US to investigate the stability of thiacloprid and its metabolites Amide-YRC 2894, 4-Hydroxy YRC 2894 Amide, and YRC 2894 Sodium Sulfonate in a variety of crops representing the water-, oil, protein, acidic- or starch- containing types over a period of 28 months at -10°C storage temperature.

Report:	[redacted]; [redacted]; 2005; M-264236-02-1
Title:	Storage stability of YRC 2894 and metabolites in various crop matrices: Analysis at 28 months
Report No.:	200120-1
Document No.:	M-264236-02-1
Guidelines:	**US EPA Residue Chemistry Test Guideline OPPTS 860.1380, Storage Stability Data**
GLP/GEP:	**yes**

Material and Methods

A freezer storage stability study was conducted to test the stability of YRC 2894 and three metabolites in soybean (forage, hay, seed), wheat (grain, hay, forage, straw), potato tuber, mustard greens, and orange fruit. Each matrix was fortified with YRC 2894, Amide-YRC 2894, 4-OH YRC 2894 Amide, and YRC 2894 Sodium Sulfonate at 0.10 mg/kg for each analyte. Samples were held in frozen storage (<-10 °C) and analysed after 28 months. The method used for analysis detects each of the four analytes separately. The samples were quantitated using HPLC-MS/MS. The samples were fortified in March 2001, resulting in between 854 to 860 days (around 28 months) of frozen storage.

Eighteen samples (5.0 g) of each matrix were weighed into 4 oz glass jars. A mixed standard solution containing YRC 2894, Amide-YRC 2894, 4-OH YRC 2894 Amide,and YRC 2894 Sodium Sulfonate (1:1:1:1, parent equivalents) was prepared in ACN/water(1:1 v/v). Twelve samples of each matrix were fortified with the mixed standard solution at 0.10 mg/kg. The remaining 6 samples were used for controls. After fortification, the solvent was allowed to evaporate before the jars were sealed. All jars were maintained in frozen storage (<-10 °C) in the freezer. At 7 months, two fortified samples and one control sample of soybean seed were removed from frozen storage. Concurrent recoveries were prepared by fortifying two fresh aliquots of the original soybean seed control matrix with the mixed YRC 2894 standard at the time of analysis. At 10 months, two fortified samples and one control sample of each matrix were removed from frozen storage. Concurrent recoveries were prepared by fortifying two fresh aliquots of each original control matrix with the mixed YRC 2894 standard at the time of analysis. At 28 months, two fortified samples and one control sample of each matrix were removed from frozen storage. Concurrent recoveries were prepared by fortifying two fresh aliquots of each original control matrix with the mixed YRC 2894 standard at the time of analysis. All values generated were reported.

Results and Conclusion

After 854 to 860 days of frozen storage at a temperature ≤ -10°C, all matrices showed <10% decomposition of YRC 2894, <25% decomposition of Amide-YRC 2894, <24% decomposition of 4-OH YRC 2894 Amide, and <14% decomposition of YRC 2894 Sodium Sulfonate. This data indicates that YRC2894, Amide-YRC 2894, 4-OH YRC 2894 Amide, and YRC 2894 Sodium Sulfonate are stable under frozen conditions for at least 854 days of frozen storage.

Also in 2005 a study was conducted in Europe to investigate the stability of thiacloprid in a variety of plant matrices representing, the high starch content with potato, the dry matrices with tobacco and wheat straw, the oily matrix with rape seed, the high water content matrix with pea with pod and the acidic matrix with currant. All these samples were stored at -18°C during approximately 24 months.

Report:	[redacted]; [redacted]; 2005; M-252414-01-1
Title:	Storage stability of YRC 2894 in/on potato (tuber), tobacco (leaf, dry), wheat (straw), rape (seed), pea (pea with pod) and currant (fruit) for 24 months
Report No.:	MR-073/03
Document No.:	M-252414-01-1
Guidelines:	
GLP/GEP:	**yes**

Material and Methods

This report describes the stability of residues of YRC2894 (thiacloprid) in fortified control samples of plant origin (potato (tuber), tobacco (leaf, dry), wheat (straw), rape (seed), pea (pea with pod) and currant (fruit)) during freezer storage for 24 months. The samples were fortified at a level of 0.20 mg/kg (2.0 mg/kg for tobacco).
The samples were stored in amber glass bottles at – 18°C or below and were analysed at nominal intervals of 0, 30, 90, 180, 360, 540 and 730 days.
Residues of YRC 2894 in/on plant material were determined by HPLC-MS/MS according to the methods 00548, 00548/E002 and 00548/E003.
The residues were extracted from 5 g plant material with a mixture of acetonitrile/water. After filtration, the extract was concentrated to the aqueous remainder and partitioned against cyclohexane/ethyl acetate using a Chromabond XTR™ column. The residues were quantified by reversed phase HPLC with MS/MS-detection using stable labelled thiacloprid as internal standard.
The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.02 mg/kg (0.20 mg/kg in/on tobacco, leaf dry) for YRC 2894 in the plant materials.
On day 0 (zero time analyses) five spiked samples and two control samples were analysed. Since these samples are recovery samples, it was not necessary to include concurrent recoveries. In addition, two recoveries for method validation, spiked at the respective LOQ, were performed.

Further samples were stored in a deep-freezer at –18°C or below. The temperature in the deep-freezer was recorded by a computer controlled system (novaPro open) during the whole storage period. The data of temperature measurements were printed out and archived in BCS-RD-D-ROCS, D-40789 Monheim, Building 6610.

At each sampling interval three fortified and five control samples were removed from the deep-freezer and allowed to reach room temperature. Subsequently, two of the control samples of each sample material were fortified with the test substances to determine the concurrent recoveries (fortification levels were at the same magnitude as the spiked storage samples). The samples were extracted and analysed concurrently with the *third* control sample and the spiked storage samples.

Results and Conclusion

After a deep-freezer storage period of 24 months, mean residue values determined for YRC 2894 were between 80 and 103% (normalised to day 0). No degradation during the deep-freezer storage could be observed.
The residues from stored samples were at a similar level as those from concurrent recovery experiments. Therefore, it can be assumed that all residues of YRC 2894 in samples of plant origin (potato (tuber), tobacco (leaf, dry), wheat (straw), rape (seed), pea (pea with pod) and currant (fruit)) are stable for at least 24 months under deep-freezer storage conditions, as shown in this study.
In the control samples, the residues of all compounds were always below 30% of the LOQ.

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Plants

In the baseline dossier, plant metabolism studies on tomatoes, apples and cotton following spray application have been presented. In this dossier, metabolism studies on spring wheat after spray application and on sunflower following seed dressing are described. In these studies the [methylene-^{14}C-label was employed. An additional study on potatoes after spray application of [thiazolidine-2-^{14}C]-labelled thiacloprid is also presented in this dossier. For thiacloprid, metabolism studies for 6 crops from 4 categories (fruit, pulses and oilseeds, cereals/grass crops and root crops) are now available.

Report:	[redacted]; [redacted]; [redacted], 2001; M-035182-01-1
Title:	Metabolism of [pyridinyl-14C-methyl]YRC2894 in spring wheat
Report No.:	MR-046/01
Document No.:	M-035182-01-1
Guidelines:	**US EPA Residue Chemistry Test Guideline OPPTS 860.1300 Nature of the Residue – Plants, Livestock; EU Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC**
GLP/GEP:	**yes**

Executive Summary

The metabolism of thiacloprid was investigated in spring wheat following two spray applications with a spray interval of 14 days and a pre-harvest interval of 21 days. The actual application conditions simulated practice conditions: Radiolabelled [methylene-^{14}C]thiacloprid was formulated as a 112.5 SE containing 100 g/L thiacloprid and 12.5 g/L of a mixing partner, which was replaced by water in the study. A computer controlled track sprayer with a flat fan nozzle was used for the two applications. In the first spray application 49.9 g a.s./ha was applied to wheat at growth stage 75 of the BBCH code (medium milk stage). The second application of 44.8 g a.s./ha followed 14 days later at growth stage 77 of the BBCH code (late milk stage). This resulted in a total annual application rate of 94.7 g a.s./ha. Wheat hay was sampled seven days after the first application. Wheat straw and grain were harvested at maturity 21 days after the second application. Hay, straw, and grain were homogenised and extracted with acetonitrile/water (1:1) and acetonitrile. The combined extracts for each sample material were partitioned with dichloromethane. All phases were chromatographed and quantitated by HPLC with radioactivity detection.

The extraction residues (solids 1) were extracted with acetonitrile/water (1:1) at 120°C under microwave assistance. After this the extraction residues of straw (solids 2) were hydrolysed with dioxane/2N HCl (9:1).
Metabolites were isolated and purified by HPLC and identified by HPLC/MS and HPLC/MS/MS and ^{1}H-NMR spectroscopy.

The total radioactive residue (TRR) concentrations (parent compound equivalents) in hay, straw and grains of wheat as well as the percentage distribution and concentrations of the identified metabolites in these matrices are summarised in the table below.

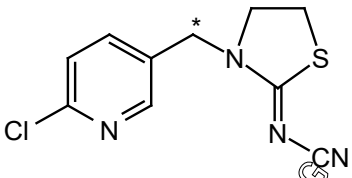
	Hay		Straw		Grain	
	% of TRR	TRR [mg/kg]	% of TRR	TRR [mg/kg]	% of TRR	TRR [mg/kg]
	100	2.04	100	12.36	100	0.21
Thiacloprid (YRC 2894)	81.4	1.66	83.4	10.30	80.9	0.17
YRC 2894 olefine	0.4	0.01	0.3	0.04	n.d.	n.d.
4-OH-YRC 2894	1.6	0.03	1.9	0.23	0.7	<0.01
YRC 2894 amide	0.2	<0.01	0.3	0.04	n.d.	n.d.
YRC 2894 hydroxyethyl diamide	0.1	<0.01	0.1	0.01	n.d.	n.d.
YRC 2894 sulfonic acid	1.2	0.03	1.0	0.13	n.d.	n.d.
YRC 2894 sulfonic acid conjugate	0.4	0.01	0.3	0.03	n.d.	n.d.
YRC 2894 diamide	0.5	0.01	0.4	0.05	n.d.	n.d.
6-CPA	0.4	0.01	0.3	0.04	n.d.	n.d.
6-CNA	1.2	0.03	2.2	0.27	n.d.	n.d.
6-CNA conjugate	1.7	0.03	1.1	0.13	1.7	<0.01
Sum identified	89.3	1.82	91.3	11.28	83.3	0.17

A total of 89.3%, 91.3% and 83.3% of the TRR was identified in hay, straw and grain, respectively. Unchanged thiacloprid was the predominant compound of the radioactive residue in all RACs amounting to 80.9 – 83.4%.
A total of 10 metabolites were identified in wheat. Each of them amounted to ≤2.2% of the TRR of the respective RAC. From the results of this study the metabolic pathway in spring wheat was deduced (see Figure 6.2.1-1). Thiacloprid was hydroxylated to 4-OH-YRC 2894. YRC 2894 olefine was formed by elimination of water from 4-OH-YRC 2894. Another portion of thiacloprid was hydrolysed at the cyano group leading to YRC 2894 amide. This compound was oxidised in the thiazolidinylidene moiety and cleaved yielding YRC 2894 sulfonic acid, which was partly conjugated with a C7 dicarboxylic acid. YRC 2894 sulfonic acid was also hydrolysed leading to YRC 2894 hydroxyethyl diamide. This metabolite was hydrolysed to YRC 2894 diamide followed by cleavage of the molecule leading to 6-CPA. The alcohol was oxidised to 6-CNA. 6-CNA was partly conjugated leading to a rare glycerol-glucuronic acid-conjugate.

I. Material and Methods

A. Materials

1. Test Material

Chemical structure		* position of the radiolabel
IUPAC name	{(2Z)-3-[(6-chloro-3-pyridinyl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide	
CAS name	Cyanamide, [3-[(6-chloro-3-pyridinyl)methyl-]-2-thiazolidinylidene]-	
CAS number	111988-49-9	
Formula	$C_{10}H_9ClN_4S$	
Molecular weight	252.7 g/mol	
Radiolabelled test material	[pyridinyl-^{14}C-methyl]YRC 2894	
Specific radioactivity	3.77 MBq/mg (102 µCi/mg)	
Chemical Purity	> 99% (HPLC and TLC)	
Radiochemical purity	> 99% (HPLC and TLC)	

B. Study Design

Experimental conditions:

Growth:

The planting container (surface area 1 m²) was filled with a sandy loam soil ("Monheim 3"). Spring wheat of the variety Thasos was sown on March 13, 2000. The climatic conditions during the vegetation period are listed in the table below.

	Average temp. (° C)	Sunshine hours (h)
March 2000	7.7	67
April 2000	13.3	164
May 2000	18.8	191
June 2000	21.7	235
July 2000	18.5	88

Application:

The application solutions were prepared by dissolving a 112.5 SE formulation of pyridinyl-^{14}C-methyl labelled thiacloprid in 40 mL of water. The application conditions simulated practice conditions of two spray applications to wheat at a target rate of 50 g a.s./ha in a spray volume of ca. 300 L/ha for each application. On June 26, 2000, at the stage of milk ripeness when the grains had reached their final size (growth stage 75 of the BBCH code), the first application (day 0) was performed. On July 10, 2000, 14 days later, the second application followed at late milk ripeness (growth stage 77 of the BBCH code). The application solutions were prepared by dissolving a 112.5 SE formulation of pyridinyl-^{14}C-methyl labelled thiacloprid in 40 mL of water. The formulation for the first application contained 5.78 mg a.s. (corresponding to 21.8 MBq) and for the second application 5.60 mg a.s. (corresponding to 21.1 MBq). A computer controlled track sprayer with a flat fan nozzle was used for application.

After each spray application, the protective plastic cover around the planting container was removed and rinsed with methanol. The washing solution was measured for radioactivity by LSC (liquid scintillation counting). The stock container in the application apparatus was also rinsed and the radioactivity determined. The amount of radiolabelled a.s. actually applied to wheat was determined by subtraction of the losses in the washing solutions. As a result 4.99 mg a.s. was actually applied with

the first application corresponding to 49.9 g a.s./ha and 4.48 mg a.s. was applied with the second application corresponding to 44.8 g a.s./ha, resulting in a total annual application rate of 94.7 g a.s./ha. The two application solutions were checked for stability by TLC and HPLC before and after the application.

Sampling and storage:
Hay was sampled seven days after the first application at BBCH 76-77. One of nine rows of wheat plants were cut. The sample material was dried at room temperature for 4 days, cut, and homogenised with liquid nitrogen. An aliquot of the homogenised sample was used for extraction. The remaining sample material was stored at about -20°C.
Grain and straw were harvested together at maturity (BBCH 89) 21 days after the second application. The wheat plants were cut close to the soil surface. The grains were collected by hand. The remaining ears and chaffs were combined with the straw sample.
Grain and straw samples were homogenised as described for hay. The homogenised samples were stored in aliquots at about -20°C. One aliquot of each sample was used for extraction.

C. Analytical Procedures

Extraction:
The homogenised sample aliquots were extracted 1x with 300 or 400 mL of acetonitrile/water (1:1, v/v) and 2x with 300 mL acetonitrile. After each extraction the extract was separated from the solids by filtration. All three extracts were combined and measured for radioactivity by LSC. The solids were air-dried yielding the solids 1. An aliquot was combusted and measured for radioactivity by LSC. All samples were treated in the same way. The results of combined extracts and solids were used for the determination of the TRR.
For further analysis the combined acetonitrile/water extracts were reduced in volume and partitioned 3x with ca. 150 mL dichloromethane yielding a dichloromethane and an aqueous phase. These phases were concentrated, measured for radioactivity by LSC and analysed by HPLC.
The total solids 1 were extracted with acetonitrile/water (1:1, v/v) for 15 min at 120°C in a microwave. The extracts were filtered and the radioactivity in the filtrate measured by LSC. The remaining solids 2 were dried at room temperature and combusted for radioactivity determination.

Hydrolysis:
The concentration of the radioactive residue in solids 2 of straw was less than 0.01 mg/kg. Therefore, an aliquot (15.4 g of 27.7 g) was hydrolysed with 200 mL 2N HCl/dioxane (1:9, v/v) at 100°C for 2 hours. The suspension was filtered and the radioactivity in the hydrolysate measured by LSC. The remaining solids (solids 3) were dried at room temperature and weighed. The radioactivity was determined. The hydrolysate was partitioned with 200 mL dichloromethane after addition of 300 mL of water yielding a dichloromethane and an aqueous phase. These phases were measured for radioactivity by LSC. Chromatography of the phases was not possible due to the high matrix load of the samples.

Quantitation:
Parent compound and metabolites in the extracts were quantified by reverse phase HPLC coupled to a radioactivity detector with a glass scintillator cell.

Measurement of radioactivity:
The measurement of the radioactivity was carried out by liquid scintillation counting (LSC). Aliquots of each liquid sample were generally measured in triplicate. Solid samples were combusted using a sample oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and radioassayed by LSC.

Identification and characterisation:

The quantification and purification of metabolites were carried out by HPLC. The identification was conducted by HPLC/MS, HPLC/MS/MS and ^{1}H-NMR spectroscopy. Details of the chromatographic and spectroscopic conditions are described in the report.

Storage stability:

Extraction and quantification of metabolites for each sample matrix were finished within 6 months from harvest. Therefore, no further stability investigations were required.

II. Results and Discussion

The total radioactive residue (TRR) concentrations (parent compound equivalents) in hay, straw and grains of wheat as well as the percentage distribution and concentrations of the identified metabolites in these matrices are summarised in Table 6.2.1-1.

Table 6.2.1- 1: Distribution of active substance and metabolites (% of total radioactive residue and parent equivalent concentration) in different matrices of spring wheat after spray application of [pyridinyl-^{14}C-methyl]thiacloprid.

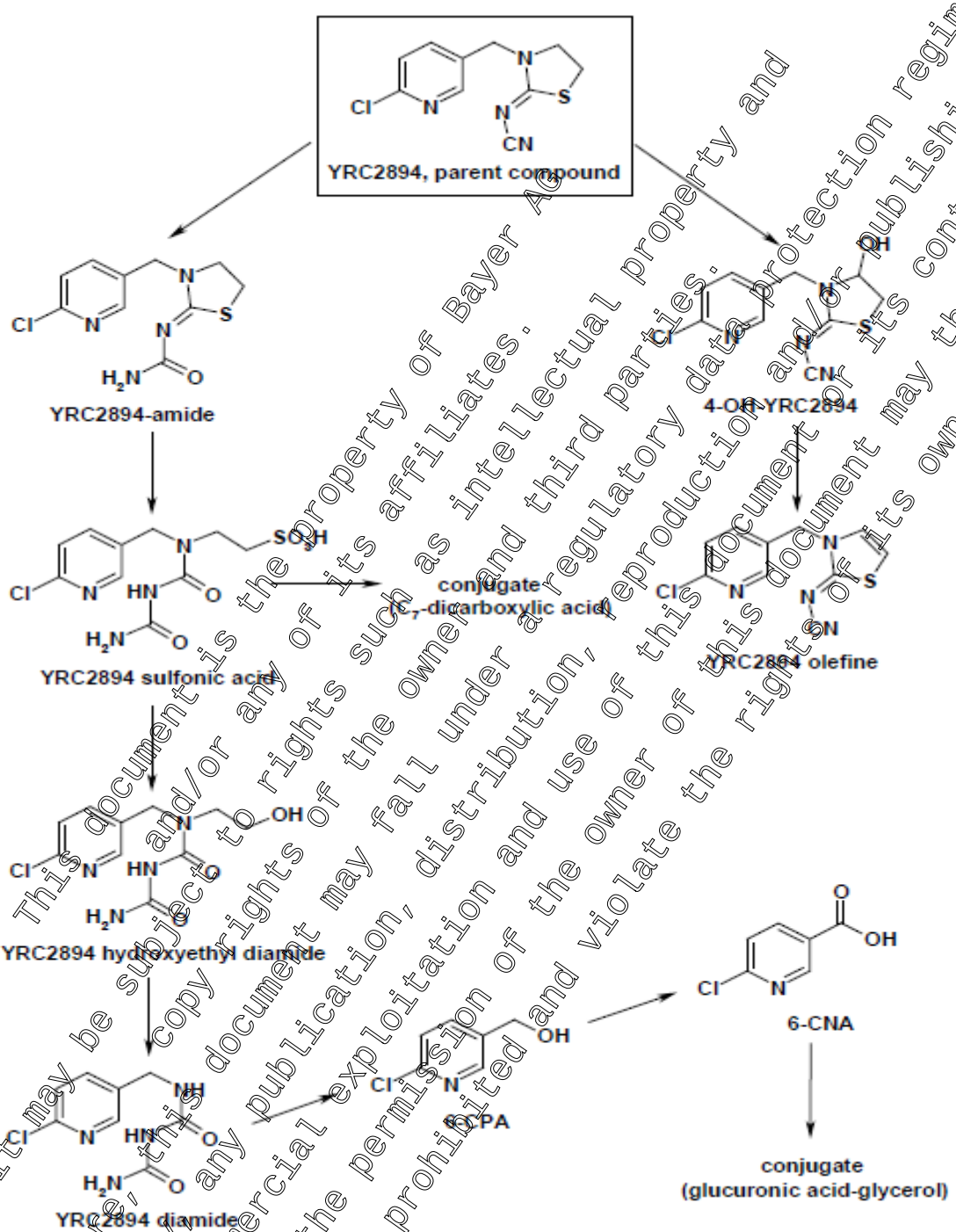
	Hay		Straw		Grain	
	% of TRR	TRR [mg/kg]	% of TRR	TRR [mg/kg]	% of TRR	TRR [mg/kg]
	100	2.04	100	12.36	100	0.21
Thiacloprid (YRC 2894)	81.4	1.66	83.4	10.30	80.9	0.17
YRC 2894 olefine	0.4	0.01	0.3	0.04	n.d.	n.d.
4-OH-YRC 2894	1.6	0.03	1.9	0.23	0.7	<0.01
YRC 2894 amide	0.2	<0.01	0.3	0.04	n.d.	n.d.
YRC 2894 hydroxyethyl diamide	0.1	<0.01	0.1	0.01	n.d.	n.d.
YRC 2894 sulfonic acid	1.2	0.03	1.0	0.13	n.d.	n.d.
YRC 2894 sulfonic acid conjugate	0.4	0.01	0.3	0.03	n.d.	n.d.
YRC 2894 diamide	0.5	0.01	0.4	0.05	n.d.	n.d.
6-CPA	0.4	0.01	0.3	0.04	n.d.	n.d.
6-CNA	1.2	0.03	2.2	0.27	n.d.	n.d.
6-CNA conjugate	1.7	0.03	1.1	0.13	1.7	<0.01
Sum identified	89.3	1.82	91.3	11.28	83.3	0.17

A total of 89.3%, 91.3% and 83.3% of the TRR was identified in hay, straw and grain, respectively.

Unchanged thiacloprid was the predominant compound of the radioactive residue in all RACs amounting to 80.9 – 83.4%.

A total of 10 metabolites were identified in wheat. Each of them amounted to ≤2.2% of the TRR of the respective RAC. From the results of this study the metabolic pathway in spring wheat was deduced (see Figure 6.2.1-1). Thiacloprid was hydroxylated to 4-OH-YRC 2894. YRC 2894 olefine was formed by elimination of water from 4-OH-YRC 2894. Another portion of thiacloprid was hydrolysed at the cyano group leading to YRC 2894 amide. This compound was oxidised in the thiazolidinylidene moiety and cleaved yielding YRC 2894 sulfonic acid, which was partly conjugated with a C7 dicarboxylic acid. YRC 2894 sulfonic acid was also hydrolysed leading to YRC 2894 hydroxyethyl diamide. This metabolite was hydrolysed to YRC 2894 diamide followed by cleavage of the molecule leading to 6-CPA. The alcohol was oxidised to 6-CNA. 6-CNA was partly conjugated leading to a rare glycerol-glucuronic acid-conjugate.

Figure 6.2.1- 1: Proposed metabolic pathway of thiacloprid in spring wheat following spray application of [pyridinyl-^{14}C-methyl]thiacloprid.



Report: [redacted]ö;[redacted]; 2008; M-308269-01-1
Title: Metabolism of [methylene-14C]thiacloprid in sunflower after seed treatment
Report No.: MEF-08/305
Document No.: M-308269-01-1
Guidelines: **US EPA OPPTS 860.1300; Canadian PMRA Ref.: DACO 6.3; OECD 501; EU 91/414/EEC amended by 96/68/EC; Japanese MAFF, 12 Nousan 8147, Appendix 2-4-1; not specified**
GLP/GEP: **yes**

Executive Summary

The metabolism of the insecticide thiacloprid was investigated in sunflower following seed dressing with [methylene-^{14}C]thiacloprid formulated as an FS 600. An aqueous dilution of the formulation was applied during the seeding process. The seeds were placed in the planting holes and the formulated a.s. was pipetted onto the seeds. Following the envisaged use pattern, seed treatment was performed using 1 mg a.s./seed, corresponding to an application rate of 80 g a.s./ha at a sowing rate of 80,000 seeds/ha. Additionally, an overdose experiment (5x) was performed to facilitate structure elucidation of the metabolites formed. The plants of both experiments were cultivated in the greenhouse.

Sunflower seeds of the 1x and the 5x overdose experiment were harvested at maturity (BBCH code 89-92) as obligatory RAC. To obtain supplementary information on the metabolic behaviour of thiacloprid applied as seed dressing, intermediate plants were collected as well when approximately eight leaves were unfolded (BBCH 18-19). Additionally, florets were plucked during blooming (BBCH 63-67). The florets contained nectar and pollen.

Total radioactive residue levels in the 1x and the 5x experiment (TRR, expressed as a.s. equivalents) are summarised in the following table:

	TRR [mg/kg]			
	Intermediate	Florets	Pollen	Seeds
1x experiment:	0.790	0.009	0.004	0.035
5x experiment	9.752	0.040	-	0.143

All RACs were extracted conventionally using acetonitrile/water mixtures. For sunflower seeds, the first extraction step was performed with n-heptane to remove fatty acids and other lipophilic compounds. At least 75% of the TRR was released by conventional solvent extraction. High portions of the TRR (at least 92% of the TRR) were extracted from sunflower intermediate and florets by acetonitrile/water mixtures. From seeds, only 72% and 77% of the TRR was extracted using acetonitrile/water mixtures. Extraction with n-heptane released additionally approx. 3% of the TRR leaving approx. 20% to 25% of the TRR unextracted in the solids. Due to the fact that the residue level in the unextracted solids of the seeds (1x experiment) was low (< 0.01 mg/kg), no additional extraction steps were performed.

Identification of metabolites was performed by HPLC and TLC co-chromatography or by spectroscopic means after isolation and purification of single metabolites. Parent compound was the main residue in the sunflower intermediate, followed by YRC 2894-amide. In sunflower seeds and florets (5x experiment), parent compound and YRC 2894-amide were still present, although in very

low concentrations. They were not detected in the florets of the 1x experiment. In the florets, 6-CPA-glucoside was the predominant metabolite. The corresponding aglycon 6-CPA is the precursor metabolite for the formation of 6-CNA and of the detected 6-CNA-conjugate, the main metabolites in seeds. 6-CNA and its corresponding conjugate were detected exclusively in seeds. In analogy to other carboxylic acids it was considered that 6-CNA - as a weak pyridine carboxylic acid - has a pronounced phloem mobility and was therefore transported selectively into the sunflower seeds as phloem sink. The same effect was also observed in the cotton metabolism study.

Minor metabolites (≤ 5% of the TRR) detected in the sunflower intermediate and in the seeds are all derived from parent compound or from YRC 2894-amide as the direct successor. Hydroxylation of the thiazolidine moiety and subsequent conjugation with a glycose (hexose) was observed for both compounds, whereby the hydroxylation of YRC 2894-amide was preferred. More details about amounts of parent compound and metabolites are shown in the following table for the 1x experiment.

	Intermediate		Florets		Seeds	
	TRR = 0.790 mg/kg		TRR = 0.009 mg/kg		TRR = 0.035 mg/kg	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Thiacloprid (YRC 2894)	42.8	0.338	---	---	7.2	0.003
6-CNA conjugate	---	---	---	---	11.9	0.004
6-CNA	---	---	---	---	10.4	0.004
6-CPA-glucoside	2.1	0.016	82.8	0.007	---	---
hydroxy-YRC 2894-amide-glycoside*	3.0	0.024	---	---	---	---
4-hydroxy-YRC 2894-amide	5.2	0.041	---	---	---	---
hydroxy-YRC 2894-glycoside*	2.8	0.022	---	---	---	---
YRC 2894-amide	33.2	0.262	---	---	6.0	0.002
4-hydroxy-YRC 2894	1.5	0.012	---	---	---	---
Total identified	90.6	0.715	82.8	0.007	35.5	0.012

* position of hydroxylation not determined by LC-MS and LC-MS/MS, assignment to “4-hydroxy” is most plausible

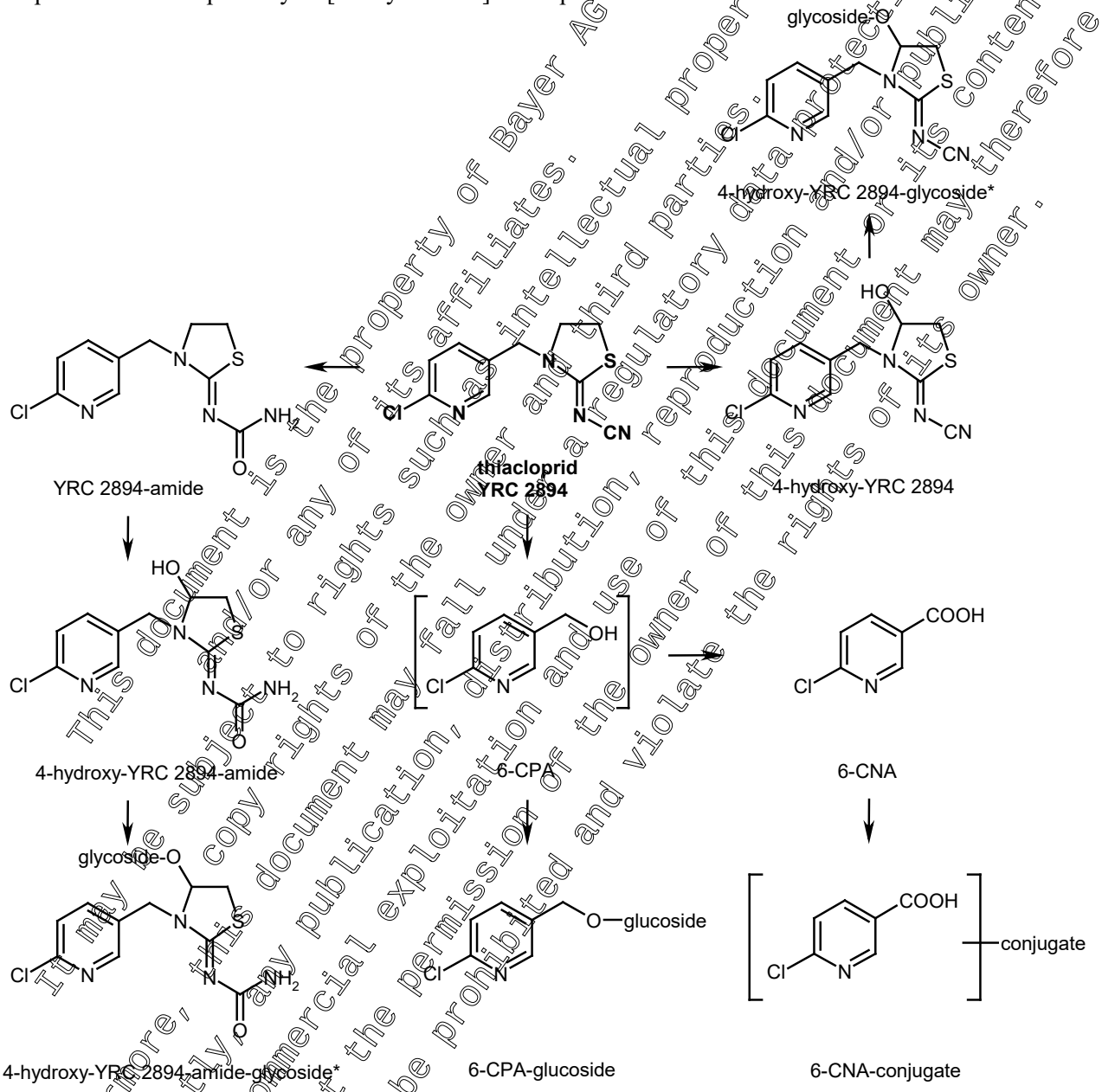
Since identification of a major metabolite in seeds (17.0% of TRR, 0.006 mg/kg) failed, an approach to determine the total residue of thiacloprid was applied. Parent compound and all metabolites containing the 6-chloropicolyl moiety were oxidised quantitatively to 6-CNA with an alkaline potassium permanganate solution. The amount of resulting 6-CNA was approx. 59% of the TRR.
The entire analytical work including extraction, HPLC analysis for metabolic profiling and identification of metabolites was completed within ca. 10 months after seed treatment. The time period between sampling of a RAC and HPLC analysis (metabolite profile) was no longer than 42 days (1.4 months). All investigations concerning identification and characterisation of single metabolites were completed not later than five months after sampling of the respective RAC. Hence, no storage investigations were necessary.

Based on the identified metabolites, the metabolic pathway of [methylene-^{14}C]thiacloprid in sunflower was proposed (see next page). The following main metabolic degradation routes were detected:

- Hydrolysis of the cyano group of the parent compound resulting in YRC 2894-amide, followed by hydroxylation of the thiazolidine moiety and subsequent conjugation with a hexose moiety

- Direct hydroxylation of the thiazolidine moiety of the parent compound and subsequent conjugation with a hexose moiety
- Oxidative cleavage of the molecule leading to 6-chloropicolyl alcohol (6-CPA) and subsequent conjugation with glucose
- or alternatively further oxidation of the alcohol to 6-chloronicotinic acid (6-CNA) and subsequent conjugation with an unidentified endocon

Proposed metabolic pathway of [methylene-^{14}C]thiacloprid in sunflower:

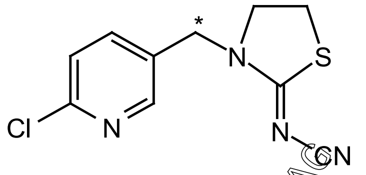


* position of hydroxylation was assumed;
an unambiguous assignment of the hydroxylation position by LC-MS and LC-MS/MS was not possible.

I. Material and Methods

A. Materials

1. Test Material

Chemical structure		* position of the radiolabel
IUPAC name	{(2Z)-3-[(6-chloro-3-pyridinyl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide	
CAS name	Cyanamide, [3-[(6-chloro-3-pyridinyl)methyl-]-2-thiazolidinylidene]-	
CAS number	111988-49-9	
Formula	$C_{10}H_9ClN_4S$	
Molecular weight	252.7 g/mol	
Radiolabelled test material	[methylene-^{14}C]thiacloprid (pyridinyl-^{14}C-methyl)thiacloprid	
Specific radioactivity	3.77 MBq/mg (102 µCi/mg)	
Chemical Purity	> 98% (HPLC)	
Radiochemical purity	> 98% (HPLC and TLC)	

B. Study Design

Test site and crop information:

The sunflower plants (variety: Pegasol) were cultivated in the greenhouse. Each plant was sown in a 30 L-planting pot with a surface area of 1164 cm². Six plants were used for the 1x- and 6 plants for the 5x experiment. The planting pots were filled with the sandy loam soil "Monheim 4". The sowing date was 21st of March, 2007, the harvest was conducted on the 6th of August, 2007. The daytime (6 a.m. to 8 p.m.) temperature in the greenhouse was kept between 19 and 20°C, during the night the temperature was lowered to 13-14°C, the light intensity during the day (6 a.m. to 8 p.m.) was ≥ 35 kLux, the relative air humidity was kept at 60%. An artificial pollination was achieved by manual distribution of the pollen to the plants using a brush.

Formulation:

For the preparation of the radioactive FS 600 formulation, the radiolabelled test substance was thoroughly ground and mixed with a blank formulation using a ball mill. The conditions needed to obtain a formulation equivalent to the commercial product were determined in pre-experiments with non-radiolabelled a.s. The resulting suspension concentrate was diluted with an adequate volume of water for seed treatment. The same aqueous dilution was used for the 1x- and the 5x experiment, only the applied volume was adapted. The aqueous seed dressing suspension was well homogenised before treating each seed.

The concentration of active substance in the diluted FS 600 formulation was 0.107 mg/µL (corresponding to 405 kBq/µL). For the 1x application, each seed was treated with a volume of 10 µL (corresponding to 4.05 MBq). Accordingly, a volume of 50 µL was used for the seed treatment in the 5x experiment (overdose experiment, corresponding to 20.23 MBq). Small aliquots were taken for the determination of radioactivity in the seed dressing suspension. Additionally, small amounts of the seed dressing suspension were analysed before and after the seed treatment by HPLC to confirm the identity and the stability of the test substance. The identity was confirmed by HPLC co-chromatography or by comparison of retention times using a non-radiolabelled authentic reference compound.

C. Identification and Characterisation of Residues

Sample handling and preparation
Sunflower intermediate:
When eight leaves were unfolded (BBCH 18-19), two intermediate plants of the 1x experiment and one intermediate plant of the 5x experiment were harvested. The plants were cut close to the soil surface and the total weight of the sample material was determined for both experiments. The plants were cut into pieces and the sample material was homogenised with liquid nitrogen. Prior to extraction, five small aliquots of the homogenised sample were analysed by combustion to estimate the TRR. Another aliquot was used for extraction and the remaining sample was stored in a freezer. The actual TRR value of the RAC was determined by adding up the radioactivity measured in the extracts and in the remaining solids.

Florets:
Sunflower inflorescences generally start flowering in circles from the outer to the inner florets. Each floret is in the male phase (stamens visible, shedding of pollen) on the first day of flowering and in the female phase (stigma visible) on the next day. As known from earlier studies, considerable amounts of nectar are only present in the female florets in the afternoon. Thus, florets were plucked in the female phase at approx. 1 p.m. from the inflorescence using tweezers. Florets of two sunflower plants were sampled for the 1x experiment and as well as for the 5x experiment.
The florets containing the nectar and pollen, were collected every day or every second day during the time of blooming (BBCH 65-67). For the 1x experiment flowering started on May 31, 2007 and was completed on June 10, 2007. The plants of the 5x experiment started flowering on June 04, 2007 and the last full flowering florets were collected on June 12, 2007. The florets of all sampling points were stored in a freezer until extraction and analysis.
Since the florets were sampled in the female phase, pollination was possible and a normal development of the seeds was observed, though not expected. Therefore, the seeds of these sunflower plants were also collected at maturity.

Pollen:
At the beginning of blooming, plastic boxes were installed underneath the inflorescences to collect the pollen shed by the sunflowers. In general, the pollen was sampled from those sunflowers from which the florets were collected. The pollen was combined with the florets for extraction and analysis. An exception was the collection of pollen of one sunflower plant of the 1x experiment on June 1 and June 4, 2007, which was cultivated for the collection of seeds. These pollen samples were collected and combusted to obtain an estimation of the radioactivity concentration in the pollen.

Seeds and rest of plant:
At BBCH 89-92, the anthocarps were separated from the rest of the plants to sample the fully ripe seeds. The remaining plants (stem and leaves) were cut close to the soil surface and then cut into small pieces. The plant pieces were combined with the emptied anthocarps and stored in a freezer for optional analysis.
The seeds were weighed, dried overnight at room temperature and weighed again. Then, they were homogenised with liquid nitrogen. Prior to extraction, five small aliquots of the homogenised sample material were analysed by combustion to estimate the TRR. Another aliquot was used for extraction and the remaining sample was stored in a freezer. The actual TRR value of the RAC was determined by adding up the radioactivity measured in the extracts and in the remaining solids.

Extraction of residues:
The samples of the homogenised RACs were exhaustively extracted with acetonitrile/water mixtures. For sunflower seeds, the first extraction step was performed with n-heptane to remove fatty acids and other lipophilic compounds. The radioactivity in each extract was determined by LS counting. The acetonitrile/water extracts were combined and subjected to a solid phase extraction (SPE) clean-up (RP18) to separate the matrix compounds. The purified extracts were concentrated and analysed by HPLC. The remaining solids were weighed and the radioactivity was determined by combustion followed by LSC. The sum of the radioactivity in the single extracts and in the solids was used for the calculation of the TRR.

Identification and quantification of parent compound and metabolites:
Parent compound and metabolites were identified by HPLC and/or TLC co-chromatography with authentic reference compounds or by spectroscopic investigations (LC-MS and LC-MS/MS). LC-MS was performed with single fractions isolated by HPLC from the extracts of sunflower intermediate and sunflower seeds of the overdose (5x) experiment. These isolated and identified metabolites were used for co-chromatographiy in all RACs of the 1x experiment. Additionally, the metabolite profiles of all RACs of the 1x experiment were compared with the corresponding RACs of the 5x experiment. Prior to the identification of intermediate metabolites by TLC co-chromatography, the extract of sunflower intermediate (1x) was fractionated and the single fractions were applied in overlapping zones with authentic reference compounds. For quantification of metabolites, the combined extracts of all plant matrices were analysed by integrating the regions of interest of the HPLC-chromatograms.

For sunflower seeds - as edible RAC - a further approach was applied. Since thiacloprid was degraded to numerous different metabolites, most of them containing the 6-chloropicolyl moiety, it was possible to determine the total residue of thiacloprid. For the determination of the total residue an aliquot of the acetonitrile/water extract of the seeds was treated with alkaline potassium permanganate solution to oxidise parent compound and all metabolites containing the 6-chloropicolyl moiety to 6-chloronicotinic acid (6-CNA). The 6-CNA was then extracted and analysed by HPLC.

D. Analytical Methodology

Radioactivity measurement:
The radioactivity measurement in the liquid samples was carried out by liquid scintillation counting. All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail prior to radioactivity determination by LSC.

Identification and characterisation:
The quantification and purification of metabolites were carried out by HPLC and TLC. The identification was conducted by HPLC/MS, HPLC/MS/MS and LC-NMR-MS. Details of the chromatographic and spectroscopic conditions are described in the report.

Storage stability:
Aliquots of sunflower intermediate and sunflower seeds were extracted and analysed by HPLC not later than 25 days after sampling. Florets were collected at several time points during a period of 9 to 11 days and stored in a freezer. Extraction followed 9 to 12 days after the last sampling date, HPLC analysis 2 to 3 days later. Thus, the maximum time period between sampling and HPLC analysis (metabolite profile) was 42 days.
In general, identification of parent compound and metabolites was based on HPLC and TLC co-chromatography or on LC-MS and LC-MS/MS analysis after isolation of the single compounds. To characterise some unknown compounds, the extracts of the sunflower intermediate sample and of

sunflower seeds were additionally treated with hydrochloric acid. The identification- and characterisation work was completed not later than 5 months after sampling of the respective RAC. Hence, no additional storage investigations were necessary to prove the stability of thiacloprid residues in the solvent extracts.

To determine the total residue of thiacloprid in seeds, a second extraction of seeds (1x experiment) was performed ca. 6 months after sampling. Comparison of the metabolite profiles recorded after the first and the second extraction showed almost identical metabolite patterns and a comparable metabolic distribution. Only the acidic compounds showed shifted retention times and other ratios indicating different pH values of the sample aliquots analysed. As a conclusion, no transformation or degradation effects occurred when storing sunflower seeds for ca. 6 months at < -18°C.

II. Results and Discussion

A. Total Radioactive Residues

The total radioactive residues (TRR values) in the 1x experiment ranged from 0.004 mg/kg in the pollen (mean value of two sampling times) to 0.790 mg/kg in the intermediate sample. Low residues were detected in the seeds (0.035 mg/kg) at harvest. Accordingly, the RACs of the 5x experiment showed higher residues and could therefore be used for the elucidation of metabolites. An overview is given in Table 6.2.1-2.

Table 6.2.1-2: [Methylene-^{14}C]thiacloprid: Total radioactive residues (TRRs) in sunflower matrices

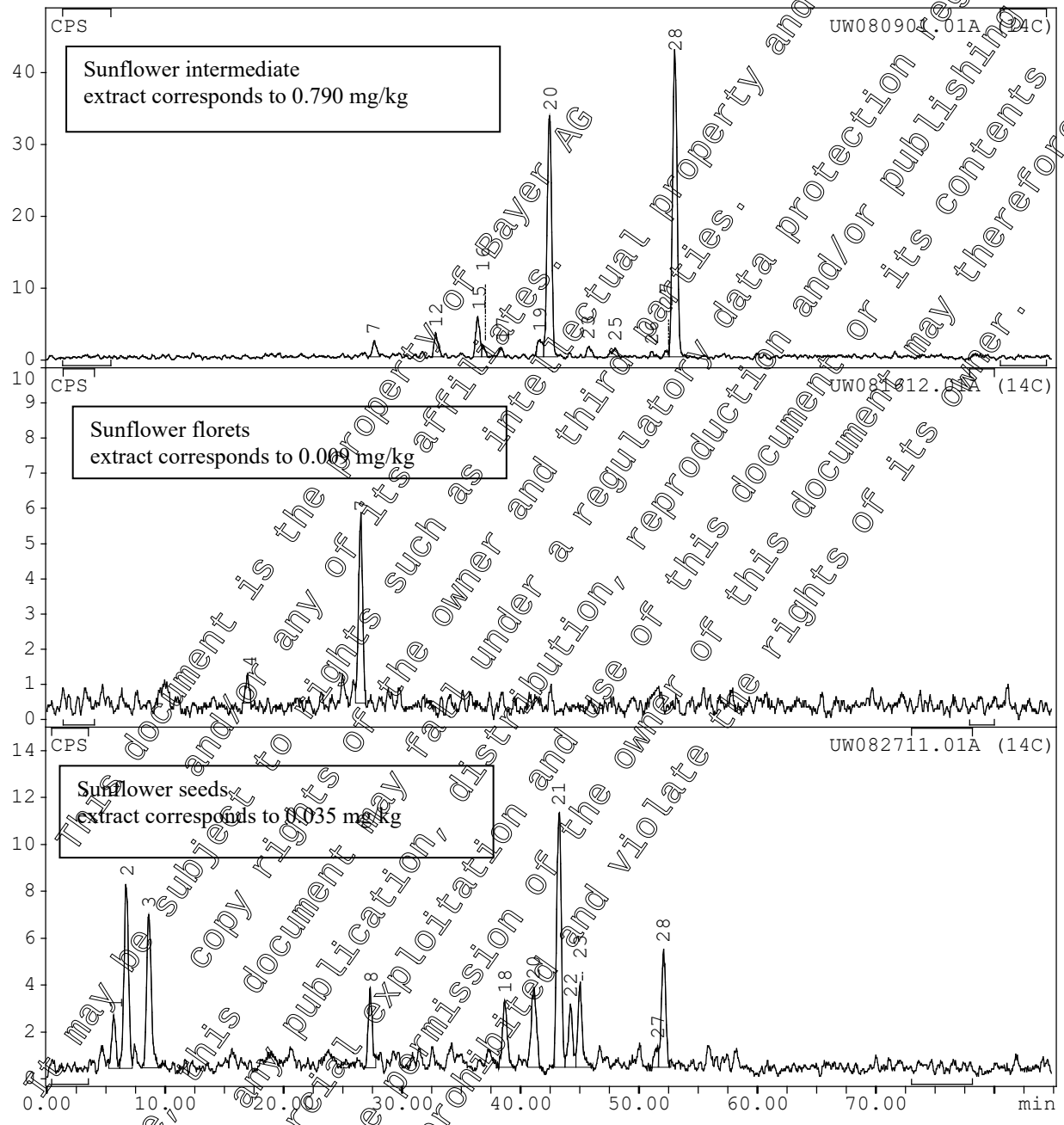
Matrix	TRR [mg a.s. equivalents per kg sample material]		PHI* [d]	
	1x exp.	5x exp.	1x exp	5x exp.
Intermediate	0.790	9.752	36	36
Florets	0.009	0.040	71-81	75-84
Pollen	0.004	n.c.	62-65	n.c.
Seeds	0.035	0.143	138	138

*: PHI: pre-harvest interval = days between seed treatment and harvest
n.c.: not collected

B. Distribution of Radioactivity in Raw Agricultural Commodities

An overview of the chromatographic patterns of the extracts of the 1x experiment is provided in Figure 6.2.1-2.

Figure 6.2.1-2: [Methylene-^{14}C]thiacloprid: Comparison of the chromatographic patterns of sunflower intermediate, florets and seeds (1x experiment, for details of the HPLC methods see report)



2	6-CNA-conjugate	19	hydroxy-YRC 2894-glycoside
3	6-CNA	20	YRC 2894-amide
7	6-CPA-glucoside	25	4-hydroxy-YRC 2894
12	hydroxy-YRC 2894-amide-glycoside	28	thiacloprid (YRC 2894)
15	4-hydroxy-YRC 2894-amide		

The acetonitrile/water extracts were analysed as soon as possible after sample preparation by HPLC. The chromatograms were integrated for quantitative evaluation.

The quantitative distribution of the identified metabolites in the extracts and solids is compiled in Table 6.2.1-3.

Table 6.2.1-3: [Methylene-^{14}C]thiacloprid: Summary of characterisation and identification of radioactive residues in the RACs of sunflower (1x experiment)

	Intermediate		**Florets**		**Seeds**	
	TRR = 0.790 mg/kg		TRR = 0.009 mg/kg		TRR = 0.035 mg/kg	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Thiacloprid (YRC 2894)	42.8	0.338	---	---	7.2	0.003
6-CNA conjugate	---	---	---	---	11.9	0.004
6-CNA	---	---	---	---	10.4	0.004
6-CPA-glucoside	2.1	0.016	82.8	0.007	---	---
hydroxy-YRC 2894-amide-glycoside*	3.0	0.024	---	---	---	---
4-hydroxy-YRC 2894-amide	5.2	0.041	---	---	---	---
hydroxy-YRC 2894-glycoside*	2.8	0.022	---	---	---	---
YRC 2894-amide	33.2	0.262	---	---	6.0	0.002
4-hydroxy-YRC 2894	1.5	0.012	---	---	---	---
Total identified	90.6	0.715	82.8	0.007	35.5	0.012

* position of hydroxylation not determined by LC-MS and LC-MS/MS, assignment to "4-hydroxy" is most plausible

At least 91.9% of the TRR was extracted by acetonitrile/water mixtures from sunflower intermediate and florets. A somewhat lower recovery was observed when extracting the seeds conventionally with acetonitrile/water mixtures, pure acetonitrile and n-heptane (in total 75.0% to 80.4% of the TRR). A very small amount of the TRR (about 3%) was released from seeds by n-heptane, indicating that no significant incorporation of radioactivity into biomolecules (fatty acids) occurred. Since the radioactive residues in the solids of the 1x experiment were < 0.01 mg/kg, no additional exhaustive extraction steps were performed.

Thiacloprid was the main compound in the sunflower intermediate accounting for approx. 43% (1x experiment) and approx. 49% (5x experiment) of the TRR. In sunflower florets and seeds, it was still representing between approx. 6% and 10% of the TRR, though it was not detected in the florets of the 1x experiment due to the low residue level. The majority of the compounds detected in the intermediate sample was also detected in florets and/or seeds. 6-CNA and its conjugate were detected exclusively in the seeds and were the major compounds in this RAC representing between approx. 6% and 12% of the TRR. The exclusive presence of these compounds in the seeds can be explained as follows: In analogy to the behaviour of other weak pyridine carboxylic acids in vascular plants it was concluded that the presence of high amounts of 6-CNA was a secondary result of accumulation of this acid in the seeds as phloem sink after being secreted from the apoplasm into the phloem as a trap compartment for weak pyridine carboxylic acids. Initial formation of 6-CNA took probably place in the leaves, or even in the soil. Due to the pronounced phloem mobility and the selective transport, 6-CNA was concentrated in the seeds so that detectable amounts resulted (0.004 mg/kg to 0.009 mg/kg). Conjugation of 6-CNA occurred most probably in the seeds, where 6-CNA was concentrated. The precursor metabolite of 6-CNA, 6-CPA, was detected as glucose conjugate in the intermediate, and also in the florets. In florets, 6-CPA-glucoside was the main metabolite accounting for approx. 59% to 83% of the TRR.

The metabolite YRC 2894-amide was detected in all three RACs representing between ca. 5% and ca. 33% of the TRR. In the intermediate sample and in the seeds it was an important precursor for further metabolic transformation reactions (hydroxylation and conjugation with a hexose). The resulting metabolites ranged from ca. 2% to 5% of the TRR. In the intermediate sample, these metabolic transformation reactions were also observed for the parent compound itself. The resulting metabolites ranged from ca. 1% to 3% of the TRR.

C. Determination and Quantification of the Radioactive Residue Determined as 6-CNA

Since thiacloprid was degraded to numerous different metabolites with partly unknown structures, it was decided to determine the so-called total residue of thiacloprid in the seeds. This approach is based on the fact that thiacloprid and all metabolites containing the 6-chloropicolyl moiety can be quantitatively oxidised with an alkaline potassium permanganate solution to 6-CNA which can be partitioned into tert.-butyl methyl ether and analysed by HPLC. Thus, after a chemical conversion step, the sum of thiacloprid and all metabolites containing the 6-chloropicolyl moiety can be detected as a single compound. According to the radioactive balances 75% to 76% of the TRR was available after conventional solvent extraction and the following clean-up step by SPE for the oxidation step. After oxidation and partitioning the oxidation products into tert.-butyl methyl ether (two partitioning steps), ca. 59% of the TRR was recovered. HPLC analysis revealed that the recovered radioactivity was only attributable to 6-CNA. Thus, approx. 59% of the TRR present in the seeds was represented by parent compound or metabolites containing the 6-chloropicolyl moiety.

D. Storage Stability

Aliquots of sunflower intermediate and sunflower seeds were extracted and analysed by HPLC within 25 days after sampling. Florets were collected at several sampling points during a period of 9 to 11 days. The florets were stored in a freezer between the different sampling dates. Extraction followed 9 to 12 days after the last sampling date, followed by HPLC analysis 2 to 3 days later. Thus, the maximum time period between sampling and HPLC analysis was 42 days.

In general, identification of parent compound and metabolites was based on HPLC and TLC co-chromatography or on LC-MS and LC-MS/MS analysis after isolation of the single compounds. To characterise unknown compounds, the extracts of the sunflower intermediate sample and of sunflower seeds were additionally treated with hydrochloric acid. All investigations concerning identification and characterisation were completed not later than 5 months after sampling of the respective RAC. Hence, storage stability investigations were unnecessary.

To determine the total residue of thiacloprid in seeds, a second extraction of sunflower seeds (1x experiment) was performed approx. six months after sampling of the RAC. Comparison of the metabolite profiles recorded after the first and the second extraction showed almost identical metabolite patterns and a comparable metabolic distribution. Only the acidic compounds showed shifted retention times and other ratios indicating different pH values of the sample aliquots analysed. Hence, no transformation or degradation effects occurred when storing sunflower seeds for approx. 6 months at ≤ -18 °C.

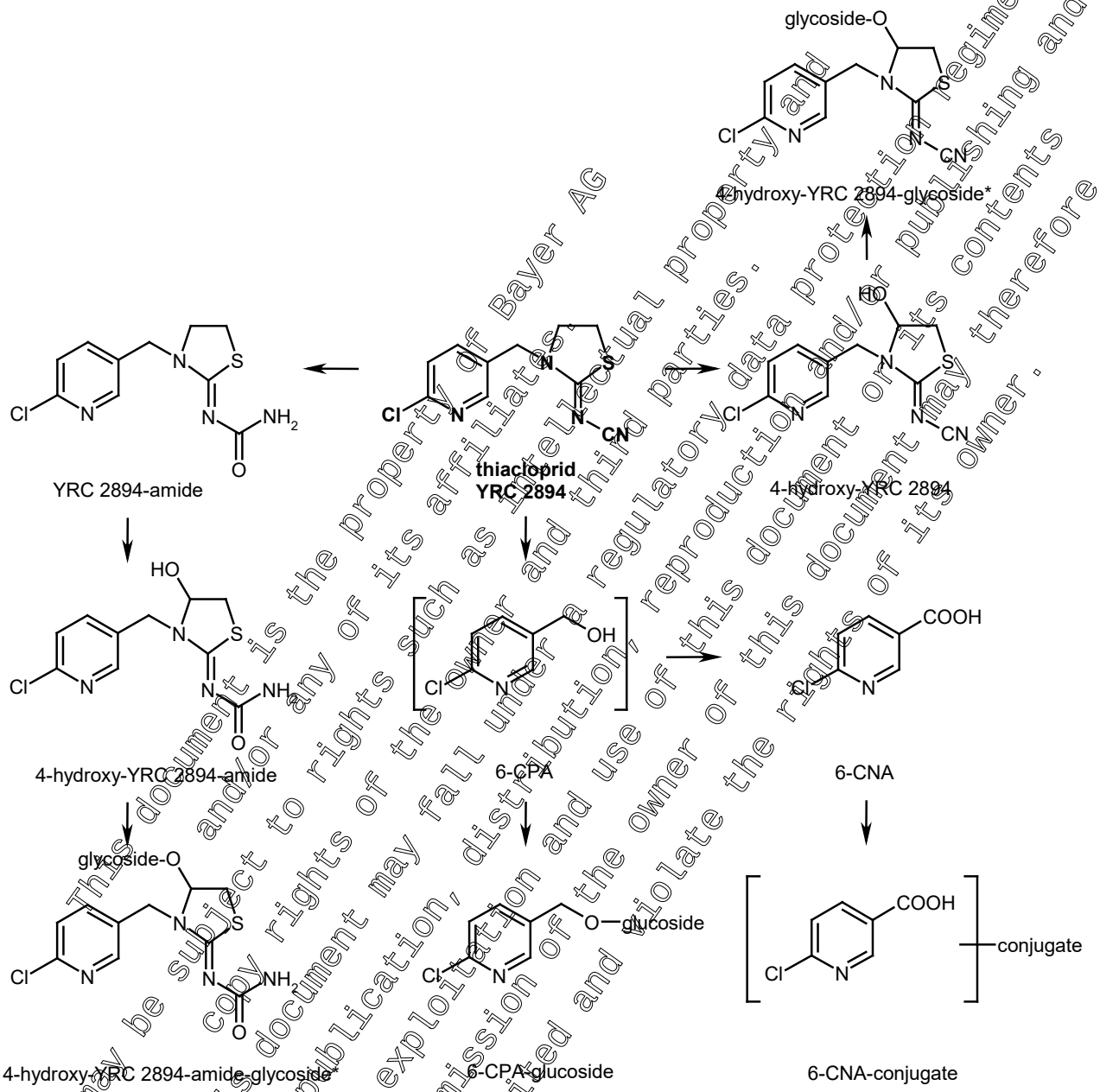
E. Proposed Metabolic Pathway of [methylene-^{14}C]thiacloprid in Sumflower

The metabolic transformation of thiacloprid was characterised by thee main routes. Major reactions were the hydrolysis of the cyano group to form the respective amide, the hydroxylation of the thiazolidine ring and the oxidative cleavage of the molecule at the methylene bridge.

Hydrolysis of the cyano group yielded YRC 2894-amide, the main metabolite in the intermediate sample. Subsequent hydroxylation of the thiazolidine moiety of the metabolite and conjugation with

hexose followed. The hydroxylation position was assigned to the 4-position in the phase I metabolite (4-hydroxy-YRC 2894-amide), but could not be determined unambiguously for the conjugate - though it seems plausible that the position of the hydroxylation was the same as in the proposed precursor molecule. YRC 2894-amide, 4-hydroxy-YRC 2894-amide and hydroxy-YRC 2894-amide-glycoside were detected in the intermediate sample and in sunflower seeds.
Hydroxylation of the thiazolidine moiety was also observed for the parent compound. The resulting metabolite 4-hydroxy-YRC 2894 was also the precursor for subsequent conjugation. These metabolites (4-hydroxy-YRC 2894 and hydroxy-YRC 2894-glycoside) were detected exclusively in the intermediate sample.
Oxidative cleavage of the molecule resulted in the formation of 6-chloropicolyl alcohol (6-CPA) which was further oxidised to 6-chloronicotinic acid (6-CNA). Both metabolites were subjected to conjugation resulting in 6-CPA-glucoside and an unknown conjugate of 6-CNA. 6-CNA and its conjugate were detected exclusively in sunflower seeds. In analogy to other pyridine carboxylic acids it was considered that 6-CNA - as weak organic acid - has a pronounced phloem mobility and was therefore transported selectively into the sunflower seeds as phloem sink. Conjugation of 6-CNA occurred most probably in the seeds, where the 6-CNA was concentrated. 6-CPA was detected only as its glucoside. which was the main compound in the florets and was also detected in small amounts in the intermediate sample, but not in the seeds.
Unchanged thiacloprid was only a prominent compound in the intermediate plants of the 1x and the 5x experiment which were sampled already 36 days after the seed treatment. In florets and mature seeds, thiacloprid accounted for less than 11% of the TRR. Due to the low residue level in the florets of the 1x experiment, no parent compound was detected. The low amount of parent compound in florets and seeds, as well as the high number of identified metabolites, indicates that thiacloprid is subjected to a quite intensive metabolic transformation in the sunflower.
Based on the results of this study the metabolic pathway of [methylene-^{14}C]thiacloprid in sunflower as shown in Figure 6.2.1-3 can be proposed.

Figure 6.2.1-3: Proposed metabolic pathway of [methylene-^{14}C]thiacloprid in sunflower after seed treatment



* position of hydroxylation was assumed; an unambiguous assignment of the hydroxylation position by LC-MS and LC-MS/MS was not possible.

F. Conclusion

The TRR in sunflower seeds was low (0.035 mg/kg) following seed treatment with [methylene-^{14}C]thiacloprid according to the envisaged use pattern (1 mg a.s./seed). The TRR in an additionally collected intermediate sample was 0.790 mg/kg. Florets containing nectar and pollen showed a very low residue level of 0.009 mg/kg. Additional collection of pollen of the 1x experiment at two time points showed that the TRR ranged from 0.003 mg/kg to 0.005 mg/kg. The TRR values in the 5x overdose experiment, performed to support identification and structure elucidation of metabolites, amounted to 0.143 mg/kg in seeds, to 9.752 mg/kg in the intermediate sample and to 0.040 mg/kg in florets, respectively.

Several metabolic routes of thiacloprid were determined in sunflowers after seed treatment. Unchanged parent compound was detected only as main compound in the intermediate sample (ca. 43% to 49% of the TRR) which was collected at a rather early time point (36 days after seed treatment). In florets of the 5x experiment, parent compound accounted for less than 11% of the TRR (0.004 mg/kg) and in the florets of the 1x experiment, no parent compound was detected. The residue level in the florets of the 1x experiment was low. Only one very minor and one major compound were detected. The main compound was identified as 6-CPA-glucoside which was formed by cleavage and subsequent conjugation. Thus, due to the loss of the thiazolidine moiety, which includes the pharmacophor (active moiety), the main metabolite in florets does not exhibit insecticidal activity. In seeds, parent compound accounted for less than 8% of the TRR in both experiments. 6-CNA and its conjugate were detected as major compounds. Both metabolites were detected exclusively in the seeds.

The major metabolic reactions of thiacloprid in sunflower were:

- hydrolysis of the cyano group to form the respective amide,
- hydroxylation of the thiazolidine moiety,
- oxidative cleavage of the molecule at the methylene bridge and
- conjugation of the hydroxylated metabolites with glycosides.

Hydrolysis of the cyano group yielded YRC 2894-amide, the main metabolite in the intermediate sample. Hydroxylation of the thiazolidine moiety followed yielding 4-hydroxy-YRC 2894-amide. Hydroxylation of the thiazolidine moiety was also observed as first metabolic step yielding 4-hydroxy-YRC 2894. Both hydroxylated metabolites were conjugated with a hexose. These metabolic paths were both observed in the intermediate sample, whereas hydroxylation of YRC 2894-amide and following conjugation with a hexose was preferred in seeds. Oxidative cleavage of the molecule forming the label-specific 6-CPA was detected only indirectly. All three RACs under investigation showed label-specific metabolites. In the intermediate sample and in florets, 6-CPA-glucoside was identified. It was the main metabolite in florets (ca. 59% and 83% of the TRR), a minor metabolite in the intermediate sample, but not detected in seeds. However, 6-CNA – formed from 6-CPA by oxidation – and a corresponding conjugate were the main metabolites detected in seeds (ca. 6% to 12% of the TRR). It was considered that 6-CNA - as weak pyridine carboxylic acid - has a pronounced phloem mobility and was therefore transported selectively into the sunflower seeds as phloem sink.

Applying the total residue method, approx. 59% of the TRR in seeds was determined as 6-CNA due to the oxidative conversion step with potassium permanganate. Thus, the main part of the extracted TRR in seeds was characterized using this total residue method.

Report:	[redacted]ö;[redacted];[redacted]; 2014; M-494284-01-1
Title:	The metabolism of [thiazolidine-2-14C] thiacloprid in potato
Report No.:	MEYRN033
Document No.:	M-494284-01-1
Guidelines:	**OECD Guideline for the Testing of Chemicals No. 501: Metabolism in Crops European Parliament and Council Regulation (EC) No 1107/2009 and Commission Regulation (EU) No 283/2013 US EPA OCSPP 860.1300 - Nature of the Residue - Plants, Livestock PMRA DACO 6.3, Metabolism/Toxicokinetics Studies, Plants; not applicable**
GLP/GEP:	**yes**

Executive Summary

The metabolism of thiacloprid was investigated in potato following three foliar applications of [thiazolidine-2-^{14}C] thiacloprid formulated as an OD 240 at a rate of 106 g a.s./ha to 107 g a.s./ha. The application rate was 1.1 times (total application rate of 320 g a.s./ha) the proposed maximum seasonal application rate of 288 g a.s./ha for European uses.

Potato tubers were harvested at maturity (BBCH growth stage 85 to 95). To obtain supplementary information on the metabolic behaviour of thiacloprid in potatoes, potato early vines (BBCH growth stage 71 to 75) and vines samples (BBCH growth stage 85 to 95) were also collected. After application of [thiazolidine-2-^{14}C] thiacloprid to the potato plants, the total radioactive residues (TRRs) for potato tubers, early vines, and vines samples were 0.057 mg/kg, 5.637 mg/kg, and 1.119 mg/kg, respectively.

The potato tubers, early vines, and vines were extracted using acetonitrile (ACN)/H_2O (4:1). Extractability from the tubers, early vines, and vines was 66% of the TRR (0.038 mg/kg), 90% of the TRR (5.054 mg/kg), and 86% of the TRR (0.961 mg/kg), respectively, using ACN/H_2O (4:1). Reflux of the extracted tuber solids with 2 N HCl and with 2 N NaOH released 31% (0.018 mg/kg) and 41% of the TRR (0.023 mg/kg), respectively.

Identification of metabolites was performed by mass spectrometry after isolation and purification of residues. Parent thiacloprid (3% of the TRR; 0.002 mg/kg) and YRC 2894 amide (5% of the TRR; 0.003 mg/kg) were identified in low concentrations in the potato tubers. Parent thiacloprid was also identified in the early vines sample (32% of the TRR; 1.852 mg/kg).

The major residue (53% of the TRR; 0.030 mg/kg) found in the potato tubers ACN/H_2O (4:1) extract was a polar component which was characterised using various techniques. No significant amount of the polar residue shifted in retention time when analysed using a Hypercarb column with four different mobile phase systems. There was also no change in the retention time of any residue component during attempted derivatisation with acetic anhydride. Shaking of the acidified and basified polar residue with methylene chloride did not result in any significant portion of the residue partitioning into the organic phase. There were no apparent changes to any component of the polar residue when subjected to β-glucosidase, protease, cellulase, α-amylase, and amyloglucosidase enzyme hydrolysis conditions. The polar residue did not match the standards [^{14}C] D-glucose, [^{14}C] urea, [^{14}C] potassium cyanate, and [^{14}C] potassium thiocyanate when analysed by thin layer chromatography (TLC). Analysis of the polar residue using a 3 K molecular weight cut off protein concentrator indicated that the residue components had a molecular weight below 3000 Da.

No significant amount of residue could be partitioned from the 2 N HCl or the 2 N NaOH reflux extracts of potato tuber ACN/water (4:1) extracted solids by using methylene chloride. HPLC analysis of the neutralised 2 N HCl reflux extract showed primarily the polar residue.

The analytical work, including extraction, chromatographic analysis for metabolic profiling, and identification of metabolites was completed within approximately 14 months following application. The interval between potato tuber sample collection and analysis of the extract by HPLC was 25 days.

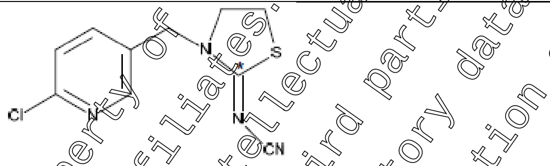
Therefore, no additional storage investigations were necessary. For potato early vines and vines sampled at harvest, the interval between sample collection and analysis of the extract by HPLC was 13 and 147 days, respectively.

Parent thiacloprid and YRC 2894 amide was identified in the potato tubers as well as in the early vines, and this is consistent with what was observed in previous metabolism studies on cotton, tomato (parent compound only), apple, spring wheat, and sunflower. The polar residue found in the potato matrices is apparently comprised of a single or multiple small molecules and is very likely not bioavailable.

I. Material and Methods

A. Materials

1. Test Material

Chemical structure		* position of the radiolabel
IUPAC name	{(2Z)-3-[(6-chloro-3-pyridinyl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide	
CAS name	Cyanamide, [3-[(6-chloro-3-pyridinyl)methyl-]-2-thiazolidinylidene]	
CAS number	111988-49-9	
Formula	$C_{10}H_9ClN_4S$	
Molecular weight	252.7 g/mol	
Radiolabelled test material	[thiazolidine-2-^{14}C]thiacloprid	
Specific radioactivity	4.12 MBq/mg (111 µCi/mg)	
Chemical Purity	99%	
Radiochemical purity	100%	

B. Study Design

Test site and crop information:

A rectangular-shaped, metal tub (surface area 1.9 m^2) was prepared by placing approximately 8 cm of gravel in the bottom and filling with sandy loam. Potato seed pieces (Red Norland 11-H-04; Gurney Seed Company; Greendale, Indiana; were planted into the tub on May 31, 2013. The tub was located in an outdoor area at the [redacted] in [redacted], NC, USA. The potato plants were fertilised, watered, and treated with maintenance chemicals as necessary to maintain healthy plant growth.

The potato early vines sample was harvested on August 7, 2013 which was after application two and prior to application three and when the potato plants were 30 to 46 cm tall and at BBCH growth stage 71 to 75. Two to three sprigs of leaves/stems were cut from each plant to make up the early vines sample. The sample was placed into a labelled plastic bag.

The potato tubers and vines samples were harvested on August 23, 2013 which was 14 days after application three and when the potato plants were 30 to 46 cm tall and at BBCH growth stage 85 to 95. Plants were cut at soil level. The vines sample was placed into a labelled plastic bag. Tubers were dug from the soil, and adhering soil was brushed off. The tubers sample was placed into a labelled plastic bag. All samples were placed into frozen storage following collection.

Formulation and application:

Three batches of [thiazolidine-2-^{14}C] thiacloprid were prepared as OD 240 formulations. These three formulated batches were used to prepare the spray solutions for the treatment of the potatoes.

To prepare a spray solution for a treatment, a batch of the formulated [thiazolidine-2-^{14}C] thiacloprid was mixed and then transferred to a 100-mL volumetric flask. The vial that contained the formulated material was rinsed four times with 2 to 3 mL of water, and each rinse was transferred to the volumetric flask. The volume of the solution in the volumetric flask was adjusted to 100 mL with water, and the solution was mixed to create the spray solution. The spray solution was radioassayed and aliquots were analysed by HPLC.

Just prior to each treatment, a plastic tent was constructed over the tub to prevent overspray during application. The spray solution was transferred from the 100-mL volumetric flask to a plastic spray bottle (946 mL for treatment one; 651 mL for treatments two and three). The volumetric flask was rinsed using a 10-mL portion of water, and the rinseate was transferred to the plastic spray bottle. The plants were sprayed as uniformly as possible. Midway through the application, approximately 0.1 ml of the spray solution analysed by HPLC. The plastic tent was removed from the tub shortly after each application. The potato plants were treated with the [thiazolidine-2-^{14}C] thiacloprid spray solutions on July 26, 2013, August 2, 2013, and August 9, 2013.

Following treatment, the 100-mL volumetric flask, spray bottle, and vial that contained the formulated [thiazolidine-2-^{14}C] thiacloprid were each rinsed three times with 2- to 3-mL portions of water, and the rinses were radioassayed.

C. Identification and Characterisation of Residues

Sample handling and preparation

Crop processing:

The potato tuber samples were cut into small pieces just prior to homogenisation. The potato early vines, vines, and tuber samples were homogenised in dry ice, poured into plastic bags and stored open in the freezer to allow the dry ice to sublime. The plastic bags were sealed and the frozen samples were stored in the freezer except during subsampling for analysis (<-9°C). Ten aliquots (0.09 to 0.12 g) of each of the homogenised RACs were radioassayed.

Extraction of potato tubers:

An aliquot of the potato tuber sample was extracted by blending several times with ACN/H_2O (4:1). After each extraction step, the extracts and solids were separated by centrifugation and filtration. The extracts were combined and radioassayed. The combined extract was subjected to a clean-up step using a previously conditioned C-18 solid phase extraction (SPE) cartridge. The cartridge was eluted with ACN/H_2O (4:1). The effluent from loading and eluting the cartridges was collected and radioassayed. The effluent was concentrated, and the concentrate was diluted with water and ACN. The sample was radioassayed, and an aliquot was analysed by C-18 reverse phase HPLC.

Analysis of polar esidues in potato tubers:

The peak at a retention time of 4.47 min ("polar peak") was isolated by manual collection into a flask during elution from the C-18 reverse phase HPLC analysis. The collected "polar peak" sample was further purified by HPLC by injection onto a Hypercarb column and collection of the peak during elution.

Aliquots of the "polar peak" sample were analysed by HPLC using a Hypercarb column and with 4 different mobile phases:

- 100 mmol KH_2PO_4 in water (solvent A) and 100 mmol KH_2PO_4 in ACN/water (solvent B);
- 5 mM NH_4OAc in 2% ACN/98% water (solvent A) and 5 mM NH_4OAc in 80% ACN/20% water (solvent B);
- Water (solvent A) and ACN (solvent B).
- 0.1% formic acid in water (solvent A) and 0.1% formic acid in ACN (solvent B);

Analysis of polar residues in potato tubers by derivatisation:
An aliquot of the "polar peak" sample was suspended in 1 mL of pyridine. To the suspension was added 5 mg of 4-dimethylaminopyridine (DMAP) and 100 µL of acetic anhydride. The suspension was stirred at room temperature for 48 h. Following concentration, the sample was dissolved in 800 µL of water and analysed by HPLC.

Analysis of polar residues in potato tubers by solvent partitioning with methylene chloride:
An aliquot of the "polar peak" sample was dissolved in 1 mL of 1 N HCl, and the solution was partitioned by shaking with $CHCl_2$. The organic phase was concentrated to dryness. A 5-mL portion of scintillation fluid was added to the concentrate, and the sample was radioassayed. An aliquot of the "polar peak" sample was dissolved in 1 mL of 30% aqueous NH_3, and the solution was partitioned by shaking with $CHCl_2$. The organic phase was concentrated to dryness. A 5-mL portion of scintillation fluid was added to the concentrate, and the sample was radioassayed.

Analysis of polar residues in potato tubers by enzymatic hydrolysis:
The "polar peak" sample was analysed using the following enzyme preparations in buffer solutions:

- β-glucosidase from almonds;
- Protease from *Streptomyces griseus*;
- Cellulase from *Aspergillus niger*;
- α-Amylase from *Bacillus licheniformis*;
- Amyloglucosidase from *Aspergillus niger*.

The samples were incubated at 37°C for ≥6 hours and subsequently analysed by HPLC. In parallel to the enzyme hydrolysis preparation, a blank assay (same preparation, but without enzyme) was conducted.

Analysis of polar residues in potato tubers by TLC:
Aliquots of the "polar peak" sample were analysed using thin-layer chromatography (TLC) along with the standards [^{14}C] D-glucose, [^{14}C] urea, [^{14}C] potassium cyanate, and [^{14}C] potassium thiocyanate.

Analysis of polar residues in potato tubers using a 3 K MWCO protein concentrator:
An aliquot of the "polar peak" sample was transferred into a 0.5 mL 3 K molecular weight cut off (MWCO) protein concentrator, and the concentrator was centrifuged at 12500 x g for 15 min. The retentate (fraction not passing through the membrane) and eluent (fraction passing through the membrane) fractions were radioassayed.

Extracted solids from potato tubers:
The ACN/H_2O (4:1) extracted tuber solids were refluxed for 8 hours with 2 N HCl. The extract was filtered, radioassayed, and partitioned with $CHCl_2$. The organic phase was radioassayed. The acid extract was neutralised using 2 N NaOH, and partitioned with $CHCl_2$. The neutralised extract and methylene chloride phase were radioassayed. The neutralised extract was concentrated, radioassayed and an aliquot was analysed by C-18 reverse phase HPLC.
The ACN/H_2O (4:1) extracted tuber solids were refluxed for 6 hours with 2 N NaOH. The extract was filtered, radioassayed, and partitioned with $CHCl_2$. The organic phase was radioassayed. The acid extract was neutralised using 37% HCl, and partitioned with methylene chloride. The neutralised extract and methylene chloride phase were radioassayed. The neutralised extract was concentrated and radioassayed. Attempt was made at analysis of an aliquot of the neutralised extract by C-18 reverse phase HPLC, but failed due to the viscosity of the sample.

Extraction of potato early vines:
An aliquot of the early vines sample was extracted by blending several times with ACN/H_2O (4:1). After each extraction step, the extracts and solids were separated by filtration. The extracts were combined and radioassayed. The combined extract was subjected to a clean-up step using a previously conditioned C-18 SPE cartridge. The cartridge was eluted with ACN/H_2O (4:1). The effluent from loading and eluting the cartridge was collected and radioassayed. The cartridge was further eluted with ACN, and the collected effluent was radioassayed. The effluents were concentrated separately, and the concentrates were each diluted with water and ACN. The samples were radioassayed, and aliquots were analysed by C-18 reverse phase HPLC.

Extraction of potato vines:
An aliquot of the vines sample was extracted by blending several times with ACN/H_2O (4:1). After each extraction step, the extracts and solids were separated by centrifugation. The extracts were combined and radioassayed. The combined extract was subjected to a clean-up step using a previously conditioned C-18 SPE cartridge. The cartridge was eluted with ACN/H_2O (4:1), and the effluent from loading and eluting the cartridges was collected and radioassayed. The effluent was concentrated and diluted with water and ACN. The sample was radioassayed, and an aliquot was analysed by C-18 reverse phase HPLC. The peak at a retention time of 4.05 min ("polar peak") was isolated by manual collection into a flask during elution from the C-18 reverse phase HPLC analysis. The collected sample was further analysed by HPLC using a Hypercarb column.

D. Analytical Methodology

Radioactivity measurement:
The radioactivity measurement in the liquid samples was carried out by liquid scintillation counting (LSC). All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail prior to radioactivity determination by LSC.

Identification and characterisation:
The quantification and purification of metabolites were carried out by HPLC and TLC. Aliquots of extracts from the potato tubers were injected onto C-18 reverse phase HPLC, and samples for mass spectral identification were isolated by manually collecting individual peaks into separate flasks. These individual samples were further purified as needed by HPLC. The purified radioactive residues were analysed by LC/MS/MS. Details of the chromatographic and spectroscopic conditions are described in the report.

II. Results and Discussion

A. Storage stability

Stability of residues in crops:

RACs and their extracts were stored frozen (<-5°C) except during handling. The potato tuber RAC was initially processed and analysed within 25 days of harvest. The potato early vines and vines RACs were initially processed and analysed within13 and 147 days of harvest, respectively. According to OECD test Guideline 501 (Metabolism in Crops) storage stability data are not normally required for samples analysed within 6 months of collection.

Stability of spray solutions:

The [thiazolidine-2-^{14}C] thiacloprid spray solutions were 100% radiochemically pure. The treating solutions were stable from the time of preparation through completion of the application procedures, i.e. no decomposition was observed.

B. Identification, Characterisation, and Distribution of Residues

Treatment rate:

The treatment rates for the three applications of [thiazolidine-2-^{14}C] thiacloprid ranged from 106 g a.s./ha to 107 g a.s./ha which was 1.1 times (total application rate of 320 g a.s./ha) the proposed maximum seasonal application rate of 288 g a.s./ha for European uses.

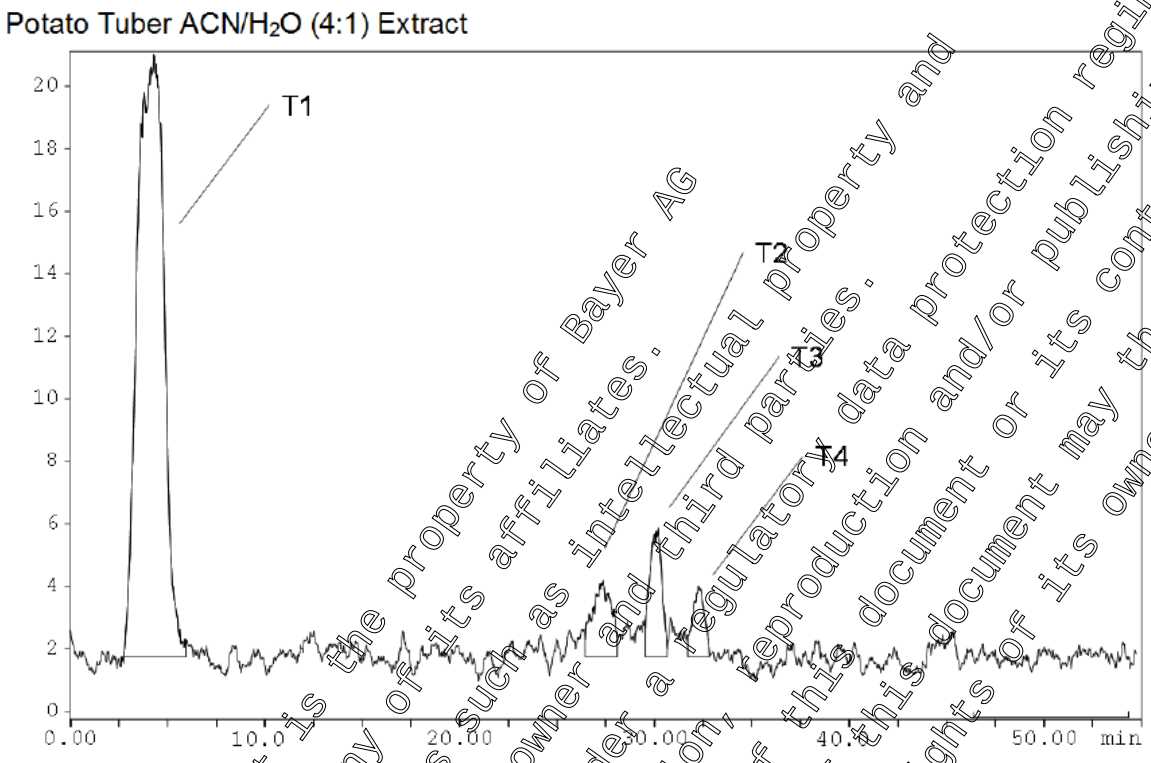
Residue levels in potato matrices:

After application of [thiazolidine-2-^{14}C] thiacloprid to the potato plants at 1.1 times the maximum seasonal rate, the TRRs for potato tubers, early vines, and vines samples were 0.057 mg/kg, 5.637 mg/kg, and 1.119 mg/kg respectively.

Extraction and characterisation of residues in potato tubers:

From potato tubers, 66% of the TRR (0.038 mg/kg) was extracted using ACN/H_2O (4:1). The HPLC profile (Figure 6.2.1-4) of the ACN/H_2O (4:1) extract showed four peaks (T1 to T4). The highest residue component in the tubers extract was peak T1 which accounted for 52% of the TRR (0.037 mg/kg). this peak at an HPLC retention time of 4.47 min ("polar peak") was analysed and characterised by various techniques as described in detail below.

Figure 6.2.1-4: HPLC of the potato tuber ACN/H_2O (4:1) extract from the [thiazolidine-2-^{14}C] thiacloprid potato metabolism study.



The "polar peak" (T1) tuber residues were characterised by HPLC using a Hypercarb column with four different mobile phase systems.

The HPLC profile of the "polar peak" (T1) tuber residues analysed by Hypercarb column using 100 mmol KH_2PO_4 in water as solvent A and 100 mmol KH_2PO_4 in ACN/water as solvent B showed six peaks. The highest residue in the profile was the most polar component (27% of the TRR; 0.015 mg/kg), with a retention time of 3.28 min.

The HPLC profile of the "polar peak" (T1) tuber residues analysed by Hypercarb column using 5 mM NH_4OAc in 2% ACN/98% water as solvent A and 5 mM NH_4OAc in 80% ACN/20% water as solvent B showed four peaks. The highest residue in the profile was again the most polar component (31% of the TRR; 0.018 mg/kg), with a retention time of 3.32 min.

The HPLC profile of the "polar peak" (T1) tuber residues analysed by Hypercarb column using water as solvent A and ACN as solvent B showed three peaks. The highest residue in the profile was the most polar component, (36% of the TRR; 0.020 mg/kg), with a retention time of 3.03 min.

The HPLC profile of the "polar peak" (T1) tuber residues analysed by Hypercarb column using 0.1% formic acid in water as solvent A and 0.1% formic acid in ACN as solvent B showed four peaks. The highest residue in the profile was the most polar component (35% of the TRR; 0.020 mg/kg), with a retention time of 2.92 min.

In conclusion, no significant portion of the polar residue shifted in retention time under the various Hypercarb HPLC conditions employed.

Characterisation of polar residues in potato tubers by derivatisation:

The treatment of the "polar peak" (T1) tuber residues with acetic anhydride and DMAP did not cause a change in the retention time of any component of the residue due to derivatisation.

Characterisation of polar residues in potato tubers by solvent partitioning with methylene chloride:

Under acidic and basic conditions, no significant amount of “polar peak” (T1) tuber residues could be partitioned using methylene chloride, i.e. <3% and <1% of the radioactivity partitioned under acidic and basic conditions, respectively.

Characterisation of polar residues in potato tubers by enzymatic hydrolysis:

The “polar peak” (T1) tuber residues were subjected to enzymatic hydrolysis using β-glucosidase, protease, cellulase, α-amylase, and amyloglucosidase. Under the enzyme hydrolysis conditions employed, there were no changes to the retention times of any component of the polar residue.

Characterisation of polar residues in potato tubers by thin-layer chromatography:

In order to prove whether the radioactivity of the polar fraction was due to incorporation into low molecular weight natural constituents of the tubers, the “polar peak” (T1) residues were co-chromatographed using TLC with the four standards [^{14}C] D-glucose, [^{14}C] urea, [^{14}C] potassium cyanate, and [^{14}C] potassium thiocyanate. The TLC elution of the “polar peak” residues did not match any of the four standards.

Characterisation of polar residues in potato tubers by 3 KDa MWCO protein concentrator:

The residue concentrations in the eluent and retentate fractions were similar (16 dpm/µL and 20 dpm/µL, respectively), indicating that the molecular weight of the “polar peak” (T1) residue was less than the cut off of 3000 Da.

Characterisation of extracted potato tuber solids:

From ACN/H_2O (4:1) extracted potato tuber solids, 31% of the TRR (0.018 mg/kg) was extracted by refluxing with 2 N HCl. Methylene chloride partitioning of the 2 N HCl extract did not result in any significant amounts of residue in the organic phase (2% of the TRR; 0.001 mg/kg). Furthermore, no significant amount of residues could be partitioned from the neutralised 2 N HCl extract using methylene chloride (5% of the TRR; 0.003 mg/kg).
By refluxing with 2 N NaOH, 41% of the TRR (0.023 mg/kg) was extracted from the ACN/H_2O (4:1) extracted potato tuber solids. Partitioning of the 2 N NaOH extract with methylene chloride did not result in any significant amounts of residue in the organic phase (1% of the TRR; 0.001 mg/kg). Additionally, no significant amount of residues could be partitioned from the neutralised 2 N NaOH extract using methylene chloride (<1% of the TRR; <0.001 mg/kg).

Extraction of residues in potato early vines:

From potato early vines, 90% of the TRR (5.054 mg/kg) was extracted while from potato vines, 86% of the TRR (0.961 mg/kg) was extracted. In both cases, a mixture of ACN/H_2O (4:1) was used.

Identification of residues in potato matrices:

Identification of the residues was accomplished by comparison of the mass spectral data to that of authentic reference standards. The mass spectra of the identified residues and reference standards were obtained in the positive ion mode.

Thiacloprid:

Peak T4 (3% of the TRR, 0.002 mg/kg), which was isolated from the ACN/H_2O (4:1) extract of potato tubers (Figure 6.2.1-4), was identified as thiacloprid (MW = 253). The LC/MS chromatogram of T4 showed a peak with a retention time of 4.58 min when filtering in an m/z range of 253.02892 to 253.03292. The retention time of T4 was very similar to that of the non-radioactive thiacloprid standard, which had a retention time of 4.60 min when filtered in the same m/z range. Additionally, the HPLC retention time of peak T4 (Rt = 32.25 min; Figure 6.2.1-4) was very similar to that of the [thiazolidine-2-^{14}C] thiacloprid standard (Rt = 32.27).

Approximately one third of the extracted radioactivity from early vines (32% of the TRR, 1.852 mg/kg) were tentatively identified as thiacloprid. The HPLC retention time (Rt = 16.73 min) of the corresponding peaks in the SPE-purified extracts was similar to that of the [thiazolidine-2-^{14}C] thiacloprid standard.

YRC 2894 Amide:

Peak T3 (5% of the TRR, 0.003 mg/kg;), which was isolated from the ACN/H_2O (4:1) extract of potato tubers (Figure 6.2.1-4), was identified as YRC 2894 amide (MW = 271). The LC/MS chromatogram of T3 showed a peak with a retention time of 3.58 min when filtering in an m/z range of 271.03949 to 271.04349. The retention time of T3 was very similar to that of the non-radioactive YRC 2894 amide standard, which had a retention time of 3.57 min when filtered in the same m/z range. Additionally, the HPLC retention time of peak T3 (Rt = 30.08 min; Figure 6.2.1-4) was similar to that of the [methylene-^{14}C] YRC 2894 amide standard (Rt = 30.15 min).

C. Conclusion

Following the treatment of potato plants with [thiazolidine-2-^{14}C] thiacloprid at a total rate of 320 g a.s./ha (1.1 times the proposed maximum seasonal field application rate of 288 g a.s./ha), the total radioactive residue observed in the potato tubers, early vines, and vines samples were 0.057 mg/kg, 5.637 mg/kg, and 1.119 mg/kg, respectively.

Extractability from the potato tubers, early vines, and vines was 66% of the TRR (0.038 mg/kg), 90% of the TRR (5.054 mg/kg), and 86% of the TRR (0.961 mg/kg), respectively, using ACN/H_2O (4:1). Reflux of the extracted tuber solids with 2 N HCl and 2 N NaOH released 31% (0.018 mg/kg) and 41% of the TRR (0.023 mg/kg), respectively. In the potato tubers, parent thiacloprid (3% of the TRR; 0.002 mg/kg) and YRC 2894 amide (5% of the TRR; 0.003 mg/kg) were identified in low concentrations. Parent thiacloprid was also identified in the early vines sample (32% of the TRR; 1.852 mg/kg). The total identification rate of residues from the potato tuber and early vines was 8% (0.005 mg/kg) and 32% (1.852 mg/kg) of the TRR, respectively. The total characterisation of residues in the potato tuber, early vines, and vines was 97% (0.056 mg/kg), 54% (3.113 mg/kg), and 85% (0.962 mg/kg) of the TRR, respectively.

The identification of parent thiacloprid and YRC 2894 amide in the potato tubers as well as in the early vines is consistent with what was observed in previous metabolism studies on cotton, tomato (only parent compound), apple, spring wheat, and sunflower. The major part of the radioactive residue found in the potato matrices is highly polar in nature. Several attempts were made to further characterise this residue. In particular, it was shown that it was not possible to derivatise any possibly existing hydroxyl groups of the residue with acetic anhydride, nor was it possible to hydrolyse it under alkaline or acidic conditions. Also the treatment with various enzymes had no effect. Co-chromatography with radioactive standards revealed that the radioactivity applied with [thiazolidine-2-^{14}C] thiacloprid was not incorporated into small endogenous molecules like glucose, urea, cyanate or thiocyanante. A molecular size exclusion experiment showed that the molecular weight of this fraction is below 3 kDaltons.

In consideration of this information the assumption seems to be justified that the polar residue found in potato matrices is apparently comprised of one or several small molecules and is not likely to be bioavailable. It should also be kept in mind that the total radioactivity concentration in tubers, which is the only relevant commodity in terms of human consumption, is rather low (0.057 mg/kg), even at the exaggerated application rate.

II. Summary of Plant Metabolism

The results of the thiacloprid metabolism studies on wheat, sunflower and potato as well as the other plant metabolism studies submitted with the baseline dossier for the relevant raw agricultural commodities are summarised in Table 6.2.1-4, the proposed metabolic pathway covering all investigated crops including the rotational crop study is shown in Figure 6.2.1-5.
In the baseline dossier, crop metabolism studies on tomatoes, apples and cotton following spray application and the rotational crop study have been presented. In this dossier, metabolism studies on spring wheat after spray application and on sunflower following seed dressing are described. In these studies the [methylene-^{14}C]-label was employed. An additional study on potatoes after spray application of [thiazolidine-2-^{14}C]-labelled thiacloprid is also presented in this dossier. For thiacloprid, metabolism studies for 6 crops from 4 categories (fruit, pulses and oilseeds, cereals/grass crops and root crops) are now available. The results show that the route of degradation is similar in all four categories independent of the application route. The unchanged parent compound is the major component of the residue in all crop groups. In commodities relevant for human consumption, no metabolite appears in quantities above 12 % of the radioactive residue. The high level of recovery and characterisation achieved in the big majority of the studies strongly supports the existing residue definition as parent compound only for MRL enforcement as well as for dietary risk assessment. The presence of high amounts of 6-chloronicotinic acid in cotton seed is due to the accumulation of this weak pyridine carboxylic acid in the seed as a phloem sink after being secreted from the apoplasm into the phloem as a sink compartment for weak acids. It is very likely that the 6-chloronicotinic acid in the seed originated from cotton leaf metabolism where it was identified as one of the metabolites. But it should also be noted that the concentration of the total radioactive residue in cotton seed and especially in sunflower seed and potatoes is rather low. The same metabolic profile was also observed in the rotational crop study which was submitted with the baseline dossier. Furthermore, all main metabolites identified in plants were also detected in the rat metabolism studies described in the baseline dossier ([redacted], O. and [redacted], W.; M-001080-01-1; (1998); [redacted], H. and [redacted], W.; M-000847-03-1; (1998)).

On the basis of these studies, the metabolic pathway of thiacloprid follows these main metabolic degradation routes:

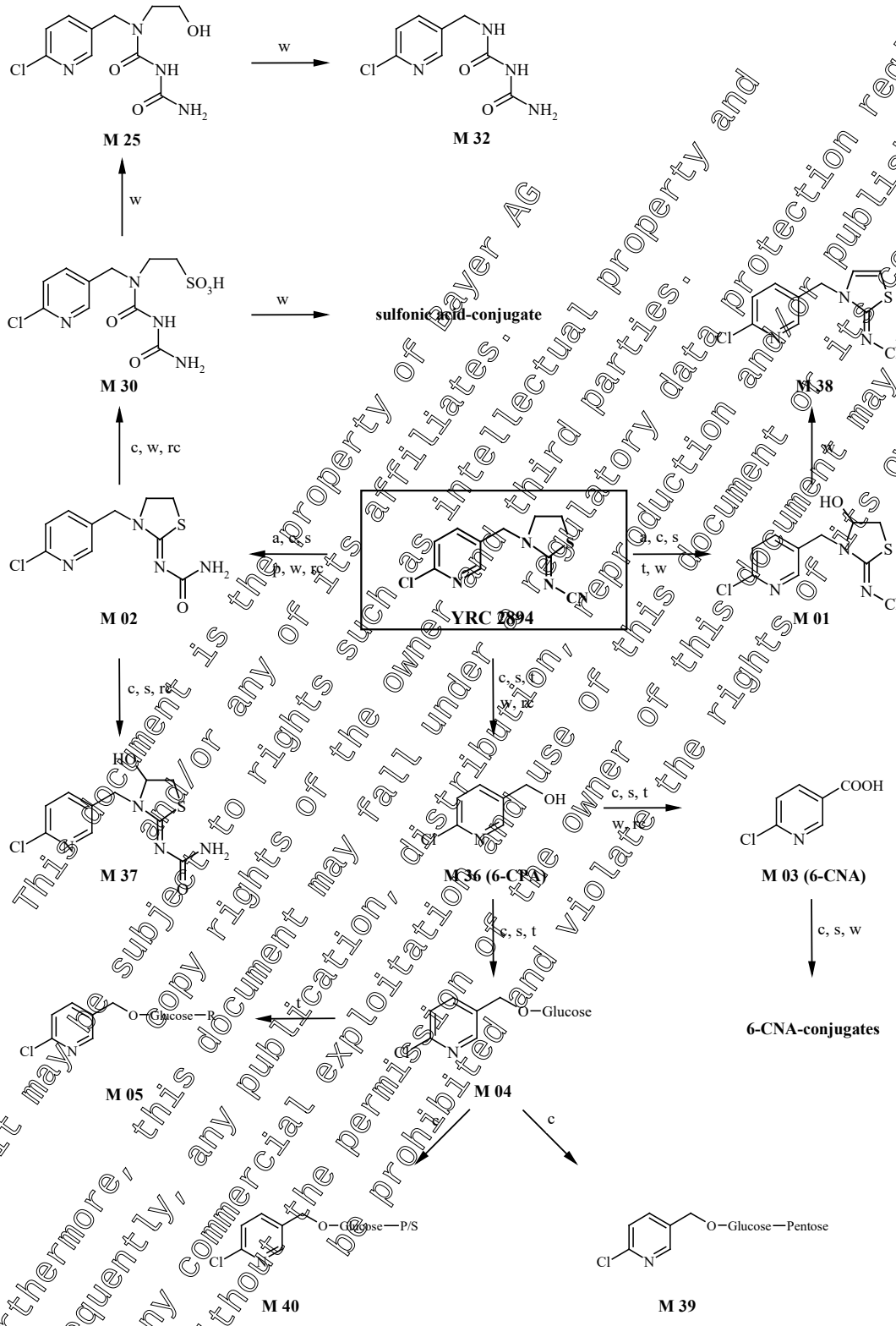
- Hydrolysis of the cyano group of the parent compound yielding YRC 2894-amide, followed by hydroxylation of the thiazolidine moiety and subsequent conjugation with a hexose moiety
- Direct hydroxylation of the thiazolidine moiety of the parent compound and subsequent conjugation with a hexose moiety
- Oxidative cleavage of the molecule leading to 6-chloropicolyl alcohol (6-CPA) and subsequent conjugation with glucose or alternatively
- further oxidation of the alcohol to 6-chloronicotinic acid (6-CNA) and subsequent conjugation with an unidentified endocon

Based on these results the residue definition for plants is proposed as parent compound only.

Table 6.2.1- 4: Distribution of active substance and metabolites (% of total radioactive residue) in different crops following application of [methylene-^{14}C]- or [thiazolidine-2-^{14}C]thiacloprid.

Labelling position		[methylene-^{14}C]									[thiazolidine-2-^{14}C]	
Application type		Spray application							Seed treatment		Spray	
Crop		Apple	Tomato	Cotton		Wheat			Sunflower		Potato	
Crop part		Fruit	Fruit	Leaves	Seed	Hay	Straw	Grain	Interm..	Seed	Leaves	Tuber
Application rate [g as/ha]		2 x 150	2 x 375	3 x ca. 210		2 x ca. 50			80		3 x 10^{2}	
Days after last application		14	14	120		7 (1st app.)	21	36	138		5 (2nd app.)	14
TRR [mg/kg]		0.74	0.94	30.35	1.12	2.04	12.36	0.21	0.79	0.04	5.64	0.66
Thiacloprid		90.8	94.4	83.9	0.6	81.4	83.4	80.9	42.8	7.2	32.0	3.0
4-OH-YRC 2894	M 01	2.2	0.4	0.8		1.6	1.9	0.7	1.5			
4-OH-YRC 2894 glycoside									2.8			
YRC 2894-amide	M 02	1.3				0.2	0.3		33.2	6.0		5.0
4-OH YRC 2894 amide	M 37			1.2					5.2			
4-OH YRC 2894 amide glycoside									3.0			
YRC 2894 olefine	M 38					0.4	0.3					
YRC 2894 diamide	M 32					0.5	0.4					
YRC 2894 hydroxyethyl diamide	M 25					0.1	0.1					
YRC 2894 sulfonic acid	M 30			0.3		1.2	1.0					
YRC 2894 sulfonic acid conjugate						0.4	0.3					
6-CNA	M 03		0.2	1.1	45.8	1.2	2.2			10.4		
6-CNA conjugate						1.7	1.1	1.7		11.9		
6-CNA-complex glucoside					29.7							
6-CPA	M 36			0.5		0.4	0.3					
6-CPA-glucoside	M 04		0.3	1.2					2.1			
6-CPA-complex glucoside	M 05			1.8	0.3							
6-CPA glycosyl pentoside	M 39			2.2								
6-CPA glycosyl phosphate/sulfate	M 40			1.4								

Figure 6.2.1-5: Proposed metabolic pathway of thiacloprid in plants.



a: apple c: cotton s: sunflower
t: tomato p: potato w: wheat
rc: rotational crop

CA 6.2.2 Poultry

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of thiacloprid.

CA 6.2.3 Lactating ruminants

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of thiacloprid.

CA 6.2.4 Pigs

Not required, the metabolism is considered similar in rats, lactating ruminant and poultry.

CA 6.2.5 Fish

At present, there are no guidance documents published in form of an update of the Commission Communications 2013/C 95/01 to fulfil the data requirement as laid down in Commission Regulation (EU) No 283/2013 of 1 March 2013. It is stated in the *"Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to regulation (EU) no 283/2013 and regulation (EU) No 284/2013"* (SANCO/10181/2013 - rev. 2.1, 13 May 2013) that in cases where agreed test methods or guidance documents are not yet available for particular data requirements, waiving of these particular data requirement points is considered acceptable. Therefore, Bayer Cropscience did not conduct a fish metabolism study. Bayer CropScience also believes that from a scientific point of view a fish metabolism study would not be very meaningful because due to the low log P_{ow} of 1.26 accumulation of parent compound and even more so of the metabolites formed in crops treated with thiacloprid in edible tissues of fish is highly unlikely. Also in consideration of this aspect the conduct of another vertebrate study was considered avoidable.

CA 6.3 Magnitude of residue trials in plants

CA 6.3.1 Oilseed rape

A summary of the residue data is presented below. The summary includes all trials reviewed during the last thiacloprid MRL EU review in the frame of Article 12 of Reg. 396/2005 (EFSA, 2014. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for thiacloprid according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2014;12(3):3617).

Crop	Region	EU critical GAP	Residues (mg/kg)	n	STMR (mg/kg)	HR (mg/kg)
Thiacloprid						
Rape	North	1x72 g/ha, PHI 30d	<0.02; 0.05; 0.05; 0.05; 0.07; 0.07; 0.08; 0.16	8	0.06	0.16
Rape	South	1x74 g/ha, PHI 45d	0.03; 0.04; 0.04; 0.09; 0.30	5	0.04	0.30

The intended GAP for the AIR dossier is the following for both European regions:

2 x 72 g a.s /ha, last application at BBCH 59, before flowering, interval of 10 days between applications

New data for AIR:

New supplementary data were generated in order to support the representative use on oil seed rape supported in this dossier.

The following studies were not evaluated during the last EU review and are submitted for review:

Northern European GAP

Report:	KCA 6.3.1/21 [redacted]; 2013; M-454920-01-1
Title:	Determination of the residues of thiacloprid in/on rape after spray application of thiacloprid OD 240 in Germany, the Netherlands and Belgium
Report No.:	01.12.2074
Document No.:	M-454920-01-1
Guidelines:	**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** **EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)** **OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial** **US EPA OCSPP Guideline No. 860.1500; not specified**
GLP/GEP:	**yes**

Report:	KCA 6.3.1/22 [redacted],; [redacted]; 2014; M-492626-01-1
Title:	Determination of the residues of thiacloprid in/on rape after spray application of thiacloprid OD 240 in United Kingdom, Germany, Belgium and the Netherlands
Report No.:	13-2096
Document No.:	M-492626-01-1
Guidelines:	**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** **EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)** **OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial** **US EPA OCSPP Guideline No. 860.1500; not applicable**
GLP/GEP:	**yes**

Material and Methods:

Nine trials were conducted in 2012 and in 2013 on rape. The intended use consisted of 2 applications of Biscaya 240 OD (240 g/L of OD formulation, an oil based dispersion concentrate formulation) at a dose rate of 72 g a.s./ha, with an interval varying from 6 to 10 days and the last application not later than BBCH 59, before flowering. The limitation of the period of application is deemed to minimize the thiacloprid residue in honey. The field samples from the year 2012 were analysed according to the LC-MS/MS method 00548/M001 with a LOQ of 0.01 mg/kg in seeds and flowers and a LOQ of 0.05 mg/kg in green material, straw and rest of plant.

The field samples from the year 2013 were analysed with LC-MS/MS method 00548/M001 and also the LC-MS/MS method 01156 for the total residue of thiacloprid. Since these trials were analysed conjointly with the corn trials where thiacloprid was applied as seed treatment, the total residue of thiacloprid was also assessed with the common moiety method 01156. In the case of foliar application the residue levels expressed as total residue thiacloprid are not relevant. For clarity sake only the results of thiacloprid are presented here below.

Findings: The procedural recoveries determined from fortified samples analysed alongside with the treated samples were satisfactory, as shown in Table 6.3.1- 1.

Table 6.3.1- 1: Recovery data for Thiacloprid

Study Number	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
12-2074 M-454920-01-1	Rape	green material	thiacloprid	1	0.05	100	100	100	100	
				1	0.5	97	97	97	97	
				1	5	98	98	98	98	
				3	overall mg/kg		97	100	98	1.6
		flower	thiacloprid	1	0.01	118	118	118	118	
				1	0.1	95	95	95	95	
				1	1	96	96	96	96	
				3	overall mg/kg		95	118	103	12.6
		pod	thiacloprid	1	0.01	105	105	105	105	
				1	0.1	97	97	97	97	
				1	1	97	97	97	97	
				3	overall mg/kg		97	105	100	4.6
		rest of plant	thiacloprid	1	0.05	98	98	98	98	
				1	0.5	89	89	89	89	
				1	5	94	94	94	94	
				3	overall mg/kg		89	98	94	4.8
		seed	thiacloprid	1	0.01	97	97	97	97	
				1	0.1	90	90	90	90	
				1	1	89	89	89	89	
				3	overall mg/kg		89	97	92	4.7
		straw	thiacloprid	1	0.05	99	99	99	99	
				1	0.5	94	94	94	94	
				1	5	85	85	85	85	

Study Number	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
				3	overall mg/kg		85	99	93	7.7
13-2096 M-492626-01-1	Rape	green material	thiacloprid	2	0.05	76; 77	76	77	77	
				1	1	98	98	98	98	
				2	2	70; 110	70	110	90	
				5	overall mg/kg		70	110	86	19.7
		flower	thiacloprid	1	0.01	90	90	90	90	
				1	0.4	105	105	105	105	
				2	overall mg/kg		90	105	98	-
		pod	thiacloprid	1	0.01	88	88	88	88	
				1	0.4	75	75	75	75	
				2	overall mg/kg		75	88	82	-
		rest of plant	thiacloprid	1	0.05	88	88	88	88	
				1	2	85	85	85	85	
				2	overall mg/kg		85	88	87	-
		seed	thiacloprid	2	0.01	85; 91	85	91	88	
				1	0.1	82	82	82	82	
				1	0.4	78	78	78	78	
				4	overall mg/kg		78	91	84	6.5
		straw	thiacloprid	2	0.05	89; 94	89	94	92	
				1	0.5	90	90	90	90	
				1	2	82	82	82	82	
				4	overall mg/kg		82	94	89	5.6

The results of the method validation are in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

Storage periodfor samples:
The maximum storage period of deep-frozen samples was 351 days for the samples belonging to study 13-2096.

Residue results:
In the following table, the application information and the residues found in/on rape samples are summarised.

Table 6.3.1- 2: Results of residue trials conducted with Thiacloprid OD 240 on rape (240 g/L thiacloprid) in Northern Europe

Study Trial No. GLP Year Document No.	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	thiacloprid (mg/kg)
12-2074 12-2074-01 GLP: yes 2012 M-454920-01-1	Rape Vision; typical of region	Germany [redacted] Europe, North	240 OD	2	0.072	0.024	59	green material	0*	0.24
									0	1.8
								flower	22	0.036
								pod	40	<0.01
								rest of plant	40	<0.05
								seed	99	<0.01
								straw	99	<0.05
12-2074 12-2074-02 GLP: yes 2012 M-454920-01-1	Rape Visby; hybrid rapeseed	Germany [redacted] Europe, North	240 OD	2	0.072	0.024	59	green material	0	1.5
								seed	104	<0.01
								straw	104	<0.05
12-2074 12-2074-03 GLP: yes 2012 M-454920-01-1	Rape Haydn; summer variety	Netherlands [redacted] Europe, North	240 OD	2	0.072	0.024	59	green material	0*	0.16
									0	1.2
								flower	13	0.029
								pod	40	<0.01
								rest of plant	40	<0.05
								seed	69	<0.01
								straw	69	<0.05
12-2074 12-2074-04 GLP: yes 2012 M-454920-01-1	Rape DK Excellium; Medium-early hybrid restored	Belgium [redacted] Europe, North	240 OD	2	0.072	0.036	59	green material	0	1.2
								seed	111	<0.01
								straw	111	<0.05
12-2074 12-2074-05 GLP: yes 2012 M-454920-01-1	Rape Visby; winter variety	Netherlands [redacted] Europe, North	240 OD	2	0.072	0.024	59	green material	0*	0.16
									0	1.6
								flower	17	0.040
								pod	40	<0.01
								rest of plant	40	<0.05
								seed	104	<0.01
								straw	104	<0.05
13-2096 13-2096-01 GLP: yes 2013 M-492626-01-1	Rape D.K.Cabernet Open Pollenator	United Kingdom [redacted] Royston	240 OD	2	0.072	0.036	57	green material	0*	0.082
									0	1.4
								flower	31	0.012**
								pod	66	<0.01
								rest of plant	66	<0.05

Study Trial No. GLP Year Document No.	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	thiacloprid (mg/kg)
		Europe, North						seed	110	<0.01
								straw	110	<0.05
13-2096 13-2096-02 GLP: yes 2013 M-492626-01-1	Rape Visby rape-winter	Germany [redacted] Europe, North	240 OD	2	0.072	0.024	59	green material	0	1.4
								seed	105	<0.01
								straw	105	<0.05
13-2096 13-2096-03 GLP: yes 2013 M-492626-01-1	Rape DK exquisite Hybrid medium early	Belgium [redacted] Europe, North	240 OD	2	0.072	0.036	59	green material	0	1.1
								seed	93	<0.01
								straw	93	<0.05
13-2096 13-2096-04 GLP: yes 2013 M-492626-01-1	Rape Pauline summer	Netherlands 1774 PE Slootdorp Europe, North	240 OD	3	0.072	0.024	59	green material	0* 0	0.39 1.8**
								flower	6	0.030
								pod	41	<0.01
								rest of plant	41	<0.05
								seed	63	<0.01
								straw	63	<0.05

* prior to last treatment
** mean value of a double reanalysis on the reserve sample

As expected the residue levels found in seeds are below the LOQ of 0.01 mg/kg, at harvest even in the straw samples the residue levels are below the LOQ of 0.05 mg/kg.

After flowering, the flowers are collected and analysed for thiacloprid content. The residue levels vary in a range 0.012 mg/kg to 0.04 mg/kg. These limited amounts would lead to residue levels in honey below the LOQ of 0.01 mg/kg.

Southern European GAP

Report: KCA 6.3.1.23 [redacted]f; 2013; M-456175-01-1
Title: Determination of the residues of thiacloprid in/on rape after spray application of thiacloprid OD 240 in Italy, Spain and southern France
Report No.: 01.12.2075
Document No.: M-456175-01-1
Guidelines: **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**
EC Guidance working document 7029/VI/95 rev.5 (1997-07-22), OECD 509 Adopted 2009-09-07
OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial
US EPA OCSPP Guideline No. 860.1500; not specified
GLP/GLP: **yes**

Report: KCA 6.3.1/24[redacted],[redacted]; 2014; M-492017-01-1
Title: Determination of the residues of thiacloprid in/on rape after spray application of thiacloprid OD 240 in Italy, southern France and Spain
Report No.: 13-2097
Document No.: M-492017-01-1
Guidelines: **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**
EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)
OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial
US EPA OCSPP Guideline No. 860.1500; not applicable
GLP/GEP: **yes**

Material and Methods:

Six trials were conducted in 2012 and 3 trials in 2013. The intended use consisted of 1 spray application of Biscaya 240 OD (240 g/L of OD formulation, an oil based dispersion concentrate formulation) and 2 spray applications for the trials conducted in 2013, at a dose rate of 72 g a.s./ha, applied no later than BBCH 59, just before flowering. The limitation of the period of application is deemed to minimize the thiacloprid residue in honey. The field samples from the year 2012 were analysed according to the LC-MS/MS method 00548/M001 with a LOQ of 0.01 mg/kg in seeds and flowers and a LOQ of 0.05 mg/kg in green material, straw and rest of plant.
The field samples from the year 2013 were analysed according to the LC-MS/MS method 00548/M001 with a LOQ of 0.01 mg/kg in seeds and flowers and a LOQ of 0.05 mg/kg in green material, straw and rest of plant. Since these trials were analysed conjointly with the corn trials where thiacloprid was applied as seed treatment, the total residue of thiacloprid was also assessed with the common moiety method 01156. In the case of foliar application the residue levels expressed as total residue thiacloprid are not relevant. For clarity sake only the results of thiacloprid are presented here below.

Findings:

The procedural recoveries determined from fortified samples analysed alongside with the treated samples were satisfactory, as shown in Table 6.3.1- 3.

Table 6.3.1- 3: Recovery data for Thiacloprid

Study Number	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
12-2075 M-456175-01-1	Rape	green material	thiacloprid	1	0.05	95	95	95	95	
				1	0.5	98	98	98	98	
				1	1	96	96	96	96	
				1	5	92	92	92	92	
				4	overall mg/kg		92	98	95	2.6
		flower	thiacloprid	3	0.01	70;72;78	70	78	73	5.7
				3	0.1	73;80;85	73	85	79	7.6
				1	0.2	81	81	81	81	
				7	overall mg/kg		70	85	77	7.1

Study Number	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
		pod	thiacloprid	1	0.01	83	83	83	83	
				2	0.2	83;78	78	83	81	
				3	overall mg/kg		78	83	81	3.5
		rest of plant	thiacloprid	1	0.05	95	95	95	95	
				1	0.5	95	95	95	95	
				1	1	89	89	89	89	
				3	overall mg/kg		89	95	93	3.4
		seed	thiacloprid	1	0.01	72	72	72	72	
				2	0.2	78;68	68	78	73	
				3	overall mg/kg		68	78	73	6.9
		straw	thiacloprid	1	0.05	65	65	65	65	
				1	0.5	78	78	78	78	
				1	5	74	74	74	74	
				3	overall mg/kg		65	78	72	9.2
13-2097 M-492017-01-1	Rape	green material	thiacloprid	2	0.05	76; 77	76	77	77	
				1	1	98	98	98	98	
				2	2	70; 110	70	110	90	
				5	overall mg/kg		70	110	86	19.7
		flower	thiacloprid	1	0.01	90	90	90	90	
				1	0.4	105	105	105	105	
				2	overall mg/kg		90	105	98	-
		pod	thiacloprid	1	0.01	88	88	88	88	
				1	0.4	75	75	75	75	
				2	overall mg/kg		75	88	82	-
		rest of plant	thiacloprid	1	0.05	88	88	88	88	
				1	2	85	85	85	85	
				3	overall mg/kg		85	88	87	
		seed	thiacloprid	2	0.01	85; 91	85	91	88	
				1	0.1	82	82	82	82	
				1	0.4	78	78	78	78	
				4	overall mg/kg		78	91	84	6.5
		straw	thiacloprid	2	0.05	89; 94	89	94	92	
				1	0.5	90	90	90	90	
				1	2	82	82	82	82	
				4	overall mg/kg		82	94	89	5.6

The results of the method validation are in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

Storage period for samples:

The maximum storage period of deep-frozen samples was 321 days for the samples belonging to study 13-2097.

Residue results:

In the following table, the application information and the residues found in/on rape seed are summarised.

Table 6.3.1- 4: Results of residue trials conducted with Thiacloprid OD 240 on rape (240 g/L thiacloprid) in Southern Europe

Study Trial No. GLP Year Document No.	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	thiacloprid (mg/kg)
12-2075 12-2075-01 GLP: yes 2012 M-456175-01-1	Rape Anaconda	Italy I-[redacted] Europe, South	240 OD	1	0.072	0.024	59	green material	0	0.46
								flower	11	0.13
								pod	40	<0.01
								rest of plant	40	<0.05
								seed	72	<0.01
								straw	72	<0.05
12-2075 12-2075-02 GLP: yes 2012 M-456175-01-1	Rape Pacifik; winterrape	Spain [redacted] (Llerona) Europe, South	240 OD	1	0.072	0.024	59	green material	0	0.98
								seed	81	<0.01
								straw	81	<0.05
12-2075 12-2075-03 GLP: yes 2012 M-456175-01-1	Rape Hybrilo x; Winter rape	France [redacted] Europe, South	240 OD	1	0.072	0.024	59	green material	0	1.1
								flower	18	0.014
								pod	41	<0.01
								rest of plant	41	<0.05
								seed	85	<0.01
								straw	85	<0.05
12-2075 12-2075-04 GLP: yes 2012 M-456175-01-1	Rape Avenir; Winter rape	Italy [redacted] (Bologna) Europe, South	240 OD	1	0.072	0.024	59	green material	0	0.61
								flower	13	0.026
								pod	38	<0.01
								rest of plant	38	<0.05
								seed	83	<0.01
								straw	83	<0.05
12-2075 12-2075-05 GLP: yes 2012 M-456175-01-1	Rape ES-Hidromel	Spain E-[redacted] Europe, South	240 OD	1	0.072	0.026	59	green material	0	1.0
								seed	70	<0.01
								straw	70	<0.05
12-2075 12-2075-06 GLP: yes 2012 M-456175-01-1	Rape Hybriock; Winter hybrid	France [redacted] Europe, South	240 OD	1	0.072	0.024	59	green material	0	1.2
								seed	102	<0.01
								straw	102	<0.05

Study Trial No. GLP Year Document No.	Crop Variety	Country	Application FL	 No	 kg/ha (a.s.)	 kg/hL (a.s.)	 GS	Residues Portion analysed	 DALT (days)	 thiacloprid (mg/kg)
13-2097 13-2097-01 GLP: yes 2013 M-492017-01-1	Rape Pulsar, Autumnal variety	Italy [redacted] di Savena - Bologna Europe, South	240 OD	2	0.072	0.024	59	green material flower pod rest of plant seed straw	0* 0 10 38 38 72 72	0.059 0.81 0.060 <0.01 <0.05 <0.01 <0.05
13-2097 13-2097-02 GLP: yes 2013 M-492017-01-1	Rape Avenir, Restored hybrid semi-dwarf	France [redacted] Europe, South	240 OD	2	0.072	0.024	57	green material seed straw	0 106 106	1.0 <0.01 <0.05
13-2097 13-2097-03 GLP: yes 2013 M-492017-01-1	Rape ES Hidromel	Spain [redacted] Europe, South	240 OD	2	0.0637 - 0.0762	0.024	59	green material flower pod rest of plant seed straw	0* 0 9 41 41 84 84	0.097 1.1 0.14 <0.01 <0.05 <0.01 <0.05

* prior to last treatment

As expected the residue levels found in seeds are below the LOQ of 0.01 mg/kg, at harvest even in the straw samples the residue levels are below the LOQ of 0.05 mg/kg.
After flowering, the flowers are collected and analysed for thiacloprid content. The residue levels vary in a range 0.014 mg/kg to 0.14 mg/kg. These limited amounts would lead to residue levels in honey below the LOQ of 0.01 mg/kg.

Conclusion
The residue trials conducted on oil seed rape spread on both European regions clearly demonstrate that the thiacloprid residue in seeds are below the LOQ of 0.01 mg/kg and in straw below the LOQ of 0.05 mg/kg.
The thiacloprid residue levels found in flowers are limited and likely to lead to residue level in honey below the LOQ of 0.01 mg/kg.

CA 6.3.2 Corn

A summary of the residue data is presented below. The summary includes all trials reviewed during the last thiacloprid MRL EU review in the frame of Article 12 of Reg. 396/2005 (EFSA, 2014. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for thiacloprid according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2014;12(3):3617).

Crop	Region	Application Scheme	Residues (mg/kg)	n	STMR (mg/kg)	HR (mg/kg)
Thiacloprid						
Maize	South	Seed treatment French GAP: 50 g a.s./unit, 1 unit=50000 grain (2.2 unit/ha)	6x<0.01	6	0.01*	0.01*
Maize forage	South	Seed treatment French GAP: 50 g a.s./unit, 1 unit=50000 grain (2.2 unit/ha)	6x<0.05	6	0.05*	0.05*

*LOQ of the method of analysis

The intended GAP for the AIR dossier is the following for both European regions:

Seed treatment 50 g ai/unit with a sowing rate of 2.2 unit/ha (1 unit=50000 seeds)

New data for AIR:

The following studies were not evaluated during the last EU review and are submitted for review:

Northern European GAP

Report:	[redacted]; [redacted]; 2009; M-328156-01-1
Title:	Determination of the residues of YRC 2894 in/on corn after seed treatment of YRC 2894 (600 FS) in the field in Germany and Belgium
Report No.:	RA-2664/07
Document No.:	M-328156-01-1
Guidelines:	**EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22); not specified**
GLP/GEP:	**yes**

In 2007, initially four trials were conducted in Northern France, Germany (2) and Belgium. Unfortunately, the French trial was destroyed accidentally. Seed treatment was performed with Thiacloprid FS 600, which is a flowable concentrate, containing 600 g/L of active substance.

Test system

Seeding rate was 2.2 units/ha (1unit = 50000 seeds). The seeds were treated with 125 mL/unit of YRC 2894 (600 FS) corresponding to 75 g ai/unit, a flowable concentrate for seed treatment, containing 50.5% w/w of thiacloprid (YRC 2894), corresponding to an application rate of 0.165 kg/ha thiacloprid (YRC 2894). To achieve the target rate of 125 mL/unit the seed treatment rate was increased to 137.5 mL/unit (10%) although all the calculations were done with the nominal rate of 125 mL/unit.
For residue analysis, samples were taken from the treated and the control material as well as from the corresponding plots. In order to obtain representative samples of the raw commodity, samples were taken at random from various parts of either treated or control plot.
Samples were taken from the control and treated material on day 0 before sowing. Samples from the control and the treated plot were also taken at a growth stage of BBCH 75, 85 and 89.
The samples of this study were analysed for the quantification of thiacloprid parent only with an LC-MS/MS method, 00548/M001/E006, with an LOQ of 0.01 mg/kg for kernel and ear without husk and a LOQ of 0.05 mg/kg in green material.

Findings
Mean concurrent recoveries were within the acceptable range of 70-110%, RSD <20% as shown in Table 6.3.2- 1. They validate the study results.

Table 6.3.2- 1: concurrent recoveries in/on maize for thiacloprid

Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
corn (green material)	0.05	87	87	--	0.05
	0.5	96	96	--	
	5	93	93	--	
	Overall Recovery (n = 3)		92	5.0	
corn (ear without husk)	0.01	75; 86	81	--	0.01
	0.1	83	83	--	
	Overall Recovery (n = 3)		81	7.0	
corn (kernel)	0.01	95; 72	84	--	0.01
	0.1	81	81	--	
	Overall Recovery (n = 3)		83	14.0	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantification
Final determination as : Thiacloprid, Residues calculated as : Thiacloprid

Storage period for samples:
The maximum storage period of deep-frozen samples was 523 days for the samples belonging to study RA-2664/07.

Residue results:
No residue above the LOQ of 0.05 mg/kg in green material was detected in any control samples. No residue above the LOQ of 0.01 mg/kg neither in ear without husk nor in kernel was detected, in any control samples.
At harvest, residues of parent thiacloprid in all kernel samples were below the LOQ of 0.01 mg/kg.

Table 6.3.2- 2: Residue of thiacloprid in trials conducted on maize after seed treatment in Northern Europe with Thiacloprid FS 600

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	kg/unit (a.s.)	g/ha (a.s.)	Residues Portion analysed	DALT (days)	thiacloprid (mg/kg)
RA-2664/07 R 2007 0715/0 0715-07 GLP: yes 2007 M-328156-01-1	Maize/ Corn Total	Germany D-[redacted] Europe, North	600 FS	0.075	Seed rate: 180 g/ha 2.4 units/ha; 1 unit=500 00 seeds	seed for sowing	0	<10
						green material	98 135	<0.05 <0.05
						ear without husk	98 135	<0.01 <0.01
						kernel	161	<0.01
RA-2664/07 R 2007 0756/8 0756-07 GLP: yes 2007 M-328156-01-1	Maize/ Corn Total	Germany D-[redacted] Europe, North	600 FS	0.075	Seed rate165 g/ha 2.2 units/ha; 1 unit=500 00 seeds	seed for sowing	0	<10
						green material	118 137	<0.05 <0.05
						ear without husk	118 137	<0.01 <0.01
						kernel	150	<0.01

RA-2664/07 R 2007 0757/6 0757-07 GLP: yes 2007 M-328156-01-1	Maize/ Corn Total	Belgium B-[redacted] Europe, North	600 FS	0.075	Seed rate: 165 g/ha 2.2 units/ha; 1 unit=50000 seeds	seed for sowing green material ear without husk kernel	0 118 135 118 135 162	<10 <0.05 <0.05 <0.01 <0.01 <0.01

Conclusion

Three supervised field trials on maize were conducted in Northern Europe during the 2007 growing season with thiacloprid FS 600, under different application conditions than the intended use pattern for Thialoprid FS 400, resulting in an application rate of 165 g/ha instead of 110 g/ha as intended. All trials were conducted under GLP.
At harvest, the thiacloprid residues in kernel were below the LOQ of 0.01 mg/kg.

According to the seed treatment metabolism study in sunflower (M-308269-01-1, KCA 6.2.2/02) presented under point 6.2.2 of this dossier, it was decided to analyse also the common moiety 6-CNA, in order to cover the majority of metabolites resulting from soil degradation after thiacloprid application onto the seeds. In 2008 five new supervised field trials were conducted in Northern Europe.

Report: [redacted]d; [redacted]; 2010; M-366150-01-1
Title: Determination of the residues of thiacloprid in/on maize/corn after seed treatment, general of thiacloprid FS 600 in the field in Belgium, France (North), Germany and United Kingdom
Report No.: 08-2024
Document No.: M-366150-01-1
Guidelines: **EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed; EC guidance working document 7029/VI/95 rev. 5 (1997-07-22); not specified**
GLP/GEP: **yes**

Test system

The purpose of the presented study was to determine the magnitude of residues of Thiacloprid and Total Residue of Thiacloprid defined as 6-Chloronicotinic Acid (6-CNA) in/on maize/corn (ear without husk, green material, kernel and treated seed) harvested after one seed treatment, general application with Thiacloprid FS 600 on maize/corn in Northern Europe.
The seed treatment was done with an application rate of 0.125 L/unit test item, containing 75 g/unit Thiacloprid active substance (a.s.) and a thousand grain weight of 211.2 g. Seed treated were sampled from the treated and the control plot before sowing, green material and ear without husk samples were taken from the control plot and treated plot at BBCH 85 (except in trial 08-2024-05 at BBCH 85–87), with additional green material samples from the treated plot at BBCH 73-79 in trials 08-2024-01, 08-2024-02 and 08-2024-05. Kernel samples were sampled from the treated and the control plot at BBCH 89 (harvest).

The samples collected were analysed for quantification of Thiacloprid parent using method 00548/M001 with a LOQ of 0.01 mg/kg in kernel and in ear without husk and a LOQ of 0.05 mg/kg in green material. The samples were also analysed using the common moiety method 01156 for quantifying the 6-CNA, with a LOQ of 0.05 mg/kg in the three matrices.

Findings

- Mean concurrent recoveries were within the acceptable range of 70-110 %, RSD<20% as shown in Table 6.3.2- 3 for thiacloprid and Table 6.3.2- 4 for 6-CNA.

Table 6.3.2- 3: Recovery Data for thiacloprid.

Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Maize/Corn, Ear Without Husk	0.01	95; 88	92	5.4	0.01
	0.1	93	93	-	
		Overall Recovery (n=3)	92	3.9	
Maize/Corn, Green Material	0.05	82; 103	93	16.1	0.05
	0.5	85	85	-	
		Overall Recovery (n = 3)	90	12.6	
Maize/Corn, Kernel	0.01	78; 83	81	4.4	0.01
	0.1	80	80	-	
		Overall Recovery (n = 3)	80	3.1	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantification
Final determination as: thiacloprid Residues calculated as: thiacloprid
Fortification level calculated as : thiacloprid

Table 6.3.2- 4: Recovery Data for 6-chloronicotinic acid.

Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Maize/Corn, Ear Without Husk	0.05	105 [a]	105	-	0.05
	0.5	91	91	-	
	5	86	86	-	
		Overall Recovery (n = 3)	**94**	**10.5**	
Maize/Corn, Green Material	0.05	96 [c]	96	-	
	0.5	88 [b]; 89	89	0.8	
	5	84	84	-	
		Overall Recovery (n = 4)	**89**	**5.6**	
Maize/Corn, Kernel	0.05	103	103	-	
	0.5	86	86	-	
	5	81	81	-	
		Overall Recovery (n = 3)	**90**	**12.8**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantification
Final determination as: 6-chloronicotinic acid Residues calculated as: thiacloprid
Fortified compound: 6-chloronicotinic acid Fortification level calculated as : thiacloprid

[a] Value corrected for residues in control sample (ref. 08-2024-01-0014E) estimating 0.005 mg/kg of 6-chloronicotinic acid. Uncorrected value was 122%.

[b] Value corrected for residues in control sample (ref. 08-2024-05-0013E) estimating 0.026 mg/kg of 6-chloronicotinic acid. Uncorrected value was 96%.

[c] Value corrected for residues in control sample (ref. 08-2024-03-0011E) estimating 0.016 mg/kg of 6-chloronicotinic acid. Uncorrected value was 148%.

- Storage period of samples:
The maximum storage period of deep-frozen samples was 611 days for the samples belonging to study 08-2024.

- Residue results:
No residue levels of thiacloprid parent above the LOQ of 0.05 mg/kg (green material) or above the LOQ of 0.01 mg/kg (kernel and ear without husk) was found in any of the control samples.
Some residue level of 6-CNA were found above the LOQ of 0.05 mg/kg in green material control samples. These residues levels found in the green material control samples (0.06 and 0.09 mg/kg) are coming from the protection treatment applied on the seeds used for the control plot. Indeed this treatment Imidacloprid, Fludioxonil and Metalaxyl-M, contains the active susbtance Imidacloprid which gives also the 6-chloronicotinic acid after its oxidation. There is no impact on the treated sample results because the protection treatment used for the treated plot did not contain Imidacloprid.
The residue levels in treated samples are summarised in Table 6.3.2- 5.

Table 6.3.2- 5: Residue of thiacloprid and total residue of thiacloprid expressed in thiacloprid, in trials conducted on maize after seed treatment in Northern Europe with Thiacloprid FS 600

Study Trial No. GLP Year Document No.	Crop Variety	Country	Application			Portion analysed	Residues		
			FS	kg/unit (a.s.)	g/ha (a.s.)		DALT (days)	thiacloprid (mg/kg)	total residue thiacloprid (mg/kg)
08-2024 08-2024-01 GLP: yes 2008 M-366150-01-1	Maize/ Corn Varial	France [redacted] Europe, North	600 FS	0.07 50 1 unit: 50000 seeds	165 Seed rate: 2.2 units/ha	green material ear without husk kernel	92 106 106 153	<0.05 <0.05 <0.01 <0.01	0.06 <0.05/0.06* <0.05 <0.05
08-2024 08-2024-02 GLP: yes 2008 M-366150-01-1	Maize/ Corn Varial	United Kingdom [redacted] Europe, North	600 FS	0.07 50 1 unit: 50000 seeds	165 Seed rate: 2.2 units/ha	green material ear without husk kernel	118 140 140 153	<0.05 <0.05 <0.01 <0.01	<0.05 <0.05 <0.05 <0.05
08-2024 08-2024-03 GLP: yes 2008 M-366150-01-1	Maize/ Corn Varial	Germany [redacted] Europe, North	600 FS	0.07 50 1 unit: 50000 seeds	165 Seed rate: 2.2 units/ha	green material ear without husk kernel	119 119 134	<0.05 <0.01 <0.01	<0.05 <0.05 <0.05
08-2024 08-2024-04 GLP: yes 2008 M-366150-01-1	Maize/ Corn Varial	Germany [redacted] Europe, North	600 FS	0.07 50 1 unit: 50000 seeds	172.5 Seed rate: 2.3 units/ha	green material ear without husk kernel	140 140 157	<0.05 <0.01 <0.01	0.06**/0.09* <0.05 <0.05

08-2024 08-2024-05 GLP: yes 2008 M-366150-01-1	Maize/ Corn Varial	Belgium [redacted] Europe, North	600 FS	0.07 50	165 Seed rate: 2.2 units/ha 1 unit: 50000 seeds	green material ear without husk kernel	124 149 149 174	<0.05 <0.05 <0.01 <0.01	<0.05 <0.05 <0.05 <0.05

* residue found in control samples
** mean of three results (0.0858, 0.0507 and 0.0576 mg/kg)

Conclusion
As expected at harvest, the seed treatment of maize with Thiacloprid FS 600 does not result in residue levels either for thiacloprid parent or total residue of thiacloprid above the respective LOQ of 0.01 mg/kg and 0.05 mg/kg in kernel.

Southern European GAP

Report: [redacted]; 2013; M-451345-01-1
Title: Determination of the residues of thiacloprid in/on maize/corn after seed treatment with thiacloprid FS 400 and subsequent cultivation in Italy and Spain
Report No.: 12-2076
Document No.: M-451345-01-1
Guidelines: **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**
EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997)
US EPA OCSPP Guideline No. 860.1500; not applicable
GLP/GEP: **yes**

Report: [redacted]; 2014; M-492370-01-1
Title: Determination of the residues of thiacloprid in/on maize/corn after seed treatment with thiacloprid FS 400 in Spain, Italy and southern France
Report No.: 13-2098
Document No.: M-492370-01-1
Guidelines: **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**
EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)
OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial
US EPA OCSPP Guideline No. 860.1500; not applicable
GLP/GEP: **yes**

Materials and methods

Eight field trials were conducted in Southern Europe during the two growing seasons 2012 and 2013 on maize after seed treatment with Thiacloprid FS 400, a flowable concentrate for seed treatment at a concentration of 400 g/L thiacloprid. The product was applied to the seeds at an application rate of 52 g a.s./unit. A unit represents 50000 seeds and the sowing rate is 2.2 units per ha. The thousand grain weitght is estimated at 384 g corresponding to a the theoretical rate of 2708 mg/kg of thiacloprid by grain. The treated seeds were analysed before sowing with a LC-MS/MS method 01176 in order to check the performance of the coating seed. The seeds were placed in a mixture of acetonitrile/water (4/1, v/v) and were shaken for at least three hours. The subsequent LC-MS/MS analysis was performed under conditions given in method 00548/M001/E006.
The sample materials analysed were green material, ear, without husk and kernel, they were collected from the treated plot and the control plot. The untreated plot was sampled before the treated plot. Samples were collected at harvest with additional samples in trials 12-2076-01 and 12-2076-03 at BBCH 75/76 and 85/86 and in trials 13-2098-01 and 13-2098-03 at BBCH 85. The field samples were analysed for thiaclorpid parent with the LC-MS/MS method 00584/M001/E006 for the year 2012 and the LC-MS/MS method 00548/M001 for the year 2013 with a LOQ of 0.01 mg/kg for ear without husk and kernel and a LOQ of 0.05 mg/kg for green material for both methods.
According to the results of the Metabolism of [methylene-^{14}C]thiacloprid in sunflower after seed treatment, it was recommended to analyse all the samples (green material, ear, without husk and kernel) with the method 01156 for the total residue of thiacloprid defined as 6-chloronicotinic acid (6-CNA) with a limit of quantification of 0.05 mg/kg expressed as thiacloprid.

Findings

The procedural recoveries determined from fortified samples analysed alongside with the treated samples were satisfactory, as shown in Table 6.3.2- 6.

Table 6.3.2- 6: Recovery data for Thiacloprid

Study Number	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
12-2076 M-451345-01-1	Maize/ Corn	ear without husk	thiacloprid	2	0.01	93;97	93	97	95	
				1	0.1	92	92	92	92	
				3	overall mg/kg		92	97	94	2.8
			total residue thiacloprid	1	0.05	95	95	95	95	
				1	0.5	81	81	81	81	
				2	overall mg/kg		81	95	88	-
		green material	thiacloprid	1	0.05	84	84	84	84	
				1	0.5	94	94	94	94	
				2	overall mg/kg		84	94	89	-
			total residue thiacloprid	1	0.05	75	75	75	75	
				1	0.5	85	85	85	85	
				2	overall mg/kg		75	85	80	-
		kernel	thiacloprid	2	0.01	99;104	99	104	102	
				2	0.1	97;103	97	103	100	

				4	overall mg/kg		97	104	101	3.3
			total residue thiacloprid	1 1	0.05 0.5	91 82	91 82	91 82	91 82	
				2	overall mg/kg		82	91	87	-
13-2098 M-492370-01-1	Maize/ Corn	ear without husk	thiacloprid	1	0.01	115	115	115	115	
				1	overall mg/kg		115	115	115	
			total residue thiacloprid	1 1	0.05 1.0	83 92	83 92	83 92	83 92	
				2	overall mg/kg		83	92	88	
		green material	thiacloprid	1 1	0.05 0.5	92 87	92 87	92 87	92 87	
				2	overall mg/kg		87	92	90	-
			total residue thiacloprid	1 1 2 3	0.05 2 5 overall mg/kg	98 78 76	98 78 76 76	98 78 76 98	98 78 76 84	14.5
		kernel	thiacloprid	1 1	0.01 0.4	105 106	105 106	105 106	105 106	
				2	overall mg/kg		105	106	106	-
			total residue thiacloprid	1 1	0.05 1	90 82	90 82	90 82	90 82	
				2	overall mg/kg		82	90	86	-

Storage period:

The maximum storage period for the deep frozen samples analysed for thiacloprid parent was 205 days belonging to the study 13-2098 and the maximum storage period for the deep frozen samples analysed for the total residue thiacloprid was 239 days for the samples belonging to study 13-2098.

Residue results:

In the following table, the application information and the residues found in/on corn are summarised. Residues of thiacloprid in all of the trials were found to be below LOQ of 0.01 mg/kg (ear without husk, kernel). Residues of total residue thiacloprid were found in all the trials below the LOQ of 0.05 mg/kg expressed as thiacloprid, except in 2 samples of green material were values sligltly above the LOQ were found.

Table 6.3.2- 7: Results of residue trials conducted with Thiacloprid FS 400 on Corn (480 g/L thiacloprid) in Southern Europe, residues for thiacloprid and for total residue of thiacloprid expressed as thiacloprid

Study Trial No. GLP Year Document No.	Crop Variety	Country	Application			Residues			
			FL	kg/unit (a.s.)	g/ha (a.s.)	Portion analysed	DALT (days)	thiacloprid (mg/kg)	total residue thiacloprid (mg/kg)
12-2076 12-2076-01 GLP: yes 2012 M-451345-01-1	Maize/ Corn Scandi	Spain [redacted] Europe, South	400 FS	0.0520	1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing	0	2790	
						ear without husk	111	<0.01	<0.05
						green material	91	<0.05	<0.05
							111	<0.05	0.079
						kernel	124	<0.01	<0.05
12-2076 12-2076-02 GLP: yes 2012 M-451345-01-1	Maize/ Corn Scandi	Spain [redacted] Europe, South	400 FS	0.0520	1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing	55	2880	
						kernel	112	<0.01	<0.05
12-2076 12-2076-03 GLP: yes 2012 M-451345-01-1	Maize/ Corn Scandi	Italy [redacted] Europe, South	400 FS	0.0520	1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing	0	2730	
						ear without husk	103	<0.01	<0.05
						green material	81	<0.05	<0.05
							103	<0.05	<0.05
						kernel	126	<0.01	<0.05
12-2076 12-2076-04 GLP: yes 2012 M-451345-01-1	Maize/ Corn Scandi	Italy [redacted] Europe, South	400 FS	0.0520	1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing	0	2640	
						kernel	133	<0.01	<0.05
13-2098 13-2098-01 GLP: yes 2013 M-492370-01-1	Maize/ Corn Scandi	Spain [redacted] Europe, South	400 FS	0.052	96.4 1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing	0	2440/1*	
						green material	89	<0.05	<0.05
							108	<0.05	0.064
						ear without husk	108	<0.01	<0.05
						kernel	131	<0.01	<0.05

Study Trial No. GLP Year Document No.	Crop Variety	Country	Application			Residues			
			FL	kg/unit (a.s.)	g/ha (a.s.)	Portion analysed	DALT (days)	thiacloprid (mg/kg)	total residue thiacloprid (mg/kg)
13-2098 13-2098-02 GLP: yes 2013 M-492370-01-1	Maize/ Corn Scandi	Italy I-[redacted] Europe, South	400 FS	0.052	98.8 1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing kernel	0 127	2500 <0.01	 <0.05
13-2098 13-2098-03 GLP: yes 2013 M-492370-01-1	Maize/ Corn Scandi	Italy I-[redacted] Europe, South	400 FS	0.052	114 1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing ear without husk green material kernel	0 111 91 111 138	2880** <0.01 <0.05 <0.05 <0.01	 <0.05 <0.05 <0.05 <0.05
13-2098 13-2098-04 GLP: yes 2013 M-492370-01-1	Maize/ Corn Scandi	France F-[redacted] Europe, South	400 FS	0.052	90.1 1 unit: 50000 seeds Seed rate: 110000 seeds/ha	seed for sowing kernel	0 182	2280 <0.01	 <0.05

* residue in control sample
** mean value (2782 and 2975 mg/kg) of a double reanalysis on a reserve sample

Conclusion

As expected, the use of thiacloprid as seed treatment leads to a situation of no residue of thiacloprid in the kernels. Since thiacloprid is applied as seed treatment the total residue of thiacloprid content was assessed by measuring the content of 6-CNA after oxidation of the extracts. In most of the samples the residue level was found below the LOQ of 0.05 mg/kg, except in 2 green material samples where 6-CNA was found slightly above the LOQ.

Both methods of analysis which were used to analyse the corn samples have a respective LOQ of 0.01 mg/kg for kernel or ear without husk and a LOQ of 0.05 mg/kg for green material. In order to check that the presence of thiacloprid in all maize matrices is below the value of 0.01 mg/kg, an in depth examination of chromatograms corresponding to green material of maize was done. The chromatograms corresponding to the green material samples were extracted from the raw data and compiled within one document (M-565717-01-1). As expected, the residue content of thiacloprid is clearly below the value of 0.01 mg/kg in all the green material samples analysed in the four studies conducted to support the use of FS 400.

CA 6.4 Feeding studies

Data/information on livestock feeding studies were reviewed during the Annex I inclusion process and were considered to be acceptable and no further data have been generated.
Thiacloprid is sought for use on oil seed rape and corn with parts of these crops being fed to livestock. Despite the fact that the residue content in thiacloprid in the feed items is below the LOQ in all samples, a worst case estimation of dietary burden was conducted using the LOQ values as input values.

The maximum dietary burdens were therefore calculated for different groups of livestock as described in the OECD Guidance Document on Residues in Livestock (ENV/JM/MONO(2013)8 dated of 04-Sep-2013). The input values for all relevant commodities are summarized in Table 6.4 - 1.

Table 6.4 - 1: Input values for the dietary burden calculation – OECD methodology

Commodity	Input value (mg/kg)	Comment
Risk assessment residue definition: thiacloprid		
Maize silage	0.05	LOQ
Maize grain	0.01	LOQ
Rape seed	0.01	LOQ

The results of the calculations are reported in Table 6.4 - 2.

Table 6.4 - 2: Results of the dietary burden calculation - OECD methodology

	Maximum dietary burden (mg/kg bw/day)	Max dietary burden (mg/kg DM)	Highest contributing commodity
Cattle - Beef	0.002	0.402	Corn forage/silage
Cattle - Dairy	0.003	0.080	Corn forage/silage
Sheep – Rams/Ewes	0.000	0.005	-
Sheep – Lambs	0.002	0.043	Corn forage/silage
Swine - Breeding	0.001	0.034	Corn forage/silage
Swine - Finishing	0.000	0.001	-
Poultry - Broiler	0.001	0.008	Corn grain
Poultry - Layer	0.001	0.022	Corn forage/silage/grain
Poultry - Turkey	0.001	0.008	-

The calculated dietary burdens for all categories of livestock were found to be far below the 1x dose level of the cow feeding study, which was set at 2 mg/kg DM in feed. According to this worst case assumption of residue level present in the feed items at the respective LOQ, no transfer of thiacloprid into animal matrices is expected for the supported uses.

CA 6.4.1 Poultry

A position paper (M-356456-01-1) was submitted to Dutch CTGB about the non relevance to conduct a poultry feeding study. There is no reasonable expectation of significant residues of thiacloprid in food items originating from poultry. This conclusion is derived from the available residue data from treated crops and the metabolism study in laying hens as well as the toxicokinetics and metabolism study in the rat.

CA 6.4.2 Ruminants

A dairy cow feeding study was previously evaluated (KCA 6.4.2/01). Therefore, no new studies were conducted.

CA 6.4.3 Pigs

The metabolic pathway of thiacloprid is similar in rats, poultry (laying hens), and ruminants (goat). Therefore, it can be expected that the metabolism in other farm animals does not differ, and thus a study in pigs is not required for this active ingredient. Hence a pig feeding study is also not necessary for this dossier.

CA 6.4.4 Fish

No metabolism study or feeding study in fish was conducted (refer to CA 6.2.5).
Currently, no test method or guidance document is available for conducting a feeding study in fish. Also, no feeding table with plant commodities for fish feeding is available. Therefore, it cannot be decided whether fish might be exposed to residues of thiacloprid in parts of plant that have been treated with thiacloprid.

In these cases, waiving of this particular data requirement is considered acceptable according to the "Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and the renewal of approval of the chemical active substance according to regulation (EU) No. 283/2013 and regulation (EU) No. 284/2013" (SANCO/10181/2013-rev.2 of 2-May-2013).

CA 6.5 Effects of processing

CA 6.5.1 Nature of the residue

The processing study (M-002136-01-1) was performed and evaluated (see Monograph Annex B from November 2000). It was designed to determine the nature and quantity of residues which might be formed during processing of raw agricultural commodities.

Report:	[redacted]; 1998; M-002136-01-1
Title:	Aqueous hydrolysis of YRC 2894 under conditions of processing studies
Report No.:	PF4364
Document No.:	M-002136-01-1
Guidelines:	
GLP/GEP:	**yes**

The effect on processing on the nature of thiacloprid was investigated in studies performed at three test conditions (20 minutes at 90°C, pH 4; 60 minutes at 100°C pH 5; 20 minutes at 120°C, pH 6) and evaluated under the peer review (United Kingdom). It was concluded that thiacloprid is stable under representative processing conditions and no formation of toxicologically relevant metabolites occurs. The residue definition remains the same for the processed commodities.

CA 6.5.2 Distribution of the residue in peel and pulp

The distribution of the residue in peel and pulp is not relevant for the supported crops.

CA 6.5.3 Magnitude of residues in processed commodities

Although the residue level was found below the LOQ of 0.01 mg/kg in rapeseed at harvest a processing study performed according a use pattern leading to residue of thiacloprid in the raw agricultural commodity is presented herebelow.

Report:	[redacted]ö; [redacted]; 2010; M-393217-01-1
Title:	Determination of the residues of thiacloprid in/on rape and the processed fractions (oil, screwpressed; pomace; extracted meal; oil, solv. extracted; crude oil; crude oil, preclarified; crude oil, neutralised and oil, refined) after spraying of YRC 2894 OD 240 in the field in Germany, France and Italy
Report No.:	01.09.3183
Document No.:	M-393217-01-1
Guidelines:	**91/414/EEC of July 15, 1991, 7029/VI/95 rev. 5 (1997-07-22); not specified**
GLP/GEP:	**yes**

Test system

The purpose of the study 09-3183 was to determine the magnitude of the residues of thiacloprid in/on rape (neutralised crude oil, preclarified crude oil, extracted meal, crude oil, refined oil, screwpressed solv. extracted oil oil, pomace, press cake meal, sample for acid determination, seed and trash) after two spraying applications with YRC 2894 OD 240, an OD formulation containing thiacloprid.
The rape seed samples to be processed and reference raw agricultural commodity (RAC) samples originate from four supervised residue trials (09-2183-01, 09-2183-02, 09-2183-05, 09-2183-06) in the conduct of study 09-2183. These trials were conducted in Europe (Germany, France and Italy) during the 2009 season. Rape seed samples to be processed were sampled approx. 30 days after the last treatment.

The analyses were conducted according to the following analytical method(s):

Table 6.5.3-1: Analytical Method.

Active Substance	Analyte(s)	Method Number	Limit of Quantitation [mg/kg]	Sample Material	Measurement Principle
Thiacloprid	Thiacloprid	00548/M001/E006	0.01	Oil*	LC-MS/MS
	Thiacloprid	00548/M001/E006	0.01	Pomace**	LC-MS/MS

*: Covers crude oil, preclarified crude oil; neutralised crude oil; screwpressed oil and solv. extracted oil.

**: Covers extracted meal.

Analyte	Final determination as:	Residues calculated as:
Thiacloprid	Thiacloprid	Thiacloprid

Processing

The processing of the rape seed samples into screwpressed oil; pomace; extracted meal; solv. extracted oil; cruide oil; preclarified crude oil; neutralised crude oil and refined oil was performed in Food Processing Laboratory (FPL) Bayer Cropscience AG in D-[redacted]. The processing procedure simulated industrial practice at a laboratory scale.

A description of the processing and flow charts can be found in paragraphs 6.5.3-1and **Fehler! Verweisquelle konnte nicht gefunden werden.**, respectively.

Fig.6.5.3-1: Flow Chart of the Preparation of Screwpressed Oil; Pomace; Solv. Extracted Oil; Crude Oil; Extracted Meal

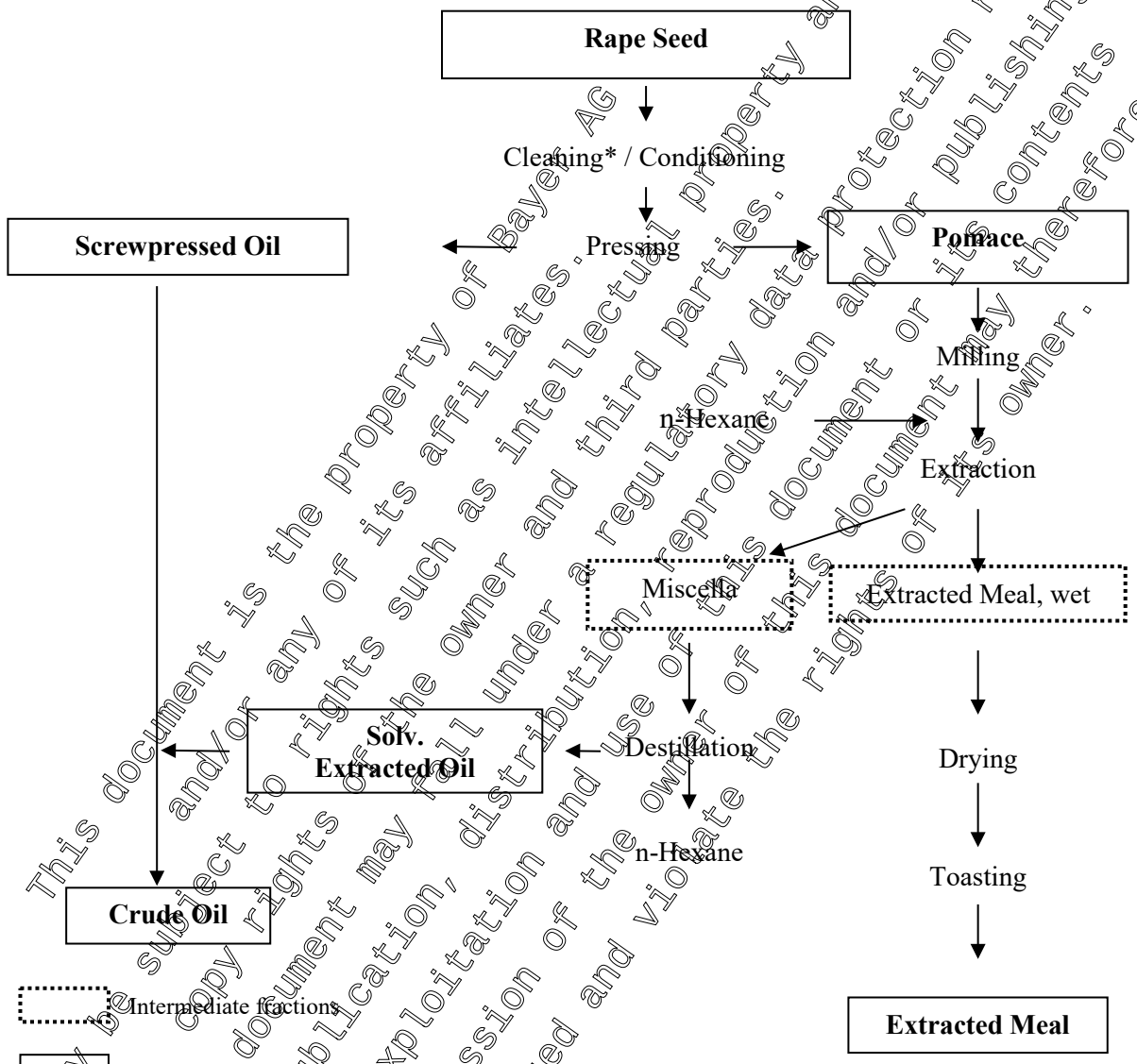
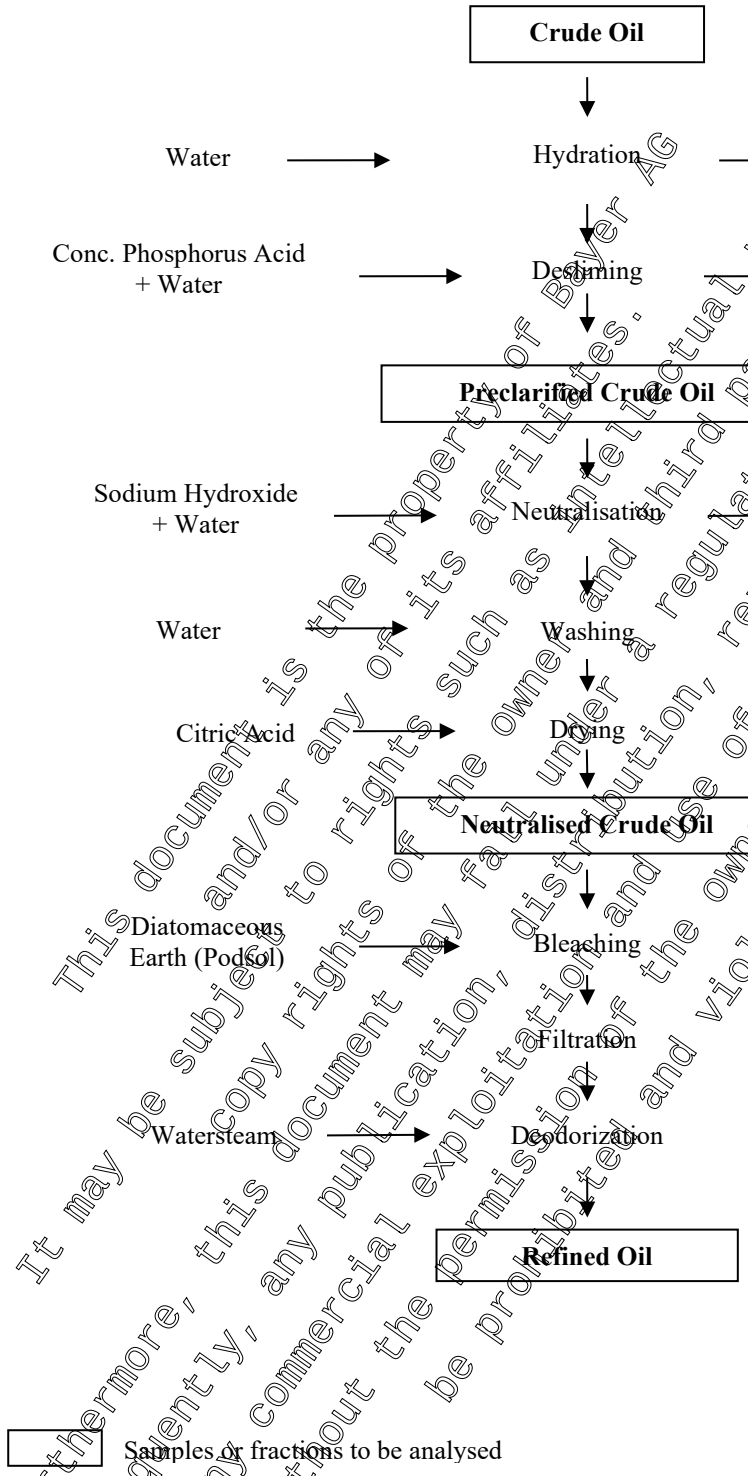


Fig. 6.5.3-2: Flow Chart of the Refinement of Crude Oil



Findings

- Mean concurrent recoveries were within the acceptable range of 70-110% and the RSD below 20% as shown in table 6.5.3-2.

Table6.5.3-2: Recovery Data for Thiacloprid.

Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Rape Oil*	0.01	94; 84; 87	85	10.1	0.01
	5.0	94; 98; 82	91	9.1	
		Overall Recovery (n = 6)	**88**	**9.4**	
Rape Pomace**	0.01	87; 86; 95	89	5.5	0.01
	5.0	93; 96; 96	95	1.8	
		Overall Recovery (n = 6)	**92**	**4.9**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantification
These recoveries were performed during the conduct of the studies 09-3182.
*: All oils (e.g. crude oil, screwpressed oil, refined oil etc) are covered by oil.
**: Extracted meal is covered by pomace.

- Residue results:

The residue levels in the RAC vary from 0.09 mg/kg to 0.18 mg/kg. Considering the low log Pow of 1.26, as expected the oil fractions were shown to be free of thiacloprid. As a consequence the thiacloprid residue are located into pomace and extracted meal.

Table6.5.3-3: Results of processing trials conducted with YRC 2894 OD 240 on Rapeseed for oil processing

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	thiacloprid (mg/kg)
09-3183 M-393217-01-1 09-3183-01 GLP: yes 2009	Rape Galileo	Germany [redacted] Europe, North	240 OD	2	0.0720	0.0240	80	seed	30	0.09
								oil, screwpressed	30	<0.01
								pomace	30	0.09
								extracted meal	30	0.13
								oil, solv. extracted	30	<0.01
								oil, crude	30	<0.01
								crude oil, preclarified	30	<0.01
								crude oil, neutralised	30	<0.01

								oil, refined	30	<0.01
09-3183MAN M-393217-01-1 09-3183-02 GLP: yes 2009	Rape Safran	France [redacted] Europe, North	240 OD	2	0.0720	0.0240	80	seed	29	0.06
								oil, screwpressed	29	<0.01
								pomace	29	0.06
								extracted meal	29	0.09
								oil, solv. extracted	29	<0.01
								oil, crude	29	<0.01
								crude oil, preclarified	29	<0.01
								crude oil, neutralised	29	<0.01
								oil, refined	29	<0.01
09-3183MAN M-393217-01-1 09-3183-05 GLP: yes 2009	Rape Corail	France [redacted] Europe, South	240 OD	2	0.0720	0.0240	78	seed	30	0.12
								oil, screwpressed	30	<0.01
								pomace	30	0.11
								extracted meal	30	0.14
								oil, solv. extracted	30	<0.01
								oil, crude	30	<0.01
								crude oil, preclarified	30	<0.01
								crude oil, neutralised	30	<0.01
								oil, refined	30	<0.01
09-3183MAN M-393217-01-1 09-3183-06 GLP: yes 2009	Rape PR46W 09	Italy [redacted] (BO) Europe, South	240 OD	2	0.0720	0.0240	80	seed	30	0.18
								oil, screwpressed	30	<0.01
								pomace	30	0.21
								extracted meal	30	0.28
								oil, solv. extracted	30	<0.01
								oil, crude	30	<0.01
								crude oil, preclarified	30	<0.01
								crude oil, neutralised	30	<0.01
								oil, refined	30	<0.01

Based on the residue levels in the treated processing products processing factors were calculated. The processing factors for the processing products are calculated according to the following equation:

$$\text{Processing Factor} = \frac{\text{Residue concentration in the processed product [mg/kg]}}{}$$

Residue concentration in the RAC [mg/kg]

RAC: Raw Agricultural Commodity.

In case residues in the RAC are ≥ LOQ but residues in the processed product are < LOQ, the processing factor is calculated as follows:

$$\text{Processing Factor} = < \left[\frac{\text{LOQ processed product [mg/kg]}}{\text{Residue concentration in the RAC [mg/kg]}} \right]$$

Transfer factors calculated for the treated processing products is given in the table below.

Table 6.5.3-4: Summary of Processing Factors.

Sample Material	Processing Factor for Thiacloprid			
	09-3183-01	09-3183-02	09-3183-05	09-3183-06
screwpressed oil	< 0.11	< 0.17	< 0.08	< 0.06
pomace	1.0	1.0	0.92	1.2
extracted meal	1.4	1.5	1.2	1.6
solv. extracted oil	< 0.11	< 0.17	< 0.08	< 0.06
crude oil	< 0.11	< 0.17	< 0.08	< 0.06
preclarified crude oil	< 0.11	< 0.17	< 0.08	< 0.06
neutralised crude oil	< 0.11	< 0.17	< 0.08	< 0.06
refined oil	< 0.11	< 0.17	< 0.08	< 0.06

Conclusion

As expected the residue of thiacloprid does not concentrate in oil. The residue is located into the pomace fraction. A concentration is observed into the extracted meal following the drying process. The supported use pattern of thiacloprid on oil seed rape in this dossier, leads to a situation of no residue into the seeds. According to the results of this processing study, no residue in oil neither in meal is expected.

CA 6.6 Residues in rotational crops

A confined rotational crop study with thiacloprid was investigated in the framework of the peer review under Directive 91/414/EEC (United Kingdom, 2002). In all rotational crops thiacloprid was never detected (below the LOQ) and the residues of the individual metabolites were generally low, less than 0.1 mg/kg. These data were peer reviewed during the Annex I inclusion process and it was concluded that the residue definition established in primary crops is also applicable to rotational crops.

The field rotational crop studies conducted in lettuce, turnip and wheat were performed with an exaggerated application rate. The residues in samples declined after each plant back interval, and at harvest no residue of thiacloprid above the LOQ was seen in all crop samples at each rotation. (M-001542-01-1)

CA 6.6.1 Metabolism in rotational crops

Please refer to CA 6.6.

CA 6.6.2 Magnitude of residues in rotational crops

Please refer to CA 6.6.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

In the baseline dossier, crop metabolism studies on tomatoes, apples and cotton following spray application and the rotational crop study have been presented. Based on these studies, the residue definition for risk assessment and enforcement purposes was established as parent compound.

Matrices	EU Residue definition		Reference
Food of plant origin	Risk assessment	thiacloprid parent	Review Report SANCO/4347/2000 13 May 2004
	Monitoring	thiacloprid parent	
Food of animal origin	Risk assessment	thiacloprid parent	
	Monitoring	thiacloprid parent	

In this dossier, metabolism studies on spring wheat after spray application and on sunflower following seed dressing are described. In these studies the [methylene-^{14}C-label was employed. An additional study on potatoes after spray application of [thiazolidine-2-^{14}C]-labelled thiacloprid is also presented in this dossier. For thiacloprid, metabolism studies for 6 crops from 4 categories (fruit, pulses and oilseeds, cereals/grass crops and root crops) are now available. The results show that the route of degradation is similar in all four categories independent of the application route. The unchanged parent compound is the major component of the residue in all crop groups. In commodities relevant for human consumption, no metabolite appears in quantities above 12 % of the radioactive residue. The high level of recovery and characterisation achieved in the big majority of the studies strongly supports the existing residue definition as parent compound only for enforcement of MRLs as well as for dietary risk assessment.

CA 6.7.2 Proposed MRLs and justification of the acceptability of the levels proposed

The representative uses supported in this dossier, do not trigger change for the existing EU-MRLs, neither for plant commodities nor for animal commodities.

CA 6.7.3 Proposed MRLs and justification of the acceptability of the levels proposed for imported products (import tolerance)

MRL settings based on imported products are not proposed with this dossier.

CA 6.8 Proposed safety intervals

There is no need to propose safety intervals.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

In order to evaluate the potential chronic exposure to thiacloprid residues through the diet, the Theoretical Maximum Dietary Intakes (TMDI) were calculated using:

- The EFSA PRIMo model (revision 2). For the evaluation of the chronic exposure the model uses 5 WHO diets relevant to the EU and 22 national diets from 13 different EU Member States.
- An ADI of 0.01 mg/kg bw/day
- The STMRs corresponding to the residue data package presented in this dossier for supporting the representative uses of thiacloprid in/on oilseed rape and on corn, respectively 0.01 mg/kg for each crop.
- Since no transfer in food of animal origin is expected according to the observed residue level resulting from the supported representative uses on corn and oilseed rape, values corresponding to LOQ of the enforcement method were used to perform the evaluation of chronic exposure.

As shown in the following table, the highest TMDI represents **4.3% of the ADI** and was calculated for the FR toddler.

Therefore, a long-term intake of residues of thiacloprid is unlikely to present a public health concern.

Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	

Toxicological end points

ADI (mg/kg bw/day):	0,01	ARfD (mg/kg bw):	0,03
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI
minimum - maximum
4

No of diets exceeding ADI: -

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
4,3	FR toddler	4,3	PRODUCTS OF ANIMAL ORIGIN		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
4,3	UK Infant	4,2	PRODUCTS OF ANIMAL ORIGIN	0,1	Maize		FRUIT (FRESH OR FROZEN)	
3,3	NL child	3,3	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize	0,0	Rape seed	
2,7	FR infant	2,7	PRODUCTS OF ANIMAL ORIGIN		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
2,4	DE child	2,4	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize	0,0	Rape seed	
2,3	UK Toddler	2,3	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize		FRUIT (FRESH OR FROZEN)	
2,2	DK child	2,2	PRODUCTS OF ANIMAL ORIGIN		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
1,8	ES child	1,8	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize		FRUIT (FRESH OR FROZEN)	
1,8	SE general population 90th percentile	1,8	PRODUCTS OF ANIMAL ORIGIN		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
1,0	WHO Cluster diet B	0,7	PRODUCTS OF ANIMAL ORIGIN	0,2	Maize	0,0	Rape seed	
1,0	WHO regional European diet	0,9	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize	0,0	Rape seed	
0,8	IE adult	0,6	PRODUCTS OF ANIMAL ORIGIN	0,2	Maize		FRUIT (FRESH OR FROZEN)	
0,8	NL general	0,8	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize	0,0	Rape seed	
0,8	ES adult	0,8	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize		FRUIT (FRESH OR FROZEN)	
0,8	WHO cluster diet E	0,7	PRODUCTS OF ANIMAL ORIGIN	0,1	Rape seed	0,1	Maize	
0,8	WHO Cluster diet F	0,7	PRODUCTS OF ANIMAL ORIGIN	0,0	Rape seed	0,0	Maize	
0,8	WHO cluster diet D	0,7	PRODUCTS OF ANIMAL ORIGIN	0,1	Maize	0,0	Rape seed	
0,8	DK adult	0,8	PRODUCTS OF ANIMAL ORIGIN	0,0	Rape seed		FRUIT (FRESH OR FROZEN)	
0,7	FI adult	0,7	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize		FRUIT (FRESH OR FROZEN)	
0,6	LT adult	0,6	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize		FRUIT (FRESH OR FROZEN)	
0,4	UK Adult	0,4	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize		FRUIT (FRESH OR FROZEN)	
0,4	FR all population	0,4	PRODUCTS OF ANIMAL ORIGIN	0,0	Rape seed		FRUIT (FRESH OR FROZEN)	
0,4	UK vegetarian	0,4	PRODUCTS OF ANIMAL ORIGIN	0,0	Maize		FRUIT (FRESH OR FROZEN)	
0,0	PT General population	0,0	Maize		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0,0	IT kids/toddler	0,0	Maize		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0,0	IT adult	0,0	Maize		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0,0	PL general population	0,0	Maize		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of is unlikely to present a public health concern.

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Acute Reference Dose (ARfD) and Dietary Exposure Calculation
In order to evaluate the potential acute exposure to thiacloprid residues through the diet, the International Estimated Short Term Intakes (IESTI) were calculated using:

- The EFSA PRIMo model (revision 2). For the evaluation of the acute exposure 19 national diets from 11 different EU Member States are used.
- An ARfD of 0.03 mg/kg bw/day
- The Highest Residue values observed for the representative uses respectively: 0.01 mg/kg for oilseed rape, 0.01 mg/kg for corn grain.

The highest IESTI represents **0.2% of the ARfD** and was calculated for corn consumed by UK infant. Therefore, a short-term intake of residues of thiacloprid is unlikely to present a public health concern.

CA 6.10 Other studies

CA 6.10.1 Effect on the residue level in pollen and bee products

Since oil seed rape is a melliferous crop, and thiacloprid has systemic property, the presence of thiacloprid residues in the aerial parts foraged by bees and the potential transfer to honey therefore needs to be investigated.
The currently registered use of thiacloprid on oil seed rape consists of 3 spray applications at 72 g/ha, the last application taking place at BBCH 65, during flowering. This use leads to residue of thiacloprid in honey which are covered by the existing EU-MRL of 0.2 mg/kg (Reg (EU) No 364/2014) in honey. The honey-MRL was set on the basis of monitoring data.

The supported use of thiacloprid on oil seed rape in this dossier consists of spray applications before flowering. The transfer of residue of thiacloprid into honey is supposed to be very limited. In order to determine if under these conditions of applications a residue transfer into honey does exist, three honey trials were conducted in 2014.
In absence of final guideline document describing supervised honey residue trials, our proposal is based on the German guidance document issued by the Federal Office for Consumer Protection and Food Safety cited into Afssa-saisine n° 2007-SA-0209- Document guide de fixation de LMR pour le miel .

The study protocol is detailed here below. The phase analytical report is available but the final report will be available by end of December 2014.

Report:	[redacted]h;[redacted];2015;M-510422-01
Title:	Determination of residues of thiacloprid OD 240B G in honey after application of thiacloprid OD 240B G just before flowering in a semi-field residue study with honeybees (Apis mellifera L.) in Winter Oilseed Rape in 2014
Report No:	S14-00167
Document No(s):	Report includes Trial Nos.: S14-00167-01 S14-00167-02 S14-00167-03 S14-00167-L1 M-510422-01-1
Guidelines:	**Guideline 1607/VI/97 (rev.2) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009, under consideration of the provisions of the Afssa saisine n°2007-SA-0209-Document guide de fixation de LMR pour le miel;**
Deviations	**none**
GLP/GEP:	**yes**

Materials and methods

The study was designed to determine the magnitude of residues of thiacloprid in honey, following exposure of honeybees under confined semi-field conditions to winter oilseed rape, treated sequentially two times at a rate of 72 g a.s./ha with Thiacloprid OD 240 B G via foliar application, respectively, just before onset of flowering.

The study comprised in total three independent trials, which were conducted on three different, spatially separated trial locations. Two trials were located in Germany (region: Baden-Württemberg; trials S14-00167-01 and S14-00167-02) and one trial was located in France (region: Alsace; trial S14-00167-03). In order to maximise exposure of the bee colonies to the treated oil seed rape plants, the honey-producing bee colonies were confined in gauze-tunnels, which were placed on thiacloprid-treated winter oil seed rape plots. One gauze-tunnel per trial on a thiacloprid untreated plot was used as control. The honey bee colonies remained in the gauze-tunnels as long as reasonably possible and until sampling of honey was completed.

The study consisted on each of the three trial locations (i.e. in each of the three trials) of two treatment groups: the test item group T with 3 replicates and an untreated control C (one replicate). The nominal application rate in each of the two sequential, pre-flowering foliar applications in the respective test item treatment groups corresponded to 72 g a.s./ha, respectively. The 2nd (last) application was conducted at imminent pre-bloom [BBCH 57-59 (trial -01 and -02), BBCH 59 (trial-03)], the 1st application was conducted between 8 (trials in Germany) and 12 (trial in France) days before the 2nd (last) application. Honey bee colonies were placed in the tunnels at the beginning of flowering [BBCH 63 (trial-01), BBCH 63-65 (trial-02 and -03)].

The target of the study was to obtain honey from each of the employed colonies, which was exclusively produced from the nectar of winter oil seed rape plants, confined in gauze-tunnels.

A colony assessment was made before the set-up in the tunnels at each site according to the following parameters:

- Colony strength (number of bees, estimation adapted to Imdorf & Gerig, 1999, and Imdorf *et al.*, 1987)
- Presence of a healthy queen (e.g. presence of eggs)
- Pollen storage area and area with nectar or honey (estimation adapted to Imdorf & Gerig, 1999, and Imdorf *et al.*, 1987)
- Area containing cells with eggs, larvae and capped cells (estimation adapted to Imdorf & Gerig, 1999, and Imdorf *et al.*, 1987)

At the colony assessment, the comb area covered with bees or containing cells with nectar, pollen, eggs, larvae, and capped cells were estimated per comb side. The total number of bees and the total

area (number of cells) containing the single brood stages, pollen and nectar was calculated for each colony. Afterwards the mean values were calculated for the test item treated group.
At the colony assessment, each comb of the colonies was assessed visually for symptoms of bee diseases by a beekeeper according to standard beekeeping practice, in particular with respect to unusual observations and clear symptoms of disease (e.g. chalk brood, sacbrood, *Nosema*, American or European foulbrood) or pests (e.g. *Varroa* sp., *Aethina tumida*, *Tropilaelaps* spp.).
The calculation of the area containing brood or food stages was based on a comb size of 800 cm² (per comb side, type of comb: Zander) and assuming 400 cells per unit of 100 cm² (3200 cells per comb side). For the calculation of colony strength 125 honeybees per fully covered unit of 100 cm² were assumed.
Honey was collected by gently pushing a spoon into the walls of storage cells, allowing the honey to flow onto the spoon. 2-4 combs were placed in the brood body shortly before set-up in the tunnel tents. The honey was preferably collected from various comb location of these combs resulting in a minimum of 20 g honey per colony (<20 g honey was sampled in S14-00167-02; C and Ta).
Honey samples were taken 34 days after start of exposure (S14-00167-01), 21 days after start of exposure (S14-00167-02) and 19 days after start of exposure (S14-00167-03) from the still confined colonies. After honey harvest, the honey samples were stored deep-frozen until being analysed for residues of thiacloprid.
Analysis of residues of thiacloprid in honey was carried out in the analytical laboratories of the Bayer CropScience AG, Germany. Honey samples were analysed for residues of thiacloprid following the provisions of the Bayer CropScience method 01155/M001. The Limit of Quantification (LOQ), defined as the lowest validated fortification level, was 1 µg/kg (1 ppb). The Limit of Detection (LOD), defined as the linearity response data of the lowest-concentration standard, was 0.3 µg/kg (0.3 ppb).

Results: Analytical phase report

Report: [redacted]; [redacted]; [redacted]; 2014; M-495580-01-1
Title: Determination of residues of thiacloprid OD 240B G in honey after application of thiacloprid OD 240B G just before flowering in a semi-field residue study with honeybees (Apis mellifera L.) in Winter Oilseed Rape in 2014
Report No.: S14-00167
Document No.: M-495580-01-1
Guidelines: **Guideline No.1607/VI/97 (2) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009**
OECD Principles of Good Laboratory Practice (OECD 1998); not applicable
GLP/GEP: **yes**

The field part of the study was performed by Eurofins Agroscience Services GmbH, 75223 Niefern-Oeschelbronn, Germany and is not documented in the raw data of this Analytical Phase Report. A final report, including besides the field part of the study and also this Analytical Phase Report, will be prepared by the study director.

All samples were stored deep-frozen at the field-site accommodation until transport. The samples were sent deep-frozen on dry ice by a professional shipping agent on 2014-06-03 and 2014-06-04. They arrived on 2014-06-04 and 2014-06-05 at the Test Site Bayer CropScience AG, Institute for Human Safety – Residue Analysis (BCS-R&D-D-HS-RA) in good and deep-frozen conditions.

All samples were stored thereafter deep-frozen in the analytical laboratory until analysis.

Samples of honey belonging to honeybees exposed to thiacloprid treated winter oil seed rape plants were analysed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

Analysis of honey samples was performed according to 01155/M001. The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 1 µg/kg. The Limit of Detection (LOD), defined as the linearity response data of the lowest-concentration standard, was 0.3 µg/kg.
The analytical method for honey was validated by running concurrent recovery experiments at the LOQ (1 µg/kg) and 10-fold LOQ (10 µg/kg). Recovery experiments were performed by spiking honey samples with defined amounts of thiacloprid, respectively. Fortification levels and recovery data are given in the following table. For the recovery experiments, untreated honey was used.

Table 6.10-1: Recoveries thiacloprid in honey samples

Matrix	Fortification Level (FL) [µg/kg]	Recoveries [%] (Single Values)				per FL		Overall	
						Mean [%]	RSD [%]	Mean [%]	RSD [%]
Honey	1	91	81	94	102	92	9.4	89	8.8
	10	79	84	88	96	87	8.3		

The individual recovery values of thiacloprid in honey were within the range of 79 to 102%, with an overall mean recovery of 89%. The corresponding relative standard deviation (RSD) was 8.8% (n = 8).

The average recoveries were within the acceptable range of 70 – 110%. RSD values were below 20%. Then the method can be considered successfully validated.
The analytical results of the honey samples belonging to the three locations, one in France and two in Germany are presented in the following table 6.10-2.

Table 6.10-2: Residues of thiacloprid in honey

Trial No. (Country)	Sample Name	Sample Type	Treatment	Residue Thiacloprid [µg/kg]
S14-00167-01 Germany	S14-00167-01-001A C	Honey	Control	<LOD
	S14-00167-01-002A T		Treated	<LOD
	S14-00167-01-003A T			<LOD
	S14-00167-01-004A T			<LOD
S14-00167-02 Germany	S14-00167-02-001A C	Honey	Control	<LOD
	S14-00167-02-002A T		Treated	1
	S14-00167-02-003A T			1
	S14-00167-02-004A T			2
S14-00167-03 France	S14-00167-03-001A C	Honey	Control	<LOD
	S14-00167-03-002A T		Treated	<LOD
	S14-00167-03-003A T			<LOD
	S14-00167-03-004A T			2
	S14-00167-03-005A T			<LOD

LOQ = 1 µg/kg and LOD = 0.3 µg/kg

No residues of thiacloprid at or above the respective limit of detection were found in any of the untreated samples taken from control. No residues of thiacloprid at or above the respective limit of detection were found in any of the treated samples taken from trial S14-00167-01. Residues of thiacloprid were found in all treated samples (Ta, Tb, Tc) taken from trial S14-00167-02. The respective concentration ranged from 1 µg a.s./kg (1 ppb) in Ta and Tb to a maximum of 2 µg a.s./kg (2 ppb) in Tc. A maximum residue level of 2 µg a.s./kg (2 ppb) was also found in one of the treated samples (Tc) taken from trial S14-00167-03.

Conclusion

Following exposure of honeybees under confined semi-field conditions to winter oilseed rape, treated sequentially two times at a nominal rate of 72 g a.s./ha with Thiacloprid, OD 240 B G via foliar application, just before onset of flowering, the maximum thiacloprid residues in honey (aged nectar), collected from the employed and still tunnel-confined colonies, accounted to 2 µg a.s./kg (2 ppb).

As expected the residue level of thiacloprid transferred into honey can be considered as negligible since in all samples it is below the threshold value of 0.01 mg/kg.