Document Title

Summary of the Residues in or on treated products, food and feed for Isoflucypram

(Code: BCS-CN88460)

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 283/2013

Document MCA

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

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[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

INTRODUCTION

Isoflucypram (CAS-No. 1255734-28-1) is a new fungicidal active substance developed by Bayer. This document supports the application for regulatory approval of Isoflucypram in Europe under Regulation (EC) No 1107/2009.

The document MCA Section 6 summarises all metabolism and residue data in or on treated products of plant and animal origin and consumer dietary risk assessments which are relevant for the approval of Isoflucypram and the proposed intended uses, including the representative uses, under Regulation (EC) No 1107/2009 in accordance with the requirements laid down in the Commission Regulation (EU) No 283/2013.

Isoflucypram is a novel broad spectrum fungicide of the chemical class of N-cyclopropyl-N-benzyl-pyrazole-carboxamides with an outstanding efficacy against the major economically important fungal diseases of cereal crops (wheat, triticale, rye, barley and oats) and excellent crop safety.

Since Isoflucypram is an SDH inhibitor and thus assigned to the FRAC resistance Group 7 the application scope of Isoflucypram-containing products on cereals with only one foliar spray at a maximum of 75 g a.s./ha supports an effective antr-resistance management strategy.

Tailor-made and broad spectrum Isoflucypram combinations show highly beneficial properties in terms of plant physiology beside the long-lasting and certain curative efficacy to control fungal diseases and to maximize the full yield potential of the cereal crops.

Details of the literature search undertaken are summarized in MCA Section 9. For Isoflucypram and its metabolites, no publication and relevant scientifically peer-reviewed open literature reference has been identified which would indicate that a side-effect on human health, the environment and non-target species may exist, which would then need to be considered in the risk assessment of this new active substance dossier.

Throughout the development of Isoflucypram the following synonyms may have been used and also referred to in individual study reports: Bayer Code: BCS-CN88460, BCS-CN88460-a.s., ´460 and the Bayer-internal short Code: ISY. All chemical substances described by either of these codes refer to the same chemical name and structural formula.

In this MCA Summary Section 6 the following codes will be used for the metabolites:

Name	Metab. No.	Standard "dossier name"
BCS-CR60082	M49	BCS-CN88460-N-methyl-cyclopropyl- pyrazole-carboxamide
BCS-DC20298	M02	BCS-CN88460-2-propanol
BCS-CY26497	M12	BCS-CN88460-carboxylic acid
BCS-CY24813	M01	BCS-CN88460-propanol
BCS-DC22055	M06	BCS-CN88460-desmethyl-propanol
BCS-CX99799	M11	BCS-CN88460-desmethyl-carboxylic acid
--	M19	BCS-CN88460-propanol-GlucA
--	M20	BCS-CN88460-2-propanol-GlucA
--	M37	BCS-CN88460-desmethyl-propanol-GlucA

CA 6.1 Storage stability of residues

As residue samples in trials with BCS-CN88460 on crops were routinely stored frozen prior to their analysis, the effects of frozen storage on the residue levels were investigated.

► PLANT MATRICES

The longest periods of frozen storage of samples from plant residue studies (field residue, processing, or rotational crop trials) are shown in Table 6.1- 1 below.

Table 6.1- 1: Periods of frozen storage (approx. -18°C) of plant-based samples (between sampling and analysis) for matrices relevant to this dossier

Sample material		Longest storage duration (days)	Study	
Crop	**Matrix**		**Report No.**	**Reference**
Barley	green material	390	15-2118 15-2117	M-583909-02-1 KCA 6.3.1/03 M-583692-02-1 KCA 6.3.1/07
	straw	350	15-2118	M-583909-02-1 KCA 6.3.1/03
	grain	396		
	beer brewer's grain brewer's malt brewer's yeast hops draff malt sprouts pearl barley pearl barley rub off	297	15-3407	M-579494-01-1 KCA 6.5.3/01
Wheat	green material	398	15-2119	M-584690-02-1 KCA 6.3.2/07
	straw	355		
	grain	402	RALNN137	M-600505-02-1 KCA 6.5.3/02
	bran flour whole meal germ middlings shorts pasta, fresh pasta, dry pasta, cooked pasta, dried and cooked gluten starch aspirated grain cooking water white bread whole meal bread	119		

Sample material		Longest storage duration (days)	Study	
Crop	Matrix		Report No.	Reference
Turnip	body	194	15-2502	M-605705-01-1 KCA 6.6.2/01
	leaf	193		
Carrot	root	300		
	leaf	299		
Lettuce	head	341		

A study was initiated to evaluate the stability of BCS-CN88460 and its metabolite BCS-CR60082 during deep freezing storage (≤ -18°C) for a period of 24 months in tomato (fruit), bean (dry seed), wheat (grain), rape (seed) and orange (fruit). The following interim report describes the effects of deep freezing storage over a period of 18 months.

Report: KCA 6.1/01; [redacted]; 2017; M-605556-01-1
Title: Storage stability of residues of BCS-CN88460 and its metabolite BCS-CR60082 in tomato (fruit), bean (dry seed), wheat (grain), rape (seed) and orange (fruit) during deep freeze storage for at least 24 months
Report No.: MR-17/244
Document No.: M-605556-01-1
Guideline(s): Commission Regulation (EU) No 283/2013 of 1 March 2013 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances [2].
US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data - August 1996 [3]
OECD Test Guideline 506, adopted 16 October 2007 [4]
Guideline deviation(s): not specified
GLP/GEP: **yes**

I. Materials and Methods

To determine the freezer storage stability of the relevant residues of BCS-CN88460 in plant materials, individual 5-g control samples of orange fruit (high acid content), tomato fruit (high water content), wheat grain (high starch content), bean dry seed (high protein content) and rape seed (high oil content) were separately spiked with either BCS-CN88460 parent compound or BCS-CR60082 at a level of 0.20 mg/kg. The samples were stored in High Density Poly Ethylene (HDPE) Nalgene containers at an average temperature of -18 °C or below. Tomato (fruit), bean (dry seed), wheat (grain) and rape (seed) were analyzed at the nominal storage intervals of 0, 3, 8, 13 and 18 months for this interim report. The samples of orange (fruit) were analyzed at the nominal storage intervals of 0 and 6 days, and 1, 3, 8, 13 and 18 months.

For orange (fruit), although the RSDs on Day 0 remained acceptable (<20%), the variability of results was higher than for the other matrices and higher compared to what was observed during the method validation. It was thus decided to increase the number of analyses for the later storage intervals and to start a second set of orange (fruit) sample in order to ensure that sufficient samples would be available to investigate the stability of residues over 24 months. The new set of orange (fruit) was separately fortified with BCS-CN88460 and BCS-CR60082 at 0.20 mg/kg. The samples stored in HDPE Nalgene containers at an average temperature of -18 °C or below, and analyzed at the nominal storage intervals of 0, 5 and 10 months for this interim report.

The results covering a storage interval 24 months will be given in the final report.

For Day 0 analysis, three stored spiked samples of each material were analyzed, alongside with one control sample and concurrent recoveries at 0.01 and 0.20 mg/kg. At later storage intervals, two stored spiked samples were analysed (3 for orange fruit) alongside with one control sample and concurrent

recoveries at 0.01 and 0.20 mg/kg. Samples used for concurrent recoveries were prepared at the same time and stored in the same way as the control samples, and spiked on the day of analysis.

Concurrent recoveries at 0.01 and 0.20 mg/kg were conducted for each sample material, at each storage interval.

Residues of BCS-CN88460 and its metabolite BCS-CR60082 were determined according to the analytical method 01475 ([redacted]; 2016; M-558986-01-1, referenced in MCA Section 4 under Point 4.1.2 (e)) which had been validated for the sample materials relevant to this study in the method 01475. Samples of orange fruit, tomato fruit, wheat grain, bean dry seed and rape seed were analyzed according to the procedure described in the method for these matrices. The LOQ was 0.01 mg/kg for BCS-CN88460 and BCS-CR60082, expressed as parent.

II. Findings

At each storage interval, apparent residues of BCS-CN88460 and BCS-CR60082 in the control samples were below 30% of the LOQ (LOQ=0.01 mg/kg) for each sample material except for BCS-CR60082 at the storage interval 6 days in orange (fruit) where the control sample was at 0.003 mg/kg. Nevertheless, this result was consider acceptable.

For BCS-CN88460, all the concurrent recovery means were within the acceptable range of 70-110% with corresponding RSDs (relative standard deviation) below 20% except the recovery mean of 111% at the storage interval 561 days for bean (dry seed). Nevertheless, this was considered acceptable since it only slightly exceeds the criteria laid down in the EU guidance SANCO/3029/99 rev. 4, but remains within the OECD guidance ENV/JM/MONO(2007)17 criteria.

For BCS-CR60082, all the recovery means were within the acceptable range of 70-110% with corresponding RSDs (relative standard deviation) < 20%.

Details on concurrent recovery data are shown in Table 6.1- 2 for BCS-CN88460 and Table 6.1- 3 for BCS-CR60082.

On Day 0, average residue recoveries of BCS-CN88460 ranged from 96-110% of the nominal spiked concentration in the stored samples; and ranged from 92-110% for BCS-CR60082. In samples analysed after approximately 18 months of frozen storage (558-563 days), storage stability recoveries ranged from 89-104% for BCS-CN88460 and 82-102% for BCS-CR60082. At all sampling dates and in all sample materials, the relevant components of the residue of BCS-CN88460 were above 70%. Even in the case of the lower values in the given ranges, there was no evidence of any continued degradation of any of the analytes in any of the sample materials. Thus, both analytes can be considered stable in all relevant plant matrix types for a period of at least 18 months (558 to 563 days).

All storage stability results are summarised below in Table 6.1- 4 for BCS-CN88460 and Table 6.1- 5 for BCS-CR60082.

The time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours for tomato fruit, bean dry seed, wheat grain and rape seed. For orange fruit and the compound BCS-CR60082, the analysis for time point T=0 was done after a maximum time of 105 hours from the beginning of preparation (most of the samples had to be rediluted from the Extract A to be analysed). This storage period is covered by the stability experiments done in method 01475 ([redacted]; 2016; M-558986-01-1, referenced in MCA Section 4 under Point 4.1.2 (e)) which indicated that Extract A are stable for at least a period of 149 hours for orange (fruit).

III. Conclusions

During a storage period of 18 months under deep-freezer conditions, BCS-CN88460 and BCS-CR60082 were stable in orange fruit (high acid content), tomato fruit (high water content), wheat grain (high starch content), bean dry seed (high protein content) and rape seed (high oil content), representing a wide array of plant-based sample materials. According to OECD guideline 506 on the

stability of pesticide residues in stored commodities, it can be concluded that residues of BCS-CN88460 and BCS-CR60082 are stable for 18 months at ≤ -18°C in all raw agricultural commodities and processed commodities.

These results validate the residue values reported in all supervised field trials and processing studies with respect to storage stability of samples frozen prior to analysis.

Table 6.1- 2: Concurrent recovery data for BCS-CN88460

Plant material	Fortification Level [mg/kg]	Date of Extraction	Storage Interval (days)	BCS-CN88460 Single Recoveries [%]			Mean [%]	RSD [%]	Standard deviation
Tomato (fruit)	0.01	2016-03-29	0	100	-	-	-	-	-
		2016-07-07	100	96	-	-	-	-	-
		2016-12-05	251	103	-	-	-	-	-
		2017-05-18	415	106	-	-	-	-	-
		2017-10-13	563	97	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**100**	**4.1**	**4.2**
Tomato (fruit)	0.20	2016-03-29	0	98	102	103	101	2.6	2.6
		2016-07-07	100	82	89	-	86	-	-
		2016-12-05	251	106	103	-	105	-	-
		2017-05-18	415	95	84	-	90	-	-
		2017-10-13	563	100	103	-	102	-	-
	Overall Mean, RSD and standard deviation [%]						**95**	**8.6**	**8.3**
Bean (dry seed)	0.01	2016-03-30	0	99	-	-	-	-	-
		2016-07-08	100	99	-	-	-	-	-
		2016-12-01	246	97	-	-	-	-	-
		2017-05-19	415	87	-	-	-	-	-
		2017-10-12	561	115	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**98**	**10.9**	**10.7**
Bean (dry seed)	0.20	2016-03-30	0	99	99	98	99	0.6	0.6
		2016-07-08	100	97	88	-	93	-	-
		2016-12-01	246	98	101	-	100	-	-
		2017-05-19	415	76	82	-	79	-	-
		2017-10-12	561	113	109	-	111		
	Overall Mean, RSD and standard deviation [%]						**96**	**11.2**	**10.8**
Wheat (grain)	0.01	2016-03-31	0	92	-	-	-	-	-
		2016-07-11	102	93	-	-	-	-	-
		2016-12-05	249	104	-	-	-	-	-
		2017-05-19	414	88	-	-	-	-	-
		2017-10-12	560	108	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**97**	**8.8**	**8.5**
Wheat (grain)	0.20	2016-03-31	0	95	94	97	95	1.6	1.5
		2016-07-11	102	94	96	-	95	-	-
		2016-12-05	249	102	102	-	102	-	-
		2017-05-19	414	97	87	-	92	-	-
		2017-10-12	560	90	103	-	97	-	-
	Overall Mean, RSD and standard deviation [%]						96	5.2	5.0
Rape (seed)	0.01	2016-03-31	0	113	-	-	-	-	-
		2016-07-12	103	93	-	-	-	-	-
		2016-12-01	245	94	-	-	-	-	-

Plant material	Fortification Level [mg/kg]	Date of Extraction	Storage Interval (days)	BCS-CN88460 Single Recoveries [%]			Mean [%]	RSD [%]	Standard deviation
		2017-05-22	417	86	-	-	-	-	-
		2017-10-10	558	97	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**97**	**10.4**	**10.0**
Rape (seed)	0.20	2016-03-31	0	100	99	96	98	2.1	2.1
		2016-07-12	103	92	83	-	88	-	-
		2016-12-01	245	98	96	-	97	-	-
		2017-05-22	417	91	93	-	92	-	-
		2017-10-10	558	87	83		85		
	Overall Mean, RSD and standard deviation [%]						**93**	**6.5**	**6.1**
Orange (fruit)	0.01	2016-03-29	0	82	-	-	-	-	-
		2016-04-04	6	82					-
		2016-05-02	34	87	-				-
		2016-07-13	106	(1)	-	-	-	-	
		2016-12-08	254	94		-			-
		2017-05-18	415	85		-			-
		2017-10-09	559	106	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**89**	**10.4**	**9.3**
Orange (fruit)	0.20	2016-03-29	0	76	101	71	83	19.4	16.1
		2016-04-04	6	92	82	94	89	7.2	6.4
		2016-05-02	34	104	77	88	90	15.1	13.6
		2016-07-13	106	92	99	88	93	6.0	5.6
		2016-12-08	254	87	83	108	93	12.5	13.4
		2017-05-18	415	89	80	96	88	9.1	8.0
		2017-10-09	559	101	109	105	105	3.8	4.0
	Overall Mean, RSD and standard deviation [%]						**92**	**12.0**	**10.9**
Orange * (fruit)	0.01	2016-12-02	0	108	-	-	-	-	-
		2017-05-18	167	93	-	-	-	-	-
		2017-10-18	315	103			-	-	-
	Overall Mean, RSD and standard deviation [%]						**101**	**7.5**	**7.6**
Orange * (fruit)	0.20	2016-12-02	0	101	106	104	104	2.4	2.5
		2017-05-18	167	94	82	89	88	6.8	6.0
		2017-10-18	315	101	102	97	100	2.6	2.6
	Overall Mean, RSD and standard deviation [%]						**97**	**8.0**	**7.8**

RSD: relative standard deviation
* New set of Orange (fruit)
Fortification as: BCS-CN88460 Determination as: BCS-CN88460 Calculated as: BCS-CN88460
(1): value not retained

Table 6.1- 3: Concurrent recovery data for BCS-CR60082

Plant material	Fortification Level [mg/kg]	Date of Extraction	Storage Interval (days)	BCS-CR60082 Single Recoveries [%]			Mean [%]	RSD [%]	Standard deviation
Tomato (fruit)	0.01	2016-03-29	0	88	-	-	-	-	-
		2016-07-07	100	101	-	-	-	-	-
		2016-12-05	251	100	-	-	-	-	-
		2017-05-18	415	97	-	-	-	-	-
		2017-10-13	563	90	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**95**	**6.2**	**5.9**
Tomato (fruit)	0.20	2016-03-29	0	94	95	93	94	1.1	1.0
		2016-07-07	100	84	86	-	85	-	-
		2016-12-05	251	102	97	-	100	-	-
		2017-05-18	415	92	82	-	87	-	-
		2017-10-13	563	95	102	-	99	-	-
	Overall Mean, RSD and standard deviation [%]						**93**	**7.1**	**6.6**
Bean (dry seed)	0.01	2016-03-30	0	84	-	-	-	-	-
		2016-07-08	100	99	-	-	-	-	-
		2016-12-01	246	87	-	-	-	-	-
		2017-05-19	415	81	-	-	-	-	-
		2017-10-12	561	110	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**92**	**13.1**	**12.1**
Bean (dry seed)	0.20	2016-03-30	0	93	94	94	94	0.6	0.6
		2016-07-08	100	94	84	-	89	-	-
		2016-12-01	246	91	95	-	93	-	-
		2017-05-19	415	74	77	-	76	-	-
		2017-10-12	561	97	94	-	96	-	-
	Overall Mean, RSD and standard deviation [%]						**90**	**8.7**	**7.8**
Wheat (grain)	0.01	2016-03-31	0	87	-	-	-	-	-
		2016-07-11	102	92	-	-	-	-	-
		2016-12-05	249	95	-	-	-	-	-
		2017-05-19	414	80	-	-	-	-	-
		2017-10-12	560	101	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**91**	**8.8**	**8.0**
Wheat (grain)	0.20	2016-03-31	0	90	93	92	92	1.7	1.5
		2016-07-11	102	89	93	-	91	-	-
		2016-12-05	249	99	97	-	98	-	-
		2017-05-19	414	89	81	-	85	-	-
		2017-10-12	560	84	90	-	87	-	-
	Overall Mean, RSD and standard deviation [%]						**91**	**5.7**	**5.2**
Rape (seed)	0.01	2016-03-31	0	116	-	-	-	-	-
		2016-07-12	103	110	-	-	-	-	-
		2016-12-01	245	91	-	-	-	-	-
		2017-05-22	417	97	-	-	-	-	-
		2017-10-10	558	110	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**105**	**9.9**	**10.4**

Plant material	Fortification Level [mg/kg]	Date of Extraction	Storage Interval (days)	BCS-CR60082 Single Recoveries [%]			Mean [%]	RSD [%]	Standard deviation
Rape (seed)	0.20	2016-03-31	0	99	94	97	97	2.6	2.5
		2016-07-12	103	87	81	-	84	-	-
		2016-12-01	245	101	94	-	98	-	-
		2017-05-22	417	92	94	-	93	-	-
		2017-10-10	558	81	81	-	81	-	-
	Overall Mean, RSD and standard deviation [%]						91	8.1	7.4
Orange (fruit)	0.01	2016-03-29	0	82	-	-	-	-	-
		2016-04-04	6	92	-	-	-	-	-
		2016-05-02	34	78	-	-	-	-	-
		2016-07-13	106	63	-	-	-	-	-
		2016-12-08	254	83	-	-	-	-	-
		2017-05-18	415	74	-	-	-	-	-
		2017-10-09	559	106	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**83**	**16.5**	**13.6**
Orange (fruit)	0.20	2016-03-29	0	75	87	67	76	13.2	10.1
		2016-04-04	6	84	65	79	76	13.0	9.8
		2016-05-02	34	93	69	81	81	14.8	12.0
		2016-07-13	106	84	87	81	84	3.6	3.0
		2016-12-08	254	80	76	96	84	12.6	10.6
		2017-05-18	415	83	78	87	83	5.5	4.5
		2017-10-09	559	92	97	101	97	4.7	4.5
	Overall Mean, RSD and standard deviation [%]						**83**	**11.6**	**9.7**
Orange* (fruit)	0.01	2016-12-02	0	93	-	-	-	-	-
		2017-05-18	167	77	-	-	-	-	-
		2017-10-13	315	91	-	-	-	-	-
	Overall Mean, RSD and standard deviation [%]						**87**	**10.0**	**8.7**
Orange* (fruit)	0.20	2016-12-02	0	97	99	94	96	3.0	2.9
		2017-05-18	167	86	78	85	83	5.3	4.3
		2017-10-13	315	107	101	100	103	3.7	3.8
	Overall Mean, RSD and standard deviation [%]						**94**	**9.8**	**9.2**

RSD: relative standard deviation
* New set of Orange (fruit)
Fortification as: BCS-CR60082 Determination as: BCS-CR60082 Calculated as: BCS-CN88460

Table 6.1- 4: Storage stability data and concurrent recovery data for BCS-CN88460

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Tomato (fruit)	0	0.197 0.199 0.207	99 99 104	101	100	101	100
	100	0.199 0.193	100 97	99	98	86	115
	251	0.198 0.204	99 102	101	100	105	96
	415	0.172 0.180	86 90	88	87	90	98
	563	0.192 0.201	96 101	99	98	102	97
Bean (dry seed)	0	0.204 0.200 0.203	102 100 102	101	100	99	103
	100	0.205 0.206	103 103	103	102	92	111
	246	0.195 0.195	98 98	98	97	100	98
	415	0.168 0.167	84 84	84	83	79	106
	561	0.208 0.206	104 103	104	102	111	93
Wheat (grain)	0	0.194 0.189 0.194	97 95 97	96	100	95	101
	102	0.187 0.189	94 94	94	98	95	99
	249	0.201 0.194	100 97	99	102	102	97
	414	0.177 0.181	89 91	90	93	92	98
	560	0.189 0.170	94 85	90	93	97	93
Rape (seed)	0	0.201 0.188 0.189	101 94 95	97	100	98	98
	103	0.160 0.194	80 97	89	92	88	101
	245	0.184 0.200	92 100	96	99	97	99
	417	0.193 0.186	97 93	95	98	92	103
	558	0.180 0.175	90 88	89	92	85	105

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Orange (fruit)	0	0.251 0.175 0.194	126 88 97	104	100	83	125
	6	0.189 0.182 0.175	95 91 88	91	88	89	102
	34	0.213 0.203 0.183	107 101 91	100	96	90	111
	106	0.121 0.173 0.186	61 87 93	80	77	93	86
	254	0.181 0.188 0.200	90 94 100	95	91	93	102
	415	0.169 0.183 0.183	84 92 92	89	86	88	101
	559	0.207 0.195 0.201	103 97 100	100	96	105	95
Orange* (fruit)	0	0.228 0.208 0.223	114 104 111	110	100	104	106
	167	0.185 0.172 0.167	93 86 83	87	80	88	99
	315	0.204 0.191 0.204	102 95 102	100	91	100	100

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%
* New set of Orange (fruit)

Table 6.1- 5: Storage stability data and concurrent recovery data for BCS-CR60082

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Average % recovery			
Tomato (fruit)	0	0.192 0.186 0.189	96 93 94	94	100	94	100
	100	0.196 0.191	98 96	97	103	85	114
	251	0.199 0.203	100 102	101	107	100	102
	415	0.175 0.174	88 87	88	93	87	101
	563	0.180 0.184	90 92	91	96	99	92
Bean (dry seed)	0	0.183 0.191 0.183	91 95 91	92	100	94	99
	100	0.189 0.203	94 102	98	106	89	110
	246	0.193 0.196	97 98	98	106	93	105
	415	0.154 0.169	77 85	81	88	76	107
	561	0.171 0.174	86 87	87	94	96	91
Wheat (grain)	0	0.188 0.179 0.181	94 90 91	92	100	92	100
	102	0.192 0.187	96 94	95	104	91	104
	249	0.208 0.207	104 104	104	113	98	106
	414	0.177 0.175	89 88	89	97	85	104
	560	0.169 0.159	85 79	82	89	87	94
Rape (seed)	0	0.227 0.227 0.208	114 113 104	110	100	97	114
	103	0.151 0.154	76 77	77	69	84	91
	245	0.199 0.191	100 95	98	88	98	100
	417	0.181 0.195	91 97	94	85	93	101
	558	0.172 0.178	86 89	88	79	81	108

Commodity	Storage Period (days)	Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Average % recovery			
Orange (fruit)	0	0.197 0.176 0.180	98 88 90	92	100	76	121
	6	0.159 0.119 0.145	80 60 72	71	77	76	93
	34	0.178 0.174 0.134	89 87 67	81	88	81	100
	106	0.190 0.172 0.185	95 86 93	91	99	84	109
	254	0.186 0.174 0.185	93 87 93	91	99	84	108
	415	0.166 0.142 0.149	83 71 74	76	83	83	92
	559	0.194 0.197 0.219	97 99 109	102	111	97	105
Orange* (fruit)	0	0.198 0.209 0.203	99 104 102	102	100	96	106
	161	0.180 0.168 0.155	90 84 77	84	82	83	101
	315	0.178 0.212 0.208	89 106 104	100	98	103	97

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%
* New set of Orange (fruit)

► ANIMAL MATRICES

Information on stability of residues is given in the hen and goat metabolism studies (see Table 6.1- 6).

During these studies, all samples and extracts were stored in a freezer at ≤-18°C or for a short time in a refrigerator. All samples of milk, eggs, excreta, edible organs and tissues were extracted within several months after sample collection. Quantitative analysis by HPLC was performed either on the day of extraction or up to six days after the start of extraction.

A second conventional extraction of liver and kidney was performed within 9-20 months after collection to be used for enzymatic cleavage experiments. Analyses of these second conventional extracts of liver and kidney showed no indication of degradation of parent compound and metabolites in the profiles when comparing to the HPLC metabolite profiles obtained after the first extraction. The storage stability of parent compound and metabolites in goat and hen liver as well as in goat kidney was exemplarily demonstrated for all other matrices.

Table 6.1- 6: Storage stability data from livestock metabolism studies

Sample	Storage temperature (°C)	Initial analysis after sample collection	Demonstrated storage stability	Document No.	Reference
Hen Liver	≤-18°C	< 5 months	20 months	M-601665-01-1	KCA 6.2.2/01
Hen Liver	≤-18°C	< 3 months	9 months	M-601667-01-1	KCA 6.2.2/02
Goat Liver	≤-18°C	Approx. 3 months	Approx. 13 months	M-604281-01-1	KCA 6.2.3/01
Goat Kidney	≤-18°C	Approx. 3 months	Approx. 14 months		
Goat Liver	≤-18°C	Approx. 3 months	11 months	M-604286-01-1	KCA 6.2.3/02

The longest storage periods for samples from animal residue studies (feeding studies) are shown in Table 6.1- 7.

Table 6.1- 7: Periods of frozen storage (≤ -18°C) of animal-based samples (between sampling and analysis) for matrices relevant to this dossier

Sample material		Analyte group*	Longest storage duration (days)	Study	
Animal	Matrix			Report No.	Annex point Document No.
ruminant	milk	A	< 30	17-8001	6.4.2/01 M-604191-02-1
	cream	A			
	whey	A			
	fat	A			
	muscle	A			
	kidney	A & B			
	liver	A & B			
poultry	eggs	A	< 30	17-8002	6.4.1/01 M-605909-01-1
	fat	A			
	muscle	A			
	liver	A & C			

* Analyte group A comprises free residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99790
Analyte group B comprises the sum of BCS-CY24813 and its conjugate M19, the sum of BCS-DC20298 and its conjugate M20
Analyte group C comprises the sum of BCS-DC22055 and its conjugate M37

In the dairy ruminant and poultry feeding studies, the analyses were done within 30 days after sample collection. Therefore further storage stability data for the relevant residues of BCS-CN88460 in all animal matrices are not necessary.

In most of the cases, the time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours in the residue studies. If not the case, the maximum storage period of extracts was covered by stability experiments conducted in the course of the analytical methods validations ([redacted]; 2016; M-558986-01-1; [redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2 (e)), in the residue study J3-2066 ([redacted], G.; 2017; M-584388-02-1) or in the residue study 15-2069 ([redacted], G.; 2017; M-584384-02-1; KCA 6.3.2). These stability experiments are summarized in the MCA Summary Section 4 and referenced under Point 4.1.2 (e) and also in Table 6.1- 8.

Table 6.1- 8: Stability of residues in extracts

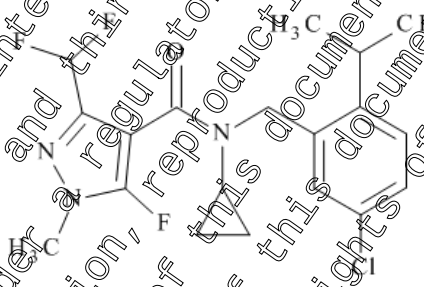
Matrices	Compound tested	Extracts	Storage conditions	Stable for at least..	Reference
Tomato (fruit)	BCS-CN88460 BCS-CR60082	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	105 hours 105 hours	M-558986-01-1 KCA 4.1.2 method 01455
Orange (fruit)	BCS-CN88460 BCS-CR60082	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	149 hours 149 hours	
Wheat (grain)	BCS-CN88460 BCS-CR60082	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	82 hours 82 hours	
Wheat (straw)	BCS-CN88460 BCS-CR60082	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	79 hours 79 hours	
Rape (seed)	BCS-CN88460 BCS-CR60082	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	54 hours 54 hours	
Bean (dry seed)	BCS-CN88460 BCS-CR60082	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	77 hours 77 hours	
Barley (grain)	BCS-CN88460	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	81 hours 81 hours	M-584388-02-1 KCA 6.3.1 study 15-2066
Barley (straw)	BCS-CN88460	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	83 hours 83 hours	
Barley (green material)	BCS-CN88460	Extract A Final extract	4 °C ± 3 °C 10 °C ± 3 °C	81 hours 81 hours	
Wheat (green material)	BCS-CN88460	Final extract	10 °C ± 3 °C	98.5 hours	M-584384-02-1 KCA 6.3.2 study 15-2069
Hen egg	BCS-CN88460	Extract A	5 °C ± 3 °C	23 days	M-599206-01-1 KCA 4.1.2 method 01511
Cow milk	BCS-DC20298	Extract A	5 °C ± 3 °C	23 days	
Cow muscle	BCS-CY26497	Extract A	5 °C ± 3 °C	23 days	
Cow fat	BCS-CY24813	Extract A	5 °C ± 3 °C	27 days	
Cow liver	BCS-DC22055	Extract A	5 °C ± 3 °C	21 days	
Cow kidney	BCS-CX99799	Extract A	5 °C ± 3 °C	22 days	
Hen liver		Extract A	5 °C ± 3 °C	26 days	
Cow kidney (hydrolyse)	BCS-DC20298 + M20	Final extract	5 °C ± 3 °C	22 days	
Cow liver (hydrolyse)	BCS-CY24813 + M19	Final extract	5 °C ± 3 °C	21 days	
Hen liver (hydrolyse)	BCS-DC22055 + M37	Final extract	5 °C ± 3 °C	26 days	

Moreover, relevant information on the stability of residues in the final or any intermediate extracts can be derived from the fortification experiments performed during sample analysis. Every analytical batch contains at least one freshly fortified sample for concurrent recovery determination. The extracts of the fortified samples and of the study samples are handled and stored in parallel. If the recoveries in the fortified samples are within acceptable ranges, the stability of the sample extracts is considered as being sufficiently proven.

Acceptable recoveries measured concurrently with each set of samples ensured the integrity of the sample extracts during the period of time between extraction and analysis.

CA 6.2 Metabolism, distribution and expression of residues

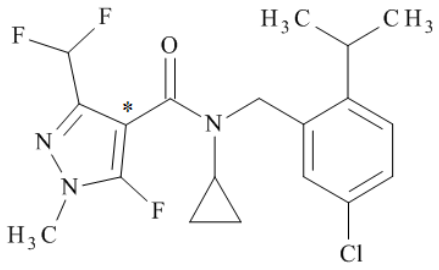
The chemical structure and nomenclature of the fungicide BCS-CN88460 (common name: isoflucypram) are provided below.

Chemical structure	
Common name	Isoflucypram
Company experimental name	BCS-CN88460
IUPAC name	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide
Molecular formula	$C_{19}H_{21}ClF_3N_3O$
Molecular weight	399.84 g/mol

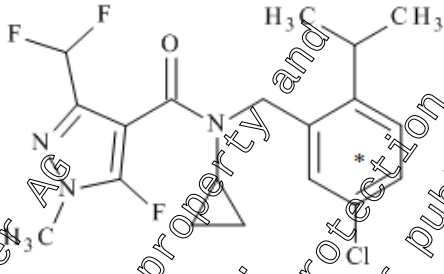
The metabolism of isoflucypram was investigated in primary crops (tomatoes, wheat, soybean, oilseed rape) after post-emergence foliar application and in confined rotational crops. The representative rotational crops were wheat, Swiss chard and turnip which were studied at three plant back intervals. The metabolism of isoflucypram in primary crops is further being investigated in potatoes after seed treatment. The potato metabolism studies are already listed in this dossier, but will be submitted after finalization of the studies.

The metabolic fate of isoflucypram has also been investigated in livestock (lactating goats and laying hens).

As isoflucypram contains separate ring systems, two different radiolabels were used in all plant and animal studies. These label positions are shown below:



[pyrazole-4-^{14}C]



[phenyl-UL-^{14}C]

Numerous metabolites were identified in the metabolism studies. The chemical structures and report names used in the summaries are given at the end of this Section and in the List of Metabolites presented in Document N3. All residue values given in mg/kg refer to parent compound equivalents if not indicated otherwise.

Metabolism in plants

The metabolism of BCS-CN88460 in **tomato fruits** was investigated after two post-emergence spray applications, at a targeted single application rate of 75 g a.s./ha. After foliar application of [pyrazole-4-^{14}C]isoflucypram or [phenyl-UL-^{14}C]isoflucypram to tomatoes, the TRR in tomato fruits was calculated based on the radioactivity in the surface wash solution and the fruit sample and amounted up to 0.170 mg a.s. equivalents/kg in total. Most of the radioactive residues (up to 75% of the TRR) were recovered in the surface wash of tomatoes. The only substance identified in rinse and fruit was unchanged isoflucypram amounting up to 98% of the TRR. Consequently, no relevant metabolisation of BCS-CN88460 occurs in tomato fruits after foliar treatment.

The metabolism of BCS-CN88460 was investigated in **wheat** plants after two spray applications using a nominal application rate of 65 g a.s./ha each. The applications were performed at the growth stage of BBCH 30 (beginning of stem elongation) and BBCH 69 (end of flowering). After post-emergence spray application of [pyrazole-4-^{14}C]isoflucypram or [phenyl-UL-^{14}C] isoflucypram to wheat plants, TRR values in wheat straw and wheat hay were high (up to 16.031 mg/kg and 4.032 mg/kg, respectively) and the TRR values in grains were moderate and amounted up to 0.385 mg/kg. Parent compound was the main component (up to 64% of the TRR) in wheat hay and straw samples and the only component in wheat grain samples (up to 92% of the TRR). Metabolites in wheat hay and straw were detected in lower amounts (equal or below 10% of the TRR) and were formed predominantly by hydroxylation at the benzyl moiety followed by hexose and malonic acid conjugation and to a lesser extend by additional demethylation. No label specific metabolism was observed in wheat.

The metabolism of BCS-CN88460 was investigated in **oilseed rape** plants after two foliar applications using a nominal application rate of 60 g a.s./ha each. The applications were performed at the growth stage of BBCH 14 (trifoliolate on the 3rd up to 5th node unfolded) and BBCH 77 (70% of pods have reached final size). After post-emergence spray application of [pyrazole-4-^{14}C]isoflucypram or [phenyl-UL-^{14}C] isoflucypram to oilseed rape, TRR values in oilseed rape intermediate harvest and mature plants were high and amounted up to 4.751 mg/kg and 4.076 mg/kg, respectively. The TRR values in oilseed rape forage and seeds were low and amounted up to 0.012 mg/kg and to 0.126 mg/kg, respectively. Parent compound BCS-CN88460 was the main component in intermediate harvest and mature plants (up to 88% of the TRR) and the only component in oilseed rape seeds (up to 74% of the TRR). The forage extract contained no residue above the limit of detection. Hexose and malonic acid conjugated metabolites after hydroxylation at the benzyl moiety were formed in oilseed

rape intermediate harvest and mature plants but were detected in low amounts (equal or below 5% of the TRR). No label specific metabolism was observed in oilseed rape.

The metabolism of BCS-CN88460 was investigated in **soybean** plants after three post-emergent plant applications using a nominal application rate of 60 g a.s./ha each. The plants were applied at three different growth stages (BBCH 14, 51 and 84). After post-emergence spray application of [pyrazole-4-^{14}C]isoflucypram or [phenyl-UL-^{14}C]isoflucypram to soybean, TRR values in soybean straw were highest and amounted up to 17.715 mg/kg. The TRR in soybean forage and soybean hay were high and amounted up to 4.371 mg/kg and 4.679 mg/kg, respectively. The TRR values in seeds were low and amounted up to 0.035 mg/kg. Parent compound BCS-CN88460 was the main residue component in soybean straw (up to 70% of the TRR), and a major residue in soybean forage and hay (up to 20% of the TRR). In soybean seeds parent compound was the only component (up to 77% of the TRR). BCS-CN88460 was moderately metabolised in soybean forage, hay and straw samples after three post-emergence applications. Besides parent compound, the following metabolites were identified: BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-homoGSH, BCS-CN88460-desfluoro-mercapto-lactic acid-OH, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA, accounting for up to 23% of the TRR. The main metabolic reaction observed was de-fluorination at position 5 of the pyrazole ring. Subsequently, conjugation with homoglutathione and degradation of the homoglutathione moiety followed by conjugation with malonic acid or degradation and desamination to mercapto lactic acid group and hydroxylation of the benzyl moiety or of the propyl group. No label specific metabolism was observed in soybean.

Overall isoflucypram is moderately metabolized in primary crops and unchanged isoflucypram was the main or a major residue in all plants investigated. In human edible commodities (tomato fruit, soybean grain, rape seed and wheat grain) no metabolites were identified and parent represented the only residue. In general, no label specific metabolisation of the active substance was observed for all primary crops. Metabolites in soybean feed items (straw, forage, hay) were different from the metabolites found in feed items from rape (intermediate harvest, mature plants) and wheat (hay, straw), involving metabolism in soybean through GST (glutathione S-transferase).

Most important metabolisation in soybean occurred at the pyrazole ring: De-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione resulted in metabolite BCS-CN88460-desfluoro homoGSH. Degradation of the homoglutathione moiety followed by conjugation with malonic acid or desamination to mercaptic acid group and hydroxylation at the benzyl moiety resulted in metabolites BCS-CN88460-desfluoro-Cys-MA, BCS-CN88460-desfluoro-mercapto-lactic acid-OH and BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc. Furthermore, glycosilation was clearly observed at the mercapto lactic acid group and formed metabolite BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc.

In feed items from rape (intermediate harvest, mature plants) and wheat (hay, straw) parent compound represented the most prominent residue. In constrast to soybean - where the main metabolic route involved metabolism through GST at the pyrazole moiety - the most important metabolisation observed in rape and wheat was hydroxylation at the benzyl moiety followed by conjugation with hexose and malonic acid. In wheat, hydroxylation at position 1 of the propyl group followed by hexose and malonic acid conjugation and to a minor extent demethylation of the pyrazole moiety formed the metabolites BCS-CN88460-propanol, BCS-CN88460-propanol-Glyc, BCS-CN88460-propanol-Glyc-MA, BCS-CN88460-desmethyl-propanol and BCS-CN88460-desmethyl-propanol-Gly-MA. In rape (intermediate harvest, mature plants) metabolites were generally minor (<5% of TRR). Besides formation of BCS-CN88460-propanol-Glyc-MA also hydroxylation and conjugation at position 2 of the propyl group and of the phenyl moiety was observed forming the metabolites BCS-CN88460-propanol-Glyc-MA, BCS-CN88460-2-propanol-Glyc-MA, BCS-CN88460-hydroxyphenyl-Gluc-MA and BCS-CN88460-hydroxyphenyl-Glyc-MA.

The primary crops represent three different crop categories (fruit, cereals and oilseeds) covering foliar application. Except for soybean, the general metabolic steps in the primary crop metabolism studies were very similar. The divergent metabolism observed for soybean is deemed specific to soybean as this was not observed in rape - which pertains to the same metabolism crop group as soybean – and for rape and wheat common metabolic reactions were observed. Therefore it is concluded that the nature of residues in primary crops after foliar application of isoflucypram is sufficiently understood and that no further studies are needed.

In the following tables the distribution of parent compound and metabolites in the different plant matrices are shown. Generally, isoflucypram forms the main or a major residue in all crops.

Table 6.2- 1: TRR values and distribution of parent compound and metabolites in tomato fruits after two foliar treatments with isoflucypram

Reference	M-597485-01-1	M-597481-01-1
Radiolabel	pyrazole-4-^{14}C	phenyl-UL-^{14}C
Sample	tomato fruits	tomato fruits
Type of application	two foliar	two foliar
Single application rate [g a.s./ha]	79 and 89	78 and 78
Total application rate [g a.s./ha]	168	156
TRR [mg/kg]	0.170	0.095
Compound	% of TRR	% of TRR
Isoflucypram	96.7	98.2
Total identified	96.7	98.2
Total characterised	3.1	1.5
Total extracted	99.8	99.8
Post extraction solids (PES)	0.2	0.2
Total	100.0	100.0

Table 6.2- 2: TRR values and distribution of parent compound and metabolites in wheat matrices after two foliar treatments with isoflucypram

Reference	M-604361-02-1			M-604358-02-1		
Radiolabel	pyrazole-4-^{14}C			phenyl-UL-^{14}C		
Type of application	two foliar			two foliar		
Single application rate [g a.s./ha]	69 and 67			64 and 66		
Total application rate [g a.s./ha]	136			130		
RAC	hay*	straw	grains	hay*	straw	grains
TRR [mg/kg]	4.032	15.536	0.385	3.040	16.031	0.284
Compound	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR
Isoflucypram	50.0	64.0	92.0	54.7	62.1	92.7
M18	2.4	3.7	--	0.8	2.3	--
M41	2.5	2.9	--	2.7	1.9	--
M21	10.3	6.7	--	7.5	5.0	--
M01	0.7	1.7	--	0.7	0.9	--
M06	--	1.0	--	--	0.3	--
Total identified	66.0	80.0	92.0	66.3	72.6	92.7
Total characterised	29.8	18.1	--	30.0	20.5	--
Not analysed	0.4	0.5	1.6	0.5	2.1	0.8
Total extracted	95.8	98.7	93.6	96.7	95.2	93.5
Post extraction solids (PES)	4.2	1.3	6.4	3.3	4.8	6.5
Accountability	100.0	100.0	100.0	100.0	100.0	100.0

* Wheat hay was harvested 1 day prior to the second application
M18: BCS-CN88460-propanol-Glyc
M41: BCS-CN88460-desmethyl-propanol-Glyc-MA
M21: BCS-CN88460-propanol-Glyc-MA
M01: BCS-CN88460-propanol
M06: BCS-CN88460-desmethyl-propanol

Table 6.2- 3: TRR values and distribution of parent compound and metabolites in oilseed rape matrices after two foliar treatments with isoflucypram

Reference	M-609378-01-1				M-609380-01-1			
Radiolabel	pyrazole-4-^{14}C				phenyl-UL-^{14}C			
Type of application	two foliar				two foliar			
Single application rate [g a.s./ha]	64 and 62				63 and 63			
Total application rate [g a.s./ha]	126				126			
RAC	inter-mediate harvest*	forage#	mature plants	seeds	inter-mediate harvest*	forage#	mature plants	seeds
TRR [mg/kg]	4.751	0.012	4.076	0.099	3.295	0.008	3.934	0.126
Compound	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR
Isoflucypram	81.9	---	88.1	71.0	84.1	---	72.0	73.6
M23	2.3	---	0.7	---	2.3	---	2.2	---
M22	2.2	---	0.9	---	1.6	---	4.6	---
M21	2.8	---	0.6	---	2.2	---	2.5	---
M24	3.1	---	1.0	---	3.8	---	3.1	---
Total identified	92.3	---	91.3	71.0	94.0	---	84.4	73.6
Total characterised	6.8	---	6.0	22.3	5.6	---	11.6	19.8
Not analysed	0.4	---	0.2	---	0.1	---	0.3	---
Total extracted	99.5	85.4	97.4	93.3	99.7	77.3	96.3	93.5
PES	0.5	14.6	2.6	6.7	0.3	22.7	3.8	6.5
Accountability	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* Oilseed rape intermediate harvest was harvested 2 days after the first application
# Oilseed rape forage was harvested 40 days after the first application
M23: BCS-CN88460-hydroxyphenyl-Gluc-MA M22: BCS-CN88460-2-propanol-Glyc-MA
M21: BCS-CN88460-propanol-Glyc-MA M24: BCS-CN88460-hydroxyphenyl-Glyc-MA

Table 6.2- 4: TRR values and distribution of parent compound and metabolites in soybean matrices after three foliar treatments with isoflucypram

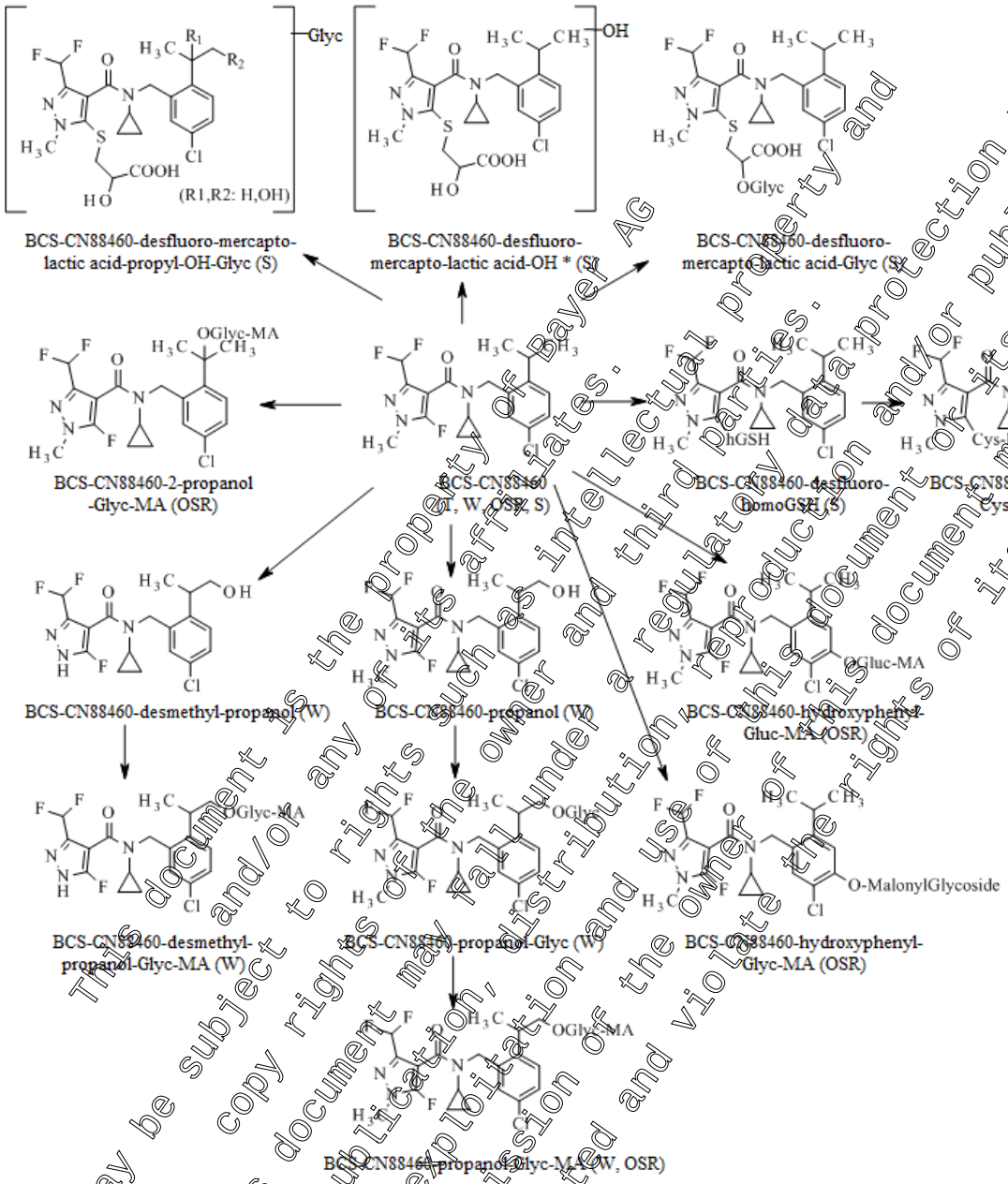
Reference	M-609373-01-1				M-609376-01-1			
Radiolabel	pyrazole-4-^{14}C				phenyl-UL-^{14}C			
Type of application	three foliar				three foliar			
Single application rate [g a.s./ha]	59, 57 and 65				54, 56 and 66			
Total application rate [g a.s./ha]	181				176			
RAC	forage*	hay#	straw	seeds	forage*	hay#	straw	seeds
TRR [mg/kg]	4.371	4.679	17.715	0.035	3.936	1.397	8.527	0.015
Compound	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR	% of TRR
Isoflucypram	18.7	10.4	64.5	76.6	19.2	10.3	70.2	69.8
M48	3.4	15.2	3.9	---	4.8	17.6	2.1	---
M44	22.9	7.8	4.8	---	20.2	7.8	2.8	---
M46	9.5	3.2	1.9	---	17.0	12.8	2.5	---
M47	3.0	11.1	3.0	---	2.7	10.7	2.8	---
M45	9.2	15.4	4.6	---	8.2	20.5	4.3	---
Total identified	66.6	63.2	82.8	76.6	72.2	69.8	84.6	69.8
Total characterised	28.9	29.7	12.8	---	32.8	23.6	10.3	---
Not analysed	1.7	1.4	0.9	11.0	1.7	1.5	1.0	---
PES	2.8	5.7	3.4	12.3	3.4	5.1	4.1	30.2
Accountability	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* Soybean forage was harvested 6 days after the first application
# Soybean hay was harvested 38 days after the second application
M48: BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc
M44: BCS-CN88460-desfluoro-homoGSH
M46: BCS-CN88460-desfluoro-mercapto-lactic acid-OH
M47: BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc
M45: BCS-CN88460-desfluoro-Cys-MA

Based on the results of the metabolism studies in primary crops, only parent compound was proposed as residue definition for data collection and measured in the residue trials presented under Point 6.3.

In the following figure, the proposed metabolic pathway for isoflucypram in primary crops (tomato, T; wheat, W; oilseed rape, OSR and soybean, S) is presented.

Figure 6.2- 1: Proposed metabolic pathway of isoflucypram in primary crops (tomato, T; wheat, W; oilseed rape, OSR and soybean, S)



* BCS-CN88460-desfluoro-mercapto-lactic acid-OH: OH position based on mass spectrometry: benzyl moiety.

Nature of the residues after processing

Isoflucypram was stable under all tested processing conditions.

Based on these results, only parent compound was proposed as residue definition for data collection and measured in the processing studies presented in this MCA Summary under Point 6.5.3.

Metabolism in livestock

The metabolism of isoflucypram in livestock animals has been studied in laying hens and lactating goats.

The TRR-values and even transfer factors for eggs and edible tissues in laying hens after dosing with [phenyl-UL-^{14}C]isoflucypram or [pyrazole-4-^{14}C]isoflucypram were very low with respect to the dose level and the dosing period of 14 days. This indicates that test compound related radioactivity does not accumulate during the time of feeding. The evaluations of the TRR-values should however consider the fact that an exaggerated dose level of up to 18 mg a.s./kg feed/day was administered. Furthermore, the fact that the entire radioactivity was detected in the excreta and the relatively high TRR in kidney and liver at sacrifice approx. 6 hours after the last administration revealed that the test compound related residues are further metabolised and finally eliminated from the hen's bodies. In fat isoflucypram represented the major residue with an amount of up to 0.010 mg/kg. In eggs and leg muscle isoflucypram was detected in low amounts, only. While in thorax muscle and liver unchanged isoflucypram was not found. Isoflucypram was metabolised most extensively in liver. The main metabolic reactions represented demethylation, hydroxylation followed by oxidation to carboxylic acid. Further metabolic reactions included conjugation with glucuronic acid.

The TRR-values and the transfer factors in milk, organs and tissues in lactating goats after dosing with [phenyl-UL-^{14}C]isoflucypram or [pyrazole-4-^{14}C]isoflucypram were very low compared to the dose level of up to 45 mg a.s./kg feed/day and a dosing period of five days. The highest TRR-value was detected for liver and was caused by the short time period of six hours between last dosing and sacrifice. It indicates the significance of this organ for metabolism. The elimination of radioactivity was mainly faecal and less than 10% of the dose was eliminated via urine, which was reflected by the low TRR-value for kidney. This excretion behaviour was similar to the findings in the ADME studies with rats. The TRR-values in the respective evening and morning milk samples showed a diurnal pattern as they declined slightly prior to the delivery of the next dose for most days. A continuous increase was observed before a residue plateau-level was reached at day three after the first administration. Isoflucypram was the main residue in fat. Isoflucypram was intensively metabolised in liver and kidney, where unchanged parent compound was only present in minor amounts. In milk and muscle unchanged parent compound was detected in low amounts but represented a major residue for both matrices. The main metabolism involves demethylation, hydroxylation followed by oxidation to carboxylic acid, and conjugation with glucuronic acid.

In the following tables the distribution of metabolites in edible organs and tissues are presented.

Table 6.2- 5: TRR values and distribution of parent compound and metabolites in laying hens after dosing with isoflucypram

Report	M-601665-01-1					M-601667-01-1				
	Eggs	Leg Muscle	Thorax Muscle	Fat	Liver	Eggs	Leg Muscle	Thorax Muscle	Fat	Liver
TRR [mg/kg]=	0.050	0.029	0.018	0.042	0.370	0.050	0.029	0.017	0.037	0.373
Dosing	14 x 1.0 mg/kg body weight					14 x 1.0 mg/kg body weight				
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Isoflucypram	3.7	2.3	---	23.6	---	6.4	2.9	---	20.1	---
M36	---	---	---	---	5.4	---	4.1	---	---	9.2
M37	---	---	---	---	6.1	3.0	3.8	---	---	10.8
M38	---	---	---	---	2.5	---	---	---	---	3.0
M07	5.2	15.0	17.9	5.1	6.9	6.2	14.2	22.3	5.9	5.6
M26	---	---	---	---	---	---	---	---	---	1.2
M11	---	12.1	12.0	---	14.4	---	19.9	20.2	7.9	21.9
M06	22.3	29.7	20.9	10.1	5.3	22.6	25.8	20.9	8.0	2.7
M12	3.4	9.1	11.0	4.8	11.9	7.2	6.6	8.6	3.0	5.8
M01	35.0	5.3	5.9	11.9	1.7	33.9	2.5	4.3	6.5	1.7
Total identified	69.6	73.5	67.6	55.5	54.3	79.2	79.8	76.3	51.5	62.0
Total characterised	23.7	19.3	24.2	37.1	45.6	13.6	13.5	16.1	40.3	37.9
PES	6.6	7.2	8.2	7.3	0.1	7.2	6.7	7.6	8.2	0.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

M36: BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA
M37: BCS-CN88460-desmethyl-propanol-N-GlucA
M38: BCS-CN88460-desmethyl-2-propanol-N-GlucA
M07: BCS-CN88460-desmethyl-1,2-propandiol
M26: BCS-CN88460-propanol-SA
M11: BCS-CN88460-desmethyl-carboxylic acid
M06 : BCS-CN88460-desmethyl-propanol
M12: BCS-CN88460-carboxylic acid
M01: BCS-CN88460-propanol

Table 6.2- 6: TRR values and distribution of parent compound and metabolites in lactating goats after dosing with isoflucypram

Report	M-604281-01-1					M-604286-01-1			
	Milk	Muscle	Fat	Liver	Kidney	Milk	Muscle	Liver	Kidney
TRR [mg/kg]=	0.015	0.036	0.104	0.717	0.189	0.013	0.011	0.348	0.183
Dosing	5 x 1.0 mg/kg body weight					5 x 1.0 mg/kg body weight			
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Isoflucypram	33.4	22.3	58.7	3.5	2.7	33.9	21.5	5.3	1.6
M50	---	---	---	---	5.8	---	---	---	---
M20	---	---	---	13.8	2.6	---	---	13.0	4.0
M25	---	---	---	---	3.6	---	---	---	6.2
M10	---	---	---	1.6	6.1	---	---	1.3	4.2
M19 (isomer 1)	---	---	---	13.1	3.1	2.3	---	8.8	3.6
M19 (isomer 2)	---	4.8	---	7.7	7.0	2.5	6.5	5.9	8.6
M11	---	---	---	0.9	4.2	---	---	0.5	3.6
M06	---	4.4	---	0.5	1.6	6.7	3.7	0.6	---
M12	---	8.1	3.3	8.9	18.0	---	9.0	4.2	6.8
M01	---	10.2	2.7	5.8	5.6	---	9.0	4.9	2.5
M02	20.3	17.9	16.8	2.6	4.2	5.0	14.2	2.8	1.4
Total identified	53.7	67.7	81.5	58.3	65.4	50.4	64.0	47.2	42.4
Total characterised	45.1	25.8	16.8	36.6	26.8	48.1	36.1	44.5	55.5
PES	1.3	6.4	1.7	5.1	7.8	1.5	n.q.	8.2	2.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

M50 BCS-CN88460-N-methyl-pyrazole-carboxylic acid
M20 BCS-CN88460-2-propanol-GlucA
M25 BCS-CN88460-propenol-GlucA
M10 BCS-CN88460-lactic acid
M19 BCS-CN88460-propanol-GlucA (isomer 1)
M19 BCS-CN88460-propanol-GlucA (isomer 2)
M11 BCS-CN88460-desmethyl-carboxylic acid
M06 BCS-CN88460-desmethyl-propanol
M12 BCS-CN88460-carboxylic acid
M01 BCS-CN88460-propanol
M02 BCS-CN88460-2-propanol

The metabolism in hens (poultry) and goat (ruminant) is similar with some minor varieties and includes:

1) hydroxylation of isoflucypram to isoflucypram-propanol, isoflucypram-2-propanol or isoflucypram-1,2-propandiol
2) further oxidation to isoflucypram-carboxylic acid or to a lactic acid group
3) conjugation of isoflucypram-propanol, isoflucypram-2-propanol or isoflucypram-1,2-propandiol with glucuronic acid or sulfate
4) demethylation of the pyrazole moiety and conjugation with glucuronic acid after demethylation
5) cleavage of the phenyl moiety in combination with cleavage of the cyclopropyl ring
6) dehydration after hydroxylation of the propyl group followed by conjugation with glucuronic acid

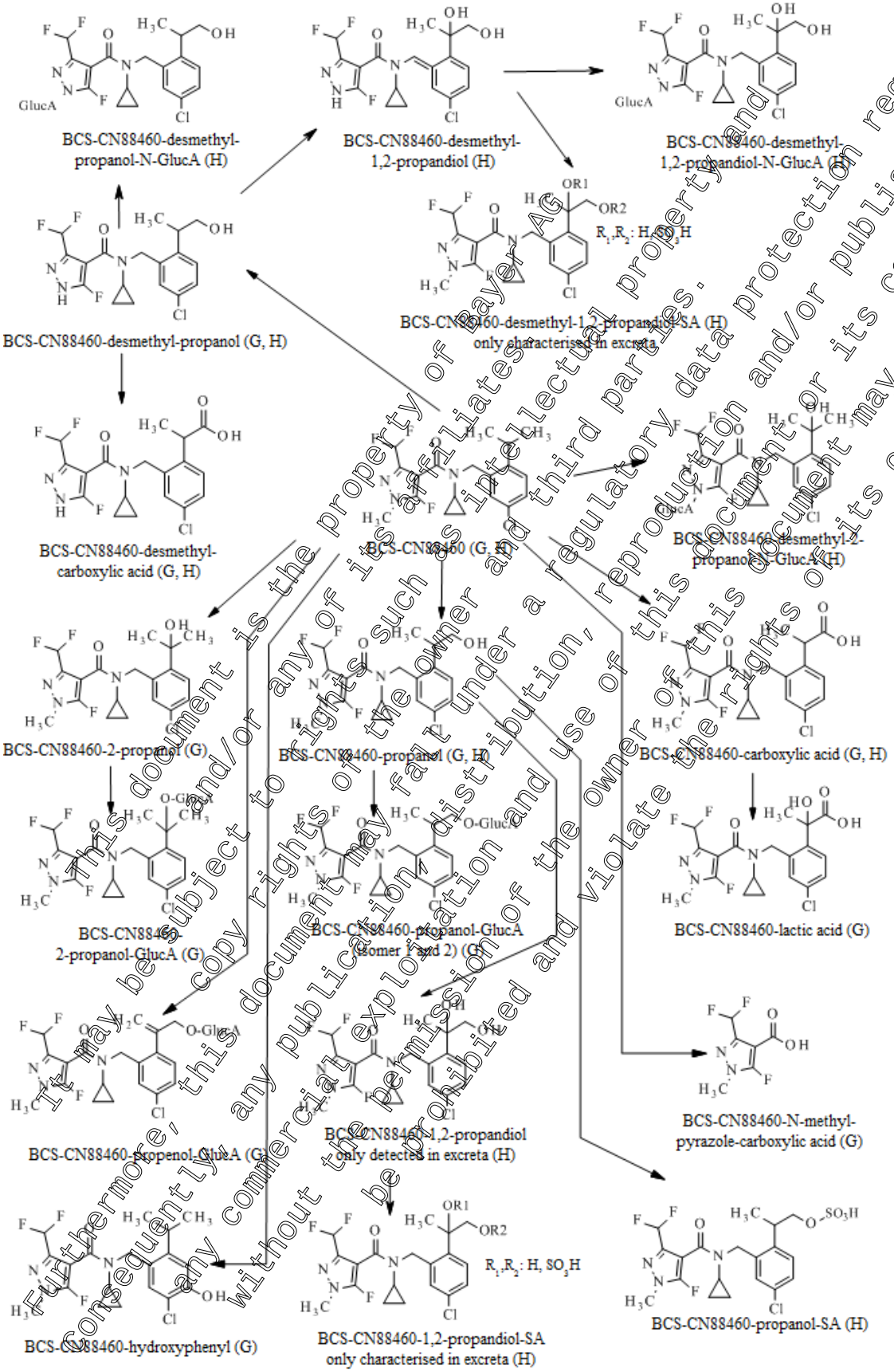
Based on the results of the metabolism studies in livestock, several compounds were proposed as residue definition for data collection and measured in the feeding studies presented under Point 6.4:

- Isoflucypram parent compound and its metabolites M01, M02, M06, M07 and M12 were individually determined in milk, eggs and all tissues
- Additionally, the sum of M02 and its conjugate M20, and the sum of M01 and its conjugate M19 were determined in cow liver and kidney.
- Moreover, the sum of M06 and its conjugate M37 was determined in hen liver.

The metabolic pathway in laying hen, lactating goat and rat is evaluated to be similar since, generally, the same metabolic steps are involved and the same metabolites are found.

In the figure below the proposed metabolic pathway for isoflucypram in poultry (H, laying hens) and ruminants (G, lactating goats) is shown.

Figure 6.2- 2: Proposed metabolic pathway of isoflucypram in livestock (hens, H; goats, G)



As the exact chemical structures of various metabolites could not be clarified in rats, some of the identified metabolites in livestock animals were not exactly observed in rats. Isoflucypram-2-propanol-GlucA is found in amount of 13% of the TRR in the liver of goats, but was not exactly detected in rat. Isoflucypram-propenol-GlucA is found in minor amounts in kidney and urine in the goats, but was not detected at all in rats. Isoflucypram-hydroxyphenyl was only detected in urine of goats, but not detected in rats, whereas other metabolites with the hydroxylated position 4 of the phenyl ring were detected in the rat.

N-glucuronic acid conjugates and conjugates with SA-group were only detected in hens. Conjugates with SA-group were only present in minor amounts, and mainly in excreta.

The general metabolic reactions for conjugation with glucuronic acid and sulphuric acid were also observed in the metabolism study with rats and could be verified by enzymatic cleavage of the conjugates to their specific aglycons. Hydroxylation and demetylation leading to the specific aglycons were observed in the metabolic pathway of the rat.

All other metabolites including metabolites detected in fish were also detected in the rat.

Metabolism in rotational crops

In the two confined rotational crop studies applied with an application rate of 198 - 202 g/ha of pyrazole and phenyl labelled isoflucypram, the residues of isoflucypram in rotational crops planted at all intervals were less than 0.08 mg/kg except in wheat hay and wheat straw of the pyrazole labelled confined rotational crop study where the content of isoflucypram was 0.114-0.220 mg/kg and 0.131-0.340 mg/kg, respectively. The TRRs in the different raw agricultural commodities (RACs) were generally low, increased slightly from the 1st to the 2nd rotation and stayed stable or declined to lower values in the 3rd rotation. The TRRs in the RACs of the phenyl labelled study were lower as the TRRs found in the study with the pyrazole label.

In the confined rotational crop study with isoflucypram labelled in the pyrazole mojety, unchanged parent compound was only detected in wheat forage, Swiss chard and turnip leaves with amounts of equal or less than 7.0% (0.003 mg/kg) of the TRR. Up to thirteen pyrazole derivative metabolites were identified. As the TRR values of the confined study was generally low, none of the identified metabolites accounted for more than 0.022 mg/kg and none of the unknown compounds accounted for more than 0.021 mg/kg.

The main metabolic reactions were the cleavage of the parent compound to BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (named as BCS-CR60082) and following conjugation of BCS-CR60082 with alanine (with or without defluorination) or the hydroxylation and defluoronation of BCS-CR60082 followed by conjugation with cysteine or glutathione. Other metabolic reactions were demethylation, hydroxylation, deamination or defluorination of BCS-CR60082, followed by conjugation with glucose, lactic acid, acetic acid, cysteine or glutathione. The glutathione group was afterwards degraded to mercapto alcohol with an additional conjugation with malonic acid.

In the confined rotational crop study with isoflucypram labelled in the phenyl mojety, unchanged parent compound was only detected in wheat forage, wheat hay and Swiss chard with amounts of equal or less than 17.0% (0.004 mg/kg) of the TRR. Due to very low TRR values in the confined study, no further metabolites were identified in the conventional extracts as none of the unknown compounds was larger than 0.009 mg/kg. No label specific metabolites were detected in the CRC study with the phenyl label.

Isoflucypram labelled in the pyrazole moiety lead to higher residues than isoflucypram labelled in the phenyl moiety. This indicates that cleavage of the molecule more likely happens in soil, followed by uptake and subsequent conjugation in the plant.

In the following tables the percent of TRR values of parent compound and metabolites in the different samples are summarised.

Table 6.2- 7: TRR values and distribution of parent compound and metabolites after spray application using [pyrazole-4-^{14}C]isoflucypram at an rate of 201.9 g a.s./ha

M-595694-02-1	DAT	TRR [mg/kg]	PC	M49	M66	M62 - i1	M62 - i2	M52	M54	M69 - i1	M56	M69 - i2	M67	M55	M57	M68	Total ident.	Total char.#	PES	Total
			% TRR																	
wheat forage	62	0.041	7.0	9.2	22.4	---	---	---	9.3	---	4.0	5.8	2.8	---	---	---	60.6	32.4	7.1	100.0
	175	0.078	1.4	7.3	25.6	---	4.2	3.9	8.7	---	8.4	10.2	---	6.9	---	---	76.6	14.4	9.0	100.0
	335	0.072	---	4.3	15.4	---	3.2	2.0	6.3	---	1.8	6.7	---	2.1	4.5	---	46.2	45.8	8.1	100.0
wheat hay	101	0.114	---	2.2	12.2	7.7	---	---	5.2	---	2.9	9.0	---	1.6	4.2	---	44.9	40.7	3.9	100.0
	233	0.220	---	1.1	13.9	3.0	5.8	5.7	6.5	---	2.3	3.2	4.8	2.5	3.2	2.0	54.0	41.6	4.3	100.0
	387	0.187	---	2.3	7.9	2.9	3.8	7.0	1.8	3.3	1.9	8.4	2.2	1.1	3.6	1.6	47.7	37.7	2.1	100.0
wheat straw	139	0.131	---	7.0	2.6	2.3	3.5	2.6	6.6	---	3.7	11.9	---	---	5.5	---	45.7	32.1	6.4	100.0
	286	0.247	---	2.8	9.1	3.6	3.4	5.0	5.5	---	3.7	5.9	---	1.1	3.8	---	44.0	51.6	4.5	100.0
	427	0.340	---	5.8	2.8	3.2	3.3	7.6	2.6	2.2	2.0	8.6	1.1	1.6	2.9	1.4	45.1	50.7	4.2	100.0
wheat grain	139	0.004*	---	---	---	---	---	---	---	---	---	---	---	---	---	---	n.a.	n.a.	n.a.	100.0
	286	0.011	---	---	7.7	---	---	---	---	---	---	---	13.4	---	---	---	21.2	59.3	19.6	100.0
	427	0.016	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	85.2	14.8	100.0
Swiss chard (im)	56	0.031	6.0	18.9	---	---	---	26.9	21.2	---	---	---	---	---	---	---	73.0	23.1	2.8	100.0
	177	0.062	0.5	9.0	1.8	6.8	---	25.7	6.3	5.2	---	---	1.2	---	---	---	56.4	38.9	4.7	100.0
	330	0.056	0.8	7.3	2.3	5.4	1.6	23.0	8.4	---	---	0.5	1.3	0.6	---	---	51.3	43.1	5.6	100.0
Swiss chard (m)	62	0.026	4.6	19.4	---	---	---	34.2	22.9	---	---	---	---	---	---	---	81.1	14.9	4.0	100.0
	189	0.062	---	3.8	1.8	12.2	---	16.4	6.2	2.5	---	---	1.9	---	---	---	44.7	49.5	5.8	100.0
	342	0.052	1.1	4.0	2.8	9.5	---	21.1	3.7	---	---	---	1.6	---	---	---	43.9	48.5	7.6	100.0
turnip roots	79	0.006*	---	---	---	---	---	---	---	---	---	---	---	---	---	---	n.a.	n.a.	n.a.	100.0
	212	0.006*	---	---	---	---	---	---	---	---	---	---	---	---	---	---	n.a.	n.a.	n.a.	100.0
	356	0.006*	---	---	---	---	---	---	---	---	---	---	---	---	---	---	n.a.	n.a.	n.a.	100.0
turnip leaves	79	0.018	4.8	18.4	---	---	13.0	7.7	25.7	---	---	---	5.9	4.6	12.3	---	92.3	---	7.7	100.0
	212	0.031	---	12.7	8.1	6.1	4.6	4.4	19.9	---	2.9	---	7.2	---	7.4	---	73.3	19.7	6.9	100.0
	356	0.026	---	9.4	4.7	5.5	2.9	2.9	13.9	---	5.0	---	5.8	1.8	3.8	---	55.4	36.9	7.7	100.0

Number	Compound name: BCS-CN88460-
PC:	(parent compound) BCS-CN88460;
M66:	cyclopropyl-pyrazole-carboxamide-Ala;
M62- i2:	cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2);
M54:	desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH;
M56:	desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc;
M67:	desfluoro-cyclopropyl-pyrazole-carboxamide-Ala;
M57:	desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA;

Number	Compound name: BCS-CN88460-
M49:	N-methyl-cyclopropyl-pyrazole-carboxamide (BCS-CR60082);
M62- i1:	cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1);
M52:	desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys;
M69- i1:	cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1);
M69- i2:	cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2);
M55:	desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys;
M68:	cyclopropyl-pyrazole-carboxamide-acetic acid

DAT: days after treatment; im: immature; m: mature; n.a.: not analysed
* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.
# Total characterised based on extraction or chromatographic behaviour.

Table 6.2- 8: TRR values and distribution of parent compound and metabolites after spray application using [phenyl-UL-^{14}C]isoflucypram at an rate of 197.7 g a.s./ha

M-595695-01-1	DAT	TRR [mg/kg]	PC	Total ident.	Total char.#	PES	Total
			% TRR				
wheat forage	62	0.023	17.0	17.0	77.9	5.2	100.0
	175	0.018	12.2	12.2	81.3	6.5	100.0
	335	0.015	5.2	5.2	84.9	9.9	100.0
wheat hay	101	0.039	1.1	1.1	91.7	7.2	100.0
	233	0.062	---	---	91.8	8.2	100.0
	387	0.036	---	---	88.4	11.7	100.0
wheat straw	139	0.051	---	---	88.6	11.5	100.0
	286	0.070	---	---	90.5	9.6	100.0
	427	0.055	---	---	90.7	9.3	100.0
wheat grain	139	0.001*	---	n.a	n.a	n.a.	100.0
	286	0.004*	---	n.a	n.a	n.a.	100.0
	427	0.003*	---	n.a.	n.a	n.a.	100.0
Swiss chard (im)	56	0.029	2.8	2.8	94.9	2.3	100.0
	177	0.016	---	---	96.4	3.6	100.0
	330	0.020	---	---	96.2	3.8	100.0
Swiss chard (m)	62	0.020	6.1	6.1	91.6	2.3	100.0
	189	0.016	---	---	96.6	3.4	100.0
	342	0.025	---	---	95.6	4.4	100.0
turnip roots	79	0.003*	---	n.a	n.a	n.a.	100.0
	212	0.009*	---	n.a	n.a	n.a.	100.0
	356	0.003*	---	n.a	n.a	n.a.	100.0
turnip leaves	79	0.004*	---	n.a	n.a	n.a.	100.0
	212	0.006*	---	n.a	n.a	n.a.	100.0
	356	0.006*	---	n.a	n.a	n.a.	100.0

PC (parent compound): BCS-CN88460;
DAT: days after treatment; im: immature; m: mature; n.a.: not analysed
* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.
# Total characterised based on extraction or chromatographic behaviour

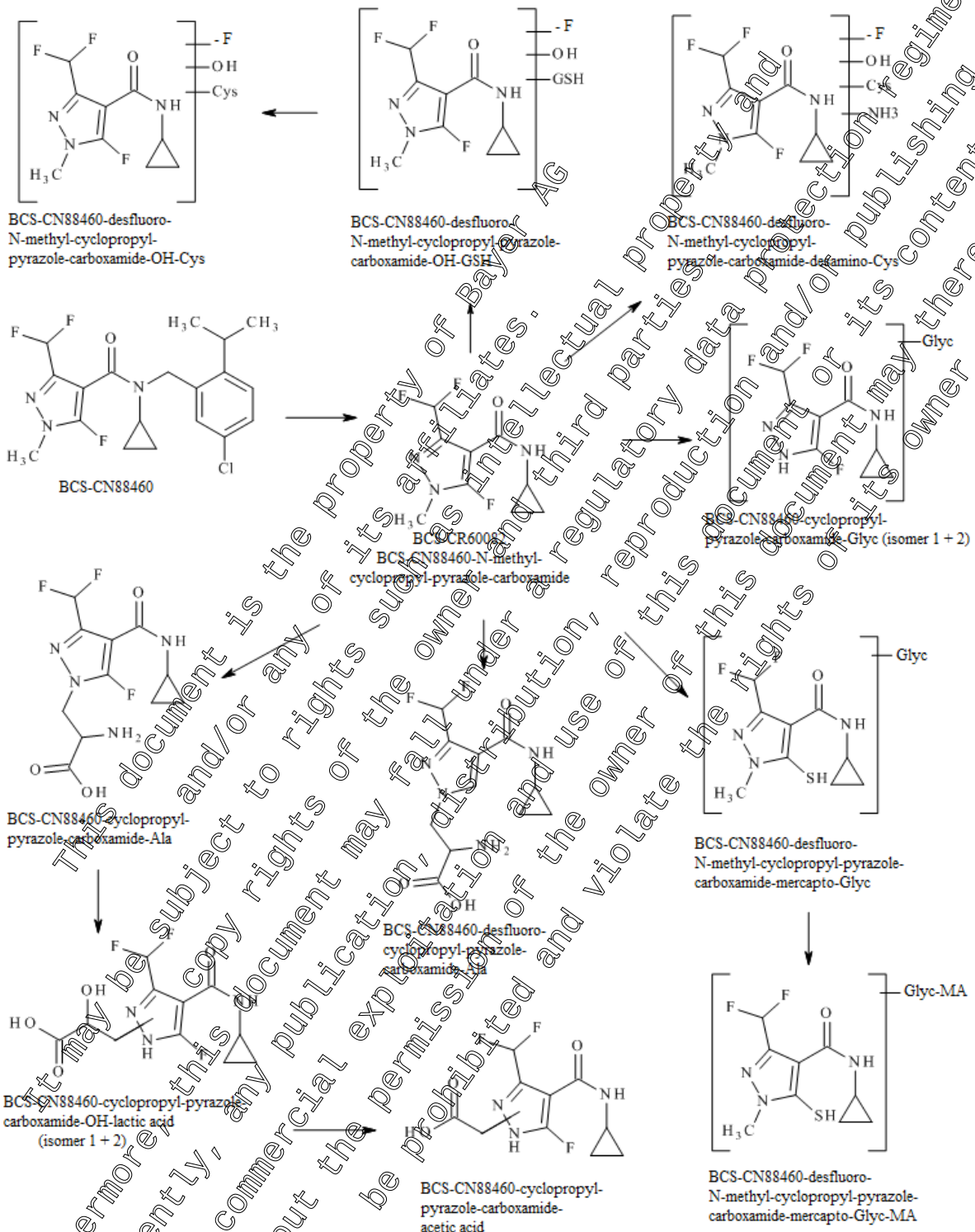
The following metabolic reactions were observed:

- cleavage of the parent compound leading to BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (BCS-CR60082)
- conjugation of BCS-CR60082 with alanine, lactic acid or acetic acid with or without defluoronation of the pyrazole ring
- demethylation of BCS-CR60082 followed by conjugation with glucose
- hydroxylation, deamination and defluoronation of BCS-CR60082 followed by conjugation with cysteine or glutathione
- defluoronation of BCS-CR60082 followed by conjugation with glucose and glutathione and degradation of the glutathione group to mercapto alcohol, additional conjugation with malonic acid

Based on these results, parent compound and its metabolite M49 were proposed as residue definition for data collection and measured in the field rotational crop studies presented under Point 6.6.2.

Based on the identified metabolites, the metabolism of BCS-CN88460 in confined rotational crops is adequately understood and the following metabolic pathway is proposed.

Figure 6.2- 3: Proposed metabolic pathway of BCS-CN88460 based on identified metabolites in confined rotational crops



CA 6.2.1 Metabolism, distribution and expression of residues in plants

The metabolism of isoflucypram in primary crops has been investigated after foliar treatment in tomatoes (fruits), wheat (cereals), oilseed rape and soybean (oilseed) using isoflucypram either labelled in the phenyl or in the pyrazole moiety as shown in the table below. The metabolism of isoflucypram is further being investigated in potatoes (root crop) after seed/tuber treatment. The potato metabolism studies are already listed in this dossier, but will be submitted after finalization of the studies.

Table 6.2.1- 1: Overview over available plant metabolism studies

Crop group	Crop	Application	Target application rate	Reference
fruits	tomato	foliar application, pyrazole-label	2 x 75 g a.s./ha	M-597485-01-1
fruits	tomato	foliar application, phenyl-label	2 x 75 g a.s./ha	M-597481-01-1
cereals	wheat	foliar application, pyrazole-label	2 x 65 g a.s./ha	M-604361-02-1
cereals	wheat	foliar application,phenyl-label	2 x 63 g a.s./ha	M-604358-02-1
oilseed	oilseed rape	foliar application, pyrazole-label	2 x 60 g a.s./ha	M-609378-01-1
oilseed	oilseed rape	foliar application, phenyl-label	2 x 60 g a.s./ha	M-609380-01-1
oilseed	soybean	foliar application, pyrazole-label	3 x 60 g a.s./ha	M-609373-01-1
oilseed	soybean	foliar application, phenyl-label	3 x 60 g a.s./ha	M-609376-01-1
root crops	potato	tuber treatment, pyrazole-label	Will be filed subsequently after finalization of the studies.	
root crops	potato	tuber treatment, phenyl-label		

Metabolism, distribution and expression of residues in tomatoes (foliar spray application)

Metabolism studies in tomatoes were conducted with [pyrazole-4-^{14}C] and [phenyl-UL-^{14}C]BCS-CN88460.

Table 6.2.1- 2: Overview over available tomato metabolism studies

Plant	Application	Target application rate	BBCH Code	Reference
tomato	two foliar spray applications, pyrazole-labelled isoflucypram	2 x 75 g a.s./ha	BBCH 14-15 and BBCH 85-86	M-597485-01-1
tomato	two foliar spray applications, phenyl-labelled isoflucypram	2 x 75 g a.s./ha	BBCH 14-15 and BBCH 85-87	M-597481-01-1

Summary of metabolism in tomatoes (foliar spray application)

The metabolism observed in tomatoes after foliar application of [pyrazole-4-^{14}C] and [phenyl-UL-^{14}C] BCS-CN88460 corresponds well between the two labels. Most of the radioactive residues were recovered in the surface wash of tomatoes. Parent compound was the main compound. No relevant metabolisation of isoflucypram occurs in tomato fruits after foliar treatment.

Report:	KCA 6.2.1/01; [redacted]; 2017; M-597485-01-1
Title:	Metabolism of [pyrazole-4-14C]BCS-CN88460 in tomato
Report No.:	EnSa-16-0959
Document No.:	M-597485-01-1
Guideline(s):	OECD Test Guideline No. 501 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 US EPA OCSPP Test Guideline No. 860.1300 JAP FAMIC-ACIS Notification 12 Nousan 8147
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of BCS-CN88460 in tomato fruits was investigated after two post-emergence spray applications. The test compound, [pyrazole-4-^{14}C]BCS-CN88460, was formulated as an EC 200 and applied to two tomato plants at growth stages BBCH 14 - 15 (four or five leaves unfolded) and BBCH 85 - 86 (50-60 % of ripe fruits), i.e. a total of two applications per plant. The total actual application rate was 27.97 mg a.s., corresponding to an application rate of 168 g a.s./ha based on a plant density of 12,000 tomato plants/ha.

Tomato fruits were harvested at the end of the fruit ripening period (BBCH 87-89). After surface washing with dichloromethane, tomato fruits were homogenised and sufficiently extracted by conventional methods with a mixture of acetonitrile/water.

The TRR in tomato fruits was calculated based on the radioactivity in the surface wash solution and the fruit sample and amounted to 0.170 mg a.s. equivalents/kg in total. The largest portion of the TRR was detected in the surface wash solution (0.125 mg/kg, 73.6% of the TRR). The fruit extract and post-extraction solids (PES) amounted to 0.045 mg/kg (26.1% of the TRR) and < 0.001 mg/kg (0.2% of the TRR), respectively.

Parent compound was the main compound in the tomato fruit sample and amounted to 0.165 mg/kg, 96.7% of the TRR (sum of surface wash solution and extracts). Only four very minor metabolites (total 0.007 mg/kg, single compound ≤ 0.003 mg/kg) were detected in the surface wash solution and tomato fruit extracts.

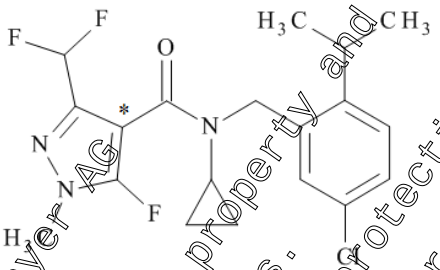
Parent compound was identified in an isolated fraction of the surface wash solution by spectroscopic methods and confirmed in extract of fruits by HPLC co-chromatography with the reference compound.

Based on these results, it can be reasonably assumed that no relevant metabolisation of [pyrazole-4-^{14}C]BCS-CN88460 occurs in tomato fruits after foliar treatment.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	
	*denotes the position of the ^{14}C-label
Radiolabel position	[pyrazole-4-^{14}C]
Specific radioactivity	4.22 MBq/mg
Radiochemical purity	> 99%
Chemical purity	99%

Formulation of the test compound

Stock solutions were prepared by dissolving of the test compound in acetonitrile. The identity of the test compound in the stock solution was confirmed by spectroscopic methods (LC-MS/MS and ^{1}H-NMR). Adequate parts of the stock solution were transferred into glass vials and evaporated to dryness. The formulation concentrate was prepared by combining the test compound with a corresponding amount of the EC 200 blank formulation. The formulation concentrate was homogenised using a vortex mixer. Afterwards, the formulation was diluted with water by stirring or swirling in order to obtain the ready-to-use application solutions.

2. Soil: "Einheitserde T", pH (CaCl$_2$) = 5.8, 15 vol.% clay

3. Plant: Tomato, variety "Philona", representative for fruiting crops

B. Study Design

1. Experimental conditions:

Two tomato plants (*Lycopersicon lycopersicum*, variety: Philona) were cultivated under natural temperature and light conditions in the vegetation area of the test facility. Plants were irrigated as needed to maintain the optimal growth conditions. Each plant was grown in its own planting container (30 L pots). The containers were filled with white moor peat and "Einheitserde T" and marked with the name of the test compound, study number and radioactivity symbol.

The application conditions simulated two spray applications each with an intended application rate of 75 g a.s./ha. The first treatment was performed at growth stage BBCH 14 - 15 (four or five leaves unfolded). The plants were treated with 100 mL of the application solutions. At the first application 55.66 MBq of the labelled test compound were applied to two tomato plants, corresponding to 79.14 g a.s./ha. At the second application (performed at BBCH 85 – 86; 50-60% of ripe fruits) 62.35 MBq of the labelled test compound were applied to two tomato plants, corresponding to 88.65 g a.s./ha.

Taking into the account the amount of radioactivity found in the rinsing solution of the sprayer equipment, a total amount of 118.01 MBq (27.97 mg) was applied onto the two tomato plants. This resulted in a total mean application rate of 168 g a.s./ha based on a plant density of 12,000 tomato plants/ha.

2. Sampling:

Tomato fruits were collected at BBCH 87 – 89, 14 days after the second treatment, weighed and subdivided into two aliquots.

For investigation of the metabolic pathway, a subset of fruits was washed by dipping the fruits into a dichloromethane bath. Afterwards the fruits were diced and homogenised. Aliquots were stored at approx. ≤ -18 °C until extraction.

C. Analytical Procedures

1. Extraction:

After surface washing with dichloromethane, the tomato fruits sample was conventionally extracted three times with mixtures of acetonitrile/water (8/2; v/v) using a high speed blender. The combined extracts were subjected to a clean-up step using an SPE RP 18 cartridge and rinsed with acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The surface wash solution and the SPE percolate and rinse were concentrated and analysed by HPLC.

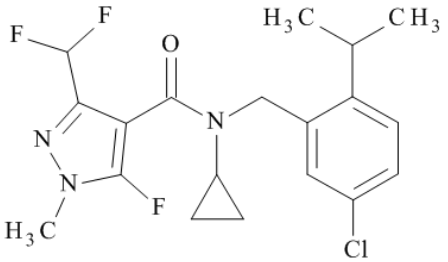
The TRR value of the fruit sample was calculated by summing up the radioactivity in the surface wash, the extract and the post extraction solids (PES) based on the weight of the sample used and the specific radioactivity.

2. Identification and characterisation:

Parent compound and metabolites were quantified in the surface wash solution and in the conventional extract of tomato fruits by HPLC analysis based on reversed phase chromatography (RP 18) with an acidic water/acetonitrile/THF gradient.

Parent compound was identified by spectroscopic methods in the surface wash and by co-chromatography with reference compound in the extract. The peaks in the individual HPLC profiles of the extracts were numbered in ascending order according to appearance in the chromatogram. Corresponding peaks in the HPLC profiles were designated with the same number. Unidentified metabolites were designated with "unknown" and numbered in ascending order. Corresponding unidentified metabolites in the different HPLC profiles were designated with the same number.

Table 6.2.1- 3: List of reference compound

Report name/ retention time in HPLC and remarks	**Chemical name (IUPAC)**	**Chemical Structure**
Parent compound (BCS CN88460)	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

Extraction and quantification of four trace metabolites and parent compound were finished within six month. Hence, no further stability investigations were conducted.

II. Results and Discussion

The metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in tomatoes was investigated after two spray applications.

The TRR value of the tomato fruit sample was calculated by summing up the radioactivity determined in the surface wash solution, the extract and the post extraction solids (PES) based on the weight of the sample used for extraction and the specific radioactivity of the test compound. The TRR for the fruit sample was low considering the application rate and amounted to 0.170 mg/kg.

Table 6.2.1- 4: TRR value in tomato fruits after foliar application of [pyrazole-4-^{14}C]BCS-CN88460

Matrix	Timing and Application	Growth stage at harvest	PHI* (days)	ppm (mg a.s. equiv./kg)
Tomato fruits	Two foliar spray applications at BBCH 14-15 and BBCH 85-86 Total application rate: 168 g a.s./ha	BBCH 87 - 89	14	0.170

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest sampling)

For tomato fruits, the main portion of the radioactivity (0.125 mg/kg, 73.6% of the TRR) was detected in the surface wash solution. Residues in the tomato fruit sample were efficiently extracted with conventional methods using acetonitrile/water (8:2; v/v) and amounted to 26.1% (0.045 mg/kg) of the TRR. The post extraction solids amounted to 0.2% (<0.001 mg/kg) of the TRR, only. There were no losses during the sample preparation and no radioactivity was observed in the distillate of the concentration procedures.

Table 6.2.1- 5: Distribution of radioactivity in the extracts of the tomato fruits after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

Sample	**tomato fruits**	
TRR [mg/kg]	**0.170**	
	% of TRR	mg/kg
Surface wash solution	73.6	0.125
Conventional extract	26.1	0.045
Losses (distillate)	---	---
Total extracted	**99.8**	**0.170**
Post extraction solids (PES)	**0.2**	**<0.001**
Accountability	**100.0**	**0.170**

Besides parent compound (0.165 mg/kg, 96.7% of the TRR), only four very minor metabolites (≤0.003 mg/kg) were detected in the surface wash solution and tomato fruit extracts.

The TRR and the distribution of parent and metabolites in tomato fruits are shown in Table 6.2.1- 6 below.

Table 6.2.1- 6: Distribution of parent compound and metabolites in the extracts of tomato fruits after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

Sample	tomato fruits	
TRR [mg/kg] =	**0.170**	
Compound (BCS-CN88460)	% TRR	mg/kg
BCS-CN88460 (parent compound)	96.7	0.165
Total identified	**96.7**	**0.165**
unknown 1	0.3	0.001
unknown 2	1.6	0.003
unknown 3	0.2	<0.001
unknown 4	1.0	0.002
Total characterised	**3.1**	**0.007**
Analysed extract(s)	99.8	0.170
Extracts not analysed	-	-
Total extracted	**99.8**	**0.170**
Post extraction solids (PES)	**0.2**	**<0.001**
Accountability	**100.0**	**0.170**

III. Conclusions

The metabolism of the fungicide isoflucypram (BCS-CN88460) was investigated in tomatoes after two post-emergence spray applications. The application rate amounted to 168 g a.s./ha. The test compound was ^{14}C-labelled at the pyrazole-4-position.

The TRR level in tomatoes was 0.170 mg/kg. The radioactive residues were efficiently recovered by surface wash with dichloromethane (73.6%, 0.125 mg/kg of the TRR) and conventional extraction with acetonitrile/water mixtures. In total, 99.8% TRR was recovered from the tomatoes.

Parent compound was the only major component in tomatoes (96.7%, 0.165 mg/kg of the TRR).

Therefore, it can be reasonably assumed that no relevant metabolisation of [pyrazole-4-^{14}C] BCS-CN88460 occurs in tomato fruits after foliar treatment.

Report: KCA 6.2.1-02; [redacted]; 2017; M-597481-01-1
Title: Metabolism of [phenyl-UL-14C]BCS-CN88460 in tomato
Report No.: EnSa-16-0960
Document No.: M-597481-01-1
Guideline(s): OECD Test Guideline No. 501
Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
US EPA OCSPP Test Guideline No. 860.1300
JAP FAMIC-ACIS Notification 12 Nousan 8147
Guideline deviation(s): none
GLP/GEP: **yes**

Executive Summary

The metabolism of BCS-CN88460 in tomato fruits was investigated after two post-emergence spray applications. The test compound, [phenyl-UL-^{14}C]BCS-CN88460, was formulated as an EC 200 and

applied to two tomato plants at growth stages BBCH 14 - 15 (four or five leaves unfolded) and BBCH 85 - 87 (50-70 % of ripe fruits), i.e. a total of two applications per plant. The total actual application rate was 26.02 mg a.s., corresponding to an application rate of 156 g a.s./ha based on a plant density of 12,000 tomato plants/ha.

Tomato fruits were harvested at the end of the fruit ripening period (BBCH 87 -89). After surface washing with dichloromethane, tomato fruits were homogenised and sufficiently extracted by conventional methods with a mixture of acetonitrile/water.

The TRR in tomato fruits was calculated based on the radioactivity in the surface wash solution and the fruit sample and amounted to 0.095 mg a.s. equivalents/kg in total. The largest portion of the TRR was detected in the surface wash solution (0.071 mg/kg, 74.6% of the TRR). The fruit extract and post-extraction solids (PES) amounted to 0.024 mg/kg (25.1% of the TRR) and < 0.001 mg/kg (0.2% of the TRR), respectively.

Parent compound was the main compound in the tomato fruit sample and amounted to 0.094 mg/kg, 98.2% of the TRR (sum of surface wash solution and extracts). Only two very minor metabolites (total 0.002 mg/kg, single compound ≤0.001 mg/kg) were detected in the surface wash solution and tomato fruit extracts.

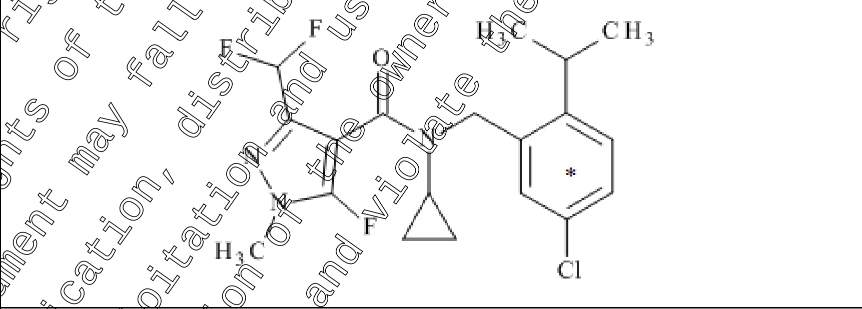
Parent compound was identified in extract of fruits by HPLC co-chromatography with reference compound.

Based on these results, it can be reasonably assumed that no relevant metabolisation of [phenyl-UL-^{14}C]BCS-CN88460 occurs in tomato fruits after foliar treatment.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * denotes the position of the ^{14}C-label
Radiolabel position	[phenyl-UL-^{14}C]
Specific radioactivity	4.13 MBq/mg
Radiochemical purity	> 99%
Chemical purity	> 99%

Formulation of the test compound

Stock solutions were prepared by dissolving of the test compound in acetonitrile. The identity of the test compound in the stock solution was confirmed by spectroscopic methods (LC-MS/MS and ^{1}H-NMR). Adequate parts of the stock solution were transferred into glass vials and evaporated to dryness. The formulation concentrate was prepared by combining the test compound with a corresponding amount of the EC 200 blank formulation. The formulation concentrate was homogenised using a vortex mixer. Afterwards, the formulation was diluted with water by stirring or swirling in order to obtain the ready-to-use application solutions.

2. Soil: "Einheitserde T", pH ($CaCl_2$) = 5.8, 15 vol.% clay

3. Plant: Tomato, variety "Philona", representative for fruiting crops

B. Study Design

1. Experimental conditions:

Two tomato plants (*Lycopersicon lycopersicum*, variety: Philona) were cultivated under natural temperature and light conditions in the vegetation area of the test facility. Plants were irrigated as needed to maintain the optimal growth conditions. Each plant was grown in its own planting container (30 L pots). The containers were filled with white moor peat, "Einheitserde T" and marked with the name of the test compound, study number and radioactivity symbol.

The application conditions simulated two spray applications each with an intended application rate of 75 g a.s./ha. The first treatment was performed at growth stage BBCH 14 - 15 (four or five leaves unfolded). The plants were treated with 100 mL of the application solutions. At the first application 53.99 MBq of the labelled test compound were applied to two tomato plants, corresponding to 78.4 g a.s./ha. At the second application (performed at BBCH 85 – 87, 50-70% of ripe fruits) 53.5 MBq of the labelled test compound were applied to two tomato plants, corresponding to 77.7 g a.s./ha.

Taking into the account the amount of radioactivity found in the rinsing solution of the sprayer equipment, a total amount of 107.49 MBq (26.02 mg) was applied onto the two tomato plants. This resulted in a total mean application rate of 156.1 g a.s./ha based on a plant density of 12,000 tomato plants/ha.

2. Sampling:

Tomato fruits were collected at BBCH 87 – 89, 14 days after the second treatment, weighed and subdivided into two aliquots.

For investigation of the metabolic pathway, a subset of fruits was washed by dipping the fruits into a dichloromethane bath. Afterwards the fruits were diced and homogenised. Aliquots were stored at approx. -18 °C until extraction.

C. Analytical Procedures

1. Extraction:

After surface washing with dichloromethane the tomato fruit sample was conventionally extracted three times with mixtures of acetonitrile/water (8/2, v/v) using a high speed blender. The combined extracts were subjected to a clean-up step using an SPE RP 18 cartridge and rinsed with acetonitrile/water (8/2, v/v) and THF/methanol (1:1, v/v). The surface wash solution and the SPE percolate and rinse were concentrated and analysed by HPLC.

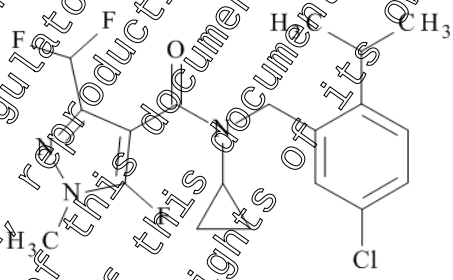
The TRR value of the fruit sample was calculated by summing up the radioactivity in the surface wash, the extract and the post extraction solids (PES) based on the weight of the sample used and the specific radioactivity.

2. Identification and characterisation:

Parent compound and metabolites were quantified in the surface wash solution and in the conventional extract of tomato fruits by HPLC analysis based on reversed phase chromatography (RP 18) with an acidic water/acetonitrile/THF gradient.

Parent compound was identified by co-chromatography with the reference compound. The peaks in the individual HPLC profiles of the extracts were numbered in ascending order according to appearance in the chromatogram. Corresponding peaks in the HPLC profiles were designated with the same number. Unidentified metabolites were designated with "unknown" and numbered in ascending order. Corresponding unidentified metabolites in the different HPLC profiles were designated with the same number.

Table 6.2.1- 7: List of reference compound

Report name/ retention time in HPLC and remarks	**Chemical name (IUPAC)**	**Chemical Structure**
Parent compound (BCS CN88460)	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

Extraction and quantification of four trace metabolites and parent compound were finished within six month. Hence, no further stability investigations were conducted.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in tomatoes was investigated after two spray applications.

The TRR value of the tomato fruit sample was calculated by summing up the radioactivity determined in the surface wash solution, the extract and the post extraction solids (PES) based on the weight of the sample used for extraction and the specific radioactivity of the test compound. The TRR for the fruit sample was low considering the application rate and amounted to 0.095 mg/kg.

Table 6.2.1- 8: TRR value in tomato fruits after foliar application of [phenyl-UL-^{14}C]BCS-CN88460

Matrix	**Timing and Application**	**Growth stage at harvest**	**PHI* (days)**	**ppm (mg a.s. equiv./kg)**
Tomato fruits	Two foliar treatments at BBCH 14-15 and BBCH 85-87 Total application rate: 156 g a.s./ha	BBCH 87 - 89	14	0.095

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

For tomato fruits, the main portion of the radioactivity (0.071 mg/kg, 74.6% of the TRR) was detected in the surface wash solution. Residues in the tomato fruit sample were efficiently extracted with conventional methods using acetonitrile/water (8/2; v/v) and amounted to 25.1% (0.024 mg/kg) of the TRR. The post extraction solids amounted to 0.2% (< 0.001 mg/kg) of the TRR, only. There were no losses during the sample preparation and no radioactivity was observed in the distillate of the concentration procedures.

The distribution of the radioactive residues is shown in the following table.

Table 6.2.1- 9: Distribution of radioactivity in the extracts of the tomato fruits after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

Sample	**tomato fruits**	
TRR [mg/kg]	**0.095**	
	% of TRR	mg/kg
Surface wash solution	74.6	0.071
Conventional extract	25.1	0.024
Losses (distillate)	---	---
Total extracted	**99.8**	**0.095**
Post extraction solids (PES)	**0.2**	**<0.001**
Accountability	**100.0**	**0.095**

Besides parent compound (0.094 mg/kg, 98.2% of the TRR), only two very minor metabolites (≤0.001 mg/kg) were detected in the surface wash solution and tomato fruit extracts.

The TRR and the distribution of parent and metabolites in tomato fruits are shown in the following table.

Table 6.2.1- 10: Distribution of parent compound and metabolites in the extracts of tomato fruits after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

Sample	**tomato fruits**	
TRR [mg/kg] =	0.095	
Compound (BCS-CN88460)	% TRR	mg/kg
BCS-CN88460 (parent compound)	98.2	0.094
Total identified	**98.2**	**0.094**
unknown 1	0.8	0.001
unknown 2	0.7	<0.001
Total characterised	**1.5**	**0.002**
Analysed extract(s)	99.8	0.095
Extracts not analysed	---	---
Total extracted	**99.8**	**0.095**
Post extraction solids (PES)	0.2	<0.001
Accountability	**100.0**	**0.095**

III. Conclusions

The metabolism of the fungicide isoflucypram (BCS-CN88460) was investigated in tomatoes after two post-emergence spray applications. The application rate amounted to 156 g a.s./ha. The test compound was ^{14}C-labelled in the phenyl-moiety.

The TRR level in tomatoes was 0.095 mg/kg. The radioactive residues were efficiently recovered by surface wash with dichloromethane (74.6%, 0.071 mg/kg of the TRR) and conventional extraction with acetonitrile/water mixtures. In total, 99.8% TRR was recovered from the tomatoes.

Parent compound was the only major component in tomatoes (98.2%, 0.094 mg/kg of the TRR).

Therefore, it can be reasonably assumed that no relevant metabolisation of [phenyl-UL-^{14}C] BCS-CN88460 occurs in tomato fruits after foliar treatment.

Metabolism, distribution and expression of residues in wheat (foliar spray application)

Metabolism studies in spring wheat after foliar application were conducted with [pyrazole-4-^{14}C] and [phenyl-UL-^{14}C]BCS-CN88460.

Table 6.2.1- 11: Overview over available wheat metabolism studies

Plant	Application	Target application rate	BBCH Code	Reference
wheat	two foliar spray applications, pyrazole-labelled isoflucypram	2 x 65 g a.s./ha	BBCH 30 and BBCH 69	M-604361-02-1
wheat	two foliar spray applications, phenyl-labelled isoflucypram	2 x 65 g a.s./ha	BBCH 30 and BBCH 69	M-604358-02-1

Summary of metabolism in wheat (foliar spray application)

The metabolite pattern corresponds well when comparing the two metabolism studies in wheat after foliar application. Parent compound BCS-CN88460 was the main residue component in wheat hay and straw and the only component in wheat grains. BCS-CN88460 was moderately metabolised in wheat after two post-emergence applications. The main metabolic reactions were hydroxylation in position 1 of the propyl group followed by conjugation with hexose and malonic acid and to a lesser extent demethylation of the pyrazole moiety.

Besides parent compound, the following metabolites were identified: BCS-CN88460-propanol-Glyc (M18), BCS-CN88460-desmethyl-propanol-Glyc-MA (M41), BCS-CN88460-propanol-Glyc-MA (M21), BCS-CN88460-propanol (M01) and BCS-CN88460-desmethyl-propanol (M06).

Report:	KCA 6.2.1/03; [redacted], M.; 2018; M-604361-02-1
Title:	Amendment no.1 to final report - Metabolism of [pyrazole-4-14C] BCS-CN88460 in wheat plants
Report No.:	S14-01087
Document No.:	M-604361-02-1
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Executive Summary

The metabolism of BCS-CN88460 was investigated in wheat plants after two spray applications. For each of the two foliar applications the test item [pyrazole-4-^{14}C]BCS-CN88460 was formulated as an aqueous EC 50 using a nominal application rate of 65 g a.s./ha each. The applications were performed at the growth stage of BBCH 30 (beginning of stem elongation) and BBCH 69 (end of flowering). The actual application rates corresponded to 69 and 67 g a.s./ha for the first and second application, respectively resulting in a total application rate of 136 g a.s./ha.

Wheat hay was harvested at BBCH 69, 1 day prior to the second application, wheat straw and grains were harvested at maturity (BBCH 89). The total radioactive residues (TRR) in wheat straw and hay were high and amounted to 15.536 mg eq/kg and 4.032 mg eq/kg, respectively. The TRR in wheat grains was moderate and amounted to 0.385 mg eq/kg.

Homogenised plant material from RACs was conventionally extracted with a mixture of acetonitrile/water (8:2; v/v). The extraction rates after conventional extraction of wheat hay, straw and grains were high and amounted to 95.8% (3.864 mg eq/kg) of the TRR for hay, 94.0% (14.604 mg eq/kg) of the TRR for straw and 93.6% (0.360 mg eq/kg) of the TRR for grain. The post extraction solids (PES) of all RACs remaining after conventional extraction amounted to ≤ 6.4% of TRR.

Solids after conventional extraction of straw were exhaustively extracted using microwave assistance with a mixture of acetonitrile/water/formic acid (50/50/1; v/v/v) releasing further 4.7% (0.727 mg eq/kg) of TRR.

Residues in the conventional extracts were analysed and quantified by HPLC. The parent compound and metabolites were either identified by co-chromatography with the reference compound or by spectroscopic analysis in isolated fractions of wheat straw. Additionally, the metabolite pattern and retention times of the current and the wheat metabolism study with the phenyl label were compared.

The parent substance BCS-CN88460 represented the most prominent residue component in all RACs accounting for 50.0% of TRR (2.016 mg eq/kg) in wheat hay, 64.0% of TRR (9.933 mg eq/kg) in wheat straw and 92.0% of TRR (0.354 mg eq/kg) in wheat grains. Besides parent compound no other metabolite was detected in the extract of grains. In wheat straw five metabolites were identified besides parent compound: BCS-CN88460-propanol-Glyc (M18), BCS-CN88460-desmethyl-propanol-Glyc-MA (M41), BCS-CN88460-propanol-Glyc-MA (M21), BCS-CN88460-propanol (M01) and BCS-CN88460-desmethyl-propanol (M06) accounting for 3.7, 2.9, 6.7, 1.7 and 1.1% of the TRR, corresponding to 0.561, 0.448, 1.042, 0.267 and 0.171 mg eq/kg, respectively. BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-CN88460-propanol were also identified in wheat hay and amounted to 2.4, 2.5, 10.3 and 0.7% of the TRR, corresponding to 0.096, 0.103, 0.414 and 0.029 mg eq/kg, respectively.

Overall, identification rates were sufficient and amounted to 80.0% of TRR for straw, 66.0% of TRR for hay and 92.0% of TRR for grains. Unknown metabolites were characterised in the extracts by their chromatographic behavior, individually accounting for equal to or less than 3.1% of the TRR.

Comparison of metabolic profiles with those of a parallel study with [phenyl-UL-^{14}C]BCS-CN88460 revealed a high correspondence and no label specific metabolite could be observed for the both labels.

More conjugates may be present among the characterised unknown metabolites in the chromatograms. Therefore, acid hydrolysis (1 M HCl, 100 °C, 1 h) of the conventional extracts of wheat hay and straw were conducted in order to analyse for hydrolysable conjugates. Major hydrolysis products detected in the acidic hydrolysates besides parent compound were the aglycons BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol. Identification rates after hydrolytic treatment increased. Based on these results it can be concluded that a significant amount of residues in the conventional extracts of hay and straw consists of conjugates of BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol.

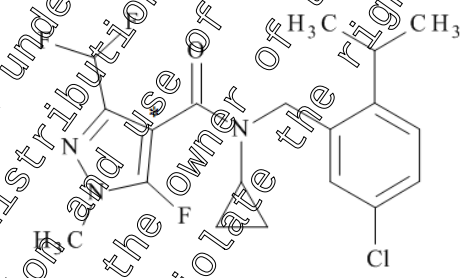
As metabolic reactions, hydroxylation of the propyl group followed by conjugation with hexose and malonic acid and the demethylation of the pyrazole moiety were observed.

Based on these results, the degradation behavior of [pyrazole-4-^{14}C]BCS-CN88460 in wheat is adequately understood and a pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * denotes the position of the ^{14}C-label
Radiolabel position	[pyrazole-4-^{14}C]
Specific radioactivity	4.22 MBq/mg
Radiochemical purity	100%
Chemical purity	> 99%

Formulation of the test compound

The test compound was formulated as an EC 50 for the experiment. The active substance [pyrazole-4-^{14}C]BCS-CN88460 was dissolved in acetonitrile. For each spray dilution, adequate parts of the stock solution were transferred into special glass vials and evaporated to dryness. Blank formulation was added and the mixtures were homogenised using a magnetic stirrer. The sample was then adjusted with water to a final volume of 100 mL of the spray dilution and homogenised by stirring.

2. Soil:

Soil characteristics			
Type	TOC	pH ($CaCl_2$)	CEC
Sandy loam	2.1%	7.23	15.5 meq/100 g

3. Plant: wheat, variety "KADRILJ", representative for cereals

B. Study Design

1. Experimental conditions:

The experiment was conducted with wheat plants based on a plant density of 5,000,000 wheat plants per hectare. A planting container with a surface area of 1 m^2 was used corresponding to the rate of 500 grains per m^2. The plants were applied at two different growth stages (BBCH 30 and 69). At both growth stages the target application rate was 65 g/ha. The planting container was filled with sandy loam soil "CUPF_soil". The plants were cultivated in the glass-roofed greenhouse of the test facility. The plants were grown similar to natural temperature and light conditions, but protected from rainfall. The plants were watered by pouring water onto the soil in the planting containers.

The wheat plants were treated with 100 mL of the aqueous spray dilutions using a controlled track sprayer with a flat fan nozzle. At the 1st application 29.1 MBq of the labelled test compound were applied, corresponding to 6.9 mg a.s. At the 2nd application 28.1 MBq of the test compound were applied, corresponding to 6.7 mg a.s. The total actual treatment rate was 69 and 67 g a.s./ha for the 1st and 2nd application, respectively. The seed density was 500 grains/m^2. After spray application onto the wheat plants of the planting container, the spray device and the protective plastic foil around the planting container were rinsed with acetonitrile/water (8/2; v/v). The actual amount applied was calculated by subtracting the losses from the radioactivity in the original application solution.

2. Sampling:

At growth stage BBCH 69 the RAC hay and at BBCH 89 the RACs straw and grains were harvested. Plant samples were collected by cutting approximately 1-2 cm above the soil level. Plants sampled at hay stage were dried in a hood for 4 days. The total weight of each sample was determined. The samples were homogenised with liquid nitrogen using a high speed blender. The sample materials were stored in a freezer (≤ -18 °C). Aliquots of the homogenates were extracted conventionally. The final TRR values of the samples were determined by summing up the radioactivity measured in the conventional extracts and in the remaining solids.

C. Analytical Procedures

1. Extraction:

Conventional extraction procedure and sample clean up:

Aliquots of the homogenised samples of wheat hay, straw and grains were conventionally extracted three times with a mixture of acetonitrile/water (8/2; v/v) using a high speed blender. After each extraction step the extracts were filtered by suction and the solids were rinsed with a small amount of the solvent mixture used for extraction. The solids were dried, homogenised aliquots subjected to combustion.

The extracts were combined and subjected to a clean-up step using a SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the

cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v). Volume and radioactivity of this fraction was also determined. Each combined percolate/rinse solution obtained from SPE purification was mixed with emulsifier and evaporated to the aqueous remainder. The final conventional extracts were then analysed by HPLC with the general profiling method. All wheat samples and extracts were stored in a freezer (≤ -18 °C).

Exhaustive extraction and corresponding clean-up:

Solids from the conventional extraction of wheat straw were exhaustively extracted two times with acetonitrile/water/formic acid (50/50/1; v/v/v) under microwave assistance at increased temperature (0 to 5 min increase to 120 °C, 5 to 20 min at 120 °C, 800 W). The microwave extracts were cooled down at room temperature, combined and concentrated by rotary evaporation. Aliquots of the extracts were centrifuged and separated into supernatant and pellet, which was dissolved in acetonitrile/water. Both extracts were further analysed HPLC.

Hydrolysis of the conventional extracts from wheat hay and straw:

Hydrolysis experiments in acidic medium were conducted with conventional extract of wheat hay and straw, to further characterise the residues. Aliquots of the conventional concentrated purified extract of wheat hay and straw were incubated with 1 M HCl and 5% acetonitrile at 100 °C for 1 hour and afterwards centrifuged. For wheat straw, the supernatant was removed and the pellet was dissolved in acetonitrile/water (1/1; v/v) whereas no pellet was formed during processing of wheat hay. Aliquots of the extracts were used for further HPLC analysis with the general profiling method. All wheat samples and extracts were stored in a freezer (≤ -18 °C).

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

Conventional and microwave extracts were analysed by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

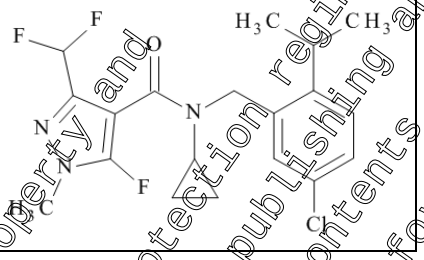
2. Identification and characterisation:

For identification of radioactive ingredients in conventional extract from wheat straw, selected major radiosignals were isolated by HPLC fractionation. Additional fractions were prepared by hydrolysis of isolated fractions by addition of HCl. The structures were identified by spectroscopic methods. Purity of isolated fractions before and after spectroscopic analysis was sufficient and each fraction was used as radiolabelled reference compound.

Metabolic profiles of all RACs were compared, as analysed by HPLC among themselves. Metabolic profiles of all RACs were compared with metabolic profiles of corresponding RACs in the wheat metabolism study with the phenyl label. Parent compound was identified in wheat grains extract by TLC and HPLC co-chromatography with non-radiolabelled and radiolabelled reference compound BCS-CN88460. Metabolic profiles of wheat hay and straw before and after hydrolysis were compared with corresponding profiles of the wheat metabolism study with the phenyl label, as analysed by HPLC.

Major metabolites and hydrolytic cleavage products from conventional extract of wheat straw in isolated fractions were identified by spectroscopic analysis. Metabolites in the conventional extract of wheat straw were confirmed by co-chromatography with the identified compounds. Unknown metabolites were characterised based on their extraction and chromatographic behavior.

Table 6.2.1- 12: List of reference compounds

Report name / other names/codes	Chemical Name (IUPAC)	Structure
Parent compound BCS-CN88460 Radiolabeled reference: S_PY_1_1000 Non-radiolabeled reference: BCS-CN88460-01-02	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

All extraction experiments with wheat hay, straw and grain and the first HPLC analyses were performed within one month after harvest of the wheat plant raw material. The stability of the stored extract of wheat hay, straw and grains was demonstrated by re-analysis of the extract by HPLC after 21, 14 and 15 months of storage.

A second analysis of wheat straw and grains was needed for analytical reasons and performed after 4 and 13 months of storage of the respective plant material after harvest. The storage stability of these samples could be demonstrated.

It was therefore concluded, that the residues in the samples were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in wheat was investigated after two spray applications. Wheat plants were treated with [pyrazole-4-^{14}C]BCS-CN88460 formulated as an EC 50 at BBCH 30 (beginning of stem elongation) and BBCH 69 (end of flowering). The actual single target application rate corresponded to 69 and 67 g a.s./ha for the first and second spray application, respectively which was slightly above the anticipated maximum application rate (2 x 65 g a.s./ha). The total application rate amounted to 136 g a.s./ha.

The TRR values of the individual RACs were determined by summing up the radioactivity determined in the combined extracts and the radioactivity in the solids. The residue levels are shown in ppm or mg active substance equivalents per kg sample material (mg a.s.equiv./kg or simplified mg eq/kg). The TRR in wheat hay and straw were high and amounted to 4.032 mg eq/kg and 15.536 mg eq/kg, respectively. The wheat grains showed a moderate TRR of 0.385 mg eq/kg.

Table 6.2.1- 13: TRR values in wheat matrices after foliar application of [pyrazole-4-^{14}C]BCS-CN88460

Matrix	Timing and Application	PHI (days)*	ppm (=mg eq/kg)
Wheat hay	1 spray application at BBCH 30, 69 g a.s./ha	27	4.032
Wheat straw	2 spray applications at BBCH 30 and BBCH 69, 69 and 67 g a.s./ha (136 g a.s./ha total)	17	15.536
Wheat grains		17	0.385

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at harvest/sampling)

Wheat hay was conventionally extracted three times with acetonitrile/water mixtures releasing 95.8% of the TRR (3.864 mg eq/kg). After purification and concentration steps 95.4% of the TRR (3.846 mg eq/kg) were analysed. Losses during sample clean up were 0.4% (0.018 mg eq/kg) of the TRR.

Wheat straw was conventionally extracted three times with acetonitrile/water mixtures releasing 94.0% of the TRR (14.604 mg eq/kg). After purification and concentration steps 93.5% of the TRR (14.521 mg eq/kg) were analysed. For wheat straw samples, a microwave extraction was performed. With this exhaustive method about 4.7% (0.727 mg eq/kg) of the TRR were extracted additionally. Finally, the residue level in the solids of wheat straw was 1.3% of the TRR (0.206 mg eq/kg). Losses during sample clean up of straw samples were 0.083 mg eq/kg.

Wheat grains were conventionally extracted three times with acetonitrile/water mixtures releasing 93.6% of the TRR (0.360 mg eq/kg). Based on the low amount of radioactivity (0.002 mg/kg) and high matrix load the third extract was not combined. After purification and concentration steps 92.0% of the TRR (0.354 mg eq/kg) were analysed. Losses during sample clean up were 0.006 mg eq/kg.

Table 6.2.1- 14: Distribution of radioactivity in the extracts of wheat matrices after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	hay		straw		grains	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		4.032		15.536		0.385
Conventional Extraction	95.8	3.864	94.0	14.604	93.6	0.360
Analysed extracts	95.4	3.846	93.5	14.521	92.0	0.354
Losses (not analysed) [#]	0.4	0.018	0.5	0.083	1.6	0.006
Microwave Extraction	--	--	4.7	0.727	--	--
Analysed extracts	--	--	4.7	0.727	--	--
Total extracted	**95.8**	**3.864**	**98.7**	**15.330**	**93.6**	**0.360**
Post extraction solids (PES)	**4.2**	**0.168**	**1.3**	**0.206**	**6.4**	**0.025**
Accountability	**100.0**	**4.032**	**100.0**	**15.536**	**100.0**	**0.385**

[#] losses during clean up, concentration, centrifugation, etc.
-- not performed

In the conventional extract from wheat hay 66.0% of the TRR (2.659 mg eq/kg) were identified in total. The parent compound was the major component representing 50.0% of the TRR (2.016 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-CN88460-propanol represented 2.4, 2.5, 10.3 and 0.7% of the TRR corresponding to 0.096, 0.103, 0.414 and 0.029 mg eq/kg, respectively.

In the conventional extract from wheat straw 77.1% of the TRR (11.969 mg eq/kg) were identified in total. The parent compound was the major component representing 62.9% of the TRR (9.761 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA, BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol represented 2.5, 2.9, 6.7, 0.7 and 1.4% of the TRR corresponding to 0.383, 0.448, 1.042, 0.116 and 0.219 mg eq/kg, respectively.

In the exhaustive extract of wheat straw 3.0% of the TRR (0.453 mg eq/kg) were further identified, consisting of parent compound and metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol representing 1.1, 1.2, 0.4 and 0.3% of the TRR corresponding to 0.172, 0.178, 0.055, 0.048 mg eq/kg, respectively. In total, 80.0% (12.422 mg eq/kg) of the TRR were identified in the conventional and exhaustive extracts of wheat straw.

Conventional extract from wheat grains contained only parent compound representing 92.0% of the TRR corresponding to 0.354 mg eq/kg.

The TRR and the distribution of parent and metabolites in wheat matrices are shown in the following table.

Table 6.2.1- 15: Distribution of parent compound and metabolites in the extracts of wheat matrices after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	wheat hay		wheat straw		wheat grains	
TRR [mg eq/kg] =	4.032		18.536		0.385	
Conventional extract						
Report name	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460 (parent compound)	50.0	2.016	52.9	9.761	92.0	0.354
BCS-CN88460-propanol-Glyc (M18)	2.4	0.096	2.5	0.383	n.d.	n.d.
BCS-CN88460-desmethyl-propanol-Glyc-MA (M41)	2.5	0.103	2.9	0.448	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	10.3	0.414	6.7	1.042	n.d.	n.d.
BCS-CN88460-propanol (M01)	0.7	0.029	1.4	0.219	n.d.	n.d.
BCS-CN88460-desmethyl-propanol (M06)	n.d.	n.d.	0.7	0.116	n.d.	n.d.
Subtotal identified	**66.0**	**2.659**	**77.1**	**11.969**	**92.0**	**0.354**
Unknown 88	n.d.	n.d.	0.6	0.086	n.d.	n.d.
Unknown 2	1.3	0.052	0.2	0.029	n.d.	n.d.
Unknown 32	1.1	0.044	0.4	0.063	n.d.	n.d.
Unknown 33	1.7	0.068	0.5	0.072	n.d.	n.d.
Unknown 34	0.9	0.036	1.5	0.230	n.d.	n.d.
Unknown 35	0.6	0.026	0.9	0.138	n.d.	n.d.
Unknown 36	1.1	0.046	n.d.	n.d.	n.d.	n.d.
Unknown 66	0.8	0.032	0.2	0.034	n.d.	n.d.
Unknown 37	1.8	0.071	n.d.	n.d.	n.d.	n.d.
Unknown 38	1.6	0.066	0.5	0.073	n.d.	n.d.
Unknown 39	1.1	0.042	1.0	0.149	n.d.	n.d.
Unknown 40	1.9	0.075	0.2	0.030	n.d.	n.d.
Unknown 64	1.3	0.051	0.1	0.018	n.d.	n.d.
Unknown 3	1.0	0.039	n.d.	n.d.	n.d.	n.d.
Unknown 23	0.9	0.037	n.d.	n.d.	n.d.	n.d.
Unknown 5	3.0	0.119	n.d.	n.d.	n.d.	n.d.
Unknown 8	2.6	0.104	1.4	0.210	n.d.	n.d.
Unknown 9	3.1	0.127	1.3	0.204	n.d.	n.d.
Unknown 42	1.5	0.059	1.1	0.168	n.d.	n.d.
Unknown 43	0.6	0.024	n.d.	n.d.	n.d.	n.d.
Unknown 44	0.5	0.021	0.7	0.104	n.d.	n.d.
Unknown 45	1.2	0.050	0.8	0.127	n.d.	n.d.
Unknown 52	n.d.	n.d.	0.2	0.038	n.d.	n.d.
Unknown 53	n.d.	n.d.	0.5	0.074	n.d.	n.d.
Unknown 54	n.d.	n.d.	0.5	0.076	n.d.	n.d.
Unknown 55	n.d.	n.d.	0.3	0.047	n.d.	n.d.
Unknown 59	n.d.	n.d.	0.5	0.083	n.d.	n.d.
Unknown 61	n.d.	n.d.	0.7	0.110	n.d.	n.d.
Unknown 62	n.d.	n.d.	0.9	0.136	n.d.	n.d.
Unknown 102	n.d.	n.d.	0.3	0.054	n.d.	n.d.
Unknown 103	n.d.	n.d.	0.3	0.054	n.d.	n.d.
Unknown 13	n.d.	n.d.	0.2	0.032	n.d.	n.d.
Unknown 14	n.d.	n.d.	0.2	0.032	n.d.	n.d.
Unknown 67	n.d.	n.d.	0.5	0.071	n.d.	n.d.
Subtotal characterised	**29.5**	**1.187**	**16.4**	**2.552**	**n.d.**	**n.d.**

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Table 6.2.1- 15 continued

	wheat hay		wheat straw		wheat grains	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Exhaustive extract *						
BCS-CN88460 (parent compound)	--	--	1.1	0.172	--	--
BCS-CN88460-propanol-Glyc (M18)	--	--	1.2	0.178	--	--
BCS-CN88460-desmethyl-propanol (M06)	--	--	0.4	0.055	--	--
BCS-CN88460-propanol (M01)	--	--	0.3	0.048	--	--
Subtotal identified	--	--	3.0	0.453	--	--
Unknown 40	--	--	0.1	0.018	--	--
Unknown 43	--	--	0.2	0.026	--	--
Unknown 3	--	--	0.5	0.085	--	--
Unknown 7	--	--	0.2	0.024	--	--
Unknown 41	--	--	0.1	0.021	--	--
Unknown 45	--	--	0.4	0.059	--	--
Unknown 50	--	--	0.3	0.043	--	--
Subtotal characterised	--	--	1.8	0.276	--	--
Total identified	**66.0**	**2.659**	**80.0**	**12.422**	**92.0**	**0.354**
Total characterised	**29.5**	**1.187**	**18.2**	**2.827**	--	--
Analysed extract(s)	95.4	3.846	98.2	15.248	92.0	0.354
Not analysed / Losses	0.4	0.018	0.5	0.083	1.6	0.006
Total extracted	**95.8**	**3.864**	**98.7**	**15.330**	**93.6**	**0.360**
Post extraction solids (PES)	**4.2**	**0.168**	**1.3**	**0.206**	**6.4**	**0.025**
Accountability	**100.0**	**4.032**	**100.0**	**15.536**	**100.0**	**0.385**

***** Given as sum of supernatant and dissolved pellet
-- not performed
n.d.: not detected

In wheat hay and straw 21 and 32 unknown metabolites were characterised in the extracts by their chromatographic behavior, individually accounting for less than 3.1% of the TRR and 0.127 mg eq/kg and 1.5% of the TRR and 0.239 mg eq/kg.

Table 6.2.1- 16: Summary of characterisation and identification of radioactive residues in wheat matrices after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	wheat hay		wheat straw		wheat grains	
TRR [mg eq/kg] =	4.032		15.536		0.385	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460 (parent compound)	50.0	2.016	64.0	9.933	92.0	0.354
BCS-CN88460-propanol-Glyc (M18)	2.4	0.096	3.7	0.561	--	--
BCS-CN88460-desmethyl-propanol-Glyc-MA (M41)	2.5	0.103	2.9	0.448	--	--
BCS-CN88460-propanol-Glyc-MA (M21)	10.3	0.414	6.7	1.042	--	--
BCS-CN88460-propanol (M01)	0.7	0.029	1.7	0.267	--	--
BCS-CN88460-desmethyl-propanol (M06)	--	--	1.1	0.171	--	--
Total identified	**66.0**	**2.659**	**80.0**	**12.422**	**92.0**	**0.354**
Number of unknown peaks	21		32		--	
Largest unknown peak	3.1	0.119	1.5	0.239	--	--
Total characterised	**29.5**	**1.187**	**18.2**	**2.827**		**--**
Analysed extract(s)	95.4	3.846	98.2	15.248	92.0	0.354
Not analysed / Losses	0.4	0.018	0.5	0.083	1.6	0.006
Total extracted	**95.8**	**3.864**	**98.7**	**15.330**	**93.6**	**0.360**
Post extraction solids (PES)	**4.2**	**0.168**	**1.3**	**0.206**	**6.4**	**0.025**
Accountability	**100.0**	**4.032**	**100.0**	**15.536**	**100.0**	**0.385**

More conjugates may be present among the characterised unknown metabolites in the chromatograms. Therefore, acid hydrolysis (1 M HCl, 100 °C, 1 h) of the conventional extracts of wheat hay and straw were conducted in order to analyse for hydrolysable conjugates.

In hydrolysed extract of wheat hay 90.3% of the TRR (3.640 mg eq/kg) were analysed. The parent compound was the major component representing 44.4% of the TRR (1.791 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-propanol-Glyc-MA, BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol represented 0.8, 0.9, 22.3 and 6.9% of the TRR corresponding to 0.031, 0.036, 0.901 and 0.277 mg eq/kg, respectively.

In hydrolysed extract of wheat straw 92.5% of the TRR (14.372 mg eq/kg) were analysed. The parent compound was the major component representing 67.0% of the TRR (10.397 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-propanol-Glyc-MA, BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol represented 0.2, 0.3, 10.5 and 3.6% of the TRR corresponding to 0.024, 0.056, 1.625 and 0.564 mg eq/kg, respectively. In contrast to hydrolysis of the hay extract, a pellet was formed during hydrolysis of the straw extract. Therefore values of analysed residues are given as sum of supernatant and dissolved pellet.

Identification rates after hydrolytic treatment increased for wheat hay from 66.0% of the TRR (2.659 mg eq/kg) before hydrolysis to 75.3% of the TRR (3.035 mg eq/kg) after hydrolysis and for wheat straw from 77.1% of the TRR (11.969 mg eq/kg) before hydrolysis to 81.6% of the TRR (12.666 mg eq/kg) after hydrolysis. Two major metabolites were formed after acidic hydrolysis as a result of de-conjugation of residues: BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol. BCS-CN88460-desmethyl-propanol in wheat hay accounted for 6.9% of the TRR (0.277 mg eq/kg) and 3.6% of the TRR (0.564 mg eq/kg) in conventional extract from wheat straw. Metabolite BCS-CN88460-propanol was detected in hydrolysed extract from wheat hay and straw accounting for 22.3% of the TRR (0.901 mg eq/kg) and 10.5% of the TRR (1.625 mg eq/kg), respectively.

Based on these results it can be concluded that a significant amount of residues in the conventional extracts of hay and straw consists of conjugates of BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol. A comparison of the distribution of parent compound and metabolites in the conventional wheat hay and straw extracts before and after hydrolysis is given in the table below.

Analogous hydrolysis experiments were performed in the parallel study with the phenyl-label showing good accordance with the current study.

Table 6.2.1- 17: Distribution of radioactive residues of parent compound and metabolites in the extracts of wheat matrices before and after hydrolysis (1 M HCl, 100 °C, 1 h)

	wheat hay				wheat straw			
	conventional extract **before** hydrolysis		conventional extract **after** hydrolysis*		conventional extract **before** hydrolysis		conventional extract **after** hydrolysis*	
Report name	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460 (parent compound)	50.0	2.016	44.4	1.791	62.9	9.761	67.0	10.397
BCS-CN88460-propanol-Glyc (M18)	2.4	0.096	0.8	0.031	2.5	0.383	0.2	0.024
BCS-CN88460-desmethyl-propanol-Glyc-MA (M41)	2.5	0.103	n.d.	n.d.	2.9	0.448	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	10.3	0.414	0.9	0.036	6.7	1.042	0.3	0.056
BCS-CN88460-propanol (M01)	0.7	0.029	22.3	0.901	1.4	0.219	10.5	1.625
BCS-CN88460-desmethyl-propanol (M06)	n.d.	n.d.	6.9	0.277	0.7	0.116	3.6	0.564
Total identified	**66.0**	**2.659**	**75.3**	**3.035**	**77.1**	**11.969**	**81.6**	**12.666**
Total characterised	**29.5**	**1.187**	**15.0**	**0.605**	**16.4**	**2.552**	**11.0**	**1.706**

***** values are given as sum of supernatant and dissolved pellet; during hydrolysis of wheat hay extract, no pellet was formed
n.d.: not detected

III. Conclusions

The metabolism of BCS-CN88460 in wheat was investigated after two foliar applications at growth stages BBCH 30 and BBCH 69. The wheat plants were treated with [pyrazole-4-^{14}C]BCS-CN88460 formulated as an EC 50 at a single application rate of 69 and 67 g a.s./ha for the first and second spray application, respectively, corresponding to a total application rate of 136 g a.s/ha. Wheat hay was harvested at BBCH 69 (1 day prior to the second application, wheat straw and grains were harvested at maturity (BBCH 89).

Residues in wheat grains were significantly lower than those in wheat hay and straw. The extraction rates of hay, straw and grains were high. Overall, identification rates in wheat hay, straw and grain were sufficient. In all RACs, parent compound BCS-CN88460 was the main residue component and the only component in wheat grains. Besides parent compound, other metabolites were identified in wheat straw and hay: BCS-CN88460-propanol-Glyc (M18), BCS-CN88460-desmethyl-propanol-Glyc-MA (M41), BCS-CN88460-propanol-Glyc-MA (M21), BCS-CN88460-propanol (M01) and BCS-CN88460-desmethyl-propanol (M06).

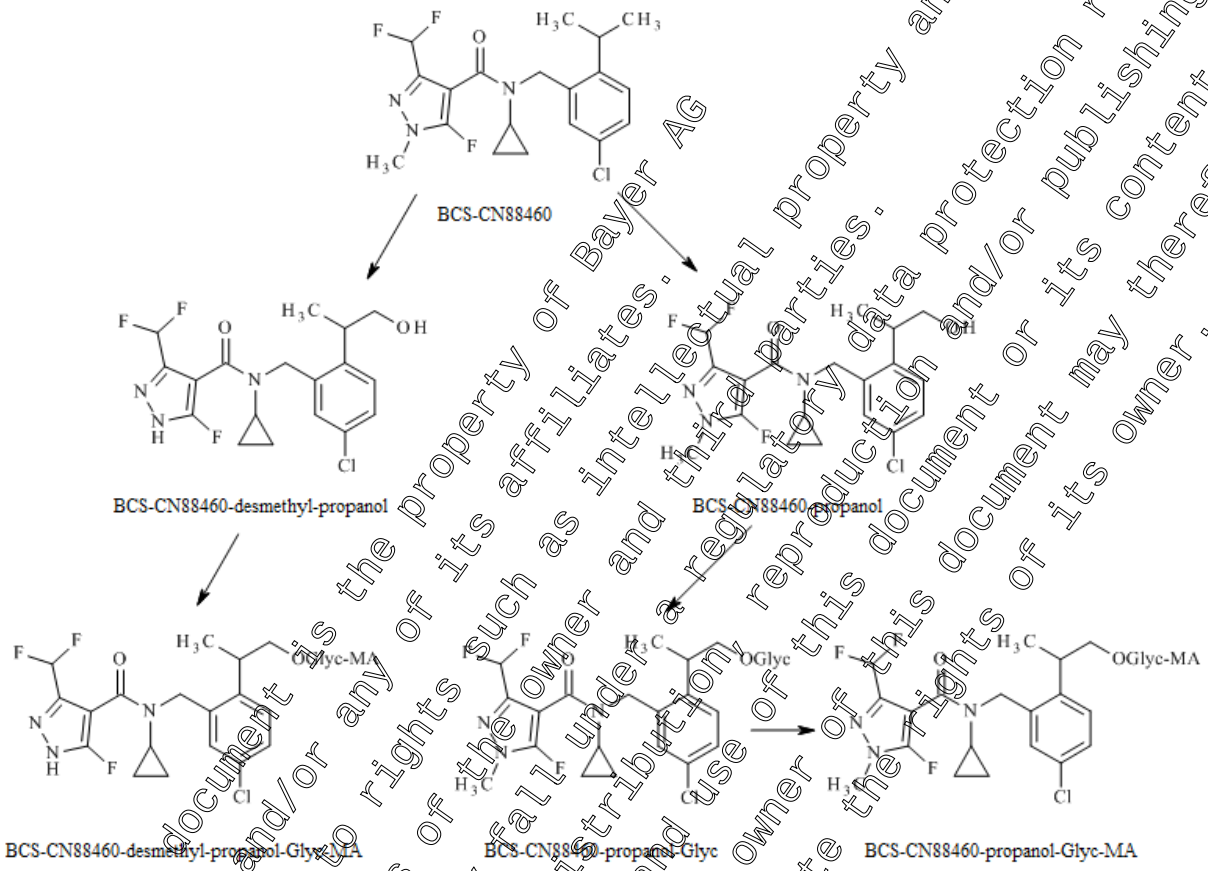
The results in the present study are in good agreement with the results with the phenyl-label. No label specific metabolites were observed. Acid hydrolysis of the conventional extracts of wheat hay and wheat straw showed cleavage of the identified conjugates to the aglycons BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol and the presence of further conjugates of the aforementioned aglycons.

The test compound [pyrazole-4-^{14}C]BCS-CN88460 was moderately metabolised in wheat after two post-emergence applications. The main metabolic reactions observed were:

- hydroxylation in position 1 of the propyl group followed by conjugation with hexose and malonic acid
- demethylation of the pyrazole moiety

Based on these results, the degradation behavior of [pyrazole-4-^{14}C]BCS-CN88460 in wheat is adequately understood.

Figure 6.2.1- 1: Proposed metabolic pathway of [pyrazole-4-^{14}C]BCS-CN88460 in wheat



Report: KCA 6.2.1.04; [redacted], M., 2018, M-604358-02-1
Title: Amendment no.1 to final report - Metabolism of [phenyl-UL-14C] BCS-CN88460 in wheat plants
Report No.: S14-01086
Document No.: M-604358-02-1
Guideline(s): OECD Test Guideline No. 501
Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
US EPA OCSPP Test Guideline No. 860.1300
JAP FAMIC-ACIS Notification 12 Nousan 8147
Guideline deviation(s): none
GLP/GEP: **yes**

Executive Summary

The metabolism of the novel fungicide BCS-CN88460 was investigated in wheat plants after two applications. For each of the two foliar applications the test item [phenyl-UL-^{14}C]BCS-CN88460 was formulated as an aqueous EC 50 using a nominal application rate of 65 g a.s./ha each. The applications were performed at the growth stage of BBCH 30 (beginning of stem elongation) and BBCH 69 (end of

flowering). The actual application rate corresponded to 64 and 66 g a.s./ha for the first and second application, respectively resulting in a total application rate of 130 g a.s./ha.

Wheat hay was harvested at BBCH 69, 1 day prior to the second application, wheat straw and grains were harvested at maturity (BBCH 89). The total radioactive residues (TRR) in wheat straw and hay were high and amounted to 16.031 mg eq/kg and 3.040 mg eq/kg, respectively. The TRR in wheat grains was moderate and amounted to 0.284 mg eq/kg.

Homogenised plant material from RACs was conventionally extracted with a mixture of acetonitrile/water (8/2, v/v). The extraction rates after conventional extraction of wheat hay, straw and grains were high and amounted to 96.7% (2.940 mg eq/kg) of the TRR for hay, 95.2% (15.264 mg eq/kg) of the TRR for straw and 93.5% (0.266 mg eq/kg) of the TRR for grains. The post extraction solids (PES) of all RACs remaining after conventional extraction amounted to ≤ 6.5% of TRR.

Residues in the conventional extracts were analysed and quantified by HPLC. The parent compound was identified by co-chromatography with the reference compound and metabolites were assigned by comparison of the metabolite pattern and retention times of the current and the wheat metabolism study with pyrazole label.

The parent substance BCS-CN88460 represented the most prominent residue component in all RACs accounting for 54.7% of TRR (1.661 mg eq/kg) in wheat hay, 62.1% of TRR (9.954 mg eq/kg) in wheat straw and 92.7% of TRR (0.264 mg eq/kg) in wheat grains. Besides parent compound no other metabolite was detected in the extract of grains. In wheat straw five metabolites were identified besides parent compound: BCS-CN88460-propanol-Glyc (M18), BCS-CN88460-desmethyl-propanol-Glyc-MA (M41), BCS-CN88460-propanol-Glyc-MA (M21), BCS-CN88460-propanol (M01) and BCS-CN88460-desmethyl-propanol (M06) accounting for 2.3, 1.9, 5.0, 0.9 and 0.3% of the TRR, corresponding to 0.373, 0.306, 0.808, 0.147 and 0.052 mg eq/kg, respectively. BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-CN88460-propanol were also identified in wheat hay and amounted to 0.8, 2.7, 7.5 and 0.7% of the TRR, corresponding to 0.023, 0.081, 0.229 and 0.021 mg eq/kg, respectively.

Overall, identification rates were sufficient and amounted to 72.6% of TRR for straw, 66.3% of TRR for hay and 92.7% of TRR for grains. In wheat hay and straw, 23 and 39 unknown metabolites were characterised in the extracts by their chromatographic behavior, individually accounting for less than 3.1% of the TRR and 0.095 mg eq/kg and 2.1% of the TRR and 0.345 mg eq/kg.

Comparison of metabolic profiles with those of a parallel study with [pyrazole-4-^{14}C]BCS-CN88460 revealed a high correspondence and no label specific metabolite could be observed for the both labels.

More conjugates might be present among the characterised unknown metabol1ites in the chromatograms. Therefore, acid hydrolysis (1 M HCl, 100 °C, 1 h) of the conventional extracts of wheat hay and straw was conducted in order to analyse for hydrolysable conjugates. Major hydrolysis products detected in the acidic hydrolysates besides parent compound were BCS-CN88460-desmethyl-propanol (M06) and BCS-CN88460-propanol (M01). Identification rates after hydrolytic treatment increased. Based on these results it can be concluded that a significant amount of residues in the conventional extracts of hay and straw consists of conjugates of BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol. Analogous hydrolysis experiments were performed in the parallel study with the pyrazole-label showing good accordance with the current study.

Storage stability was demonstrated for residues in wheat hay and straw by extraction and comparison of extracts for up to 30 months.

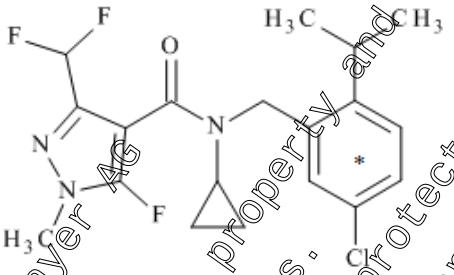
As metabolic reactions, hydroxylation of the propyl group followed by conjugation with hexose and malonic acid and the demethylation of the pyrazole moiety were observed.

Based on these results, the degradation behavior of [phenyl-UL-^{14}C]BCS-CN88460 in wheat is adequately understood and a pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	
	*denotes the position of the ^{14}C-label
Radiolabel position	[phenyl-UL-^{14}C]
Specific radioactivity	4.13 MBq/mg
Radiochemical purity	100%
Chemical purity	> 98%

Formulation of the test compound

A stock solution of the test compound was prepared by dissolving the test compound in acetonitrile to give a concentration of about 5 mg/mL. The purity was checked by HPLC with radiodetection and was 100%. The test compound was formulated as an EC 50 for the experiment. The active substance [phenyl-UL-^{14}C]BCS-CN88460 was dissolved in acetonitrile. For each spray dilution, adequate parts of the stock solution were transferred into special glass vials and evaporated to dryness. Blank formulation was added and the mixtures were homogenised using a magnetic stirrer. The sample was then adjusted with water to a final volume of 100 mL of the spray dilution and homogenised by stirring.

2. Soil:

Soil characteristics			
Type	TOC	pH ($CaCl_2$)	CEC
Sandy loam	2.1%	7.23	15.5 meq/100 g

3. Plant: wheat, variety "KADRILJ", representative for cereals

B. Study Design

1. Experimental conditions:

The experiment was conducted with wheat plants based on a plant density of 5,000,000 wheat plants per hectare. A planting container with a surface area of 1 m^2 was used corresponding to the rate of 500 grains per m^2. The plants were applied at two different growth stages (BBCH 30 and 69). The experiment was conducted, representing the intended application type for BCS-CN88460. The formulated test compound was applied to the wheat plants at a target rate of 2 x 65 g a.s./ha. The target rate corresponds to the anticipated maximum application rates for the use type. The planting container was filled with sandy loam soil "CUPF_soil". The plants were cultivated in the glass-roofed greenhouse of the test facility. The plants were grown similar to natural temperature and light conditions, but protected from rainfall. The plants were watered by pouring water onto the soil in the planting containers.

The wheat plants were treated with 100 mL of the aqueous spray dilutions as a spray using a controlled track sprayer with a flat fan nozzle. At the 1st application 26.3 MBq of the labelled test compound were applied, corresponding to 6.4 mg a.s.. At the 2nd application 27.1 MBq were applied, corresponding to 6.6 mg a.s. The total actual treatment rate was 64 and 66 g a.s./ha. The seed density was 500 grains/m^2. Calculations for losses during application are taken into account: After spraying the spray dilution onto the wheat plants of the planting container, the spray device and the protective plastic foil around the planting container were rinsed with acetonitrile/water (8/2; v/v). The actual amount applied was calculated by subtracting the losses from the radioactivity in the original application solution.

2. Sampling:

At growth stage BBCH 69 the RAC hay and at BBCH 89 the RACs straw and grains were harvested. Plant samples were collected by cutting approximately 1-2 cm above the soil level. Plants sampled at hay stage were dried in a flue for 4 days. The total weight of each sample was determined. The samples were homogenised with liquid nitrogen using a high speed blender. The sample materials were stored in a freezer (≤ -18 °C). Aliquots of the homogenates were extracted conventionally. The final TRR values of the samples were determined by summing up the radioactivity measured in the conventional extracts and in the remaining solids.

C. Analytical Procedures

1. Extraction:

Conventional extraction procedure and sample clean up:

Aliquots of the homogenised samples of wheat hay, straw and grains were extracted three times with a mixture of acetonitrile/water (8/2; v/v) using a high speed blender. After each extraction step, the extracts were filtered by suction and the solids were rinsed with a small amount of the solvent mixture used for extraction. The solids were dried, homogenised aliquots subjected to combustion.

The extracts were combined and subjected to a clean-up step using a SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v). Volume and radioactivity of this fraction was also determined. Each combined percolate/rinse solution obtained from SPE purification was mixed with emulsifier and evaporated to the aqueous remainder. The final conventional extracts were analysed by HPLC. All wheat samples and extracts were stored in a freezer (≤ -18 °C).

<u>Hydrolysis of the conventional extracts from wheat hay and straw:</u>

Hydrolysis experiments in acidic medium were conducted with conventional extracts of wheat hay and straw, to further characterise the residues. Aliquots of the conventional concentrated purified extract of wheat hay and straw were incubated with 1 M HCl and 5% acetonitrile at 100 °C for 1 hour and afterwards centrifuged. For wheat straw, the supernatant was removed and the pellet was dissolved in acetonitrile/water (1/1; v/v) whereas no pellet was formed during processing of wheat hay. Aliquots of the extracts were analysed by HPLC.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

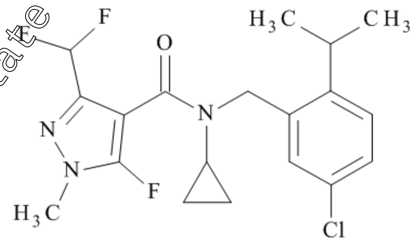
Parent compound and metabolites were quantified in the extracts by HPLC with radiodetection based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

2. Identification and characterisation:

Metabolic profiles of all RACs were compared, as analysed by HPLC among themselves. Metabolic profiles of all RACs were compared with metabolic profiles of corresponding RACs in the wheat metabolism study with the pyrazole label in which the major metabolites were identified spectroscopically. Parent compound was identified in wheat grains extract by TLC and HPLC co-chromatography with non-radiolabelled and radiolabelled reference compound BCS-CN88460. Metabolic profiles of wheat hay and straw before and after hydrolysis were compared with corresponding profiles of the wheat metabolism study with the pyrazole label, as analysed by HPLC.

Unknown metabolites were characterised based on their extraction and chromatographic behavior.

Table 6.2.1- 18: List of reference compound

Report name / other names/codes	**Chemical Name (IUPAC)**	**Structure**
Parent compound **BCS-CN88460** Radiolabeled reference: S_PH_1_1000 Non-radiolabeled reference: BCS-CN88460-01-02	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

All extraction experiments with wheat hay and straw and the first HPLC analyses were performed within one month after harvest of the wheat plant raw material. The stability of the stored extract of wheat hay, straw and grains was demonstrated by re-analysis of the extract by HPLC after 30, 28 and 15 months of storage.

A second analysis of wheat straw and grains was needed for analytical reasons and performed after 30 months of storage of the respective plant material after harvest. The storage stability of these samples could be demonstrated.

It was therefore concluded, that the residues in the samples were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in wheat was investigated after two spray applications. Wheat plants were treated with [phenyl-UL-^{14}C]BCS-CN88460 formulated as an EC 50 at BBCH 30 (beginning of stem elongation) and BBCH 69 (end of flowering). The actual single target application rate corresponded to 64 and 66 g a.s./ha for the first and second spray application, respectively which was slightly above the anticipated maximum application rate. The total application rate amounted to 130 g a.s./ha.

The TRR values of the individual RACs were determined by summing up the radioactivity determined in the combined extracts and the radioactivity in the solids. The residue levels are shown in ppm or mg active substance equivalents per kg sample material (mg a.s.equiv./kg or simplified mg eq/kg). The TRR in wheat hay and straw were high and amounted to 3.040 mg eq/kg and 16.031 mg eq/kg, respectively. The wheat grains showed a moderate TRR of 0.284 mg eq/kg.

Table 6.2.1- 19: TRR values in wheat matrices after foliar application of [phenyl-UL-^{14}C]BCS-CN88460

Matrix	Timing and Application	PHI (days)*	ppm (=mg eq/kg)
Wheat hay	1 spray application at BBCH 30, 64 g a.s./ha	32	3.040
Wheat straw	2 spray applications at BBCH 30 and BBCH 69, 64 and 66 g a.s./ha (130 g a.s./ha total)	18	16.031
Wheat grains		18	0.284

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at harvest/sampling)

Wheat hay was conventionally extracted three times with acetonitrile/water mixtures releasing 96.7% of the TRR (2.940 mg eq/kg). After purification and concentration steps 96.2% of the TRR (2.925 mg eq/kg) were analysed. Losses during sample clean up were 0.5% of TRR (0.015 mg eq/kg).

Wheat straw was conventionally extracted three times with acetonitrile/water mixtures releasing 95.2% of the TRR (15.264 mg eq/kg). After purification and concentration steps 93.1% of the TRR (14.922 mg eq/kg) were analysed. Losses during sample clean up of straw samples were 2.1% of TRR (0.342 mg eq/kg).

Wheat grains were conventionally extracted three times with acetonitrile/water mixtures releasing 93.5% of the TRR (0.266 mg eq/kg). Based on the low amount of radioactivity (0.001 mg/kg) and high matrix load the third extract was not combined. After purification and concentration steps 92.7% of the TRR (0.264 mg eq/kg) were analysed. Losses during sample clean up were 0.8% of TRR (0.002 mg eq/kg).

The distribution of the radioactive residues is shown in the following table.

Table 6.2.1- 20: Distribution of radioactivity in the extracts of wheat matrices after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

Sample	hay		straw		grains	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		**3.040**		**16.031**		**0.284**
Conventional Extraction	96.7	2.940	95.2	15.264	93.5	0.266
Analysed extracts	96.2	2.925	93.1	14.922	92.7	0.264
Losses (not analysed)[#]	0.5	0.015	2.1	0.342	0.8	0.002
Total extracted	**96.7**	**2.940**	**95.2**	**15.264**	**93.5**	**0.266**
Post extraction solids (PES)	**3.3**	**0.099**	**4.8**	**0.767**	**6.5**	**0.019**
Accountability	**100.0**	**3.040**	**100.0**	**16.031**	**100.0**	**0.284**

[#] losses during clean up, concentration, centrifugation etc.

In the conventional extract from wheat hay 66.3% of the TRR (2.015 mg eq/kg) were identified in total. The parent compound was the major component representing 54.7% of the TRR (1.661 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-CN88460-propanol represented 0.8, 2.7, 7.5 and 0.7% of the TRR corresponding to 0.023, 0.081, 0.229 and 0.021 mg eq/kg, respectively.

In the conventional extract from wheat straw 72.6% of the TRR (11.640 mg eq/kg) were identified in total. The parent compound was the major component representing 62.1% of the TRR (9.954 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA, BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol represented 2.3, 1.9, 5.0, 0.3 and 0.9% of the TRR corresponding to 0.373, 0.306, 0.808, 0.052 and 0.147 mg eq/kg, respectively.

The conventional extract from wheat grains contained only parent compound representing 92.7% of the TRR corresponding to 0.264 mg eq/kg. The compound was identified by co-chromatography using HPLC and TLC.

The TRR and the distribution of parent and metabolites in wheat matrices are shown in the following table.

Table 6.2.1- 21: Distribution of parent compound and metabolites in the extracts of wheat matrices after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

	wheat hay		wheat straw		wheat grains	
TRR [mg eq/kg] =		3.040		16.031		0.284
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460 (parent compound)	54.7	1.661	62.1	9.954	92.7	0.264
BCS-CN88460-propanol-Glyc (M18)	0.8	0.023	2.3	0.373	n.d.	n.d.
BCS-CN88460-desmethyl-propanol-Glyc-MA (M41)	2.7	0.081	1.9	0.306	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	7.5	0.229	5.0	0.808	n.d.	n.d.
BCS-CN88460-propanol (M01)	0.7	0.021	0.9	0.147	n.d.	n.d.
BCS-CN88460-desmethyl-propanol (M06)	n.d.	n.d.	0.3	0.052	n.d.	n.d.
Total identified	**66.3**	**2.015**	**72.6**	**11.640**	**92.7**	**0.264**
Unknown 2	0.7	0.020	0.4	0.069	n.d.	n.d.
Unknown 72	1.2	0.035	n.d.	n.d.	n.d.	n.d.
Unknown 74	0.7	0.022	n.d.	n.d.	n.d.	n.d.
Unknown 28	n.d.	n.d.	0.3	0.048	n.d.	n.d.
Unknown 29	n.d.	n.d.	0.3	0.050	n.d.	n.d.
Unknown 30	n.d.	n.d.	0.7	0.115	n.d.	n.d.
Unknown 31	n.d.	n.d.	0.4	0.059	n.d.	n.d.
Unknown 32	1.5	0.046	0.7	0.104	n.d.	n.d.
Unknown 33	0.9	0.027	0.4	0.057	n.d.	n.d.
Unknown 34	0.9	0.028	n.d.	n.d.	n.d.	n.d.
Unknown 35	0.6	0.018	n.d.	n.d.	n.d.	n.d.
Unknown 36	1.1	0.034	n.d.	n.d.	n.d.	n.d.
Unknown 37	1.6	0.048	0.8	0.126	n.d.	n.d.
Unknown 77	1.1	0.032	n.d.	n.d.	n.d.	n.d.
Unknown 38	n.d.	n.d.	0.4	0.063	n.d.	n.d.
Unknown 39	1.0	0.030	0.4	0.067	n.d.	n.d.
Unknown 79	n.d.	n.d.	0.2	0.038	n.d.	n.d.
Unknown 40	1.8	0.055	0.5	0.077	n.d.	n.d.
Unknown 81	0.7	0.022	n.d.	n.d.	n.d.	n.d.
Unknown 64	1.2	0.037	n.d.	n.d.	n.d.	n.d.
Unknown 3	0.9	0.027	1.5	0.248	n.d.	n.d.
Unknown 23	n.d.	n.d.	1.6	0.251	n.d.	n.d.
Unknown 5	0.7	0.022	n.d.	n.d.	n.d.	n.d.
Unknown 7	3.1	0.095	1.0	0.162	n.d.	n.d.
Unknown 41	1.8	0.055	0.6	0.104	n.d.	n.d.
Unknown 9	3.0	0.090	2.1	0.345	n.d.	n.d.
Unknown 42	1.2	0.037	0.4	0.067	n.d.	n.d.
Unknown 43	1.0	0.032	0.3	0.054	n.d.	n.d.

continued on next page

Table 6.2.1- 21: continued

	wheat hay		wheat straw		wheat grains	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Unknown 45	n.d.	n.d.	0.3	0.054	n.d.	n.d.
Unknown 84	n.d.	n.d.	0.2	0.040	n.d.	n.d.
Unknown 46	n.d.	n.d.	0.2	0.034	n.d.	n.d.
Unknown 47	n.d.	n.d.	0.2	0.036	n.d.	n.d.
Unknown 48	n.d.	n.d.	0.4	0.060	n.d.	n.d.
Unknown 50	n.d.	n.d.	0.2	0.036	n.d.	n.d.
Unknown 51	n.d.	n.d.	0.3	0.050	n.d.	n.d.
Unknown 52	n.d.	n.d.	0.2	0.035	n.d.	n.d.
Unknown 53	n.d.	n.d.	0.2	0.031	n.d.	n.d.
Unknown 54	n.d.	n.d.	0.2	0.032	n.d.	n.d.
Unknown 55	n.d.	n.d.	0.4	0.060	n.d.	n.d.
Unknown 57	n.d.	n.d.	0.4	0.070	n.d.	n.d.
Unknown 58	n.d.	n.d.	0.4	0.058	n.d.	n.d.
Unknown 61	n.d.	n.d.	0.4	0.061	n.d.	n.d.
Unknown 11	1.0	0.030	0.5	0.085	n.d.	n.d.
Unknown 12	n.d.	n.d.	0.8	0.121	n.d.	n.d.
Unknown 62	n.d.	n.d.	0.5	0.083	n.d.	n.d.
Unknown 63	n.d.	n.d.	0.5	0.084	n.d.	n.d.
Unknown 13	n.d.	n.d.	0.3	0.048	n.d.	n.d.
Unknown 14	2.2	0.068	0.6	0.103	n.d.	n.d.
Total characterised	**30.0**	**0.910**	**20.5**	**3.281**	n.d.	n.d.
Analysed extract(s)	96.2	2.925	93.1	14.922	92.7	0.264
Not analysed / Losses	0.5	0.015	2.1	0.342	0.8	0.002
Total extracted	**96.7**	**2.940**	**95.2**	**15.264**	**93.5**	**0.266**
Post extraction solids (PES)	**3.3**	**0.099**	**4.8**	**0.767**	**6.5**	**0.019**
Accountability	**100.0**	**3.040**	**100.0**	**16.031**	**100.0**	**0.284**

n.d. not detected

In wheat hay and straw 23 and 39 unknown metabolites were characterised in the extracts, individually accounting for less than 3.1% of the TRR and 0.095 mg eq/kg and 2.1% of the TRR and 0.345 mg eq/kg.

Table 6.2.1- 22: Summary of characterisation and identification of radioactive residues in wheat matrices after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

	wheat hay		wheat straw		wheat grains	
TRR [mg eq/kg] =	3.040		16.031		0.284	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460 (parent compound)	54.7	1.661	62.1	9.954	92.7	0.264
BCS-CN88460-propanol-Glyc (M18)	0.8	0.023	2.3	0.373	n.d.	n.d.
BCS-CN88460-desmethyl-propanol-Glyc-MA (M41)	2.7	0.081	1.9	0.306	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	7.5	0.229	5.0	0.808	n.d.	n.d.
BCS-CN88460-propanol (M01)	0.7	0.021	0.9	0.147	n.d.	n.d.
BCS-CN88460-desmethyl-propanol (M06)	n.d.	n.d.	0.3	0.052	n.d.	n.d.
Total identified	**66.3**	**2.015**	**72.6**	**11.640**	**92.7**	**0.264**
Number of unknown peaks	23		39		n.d.	
Largest unknown peak	3.1	0.095	2.10	0.345	n.d.	
Total characterised	**30.0**	**0.910**	**20.5**	**3.281**	n.d.	
Analysed extract(s)	96.2	2.925	93.1	14.922	92.7	0.264
Not analysed / Losses	0.5	0.015	2.1	0.342	0.8	0.002
Total extracted	**96.7**	**2.940**	**95.2**	**15.264**	**93.5**	**0.266**
Post extraction solids (PES)	**3.3**	**0.099**	**4.8**	**0.767**	**6.5**	**0.019**
Accountability	**100.0**	**3.040**	**100.0**	**16.031**	**100.0**	**0.284**

n.d. not detected

More conjugates might be present among the characterised unknown metabolites in the chromatograms. Therefore, acid hydrolysis (1 M HCl, 100 °C, 1 h) of the conventional extracts of wheat hay and straw were conducted in order to analyse for hydrolysable conjugates.

In hydrolysed extract of wheat hay 95.2% of the TRR (2.893 mg eq/kg) were analysed. The parent compound was the major component representing 49.6% of the TRR (1.508 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol represented 0.6, 21.1 and 6.7% of the TRR corresponding to 0.019, 0.642 and 0.204 mg eq/kg, respectively.

In hydrolysed extract of wheat straw 92.5% of the TRR (14.832 mg eq/kg) were analysed. The parent compound was the major component representing 60.8% of the TRR (9.751 mg eq/kg), whereas the metabolites BCS-CN88460-propanol-Glyc, BCS-CN88460-desmethyl-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA, BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol represented 0.3 0.3, 0.1, 12.7 and 4.0% of the TRR corresponding to 0.053, 0.054, 0.022, 2.046 and 0.644 mg eq/kg, respectively. In contrast to hydrolysis of the hay extract, a pellet was formed during hydrolysis of the straw extract. Therefore values of analysed residues are given as sum of supernatant and dissolved pellet.

Identification rates after hydrolytic treatment increased for wheat hay from 66.3% of the TRR (2.015 mg eq/kg) before hydrolysis to 78.0% of the TRR (2.373 mg eq/kg) after hydrolysis and for wheat straw from 72.6% of the TRR (11.640 mg eq/kg) before hydrolysis to 78.2% of the TRR (12.570 mg eq/kg) after hydrolysis. Two major metabolites were formed after acidic hydrolysis as a result of de-conjugation of residues: BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol. BCS-CN88460-desmethyl-propanol in wheat hay accounted for 6.7% of the TRR (0.204 mg eq/kg) and 4.0% of the TRR (0.644 mg eq/kg) in the hydrolysed extract from wheat straw. Metabolite BCS-CN88460-propanol was detected in hydrolysed extract from wheat hay and straw accounting for 21.1% of the TRR (0.642 mg eq/kg) and 12.7% of the TRR (2.046 mg eq/kg), respectively.

Based on these results it can be concluded that a significant amount of residues in the conventional extracts of hay and straw consists of conjugates of BCS-CN88460-desmethyl-propanol (M06) and

BCS-CN88460-propanol (M01). A comparison of the distribution of parent compound and metabolites in the conventional wheat hay and straw extracts before and after hydrolysis is given in the table below.

Analogous hydrolysis experiments were performed in the parallel study with the pyrazole-label showing good accordance with the current study.

Table 6.2.1- 23: Distribution of radioactive residues of parent compound and metabolites in the extracts of wheat matrices before and after hydrolysis (1 M HCl, 100 °C, 1 h)

	wheat hay				**wheat straw**			
	conventional extract **before** hydrolysis		conventional extract **after** hydrolysis*		conventional extract **before** hydrolysis		conventional extract **after** hydrolysis*	
Report name	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460 (parent compound)	24.7	1.661	49.6	1.508	62.1	9.954	60.8	9.751
BCS-CN88460-propanol-Glyc (M18)	0.8	0.023	0.6	0.019	2.3	0.373	0.3	0.053
BCS-CN88460-desmethyl-propanol-Glyc-MA (M41)	2.7	0.081	n.d.	n.d.	1.9	0.306	0.3	0.054
BCS-CN88460-propanol-Glyc-MA (M21)	7.5	0.229	n.d.	n.d.	5.0	0.808	0.1	0.022
BCS-CN88460-propanol (M01)	0.7	0.021	21.1	0.642	0.9	0.147	12.7	2.046
BCS-CN88460-desmethyl-propanol (M06)	n.d.	n.d.	6.9	0.204	0.3	0.052	4.0	0.644
Total identified	**66.3**	**2.015**	**78.0**	**2.373**	**72.6**	**11.640**	**78.2**	**12.570**
Total characterised	**30.0**	**0.910**	**17.2**	**0.520**	**20.5**	**3.281**	**14.3**	**2.262**

***** values are given as sum of supernatant and dissolved pellet; during hydrolysis of wheat hay extract, no pellet was formed.
n.d.: not detected

III. Conclusions

The metabolism of BCS-CN88460 in wheat was investigated after two foliar applications at growth stages BBCH 30 and BBCH 69. The wheat plants were treated with [phenyl-UL-^{14}C]BCS-CN88460 formulated as an EC 50 at a single application rate of 64 and 66 g a.s./ha for the first and second spray application, respectively, corresponding to a total application rate of 130 g a.s/ha. Wheat hay was harvested at BBCH 69 1 day prior to the second application, wheat straw and grains were harvested at maturity (BBCH 89).

Residues in wheat grains were significantly lower than those in wheat hay and straw. The extraction rates of hay, straw and grains were high. Overall, identification rates in wheat hay, straw and grain were sufficient. In all RACs, parent compound BCS-CN88460 was the main residue component and the only component in wheat grains. Besides parent compound, other metabolites were identified in wheat straw and hay: BCS-CN88460-propanol-Glyc (M18), BCS-CN88460-desmethyl-propanol-Glyc-MA (M41), BCS-CN88460-propanol-Glyc-MA (M21), BCS-CN88460-propanol (M01) and BCS-CN88460-desmethyl-propanol (M06).

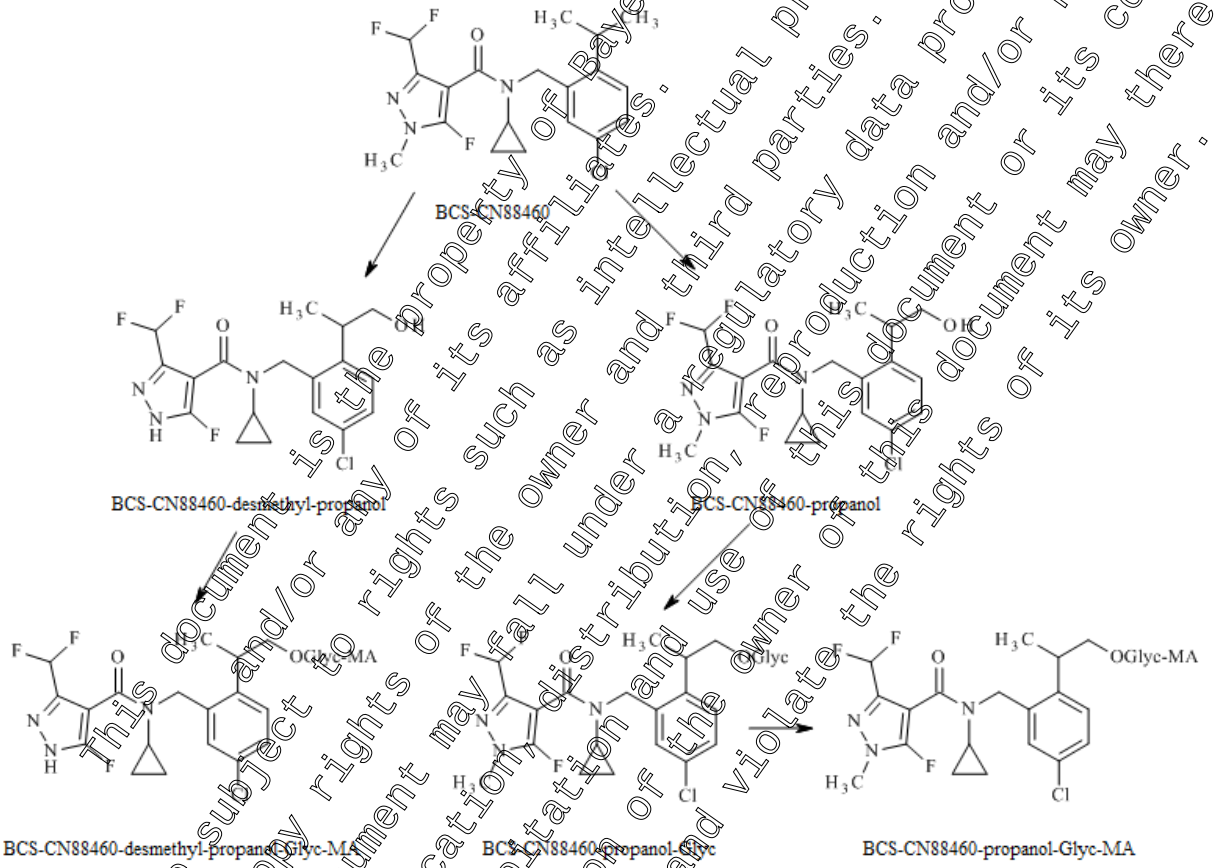
The results in the present study are in good agreement with the results with the pyrazole-label. No label specific metabolites were observed. Acid hydrolysis of the conventional extracts of wheat hay and wheat straw showed cleavage of the identified conjugates to the aglycons BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol and the presence of further conjugates of the aforementioned aglycons.

The test compound [phenyl-UL-^{14}C]BCS-CN88460 was moderately metabolised in wheat after two post-emergence applications. The main metabolic reactions are listed below:

- hydroxylation in position 1 of the propyl group followed by conjugation with hexose and malonic acid
- demethylation of the pyrazole moiety

Based on these results, the degradation behavior of [phenyl-UL-^{14}C]BCS-CN88460 in wheat is adequately understood and a metabolic pathway is proposed in the figure below.

Figure 6.2.1- 2: Proposed metabolic pathway of [phenyl-UL-^{14}C]BCS-CN88460 in wheat



Metabolism, distribution and expression of residues in potatoes (tuber treatment)

Metabolism studies in potatoes after tuber/seed treatment will be conducted with [pyrazole-4-^{14}C] and [phenyl-UL-^{14}C]BCS-CN88460. The studies are already listed in this dossier but will be filed subsequently after finalization of the studies.

Table 6.2.1- 24: Overview over potatoes metabolism studies

Plant	Application	Target application rate	BBCH Code	Reference
potato	tuber treatment, pyrazole-labelled isoflucypram	Will be provided after finalization of the study	BBCH 00	Will be provided after finalization of the study
potato	tuber treatment, phenyl-labelled isoflucypram		BBCH 00	

Report:
Title: Metabolism of [pyrazole-4-^{14}C] BCS-CN88460 in Potato after Seed Treatment
Report No.: S17-01394.
Document No.:
Guideline(s): Will be provided after finalization of the study.
Guideline deviation(s):
GLP/GEP: yes

Report:
Title: Metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in Potato after Seed Treatment
Report No.: S17-01392
Document No.:
Guideline(s): Will be provided after finalization of the study.
Guideline deviation(s):
GLP/GEP: yes

Metabolism, distribution and expression of residues in oilseed rape (foliar spray application)

Metabolism studies in oilseed rape were conducted with [pyrazole-4-^{14}C] and [phenyl-UL-^{14}C]BCS-CN88460.

Table 6.2.1- 25: Overview over available oilseed rape metabolism studies

Plant	Application	Target application rate	BBCH Code	Reference
oilseed rape	two foliar spray applications, pyrazole-labelled isoflucypram	2 x 60 g a.s./ha	BBCH 14 and BBCH 77	M-609378-01-1
oilseed rape	two foliar spray applications, phenyl-labelled isoflucypram	2 x 60 g a.s./ha	BBCH 14 and BBCH 77	M-609380-01-1

Summary of metabolism in oilseed rape (foliar spray application)

The metabolite pattern corresponds well when comparing the two metabolism studies in oilseed rape after foliar application. Parent compound BCS-CN88460 was the main residue component in all RACs except in forage extract which contained no residue above the limit of detection. Parent compound was the only component in oilseed rape seeds. BCS-CN88460 was moderately metabolised in oilseed rape intermediate harvest and mature plants samples after two post-emergence applications. The main metabolic reactions were hydroxylation in position 1 or 2 of the propyl group of the phenyl ring followed by conjugation with hexose and malonic acid and hydroxylation in position 4 of the phenyl moiety followed by conjugation with hexose and malonic acid.

Besides parent compound, the following metabolites were identified: BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24).

Report:	KCA 6.2.1/05; [redacted], K, 2017, M-609378-01-1
Title:	Metabolism of [pyrazole-4-14C]-BCS-CN88460 in oilseed rape
Report No.:	S16-01038
Document No.:	M-609378-01-1
Guideline(s):	OECD Test Guideline No. 501 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 US EPA OCSPP Test Guideline No. 860.1300 JAP FAMIC-ACIS Notification 12 Nousan 8147
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of BCS-CN88460 was investigated in oilseed rape plants after two foliar applications. For each of the two foliar applications the test item [pyrazole-4-^{14}C]BCS-CN88460 was formulated as an aqueous EC 50 using a nominal application rate of 60 g a.s./ha each. The applications were performed at the growth stage of BBCH 14 (trifoliolate on the 3rd up to 5th node unfolded) and BBCH 77 (70% of pods have reached final size). The actual application rates corresponded to 64 and 62 g a.s./ha for the first and second application, respectively resulting in a total application rate of 126 g a.s./ha.

Oilseed rape intermediate harvest was harvested at BBCH 30, 2 days after the first application, forage at BBCH 55, 40 days after the first application, and mature plants and seeds were harvested at BBCH 89, 21 days after the second application. The total radioactive residues (TRR) in intermediate harvest and mature plants were high and amounted to 4.751 mg eq/kg and 4.076 mg eq/kg, respectively. The TRR in forage was low due to the increase of plant mass from first application at BBCH 14 until sampling of forage at BBCH 55 and amounted to 0.012 mg eq/kg. The TRR in seeds was low and amounted to 0.099 mg eq/kg.

Homogenised plant material from RACs was conventionally extracted with a mixture of acetonitrile/water (8/2; v/v). The extraction rates after conventional extraction of intermediate harvest, forage, mature plants and seeds were high and amounted to 99.5% (4.730 mg eq/kg) of the TRR for intermediate harvest, 85.4% (0.010 mg eq/kg) of the TRR for forage, 97.4% (3.970 mg eq/kg) of the TRR for mature plants and 71.0% (0.070 mg eq/kg) of the TRR for seeds.

Solids after conventional extraction of seeds were exhaustively extracted using microwave assistance with a mixture of acetonitrile/water/formic acid (50/50/1; v/v/v) releasing further 9.8% (0.010 mg eq/kg) of TRR. The post extraction solids of seeds after microwave extraction were subjected to consecutive enzyme digestion with cellulase and amylase, which released further 1.5% of the TRR

(0.002 mg eq/kg). Subsequent extraction under acidic conditions with HCl released 11.0% of the TRR (0.011 mg eq/kg). 8.1% of the TRR (0.008 mg eq/kg) of this extract were further characterised by partitioning with ethylacetate. In the unpolar ethylacetate fraction remained 5.4% (0.005 mg eq/kg) of the residues and 2.7% of TRR (0.003 mg eq/kg) remained in the water phase.

The post extraction solids after conventional and exhaustive extractions accounted for 0.5% of the TRR (0.022 mg eq/kg), 14.6% of the TRR (0.002 mg eq/kg), 2.6% of the TRR (0.106 mg eq/kg) and 6.7% of the TRR (0.006 mg eq/kg) for intermediate harvest, forage, mature plants and seeds, respectively.

Residues in the conventional extracts were analysed and quantified by HPLC. The parent compound and metabolites were either identified by co-chromatography with the reference compound or by spectroscopic analysis in isolated fractions of intermediate harvest. Additionally, the metabolite pattern and retention times of the current and the oilseed rape metabolism study with the phenyl label were compared.

The forage extract contained no residue above the limit of detection. Parent compound BCS-CN88460 represented the most prominent residue component in all RACs, accounting for 81.9% of TRR (3.890 mg eq/kg) in intermediate harvest, 88.1% of TRR (3.589 mg eq/kg) in mature plants and 71.0% of TRR (0.070 mg eq/kg) in seeds. Parent compound was the only component detected in the extract of seeds.

Besides parent compound four metabolites were identified in intermediate harvest and mature plants.

In intermediate harvest, BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) accounted for 2.3, 2.2, 2.8 and 3.1% of the TRR corresponding to 0.109, 0.106, 0.131 and 0.148 mg eq/kg, respectively.

In mature plants BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) accounted for 0.7, 0.9, 0.6 and 1.0% of the TRR corresponding to 0.027, 0.038, 0.025 and 0.040 mg eq/kg, respectively.

Overall, identification rates were high and amounted to 92.3% of TRR for intermediate harvest, 91.3% of TRR for mature plants and 71.0% of TRR for seeds. In intermediate harvest, 22 unknown metabolites were characterised in the extracts, individually accounting for equal or less than 1.5% of the TRR and 0.072 mg eq/kg and 16 were characterised in mature plants, individually accounting for equal or less than 1.3% of the TRR and 0.054 mg eq/kg.

Comparison of metabolic profiles with those of a parallel study with [phenyl-UL-^{14}C]BCS-CN88460 revealed a high correspondence and no label specific metabolite could be observed for the both labels.

Acid hydrolysis (1 N HCl, 100 °C, 1 h) of the conventional extract of intermediate harvest was performed in order to analyse for hydrolysable conjugates. Comparison of metabolic profiles before and after hydrolysis indicated cleavage of the identified conjugates BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) to less polar compounds. Analogous hydrolysis experiments were performed in a parallel study with the phenyl-label showing good accordance with the current study.

All conventional and exhaustive extraction experiments of the raw agricultural commodities and the first HPLC analyses were performed within three months after harvest of the oilseed rape samples.

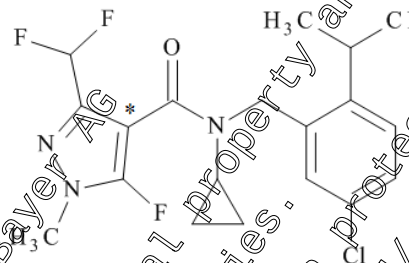
As metabolic reactions, hydroxylation in position 1 or 2 of the propyl group of the phenyl ring followed by conjugation with hexose and malonic acid and hydroxylation in position 4 of the phenyl moiety followed by conjugation with hexose and malonic acid were observed.

Based on these results, the degradation behavior of [pyrazole-4-^{14}C]BCS-CN88460 in oilseed rape is adequately understood and a pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	
	* denotes the position of the ^{14}C-label
Radiolabel position	[pyrazole-4-^{14}C]
Specific radioactivity	2.11 MBq/mg
Radiochemical purity	> 98% (HPLC), > 99% (TLC)
Chemical purity	> 98%

Formulation of the test compound

The test compound was diluted as a 50:50 mixture of radiolabelled and non-radiolabelled test compound resulting in a calculated final specific activity for the test item of 2.11 MBq/mg. A stock solution of the test compound was prepared by dissolving the test compound in acetonitrile. The purity in the stock solution for both applications and the identity of the test compound in the application dilutions was checked by HPLC with radiodetection and was above 98%

The test compound was formulated as an EC 50 for the experiment and therefore [pyrazole-4-^{14}C] BCS-CN88460 was dissolved in acetonitrile. For each of the two spray dilutions, adequate parts of the stock solution were transferred into glass vials and evaporated to dryness. Blank formulation was added and the mixtures were homogenised using a magnetic stirrer. The sample was then adjusted with water to a final volume of 100 mL of the spray dilution and homogenised by stirring.

2. Soil:

Soil characteristics			
Type	TOC	pH ($CaCl_2$)	CEC
Sandy loam	2.37%	7.48	20.9 meq/100 g

3. Plant: oilseed rape, variety "JERRY", representative for oilseeds

B. Study Design

1. Experimental conditions:

The experiment was conducted with oilseed rape plants (variety: JERRY) at the rate of 275 seeds per m^2 in a planting container with a surface area of 1 m^2. The planting container was filled with sandy loam soil. The plants were cultivated in the glass-roofed greenhouse of the test facility and were grown similar to natural temperature and light conditions, but protected from rainfall. They were watered by pouring onto the soil in the planting containers. The plants were applied at two different growth stages (BBCH 14 and 77). For both applications the target single application rate was 60 g a.s./ha. The target rate corresponds to the anticipated maximum application rate for the use type.

For each application, the plants were treated with 100 mL of the aqueous spray dilutions using a controlled track sprayer with a flat fan nozzle. To avoid contamination of the surrounding area by drift, the plants in the planting container were enclosed with a foil housing. After spraying the spray dilution onto the oilseed rape plants in the planting container, the spray device and the protective plastic foil around the planting container were rinsed with acetonitrile/water (8/2; v/v). The actual amount applied was calculated by subtracting the losses from the radioactivity in the original application solution. At the 1st application 13.5 MBq of the labelled test compound were applied, corresponding to 6.4 mg a.s. At the 2nd application 13.1 MBq of the test compound were applied, corresponding to 6.2 mg a.s. The actual single treatment rates were 64 and 62 g a.s./ha, corresponding to a total actual application rate of 126 g a.s./ha.

2. Sampling:

Two days after the first application (at BBCH 30), the RAC intermediate harvest and 40 days after the first application at BBCH 85, the RAC forage was harvested. The RACs seeds and mature plant (= rest of plant including pods without seeds) were harvested at BBCH 89, 21 days after the 2nd application.

Intermediate harvest, forage and mature plants were sampled by cut-off of the plants 1-2 cm above the soil surface. Seeds were isolated from mature plants by hand. The empty pods were combined with the mature plant sample.

The total weight of each sample was determined. The samples were homogenised with liquid nitrogen using a high speed blender. The sample materials were stored in a freezer (≤ -18 °C). Aliquots of the homogenates were extracted. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids.

C. Analytical Procedures

1. Extraction:

Conventional extraction procedure and sample clean up:

For conventional extraction of oilseed rape intermediate harvest, forage, mature plants and seeds, aliquots of the homogenised samples were extracted three times with a mixture of acetonitrile/water (8/2; v/v) using a high speed blender. After each extraction step, the extracts were filtered by suction and the solids were rinsed with a small amount of the solvent mixture used for extraction. The solids were dried and aliquots were subjected to combustion.

The extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v).

Each combined percolate/rinse solution obtained from SPE purification was mixed with emulsifier and evaporated to the aqueous remainder. The final conventional extracts were analysed by HPLC with the general profiling method.

Exhaustive extraction and corresponding clean-up:

Solids from the conventional extraction of oilseed rape seeds were exhaustively extracted two times with acetonitrile/water/formic acid (50/50/1; v/v/v) under microwave assistance at increased temperature (0 to 5 min increase to 120 °C, 5 to 20 min at 120 °C, 800 W). The microwave extracts were cooled down at room temperature. After each extraction step, extract and solids were filtrated by suction and centrifuged and finally the extracts were combined.

Aliquots of the combined extract were subjected to a clean-up step using a SPE RP 18 cartridge, which was rinsed with methanol and water beforehand. The flow-through fraction was collected and the cartridge was rinsed with acetonitrile/water (8/2; v/v). Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v).

The flow-through fraction and the rinse obtained from SPE purification were combined and mixed with emulsifier and evaporated to the aqueous remainder. The final exhaustive extract was analysed by HPLC with the general profiling method. The extract was stored in a freezer (≤ -18 °C).

Release of residues upon enzymatic digestion:

Solids of oilseed rape seeds after exhaustive extraction were further incubated with cellulase in sodium acetate buffer (0.1 M) to release radioactive residues. The solids were autoclaved in buffer (121 °C, 2 bar vapor pressure) for 2 hours. After autoclaving the sample was cooled down at room temperature and the buffer was set to pH 5 by use of acetic acid. The solution was mixed with 100 mg cellulose, incubated for 24 hours in a water bath at 37 °C and centrifuged.

Solids of oilseed rape seeds remaining after cellulase treatment were further incubated with amylase in sodium acetate buffer (0.1 M) to specifically release radioactive residues previously assimilated to carbohydrates. The solids were autoclaved in buffer (121 °C, 2 bar vapour pressure) for 2 hours. After autoclaving the sample was cooled down to 20 °C and the buffer was set to pH 5 by use of acetic acid. The solution was mixed with 50.0 mg amylase (1.9 units/mg), incubated for 24 hours in a water bath at 37 °C and centrifuged.

Release of residues upon acidic extraction with HCl:

Solids of oilseed rape seeds after enzymatic digestion with cellulase and amylase (approx. 5 g) were further extracted with HCl (5 M, 30 mL) to release radioactive residues. After addition of the HCl solution, the mixture was incubated for 60 minutes at 120°C under microwave assistance.

Residues contained in the combined extracts obtained by acidic extraction were characterised by partitioning. Therefore, the complete extract was neutralized by addition of 10M NaOH and mixed with ethylacetate (1/1; v/v) in a separatory funnel and shaken by hand. Ethylacetate and water phase were separated. The water phase was again mixed with ethylacetate (1/1; v/v) in a separatory funnel and the procedure was repeated.

Hydrolysis of the conventional extracts from oilseed rape intermediate harvest:

Hydrolysis experiments in acidic medium (1 N HCl) were conducted with concentrated conventional extract from intermediate harvest. Therefore, the final purified extract was mixed with 10 N HCl to obtain a concentration of approximately 1 N HCl and incubated 100 °C for 1 hour. Afterwards, the mixture was adjusted to pH 7 with 10 N NaOH and analysed by HPLC.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

Conventional and microwave extracts were analysed by HPLC with radiodetection based on reversed phase chromatography using an acidic water/acetonitrile/tetrahydrofurane gradient.

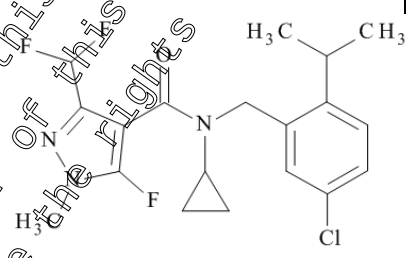
2. Identification and characterisation:

For identification of radioactive ingredients in conventional extract from intermediate harvest, selected major radiosignals were isolated as fractions of elutent by HPLC fractionation. Isolated fractions were identified by spectroscopic analysis as following metabolites: BCS-CN88460-hydroxyphenyl-Gluc-MA, BCS-CN88460-2-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-CN88460-hydroxyphenyl-Glyc-MA.

Metabolic profiles of all RACs were compared, as analysed by HPLC among themselves. Metabolic profiles of all RACs were compared with metabolic profiles of corresponding RACs in the oilseed rape metabolism study with the phenyl label. Parent compound was identified in oilseed rape seeds extract by TLC and HPLC co-chromatography with the test compound. Metabolic profiles of oilseed rape intermediate harvest extract before and after acid hydrolysis were compared with corresponding profiles of the oilseed rape metabolism study with the phenyl label, as analysed by HPLC.

Unknown metabolites were characterised based on their extraction and chromatographic behavior.

Table 6.2.1- 26: List of reference compound

Report name / other names/codes	**Chemical Name (IUPAC)**	**Structure and formula**
Parent compound BCS-CN88460 Reference: M-00002258 S1000	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

All conventional extraction experiments of the raw agricultural commodities were performed within two months after harvest of the oilseed rape samples. The exhaustive extraction of seeds was performed within three months after harvest. All first quantitative analyses by HPLC were performed within two days after the start of extraction.

It was therefore concluded, that the residues in the samples were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in oilseed rape was investigated after two spray applications.

Oilseed rape plants were treated with [pyrazole-4-^{14}C]BCS-CN88460 formulated as an EC 50 at BBCH 14 (trifoliolate on the 3rd up to 5th node unfolded) and BBCH 77 (70% of pods have reached final size). The actual single application rate corresponded to 64 and 62 g a.s./ha which was slightly above the anticipated maximum application rate (2 x 60 g a.s./ha). The total application rate amounted to 126 g a.s./ha.

The TRR values of the individual RACs were determined by summing up the radioactivity determined in the combined extracts and the radioactivity in the solids. The residue levels are shown in mg active substance equivalents per kg sample material (mg a.s.equiv./kg or simplified mg eq/kg).

TRR values in intermediate harvest and mature plants were high and amounted to 4.751 mg eq/kg and 4.076 mg eq/kg, respectively. The TRR in forage and seeds were low and amounted to 0.012 mg eq/kg and 0.099 mg eq/kg.

The high TRRs found for intermediate harvest and mature plants are due to harvest shortly after the first foliar application (PHI = 2 d) in case of intermediate harvest or due to sampling after two foliar applications in case of mature plants. For forage, the low TRR can be ascribed to the increase of plant mass from the 1st foliar application at BBCH 14 to sampling 40 days after 1st application at BBCH 55.

Table 6.2.1- 27: TRR values in oilseed rape matrices after foliar application of [pyrazole-4-^{14}C]BCS-CN88460

Matrix	Timing and Application	PHI (days)*	ppm (=mg eq/kg)
Intermediate Harvest	1 Spray application at BBCH 14, 64 g a.s./ha	2	4.751
Forage		40	0.012
Mature Plants	2 Spray applications at BBCH 14 and BBCH 77, 64 and 62 g a.s./ha (126 g a.s./ha total)	21	4.076
Seeds		21	0.099

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at harvest/sampling)

Intermediate harvest was conventionally extracted three times with acetonitrile/water mixtures releasing 99.5% of the TRR (4.730 mg eq/kg). Losses during sample clean up accounted for 0.4% of the TRR (0.020 mg eq/kg). After concentration and purification steps 99.1% of the TRR (4.710 mg eq/kg) were analysed. The post extraction solids amounted to 0.5% (0.022 mg eq/kg) of the TRR, only.

Forage was conventionally extracted three times with acetonitrile/water mixtures releasing 85.4% of the TRR (0.010 mg eq/kg). No losses of RA occurred during sample clean up and 85.4% of the TRR (0.010 mg eq/kg) were analysed. The post extraction solids amounted to 14.6% of the TRR (0.002 mg eq/kg), only.

Mature plants were conventionally extracted three times with acetonitrile/water mixtures releasing 97.4% of the TRR (3.970 mg eq/kg). Losses of RA during sample clean up accounted for 0.2% of TRR and 0.006 mg eq/kg). After concentration and purification steps 97.3% of the TRR (3.964 mg eq/kg) were analysed. The post extraction solids contained 2.6% (0.106 mg eq/kg) of the TRR.

Seeds were conventionally extracted three times with acetonitrile/water mixtures releasing 71.0% of the TRR (0.070 mg eq/kg). No losses of RA occurred during sample clean up and 71.0% of the TRR (0.070 mg eq/kg) were analysed. The post extraction solids after conventional extraction accounted for 29.0% of TRR (0.029 mg eq/kg).

The PES of seeds were subjected to exhaustive extraction under microwave support. This treatment released 9.8% of the TRR (0.010 mq eq/kg). Losses during sample clean up accounted for 2.2% of the TRR (0.002 mg eq/kg) and 7.6% of the TRR (0.008 mg eq/kg) were analysed by HPLC. Additionally, the solids remaining after exhaustive extraction were incubated consecutively with cellulase and amylase to release further residues. This digestion released 1.5% of the TRR (0.002 mg eq/kg).

Subsequent acidic extraction additionally released 11.0% of the TRR (0.011 mg eq/kg). Losses of RA during neutralization of the acidic extract accounted for 2.9% of TRR (0.003 mg eq/kg). The neutralized extract was further characterised by partitioning with ethylacetate. 5.4% of the TRR

(0.005 mg eq/kg) was detected within the ethylacetate fraction and 2.7% of the TRR (0.003 mg eq/kg) were detected within the water phase.

In total 93.3% of the TRR (0.093 mg eq/kg) was extracted from seeds. Total losses of RA accounted for 5.1% of the TRR (0.005 mg eq/kg). The post extraction solids amounted to 6.7% (0.006 mg eq/kg) of the TRR.

The distribution of the radioactive residues is shown in the following table.

Table 6.2.1- 28: Distribution of radioactivity in the extracts of oilseed rape matrices after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	intermediate harvest		forage		mature plants		seeds	
TRR [mg eq/kg] =	4.751		0.012		4.076		0.099	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	**99.5**	**4.730**	**85.4**	**0.010**	**97.4**	**3.970**	**71.0**	**0.070**
Analysed extracts	99.1	4.710	85.4	0.010	97.3	3.964	71.0	0.070
Not analysed	0.4	0.020	n.q.	n.q.	0.2	0.006	n.q.	n.q.
Exhaustive extraction	--	--	--	--	--	--	**9.8**	**0.010**
Analysed extracts*	--	--	--	--	--	--	7.6	0.008
Not analysed	--	--	--	--	--	--	2.2	0.002
Enzymatic digestion	--	--	--	--	--	--	**1.5**	**0.002**
Acidic Extraction and partitioning	--	--	--	--	--	--	**11.0**	**0.011**
Ethylacetate phase	--	--	--	--	--	--	5.4	0.005
Water phase	--	--	--	--	--	--	2.7	0.003
Not analysed							2.9	0.003
Total extracted	**99.5**	**4.730**	**85.4**	**0.010**	**97.4**	**3.970**	**93.3**	**0.093**
Post extraction solids (PES)	**0.5**	**0.022**	**14.6**	**0.002**	**2.6**	**0.106**	**6.7**	**0.006**
Accountability	**100.0**	**4.751**	**100.0**	**0.012**	**100.0**	**4.076**	**100.0**	**0.099**

-- not applicable
n.q. not quantified (< LOQ)
* no individual peak above detection limit was observed in the HPLC chromatogram of the exhaustive extract of seeds

Comparison of chromatograms from conventional extracts of intermediate harvest and mature plants of the current study among themselves indicated a high grade of comparability. Conventional extract of forage contained no individual radiosignal above the background noise whereas conventional extract from seeds contained only the test compound.

The comparison of profiles of all RACs to corresponding profiles of the parallel study performed with [phenyl-UL-^{14}C]BCS-CN88460 revealed that no label specific metabolism could be observed.

In conventional extracts from intermediate harvest 92.3% of the TRR (4.384 mg eq/kg) were identified in total. The parent compound was by far the major component representing 81.9% of the TRR (3.890 mg eq/kg), whereas the BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) represented 2.3, 2.2, 2.8 and 3.1% of the TRR corresponding to 0.109, 0.106, 0.131 and 0.148 mg eq/kg, respectively.

Acid hydrolysis (1 N HCl, 100 °C, 1 h) of the conventional extract of intermediate harvest was performed in order to analyse for hydrolysable conjugates. Comparison of metabolic profiles before and after hydrolysis indicated cleavage of the identified conjugates BCS-CN88460-hydroxyphenyl-Gluc-MA, BCS-CN88460-2-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-

CN88460-hydroxyphenyl-Glyc-MA to less polar compounds. Analogous hydrolysis experiments were performed in the parallel study with the phenyl-label showing good accordance with the current study.

In conventional extracts from forage 85.4% of the TRR was analysed in total (0.010 mg eq/kg). No individual radiosignal was above the background noise due to low amounts of total radioactive ingredients in the extract.

In conventional extracts from mature plants 91.3% of the TRR (3.719 mg eq/kg) were identified in total. The parent compound was by far the major component representing 88.1% of the TRR (3.589 mg eq/kg), whereas the metabolites BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) represented 0.7, 0.9, 0.6 and 1.0% of the TRR corresponding to 0.027, 0.038, 0.025 and 0.040 mg eq/kg, respectively.

In conventional extracts from seeds 71.0% of the TRR (0.070 mg eq/kg) were identified. The parent compound was the only component representing 71.0% of the TRR (0.070 mg eq/kg). Parent compound was identified by HPLC and TLC co-chromatography with the test item as radiolabelled reference compound.

Exhaustive extraction of seeds released further 9.8 % of the TRR (0.010 mg eq/kg) and 7.6 % of the TRR (0.008 mg eq/kg) were analysed by HPLC. No individual radiosignal was above the background noise due to low amounts of total radioactive ingredients in the exhaustive extract. Conventional extract of seeds treated under microwave extraction conditions in comparison of the corresponding conventional extract and exhaustive extract, showed that degradation of parent compound by this treatment was neglectable.

The TRR and the distribution of parent and metabolites in oilseed rape matrices are shown in the following table.

Table 6.2.1- 29: Distribution of parent compound and metabolites the extracts of oilseed rape matrices after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	intermediate harvest		forage		mature plants		seeds	
TRR [mg eq/kg] =		4.751		0.012		4.076		0.099
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	***99.5***	***4.730***	***85.4***	***0.010***	***97.4***	***3.970***	***71.0***	***0.070***
BCS-CN88460 (parent compound)	81.9	3.890	n.d.	n.d.	88.1	3.589	71.0	0.070
BCS-CN88460-hydroxyphenyl-Gluc-MA (M23)	2.3	0.109	n.d.	n.d.	0.7	0.027	n.d.	n.d.
BCS-CN88460-2-propanol-Glyc-MA (M22)	2.2	0.106	n.d.	n.d.	0.9	0.038	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	2.8	0.131	n.d.	n.d.	0.6	0.025	n.d.	n.d.
BCS-CN88460-hydroxyphenyl-Glyc-MA (M24)	3.1	0.148	n.d.	n.d.	1.0	0.040	n.d.	n.d.
Total identified	**92.3**	**4.384**	**n.d.**	**n.d.**	**91.3**	**3.719**	**71.0**	**0.070**
Unknown 1	n.d.	n.d.	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 2	0.1	0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 3	n.d.	n.d.	n.d.	n.d.	0.4	0.018	n.d.	n.d.
Unknown 4	0.1	0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 5	0.1	0.007	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 6	0.1	0.005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 7	0.2	0.010	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 8	0.3	0.013	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 9	0.2	0.009	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 10	0.2	0.007	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 11	0.2	0.008	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 12	0.4	0.018	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 13	0.2	0.012	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 14	0.2	0.012	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 15	n.d.	n.d.	n.d.	n.d.	0.1	0.005	n.d.	n.d.
Unknown 16	n.d.	n.d.	n.d.	n.d.	0.5	0.022	n.d.	n.d.
Unknown 17	0.9	0.045	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 18	0.1	0.005	n.d.	n.d.	0.1	0.006	n.d.	n.d.
Unknown 20	n.d.	n.d.	n.d.	n.d.	0.8	0.034	n.d.	n.d.
Unknown 22	n.d.	n.d.	n.d.	n.d.	0.2	0.010	n.d.	n.d.
Unknown 23	0.9	0.044	n.d.	n.d.	0.2	0.010	n.d.	n.d.
Unknown 26	0.7	0.034	n.d.	n.d.	0.1	0.005	n.d.	n.d.
Unknown 27	n.d.	n.d.	n.d.	n.d.	0.5	0.020	n.d.	n.d.
Unknown 28	0.1	0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 29	0.3	0.012	n.d.	n.d.	0.5	0.018	n.d.	n.d.
Unknown 30	0.2	0.008	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 31	n.d.	n.d.	n.d.	n.d.	0.3	0.012	n.d.	n.d.
Unknown 32	0.1	0.005	n.d.	n.d.	0.4	0.017	n.d.	n.d.
Unknown 33	0.2	0.010	n.d.	n.d.	0.3	0.014	n.d.	n.d.
Unknown 34	1.5	0.072	n.d.	n.d.	1.3	0.054	n.d.	n.d.
Unknown 36	n.d.	n.d.	n.d.	n.d.	0.1	0.002	n.d.	n.d.
Characterised by HPLC	**6.8**	**0.326**	**--**	**--**	**6.0**	**0.245**	**--**	**--**
Total not analysed of conventional extraction	**0.4**	**0.020**	**--**	**--**	**0.2**	**0.006**	**--**	**--**

continued on next page

Table 6.2.1- 29 continued

	intermediate harvest		forage		mature plants		seeds	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Exhaustive extraction	--	--	--	--	--	--	***9.8***	***0.010***
*Analysed by HPLC**	--	--	--	--	--	--	*7.6*	*0.008*
Not analysed	--	--	--	--	--	--	*2.2*	*0.002*
Enzymatic digestion	--	--	--	--	--	--	***1.5***	***0.002***
Acidic extraction and partitioning	--	--	--	--	--	--	***11.0***	***0.011***
Ethylacetate phase	--	--	--	--	--	--	*5.4*	*0.005*
Water phase	--	--	--	--	--	--	*2.7*	*0.003*
Not analysed by partition	--	--	--	--	--	--	*2.9*	*0.003*
Total characterised**	**6.8**	**0.326**	--	--	**6.0**	**0.245**	**22.3**	**0.023**
Total extracted	**99.5**	**4.730**	**85.4**	**0.010**	**97.4**	**3.970**	**93.3**	**0.093**
Post extraction solids (PES)	**0.5**	**0.022**	**14.6**	**0.002**	**2.6**	**0.106**	**6.7**	**0.006**
Accountability	**100.0**	**4.754**	**100.0**	**0.012**	**100.0**	**4.076**	**100.0**	**0.099**

* no individual peak above detection limit was observed in the HPLC chromatogram of the exhaustive extract of seeds
**by chromatographic and/or extraction behaviour
n.d. not detected
-- not applicable

In conventional extract of intermediate harvest, 22 unknown metabolites were characterised, individually accounting for equal or less than 1.5% of the TRR and 0.072 mg eq/kg and 16 were characterised in mature plants, individually accounting for equal or less than 1.3% of the TRR and 0.054 mg eq/kg.

Table 6.2.1- 30: Summary of characterisation and identification of radioactive residues in oilseed rape matrices after two foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	intermediate harvest		forage		mature plants		seeds	
TRR [mg eq/kg]		4.751		0.012		4.076		0.099
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	*99.5*	*4.730*	*85.4*	*0.010*	*97.4*	*3.970*	*71.0*	*0.070*
BCS-CN88460 (parent compound)	81.9	3.890	n.d.	n.d.	88.1	3.589	71.0	0.070
BCS-CN88460-hydroxyphenyl-Gluc-MA (M23)	2.3	0.109	n.d.	n.d.	0.7	0.027	n.d.	n.d.
BCS-CN88460-2-propanol-Glyc-MA (M22)	2.2	0.106	n.d.	n.d.	0.9	0.038	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	2.8	0.131	n.d.	n.d.	0.6	0.026	n.d.	n.d.
BCS-CN88460-hydroxyphenyl-Glyc-MA (M24)	3.1	0.148	n.d.	n.d.	1.0	0.040	n.d.	n.d.
Total identified	**92.3**	**4.384**	--	--	**91.3**	**3.719**	**71.0**	**0.070**
Characterised in the conventional extract by HPLC	**6.8**	**0.326**	--	--	**6.0**	**0.245**	--	--
Number of unknown peaks	22				16		--	
Largest unknown peak	1.5	0.072	--	--	1.3	0.054	--	--
Total not analysed of conventional extraction	0.4	0.020	--	--	0.1	0.006	--	--
Exhaustive extraction	--	--	--	--	--	--	***9.8***	***0.010***
*Analysed by HPLC**	--	--	--	--	--	--	*7.6*	*0.008*
Not analysed	--	--	--	--	--	--	*2.2*	*0.002*
Enzymatic digestion	--	--	--	--	--	--	***1.5***	***0.002***
Acidic extraction and partitioning	--	--	--	--	--	--	***11.0***	***0.011***
Ethylacetate phase	--	--	--	--	--	--	*5.4*	*0.005*
Water phase	--	--	--	--	--	--	*2.7*	*0.003*
Not analysed by partition	--	--	--	--	--	--	*2.9*	*0.003*
Total characterised**	**6.8**	**0.326**	--	--	**6.0**	**0.245**	**22.3**	**0.023**
Total extracted	**99.5**	**4.730**	**85.4**	**0.010**	**97.4**	**3.970**	**93.3**	**0.093**
Post extraction solids (PES)	**0.5**	**0.022**	**14.6**	**0.002**	**2.6**	**0.106**	**6.7**	**0.006**
Accountability	**100.0**	**4.751**	**100.0**	**0.012**	**100.0**	**4.076**	**100.0**	**0.099**

* no individual peak above detection limit was observed in the HPLC chromatogram of the exhaustive extract of seeds
**by chromatographic and/or extraction behavior
n.d. not detected
-- not applicable

III. Conclusions

The metabolism of BCS-CN88460 in oilseed rape was investigated after two foliar applications at BBCH 14 and BBCH 77. The oilseed rape plants were treated with [pyrazole-4-^{14}C]BCS-CN88460 formulated as an EC 50 at a single application rate of 64 and 62 g a.s./ha for the first and second spray application, respectively, corresponding to a total application rate of 126 g a.s/ha. Oilseed rape intermediate harvest was harvested at BBCH 30, 2 days after the first application and forage was harvested at BBCH 55, 40 days after the first application. Mature plants and seeds were harvested at BBCH 89, 21 days after the second application.

Residues in forage and seeds were significantly lower than those in intermediate harvest and mature plants. The extraction rates of intermediate harvest, forage, mature plants and seeds were high. Overall, identification rates for intermediate harvest, mature plants and seeds were high. Parent

compound BCS-CN88460 was the main residue component in intermediate harvest and mature plant and the only component in seeds. Conventional extract of forage contained no residues above the limit of detection of the analytical method. Besides parent compound, four metabolites were identified in intermediate harvest and mature plants: BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24).

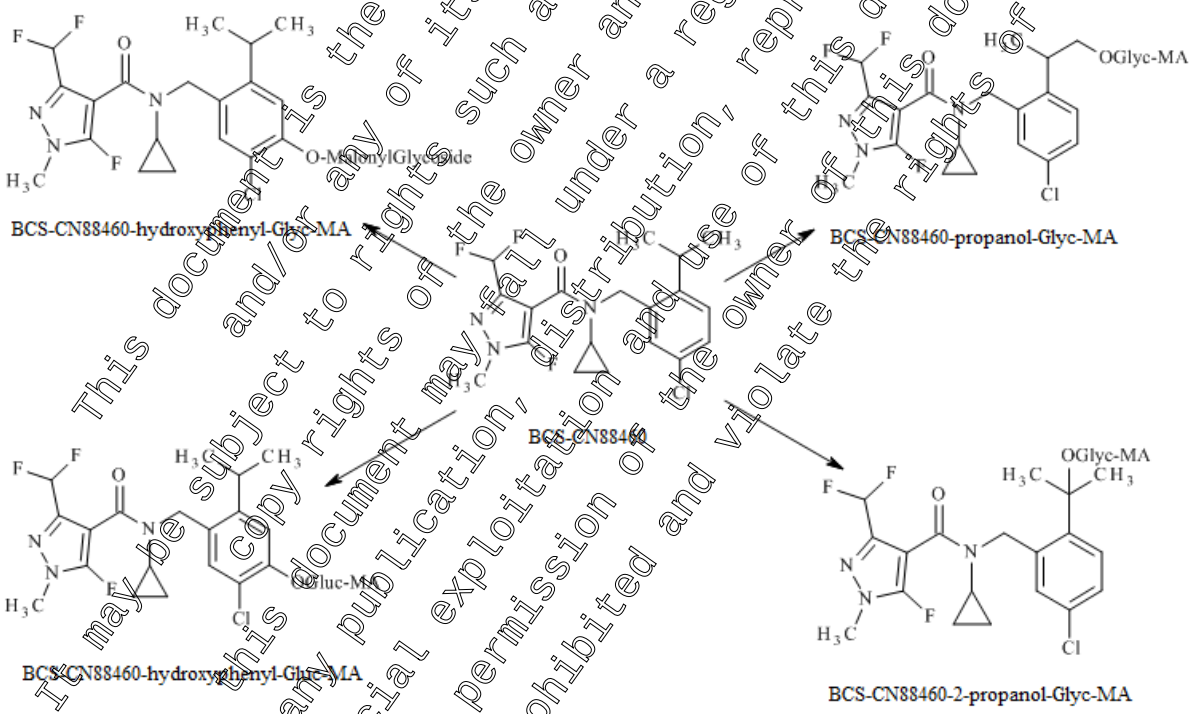
The results in the present study are in good agreement with the results with the phenyl-label. No label specific metabolites were observed.

The test compound [pyrazole-4-^{14}C]BCS-CN88460 was moderately metabolised in oilseed rape after two foliar applications. The main metabolic reactions observed were:

- Hydroxylation in position 1 or 2 of the propyl group of the phenyl ring followed by conjugation with hexose and malonic acid
- Hydroxylation in position 4 of the phenyl moiety followed by conjugation with hexose and malonic acid

Based on these results, the degradation behavior of [pyrazole-4-^{14}C]BCS-CN88460 in oilseed rape is adequately understood and a metabolic pathway is proposed in the figure below.

Figure 6.2.1- 3: Proposed metabolic pathway of [pyrazole-4-^{14}C]BCS-CN88460 in oilseed rape



Report:	KCA 6.2.1/06; [redacted], J.; 2017; M-609380-01-1
Title:	Metabolism of [phenyl-UL-14C]-BCS-CN88460 in oilseed rape
Report No.:	S16-01044
Document No.:	M-609380-01-1
Guideline(s):	OECD Test Guideline No. 501 EPA OCSPP Harmonized Test Guideline 860.1300 Nature of the Residue - Plants, Livestock (August 1996) PMRA Regulatory Directive Dir98-02 - Residue Chemistry Guidelines, Section 2, Nature of the Residue - Plants, Livestock JMAFF guideline 12 Nousan No 8147 requirement 2-4-1 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/EECCompletion Date
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of BCS-CN88460 was investigated in oilseed rape plants after two foliar applications. For each of the two foliar applications the test item [phenyl-UL-^{14}C]BCS-CN88460 was formulated as an aqueous EC 50 using a nominal application rate of 60 g a.s./ha each. The applications were performed at the growth stage of BBCH 14 (trifoliolate on the 3rd up to 5th node unfolded) and BBCH 77 (70% of pods have reached final size). The actual application rates corresponded to 63 and 63 g a.s./ha for the first and second application, respectively resulting in a total application rate of 126 g a.s./ha.

Oilseed rape intermediate harvest was harvested at BBCH 30, 2 days after the first application, forage at BBCH 55, 40 days after the first application, and mature plants and seeds were harvested at BBCH 89, 21 days after the second application. The total radioactive residues (TRR) in intermediate harvest and mature plants were high and amounted to 3.295 mg eq/kg and 3.934 mg eq/kg, respectively. The TRR in forage was low due to the increase of plant mass from first application at BBCH 14 until sampling of forage at BBCH 55 and amounted to 0.008 mg eq/kg. The TRR in seeds was low and amounted to 0.126 mg eq/kg.

Homogenised plant material from RACs was conventionally extracted with a mixture of acetonitrile/water (8/2, v/v). The extraction rates after conventional extraction of intermediate harvest, forage, mature plants and seeds were high and amounted to 99.7% (3.285 mg eq/kg) of the TRR for intermediate harvest, 77.3% (0.006 mg eq/kg) of the TRR for forage, 96.2% (3.786 mg eq/kg) of the TRR for mature plants and 73.6% (0.093 mg eq/kg) of the TRR for seeds.

Solids after conventional extraction of seeds were exhaustively extracted using microwave assistance with a mixture of acetonitrile/water/formic acid (50/50/1; v/v/v) releasing further 10.6% (0.013 mg eq/kg) of TRR. The post extraction solids of seeds after microwave extraction were subjected to consecutive enzyme digestion with cellulase and amylase, which released further 1.3% of the TRR (0.002 mg eq/kg). Subsequent extraction under acidic conditions with HCl released 7.9% of the TRR (0.010 mg eq/kg). 6.5% of the TRR (0.010 mg eq/kg) of this extract were further characterised by partitioning with ethyl acetate. In the unpolar ethyl acetate fraction remained 4.4% (0.007 mg eq/kg) of the residues and 2.1% of TRR (0.003 mg eq/kg) remained in the water phase.

The post extraction solids after conventional and exhaustive extractions accounted for 0.3% of the TRR (0.010 mg eq/kg), 22.7% of the TRR (0.002 mg eq/kg), 3.8% of the TRR (0.148 mg eq/kg) and 6.5% of the TRR (0.008 mg eq/kg) for intermediate harvest, forage, mature plants and seeds, respectively.

Residues in the conventional extracts were analysed and quantified by HPLC. The parent compound was identified by co-chromatography with the reference compound and metabolites were assigned by

comparison of the metabolite pattern and retention times of the current and the oilseed rape metabolism study with the pyrazole label.

The forage extract contained no residue above the limit of detection. Parent compound BCS-CN88460 represented the most prominent residue component in all RACs, accounting for 84.1% of TRR (2.770 mg eq/kg) in intermediate harvest, 72.0% of TRR (2.831 mg eq/kg) in mature plants and 73.6% of TRR (0.093 mg eq/kg) in seeds. Parent compound was the only component detected in the extract of seeds.

Besides parent compound four metabolites were identified in intermediate harvest and mature plants.

In intermediate harvest, BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) accounted for 2.3, 1.6, 2.2, and 3.8% of the TRR corresponding to 0.077, 0.052, 0.071 and 0.126 mg eq/kg, respectively.

In mature plants BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) accounted for 2.2, 4.6, 2.5, and 3.1% of the TRR corresponding to 0.087, 0.181, 0.097 and 0.122 mg eq/kg, respectively.

Overall, identification rates were high and amounted to 94.0% of TRR for intermediate harvest, 84.4% of TRR for mature plants and 73.6% of TRR for seeds. In intermediate harvest, 23 unknown metabolites were characterised in the extracts, individually accounting for equal or less than 1.2% of the TRR and 0.038 mg eq/kg and 39 were characterised in mature plants, individually accounting for equal or less than 1.3% of the TRR and 0.050 mg eq/kg.

Comparison of metabolic profiles with those of a parallel study with [pyrazole-4-^{14}C]BCS-CN88460 revealed a high correspondence and no label specific metabolite could be observed for the both labels.

Acid hydrolysis (1 N HCl, 100 °C, 1 h) of the conventional extract of intermediate harvest was performed in order to analyse for hydrolysable conjugates. Comparison of metabolic profiles before and after hydrolysis indicated cleavage of the identified conjugates BCS-CN88460-hydroxyphenyl-Gluc-MA, BCS-CN88460-2-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-CN88460-hydroxyphenyl-Glyc-MA to less polar compounds. Analogous hydrolysis experiments were performed in the parallel study with the pyrazole-label showing good accordance with the current study.

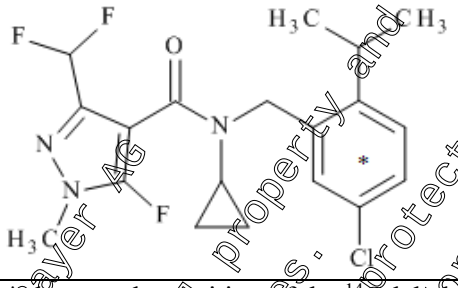
As metabolic reactions, hydroxylation in position 1 or 2 of the propyl group of the phenyl ring followed by conjugation with hexose and malonic acid and the hydroxylation in position 4 of the phenyl moiety followed by conjugation with hexose and malonic acid were observed.

Based on these results, the degradation behavior of [phenyl-UL-^{14}C]BCS-CN88460 in oilseed rape is adequately understood and is proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	
	* denotes the position of the ^{14}C-label
Radiolabel position	[phenyl-UL-^{14}C]
Specific radioactivity	2.07 MBq/mg
Radiochemical purity	> 98%
Chemical purity	> 98%

Formulation of the test compound

The test compound was diluted as a 50:50 mixture of radiolabelled and non-radiolabelled test compound resulting in calculated a final specific activity for the test item of 2.07 MBq/mg. A stock solution of the test compound was prepared by dissolving the test compound in acetonitrile. The purity in the stock solution for both applications and the identity of the test compound in the application dilutions was checked by HPLC with radiodetection and was above 98%

The test compound was formulated as an EC 50 for the experiment and therefore [phenyl-UL-^{14}C] BCS-CN88460 was dissolved in acetonitrile. For each of the two spray dilutions, adequate parts of the stock solution were transferred into glass vials and evaporated to dryness. Blank formulation was added and the mixtures were homogenised using a magnetic stirrer. The sample was then adjusted with water to a final volume of 100 mL of the spray dilution and homogenised by stirring.

2. Soil:

Soil characteristics			
Type	TOC	pH ($CaCl_2$)	CEC
Sandy loam	2.37%	7.48	20.9 meq/100 g

3. Plant: oilseed rape, variety "JERRY", representative for oilseeds

B. Study Design

1. Experimental conditions:

The experiment was conducted with oilseed rape plants (variety: JERRY) at the rate of 275 seeds per m^2 in a planting container with a surface area of 1 m^2. The planting container was filled with sandy loam soil. The plants were cultivated in the glass-roofed greenhouse of the test facility and were grown similar to natural temperature and light conditions, but protected from rainfall. They were watered by pouring onto the soil in the planting containers. The plants were applied at two different growth stages (BBCH 14 and 77). For both applications the target single application rate was 60 g a.s./ha. The target rate corresponds to the anticipated maximum application rate for the use type.

For each application, the plants were treated with 100 mL of the aqueous spray dilutions using a controlled track sprayer with a flat fan nozzle. To avoid contamination of the surrounding area by drift, the plants in the planting container were enclosed with a foil housing. After spraying the spray dilution onto the oilseed rape plants in the planting container, the spray device and the protective plastic foil around the planting container were rinsed with acetonitrile/water (8/2; v/v). The actual amount applied was calculated by subtracting the losses from the radioactivity in the original application solution. At the 1st application 13.0 MBq of the labelled test compound were applied, corresponding to to 6.3 mg a.s.. At the 2nd application 13.1 MBq of the labelled test compound were applied, corresponding to 6.3 mg a.s. The actual single treatment rates were 63 and 63 g a.s./ha, corresponding to a total application rate of 126 g a.s./ha.

2. Sampling:

At growth stage BBCH 30, 2 days after the first application, the RAC intermediate harvest and at BBCH 55, 40 days after the first application, the RAC forage was harvested. The RACs seeds and mature plant (= rest of plant including pods without seeds) were harvested at BBCH 89, 21 days after the 2nd application.

Intermediate harvest, forage and mature plants were sampled by cut-off of the plants 1-2 cm above the soil surface. Seeds were isolated from mature plants by hand. The empty pods were combined with the mature plant sample.

The total weight of each sample was determined. The samples were homogenised with liquid nitrogen using a high speed blender. The sample materials were stored in a freezer (≤ -18 °C). Aliquots of the homogenates were extracted. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids.

C. Analytical Procedures

1. Extraction:

Conventional extraction procedure and sample clean up:

For conventional extraction of oilseed rape intermediate harvest, forage, mature plants and seeds, aliquots of the homogenised samples were extracted three times with a mixture of acetonitrile/water (8/2; v/v) using a high speed blender. After each extraction step, the extracts were filtered by suction and the solids were rinsed with a small amount of the solvent mixture used for extraction. The solids were dried, aliquots subjected to combustion.

The extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v).

Each combined percolate/rinse solution obtained from SPE purification was mixed with emulsifier and evaporated to the aqueous remainder. The final conventional extracts were analysed by HPLC with the general profiling method.

Exhaustive extraction and corresponding clean up:

Solids from the conventional extraction of oilseed rape seeds were exhaustively extracted two times with acetonitrile/water/formic acid (50/50/1; v/v/v) under microwave assistance at increased temperature (0 to 5 min increase to 120 °C, 5 to 20 min at 120 °C, 800 W). The microwave extracts were cooled down at room temperature. After each extraction step, extract and solids were filtrated by suction and centrifuged and finally the extracts were combined.

Aliquots of the combined extract were subjected to a clean-up step using a SPE RP 18, which was rinsed with methanol and water beforehand. The flow-through fraction was collected and the cartridge was rinsed with acetonitrile/water (8/2; v/v). Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v).

The flow-through fraction and the rinse obtained from SPE purification were combined and mixed with emulsifier and evaporated to the aqueous remainder. The final exhaustive extract was analysed by HPLC with the general profiling method. The extracts were stored in a freezer (≤ -18 °C).

Release of residues upon enzymatic digestion:

Solids of oilseed rape seeds after exhaustive extraction were further incubated with cellulase in sodium acetate buffer (0.1 M) to release radioactive residues. The solids were autoclaved in buffer (121 °C, 2 bar vapour pressure) for 2 hours. After autoclaving the sample was cooled down at room temperature and the buffer was set to pH 5 by use of acetic acid. The solution was mixed with 100 mg cellulase and incubated for 24 hours in a water bath at 37 °C and centrifuged.

Solids of oilseed rape seeds remaining after cellulase treatment were further incubated with amylase in sodium acetate buffer (0.1 M) to specifically release radioactive residues previously assimilated to carbohydrates. The solids were autoclaved in buffer (121 °C, 2 bar vapor pressure) for 2 hours. After autoclaving the sample was cooled down to 20 °C and the buffer was set to pH 5 by use of acetic acid. The solution was mixed with 50.0 mg amylase (1.9 units/mg) and incubated for 24 hours in a water bath at 37 °C and centrifuged. Radioactivity in remaining solids was determined by combustion.

Release of residues upon acidic extraction with HCl:

Solids of oilseed rape seeds after enzymatic digestion with cellulase and amylase (approx. 5 g) were further extracted with HCl (5 M, 30 mL) to release radioactive residues. After addition of the HCl solution, the mixture was incubated for 60 minutes at 120°C under microwave assistance and centrifuged. Radioactivity in remaining solids was determined by combustion.

Residues contained in the combined extracts obtained by acidic extraction were characterised by partitioning. Therefore, the complete extract was neutralized by addition of 10M NaOH and mixed with ethylacetate (1/1; v/v) in a separatory funnel and shaken by hand. Ethylacetate and water phase were separated. The water phase was again mixed with ethylacetate (1/1; v/v) in a separatory funnel and the procedure was repeated.

Hydrolysis of the conventional extracts:

Hydrolysis experiments in acidic medium (1 N HCl) were conducted with concentrated conventional extract from intermediate harvest. Therefore, the final purified extract was mixed with 10 N HCl to obtain a concentration of approximately 1 N HCl and incubated 100 °C for 1 hour. Afterwards, the mixture was adjusted to pH 7 with 10 N NaOH and analysed by HPLC.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

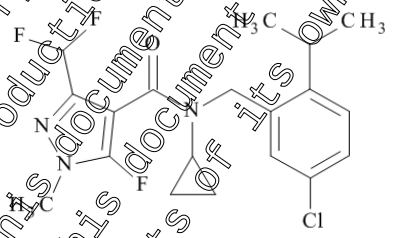
Conventional and microwave extracts were analysed by HPLC with radiodetection based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

2. Identification and characterisation:

Metabolic profiles of all RACs were compared, as analysed by HPLC among themselves. Metabolic profiles of all RACs were compared with metabolic profiles of corresponding RACs in the oilseed rape metabolism study with the pyrazole label in which the major metabolites were identified spectroscopically. Parent compound was identified in oilseed rape seeds extract by TLC and HPLC co-chromatography with the test compound. Metabolic profiles of oilseed rape intermediate harvest extract before and after hydrolysis were compared with corresponding profiles of the wheat metabolism study with the pyrazole label, as analysed by HPLC.

Unknown metabolites were characterised based on their extraction and chromatographic behavior.

Table 6.2.1- 31: List of reference compound

Report name / other names/codes	**Chemical Name (IUPAC)**	**Structure and formula**
Parent compound BCS-CN88460 Radiolabeled reference: M-00002258 S1000	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

All conventional extraction experiments of the raw agricultural commodities were performed within two months after harvest of the oilseed rape samples. The exhaustive extraction of seeds was performed within three months after harvest. All first quantitative analyses by HPLC of the conventional extracts were performed within one day after the start of extraction. For the exhaustive extract of seeds, not showing any radiosignal above the background noise, first HPLC analysis was performed within ten days after start of the extraction and degradation due to microwave treatment could be excluded.

It was therefore concluded, that the residues in the samples were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in oilseed rape was investigated after two spray applications. Oilseed rape plants were treated with [phenyl-UL-^{14}C]BCS-CN88460 formulated as an EC 50 at BBCH 14 (trifoliolate on the 3rd up to 5th node unfolded) and BBCH 77 (70% of pods have reached final size). The actual single application rate corresponded to 63 and 63 g a.s./ha which was slightly above the anticipated maximum application rate (2 x 60g a.s./ha). The total application rate amounted to 126 g a.s./ha.

The TRR values of the individual RACs were determined by summing up the radioactivity determined in the combined extracts and the radioactivity in the solids. The residue levels are shown in mg active substance equivalents per kg sample material (mg a.s.equiv./kg or simplified mg eq/kg).

TRR values in intermediate harvest and mature plants were high and amounted to 3.295 mg eq/kg and 3.934 mg eq/kg, respectively. The TRR in forage and seeds were low and amounted to 0.008 mg eq/kg and 0.126 mg eq/kg.

The high TRRs found for intermediate harvest and mature plants are due to harvest shortly after the first foliar application (PHI = 2 d) in case of intermediate harvest or due to sampling after two foliar applications in case of mature plants. For forage, the low TRR can be ascribed to the increase of plant mass from the 1st foliar application at BBCH 14 to sampling 40 days after 1st application at BBCH 55.

Table 6.2.1- 32: TRR values in oilseed rape matrices after foliar application of [phenyl-UL-^{14}C]BCS-CN88460

Matrix	Timing and Application	PHI (days)*	ppm (=mg eq/kg)
Intermediate Harvest	1 Spray application at BBCH 14, 63 g a.s./ha	2	3.295
Forage		40	0.008
Mature Plants	2 Spray applications at BBCH 14 and BBCH 77, 63 and 63 g a.s./ha (126 g a.s./ha total)	21	3.934
Seeds		21	0.126

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at harvest/sampling)

Intermediate harvest was conventionally extracted three times with acetonitrile/water mixtures releasing 99.7% of the TRR (3.285 mg eq/kg). Losses during sample clean up accounted for 0.1% of the TRR (0.004 mg eq/kg). After concentration and purification steps 99.6% of the TRR (3.281 mg eq/kg) were analysed. The post extraction solids amounted to 0.3% (0.010 mg eq/kg) of the TRR, only.

Forage was conventionally extracted three times with acetonitrile/water mixtures releasing 77.3% of the TRR (0.006 mg eq/kg). No losses of RA occurred during sample clean up and 77.3% of the TRR (0.006 mg eq/kg) were analysed. The post extraction solids amounted to 22.7% of the TRR (0.002 mg eq/kg), only.

Mature plants were conventionally extracted three times with acetonitrile/water mixtures releasing 96.2% of the TRR (3.786 mg eq/kg). Losses during sample clean up accounted for 0.2% of the TRR (0.010 mg eq/kg). After concentration and purification steps 96.0% of the TRR (3.776 mg eq/kg) were analysed. The post extraction solids contained 3.8% (0.148 mg eq/kg) of the TRR.

Seeds were conventionally extracted three times with acetonitrile/water mixtures releasing 73.6% of the TRR (0.093 mg eq/kg). No losses of RA occurred during sample clean up and 77.3% of the TRR (0.093 mg eq/kg) were analysed. The post extraction solids after conventional extraction accounted for 26.4% of the TRR (0.033 mg eq/kg).

The PES of seeds were subjected to exhaustive extraction under microwave support. This treatment released 10.6% of the TRR (0.013 mq eq/kg). Losses during sample clean up accounted for 1.0% of the TRR (0.001 mg eq/kg) and 9.6% of the TRR (0.012 mg eq/kg) were analysed by HPLC. Additionally, the solids remaining after exhaustive extraction were incubated consecutively with cellulase and amylase to release further residues. These extractions released 1.3% of the TRR (0.002 mg eq/kg).

Subsequent acidic extraction additionally released 7.9% of the TRR (0.010 mg eq/kg). Losses of RA during neutralization of the acidic extract accounted for 1.4% of the TRR (<0.001 mg eq/kg). The neutralized extract was further characterised by partitioning with ethylacetate. 4.4% of the TRR (0.007 mg eq/kg) was detected within the ethylacetate fraction and 2.1% of the TRR (0.003 mg eq/kg) was detected in the water phase.

In total 93.5% of the TRR (0.118 mg eq/kg) was extracted from seeds. Total losses of RA accounted for 2.4% of the TRR (0.001 mg eq/kg). The post extraction solids amounted to 6.5% (0.008 mg eq/kg) of the TRR.

The distribution of the radioactive residues is shown in the following table.

Table 6.2.1- 33: Distribution of radioactivity in the extracts of oilseed rape matrices after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

	intermediate harvest		forage		mature plants		seeds	
TRR [mg eq/kg] =	3.295		0.008		3.934		0.126	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	**99.7**	**3.285**	**77.3**	**0.006**	**96.2**	**3.786**	**73.6**	**0.093**
Analysed extracts	99.6	3.281	77.3	0.006	96.0	3.776	73.6	0.093
Not analysed	0.1	0.004	n.q.	n.q.	0.2	0.010	n.q.	n.q.
Exhaustive extraction	--	--	--	--	--	--	**10.6**	**0.013**
Analysed extracts*	--	--	--	--	--	--	9.6	0.012
Not analysed	--	--	--	--	--	--	1.0	0.001
Enzymatic digestion	--	--	--	--	--	--	**1.3**	**0.002**
Acidic Extraction and partitioning	--	--	--	--	--	--	**7.9**	**0.010**
Ethylacetate phase	--	--	--	--	--	--	4.4	0.007
Water phase	--	--	--	--	--	--	2.1	0.003
Not analysed							1.4	<0.001
Total extracted	**99.7**	**3.285**	**77.3**	**0.006**	**96.2**	**3.786**	**93.5**	**0.118**
Post extraction solids (PES)	**0.3**	**0.010**	**22.7**	**0.002**	**3.8**	**0.148**	**6.5**	**0.008**
Balance	**100.0**	**3.295**	**100.0**	**0.008**	**100.0**	**3.934**	**100.0**	**0.126**

-- not applicable
n.q. not quantified (< LOQ)
* no individual peak above detection limit was observed in the HPLC chromatogram of the exhaustive extract of seeds

Comparison of chromatograms from conventional extracts of intermediate harvest and mature plants of the current study among themselves indicated a high grade of comparability. Conventional extract of forage contained no individual radiosignal above the background noise whereas conventional extract from seeds contained only the test compound.

The comparison of profiles of all RACs to corresponding profiles of the parallel study performed with [pyrazole-4-^{14}C]BCS-CN88460 revealed that no label specific metabolism could be observed.

In conventional extracts from intermediate harvest 94.0% of the TRR (3.096 mg eq/kg) were identified in total. The parent compound was the major component representing 84.1% of the TRR

(2.770 mg eq/kg), whereas the metabolites BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) represented 2.3, 1.6, 2.2 and 3.8% of the TRR corresponding to 0.077, 0.052, 0.071 and 0.126 mg eq/kg, respectively.

Acid hydrolysis (1 N HCl, 100 °C, 1 h) of the conventional extract of intermediate harvest was performed in order to analyse for hydrolysable conjugates. Comparison of metabolic profiles before and after hydrolysis indicated cleavage of the identified conjugates BCS-CN88460-hydroxyphenyl-Gluc-MA, BCS-CN88460-2-propanol-Glyc-MA, BCS-CN88460-propanol-Glyc-MA and BCS-CN88460-hydroxyphenyl-Glyc-MA to less polar compounds. Analogous hydrolysis experiments were performed in the parallel study with the pyrazole-label showing good accordance with the current study.

In conventional extracts from forage 77.3% of the TRR was analysed in total (0.006 mg eq/kg). No individual radiosignal was above the background noise due to very low amounts of total radioactive ingredients in the extract.

In conventional extracts from mature plants 84.4% of the TRR (3.318 mg eq/kg) were analysed in total. The parent compound was by far the major component representing 72.0% of the TRR (2.831 mg eq/kg), whereas the metabolites BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) represented 2.2, 4.6, 2.5 and 3.1% of the TRR corresponding to 0.087, 0.181, 0.097 and 0.122 mg eq/kg, respectively.

In conventional extracts from seeds 73.6% of the TRR (0.093 mg eq/kg) were identified. The parent compound was the only component representing 73.6% of the TRR (0.093 mg eq/kg). Parent compound was identified by HPLC and TLC co-chromatography with the test item as radiolabelled reference compound.

Exhaustive extraction of seeds released further 10.6 % of the TRR (0.013 mg eq/kg) and 9.6 % of the TRR (0.012 mg eq/kg) were analysed by HPLC. No individual radiosignal was above the background noise due to low amounts of total radioactive ingredients in the exhaustive extract. Conventional extract of seeds treated under microwave extraction conditions in comparison of the corresponding conventional extract and exhaustive extract, showed that degradation of parent compound by this treatment was neglectable.

The TRR and the distribution of parent and metabolites in oilseed rape matrices are shown in the following table.

Table 6.2.1- 34: Distribution of parent compound and metabolites in the extracts of oilseed rape matrices after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

	intermediate harvest		forage		mature plants		seeds	
TRR [mg eq/kg] =		3.295		0.008		3.934		0.126
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	***99.7***	***3.285***	***77.3***	***0.006***	***96.2***	***3.876***	***73.6***	***0.093***
BCS-CN88460 (parent compound)	84.1	2.770	n.d.	n.d.	72.0	2.831	73.6	0.093
BCS-CN88460-hydroxyphenyl-Gluc-MA (M23)	2.3	0.077	n.d.	n.d.	2.2	0.087	n.d.	n.d.
BCS-CN88460-2-propanol-Glyc-MA (M22)	1.6	0.052	n.d.	n.d.	4.6	0.181	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	2.2	0.071	n.d.	n.d.	2.5	0.097	n.d.	n.d.
BCS-CN88460-hydroxyphenyl-Glyc-MA (M24)	3.8	0.126	n.d.	n.d.	3.1	0.122	n.d.	n.d.
Total identified	**94.0**	**3.096**	**n.d.**	**n.d.**	**84.4**	**3.318**	**73.6**	**0.093**
Unknown 1	n.d.	n.d.	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 2	0.1	0.004	n.d.	n.d.	0.1	0.003	n.d.	n.d.
Unknown 3	n.d.	n.d.	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 4	0.1	0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 5	0.2	0.006	n.d.	n.d.	0.2	0.006	n.d.	n.d.
Unknown 6	0.1	0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 7	n.d.	n.d.	n.d.	n.d.	0.1	0.005	n.d.	n.d.
Unknown 8	0.1	0.004	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 9	0.2	0.007	n.d.	n.d.	0.2	0.008	n.d.	n.d.
Unknown 10	n.d.	n.d.	n.d.	n.d.	<0.1	0.001	n.d.	n.d.
Unknown 11	n.d.	n.d.	n.d.	n.d.	<0.1	0.002	n.d.	n.d.
Unknown 12	0.1	0.004	n.d.	n.d.	0.1	0.003	n.d.	n.d.
Unknown 13	n.d.	n.d.	n.d.	n.d.	0.2	0.008	n.d.	n.d.
Unknown 14	0.1	0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 15	n.d.	n.d.	n.d.	n.d.	0.2	0.006	n.d.	n.d.
Unknown 16	n.d.	n.d.	n.d.	n.d.	0.1	0.003	n.d.	n.d.
Unknown 17	0.3	0.011	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 18	n.d.	n.d.	n.d.	n.d.	0.1	0.003	n.d.	n.d.
Unknown 19	0.3	0.010	n.d.	n.d.	0.1	0.002	n.d.	n.d.
Unknown 20	n.d.	n.d.	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 21	n.d.	n.d.	n.d.	n.d.	0.1	0.003	n.d.	n.d.
Unknown 22	0.3	0.010	n.d.	n.d.	0.2	0.009	n.d.	n.d.
Unknown 23	n.d.	n.d.	n.d.	n.d.	0.2	0.008	n.d.	n.d.
Unknown 24	0.1	0.004	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 25	0.2	0.007	n.d.	n.d.	0.8	0.032	n.d.	n.d.
Unknown 26	0.4	0.012	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 27	0.1	0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 28	0.1	0.005	n.d.	n.d.	1.0	0.040	n.d.	n.d.
Unknown 29	0.1	0.003	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 32	n.d.	n.d.	n.d.	n.d.	0.7	0.026	n.d.	n.d.
Unknown 33	0.7	0.022	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 36	0.4	0.013	n.d.	n.d.	0.6	0.024	n.d.	n.d.
Unknown 37	n.d.	n.d.	n.d.	n.d.	0.3	0.014	n.d.	n.d.
Unknown 38	n.d.	n.d.	n.d.	n.d.	0.1	0.002	n.d.	n.d.
Unknown 39	n.d.	n.d.	n.d.	n.d.	0.1	0.005	n.d.	n.d.
Unknown 40	n.d.	n.d.	n.d.	n.d.	0.2	0.007	n.d.	n.d.

continued on next page

Table 6.2.1- 34 continued

	intermediate harvest		forage		mature plants		seeds	
Unknown 41	n.d.	n.d.	n.d.	n.d.	0.1	0.004	n.d.	n.d.
Unknown 42	n.d.	n.d.	n.d.	n.d.	0.2	0.008	n.d.	n.d.
Unknown 43	n.d.	n.d.	n.d.	n.d.	0.2	0.007	n.d.	n.d.
Unknown 44	n.d.	n.d.	n.d.	n.d.	0.6	0.023	n.d.	n.d.
Unknown 45	0.2	0.007	n.d.	n.d.	1.3	0.050	n.d.	n.d.
Unknown 46	n.d.	n.d.	n.d.	n.d.	0.7	0.026	n.d.	n.d.
Unknown 47	0.1	0.003	n.d.	n.d.	0.9	0.035	n.d.	n.d.
Unknown 48	0.1	0.004	n.d.	n.d.	0.2	0.009	n.d.	n.d.
Unknown 49	n.d.	n.d.	n.d.	n.d.	0.1	0.003	n.d.	n.d.
Unknown 50	n.d.	n.d.	n.d.	n.d.	0.3	0.013	n.d.	n.d.
Unknown 51	1.2	0.038	n.d.	n.d.	1.0	0.038	n.d.	n.d.
Characterised by HPLC	**5.6**	**0.185**	**n.d.**	**n.d.**	**11.6**	**0.458**	**--**	**--**
Total not analysed of conventional extraction	0.1	0.004	--	--	0.2	0.010	--	--
Exhaustive extraction	**--**	**--**	**--**	**--**	**--**	**--**	***10.6***	***0.013***
*Analysed by HPLC**	--	--	--	--	--	--	*9.6*	*0.012*
Not analysed							*1.0*	*0.001*
Enzymatic digestion	**--**	**--**	**--**	**--**	**--**	**--**	***1.3***	***0.002***
Acidic extraction and partitioning	**--**	**--**	**--**	**--**	**--**	**--**	***7.9***	***0.010***
Ethylacetate phase	--	--	--	--	--	--	*4.4*	*0.005*
Water phase	--	--	--	--	--	--	*2.1*	*0.003*
Not analysed by partition	--	--	--	--	--	--	*1.4*	*0.002*
Total characterised**	**5.6**	**0.185**	**--**	**--**	**11.6**	**0.458**	**19.8**	**0.025**
Total extracted	**99.7**	**3.285**	**77.3**	**0.006**	**96.2**	**3.786**	**93.5**	**0.118**
Post extraction solids (PES)	**0.3**	**0.010**	**22.7**	**0.002**	**3.8**	**0.148**	**6.5**	**0.008**
Accountability	**100.0**	**3.295**	**100.0**	**0.008**	**100.0**	**3.934**	**100.0**	**0.126**

* no individual peak above detection limit was observed in the HPLC chromatogram of the exhaustive extract of seeds
**by chromatographic and/or extraction behaviour
n.d. not detected
-- not applicable

In conventional extract of intermediate harvest, 23 unknown metabolites were characterised, individually accounting for equal or less than 1.2% of the TRR and 0.038 mg eq/kg and 39 were characterised in mature plants, individually accounting for equal or less than 1.3% of the TRR and 0.050 mg eq/kg.

Table 6.2.1- 35: Summary of characterisation and identification of radioactive residues in oilseed rape matrices after two foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

	intermediate harvest		forage		mature plants		seeds	
TRR [mg eq/kg]		3.295		0.008		3.934		0.126
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	***99.7***	***3.285***	***77.3***	***0.006***	***96.2***	***3.786***	***73.6***	***0.093***
BCS-CN88460 (parent compound)	84.1	2.770	n.d.	n.d.	72.0	2.831	73.6	0.093
BCS-CN88460-hydroxyphenyl-Gluc-MA (M23)	2.3	0.077	n.d.	n.d.	2.2	0.087	n.d.	n.d.
BCS-CN88460-2-propanol-Glyc-MA (M22)	1.6	0.052	n.d.	n.d.	4.6	0.181	n.d.	n.d.
BCS-CN88460-propanol-Glyc-MA (M21)	2.2	0.071	n.d.	n.d.	2.5	0.097	n.d.	n.d.
BCS-CN88460-hydroxyphenyl-Glyc-MA (M24)	3.8	0.126	n.d.	n.d.	3.1	0.122	n.d.	n.d.
Total identified	**94.0**	**3.096**	--	--	**84.4**	**3.318**	**73.6**	**0.093**
Characterised in the conventional extract by HPLC	**5.6**	**0.185**	--	--	**11.6**	**0.458**	--	--.
Number of unknown peaks	23				39		--	
Largest unknown peak	1.2	0.038	--	--	1.3	0.050	--	--
Total not analysed of conventional extraction	**0.1**	**0.004**	--	--	**0.2**	**0.010**	--	--
Exhaustive extraction	--	--	--	--	--	--	***10.6***	***0.013***
*Analysed by HPLC**	--	--	--	--	--	--	*9.6*	*0.012*
Not analysed							*1.0*	*0.001*
Enzymatic digestion	--	--	--	--	--	--	***1.3***	***0.002***
Acidic extraction and partitioning	--	--	--	--	--	--	***7.9***	***0.010***
Ethylacetate phase	--	--	--	--	--	--	*4.4*	*0.007*
Water phase	--	--	--	--	--	--	*2.1*	*0.003*
Not analysed by partition							*1.4*	*<0.001*
Total characterised**	**5.6**	**0.185**	--	--	**11.6**	**0.458**	**19.8**	**0.025**
Total extracted	**99.7**	**3.285**	**77.3**	**0.006**	**96.2**	**3.786**	**93.5**	**0.118**
Post extraction solids (PES)	**0.3**	**0.010**	**22.7**	**0.002**	**3.8**	**0.148**	**6.5**	**0.008**
Accountability	**100.0**	**3.295**	**100.0**	**0.008**	**100.0**	**3.934**	**100.0**	**0.126**

* no individual peak above detection limit was observed in the HPLC chromatogram of the exhaustive extract of seeds
**by chromatographic and/or extraction behavior
n.d. not detected
-- not applicable

III. Conclusions

The metabolism of BCS-CN88460 in oilseed rape was investigated after two foliar applications at BBCH 14 and BBCH 77. The oilseed rape plants were treated with [phenyl-UL-^{14}C]BCS-CN88460 formulated as an EC 50 at a single application rate of 63 and 63 g a.s./ha for the first and second spray application, respectively, corresponding to a total application rate of 126 g a.s/ha. The oilseed rape intermediate harvest was harvested at BBCH 30, 2 days after the first application and forage was harvested at BBCH 55, 40 days after the first application. Mature plants and seeds were harvested at BBCH 89, 21 days after the second application.

Residues in forage and seeds were significantly lower than those in intermediate harvest and mature plants. The extraction rates of intermediate harvest, forage, mature plants and seeds were high. Overall, identification rates for intermediate harvest, mature plants and seeds were high. Parent compound BCS-CN88460 was the main residue component in intermediate harvest and mature plant and the only component in seeds. Conventional extract of forage contained no residues above the limit of detection of the analytical method.

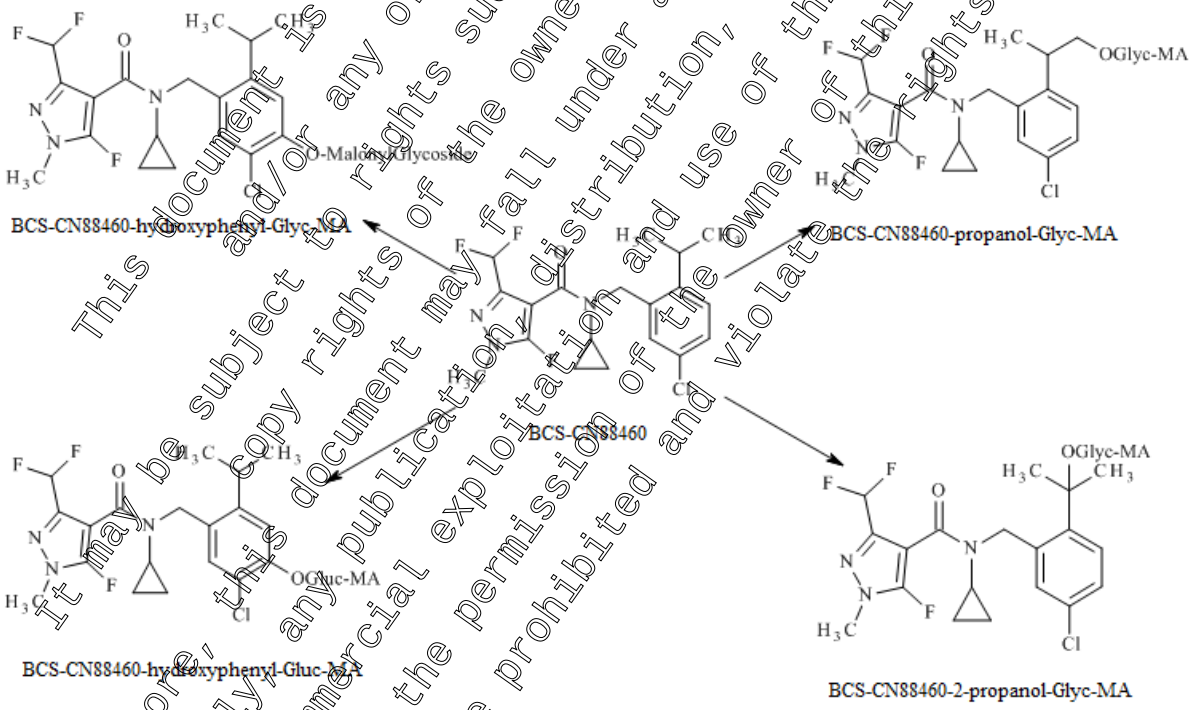
Besides parent compound, four metabolites were identified in intermediate harvest and mature plants: BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-2-propanol-Glyc-MA (M22), BCS-CN88460-propanol-Glyc-MA (M21) and BCS-CN88460-hydroxyphenyl-Glyc-MA (M24). The results in the present study are in good agreement with the results with the pyrazole-label. No label specific metabolites were observed.

The test compound [phenyl-UL-^{14}C]BCS-CN88460 was moderately metabolised in oilseed rape after two foliar applications. The main metabolic reactions observed were:

- Hydroxylation in position 1 or 2 of the propyl group of the phenyl ring followed by conjugation with hexose and malonic acid
- Hydroxylation in position 4 of the phenyl moiety followed by conjugation with hexose and malonic acid

Based on these results, the degradation behavior of [phenyl-UL-^{14}C]BCS-CN88460 in oilseed rape is adequately understood and a metabolic pathway is proposed in the figure below.

Figure 6.2.1- 4: Proposed metabolic pathway of [phenyl-UL-^{14}C]BCS-CN88460 in oilseed rape



Metabolism, distribution and expression of residues in soybean (foliar spray application)

Metabolism studies in soybean after foliar application were conducted with [pyrazole-4-^{14}C] and [phenyl-UL-^{14}C]BCS-CN88460.

Table 6.2.1- 36: Overview over available soybean metabolism studies

Plant	Application	Target application rate	BBCH Code	Reference
soybean	three foliar spray applications, pyrazole-labelled isoflucypram	3 x 60 g a.s./ha	BBCH 14, BBCH 51 and BBCH 84	M-609373-01-1
soybean	three foliar spray applications, phenyl-labelled isoflucypram	3 x 60 g a.s./ha	BBCH 14, BBCH 51 and BBCH 85	M-609376-01-1

Summary of metabolism in soybean (foliar spray application)

The metabolite pattern corresponds well when comparing the two metabolism studies in soybean after foliar application. Parent compound BCS-CN88460 was the main residue component in soybean straw and soybean seeds and a major residue in soybean forage and hay. Parent compound was the only component in soybean seeds. BCS-CN88460 was moderately metabolised in soybean forage, hay and straw samples after three post-emergence applications. The main metabolic reactions were de-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione and degradation of the homoglutathione moiety followed by conjugation with malonic acid or degradation and desamination to mercapto lactic acid group and hydroxylation of the benzyl moiety or of the propyl group were observed. Furthermore glycosilation was clearly observed at the mercapto lactic acid group.

Besides parent compound, the following metabolites were identified: BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48), BCS-CN88460-desfluoro-homoGSH (M44), BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46), BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47) and BCS-CN88460-desfluoro-Cys-MA (M45).

Report: KCA 6.2.1/07; [redacted], M., 2017; M-609373-01-1
Title: Metabolism of [pyrazole-4-14C]-BCS-CN88460 in soybean plants
Report No.: S14-01089
Document No.: M-609373-01-1
Guideline(s): OECD Test Guideline No. 501
Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107 /2009
US EPA OCSPP Test Guideline No. 860.1300
JAP FAMIC-ACIS Notification 12 Nousan 8147
Guideline deviation(s): none
GLP/GEP: yes

Executive Summary

The metabolism of BCS-CN88460 was investigated in soybean plants after three post-emergent spray applications. For each of the three foliar applications, the test item [pyrazole-4-^{14}C]BCS-CN88460 was formulated and applied as aqueous EC 50 using a nominal application rate of 60 g a.s./ha each. The actual single application rates corresponded to 59, 57 and 65 g BCS-CN88460/ha resulting in a total actual application rate of 181 g a.s./ha.

Soybean forage was harvested at BBCH stage 49 after the first application (PHI = 5d), hay at BBCH stage 77 after the 2nd application (PHI = 39d) and straw and seeds at BBCH stage 96, 21d after the 3rd application. The radioactive residues of soybean forage, hay, straw and seeds were determined by summing up the extractable and unextractable radioactivity. Soybean seeds contained significantly lower residue amounts than forage, hay and straw. The total radioactive residue (TRR) in soybean straw was highest and amounted to 17.715 mg eq/kg. The TRR in soybean forage and soybean hay were high and amounted to 4.371 mg eq/kg and 4.679 mg eq/kg, respectively. The TRR in seeds was low and amounted to 0.035 mg eq/kg.

Homogenised plant material from RACs was conventionally extracted with acetonitrile/water mixtures. Overall, the extraction rates after conventional extraction of soybean forage, hay, straw and seeds were high and amounted to 92.1, 87.4, 94.1 and 87.7% of the TRR (4.026, 4.091, 16.669 and 0.031 mg eq/kg), respectively. Exhaustive extract of soybean forage, hay and straw increased the extraction rate slightly: 5.1% of the TRR (0.222 mg eq/kg), 6.9% of the TRR (0.321 mg eq/kg) and 2.5% of the TRR (0.441 mg eq/kg) were released by this treatment, respectively.

The post extraction solids after conventional and exhaustive extractions accounted for 2.8% of the TRR (0.123 mg eq/kg), 5.7% of the TRR (0.266 mg eq/kg), 3.4% of the TRR (0.605 mg eq/kg) and 12.3% of the TRR (0.004 mg eq/kg) for soybean forage, hay, straw and seeds, respectively.

Residues in the conventional extracts of all RACs and in the exhaustive extracts of forage and straw were analysed and quantified by HPLC. The parent compound and metabolites were either identified by co-chromatography with the reference compound or by spectroscopic analysis in isolated fractions fractions from the conventional extract of soybean hay. Additionally, the metabolite pattern and retention times of the current and the soybean metabolism study with the phenyl label were compared.

Profiles of forage, hay and straw showed a high grade of comparability among themselves concerning metabolization of the test item whereas the extract from soybean seeds contained only the parent compound.

The comparison of metabolic profiles of conventional extracts of all RACs with those of a parallel study with [phenyl-UL-^{14}C]BCS-CN88460 revealed a high grade of comparability of the metabolization of the test compound in soybean and no label specific metabolite could be observed for the both labels.

A major component in all RACs was the test compound BCS-CN88460 accounting for 18.7% of the TRR (0.819 mg eq/kg) in soybean forage, 10.4% of the TRR (0.487 mg eq/kg) in soybean hay, 64.5% of the TRR (11.424 mg eq/kg) in soybean straw and 76.6% of the TRR (0.027 mg eq/kg) in soybean seeds. Besides parent compound no other metabolite was detected in the extract of soybean seeds.

Besides parent compound five metabolites were identified soybean forage, hay and straw.

BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48) accounted for 3.4, 15.2 and 3.9% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.147, 0.711 and 0.690 mg eq/kg, respectively. BCS-CN88460-desfluoro-homoGSH (M44) accounted for 22.9, 7.8 and 4.8% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.997, 0.366 and 0.857 mg eq/kg, respectively. BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46) accounted for 9.5, 3.2 and 1.9% of the TRR in extracts from soybean forage, hay and straw, corresponding to 0.415, 0.151 and 0.337 mg eq/kg, respectively. BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47) accounted for 3.0, 11.1 and 3.0% of the TRR in extracts from soybean forage, hay and straw, corresponding to 0.129, 0.520 and 0.533 mg eq/kg, respectively. BCS-CN88460-desfluoro-Cys-MA (M45) accounted for 9.2, 15.4 and 4.6% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.400, 0.723 and 0.828 mg eq/kg, respectively.

Overall, identification rates were sufficient and amounted to 66.6% of TRR for forage, 63.2% of TRR for hay, 82.8% of the TRR for straw and 76.6% of TRR for seeds. In soybean forage, hay and straw, 17, 13 and 20 unknown metabolites were characterised in the extracts by their chromatographic behaviour, individually accounting for less than 3.9% of the TRR (0.171 mg eq/kg), 4.4% of the TRR (0.208 mg eq/kg) and 1.9% of the TRR (0.329 mg eq/kg) for forage, hay and straw, respectively.

All initial profiles of conventional extracts of the raw agricultural commodities were performed within 6 months after harvest. Furthermore, storage stability of residues in stored sample matrix and extracts could be demonstrated exemplarily for soybean hay and straw for 18 and 27 months, respectively, in the course of a parallel study with the phenyl-label which showed a high grade of comparability of the metabolic profiles of the individual RACs.

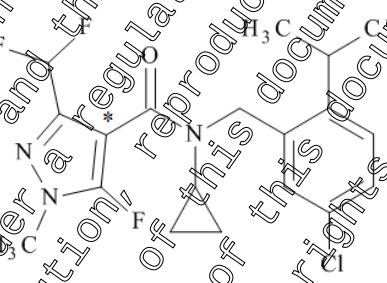
As metabolic reactions, de-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione and degradation of the homoglutathione moiety followed by conjugation with malonic acid or degradation and desamination to mercapto lactic acid group and hydroxylation of the benzyl moiety or of the propyl group were observed. Furthermore glycosilation was clearly observed at the mercapto lactic acid group.

Based on these results, the degradation behavior of [pyrazole-4-^{14}C]BCS-CN88460 in soybean is adequately understood and a pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	
	* denotes the position of the ^{14}C-label
Radiolabel position	[pyrazole-4-^{14}C]
Specific radioactivity	4.22 MBq/mg
Radiochemical purity	> 98% (HPLC)
Chemical purity	> 99%

Formulation of the test compound

A stock solution of the test compound was prepared by dissolving the test compound in acetonitrile to give a concentration of about 5 mg/mL. The purity in the stock solution and the identity of the test compound was checked by HPLC with radiodetection and was 100%

The test compound was formulated as an EC 50 for the experiment and therefore [pyrazole-4-^{14}C] BCS-CN88460 was dissolved in acetonitrile. For each of the three spray dilutions, adequate parts of the stock solution were transferred into glass vials and evaporated to dryness. Blank formulation was added and the mixtures were homogenised using a magnetic stirrer. The sample was then adjusted with water to a final volume of 50 mL (1st and 2nd application) and 100 mL (3rd application) of the spray dilution and homogenised by stirring.

2. Soil:

Soil characteristics			
Type	TOC	pH ($CaCl_2$)	CEC
Sandy loam	2.1%	7.23	15.5 meq/100 g

3. Plant: soybean, variety “Amandine”, representative for oilseeds

B. Study Design

1. Experimental conditions:

The experiment was conducted with soybean plants (variety: Amandine) based on a plant density of 800,000 soybean plants per hectare. A planting container with a surface area of 1 m² was used corresponding to the rate of 80 seeds per m². The plants were cultivated in the glass-roofed greenhouse of the test facility and grown similar to natural temperature and light conditions, but protected from rainfall. They were watered by pouring onto the soil in the planting containers. The plants were applied at three different growth stages (BBCH 14, 71 and 84). At all these growth stages the target single application rate was 60 g a.s./ha. The target rate corresponds to the anticipated maximum application rates for the use type.

For each application, the soybean plants were treated with 50 mL (1st and 2nd application) and 100 mL (3rd application) of the aqueous spray dilutions using a controlled track sprayer with a flat fan nozzle. To avoid contamination of the surrounding area by drift, the plants in the planting container were enclosed with a foil housing. After spraying the spray dilution onto the oilseed rape plants in the planting container, the spray device and the protective plastic foil around the planting container were rinsed with acetonitrile/water (8/2; v/v). The actual amount applied was calculated by subtracting the losses from the radioactivity in the original application solution. At the 1st application 24.8 MBq of the labelled test comound were applied, corresponding to 5.9 mg a.s.. At the 2nd application 23.9 MBq of the test compound were applied, corresponding to 5.7 mg a.s.. At the 3rd application 27.3 MBq of the test compound were applied, corresponding to 6.5 mg a.s.. The actual single application rates were 59, 57 and 65 g a.s./ha resulting in a total actual application rate of 181 g a.s./ha.

2. Sampling:

At growth stage BBCH 49 the RAC forage, at growth stage BBCH 77 the RAC hay and at BBCH 96 the RACs straw and seeds were harvested. Plant samples were collected by cutting approximately 1-2 cm above the soil level. Plants sampled at hay stage were dried in a hood for 4 days.

The total weight of each sample was determined. The samples were homogenised with liquid nitrogen using a high speed blender. The sample materials were stored in a freezer (≤ -18 °C). Aliquots of the homogenates were extracted. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids.

C. Analytical Procedures

1. Extraction:

Conventional extraction procedure and sample clean up:

Aliquots of the homogenised samples of soybean forage, hay, straw and seeds were extracted three times with a mixture of acetonitrile/water (8/2; v/v) using a high speed blender. After each extraction step, the extracts were filtered by suction and the solids were rinsed with a small amount of the solvent mixture used for extraction. The solids were dried and aliquots were subjected to combustion and LSC.

The extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v).

Each combined percolate/rinse solution obtained from SPE purification was evaporated to the aqueous

remainder. The final conventional extracts were analysed by HPLC with the general profiling method. All soybean samples and extracts were stored in a freezer (≤ -18 °C).

Exhaustive extraction and corresponding clean-up:

Solids from the conventional extraction of soybean forage, hay and straw were exhaustively extracted two times with acetonitrile/water/formic acid (50/50/1; v/v/v) under microwave assistance at increased temperature (0 to 5 min increase to 120 °C, 5 to 20 min at 120 °C, 800 W). The microwave extracts were cooled down at room temperature.

For forage and hay, the individual extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v). Each combined percolate/rinse solution obtained from SPE purification was mixed with emulsifier and evaporated to the aqueous remainder.

The exhaustive extracts of straw were directly concentrated by evaporation to the aqueous remainder. The final exhaustive extract of soybean hay was partitioned for characterisation. The final exhaustive extracts of forage and straw were analysed by HPLC with the general profiling method. The extracts were stored in a freezer (≤ -18 °C).

Characterisation of residues by partitioning

Radioactivity released from soybean hay by exhaustive extraction under microwave assistance was characterised by partitioning. Therefore, two milliliters of the concentrate obtained during exhaustive extraction and after purification were mixed with 8 mL water and 10 mL ethylacetate in a centrifuge tube. The mixture was incubated 30 min by 150 rpm on a flatbed shaker. Four gram anhydrous magnesium sulphate, one gram sodium chloride, one gram trisodium citrate and 0.5 gram disodium citrate sesquihydrate were added to remove water and adjust the pH value. After addition of the salt mixture, the tube was immediately shaken vigorously by hands for 10 seconds and with a flatbed shaker for at least one minute and afterwards centrifuged. Ethylacetate and water phase were separated, their volume was determined.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

Conventional and microwave extracts were analysed by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

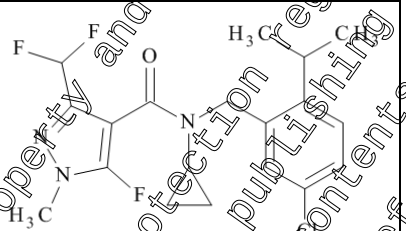
2. Identification and characterisation:

For identification of radioactive ingredients in conventional extract from soybean hay, selected major radiosignals were isolated as fractions of eluent by HPLC fractionation. Isolated fractions were identified by spectroscopic analysis as following metabolites: BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48), BCS-CN88460-desfluoro-homoGSH (M44), BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46), BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47) and BCS-CN88460-desfluoro-Cys-MA (M45).

Metabolic profiles from the conventional and exhaustive extracts of all RACs were compared, as analysed by HPLC among themselves. Metabolic profiles of all RACs were compared with metabolic profiles of corresponding RACs in the soybean metabolism study with the phenyl label. Parent compound was identified in the extract of soybean seeds by TLC co-chromatography with the radiolabelled test compound. Metabolites after structure elucidation in the conventional extract of soybean hay were re-assigned.

Unknown metabolites were characterised based on their extraction and chromatographic behavior.

Table 6.2.1- 37: List of reference compound

Report name / other names/codes	**Chemical Name (IUPAC)**	**Structure and formula**
Parent compound BCS-CN88460 Reference: S_PY_1_1000	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

The initial profiles of the conventional extracts of soybean forage, hay, straw and seeds were analysed within 6 months after harvest of the soybean plant raw material. The stability of residues in the stored sample matrices and extracts were demonstrated in the parallel study with the phenyl label. As the profiles of the respective RACs were highly comparable between the parallel studies and no label specific metabolites were observed it was concluded, that the residues in the samples were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in soybean was investigated after three spray applications.

Soybean plants in a 1 m² container were treated with [pyrazole-4-^{14}C]BCS-CN88460 formulated as an EC 50 at BBCH 14 (beginning of stem elongation), BBCH 51 (first flower buds visible) and BBCH 84 (about 40% of pods are ripe). The actual single application rates corresponded to 59, 57 and 65 g a.s./ha. The total application rate amounted to 181 g a.s./ha, corresponding well to the anticipated maximum application rate.

The TRR values of the individual RACs were determined by summing up the radioactivity determined in the combined extracts and the radioactivity in the solids. The residue levels are shown in mg active substance equivalents per kg sample material (mg a.s.equiv./kg or simplified mg eq/kg).

The TRR in soybean straw was high and amounted to 17.715 mg eq/kg. Soybean forage and hay contained a TRR of 4.371 mg eq/kg and 4.679 mg eq/kg, respectively. The TRR determined for soybean seeds was low and amounted to 0.035 mg eq/kg.

The residue values in the parallel study with the phenyl label were comparable for forage but significantly lower for hay, straw and seeds. This is due to differences in plant growth during cultivation of both studies resulting in vegetation differences during the second and third application.

Table 6.2.1- 38: TRR values in soybean matrices after foliar application of [pyrazole-4-^{14}C]BCS-CN88460

Matrix	Timing and Application	PHI (days)*	ppm (= mg eq/kg)
Soybean forage	1 spray application at BBCH 14, 59 g a.s./ha	5	4.371
Soybean hay	2 spray applications at BBCH 14 and 51, 59 and 57 g a.s./ha (116 g a.s./ha, total)	39	4.679
Soybean straw	3 spray applications at BBCH 14, 51 and 84, 59, 57 and 65 g a.s./ha (181 g a.s./ha; in total)	21	17.715
Soybean seeds		21	0.035

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at harvest/sampling)

Soybean forage was conventionally extracted three times with acetonitrile/water mixtures releasing 92.1% of the TRR (4.026 mg eq/kg). After concentration and purification steps 91.1% of the TRR (3.981 mg eq/kg) were analysed by HPLC. For soybean forage, a microwave extraction was performed. With this exhaustive method 5.1% (0.222 mg eq/kg) of the TRR were further extracted and 4.4% of the TRR (0.193 mg eq/kg) analysed by HPLC, additionally. The residue level in the solids of soybean forage after conventional and exhaustive extraction amounted to 2.8% of the TRR and 0.123 mg eq/kg. Losses during sample clean up of forage samples were 1.7% of the TRR (0.074 mg eq/kg).

Soybean hay was conventionally extracted three times with acetonitrile/water mixtures releasing 87.4% of the TRR (4.091 mg eq/kg). After concentration and purification steps 86.5% of the TRR (4.048 mg eq/kg) were analysed by HPLC. Exhaustive extraction of soybean hay, releasing 6.9% of the TRR and 0.321 mg eq/kg, was further characterised by partitioning of the exhaustive extract using ethylacetate accounting for 6.4% of the TRR (0.299 mg eq/kg) after purification. Complete radioactivity of the exhaustive extract was found in the ethylacetate phase after partitioning. This is in good accordance to results from the partitioning of experiment performed with soybean hay exhaustive extract in a parallel study with [phenyl-UL-^{14}C]BCS-CN88460. Since the complete radioactivity of the exhaustive extracts of soybean hay in both studies was found in the ethylacetate phase, it can be assumed that the residues were highly unpolar and likely ascribable to radioactivity derived from parent compound. The residue level in the solids of soybean hay after conventional and exhaustive extraction amounted to 5.7% of the TRR and 0.266 mg eq/kg. Losses during sample clean up were 1.4% of the TRR (0.065 mg eq/kg).

Soybean straw was conventionally extracted three times with acetonitrile/water mixtures releasing 94.1% of the TRR (16.669 mg eq/kg). After concentration and purification steps, 93.2% of the TRR (16.516 mg eq/kg) were analysed by HPLC. For soybean straw samples, a microwave extraction was performed. With this method 2.5% (0.441 mg eq/kg) were extracted and 2.4% of the TRR (0.432 mg eq/kg) analysed by HPLC, additionally. The residue level in the solids of soybean straw after conventional and exhaustive extraction amounted to 3.4% of the TRR and 0.605 mg eq/kg. Losses during sample clean up were 0.9% of the TRR (0.163 mg eq/kg).

Soybean seeds were conventionally extracted three times with acetonitrile/water mixtures releasing 87.7% of the TRR (0.031 mg eq/kg). After concentration and purification steps 76.6% of the TRR (0.027 mg eq/kg) were analysed by HPLC. Losses during sample clean up were 11.0% of the TRR (0.004 mg eq/kg). The residue level in the solids of soybean seeds after conventional extraction amounted to 12.3% of the TRR and 0.004 mg eq/kg.

The distribution of the radioactive residues is shown in the following table.

Table 6.2.1- 39: Distribution of radioactivity in the extracts of soybean matrices after three foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	soybean forage		soybean hay		soybean straw		soybean seeds	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	**92.1**	**4.026**	**87.4**	**4.091**	**94.1**	**16.669**	**87.7**	**0.031**
Analysed extracts (HPLC)	91.1	3.981	86.5	4.048	93.2	16.515	76.6	0.027
Losses (not analysed)[#]	1.0	0.045	0.9	0.044	0.9	0.154	11.0	0.004
Exhaustive extraction	**5.1**	**0.222**	**6.9**	**0.321**	**2.5**	**0.441**	**--**	**--**
Analysed extracts (HPLC)	4.4	0.193	--	--	2.4	0.432	--	--
Partitioning of purified exhaustive extract	--	--	6.4	0.299	--	--	--	--
Ethylacetate phase	--	--	6.4	0.299	--	--	--	--
Water phase	--	--	n.q.	n.q.	--	--	--	--
Losses (not analysed)[#]	0.7	0.029	0.5	0.022	0.1	0.009	--	--
Total extracted	**97.2**	**4.248**	**94.3**	**4.413**	**96.6**	**17.110**	**87.7**	**0.031**
Post extraction solids (PES)	**2.8**	**0.123**	**5.7**	**0.266**	**3.4**	**0.605**	**12.3**	**0.004**
Accountability	**100.0**	**4.371**	**100.0**	**4.679**	**100.0**	**17.715**	**100.0**	**0.035**

[#] losses during clean up, concentration, degreasing, centrifugation, etc.
-- not performed
n.q.: not quantified

Comparison of profiles of all RACs to corresponding profiles of the parallel study performed with [phenyl-UL-^{14}C]BCS-CN88460 showed good correspondence to each other and revealed that no label specific metabolism could be observed for the pyrazole label.

In the parallel study with the phenyl-labelled test compound, chromatograms obtained after exhaustive extraction were compared to the profile of conventional extract of soybean straw treated under microwave conditions. This experiment demonstrated that the parent compound and the major metabolites were not degraded by this form of extraction.

In conventional extract from soybean forage 64.3% of the TRR (2.847 mg eq/kg) were identified in total. The parent compound and BCS-CN88460-desfluoro-homoGSH were major component representing 17.6% of the TRR (0.770 mg eq/kg) and 21.9% of the TRR (0.955 mg eq/kg), respectively. The metabolites BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-mercapto-lactic acid-OH, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA represented 3.4, 9.5, 3.0 and 9.2% of the TRR corresponding to 0.147, 0.415, 0.129 and 0.400 mg eq/kg, respectively. In exhaustive extract 2.1% of the TRR (0.091 mg eq/kg) were further identified. The parent compound and the metabolite BCS-CN88460-desfluoro-homoGSH represented 1.1 and 1.0% of the TRR corresponding to 0.049 and 0.042 mg eq/kg, respectively.

In conventional extract from soybean hay 63.2% of the TRR (2.958 mg eq/kg) were identified in total. The parent compound and the metabolites BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA were major components representing 10.4, 15.2, 11.1 and 15.4% of the TRR corresponding to 0.487, 0.711, 0.520 and 0.723 mg eq/kg, respectively. The metabolites BCS-CN88460-desfluoro-homoGSH and BCS-CN88460-desfluoro-mercapto-lactic acid-OH represented 7.8 and 3.2% of the TRR corresponding to 0.366 and 0.151 mg eq/kg, respectively. Residues in the exhaustive extract of soybean hay were characterised by partitioning using ethylacetate. Complete radioactivity of the exhaustive extract was found in the ethylacetate phase after partitioning. This is in good accordance to results from the partitioning experiment performed with soybean hay exhaustive extract in a parallel study with [phenyl-UL-^{14}C]BCS-CN88460. Since the complete radioactivity of the exhaustive extracts of soybean hay in both studies was found in the ethylacetate phase, it can be assumed that the residues were highly unpolar and likely ascribable to radioactivity derived from parent compound.

In conventional extract from soybean straw 81.2% of the TRR (14.380 mg eq/kg) were identified in total. The parent compound was by far the major component representing 63.6% of the TRR (11.262 mg eq/kg), whereas the metabolites BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-homoGSH, BCS-CN88460-desfluoro-mercapto-lactic acid-OH, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA represented 3.8, 4.8, 1.5, 3.0 and 4.4% of the TRR corresponding to 0.667, 0.857, 0.272, 0.533 and 0.788 mg eq/kg, respectively. In the exhaustive extract of straw further 1.6% of the TRR (0.288 mg eq/kg) were identified. The parent compound and the metabolites BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-mercapto-lactic acid-OH and BCS-CN88460-desfluoro-Cys-MA represented 0.9, 0.1, 0.4 and 0.2% of the TRR corresponding to 0.162, 0.023, 0.065 and 0.040 mg eq/kg, respectively.

Conventional extract from soybean seeds contained only parent compound representing 76.6% of the TRR corresponding to 0.027 mg eq/kg. The compound was identified by TLC co-chromatography of the parent compound fraction isolated from conventional extract of soybean seeds with the non-radiolabelled reference compound BCS-CN88460.

The TRR and the distribution of parent and metabolites in soybean matrices are shown in the following table.

Table 6.2.1- 40: Distribution of parent compound and metabolites the extracts of soybean matrices after three foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	soybean forage		soybean hay		soybean straw		soybean seeds	
TRR [mg eq/kg] =		4.371		4.679		17.715		0.035
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	***92.1***	***4.026***	***87.4***	***4.091***	***94.1***	***16.669***	***87.7***	***0.031***
BCS-CN88460	17.6	0.770	10.4	0.487	63.6	11.262	76.6	0.027
BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48)	3.4	0.147	15.2	0.711	3.8	0.667	n.d.	n.d.
BCS-CN88460-desfluoro-homoGSH (M44)	21.9	0.955	7.8	0.366	4.8	0.857	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46)	9.5	0.415	3.2	0.151	1.5	0.272	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47)	3.0	0.129	11.1	0.520	3.0	0.533	n.d.	n.d.
BCS-CN88460-desfluoro-Cys-MA (M45)	9.2	0.400	15.4	0.723	4.4	0.788	n.d.	n.d.
Subtotal identified	**64.5**	**2.817**	**63.2**	**2.958**	**81.2**	**14.380**	**76.6**	**0.027**
Unknown 1	n.d.	n.d.	n.d.	n.d.	0.3	0.060	n.d.	n.d.
Unknown 2	n.d.	n.d.	0.5	0.025	n.d.	n.d.	n.d.	n.d.
Unknown 3	0.8	0.037	1.5	0.069	n.d.	n.d.	n.d.	n.d.
Unknown 4	n.d.	n.d.	1.2	0.055	n.d.	n.d.	n.d.	n.d.
Unknown 5	n.d.	n.d.	0.7	0.033	n.d.	n.d.	n.d.	n.d.
Unknown 6	n.d.	n.d.	n.d.	n.d.	0.7	0.119	n.d.	n.d.
Unknown 7	0.9	0.041	4.4	0.208	n.d.	n.d.	n.d.	n.d.
Unknown 9	0.9	0.040	3.5	0.162	n.d.	n.d.	n.d.	n.d.
Unknown 11	0.9	0.039	1.5	0.071	n.d.	n.d.	n.d.	n.d.
Unknown 12	n.d.	n.d.	1.3	0.060	n.d.	n.d.	n.d.	n.d.
Unknown 15	3.1	0.136	n.d.	n.d.	1.9	0.329	n.d.	n.d.
Unknown 17	n.d.	n.d.	n.d.	n.d.	0.9	0.164	n.d.	n.d.
Unknown 19	2.0	0.087	1.2	0.058	0.7	0.124	n.d.	n.d.
Unknown 22	n.d.	n.d.	n.d.	n.d.	0.6	0.105	n.d.	n.d.
Unknown 23	n.d.	n.d.	n.d.	n.d.	0.6	0.098	n.d.	n.d.
Unknown 24	0.8	0.037	n.d.	n.d.	0.8	0.138	n.d.	n.d.
Unknown 25	n.d.	n.d.	2.2	0.102	1.5	0.268	n.d.	n.d.
Unknown 26	n.d.	n.d.	n.d.	n.d.	0.6	0.110	n.d.	n.d.
Unknown 27	1.1	0.049	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 29	n.d.	n.d.	2.5	0.115	0.7	0.125	n.d.	n.d.
Unknown 30	1.1	0.049	1.1	0.053	n.d.	n.d.	n.d.	n.d.
Unknown 33	n.d.	n.d.	n.d.	n.d.	0.4	0.079	n.d.	n.d.
Unknown 37	n.d.	n.d.	n.d.	n.d.	1.0	0.177	n.d.	n.d.
Unknown 38	3.9	0.171	1.7	0.081	n.d.	n.d.	n.d.	n.d.
Unknown 39	n.d.	n.d.	n.d.	n.d.	0.4	0.075	n.d.	n.d.
Unknown 40	2.4	0.106	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 42	n.d.	n.d.	n.d.	n.d.	0.4	0.063	n.d.	n.d.
Unknown 43	n.d.	n.d.	n.d.	n.d.	0.6	0.100	n.d.	n.d.
Unknown 44	3.0	0.133	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 45	1.5	0.068	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 46	1.8	0.078	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 47	2.2	0.095	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Subtotal characterised by HPLC	**26.6**	**1.164**	**23.3**	**1.090**	**12.0**	**2.135**	**n.d.**	**n.d.**

continued on next page

Table 6.2.1- 40 continued

	soybean forage		soybean hay		soybean straw		soybean seeds	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Exhaustive extraction	***5.1***	***0.222***	***6.9***	***0.321***	***2.5***	***0.441***	**--**	**--**
BCS-CN88460	1.1	0.049	--	--	0.9	0.162	--	--
BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48)	n.d.	n.d.	--	--	0.1	0.023	--	--
BCS-CN88460-desfluoro-homoGSH (M44)	1.0	0.042	--	--	n.d.	n.d.	--	--
BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46)	n.d.	n.d.	--	--	0.4	0.065	--	--
BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47)	n.d.	n.d.	--	--	n.d.	n.d.	--	--
BCS-CN88460-desfluoro-Cys-MA (M45)	n.d.	n.d.	--	--	0.2	0.040	--	--
Subtotal identified	**2.1**	**0.091**	--	--	**1.6**	**0.288**	--	--
Unknown 10	1.2	0.051	--	--	n.d.	n.d.	--	--
Unknown 14	n.d.	n.d.	--	--	0.1	0.024	--	--
Unknown 32	n.d.	n.d.	--	--	0.2	0.028	--	--
Unknown 34	n.d.	n.d.	--	--	0.1	0.025	--	--
Unknown 39	n.d.	n.d.	--	--	0.1	0.018	--	--
Unknown 41	1.2	0.051	--	--	0.3	0.050	--	--
Subtotal characterised by HPLC	**2.3**	**0.102**	--	--	**0.8**	**0.144**	--	--
Partitioning of purified exhaustive extract								
Ethylacetate phase	--	--	*6.4*	*0.299*	--	--	--	--
Water phase	--	--	*n.q.*	*n.q.*	--	--	--	--
Subtotal characterised by partitioning	**--**	**--**	***6.4***	***0.299***	**--**	**--**	**--**	**--**
Total not analysed / Losses	**1.7**	**0.074**	**1.4**	**0.065**	**0.9**	**0.163**	**11.0**	**0.004**
Total identified	**66.6**	**2.908**	**63.2**	**2.985**	**82.8**	**14.668**	**76.6**	**0.027**
Total characterised	**28.9**	**1.266**	**29.7**	**1.389**	**12.8**	**2.279**	**n.d.**	**n.d.**
Total extracted	**97.2**	**4.248**	**94.3**	**4.413**	**96.6**	**17.110**	**87.7**	**0.031**
Post extraction solids (PES)	**2.8**	**0.123**	**5.7**	**0.266**	**3.4**	**0.605**	**12.3**	**0.004**
Accountability	**100.0**	**4.371**	**100.0**	**4.679**	**100.0**	**17.715**	**100.0**	**0.035**

n.d.: not detected
-- not performed
n.q.: not quantified

Overall, identification rates were sufficient and amounted to 66.6% of TRR for forage, 63.2% of TRR for hay, 82.8% of the TRR for straw and 76.6% of TRR for seeds. In soybean forage, hay and straw, 17, 13 and 20 unknown metabolites were characterised in the extracts by their chromatographic behaviour, individually accounting for less than 3.9% of the TRR (0.171 mg eq/kg), 4.4% of the TRR (0.208 mg eq/kg) and 1.9% of the TRR (0.329 mg eq/kg).

Table 6.2.1- 41: Summary of characterisation and identification of radioactive residues in soybean matrices after three foliar applications of [pyrazole-4-^{14}C]BCS-CN88460

	soybean forage		soybean hay		soybean straw		soybean seeds	
TRR [mg eq/kg] =	4.371		4.679		17.715		0.035	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460	18.7	0.819	10.4	0.487	64.5	11.424	76.6	0.027
BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48)	3.4	0.147	15.2	0.711	3.9	0.690	n.d.	n.d.
BCS-CN88460-desfluoro-homoGSH (M44)	22.9	0.997	7.8	0.366	4.8	0.857	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46)	9.5	0.415	3.2	0.151	1.9	0.337	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47)	3.0	0.129	11.1	0.520	3.0	0.533	n.d.	n.d.
BCS-CN88460-desfluoro-Cys-MA (M45)	9.2	0.400	15.4	0.723	4.6	0.828	n.d.	n.d.
Total identified	**66.6**	**2.908**	**63.2**	**2.958**	**82.8**	**14.668**	**76.6**	**0.027**
Number of unknown peaks	17		13		20		0	
Largest unknown peak	3.9	0.171	4.4	0.208	1.9	0.329	n.d.	n.d.
Subtotal characterised by HPLC	**28.9**	**1.266**	**23.3**	**1.090**	**12.8**	**2.279**	**--**	**--**
Subtotal characterised by partitioning of exhaustive extract	**--**	**--**	***6.4***	***0.299***	**--**	**--**	**--**	**--**
Total characterised	**28.9**	**1.266**	**29.7**	**1.389**	**12.8**	**2.279**	**n.d.**	**n.d.**
Not analysed / Losses	**1.7**	**0.074**	**1.4**	**0.065**	**0.9**	**0.163**	**11.0**	**0.004**
Total extracted	**97.2**	**4.248**	**94.3**	**4.413**	**96.6**	**17.110**	**87.7**	**0.031**
Post extraction solids (PES)	**2.8**	**0.123**	**5.7**	**0.266**	**3.4**	**0.605**	**12.3**	**0.004**
Accountability	**100.0**	**4.371**	**100.0**	**4.679**	**100.0**	**17.715**	**100.0**	**0.035**

n.d.: not detected
-- not performed

III. Conclusions

The metabolism of BCS-CN88460 in soybean plants was investigated after three foliar applications at growth stages BBCH 14, BBCH 51 and BBCH 84. The soybean plants were treated with [pyrazole-4-^{14}C]BCS-CN88460 formulated as an EC 50 at a single application rate of 59, 57 and 65 g a.s./ha for the first, second and third spray application, respectively, corresponding to a total application rate of 181 g a.s./ha. Soybean forage was harvested at BBCH 49, soybean hay at BBCH 77, soybean straw and seeds were harvested at BBCH 96.

The residues in soybean forage, hay and straw were high compared to very low residues in seed. The main portion of residues of forage, hay, straw and seeds were released by conventional extraction. Minor amounts were additionally released for forage, hay and straw by exhaustive extraction. Overall, identification rates in soybean forage hay, straw and seeds were sufficient. Parent compound BCS-CN88460 was the only component in soybean seeds.

In soybean forage, hay and straw parent compound was a major component besides the identified major metabolites: BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48), BCS-CN88460-desfluoro-homoGSH (M44), BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46), BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47) and BCS-CN88460-desfluoro-Cys-MA (M45).

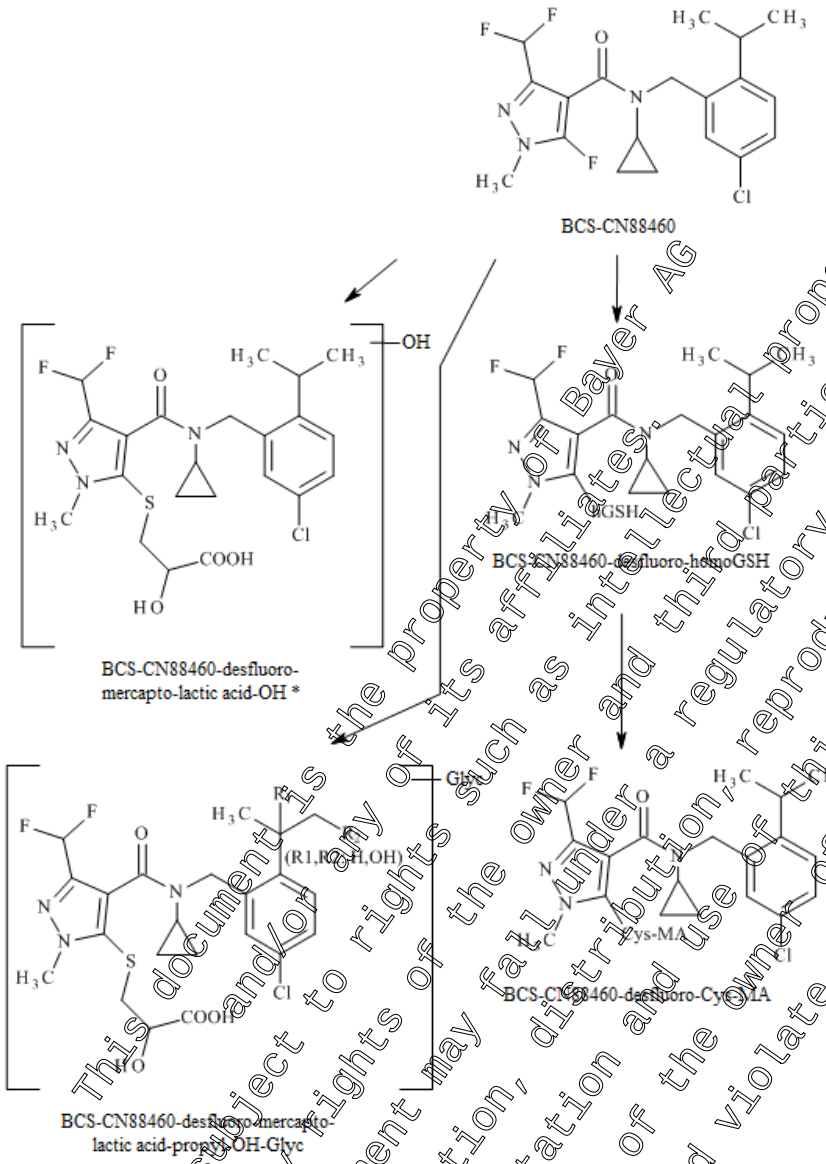
No label specific metabolites were observed. The storage stability of matrices and extracts was demonstrated in the parallel study with the phenyl label.

The main metabolic reactions observed were:

- de-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione. Degradation of the homoglutathione moiety followed by conjugation with malonic acid leading to BCS-CN88460-desfluoro-Cys-MA
- de-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione, degradation and desamination to mercapto lactic acid group and hydroxylation of the benzyl moiety or of the propyl group
- glycosilation was clearly observed at the mercapto lactic acid group and could not be located for metabolite BCS-CN88460-desfluoro-mercapto lactic acid-propyl-OH-Glyc

The results of the present study are in good agreement with the results with the phenyl-label. Based on these results, the degradation behaviour of [pyrazole-4-^{14}C]BCS-CN88460 in soybean is adequately understood and a metabolic pathway is proposed in the figure below.

Figure 6.2.1- 5: Proposed metabolic pathway of [pyrazole-4-^{14}C]BCS-CN88460 in soybean



* BCS-CN88460-desfluoro-mercapto-lactic acid-OH: OH position based on mass spectrometry: benzyl moiety.

Report:	KCA 6.2.1/08; [redacted], M.; 2017; M-609376-01-1
Title:	Metabolism of [phenyl-UL-14C]-BCS-CN88460 in soybean plants
Report No.:	S14-01090
Document No.:	M-609376-01-1
Guideline(s):	OECD Test Guideline No. 501 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 US EPA OCSPP Test Guideline No. 860.1300 JAP FAMIC-ACIS Notification 12 Nousan 8147
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of BCS-CN88460 was investigated in soybean plants after three post-emergent spray applications. For each of the three foliar applications, the test item [phenyl-UL-^{14}C]BCS-CN88460 was formulated and applied as aqueous EC 50 using a nominal application rate of 60 g a.s./ha each. The actual single application rates corresponded to 54, 56 and 66 g a.s./ha resulting in a total actual application rate of 176 g a.s./ha.

Soybean forage was harvested at BBCH 49 after the first application (PHI = 6d), hay at BBCH 77 after the 2nd application (PHI = 38d) and straw and seeds at BBCH 96, 21d after the 3rd application. The radioactive residues of soybean forage, hay, straw and seeds were determined by summing up the extractable and unextractable radioactivity. Soybean seeds contained significantly lower residue amounts than forage, hay and straw. The total radioactive residue (TRR) in soybean straw was highest and amounted to 8.527 mg eq/kg. The TRR in soybean forage and soybean hay were high and amounted to 3.936 mg eq/kg and 1.397 mg eq/kg, respectively. The TRR in seeds was low and amounted to 0.015 mg eq/kg.

Homogenised plant material from RACs was conventionally extracted with acetonitrile/water mixtures. Overall, the extraction rates after conventional extraction of soybean forage, hay, straw and seeds were high and amounted to 91.4, 88.6, 92.8 and 69.8% of the TRR (3.597, 1.238, 7.914 and 0.011 mg eq/kg), respectively. Exhaustive extract of soybean forage, hay and straw increased the extraction rate slightly: 5.2% of the TRR (0.205 mg eq/kg), 6.3% of the TRR (0.088 mg eq/kg) and 3.1% of the TRR (0.264 mg eq/kg) were released by this treatment, respectively.

The post extraction solids after conventional and exhaustive extractions accounted for 3.4% of the TRR (0.134 mg eq/kg), 5.1% of the TRR (0.071 mg eq/kg), 4.1% of the TRR (0.349 mg eq/kg) and 30.2% of the TRR (0.005 mg eq/kg) for soybean forage, hay, straw and seeds, respectively.

Residues in the conventional extracts of all RACs and in the exhaustive extracts of forage and straw were analysed and quantified by HPLC. The parent compound was identified by co-chromatography with the reference compound and metabolites were assigned by comparison of the metabolite pattern and retention times of the current and the soybean metabolism study with the pyrazole label.

Profiles of forage, hay and straw showed a high grade of comparability among themselves concerning metabolisation of the test item whereas the extract from soybean seeds contained only the parent compound.

The comparison of metabolic profiles of conventional extracts of all RACs with those of a parallel study with [pyrazole-4-^{14}C]BCS-CN88460 revealed a high grade of comparability of the metabolisation of the test compound in soybean and no label specific metabolite could be observed for the both labels.

A major component in all RACs was the test compound BCS-CN88460 accounting for 19.2% of the TRR (0.756 mg eq/kg) in soybean forage, 10.3% of the TRR (0.144 mg eq/kg) in soybean hay, 70.2% of the TRR (5.983 mg eq/kg) in soybean straw and 69.8% of the TRR (0.011 mg eq/kg) in soybean seeds. Besides parent compound no other metabolite was detected in the extract of soybean seeds.

Besides parent compound five metabolites were identified soybean forage, hay and straw.

BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48) accounted for 4.8, 17.6 and 2.1% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.187, 0.246 and 0.177 mg eq/kg, respectively. BCS-CN88460-desfluoro-homoGSH (M44) accounted for 20.2, 7.8 and 2.8% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.793, 0.109 and 0.235 mg eq/kg, respectively. BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46) accounted for 17.0, 2.8 and 2.5% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.672, 0.040 and 0.215 mg eq/kg, respectively. BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47) accounted for 2.7, 10.7 and 2.8% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.107, 0.150 and 0.235 mg eq/kg, respectively. BCS-CN88460-desfluoro-Cys-MA (M45) accounted for 8.2, 20.5 and 4.3% of the TRR in extracts from soybean forage hay and straw, corresponding to 0.323, 0.286 and 0.370 mg eq/kg, respectively.

Overall, identification rates were sufficient and amounted to 72.2% of TRR for forage, 69.8% of TRR for hay, 84.6% of the TRR for straw and 69.8% of TRR for seeds. In soybean forage, hay and straw 14, 5 and 20 unknown metabolites were characterised in the extracts by their chromatographic behaviour, individually accounting for less than 3.0% of the TRR (0.116 mg eq/kg), 0.1% of the TRR (0.086 mg eq/kg) and 2.8% of the TRR (0.230 mg eq/kg) for forage, hay and straw, respectively.

All initial profiles of conventional extracts of the raw agricultural commodities were performed within 6 months after harvest. Storage stability of matrix and extract samples was demonstrated exemplarily for soybean hay and straw for 18 and 27 months, respectively.

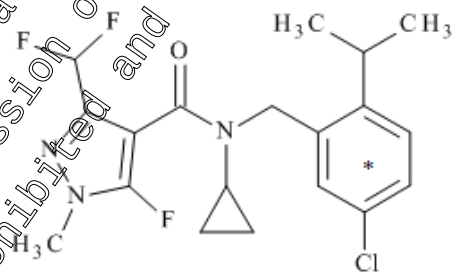
As metabolic reactions, de-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione and degradation of the homoglutathione moiety followed by conjugation with malonic acid or degradation and desamination to mercapto lactic acid group and hydroxylation of the benzyl moiety or of the propyl group were observed. Furthermore glycosilation was clearly observed at the mercapto lactic acid group.

Based on these results, the degradation behavior of [phenyl-UL-^{14}C]BCS-CN88460 in soybean is adequately understood and a pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * denotes the position of the ^{14}C-label
Radiolabel position	[phenyl-UL-^{14}C]
Specific radioactivity	4.13 MBq/mg
Radiochemical purity	> 98%
Chemical purity	> 98%

Formulation of the test compound

A stock solution of the test compound was prepared by dissolving the test compound in acetonitrile to give a concentration of about 5 mg/mL. The purity in the stock solution was checked by HPLC with radiodetection and was 100%

The test compound was formulated as an EC 50 for the experiment and therefore [phenyl-UL-^{14}C] BCS-CN88460 was dissolved in acetonitrile. For each of the three spray dilutions, adequate parts of the stock solution were transferred into glass vials and evaporated to dryness. Blank formulation was added and the mixtures were homogenised using a magnetic stirrer. The sample was then adjusted with water to a final volume of 50 mL (1st and 2nd application) and 100 mL (3rd application) of the spray dilution and homogenised by stirring.

2. Soil:

Soil characteristics			
Type	TOC	pH (CaCl$_2$)	CEC
Sandy loam	2.1 %	7.23	15.5 meq/100 g

3. Plant: soybean, variety "Amandine", representative for oilseeds

B. Study Design

1. Experimental conditions:

The experiment was conducted with soybean plants (variety: Amandine) based on a plant density of 800,000 soybean plants per hectare. A planting container with a surface area of 1 m^2 was used corresponding to the rate of 80 seeds per m^2. The plants were cultivated in the glass-roofed greenhouse ([redacted]) of the test facility and were grown similar to natural temperature and light conditions, but protected from rainfall. They were watered by pouring onto the soil in the planting containers. The plants were applied at three different growth stages (BBCH 14, 51 and 85). At all these growth stages the target single application rate was 60 g a.s./ha. The target rate corresponds to the anticipated maximum application rates for the use type.

For each application, the soybean plants were treated with 50 mL (1st and 2nd application) and 100 mL (3rd application) of the aqueous spray dilutions using a controlled track sprayer with a flat fan nozzle. To avoid contamination of the surrounding area by drift, the plants in the planting container were enclosed with a foil housing. After spraying the spray dilution onto the oilseed rape plants in the planting container, the spray device and the protective plastic foil around the planting container were rinsed with acetonitrile/water (8/2; v/v). The actual amount applied was calculated by subtracting the losses from the radioactivity in the original application solution. At the 1st application 22.2 MBq of the labelled test comound were applied, corresponding to 5.4 mg a.s.. At the 2nd application 23.1 MBq of the test compound were applied, corresponding to 5.6 mg a.s.. At the 3rd application 27.3 MBq of the test compound were applied, corresponding to 6.6 mg a.s.. The actual single application rates were 54, 56 and 66 g a.s./ha, resulting in a total actual application rate of 176 g a.s./ha.

2. Sampling:

At growth stage BBCH 49 the RAC forage, at growth stage BBCH 77 the RAC hay and at BBCH 96 the RACs straw and seeds were harvested. Plant samples were collected by cutting approximately 1-2 cm above the soil level. Plants sampled at hay stage were dried in a hood for 4 days.

The total weight of each sample was determined. The samples were homogenised with liquid nitrogen using a high speed blender. The sample materials were stored in a freezer (≤ -18 °C). Aliquots of the

homogenates were extracted. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids.

C. Analytical Procedures

1. Extractiom:

Conventional extraction procedure and sample clean up:

Aliquots of the homogenised samples of soybean forage, hay, straw and seeds were extracted three times with a mixture of acetonitrile/water (8/2; v/v) using a high speed blender. After each extraction step, the extracts were filtered by suction and the solids were rinsed with a small amount of the solvent mixture used for extraction. The solids were dried and aliquots were subjected to combustion and LSC.

The extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v).

Each combined percolate/rinse solution obtained from SPE purification was evaporated to the aqueous remainder. The final conventional extracts were analysed by HPLC with the general profiling method. All soybean samples and extracts were stored in a freezer (≤ -18 °C).

Exhaustive extraction and corresponding clean-up:

Solids from the conventional extraction of soybean forage, hay and straw were exhaustively extracted two times with acetonitrile/water/formic acid (50/50/1; v/v/v) under microwave assistance at increased temperature (0 to 5 min increase to 120 °C, 5 to 20 min at 120 °C, 800 W). The microwave extracts were cooled down at room temperature.

For forage and hay, the individual extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge, which was rinsed with methanol and water and conditioned with acetonitrile/water (8/2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (8/2; v/v). The percolate and the rinse were combined. Less polar fractions on the cartridge were eluted by rinsing the cartridge with methanol/tetrahydrofurane (1/1; v/v). Each combined percolate/rinse solution obtained from SPE purification was mixed with emulsifier and evaporated to the aqueous remainder.

The exhaustive extracts of straw were directly concentrated by evaporation to the aqueous remainder. The final exhaustive extract of soybean hay was partitioned for characterisation. The final exhaustive extracts of forage and straw were analysed by HPLC with the general profiling method. The extracts were stored in a freezer (≤ -18 °C).

Characterisation of residues by partitioning:

Radioactivity released from soybean hay by exhaustive extraction under microwave assistance was characterised by partitioning. Therefore, two milliliters of the concentrate obtained during exhaustive extraction and after purification were mixed with 8 mL water and 10 mL ethylacetate in a centrifuge tube. The mixture was incubated 30 min by 150 rpm on a flatbed shaker. Four gram anhydrous magnesium sulphate, one gram sodium chloride, one gram trisodium citrate and 0.5 gram disodium citrate sesquihydrate were added to remove water and adjust the pH value. After addition of the salt mixture, the tube was immediately shaken vigorously by hands for 10 seconds and with a flatbed shaker for at least one minute and afterwards centrifuged. Ethylacetate and water phase were separated, their volume was determined. An aliquot of the concentrated ethylacetate phase was analysed by HPLC.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/

scintillation cocktail mixture and the radioactivity was measured by LSC.

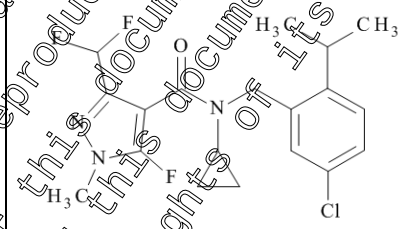
Conventional and microwave extracts were analysed by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

2. Identification and characterisation:

Metabolic profiles from the conventional and exhaustive extracts of all RACs were compared, as analysed by HPLC among themselves. Metabolic profiles of all RACs were compared with metabolic profiles of corresponding RACs in the soybean metabolism study with the pyrazole label in which the major metabolites were identified spectroscopically. Parent compound was identified in soybean seeds extract by TLC co-chromatography with the test compound.

Unknown metabolites were characterised based on their extraction and chromatographic behavior.

Table 6.2.1- 42: List of reference compound

Report name / other names/codes	**Chemical Name (IUPAC)**	**Structure and formula**
Parent compound BCS-CN88460 Radiolabelled reference: S_PH_1_1000 Non-radiolabelled reference: BCS-CN88460-01-02	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

3. Storage stability:

The initial profiles of the conventional extracts of soybean forage, hay, straw and seeds were analysed within 6 months after harvest of the soybean plant raw material. The stability of the residues in the stored sample matrices and extracts were exemplarily demonstrated in of soybean hay and straw by re-extraction followed by HPLC after 18 and 27 months of storage, respectively.

The comparison of the respective HPLC chromatograms revealed that the profiles of the extracts did not significantly change after storage of the material. Therefore, the residues were sufficiently stable during the experimental period of the study and the chromatograms represent the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in soybean was investigated after three spray applications.

Soybean plants in a 1 m^2 container were treated with [phenyl-UL-^{14}C]BCS-CN88460 formulated as an EC 50 at BBCH 14 (beginning of stem elongation), BBCH 51 (first flower buds visible) and BBCH 85 (about 50% of pods are ripe). The actual single application rate corresponded to 54, 56 and 66 g a.s./ha. The total application rate amounted to 176 g a.s./ha, which was slightly below the anticipated maximum application rate of 180 g a.s./ha.

The TRR values of the individual RACs were determined by summing up the radioactivity determined in the combined extracts and the radioactivity in the solids. The residue levels are shown in mg active substance equivalents per kg sample material (mg a.s.equiv./kg or simplified mg eq/kg).

The TRR in soybean straw was high and amounted to 8.527 mg eq/kg. Soybean forage and hay contained a TRR of 3.936 mg eq/kg and 1.397 mg eq/kg, respectively. The TRR determined for soybean seeds was low and amounted to 0.015 mg eq/kg.

The residue values in the parallel study with the pyrazole label were comparable for forage but significantly higher for hay, straw and seeds. This is due to differences in plant growth during cultivation of both studies resulting in vegetation differences during the second and third application.

Table 6.2.1- 43: TRR values in soybean matrices after foliar application of [phenyl-UL-^{14}C]BCS-CN88460

Matrix	Timing and Application	PHI (days)*	ppm (= mg eq/kg)
Soybean forage	1 spray application at BBCH 14, 54 g a.s./ha	56	3.936
Soybean hay	2 spray applications at BBCH 14 and 51, 54 and 56 g a.s./ha (110 g a.s./ha, total)	38	1.397
Soybean straw	3 spray applications at BBCH 14, 51 and 85, 54, 56 and 66 g a.s./ha (176 g a.s./ha; in total)	21	8.527
Soybean seeds		21	0.015

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at harvest/sampling)

Soybean forage was conventionally extracted three times with acetonitrile/water mixtures releasing 91.4% of the TRR (3.597 mg eq/kg). After concentration and purification steps 90.4% of the TRR (3.557 mg eq/kg) were analysed by HPLC. For soybean forage, a microwave extraction was performed. With this exhaustive method 5.2% (0.205 mg eq/kg) of the TRR were further extracted and 4.5% of the TRR (0.176 mg eq/kg) analysed by HPLC, additionally. The residue level in the solids of soybean forage after conventional and exhaustive extraction amounted to 3.4% of the TRR and 0.134 mg eq/kg. Losses during sample clean up of forage samples were 1.7% of the TRR (0.069 mg eq/kg).

Soybean hay was conventionally extracted three times with acetonitrile/water mixtures releasing 88.6% of the TRR (1.238 mg eq/kg). After concentration and purification steps 87.5% of the TRR (1.222 mg eq/kg) were analysed by HPLC. Exhaustive extraction of soybean hay, releasing 6.3% of the TRR and 0.088 mg eq/kg, was further characterized by partitioning of the exhaustive extract using ethylacetate accounting for 5.9% of the TRR (0.082 mg eq/kg) after purification. Complete radioactivity of the exhaustive extract was found in the ethylacetate phase after partitioning. This is in good accordance to results from the partitioning of experiment performed with soybean hay exhaustive extract in a parallel study with [pyrazole-4-^{14}C]BCS-CN88460. Since the complete radioactivity of the exhaustive extracts of soybean hay in both studies was found in the ethylacetate phase, it can be assumed that the residues were highly unpolar and likely ascribable to radioactivity derived from parent compound. An aliquot of the concentrated ethylacetate phase was further analyzed by HPLC but not peak above detection limit was detected due to low radioactivity concentrations and high matrix content of the sample. The residue level in the solids of soybean hay after conventional and exhaustive extraction amounted to 3.1% of the TRR and 0.071 mg eq/kg. Losses during sample clean up were 1.5% of the TRR (0.022 mg eq/kg).

Soybean straw was conventionally extracted three times with acetonitrile/water mixtures releasing 92.8% of the TRR (7.914 mg eq/kg). After concentration and purification steps 91.8% of the TRR (7.830 mg eq/kg) were analysed by HPLC. For soybean straw samples, a microwave extraction was performed. With this method 3.1% (0.264 mg eq/kg) were extracted and 3.1% of the TRR (0.264 mg eq/kg) analysed by HPLC, additionally. The residue level in the solids of soybean straw after conventional and exhaustive extraction amounted to 4.1% of the TRR and 0.349 mg eq/kg. Losses during sample clean up were 1.0% of the TRR (0.084 mg eq/kg).

Soybean seeds were conventionally extracted three times with acetonitrile/water mixtures releasing 69.8% of the TRR (0.011 mg eq/kg). No losses during concentration and purification steps occurred

and all of the residues in the extract (69.8% of the TRR, 0.011 mg eq/kg) were quantitatively analysed by HPLC. The residue level in the solids of soybean seeds after conventional extraction amounted to 30.2% of the TRR and 0.005 mg eq/kg.

The distribution of the radioactive residues is shown in the following table.

Table 6.2.1- 44: Distribution of radioactivity in the extracts of soybean matrices after three foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

	soybean forage		soybean hay		soybean straw		soybean seeds	
TRR [mg eq/kg] =	3.936		1.397		8.527		0.015	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	**91.4**	**3.597**	**88.6**	**1.238**	**92.8**	**7.914**	**69.8**	**0.011**
Analysed extracts (HPLC)	90.4	3.557	87.5	1.222	91.8	7.830	69.8	0.011
Losses (not analysed)[#]	1.0	0.040	1.1	0.016	1.0	0.084	n.q.	n.q.
Exhaustive extraction	**5.2**	**0.205**	**6.3**	**0.088**	**3.1**	**0.264**	--	--
Analysed extracts (HPLC)	4.5	0.176	n.q.	n.q.	3.1	0.264	--	--
Partitioning of purified exhaustive extract	--	--	5.9	0.082	--	--	--	--
Ethylacetate phase	--	--	5.9	0.082	--	--	--	--
Water phase	--	--	n.q.	n.q.	--	--	--	--
Losses (not analysed)[#]	0.7	0.029	0.4	0.006	n.q.	n.q.	--	--
Total extracted	**96.6**	**3.802**	**93.9**	**1.326**	**95.9**	**8.178**	**69.8**	**0.011**
Post extraction solids (PES)	**3.4**	**0.134**	**5.1**	**0.071**	**4.1**	**0.349**	**30.2**	**0.005**
Accountability	**100.0**	**3.936**	**100.0**	**1.397**	**100.0**	**8.527**	**100.0**	**0.015**

[#] losses during clean up, concentration, degreasing, centrifugation, etc.
-- not performed
n.q.: not quantified

Comparison of profiles of all RACs to corresponding profiles of the parallel study performed with [pyrazole-4-^{14}C]BCS-CN88460 showed good correspondence to each other and revealed that no label specific metabolism could be observed for the pyrazole label.

Chromatograms of conventional extracts of soybean straw were compared to the profile of conventional extract of soybean straw treated under microwave conditions. This experiment demonstrated that the parent compound and the major metabolites were not degraded by this form of extraction.

In conventional extract from soybean forage 69.4% of the TRR (2.729 mg eq/kg) were identified in total. The parent compound, BCS-CN88460-desfluoro-homoGSH and BCS-CN88460-desfluoro-mercapto-lactic acid-OH were major components representing 17.9, 19.6 and 16.7% of the TRR corresponding to 0.703, 0.770 and 0.658 mg eq/kg, respectively. The metabolites BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA represented 4.8, 2.7 and 7.7% of the TRR corresponding to 0.187, 0.107 and 0.304 mg eq/kg, respectively. In the exhaustive extract of forage 2.8% of the TRR (0.108 mg eq/kg) were further identified. The parent compound and the metabolites BCS-CN88460-desfluoro-homoGSH, BCS-CN88460-desfluoro-mercapto-lactic acid-OH and BCS-CN88460-desfluoro-Cys-MA represented 1.3, 0.6, 0.3 and 0.5% of the TRR corresponding to 0.053, 0.023, 0.014 and 0.019 mg eq/kg, respectively.

In conventional extract from soybean hay 69.8% of the TRR (0.974 mg eq/kg) were identified in total. The parent compound, BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA were major components representing 10.3, 17.6, 10.7 and 20.5% of the TRR corresponding to 0.144, 0.246, 0.150

and 0.286 mg eq/kg, respectively. The metabolites BCS-CN88460-desfluoro-homoGSH and BCS-CN88460-desfluoro-mercapto-lactic acid-OH represented 7.8 and 2.8% of the TRR corresponding to 0.109 and 0.040 mg eq/kg, respectively. Residues in the exhaustive extract of soybean hay were characterized by partitioning using ethylacetate. Complete radioactivity of the exhaustive extract was found in the ethylacetate phase after partitioning. This indicates the residues contained and previously extracted under microwave assistance were highly unpolar residues and likely ascribable to radioactivity derived from parent compound. Exhaustive extract of soybean hay was partitioned using ethylacetate. Radioactivity in the concentrated ethylacetate phase was analysed by HPLC but no single ingredient in the extract was detected above the limit of detection due to low radioactivity concentration and high matrix content of the sample.

In conventional extract from soybean straw 83.3% of the TRR (7.107 mg eq/kg) were identified in total. The parent compound was by far the major component representing 69.6% of the TRR (5.934 mg eq/kg), whereas the metabolites BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc, BCS-CN88460-desfluoro-homoGSH, BCS-CN88460-desfluoro-mercapto-lactic acid-OH, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA represented 2.1, 2.5, 2.4, 2.7 and 4.1% of the TRR corresponding to 0.177, 0.211, 0.206, 0.228 and 0.350 mg eq/kg, respectively. In the exhaustive extract of straw further 3.1% of the TRR (0.264 mg eq/kg) were identified. The parent compound and the metabolites BCS-CN88460-desfluoro-homoGSH, BCS-CN88460-desfluoro-mercapto-lactic acid-OH, BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc and BCS-CN88460-desfluoro-Cys-MA represented 0.6, 0.3, 0.1, 0.1 and 0.2% of the TRR corresponding to 0.049, 0.024, 0.009, 0.007 and 0.020 mg eq/kg, respectively.

Conventional extract from soybean seeds contained only parent compound representing 69.8% of the TRR corresponding to 0.011 mg eq/kg. The compound was identified by TLC co-chromatography of the parent compound fraction isolated from conventional extract of soybean seeds with the non-radiolabelled reference compound BCS-CN88460.

The TRR and the distribution of parent and metabolites in soybean matrices are shown in the following table.

Table 6.2.1- 45: Distribution of parent compound and metabolites the extracts of soybean matrices after three foliar applications of [phenyl-UL-^{14}C]BCS-CN88460

	soybean forage		soybean hay		soybean straw		soybean seeds	
TRR [mg eq/kg] =		3.936		1.397		8.527		0.015
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Conventional extraction	***91.4***	***3.597***	***88.6***	***1.238***	***92.8***	***7.914***	***69.8***	***0.011***
BCS-CN88460	17.9	0.703	10.3	0.144	69.6	5.934	69.8	0.011
BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48)	4.8	0.187	17.6	0.246	2.1	0.177	n.d.	n.d.
BCS-CN88460-desfluoro-homoGSH (M44)	19.6	0.770	7.8	0.109	2.5	0.211	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46)	16.7	0.658	2.8	0.040	2.4	0.206	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47)	2.7	0.107	10.7	0.150	2.7	0.228	n.d.	n.d.
BCS-CN88460-desfluoro-Cys-MA (M45)	7.7	0.304	20.5	0.286	4.1	0.350	n.d.	n.d.
Subtotal identified	**69.4**	**2.729**	**69.8**	**0.974**	**83.3**	**7.107**	**69.8**	**0.011**
Unknown 3	1.2	0.047	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 7	1.7	0.069	6.2	0.086	n.d.	n.d.	n.d.	n.d.
Unknown 9	0.8	0.033	4.6	0.065	n.d.	n.d.	n.d.	n.d.
Unknown 11	1.1	0.042	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 13	1.5	0.057	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 14	n.d.	n.d.	n.d.	n.d.	0.9	0.072	n.d.	n.d.
Unknown 15	3.0	0.116	2.7	0.038	2.8	0.230	n.d.	n.d.
Unknown 19	2.5	0.099	2.4	0.033	n.d.	n.d.	n.d.	n.d.
Unknown 24	0.8	0.031	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 25	n.d.	n.d.	1.9	0.026	n.d.	n.d.	n.d.	n.d.
Unknown 26	n.d.	n.d.	n.d.	n.d.	0.8	0.064	n.d.	n.d.
Unknown 27	1.3	0.049	n.d.	n.d.	0.8	0.068	n.d.	n.d.
Unknown 29	1.1	0.044	n.d.	n.d.	0.7	0.057	n.d.	n.d.
Unknown 30	1.1	0.042	n.d.	n.d.	1.0	0.083	n.d.	n.d.
Unknown 38	2.9	0.114	n.d.	n.d.	1.3	0.103	n.d.	n.d.
Unknown 39	n.d.	n.d.	n.d.	n.d.	0.6	0.047	n.d.	n.d.
Unknown 40	0.8	0.033	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown 44	1.3	0.052	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Subtotal characterised by HPLC	**21.0**	**0.828**	**17.7**	**0.248**	**8.5**	**0.723**	**n.d.**	**n.d.**

continued on next page

Table 6.2.1- 45 continued

	soybean forage		soybean hay		soybean straw		soybean seeds	
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Exhaustive extraction	***5.2***	***0.205***	***6.3***	***0.088***	***3.1***	***0.264***	**--**	**--**
BCS-CN88460	1.3	0.053	n.d.	n.d.	0.6	0.049	--	--
BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	--	--
BCS-CN88460-desfluoro-homoGSH (M44)	0.6	0.023	n.d.	n.d.	0.3	0.024	--	--
BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46)	0.3	0.014	n.d.	n.d.	0.1	0.009	--	--
BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47)	n.d.	n.d.	n.d.	n.d.	0.1	0.007	--	--
BCS-CN88460-desfluoro-Cys-MA (M45)	0.5	0.019	n.d.	n.d.	0.2	0.020	--	--
Subtotal identified	**2.8**	**0.108**	**n.d.**	**n.d.**	**1.3**	**0.109**	**--**	**--**
Unknown 7	0.8	0.032	n.d.	n.d.	n.d.	n.d.	--	--
Unknown 13	n.d.	n.d.	n.d.	n.d.	0.1	0.010	--	--
Unknown 21	n.d.	n.d.	n.d.	n.d.	0.1	0.010	--	--
Unknown 23	n.d.	n.d.	n.d.	n.d.	0.1	0.009	--	--
Unknown 30	n.d.	n.d.	n.d.	n.d.	0.1	0.005	--	--
Unknown 31	n.d.	n.d.	n.d.	n.d.	0.1	0.007	--	--
Unknown 32	n.d.	n.d.	n.d.	n.d.	0.1	0.008	--	--
Unknown 34	n.d.	n.d.	n.d.	n.d.	0.1	0.011	--	--
Unknown 35	n.d.	n.d.	n.d.	n.d.	0.1	0.007	--	--
Unknown 37	n.d.	n.d.	n.d.	n.d.	0.1	0.012	--	--
Unknown 38	n.d.	n.d.	n.d.	n.d.	0.1	0.010	--	--
Unknown 39	n.d.	n.d.	n.d.	n.d.	0.1	0.007	--	--
Unknown 40	n.d.	n.d.	n.d.	n.d.	0.1	0.009	--	--
Unknown 41	0.9	0.036	n.d.	n.d.	0.3	0.025	--	--
Unknown 46	n.d.	n.d.	n.d.	n.d.	0.2	0.013	--	--
Unknown 47	n.d.	n.d.	n.d.	n.d.	0.1	0.011	--	--
Subtotal characterised by HPLC	**1.7**	**0.068**	**--**	**--**	**1.8**	**0.155**	**--**	**--**
Partitioning of purified exhaustive extract								
Ethylacetate phase	--	--	*5.9*	*0.082*	--	--	--	--
Water phase	--	--	*n.q.*	*n.q.*	--	--	--	--
Subtotal characterised by partitioning	**--**	**--**	***5.9***	***0.082***	**--**	**--**	**--**	**--**
Total not analysed / Losses	**1.7**	**0.069**	**1.5**	**0.022**	**1.0**	**0.084**	**n.q.**	**n.q.**
Total identified	**72.2**	**2.837**	**69.8**	**0.974**	**84.6**	**7.216**	**69.8**	**0.011**
Total characterised	**22.8**	**0.896**	**23.6**	**0.330**	**10.3**	**0.878**	**n.d.**	**n.d.**
Total extracted	**96.6**	**3.802**	**94.9**	**1.326**	**95.9**	**8.178**	**69.8**	**0.011**
Post extraction solids (PES)	**3.4**	**0.134**	**5.1**	**0.071**	**4.1**	**0.349**	**30.2**	**0.005**
Accountability	**100.0**	**3.936**	**100.0**	**1.397**	**100.0**	**8.527**	**100.0**	**0.015**

n.d.: not detected
-- not performed
n.q.: not quantified

Overall, identification rates were sufficient and amounted to 72.2% of TRR for forage, 69.8% of TRR for hay, 84.6% of the TRR for straw and 69.8% of TRR for seeds. In soybean forage, hay and straw, 14, 5 and 20 unknown metabolites were characterised in the extracts by their chromatographic behaviour, individually accounting for less than 3.0% of the TRR (0.116 mg eq/kg), 6.1% of the TRR (0.086 mg eq/kg) and 2.8% of the TRR (0.230 mg eq/kg).

Table 6.2.1- 46: Summary of characterisation and identification of radioactive residues in soybean matrices after three foliar applications of [phenyl-UL-4-^{14}C]BCS-CN88460

	soybean forage		soybean hay		soybean straw		soybean seeds	
TRR [mg eq/kg] =		3.936		1.397		8.527		0.015
Compound	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BCS-CN88460	19.2	0.756	10.3	0.144	70.2	5.983	69.8	0.011
BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48)	4.8	0.187	17.6	0.246	2.1	0.177	n.d.	n.d.
BCS-CN88460-desfluoro-homoGSH (M44)	20.2	0.793	7.8	0.109	2.8	0.235	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46)	17.0	0.672	2.8	0.040	2.5	0.215	n.d.	n.d.
BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47)	2.7	0.107	10.7	0.150	2.8	0.235	n.d.	n.d.
BCS-CN88460-desfluoro-Cys-MA (M45)	8.2	0.323	20.5	0.286	4.3	0.370	n.d.	n.d.
Total identified	**72.2**	**2.837**	**69.8**	**0.974**	**84.6**	**7.216**	**69.8**	**0.011**
Number of unknown peaks	14		5		20		0	
Largest unknown peak	3.0	0.116	6.1	0.086	2.8	0.230	n.d.	n.d.
Subtotal characterised by HPLC	**22.8**	**0.896**	**17.7**	**0.248**	**10.3**	**0.878**	**n.d.**	**n.d.**
Subtotal characterized by partitioning of exhaustive extract	**--**	**--**	***5.9***	***0.082***	**--**	**--**	**--**	**--**
Total characterised	**22.8**	**0.896**	**23.6**	**0.330**	**10.3**	**0.878**	**--**	**--**
Not analysed / Losses	**1.7**	**0.069**	**1.5**	**0.022**	**1.0**	**0.084**	**n.q.**	**n.q.**
Total extracted	**96.6**	**3.802**	**94.9**	**1.326**	**95.9**	**8.178**	**69.8**	**0.011**
Post extraction solids (PES)	**3.4**	**0.134**	**5.1**	**0.071**	**4.1**	**0.349**	**30.2**	**0.005**
Accountability	**100.0**	**3.936**	**100.0**	**1.397**	**100.0**	**8.527**	**100.0**	**0.015**

n.d.: not detected
-- not performed

III. Conclusions

The metabolism of BCS-CN88460 in soybean plants was investigated after three foliar applications at growth stages BBCH 14, BBCH 51 and BBCH 85. The soybean plants were treated with [phenyl-UL-^{14}C] BCS-CN88460 formulated as an EC 50 at a single application rate of 54, 56 and 66 g a.s./ha for the first, second and third spray application, respectively, corresponding to a total application rate of 176 g a.s/ha. Soybean forage was harvested at BBCH 49, soybean hay at BBCH 77, soybean straw and seeds were harvested at BBCH 96.

Residues in soybean forage, hay and straw were high compared to very low residues in seeds. The main portion of residues of forage, hay, straw and seeds were released by conventional extraction.

Minor amounts were additionally released for forage, hay and straw by exhaustive extraction. Overall, identification rates in soybean forage hay, straw and seeds were sufficient. Parent compound BCS-CN88460 was the only component in soybean seeds.

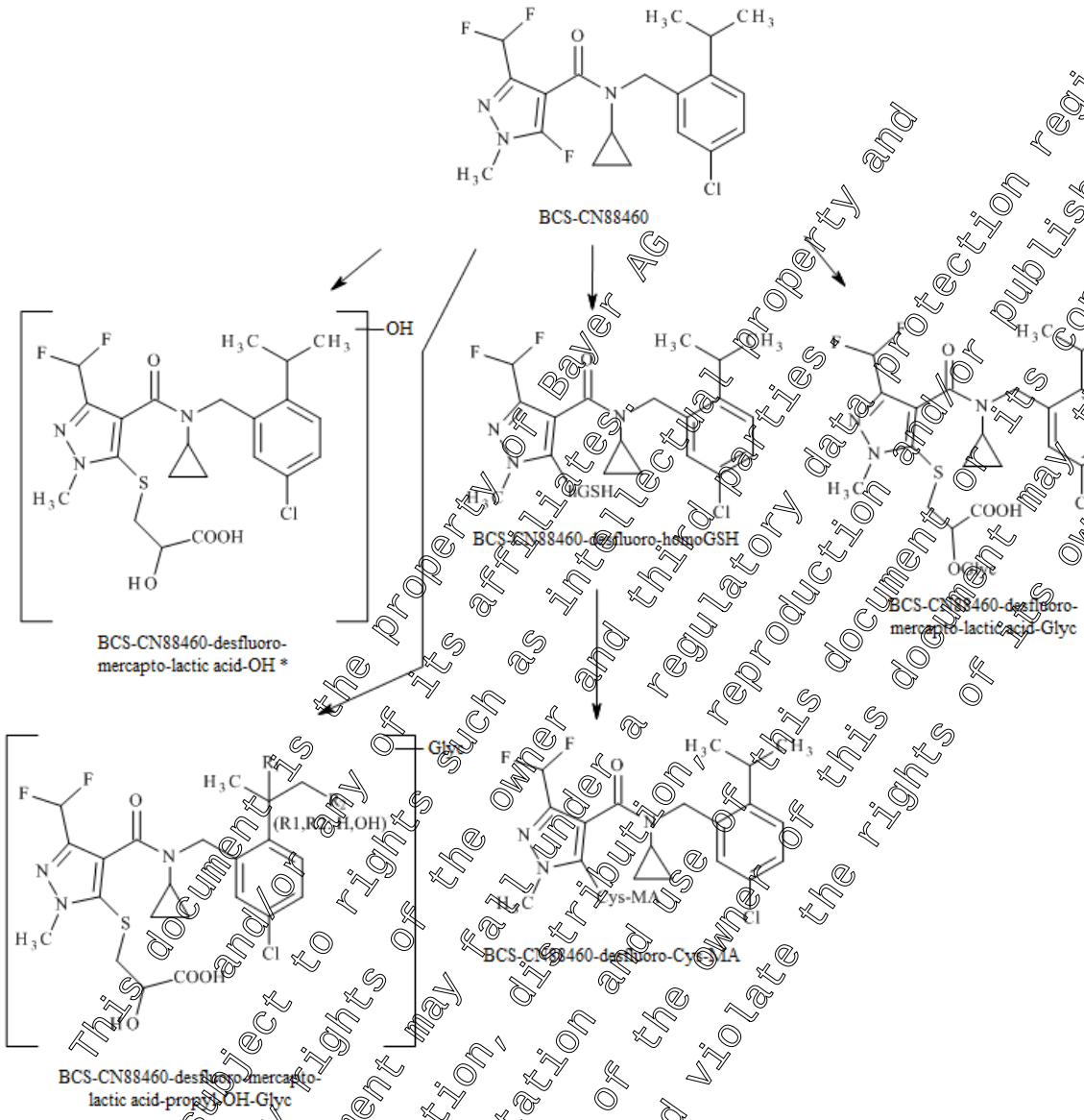
In soybean forage, hay and straw parent compound was a major component besides the identified major metabolites: BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (M48), BCS-CN88460-desfluoro-homoGSH (M44), BCS-CN88460-desfluoro-mercapto-lactic acid-OH (M46), BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (M47) and BCS-CN88460-desfluoro-Cys-MA (M45). No label specific metabolites were observed. Storage stability of matrices and extracts was demonstrated for a period of 18 and 27 months.

The main metabolic reactions observed were:

- de-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione. Degradation of the homoglutathione moiety followed by conjugation with malonic acid leading to BCS-CN88460-desfluoro-Cys-MA
- de-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione, degradation and desamination to mercapto lactic acid group and hydroxylation of the benzyl moiety or of the propyl group
- glycosilation was clearly observed at the mercapto lactic acid group and could not be located for metabolite BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc.

Based on these results, the degradation behavior of [phenyl-UL-^{14}C]BCS-CN88460 in soybean is adequately understood and a metabolic pathway is proposed in the figure below.

Figure 6.2.1- 6: Proposed metabolic pathway of [phenyl-UL-^{14}C]BCS-CN88460 in soybean



* BCS-CN88460-desfluoro-mercapto-lactic acid-OH: OH position based on mass spectrometry: benzyl moiety.

CA 6.2.2 Poultry

The metabolism of the fungicide isoflucypram in laying hens was investigated after administration with isoflucypram either labelled in the phenyl or in the pyrazole moiety. Parent compound and metabolites were isolated from the eggs (6 - 13 days) and the extract of excreta and identified in the isolated fractions by spectroscopic methods in the study with the pyrazole-label and assigned to the metabolites in the laying hen study with phenyl-UL-^{14}C label by comparison of metabolite profiles and retention times.

Table 6.2.2- 1: Overview over available poultry metabolism studies

Poultry	Application	Dose	Reference
Laying hen	14 daily administrations of pyrazole-labelled isoflucypram in aqueous 0.5% Tragacanth suspension via gavage	1.0 mg/kg bw	M-601665-01-1
Laying hen	14 daily administrations of phenyl-labelled isoflucypram in aqueous 0.5% Tragacanth suspension via gavage	1.0 mg/kg bw	M-601667-01-1

Summary of metabolism in poultry

The metabolite pattern corresponds well when comparing the two metabolism studies in laying hens. Isoflucypram was metabolised extensively in liver of hens. With the exception of fat, only low amounts of the parent compound were detected in the edible tissues and eggs. The major metabolites detected were BCS-CN88460-desmethyl-1,2-propandiol (M07), BCS-CN88460-desmethyl-carboxylic acid (M11), BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-carboxylic acid (M12) and BCS-CN88460-propanol (M01). The main metabolic reactions were hydroxylations with further oxidation or demethylation of the pyrazole moiety.

Report: KCA 6.2.2/01, [redacted], R.; [redacted], A.K.; [redacted], F.; 2017, M-601665-01-1
Title: [Pyrazole-4-^{14}C]BCS-CN88460: Metabolism in the laying hen
Report No.: Ensa-17-0307
Document No.: M-601665-01-1
Guideline(s): OECD Test Guideline No. 503 Regulation (EC) No 1107/2009 amended by the Commission Regulation (EU) No 283/2013 (Europe)
US EPA OCSPP 860.1300
Guideline deviation(s): none
GLP/GEP: yes

Executive Summary

The metabolism and excretion of [pyrazole-4-^{14}C]BCS-CN88460 was investigated in laying hens as a model for poultry. The test compound was orally administered to six hens as aqueous 0.5% Tragacanth suspension at an intended dose rate of 1 mg per kg body weight. Based on the daily feed consumption, the dose level corresponded up to 16.57 mg a.s./kg dry feed/day. The hens received 14 doses at 24-hour intervals in the morning and were sacrificed approx. 6 hours after the last dosing. Throughout the experiment, the hens were housed in metabolism cages, which permitted separate collection of eggs and excreta. The eggs were collected once daily and before sacrifice. Total radioactive residues (TRR) were determined in each egg (mixed sample from egg white and yolk) and in dissected organs and tissues (muscle, fat, liver, kidney, skin and eggs from ovary/ oviduct) at sacrifice. The total radioactivity (% of total dose administered) was additionally determined in each excreta sample.

Recovery and Elimination of Radioactivity

The overall recovery amounted to 96% of the total dose and up to the time of sacrifice the excretion accounted for up to 95.8% of the total dose. After the third administration the daily excretion rate was on a more or less constant level of about 6.3 to 7.7% within 24 hours. The remaining amount of radioactivity (approx. 4%) was expected to still be present in the gastrointestinal tract at sacrifice, because of the short period of time between last administration and sacrifice (approx. 6 hours).

An average amount of approx. 0.12% of the total dose was measured in the eggs. At sacrifice radioactive residues in the organs and tissues dissected from the bodies were calculated or estimated to be about 0.22% of the total dose.

Total Radioactive Residues in Eggs, Organs and Tissues

The TRR-values and transfer factors for eggs and organs and tissues were very low compared to the dose level of 16.57 mg a.s. /kg feed/day and a dosing period of 14 days. The TRR-values in eggs ranged from 0.029 mg/kg at day two to 0.057 mg/kg at sacrifice. Following a li[redacted] increase a residue plateau-level of 0.050 mg/kg was reached at day six after the first administration.

Regarding organs and tissues, the TRR-values amounted to 0.370 mg/kg in liver, 0.391 mg/kg in kidney, 0.042 mg/kg in subcutaneous fat, 0.075 mg/kg in skin, 0.029 mg/kg in leg muscle and 0.018 mg/kg in thorax muscle.

Metabolism

The majority of the residues in the eggs as well as organs and tissues were efficiently extracted (83.9% to 93.4%) using acetonitrile/water mixtures. In case of liver, the solids after conventional extraction were exhaustively extracted with microwave treatment. Only up to 8.2% of the TRR or 0.003 mg/kg of the residues remained in the post extraction solids (PES).

For sample preparation, the extracts were partitioned against n-heptane except the extract from fat. Very low amounts of radioactivity were recovered in the n-heptane phases and amounted to ≤1.3% (0.001 mg/kg) of the TRR.

Metabolites were isolated from extracts of eggs and excreta and identified by spectroscopic investigations. Parent compound and metabolites were further identified based on co-chromatography with the isolated metabolites and by comparison of the metabolite pattern and retention times. In addition, the assignment of parent compound and metabolites in the current study was performed based on a comparison of the extracts from excreta of the current study and the hen metabolism study with the phenyl label.

The identification rates amounted to 69.6% of the TRR for eggs, 73.5% for leg muscle, 67.6% for thorax muscle, 55.5% for fat and 54.3% for liver.

Parent compound was only detected in eggs, leg muscle and fat and amounted to 0.002 mg/kg (3.7% of the TRR) for eggs, 0.001 mg/kg (2.3% of the TRR) for leg muscle and to 0.010 mg/kg (23.6% of the TRR) for fat.

Metabolites BCS-CN88460-desmethyl-1,2-propandiol (M07), BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-carboxylic acid (M12) and BCS-CN88460-propanol (M01) were detected in all edible materials and eggs.

The amount of BCS-CN88460-desmethyl-1,2-propandiol ranged from 5.1% to 17.9% of the TRR and it represented a major residue (>10% of the TRR) in leg and thorax muscle. The amount of BCS-CN88460-desmethyl-propanol (representing a major compound in eggs, both muscles and fat) ranged from 5.3% to 29.7% of the TRR and the amount of BCS-CN88460-carboxylic acid (representing a major compound in thorax muscle and liver) ranged from 3.4% to 11.9% of the TRR. The amount of BCS-CN88460-propanol (representing a major residue in eggs and fat) ranged from 1.7% to 35.5% of the TRR.

BCS-CN88460-desmethyl-carboxylic acid (M11) was only detected in leg and thorax muscle and liver accounting for between 12.0% and 14.4% of the TRR.

BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA (M36), BCS-CN88460-desmethyl-propanol-N-GlucA (M37) and BCS-CN88460-desmethyl-2-propanol-N-GlucA (M38) were only detected in liver, accounting for 5.4%, 6.1% and 2.5% of the TRR, respectively.

More metabolites in the matrices may be present as indicated by broad non-resolved zones in the chromatograms. All unknown metabolites in the extracts were characterised by their extraction and chromatographic behaviour and amounted to each ≤19.5% of the TRR or 0.004 mg/kg.

The metabolic profile of excreta from day 1 was similar to the profiles of edible materials, especially liver, with the difference that metabolites BCS-CN88460-desmethyl-1,2-propandiol-SA (M42), BCS-CN88460-1,2-propandiol-SA (M27), BCS-CN88460-1,2-propandiol (M03) and BCS-CN88460-propanol-SA (M26) were only detected in excreta.

A summary of the distribution of parent compound and metabolites for edible materials is provided in the table overleaf.

Table 6.2.2- 2: Overview Radioactive residues of parent compound and metabolites in eggs and edible organs and tissues of laying hens following oral administration of 14 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.04 mg/kg

Sample		Eggs		Muscle Leg		Muscle Thorax		Fat		Liver	
TRR [mg/kg]		**0.050**		**0.029**		**0.018**		**0.042**		**0.370**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***93.4***	***0.047***	***92.8***	***0.027***	***91.8***	***0.017***	***92.7***	***0.039***	***83.9***	***0.310***
37	parent compound	3.7	0.002	2.3	0.001	---	---	23.6	0.010	---	---
6	desmethyl-1,2-propandiol-N-GlucA (M36)	---	---	---	---	---	---	---	---	5.4	0.020
13	desmethyl-propanol-N-GlucA (M37)	---	---	---	---	---	---	---	---	6.1	0.023
14	desmethyl-2-propanol-N-GlucA (M38)	---	---	---	---	---	---	---	---	2.5	0.009
17	desmethyl-1,2-propandiol (M07)	5.2	0.003	15.0	0.004	17.9	0.003	5.4	0.002	6.9	0.025
27	desmethyl-carboxylic acid (M11)	---	---	12.1	0.004	12.0	0.002	---	---	14.4	0.053
29	desmethyl-propanol (M06)	22.3	0.011	29.7	0.009	20.9	0.004	10.1	0.004	5.3	0.020
31	carboxylic acid (M12)	3.4	0.002	9.1	0.003	11.0	0.002	4.8	0.002	11.9	0.044
32	propanol (M01)	35.0	0.018	5.3	0.002	5.9	0.001	11.9	0.005	1.7	0.006
Total identified		**69.6**	**0.035**	**73.5**	**0.021**	**67.6**	**0.012**	**55.5**	**0.023**	**54.3**	**0.201**
Characterised by HPLC		*22.4*	*0.011*	*18.7*	*0.005*	*24.2*	*0.004*	*37.1*	*0.016*	*29.0*	*0.107*
Characterised in n-heptane phase		*1.3*	*0.001*	*0.6*	*0.002*	---	---	---	---	*0.6*	*0.002*
Exhaustive extraction		---	---	---	---	---	---	---	---	***16.0***	***0.060***
- ACN/water extract		---	---	---	---	---	---	---	---	4.5	0.017
- 0.1 M HCL extract		---	---	---	---	---	---	---	---	11.5	0.043
Total characterised		23.7	0.012	19.3	0.005	24.2	0.004	37.1	0.016	45.6	0.169
Total extractable		**93.4**	**0.047**	**92.8**	**0.027**	**91.8**	**0.017**	**92.7**	**0.039**	**99.9**	**0.370**
Unextractable (PES)		6.6	0.003	7.2	0.002	8.2	0.001	7.3	0.003	0.1	<0.001
Accountability		**100.0**	**0.050**	**100.0**	**0.029**	**100.0**	**0.018**	**100.0**	**0.042**	**100.0**	**0.370**

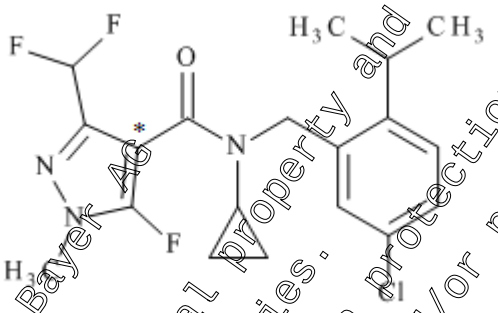
The main metabolic reactions were the hydroxylation in the propyl group of the phenyl ring and demethylation of the pyrazole moiety. Hydroxylation in the propyl group was leading to mono- or dihydroxy compounds. Conjugation with glucuronic acid was observed after demethylation of the pyrazole moiety and conjugation with sulphuric acid after hydroxylation in position 1 of the propyl group. Another metabolic reaction was further oxidation of BCS-CN88460-propanol to BCS-CN88460-carboxylic acid.

Based on the results the metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in the laying hen is considered as adequately understood and a metabolic pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Materials

Chemical structure	 * denotes the ^{14}C-label position
Radiolabelled test material	[pyrazole-4-^{14}C]BCS-CN88460
Specific radioactivity	4.22 MBq/mg = 2.53 x 10^{8} dpm/mg
Radiochemical purity	>98% (HPLC), >99% (TLC)
Chemical purity	>98% (HPLC)
Dose level	14 oral doses of 16.57 mg a.s./kg feed/day (1.04 mg a.s./kg bw/day)
Vehicle	0.5 % aqueous Tragacanth® suspension

2. Test Animals

Species	Laying hen (*Gallus gallus domesticus*)
Strain	"LB Lohmann White"
Breeder	[redacted]
Animal numbers	8 animals in total, from which 6 (no's 403 - 408) were chosen for the test. The hens were selected by maximum egg production
Mean body weights	1.54 kg at delivery (2015-01-15) 1.63 kg at the first administration (2015-01-28) 1.68 kg at sacrifice (2015-02-10)
Identification	During the acclimation period, the animals were identified by individual cage cards (1 – 8). During the testing period an individual animal number (see above) was allocated on the cage cards and additionally by foot ring.
Acclimation period	13 days
Husbandry	Conventional hygienic conditions in air-conditioned rooms
Housing	During the acclimation period and the whole testing period, the hens were kept individually in electro-polished stainless steel metabolism cages for laying hens, supplied by [redacted]. These cages allow almost separate and quantitative collection of excreta and eggs.
Dietary regimen	During the whole residence time, the hens were fed with "RWZ-LegeGold Mehl", a pulverised chicken feed. This feed was not a certified diet, i.e. it was not checked for contamination according to current standards. The feed was supplemented by eggshells during the acclimation period. The feed consumption during the testing period was recorded by back-weighing. Tap water from the local mains supply was given *ad libitum* during the whole residence time.
Environmental conditions	Temperature: 19 – 21°C Relative humidity: 20 – 38% Photoperiod: alternating 16- to 8-hours light / dark cycles Air change: 10 – 15 times per hour

B. Study Design

Preparation of the Test Item for Administration

The radiolabelled test compound was delivered in solid form and dissolved in 50 mL acetonitrile for preparation of the stock solution. The radiochemical purity amounted to >98% as assured by HPLC. The identity of the test compound was confirmed by LC/MS/MS.

Four administration suspensions were prepared and each suspension was applied for three to four administrations. Definite volumes of the stock solution were concentrated to [redacted] dryness by a gentle stream of nitrogen gas. Afterwards, the residue was suspended in a 0.5% aqueous Tragacanth suspension. Aliquots were taken for determination of the total radioactivity by LSC. The suspensions were kept permanently under stirring at + 5 °C in a cooling cabinet until the administrations at which they were stirred at room temperature and were proved to be stable until the last dose.

Dosing

All oral administrations were performed by gavage using a syringe attached to an animal-feeding knob cannula once daily for 14 consecutive days in the morning in relation to the individual body weights. The laying hens received on each day an average amount of 1.69 mg ^{14}C-BCS-CN88460, which corresponded to 7.13 MBq (mean per animal and day). The total administered average amount and radioactivity accounted for 23.70 mg and 100.01 MBq, respectively. The administration volume was 1.0 mL/kg body weight.

The total amount of radioactivity administered to each animal served as reference value (A_0 = 100%) for the percentage calculation of the total radioactivity in the biological samples.

Based upon the experimentally determined daily feed consumption during the testing period of 102 g dry feed per day (= 6.12% of the body weight), the dose of 1.04 mg a.s./kg bw corresponded to a concentration of 16.57 mg a.s./kg dry feed per day in the diet. This dose was tolerated without any observable toxicological effects.

Collection of eggs

The egg production of the laying hens was checked during the acclimation period (beginning of the laying phase) and during the testing period. Assuming an average laying rate of 314 eggs per hen and year the mean egg production during the acclimation period was 109% and 123% during the testing period. Therefore, the egg production exceeded the target value for laying hens being in good egg production.

During the test, the cages were inspected for egg production once daily (in the morning before administration) and the number of eggs was recorded for all hens. After removal of the shells, the contents of each egg were weighed and thoroughly mixed afterwards. Aliquots of each homogenate were mixed with scintillator for determination of the radioactivity by LSC. The remaining samples were stored in a freezer until start of metabolite analysis.

Collection of excreta

The excreta of each hen were collected from the collecting tins as far as possible quantitatively in daily intervals until sacrifice. Individual samples were weighed after water was added. Afterwards, the individual samples were homogenised. Aliquots of each sample were processed for radioactivity measurement by combustion / LSC. The remaining samples were stored in a freezer until start of metabolite analysis.

Sacrifice

The animals were sacrificed approx. 6 hours after the last administration, a time distance that is consistent with normal slaughtering practices. Each laying hen was transferred into a special cage, weighed and anaesthetized using carbon dioxide gas. Under general anaesthesia the animals were sacrificed by decapitation followed by exsanguination.

Preparation of organs and tissues

After exsanguination, the following edible organs and tissues were dissected: muscle (leg and thorax), fat (subcutaneous), liver (without gall bladder), skin (without subcutaneous fat), kidneys and eggs from the ovary and oviduct.

The organs or tissue samples were transferred into tared weight plastic vessels. After determination and recording of the individual weights, muscle, fat, liver, skin, kidney samples and eggs dissected from the ovary and oviduct were passed several times through a mincing machine in half-frozen state or blended with an Ultra Turrax in thawed state. Aliquots of the individual organ and tissue samples were combusted and the radioactivity measured by LSC.

The organ and tissue samples of the six hens were pooled separately for each sample type, divided in suitable portions that were stored frozen at ≤ -18 °C until the start of metabolite analysis. For skin, the remaining homogenates of kidneys and eggs from the ovary/oviduct, metabolite analysis was optional.

All individual samples were identified with a specific sample number. The individual excreta, egg, as well as organ and tissue samples were kept frozen at ≤ -18 °C at all times except during aliquotation for analysis. During the analytical work the samples and extracts of samples were stored either in a freezer at ≤ -18 °C or for a short period in a refrigerator at +4 °C.

Radioactivity measurement

The radioactivity measurement in liquid samples was carried out by liquid scintillation counting (LSC). The solid samples were either dissolved in BIOLUTE S and radioactivity determined by LSC or combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

C. Analytical Procedures

Sample Extraction and Analysis of Extracts

Aliquot samples from eggs, muscle, fat, liver and excreta were conventionally extracted three times with a mixture of acetonitrile/ water (8/2; v/v) using a Polytron homogeniser. For sample preparation the combined conventional extracts from eggs, muscle, liver and excreta were partitioned against n-heptane. The purified conventional extracts were concentrated by rotary evaporation and subjected to HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

Solids of liver from the first conventional extraction were exhaustively extracted twice with acetonitrile/water (8/2; v/v) using microwave assistance followed by microwave treatment with 0.1 M hydrochloric acid. The exhaustive extracts were characterised using TLC analysis.

Aliquots of the conventional liver extracts were incubated for 96 hours at 37°C with a defined amount of ß-glucuronidase/arylsulfatase. After incubation, the enzymatic suspensions were purified and analysed by HPLC.

Metabolite analysis

Parent compound and metabolites were quantified in the extracts by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

The following strategy was used for identification of the parent compound and metabolites:
All metabolites were assigned based on a comparison of the metabolite profiles and retention times. Metabolites were isolated from extracts of egg pool and excreta and used as reference compounds after structure elucidation. Parent compound and metabolites were identified by HPLC co-chromatography with radiolabelled reference compounds in selected samples. The conventional extract of liver was enzymatically digested. The glucuronic acid and sulphate conjugates of BCS-CN88460 were thereby digested and converted into the corresponding BCS-CN88460-aglycons.

The HPLC profile of excreta was compared to the profile of excreta from the phenyl labelled hen metabolism study conducted in parallel to demonstrate the comparability.

Unknown metabolites were characterised based on their extraction and chromatographic behaviour.

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The overall recovery accounted for 96.14% of the total dose and up to the time of sacrifice, 95.8% of the total dose was excreted. After the third administration the daily excretion rate was on a more or less constant level of about 6.3 to 7.7% within 24 hours. The remaining amount of radioactivity (approx. 4%) was expected to still be present in the gastrointestinal tract at sacrifice, due to the short period of time between last administration and sacrifice (approx. six hours). An average amount of 0.12% of the total dose was measured in the eggs. At sacrifice, the radioactive residues in the organs and tissues dissected from the bodies were calculated or estimated to be about 0.22% of the total dose.

All TRRs for eggs and dissected organs and tissues were calculated as radioactivity originating from the radiolabelled test compound. Therefore, all concentration data in this report represent active substance equivalents (mg a.s. equiv./kg$_{sample}$).

Table 6.2.2- 3: Overview Distribution of residues in liver, kidney, eggs, muscle, skin and fat of laying hens following oral administration of 14 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.04 mg/kg

Sample	Collection time	TRR [mg/kg] *)	Transfer factor **)	Percent of total dose administered
Liver	approx. 6 h after last admin.	0.370	0.022	0.07
Kidney		0.390	0.024	0.02
Eggs from ovary/oviduct		0.076	0.005	0.01
Total skeletal muscle #)		0.023	0.001	0.07
Total body skin #)		0.075	0.005	0.02
Total body fat #)		0.042	0.003	0.04
Total of organs/tissues		-----	-----	0.22
Eggs, total	day 1 – 13.25	0.044	0.003	0.12
Eggs, plateau-level	day 6 – 13	0.050	0.003	-----
Excreta, total	day 1 – 13.25	-----	-----	95.79
Total Recovery				**96.14**
Feeding level	16.57	[mg a.s. /kg dry feed/day		

#) Percentage values were calculated from the body weights at sacrifice, assuming 40%, 12% and 4% of the body weight for total skeletal muscle, fat, or skin (without subcutaneous fat), respectively.

*) weighted mean TRR-values of the individual animals

**) The transfer factor was calculated by dividing the TRR-value of the respective sample by the feeding level (milligrams of a.s. per kilograms dry feed on each day).

B. Levels and Time Course of Total Radioactive Residues in Eggs

The TRR-values in eggs ranged from 0.029 mg/kg at day two to 0.057 mg/kg at sacrifice. Following a li[redacted] increase a residue plateau-level of 0.050 mg/kg was reached at day six after the first administration.

Table 6.2.2- 4: Overview Time course of total radioactivity in eggs following oral administration of 14 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.04 mg/kg

Animal no's.	Time after the first admin. [d]	Admin. no.	Cumulative secretion [% of total dose admin.] (mean)	TRR [mg/kg] (mean)
403 - 408	0	1	-----	----- #
	1	2	n.c.	n.c.
	2	3	0.01	0.029
	3	4	0.01	0.029
	4	5	0.02	0.038
	5	6	0.03	0.041
	6	7	0.04	0.053
	7	8	0.05	0.054
	8	9	0.05	0.049
	9	10	0.06	0.051
	10	11	0.07	0.049
	11	12	0.08	0.050
	12	13	0.09	0.047
	13	14	0.10	0.049
	13.25	---	0.12	0.057
Mean *				0.044

----- # no egg collected
* weighted mean-value
n.c. not calculated
TRR, plateau-level (day 4 – 13): **0.050** mg/kg

C. Total Radioactive Residues in Dissected Organs and Tissues

The highest TRR-value was determined in kidney (0.390 mg/kg; 0.02% of total admin. dose) followed by liver (0.370 mg/kg; 0.07% of total admin. dose) indicating the significance of these organs for metabolism and excretion. The TRR-value of the eggs collected from the ovary and oviduct at sacrifice (0.076 mg/kg) was by a factor of 1.3 higher than the levels of the laid eggs collected at sacrifice (0.057 mg/kg). This showed that the egg yolk was a preferential site for secretion of test compound related radioactivity. Lower TRR-values were detected for subcutaneous fat (0.042 mg/kg), skin (0.075 mg/kg) and total skeletal muscle (0.029 mg/kg). The TRR-values of the total subcutaneous fat, skin and muscle corresponded to about 0.04%, 0.02% and 0.07% of the total dose assuming values of 12%, 4% and 40% of the body weight for these tissues, respectively.

D. Extraction Efficiency of Residues

The majority of the residues in the eggs as well as organs and tissues were efficiently extracted (83.9% to 93.4%) using acetonitrile/water mixtures. In case of liver, the solids after conventional extraction were further extracted using microwave treatment. Only up to 8.2% of the TRR or 0.003 mg/kg of the residues remained in the post extraction solids (PES).

For sample preparation, the extracts were partitioned against n-heptane except the extract from fat. Very low amounts of radioactivity were recovered in the n-heptane phases amounting to ≤1.3% (0.001 mg/kg) of the TRR. Concentration procedures of the aqueous phases caused no losses, so all of the residues in the aqueous phases were quantitatively analysed by HPLC.

A summary of the extraction efficiency is shown in the table below.

Table 6.2.2- 5: Overview Extraction efficiency of eggs, muscle, fat and liver samples following oral administration of 14 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.04 mg/kg

Sample	Eggs (day 4 – 13)		Muscle Leg		Muscle Thorax		Fat		Liver	
TRR [mg/kg]	**0.050**		**0.029**		**0.018**		**0.042**		**0.370**	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	93.4	0.047	92.8	0.027	91.8	0.017	92.7	0.039	83.9	0.310
Exhaustive extraction	---	---	---	---	---	---	---	---	16.0	0.060
Total extracted	93.4	0.047	92.8	0.027	91.8	0.017	92.7	0.039	99.9	0.370
Post-extraction solids (PES)	6.6	0.003	7.2	0.002	8.2	0.001	7.3	0.003	0.1	<0.001
Accountability	100.0	0.050	100.0	0.029	100.0	0.018	100.0	0.042	100.0	0.370

E. Quantification, Identification and Characterisation of Residues

Parent compound and metabolites were quantified in the conventional extracts by HPLC chromatography based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. Metabolites in the extracts were assigned to each other by comparison of the metabolite profiles and their retention times. Corresponding metabolites were named with the same peak ID.

The identification of metabolites was performed in isolated fractions from the extract of eggs and excreta by spectroscopic methods and by enzymatic cleavage of selected conjugates. The identified metabolites and aglycons in the isolated fractions were used as reference compounds. BCS-CN88460 metabolites were identified by co-chromatography with radiolabelled reference compounds. Metabolites in edible organs and tissues were assigned by comparison of the metabolic profiles and retention times. In addition, the assignment of parent compound and metabolites in the current study was performed based on a comparison of the extracts from excreta of the current study and the hen metabolism study with the phenyl label.

F. Distribution of Parent Compound and Metabolites in Eggs, Organs and Tissues

The identification rates amounted to 69.6% of the TRR for eggs, 73.5% for leg muscle, 67.6% for thorax muscle, 55.5% for fat, 54.3% for liver and 60.1% for excreta.

Parent compound was only detected in eggs, leg muscle and fat and amounted to 0.002 mg/kg (3.7% of the TRR) for eggs, 0.001 mg/kg (2.3% of the TRR) for leg muscle and to 0.010 mg/kg (23.6% of the TRR) for fat.

Metabolites in eggs

Metabolites BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol were the main residues in eggs and accounted for 35.0% (0.018 mg/kg) of the TRR and 22.3% (0.011 mg/kg) of the TRR, respectively. Other prominent metabolites in eggs were BCS-CN88460-desmethyl-1,2-propandiol, BCS-CN88460-carboxylic acid and parent compound, each ≤5.2% of TRR.

Metabolites in leg and thorax muscle

The main residues in leg muscle were BCS-CN88460-desmethyl-propanol (29.7% (0.009 mg/kg) of the TRR), BCS-CN88460-desmethyl-1.2-propandiol (15.0% (0.004 mg/kg) of the TRR) and BCS-CN88460-desmethyl-carboxylic acid (12.1% (0.004 mg/kg) of the TRR). These three main metabolites were also detected in the thorax muscle and amounted between 20.9% and 12.0% of the TRR. BCS-CN88460-carboxylic acid was also a main residue in thorax muscle with 11.0% of the TRR (0.002 mg/kg) which was a prominent metabolite in leg muscle amounting to 9.1% of the TRR. Another prominent metabolite in muscle was BCS-CN88460-propanol, amounting to 5.3% and 5.9% of the TRR in leg and thorax muscle, respectively.

Metabolites in fat

Besides parent compound as the main residue in fat, two main metabolites were identified, namely BCS-CN88460-propanol with 11.9% of the TRR (0.005 mg/kg) and BCS-CN88460-desmethyl-propanol with 10.1% of the TRR (0.004 mg/kg). Other prominent metabolites were identified and named as BCS-CN88460-desmethyl-1,2-propandiol and BCS-CN88460-carboxylic acid. These two metabolites amounted to between 4.8% and 5.1% of the TRR.

Metabolites in liver

The main residue in liver were identified as BCS-CN88460-desmethyl-carboxylic acid (14.4% (0.053 mg/kg) of the TRR) and BCS-CN88460-carboxylic acid (11.9% (0.044 mg/kg) of the TRR). Prominent metabolites in the liver were BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA, BCS-CN88460-desmethyl-propanol-N-GlucA, BCS-CN88460-desmethyl-1.2-propandiol and BCS-CN88460-desmethyl-propanol and amounted to between 5.3% and 6.9% of the TRR. Two minor metabolites named as BCS-CN88460-desmethyl-2-propanol-N-GlucA and BCS-CN88460-propanol were detected (both ≤2.5 of TRR).

More metabolites in the matrices may be present as indicated by broad non-resolved zones in the chromatograms. All unknown metabolites in the extracts were characterised by their extraction and chromatographic behaviour and amounted to each ≤19.5% of the TRR or 0.004 mg/kg.

The distribution of the parent compound and metabolites in milk, organs and tissues is summarised in the table below.

Table 6.2.2- 6: **Overview Radioactive residues of parent compound and metabolites in eggs and edible organs and tissues of laying hens following oral administration of 14 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.04 mg/kg**

Sample		Eggs		Muscle Leg		Muscle Thorax		Fat		Liver	
TRR [mg/kg]		**0.050**		**0.029**		**0.018**		**0.042**		**0.370**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***93.4***	***0.047***	***92.8***	***0.027***	***91.8***	***0.017***	***92.7***	***0.039***	***83.9***	***0.310***
37	parent compound	3.7	0.002	2.3	0.001	---	---	23.6	0.010	---	---
6	desmethyl-1,2-propandiol-N-GlucA (M36)	---	---	---	---	---	---	---	---	5.4	0.020
13	desmethyl-propanol-N-GlucA (M37)	---	---	---	---	---	---	---	---	6.1	0.023
14	desmethyl-2-propanol-N-GlucA (M38)	---	---	---	---	---	---	---	---	2.5	0.009
17	desmethyl-1,2-propandiol (M07)	5.2	0.003	15.0	0.004	17.9	0.003	5.1	0.002	6.9	0.025
27	desmethyl-carboxylic acid (M11)	---	---	12.1	0.004	12.0	0.002	---	---	14.4	0.053
29	desmethyl-propanol (M06)	22.3	0.011	29.7	0.009	20.9	0.004	10.1	0.004	5.3	0.020
31	carboxylic acid (M12)	3.4	0.002	9.1	0.003	11.0	0.002	4.8	0.002	11.9	0.044
32	propanol (M01)	35.0	0.018	5.3	0.002	5.9	0.001	11.9	0.005	1.7	0.006
Total identified		**69.6**	**0.035**	**73.5**	**0.021**	**67.6**	**0.012**	**55.5**	**0.023**	**54.3**	**0.201**
Characterised in the conventional extract by HPLC		*22.4*	*0.011*	*18.7*	*0.005*	*24.2*	*0.004*	*37.1*	*0.016*	*29.0*	*0.107*
Number of unknown peaks		5		4		2		6		18	
Largest unknown peak		7.7	0.004	9.9	0.003	19.5	0.004	11.2	0.005	8.4	0.031
Characterised by partition (n-heptane phase)		*1.3*	*0.001*	*0.6*	*<0.001*	---	---	---	---	*0.6*	*0.002*
Exhaustive extraction		---	---	---	---	---	---	---	---	***16.0***	***0.060***
- ACN/water extract		---	---	---	---	---	---	---	---	*4.5*	*0.017*
Number of unknown peaks		---	---	---	---	---	---	---	---	5	
Largest unknown peak		---	---	---	---	---	---	---	---	2.8	0.010
- 0.1 M HCL extract		---	---	---	---	---	---	---	---	*11.5*	*0.043*
Number of unknown peaks		---	---	---	---	---	---	---	---	4	
Largest of unknown peak		---	---	---	---	---	---	---	---	8.2	0.031
Total characterised		33.7	0.012	19.3	0.005	24.2	0.004	37.1	0.016	45.6	0.169
Total extractable		**93.4**	**0.047**	**92.8**	**0.027**	**91.8**	**0.017**	**92.7**	**0.039**	**99.9**	**0.370**
Unextractable (PES)		6.6	0.003	7.2	0.002	8.2	0.001	7.3	0.003	0.1	<0.001
Accountability		**100.0**	**0.050**	**100.0**	**0.029**	**100.0**	**0.018**	**100.0**	**0.042**	**100.0**	**0.370**

Conjugates in the liver like BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA, BCS-CN88460-desmethyl-propanol-N-GlucA and BCS-CN88460-desmethyl-2-propanol-N-GlucA could be enzymatically cleaved to their aglycons. The cleavage of some unknown conjugates in non-resolved zones resulted in higher amounts of the aglycons. None of the unknown compounds after cleavage accounted for more than 0.010 mg/kg (2.6% of the TRR) after enzymatic cleavage. The following main aglycons could be clearly identified after enzymatic cleavage: BCS-CN88460-desmethyl-1,2-propandiol and BCS-CN88460-desmethyl-propanol.

Table 6.2.2- 7: Overview Radioactive residues of parent compound and metabolites in liver samples of first and second conventional extraction and after enzymatic cleavage for 96 h – experiment 1 and 2

Sample		Liver- 1st conventional extraction		Liver- 2nd conventional extraction		Liver- enzymatic cleavage of 2nd conventional extract - experiment 1		Liver- enzymatic cleavage of 2nd conventional extract - experiment 2	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Extract used for HPLC analysis		**83.3**	**0.308**	**83.3**	**0.308**	**75.2**	**0.278**	**75.2**	**0.278**
6	desmethyl-1,2-propandiol-N-GlucA (M36)	3.4	0.020	5.3	0.019	0.5	0.002	0.7	0.003
13	desmethyl-propanol-N-GlucA (M37)	6.4	0.023	5.6	0.021	--	--	---	---
14	desmethyl-2-propanol-N-GlucA (M38)	2.5	0.009	2.2	0.008	0.5	0.002	0.5	0.002
17	desmethyl-1,2-propandiol (M07)	6.9	0.025	7.6	0.028	11.6	0.043	11.3	0.042
27	desmethyl-carboxylic acid (M11)	14.4	0.053	14.5	0.054	17.4	0.065	17.0	0.063
29	desmethyl-propanol (M06)	5.3	0.020	5.1	0.019	14.7	0.054	14.5	0.054
31	carboxylic acid (M12)	11.9	0.044	11.9	0.044	16.4	0.061	16.8	0.062
32	propanol (M01)	1.7	0.006	2.1	0.008	1.5	0.006	1.8	0.007
Total identified		**54.3**	**0.201**	**54.1**	**0.200**	**62.6**	**0.232**	**62.7**	**0.232**
Total characterised		29.0	0.107	29.2	0.108	12.6	0.046	12.5	0.046
Accountability		83.3	0.308	83.3	0.308	75.2	0.278	75.2	0.278

Metabolites in Excreta

The excreta extract of day 1 was analysed by HPLC. Parent compound accounted for 2.6% of the dose. BCS-CN88460-desmethyl-carboxylic acid and BCS-CN88460-carboxylic acid were the most prominent compound, accounting for 17.7% and 15.4% of the dose, respectively. Further eight identified metabolites ranged from 1.4% to 8.6% of the dose.

Generally, metabolites in the extract from excreta were the same as in eggs, organs and tissues, except for metabolites BCS-CN88460-desmethyl-1,2-propandiol-SA, BCS-CN88460-1,2-propandiol-SA and BCS-CN88460-1,2-propandiol which accounted for equal or less than 8.6% of the dose, respectively.

G. Storage Stability of Residues

All samples of eggs, excreta, edible organs and tissues were extracted within five months after sample collection. Quantitative analysis by HPLC was performed either on the day of extraction or up to four days after the start of extraction. A second conventional extraction of liver was performed approx. 20 months after sampling. The extract was used for enzymatic cleavage experiments. The storage stability was exemplarily demonstrated for these liver samples. It was therefore concluded, that the metabolic profiles represent the residues in the matrices and analysed samples at sacrifice.

III. Conclusion

The metabolic behaviour of [pyrazole-4-^{14}C]BCS-CN88460 in the laying hen can be characterised by the following observations:

The TRR-values and even transfer factors for eggs and edible tissues were very low with respect to the dose level and the dosing period of 14 days. This indicates that test compound related radioactivity does not accumulate during the time of feeding. The evaluations of the TRR-values should however consider the fact that an exaggerated dose level of 16.57 mg a.s./kg feed/day was administered. Furthermore, the fact that the entire radioactivity was detected in the excreta and the relatively high TRR in kidney and liver at sacrifice approx. 6 hours after the last administration revealed that the test compound related residues are further metabolised and finally eliminated from the hen's bodies. A residue plateau level in eggs was clearly reached during the course of the experiment approx. at day six after the first administration.

The radioactive residues were extracted with conventional methods from eggs and tissues; extraction rates ranged from 83.9% to 93.4%. Extraction with conventional and exhaustive methods from edible organs accounted for 99.9% for the liver.

The identification rates in eggs, and edible organs and tissues ranged between 54.3% and 73.5% for the TRR.

Parent compound was detected as major compound in fat and minor in eggs, leg and muscle.

Overall eight metabolites were identified in eggs, edible organs and tissues. Metabolites BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-propanol (M01), BCS-CN88460-desmethyl-carboxylic acid (M11), BCS-CN88460-carboxylic acid (M12) and BCS-CN8840-desmethyl-1,2-propandiol (M07) were major residues in eggs, leg muscle, thorax muscle, fat and liver. Prominent metabolites BCS-CN88460-desmethyl-propanol-N-GlucA (M37) and BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA (M36) were found in liver, only. Minor metabolites in liver were BCS-CN88460-propanol (M01) and BCS-CN88460-desmethyl-2-propanol-N-GlucA (M38).

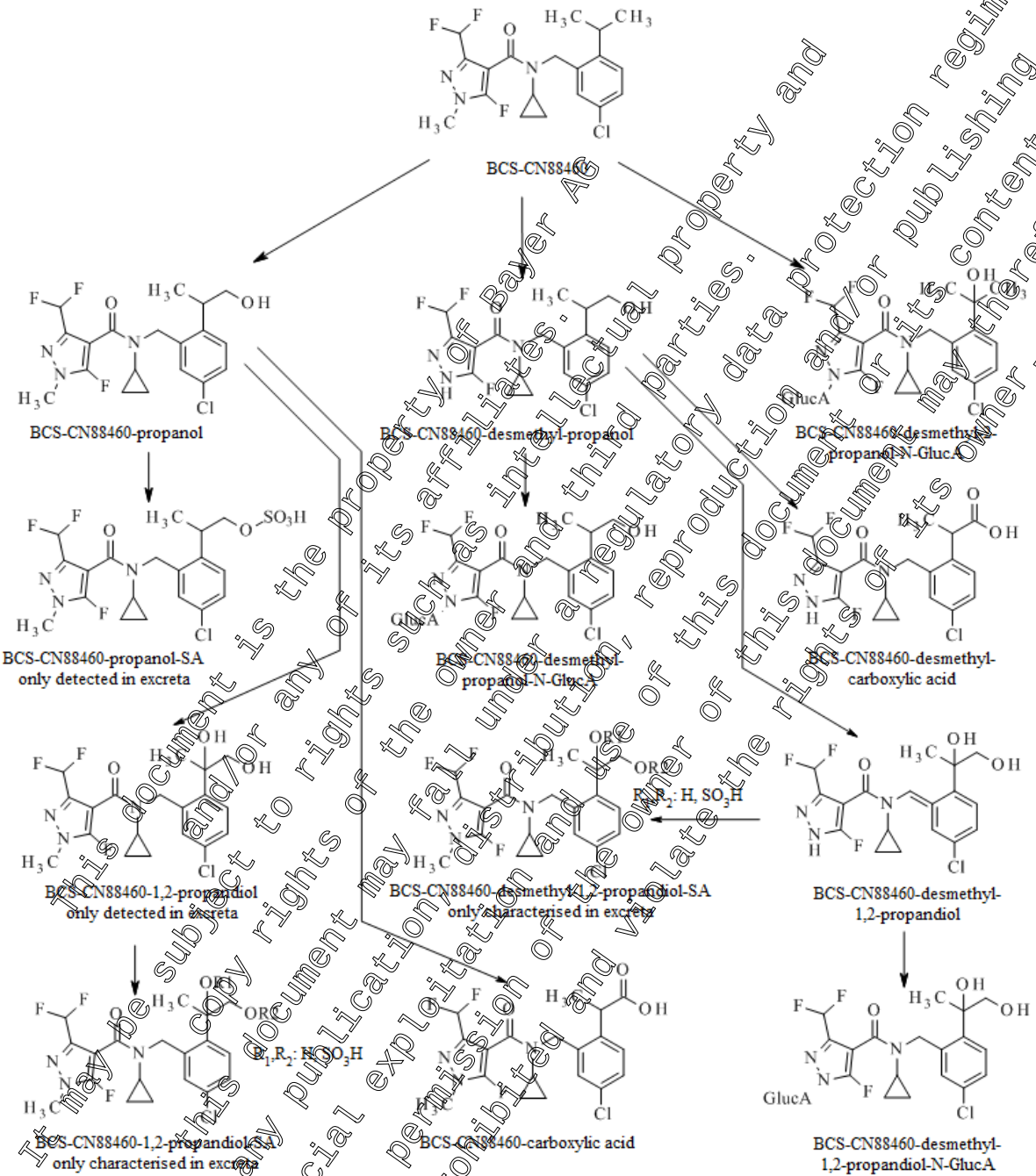
The metabolic profile of excreta from day 1 was similar to the profiles of edible materials, especially liver, with the difference that metabolites BCS-CN88460-desmethyl-1,2-propandiol-SA (M42), BCS-CN88460-1,2-propandiol-SA (M27), BCS-CN88460-1,2-propandiol (M03) and BCS-CN88460-propanol-SA (M26) were present.

The principal metabolic reactions of [pyrazole-4-^{14}C]BCS-CN88460 in the laying hen are listed below:

- demethylation of the pyrazole moiety
- conjugation with glucuronic acid after demethylation of the pyrazole moiety
- hydroxylation in position 1 and/or position 2 of the propyl group
- further oxidation of the 1-propanol group leading to a carboxylic acid group
- conjugation with sulphuric acid after hydroxylation in position 1 or position 2 of the propyl group

Based on these results, the metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in the laying hen is considered as sufficiently understood and a metabolic pathway is proposed in the figure below.

Figure 6.2.2- 1: Proposed metabolic pathway of [pyrazole-4-^{14}C]BCS-CN88460 in laying hens



Report:	KCA 6.2.2/02; [redacted], R.; [redacted]; [redacted], F.; 2017; M-601667-01-1
Title:	[Phenyl-UL-14C]BCS-CN88460: Metabolism in the laying hen
Report No.:	EnSa-17-0306
Document No.:	M-601667-01-1
Guideline(s):	OECD Test Guideline No. 503 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 US EPA OCSPP Test Guideline No. 860.1300
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism and excretion of [phenyl-UL-^{14}C]BCS-CN88460 was investigated in laying hens as a model for poultry. The test compound was orally administered to six hens as aqueous 0.5% Tragacanth suspension at an intended dose rate of 1 mg per kg body weight. Based on the daily feed consumption, the dose level corresponded up to 18.12 mg a.s./kg dry feed/day. The hens received 14 doses at 24-hour intervals in the morning and were sacrificed approx. 6 hours after the last dosing. Throughout the experiment, the hens were housed in metabolism cages, which permitted separate collection of eggs and excreta. The eggs were collected once daily and before sacrifice. Total radioactive residues (TRR) were determined in each egg (mixed sample from egg white and yolk) and in dissected organs and tissues (muscle, fat, liver, kidney, skin and eggs from ovary / oviduct) at sacrifice. The total radioactivity (% of total dose administered) was additionally determined in each excreta sample.

Recovery and Elimination of Radioactivity

The overall recovery amounted to 103.36% of the total dose and up to the time of sacrifice the excretion accounted for up to 102.97% of the total dose. After the third administration the daily excretion rate was on a more or less constant level of about 7.1 to 8.8% within 24 hours.

An average amount of approx. 0.14% of the total dose was measured in the eggs. At sacrifice, radioactive residues in the organs and tissues dissected from the bodies were calculated to be about 0.24% of the total dose.

Total Radioactive Residues in Eggs, Organs and Tissues

The TRR-values and transfer factors for eggs and organs and tissues were very low compared to the dose level of 18.12 mg a.s. /kg feed/day and a dosing period of 14 days.

The TRR-values in eggs ranged from 0.032 mg/kg at day three to 0.066 mg/kg at sacrifice. Following a li[redacted] increase a residue plateau-level of 0.050 mg/kg was reached at day four after the first administration.

Regarding organs and tissues the TRR-values amounted to 0.373 mg/kg in liver, 0.360 mg/kg in kidney, 0.047 mg/kg in subcutaneous fat, 0.109 mg/kg in skin, 0.029 mg/kg in leg muscle and 0.017 mg/kg in thorax muscle.

Metabolism

The majority of the residues in the eggs as well as organs and tissues were efficiently extracted (85.4% to 93.3%) using acetonitrile/water (8/2; v/v) mixtures. In case of liver, the solids after conventional extraction were exhaustively extracted using acetonitrile/water (1/1; v/v) mixtures and 0.1 M hydrochloric acid with microwave treatment. Only up to 8.2% of the TRR (0.004 mg/kg) of the residues remained in the post extraction solids (PES).

For sample preparation the extracts were partitioned against n-heptane except the extract from fat. Very low amounts of radioactivity were recovered in the n-heptane phases and amounted to ≤1.2% (0.001 mg/kg) of the TRR.

Parent compound and metabolites were identified based on co-chromatography with reference compounds or by comparison of the metabolite pattern and retention times. Reference compounds were taken from the hen metabolism study with the pyrazole label or the goat metabolism study with the phenyl label.

The identification rates amounted to 79.2% of the TRR for eggs, 79.8% for leg muscle, 76.3% for thorax muscle, 51.5% for fat and 62.0% for liver.

Parent compound was only detected in eggs, leg muscle and fat and amounted to 0.003 mg/kg (6.4% of the TRR) for eggs, 0.001 mg/kg (2.9% of the TRR) for leg muscle and to 0.009 mg/kg (20.1% of the TRR) for fat.

Metabolites BCS-CN88460-desmethyl-1,2-propandiol (M07), BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-carboxylic acid (M12) and BCS-CN88460-propanol (M01) were detected in all edible materials and eggs.

The amount of BCS-CN88460-desmethyl-1,2-propandiol ranged from 5.6% to 22.3% of the TRR and it represented a major residue (>10% of the TRR) in leg and thorax muscle. The amount of BCS-CN88460-desmethyl-propanol (representing a maior compound in eggs and both muscles) ranged from 2.7% to 25.8% of the TRR and the amount of BCS-CN88460-propanol (representing a major compound in eggs) ranged from 1.7% to 33.9% of the TRR, while the amount of BCS-CN88460-carboxylic acid ranged from 3.0% to 8.6% of the TRR. BCS-CN88460-desmethyl-carboxylic acid was detected as major residue in muscle and liver, while it was detected in minor amounts in fat. Its amount ranged thereby from 7.9% to 21.9% of the TRR.

BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA (M36) was detected in leg muscle and liver, accounting for 4.1% to 9.2% of the TRR, respectively. BCS-CN88460-desmethyl-propanol-N-GlucA (M37) was detected in eggs, leg muscle and liver, ranging from 3.0% to 10.8% of the TRR.

Metabolites BCS-CN88460-desmethyl-2-propanol-N-GlucA (M38) and BCS-CN88460-propanol-SA (M26) were only detected in liver (both equal or less than 3.0% of the TRR).

The metabolic profile of excreta from day 1 was similar to the profiles of edible materials, especially liver, with the difference that metabolites BCS-CN88460-desmethyl-1,2-propandiol-SA (M42), BCS-CN88460-1,2-propandiol-SA (M27) and BCS-CN88460-1,2-propandiol (M03) were only detected in excreta.

A summary of the distribution of parent compound and metabolites for edible materials is provided in the table overleaf.

Table 6.2.2- 8: Overview Radioactive residues of parent compound and metabolites in eggs and edible organs and tissues of laying hens following oral administration of 14 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.01 mg/kg

Sample		Eggs (day 4 – 13)		Muscle Leg		Muscle Thorax		Fat		Liver	
TRR [mg/kg]		**0.050**		**0.029**		**0.017**		**0.047**		**0.373**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***92.8***	***0.046***	***93.3***	***0.027***	***92.4***	***0.016***	***91.8***	***0.043***	***85.4***	***0.318***
42	parent compound	6.4	0.003	2.9	0.001	---	---	20.1	0.009	---	---
7	desmethyl-1,2-propandiol-N-GlucA (M36)	---	---	4.1	0.001	---	---	---	---	9.2	0.034
18	desmethyl-propanol-N-GlucA (M37)	3.0	0.002	3.8	0.001	---	---	---	---	10.8	0.040
19	desmethyl-2-propanol-N-GlucA (M38)	---	---	---	---	---	---	---	---	3.0	0.011
22	desmethyl-1,2-propandiol (M07)	6.2	0.003	14.2	0.004	22.3	0.004	5.9	0.003	5.6	0.021
29	propanol-SA (M26)	---	---	---	---	---	---	---	---	1.2	0.005
33	desmethyl-carboxylic acid (M11)	---	---	19.9	0.006	20.2	0.003	7.9	0.004	21.9	0.082
35	desmethyl-propanol (M06)	22.6	0.011	25.8	0.007	20.9	0.004	8.0	0.004	2.7	0.010
37	carboxylic acid (M12)	7.2	0.004	6.6	0.002	8.6	0.001	3.0	0.001	5.8	0.022
38	propanol (M01)	33.9	0.017	2.5	0.001	4.3	0.001	6.5	0.003	1.7	0.006
Total identified		**79.2**	**0.040**	**79.8**	**0.023**	**76.3**	**0.013**	**51.5**	**0.024**	**62.0**	**0.231**
Characterised by HPLC		*12.4*	*0.006*	*13.2*	*0.004*	*16.1*	*0.003*	*40.3*	*0.019*	*23.1*	*0.086*
Characterised in n-heptane phase		*1.2*	*0.001*	*0.4*	*<0.001*	---	---	---	---	*0.3*	*0.001*
Exhaustive extraction		---	---	---	---	---	---	---	---	***14.5***	***0.054***
-ACN/water extract		---	---	---	---	---	---	---	---	4.8	0.018
-0.1 M HCL extract		---	---	---	---	---	---	---	---	9.6	0.036
Total characterised		13.6	0.007	13.5	0.004	16.1	0.003	40.3	0.019	37.9	0.142
Total extractable		**92.8**	**0.046**	**93.3**	**0.027**	**92.4**	**0.016**	**91.8**	**0.043**	**99.9**	**0.373**
Unextractable (PES)		**7.2**	**0.004**	**6.7**	**0.002**	**7.6**	**0.001**	**8.2**	**0.004**	**0.1**	**<0.001**
Accountability		**100.0**	**0.050**	**100.0**	**0.029**	**100.0**	**0.017**	**100.0**	**0.047**	**100.0**	**0.373**

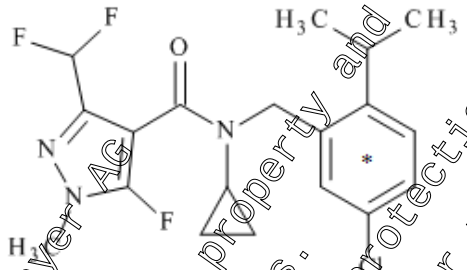
The main metabolic reactions were the demethylation of the pyrazole moiety and hydroxylation in the propyl group of the phenyl ring. Hydroxylation in the propyl group was leading to mono- or dihydroxy compounds. Conjugation with glucuronic acid was observed after demethylation of the pyrazole moiety and conjugation with sulphuric acid after hydroxylation in position 1 of the propyl group. Another metabolic reaction was further oxidation of BCS-CN88460-propanol to BCS-CN88460-carboxylic acid.

Based on the results the metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in the laying hen is considered as adequately understood and a metabolic pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material

Chemical structure	 * denotes the ^{14}C-label position
Radiolabelled test material	[phenyl-UL-^{14}C]BCS-CN88460
Specific radioactivity	4.13 MBq/mg = 2.48 x 10^8 dpm/mg
Radiochemical purity	>98% (HPLC)
Chemical purity	>98% (HPLC)
Dose level	14 oral doses of 18.12 mg a.s./kg feed/day (1 mg a.s./kg bw/day)
Vehicle	0.5 % aqueous Tragacanth® suspension

2. Test Animals

Species	Laying hen (*Gallus gallus domesticus*)
Strain	"LB Lohmann Brown"
Breeder	[redacted]
Animal numbers	8 animals in total, from which 6 (no's 493 – 498) were chosen for the test. The hens were selected by maximum egg production.
Mean body weights	1.83 kg at delivery (2015-10-06) 1.82 kg at the first administration (2015-10-23) 1.80 kg at sacrifice (2015-11-03)
Identification	During the acclimation period, the animals were identified by individual cage cards (1 – 8). During the testing period an individual animal number (see above) was allocated on the cage cards and additionally by foot ring.
Acclimation period	15 days
Husbandry	Conventional hygienic conditions in air-conditioned rooms
Housing	During the acclimation period and the whole testing period, the hens were kept individually in electro-polished stainless steel metabolism cages for laying hens, supplied by [redacted]. These cages allow almost separate and quantitative collection of excreta and eggs.
Dietary regimen	During the whole residence time, the hens were fed with "RWZ-LegeGold Mehl", a pulverised chicken feed. This feed was not a certified diet, i.e. it was not checked for contamination according to current standards. The feed was supplemented by eggshells during the acclimation period. The feed consumption during the testing period was recorded by back-weighing. Tap water from the local mains supply was given *ad libitum* during the whole residence time.
Environmental conditions	Temperature: 19 – 23°C Relative humidity: 23 – 78% Photoperiod: alternating 16- to 8-hours light / dark cycles Air change: 10 – 15 times per hour

B. Study Design

Preparation of the Test Item for Administration

The radiolabelled test compound was delivered in solid form and dissolved in 25 mL acetonitrile for preparation of the stock solution. The radiochemical purity amounted to >98% as assured by HPLC. The identity of the test compound was confirmed by LC/MS/MS.

Four administration suspensions were prepared and each suspension was applied for three to four administrations. Definite volumes of the stock solution were concentrated to [redacted] dryness by a gentle stream of nitrogen gas. Afterwards, the residue was suspended in a 0.5% aqueous Tragacanth suspension. Aliquots were taken for determination of the total radioactivity by LSC. The suspensions were kept permanently under stirring at + 5 °C in a cooling cabinet until the administrations at which they were stirred at room temperature and were proved to be stable until the last dose.

Dosing

All oral administrations were performed by gavage using a syringe attached to an animal-feeding knob cannula once daily for 14 consecutive days in the morning in relation to the individual body weights. The laying hens received on each day an average amount of 1.83 mg ^{14}C-BCS-CN88460, which corresponded to 7.56 MBq (mean per animal and day). The total administered average amount and radioactivity accounted for 25.56 mg and 105.56 MBq, respectively. The administration volume was 1.0 mL/kg body weight.

The total amount of radioactivity administered to each animal served as reference value (A0 = 100%) for the percentage calculation of the total radioactivity in the biological samples.

Based upon the experimentally determined daily feed consumption during the testing period of 101 g dry feed per day (= 5.61% of the body weight), the dose of 1.01 mg a.s./kg bw corresponded to a concentration of 18.12 mg a.s./kg dry feed per day in the diet. This dose was tolerated without any observable toxicological effects.

Collection of eggs

The egg production of the laying hens was checked during the acclimation period (beginning of the laying phase) and during the testing period. Assuming an average laying rate of 314 eggs per hen and year the mean egg production during the acclimation period was 113% and 121% during the testing period. Therefore, the egg production exceeded the target value for laying hens being in good egg production.

During the test, the grates of the cages were inspected for egg production once daily and the number of eggs was recorded for all hens. After removal of the shells, the contents of each egg were weighed and thoroughly mixed afterwards. Aliquots of each homogenate were mixed with scintillator for determination of the radioactivity by LSC. The remaining samples were stored in a freezer until start of metabolite analysis.

Collection of excreta

The excreta of each hen were collected from the collecting tins as far as possible quantitatively in daily intervals until sacrifice. Individual samples were weighed after water was added. Afterwards, the individual samples were homogenised. Aliquots of each sample were processed for radioactivity measurement by combustion / LSC. The remaining samples were stored in a freezer until start of metabolite analysis.

Sacrifice

The animals were sacrificed approx. 6 hours after the last administration, a time distance that is consistent with normal slaughtering practices. Each laying hen was transferred into a special cage, weighed and anaesthetized using carbon dioxide gas. Under general anaesthesia the animals were sacrificed by decapitation followed by exsanguination.

Preparation of organs and tissues

After exsanguination, the following edible organs and tissues were dissected: muscle (leg and thorax), fat (subcutaneous), liver (without gall bladder), skin (without subcutaneous fat), kidneys and eggs from the ovary and oviduct.

The organs or tissue samples were transferred into tared weight plastic vessels. After determination and recording of the individual weights, muscle, fat, liver, skin, kidney samples and eggs dissected from the ovary and oviduct were passed several times through a mincing machine in half-frozen state or blended with an Ultra Turrax in thawed state. Aliquots of the individual organ and tissue samples were combusted and the radioactivity measured by LSC.

The organ and tissue samples of the six hens were pooled separately for each sample type, divided in suitable portions that were stored frozen at ≤ -18 °C until the start of metabolite analysis. For skin, the remaining homogenates of kidneys and eggs from the ovary/oviduct, metabolite analysis was optional.

All individual samples were identified with a specific sample number. The individual excreta, egg, as well as organ and tissue samples were kept frozen at ≤ -18 °C at all times except during aliquotation for analysis. During the analytical work the samples and extracts of samples were stored either in a freezer at ≤ -18 °C or for a short period in a refrigerator at +4 °C.

Radioactivity measurement

The radioactivity measurement in liquid samples was carried out by liquid scintillation counting (LSC). The solid samples were either dissolved in BIOLUTE S and radioactivity determined by LSC or combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

C. Analytical Procedures

Sample Extraction and Analysis of Extracts

Aliquot samples from eggs, muscle, fat, liver and excreta were conventionally extracted three times with a mixture of acetonitrile/ water (8/2; v/v) using a Polytron homogeniser. For sample preparation the combined conventional extracts from eggs, muscle, liver and excreta were partitioned against n-heptane. The purified conventional extracts were concentrated by rotary evaporation and subjected to HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

Solids of liver from the first conventional extraction were exhaustively extracted twice with acetonitrile/water (8/2; v/v) using microwave assistance followed by further treatment with microwave and 0.1 M hydrochloric acid. All exhaustive extracts were characterised using TLC analysis.

Aliquots of the conventional liver extracts were incubated for 96 hours at 37°C with a defined amount of ß-glucuronidase/arylsulfatase. After incubation, the enzymatic suspensions were purified and analysed by HPLC.

Metabolite analysis

Parent compound and metabolites were quantified in the extracts by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

The following strategy was used for identification of the parent compound and metabolites:
All metabolites were assigned based on a comparison of the metabolite profiles and retention times analysed in the hen metabolism study after administration of pyrazole labelled BCS-CN88460. Parent compound and metabolites were identified in selected samples by HPLC or TLC co-chromatography with radiolabelled reference compounds taken from the hen metabolism with the pyrazole label or from the goat metabolism study with the phenyl label. The assignment of parent compound and metabolites in all samples was then achieved by comparison of HPLC metabolite profiles of the analysed samples among each other. The conventional extract of liver was enzymatically digested.

The glucuronic acid and sulphate conjugates were thereby digested and converted into their corresponding aglycons.

Unknown metabolites were characterised based on their extraction and chromatographic behaviour.

An overview on the reference compounds is given in Table 6.2.2- 9 .

Table 6.2.2- 9: Overview List of reference compounds

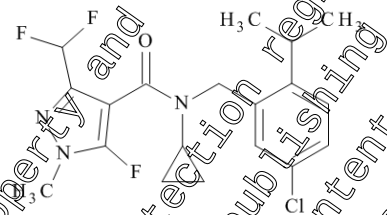
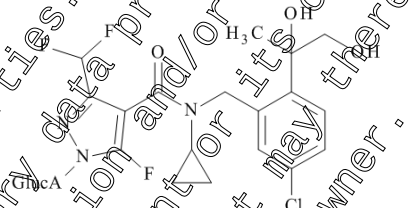
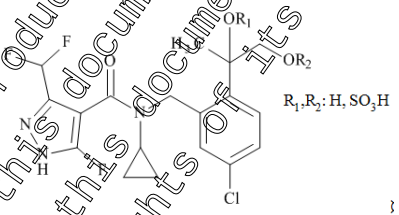
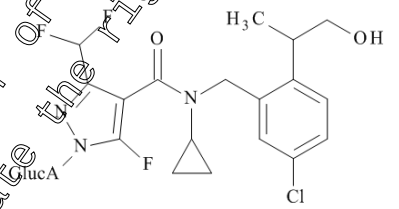
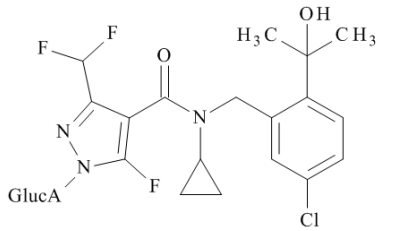
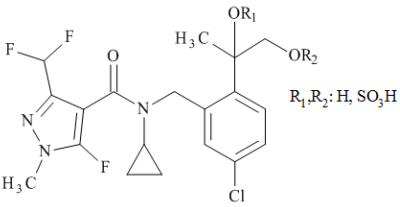
Report name/ other names/codes	**Chemical Name (IUPAC)**	**Chemical Structure**
Parent compound ID: BN5005A	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	
BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA ID: BN4115A	N-[5-chloro-2-(1,2-dihydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(glucopyranuronosyl)-1H-pyrazole-4-carboxamide	
BCS-CN88460-desmethyl-1,2-propandiol-SA ID: BN4115B	not available	
BCS-CN88460-desmethyl-propanol-N-GlucA ID: BN4115C	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(glucopyranuronosyl)-1H-pyrazole-4-carboxamide	
BCS-CN88460-desmethyl-2-propanol-N-GlucA ID: BN4115D	N-[5-chloro-2-(2-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(glucopyranuronosyl)-1H-pyrazole-4-carboxamide	
BCS-CN88460-1,2-propandiol-SA ID: BN4115E	not available	

Table is continued on the next page

Table 6.2.2- 9 continued

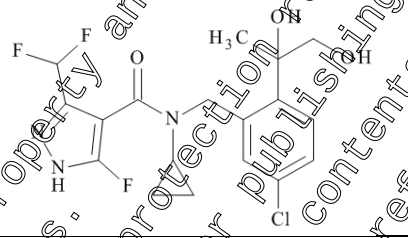
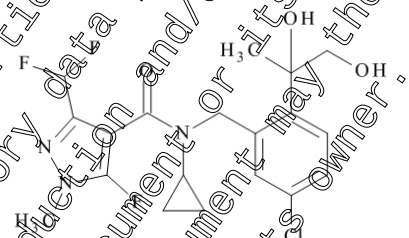
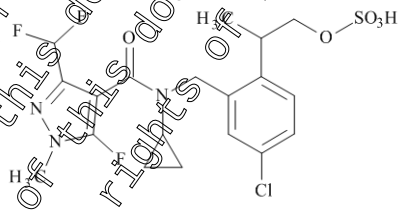
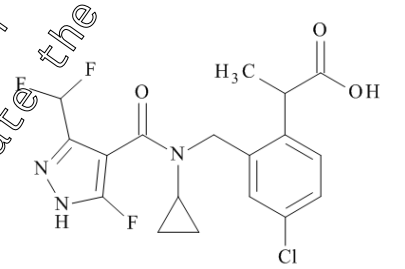
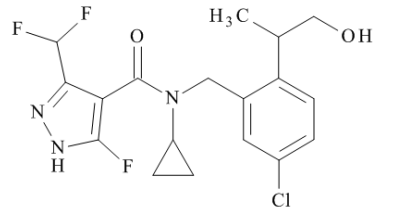
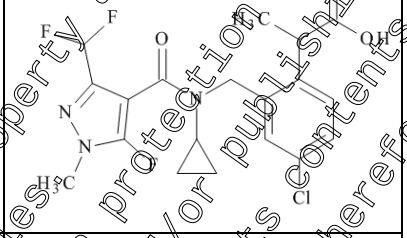
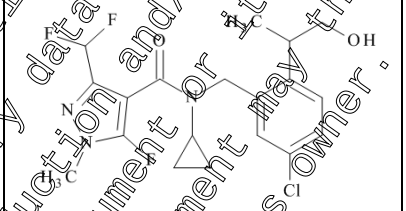
Report name/ other names/codes	Chemical Name (IUPAC)	Chemical Structure
BCS-CN88460-desmethyl-1,2-propandiol ID: BN4115X	N-[5-chloro-2-(1,2-dihydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide	
BCS-CN88460-1,2-propandiol ID: BN4115E2	N-[5-chloro-2-(1,2-dihydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	
BCS-CN88460-propanol-SA ID: BN4114A	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl} propyl hydrogen sulfate	
BCS-CN88460-desmethyl-carboxylic acid BCS-CX99799 ID: BN4114B	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl} propanoic acid	
BCS-CN88460-desmethyl-propanol BCS-DC22055 ID: BN4114D and ID: YK0614E	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide	

Table is continued on the next page

Table 6.2.2- 9 continued

Report name/ other names/codes	**Chemical Name (IUPAC)**	**Chemical Structure**
BCS-CN88460-carboxylic acid/ BCS-CY26497 ID: BN4114F	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid	
BCS-CN88460-propanol BCS-CY24813 ID: BN4183B	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The overall recovery accounted for 103.36% of the total dose and up to the time of sacrifice, 102.97% of the total dose was excreted. After the third administration the daily excretion rate was on a more or less constant level of about 7.1 to 8.8 % within 24 hours. An average amount of 0.14% of the total dose was measured in the eggs. At sacrifice, the radioactive residues in the organs and tissues dissected from the bodies were calculated or estimated to be about 0.24% of the total dose.

All TRRs for eggs and dissected organs and tissues were calculated as radioactivity originating from the radiolabelled test compound. Therefore, all concentration data in this report represent active substance equivalents (mg a.s. equiv. /kg_{sample}).

Table 6.2.2- 10: Distribution of residues in liver, kidney, eggs, muscle, skin and fat of laying hens following oral administration of 14 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.01 mg/kg

Sample	Collection time	TRR [mg/kg] *)	Transfer factor **)	Percent of total dose administered
Liver	approx. 6 h after last admin.	0.373	0.021	0.07
Kidney		0.360	0.020	0.01
Eggs from ovary/oviduct		0.096	0.005	0.02
Total skeletal muscle #)		0.024	0.001	0.07
Total body skin #)		0.109	0.006	0.03
Total body fat #)		0.047	0.003	0.04
Total of organs/tissues		-----	-----	0.24
Eggs, total	day 1 – 13.25	0.047	0.003	0.14
Eggs, plateau-level	day 4 – 13	0.050	0.003	-----
Excreta, total	day 1 – 13.25	-----	-----	102.97
Total Recovery				**103.36**
Feeding level	18.12	[mg a.s. /kg dry feed/day]		

#) Percentage values were calculated from the body weights at sacrifice, assuming 40%, 12% and 4% of the body weight for total skeletal muscle, fat, or skin (without subcutaneous fat), respectively.
*) weighted mean TRR-values of the individual animals
**) The transfer factor was calculated by dividing the TRR-value of the respective sample by the feeding level (milligrams of a.s. per kilograms dry feed on each day).

B. Levels and Time Course of Total Radioactive Residues in Eggs

The TRR-values in eggs ranged from 0.032 mg/kg at day three to 0.066 mg/kg at sacrifice. Following a li[redacted] increase a residue plateau-level of 0.050 mg/kg was reached at day four after the first administration.

Table 6.2.2- 11: Time course of total radioactivity in eggs following oral administration of 14 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.01 mg/kg

Animal no's.	Time after the first admin. [d]	Admin. no.	Cumulative secretion [% of total dose admin.] (mean)	TRR [mg/kg] (mean)
493 - 498	0	1	----- #	----- #
	1	2	0.00	n.c.
	2	3	0.01	0.048
	3	4	0.02	0.032
	4	5	0.03	0.043
	5	6	0.04	0.047
	6	7	0.05	0.050
	7	8	0.07	0.062
	8	9	0.08	0.054
	9	10	0.09	0.054
	10	11	0.10	0.051
	11	12	0.11	0.045
	12	13	0.12	0.045
	13	14	0.13	0.047
	13.25	---	0.14	0.066
Mean *				0.047

----- # no egg collected
* weighted mean-value
n.c. not calculated
TRR, plateau-level (day 4 – 13): **0.050** mg/kg

C. Total Radioactive Residues in Dissected Organs and Tissues

The highest TRR-value was determined in liver (0.373 mg/kg; 0.07% of total admin. dose) followed by kidney (0.360 mg/kg; 0.01% of total admin. dose) indicating the significance of these organs for metabolism and excretion. The TRR-value of the eggs collected from the ovary and oviduct at sacrifice (0.096 mg/kg) was by a factor of 1.5 higher than the levels of the laid eggs collected at sacrifice (0.066 mg/kg). This showed that the egg yolk was a preferential site for secretion of test compound related radioactivity. Lower TRR-values were detected for subcutaneous fat (0.047 mg/kg), skin (0.109 mg/kg) and total skeletal muscle (0.024 mg/kg). The TRR-values of the total subcutaneous fat, skin and muscle corresponded to about 0.04%, 0.03% and 0.07% of the total dose assuming values of 12%, 4% and 40% of the body weight for these tissues, respectively.

D. Extraction Efficiency of Residues

The majority of the residues in the eggs as well as organs and tissues were efficiently extracted (85.4% to 93.3%) using acetonitrile/water mixtures. In case of liver, the solids after conventional extraction were further extracted using microwave treatment releasing 14.5% of the TRR (0.054 mg/kg). Only up to 8.2% of the TRR (0.004 mg/kg) of the residues remained in the post extraction solids (PES).

For sample preparation, the extracts were partitioned against n-heptane except the extract from fat. Very low amounts of radioactivity were recovered in the n-heptane phases amounting to ≤1.2% (0.001 mg/kg) of the TRR. Concentration procedures of the aqueous phases caused no losses, so all of the residues in the aqueous phases were quantitatively analysed by HPLC.

A summary of the extraction efficiency is shown in the table below.

Table 6.2.2- 12: Extraction efficiency of eggs, muscle, fat and liver samples following oral administration of 14 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.01 mg/kg

Sample	Eggs (day 4 – 13)		Muscle Leg		Muscle Thorax		Fat		Liver	
TRR [mg/kg]	**0.050**		**0.029**		**0.017**		**0.047**		**0.373**	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	92.8	0.046	93.3	0.027	92.4	0.016	91.8	0.043	85.4	0.318
Exhaustive extraction	---	---	---	---	---	---	---	---	14.5	0.054
Total extracted	92.8	0.046	93.3	0.027	92.4	0.016	91.8	0.043	99.9	0.373
Post-extraction solids (PES)	7.2	0.004	6.7	0.002	7.6	0.001	8.2	0.004	0.1	<0.001
Accountability	100.0	0.050	100.0	0.029	100.0	0.017	100.0	0.047	100.0	0.373

E. Quantification, Identification and Characterisation of Residues

Parent compound and metabolites were quantified in the conventional extracts by HPLC chromatography based on reversed-phase chromatography using an acidic water/acetonitrile/THF gradient. Metabolites in the extracts were assigned to each other by comparison of the metabolite profiles and their retention times. Corresponding metabolites were named with the same peak ID.

BCS-CN88460 metabolites were identified by co-chromatography with radiolabelled reference compounds taken from the hen metabolism study after administration of pyrazole labelled BCS-CN88460 or from the goat metabolism study after administration of phenyl labelled BCS-CN88460. Metabolites in other organs and tissues were assigned by comparison of the metabolic profiles and retention times. In addition, the assignment of parent compound and metabolites in the current study was performed based on a comparison of the retention times and metabolic profile of the current study and the hen metabolism study with the pyrazole label.

F. Distribution of Parent Compound and Metabolites in Eggs, Organs and Tissues

The identification rates amounted to 79.2% of the TRR for eggs, 79.8% for leg muscle, 76.3% for thorax muscle, 51.5% for fat and 62.0% for liver.

Parent compound was only detected in eggs, leg muscle and fat and amounted to 0.003 mg/kg (6.4% of the TRR) for eggs, 0.001 mg/kg (2.9% of the TRR) for leg muscle and to 0.009 mg/kg (20.1% of the TRR) for fat.

Metabolites in eggs (day 4 – 13)

Metabolites BCS-CN88460-propanol and BCS-CN88460-desmethyl-propanol were the main residues in eggs and accounted for 0.017 mg/kg (33.9% of the TRR) and 0.011 mg/kg (22.6% of the TRR), respectively. Other prominent metabolites in eggs were BCS-CN88460-desmethyl-propanol-N-GlucA, BCS-CN88460-desmethyl-1,2-propandiol and BCS-CN88460-carboxylic acid, each ≤7.2% of TRR.

Metabolites in leg and thorax muscle

The main residues in leg muscle were BCS-CN88460-desmethyl-propanol (25.8 % (0.007 mg/kg) of the TRR), BCS-CN88460-desmethyl-carboxylic acid (19.9% (0.006 mg/kg) of the TRR) and BCS-CN88460-desmethyl-1.2-propandiol (14.2% (0.004 mg/kg) of the TRR). These three main metabolites were also detected in the thorax muscle and amounted between 20.2% and 22.3% of the TRR. BCS-CN88460-carboxylic acid was a prominent metabolite in leg and thorax muscle and amounted to 6.6% and 8.6% of the TRR, respectively. Minor metabolites in muscle were BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA, BCS-CN88460-desmethyl-propanol-N-GlucA (both only detected in leg muscle) and BCS-CN88460-propanol (all ≤4.3% of TRR).

Metabolites in fat

Besides parent compound as the main residue in fat, five abundant metabolites were identified and named as BCS-CN88460-desmethyl-1,2-propandiol, BCS-CN88460-desmethyl-carboxylic acid, BCS-CN88460-desmethyl-propanol, BCS-CN88460-carboxylic acid and BCS-CN88460-propanol. These five metabolites amounted between 3.0% and 8.0% of the TRR.

Metabolites in liver

The main residue in liver was identified as BCS-CN88460-desmethyl-carboxylic acid (21.9% (0.082 mg/kg) of the TRR). Prominent metabolites in the liver were BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA, BCS-CN88460-desmethyl-propanol-N-GlucA, BCS-CN88460-desmethyl-1,2-propandiol and BCS-CN88460-carboxylic acid and amounted between 5.5% and 10.8% of the TRR. Four minor metabolites named as BCS-CN88460-desmethyl-2-propanol-N-GlucA, BCS-CN88460-propanol-SA, BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol were detected (all ≤3.0 of TRR).

More metabolites in the matrices may be present as indicated by broad non-resolved zones in the chromatograms. All unknown metabolites in the extracts were characterised by their extraction and chromatographic behaviour and amounted to each ≤10.0% of the TRR.

The distribution of the parent compound and metabolites in milk, organs and tissues is summarised in the table below.

Table 6.2.2- 13: Radioactive residues of parent compound and metabolites in eggs and edible organs and tissues of laying hens following oral administration of 14 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.01 mg/kg

Sample		Eggs (day 4 – 13)		Muscle Leg		Muscle Thorax		Fat		Liver	
TRR [mg/kg]		**0.050**		**0.029**		**0.017**		**0.047**		**0.373**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***92.8***	***0.046***	***93.3***	***0.027***	***92.4***	***0.016***	***91.8***	***0.043***	***85.4***	***0.318***
42	parent compound	6.4	0.003	2.9	0.001	---	---	20.1	0.009	---	---
7	desmethyl-1,2-propandiol-N-GlucA (M36)	---	---	4.1	0.001	---	---	---	---	9.2	0.034
18	desmethyl-propanol-N-GlucA (M37)	3.0	0.002	3.8	0.001	---	---	---	---	10.8	0.040
19	desmethyl-2-propanol-N-GlucA (M38)	---	---	---	---	---	---	---	---	3.0	0.011
22	desmethyl-1,2-propandiol (M07)	6.2	0.003	14.2	0.004	22.3	0.004	5.9	0.003	5.6	0.021
29	propanol-SA (M26)	---	---	---	---	---	---	---	---	1.2	0.005
33	desmethyl-carboxylic acid (M11)	---	---	19.9	0.006	20.2	0.003	7.9	0.004	21.9	0.082
35	desmethyl-propanol (M06)	22.6	0.011	25.8	0.007	20.9	0.004	8.0	0.004	2.7	0.010
37	carboxylic acid (M12)	7.2	0.004	6.6	0.002	8.6	0.001	3.0	0.001	5.8	0.022
38	propanol (M01)	33.9	0.017	2.5	0.001	4.3	0.001	6.5	0.003	1.7	0.006
Total identified		**79.2**	**0.040**	**79.8**	**0.023**	**76.3**	**0.013**	**51.5**	**0.024**	**62.0**	**0.231**
Characterised in the conventional extract by HPLC		*12.3*	*0.006*	*13.2*	*0.004*	*16.1*	*0.003*	*40.3*	*0.019*	*23.1*	*0.086*
Number of unknown peaks		3		3		2		7		17	
Largest unknown peak		4.9	0.002	6.7	0.002	8.5	0.001	10.0	0.005	8.0	0.030
Characterised by partition (n-heptane phase)		*1.2*	*0.001*	*0.4*	*0.001*	---	---	---	---	*0.3*	*0.001*
Exhaustive extraction		---	---	---	---	---	---	---	---	***14.5***	***0.054***
- ACN/water extract		---	---	---	---	---	---	---	---	*4.8*	*0.018*
Number of unknown peaks		---	---	---	---	---	---	---	---	5	
Largest unknown peak		---	---	---	---	---	---	---	---	2.4	0.009
- 0.1 M HCL extract		---	---	---	---	---	---	---	---	*9.6*	*0.036*
Number of unknown peaks		---	---	---	---	---	---	---	---	4	
Largest of unknown peak		---	---	---	---	---	---	---	---	6.6	0.025
Total characterised		13.6	0.007	13.5	0.004	16.1	0.003	40.3	0.019	37.9	0.142
Total extractable		**92.8**	**0.046**	**93.3**	**0.027**	**92.4**	**0.016**	**91.8**	**0.043**	**99.9**	**0.373**
Unextractable (PES)		7.2	0.004	6.7	0.002	7.6	0.001	8.2	0.004	0.1	<0.001
Accountability		**100.0**	**0.050**	**100.0**	**0.029**	**100.0**	**0.017**	**100.0**	**0.047**	**100.0**	**0.373**

Conjugates in the liver like BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA, BCS-CN88460-desmethyl-propanol-N-GlucA, BCS-CN88460-desmethyl-2-propanol-N-GlucA and BCS-CN88460-propanol-SA could be enzymatically cleaved to their aglycons. The cleavage of some unknown conjugates in non-resolved zones resulted in higher amounts of the aglycons. None of the unknown compounds after cleavage accounted for more than 5.4% (0.020 mg/kg) of the TRR. The following main aglycons could be clearly identified after enzymatic cleavage: BCS-CN88460-desmethyl-1,2-propandiol and BCS-CN88460-desmethyl-propanol.

Table 6.2.2- 14: Radioactive residues of parent compound and metabolites in liver samples of first and second conventional extraction and after enzymatic cleavage for 96 h

Sample		Liver- 1st conventional extraction		Liver- 2nd conventional extraction		Enzymatic cleavage of five aliquots from the 2nd liver extract			
						mean values		standard deviation	
Peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Extract used for HPLC analysis		**85.1**	**0.317**	**84.6**	**0.315**	**78.3**	**0.292**	**1.0**	**0.004**
7	desmethyl-1,2-propandiol-N-GlucA (M36)	9.2	0.034	9.5	0.035	1.4	0.005	0.1	0.000
18	desmethyl-propanol-N-GlucA (M37)	10.8	0.040	11.1	0.041	0.8	0.003	0.1	0.000
19	desmethyl-2-propanol-N-GlucA (M38)	3.0	0.011	3.4	0.013	0.6	0.002	0.2	0.001
22	desmethyl-1,2-propandiol (M07)	5.6	0.021	4.3	0.016	11.0	0.041	0.4	0.001
29	propanol-SA (M26)	1.2	0.005	1.3	0.005	---	---	---	---
33	desmethyl-carboxylic acid (M11)	21.9	0.082	21.1	0.079	25.7	0.096	0.8	0.003
35	desmethyl-propanol (M06)	2.7	0.010	2.5	0.009	14.1	0.053	0.4	0.002
37	carboxylic acid (M12)	5.8	0.022	6.9	0.026	9.0	0.033	0.6	0.002
38	propanol (M01)	1.7	0.006	1.2	0.004	1.7	0.006	0.2	0.001
Total identified		**62.0**	**0.231**	**61.3**	**0.229**	**64.4**	**0.240**	**1.3**	**0.005**
Total characterised		23.1	0.086	22.8	0.085	14.0	0.052	0.6	0.002
Accountability		85.1	0.317	84.1	0.314	78.4	0.292	1.0	0.004

Metabolites in Excreta

The excreta extract of day 1 was analysed by HPLC. Parent compound accounted for 1.2% of the dose. BCS-CN88460-desmethyl-carboxylic acid was the most prominent compound and amounted to 20.0% of the dose. Further nine identified metabolites ranged from 0.8% to 9.6% of the dose.

Generally, metabolites in the extract from excreta were the same as in eggs, organs and tissues, except for metabolites BCS-CN88460-desmethyl-1,2-propandiol-SA, BCS-CN88460-1,2-propandiol-SA and BCS-CN88460-1,2-propandiol which accounted for equal or less than 7.2% of the dose, respectively.

G. Storage Stability of Residues

All samples of eggs, excreta, edible organs and tissues were extracted within three months after sample collection. Quantitative analysis by HPLC was performed either on the day of extraction or up to one day after the start of extraction.

A second conventional extraction of liver was performed approx. 9 months after sampling. The extract was used for enzymatic cleavage experiments. The storage stability was exemplarily demonstrated for these liver samples.

It was therefore concluded, that the metabolic profiles represent the residues in the matrices and analysed samples at sacrifice.

III. Conclusion

The metabolic behaviour of [phenyl-UL-^{14}C]BCS-CN88460 in the laying hen can be characterised by the following observations:

The TRR-values and even the transfer factors in eggs and edible tissues were low with respect to the dose level and the dosing period of 14 days. This indicates that test compound-related radioactivity does not accumulate during the time of feeding. The evaluations of the TRR-values should however consider the fact that an exaggerated dose level of 18.12 mg a.s./kg feed/day was administered. Furthermore, the fact that the entire radioactivity was detected in the excreta and the relatively high TRR in kidney and liver at sacrifice approx. 6 hours after the last administration revealed that the test compound related residues are further metabolised and finally eliminated from the hen's bodies. A residue plateau level in eggs was reached during the course of the experiment at day four after the first administration.

The radioactive residues were extracted with conventional methods from eggs and tissues; extraction rates ranged from 91.8% to 93.3%. Extraction with conventional and exhaustive methods from edible organs accounted for 99.9% for the liver.

The identification rates in eggs, edible organs and tissues ranged between 51.5% and 79.8% of the TRR.

Parent compound was detected in eggs, leg muscle and fat.

Overall nine metabolites were identified in eggs, edible organs and tissues. Metabolites BCS-CN88460-propanol (M01), BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-desmethyl-carboxylic acid (M11), BCS-CN88460-desmethyl-1,2-propandiol (M07) and BCS-CN88460-carboxylic acid (M12) were major residues in eggs, leg muscle, thorax muscle, fat and liver. In liver, metabolites BCS-CN88460-desmethyl-propanol-N-GlucA (M37) and BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA (M36) were further prominent metabolites. Minor metabolites were BCS-CN88460-desmethyl-2-propanol-N-GlucA (M38) and BCS-CN88460-propanol-SA (M26).

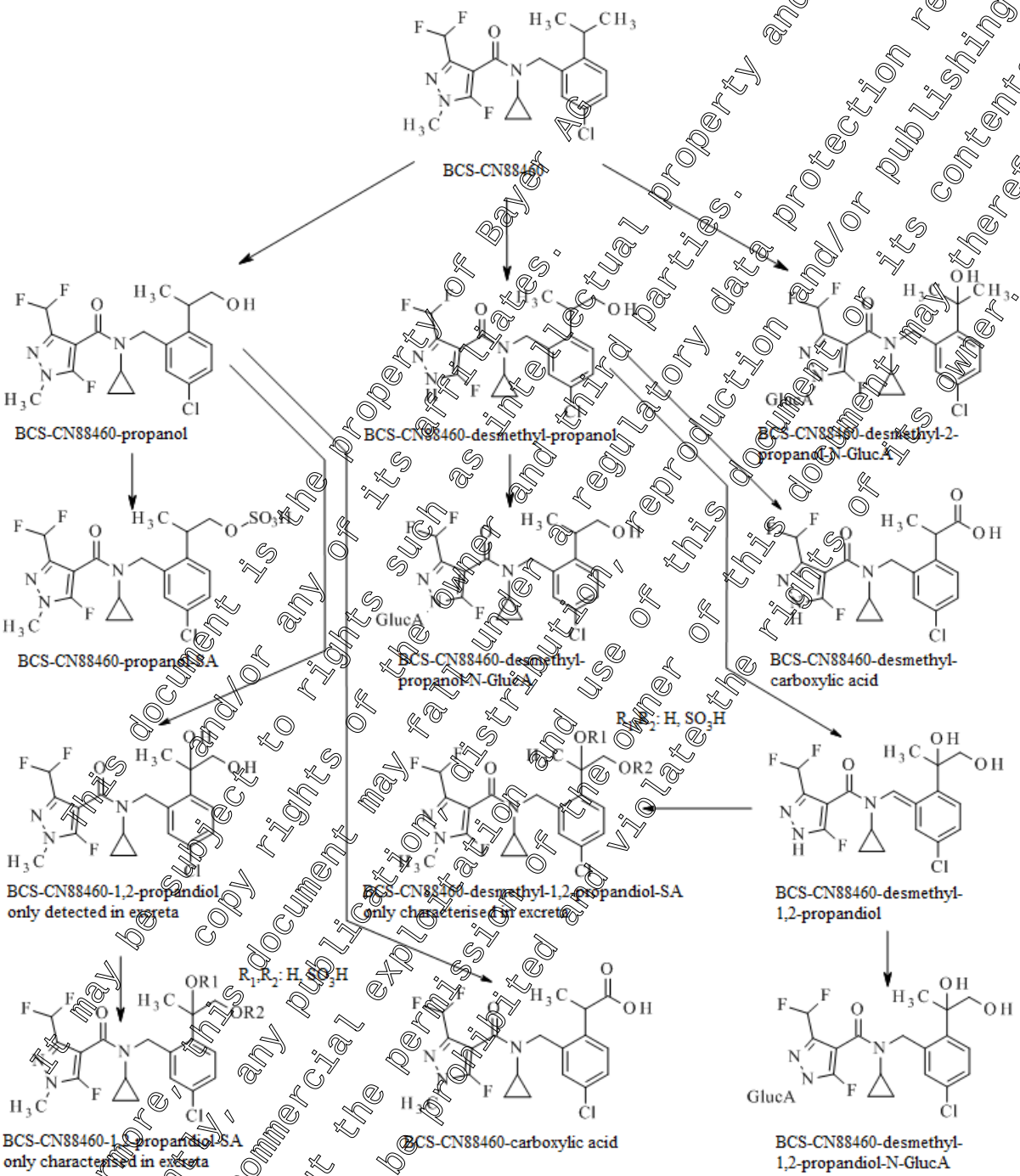
The metabolic profile of excreta from day 1 was similar to the profiles of edible materials, especially liver, with the difference that metabolites BCS-CN88460-desmethyl-1,2-propandiol-SA (M42), BCS-CN88460-1,2-propandiol-SA (M27) and BCS-CN88460-1,2-propandiol (M03) were present.

The principal metabolic reactions of [phenyl-UL-^{14}C]BCS-CN88460 in the laying hen are listed below:

- demethylation of the pyrazole moiety
- conjugation with glucuronic acid after demethylation of the pyrazole moiety
- hydroxylation in position 1 and position 2 of the propyl group in the phenyl ring
- further oxidation of the 1-propanol group was leading to a carboxylic acid group
- conjugation with sulphuric acid after hydroxylation in position 1 or position 2 of the propyl group

Based on these results, the metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in the laying hen is considered as sufficiently understood and a metabolic pathway is proposed in the figure below.

Figure 6.2.2- 2: Proposed metabolic pathway of [phenyl-UL-^{14}C]BCS-CN88460 in laying hens



CA 6.2.3 Lactating ruminants

The metabolism of the fungicide isoflucypram in lactating goats was investigated after administration with isoflucypram either labelled in the pyrazole or in the phenyl moiety.

Table 6.2.3- 1: Overview over available lactating goats metabolism studies

Poultry	Application	Dose	Reference
Lactating goat	5 daily doses over 5 days of pyrazole-labelled isoflucypram via gelatine capsules	1.0 mg/kg bw	M-604281-01-1
Lactating goat	5 daily doses over 5 days of phenyl-labelled isoflucypram via gelatine capsules	1.0 mg/kg bw	M-604286-01-1

Summary of metabolism in ruminants

The metabolite pattern corresponds well when comparing the two metabolism studies in lactating goats. Parent compound was detected in milk, muscle, fat, liver and kidney. It was a major compound in milk, muscle and fat and a minor compound in liver and kidney. BCS-CN88460-2-propanol (M02), BCS-CN88460-propanol (M01), BCS-CN88460-2-propanol-GlucA (M20), BCS-CN88460-propanol-GlucA (isomer 1, M19 - isomer 1) and BCS-CN88460-carboxylic acid (M12) were major metabolites. The metabolic reactions were hydroxylation in position 1 and/or position 2 of the propyl group following conjugation with glucuronic acid.

Report: KCA 6.2.3/01; [redacted], R.; [redacted]; [redacted], A.-K.; [redacted], F.; 2017; M-604281-01-1
Title: [Pyrazole-4-[14C]BCS-CN88460 - Metabolism in the lactating goat
Report No.: EnSa-17-0309
Document No.: M-604281-01-1
Guideline(s): OECD Test Guideline No. 503 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
US EPA OCSPP Test Guideline No. 860.1300
Guideline deviation(s): none
GLP/GEP: yes

Executive Summary

The metabolism and excretion of [pyrazole-4-^{14}C]BCS-CN88460 was investigated in the lactating goat as a model for ruminants. The test compound was orally administered to a lactating goat in gelatine capsules at a dose of approx. 1 mg per kg body weight. Based on the daily feed consumption, the dose level corresponded to 45.19 mg a.s./kg dry feed/day. The goat received five doses at 24-hour intervals in the morning after milking and was sacrificed approx. 6 hours after the last dosing.

Throughout the experiment, the goat was housed in a metabolism cage, which permitted separate collection of urine and faeces. The goat was milked in the morning immediately prior to each administration, about eight hours later in the afternoon and approx. 1 hour before sacrifice. The total radioactive residues (TRRs) were determined in each milk sample and in dissected organs and tissues (muscle, fat, liver and kidney) at sacrifice. The total radioactivity (% of total dose administered) was additionally determined in each urine and faeces sample.

Recovery and Elimination of Radioactivity

The overall recovery accounted for approx. 64% of the total dose. The remaining amount of radioactivity (approx. 36%) was expected to still be present in the non-edible part of the animal body and especially in the gastrointestinal tract at sacrifice due to the short time period between last administration and sacrifice (approx. six hours).

An amount of approx. 0.03% of the total dose was secreted with the milk, only. At sacrifice, radioactive residues in the edible organs and tissues dissected from the body were calculated to be approx. 0.72% of the total dose and were very low.

Up to the time of sacrifice, approx. 6.9% of the total dose was excreted with the urine and approx. 56.1% with faeces. The daily renal excretion of the radioactivity started shortly after the first dosing before the daily renal excretion rate reached a more or less constant level with round about 1.1% to 1.9%. The daily faecal excretion of the radioactivity started after the first dosing. The daily faecal excretion rate exhibited a more or less constant gradient and amounted to 10.4% after the fifth dosing. The cumulative renal and faecal excretion was characterised by a li[redacted] increase from 24 h until sacrifice.

Total Radioactive Residues in Milk, Organs and Tissues

The TRR-values and transfer factors for milk, organs and tissues were very low compared to the dose level of 45.19 mg a.s./kg feed/day and a dosing period of five days.

The TRR-values in milk samples ranged from 0.009 mg/kg to 0.021 mg/kg after the first and third administration, respectively. The time course of TRR-values of the evening and morning milk samples indicated a diurnal pattern for the testing period. The radioactive residues increased significantly during the eight-hour period after each administration.

Regarding organs and tissues, the TRR-values amounted to 0.038 mg/kg for muscle (composite of round and loin muscle), 0.102 mg/kg for fat (composite of perirenal and omental fat), 0.717 mg/kg for liver and 0.189 mg/kg for kidney.

Metabolism

The majority of the residues in the milk as well as organs and tissues were efficiently extracted (extraction rates between 89.2% and 98.7%) using acetonitrile/water (8/2; v/v) mixtures. In case of muscle and liver, the solids after conventional extraction were exhaustively extracted using acetonitrile/water mixtures with microwave treatment. Up to 7.8% of the TRR or 0.036 mg/kg of the residues remained in the post extraction solids (PES). After partitioning of the conventional extracts against n-heptane very low amounts of radioactivity were detected in the organic phases amounting to ≤1.4% of the TRR, only.

Parent compound and metabolites were identified based on co-chromatography with isolated metabolites and reference compounds or by comparison of the metabolite pattern and retention times. Metabolites were isolated from urine and identified by spectroscopic investigations.

The identification rates amounted to 53.7% of the TRR for milk, 67.7% for muscle, 81.5% for fat, 58.3% for liver, and 65.4% for kidney.

Parent compound was detected in milk, muscle, fat, liver and kidney. It was the major compound in milk, muscle and fat and a minor compound in liver and kidney. Overall up to eleven metabolites were identified.

Metabolite BCS-CN88460-2-propanol (M02) was detected in all matrices, amounting from 2.6% to 20.3% of the TRR and represented a major residue (>10% of the TRR) in milk, muscle and fat. Metabolites BCS-CN88460-carboxylic acid (M12, representing a major metabolite in kidney) and BCS-CN88460-propanol (M01, representing a major metabolite in muscle) were detected in muscle, fat, liver and kidney. The amount of BCS-CN88460-carboxylic acid (M12) amounted between 3.3% and 18.0% of the TRR, while the amount of BCS-CN88460-propanol (M01) amounted between 2.7% and 10.2% of the TRR. BCS-CN88460-2-propanol-GlucA (M20) and BCS-CN88460-propanol-GlucA

(isomer 1, M19 – isomer 1) (both representing a major metabolite in liver) were detected in liver and kidney and ranged between 2.6% and 13.8% of the TRR. Metabolites BCS-CN88460-propanol-GlucA (isomer 2, M19 – isomer 2) and BCS-CN88460-desmethyl-propanol (M06) were detected in muscle, liver and kidney and their amount ranged between 0.5% and 7.7% of the TRR. BCS-CN88460-lactic acid (M10) and BCS-CN88460-desmethyl-carboxylic acid (M11) were identified in liver and kidney, BCS-CN88460-N-methyl-pyrazole-carboxylic acid (M50) and BCS-CN88460-propenol-GlucA (M25) were detected in kidney, only. All metabolites were minor abundant and accounted for equal or less than 6.1% of the TRR.

More metabolites in the matrices may be present as indicated by broad non-resolved zones in the chromatograms. All unknown metabolites in the extracts were characterised by their extraction and chromatographic behaviour and amounted to ≤14.3% of the TRR.

The metabolic profiles of urine and faeces were similar to the profiles of edible materials, especially liver and kidney, except that parent compound was not present in the sample of urine.

A summary of the distribution of parent compound and metabolites for edible materials is provided in the following table:

Table 6.2.3- 2: Radioactive residues of parent compound and metabolites in milk and edible organs of lactating goats following oral administration of 5 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample		Milk		Muscle		Fat		Liver		Kidney	
TRR [mg/kg]		**0.015**		**0.036**		**0.104**		**0.717**		**0.189**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***98.7***	***0.015***	***89.2***	***0.032***	***98.3***	***0.102***	***90.1***	***0.646***	***92.2***	***0.174***
39	parent compound	33.4	0.005	22.3	0.008	58.7	0.061	3.5	0.025	2.7	0.005
4	N-methyl-pyrazole-carboxylic acid (M50)	---	---	---	---	---	---	---	---	5.8	0.011
25	2-propanol-GlucA* (M20)	---	---	---	---	---	---	13.8	0.099	2.6	0.005
25	propenol-GlucA* (M25)	---	---	---	---	---	---	---	---	3.6	0.007
26	lactic acid (M10)	---	---	---	---	---	---	1.6	0.011	6.1	0.012
27	propanol-GlucA (isomer 1) (M19, isomer 1)	---	---	---	---	---	---	13.1	0.094	3.1	0.006
28	propanol-GlucA (isomer 2) (M19, isomer 2)	---	---	4.8	0.002	---	---	7.7	0.055	7.0	0.013
30	desmethyl-carboxylic acid (M11)	---	---	---	---	---	---	0.9	0.007	4.2	0.008
31	desmethyl-propanol (M06)	---	---	4.4	0.002	---	---	0.5	0.003	1.6	0.003
33	carboxylic acid (M12)	---	---	8.1	0.003	3.3	0.003	8.9	0.064	18.0	0.034
34	propanol (M01)	---	---	10.2	0.004	2.7	0.003	5.8	0.042	5.6	0.011
36	2-propanol (M02)	20.3	0.003	17.9	0.006	16.8	0.017	2.6	0.019	4.2	0.008
Total identified		**53.7**	**0.008**	**67.7**	**0.024**	**81.5**	**0.085**	**58.3**	**0.418**	**65.4**	**0.123**
Characterised by HPLC		*42.7*	*0.006*	*20.6*	*0.008*	*16.1*	*0.017*	*31.5*	*0.226*	*26.8*	*0.050*
Characterised in organic phase		*1.4*	*<0.001*	*0.9*	*<0.001*	*0.8*	*0.001*	*0.3*	*0.002*	*n.q.*	*n.q.*
Exhaustive extraction		---	---	***4.3***	***0.002***	---	---	***4.8***	***0.035***	---	---
- ACN/water extract		---	---	4.3	0.002	---	---	2.1	0.015	---	---
- HCl extract		---	---	---	---	---	---	2.8	0.020	---	---
Total characterised		44.1	0.006	25.8	0.009	16.8	0.017	36.6	0.262	26.8	0.050
Sum of losses		1.0	0.001	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Total extractable		**98.7**	**0.015**	**93.6**	**0.034**	**98.3**	**0.102**	**94.9**	**0.681**	**92.2**	**0.174**
Post extraction solids (PES)		1.3	<0.001	6.4	0.002	1.7	0.002	5.1	0.036	7.8	0.015
Accountability		**100.0**	**0.015**	**100.0**	**0.036**	**100.0**	**0.104**	**100.0**	**0.717**	**100.0**	**0.189**

* BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propenol-GlucA were co-eluting in the kidney and were subquantified by LC.

The main metabolic reaction was the hydroxylation in the propyl group of the phenyl ring. Conjugation with glucuronic acid was observed after hydroxylation in position 1 or 2 of the propyl group. Another important metabolic reaction was the carboxylation of the 1-propanol group, leading to a carboxylic acid or with a hydroxy group in position 2 of the propyl group to a lactic acid. Minor

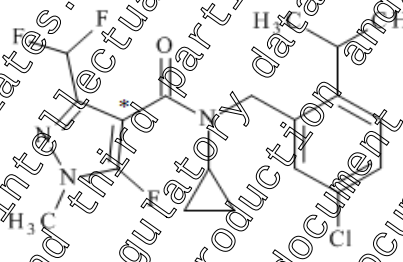
metabolic reactions were the demethylation of the pyrazole moiety, the cleavage of the phenyl moiety in combination with cleavage of the cyclopropyl ring and the dehydration after hydroxylation in position 1 and 2 of the propyl group followed by conjugation with glucuronic acid.

Based on the results the metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in the lactating goat is considered as adequately understood and a metabolic pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material

Chemical structure	 * denotes the ^{14}C-label position	
Radiolabelled test material	[Pyrazole-4-^{14}C]BCS-CN88460	
Specific radioactivity used for administration	4.22 MBq/mg = 2.53 x 10^8 dpm/mg (delivered sample before radiodilution)	2.11 MBq/mg = 1.27 x 10^8 dpm/mg (sample after radiodilution)
Radiochemical purity	~98% (HPLC)	
Non-labelled test material	BCS-CN88460	
Chemical purity	98.4%	
Dose level	5 daily oral doses of 1 mg/kg bw	
Vehicle	Gelatine capsule	

2. Test Animals

Species	Lactating goat (*Capra hircus*)
Strain	"Weiße Deutsche Edelziege"
Breeding facility	[redacted]
Body weight	99 kg at delivery (2015-06-08) 89 kg at first administration (2015-06-15) 90 kg at sacrifice (2015-06-19)
Acclimatization	7 days
Identification	On arrival, the goat was arbitrarily allocated a stock number, or supplier's number. During the acclimation and testing period an individual animal number was allocated.
Husbandry	Conventional hygienic conditions in air-conditioned rooms
Housing	During the acclimation period, the goat was kept in a raised stall with a metal grid as base and straw and hay as bedding. During the whole testing period, the goat was kept in an electro-polished stainless steel metabolism cage for farm animals (goat, sheep, and pig), supplied by [redacted]. With this cage, an almost separate and quantitative collection of urine and faeces was possible. The cage was equipped with a variable-restraining device.
Dietary regimen	During the whole residence time, the goat was fed *ad libitum* with ruminant feed ("Raiffeisen Lamm-Gold" supplied by [redacted]). It was supplemented by hay (during the acclimation period only), hay pellets and carrots. The feed was not a certified diet, i.e. it was not checked for contamination according to current standards. The feed consumption was recorded by back-weighing during the experiment. Tap water from the local mains supply was given *ad libitum* during the whole residence time.

B. Study Design

Preparation of the Test Compound for Administration

The radiolabelled test compound was delivered in solid form. It was diluted in a ratio of 1:1 with the non-radiolabelled test compound. In total, five gelatine capsules containing the solid test compound were prepared and stored in a freezer at -18 °C. The identity confirmation of the test compound was proven by LC-MS/MS. In order to demonstrate the stability of the solid test compound in the capsules, a small portion of the test compound was stored together with the capsules until the last administration. Aliquots of the small portion were analysed for stability and purity of the test compound after the first and the last administration by HPLC. In both cases, the purity amounted to >98%.

Dosing

All oral administrations were performed using a capsule applicator once daily for five consecutive days in the morning after milking. Each gelatine capsule contained an average amount of 89.17 mg, which corresponded to 188 MBq. The total administered amount and radioactivity accounted for 445.87 mg and 941 MBq, respectively.

The total amount of radioactivity administered to the animal served as reference-value (A_0 = 100%) for the percentage calculation of the total radioactivity in the biological samples.

Based upon the experimentally determined daily feed consumption during the testing period of 1,974 g dry feed per day (= 2.22% of the body weight), the dose of 1.0 mg a.s./kg bw corresponded to a concentration of 45.19 mg a.s./kg dry feed per day in the diet. This dose was tolerated without any observable toxicological effects.

Sampling of milk, urine and faeces during the in-life phase

The goat was milked in the morning immediately prior to each administration, about eight hours later in the afternoon and directly before the scheduled termination. After weighing, aliquots were taken from each sample for radioactivity measurement by LSC.

Urine and faeces samples were collected in plastic vessels or collecting grids as quantitatively as possible under dry ice cooling in intervals of 24 hours after the administrations 1 to 4 and 6 hours after the last administration. The vessels were exchanged immediately before the next administration. The collection funnel was rinsed with deionised water into the urine vessel of the respective collection period. After determination of the total volumes, aliquots of each urine sample were used for radioactivity measurement by LSC. For collection of faeces, the collecting grid was cleaned prior to each administration. After determination and recording of the individual weights each faeces fraction was passed several times through a mincing machine in half-frozen state. Aliquots of each faeces fraction were combusted and the radioactivity measured by LSC.

Sacrifice and dissection of organs and tissues

The animal was sacrificed approx. 6 hours after the last administration, a time interval that is consistent with normal slaughtering practices. The animal was at first sedated by an intramuscular mixed injection of Xylazin/Rompun (2%; 0.2 mg/kg bw) and Ketamin (10%; 5 mg/kg bw) and afterwards anaesthetised by an intravenous dose of about 40 mg/kg bw Pentobarbital-Na (Narcoren®). Under deep anaesthesia, the animal was then exsanguinated by transection the jugular vein and finally terminated by intracardiac injection with approx. 10 mL of the veterinary drug "T 61®". Following exsanguination, the following edible organs and tissues were dissected: muscle (round and loin), fat (omental and perirenal), liver (without gall bladder) and kidneys.

Preparation of Organs and Tissues

The organs or tissue samples were transferred into tared plastic vessels. After determination and recording of the individual weights, muscle, fat, liver and kidney samples were passed several times through a mincing machine in half-frozen state. Aliquots of the individual organ and tissue samples were combusted and the radioactivity measured by LSC. All samples were stored at ≤ -18 °C until the start of metabolite analysis.

For metabolism investigations, pooled samples of milk (collected from 32 h until 101 h), muscle (loin and round muscle) and fat (perirenal and omental) were prepared. Liver, kidney and faeces (0 - 24 h) were homogenised and aliquots were stored frozen until start of extraction. The radioactivity and TRR-values of the samples were calculated from the in-life data

C. Analytical Procedures

Sample Extraction and Analysis of Extracts

Aliquots from milk, muscle, fat, liver, kidney and faeces were conventionally extracted three times with a mixture of acetonitrile/water (8/2; v/v) using a Polytron homogeniser. Additionally milk was extracted once with THF and once with acetonitrile/water (3/7; v/v). Minor radioactivity could be released by these additional extractions, only. The combined conventional extracts were partitioned against n-heptane.

The aqueous phases were concentrated by rotary evaporation and subjected to HPLC analysis based on reversed phase chromatography with an acidic water/acetonitrile/THF gradient.

Solids of muscle from the conventional extraction were exhaustively extracted with acetonitrile/water (1/1; v/v) using microwave assistance. Solids of liver from the first conventional extraction were exhaustively extracted twice with acetonitrile/water (8/2; v/v) followed by acetonitrile/water (1/1; v/v) and by 0.1 M HCl using microwave assistance. The metabolites in the exhaustive extract were further characterised using TLC analysis.

Aliquots of the conventional liver extracts and kidney extracts were incubated for 20 hours at 37°C with a defined amount of ß-glucuronidase/arylsulfatase. After incubation, the enzymatic suspensions were purified and analysed by HPLC.

Determination of the Radioactivity in the Cream Fraction of Milk

To evaluate the radioactivity in the cream fraction of milk, an aliquot of the milk sample was partitioned against n-heptane.

Radioactivity measurement

The radioactivity measurement in liquid samples was carried out by liquid scintillation counting (LSC). The solid samples were either dissolved in BIOLUTE S or combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

Metabolite analysis

Parent compound and metabolites were quantified in the extracts by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

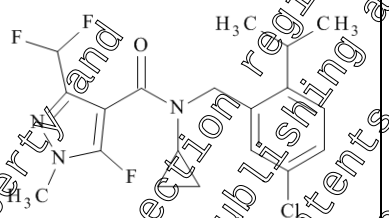
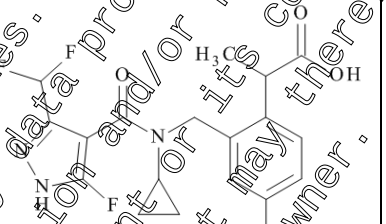
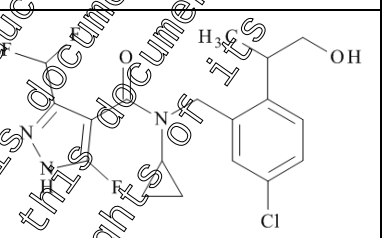
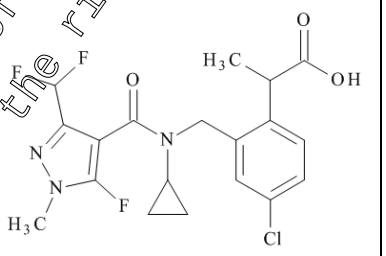
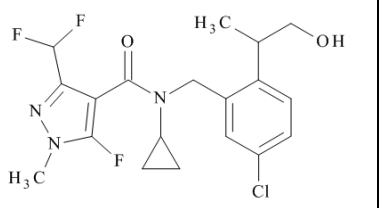
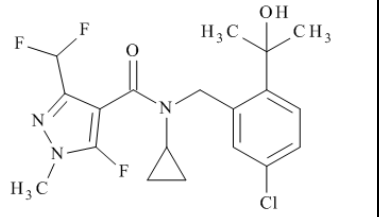
The peaks in the individual HPLC profiles were numbered increasingly in the order of elution in the HPLC-profiling methods. Corresponding metabolites were named with the same peak-ID.

The assignment of parent compound and metabolites was achieved by comparison of HPLC metabolite profiles of the analysed samples among each other and compared to the goat metabolism study with the phenyl label. Furthermore, metabolites were isolated from urine by HPLC. They were identified in the isolated fractions by spectroscopic methods. Other metabolites were identified by chromatographic comparison of the metabolic profile or by co-chromatography with radiolabelled test and reference compounds in selected samples. The conventional extracts of liver and kidney were enzymatically digested. The glucuronic acid conjugates were thereby digested and converted into their corresponding aglycons.

Unknown metabolites were characterised based on their extraction and chromatographic behaviour.

An overview on the reference compounds is given in Table 6.2.3-3.

Table 6.2.3- 3: List of reference compounds

Report name/ other names/codes	Chemical Name (IUPAC)	Chemical Structure
BCS-CN88460 (parent compound) ID: BN4106A1	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	
BCS-CN88460-desmethyl-carboxylic acid ID: BN4114B1	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid	
BCS-CN88460-desmethyl-propanol ID: YK0614E2	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide	
BCS-CN88460-carboxylic acid ID: BN4114F1	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid	
BCS-CN88460-propanol ID: BN4183B	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	
BCS-CN88460-2-propanol ID: YK0614H2	N-[5-chloro-2-(2-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The overall recovery in the lactating goat after administration of a mean daily dose of 1.0 mg [pyrazole-4-^{14}C]BCS-CN88460 per kg body weight (according to 45.19 mg a.s./kg feed/day) on five consecutive days accounted for 63.78% of the total dose. The remaining amount of radioactivity (approx. 36%) was expected to still be present in the gastrointestinal tract at sacrifice, due to the short period between last administration and sacrifice (approx. 6 hours).

Table 6.2.3- 4: Recovery of radioactivity in dissected organs and tissues, milk, and excreta of lactating goats following oral administration of 5 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Biological matrix	Percent of total dose administered
Liver	0.23
Kidney	0.01
Total body muscle	0.23
Total body fat	0.25
Total of organs/tissues	0.72
Milk, 0 – 101 h	0.03
Urine, 0 – 102 h (plus funnel rinsing)	6.94
Faeces, 0 – 102 h	56.09
Total excreted	63.03
Total Recovery	63.78

An amount of approx. 0.03% of the total dose was secreted with the milk, only. At sacrifice, radioactive residues in the organs and tissues dissected from the body were calculated or estimated to be 0.72% of the total dose.

Up to the time of sacrifice, 6.94% of the total dose was excreted with the urine and 56.09% with faeces (see table above). The daily renal excretion of the radioactivity started shortly after the first dosing before the daily renal excretion rate reached a more or less constant level with round about 1.1% to 1.9%. The daily faecal excretion of the radioactivity started after the first dosing. The daily faecal excretion rate exhibited a more or less constant gradient and the faecal excretion reached its maximum after the fifth dosing with 19.4%. The cumulative renal and faecal excretion was characterised by a li[redacted] increase from 24 h until sacrifice

Table 6.2.3- 5: Distribution of residues in liver, kidney, muscle, fat and milk of lactating goats following oral administration of 5 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Organ/Tissue	Collection Time	Fresh weight [g]	TRR [mg/kg]	Transfer factor [TF] ***)	Percent of total dose administered
Liver	6 h after last admin.	1,440.86	0.717	0.016	0.23
Kidney	6 h after last admin.	244.43	0.189	0.004	0.01
Round muscle (sample)	6 h after last admin.	3,340.21	0.035	-----	-----
Loin muscle (sample)		292.03	0.041	-----	-----
Total body muscle **)		27,000.00	0.038	0.001	0.23
Perirenal fat (sample)	6 h after last admin.	778.43	0.095	-----	-----
Omental fat (sample)		1,145.64	0.110	-----	-----
Total body fat **)		10,800.00	0.102	0.002	0.25
Total of organs/tissues					0.72
Milk, total	day 1 to 5	10,014.26	0.014	≤0.001	0.03
Milk, plateau-level	day 3-4	4,584.45	0.015	≤0.001	0.02
Feeding level		45.19	[mg a.s./kg dry feed/day]		

*) Percentage values and fresh weights were calculated from the body weight at sacrifice, assuming 30% and 12% of the body weight for total body muscle and total body fat, respectively.

**) weighted mean TRR-values from the two types of muscle and fat

***) The transfer factor was calculated by dividing the TRR-value of the respective sample by the feeding level (milligrams of a.s. per kilograms dry feed for each day).

B. Levels and Time Course of Total Radioactive Residues in Milk

The radioactivity levels measured in milk samples and the calculated amounts are shown in the table below. The TRR-values in milk samples ranged from 0.009 mg/kg at 24 hours after the first administration to 0.021 mg/kg approx. 8 hours after the third administration and approx. 1 h before sacrifice. The time course of TRR-values of the evening and morning milk indicated a diurnal pattern for the testing period. The radioactive residues increased significantly during the eight-hour period after each administration. A residue plateau-level of 0.015 mg/kg was reached at about day 3-4 after the first administration. This value was calculated from the evening sample of day 3, both samples of day 4 and the morning sample of day 5.

Table 6.2.3- 6: Time course of total radioactivity in the milk of lactating goats following oral administration of 5 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Time after the first admin. [h]	Admin. no.	Cumulative secretion [% of total dose admin.]	Secretion per day [% of total dose admin.]	TRR [mg/kg]
0	1	-----	-----	-----
8		0.0019		0.009
24		0.0048	0.005	0.009
24	2	-----	-----	-----
32		0.0089		0.020
48		0.0126	0.008	0.011
48	3	-----	-----	-----
56		0.0171		0.021
72		0.0202	0.008	0.011
72	4	-----	-----	-----
80		0.0238		0.018
96		0.0277	0.008	0.012
96	5	-----	-----	-----
101		0.0305	0.003	0.021

C. Total Radioactive Residues in the Dissected Organs and Tissues

The highest TRR-value was determined in liver (0.717 mg/kg; 0.23% of total admin. dose) indicating the significance of this organ for metabolism. The TRR-value for kidney accounted for 0.189 mg/kg (0.01% of total admin. dose) and demonstrated that test compound related radioactivity partly was eliminated from the animal via urinary excretion. For body fat, the TRR-value amounted to 0.102 mg/kg (0.25% of total admin. dose assuming 12% of the body weight for this tissue). The lowest TRR-value was determined in muscle (0.038 mg/kg; 0.23% of total admin. dose assuming a value of 30% of the body weight for this tissue).

D. Extraction Efficiency of Residues

The majority of the residues in the milk as well as organs and tissues were efficiently extracted (89.2% to 98.7%) using acetonitrile/water (8/2; v/v) mixtures. In case of muscle and liver, the solids after conventional extraction were exhaustively extracted using acetonitrile/water mixtures with microwave treatment. Only up to 7.8% of the TRR or 0.036 mg/kg of the residues remained in the post extraction solids (PES) of liver and kidney. For sample preparation the extracts were partitioned against n-heptane. Very low amounts of radioactivity were recovered in the organic phase of the extracts amounting to ≤1.4% or ≤0.001 mg/kg of the TRR. Concentration procedures of the aqueous phases caused no losses so all of the residues in the aqueous phases were quantitatively analysed by HPLC.

A summary of the extraction efficiency is shown in the table below.

Table 6.2.3- 7: Extraction efficiency of milk, muscle, fat, liver and kidney samples of lactating goats following oral administration of 5 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample	Milk (32 – 101 h)		Muscle		Fat		Liver		Kidney	
TRR [mg/kg]	0.015		0.036		0.104		0.717		0.189	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	98.7	0.015	89.2	0.032	98.3	0.102	90.1	0.646	92.2	0.174
Exhaustive extraction	---	---	4.3	0.002	---	---	4.8	0.035	---	---
Total extracted	98.7	0.015	93.6	0.034	98.3	0.102	94.9	0.681	92.2	0.174
Post-extraction solids (PES)	1.3	<0.001	6.4	0.002	1.7	0.002	5.1	0.036	7.8	0.015
Accountability	100.0	0.015	100.0	0.036	100.0	0.104	100.0	0.717	100.0	0.189

E. Quantification, Identification and Characterisation of Residues

Quantification of Parent Compound and Metabolites

Parent compound and metabolites were quantified in the extracts as well as in the sample of urine (0-24 h) by HPLC-chromatography based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. Metabolites in the exhaustive extracts from muscle and liver as well as co-eluting metabolites in a fraction of kidney were quantified by TLC.

Metabolites in the extracts as well as in the sample of urine (0-24 h) were assigned to each other by comparison of the metabolite profiles and their retention times. Corresponding metabolites were named with the same peak ID. Detailed information can be found in the report.

Isolation and Identification of Parent Compound and Metabolites

The assignment of parent compound and metabolites was achieved by comparison of HPLC metabolite profiles of the analysed samples among each other and by comparison of the metabolite profiles of the current study with the profiles of the goat metabolism study of the phenyl label.

Metabolites were isolated from urine (24 - 48 h) by HPLC. They were identified in the isolated fractions by spectroscopic methods. In addition parent compound and metabolites were identified in urine (0 - 24 h), in the conventional extracts of milk, muscle and fat as well as in isolated fractions from milk, muscle, fat, liver and kidney by HPLC co-chromatography with radiolabelled reference compounds taken from the hen metabolism study with the pyrazole label or from the goat metabolism study with the phenyl label. Conjugates were subjected to enzymatic cleavage with an aqueous solution of ß-glucuronidase/sulfatase before HPLC-analysis.

F. Distribution of Parent Compound and Metabolites in Milk, Organs and Tissues

The identification rates amounted to 53.7% of the TRR for milk, 67.7% for muscle, 81.5% for fat, 58.3% for liver, and 65.4% for kidney.

Parent compound was detected in milk, muscle, fat, liver and kidney and amounted to 0.005 mg/kg (33.4% of the TRR) for milk, 0.008 mg/kg (22.3% of the TRR) for muscle, 0.061 mg/kg (58.7% of the TRR) for fat, 0.025 mg/kg (3.5% of the TRR) for liver and 0.005 mg/kg (2.7% of the TRR) for kidney.

Metabolites in milk

Besides parent compound as the main residue in milk, BCS-CN88460-2-propanol was the major metabolite (20.3% (0.003 mg/kg) of the TRR).

Metabolites in muscle

Besides parent compound as the main residue, BCS-CN88460-2-propanol was the major metabolite and accounted for 17.9% (0.006 mg/kg) of the TRR. Other prominent metabolites were BCS-CN88460-propanol (10.2% (0.004 mg/kg) of the TRR) and BCS-CN88460-carboxylic acid (8.1% (0.003 mg/kg) of the TRR). BCS-CN88460-desmethyl-propanol (4.4% (0.002 mg/kg) of the TRR) and BCS-CN88460-propanol-GlucA (isomer 2) (4.8% (0.002 mg/kg) of the TRR) were minor compounds.

Metabolites in fat

Besides parent compound, the major metabolite was BCS-CN88460-2-propanol accounting for 16.8% (0.017 mg/kg) of the TRR. BCS-CN88460-carboxylic acid (3.3% (0.003 mg/kg) of the TRR) and BCS-CN88460-propanol (2.7% (0.003 mg/kg) of the TRR) were minor compounds in fat.

Metabolites in liver

BCS-CN88460-2-propanol-GlucA was the main residue in liver and accounted for 0.099 mg/kg (13.8% of the TRR). Other prominent metabolites were BCS-CN88460-propanol-GlucA (isomer 1 and 2) accounting for 0.094 mg/kg (13.1% of the TRR) and 0.055 mg/kg (7.7% of the TRR), respectively, BCS-CN88460-carboxylic acid accounting for 8.9% (0.064 mg/kg) of the TRR, as well as BCS-CN88460-propanol accounting for 0.042 mg/kg (5.8% of the TRR). The four metabolites BCS-CN88460-lactic acid, BCS-CN88460-desmethyl-carboxylic acid, BCS-CN88460-desmethyl-propanol and BCS-CN88460-2-propanol were minor metabolites and ranged between 0.003 mg/kg (0.5% of the TRR) and 0.019 mg/kg (2.6% of the TRR).

Metabolites in kidney

BCS-CN88460-carboxylic acid was the main metabolite in kidney, accounting for 0.034 mg/kg (18.0% of the TRR). Other prominent metabolites in kidney were BCS-CN88460-N-methyl-pyrazole-carboxylic acid (5.8% (0.011 mg/kg) of the TRR), BCS-CN88460-lactic acid (6.1% (0.012 mg/kg) of the TRR), BCS-CN88460-propanol-GlucA (isomer 2) (7.0% (0.013 mg/kg) of the TRR) and BCS-CN88460-propanol (5.6% (0.011 mg/kg) of the TRR). The two minor co-eluting metabolites BCS-CN88460-2-propanol-GlucA (2.6% (0.005 mg/kg) of the TRR) and BCS-CN88460-propenol-GlucA (3.6% (0.007 mg/kg) of the TRR) were subquantified by TLC. Four metabolites BCS-CN88460-propanol-GluA (isomer 1), BCS-CN88460-desmethyl-carboxylic acid, BCS-CN88460-desmethyl-propanol and BCS-CN88460-2-propanol were detected in minor amounts (≤4.2% of the TRR).

More metabolites in the matrices may be present as indicated by broad non-resolved zones in the chromatograms. All unknown metabolites in the extracts were characterised by their extraction and chromatographic behaviour and amounted to each ≤14.3% of the TRR.

The distribution of the parent compound and metabolites in milk, organs and tissues is summarised in the table below.

Table 6.2.3- 8: Radioactive residues of parent compound and metabolites in milk and edible organs of lactating goats following oral administration of 5 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample		Milk		Muscle		Fat		Liver		Kidney	
TRR [mg/kg]		**0.015**		**0.036**		**0.104**		**0.717**		**0.189**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***98.7***	***0.015***	***89.2***	***0.032***	***98.3***	***0.102***	***90.1***	***0.646***	***92.2***	***0.174***
39	parent compound	33.4	0.005	22.3	0.008	58.7	0.061	3.5	0.025	2.7	0.005
4	N-methyl-pyrazole-carboxylic acid (M50)	---	---	---	---	---	---	---	---	5.8	0.011
25	2-propanol-GlucA* (M20)	---	---	---	---	---	---	13.8	0.099	2.6	0.005
25	propenol-GlucA* (M25)	---	---	---	---	---	---	---	---	3.6	0.007
26	lactic acid (M10)	---	---	---	---	---	---	1.6	0.011	6.1	0.012
27	propanol-GlucA (isomer 1) (M19, isomer 1)	---	---	---	---	---	---	13.1	0.094	3.1	0.006
28	propanol-GlucA (isomer 2) (M19, isomer 2)	---	---	4.8	0.002	---	---	7.7	0.055	7.0	0.013
30	desmethyl-carboxylic acid (M11)	---	---	---	---	---	---	0.9	0.007	4.2	0.008
31	desmethyl-propanol (M06)	---	---	4.4	0.002	---	---	0.5	0.003	1.6	0.003
33	carboxylic acid (M12)	---	---	8.1	0.003	3.3	0.003	8.9	0.064	18.0	0.034
34	propanol (M01)	---	---	10.2	0.004	2.7	0.003	5.8	0.042	5.6	0.011
36	2-propanol (M02)	20.3	0.003	17.9	0.006	16.8	0.017	2.6	0.019	4.2	0.008
Total identified		**53.7**	**0.008**	**67.7**	**0.024**	**81.5**	**0.085**	**58.3**	**0.418**	**65.4**	**0.123**
Characterised by HPLC		*42.7*	*0.006*	*20.6*	*0.008*	*16.1*	*0.017*	*31.5*	*0.226*	*26.8*	*0.050*
Number of unknown peaks		5		5		4		17		16	
Largest unknown peak		14.5	0.002	6.8	0.002	7.5	0.008	4.0	0.029	5.7	0.011
Characterised by partition (organic phase)		*1.4*	*<0.001*	*0.9*	*0.001*	*0.8*	*0.001*	*0.3*	*0.002*	*n.q.*	*n.q.*
Exhaustive extraction		---	---	***4.3***	***0.002***	---	---	***4.8***	***0.035***	---	---
- ACN/water extract		---	---	*4.3*	*0.002*	---	---	*2.1*	*0.015*	---	---
Number of unknown peaks		---		5		---		11		---	
Largest unknown peak		---	---	1.0	0.001	---	---	0.9	0.007	---	---
- HCl extract		---	---	---	---	---	---	*2.8*	*0.020*	---	---
Number of unknown peaks		---		---		---		4		---	
Largest unknown peak		---	---	---	---	---	---	2.3	0.017	---	---
Total characterised		**44.1**	**0.006**	**25.8**	**0.009**	**16.8**	**0.017**	**36.6**	**0.262**	**26.8**	**0.050**
Sum of losses		1.0	<0.001	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Total extractable		**98.7**	**0.015**	**93.6**	**0.034**	**98.3**	**0.102**	**94.9**	**0.681**	**92.2**	**0.174**
Post extraction solids (PES)		1.3	<0.001	6.4	0.002	1.7	0.002	5.1	0.036	7.8	0.015
Accountability		**100.0**	**0.015**	**100.0**	**0.036**	**100.0**	**0.104**	**100.0**	**0.717**	**100.0**	**0.189**

* BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propenol-GlucA were co-eluting in the kidney and were subquantified by TLC.

The distribution of radioactivity in milk between the cream fraction and the remaining skimmed milk showed that the major portion of the radioactivity was found in the fraction of skimmed milk (see below).

Table 6.2.3- 9: Distribution of radioactivity in milk between cream and skimmed milk fractions

Sample description	% of TRR	mg/kg
n-heptane phase (cream)	13.3	0.002
aqueous phase (skimmed milk)	86.7	0.013
Total	100.0	0.015

Conjugates in the liver and kidney like BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propanol-GlucA (both isomer 1 and 2) could be enzymatically cleaved to their aglycons (see tables below). The cleavage of some unknown conjugates in non-resolved zones resulted in higher amounts of the aglycons. None of the unknown compounds after cleavage of the liver and kidney extracts accounted for more than 9.6% (0.069 mg/kg) of the TRR (mean of all three replicates) or 9.3% (0.018 mg/kg) of the TRR, respectively. The following main aglycons could be clearly identified after enzymatic cleavage: BCS-CN88460-2-propanol and BCS-CN88460-propanol.

Table 6.2.3- 10: Radioactive residues of parent compound and metabolites in liver samples of first and second conventional extraction and after enzymatic cleavage for 20 h

Sample		**Liver- 1st conventional extraction**		**Liver- 2nd conventional extraction**		**Enzymatic cleavage of three aliquots of liver extracts**			
						mean values		**standard deviation**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Extract used for HPLC		**89.8**	**0.644**	**90.2**	**0.647**	**81.5**	**0.585**	**1.2**	**0.009**
39	parent compound	3.5	0.025	5.0	0.036	6.1	0.044	0.5	0.004
25	2-propanol-GlucA (M20)	13.8	0.099	10.9	0.078	0.9	0.007	0.2	0.002
26	lactic acid (M10)	1.6	0.011	2.6	0.019	0.7	0.005	0.1	0.001
27	propanol-GlucA (isomer 1) (M19, isomer 1)	13.1	0.094	11.1	0.080	---	---	---	---
28	propanol-GlucA (isomer 2) (M19, isomer 2)	7.7	0.055	7.0	0.050	---	---	---	---
30	desmethyl-carboxylic acid (M11)	0.9	0.007	0.8	0.006	2.2	0.015	0.1	0.001
31	desmethyl-propanol (M06)	0.5	0.003	0.7	0.005	4.7	0.033	0.2	0.001
33	carboxylic acid (M12)	8.9	0.064	8.2	0.059	11.7	0.084	0.5	0.004
34	propanol (M01)	5.8	0.042	7.4	0.053	20.8	0.149	0.8	0.006
36	2-propanol (M02)	2.6	0.019	3.7	0.026	11.9	0.085	0.7	0.005
Total identified		**58.3**	**0.418**	**57.4**	**0.412**	**58.9**	**0.423**	**1.1**	**0.008**
Total characterised		31.5	0.226	32.8	0.235	22.6	0.162	1.2	0.009
Accountability		89.8	0.644	90.2	0.647	81.5	0.585	1.2	0.009

Table 6.2.3- 11: Radioactive residues of parent compound and metabolites in kidney samples of first and second conventional extraction and after enzymatic cleavage for 20 h

Sample		Kidney- 1st conventional extraction		Kidney- 2nd conventional extraction		Kidney- enzymatic cleavage of conventional extract - experiment 1		Kidney- enzymatic cleavage of conventional extract- experiment 2	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Extract used for HPLC		**92.2**	**0.174**	**91.8**	**0.173**	**82.1**	**0.155**	**82.1**	**0.155**
39	parent compound	2.7	0.005	3.6	0.007	3.0	0.006	2.2	0.004
4	N-methyl-pyrazole-carboxylic acid (M50)	5.8	0.011	4.8	0.009	1.8	0.003	1.9	0.004
25	2-propanol-GlucA (M20)	7.3	0.014	6.5	0.012	---	---	---	---
26	lactic acid (M10)	6.1	0.012	3.3	0.006	3.0	0.006	3.7	0.007
27	propanol-GlucA (isomer 1) (M19, isomer 1)	5.1	0.096	3.6	0.007	---	---	---	---
28	propanol-GlucA (isomer 2) (M19, isomer 2)	7.0	0.013	7.4	0.014	---	---	---	---
30	desmethyl-carboxylic acid (M11)	4.2	0.008	3.5	0.007	3.8	0.007	4.6	0.009
31	desmethyl-propanol (M06)	1.6	0.003	1.4	0.003	4.9	0.009	4.3	0.008
33	carboxylic acid (M12)	18.0	0.034	18.3	0.035	23.6	0.044	22.7	0.043
34	propanol (M01)	5.6	0.011	3.2	0.006	10.6	0.020	10.8	0.020
36	2-propanol (M02)	4.2	0.008	4.5	0.008	6.6	0.013	6.8	0.013
Total identified		**65.4**	**0.123**	**60.1**	**0.113**	**57.3**	**0.108**	**57.0**	**0.108**
Total characterised		26.8	0.050	31.7	0.060	24.8	0.047	25.1	0.047
Accountability		92.2	0.174	91.8	0.173	82.1	0.155	82.1	0.155

Distribution of Metabolites in Urine and Faeces

The metabolic profile of urine (0 - 24 h) and faeces (0 - 24 h) were similar to the profiles of edible materials, especially liver and kidney, except that parent compound was not present in the sample of urine. In faeces, parent compound was the main residue (8.4% of the first dose). All identified metabolites in urine and faeces accounted for ≤1.3% of the first dose.

The distribution of the parent compound and metabolites in urine and faeces is summarized in the table below.

Table 6.2.3- 12: Radioactive residues of parent compound and metabolites in non-edible samples of lactating goat following oral administration of 5 daily doses of [pyrazole-4-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample		Faeces (0 - 24 h)	Urine (0 – 24 h)
Peak ID	Compound (Report name) BCS-CN88460-	% of dose in the sample	% of dose in the sample
39	parent compound	8.4	1.1
4	N-methyl-pyrazole-carboxylic acid (M50)	---	0.6
25	2-propanol-GlucA (M20)	<0.1	0.3*
25	propenol-GlucA (M25)	---	0.2*
26	lactic acid (M10)	0.1	0.4
27	propanol-GlucA (isomer 1) (M19, isomer 1)	---	0.2
28	propanol-GlucA (isomer 2) (M19, isomer 2)	0.5	0.6
30	desmethyl-carboxylic acid (M11)	0.3	0.3
31	desmethyl-propanol (M06)	0.3	---
33	carboxylic acid (M12)	1.0	1.1
34	propanol (M01)	1.3	---
36	2-propanol (M02)	0.7	---
Sum identified		**12.7**	**3.7**
Subtotal characterised by HPLC		0.4	1.7
number of unknown peaks		*2*	*15*
largest unknown peak		*0.3*	*0.3*
Characterised by partitioning (n-heptane phase)		0.1	---
Total characterised		**0.5**	**1.7**
Sum of losses		---	---
Total extracted		**13.2**	**---**
Post extraction solids (PES)		0.5	---
Total		**13.8**	**5.4**

* BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propenol-GlucA co-eluted in HPLC-analysis with the profiling method BCS460_1. Subquantification in the fraction of the sample of urine resulted in a ratio of 60.6% of BCS-CN88460-2-propanol-GlucA to 39.4% of BCS-CN88460-propenol-GlucA.

G. Storage Stability of Residues

During the study, all samples and extracts were stored in a freezer at ≤ -18 °C or for a short time in a refrigerator. All samples of milk and edible organs and tissues were extracted within three months after sample collection. The first metabolite profile was recorded not later than six days after the start of the extraction and sample preparation.

A second conventional extraction of milk was performed approx. 15 months after sampling, a second conventional extraction of liver was performed approx. 13 months after sampling and a second conventional extraction of kidney was performed approx. 14 months after sampling. From the stored milk sample the distribution of radioactivity in the fractions of skimmed milk and cream was determined. The extracts of liver and kidney were used for enzymatic cleavage experiments. The storage stability was exemplarily demonstrated for these samples. It was therefore concluded, that the metabolic profiles represent the residues in the matrices and analysed samples at sacrifice.

III. Conclusion

The metabolic behaviour of [pyrazole-4-^{14}C]BCS-CN88460 in the lactating goat can be characterised by the following observations:

The TRR-values and the transfer factors in milk, organs and tissues were very low compared to the dose level of 45.19 mg a.s./kg feed/day and a dosing period of five days. The highest TRR-value was detected for liver and was caused by the short time period of 6 hours between last dosing and sacrifice. It indicates the significance of this organ for metabolism. The significantly lower TRR-value for kidney shows that no residues were retained in this tissue and reflects the low amount of radioactivity, which was excreted by the urine. The TRR-values in the respective evening and morning milk samples showed a diurnal pattern as they declined slightly prior to the delivery of the next dose for most days. A continuous increase was observed before a residue plateau-level was reached at day three after the first administration.

The elimination of radioactivity was mainly faecal (56.1% of the dose) and only 6.9% were eliminated via urine. This excretion behaviour was similar to the findings in the ADME studies with rats.

The radioactive residues were efficiently extracted from milk as well as from edible organs and tissues; extraction rates ranged from 92.2% to 98.7%.

The identification rates ranged between 53.7% and 81.5% for the TRR in milk, and edible organs and tissues.

Parent compound was detected in milk, muscle, fat, liver and kidney. Parent compound was a major compound in milk, muscle and fat and a minor compound in liver and kidney. Overall up to eleven metabolites were identified.

BCS-CN88460-2-propanol (M02) was detected as a major metabolite in milk (32 - 101 h), muscle and fat, BCS-CN88460-propanol (M01) was detected as a major metabolite in muscle, BCS-CN88460-2-propanol-GlucA (M20) and BCS-CN88460-propanol-GlucA (isomer 1, M19 – isomer 1) as major metabolites in liver and BCS-CN88460-carboxylic acid (M12) as a major metabolite in kidney. Further abundant metabolites were BCS-CN88460-propanol-GlucA (isomer 2, M19 – isomer 2) in liver and kidney, BCS-CN88460-lactic acid (M10) in kidney, BCS-CN88460-N-methyl-pyrazole-carboxylic acid in kidney (M50), BCS-CN88460-carboxylic acid (M12) in muscle and liver and BCS-CN88460-propanol (M01) in liver and kidney. BCS-CN88460-desmethyl-carboxylic (M11) was a minor compound in liver and kidney. BCS-CN88460-desmethyl propanol (M06) was a minor metabolite in muscle, liver and kidney. BCS-CN88460-propenol-GlucA (M25) was only detected as a minor metabolite in kidney and in urine.

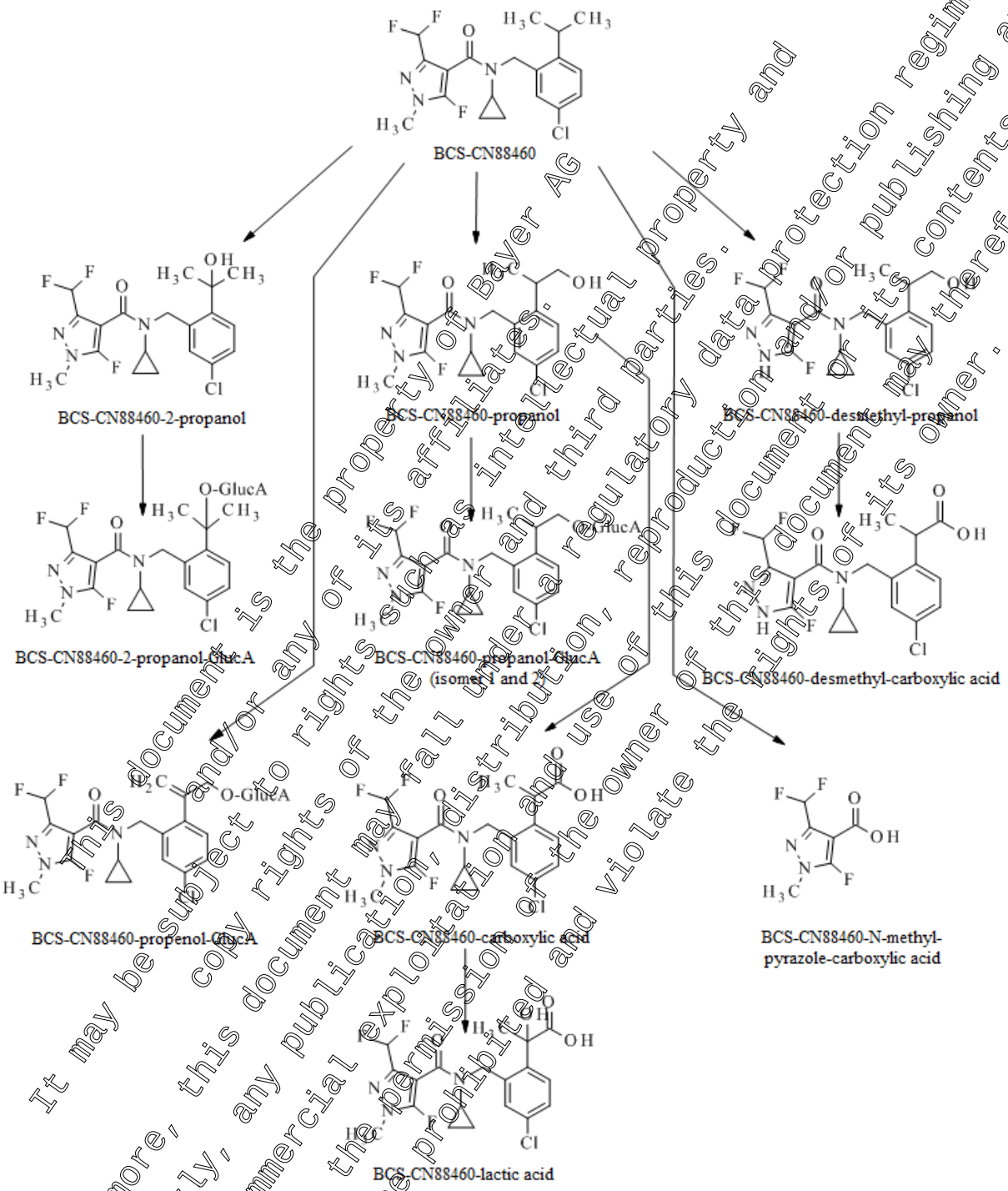
The metabolic profiles of urine and faeces were similar to the profiles of edible materials, especially liver and kidney, except that parent compound was not present in the sample of urine.

The principal metabolic reactions of [pyrazole-4-^{14}C]BCS-CN88460 in the lactating goat are listed below:

- hydroxylation in position 1 or position 2 of the propyl group in the phenyl ring
- conjugation with glucuronic acid
- oxidation of the 1-propanol group was leading to carboxylic acid or with a hydroxy group in position 2 to lactic acid
- demethylation of the pyrazole moiety
- cleavage of the phenyl moiety in combination with cleavage of the cyclopropyl ring leading to BCS-CN88460-N-methyl-pyrazole-carboxylic acid
- dehydration after hydroxylation in position 1 and 2 of the propyl group followed by conjugation with glucuronic acid

Based on these results, the metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in the lactating goat is considered as sufficiently understood and a metabolic pathway is proposed.

Figure 6.2.3- 1: Proposed metabolic pathway of [pyrazole-4-^{14}C]BCS-CN88460 in the lactating goat .



Report:	KCA 6.2.3/02; [redacted], R.; [redacted], A.-K.; [redacted]; [redacted], F.; 2017; M-604286-01-1
Title:	[Phenyl-UL-14C]BCS-CN88460 - Metabolism in the lactating goat
Report No.:	EnSa-17-0308
Document No.:	M-604286-01-1
Guideline(s):	OECD Test Guideline No. 503 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 US EPA OCSPP Test Guideline No. 860.1300
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism and excretion of [phenyl-UL-^{14}C]BCS-CN88460 was investigated in the lactating goat as a model for ruminants. The test compound was orally administered to a lactating goat in gelatine capsules at a dose of approx. 1 mg per kg body weight. Based on the daily feed consumption, the dose level corresponded to 20.57 mg a.s./kg dry feed/day. The goat received five doses at 24-hour intervals in the morning after milking and was sacrificed approx. 6 hours after the last dosing.

Throughout the experiment, the goat was housed in a metabolism cage, which permitted separate collection of urine and faeces. The goat was milked in the morning immediately prior to each administration, about eight hours later in the afternoon and approx. 1 hour before sacrifice. The total radioactive residues (TRRs) were determined in each milk sample and in dissected organs and tissues (muscle, liver and kidney) at sacrifice. Omental fat and perirenal fat could not be found. The goat was relatively young and fatless. The total radioactivity (% of total dose administered) were additionally determined in each urine and faeces sample.

Recovery and Elimination of Radioactivity

The overall recovery accounted for approx. 51% of the total dose. The remaining amount of radioactivity was expected to still be present in the non-edible part of the animal body and especially in the gastrointestinal tract.

An amount of approx. 0.06% of the total dose was secreted with the milk, only. At sacrifice, radioactive residues in the edible organs and tissues dissected from the body were calculated to be approx. 0.27% of the total dose and were very low.

Up to the time of sacrifice, approx. 9.8% of the total dose was excreted with the urine and approx. 40.8% with faeces. The daily renal excretion of the radioactivity started shortly after the first dosing before the daily renal excretion rate reached a more or less constant level with 1.2% to 3.6%. The daily faecal excretion of the radioactivity started after the first dosing. After the third dosing, the excretion rate amounted to approx. 17.5% and the daily faecal excretion rate exhibited a more or less constant gradient. The cumulative renal and faecal excretion was characterised by a li[redacted] increase from day 3 until sacrifice.

Total Radioactive Residues in Milk, Organs and Tissues

The TRR-values and transfer factors for milk, organs and tissues were very low compared to the dose level of 20.57 mg a.s./kg feed/day and a dosing period of five days. The TRR-values in milk samples were very low and ranged from 0.008 mg/kg after the first administration to 0.016 mg/kg after the fourth administration. The time course of TRR-values of the evening and morning milk samples indicated a diurnal pattern for the testing period as a whole. The radioactive residues increased significantly during the eight-hour period after each administration followed by a small decrease measured prior to the delivery of the next dose.

Regarding organs and tissues, the TRR-values amounted to 0.011 mg/kg for muscle (composite of round and loin muscle), 0.348 mg/kg for liver and 0.183 mg/kg for kidney.

Metabolism

The majority of the residues in the milk as well as organs and tissues were efficiently extracted (extraction rates between 88.3% and 100.0%) using acetonitrile/water mixtures. In case of liver, the solids after conventional extraction were exhaustively extracted using acetonitrile/water (1/1, v/v) mixtures with microwave treatment. Up to 8.2% of the TRR (0.029 mg/kg) of the residues remained in the post extraction solids (PES).

For sample preparation the extracts of liver and kidney were partitioned against n-heptane. The extracts of milk and muscle were purified with SPE cartridges and a subsequent phase separation with NaCl. Very low amounts of radioactivity were recovered in the organic phases amounting to ≤0.8% of the TRR. Concentration procedures of the aqueous phases caused minor losses of radioactivity amounting to ≤6.3% of the TRR.

Parent compound and metabolites were identified based on co-chromatography with isolated metabolites and reference compounds or by comparison of the metabolite pattern and retention times. Metabolites were isolated from urine and identified by spectroscopic investigations.

The identification rates amounted to 50.4% of the TRR for milk, 64.0% for muscle, 47.2% for liver, and 42.4% for kidney.

Parent compound was detected in milk, muscle, liver and kidney. It was a major compound in milk and muscle and a prominent compound in liver. Overall up to ten metabolites were identified.

Metabolites BCS-CN88460-2-propanol (M02) and BCS-CN88460-propanol-GlucA (isomer 2, M19 – isomer 2) were detected in all matrices. BCS-CN88460-2-propanol represented a major residue in muscle and amounted between 1.4% and 14.2% of the TRR for all matrices, while BCS-CN88460-propanol-GlucA (isomer 2) amounted between 2.5% and 8.6% of the TRR. Metabolites BCS-CN88460-carboxylic acid (M12) and BCS-CN88460-propanol (M01) were identified in muscle, liver and kidney and ranged between 2.5% and 9.0% of the TRR. BCS-CN88460-2-propanol-GlucA (M20), BCS-CN88460-lactic acid (M10) and BCS-CN88460-desmethyl-carboxylic acid (M11) were detected in liver and kidney and accounted for between 0.5% and 13.0% of the TRR. BCS-CN88460-propanol-GlucA (isomer 1, M19 – isomer 1) was identified in milk, liver and kidney and its amount ranged from 2.3% to 8.8% of the TRR. BCS-CN88460-desmethyl-propanol (M06) was detected in milk, muscle and liver and amounted between 0.6% and 6.7% of the TRR. BCS-CN88460-propenol-GlucA (M25) was detected in kidney and accounted for 6.2% of the TRR.

More metabolites in the matrices may be present as indicated by broad non-resolved zones in the chromatograms. All unknown metabolites in the extracts were characterised by their chromatographic behaviour and amounted to ≤38.6% of the TRR but to very low mg/kg values (0.005 mg/kg).

The metabolic profiles of urine (0 - 24 h) and faeces (0 - 24 h) were similar to the profiles of edible materials, especially liver and kidney, except that parent compound was not present in the sample of urine.

A summary of the distribution of parent compound and metabolites for edible materials is provided in the following table:

Table 6.2.3- 13: Radioactive residues of parent compound and metabolites in milk and edible organs of lactating goats following oral administration of 5 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample		Milk		Muscle		Liver		Kidney	
TRR [mg/kg]		**0.013**		**0.011**		**0.348**		**0.183**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***98.5***	***0.013***	***100.0***	***0.011***	***88.3***	***0.307***	***97.9***	***0.179***
38	parent compound	33.9	0.004	21.5	0.002	5.3	0.018	1.6	0.003
20	2-propanol-GlucA (M20)	---	---	---	---	13.0	0.043	4.0*	0.007
20	propenol-GlucA (M25)	---	---	---	---	---	---	6.2*	0.011
21	lactic acid (M10)	---	---	---	---	1.3	0.005	4.2	0.008
22	propanol-GlucA (isomer 1) (M19, isomer 1)	2.3	<0.001	---	---	8.8	0.031	3.6	0.007
23	propanol-GlucA (isomer 2) (M19, isomer 2)	2.5	<0.001	6.5	0.001	5.9	0.021	8.6	0.016
27	desmethyl-carboxylic acid (M11)	---	---	---	---	0.5	0.002	3.6	0.007
28	desmethyl-propanol (M06)	6.7	0.001	3.1	<0.001	0.6	0.002	---	---
30	carboxylic acid (M12)	---	---	9.0	0.001	4.2	0.015	6.8	0.012
31	propanol (M01)	---	---	9.0	0.001	4.9	0.017	2.5	0.004
34	2-propanol (M02)	5.0	0.001	14.2	0.002	2.8	0.010	1.4	0.003
Total identified		**50.4**	**0.007**	**64.0**	**0.007**	**47.2**	**0.164**	**42.4**	**0.078**
Characterised by HPLC		*47.3*	*0.006*	*29.8*	*0.003*	*37.8*	*0.132*	*54.4*	*0.100*
Exhaustive extraction (ACN/water)		---	---	---	---	***3.5***	***0.012***	---	---
Total characterised		47.3	0.006	29.8	0.003	41.3	0.144	54.4	0.100
Sum of losses		0.8	<0.001	6.3	0.001	3.2	0.011	1.1	0.002
Total extractable		**98.5**	**0.013**	**100.0**	**0.011**	**91.8**	**0.319**	**97.9**	**0.179**
Post extraction solids (PES)		1.5	<0.001	n.q.	n.q.	8.2	0.029	2.1	0.004
Accountability		**100.0**	**0.013**	**100.0**	**0.011**	**100.0**	**0.348**	**100.0**	**0.183**

* BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propenol-GlucA were co-eluting in the chromatography of the extract of kidney and were subquantified by TLC.

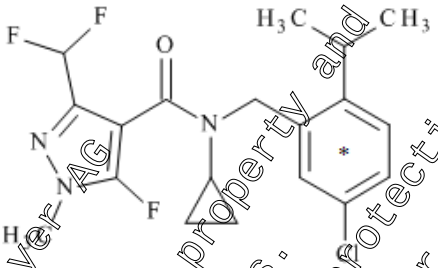
The main metabolic reaction was the hydroxylation in the propyl group of the phenyl ring. Conjugation with glucuronic acid was observed after hydroxylation in position 1 or 2 of the propyl group. Another important metabolic reaction was the carboxylation of the 1-propanol group, leading to a carboxylic acid or with a hydroxy group in position 2 of the propyl group to a lactic acid group. Minor metabolic reactions were the demethylation of the pyrazole moiety, hydroxylation in position 4 of the phenyl moiety and the dehydration after hydroxylation in position 1 and 2 of the propyl group followed by conjugation with glucuronic acid.

Based on the results the metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in the lactating goat is considered as adequately understood and a metabolic pathway is proposed.

I. Materials and Methods

A. Materials

1. Test Material

Chemical structure	 * denotes the ^{14}C-label position
Radiolabelled test material	[Phenyl-UL-^{14}C]BCS-CN88460
Specific radioactivity used for administration	4.13 MBq/mg = 2.48 x 10^8 dpm/mg (delivered sample before radiodilution) | 2.065 MBq/mg =1.24 x 10^8 dpm/mg (sample after radiodilution)
Radiochemical purity	>98% (HPLC)
Non-labelled test material	BCS-CN88460
Chemical purity	99.4%
Dose level	5 daily oral doses of 1 mg/kg bw
Vehicle	Capsule

2. Test Animals

Species	Lactating goat (*Capra hircus*)
Strain	"Weiße Deutsche Edelziege"
Breeding facility	[redacted]
Body weight	54.8 kg at delivery (2015-08-10) 47.3 kg at first administration (2015-08-17) 45.5 kg at sacrifice (2015-08-21)
Acclimatization	7 days
Identification	On arrival, the goat was arbitrarily allocated a stock number, or supplier's number. During the acclimation and testing period an individual animal number was allocated.
Husbandry	Conventional hygienic conditions in air-conditioned rooms
Housing	During the acclimation period, the goat was kept in a raised stall with a metal grid as base and straw and hay as bedding. During the whole testing period, the goat was kept in an electro-polished stainless steel metabolism cage for farm animals (goat, sheep, and pig), supplied by [redacted]. With this cage, an almost separate and quantitative collection of urine and faeces was possible. The cage was equipped with a variable-restraining device.
Dietary regimen	During the whole residence time, the goat was fed *ad libitum* with ruminant feed ("Raiffeisen Lamm-Gold" supplied by [redacted]). It was supplemented by hay (during the acclimation period only), hay pellets and carrots. The feed was not a certified diet, i.e. it was not checked for contamination according to current standards. The feed consumption was recorded by back-weighing during the experiment. Tap water from the local mains supply was given *ad libitum* during the whole residence time.

B. Study Design

Preparation of the Test Compound for Administration

The radiolabelled test compound was delivered in solid form. It was diluted in a ratio of 1:1 with the non-radiolabelled test compound. An amount of approx. 47.3 mg of this diluted test compound was taken for preparation of each administration capsule. In total, five gelatine capsules containing the solid test compound were prepared and stored in a freezer at ≤ -18 °C. A small portion of the test compound was dissolved in acetonitrile from which an aliquot was taken for identity confirmation of the test compound by LC-MS/MS.

In order to demonstrate the stability of the solid test compound in the capsules, a small portion of the test compound was stored together with the capsules until the last administration. Aliquots of the small portion were analysed for stability and purity of the test compound after the first and the last administration by HPLC. In both cases, the purity amounted to >98%.

Dosing

All oral administrations were performed using a capsule applicator once daily for five consecutive days in the morning after milking. Each gelatine capsule contained an average amount of 47.31 mg, which corresponded to 97.7MBq. The total administered amount and radioactivity accounted for 236.56 mg and 488 MBq, respectively.

The total amount of radioactivity administered to the animal served as reference-value (A_0 = 100%) for the percentage calculation of the total radioactivity in the biological samples.

Based upon the experimentally determined daily feed consumption during the testing period of 2,300 g dry feed per day (= 4.86% of the body weight), the dose of 1.0 mg a.s./kg bw corresponded to a concentration of 20.57 mg a.s./kg dry feed per day in the diet. This dose was tolerated without any observable toxicological effects.

Sampling of milk, urine and faeces during the in-life phase

The goat was milked in the morning immediately prior to each administration, about eight hours later in the afternoon and directly before the scheduled termination. After weighing, aliquots were taken from each sample for radioactivity measurement by LSC.

Urine and faeces samples were collected in plastic vessels or collecting grids as quantitatively as possible under dry ice cooling in intervals of 24 hours after the administrations 1 to 4 and 6 hours after the last administration. The vessels were exchanged immediately before the next administration. The collection funnel was rinsed with deionised water into the urine vessel of the respective collection period. After determination of the total volumes, aliquots of each urine sample were used for radioactivity measurement by LSC. For collection of faeces, the collecting grid was cleaned prior to each administration. After determination and recording of the individual weights each faeces fraction was passed several times through a mincing machine in half-frozen state. Aliquots of each faeces fraction were combusted and the radioactivity measured by LSC.

Sacrifice and dissection of organs and tissues

The animal was sacrificed approx. 6 hours after the last administration, a time interval that is consistent with normal slaughtering practices. The animal was at first sedated by an intramuscular mixed injection of Xylazin/Rompun (2%; 0.2 mg/kg bw) and Ketamin (10%; 5 mg/kg bw) and afterwards anaesthetised by an intravenous dose of about 40 mg/kg bw Pentobarbital-Na (Narcoren®). Under deep anaesthesia, the animal was then exsanguinated by transection the jugular vein and finally terminated by intracardiac injection with approx. 10 mL of the veterinary drug "T 61®". Following exsanguination, the following edible organs and tissues were dissected: muscle (round and loin), liver (without gall bladder) and kidneys. Omental fat and perirenal fat could not be found. The goat was relatively young and fatless.

Preparation of Organs and Tissues

The organs or tissue samples were transferred into tared plastic vessels. After determination and recording of the individual weights, muscle, liver and kidney samples were passed several times through a mincing machine in half-frozen state. Aliquots of the individual organ and tissue samples were combusted and the radioactivity measured by LSC. All samples were stored at -18 °C until the start of metabolite analysis.

For metabolism investigations, pooled samples of milk (collected from 32 h until 101 h) and muscle (loin and round muscle) were prepared. Liver, kidney and faeces (0 - 24 h) were homogenised and aliquots were stored frozen until start of extraction. The radioactivity and TRR-values of the samples were calculated from the in-life data

C. Analytical Procedures

Sample Extraction and Analysis of Extracts

Aliquots from liver, kidney and faeces were conventionally extracted three times, aliquots from milk and muscle were extracted two times, with a mixture of acetonitrile/ water (8/2; v/v) using a Polytron homogeniser. The combined extracts of liver, kidney and faeces were partitioned against n-heptane, the combined extracts of milk and muscle were purified using a C18 cartridge. The purified extracts were concentrated by rotary evaporation and subjected to HPLC analysis based on reversed phase chromatography with an acidic water/acetonitrile/THF gradient.

Solids of liver from the conventional extraction were exhaustively extracted twice with acetonitrile/water (1/1, v/v) using microwave assistance. The metabolites in the combined exhaustive extracts were further characterised using TLC analysis.

Aliquots of the conventional liver and kidney extracts were incubated for 20 hours at 37°C with a defined amount of ß-glucuronidase/arylsulfatase. After incubation, the enzymatic suspensions were purified and analysed by HPLC.

Determination of the Radioactivity in the Cream Fraction of Milk

To evaluate the radioactivity in the cream fraction of milk, an aliquot of the milk sample was partitioned against n-heptane.

Radioactivity measurement

The radioactivity measurement in liquid samples was carried out by liquid scintillation counting (LSC). The solid samples were either dissolved in BIOLUTE S or combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

Metabolite analysis

Parent compound and metabolites were quantified in the extracts by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. The peaks in the individual HPLC profiles were numbered increasingly in the order of elution in the HPLC-profiling methods. Corresponding metabolites were named with the same peak-ID.

The assignment of parent compound and metabolites was achieved by comparison of HPLC metabolite profiles of the analysed samples among each other and compared to the goat metabolism study with the pyrazole label. Furthermore, metabolites were isolated from urine by HPLC. They were identified in the isolated fractions by spectroscopic methods. Other metabolites were identified by chromatographic comparison of the metabolic profile or by co-chromatography with radiolabelled test and reference compounds in selected samples. The conventional extracts of liver and kidney were enzymatically digested. The glucuronic acid conjugates were thereby digested and converted into their corresponding aglycons.

Unknown metabolites were characterised based on their extraction and chromatographic behaviour.

An overview on the reference compounds is given in Table 6.2.3.-14.

Table 6.2.3- 14: List of reference compounds

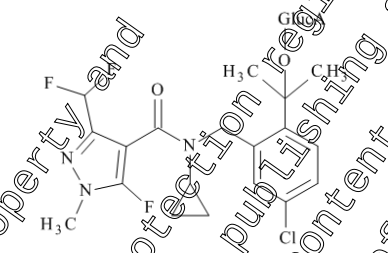
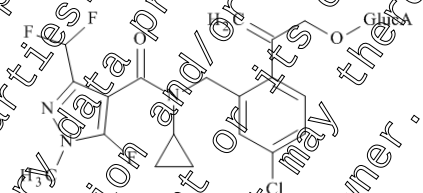
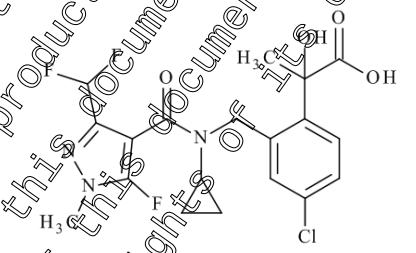
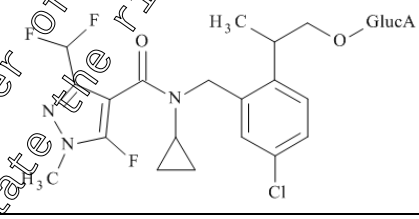
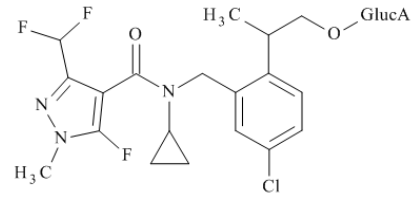
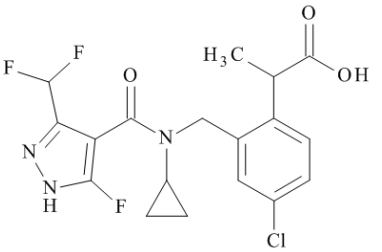
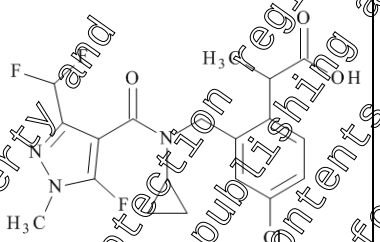
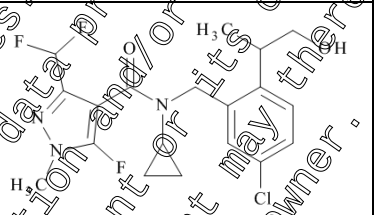
Report name/ other names/codes	Chemical Name (IUPAC)	Chemical Structure
BCS-CN88460-2-propanol-GlucA ID: YK0413E	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propan-2-yl beta-D-glucopyranosiduronic acid	
BCS-CN88460-propenol-GlucA ID: YK0413F	2-{4-chloro-2-[(cycloprop yl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}prop-2-en-1-yl beta-D-glucopyranosiduronic acid	
BCS-CN88460-lactic acid ID: YK0413C	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}-2-hydroxypropanoic acid	
BCS-CN88460-propanol-GlucA (isomer 1) ID: YK0413B	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl glucopyranosiduronic acid	
BCS-CN88460-propanol-GlucA (isomer 2) ID: YK0413D	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl glucopyranosiduronic acid	
BCS-CN88460-desmethyl-carboxylic acid ID: BN4114B	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid	

Table is continued on the next page.

Table 6.2.3- 14 continued

Report name/ other names/codes	Chemical Name (IUPAC)	Chemical Structure
BCS-CN88460-carboxylic acid/ BCS-CY26497 ID: BN4114F	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid	
BCS-CN88460-propanol ID: BN4183B	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The overall recovery in the lactating goat after administration of a mean daily dose of 1.0 mg [phenyl-UL-^{14}C]BCS-CN88460 per kg body weight (according to 20.57 mg a.s./kg feed/day) on five consecutive days accounted for approx. 51% of the total dose . The remaining amount of radioactivity was expected to still be present in the non-edible part of the animal body and especially in the gastrointestinal tract.

Table 6.2.3-15: Recovery of radioactivity in dissected organs and tissues, milk, and excreta of lactating goats following oral administration of 5 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Biological matrix	Percent of total dose administered
Liver	0.19
Kidney	0.01
Total body muscle	0.06
Total of organs/tissues	0.27
Milk, 0 – 102 h	0.06
Urine, 0 – 102 h (plus funnel rinsing)	9.81
Faeces, 0 – 102 h	40.78
Total excreted	50.59
Total Recovery	50.91

An amount of approx. 0.06% of the total dose was secreted with the milk, only. At sacrifice, radioactive residues in the organs and tissues dissected from the body were calculated to be 0.27% of the total dose.

Up to the time of sacrifice, 9.81% of the total dose was excreted with the urine and 40.78% with faeces (see table above). The daily renal excretion of the radioactivity started shortly after the first dosing before the daily renal excretion rate reached a more or less constant level with round about 1.2% to 3.6%. The daily faecal excretion of the radioactivity started after the first dosing. After the second dosing, the faecal excretion rate exhibited a more or less constant gradient and the faecal excretion reached its maximum with 17.5% after the fourth dosing. The cumulative renal and faecal excretion was characterised by a li[redacted] increase from day 3 until sacrifice.

Table 6.2.3- 16: Distribution of residues in liver, kidney, muscle and milk of lactating goats following oral administration of 5 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Organ/Tissue	Collection Time	Fresh weight [g]	TRR [mg/kg]	Transfer factor [TF] ***)	Percent of total dose administered
Liver	6 h after last admin.	1,321.66	0.348	0.017	0.19
Kidney	6 h after last admin.	155.38	0.183	0.009	0.01
Round muscle (sample)	6 h after last admin.	1,922.53	0.011	-----	-----
Loin muscle (sample)		140.80	0.010	-----	-----
Total body muscle **)		13,650.00	0.011	0.001	0.06
Total of organs/tissues					0.27
Milk, total	day 1 to 5	11,321.80	0.011	0.001	0.06
Milk, plateau-level	day 3-4	5,873.21	0.012	0.001	0.03
Feeding level		20.57	[mg a.s./kg dry feed/day]		

*) Percentage values and fresh weights were calculated from the body weight at sacrifice, assuming 30% of the body weight for total body muscle.
**) weighted mean TRR-values from the two types of muscle
***) The transfer factor was calculated by dividing the TRR-value of the respective sample by the feeding level (milligrams of a.s. per kilograms dry feed for each day).

B. Levels and Time Course of Total Radioactive Residues in Milk

The radioactivity levels measured in milk samples and the calculated amounts are shown in the table below. The TRR-values in milk samples ranged from 0.008 mg/kg after the first administration to 0.016 mg/kg after the fourth administration. The time course of TRR-values of the evening and morning milk indicated a diurnal pattern for the testing period. The radioactive residues increased significantly during the eight-hour period after the first three administrations.

A residue plateau-level of 0.012 mg/kg was reached at about day 3-4 after the first administration. This value was calculated from the evening sample of day 3, both samples of day 4 and the morning sample of day 5.

Table 6.2.3- 17: Time course of total radioactivity in the milk of lactating goats following oral administration of 5 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Time after the first admin. [h]	Admin. no.	Cumulative secretion [% of total dose admin.]	Secretion per day [% of total dose admin.]	TRR [mg/kg]
0	1	-----	-----	-----
8		0.0042		0.008
24		0.0082	0.008	0.008
24	2	-----	-----	-----
32		0.0125		0.015
48		0.0201	0.012	0.012
48	3	-----	-----	-----
56		0.0263		0.015
72		0.0347	0.015	0.011
72	4	-----	-----	-----
80		0.0419		0.016
96		0.0499	0.015	0.009
96	5	-----	-----	-----
101		0.0550	0.005	0.015

C. Total Radioactive Residues in the Dissected Organs and Tissues

The highest TRR-value was determined in liver (0.348 mg/kg; 0.19% of total admin. dose) indicating the significance of this organ for metabolism. The TRR-value for kidney accounted for 0.183 mg/kg (0.01% of total admin. dose) and demonstrated that test compound related radioactivity partly was eliminated from the animal via urinary excretion. The lowest TRR-value was determined in muscle (0.011 mg/kg; 0.06% of total admin. dose assuming a value of 30% of the body weight for this tissue).

D. Extraction Efficiency of Residues

The majority of the residues in the milk as well as organs and tissues were efficiently extracted (88.3% to 100.0%) using acetonitrile/water mixtures. In case of liver, the solids after conventional extraction were exhaustively extracted using acetonitrile/water mixtures with microwave treatment. Up to 8.2% of the TRR (0.029 mg/kg) remained in the post extraction solids (PES). After clean-up and concentration procedure the main portion of the extracted radioactivity was quantitatively analysed by HPLC.

A summary of the extraction efficiency is shown in the table below.

Table 6.2.3- 18: Extraction efficiency of milk, muscle, liver and kidney samples of lactating goats following oral administration of 5 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample	Milk (32 – 101 h)		Muscle		Liver		Kidney	
TRR [mg/kg]	**0.013**		**0.011**		**0.348**		**0.183**	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	98.5	0.013	100.0	0.011	88.3	0.307	97.9	0.179
Exhaustive extraction	---	---	---	---	3.5	0.012	---	---
Total extracted	98.5	0.013	100.0	0.011	91.8	0.319	97.9	0.179
Post-extraction solids (PES)	1.5	<0.001	n.q.	n.q.	8.2	0.029	2.1	0.004
Accountability	100.0	0.013	100.0	0.011	100.0	0.348	100.0	0.183

E. Quantification, Identification and Characterisation of Residues

Quantification of Parent Compound and Metabolites

Parent compound and metabolites were quantified in the conventional extracts of milk, edible organs, tissues and faeces as well as in urine (0 - 24 h) by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. Metabolites in the exhaustive extract of liver as well as co-eluting metabolites in an fraction of kidney were quantified by TLC.

Metabolites in the extracts as well as in the sample of urine (0 - 24 h) were assigned to each other by comparison of the metabolite profiles and their retention times. Corresponding metabolites were named with the same peak ID. Detailed information can be found in the report.

Isolation and Identification of Parent Compound and Metabolites

The assignment of parent compound and metabolites was achieved by comparison of HPLC metabolite profiles of the analysed samples among each other and by comparison of the metabolite profiles of the current study with the profiles of the goat metabolism study of the pyrazole label.

Metabolites were isolated from urine (24 - 48 h) by HPLC. They were identified in the isolated fractions by spectroscopic methods. In addition parent compound and metabolites were identified in urine (0 - 24 h), in the conventional extracts of milk and muscle as well as in isolated fractions from milk, muscle, liver and kidney by HPLC co-chromatography with radiolabelled reference compounds taken from the hen metabolism study with the pyrazole label or from the goat metabolism study with the yrazole label label. Conjugates were subjected to enzymatic cleavage with an aqueous solution of ß-glucuronidase/sulfatase before HPLC-analysis.

F. Distribution of Parent Compound and Metabolites in Milk, Organs and Tissues

The identification rates amounted to 50.4% of the TRR for milk, 64.0% for muscle, 47.2% for liver, and 42.4% for kidney.

Parent compound was detected in milk, muscle, liver and kidney and amounted to 0.004 mg/kg (33.9% of the TRR) for milk, 0.002 mg/kg (21.5% of the TRR) for muscle, 0.018 mg/kg (5.3% of the TRR) for liver and 0.003 mg/kg (1.6% of the TRR) for kidney.

Metabolites in milk

Besides parent compound as the main residue in milk, BCS-CN88460-desmethyl-propanol was the major metabolite (6.7% (0.001 mg/kg) of the TRR). Metabolites BCS-CN88460-2-propanol and BCS-CN88460-propanol-GlucA (isomer 1 and 2) were minor metabolites (all ≤5.0% (0.001 mg/kg) of the TRR).

Metabolites in muscle

Besides parent compound as the main residue in muscle, BCS-CN88460-2-propanol was the major metabolite accounting for 14.2% (0.002 mg/kg) of the TRR. Other prominent metabolites were BCS-CN88460-propanol-GlucA (isomer 2) (6.5% (0.001 mg/kg) of the TRR), BCS-CN88460-carboxylic acid (9.0% (0.001 mg/kg) of the TRR) and BCS-CN88460-propanol (9.0% (0.001 mg/kg) of the TRR). BCS-CN88460-desmethyl-propanol was present in minor amounts (3.7% (<0.001 mg/kg) of the TRR).

Metabolites in liver

BCS-CN88460-2-propanol-GlucA was the main residue in liver and accounted for 0.045 mg/kg (13.0% of the TRR). Other prominent metabolites BCS-CN88460-propanol-GlucA (isomer 1 and 2) accounting for 0.031 mg/kg (8.8% of the TRR) and 0.021 mg/kg (5.9% of the TRR), respectively. The six metabolites BCS-CN88460-lactic acid, BCS-CN88460-desmethyl-carboxylic acid, BCS-CN88460-desmethyl-propanol, BCS-CN88460-carboxylic acid, BCS-CN88460-propanol and BCS-CN88460-2-propanol were minor metabolites, amounting between 0.002 mg/kg (0.5% of the TRR) and 0.017 mg/kg (4.9% of the TRR).

Metabolites in kidney

Prominent metabolites in kidney were BCS-CN88460-carboxylic acid and BCS-CN88460-propanol-GlucA (isomer 2). They accounted for 0.012 mg/kg (6.8% of the TRR) and 0.016 mg/kg (8.6% of the TRR), respectively. Two further abundant metabolites in kidney were identified as BCS-CN88460-2-propanol-GlucA (4.0% (0.007 mg/kg) of the TRR) and BCS-CN88460-propenol-GlucA (6.2% (0.011 mg/kg) of the TRR). The five minor metabolites BCS-CN88460-lactic acid, BCS-CN88460-propanol-GlucA (isomer 1), BCS-CN88460-desmethyl-carboxylic acid, BCS-CN88460-propanol and BCS-CN88460-2-propanol were detected (≤0.008 mg/kg or ≤4.2% of the TRR).

More metabolites in the matrices may be present as indicated by broad non-resolved zones in the chromatograms. All unknown metabolites in the extracts were characterised by their extraction and chromatographic behaviour and amounted to each ≤3.8.6% of the TRR but to very low mg/kg amounts (0.005 mg/kg).

The distribution of the parent compound and metabolites in milk, organs and tissues is summarised in the table below.

Table 6.2.3- 19: Radioactive residues of parent compound and metabolites in milk and edible organs of lactating goats following oral administration of 5 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample		Milk		Muscle		Liver		Kidney	
TRR [mg/kg]		**0.013**		**0.011**		**0.348**		**0.183**	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		***98.5***	***0.013***	***100.0***	***0.011***	***88.3***	***0.307***	***97.9***	***0.179***
38	parent compound	33.9	0.004	21.5	0.002	5.3	0.018	1.6	0.003
20	2-propanol-GlucA (M20)	---	---	---	---	13.0	0.043	4.0*	0.007
20	propenol-GlucA (M25)	---	---	---	---	---	---	6.2*	0.011
21	lactic acid (M10)	---	---	---	---	1.3	0.005	4.2	0.008
22	propanol-GlucA (isomer 1) (M19, isomer 1)	2.3	<0.001	---	---	8.8	0.031	3.6	0.007
23	propanol-GlucA (isomer 2) (M19, isomer 2)	2.5	<0.001	6.5	0.001	5.9	0.021	8.6	0.016
27	desmethyl-carboxylic acid (M11)	---	---	---	---	0.5	0.002	3.6	0.007
28	desmethyl-propanol (M06)	6.7	0.001	3.2	<0.001	0.6	0.002	---	---
30	carboxylic acid (M12)	---	---	9.0	0.001	4.2	0.015	6.8	0.012
31	propanol (M01)	---	---	9.0	0.001	4.9	0.017	2.5	0.004
34	2-propanol (M02)	5.0	0.001	14.2	0.002	2.8	0.010	1.4	0.003
Total identified		**50.4**	**0.007**	**64.0**	**0.007**	**47.2**	**0.164**	**42.4**	**0.078**
Characterised in the conventional extract by HPLC		47.3	0.006	29.8	0.003	37.8	0.132	54.4	0.100
Number of unknown peaks		3		2		18		21	
Largest unknown peak		38.6	0.005	25.0	0.003	6.7	0.023	9.1	0.017
Exhaustive extraction using ACN/water		---	---	---	---	***3.5***	***0.012***	---	---
Number of unknown peaks		---		---		5		---	
Largest unknown peak		---	---	---	---	1.7	0.006	---	---
Total characterised		47.3	0.006	29.8	0.003	41.3	0.144	54.4	0.100
Sum of losses		0.8	<0.001	6.1	0.001	3.2	0.011	1.1	0.002
Total extractable		**98.5**	**0.013**	**100.0**	**0.011**	**91.8**	**0.319**	**97.9**	**0.179**
Unextractable (PES)		1.5	<0.001	n.q.	n.q.	8.2	0.029	2.1	0.004
Accountability		**100.0**	**0.013**	**100.0**	**0.011**	**100.0**	**0.348**	**100.0**	**0.183**

* BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propenol-GlucA were co-eluting in the kidney and were subquantified by TLC.

The distribution of radioactivity in milk between the cream fraction and the remaining skimmed milk showed that the radioactivity was distributed evenly between the fractions of cream and skimmed milk (see table below).

Table 6.2.3- 20: Distribution of radioactivity in milk between cream and skimmed milk fractions

Sample description	% of TRR	mg/kg
n-heptane phase (cream)	49.7	0.006
aqueous phase (skimmed milk)	50.3	0.007
Total:	100.0	0.013

Conjugates in the liver and kidney like BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propanol-GlucA (both isomers 1 and 2) could be enzymatically cleaved to their aglycons. The cleavage of some unknown conjugates in non-resolved zones resulted in higher amounts of the aglycons. None of the unknown compounds after cleavage of the liver and kidney extract accounted for more than 9.9% (0.035 mg/kg) of the TRR (mean of all five replicates) or 6.9% (0.013 mg/kg) of the TRR, respectively. The following main aglycons could be clearly identified after enzymatic cleavage: BCS-CN88460-2-propanol and BCS-CN88460-propanol.

Table 6.2.3- 21: Radioactive residues of parent compound and metabolites in liver samples of first and second conventional extraction and after enzymatic cleavage for 20 h

Sample		Liver- 1st conventional extraction		Liver- 2nd conventional extraction		Enzymatic cleavage of five aliquots of liver extracts			
						mean values		standard deviation	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Extract used for HPLC		**85.1**	**0.296**	**85.5**	**0.298**	**80.5**	**0.280**	**0.2**	**0.001**
38	parent	5.3	0.018	5.6	0.020	4.7	0.016	0.4	0.001
20	2-propanol-GlucA (M20)	13.0	0.045	13.2	0.046	1.3	0.005	0.3	0.001
21	lactic acid (M10)	1.3	0.005	1.2	0.004	0.7	0.002	0.1	<0.001
22	propanol-GlucA (isomer 1) (M19, isomer 1)	8.8	0.031	8.9	0.031	---	---	---	---
23	propanol-GlucA (isomer 2) (M19, isomer 2)	5.9	0.021	8.2	0.029	---	---	---	---
27	desmethyl-carboxylic acid (M11)	0.5	0.002	0.6	0.002	1.5	0.005	0.2	0.001
28	desmethyl-propanol (M06)	0.6	0.002	0.7	0.002	4.3	0.015	0.2	0.001
30	carboxylic acid (M12)	4.2	0.015	4.3	0.015	9.6	0.033	0.4	0.001
31	propanol (M01)	4.9	0.017	5.7	0.020	18.4	0.064	0.6	0.002
34	2-propanol (M02)	2.8	0.010	2.5	0.009	13.4	0.047	0.5	0.002
Total identified		**47.2**	**0.164**	**50.9**	**0.177**	**53.8**	**0.187**	**2.7**	**0.010**
Total characterised		37.8	0.132	34.6	0.121	26.7	0.093	2.5	0.009
Accountability		85.1	0.296	85.5	0.298	80.5	0.280	5.3	0.018

Table 6.2.3- 22: Radioactive residues of parent compound and metabolites in kidney samples of first and second conventional extraction and after enzymatic cleavage for 20 h

Sample		Kidney-conventional extraction		Kidney-enzymatic cleavage of conventional extract - experiment 1		Kidney-enzymatic cleavage of conventional extract - experiment 2		Kidney-enzymatic cleavage of conventional extract - experiment 3	
peak ID	Compound (Report name) BCS-CN88460-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Extract used for HPLC		**96.8**	**0.177**	**94.4**	**0.173**	**93.7**	**0.172**	**94.5**	**0.173**
38	parent	1.6	0.003	1.6	0.003	1.2	0.002	1.2	0.002
20	2-propanol-GlucA* (M20)	10.2	0.019	1.1	0.002	1.5	0.003	1.4	0.003
21	lactic acid (M10)	4.2	0.008	2.0	0.004	1.8	0.004	1.7	0.003
22	propanol-GlucA (isomer 1) (M19, isomer 1)	3.6	0.007	0.3	0.001	0.5	0.001	0.1	<0.001
23	propanol-GlucA (isomer 2) (M19, isomer 2)	8.6	0.016	---	---	---	---	---	---
27	desmethyl-carboxylic acid (M11)	3.6	0.007	6.4	0.012	6.0	0.011	6.7	0.012
28	desmethyl-propanol (M06)	---	---	4.9	0.009	5.0	0.009	5.2	0.010
30	carboxylic acid (M12)	6.8	0.012	12.4	0.023	12.6	0.023	13.2	0.024
31	propanol (M01)	2.5	0.004	20.2	0.037	20.8	0.038	19.1	0.035
34	2-propanol (M02)	1.4	0.003	5.1	0.009	5.8	0.011	5.7	0.010
Total identified		**42.4**	**0.078**	**54.0**	**0.099**	**55.5**	**0.101**	**54.2**	**0.099**
Total characterised		54.4	0.100	40.4	0.074	38.3	0.070	40.3	0.074
Accountability		96.8	0.177	94.4	0.173	93.7	0.172	94.5	0.173

* BCS-CN88460-2-propanol-GlucA and BCS-CN88460-propenol-GlucA were only detected in kidney in a ratio of 6/4.

Distribution of Metabolites in Urine and Faeces

The metabolic profile of urine (0 - 24 h) and faeces (0 - 24 h) were similar to the profiles of edible materials, especially liver and kidney, except that parent compound was not present in the sample of urine. In faeces, parent compound was the main residue (8.0% of the first dose). All identified metabolites in urine and faeces accounted for ≤1.2% of the first dose.

The distribution of the parent compound and metabolites in urine and faeces is summarized in the table below.

Table 6.2.3- 23: Radioactive residues of parent compound and metabolites in non-edible samples of lactating goat following oral administration of 5 daily doses of [phenyl-UL-^{14}C]BCS-CN88460 at a dose rate of 1.0 mg/kg

Sample		Faeces (0 - 24 h)	Urine (0 – 24 h)
Peak ID	Compound (Report name) BCS-CN88460-	% of dose in the sample	% of dose in the sample
38	parent compound	8.0	---
20	2-propanol-GlucA (M20) and propenol-GlucA (M25) (co-elution)	---	0.6
21	lactic acid (M10)	---	0.2
22	propanol-GlucA (isomer 1) (M19, isomer 1)	---	0.3
23	propanol-GlucA (isomer 2) (M19, isomer 2)	0.4	0.7
27	desmethyl-carboxylic acid (M11)	0.1	0.2
28	desmethyl-propanol (M06)	0.3	0.1
30	carboxylic acid (M12)	0.7	0.8
31	propanol (M01)	1.2	0.7
34	2-propanol (M02)	0.2	---
34	2-propanol and hydroxyphenyl (M04) (co-elution)	---	0.1
Sum identified		**11.4**	**3.6**
Subtotal characterised by HPLC		*0.5*	*2.6*
number of unknown peaks		*4*	*19*
largest unknown peak		*0.2*	*0.3*
Sum characterised		**0.5**	**2.6**
Sum of losses		0.5	---
Total extracted		**12.4**	**---**
Post extraction solids (PES)		0.4	---
Total		**12.8**	**6.2**

G. Storage Stability of Residues

During the study, all samples and extracts were stored in a freezer at ≤ -18 °C or for a short time in a refrigerator. All samples of milk, and edible organs and tissues were extracted within four months after sample collection. The first metabolite profile was recorded not later than four days after the start of the extraction and sample preparation.

The storage stability was exemplarily demonstrated for all other matrices. A second conventional extraction of liver was performed approx. 11 months after sampling. The extract was used for enzymatic cleavage experiments.

III. Conclusion

The metabolic behaviour of [phenyl-UL-^{14}C]BCS-CN88460 in the lactating goat can be characterised by the following observations:

The TRR-values and the transfer factors in milk, organs and tissues were very low compared to the dose level of 20.57 mg a.s./kg feed/day and a dosing period of five days. The highest TRR-value was detected for liver and was caused by the short time period of 6 hours between last dosing and sacrifice. It indicates the significance of this organ for metabolism. The significantly lower TRR-value for kidney shows that no residues were retained in this tissue and reflects the low amount of radioactivity, which was excreted by the urine. The TRR-values in the respective evening and morning milk samples

showed a diurnal pattern as they declined slightly prior to the delivery of the next dose for most days. A continuous increase was observed before a residue plateau-level was reached at day three after the first administration.

The elimination of radioactivity was mainly faecal and only 9.8% were eliminated via urine. This excretion behaviour was similar to the findings in the ADME studies with rats.

The radioactive residues were efficiently extracted from milk as well as from edible organs and tissues; extraction rates ranged from 88.3% to 100.0%.

The identification rates of parent compound and metabolites in milk, edible organs and tissues ranged between 42.4% and 64.0% of the TRR.

Parent compound was a major compound in milk and muscle, a prominent compound in liver and a minor compound in kidney. Overall up to ten metabolites were identified.

BCS-CN88460-2-propanol (M02) was detected as a major metabolite in muscle, BCS-CN88460-desmethyl-propanol (M06) as a major metabolite in milk (32 – 101 h), BCS-CN88460-2-propanol-GlucA (M20) as a major metabolite in liver and BCS-CN88460-propanol-GlucA (isomer 2, M19 – isomer 2) as a major metabolite in kidney. Further abundant metabolites were BCS-CN88460-propanol-GlucA (isomer 1, M19 – isomer 1) in liver and kidney, BCS-CN88460-propanol-GlucA (isomer 2, M19 – isomer 2) in muscle, liver and kidney, BCS-CN88460-carboxylic acid (M12) in muscle, liver and kidney and BCS-CN88460-propanol (M01) in muscle and liver. BCS-CN88460-lactic acid (M10) and BCS-CN88460-desmethyl-carboxylic acid (M11) were minor metabolites in liver and kidney. BCS-CN88460-propenol-GlucA (M25) was only detected as a minor metabolite in kidney and in urine.

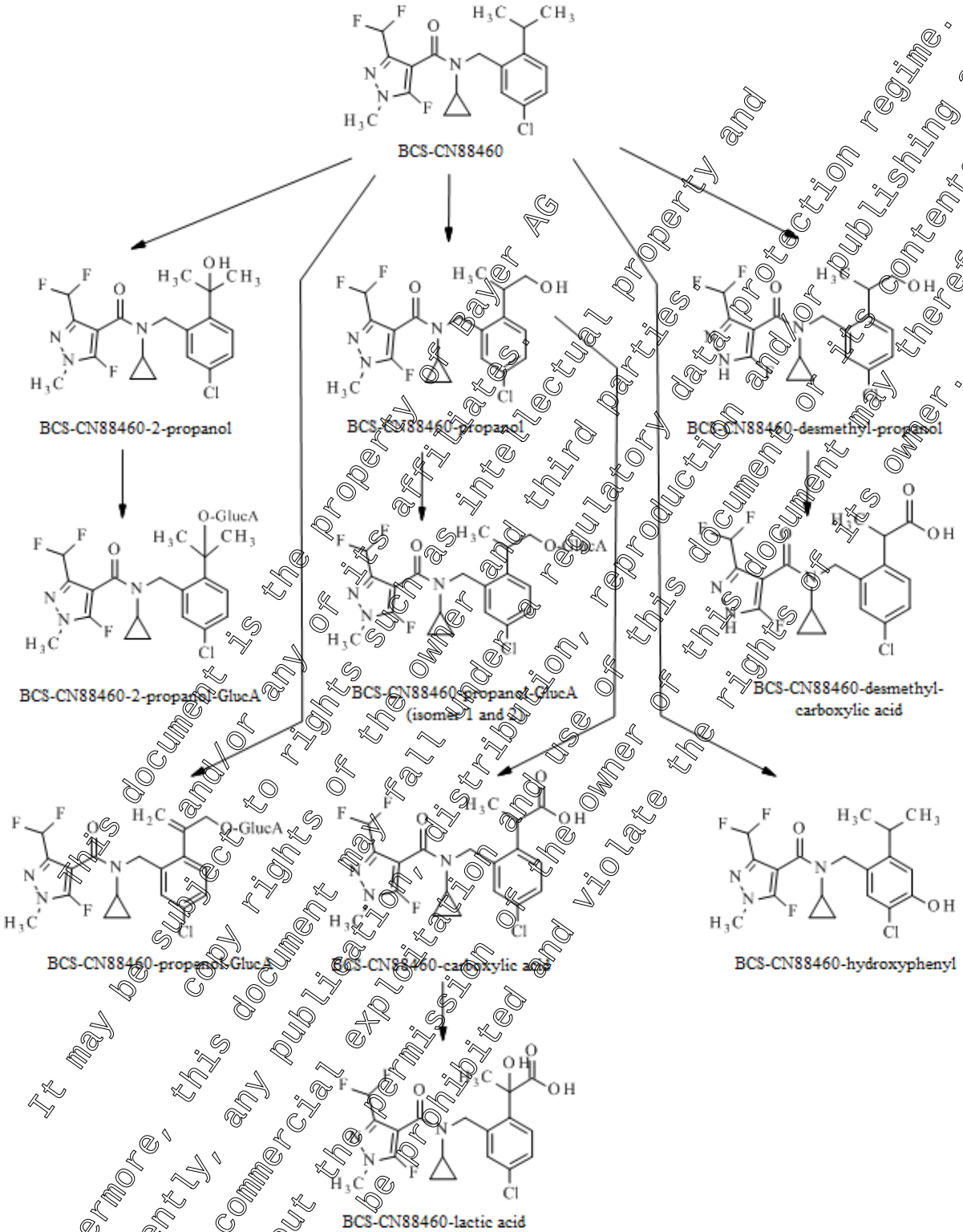
The metabolic profile of urine and faeces were similar to the profiles of edible materials, especially liver and kidney.

The principal metabolic reactions of [phenyl-UL-^{14}C]BCS-CN88460 in the lactating goat are listed below:

- hydroxylation in position 1 and position 2 of the propyl group on the phenyl ring
- conjugation with glucuronic acid
- oxidation of the 1-propanol group was leading to a carboxylic acid group or with a hydroxy group in position 2 to lactic acid
- demethylation of the pyrazole moiety
- hydroxylation in position 4 of the phenyl moiety
- dehydration after hydroxylation in position 1 and 2 of the propyl group followed by conjugation with glucuronic acid

Based on these results, the metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in the lactating goat is considered as sufficiently understood and a metabolic pathway is proposed.

Figure 6.2.3- 2: Proposed metabolic pathway of [phenyl-UL-^{14}C]BCS-CN88460 in the lactating goat



CA 6.2.4 Pigs

As the metabolic pathways in ruminants and hens were similar to the metabolic pathway in rats, no metabolism study in pigs has been submitted.

CA 6.2.5 Fish

As outlined in the EU-Directive 91/414/EEC, the EU Aquatic Guidance Document, as well as in EPA and PMRA guidelines, a log P_{ow} >3 should be used as a general trigger for a fish bioconcentration study. The whole study is summarised under CA 8.2.2.3/01 in MCA, Section 8. The nature of the residue in fish was investigated using [pyrazole-4-^{14}C] BCS-CN88460 and is summarised here in more detail (Appendix VI of the study report).

Table 6.2.5- 1: Overview over available fish bioconcentration and metabolism studies

Fish	Exposure	Concentration	Reference
Bluegill sunfish	continuous exposure for 42 days of pyrazole-labelled isoflucypram	5 µg/l	M-610008-01-1

Report: KCA 6.2.5/01; [redacted], K., [redacted], B., 2017; M-610008-01-1
Title: [pyrazole-4-14C] BCS-CN88460 - Aqueous exposure bioconcentration fish test and biotransformation in fish (Lepomis macrochirus)
Report No.: EBLNN359
Document No.: M-610008-01-1
Guideline(s): EU Directive 91/414/EEC; Regulation 1107/2009 (Europe); OECD Test Guideline 305; US EPA OCSPP 850.1730
Guideline deviation(s): According to the guideline and the study protocol, the measurement of the total organic carbon (TOC) will be performed 48 and 24 hours prior to test initiation. However, due to scheduling issues the TOC content was measured 72 hours and 24 hours prior to test initiation. This deviation has no negative impact on the outcome of the study, since there results of both measurements were reasonable (< 2 mg/L)
GLP/GEP: yes

Executive summary

The metabolism of BCS-CN88460 in bluegill sunfish was investigated as part of the fish bioconcentration study. The test compound was radiolabelled in the pyrazole-4 moiety.

The analysis of stock solutions of the test compound from all aquariums showed that [pyrazole-4-^{14}C] BCS-CN88460 was stable in the stock solutions during the exposure phase.

Water samples were collected during the exposure period of 28 days for aquarium C and 14 days for aquarium D. All water samples taken after day 28 were not further analysed due to low radioactivity. The radioactive residue was extracted from water samples by solid phase extraction (SPE) with RP18. The residues were eluted with acetonitrile and methanol/tetrahydrofurane, concentrated and analysed by HPLC with radiodetection. The radioactive concentrations in the water ranged from 3.9 to 6.4 µg/L. Parent compound was the main compound in the water samples and amounted to ≥ 98.2% in aquarium C and ≥ 90.9% in aquarium D. The following minor metabolites were detected in the water samples: conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid, BCS-CN88460-desmethyl-

GlucA (isomer 1), BCS-CN88460-cyclopropyl-pyrazole-carboxamide, BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol. Each of these metabolites accounted to ≤ 0.1 µg/L.

Total Radioactive Residues in Edible and Viscera Fish Parts

Edible parts and viscera of fish sampled on Day 7 and 14 from aquarium D (metabolism test with 5 µg/L) were conventionally extracted with acetonitrile/water mixtures. The TRRs were moderate for edible fish parts and amounted to 0.565 mg/kg for day 7 and 0.567 mg/kg for day 14. In viscera fish parts the TRR was 3.955 mg/kg on Day 7 and 3.365 mg/kg on Day 14. The extraction rates amounted between 92.8 and 98.7%. The post extraction solids (PES) amounted to 5.8% (0.033 mg/kg) of TRR for edibles on Day 7, 7.2% (0.041 mg/kg) of TRR for edibles on Day 14, 1.3% (0.053 mg/kg) of TRR for viscera on Day 7 and 1.7% (0.058 mg/kg) of TRR for viscera on Day 14.

Metabolism

Parent compound and metabolites were analysed and quantified in the concentrated extracts by HPLC with radiodetection. They were assigned to each other in the profiles based on the metabolite pattern and their retention times. Parent compound and metabolites were identified in isolated fractions by HPLC- and TLC-co-chromatography with radiolabelled reference compounds, which were identified by structure elucidaton in the ADME studies with rats.

Parent compound was a prominent compound in edible parts of fish and amounted to 16.7% (0.095 mg/kg) of TRR for day 7 and 19.2% (0.109 mg/kg) of TRR for day 14. In viscera parts of fish parent compound was detected with 5.1% (0.201 mg/kg) of TRR for day 7 and 9.7% (0.326 mg/kg) of TRR for day 14.

The main metabolite in edible fish parts was BCS-CN88460-cyclopropyl-pyrazole-carboxamide (M58) and amounted to approx. 31% of the TRR for day 7 and 14. At day 14 the conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid was detected with 15.1% (0.086 mg/kg) of TRR and BCS-CN88460-propanol (M01) with 10.0% (0.057 mg/kg) of the TRR. The conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid could be cleaved to its aglycon with hydrochloric acid at elevated temperatures. Further metabolites in edible fish parts were BCS-CN88460-N-methyl-pyrazole-carboxylic acid (M50), BCS-CN88460-desmethyl (M13), BCS-CN88460-desmethyl-propanol (M06) and conjugates like BCS-CN88460-desmethyl-propanol-GlucA (isomer 1, M31), BCS-CN88460-propanol-GlucA (isomer 1 and 2, M19) and BCS-CN88460-desmethyl-GlucA (isomer 1, M35). They amounted to ≤ 5.0% (0.028 mg/kg) of TRR.

Beside parent compound the major part of radioactivity in viscera of fish was represented by the two metabolites BCS-CN88460-propanol-GlucA (isomer 1 and 2, M19) and amounted to in sum approx. 40% of the TRR. Other metabolites in viscera fish tissues were BCS-CN88460-N-methyl-pyrazole-carboxylic acid (M50), conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid, BCS-CN88460-cyclopropyl-pyrazole-carboxamide (M58), BCS-CN88460-desmethyl-propanol-GlucA (isomer 1, M31), BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-propanol (M01), BCS-CN88460-desmethyl-GlucA (isomer 1, M35) and BCS-CN88460-desmethyl (M13). These metabolites amounted to ≤ 6.6% (0.262 mg/kg) of TRR.

Unknown metabolites in all fish tissues were characterised by their extraction and chromatographic behaviour and amounted to ≤ 3.4% (0.019 mg/kg) of TRR for edible fish parts and ≤ 5.4% (0.213 mg/kg) of TRR for viscera fish parts. Details of the distribution of parent compound and metabolites in fish tissues are presented below:

Table 6.2.5- 2: Radioactive residues of parent compound and metabolites in edible and viscera tissues of fish after 14 days exposure of [pyrazole-4-^{14}C]BCS-CN88460 to bluegill sunfish

	edible parts of fish				viscera of fish			
	day 7		day 14		day 7		day 14	
TRR [mg/kg] =	**0.565**		**0.567**		**3.955**		**3.365**	
Compound/fraction	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extract	**94.2**	**0.532**	**92.8**	**0.527**	**98.7**	**3.901**	**98.3**	**3.307**
BCS-CN88460-N-methyl-pyrazole-carboxylic acid (M50)	3.3	0.019	---	---	3.4	0.135	4.5	0.152
BCS-CN88460-cyclopropyl-pyrazole-carboxamide (M58)	30.9	0.175	30.4	0.173	5.8	0.229	5.9	0.197
Conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid **	7.3	0.041	15.1	0.086	1.2	0.049	2.1	0.072
BCS-CN88460-desmethyl-propanol-GlucA (isomer 1) (M31, isomer 1))	2.0	0.011	1.0	0.006	5.2	0.204	5.9	0.198
BCS-CN88460-propanol-GlucA (isomer 1) (M19, isomer 1)	4.6	0.026	3.9	0.022	23.0	0.909	23.5	0.792
BCS-CN88460-propanol-GlucA (isomer 2) (M19, isomer 2)	5.0	0.028	3.0	0.017	16.3	0.643	17.2	0.580
BCS-CN88460-desmethyl-propanol (M06)	1.9	0.011	---	---	3.5	0.137	1.1	0.037
BCS-CN88460-propanol (M01)	6.3	0.036	10.0	0.057	3.1	0.123	4.0	0.135
BCS-CN88460-desmethyl-GlucA (isomer 1) (M35, isomer 1)	1.8	0.010	1.4	0.008	6.6	0.262	2.6	0.086
BCS-CN88460-desmethyl (M13)	1.7	0.010	3.3	0.019	0.5	0.018	1.2	0.039
Parent compound	16.7	0.095	19.2	0.109	5.1	0.201	9.7	0.326
Total identified	**81.6**	**0.461**	**87.3**	**0.495**	**73.6**	**2.910**	**77.8**	**2.616**
Number of unknown compounds	8		3		11		11	
Amount of the largest unknown compound	3.4	0.019	2.5	0.014	5.4	0.213	4.2	0.142
Total characterised *	**12.6**	**0.071**	**5.5**	**0.031**	**25.1**	**0.991**	**20.5**	**0.691**
Total extracted	**94.2**	**0.532**	**92.8**	**0.527**	**98.7**	**3.901**	**98.3**	**3.307**
Post extraction solids (PES)	5.8	0.033	7.2	0.041	1.3	0.053	1.7	0.058
Accountability	**100.0**	**0.565**	**100.0**	**0.567**	**100.0**	**3.955**	**100.0**	**3.365**

* Unknown metabolites were characterised based on their extraction and chromatographic behaviour.

** The aglycon BCS-CN88460-N-methyl-pyrazole-carboxylic acid could be clearly identified after acidic cleavage of the conjugate.

The principal metabolic reactions of [pyrazole-4-^{14}C]BCS-CN88460 in fish are listed below:

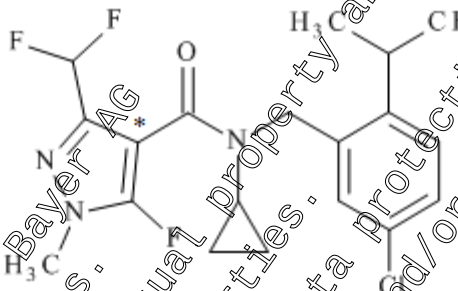
- demethylation of the pyrazole moiety was leading to desmethyl compounds
- hydroxylation in position 1 of the propyl group was leading to propanol compounds
- conjugation with glucuronic acid after hydroxylation in position 1 of the propyl group or via nitrogen was leading to several glucuronic acid-conjugates
- cleavage of the phenyl moiety was leading to the cyclopropyl-pyrazole-carboxamide compound
- cleavage of the phenyl moiety in combination with cleavage of the cyclopropyl ring and further followed by oxidation was leading to a N-methyl-pyrazole-carboxylic acid compound
- conjugation of BCS-CN88460-N-methyl-pyrazole-carboxylic acid was also observed

On the basis of the results of this study it was concluded that [pyrazole-4-^{14}C]BCS-CN88460 was stable in water and intensely metabolised in fish and a pathway of [pyrazole-4-^{14}C]BCS-CN88460 in fish is proposed.

I. Materials and Methods

A. Materials

1. Test Material

Chemical structure	 * denotes the ^{14}C-label position
Radiolabelled test material	[Pyrazole-4-^{14}C]BCS-CN88460
Specific radioactivity	4.22 MBq/mg
Radiochemical purity	>99% (HPLC)
Chemical purity	>99% (HPLC)
Non-labelled test material	BCS-CN88460
Chemical purity	94.2%

B. Study Design

Preparation of the Test Compound for Administration

For the experiments the radiolabelled test compound was diluted with appropriate amounts of non-radiolabelled test compound, yielding 2 L of stock solution for each of aquarium B and C and 1.4 L of stock solution for aquarium D. The stock solution for each individual aquarium was prepared by using dimethylformamide (DMF) as solvent.

The purity of the test compound in the stock solutions was checked before application by HPLC. In addition, an aliquot of stock solutions B and C were taken on Day 28 after application and an aliquot of stock solution D on Day 14 after application. After a storage period of the aliquots for approx. 2 months at approx. -18°C, the stability of the test compound was checked by HPLC. In all cases the purity of the test compound was ≥ 99.29%.

Water samples

Water samples were taken from day 0, 1, 28 and 29 for aquarium C, and from day 7 and 14 for aquarium D.

Fish samples

For analysis of parent compound and metabolites from aquarium D (nominal test concentration of 5.0 µg [pyrazole-4-^{14}C]BCS-CN88460 / L water) a number of 15 fishes were sampled after an exposure period of 7 and 14 days, respectively. Fishes were dissected into edible tissues (fillet, body muscle, skin and skeleton) and viscera / non-edible parts (viscera = head, fins and internal organs). The coarse pieces of the edibles or viscera of each day were combined and homogenised with a high speed blender. From these samples, a sub-sample was taken for extraction and analysis of parent compound and metabolites.

C. Analytical Procedures

Sample Extraction and Preparation for Chromatographic Analysis

Preparation of water samples

Each individual water sample (1000 mL) were applied to a pre-conditioned RP18 solid phase extraction (SPE) cartridge (to concentrate the radioactive compounds from the water sample). The aqueous flow through was collected and the retained radioactivity on the RP18 phase was eluted with approx. 500 mL acetonitrile followed by approx. 250 mL methanol/tetrahydrofurane (1/1; v/v). The acetonitrile eluates were concentrated to an aqueous remainder using a rotary evaporator (bath temperature approx. 35 °C). Each remainder was diluted with acetonitrile/water (1/3; v/v) yielding the final extracts. The final extracts and the other eluates were stored in a freezer (≤ 18 °C).

The radioactivity in the flow through fraction, eluates, concentrates and distillates was determined by LSC. The total radioactive residue (TRR) of each water sample was determined by summing up the radioactivity measured in the flow through fraction and the eluates based on the volume of the sample and the specific radioactivity of the test compound.

Extraction and Preparation of Fish Samples

Samples of the edible parts of fish and viscera were extracted successively three times with acetonitrile/water (8/2; v/v) using a high speed homogeniser. Solids and extracts were separated by centrifugation. Extracts were combined and concentrated to an aqueous remainder using a rotary evaporator (bath temperature approx. 35 °C). The remainder was diluted with acetonitrile/water (1/1; v/v) yielding the final extract. Radioactivity in the extracts was determined by LSC after volume measurement. Radioactive residues in the remaining solids were determined by combustion of aliquots followed by LSC.

The TRR (Total Radioactive Residue) in fish samples was calculated by summing up the radioactivity measured in the extracts and PES (post extraction solids) based on the used sample amount and the specific radioactivity of the test compound.

Radioactivity measurement

The radioactivity measurement in liquid samples was carried out by liquid scintillation counting (LSC). The solid samples combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

Metabolite analysis

Parent compound and metabolites were quantified in the extracts by HPLC based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

They were assigned to each other in the profiles based on the metabolite pattern and their retention times. Parent compound and metabolites were identified in isolated fractions by HPLC- and TLC co-chromatography with radiolabelled reference compounds taken from ADME studies with rats.

Table 6.2.5- 3: List of reference compounds

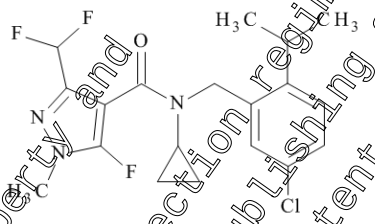
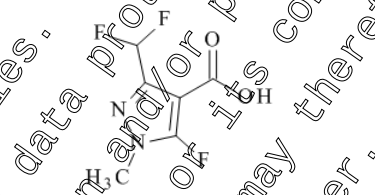
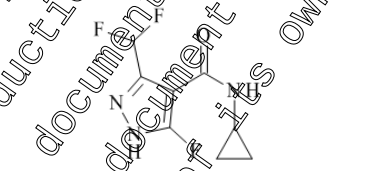
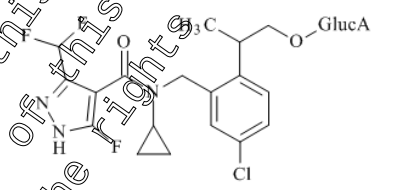
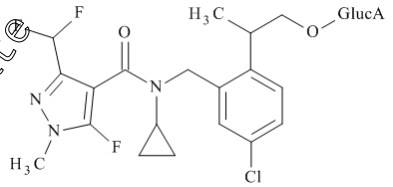
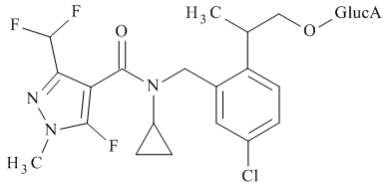
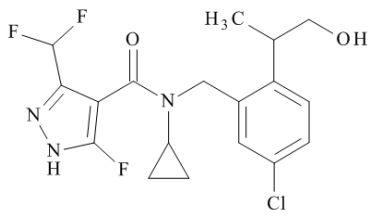
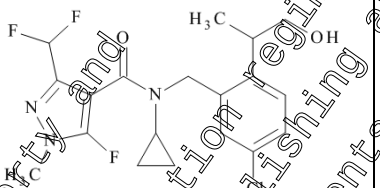
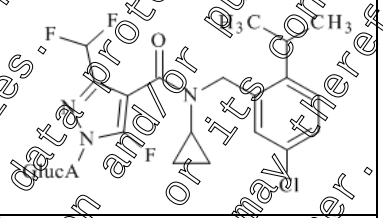
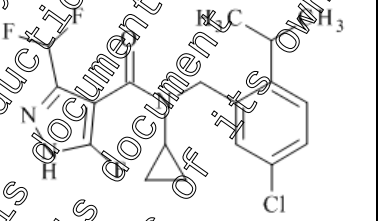
Report name/	Chemical name (IUPAC)	Chemical Structure
Parent compound (BCS-CN88460) Sample ID: PP01SSV	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	
BCS-CN88460-N-methyl-pyrazole-carboxylic acid (BCS-AB72918 or BCS-CR73065) Sample ID: HF7708I	3-(difluoromethyl)-5-fluoro-1-methyl-pyrazole-4-carboxylic acid	
BCS-CN88460-cyclopropyl-pyrazole-carboxamide (BCS-CX99798) Sample ID: HF7708J	N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide	
BCS-CN88460-desmethyl-propanol-GlucA (isomer 1) Sample ID: HF76B36A	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl glucopyranosiduronic acid	
BCS-CN88460-propanol-GlucA (isomer 1) Sample ID: HF76B37	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl glucopyranosiduronic acid	
BCS-CN88460-propanol-GlucA (isomer 2) Sample ID: HF76B38	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl glucopyranosiduronic acid	
BCS-CN88460-desmethyl-propanol (BCS-DC23055) Sample ID: HF79F21	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide	

Table is continued on next page.

Table 6.2.5- 3 continued

Report name/	Chemical name (IUPAC)	Chemical Structure
BCS-CN88460-propanol (BCS-CY24813) Sample ID: HF8614M	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide	
BCS-CN88460-desmethyl-GlucA (isomer 1) Sample ID: HF76B49A	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(beta-D-glucopyranuronosyl)-1H-pyrazole-4-carboxamide	
BCS-CN88460-desmethyl Sample ID: KM9413K	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide	

II. Results and Discussion

Water Samples

The radioactive concentrations in the water samples collected during the exposure period of 28 days for aquarium C and 14 days for aquarium D ranged from 3.9 to 6.4 µg/L. All samples taken after day 28 were not further analysed due to low radioactivity.

Parent compound was the main component in the water samples and amounted to ≥ 98.2% in water samples of aquarium C and ≥ 90.9% in water samples of aquarium D. The following minor metabolites were detected in the water samples: conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid, BCS-CN88460-desmethyl-GlucA (isomer 1), BCS-CN88460-cyclopropyl-pyrazole-carboxamide, BCS-CN88460-desmethyl-propanol and BCS-CN88460-propanol. Each of these metabolites accounted to ≤ 0.1 µg/L.

Table 6.2.5- 4: Analyses of water samples of aquarium C and D

water in aquarium C						
	day 0		day 1		day 28	
TRR [µg/L] =		3.9		4.1		5.0
Compound	% TRR	µg/L	% TRR	µg/L	% TRR	µg/L
BCS-CN88460 (parent compound)	100.0	3.9	98.2	4.1	98.2	4.9
Conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid *	---	---	1.8	0.1	---	---
BCS-CN88460-desmethyl-GlucA (isomer 1) (M35, isomer 1)	---	---	---	---	1.8	0.1
Total identified	**100.0**	**3.9**	**100.0**	**4.1**	**100.0**	**5.0**
Extracts analysed	100.0	3.9	100.0	4.1	100.0	5.0
Extracts not analysed	---	---	---	---	---	---
Accountability	**100.0**	**3.9**	**100.0**	**4.1**	**100.0**	**5.0**

water in aquarium D				
	day 7		day 14	
TRR [µg/L] =		4.2		6.4
Compound	% TRR	µg/L	% TRR	µg/L
BCS-CN88460 (parent compound)	90.9	3.9	97.4	6.3
BCS-CN88460-cyclopropyl-pyrazole-carboxamide (M58)	1.3	0.1	0.6	<0.1
Conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid *	3.0	0.1	1.7	0.1
BCS-CN88460-desmethyl-propanol (M06)	0.7	<0.1	---	---
BCS-CN88460-propanol (M01)	1.4	0.1	---	---
BCS-CN88460-desmethyl-GlucA (isomer 1) (M35, isomer 1)	1.0	<0.1	0.4	<0.1
Total identified	**98.2**	**4.1**	**100.0**	**6.4**
unknown	0.6	0.1	---	---
Total characterised	**0.6**	**<0.1**	**---**	**---**
Extracts analysed	98.9	4.2	100.0	6.4
Extracts not analysed	1.1	<0.1	---	---
Accountability	**100.0**	**4.2**	**100.0**	**6.4**

* The aglycone BCS-CN88460-N-methyl-pyrazole-carboxylic acid could be clearly identified after acidic cleavage of the conjugate.

Distribution of Radioactivity in Fish Samples

Edible parts and viscera of fish sampled on Day 7 and 14 from aquarium D (metabolism test with 5 µg/L) were conventionally extracted with acetonitrile/water mixtures. The TRRs were moderate for edible fish parts and amounted to 0.565 mg/kg for day 7 and 0.567 mg/kg for day 14. In viscera fish parts the TRR was 3.955 mg/kg on Day 7 and 3.365 mg/kg on Day 14.

The extraction rates amounted between 92.8 and 98.7%. The post extraction solids (PES) amounted to 5.8% (0.033 mg/kg) of TRR for edibles on Day 7, 7.2% (0.041 mg/kg) of TRR for edibles on Day 14, 1.3% (0.053 mg/kg) of TRR for viscera on Day 7 and 1.7% (0.058 mg/kg) of TRR for viscera on Day 14.

Distribution of Parent compound and Metabolites in Fish Samples

Parent compound was a prominent compound in edible parts of fish and amounted to 16.7% (0.095 mg/kg) of TRR for day 7 and 19.2% (0.109 mg/kg) of TRR for day 14. In viscera parts of fish parent compound was detected with 5.1% (0.201 mg/kg) of TRR for day 7 and 9.7% (0.326 mg/kg) of TRR for day 14.

BCS-CN88460 was intensely metabolised in the fish. The main metabolite in edible fish parts was BCS-CN88460-cyclopropyl-pyrazole-carboxamide and amounted to approx. 31% of the TRR for day 7 and 14. At day 14 the conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid was detected with 15.1% (0.086 mg/kg) of TRR and BCS-CN88460-propanol with 10.0% (0.057 mg/kg) of the TRR. The conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid could be cleaved to its aglycon with hydrochloric acid at elevated temperatures. Further metabolites in edible fish parts were BCS-CN88460-N-methyl-pyrazole-carboxylic acid, BCS-CN88460-desmethyl, BCS-CN88460-desmethyl-propanol and conjugates like BCS-CN88460-desmethyl-propanol-GlucA (isomer 1), BCS-CN88460-propanol-GlucA (isomer 1 and 2) and BCS-CN88460-desmethyl-GlucA (isomer 1). They amounted to ≤ 5.0% (0.028 mg/kg) of TRR.

Besides parent compound, the major part of radioactivity in viscera of fish was represented by the two metabolites BCS-CN88460-propanol-GlucA (isomer 1 and 2) and amounted to in sum approx. 40% of the TRR. Other metabolites in viscera fish tissues were BCS-CN88460-N-methyl-pyrazole-carboxylic acid, conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid, BCS-CN88460-cyclopropyl-pyrazole-carboxamide, BCS-CN88460-desmethyl-propanol-GlucA (isomer 1), BCS-CN88460-desmethyl-propanol, BCS-CN88460-propanol, BCS-CN88460-desmethyl-GlucA (isomer 1) and BCS-CN88460-desmethyl. These metabolites amounted to ≤ 6.6% (0.262 mg/kg) of TRR.

Unknown metabolites in all fish tissues were characterised by their extraction and chromatographic behaviour and amounted to ≤ 3.4% (0.019 mg/kg) of TRR for edible fish parts and ≤ 5.4% (0.213 mg/kg) of TRR for viscera fish parts.

Table 6.2.5- 5: Quantitative distribution of parent compound and metabolites in the edible and viscera tissues of fish after 14 days exposure of [pyrazole-4-^{14}C]BCS-CN88460 to bluegill sunfish

	edible parts of fish				viscera of fish			
	day 7		day 14		day 7		day 14	
TRR [mg/kg] =	**0.565**		**0.567**		**3.955**		**3.365**	
Compound/fraction	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extract	**94.2**	**0.532**	**92.8**	**0.527**	**98.7**	**3.901**	**98.3**	**3.307**
BCS-CN88460-N-methyl-pyrazole-carboxylic acid (M50)	3.3	0.019	---	---	3.4	0.135	4.5	0.152
BCS-CN88460-cyclopropyl-pyrazole-carboxamide (M58)	30.9	0.175	30.4	0.173	5.8	0.229	5.9	0.197
Conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid **	7.3	0.041	15.1	0.086	1.2	0.049	2.1	0.072
BCS-CN88460-desmethyl-propanol-GlucA (isomer 1) (M31, isomer 1))	2.0	0.011	1.0	0.006	5.2	0.204	5.9	0.198
BCS-CN88460-propanol-GlucA (isomer 1) (M19, isomer 1)	4.6	0.026	3.9	0.022	23.0	0.909	23.5	0.792
BCS-CN88460-propanol-GlucA (isomer 2) (M19, isomer 2)	5.0	0.028	3.0	0.017	16.3	0.643	17.3	0.580
BCS-CN88460-desmethyl-propanol (M06)	1.9	0.011	---	---	3.5	0.137	1.1	0.037
BCS-CN88460-propanol (M01)	6.3	0.036	10.0	0.057	3.1	0.123	4.0	0.135
BCS-CN88460-desmethyl-GlucA (isomer 1) (M35, isomer 1)	1.8	0.010	1.4	0.008	6.6	0.262	2.6	0.086
BCS-CN88460-desmethyl (M13)	1.7	0.010	3.3	0.019	0.5	0.018	1.2	0.039
Parent compound	16.7	0.095	19.2	0.109	5.1	0.201	9.7	0.326
Total identified	**81.6**	**0.461**	**87.3**	**0.495**	**73.6**	**2.910**	**77.8**	**2.616**
Number of unknown compounds	8		3		11		11	
Amount of the largest unknown compound	3.4	0.019	2.5	0.014	5.4	0.213	4.2	0.142
Total characterised *	**12.6**	**0.071**	**5.5**	**0.031**	**25.1**	**0.991**	**20.5**	**0.691**
Total extracted	**94.2**	**0.532**	**92.8**	**0.527**	**98.7**	**3.901**	**98.3**	**3.307**
Post extraction solids (PES)	5.8	0.033	7.2	0.041	1.3	0.053	1.7	0.058
Accountability	**100.0**	**0.565**	**100.0**	**0.567**	**100.0**	**3.955**	**100.0**	**3.365**

* Unknown metabolites were characterised based on their extraction and chromatographic behaviour.
** The aglycone BCS-CN88460-N-methyl-pyrazole-carboxylic acid could be clearly identified after acidic cleavage of the conjugate.

III. Conclusion

The metabolism of BCS-CN88460 in bluegill sunfish was investigated as part of the fish bioconcentration study. The test compound was radiolabelled in the pyrazole-4 moiety. BCS-CN88460 was stable in the fish water during the testing period.

After exposure of the fishes for 7 days and for 14 days with [pyrazole-4-^{14}C]BCS-CN88460 at a concentration of 5.0 µg/L the total radioactive residues (TRRs) in the edible parts (0.565 mg/kg for day 7 and 0.567 mg/kg for day 14) were moderate. In viscera parts the TRRs amounted to 3.955 mg/kg for day 7 and 3.365 mg/kg for day 14.

Residues from edible and viscera fish parts could be sufficiently extracted with conventional methods. BCS-CN88460 was intensely metabolised in the fish.

Parent compound was detected in edible and viscera fish parts. Besides parent compound as a major compound in edibles, the metabolite BCS-CN88460-cyclopropyl-pyrazole-carboxamide (M58) was

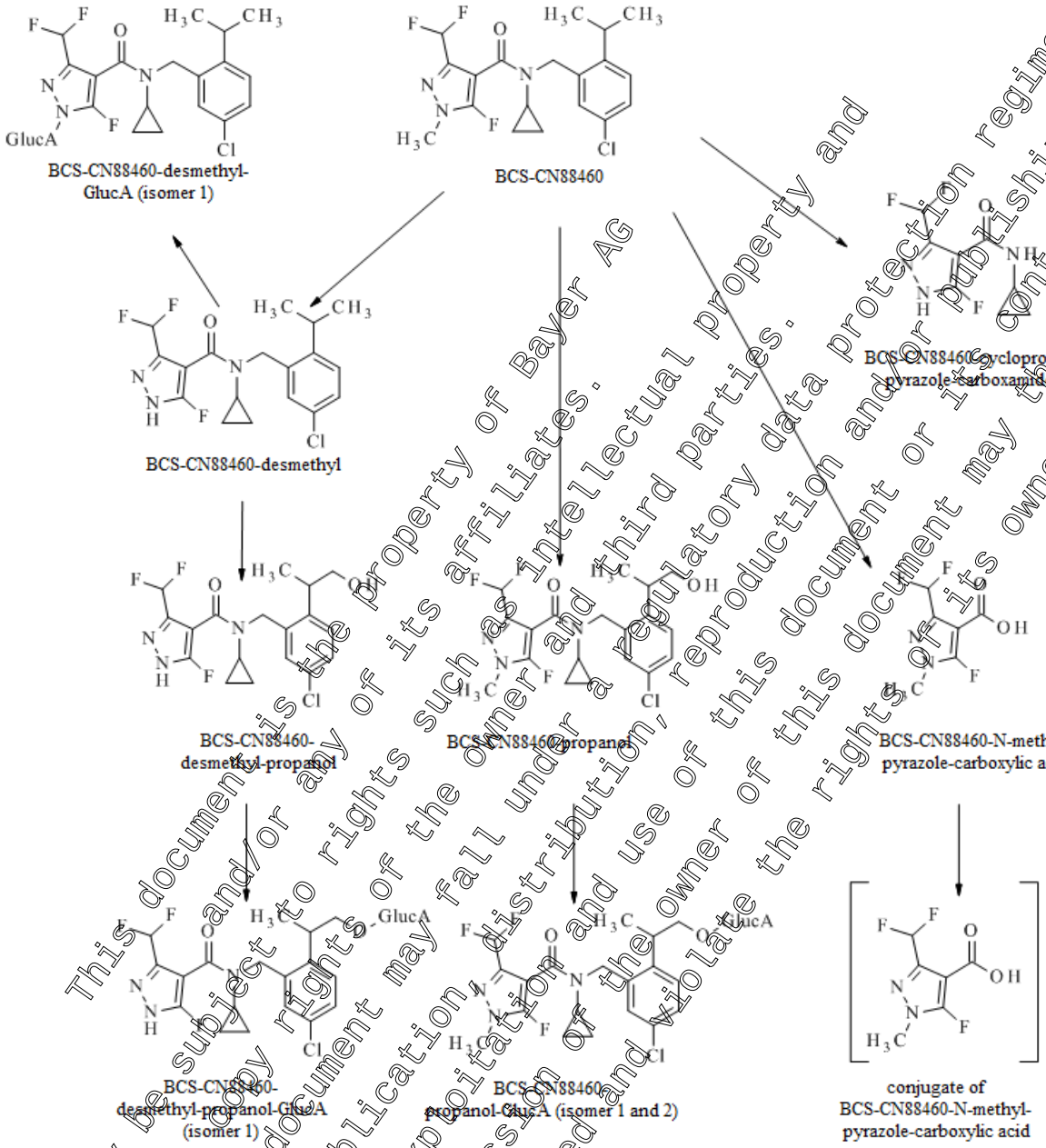
found with higher amounts. Metabolites BCS-CN88460-propanol-GlucA (M19, isomer 1 and 2) were found as main compound (in sum approx. 41% of TRR) in viscera fish parts.

Further metabolites in the fish parts were BCS-CN88460-N-methyl-pyrazole-carboxylic acid (M30), conjugate of BCS-CN88460-N-methyl-pyrazole-carboxylic acid, BCS-CN88460-desmethyl-propanol-GlucA (M31, isomer 1), BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-propanol (M01), BCS-CN88460-desmethyl-GlucA (M35, isomer 1) and BCS-CN88460-desmethyl (M13).

The principal metabolic reactions of [pyrazole-4-^{14}C]BCS-CN88460 in fish are the demethylation of the pyrazole moiety, the hydroxylation in position 1 of the propyl group, conjugation with glucuronic acid after hydroxylation or via nitrogen, cleavage of the phenyl moiety and cleavage of the phenyl moiety in combination with cleavage of the cyclopropyl ring followed by oxidation. Conjugation of BCS-CN88460-N-methyl-pyrazole-carboxylic acid was also observed.

Based on the results, it is concluded that the metabolic behaviour, as well the pathway of [pyrazole-4-^{14}C]BCS-CN88460 in fish are sufficiently understood. The following pathway of isoflucypram in bluegill sunfish is proposed:

Figure 6.2.5- 1: Proposed metabolic pathway of isoflucypram in bluegill sunfish



CA 6.3 Magnitude of residue trials in plants

CA 6.3.1 Barley

BCS-CN88460 (common name: isoflucypram) is to be registered in Europe for use in barley and oat. European residue data in barley are therefore presented below to support the intended "representative uses".

As allowed by the EU Guidance document on comparability, extrapolation, group tolerances and data requirements for setting MRLs (SANCO 7525/VI/95 rev. 10.2 of 23 September 2016), the extrapolation of the barley residue data to oat is requested.

Information on the intended use pattern (GAP) is summarised in Table 6.3.1- 1.

Table 6.3.1- 1: Use patterns (critical GAP) for the spray application of BCS-CN88460-containing formulations in/on barley and oat in European fields (northern and southern residue regions)

Description	F/G	No. of appls.	Growth stage at application (BBCH Code)	Application rate per treatment (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)
Barley, oat *	F**	1	30-61	75	100-400		NA

* agricultural use based on EC formulations (BCS-CN88460 EC 050 and Prothioconazole & BCS-CN88460 EC 150)
** uses in both the northern and southern residue regions (EU-N and EU-S)
NA: not applicable. The application timing is defined by the growth stage at application.

In order to support the EU "representative use" of BCS-CN88460, sets of GLP trials were conducted in northern and southern European fields in 2015 and 2016. In northern and in southern European field-grown barley, BCS-CN88460 was applied once at the latest intended growth stage of BBCH 61.

Three EC formulations containing BCS-CN88460 were tested with similar application rates:

- BCS-CN88460 EC 050 containing 50 g BCS-CN88460 /L
  Tested application rate: 75 g BCS-CN88460 /ha.
- Prothioconazole & BCS-CN88460 EC 150 containing 50 g BCS-CN88460 /L
  Tested application rate: 75 g BCS-CN88460 /ha.
- Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 containing 50 g BCS-CN88460 /L
  Tested application rate: 62.5 g BCS-CN88460 /ha (within + or – 25% of the 75 g as/ha application rate).

In some cases the EC 050 and the EC 150 formulations were tested in side-by-side plots at the same location so that these trials cannot be considered all as independent trials.

The number of independent trials conducted (incl. information on geographical region and vegetation period) is summarised below in Table 6.3.1- 2.

Table 6.3.1- 2: Overview of European residue trials conducted in barley per geographical "residue region" and vegetation period

Crop	Region	No. of independent trials			Report No. (Formulation)	Document number	Reference
		Veget. period		Σ			
		2015	2016				
Barley	EU-N	9	4	13	15-2110 (EC 050) 15-2113 (EC150) 15-2118 (EC250) 16-2051 (EC150)	M-585588-02-1 M-580046-02-1 M-583909-02-1 M-589582-02-1	KCA 6.3.1/01 KCA 6.3.1/02 KCA 6.3.1/03 KCA 6.3.1/04
	EU-S	8	4	12	15-2066 (EC 050) 15-2114 (EC150) 15-2117 (EC250) 16-2052 (EC150)	M-584388-02-1 M-580022-02-1 M-583692-02-1 M-589554-02-1	KCA 6.3.1/05 KCA 6.3.1/06 KCA 6.3.1/07 KCA 6.3.1/08

EU-N = northern EU field EU-S = southern EU field
FL : formulation
EC 050: BCS-CN88460 EC 050 containing 50 g BCS-CN88460 /L
EC 150: Prothioconazole & BCS-CN88460 EC 150 containing 50 g BCS-CN88460 /L
EC 250: Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 containing 50 g BCS-CN88460 /L

Northern Europe (residue region)

Report: KCA 6.3.1/01; [redacted], G.; 2017; M-585588-02-1
Title: Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on winter and spring barley after spray application of BCS-CN88460 EC 050 in the Netherlands, Germany, northern France and the United Kingdom
Report No.: 15-2110
Document No.: M-585588-02-1
Guideline(s): 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
OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009)
US EPA OCSPP 860.1500, Crop Field Trial
Guideline deviation(s): none
GLP/GEP: yes

Report: KCA 6.3.1/02; [redacted]; 2017; M-580046-02-1
Title: Amendment no. 1: Determination of the residues of BCS-CN88460 and prothioconazole in/on barley after spray application of prothioconazole & BCS-CN88460 EC 150 in the Netherlands, Germany, northern France and United Kingdom
Report No.: 15-2113
Document No.: M-580046-02-1
Guideline(s): 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
OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009)
US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s): None
GLP/GEP: yes

Report:	KCA 6.3.1/03; [redacted], G.; 2017; M-583909-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460, prothioconazole and tebuconazole in/on winter barley and spring barley after spray application of prothioconazole & tebuconazole & BCS-CN88460 EC 250 in the United Kingdom, northern France, Hungary and Czech Republic
Report No.:	15-2118
Document No.:	M-583909-02-1
Guideline(s):	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 OECD 509 Adopted 2009-09-07, OECD Guideline for the Testing of Chemicals, Crop Field Trial US EPA OCSPP Guideline No 860.1500
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 6.3.1/04; [redacted]; 2017; M-589582-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 and prothioconazole in/on winter barley and spring barley after spray application of prothioconazole & BCS-CN88460 EC 150 in the United Kingdom, Germany, northern France and the Netherlands
Report No.:	16-2051
Document No.:	M-589582-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals 'Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and Methods

Thirteen independent field residue trials were conducted on barley (with both spring and winter varieties) in the northern European residue region as follows:

In 2015, 4 supervised residue trials (the Netherlands, Germany, France and the United Kingdom) were conducted to support the use of BCS-CN88460 EC 050 in/on winter and spring barley ([redacted], G.; 2017; M-585588-02-1; KCA 6.3.1). One spray application was made at a nominal growth stage of BBCH 61 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 200-400 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing, except in the trial 15-2110-02 where the application was done at growth stage BBCH 59 instead of 61. This is considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

4 additional supervised residue trials (Netherlands, Germany, France and the United Kingdom) were conducted with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on winter and spring barley ([redacted], 2017; M-580046-02-1; KCA 6.3.1). One spray application was made at a nominal growth stage of BBCH 61 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 200-400 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing, except in the trial 15-2113-02 where the application was done at growth stage BBCH 59 instead of 61. This is considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

Trials 15-2113-02, 15-2113-03 and 15-2113-04 were located side by side with trials 15-2110-02, 15-2110-03 and 15-2110-04. Thus, for the studies 15-2110 and 15-2113, only 5 trials are considered as independent trials.

Four further supervised residue trials were carried out in 2015, in the United Kingdom, France, Hungary and Czech Republic with the formulation Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 in/on winter and spring barley ([redacted], G.; 2017; M-583909-02-1; KCA 6.3.1). One spray application was made at a nominal growth stage of BBCH 61 and a nominal rate of 1.25 L/ha, corresponding to 62.5 g/ha BCS-CN88460 a.s.; the water rate was 200- 300 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing.

Four further supervised residue trials were carried out in 2016 with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on spring and winter barley, in the United Kingdom, Germany, France and the Netherlands, to complete the data package ([redacted]; 2017; M-589582-02-1; KCA 6.3.1). One spray application was made at a nominal growth stage of BBCH 61 and a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS- CN88460 a.s.; the water rate was 200- 400 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing.

In all these trials, samples of green material (whole plants without roots) were taken immediately after the application and at several intervals thereafter (up to 28 days after treatment). It was ensured that one of these samplings be taken at BBCH 75. Furthermore, samples of grain and straw were collected at the commercial harvest (BBCH 89).

Each field sample was placed in doubled labeled bags and stored deep-frozen within 24 hours after sampling and until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer AG - Crop Science Division in [redacted] am Rhein, Germany. All field samples were shipped at a temperature of -18°C or below under monitored conditions during shipment and arrived at PVTL in good condition. The field samples were stored in a freezer at -18°C or below until preparation of the examination samples.

For the trial 15-2118-04, the maximum temperature during the two shipments of samples to PVTL was -13°C and -7°C. Since the shipments were sufficiently short (less than 24 h) this deviation is not expected to adversely impact the quality of the study results (for further information see Appendix 8 of the respective report).

For the preparation of examination samples the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at -18°C or below until analysis.

The samples were analysed for the parent compound using analytical method 01475 ([redacted]; 2016; M-558986-01-1; referenced in MCA Section 4 under Point 4.1.2) which was validated prior to the residue analysis of the samples. Additional validation recoveries were conducted for barley (grain, straw and green material) in the study 15-2066. The samples of grain and straw were analyzed according to the procedure described in the method for dry matrices (with a soaking step with water before extraction) and the green material samples were prepared according to the procedure for higher-water containing commodities (no soaking step before extraction). The LOQ was 0.01 mg/kg for parent.

II. Findings

In each study, concurrent recoveries were obtained from samples of green material, grain and straw. The recovery samples were spiked at levels of 0.01 mg/kg and up to 4 mg/kg, in order to adequately cover the residue levels found in the treated samples. Details of concurrent recovery data are shown in Table 6.3.1-3. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

No residues above the LOQs were found in the control samples. The detailed results obtained for barley treated samples in northern Europe are summarised below in Table 6.3.1- 4. The results were not corrected for concurrent recoveries.

The analyses were done after a maximum storage period of 390 days. In most of the cases, the time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours. If not the case, the maximum storage period of extracts (30 hours for green material and 55 hours for straw) was covered by stability experiments conducted in the course of the residue study 15-2066 ([redacted], G.; 2017; M-584388-02-1; KCA 6.3.1). See Table 6.1- 8.

III. Conclusions (barley, northern Europe)

In order to support the use in the EU of BCS-CN88460 in barley, 13 independent trials were conducted in the northern European residue region in the years 2015-2016. BCS-CN88460 was applied once at an active substance rate of 62.5 or 75 g/ha per treatment. All applications were at the required rates, and all trials were conducted according to GLP.

Samples were analysed for the residues of BCS-CN88460 parent compound. The results of the trials presented above demonstrate that:

- Residues of BCS-CN88460 dissipated rapidly in green material, from levels of 0.87-2.4 mg/kg on Day 0 after the treatment to 0.051-0.56 mg/kg on Day 28.
- At harvest, residues in grain ranged from <0.01 mg/kg to 0.041 mg/kg and residues in straw ranged from 0.049 to 1.2 mg/kg.

Table 6.3.1- 3: Concurrent recovery data for BCS-CN88460 in barley

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
15-2110	green material	1	0.01	96[1]	-	-	-	-
		1	0.1	97[2]	-	-	-	-
		1	2.5	94[1]	-	-	-	-
		3	**overall**		**94**	**97**	**96**	**1.5**
	grain	2	0.01	92[1]; 96[1]	92	96	94	-
		1	0.1	94[2]	-	-	-	-
		3	**overall**		**92**	**96**	**94**	**1.9**
	straw	1	0.01	114[1]	-	-	-	-
		1	0.1	100[2]	-	-	-	-
		1	2.0	93[2]	-	-	-	-
		3	**overall**		**93**	**114**	**102**	**10.9**
15-2113	green material	2	0.01	98; 99	98	99	99	-
		1	0.1	96	-	-	-	-
		1	2.5	93	-	-	-	-
		4	**overall**		**93**	**99**	**97**	**2.7**
	grain	1	0.01	97	-	-	-	-
		2	0.1	101; 102	101	102	-	-
		3	**overall**		**97**	**102**	**100**	**2.6**
	straw	1	0.01	104	104	-	-	-
		1	0.1	97	97	-	-	-
		1	2.0	99	99	-	-	-
		3	**overall**		**97**	**104**	**100**	**3.6**

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
15-2118	green material	1	0.01	98(2)	-	-	-	-
		1	0.1	106(2)	-	-	-	-
		1	2.0	98(1)	-	-	-	-
		1	2.5	94(1)	-	-	-	-
		4	**overall**		**94**	**106**	**99**	**5.1**
	grain	2	0.01	95(2);111(2)	95	111	103	-
		2	0.1	111(1);107(2)	107	111	109	-
		4	**overall**		**95**	**111**	**106**	**7.1**
	straw	1	0.01	93(2)	-	-	-	-
		1	0.1	101(2)	-	-	-	-
		1	2.0	85(1)	-	-	-	-
		3	**overall**		**85**	**101**	**93**	**8.6**
16-2051	green material	4	0.01	94; 86; 79; 85	79	94	86	7.2
		2	0.1	93; 95	93	95	94	-
		1	1.0	94	-	-	-	-
		1	4.0	95	-	-	-	-
		8	**overall**		**79**	**95**	**90**	**6.7**
	grain	3	0.01	83; 90; 94	83	94	89	6.3
		2	0.1	94; 101	94	101	98	-
		5	**overall**		**83**	**101**	**92**	**7.1**
	straw	1	0.01	96	-	-	-	-
		1	0.1	97	-	-	-	-
		1	1.0	84	-	-	-	-
		3	**overall**		**84**	**97**	**92**	**7.8**

RSD: relative standard deviation

(1) : recovery conducted with spring barley control sample

(2): recovery conducted with winter barley control sample

Table 6.3.1- 4: Detailed results of the barley residue trials in northern Europe

When trials were conducted in parallel plots, the highest residue level from the 2 plots was selected (underlined values).
Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2110-01 Netherlands [redacted] Europe, North F 2015	Spring barley/ Odyssey (malting barley middle early)	1) 23.03.2015 2) 20.06.2015 - 06.07.2015 3) 01.08.2015 - 20.08.2015	75	400	18.8	26.06.2015 -	61	Green material Grain Straw	61 61 75 77 83 89 89	1.8 0.41 0.20 0.12 0.12 <u>0.01</u> <u>0.11</u>	0 7 14 21 28 55 55	(f) 15-2110 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 264 days green material: 283 days grain: 231 days
15-2113-01 Netherlands [redacted] Europe, North F 2015	Spring Barley/Triple	1) 23.04.2015 2) 29.06.2015 - 06.07.2015 3) 15.08.2015 – 01.09.2015	75	400	18.8	29.06.2015 -	61	Green material Grain Straw	61 65 73 75 75 77 89 89	1.8 0.41 0.23 0.12 0.11 0.051 <u><0.01</u> <u>0.049</u>	0 7 14 16 21 28 61 61	(f) 15-2113 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 241 days green material: 283 days grain: 241 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1 BCS-CN88460		
	(a)	(b)				(c)					(d)	(e)
15-2110-02 Germany [redacted] Europe, North F 2015	Spring Barley/Streif	1) 13.03.2015 2) 12.06.2015 - 22.06.2015 3) 01.08.2015 - 31.08.2015	75	300	25	05.06.2015 0	59	Green material	59 61 65 75 77 83 89	2.4 0.98 0.33 0.22 0.18 0.092 <0.01	0 7 14 19 21 28 68	(f) 15-2110 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 272 days green material: 304 days grain: 239 days
								Grain Straw	89	0.42	68	
15-2113-02 Germany [redacted] Europe, North F 2015	Spring Barley/Streif	1) 13.03.2015 2) 12.06.2015 - 22.06.2015 3) 01.08.2015 – 31.08.2015	75	300	25	05.06.2015 0	59	Green material	59 61 65 75 77 83 89	2.0 1.1 0.55 0.39 0.28 0.20 <u>≤0.01</u>	0 7 14 19 21 28 68	(f) 15-2113 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 258 days green material: 307 days grain: 258 days
								Grain Straw	89	<u>0.24</u>	68	

These 2 trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2110-03 France [redacted] Europe, North F 2015	Winter Barley/ Etincel	1) 14.10.2014 2) 27.04.2015 – 05.05.2015 3) 25.06.2015 – 05.07.2015	75	300	25	28.04.2015	61	Green material	61 69 71 73 75 Grain Straw	1.1 0.18 0.16 0.091 0.098 <0.01 0.18	0 7 14 21 28 62 62	(f) 15-2110 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 316 days green material: 342 days grain: 283 days
15-2113-03 France [redacted] Europe, North F 2015	Winter Barley/ Etincel	1) 14.10.2014 2) 27.04.2015 – 05.05.2015 3) 25.06.2015 – 05.07.2015	75	300	25	28.04.2015	61	Green material	61 69 71 73 75 Grain Straw	1.1 0.18 0.11 0.080 0.051 <u><0.01</u> <u>0.20</u>	0 7 14 21 28 62 62	(f) 15-2113 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 302 days green material: 345 days grain: 302 days

These 2 trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460 (d)		(e)
15-2110-04 United Kingdom [redacted] Europe, North F 2015	Winter Barley/ Glacier	1) 12.11.2014 2) 21.05.2015 – 01.06.2015 3) 01.08.2015 – 15.08.2015	75	200	37.5	21.05.2015	61	Green material	61 69 69 71 73 75 89 89	1.7 1.2 0.59 0.35 0.22 0.18 <0.01 0.60	0 7 14 20 28 33 77 77	(f) 15-2110 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 281 days green material: 319 days grain: 245 days
								Grain Straw				
15-2113-04 United Kingdom [redacted] Europe, North F 2015	Winter Barley/ Glacier	1) 12.11.2014 2) 21.05.2015 – 01.06.2015 3) 01.08.2015 – 15.08.2015	75	200	37.5	21.05.2015	61	Green material	61 69 69 71 73 75 89 89	1.7 1.2 0.69 0.46 0.26 0.28 <0.01 0.94	0 7 14 20 28 33 77 77	(f) 15-2113 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 264 days green material: 322 days grain: 264 days
								Grain Straw				

These 2 trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undersline)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460 (d)		(e)
15-2118-01 United Kingdom [redacted], PE12 9PQ Europe, North F 2015	Winter Barley/Cassia	1) 02.09.2014 2) 20.05.2015 – 01.06.2015 3) 23.07.2015	62.5	200	31.3	21.05.2015	61	Green material	61 65 71 75 83 89 89	1.2 0.74 0.25 0.16 0.075 <0.01 0.16	0 8 13 21 28 63 63	(f) 15-2118 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 320 days green material: 368 days grain: 306 days
15-2118-02 France [redacted] Bourgogne Europe, North F 2015	Winter Barley/ Esterel	1) 03.10.2014 2) 29.04.2015 – 06.05.2015 3) 23.06.2015	62.5	300	20.8	29.04.2015	61	Green material Grain Straw	61 69 75 85 87 89 89	1.2 0.12 0.14 0.095 0.094 0.020 0.51	0 7 14 21 28 55 55	(f) 15-2118 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 350 days green material: 390 days grain: 336 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS.CN88460	(d)	(e)
15-2118-03 Hungary [redacted] Europe, North F 2015	Spring Barley/ Mandolina	1)13.03.2015 2) 30.05. 2015 – 15.06.2015 3)10.07.2015	62.5	300	20.8	05.06.2015	61	Green material	61 73 75 83 85 Grain Straw	0.87 0.53 0.31 0.35 0.55 <0.013 0.96	0 7 14 21 28 35 35	(f) 15-2118 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 333 days green material: 353 days grain: 319 days
15-2118-04 Czech Republic [redacted] Europe, North F 2015	Spring Barley/ Kangoo	1) 18.04.2015 2) 12.06.2015 – 19.06.2015 3) 05.08.2015	62.5	300	20.8	12.06.2015	61	Green material	61 69 75 83 85 Grain Straw	2.4 1.5 0.91 0.63 0.56 <0.01 1.2	0 7 14 20 28 55 55	(f) 15-2118 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 306 days green material: 346 days grain: 292 days

These two trials were not conducted in side-by-side plots. They are independent trials.

For the trial 15-2118-04, the maximum temperature during the two shipments of samples to PVTL was -13°C and -7°C. This deviation was considered acceptable (see Appendix 8 of the respective report).

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)

- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS.CN88460 (d)		(e)
16-2051-01 United Kingdom [redacted] Europe, North F 2016	Spring Barley/ Odyssey	1) 08.04.2016 2) 19.06.2016 - 27.06.2017 3) 01.08.2016 – 22.08.2016-	75	200	37.5	19.06.2016	61	Green material	61 69 73 75 75 83 Grain Straw	2.4 0.25 0.16 0.10 0.13 0.090 <u>0.01</u> <u>0.40</u>	0 8 15 22 22 29 57 57	(f) 16-2051 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 190 days green material: 248 days grain: 186 days
16-2051-02 Germany [redacted] Europe, North F 2016	Spring Barley/Vespa	1) 04.04.2016 2) 16.06.2016 – 21.06.2016 3) 01.08.2016 – 31.08.2016	75	300	25	16.06.2016	61	Green material	61 71 75 75 77 83 Grain Straw	2.4 0.53 0.17 0.17 0.13 0.10 <u>< 0.01</u> <u>0.32</u>	0 7 14 14 21 28 60 60	(f) 16-2051 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 190 days green material: 251 days grain: 186 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
16-2051-03 France [redacted] Europe, North F 2016	Winter Barley/Obit	1) 01.10.2015 2) 06.05.2016 – 13.05.2016 3) 25.06.2016 – 10.07.2016	75	300	25	06.05.2016	61	Green material	61 69 71 73 75 Grain Straw	1.4 0.45 0.30 0.18 0.11 < 0.01 0.13	0 7 14 21 27 53 53	(f) 16-2051 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 238 days green material: 292 days grain: 234 days
16-2051-04 The Netherlands [redacted] Europe, North F 2016	Winter Barley/ Quadrigo	1) 15.10.2015 2) 31.05.2016 – 15.06.2016 3) 07.07.2016 – 15.07.2016	75	400	18.8	31.05.2016	61	Green material	61 65 69 71 83 86 Grain Straw	1.6 0.97 0.83 0.54 0.39 0.41 0.041 0.44	0 7 14 21 28 34 37 37	(f) 16-2051 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 229 days green material: 267 days grain: 229 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undersine)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Southern Europe

Report:	KCA 6.3.1/05; [redacted], G.; 2017; M-584388-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on barley after spray application of BCS-CN88460 EC 050 in Portugal, southern France and Spain
Report No.:	15-2066
Document No.:	M-584388-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 6.3.1/06; [redacted]; 2017; M-580022-02-1
Title:	Amendment no. 1: Determination of the residues of BCS-CN88460 and prothioconazole in/on barley after spray application of prothioconazole & BCS-CN88460 EC 150 in Portugal, southern France and Spain
Report No.:	15-2114
Document No.:	M-580022-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	None
GLP/GEP:	**yes**

Report:	KCA 6.3.1/07; [redacted], G.; 2017; M-583692-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460, prothioconazole and tebuconazole in/on barley after spray application of prothioconazole & tebuconazole & BCS-CN88460 EC 250 in southern France, Italy, Spain and Portugal
Report No.:	15-2117
Document No.:	M-583692-02-1
Guideline(s):	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 OECD 509 Adopted 2009-09-07, OECD Guideline for the Testing of Chemicals, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 6.3.1/08; [redacted]; 2017; M-589554-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 and prothioconazole in/on barley after spray application of prothioconazole & BCS-CN88460 EC 150 in Portugal, southern France and Spain
Report No.:	16-2052
Document No.:	M-589554-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	yes, see report
GLP/GEP:	**yes**

I. Materials and Methods

Twelve independent field residue trials were conducted on barley (with both spring and winter varieties) in the southern European residue region, as follows:

In 2015, 4 supervised residue trials (Portugal, Spain and southern France) were conducted to support the use of BCS-CN88460 EC 050 in/on barley ([redacted] G.; 2017; M-584388-02-1; KCA 6.3.1). One spray application was made at a nominal growth stage of BBCH 61 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 300 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing except for the trial 15-2066-03 where the application was done at BBCH 53 instead of 61. This deviation is considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

4 additional supervised residue trials (Portugal, Spain and southern France) were conducted with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on barley ([redacted]; 2017; M-580022-02-1; KCA 6.3.1). One spray application was made at a nominal growth stage of BBCH 61 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 300 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing except for the trial 15-2114-03 where the application was done at BBCH 53 instead of 61. This deviation is considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

Trials 15-2066-01, 15-2066-02, 15-2066-03 and 15-2066-04 were located side by side with trials 15-2114-01, 15-2114-02, 15-2114-03 and 15-2114-04, respectively. Thus, for the studies 15-2066 and 15-2114, only 4 trials are considered as independent trials.

In trial 15-2066-02 and 15-2114-02 a rainfall happened within 6 hours after application. Day 0 sampling in the treated plot was collected before rainfall in trial 15-2066-02 and after rainfall in trial 15-2114-02. An impact on the residue levels cannot completely be excluded. On the other hand, this rainfall event reflects what can happen in practice. Therefore these trials are considered acceptable for MRL setting.

Four further supervised residue trials were carried out in 2015, in southern France, Italy, Spain and Portugal, with the formulation Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 in/on barley ([redacted], G., 2017; M-583692-02-1; KCA 6.3.1). One spray application was made at a nominal growth stage of BBCH 61 and a nominal rate of 1.25 L/ha, corresponding to 62.5 g/ha BCS-CN88460 a.s.; the water rate was 300-400 L/ha, reflecting local practice in the trial regions. Again, all treatments were made at the scheduled rates.

Four further supervised residue trials were carried out in 2016 with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on barley, in Portugal, southern France and Spain, to complete the data package ([redacted]; 2017; M-589554-02-1; KCA 6.3.1). One spray application was made at a

nominal growth stage of BBCH 61 and a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 300 L/ha, reflecting local practice in the trial regions. Again, all treatments were made at the scheduled rates.

In all these trials, samples of green material (whole plants without roots) were taken immediately after the application and at several intervals thereafter (up to 28/27 days after treatment). It was ensured that one of these samplings be taken at BBCH 75. Furthermore, samples of grain and straw were collected at the commercial harvest (BBCH 89).

In the trial 16-2052-03 the grain sample weight was 0.96 kg instead of 1 kg required in the protocol and OECD guideline 509. Nevertheless, the sampling was done randomly over the whole plot and the deviation from the targeted sample weight is low (4%). The collected sample is still considered representative of the plot and this deviation is deemed acceptable.

Each field sample was placed in doubled labeled bags and stored deep-frozen within 24 hours after sampling and until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer AG - Crop Science Division in [redacted] am Rhein, Germany. All field samples were shipped at a temperature of -18°C or below under monitored conditions during shipment and arrived at PVTL in good condition. The field samples were stored in a freezer at -18°C or below until preparation of the examination samples.

During the storage of the samples of the trials 15-2117-03 and 15-2117-04 the temperature rose above -18°C, reaching a maximum of -18.8°C and -15.3°C respectively. During the samples storage of the samples of the trial 16-2052-01 the temperature rose above -18°C for a short period, reaching a maximum of -9°C. Nevertheless, it was considered unlikely that these deviations have a negative impact on the quality of the study. For further details see Appendix 8 of the respective reports.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at -18°C or below until analysis.

The samples were analysed for the parent compound using analytical method 01475 ([redacted]; 2016; M-558986-01-1 referenced in MCA 4 Point 4.1.2) which was validated prior to the residue analysis of the samples. Additional validation recoveries were conducted for barley (grain, straw and green material) in the study 15-2066. The samples of grain and straw were analyzed according to the procedure described in the method for dry matrices (with a soaking step with water before extraction) and the green material samples were prepared according to the procedure for higher-water containing commodities (no soaking step before extraction). The LOQ was 0.01 mg/kg for parent.

II. Findings

In each study, concurrent recoveries were obtained from samples of green material, grain and straw. The recovery samples were spiked at levels of LOQ (0.01 mg/kg) up to 5 mg/kg in order to adequately cover the residue levels found in the treated samples. Details of concurrent recovery data are shown in Table 6.3.1- 5. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

No residues above the LOQs were found in the control samples. The detailed results obtained for barley treated samples are summarised below in Table 6.3.1- 6. The results were not corrected for concurrent recoveries.

The analyses were done after a maximum storage period of 390 days. In most of the cases, the time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours. If not the case, the maximum storage period of extracts (80.4 hours for green material and 76 hours for straw) was covered by stability experiments conducted in the course of the residue study 15-2066 ([redacted], G.; 2017; M-584388-02-1; KCA 6.3.1). See Table 6.1- 8.

III. Conclusions (barley, southern Europe)

In order to support the use in the EU of BCS-CN88460 in barley, 12 independent trials were conducted in the southern European residue region in the years 2015-2016. BCS-CN88460 was applied once at an active substance rate of 62.5 or 75 g/ha per treatment. All applications were at the required rates, and all trials were conducted according to GLP.

Samples were analysed for the residues of BCS-CN88460 parent compound. The results of the trials presented above demonstrate that:

- Residues of BCS-CN88460 dissipated rapidly in green material, from levels of 0.87-3.1 mg/kg on Day 0 after the treatment to 0.048 - 1.4 mg/kg on Day 28/27.
- At harvest, residues in grain ranged from <0.01 mg/kg to 0.037 mg/kg and residues in straw ranged from 0.021 to 3.1 mg/kg.

Table 6.3.1- 5: Concurrent recovery data for BCS-CN88460 in barley

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
15-2114	green material	2	0.01	97; 98	97	98	98	-
		1	0.10	95	-	-	-	-
		1	1.0	100	-	-	-	-
		1	2.0	92	-	-	-	-
		5	**overall**		**92**	**100**	**96**	**3.2**
	grain	3	0.01	90; 95; 98	90	98	94	4.3
		1	0.10	96	-	-	-	-
		4	**overall**		**90**	**98**	**95**	**3.6**
	straw	1	0.01	91	-	-	-	-
		1	0.10	95	-	-	-	-
		1	2.0	96	-	-	-	-
		3	**overall**		**91**	**96**	**94**	**2.8**
15-2066	green material	4	0.01	81; 85; 91; 102	81	102	90	10.2
		4	0.10	80; 81; 93; 98	80	98	88	10.1
		1	1.0	96	-	-	-	-
		1	2.0	97	-	-	-	-
		10	**overall**		**80**	**98**	**90**	**8.9**
	grain	4	0.01	94; 95; 96; 98	94	98	96	1.8
		4	0.10	100; 100; 101; 104	100	104	101	1.9
		8	**overall**		**94**	**104**	**99**	**3.4**
	straw	3	0.01	97; 98; 121	97	121	105	12.9
		3	0.10	89; 100; 101	89	101	97	6.9
		1	2.0	101	-	-	-	-
		7	**overall**		**89**	**121**	**101**	**9.7**

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
15-2117	green material	1	0.01	103	-	-	-	-
		1	0.10	105	-	-	-	-
		1	2.0	96	-	-	-	-
		1	2.5	84	-	-	-	-
		4	**overall**		**84**	**105**	**97**	**9.8**
	grain	2	0.01	95; 100	95	100	98	
		1	0.10	105	-	-	-	-
		4	**overall**		**95**	**105**	**100**	**5.0**
	straw	1	0.01	102	-	-	-	-
		1	0.10	96	-	-	-	-
		1	2.0	92	-	-	-	-
		1	5.0	77	-	-	-	-
		4	**overall**		**77**	**102**	**92**	**11.6**
16-2052	green material	5	0.01	83; 87; 98; 98; 100	83	100	93	8.2
		2	0.10	95; 97	95	97	96	-
		1	1.0	96	-	-	-	-
		1	2.0	93	-	-	-	-
		1	4.0	96	-	-	-	-
		10	**overall**		**83**	**100**	**94**	**5.7**
	grain	4	0.01	91; 93; 94; 105	91	105	96	6.6
		1	0.10	90	-	-	-	-
		5	**overall**		**90**	**105**	**95**	**6.4**
	straw	1	0.01	99	-	-	-	-
		1	0.10	97	-	-	-	-
		1	2.0	101	-	-	-	-
		3	**overall**		**97**	**101**	**99**	**2.0**

RSD: relative standard deviation

Table 6.3.1- 6: Detailed results of the barley residue trials in southern Europe

When trials were conducted in parallel plots, the highest residue level from the 2 plots was selected (underlined values).
Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1 BCS-CN88460		
	(a)	(b)				(c)					(d)	(e)
15-2114-01 Portugal [redacted] Europe, South F 2015	Winter Barley/ Pewter	1) 28.12.2014 2) 16.04.2015 – 25.04.2015 3) 15.06.2015 – 15.07.2015	75	300	25	17.04.2015	61	Green material	61 65 71 73 75 Grain Straw	<1 1.4 0.50 0.42 0.29 <0.01 1.0	0 6 14 21 28 60 60	(f) 15-2114 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 262 days green material: 332 days grain: 261 days
15-2066-01 Portugal [redacted] Europe, South F 2015	Winter Barley/ Pewter	1) 28.12.2014 2) 16.04.2015 – 25.04.2015 3) 15.06.2015 – 15.07.2015	75	300	25	17.04.2015	61	Green material	61 65 71 73 75 Grain Straw	1.9 1.5 0.52 0.37 0.23 <0.01 0.85	0 6 14 21 28 60 60	(f) 15-2066 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 238 days green material: 326 days grain: 238 days

These 2 trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2114-02 France [redacted] Europe, South F 2015	Winter Barley/ Baraka	1) 16.10.2014 2) 15.04.2015 – 20.04.2015 3) 20.06.2015 – 30.06.2015	75	300	25	16.04.2015	61	Green material	61 69 71 75 77 Grain Straw	1.0 ** 0.24 0.15 0.10 0.074 <0.01 0.16	0 7 14 21 27 69 69	(f) 15-2114 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 254 days green material: 356 days grain: 253 days
15-2066-02 France [redacted] Europe, South F 2015	Winter Barley/ Baraka	1) 16.10.2014 2) 15.04.2015 – 20.04.2015 3) 20.06.2015 – 30.06.2015	75	300	25	16.04.2015	61	Green material	61 69 71 75 77 Grain Straw	1.6 * 0.28 0.14 0.091 0.076 <0.01 0.14	0 7 14 21 27 69 69	(f) 15-2066 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 230 days green material: 327 days grain: 230 days

These 2 trials were conducted in side-by-side plots at the same location. Although rainfall occurred after application, these trials are considered acceptable for MRL setting.

* sample collected before the rainfall after application ** sample collected after the rainfall after application

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2114-03 Spain [redacted] Europe, South F 2015	Malting Barley/ Traveler	1) 18.12.2014 2) 07.04.2015 – 14.04.2015 3) 25.05.2015 – 25.06.2015	75	300	25	31.03.2015	53	Green material	53 61 71 75 75 83 89 89	1.6 1.1 0.65 0.27 0.32 0.30 0.01 0.29	0 7 14 21 22 28 57 57	(f) 15-2114 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 282 days green material: 350 days grain: 281 days
15-2066-03 Spain [redacted] Europe, South F 2015	Malting Barley/ Traveler	1) 18.12.2014 2) 07.04.2015 – 14.04.2015 3) 25.05.2015 – 25.06.2015	75	300	25	31.03.2015	53	Green material	53 61 71 75 75 83 89 89	2.0 1.2 0.55 0.28 0.25 0.20 <0.01 0.22	0 7 14 21 22 28 57 57	(f) 15-2066 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 258 days green material: 343 days grain: 258 days

These 2 trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2114-04 France [redacted] Europe, South F 2015	Winter Barley/Cacia	1) 10.11.2014 2) 05.05.2015 - 12.05.2015 3) 19.06.2015- 02.07.2015	75	300	25	05.05.2015	61	Green material	61 69 73 75 77 83 Grain Straw	1.8 1.1 0.42 0.45 0.37 0.47 0.012 <u>0.96</u>	0 7 14 17 21 28 45 45	(f) 15-2114 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 259 days green material: 315 days grain: 259 days
15-2066-04 France [redacted] Europe, South F 2015	Winter Barley/Cacia	1) 10.11.2014 2) 05.05.2015 – 12.05.2015 3) 19.06.2015 – 02.07.2015	75	300	25	05.05.2015	61	Green material	61 69 73 75 77 83 Grain Straw	1.8 1.0 0.28 0.39 0.37 0.41 <u>0.022</u> 0.65	0 7 14 17 21 28 45 45	(f) 15-2066 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 235 days green material: 308 days grain: 269 days

These 2 trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2117-01 France [redacted] Europe, South F 2015	Barley/Jallon	1) 27.10.2014 2) 23.04.2015 – 30.04.2015 3) 11.06.2015	62.5	300	20.8	24.04.2015	61	Green material	61 71 75 77 83	0.87 0.14 0.084# 0.065 0.050	0 7 13 21 28	(f) 15-2117 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 344 days green material: 390 days grain: 336 days # this value is a mean value
								Grain Straw	89 89	<0.01 0.13	48 48	
15-2117-02 Italy [redacted] (CN) Europe, South F 2015	Barley/Sfera	1) 20.10.2014 2) 08.05.2015 31.05.2015 3) 15.06.2015 – 30.06.2015	62.5	350	17.9	11.05.2015	61	Green material	61 71 75 77 87	1.1 0.75 0.31 0.24 0.34	0 7 14 21 28	(f) 15-2117 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 340 days green material: 373 days grain: 332 days
								Grain Straw	89 89	0.037 0.29	35 35	

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2117-03 Spain [redacted] (Albacete) Europe, South F 2015	Barley/ Shakira	1) 28.01.2015 2) May 2015 3) 30.06.2015	62.5	300	20.8	14.05.2015	61	Green material	61 71 75 77 77 Grain Straw	1.3 0.20 0.11 0.054 0.048 <u>0.01</u> <u>0.021</u>	0 7 13 20 27 46 46	(f) 15-2117 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 326 days green material: 370 days grain: 318 days
15-2117-04 Portugal [redacted] (Ribatejo) Europe, South F 2015	Barley/ Pewter	1) 09.03.2015 2) May 2015 3) July 2015	62.5	400	15.6	21.05.2015	61	Green material	61 75 77 83 83 Grain Straw	1.7 0.83 0.83 1.4 1.4 <u>0.027</u> <u>3.1</u>	0 8 14 20 27 41 41	(f) 15-2117 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2066 (l) straw: 334 days green material: 372 days grain: 316 days

These two trials were not conducted in side-by-side plots. They are independent trials.

During the storage of the samples of the trials 15-2117-03 and 15-2117-04 the temperature rose above -18°C, reaching a maximum of -15.8°C and -15.3°C, respectively. This deviation was considered acceptable (see Appendix 8 of the respective reports).

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
16-2052-01 Portugal [redacted] Europe, South F 2016	Barley/ Pewter	1) 11.12.2015 2) 07.04.2016 - 17.04.2016 3) 15.06.2016 – 15.07.2016	75	300	25	08.04.2016	61	Green material	61 65 71 73 75	1.4 0.37 0.28 0.16 0.079	0 7 14 21 28	(f) 16-2052 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain Straw	89 89	<0.01 * 0.24 *	67 67	(k) Method Validation Data in 01475 and study 15-2066 (l) straw: 188 days green material: 251 days grain: 118 days
16-2052-02 France [redacted] Europe, South F 2016	Winter Barley/ Augusta	1) 17.11.2015 2) 25.04.2016 – 30.04.2016 3) 15.06.2016 – 20.06.2016	75	300	25	22.04.2016	61	Green material	61 69 71 73 75	1.8 1.3 0.69 0.30 0.23	0 7 12 20 27	(f) 16-2052 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain Straw	89 89	<0.01 0.31	54 54	(k) Method Validation Data in 01475 and study 15-2066 (l) straw: 187 days green material: 237 days grain: 117 days

These two trials were not conducted in side-by-side plots. They are independent trials.

* samples affected by a temperature deviation during the shipment from the field to PVTL. This deviation was nervertheless considered acceptable (see Appendix 8 of the report)

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
16-2052-03 Spain [redacted] Europe, South F 2016	Malting Barley/ Odyssey	1) 10.12.2015 2) 14.04.2016 – 20.04.2016 3) 25.05.2016 – 30.06.2016	75	300	25	14.04.2016	61	Green material	61 71 73 75 77	0.1 0.90 0.89 0.95 0.32	0 7 14 21 28	(f) 16-2052 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain Straw	89 89	< 0.01 * 0.85	56 57	(k) Method Validation Data in 01475 and study 15-2066 (l) straw: 192 days green material: 246 days grain: 122 days
16-2052-04 France [redacted] Europe, South F 2016	Winter Barley/Cacia	1) 20.10.2015 2) 03.05.2016 10.05.2016 3) 20.06.2016 – 01.07.2016	75	300	25	03.05.2016	61	Green material	61 71 73 77 83	0.94 0.55 0.30 0.23 0.091	0 8 14 21 28	(f) 16-2052 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain Straw	89 89	< 0.01 0.18	49 49	(k) Method Validation Data in 01475 and study 15-2066 (l) straw: 181 days green material: 232 days grain: 111 days

These two trials were not conducted in side-by-side plots. They are independent trials.

* The grain sample weight was 0.96 kg instead of 1 kg required by OECD guideline 509. The collected sample is still considered representative of the plot and this deviation is deemed acceptable (see Appendix 8 of the report).

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Comparison of the barley results for the three formulations tested

Since residues in grain at harvest are often <0.01 mg/kg, the comparison of the results for the different formulations used was done using the results in straw at harvest. The data sets 1, 2 and 3 were compared to each other's using the statistical tests: Kruskal-Wallis H-Test and Mann-Whitney U-test, $\alpha = 0.05$. The same approach was done for the data sets 4, 5 and 6.

Dataset 1	Dataset 2	Dataset 3	Dataset 4	Dataset 5	Dataset 6
EC050 EUN	EC150 EUN	EC250 EUN	EC050 EUS	EC150 EUS	EC250 EUS
0.11	0.049	0.16	1.0	0.85	0.13
0.12	0.24	0.51	0.16	0.14	0.29
0.18	0.20	0.96	0.29	0.22	0.021
0.60	0.94	1.2	0.96	0.65	3.1
	0.40			0.24	
	0.32			0.31	
	0.13			0.85	
	0.44			0.18	

EUN: northern Europe EUS: southern Europe

The results of these statistical tests indicate that the populations from the data sets 1, 2 and 3 are not significantly different from each other's. The same applies for the datasets 4, 5 and 6.

The residue data for the different formulations can thus be combined in the following table to establish MRLs. When trials were conducted in side-by-side plots, the highest residue level from the 2 plots was selected. The selected data are compiled in Table 6.3.1-7.

Table 6.3.1-7: Summary of BCS-CN88460 residue data for barley trials

Crop	Northern/ Southern field or glasshouse	Trial results relevant to the critical GAP	STMR (a)	HR (b)
Barley Grain	Northern	10 × <0.01; 0.013; 0.020; 0.041	<0.01	0.041
	Southern	9 × <0.01; 0.022; 0.027; 0.037	<0.01	0.037
Barley Straw	Northern	0.049; 0.11; 0.13; 0.16; 0.20; 0.24; 0.32; 0.40; 0.44; 0.51; 0.94; 0.96; 1.2	0.32	1.2
	Southern	0.021; 0.13; 0.16; 0.18; 0.24; 0.29; 0.29; 0.31; 0.85; 0.96; 1.0; 3.1	0.29	3.1

(a) Supervised Trials Median Residue: i.e. the median residue level estimated on the basis of supervised trials relating to the critical GAP
(b) Highest residue

The results of the statistical tests Kruskal-Wallis H-Test and Mann-Whitney U-test, $\alpha = 0.05$ indicate that the straw residue data from northern Europe are not significantly different from the straw residue data in southern Europe.

CA 6.3.2 Wheat

BCS-CN88460 (common name: isoflucypram) is to be registered in Europe for use in wheat, Durum wheat, rye, spelt and triticale. European residue data in wheat are therefore presented below to support the intended "representative uses".

As allowed by the EU Guidance document on comparability, extrapolation, group tolerances and data requirements for setting MRLs (SANCO 7525/VI/95 rev. 10.2 of 23 September 2016), the extrapolation of the wheat residue data to rye, spelt and triticale is requested.

Information on the intended use pattern (GAP) is summarised in Table 6.3.2- 1.

Table 6.3.2- 1: Use patterns (critical GAP) for the spray application of BCS-CN88460-containing formulations in/on wheat, Durum wheat, rye, spelt and triticale in European fields (northern and southern residue regions)

Description	F/G	No. of appls.	Growth stage at application (BBCH Code)	Application rate per treatment (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)
Wheat, Durum wheat, rye, spelt and triticale *	F**	1	30-69	75	100-400	-	NA

* agricultural use based on EC formulations (BCS-CN88460 EC 050 and Prothioconazole & BCS-CN88460 EC 150)
** uses in both the northern and southern residue regions (EU-N and EU-S)
NA: not applicable. The application timing is defined by the growth stage at application.

In order to support the EU "representative use" of BCS-CN88460, sets of GLP trials were conducted in northern and southern European fields in 2015 and 2016. In northern and in southern European field-grown wheat, BCS-CN88460 was applied once at the latest intended growth stage of BBCH 69.

Three EC formulations containing BCS-CN88460 were tested with similar application rates:

- BCS-CN88460 EC 050 containing 50 g BCS-CN88460 /L
  Tested application rate: 75 g BCS-CN88460 /ha.
- Prothioconazole & BCS-CN88460 EC 150 containing 50 g BCS-CN88460 /L
  Tested application rate: 75 g BCS-CN88460 /ha.
- Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 containing 50 g BCS-CN88460 /L
  Tested application rate: 62.5 g BCS-CN88460 /ha (within + or – 25% of the 75 g a.s./ha application rate).

In some cases the EC050 and the EC 150 formulations were tested in side-by-side plots at the same location so that these trials cannot be considered as independent trials.

The number of independent trials conducted (incl. information on geographical region and vegetation period) is summarised below in Table 6.3.2- 2.

Table 6.3.2- 2: Overview of European residue trials conducted in barley per geographical "residue region" and vegetation period

Crop	Region	No. of independent trials			Report No. (Formulation)	Document number	Reference
		Veget. period		Σ			
		2015	2016				
Wheat	EU-N	8	4	12	15-2111 (EC 050) 15-2115 (EC 150) 15-2120 (EC 250) 16-2053 (EC 150)	M-586570-02-1 M-578221-03-1 M-584680-03-1 M-593778-02-1	KCA 6.3.2/01 KCA 6.3.2/02 KCA 6.3.2/03 KCA 6.3.2/04
	EU-S	8	5	13	15-2069 (EC 050) 15-2116 (EC 150) 15-2119 (EC 250) 16-2054 (EC 150)	M-584384-02-1 M-580537-03-1 M-584690-02-1 M-594320-02-1	KCA 6.3.2/05 KCA 6.3.2/06 KCA 6.3.2/07 KCA 6.3.2/08

EU-N = northern EU field EU-S = southern EU field
FL : formulation
EC 050: BCS-CN88460 EC 050 containing 50 g BCS-CN88460 /L
EC 150: Prothioconazole & BCS-CN88460 EC 150 containing 50 g BCS-CN88460 /L
EC 250: Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 containing 50 g BCS-CN88460 /L

Northern Europe (residue region)

Report: KCA 6.3.2/01; [redacted], G.; 2017; M-586570-02-1
Title: Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on spring and winter wheat after spray application of BCS-CN88460 EC 050 in northern France, the United Kingdom, the Netherlands and Germany
Report No.: 15-2111
Document No.: M-586570-02-1
Guideline(s): 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
OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009)
US EPA OCSPP 860.1500 Crop Field Trial
Guideline deviation(s): none
GLP/GEP: **yes**

Report: KCA 6.3.2/02; [redacted]; 2017; M-578221-03-1
Title: Amendment no. 2: Determination of the residues of BCS-CN88460 and prothioconazole in/on wheat after spray application of prothioconazole & BCS-CN88460 EC 150 in northern France, United Kingdom, the Netherlands and Germany
Report No.: 15-2115
Document No.: M-578221-03-1
Guideline(s): 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
OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009)
US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s): Yes (see report)
GLP/GEP: **yes**

Report:	KCA 6.3.2/03; [redacted], G.; 2017; M-584680-03-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460, prothioconazole and tebuconazole in/on spring wheat and winter wheat after spray application of prothioconazole & tebuconazole & BCS-CN88460 EC 250 in United Kingdom, Hungary, northern France and Poland
Report No.:	15-2120
Document No.:	M-584680-03-1
Guideline(s):	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 OECD 509 Adopted 2009-09-07, OECD Guideline for the Testing of Chemicals, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Guideline deviation(s):	yes, see report
GLP/GEP:	**yes**

Report:	KCA 6.3.2/04; [redacted]; 2017; M-593778-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 and prothioconazole in/on winter wheat and spring wheat after spray application of prothioconazole & BCS-CN88460 EC 150 in northern France, Belgium, the Netherlands and Germany
Report No.:	16-2053
Document No.:	M-593778-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and Methods

Twelve independent field residue trials were conducted on wheat (with both spring and winter varieties) in the northern European residue region, as follows:

In 2015, 4 supervised residue trials (northern France, the United Kingdom, the Netherlands and Germany) were conducted to support the use of BCS-CN88460 EC 050 in/on spring and winter wheat ([redacted], G.; 2017; M-586570-02-1; KCA 6.3.2). One spray application was made at a nominal growth stage of BBCH 69 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 200-400 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing.

4 additional supervised residue trials (northern France, the United Kingdom, the Netherlands and Germany) were conducted with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on spring and winter wheat ([redacted]; 2017; M-578221-03-1; KCA 6.3.2). One spray application was made at a nominal growth stage of BBCH 69 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 200 - 400 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing.

Trials 15-2111-01, 15-2111-02, 15-2111-03 and 15-2111-04 were located side by side with trials 15-2115-01, 15-2115-02, 15-2115-03 and 15-2115-04. Thus, for the studies 15-2111 and 15-2115, only 4 trials are considered as independent trials.

Four further supervised residue trials were carried out in 2015, in the United Kingdom, Hungary, France and Poland with the formulation Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 in/on spring and winter wheat ([redacted], G.; 2017; M-584680-03-1; KCA 6.3.2). One spray application

was made at a nominal growth stage of BBCH 69 and a nominal rate of 1.25 L/ha, corresponding to 62.5 g/ha BCS-CN88460 a.s.; the water rate was 200-300 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing.

Four further supervised residue trials were carried out in 2016 with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on winter and spring wheat (northern France, Belgium, the Netherlands and Germany) to complete the data package ([redacted]; 2017; M-593778-02-1; KCA 6.3.2). One spray application was made at a nominal growth stage of BBCH 69 and a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 300 - 400 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing, except in the trial 16-2053-02 where the application was done at growth stage BBCH 65 instead of 69. This deviation is considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

In all these trials, samples of green material (whole plants without roots) were taken immediately after the application and at several intervals thereafter (up to 28/29 days after treatment). Furthermore, samples of grain and straw were collected at the commercial harvest (BBCH 89).

Each field sample was placed in doubled labeled bags and stored deep-frozen within 24 hours after sampling and until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer AG - Crop Science Division in [redacted] am Rhein, Germany. All field samples were shipped at a temperature of -18°C or below under monitored conditions during shipment and arrived at PVTL in good condition. The field samples were stored in a freezer at -18°C or below until preparation of the examination samples.

For the preparation of examination samples the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at -18°C or below until analysis.

The samples were analysed for the parent compound using analytical method 01475 ([redacted]; 2016; M-558986-01-1; referenced in MCA Section 4 under Point 4.1.2) which was validated prior to the residue analysis of the samples. Additional validation recoveries were conducted for wheat (grain, straw and green material) in the study 15-2069. The samples of grain and straw were analyzed according to the procedure described in the method for dry matrices (with a soaking step with water before extraction) and the green material samples were prepared according to the procedure for higher-water containing commodities (no soaking step before extraction). The LOQ was 0.01 mg/kg for parent.

II. Findings

In each study, concurrent recoveries were obtained from samples of green material, grain and straw. The recovery samples were spiked at levels of 0.01 mg/kg and up to 4 mg/kg, in order to adequately cover the residue levels found in the treated samples. Details of concurrent recovery data are shown in Table 6.3.2- 3. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

No residues above the LOQs were found in the control samples. The detailed results obtained for wheat treated samples in northern Europe are summarised below in Table 6.3.2- 4. The results were not corrected for concurrent recoveries.

The analyses were done after a maximum storage period of 381 days. In most of the cases, the time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours. If not the case, the maximum storage period of extracts (38.5 hours for green material) was covered by stability experiments conducted in the course of the residue study 15-2069 ([redacted], G.; 2017; M-584384-02-1; KCA 6.3.2). See Table 6.1- 8.

III. Conclusions (wheat, northern Europe)

In order to support the use in the EU of BCS-CN88460 in wheat, 12 independent trials were conducted in the northern European residue region in the years 2015-2016. BCS-CN88460 was applied once at an active substance rate of 75 g/ha per treatment. All applications were at the required rates and all trials were conducted according to GLP.

Samples were analysed for the residues of BCS-CN88460 parent compound. The results of the trials presented above demonstrate that:

- Residues of BCS-CN88460 dissipated rapidly in green material, from levels of 0.97 - 2.1 mg/kg on Day 0 after the treatment to 0.065 - 0.87 mg/kg on Day 28/29.
- At harvest, residues in grain were all <0.01 mg/kg and residues in straw ranged from 0.054 to 3.6 mg/kg.

Table 6.3.2- 3: Concurrent recovery data for BCS-CN88460 in wheat

Study	Portion analysed		Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
15-2111	green material	3	0.01	99[(1)]; 117[(2)]; 102[(2)]	99	117	105	9.1
		2	0.10	97[(1)]; 98[(1)]	97	98	98	
		1	2.5	99[(1)]				
		6	**overall**		**97**	**117**	**102**	**7.4**
	grain	2	0.01	101[(2)]; 104[(2)]	101	104	103	
		1	0.10	107[(1)]				
		3	**overall**		**101**	**107**	**104**	**2.9**
	straw	1	0.01	92[(2)]	-	-	-	-
		1	0.10	98[(2)]	-	-	-	-
		1	2.0	80[(1)]	-	-	-	-
		3	**overall**		**80**	**98**	**90**	**10.2**
15-2115	green material	1	0.01	86	-	-	-	-
		1	0.10	85	-	-	-	-
		1	2.5	97	-	-	-	-
		3	**overall**		**85**	**97**	**89**	**7.5**
	grain	2	0.01	91; 92	91	92	92	-
		1	0.10	102	102	-	-	-
		3	**overall**		**91**	**102**	**95**	**6.4**
	straw	1	0.01	98	98	-	-	-
		1	0.10	105	105	-	-	-
		1	2.0	96	96	-	-	-
		3	**overall**		**96**	**105**	**100**	**4.7**
15-2120	green material	2	0.01	81[(2)]; 98[(2)]	81	98	90	-
		2	0.10	111[(1)]; 112[(2)]	111	112	112	-
		1	2.0	83[(1)]	83	-	-	-
		1	3.0	95[(2)]	95	-	-	-
		6	**overall**		**81**	**112**	**97**	**13.7**
	grain	2	0.01	99[(2)]; 100[(2)]	99	100	100	-
		3	0.10	95[(1)]; 113[(1)]; 116[(1)]	95	116	108	10.5
		5	**overall**		**95**	**116**	**105**	**8.6**

Study	Portion analysed		Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
	straw	1	0.01	97(2)	-	-	-	-
		3	0.10	109(2); 112(2); 116(2)	109	116	112	3.1
		1	3.0	94(1)	94	-	-	-
		1	4.0	99(2)	99	-		-
		6	**overall**		**94**	**116**	**105**	**8.6**
16-2053	green material	2	0.01	90; 95	90	95	93	-
		1	0.10	94	-	-	-	
		1	4.0	97	-	-	-	-
		4	**overall**		**90**	**97**	**94**	**3.1**
	grain	1	0.01	93	-	-	-	-
		2	0.10	91; 96	91	96	94	-
		3	**overall**		**91**	**96**	93	2.7
	straw		0.01	93	-	-	-	-
			0.10	93	-	-	-	-
			4.0	94	94	-	-	-
			overall		**93**	**95**	**93**	**0.6**

RSD: relative standard deviation
(1): recovery conducted with spring wheat control sample
(2): recovery conducted with winter wheat control sample

Table 6.3.2- 4: Detailed results of the wheat residue trials in northern Europe

When trials were conducted in parallel plots, the highest residue level from the 2 plots was selected (underlined values).
Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1 BCS-CN88460		
	(a)	(b)				(c)					(d)	(e)
15-2111-01 France [redacted] Europe, North F 2015	Winter Wheat/ Rubisko	1) 17.10.2014 2) 15.05.2015 – 22.05.2015 3) 01.07.2015 – 10.07.2015	75	300	25	22.05.2015	69	Green material Grain Straw	69 71 75 77 83 89 89	1.6 1.3 0.92 0.53 0.57 <u><0.01</u> <u>0.94</u>	0 7 14 21 28 45 45	(f) 15-2111 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 269 days green material: 300 days grain: 220 days
15-2115-01 France [redacted] Europe, North F 2015	Winter Wheat/ Rubisko	1) 17.10.2014 2) 15.05.2015 – 22.05.2015 3) 01.07.2015 – 10.07.2015	75	300	25	22.05.2015	69	Green material Grain Straw	69 71 75 77 83 89 89	1.0 0.61 0.79 0.46 0.49 <0.01 0.84	0 7 14 21 28 45 45	(f) 15-2115 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 329 days green material: 371 days grain: 311 days

These two trials were conducted in side-by-side plot at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2111-02 United Kingdom [redacted] Europe, North F 2015	Winter Wheat/ KWS Cashel	1) 12.11.2014 2) 09.06.2015 – 23.06.2015 3) 03.08.2015 – 17.08.2015	75	200	37.5	23.06.2015	69	Green material	69 73 75 77 85	1.8 1.4 0.62 0.87 0.73	0 7 14 22 29	f) 15-2111 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 239 days green material: 269 days grain: 190 days
								Grain Straw	89 89	<u>0.01</u> <u>1.7</u>	43 43	
15-2115-02 United Kingdom [redacted] Europe, North F 2015	Winter Wheat/ KWS Cashel	1) 12.11.2014 2) 09.06.2015 – 23.06.2015 3) 03.08.2015 – 17.08.2015	75	200	37.5	23.06.2015	69	Green material	69 73 75 77 85	0.97 0.72 0.48 0.54 0.49	0 7 14 22 29	(f) 15-2115 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 299 days green material: 339 days grain: 281 days
								Grain Straw	89 89	<0.01 1.2	43 43	

These two trials were conducted in side-by-side plot at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2111-03 Netherlands [redacted] Europe, North F 2015	Spring Wheat/ Quintes	1) 23.04.2015 2) 19.06.2015 - 03.07.2015 3) 15.08.2015 – 15.09.2015	75	400	18.8	03.07.2015	69	Green material	69 71 75 76 77 Grain Straw	2.0 0.87 0.37 0.24 0.12 0.01 0.074	0 7 14 21 28 68 68	f) 15-2111 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 204 days green material: 276 days grain: 155 days
15-2115-03 Netherlands [redacted] Europe, North F 2015	Spring Wheat/ Quintes	1) 23.04.2015 2) 25.06.2015 – 03.07.2015 3) 15.08.2015 – 15.09.2015	75	400	18.8	03.07.2015	69	Green material	69 71 75 76 77 Grain Straw	1.5 0.74 0.40 0.30 0.15 <0.01 0.12	0 7 14 21 28 68 68	(f) 15-2115 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 264 days green material: 329 days grain: 246 days

These two trials were conducted in side-by-side plot at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1 BCS-CN88460		
	(a)	(b)				(c)					(d)	(e)
15-2111-04 Germany [redacted] Europe, North F 2015	Spring Wheat/ KWS Chamsin	1) 13.03.2015 2) 17.06.2015 – 24.06.2015 3) 01.08.2015 – 31.08.2015	75	300	25	24.06.2015	69	Green material	69 71 75 83 85 Grain Straw	2.1 1.4 1.2 0.52 0.44 0.01 0.43	0 7 14 21 28 49 49	f) 15-2111 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 232 days green material: 279 days grain: 183 days
15-2115-04 Germany [redacted] Europe, North F 2015	Spring Wheat/ KWS Chamsin	1) 13.03.2015 2) 17.06.2015 – 24.06.2015 3) 01.08.2015 – 31.08.2015	75	300	25	24.06.2015	69	Green material	69 71 75 83 85 Grain Straw	2.1 1.5 1.1 0.78 0.61 <0.01 0.82	0 7 14 21 28 49 49	(f) 15-2115 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 292 days green material: 338 days grain: 274 days

These two trials were conducted in side-by-side plot at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2120-01 United Kingdom [redacted] Europe, North F 2015	Winter Wheat/ Skyfall	1) 06.10.2014 2) 09.06.2015 - 24.06.2015 3) 14.08.2015	62.5	200	31.3	24.06.2015	69	Green material	69 71 75 83 83 Grain Straw	1.0 0.88 0.42 0.34 0.20 <0.01 <u>0.38</u>	0 6 14 20 28 49 49	(f) 15-2120 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 309 days green material: 362 days grain: 309 days
15-2120-02 Hungary [redacted] Europe, North F 2015	Winter Wheat/ GK Szala	1) 15.10.2014 2) 15.05.2015 – 30.05.2015 3) 09.07.2015	62.5	300	20.8	05.06.2015	69	Green material	69 75 83 83 87 Grain Straw	1.3 0.95 1.2 1.3 0.70 <u><0.01</u> <u>3.6</u>	0 7 14 21 28 34 34	(f) 15-2120 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 343 days green material: 381 days grain: 343 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1 BCS-CN88460		
	(a)	(b)				(c)					(d)	(e)
15-2120-03 France [redacted] Europe, North F 2015	Spring Wheat/ Togano	1) 13.03.2015 2) 08.06.2015 – 16.06.2015 3) 09.07.2015	62.5	300	20.8	16.06.2015	69	Green material	69 75 81 87 Grain Straw	2.0 0.4 1.5 2.5 <0.01 <3.3	0 7 14 21 24 24	(f) 15-2120 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 343 days green material: 372 days grain: 343 days
15-2120-04 Poland [redacted] Europe, North F 2015	Spring Wheat/Tybalt	1) 10.04.2015 2) 30.06.2015 – 03.07.2015 3) 04.08.2015	62.5	200	31.3	29.06.2015	69	Green material	69 73 77 82 85 Grain Straw	1.4 0.91 0.58 0.66 0.84 <0.01 1.5	0 7 14 21 28 36 36	(f) 15-2120 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 317 days green material: 358 days grain: 317 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
16-2053-01 France [redacted] Europe, North F 2016	Winter Wheat/ Sy Moisson	1) 20.10.2015 2) 13.05.2016 – 20.05.2016 3) 10.07.2016 – 20.07.2016	75	300	25	20.05.2016	69	Green material	69 71 71 75 77 Grain Straw	1.7 0.55 0.23 0.21 0.14 89 <0.01 89 0.19	0 7 13 20 28 52 52	(f) 16-2053 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 233 days green material: 291 days grain: 232 days
16-2053-02 Belgium [redacted] Europe, North F 2016	Winter Wheat/ Rubisko	1) 07.11.2015 2) 05.06.2016 – 15.06.2015 3) 08.08.2016 – 15.08.2016	75	300	25	10.06.2016	65	Green material	65 71 75 77 83 Grain Straw	1.2 0.38 0.16 0.12 0.087 89 <0.01 89 0.054	0 7 14 21 28 60 60	(f) 16-2053 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 204 days green material: 269 days grain: 203 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application / Label pre-harvest interval, PHI (underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1 BCS-CN88460		
	(a)	(b)				(c)					(d)	(e)
16-2053-03 The Netherlands [redacted] Europe, North F 2016	Spring Wheat/ Tybalt	1) 17.03.2016 2) 07.06.2016 – 21.06.2016 3) 15.08.2016 – 25.08.2016	75	400	18.8	20.06.2016	69	Green material	69 69 73 85 85 Grain Straw	1.7 0.73 0.48 0.32 0.37 ≤ 0.01 0.40	0 7 14 21 28 57 57	(f) 16-2053 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 196 days green material: 258 days grain: 195 days
16-2053-04 Germany [redacted] Europe, North F 2016	Spring Wheat/ Tybalt	1) 15.03.2016 2) 10.06.2016 – 16.06.2016 3) 15.08.2016 – 31.08.2016	75	300	25	16.06.2016*	69	Green material Grain Straw	69 73 75 77 83 89 89	1.8 0.31 0.13 0.081 0.065 ≤ 0.01 0.071	0 7 14 21 28 64 64	* Trial was covered with tarpaulin for 24 hours to protect the crop against a forcasted rain (f) 16-2053 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 194 days green material: 263 days grain: 193 days

These two trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Southern Europe (residue region)

Report:	KCA 6.3.2/05; [redacted], G.; 2017; M-584384-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on wheat and durum after spray application of BCS-CN88460 EC 050 in Portugal, southern France and Spain
Report No.:	15-2069
Document No.:	M-584384-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Guideline deviation(s):	yes, see report
GLP/GEP:	**yes**

Report:	KCA 6.3.2/06; [redacted], 2017; M-580537-03-1
Title:	Amendment no. 2: Determination of the residues of BCS-CN88460 and prothioconazole in/on wheat after spray application of prothioconazole & BCS-CN88460 EC 150 in the field in Portugal, southern France and Spain
Report No.:	15-2116
Document No.:	M-580537-03-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	None
GLP/GEP:	**yes**

Report:	KCA 6.3.2/07; [redacted], G.; 2017; M-584690-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460, prothioconazole and tebuconazole in/on wheat and durum wheat after spray application of prothioconazole & tebuconazole & BCS-CN88460 EC 250 in southern France, Spain, Portugal and Italy
Report No.:	15-2119
Document No.:	M-584690-02-1
Guideline(s):	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 OECD 509 Adopted 2009-09-07, OECD Guideline for the Testing of Chemicals, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 6.3.2/08; [redacted]; 2017; M-594320-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 and prothioconazole in/on wheat and wheat, durum after spray application of Prothioconazole & BCS-CN88460 EC 150 in Italy, Spain, southern France and Greece
Report No.:	16-2054
Document No.:	M-594320-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	yes, see report
GLP/GEP:	**yes**

I. Materials and Methods

Thirteen independent field residue trials were conducted on wheat (including durum varieties) in the southern European residue region, as follows:

In 2015, 4 supervised residue trials (southern France, Portugal and Spain) were conducted to support the use of BCS-CN88460 EC 050 in/on wheat and durum wheat ([redacted], G., 2017; M-584384-02-1; KCA 6.3.2). One spray application was made at a nominal growth stage of BBCH 69 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 300 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing, except in the trial 15-2069-04 where the application was done at growth stage BBCH 65 instead of 69. This deviation is considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

4 additional supervised residue trials (southern France, Portugal and Spain) were conducted with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on wheat ([redacted]; 2017; M-580537-03-1; KCA 6.3.2). One spray application was made at a nominal growth stage of BBCH 69 and at a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 300 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing, except in the trial 15-2116-04 where the application was done at growth stage BBCH 65 instead of 69. This deviation is considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

Trials 15-2069-01, 15-2069-02, 15-2069-03 and 15-2069-04 were located side by side with trials 15-2116-01, 15-2116-02, 15-2116-03 and 15-2116-04. Application dates, sampling dates and varieties slightly differ between 15-2069-04 and 15-2116-04 due to the fact that 15-2116-04 had to be restarted. Nevertheless, both trials were located very close to each other (Bayer CropScience Research Farm, Brenes, Spain). The cultivation practices (same PI Field, same equipment used), the weather conditions, the PHI and the types of soil are not enough different to consider these trials as independent. Thus, for the studies 15-2069 and 15-2116, only 4 trials are considered as independent trials.

Four further supervised residue trials were carried out in 2015, in southern France, Spain, Portugal and Italy with the formulation Prothioconazole & Tebuconazole & BCS-CN88460 EC 250 in/on wheat and durum wheat ([redacted]; 2017; M-584690-02-1; KCA 6.3.2). One spray application was made at a nominal growth stage of BBCH 69 and a nominal rate of 1.25 L/ha, corresponding to 62.5 g/ha BCS-CN88460 a.s.; the water rate was 300 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing except in the trial 15-2119-01 where the application was 5.9% overdosed.

Five further supervised residue trials were carried out in 2016, in southern France, Italy, Spain and Greece with the formulation Prothioconazole & BCS-CN88460 EC 150 in/on wheat to complete the data package ([redacted]; 2017; M-594320-02-1; KCA 6.3.2). One spray application was made

at a nominal growth stage of BBCH 69 and a nominal rate of 1.5 L/ha, corresponding to 75 g/ha BCS-CN88460 a.s.; the water rate was 300 - 400 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates and scheduled application timing.

In all these trials, samples of green material (whole plants without roots) were taken immediately after the application and at several intervals thereafter (up to 28/29 days after treatment). Furthermore, samples of grain and straw were collected at the commercial harvest (BBCH 89).

In two trials (15-2069-03 and 15-2116-03) samples of grain and straw were not collected since plots had been harvested by mistake (see Appendix 8 of the respective reports).

Each field sample was placed in doubled labeled bags and stored deep-frozen within 24 hours after sampling and until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer AG - Crop Science Division in [redacted] am Rhein, Germany. All field samples were shipped at a temperature of -18°C or below under monitored conditions during shipment and arrived at PVTL in good condition. The field samples were stored in a freezer at -18°C or below until preparation of the examination samples.

In the trials 15-2119-02 and 15-2119-03, the temperature temporarily increased above -18°C during storage but this did not affect the integrity of the samples. Similarly, in trial 16-2054-01 some samples were transported temporarily above -18°C. These deviations were considered acceptable. For further details see Appendix 8 of the respective reports.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at -18°C or below until analysis.

The samples were analysed for the parent compound using analytical method 01475 ([redacted]; 2016; M-558986-01-1; referenced in MCA 4 Point 4.1.2) which was validated prior to the residue analysis of the samples. Additional validation recoveries were conducted for wheat (grain, straw and green material) in the study 15-2069. The samples of grain and straw were analyzed according to the procedure described in the method for dry matrices (with a soaking step with water before extraction) and the green material samples were prepared according to the procedure for higher-water containing commodities (no soaking step before extraction). The LOQ was 0.01 mg/kg for parent.

II. Findings

In each study, concurrent recoveries were obtained from samples of green material, grain and straw. The recovery samples were spiked at levels of 0.01 mg/kg and up to 8 mg/kg, in order to adequately cover the residue levels found in the treated samples. Details of concurrent recovery data are shown in Table 6.3.2- 5. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

No residues above the LOQs were found in the control samples. The detailed results obtained for wheat treated samples are summarised below in Table 6.3.2- 6. The results were not corrected for concurrent recoveries.

The analyses were done after a maximum storage period of 398 days. In most of the cases, the time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours. If not the case, the maximum storage period of extracts (30.5 hours for grain, 39 hours for green material and 76 hours for straw) was covered by stability experiments conducted in the method 01475 ([redacted]; 2016; M-558986-01-1; referenced in MCA Section 4 under Point 4.1.2), or by additional experiments conducted in the course of the residue study 15-2069 ([redacted], G.; 2017; M-584384-02-1; KCA 6.3.2). See Table 6.1- 8.

III. Conclusions (wheat, Southern Europe)

In order to support the use in the EU of BCS-CN88460 in wheat, 12 independent trials with samplings of grain and straw were conducted in the southern European residue region in the years 2015-2016. BCS-CN88460 was applied once at an active substance rate of 62.5 or 75 g/ha per treatment. All applications were at the required rates, and all trials were conducted according to GLP.

Samples were analysed for the residues of BCS-CN88460 parent compound. The results of the trials presented above demonstrate that:

- Residues of BCS-CN88460 dissipated rapidly in green material, from levels of 0.76-2.5 mg/kg on Day 0 after the treatment to 0.14-1.5 mg/kg on Day 27-29.
- At harvest, residues in grain were most of the time <0.01 mg/kg (only in one trial residue was at 0.042 mg/kg and residues in straw ranged from 0.22 to 2.4 mg/kg.

Table 6.3.2- 5: Concurrent recovery data for BCS-CN88460 in wheat

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
15-2069	green material	4	0.01	86(1); 97(2); 104(2); 96(2)	89	104	97	6.1
		5	0.10	100(1); 100(2); 95(2); 96(2); 98(2)	95	100	98	2.5
		1	2.0	101(1)	101			-
		1	2.5	101(2)	101	-	-	-
		11	**overall**		**89**	**104**	**98**	**4.1**
	grain	2	0.01	97(1); 91(1)	91	97	94	-
		1	0.10	107(2)	-	-	-	-
			overall		**91**	**107**	**98**	**8.2**
	straw	1	0.01	104(1)	-	-	-	-
		1	0.10	101(1)	-	-	-	-
		1	2.0	105(2)	-	-	-	-
			overall		**101**	**105**	**103**	**2.0**
15-2116	green material	1	0.01	95	-	-	-	-
		1	0.10	101	-	-	-	-
		1	1.0	96	-	-	-	-
		1	2.0	104	-	-	-	-
			overall		**95**	**104**	**99**	**4.3**
	grain	2	0.01	99; 96	96	99	98	-
		1	0.10	99	-	-	-	-
		3	**overall**		**96**	**99**	**98**	**1.8**
	straw	1	0.01	93	-	-	-	-
		1	0.10	98	-	-	-	-
		1	2.0	100	-	-	-	-
			overall		**93**	**100**	**97**	**3.7**
15-2119	green material	1	0.01	94; 102	94	102	98	-
		1	0.10	103	-	-	-	-
		1	3.0	82	-	-	-	-
		3	**overall**		**82**	**103**	**95**	**10.2**
	grain	1	0.01	98				-
		2	0.10	95; 95	95	95	95	-
		3	**overall**		**95**	**98**	**96**	**1.8**

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
	straw	1	0.01	100	-	-	-	-
		1	0.10	103	-	-	-	-
		1	2.0	91	-	-	-	-
		1	2.5	94	-	-	-	-
		4	**overall**		**91**	**103**	**97**	**5.6**
16-2054	green material	1	0.01	82	-	-	-	-
		1	1.0	91	-	-	-	-
		1	8.0	96	-	-	-	-
		3	**overall**		**82**	**96**	**90**	**7.9**
	grain	1	0.01	83	-	-	-	-
		1	0.10	92; 92	-	-	-	-
		2	**overall**		**83**	**92**	**89**	**5.8**
	straw	1	0.01	98	-	-	-	-
		1	0.10	92	-	-	-	-
		1	2.0	93	-	-	-	-
		1	3.0	92	-	-	-	-
		4	**overall**		**92**	**98**	**94**	**3.1**

RSD: relative standard deviation

(1): recovery conducted with wheat control sample

(2): recovery conducted with durum wheat control sample

Table 6.3.2- 6: Detailed results of the wheat residue trials in southern Europe

When trials were conducted in parallel plots, the highest residue level from the 2 plots was selected (underlined values).
Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2069-01 Portugal [redacted] Europe, South F 2015	Winter Wheat/Jordão	1) 16.12.2014 2) 01.05.2015 – 11.05.2015 3) 15.06.2015 - 15.07.2015	75	300	25	11.05.2015	69	Green material	69 71 73 75 83 Grain Straw	1.6 0.90 0.66 0.63 0.57 <0.01 1.6	0 7 14 21 28 45 45	(f) 15-2069 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 225 days green material: 304 days grain: 224 days
15-2116-01 Portugal [redacted] Europe, South F 2015	Winter Wheat/Jordão	1) 16.12.2014 2) 01.05.2015 – 11.05.2015 3) 15.06.2015 – 15.07.2015	75	300	25	11.05.2015	69	Green material	69 71 73 75 83 Grain Straw	1.1 0.80 0.66 0.57 0.83 <u><0.01</u> <u>1.9</u>	0 7 14 21 28 45 45	(f) 15-2116 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 256 days green material: 309 days grain: 256 days

These two trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2069-02 France [redacted] Europe, South F 2015	Winter Wheat/ Aubusson	1)16.10.2014 2) 02.05.2015 – 05.05.2015 3) 20.06.2015 – 02.07.2015	75	300	25	07.05.2015	69	Green material	69 71 75 77 83	1.7 0.98 0.92 0.83 0.85	0 6 14 21 28	(f) 15-2069 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 225 days green material: 308 days grain: 224 days
								Grain Straw	89 89	<0.01 0.71	49 49	
15-2116-02 France [redacted] Europe, South F 2015	Winter Wheat/ Aubusson	1)16.10.2014 2) 02.05.2015 – 08.05.2015 3) 20.06.2015 – 02.07.2015	75	300	25	07.05.2015	69	Green material	69 71 75 77 83	1.6 1.1 0.68 0.88 0.84	0 6 14 21 28	(f) 15-2116 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 256 days green material: 321 days grain: 256 days
								Grain Straw	89 89	<u><0.01</u> <u>0.87</u>	49 49	

These two trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460 (d)		(e)
15-2069-03 France [redacted] Europe, South F 2015	Winter Wheat/ Arezzo	1) 28.10.2014 2) 07.05.2015 – 15.05.2015 3) 25.06.2015 – 07.07.2015	75	300	25	13.05.2015	69	Green material	69 71 73 77 83	1.3 0.50 0.42 0.30 0.15	0 7 14 21 28	(f) 15-2069 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) green material: 306 days
15-2116-03 France [redacted] Europe, South F 2015	Winter Wheat/ Arezzo	1) 28.10.2014 2) 07.05.2015 – 15.05.2015 3) 25.06.2015 – 07.07.2015	75	300	25	13.05.2015	69	Green material	69 73 77 83	1.3 0.48 0.39 0.38 0.20	0 7 14 21 28	(f) 15-2116 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) green material: 307 days

These two trials were conducted in side-by-side plots at the same location.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460 (d)		(e)
15-2069-04 Spain [redacted] Europe, South F 2015	Durum Wheat/Vitron	1) 12.01.2015 2) 10.04.2015 – 20.04.2015 3) 01.06.2015 – 30.06.2015	75	300	25	16.04.2015	65	Green material	65 77 83 85 85 89 89	2.3 1.9 0.89 0.66 0.67 <0.01 1.2	0 7 14 21 28 53 53	(f) 15-2069 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 242 days green material: 337 days grain: 241 days
								Grain Straw				
15-2116-04 Spain [redacted] Europe, South F 2015	Durum Wheat/ Euroduro	1) 05.02.2015 2) 23.04.2015 – 01.05.2015 3) 01.06.2015 – 30.06.2015	75	300	25	27.04.2015	65	Green material	65 73 83 85 87 89 89	2.2 1.6 0.97 0.74 0.78 <u>0.01</u> <u>1.9</u>	0 7 14 21 28 51 51	(f) 15-2116 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 264 days green material: 323 days grain: 264 days
								Grain Straw				

Application dates, sampling dates and varieties slightly differ between 15-2069-04 and 15-2116-04 due to the fact that 15-2116-04 had to be restarted. Nevertheless, both trials were located very close to each other (Bayer CropScience Research Farm, Brenes, Spain). The cultivation practices (same PI Field, same equipment used), the weather conditions, the PHI and the types of soil are not enough different to consider these trials as independent.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2119-01 France [redacted] (Languedoc Roussillon) Europe, South F 2015	Wheat/ P22R58	1) 29.10.2014 2) 23.04.2015 – 30.04.2015 3) 03.07.2015	66	318	20.8	29.04.2015	69	Green material	69 71 75 83 85	1.5 0.97 0.70 0.56 0.51	0 7 14 21 29	(f) 15-2119 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 340 days green material: 398 days grain: 333 days
								Grain Straw	89 89	<0.01 1.4	65 65	
15-2119-02 Spain [redacted] (Albacete) Europe, South F 2015	Wheat/Sarina	1) 20.02.2015 2) May 2015 3) June 2015	62.5	300	20.8	20.05.2015	69	Green material	69 71 75 77 83	1.1 0.28 0.17 0.16 0.19	0 7 14 21 28	(f) 15-2119 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 344 days green material: 377 days grain: 337 days
								Grain Straw	89 89	<0.01 0.33	40 40	

These two trials were not conducted in side-by-side plots. They are independent trials.

In the trials 15-2119-02, the temperature temporarily increased above -18°C during storage but this did not affect the integrity of the samples. This deviation was considered acceptable. For further details see Appendix 8 of the respective report.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
15-2119-03 Portugal [redacted] (Ribatejo) Europe, South F 2015	Wheat/ Valbona	1) 12.12.2014 2) May 2015 3) June 2015	62.5	300	20.8	08.05.2015	69	Green material	69 71 75 77 85	0.76 0.59 0.74 1.2 1.5	0 7 13 21 27	(f) 15-2119 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 355 days green material: 389 days grain: 348 days
								Grain	89	0.042	41	
								Straw	89	2.3	41	
15-2119-04 Italy [redacted] Europe, South F 2015	Durum Wheat/Duilio	1) 22.11.2014 2) 07.05.2015 – 12.05.2015 3) 15.06.2015 – 30.06.2015	62.5	300	20.8	12.05.2015	69	Green material	69 73 75 77 85	2.5 0.79 1.1 0.84 1.1	0 7 14 20 28	(f) 15-2119 (g) EC (250 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 354 days green material: 385 days grain: 347 days
								Grain	89	<0.01	38	
								Straw	89	1.8	38	

These two trials were not conducted in side-by-side plots. They are independent trials.

In the trial 15-2119-03, the temperature temporarily increased above -18°C during storage but this did not affect the integrity of the samples. This deviation was considered acceptable. For further details see Appendix 8 of the respective report.

- (a) According to CODEX Classification Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
16-2054-01 Italy [redacted] Europe, South F 2015	Durum Wheat/ Anco Marzio	1)30.12.2015 2)10.04.2016 – 20.04.2016 3) 01.06.2016 – 30.06.2016	75	400	18.8	15.04.2016	69	Green material	69 71 83 85 87	1.2 1.1 0.88 0.46 0.70	0 7 14 21 28	(f) 16-2054 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain Straw	89 89	< 0.01 * 1.6 *	49 49	(k) Method Validation Data in 01475 and study 15-2069 (l) straw: 283 days green material: 332 days grain: 278 days
16-2054-02 Spain [redacted] Europe, South F 2015	Wheat/ Artur Nick	1) 19.01.2016 2) 10.04.2016 – 21.04.2016 3) 01.06.2016 – 30.06.2016	75	300	25	21.04.2016	69	Green material	69 71 75 79 85	1.7 1.2 0.89 0.22 0.21	0 7 14 21 28	(f) 16-2054 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain Straw	89 89	< 0.01 1.3	49 49	(k) Method Validation Data in 01475 and study 15-2069 (l) straw: 277 days green material: 326 days grain: 272 days

These two trials were not conducted in side-by-side plots. They are independent trials.

* samples transported temporarily above -18°C. This deviation was considered acceptable. For further details see Appendix 8 of the respective report.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460	(d)	(e)
16-2054-03 France [redacted] Europe, South F 2015	Winter Wheat/ Calabro	1)16.11.2015 2) 10.05.2016 – 16.05.2016 3) 25.06.2015 – 30.06.2016	75	300	25	13.05.2016	65	Green material	65 71 75 77 83	1.7 1.3 1.8 0.78 0.91	0 6 14 21 28	(f) 16-2054 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain	89	< 0.01	45	(k) Method Validation Data in 01475 and study 15-2069 (l) straw: 259 days, green material: 304 days, grain: 254 days
								Straw	89	2.4	45	
16-2054-04 France [redacted] Europe, South F 2015	Winter Wheat/ Orgrain	1) 19.10.2015 2) 10.05.2016 – 17.05.2016 3) 05.07.2016 – 15.07.2016	75	300	25	17.05.2016	69	Green material	69 71 71 75 77	1.1 0.51 0.27 0.23 0.14	0 6 14 21 28	(f) 16-2054 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg
								Grain	89	< 0.01	52	(k) Method Validation Data in 01475 and study 15-2069 (l) straw: 248 days, green material: 300 days, grain: 243 days
								Straw	89	0.22	52	

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Growth stage at sampling	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Analyte 1		
	(a)	(b)				(c)				BCS-CN88460 (d)		(e)
16-2054-05 Greece [redacted] Europe, South F 2015	Winter Wheat/ Achilleas (early-mid maturity)	1) 06.12.2015 2) 07.05.2016 – 12.05.2016 3) 04.07.2016 – 04.07.2016	75	300	25	12.05.2016	65	Green material	65 71 73 77 83	1.6 0.45 0.46 0.18 0.20	0 7 14 21 28	(f) 16-2054 (g) EC (150 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2069 (l) straw: 252 days green material: 305 days grain: 247 days
								Grain	89	< 0.01	53	
								Straw	89	0.41	53	

These last three trials were not conducted in side-by-side plots. They are independent trials.

- (a) According to CODEX classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Comparison of the wheat results for the three formulations tested

Since residues in grain at harvest are often <0.01 mg/kg, the comparison of the results for the different formulations used was done using the results in straw at harvest. The data sets 1, 2 and 3 were compared to each other's using the statistical tests: Kruskal-Wallis H-Test and Mann-Whitney U-test, $\alpha = 0.05$. The same approach was done for the data sets 4, 5 and 6.

Dataset 1	Dataset 2	Dataset 3	Dataset 4	Dataset 5	Dataset 6
EC050 EUN	EC150 EUN	EC250 EUN	EC050 EUS	EC150 EUS	EC250 EUS
0.94	0.84	0.38	1.6	1.9	1.4
1.7	1.2	3.6	0.71	0.87	0.33
0.074	0.12	3.3	1.2	1.9	2.3
0.43	0.82	1.5		1.6	1.8
	0.19			1.3	
	0.054			2.4	
	0.40			0.22	
	0.071			0.41	

EUN: northern Europe EUS: southern Europe

The results of these statistical tests indicate that the populations from the data sets 1, 2 and 3 are not significantly different from each other's. The same applies for the datasets 4, 5 and 6.

The residue data for the different formulations can thus be combined in the following table to establish MRLs. When trials were conducted in side-by-side plots, the highest residue level from the 2 plots was selected. The selected data are compiled in Table 6.3.2- 7.

Table 6.3.2- 7: Summary of BCS-CN88460 residue data for wheat trials

Crop	Northern/ Southern field or glasshouse	Trial results relevant to the critical GAP	STMR (a)	HR (b)
Wheat Grain	Northern	12 × <0.01	<0.01	<0.01
	Southern	11 × <0.010; 0.043	<0.01	0.042
Wheat Straw	Northern	0.054; 0.071; 0.12; 0.19; 0.38; 0.40; 0.82; 0.94; 1.5; 1.7; 3.3; 3.6	0.61	3.6
	Southern	0.22; 0.33; 0.41; 0.87; 1.3; 1.4; 1.6; 1.8; 1.9; 1.9; 2.3; 2.4	1.5	2.4

(a) Supervised Trials Median Residue i.e. the median residue level estimated on the basis of supervised trials relating to the critical GAP
(b) Highest residue

The results of the statistical tests Kruskal-Wallis H-Test and Mann-Whitney U-test, $\alpha = 0.05$ indicate that the straw residue data from northern Europe are not significantly different from the straw residue data in southern Europe.

CA 6.4 Feeding studies

European dietary burden calculations

BCS-CN88460 is sought for use on cereals which parts of this crop might be fed to livestock (grain, straw and by-products of grain).

The 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) and using the Excel spreadsheet dated of 2017 available in the EU Commission website (pesticides_mrl_guidelines_animal_model_2017.xls).

Based on the proposed plant residue definition for risk assessment (BCS-CN88460 parent compound), input values were derived from the residue data as summarised under CA 6.3.1 and 6.3.2. These input data are summarized Table 6.4- 1.

Table 6.4- 1: Input residue data for livestock dietary burden calculations

Feed commodity	Median dietary burden		Maximum dietary burden	
	(mg/kg)	Comment	(mg/kg)	Comment
Barley straw	0.29	STMR from N+SEU	3.1	HR from N+SEU
Oat straw				
Rye straw	0.29	STMR from N+SEU	3.6	HR from N+SEU
Triticale straw				
Wheat straw				
Barley grain	0.01	STMR from N+SEU	0.01	STMR from N+SEU
Oat grain				
Rye grain	0.01	STMR from SEU	0.01	STMR from SEU
Triticale grain				
Wheat grain				
Brewer's grain	0.007	STMR-P (0.01 x 0.67 [a])	0.067	STMR-P (0.01 x 0.67 [a])
Distiller's grain	0.033	STMR-P (0.01 x 3.3 [b])	0.033	STMR-P (0.01 x 3.3 [b])
Wheat gluten	0.011	STMR-P (0.01 x 1.1 [c])	0.011	STMR-P (0.01 x 1.1 [c])
Wheat milled by-products	0.014	STMR-P (0.01 x 1.4 [c d])	0.014	STMR-P (0.01 x 1.4 [c d])

NEU: northern Europe, SEU: southern Europe, N+SEU: northern and southern Europe
HR highest residues
STMR supervised trials median residue
[a] processing factor P derived from M-579494-01-1; KCA 6.5.3
[b] default processing factor P
[c] processing factor P derived from M-600503-02-1; KCA 6.5.3
[d] highest value from wheat middlings (<0.73), bran (1.4), and shorts (0.98)

Table 6.4- 2: Anticipated dietary burden for BCS-CN88460 residues based on EU residue data and OECD guideline

Animals	Dietary burden expressed in mg/kg bw/d		Above 0.004 mg /kg bw	Dietary burden expressed in mg/kg DM		Highest contributing commodities	
	Median	Maximum		Median	Maximum		
Cattle (Beef)	0.003	0.025	**Yes**	0.11	1.05	Barley	straw
Cattle (Dairy)	0.004	**0.041**	**Yes**	0.11	**1.05**	Barley	straw
Sheep (Ram/Ewe)	0.007	0.070	**Yes**	0.20	2.10	Barley	straw
Sheep (Lamb)	0.009	**0.089**	**Yes**	0.20	**2.10**	Barley	straw
Swine (Breeding)	0.000	0.000	No	0.01	0.01	Wheat	Milled bypdt
Swine (Finishing)	0.000	0.000	No	0.01	0.01	Wheat	Milled bypdt
Poultry (Broiler)	0.001	0.001	No	0.01	0.01	Distiller's grain	dried
Poultry (Layer)	0.003	**0.029**	**Yes**	0.05	**0.42**	Wheat	straw
Poultry (Turkey)	0.001	0.001	No	0.01	0.01	Distiller's grain	dried

Livestock feeding studies were conducted on dairy cow and poultry hens. Multi-region livestock diet calculations were conducted in order to conduct the studies in a manner appropriate to the entire scope of BCS-CN88460 use, allowing data to be generated in a fashion such that, for animal welfare considerations, a low number of animals will be used, while yielding valid data to evaluate expected residue levels in all key animal tissues and products.

The test substance used in the feeding studies should be representative of the residue in the feedstuffs. In the case of the new fungicide BCS-CN88460 by far the major part of the residue in plants is formed by parent compound BCS-CN88460.

Based on the results of the metabolism studies several compounds were measured in the feeding studies:

- In milk, eggs and all tissues free residues of BCS-CN88460 parent compound and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24819, BCS-DC22055 and BCS-CX99799 were individually determined.
- Additionally, the sum of BCS-DC20298 and its conjugate M20, and the sum of BCS-CY24813 and its conjugate M19 were determined in cow liver and kidney.
- Moreover, the sum of BCS-DC22055 and its conjugate M37 was determined in hen liver.

In the following sections (CA 6.4.1 and 6.4.2), the study data will be presented.

General remark:

In this summary section (CA 6.4), the following codes will be used for the metabolites:

Name	*Metab. No.*	*Standard "dossier name"*
BCS-DC20298	M02	BCS-CN88460-2-propanol
BCS-CY26497	M12	BCS-CN88460-carboxylic acid
BCS-CY24813	M01	BCS-CN88460-propanol
BCS-DC22055	M06	BCS-CN88460-desmethyl-propanol
BCS-CX99799	M11	BCS-CN88460-desmethyl-carboxylic acid
--	M20	BCS-CN88460-2-propanol-Gluc-A
--	M19	BCS-CN88460-propanol-GlucA
--	M37	BCS-CN88460-desmethyl-propanol-N-GlucA

CA 6.4.1 Poultry

Report:	KCA 6.4.1/01; [redacted]; [redacted], E.; 2017; M-605909-01-1
Title:	BCS-CN88460: Feeding study with laying hens
Report No.:	M-605909-01-1
Document No.:	M-605909-01-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals (Test Guideline 505 Residues in Livestock, adopted 2007-01-08) US: EPA Residue Chemistry Test Guidelines OCSPP 860.1000 Background OCSPP 860.1480 Meat, milk, poultry and Egg EU: EU Directive EC 91/414, Appendix G, 7031/VI/95 rev. 4 Livestock feeding studies
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and Methods

Test system, dosing

After an acclimatization phase of about 2 weeks, forty-two laying hens (*Gallus gallus domesticus*) were dosed orally, via gelatin capsules, for 28 consecutive days with BCS-CN88460 at dose rates of 0 mg/kg bw/day (control; 2 subgroups, 6 hens), 0.03 mg/kg bw/day (1X EU dose group; 3 subgroups, 9 hens), 0.12 mg/kg bw/day (4X EU dose group; 3 subgroups, 9 hens) and 0.48 mg/kg bw/day (16X EU dose group; 3 subgroups, 9 hens). An additional group 16XE (3 subgroups, 9 hens) was dosed at the rate of 0.48 mg/kg bw/day for 28 consecutive days simultaneously with the animals from dose group 16X. Thereafter, dosing was stopped and the animals were kept alive for further 4, 7 or 14 days in order to investigate the depuration of residues of BCS-CN88460 in eggs and tissues after the end of dosing.

The exact amounts of test item to be administered daily to each hen were calculated weekly based on the body weight determined on the Thursday preceding the week of dosing (starting on Monday). Based on this procedure 5 batches of capsules were prepared for each animal (one batch per calendar week). C.E.R. groupe prepared a dosing solution for each dose level by dissolving the test item in methanol. Depending on the animal weight and dose group, a suitable volume of about 50 µL of the corresponding test item solution was pipetted into gelatine capsules filled with cellulose powder to absorb the solvent and prevent soaking of the capsule itself. The capsule was then left open for minimum 30 min to allow the solvent to evaporate and after that it was closed. The capsules and the dosing solutions were stored in a fridge at +2°C to +8°C at the animal unit of the in-life test site until use. The BCS-CN88460 content of the dosing solutions and capsules was verified by the residue analytical team at BAG-CS-HS-RA directly after preparation and at appropriate intervals during and at the end of their storage at C.E.R.. The analyses were conducted using method 01511 ([redacted]; [redacted]; [redacted] 2017, M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2).

The hens were fed with non-supplemented commercial laying hen meal (Avi Pondeuse Farine, 88+/-1% dry matter). The feed was screened for residues of BCS-CN88460 with an LOQ of 0.01 mg/kg according to method 01475 ([redacted]; 2016; M-558986-01-1; referenced in MCA 4 Point 4.1.2). The amount of feed consumed was monitored daily. The hens were allowed *ad libitum* access to tap water. The dose rates employed in the study are summarized below in Table 6.4.1- 1.

Table 6.4.1- 1: Summary of actual BCS-CN88460 dose administration.

Dose groups	Sub-groups	Number of hens	Dose levels	
			per animal (mg/kg bw/day)	in feed (mg/kg DM)*
control	A1, A2	6	0	0
1X dose	B1, B2, B3	9	0.03	0.530
4X dose	C1, C2, C3	9	0.12	2.119
16X dose	D1, D2, D3	9	0.48	8.698
16XE dose	E1, E2, E3	9	0.48	8.140

DM: dry matter
*: Actual dose based on average feed consumption data collected from the study

The hens were dosed daily during 28 consecutive days via capsules given *per os* in the mornings just prior to the feeding period. The control animal received a placebo (empty capsule) concurrently with the treated animals.

Sampling

For the purpose of this study, a "Study Day" period was defined from morning inspection, feeding and watering to sampling of eggs on the following morning. The changeover to the following study day took place after sampling of eggs.

Eggs were collected thrice before the first dosing, at least every third day during the first three weeks of dosage and twice during the last week of dosage. Egg samples for the depuration dose group E were collected once before the dosing phase and then at least twice again starting on the last week of the dosing phase again until the final slaughtering.

The eggs were collected in the evening and the following morning, if available. The eggs were shipped on the same day to the sample preparation and logistic lab (PVTL) at Bayer AG - Crop Science Division, [redacted]. Upon arrival at PVTL, all eggs of each sub-group were mixed (without shell), chopped with dry-ice and stored at ≤ -18°C until analysis.

The eggs samples collected on the overall study day 30 of dosing from the 0X (sub-group A2) and from the 16X group animals were separated into yolk and egg white.

On the day after the final dosing (less than 6 hours after the final dose), the hens were weighed, anaesthetized with an electric shock followed by immediate exsanguination by decapitation.

Liver (entire organ), muscle (approximately equal sized pieces of leg and breast, trimmed of adherent connective tissue and fat) and fat with adhering skin (overlaying skin with subcutaneous fat and abdominal fat) were taken for analysis. The tissue samples (refrigerated with dry ice) were shipped to PVTL within 24 h of sampling. Upon arrival at PVTL the tissue samples were chopped together with dry ice by means of a meat chopper and stored at ≤ -18°C until analysis.

Twelve hens (3 from the control group and 9 from the 16XE group) entered into a 14-day depuration phase, following the administration of the final dose. Egg and tissue samples were collected on study days 35, 38, and 45 for analysis.

Analysis

Tissues and eggs samples were analyzed for free residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CX26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 by high performance liquid chromatography-electrospray ionization / tandem mass spectrometry (HPLC-MS/MS) using isotopically labeled internal standards. The analyses were conducted according to the method 01511([redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2).

In addition the sum of BCS-DC22055 and its conjugate M37 was determined in liver, after a hydrolysis step, according to the method 01511 ([redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2).

The method 01511 was validated prior to sample analysis in a separate study ([redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2). Concurrent recoveries were performed during sample analysis to demonstrate acceptable method performance. The Limit of Quantitation (LOQ) for BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 was 0.01 mg/kg per analyte expressed as BCS-CN88460 in all the matrices. The LOQ for the sum of BCS-DC22055 and its conjugate M37 in liver was 0.01 mg/kg expressed as BCS-CN88460.

II. Findings

Dose verification and storage stability

The dosing solutions for the preparation of the capsules of the different batches were analyzed for BCS-CN88460 in order to determine the content and homogeneity as well as the storage stability of BCS-CN88460 for the duration of use in the in-life phase of the study. The dose preparation was accurate and the capsules were shown to be stable over the course of the study.

Analysis of feedstuff

No residues of BCS-CN88460 were found above the LOQ (0.01 mg/kg) in the feedstuff. Concurrent recoveries were in the acceptable range of 70-110% with Relative Standard Deviations RSDs <20% (see Table 6.4.1- 2).

In-life observations

Feed consumption, body weights, and egg production were not adversely affected by treatment with BCS-CN88460. The appearance and the behavior of all birds were observed once daily throughout the study. Nothing special was observed. Hens were healthy during the whole study.

Analysis of eggs and tissues

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 70-110%, with relative standard deviations <20%. In few cases the recovery means were slightly above 110%, or the RSD was slightly above 20%. Nevertheless, the obtained results were considered acceptable since they meet the criteria laid down in the OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17. Details of recovery data are shown in Table 6.4.1-3 to Table 6.4.1- 8.

The control samples of eggs and tissues were analyzed concurrently with the treated samples. The residues of BCS-CN88460 and its metabolites were below the respective LOQ of 0.01 mg/kg in all the control samples.

In the eggs samples, no free residues of BCS-CN88460, BCS-DC20298, BCS-CY26497, BCSDC22055 and BCS-CX99799 were found above the LOQ of 0.01 mg/kg at any dose. Quantifiable residues above the LOQ (0.01 mg/kg) were only found for BCS-CY24813 of the highest dose group 16X and 16XE. The highest residue level of BCS-CY24813 in eggs was 0.020 mg/kg. The plateau concentration in eggs was reached after approximately 9-11 days.

The eggs samples collected on the overall study day 30 of dosing from the 16X group animals were separated by centrifugation into egg white and yolk. Both, the yolk and the egg white samples did not contain any residues of BCS-CN88460 or its metabolites above the LOQ (0.01 mg/kg).

The residues found in the eggs samples are summarized in Table 6.4.1- 9 and the results for egg white and yolk in Table 6.4.1- 10. For the calculation of the mean residues, in case one or two individual

values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues <0.01 mg/kg as being equal to 0.01 mg/kg. This approach differs from what is reported in the study.

In the fat with adhering skin and muscle samples at all doses, free residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 were found below the LOQ of 0.01 mg/kg.

In the liver samples at all doses, free residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY24813 and BCS-DC22055 were found below the LOQ of 0.01 mg/kg.

Free residues of BCS-CY26497 and BCS-CX99799 reached levels of up to 0.040 mg/kg and 0.11 mg/kg, respectively, in the samples from the 16X group. Residues for the sum of BCS-DC22055 and its conjugate M37 reached levels of up to 0.079 mg/kg in the samples of the16X group.

Overall residues of BCS-CN88460 were not quantifiable in eggs and tissues. The free residues of BCS-CY26497, BCS-CY24813 and BCS-CX99799 as well as free and conjugated BCS-DC22055 quantified in eggs and liver were found to increase li[redacted]ly with the dose level of BCS-CN88460.

After a depuration phase of 4, 7 and 14 days, all measured residues of BCS-CN88460 and its metabolites had declined to below the LOQ of 0.01 mg/kg in eggs and tissues.

Detailed results on the residue levels found in tissues are summarized in Table 6.4.1- 11. For the calculation of the mean residues, in case one or two individual values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues <0.01 mg/kg as being equal to 0.01 mg/kg. This approach differs from what is reported in the study.

All the analyses were conducted within less than 30 days after sample collection and the samples that were not analyzed within 24 h of sampling were stored deep frozen until analyses.

III. Conclusions

A feeding study was conducted with BCS-CN88460 on poultry in order to elucidate the levels of relevant residues in poulty tissues and in eggs.

BCS-CN88460 was administered orally (via capsule) to laying hens for 28 consecutive days at average dose rates of 0.03 mg/kg bw/d (1X EU dose), 0.12 mg/kg bw/d (4X) and 0.48 mg/kg bw/d (16X). Feed consumption, body weights, and egg production were not adversely affected by compound administration.

Prior to sacrifice, residues in eggs were measured at various intervals. After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the free residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 in all matrices. In addition, the sum of BCS-DC22055 and its conjugate M37 was determined in liver.

Overall, free residues of BCS-CN88460 parent, BCS-DC20298 and BCS-DC22055 were below the LOQ in eggs and tissues for all doses. Residues of BCS-CY26497, BCS-CY24813 and BCS-CX99799 as well as the sum of BCS-DC22055 and its conjugate M37, increase li[redacted]ly with the dose level of BCS-CN88460. In the eggs, residues above the LOQ of 0.01 mg/kg were only found for BCS-CY24813 in the samples from the highest dose group of 16X (maximum 0.02 mg/kg). The plateau concentration in eggs was reached after approximately 9-11 days.

After a depuration phase of 4, 7 and 14 days, all measured residues of BCS-CN88460 and its metabolites had declined to below the LOQ of 0.01 mg/kg in eggs and tissues.

The residue data provided in this study are suitable for regulatory purposes.

Table 6.4.1- 2: Concurrent recovery data for BCS-CN88460 in feedstuff and poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
feed	0.01	106, 104, 105, 95	103	4.9	0.01
	0.10	105, 104, 107, 106	106	1.2	
		Overall recovery (n = 8)	104	3.6	
hen / egg (complete)	0.01	94; 96; 97; 98; 99; 99; 100; 100; 100; 100; 100; 101; 102; 102; 103; 104; 105; 106; 107; 107; 108; 108; 112; 114; 115; 117; 118	104	6.2	0.01
	0.10	91; 99; 99; 99; 100; 101; 101; 102; 102; 102; 103; 103; 103; 103; 103; 103; 104; 104; 105; 105; 105; 106; 107; 108; 110; 112; 113	103	4.3	
		Overall recovery (n = 34)	**104**	**5.3**	
hen / egg whit**e	0.01	99; 101; 105; 114	105	6.4	0.01
	0.10	92; 99; 100; 103	99	4.7	
		Overall recovery (n = 8)	**102**	**6.2**	
hen / egg yolk**	0.01	105; 112; 113; 116	112*	4.2	0.01
	0.10	102; 104; 105; 109	105	2.8	
		Overall recovery (n = 8)	**108**	**4.6**	
hen / fat	0.01	103; 103; 105; 107; 116	107	5.1	0.01
	0.10	104	-	-	
	1.0	90; 91; 93; 93	92	1.6	
		Overall recovery (n = 10)	**101**	**8.4**	
hen / liver	0.01	106; 110; 114	110	3.6	0.01
	0.10	109	-	-	
	1.0	93; 95	94	-	
		Overall recovery (n = 6)	**105**	**8.2**	
hen / muscle	0.01	106; 110; 110; 112; 113; 118	112*	3.6	0.01
	0.10	101; 104; 107; 108	105	3.0	
		Overall recovery (n = 10)	**109**	**4.4**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CN88460, determined as BCS-CN88460 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8002.

* These average recoveries are considered acceptable according to OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17. Besides,there were found no residues above the LOQ (0.01 mg/kg) these matrices.

** For these recoveries external control material (Raiffeisenmarkt) has been used

Table 6.4.1- 3: Concurrent recovery data for BCS-DC20298 in poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
hen / egg (complete)	0.01	77; 83; 85; 87; 89; 91; 92; 93; 97; 98; 100; 100; 101; 102; 106; 107; 108; 110; 111; 112; 117; 124*; 125*; 126*; 127*; 129*; 136*	105	15.0	0.01
	0.10	82; 84; 90; 91; 91; 92; 96; 96; 97; 101; 103; 103; 104; 105; 105; 105; 106; 106; 110; 110; 111; 111; 114; 115; 116; 117; 125*	103	10.2	
		Overall recovery (n = 54)	**104**	**12.8**	
hen / egg white***	0.01	75; 96; 98; 99	92	12.4	0.01
	0.10	89; 93; 99; 106	97	7.7	
		Overall recovery (n = 8)	**94**	**9.8**	
hen / egg yolk***	0.01	94; 107; 108; 113	106	7.7	0.01
	0.10	98; 104; 110; 114	107	6.6	
		Overall recovery (n = 8)	**106**	**6.6**	
hen / fat	0.01	69; 70; 89; 98; 102	86	18.0	0.01
	0.10	109	-	-	
	1.0	89; 90; 94; 102	94	6.3	
		Overall recovery (n = 10)	**91**	**14.4**	
hen / liver	0.01	83; 121*; 124*	109	20.9**	0.01
	0.10	106	-	-	
	1.0	89; 102	96	-	
		Overall recovery (n = 6)	**104**	**15.8**	
hen / muscle	0.01	79; 105; 106; 109; 112; 128*	107	14.9	0.01
	0.10	102; 102; 103; 104	103	0.9	
		Overall recovery (n = 10)	**105**	**11.4**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-DC20298, determined as BCS-DC20298 and calculated as BCS-CN88460

These recoveries were performed during the conduct of the study 17-8002.

* These recoveries are considered acceptable, because they were not identified as outliers by a Grubbs outlier test with a level of significance of 95%

** This RSD-value is considered acceptable according to OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17. Besides, there were found no residues above the LOQ (0.01 mg/kg) in this matrix

*** For these recoveries external control material (Raiffeisenmarkt) has been used

Table 6.4.1- 4: Concurrent recovery data for BCS-CY26497 in poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
hen / egg (complete)	0.01	93; 94; 94; 95; 95; 96; 97; 97; 97; 98; 98; 98; 99; 101; 102; 103; 104; 105; 106; 106; 107; 107; 108; 109; 111; 111; 116	102	6.1	0.01
	0.10	93; 94; 94; 96; 96; 97; 98; 98; 99; 99; 101; 101; 102; 102; 102; 103; 103; 103; 103; 103; 105; 108; 108; 109; 111; 112	102	5.1	
		Overall recovery (n = 53)	**102**	**5.6**	
hen / egg white**	0.01	108; 110; 110; 114	111*	2.3	0.01
	0.10	90; 99; 104; 110	101	8.4	
		Overall recovery (n = 8)	**106**	**7.4**	
hen / egg yolk**	0.01	106; 107; 108; 110	108	1.6	0.01
	0.10	98; 99; 100; 107	101	4.0	
		Overall recovery (n = 8)	**104**	**4.4**	
hen / fat	0.01	91; 98; 106; 108; 113	103	8.4	0.01
	0.10	112	-	-	
	1.0	95; 95; 98; 98	97	1.8	
		Overall recovery (n = 10)	**101**	**7.6**	
hen / liver	0.01	102; 112; 115	110	6.2	0.01
	0.10	112	-	-	
	1.0	98; 104	101	-	
		Overall recovery (n = 6)	**107**	**6.3**	
hen / muscle	0.01	102; 103; 105; 107; 113; 113	107	4.5	0.01
	0.10	93; 101; 103; 107	101	5.8	
		Overall recovery (n = 10)	**105**	**5.6**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CY26497, determined as BCS-CY26497 and calculated as BCS-CN88460

These recoveries were performed during the conduct of the study 17-8002.

* This average recovery is considered acceptable according to OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17. Besides, there were found no residues above the LOQ (0.01 mg/kg) this matrix.

** For these recoveries external control material (Raiffeisenmarkt) has been used

Table 6.4.1- 5: Concurrent recovery data for BCS-CY24813 in poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
hen / egg (complete)	0.01	86; 89; 95; 95; 97; 97; 98; 98; 98; 99; 99; 100; 102; 103; 103; 103; 106; 106; 107; 107; 108; 108; 111; 116; 119; 119; 127*	104	8.9	0.01
	0.10	89; 94; 94; 96; 96; 98; 98; 99; 100; 100; 100; 100; 101; 101; 102; 102; 102; 103; 103; 103; 103; 105; 105; 106; 107; 107; 108	101	4.4	
		Overall recovery (n = 54)	**102**	**7.2**	
hen / egg white**	0.01	91; 106; 110; 118	106	10.7	0.01
	0.10	99; 102; 103; 109	103	4.1	
		Overall recovery (n = 8)	**105**	**7.7**	
hen / egg yolk**	0.01	102; 103; 107; 113	106	4.7	0.01
	0.10	105; 105; 108; 111	107	2.7	
		Overall recovery (n = 8)	**107**	**3.6**	
hen / fat	0.01	96; 100; 106; 107; 108	103	5.0	0.01
	0.10	110	-	-	
	1.0	92; 95; 95; 101	96	3.9	
		Overall recovery (n = 10)	**101**	**6.3**	
hen / liver	0.01	104; 104; 118	109	7.4	0.01
	0.10	105	-	-	
	1.0	98; 102	100	-	
		Overall recovery (n = 6)	**105**	**6.4**	
hen / muscle	0.01	96; 99; 99; 104; 105; 117	103	7.3	0.01
	0.10	99; 102; 104; 106	103	2.9	
		Overall recovery (n = 10)	**103**	**5.7**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CY24813, determined as BCS-CY24813 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8002.

* This recoveries is considered acceptable, because it was not identified as outliers by a Grubbs outlier test with a level of significance of 95%

** For these recoveries external control material (Raiffeisenmarkt) has been used

Table 6.4.1- 6: Concurrent recovery data for BCS-DC22055 in poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
hen / egg (complete)	0.01	79; 83; 83; 86; 87; 93; 94; 95; 96; 98; 98; 101; 102; 104; 107; 107; 108; 109; 109; 112; 112; 113; 117; 118; 120; 123**; 123**	103	12.3	0.01
	0.10	89; 90; 90; 91; 94; 97; 98; 98; 99; 99; 101; 101; 103; 103; 105; 105; 105; 105; 106; 106; 107; 109; 109; 114; 121**; 126**; 130**	104	9.8	
		Overall recovery (n = 54)	**103**	**11.0**	
hen / egg white***	0.01	102; 104; 105; 113	106	4.6	0.01
	0.10	91; 98; 98; 104	98	5.4	
		Overall recovery (n = 8)	**102**	**6.3**	
hen / egg yolk***	0.01	96; 107; 112; 112	107	7.1	0.01
	0.10	101; 102; 103; 110	104	3.9	
		Overall recovery (n = 8)	**105**	**5.5**	
hen / fat	0.01	90; 100; 105; 109; 116	104	9.4	0.01
	0.10	90	-		
	1.0	96; 99; 102; 104	100	3.5	
		Overall recovery (n = 10)	**101**	**8.0**	
hen / liver	0.01	97; 105; 125**	109	13.2	0.01
	0.10	103	-	-	
	1.0	86; 89	88	-	
		Overall recovery (n = 6)	**101**	**13.9-**	
hen / muscle	0.01	106; 108; 109; 110; 112; 120	111*	4.4	0.01
	0.10	97; 98; 100; 109	101	5.4	
		Overall recovery (n = 10)	**107**	**6.6**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-DC22055, determined as BCS-DC22055 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8002

* These average recoveries are considered acceptable according to OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17. Besides, there were found no residues above the LOQ (0.01 mg/kg) in these matrices.

** These recoveries are considered acceptable because they were not identified as outliers by a Grubbs outlier test with a level of significance of 95%

*** For these recoveries external control material (Raiffeisenmarkt) has been used

Table 6.4.1- 7: Concurrent recovery data for BCS-CX99799 in poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
hen / egg (complete)	0.01	74; 76; 80; 80; 89; 93; 93; 93; 94; 95; 95; 97; 98; 100; 100; 100; 103; 104; 105; 106; 107; 110; 111; 112; 116; 116; 128**	99	12.8	0.01
	0.10	75; 81; 82; 85; 91; 91; 94; 95; 95; 96; 96; 97; 98; 99; 99; 101; 101; 101; 101; 102; 103; 104; 105; 108; 109; 110; 121**	98	9.9	
		Overall recovery (n = 54)	**98**	**11.4**	
hen / egg white***	0.01	97; 108; 113; 114	108	7.2	0.01
	0.10	87; 92; 98; 112	97	11.1	
		Overall recovery (n = 8)	**103**	**10.2**	
hen / egg yolk***	0.01	103; 109; 111; 123**	112*	7.5	0.01
	0.10	95; 96; 99; 100	98	2.4	
		Overall recovery (n = 8)	**105**	**9.0**	
hen / fat	0.01	89; 95; 97; 97; 115	99	9.9	0.01
	0.10	98	-	-	
	1.0	87; 89; 91; 97	91	4.7	
		Overall recovery (n = 10)	**96**	**8.3**	
hen / liver	0.01	87; 93; 97	92	5.5	0.01
	0.10	117	-	-	
	1.0	93; 96	95	-	
		Overall recovery (n = 6)	**97**	**10.6**	
hen / muscle	0.01	100; 103; 103; 107; 108; 119	107	6.3	0.01
	0.10	96; 97; 97; 109	100	6.2	
		Overall recovery (n = 10)	**104**	**6.8**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CX99799, determined as BCS-CX90799 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8002

* These average recoveries are considered acceptable according to OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17. Besides, there were found no residues above the LOQ (0.01 mg/kg) these matrices.

** These recoveries are considered acceptable because they were not identified as outliers by a Grubbs outlier test with a level of significance of 95%.

*** For these recoveries external control material (Raiffeisenmarkt) has been used

Table 6.4.1- 8: Concurrent recovery data for free and conjugated BCS-DC22055 in poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
hen / liver	0.01	103; 111; 122*	112**	8.5	0.01
	0.10	115	-	-	
	1.0	75; 85	80	-	
		Overall recovery (n = 6)	**102**	**17.9**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-DC22055, determined as free and conjugated BCS-DC22055 and calculated as BCS-CN58460
These recoveries were performed during the conduct of the study 17-8002

* This recovery is considered acceptable, because it was not identified as outliers by a Grubbs outlier test with a level of significance of 95%

** These average recoveries are considered acceptable according to OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17.

Table 6.4.1- 9: Residue levels in eggs (mean of 3 sub-groups)

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) **					
		BCS-CN88460	BCS-DC20298	BCS-CY26497	BCS-CY24813	BCS-DC22055	BCS-CX99799
1X 0.03 mg/kg bw/d 0.530 mg/kg DM Sub-groups: B1, B2, B3	Pre-dosing -19	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -12	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	3	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	4	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	8	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	9	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	11	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	14	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	21	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	23	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) **					
		BCS-CN88460	BCS-DC20298	BCS-CY26497	BCS-CY24813	BCS-DC22055	BCS-CX99799
4X 0.12 mg/kg bw/d 2.119 mg/kg DM Sub-groups: C1, C2, C3	Pre-dosing -19	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -12	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	2	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	3	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	4	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	8	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	9	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	11	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	14	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	16	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	21	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	23	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) **					
		BCS-CN88460	**BCS-DC20298**	**BCS-CY26497**	**BCS-CY24813**	**BCS-DC22055**	**BCS-CX99799**
16X 0.48 mg/kg bw/d 8.698 mg/kg DM Sub-groups: D1, D2, D3	Pre-dosing -19	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -12	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	3	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	4	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	8	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	9	< 0.01	< 0.01	< 0.01	**0.012** (< 0.01; < 0.01; **0.015**)	< 0.01	< 0.01
	11	< 0.01	< 0.01	< 0.01	**0.012** (**0.014; 0.012**; < 0.01)	< 0.01	< 0.01
	14	< 0.01	< 0.01	< 0.01	**0.011** (< 0.01; < 0.01; **0.014**)	< 0.01	< 0.01
	16	< 0.01	< 0.01	< 0.01	**0.014** (< 0.01; **0.020; 0.013**)	< 0.01	< 0.01
	21	< 0.01	< 0.01	< 0.01	**0.012** (< 0.01; **0.017**;< 0.01)	< 0.01	< 0.01
	23	< 0.01	< 0.01	< 0.01	< 0.01 (< 0.01; < 0.01; < 0.01)	< 0.01	< 0.01
	31	< 0.01	< 0.01	< 0.01	<0.01 (< 0.01; < 0.01; < 0.01)	< 0.01	< 0.01

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) **					
		BCS-CN88460	**BCS-DC20298**	**BCS-CY26497**	**BCS-CY24813**	**BCS-DC22055**	**BCS-CX99799**
16XE depuration group 0.48 mg/kg bw/d 8.140 mg/kg DM Sub-groups: E1, E2, E3	Pre-dosing -19	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -12	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	Pre-dosing -5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	21	< 0.01	< 0.01	< 0.01	**0.010** (< 0.01; **0.011**; <0.01)	< 0.01	< 0.01
	23	< 0.01	< 0.01	< 0.01	**0.011** (< 0.01; **0.013**; <0.01)	< 0.01	< 0.01
	31	< 0.01	< 0.01	< 0.01	< 0.01 (< 0.01; < 0.01; <0.01)	< 0.01	< 0.01
	35 4d depuration	< 0.01	< 0.01	< 0.01	< 0.01 (< 0.01; < 0.01; <0.01)	< 0.01	< 0.01
	38 7d depuration	< 0.01***	< 0.01***	< 0.01***	< 0.01*** (n.a.; < 0.01; < 0.01)	< 0.01***	< 0.01***
	45 14d depuration	< 0.01****	< 0.01****	< 0.01****	< 0.01**** (n.a.; n.a.; < 0.01)	< 0.01****	< 0.01****

* overall study day

** Mean of the 3 sub-groups calculated based on the unrounded residue results. For the calculation of the mean residues, in case one or two individual values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues <0.01 mg/kg as being equal to 0.01 mg/kg. This approach differs from what is reported in the study.

*** Mean value of 2 sub-groups

**** Value from one sub-group since only one sub-group 16XE remained alive on overall study day 45

n.a. not applicable

All metabolite residues expressed in parent compound equivalents

LOQ = 0.01 mg/kg for each compound

Table 6.4.1- 10: Residue levels in yolk and egg white (mean of 3 sub-groups **)

Group Dose	Eggs Sampling Time (Day)*	Sample material	BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	BCS-CX26497 [mg/kg]	BCS-CX24813 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
16X	30	yolk	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
0.48 mg/kg bw/d	30	egg white	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
8.698 mg/kg DM	31	eggs	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Sub-groups: D1, D2, D3								

* overall study day
** Mean of the 3 sub-groups.
All metabolite residues expressed in parent compound equivalents
LOQ = 0.01 mg/kg for each compound

Table 6.4.1- 11: Residue levels in poultry tissues

Group	Dose (mg/kg bw/d)	Dose (mg/kd DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg) **						
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	BCS-DC22055 [mg/kg]	Sum of BCS-DC22055 and M37 [mg/kg]	BCS-CX99799 [mg/kg]
Poultry fat											
1X	0.03	0.530	B1	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			B2	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			B3	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
4X	0.12	2.119	C1	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			C2	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			C3	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
16X	0.48	8.698	D1	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			D2	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			D3	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
16XE depuration	0.48	8.140	E1	35	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			E2	38	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			E3	45	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01

Group	Dose (mg/kg bw/d)	Dose (mg/kd DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg) **						
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	BCS-DC22055 [mg/kg]	Sum of BCS-DC22055 and M37 [mg/kg]	BCS-CX99799 [mg/kg]
Poultry liver											
1X	0.03	0.530	B1	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			B2	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			B3	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
4X	0.12	2.119	C1	30	< 0.01	< 0.01	**0.015**	< 0.01	< 0.01	**0.012**	**0.045**
			C2	30	< 0.01	< 0.01	**0.012**	< 0.01	< 0.01	**0.021**	**0.023**
			C3	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	**0.015**	**0.010**
				mean	*< 0.01*	*< 0.01*	***0.012***	*< 0.01*	*< 0.01*	***0.016***	***0.026***
				median	*< 0.01*	*< 0.01*	***0.012***	*< 0.01*	*< 0.01*	***0.015***	***0.023***
16X	0.48	8.698	D1	30	< 0.01	< 0.01	**0.040**	< 0.01	< 0.01	**0.053**	**0.097**
			D2	30	< 0.01	< 0.01	**0.035**	< 0.01	< 0.01	**0.059**	**0.11**
			D3	30	< 0.01	< 0.01	**0.019**	< 0.01	< 0.01	**0.079**	**0.061**
				mean	*< 0.01*	*< 0.01*	***0.031***	*< 0.01*	*< 0.01*	***0.064***	***0.089***
				median	*< 0.01*	*< 0.01*	***0.035***	*< 0.01*	*< 0.01*	***0.059***	***0.097***
16XE depuration	0.48	8.140	E1	35	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			E2	38	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			E3	45	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Group	Dose (mg/kg bw/d)	Dose (mg/kd DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg) **						
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	BCS-DC22055 [mg/kg]	Sum of BCS-DC22055 and M37 [mg/kg]	BCS-CX99799 [mg/kg]
Poultry muscle											
1X	0.03	0.530	B1	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			B2	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			B3	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
4X	0.12	2.119	C1	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			C2	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			C3	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
16X	0.48	8.698	D1	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			D2	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			D3	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*
16XE depuration	0.48	8.140	E1	35	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			E2	38	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01
			E3	45	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	NA	< 0.01

NA Not analyzed
* overall study day
** All metabolite residues expressed in parent compound equivalents. Mean values are calculated based on the unrounded residue values and, in case one or two individual values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues < 0.01 mg/kg as being equal to 0.01 mg/kg. This approach differs from what is reported in the study.
LOQ = 0.01 mg/kg

CA 6.4.2 Ruminants

Report:	KCA 6.4.2/01; [redacted]; 2017; M-604191-02-1
Title:	Amendment no. 1: BCS-CN88460: Feeding study with dairy cows
Report No.:	17-8001
Document No.:	M-604191-02-1
Guideline(s):	OECD Guidelines for the Testing of Chemicals (Test Guideline 505 Residues in Livestock, adopted 2007-01-08) US: EPA Residue Chemistry Test Guidelines OCSPP 860.1000 Background OCSPP 860.1480 Meat, milk, poultry and eggs
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and Methods

Test system, dosing

After an acclimatization phase of about 3 weeks, eighteen healthy dairy cows (Holstein Frisian black) were dosed orally, via gelatine capsules, for 28 consecutive days with BCS-CN88460 at dose rates of. 0 mg/kg bw/day (control, 3 cows), 0.05 mg/kg bw/day (1X dose group, 3 cows), 0.15 mg/kg bw/day (3X dose group, 3 cows), 0.5 mg/kg bw/day (10 X dose group , 3 cows) and 1.5 mg/kg bw/day (30X dose group, 3 cows). An additional group 30XE (3 cows) was dosed at the rate of 1.5 mg/kg bw/day for 28 consecutive days simultaneously with the animals from dose group 30X. Thereafter, dosing was stopped and the animals were kept alive for further 4, 7 or 14 days in order to investigate the depuration of residues of BCS-CN88460 in milk and tissues after the end of dosing.

The exact amounts of test item to be administered daily to each cow were calculated based on the body weights measured at the beginning of the second week of the acclimatization period. Capsules were prepared at the test facility in [redacted]. The test item was weighed into capsules, which were then closed and placed in another slightly larger capsule adapted to the size of the bolus applicator. The prepared capsules were filled in bottles and handed over to Dr. Stephan Groeger (PI of in-life phase) At the in-life test site the capsules were stored at ambient temperature in the dark. A representative number of capsules were analyzed using method 01511 ([redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2) after preparation to verify the amount of technical BCS-CN88460. Further capsules were analysed 41 days after the final administration to determine the stability of the test item during the in-life phase.

The cows were fed with a combination of cob mix and feed concentrate for dairy cattle, which were supplemented with minerals (Cobs mixture prepared by AGRO-COBS, batch 10350000301216 (Prepared by AGROBS GmbH, Angerbreite 27, 82541 Münsing-Degerndorf, Germany). The feed was screened for residues of BCS-CN88460 with an LOQ of 0.01 mg/kg (expressed as BCS-CN88460) according to method 01435 ([redacted]; 2016; M-558986-01-1; referenced in MCA 4 Point 4.1.2). The amount of feed consumed was monitored daily. The cows were allowed *ad libitum* access to tap water. The dose rates employed in the study are summarized below in Table 6.4.2- 1.

Table 6.4.2- 1: Summary of actual BCS-CN88460 dose administration.

Dose groups	Number of cows	Dose levels	
		per animal (mg/kg bw/day)	in feed (mg/kg DM) *
Control (0X)	3	0	0
1X dose	3	0.05	1.61
3X dose	3	0.15	4.18
10X dose	3	0.5	15.54
30X dose	3	1.5	48.13
30XE dose	3	1.5	47.13

DM: dry matter
*: Actual dose based on average feed consumption data collected from the study

The cows were dosed daily during 28 consecutive days via capsules using a pill gun every evening after feeding and milking. The control animal received a placebo (an empty capsule) concurrently with the treated animals.

Sampling

Milk samples of the animals of the groups 0X, 1X, 3X, 10X, 30X and 30XE were taken twice before the 1st application, at least every third day during the first three weeks of dosage and twice during the last week of dosage.

The analytical samples per day consisted of about 1 L of the evening milk and 1 L of the morning milk. The analytical milk sample is then a mixture of both the evening and the morning milk sample, respecting the proportion of the produced milk quantity from the evening and morning. An aliquot was subjected to the residue analysis while the remainder was chopped with dry-ice and stored at ≤ - 18°C.

The milk samples collected on the overall study day 31 of dosing from the 0X (animal H940) and from the 30X group animals were separated by centrifugation into whey (whey) and cream.

The diagnostic slaughtering of the animals took place on the day after the final application less than 24 hours after the final dose. The animals were terminated by stunning via a captive bolt gun followed immediately by exsanguination through severing the blood vessels of the neck. After termination, the animals were eviscerated and tissue samples were taken.

Kidneys, samples of liver (without gall bladder), perirenal fat, mesenteric fat, subcutaneous fat and muscle samples (right flank, right hind leg and right loin) were collected at necropsy. Tissue samples were cut in cubes of approximately 5 cm edge length. The liver was cut in cubes and a representative sample of approximately 500 g was taken thereafter. Kidneys were processed in the same way. Different muscle samples were pooled. Fat tissue samples were kept separately. All tissue samples were immediately weighed packed into plastic bags and transported to the analytical laboratory. Upon arrival at the laboratory facility the tissue samples, were chopped with dry ice. All samples were deep-frozen within 24h after sampling and stored at ≤ - 18°C until analysis. One portion of the fat samples was sent deep-frozen to [redacted] to determine the actual fat content.

The three dairy cows of the 30XE group were kept alive for 4 - 14 days after the last dosing in order to investigate the depuration of residues in milk and tissues. During the depuration phase milk was collected periodically. At sacrifice samples of liver, muscle, kidney and fat (perirenal, subcutaneous and mesenteric) were taken for analysis.

Analysis

Tissues and milk samples were analyzed for free residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 by high performance liquid chromatography-electrospray ionization / tandem mass spectrometry (HPLC-MS/MS) using isotopically labeled internal standards. The analyses were conducted according to the

method 01511([redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2).

In addition the sum of BCS-DC20298 and its conjugate M20 and the sum of BCS-CY24813 and its conjugate M19 were determined in liver and kidney, after a hydrolysis step, according to the method 01511 ([redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2).

The method 01511 was validated prior to sample analysis in a separate study ([redacted]; [redacted]; [redacted]; 2017; M-599206-01-1; referenced in MCA Section 4 under Point 4.1.2). Concurrent recoveries were performed during sample analysis to demonstrate acceptable method performance. The Limit of Quantitation (LOQ) for BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 was 0.01 mg/kg per analyte expressed as BCS-CN88460 in all tissues and 0.005 mg/kg in milk, cream and whey. The LOQ for the sum of BCS-DC20298 and its conjugate M20 in liver and kidney was 0.01 mg/kg expressed as BCS-CN88460. The LOQ for the sum of BCS-CY24813 and its conjugate M19 in liver and kidney was 0.01 mg/kg expressed as BCS-CN88460.

II. Findings

Dose verification and storage stability

Upon analysis by LC-MS/MS immediately after preparation the actual concentration of BCSCN88460 in the capsules ranged between 88% and 100% of the nominal content (average of 3 capsules per dose group), demonstrating an accurate preparation.

The stability of BCS-CN88460 in the capsules during storage for the time of the in-life phase was investigated by analyzing capsules stored at the in-life test site under the same conditions as the capsules administered to the cows. This analysis was performed 41 days after the preparation of the capsules (4 days after the final administration). The analysed set comprised 5 capsules of the 1X group and 5 capsules of the 30X group. The actual concentration of BCSCN88460 was 104% and 100% of the nominal content in the capsules of the 1X and 30X group, respectively. Therefore, the capsules were shown to be stable over the course of the study.

Analysis of feedstuff

No residues of BCS-CN88460 above the LOQ (0.01 mg/kg) were found in the feedstuff. Concurrent recoveries were in the acceptable range of 70-110% with Relative Standard Deviations RSDs <20% (see Table 6.4.2- 2).

In-life observations

In general the feed consumption was higher during the dosing period compared to the predosing period. It is very unlikely that BCS-CN88460 did increase feed intake. It is more likely that cows were still adapting to the feeding and husbandry system when dosing started. The data given indicate that the mean calculated energy demand was not fully met by the feed consumed. But since cows were not ketotic (no findings in repeated physical examinations), milk yields were stable, and cows did not loose body weight in the course of the study it is concluded that energy supply was met adequately.

Analysis of milk, milk products and tissues

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 70-110%, with relative standard deviations <20%. In few cases the recovery means were slightly above 110%, or the RSD was slightly above 20%. Nevertheless, the obtained results were considered acceptable according to the criteria laid down in the OECD Guidance document on pesticide residue analytical methods ENV/JM/MONO(2007)17. Details of recovery data are shown in Table 6.4.2- 2 to. Table 6.4.2- 9.

The control samples of milk and tissues were analyzed concurrently with the treated samples. The residues of BCS-CN88460 and its metabolites were below the respective LOQ of 0.005 mg/kg and 0.01 mg/kg of the control milk and tissue samples.

In the milk samples, quantifiable residues above the LOQ (0.005 mg/kg) were only found for BCS-CN88460 in the highest tested dose group (30X and 30XE). The highest residue level of BCS-CN88460 in milk was 0.013 mg/kg. The plateau concentration in milk was reached after approximately 9 days. No residues of metabolites above the LOQ of 0.005 mg/kg were found in any dose groups.

The milk samples collected on the overall study day 30 of dosing from the 30X group animals were separated by centrifugation into skim milk (whey) and cream. The cream samples were found to contain up to 0.15 mg/kg of BCS-CN88460, 0.009 mg/kg of BCS-DC20298 and 0.005 mg/kg of BCS-CY24813 while the residues of BCS-CY26497, BCS-DC22055 and BCS-CX99799 were less than the LOQ of 0.005 mg/kg. The skim milk (whey) samples showed no residues of BCS-CN88460 or its metabolites above the LOQ of 0.005 mg/kg.

The residues found in the milk samples are summarized in Table 6.4.2- 10 and the results for skim milk and cream in Table 6.4.2- 11. For the calculation of the mean residues, in case one or two individual values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues <0.005 mg/kg as being equal to 0.005 mg/kg. This approach differs from what is reported in the study.

In muscle, no residues of BCS-CN88460 and its metabolites were found above the LOQ of 0.01 mg/kg at any dose.

Free residues of BCS-DC20298, BCS-CY24813 and BCS-DC22055 at or above the LOQ of 0.01 mg/kg were not found in any of the tissue samples of any dose group.

The residues of BCS-CN88460 were found to be < 0.01 mg/kg in the tissue samples of the 0X-, and 1X-groups. Residues of BCS-CN88460 above the LOQ were found in perirenal fat up to 0.087 mg/kg in the samples of the 3X, 10X, 30X and 30XE-group (HD953; 5 days of depuration).

Residues of BCS-CN88460 in kidney were only found in the 30X group up to 0.011 mg/kg. Residues of BCS-CN88460 in liver were only found in the 10X, 30X-group up to 0.02 mg/kg.

Residues of BCS-CY26497 were found to be < 0.01 mg/kg in the tissue samples of the 0X, 1X and 3X groups. Residues of BCS-CY26497 were found in liver up to 0.037 mg/kg only in the 30X group. Residues of BCS-CY26497 in kidney were found up to 0.074 mg/kg in the 10X and 30X group.

Residues of BCS-CX99799 were found to be < 0.01 mg/kg in the tissue samples of the 0X, 1X, 3X and 10X groups. Residues of BCS-CX99799 were found in liver up to 0.014 mg/kg only in the 30X-group.

Residues of BCS-CX99799 in kidney were found up to 0.023 mg/kg in the 30X-group.

The residues for the sum of BCS-DC20298 and M20 were found to be < 0.01 mg/kg in kidney at any dose and <0.01 mg/kg in liver of the 0X, 1X and 3X groups. The sum of BCS-DC20298 and M20 were found up to 0.091 mg/kg in liver in the 10X and 30X-groups.

The residues for the sum of BCS-CY24813 and its conjugate M19 were found to be < 0.01 mg/kg in kidney of the 0X, 1X, 3X and 10X groups and < 0.01 mg/kg in liver of the 0X and 1X groups. Free and conjugated residues of BCS-CY24813 were found up to 0.15 mg/kg in liver in the 3X, 10X and 30X-groups and up to 0.016 mg/kg in kidney of the 30X-group.

Overall, residues of BCS-CN88460 above the LOQ were found only in milk, fat, kidney and liver. Residues of BCS-CY26497 and BCS-CX99799 were found only in kidney and liver and free and conjugated residues of BCS-DC20298 and BCS-CY24813 were found in liver and kidney. The free residues of BCS-CN88460, BCS-CY26497 and BCS-CX99799 as well as free and conjugated residues of BCS-DC20298 and BCS-CY24813 in fat, liver and kidney were found to increase li[redacted]ly with the dose level of BCS-CN88460.

After a depuration phase of 4 days, the measured residues of BCS-CN88460 had declined to below the LOQ of 0.005 mg/kg in milk and 0.01 mg/kg in tissues except for BCS-CN88460 in fat (0.014 mg/kg).

After a depuration phase of 7 and 14 days, all measured residues were found to be below their respective LOQ in all samples.

Detailed results on the residue levels found in tissues are summarized in Table 6.4.2- 12. For the calculation of the mean residues, in case one or two individual values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues <0.01 mg/kg as being equal to 0.01 mg/kg. This approach differs from what is reported in the study.

All the analyses were conducted within less than 30 days of sampling and the samples that were not analyzed within 24 h of sampling were stored deep frozen until analyses.

III. Conclusions

A feeding study was conducted with BCS-CN88460 on cows in order to elucidate the levels of relevant residues in cow tissues and in milk.

BCS-CN88460 was administered orally (via capsule) to cows for 28 consecutive days at average dose rates of BCS-CN88460 at 0.05 mg/kg bw/day test item for the dose group 1X, 0.15 mg/kg bw/day for the dose group 3X, 0.5 mg/kg bw/day for the dose group 10X and 1.5 mg/kg bw/day for the dose groups 30X and 30XE. Feed consumption, body weights, and milk production were not adversely affected by compound administration.

Prior to sacrifice, residues in milk were measured at various intervals. After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the free residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 in all matrices. In addition the sum of BCS-DC20298 and its conjugate M20 and the sum of BCS-CY24813 and its conjugate M19 were determined in liver and kidney.

Overall, residues of BCS-CN88460 above the LOQ were found only in milk, fat, kidney and liver. Residues of BCS-CY26497 and BCS-CX99799 were found only in kidney and liver and free and conjugated residues of BCS-DC20298 and BCS-CY24813 were found in liver and kidney. The free residues of BCS-CN88460, BCS-CY26497 and BCS-CX99799 as well as free and conjugated residues of BCS-DC20298 and BCS-CY24813 in fat, liver and kidney were found to increase li[redacted]ly with the dose level of BCS-CN88460.

Residues of BCS-CN88460 were found in milk samples only in the 30X and 30XE groups up to 0.013 mg/kg, in cream samples up to 0.15 mg/kg in the 30X-group. The plateau concentration in milk was reached after approximately 9 days.

After a depuration phase of 4 days, the measured residues of BCS-CN88460 had declined to below the LOQ of 0.005 mg/kg in milk and 0.01 mg/kg in tissues except for BCS-CN88460 in fat (0.014 mg/kg).

After a depuration phase of 7 and 14 days, all measured residues were found to be below their respective LOQ in all samples.

The residue data provided in this study are suitable for regulatory purposes.

Table 6.4.2- 2: Concurrent recovery data for BCS-CN88460 in feedstuff and cattle matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
Feedstuff / Cobs mixture	0.01	88; 80; 97; 93	90	8.2	0.01
	0.10	97; 105; 102; 100	101	3.3	
		Overall recovery (n = 8)	95	8.5	
Feedstuff / Dairy Cattle Concentrate	0.01	97; 99; 96	97	1.6	
	0.10	103; 101; 101	102	1.1	
		Overall recovery (n = 6)	100	2.7	
Feedstuff / Mineral feed	0.01	106; 98; 100	101	4.1	
	0.10	109; 107; 108	108	0.9	
		Overall recovery (n = 6)	105	4.3	
cattle / milk	0.005	91; 91; 92; 93; 95; 95; 96; 97; 98; 98; 98; 98; 99; 100; 100; 100; 100; 100; 101; 102; 102; 102; 103; 103; 103; 103; 104; 104; 104; 104; 104; 104; 105; 105; 105; 105; 105; 107; 107; 107; 107; 108; 109; 110; 110; 111; 112; 116	102	5.4	0.005
	0.05	102; 103; 103; 105; 106; 107; 107; 108; 108; 108; 108; 109; 109; 109; 109; 110; 110; 110; 110; 110; 110; 110; 111; 112; 112; 112; 113; 113; 114; 114; 114; 115; 115; 116	110	3.2	
		Overall recovery (n = 82)	**105**	**5.7**	
cattle / cream	0.005	102; 102; 103; 106; 107; 108	105	2.5	0.005
	0.20	105; 110; 112	109	3.3	
		Overall recovery (n = 9)	**106**	**3.3**	
cattle / skim milk (whey)	0.005	100; 101; 104; 104; 106; 112	105	4.1	0.005
	0.05	103; 110; 111	108	4.0	
		Overall recovery (n = 9)	**106**	**4.2**	
cattle / muscle	0.01	103; 103; 109; 120	109	7.4	0.01
	0.25	105; 108; 111; 113	109	3.2	
		Overall recovery (n = 8)	**109**	**5.3**	
cattle / fat, mesenteric	0.01	93; 95; 107; 118	103	11.2	0.01
	0.25	100; 102; 103; 108	103	3.3	
		Overall recovery (n = 8)	**103**	**7.7**	
cattle / fat, perirenal	0.01	96; 96; 98; 101	98	2.4	0.01
	0.25	99; 101; 101; 104	101	2.0	
		Overall recovery (n = 8)	**100**	**2.8**	

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / fat, subcutanenous	0.01	93; 97; 101; 104	99	4.8	0.01
	0.25	98; 100; 103; 106	102	3.4	
		Overall recovery (n = 8)	**100**	**4.2**	
cattle / kidney	0.01	97; 100; 104; 105	102	3.6	0.01
	0.25	104; 105; 105; 108	106	1.6	
		Overall recovery (n = 8)	**104**	**3.3**	
cattle / liver	0.01	96; 103; 106	102	5.0	**0.01**
	0.25	102; 103; 105; 106	104	1.8	
		Overall recovery (n = 7)	**103**	**3.4**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CN88460, determined as BCS-CN88460 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8001.

Table 6.4.2- 3: Concurrent recovery data for BCS-DC20298 in cattle matrices

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / milk	0.005	62; 63; 64; 68; 69; 73; 74; 75; 77; 79; 87; 90; 94; 94; 95; 96; 100; 100; 104; 105; 105; 105; 105; 106; 106; 107; 108; 110; 110; 110; 111; 113; 115; 116; 116; 120; 120; 121*; 128*; 129*; 133*; 136*; 138*; 139*; 139*; 150*	104	21.7****	0.005
	0.05	70; 70; 73; 81; 83; 83; 83; 83; 88; 88; 89; 89; 91; 91; 94; 95; 97; 98; 99; 101; 102; 103; 104; 104; 106; 106; 107; 107; 119; 119; 119; 123*; 132*	97	15.6	
		Overall recovery (n = 79)	**101**	**19.8**	
cattle / cream	0.005	107; 108; 109; 113; 115; 116	111*	3.4	0.005
	0.20	106; 107; 110	108	1.9	
		Overall recovery (n = 9)	**110**	**3.4**	
cattle / skim milk (whey)	0.005	96; 97; 99; 102; 104; 106	101	4.0	0.005
	0.05	107; 111; 116	111**	4.1	
		Overall recovery (n = 9)	**104**	**6.3**	
cattle / muscle	0.01	80; 104; 112	99	16.9	0.01
	0.25	81; 93; 108; 108	98	13.4	
		Overall recovery (n = 7)	**98**	**13.6**	
cattle / fat, mesenteric	0.01	65; 77; 93; 101	84	19.2	0.01
	0.25	91; 100; 101; 102	99	5.1	
		Overall recovery (n = 8)	**91**	**14.8**	
cattle / fat, perirenal	0.01	93; 103; 110; 119	106	10.3	0.01
	0.25	94; 100; 100; 106	100	4.9	
		Overall recovery (n = 8)	**103**	**8.3**	

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / fat, subcutaneous	0.01	79; 96; 104; 107	97	13.0	0.01
	0.25	96; 97; 108; 111	103	7.4	
		Overall recovery (n = 8)	**100**	**10.2**	
cattle / kidney	0.01	114; 113; 96	108	9.4	0.01
	0.25	93; 102; 98; 108	100	6.3	
		Overall recovery (n = 7)	**103**	**8.1**	
cattle / liver	0.01	82; 100; 112; 109	101	13.4	0.01
	0.20	105; 102; 108; 102	104	2.8	
		Overall recovery (n = 8)	**103**	**9.0**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-DC20298, determined as BCS-DC20298 and calculated as BCS-CN88469

These recoveries were performed during the conduct of the study 17-8061.

* These recoveries are considered acceptable, because they were not identified as outliers by a Grubbs outlier test with a level of significance of 95%.

** This average recovery is considered acceptable, because it is only slightly exceeding the range and because it is considered acceptable according to OECD guideline ENV/JM/MONO(2007)17.

*** This average recovery is considered acceptable, because there were found no residues of BCSDC20298 in this sample material.

**** This RSD is considered acceptable, because it is only slightly exceeding the range of 20% and because this value is considered acceptable according to OECD guideline ENV/JM/MONO(2007)17 at this fortification level.

Table 6.4.2- 4: Concurrent recovery data for BCS-CY26497 in cattle matrices

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / milk	0.005	85; 90; 90; 92; 93; 93; 94; 94; 94; 97; 97; 97; 97; 98; 100; 101; 101; 101; 102; 102; 102; 103; 104; 104; 105; 105; 105; 105; 106; 106; 106; 106; 107; 107; 107; 107; 107; 107; 108; 108; 108; 111; 112; 114; 115; 116; 118; 125**	103	7.7	0.005
	0.05	96; 97; 99; 101; 102; 102; 104; 105; 105; 106; 106; 107; 107; 110; 110; 110; 110; 111; 111; 112; 112; 112; 112; 113; 113; 114; 116; 116; 116; 116; 117; 124**	109	5.7	
		Overall recovery (n = 81)	**106**	**7.4**	
cattle / cream	0.005	104; 106; 107; 111; 116; 119	111*	5.4	0.005
	0.20	107; 116; 119	114*	5.5	
		Overall recovery (n = 9)	**112***	**5.3**	
cattle / skim milk (whey)	0.005	94; 105; 106; 106; 109; 110	105	5.5	0.005
	0.05	102; 111; 115	109	6.1	
		Overall recovery (n = 9)	**106**	**5.7**	
cattle / muscle	0.01	108; 109; 111; 111	110	1.4	0.01
	0.25	104; 110; 100; 116	110	4.5	
		Overall recovery (n = 8)	**110**	**3.1**	
cattle / fat, mesenteric	0.01	103; 103; 103; 108	104	2.4	0.01
	0.25	97; 100; 100; 102	100	2.1	
		Overall recovery (n = 8)	**102**	**3.1**	
cattle / fat, perirenal	0.01	93; 93; 94; 98	95	2.5	0.01
	0.25	98; 99; 103; 107	102	4.0	
		Overall recovery (n = 8)	**98**	**5.1**	
cattle / fat, subcutaneous	0.01	99; 101; 111; 111	106	6.1	0.01
	0.25	94; 98; 104; 112	102	7.7	
		Overall recovery (n = 8)	**104**	**6.6**	
cattle / kidney	0.01	96; 99; 99; 103	99	2.9	0.01
	0.25	95; 100; 115; 117	107	10.2	
		Overall recovery (n = 8)	**103**	**8.2**	
cattle / liver	0.01	99; 103; 110; 112	106	5.7	0.01
	0.25	88; 96; 100; 112	99	10.1	
		Overall recovery (n = 8)	**103**	**8.3**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CY26497, determined as BCS-CY26497 and calculated as BCS-CN88460

These recoveries were performed during the conduct of the study 17-8001.

* These average recoveries are considered acceptable, because there were found no residues of BCS-CY26497 in the sample material cream

** These recoveries are considered acceptable, because they were not identified as outliers by a Grubbs outlier test with a level of significance of 95%.

Table 6.4.2- 5: Concurrent recovery data for BCS-CY24813 in cattle matrices

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / milk	0.005	87; 87; 89; 95; 95; 97; 97; 97; 98; 99; 99; 99; 100; 100; 101; 101; 102; 103; 103; 103; 103; 104; 104; 104; 105; 106; 106; 107; 107; 107; 107; 108; 108; 108; 108; 108; 108; 108; 109; 110; 110; 110; 111; 112; 112; 112; 115; 116	104	6.4	0.005
	0.05	88; 100; 101; 103; 103; 105; 105; 105; 106; 106; 106; 106; 106; 106; 106; 107; 107; 107; 109; 109; 110; 110; 111; 111; 111; 111; 112; 113; 113; 114; 114; 116; 118; 118; 119	108	6.5	
		Overall recovery (n = 82)	**106**	**6.4**	
cattle / skim milk (whey)	0.005	95; 98; 102; 105; 111; 112	104	6.6	0.005
	0.05	103; 104; 110	106	3.6	
		Overall recovery (n = 9)	**104**	**5.6**	
cattle / cream	0.005	99; 102; 103; 103; 105; 106	103	2.4	0.005
	0.20	100; 102; 109	104	4.6	
		Overall recovery (n = 9)	**103**	**3.0**	
cattle / muscle	0.01	94; 99; 104; 109	102	6.4	0.01
	0.25	98; 101; 102; 107	102	3.7	
		Overall recovery (n = 8)	**102**	**4.8**	
cattle / fat, mesenteric	0.01	93; 97; 99; 113	101	8.1	0.01
	0.25	96; 98; 106; 106	102	5.2	
		Overall recovery (n = 8)	**101**	**6.3**	
cattle / fat, perirenal	0.01	101; 104; 105; 107	104	2.4	0.01
	0.25	97; 104; 105; 108	104	4.5	
		Overall recovery (n = 8)	**104**	**3.4**	
cattle / fat, subcutaneous	0.01	93; 101; 103; 106	101	6.0	0.01
	0.25	91; 100; 100; 105	99	5.9	
		Overall recovery (n = 8)	**100**	**5.6**	
cattle / kidney	0.01	90; 96; 104; 94	96	6.1	0.01
	0.25	94; 95 ; 95; 96	95	0.9	
		Overall recovery (n = 8)	**96**	**4.1**	
cattle / liver	0.01	101; 102; 111; 110	106	4.9	0.01
	0.25	101; 108;106; 102	104	3.2	
		Overall recovery (n = 8)	**105**	**4.0**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CY24813, determined as BCS-CY24813 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8001.

Table 6.4.2- 6: Concurrent recovery data for BCS-DC22055 in cattle matrices

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / milk	0.005	83; 86; 88; 90; 90; 90; 92; 92; 94; 94; 94; 95; 95; 95; 96; 96; 97; 97; 97; 98; 98; 99; 100; 101; 102; 103; 103; 103; 103; 103; 103; 103; 104; 105; 105; 106; 107; 108; 108; 109; 110; 111; 111; 113; 115; 117; 123*; 125*	101	8.9	0.005
	0.05	92; 93; 95; 95; 95; 96; 96; 97; 97; 100; 100; 100; 102; 103; 104; 104; 104; 106; 106; 106; 106; 106; 106; 108; 109; 109; 110; 110; 110; 110; 111; 112; 115; 121*	104	6.6	
		Overall recovery (n = 82)	**102**	**8.1**	
cattle / cream	0.005	96; 100; 100; 112; 112; 112	105	7.1	0.005
	0.20	93; 99; 104	99	5.6	
		Overall recovery (n = 9)	**103**	**7.1**	
cattle / skim milk (whey)	0.005	100; 102; 107; 107; 109; 113	106	4.4	0.005
	0.05	95; 102; 104	100	4.7	
		Overall recovery (n = 9)	**104**	**5.1**	
cattle / muscle	0.01	96; 101; 103; 103	101	3.3	0.01
	0.25	97; 99; 106; 109	103	5.5	
		Overall recovery (n = 8)	**102**	**4.4**	
cattle / fat, mesenteric	0.01	99; 99; 106; 107	103	4.2	0.01
	0.25	93; 99; 105; 106	101	6.0	
		Overall recovery (n = 8)	**102**	**4.9**	
cattle / fat, perirenal	0.01	92; 95; 101; 108	99	7.1	0.01
	0.25	94; 96; 101; 102	98	3.9	
		Overall recovery (n = 8)	**99**	**5.4**	
cattle / fat, subcutaneous	0.01	91; 98; 100; 103	98	5.2	0.01
	0.25	93; 95; 102; 110	100	7.7	
		Overall recovery (n = 8)	**99**	**6.2**	
cattle / kidney	0.01	94; 103; 105; 110	103	6.5	0.01
	0.25	100; 105; 106; 107	105	3.0	
		Overall recovery (n = 8)	**104**	**4.7**	
cattle / liver	0.01	95; 98; 101; 113	102	7.8	0.01
	0.25	94; 99; 102; 108	101	5.8	
		Overall recovery (n = 8)	**101**	**6.4**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-DC22055, determined as BCS-DC22055 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8001

* These recoveries are considered acceptable, because they were not identified as outliers by a Grubbs outlier test with a level of significance of 95%.

Table 6.4.2- 7: Concurrent recovery data for BCS-CX99799 in cattle matrices

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / milk	0.005	83; 86; 89; 89; 91; 94; 95; 95; 95; 96; 96; 97; 98; 99; 99; 100; 101; 101; 101; 102; 103; 104; 105; 106; 106; 106; 107; 108; 108; 108; 108; 108; 109; 111; 112; 113; 113; 113; 113; 113; 115; 118; 119; 121**; 121**; 122**; 122**; 127**	105	9.7	0.005
	0.05	89; 91; 92; 95; 95; 96; 97; 97; 97; 98; 98; 99; 100; 100; 103; 103; 103; 103; 104; 106; 106; 108; 108; 108; 108; 109; 110; 110; 111; 112; 113; 114; 114; 120	103	7.5	
		Overall recovery (n = 82)	**104**	**8.8**	
cattle / cream	0.005	106; 108; 110; 113; 118	111*	4.2	0.005
	0.20	85; 91; 110	95	13.7	
		Overall recovery (n = 8)	**105**	**10.7**	
cattle / skim milk (whey)	0.005	103; 104; 110; 110; 110; 115	109	4.1	0.005
	0.05	98; 102; 104	101	3.0	
		Overall recovery (n = 9)	**106**	**5.0**	
cattle / muscle	0.01	88; 96; 112; 114	103	12.3	0.01
	0.25	111; 95; 99; 103	102	6.7	
		Overall recovery (n = 8)	**102**	**9.2**	
cattle / fat, mesenteric	0.01	88; 92; 114	98	14.3	0.01
	0.25	92; 92; 93; 93	93	0.6	
		Overall recovery (n = 7)	**95**	**9.1**	
cattle / fat, perirenal	0.01	64; 72; 108; 109	88	26.8**	0.01
	0.25	81; 71; 102; 106	85	26.3**	
		Overall recovery (n = 8)	**87**	**24.7**	
cattle / fat, subcutaneous	0.01	100; 107; 109; 112	107	4.8	0.01
	0.25	86; 92; 93; 96	92	4.6	
		Overall recovery (n = 8)	**99**	**9.3**	
cattle / kidney	0.01	91; 102; 105; 105	101	6.6	0.01
	0.25	97; 99; 100; 104	100	2.9	
		Overall recovery (n = 8)	**100**	**4.8**	
cattle / liver	0.01	89; 96; 99; 104	97	6.5	0.01
	0.25	104; 104; 106; 112	107	3.6	
		Overall recovery (n = 8)	**102**	**6.9**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CX99799, determined as BCS-CX99799 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8001
* These average recoveries are considered acceptable, because there were found no residues of BCS-CX99799 in the sample material cream
** These recoveries are considered acceptable, because they were not identified as outliers by a Grubbs outlier test with a level of significance of 95%.

Table 6.4.2- 8: Concurrent recovery data for free and conjugated BCS-DC20298

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / kidney	0.01	85; 105; 111; 117	105	13.3	0.01
	0.25	106; 111; 105; 106	107	2.5	
		Overall recovery (n = 8)	**106**	**8.9**	
cattle / liver	0.01	114; 81; 74; 111	95	21.5*	0.01
	0.20	98; 105; 104; 102	102	3.0	
		Overall recovery (n = 8)	**99**	**14.3**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-DC20298, determined as free and conjugated BCS-DC20298 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8001

* This RSD value is considered acceptable, because it is only slightly exceeding the range and because it is considered acceptable according to OECD guideline ENV/JM/MONO(2007)17.

Table 6.4.2- 9: Concurrent recovery data for free and conjugated BCS-CY24813

Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
cattle / kidney	0.01	96; 106; 83; 94	95	10.0	0.01
	0.25	98; 112; 99; 103	101	9.1	
		Overall recovery (n = 8)	**98**	**9.4**	
cattle / liver	0.01	94; 95; 96; 110	99	7.6	0.01
	0.25	96; 98; 109; 110	103	7.0	
		Overall recovery (n = 8)	**101**	**7.2**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CY24813, determined as free and conjugated BCS-CY24813 and calculated as BCS-CN88460
These recoveries were performed during the conduct of the study 17-8001

Table 6.4.2- 10: Residue levels in milk (mean of 3 cows)

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 cows (individual values) [a]					
		BCS-CN88460	BCS-DC20298	BCS-CY26497	BCS-CY24813	BCS-DC22055	BCS-GX99799
1X 0.05 mg/kg bw/d 1.61 mg/kg DM	Pre-dosing -19	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -12	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -5	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	4	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	7	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	9	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	11	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	14	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	16	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	18	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	21	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	23	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	29	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
3X 0.15 mg/kg bw/d 4.18 mg/kg DM	Pre-dosing -19	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -12	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -5	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	2	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	4	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	7	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	9	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	11	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	14	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	16	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	18	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	21	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	23	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	30	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 cows (individual values) [a]					
		BCS-CN88460	BCS-DC20298	BCS-CY26497	BCS-CY24813	BCS-DC22055	BCS-CX99799
10X 0.5 mg/kg bw/d 15.54 mg/kg DM	Pre-dosing -19	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -12	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -5	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	3	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	4	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	7	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	9	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	11	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	14	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	16	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	18	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	21	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	23	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	31	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 cows (individual values) [a]					
		BCS-CN88460	BCS-DC20298	BCS-CY26497	BCS-CY24813	BCS-DC22055	BCS-CX99799
30X 1.5 mg/kg bw/d 48.13 mg/kg DM	Pre-dosing -19	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -12	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -5	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	4	< 0.005 (< 0.005; < 0.005; < 0.005)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	7	**0.006** (0.006; 0.006; 0.007)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	9	**0.007** (0.008; 0.006; 0.008)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	11	**0.009** (0.008; 0.005; 0.013)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	14	**0.009** (0.010; 0.006; 0.011)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	16	**0.007** (0.007; 0.006; 0.009)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	18	**0.008** (0.008; 0.006; 0.009)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	21	**0.008** (0.008; 0.007; 0.008)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	23	**0.008** (0.008; 0.008; 0.008)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	31	**0.007** (0.007; 0.006; 0.009)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	32	**0.008** (0.008; 0.007; 0.009)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

Group Dose	Sampling day *	Residue levels of individual analytes (mg/kg) Mean of 3 cows (individual values) [a]					
		BCS-CN88460	BCS-DC20298	BCS-CY26497	BCS-CY24813	BCS-DC22055	BCS-CX99799
30XE Depuration group 1.5 mg/kg bw/d 47.13mg/kg DM	Pre-dosing -19	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -12	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	Pre-dosing -5	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	32	**0.008** (0.007; 0.009; 0.007)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	35 4d depuration	< 0.005 (<0.005; < 0.005; < 0.005)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	38 7d depuration	< 0.005*** (n.a.; < 0.005; <0.005)	< 0.005***	< 0.005***	< 0.005***	< 0.005***	< 0.005***
	45 14d depuration	< 0.005**** (n.a.; n.a.; <0.005)	< 0.005****	< 0.005****	< 0.005****	< 0.005****	< 0.005****

[a] – mean value of three animals calculated based on unrounded residue results. For the calculation of the mean residues, in case one or two individual values are <LOQ and the others < LOQ, it was deemed appropriate to consider residues <0.005 mg/kg as being equal to 0.005 mg/kg. This approach differs from what is reported in the study.

* overall study day

*** mean value of 2 individual residue results (animal H954 & H955)

**** Since only one cattle of dose group 30XE remained alive on overall study day 45 (H955), no mean values were calculated.

n.a. not applicable

All metabolite residues expressed in parent compound equivalents

LOQ = 0.005 mg/kg

Table 6.4.2- 11: Residue levels in milk and milk products

Animal No.	Sample no.	Milk sampling time (Day)**	Sample material	BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
H950 (30X)	0161E	31	milk	**0.007**	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	0160E	31	cream	**0.11**	**0.006**	< 0.005	< 0.005	< 0.005	< 0.005
	0162E	31	skim milk (whey)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
H951 (30X)	0164E	31	milk	**0.006**	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	0163E	31	cream	**0.11**	**0.006**	< 0.005	**0.005**	< 0.005	< 0.005
	0165E	31	skim milk (whey)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
H938 (30X)	0167E	31	milk	**0.009**	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
	0166E	31	cream	**0.15**	**0.009**	< 0.005	< 0.005	< 0.005	< 0.005
	0168E	31	skim milk (whey)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

LOQ = 0.005 mg/kg
* These results are the average of multiple measurements (single values see Appendix 10 of the study report).
** Overall study day

Table 6.4.2- 12: Residue levels in ruminant tissues

Group	Dose (mg/kg bw/d)	Dose (mg/kg d DM)	Animal No.	Sampling Time (Day) *	Residue levels (mg/kg)**							
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	Sum of BCS-DC20298 and M20 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	Sum of BCS-CY24813 and M19 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
Muscle												
1X	0.05	1.61	H941	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H942	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H943	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	NA	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
3X	0.15	4.18	H944	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H945	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H946	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
10X	0.5	15.54	H947	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H948	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H949	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30X	1.5	48.13	H950	31	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H951	31	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H938	31	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30XE depuration	1.5	47.13	H953	35	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H954	38	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H955	45	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01

Group	Dose (mg/kg bw/d)	Dose (mg/kg d DM)	Animal No.	Sampling Time (Day) *	Residue levels (mg/kg)**							
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	Sum of BCS-DC20298 and M20 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	Sum of BCS-CY24813 and M19 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
Kidney												
1X	0.05	1.61	H941	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H942	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H943	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
3X	0.15	4.18	H944	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H945	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H946	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
10X	0.5	15.54	H947	30	< 0.01	< 0.01	< 0.01	**0.024**	< 0.01	< 0.01	< 0.01	< 0.01
			H948	30	< 0.01	< 0.01	< 0.01	**0.021**	< 0.01	< 0.01	< 0.01	< 0.01
			H949	30	< 0.01	< 0.01	< 0.01	**0.021**	< 0.01	< 0.01	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	***0.022***	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	***0.021***	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
30X	1.5	48.13	H950	31	**0.011**	< 0.01	< 0.01	**0.063**	< 0.01	**0.016**	< 0.01	**0.020**
			H951	31	< 0.01	< 0.01	< 0.01	**0.074**	< 0.01	**0.015**	< 0.01	**0.023**
			H938	31	< 0.01	< 0.01	< 0.01	**0.050**	< 0.01	**0.016**	< 0.01	**0.014**
				mean	***0.01***	*< 0.01*	*< 0.01*	***0.062***	*< 0.01*	***0.016***	*< 0.01*	***0.019***
				median	*< 0.01*	*< 0.01*	*< 0.01*	***0.063***	*< 0.01*	***0.016***	*< 0.01*	***0.020***
30XE depuration	1.5	47.13	H953	35	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H954	38	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H955	45	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Group	Dose (mg/kg bw/d)	Dose (mg/kg d DM)	Animal No.	Sampling Time (Day) *	Residue levels (mg/kg)**							
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	Sum of BCS-DC20298 and M20 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	Sum of BCS-CY24813 and M19 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
Liver												
1X	0.05	1.61	H941	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H942	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H943	29	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
3X	0.15	4.18	H944	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H945	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.011	< 0.01	< 0.01
			H946	30	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*	*< 0.01*
10X	0.5	15.54	H947	30	< 0.01	< 0.01	**0.030**	< 0.01	< 0.01	**0.040**	< 0.01	< 0.01
			H948	30	**0.013**	< 0.01	**0.031**	< 0.01	< 0.01	**0.033**	< 0.01	< 0.01
			H949	30	< 0.01	< 0.01	**0.019**	< 0.01	< 0.01	**0.036**	< 0.01	< 0.01
				mean	***0.011***	*< 0.01*	***0.027***	*< 0.01*	*< 0.01*	***0.036***	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	***0.030***	*< 0.01*	*< 0.01*	***0.036***	*< 0.01*	*< 0.01*
30X	1.5	48.13	H950	31	**0.018**	< 0.01	**0.091**	**0.032**	< 0.01	**0.12**	< 0.01	**0.014**
			H951	31	**0.020**	< 0.01	**0.065**	**0.037**	< 0.01	**0.15**	< 0.01	**0.010**
			H938	31	< 0.01	< 0.01	**0.052**	**0.016**	< 0.01	**0.064**	< 0.01	< 0.01
				mean	***0.016***	*< 0.01*	***0.069***	***0.028***	*< 0.01*	***0.11***	*< 0.01*	***0.011***
				median	***0.018***	*< 0.01*	***0.065***	***0.032***	*< 0.01*	***0.12***	*< 0.01*	***0.010***
30XE depuration	1.5	47.13	H953	35	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H954	38	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
			H955	45	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Group	Dose (mg/kg bw/d)	Dose (mg/kg d DM)	Animal No.	Sampling Time (Day) *	Residue levels (mg/kg)**							
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	Sum of BCS-DC20298 and M20 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	Sum of BCS-CY24813 and M19 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
Fat, mesenteric												
1X	0.05	1.61	H941	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H942	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H943	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*NA*	*< 0.01*
3X	0.15	4.18	H944	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H945	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H946	30	**0.010**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	***0.01***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*NA*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*NA*	*< 0.01*
10X	0.5	15.54	H947	30	**0.037**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H948	30	**0.041**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H949	30	**0.020**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	***0.033***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	***0.037***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30X	1.5	48.13	H950	31	**0.085**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H951	31	**0.077**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H938	31	**0.068**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	***0.077***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	***0.077***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30XE depu-ration	1.5	47.13	H953	35	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H954	38	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H955	45	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01

Group	Dose (mg/kg bw/d)	Dose (mg/kg d DM)	Animal No.	Sampling Time (Day) *	Residue levels (mg/kg)**							
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	Sum of BCS-DC20298 and M20 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	Sum of BCS-CY24813 and M19 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
Fat, perirenal												
1X	0.05	1.61	H941	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H942	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H943	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
3X	0.15	4.18	H944	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H945	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H946	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
10X	0.5	15.54	H947	30	**0.039**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H948	30	**0.041**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H949	30	**0.021**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	***0.034***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	***0.039***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30X	1.5	48.13	H950	31	**0.080**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H951	31	**0.087**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H938	31	**0.075**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	***0.081***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	***0.080***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30XE depuration	1.5	47.13	H953	35	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H954	38	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H955	45	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01

Group	Dose (mg/kg bw/d)	Dose (mg/kg d DM)	Animal No.	Sampling Time (Day) *	Residue levels (mg/kg)**							
					BCS-CN88460 [mg/kg]	BCS-DC20298 [mg/kg]	Sum of BCS-DC20298 and M20 [mg/kg]	BCS-CY26497 [mg/kg]	BCS-CY24813 [mg/kg]	Sum of BCS-CY24813 and M19 [mg/kg]	BCS-DC22055 [mg/kg]	BCS-CX99799 [mg/kg]
Fat, subcutaneous												
1X	0.05	1.61	H941	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H942	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H943	29	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
3X	0.15	4.18	H944	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H945	30	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H946	30	< 0.01	0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	*< 0.01*	*0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
10X	0.5	15.54	H947	30	**0.032**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H948	30	**0.028**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H949	30	**0.013**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	***0.024***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	***0.028***	*0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30X	1.5	48.13	H950	31	**0.051**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H951	31	**0.057**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H938	31	**0.066**	0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
				mean	***0.058***	*< 0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
				median	***0.057***	*0.01*	*NA*	*< 0.01*	*< 0.01*	*NA*	*< 0.01*	*< 0.01*
30XE depuration	1.5	47.13	H953	35	**0.014**	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H954	38	< 0.01	< 0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01
			H955	45	< 0.01	0.01	NA	< 0.01	< 0.01	NA	< 0.01	< 0.01

NA Not analyzed
*overall study day
** All metabolite residues expressed in parent compound equivalents. Mean values are calculated based on the unrounded residue values and, in case one or two individual values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues <0.01 mg/kg as being equal to 0.01 mg/kg. This approach differs from what is reported in the study.
LOQ = 0.01 mg/kg

CA 6.4.3 Pigs

The maximum dietary burden for pigs remains <0.004 mg/kg bw/day. Besides, the metabolic pathways do not differ significantly in the rat as compared to ruminants (cf CA 6.2.4). Therefore, a feeding study in pigs is not required.

CA 6.4.4 Fish

No residue study in fish was conducted. Currently, no test method or Guidance document is available for conducting such study. 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

Report:	KCA 6.5.1/01; [redacted]; [redacted]; 2017; M-594825-01-1
Title:	Nature of the residues of [pyrazole-4-14C]BCS-CN88460 and [phenyl-UL-14C]BCS-CN88460 in processed commodities - High temperature hydrolysis
Report No.:	EnSa-16-0135
Document No.:	M-594825-01-1
Guideline(s):	OECD Guideline for the Testing of Chemicals 507 Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, adopted 2007-10-16 Regulation (EC) No 1107/2009 amended by Commission Regulation (EU) No 283/2013 (Europe) US EPA OCSPP not applicable
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The hydrolytic degradation behaviour of [pyrazole-4-^{14}C]BCS-CN88460 and [phenyl-UL-^{14}C]BCS-CN88460 under conditions representative for food processing operations was investigated. The following conditions were tested:

Pasteurisation:	90 °C at pH 4 for 20 min
Baking, brewing, boiling:	100 °C at pH 5 for 60 min
Sterilisation:	120 °C at pH 6 for 20 min

BCS-CN88460 was predominantly stable under all tested conditions. The identification rates were very high and in the range of 98.0% to 99.1%. Degradation products detected under the tested

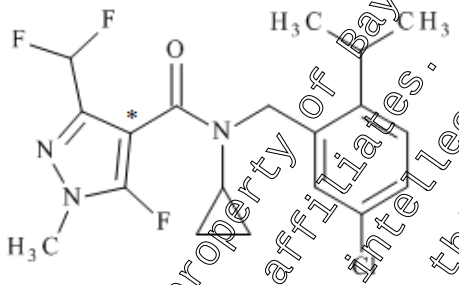
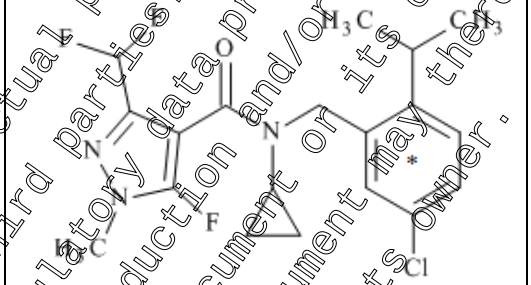
conditions representatively for food processing were very minor (≤ 0.5% and ≤ 0.005 mg/L) and were not further investigated.

All material balances were in the range of 104.2 to 110.5% demonstrating that no volatile degradation products were formed.

I. Materials and Methods

A. Materials

Table 6.5.1- 1: List of test materials

Chemical structure		
Radiolabel position	[pyrazole-4-^{14}C]	[phenyl-UL-^{14}C]
Radiochemical purity	99% (HPLC), >99% (TLC)	>98% (HPLC), >98% (TLC)
Chemical purity (%)	99% (HPLC)	> 99% (HPLC)
Specific activity	3.90 MBq/mg 234,000,000 dpm/mg	4.13 MBq/mg 247,800,000 dpm/mg

B. Study Design

Preparation of radiolabelled Stock and Test Solutions:

The [pyrazole-4-^{14}C] and [phenyl-UL-^{14}C] labelled test compound was each dissolved in acetonitrile. An aliquot of each solution was filled up to 10 mL with acetonitrile. An aliquot of the diluted solution (4 mL) was again further diluted with 6 mL acetonitrile yielding the respective stock solutions.

The targeted application rate in this study was approx. 1 mg test compound/L buffer solution. Therefore, both pyrazole- and phenyl-labelled test compounds were prepared as stock solution dissolved in acetonitrile and aliquots thereof containing the respective amount of test compound for the target concentration were added to a respective amount of buffer solution assuring for final test solutions with acetonitrile content < 1%.
After preparation of each final test solution, aliquots were taken for LS and HPLC analyses and the remaining aliquot (7 mL) of the test solution in the test vial was exposed to the respective processing condition.

Experimental conditions:

Based on different processing operations, tests for each test compound were carried out with sterilised, citrate buffered drinking water at three different pH levels and three different temperatures: pH 4 / 90°C, pH 5 / 100°C and pH 6 / 120°C. The treatment duration was 20 min, 60 min and 20 min for the three scenarios, respectively. The tests at 90°C and 100°C were carried out using a Reacti Therm heating/stirring module. The tests at 120°C were performed in an autoclave. The intended test periods listed in the table above do not include the time until reaching the test temperature or the ambient

temperature after test termination. The temperature was recorded in a separate vial filled with 7 mL buffer.

The vials of the test solutions were closed with a septum and crimp top and placed in a dry block heater or an autoclave. The vials were weighed before and after hydrolysis to correct for possible losses by evaporation of water.

Sampling:

After termination of the test, the test samples were cooled to room temperature as rapidly as possible and the pH values of the samples were measured at room temperature. The samples were transferred to a measuring cylinder and the test vials were washed with a defined amount of acetonitrile to release any possible remaining radioactivity in the test vial. The wash solution was then added to the samples and the solution was sonicated.

C. Analytical Procedures

The radioactivity content of each vessel was determined by LSC (three aliquots of each test solution, before and after the test). For HPLC profiling of the test solutions at test termination, a further aliquot of each test solution was mixed with a defined amount of emulgator to facilitate HPLC sample preparation and minimization of surface adhesion. The mixtures were sonicated and concentrated using a rotary evaporator. Defined amount of water was added and the mixture was sonicated for homogenisation. The volume was determined, aliquots were again taken for the determination of the radioactivity content by LS measurement to check for possible losses during HPLC sample preparation and aliquots were taken for HPLC profiling of the test solution at test time after hydrolysis. Recoveries for HPLC sample preparations determined for all test solutions ranged from 99.0% to 103.2% confirming that no radioactivity was lost during HPLC sample preparation.

Aliquots of all samples were analysed and quantified by HPLC with radiodetection by reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

Parent compound was identified in both pyrazole- and phenyl-labelled test solution pH 5 after processing by LC-MS and LC-MS/MS analysis and by HPLC co-chromatography with the non-radiolabelled reference compound. Furthermore, the assignment and identification of parent compound was achieved by comparison of HPLC metabolite profiles of the sample extracts of the current study with each other.

II. Results and Discussion

Material Balance

For each test, the concentration of the test compound was calculated from the radioactivity concentrations determined in the test solutions before and after incubation and a material balance was established for all tests. The amount of acetonitrile in the test solutions accounted for 0.7% for the tests with the pyrazole-label and for 0.9% for the tests with the phenyl-label.

Based on the results of the LSC measurements of the test solutions after hydrolysis, the concentration of the test compound applied was ~ 0.970 mg/L for both the tests with the pyrazole-labelled test compound and for the phenyl-labelled test compound. All material balances were in the range of 104.2% to 110.5% demonstrating that no radioactivity dissipated from the test systems. Detailed results for measured radioactivity balances are provided below.

Table 6.5.1- 2: Material Balance of Radioactivity in all Samples

Test item	Processing medium	Measurement pre-processing	Measurement post-processing	Recovery [%]
		mg/L		
[pyrazole-4-^{14}C] BCS-CN88460	pH 4	0.894	0.970	108.6
	pH 5	0.933	0.972	104.2
	pH 6	0.922	0.981	106.4
[phenyl-UL-^{14}C] BCS-CN88460	pH 4	0.930	1.027	110.5
	pH 5	0.928	0.987	106.3
	pH 6	0.928	0.972	104.8

The concentration in the test solutions at test termination were calculated using the following formula:

$$\text{Concentration after hydrolysis [mg/L]} = \frac{\text{Total dpm}_{\text{sample after hydrolysis}}}{\text{SA} * \text{Total Volume}_{\text{sample before hydrolysis}}} * 1000$$

Aliquots of all test solutions were anabysed by HPLC before and after hydrolysis. HPLC profiling of the test solutions of both labels showed that BCS-CN88460 was predominantly stable under the tested conditions representatively for food processing.

For all tests, almost complete recovery of the parent compound was observed (≥ 98.0%). Degradation products detected under the tested conditions representatively for food processing were very minor (≤0.5% and ≤0.005 mg/L) and were not further investigated (see tables below).

Identification rates of parent compound were very high and in the range of 98.0% to 99.1% of the radioactivity in the test solutions.

Table 6.5.1- 3: Radioactive residues of parent compound and hydrolysis products in the [pyrazole-4-^{14}C] BCS-CN88460 test solution under the different processing conditions

[pyrazole-4-^{14}C]BCS-CN88460	**Processing Conditions**					
Report name	**pH 4 / 90 °C / 20 min**		**pH 5 / 100 °C / 60 min**		**pH 6 / 120 °C / 20 min**	
	Area [%]	**mg/L**	**Area [%]**	**mg/L**	**Area [%]**	**mg/L**
parent compound BCS-CN88460	**99.10**	**0.962**	**98.66**	**0.959**	**99.05**	**0.971**
Total identified:	**99.10**	**0.962**	**98.66**	**0.959**	**99.05**	**0.971**
Total characterised:	0.90	0.009	1.35	0.013	0.95	0.009
Number of unknown peaks	*6*		*7*		*4*	
Largest unknown peak	*0.26*	*0.002*	*0.34*	*0.003*	*0.51*	*0.005*
Accountability:	**100.0**	**0.970**	**100.0**	**0.972**	**100.0**	**0.981**

Table 6.5.1- 4: Radioactive residues of parent compound and hydrolysis products in the [phenyl-UL-^{14}C] BCS-CN88460 test solution under the different processing conditions

[phenyl-UL-^{14}C]BCS-CN88460	Processing Conditions					
Report name	**pH 4 / 90 °C / 20 min**		**pH 5 / 100 °C / 60 min**		**pH 6 / 120 °C / 20 min**	
	Area [%]	**mg/L**	**Area [%]**	**mg/L**	**Area [%]**	**mg/L**
parent compound BCS-CN88460	**98.65**	**1.013**	**98.03**	**0.968**	**98.74**	**0.960**
Total identified:	**98.65**	**1.013**	**98.03**	**0.968**	**98.74**	**0.960**
Total characterised:	1.35	0.014	1.95	0.019	1.26	0.012
Number of unknown peaks	*6*		*11*		*7*	
Largest unknown peak	*0.33*	*0.003*	*0.32*	*0.009*	*0.42*	*0.004*
Accountability:	**100.0**	**1.027**	**100.0**	**0.987**	**100.0**	**0.972**

III.Conclusions

The hydrolytic degradation behaviour of [pyrazole 4-^{14}C]BCS-CN88460 and [phenyl-UL-^{14}C]BCS-CN88460 under conditions representative for food processing operations (pasteurization, baking, brewing, boiling and sterilization) was investigated.

BCS-CN88460 was predominantly stable under all tested conditions. The identification rates were very high. Degradation products detected under the tested conditions representatively for food processing were very minor (≤ 0.5% and ≤ 0.005 mg/L) and were not further investigated.

All material balances were in the range of 104.2 to 110.5% demonstrating that no volatile degradation products were formed.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

The distribution of residues of BCS-CN88460 between peel and pulp is not relevant for crops like cereals.

CA 6.5.3 Magnitude of residues in processed commodities

According to the data requirements of the EU Regulation (EC) No 1107/2009, studies investigating the magnitude of residues in processed commodities of barley are not required because:

- The residues in cereal grains are < 0.1 mg/kg (maximum 0.042 mg/kg for wheat grain)
- The contribution of barley grain or wheat grain to the theoretical maximum daily intake (TMDI) is ≤ 10 % of the ADI (actually maximum of 0.7% for wheat grain using the proposed MRL of 0.05 mg/kg) and the estimated daily intake is ≤ 10 % of the ARfD (actually maximum of 0.06% for wheat grain using the proposed MRL of 0.05 mg/kg) for any European consumer group diet

Nevertheless representative processing studies on barley and wheat were conducted with field samples collected from supervised residue trials conducted in Europe or Northern America and are summarized below.

Barley

Report:	KCA 6.5.3/01; [redacted]; [redacted]; 2017; M-579494-01-1
Title:	Determination of the residues of BCS-CN88460 in/on barley and the processed fractions (malt sprouts; brewer's malt; brewer's grain; hops draff; brewer's yeast; beer; pearl barley rub off and pearl barley) after spray application of BCS-CN88460 EC 050 in the field in the Netherlands and Spain
Report No.:	15-3407
Document No.:	M-579494-01-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) OECD Guideline for the Testing of Chemicals, Magnitude of the Pesticide Residues in Processed Commodities, (TG 508 published in October 2008) OECD Guidance document on magnitude of pesticide residues in processed commodities, ENV/JM/MONO(2008)23 US EPA OCSPP 860.1500, Crop Field Trial US EPA OCSPP 860.1520, Processed Food/Feed
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and Methods

The study included two supervised residue trials with barley, conducted in the field in southern Europe (Spain) and northern Europe (the Netherlands) in the 2015 season in order to determine the magnitude of the residues of BCS-CN88460 in/on barley grain and their processed fractions for the processing of beer (malt sprouts, brewer's malt, brewer's grain, hops draff, brewer's yeast and beer) and pearl barley (pearl barley rub off and pearl barley).

Field part

In the field trials BCS-CN88460 EC 050 was sprayed once at a nominal growth stage of BBCH 61 and at a nominal rate of 7.5 L /ha, corresponding to 375 g a.s./ha. The water rate was of 300-400 L/ha, reflecting local practice in the trial regions. The application was performed with an exaggerated dose rate (5X) to attempt to generate a commodity with quantifiable residues. All treatments were made at the scheduled rates. In the trial 15-3407-02 the application was done at growth stage BBCH 53 instead of BBCH 61. Nevertheless this deviation was considered acceptable since the timing between application and harvest is not expected to be significantly impacted.

Barley grain samples were sampled at BBCH 89, 91 and 58 days after the last treatment (DALT) in trials 15-3407-01 and 15-3407-02, respectively.

Barley grain samples for the processing of grain into beer consisted of at least 25 kg and samples intended for the processing of barley grain into pearl barley consisted of at least 5 kg sample material. The specimens for processing were sent after sampling to the processing test site at [redacted]) under ambient conditions.

Furthermore, barley grain samples of at least 1 kg were taken (sampling called “barley grain”) to determine the residues at harvest. They were stored deep-frozen within 24 hours after sampling, until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer AG – Crop Science Division, formerly Bayer CropScience AG, in [redacted] [redacted] am Rhein, Germany.

Finally, barley grain samples were taken in the field (samplings called “grain, stored” and also referenced as Raw Agricultural Commodity (RAC)) at the same time as the samples for processing,

stored and shipped under the same conditions (ambient temperature) as the samples for processing and deep frozen at ≤ 18°C at the very time when the processing started.

Processing procedures

The cleaning of barley grain samples took place at the processing test site at [redacted]. The malting process for the production of beer was performed under the responsibility of [redacted] at Versuchs- und Lehranstalt fuer [redacted] and the subsequent brewing process was conducted by [redacted]. The processing to pearl barley took place at Technische [redacted] under the responsibility of [redacted]

The processing simulates industrial practice at a laboratory scale. The processing of spring barley grain into the processed fractions (malt sprouts; brewer's malt; brewer's grain; hops draff; brewer's yeast; beer; pearl barley rub off and pearl barley) was performed simulating the common industrial processes.

Cleaning:

All field specimens for processing were cleaned which allows the separation of soil particles and other contaminations from the grain in a steady air flow.

Before processing start the corresponding fresh grain samples were deep-frozen, identified as **grain, stored (RAC)** samples and stored deep frozen at ≤-18ºC until analysis.

Malting (for the processing of beer):

Sieving

Before malting was started, the cleaned grain samples were sieved (sieve mesh 2.5 mm). Field samples for processing into beer were then shipped by car at ambient temperature to Versuchs- und Lehranstalt fuer [redacted]).

Steeping

The steeping process was conducted as a combined wet and dry steeping. Sieved barley grain was transferred in a special steeping vessel. During steeping water is supplied to the interior of the kernel. As a result the enzymes become active and germination begins. Water uptake depends on the steeping time, steeping temperature, kernel size, barley variety and harvest year.

Germination

The processes during germination can be divided into growth processes, enzyme formation and metabolic changes. Towards the end of steeping the rootlets break through the base of the corn and become visible. Activation of enzymes and formation of new enzymes are essential processes during germination (starch degrading enzymes, cytolytic enzymes, protein degrading enzymes and phosphoric acid splitting enzymes). For proper performance of germination it is necessary to control the duration of germination, the mean temperature of wet air and the relative humidity of the air around the kernels. During the intensive respiration the steeped good was turned over continuously.

Kiln-drying

After germination, the life processes are terminated by kilning. During kilning the water content of green malt is lowered down to < 10%, germination and modification are stopped; colour and flavour compounds are formed. The malt becomes stable and storable. Kiln-drying was conducted in a dry chamber. After kiln-drying the germs (= malt sprouts) were removed mechanically by a trimmer. **Brewer's malt** and **malt sprouts** were sampled immediately after end of malting and were transported

to [redacted]. Until brewing the malt was stored at room temperature at [redacted]

Brewing (processing of malt to beer):

The brewer's malt specimens for brewing processing were shipped by car at ambient temperature to the processing location at [redacted]).

Mashing

Mashing is the homogeneous mixing of ground malt and water according to a definite temperature time regime (mash program). The main purpose of mashing (the dissolution and enzymatic conversion of ingredients) is to form as much extract and as good an extract as possible. Before mashing, the brewer's malt was dry milled in a special malt mill. The crushed malt was mixed with brew water. To produce "Pilsener beer", mashing was conducted in a heatable tun where the mash was heated up to 76°C.

Lautering: Wort extraction and separation

After mashing, the wort was separated from the insoluble malt components (brewer's grain). The extract remaining in the brewer's grain was extracted by washing with hot water (first filter runnings). The wort separation was done using a refining vat. After separation, the **brewer's grain** was sampled and immediately deep-frozen.

Wort boiling and conditioning

After addition of hop pellets, the separated wort was boiled (about 90 min at normal pressure). This process deactivates the malt enzymes, sterilizes the wort, extracts and isomerises the essential components of the hops, precipitates high molecular proteins (called „Bruch") and expels unwanted aromatic substances.

After boiling, the flocs (hops draff) were separated in a whirlpool causing the sludge to deposit on the bottom in the shape of a cone. For cooling and ventilating the wort an intra-plant circulation was used. By adding oxygen (intra-plant circulation) the conditions for the start of the fermentation were prepared. **Hops draff** was sampled and immediately deep frozen.

Fermentation and maturation

The pure culture yeast ferments sugar of the wort to alcohol and CO_2, but in the course of the yeast metabolism also unwanted by-products are formed (diacetyl, higher alcohols and others). In the pilot plant the classical primary fermentation (low fermentation) was carried out in bottom fermentation containers. The fermentation temperature was 9 °C. Fermentation heat was dissipated by means of room ventilation.

The duration of main fermentation depends on temperature, on starting extract concentration of the finished wort, on the ratio of non-fermentable sugars to the extract, on the final attenuation and on the yeast cell number. As soon as the extract content of the fermented young beer was 2 % higher than the final attenuation, the storing time began. Before maturation the young beer was cooled down.

During the main fermentation the yeast deposits on the tank bottom and was sampled as **brewer's yeast** and immediately deep-frozen.

At the beginning of maturation the young beer was stored at room temperature (warm maturation to break down the diacetyl) in casks. Then the young beer was stored under pressure (approx. 0.7 – 2.1 bar) at 2 °C (cold maturation) for approx. 4 weeks.

In this time the remaining extract was fermented. Unwanted flavour and odorous substances were decomposed or expelled. Sludge particles and yeast settled at the bottom.

The rack beer was filtered using a special filter combination. During filtration all organisms harming the beer (bacteria and yeast) were removed and sludge particles were separated.

The final product **beer** was sampled and immediately deep-frozen.

The processed products (brewer's grain, hops draff, brewer´s yeast and beer) were kept in frozen stage (at or below -18 °C within 24 h after end of sampling) and shipped under deep-frozen conditions from fermtec GmbH to [redacted] and stored deep-frozen at <-18°C until analysis.

Pearl barley production:

The processing part for the preparation of pearl barley was conducted at TU [redacted]; [redacted], D-[redacted]. The specimens of cleaned barley grain, for processing into pearl barley, were shipped by car at ambient temperature from [redacted] to the processing location TU [redacted].

Conditioning

Before beginning of pearl barley production, an optimal moisture content of barley grain of approx. 14% was achieved. In order to obtain acceptable milling results a moisture content of up to 16 % was possible. Therefore the grain was not dried or damped because the optimal moisture content was already achieved.

Hulling

The corresponding samples were hulled using a vertical hulling machine. Each sample was hulled until the stipulated abrasion for pearl barley (30 - 35%) was reached. The degree of abrasion (pearling dust/bran and flour) was determined by the proportion of pearl barley with respect to the total portion of cleaned grain used for hulling process. **Peard barley** and **pearl barley rub off** were sampled.

The processed products (pearl barley, pearl barley rub off) were shipped at ambient temperature to [redacted] and stored deep frozen at -18°C until analysis.

The processes are illustrated in Diagram 6.5.3- 1 to Diagram 6.5.3- 3.

During processing of barley grain, laboratory samples of the various processing fractions taken at specific processing steps were immediately deep frozen and stored deep frozen at the processing test site until dispatch to PVTL. Upon reception at PVTL, samples of grain and processed commodities were stored in a freezer at -18°C or below until preparation of the examination samples. For the preparation of examination samples, the samples of grain and processed commodities were shredded and homogenized with dry ice in a cutter and stored at -18°C or below until analysis.

Residue analysis

The samples were analysed for the parent compound using analytical method 01475 ([redacted]; 2016; M-558986-01-1; referenced in MCA Section 4 under Point 4.1.2) which was validated prior to the residue analysis of the samples. Additional validation recoveries were conducted for barley (grain) and barley (beer and brewer's yeast) in the studies 15-2066 and 15-3407, respectively. The samples of barley (grain), barley (brewer's grain), barley (brewer's malt), barley (brewer's yeast), barley (grain stored), barley (hops draff), barley (malt sprouts), barley (pearl) and barley (pearl rub off) were analyzed according to the procedure described in the method for dry matrices (with a soaking step with water before extraction) and the barley (beer) samples were prepared according to the procedure for higher-water containing commodities (no soaking step before extraction). The LOQ was 0.01 mg/kg for parent for all sample materials.

Diagram 6.5.3- 1: Industrial processing of grain to malt

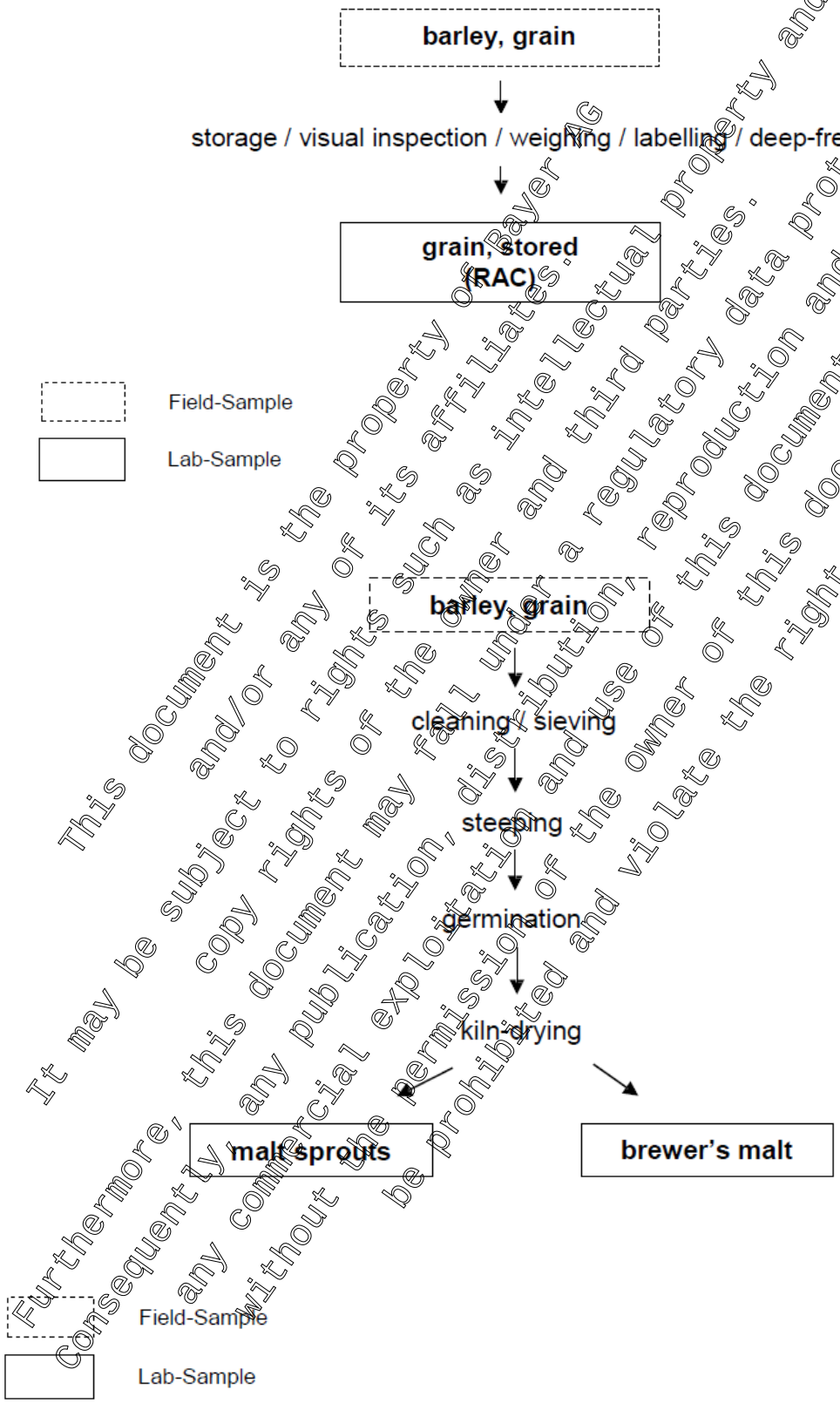


Diagram 6.5.3- 2: Industrial processing of malt to beer

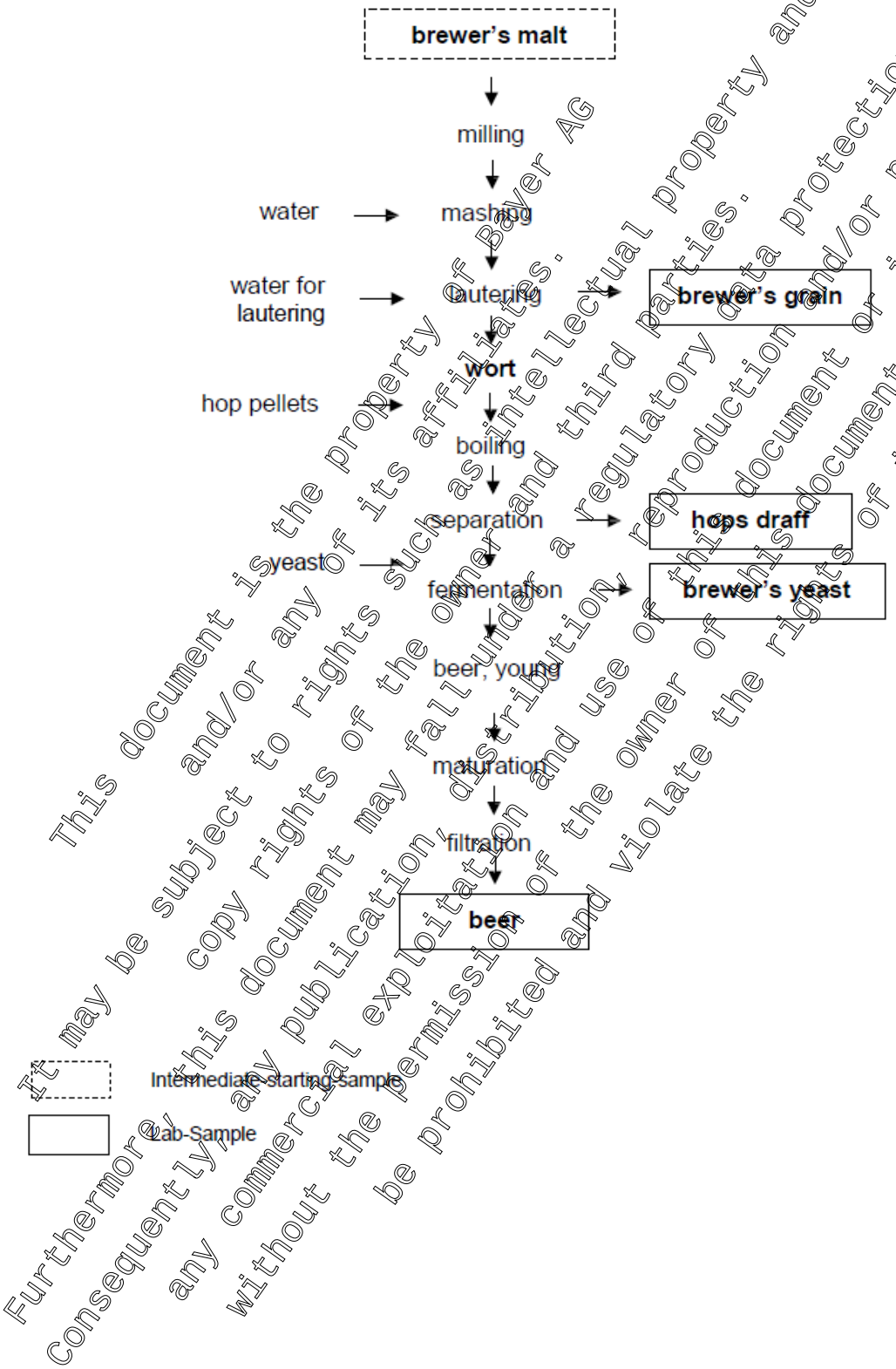
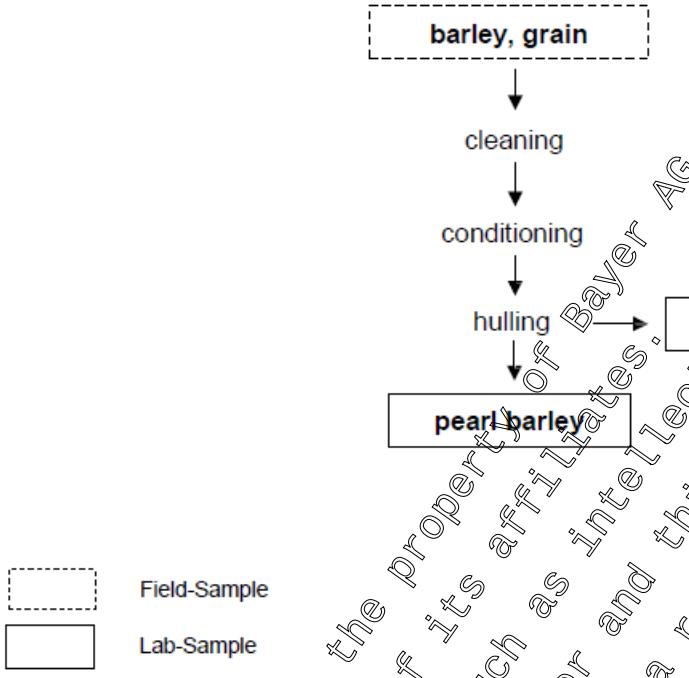


Diagram 6.5.3- 3: Industrial processing of spring barley to pearl barley



II. Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The sample materials barley brewer's grain, brewer's malt grain stored, malt sprouts, barley pearl and barley pearl rub off are considered equivalents to barley grain sample and the sample material barley hops draff is considered equivalent to sample barley brewer's yeast.

Details on concurrent recovery data are shown in Table 6.5.3- 1. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

The levels of residues of BCS-CN88460 in the treated samples are summarized in Table 6.5.3- 2. No residues above the LOQ were found in the control samples. The results were not corrected for concurrent recoveries.

Residues of BCS-CN88460 in barley grain at harvest were found at 0.012 mg/kg in the first trial (the Netherlands) but < 0.01 mg/kg in the second trial (Spain).

The mean residues levels in the stored, barley grain (RAC for the calculation of the processing factors) were 0.015 mg/kg and < 0.01 mg/kg in the first trial (the Netherlands) and second trial (Spain), respectively.

The levels of residue of BCS-CN88460 were < 0.01 mg/kg in all processed fractions except pearl barley rub off (0.032 mg/kg in the Dutch trial and 0.017 mg/kg in the Spanish trial).

The processing factors were calculated based on the residue levels in the treated processed fractions and the mean residue level of the rounded individual residues of the two RAC specimens for the corresponding processing procedure ("grain stored").When residues in the RAC and in the processed

fractions were both <0.01 mg/kg a processing factor could not be calculated. The proposed processing factors are summarised below in Table 6.5.3- 3.

The analyses were done after a maximum frozen storage period of 396 days (grain) and 397 days (processed commodities). The time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours.

III. Conclusions

Two residue trials were conducted in northern and southern Europe in 2015. Barley was treated at a growth stage of BBCH 61 (53 in one trial) with an exaggerated dose rate (5X; 375 g a.s./ha) to attempt to generate a commodity with quantifiable residues. All applications were at the required rates.

Barley grain was processed in order to obtain beer and pearl barley. The samples (RAC and processed fractions) were analysed for the residues of BCS-CN88460 parent compound.

The study was conducted according to GLP.

The results of the study clearly indicate that residues of BCS-CN88460 are diluted by the brewing processing.

When barley grain are processed into pearl barley, residues of BCS-CN88460 remain to a large extent in pearl barley rub-off and can be removed from barley grain by hulling, resulting in lower residues in the end product, pearl barley.

Table 6.5.3- 1: Concurrent recovery data for BCS-CN88460 in barley and barley matrices

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
15-3407	Beer	4	0.01	81; 92; 93; 98	81	98	91	7.9
		3	0.1	88; 102; 105	88	105	98	9.2
		7	**Overall**	-	**81**	**105**	**94**	**8.8**
	Grain[1] Brewer's grain[2] Grain stored [3] Brewer's malt[4] Malt sprouts[5] Pearl[6] Pearl rub off[7]	8	0.01	98[2]; 98[4]; 95[3]; 102[1]; 102[1]; 90[5]; 110[7]; 117[6]	90	117	102	8.4
		2	0.1	113[1]; 101[1]	101	113	107	-
		10	**Overall**		**90**	**117**	**103**	**8.2**
	Brewer's yeast [8] Hops draff[9]	5	0.01	112[8]; 104[8]; 106[8]; 106[8]; 97[9]	97	112	105	5.1
		3	0.1	101[8]; 102[8]; 97[8]	97	102	100	2.6
		8	**Overall**	-	**97**	**112**	**103**	**4.9**

RSD: Relative standard deviation
Indexes at the values of recoveries correspond to the indexes at the commodities (given in the column – "portion analysed")

Remark: sample material barley brewer's malt; brewer's grain; grain stored, malt sprouts, pearl barley; and pearl barley rub-off are considered equivalents to barley grain and sample material hops draff is considered equivalent to brewer's yeast.

Table 6.5.3- 2: Detailed results of the barley processing studies

Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Analyte 1		
	(a)	(b)				(c)			BCS-CN88460	(d)	(e)
15-3407-01 15-3407-01-T Netherlands [redacted] Europe, North F 2015	Barley Triple; summer	1) 23.04.2015 2) 29.06.2015 - 06.07.2015 3) 15.08.2015 - 01.09.2015	375	400	93.8	29.06.2015/1	61	Grain *	0.012	61	(f) 15-3407 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and in study 15-3407 (l) grain: 303 days grain, stored: 299 days malt sprouts: 161 days brewer's malt: 161 days brewer's grain: 151 days hops draff: 161 days brewer's yeast: 152 days beer: 115 days pearl barley rub off: 297 days pearl barley: 297 days
								Beer processing			
								Grain, stored **	0.018 0.012 **mean: 0.015**	61	
								Malt sprouts	<0.01	61	
								Brewer's malt	<0.01	61	
								Brewer's grain	<0.01	61	
								Hops draff	<0.01	61	
								Brewer's yeast	<0.01	61	
								Beer	<0.01	61	
								Pearl barley processing			
								Grain, stored **	0.016 0.013 **mean: 0.015**	61	
								Pearl barley rub off	0.032	61	
								Pearl barley	<0.01	61	

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study refererence
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max): Grain: between deep-freezing (in the field) and last extraction date
  Grain, stored: between deep-freezing (at start of the processing) and date of last extraction
  Processed commodities: between their deep-freezing and date of last extraction
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg)	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Analyte 1		
	(a)	(b)				(c)			BCS-CN88460 (d)		(e)
15-3407-02 15-3407-02-T Spain [redacted] Europe, South F 2015	Barley Traveler; Malting barley	1) 18.12.2014 2) 07.04.2015 - 14.04.2015 3) 25.05.2015 - 25.06.2015	375	300	125	31.03.2015/0	53	Grain *	<0.01	58	(e) 15-3407 (g) EC, 250 g/L (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and in study 15-3407 (l) grain: 396 days grain, stored: 321 days malt sprouts: 161 days brewer's malt: 161 days brewer's grain: 149 days hops draff: 159 days brewer's yeast: 151 days beer: 114 days pearl barley rub off: 297 days pearl barley: 297 days
								Beer processing			
								Grain, stored **	<0.01 <0.01	58	
								Malt sprouts	<0.01	58	
								Brewer's malt	<0.01	58	
								Brewer's grain	<0.01	58	
								Hops draff	<0.01	58	
								Brewer's yeast	<0.01	58	
								Beer	<0.01	58	
								Pearl barley processing			
								Grain, stored **	<0.01 <0.01	58	
								Pearl barley rub off	0.017	58	
								Pearl barley	<0.01	58	

* “Grain” are samples taken in the field, deep-frozen within 24 hours and stored at ≤ -18°C until analysis.
** “Grain, stored” are samples taken in the field at the same time as the samples for processing, stored and shipped at ambient temperature, under the same conditions as the samples for processing and deep-frozen at ≤ -18°C at the very time the processing started. This is the RAC reference for the processing factor calculations.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max): Grain: between deep-freezing (in the field) and last extraction date; Grain, stored: between deep-freezing (at start of the processing) and date of last extraction; Processed commodities: between their deep-freezing and date of last extraction
- * prior to last treatment
- ** residue in control
- # no data available

Table 6.5.3- 3: Proposed processing factors for barley
Tested GAP: 1 x 375 g as/ha at BBCH 61 or 53

Crop, Processed commodity	Number of trials	Trial number	Residues (mg/kg)	Individual processing factors (a)	Proposed processing factor PF
Barley, grain (RAC)	*2*	*15-3407-01* *15-3407-02*	*0.015 (61 days after treatment)* *<0.01 (58 days after treatment)*	-	-
Barley, malt sprouts	2	15-3407-01 15-3407-02	<0.01 <0.01	< 0.67 n.c.	< 0.67
Barley, brewer's malt	2	15-3407-01 15-3407-02	<0.01 <0.01	< 0.67 n.c.	< 0.67
Barley, brewer's grain	2	15-3407-01 15-3407-02	<0.01 <0.01	0.67 n.c.	0.67
Barley, hops draff	2	15-3407-01 15-3407-02	<0.01 <0.01	< 0.67 n.c.	< 0.67
Barley, brewer's yeast	2	15-3407-01 15-3407-02	<0.01 <0.01	< 0.67 n.c.	< 0.67
Barley, beer	2	15-3407-01 15-3407-02	<0.01 <0.01	< 0.67 n.c.	**< 0.67**
Barley, pearl barley rub off	2	15-3407-01 15-3407-02	0.032 0.017	2.1 > 1.7	2.1
Barley, pearl barley	2	15-3407-01 15-3407-02	<0.01 <0.01	< 0.67 n.c.	**< 0.67**

(a): Processing factors (PF) = residues in processed commodities / residues (mean) in the RAC collected at the processing lab ("grain stored")

n.c .: No processing factor can be calculated (residues <LOQ in RAC and in processed commodity)

< value: in case the mean residue level in the RAC is ≥ LOQ but residues in the processed commodity are < LOQ, the processing factor is calculated as to be below the worst case value calculated with the LOQ of the processed commodity

> value: in case the mean residue level in the RAC is < LOQ but residues in the processed commodity are ≥ LOQ, the processing factor is calculated as to be above the value calculated with the LOQ of the RAC

Wheat

Report:	KCA 6.5.3/02; [redacted], A. M.; 2017; M-600505-02-1
Title:	BCS-CN88460: Magnitude of residues in/on wheat processed fractions following treatment with BCS-CN88460 EC50
Report No.:	RALNN137
Document No.:	M-600505-02-1
Guideline(s):	PMRA DACO 7.4.1, Supervised Residue Trial Study PMRA DACO 7.4.5, Processed Food/Feed OECD Test Guideline 509: Crop Field Trial
Guideline deviation(s):	None
GLP/GEP:	**yes**

I. Materials and Methods

The study included two supervised residue trials with wheat, conducted in the field in Canada in the 2015 season in order to measure the potential concentration of BCS-CN88460 residues in the wheat processed products of grain, aspirated grain, middlings, germ, flour, shorts, bran, wheat white bread, whole meal flour, whole meal, bread, gluten, starch, fresh pasta, cooked pasta, cooking water, dry pasta, dried and cooked pasta.

Field part

In these two trials (C1101-15PA and C1102-15PA) one untreated control plot (UTC) was used for the generation of the control samples (untreated samples).

The study design included two treated plots for each trial. The application rate in the TRTD1 plot was made at 3X the maximum seasonal label use rate. The application rate in the TRTD2 plot was made at 5X the maximum seasonal label use rate. Only the treated wheat from the TRTD2 plot was harvested, the TRTD1 plot was not used, and no further data were reported from this plot.

In each field trial, the treated plot TRTD2 was sprayed once at BBCH 69 (end of flowering) with BCS-CN88460 EC50 at an application rate of 375 g a.s./ha using a spray volume of 120-122 L/ha. The applications were made using ground-based equipment with no adjuvant. The application was performed with an exaggerated dose rate (5X) to attempt to generate a commodity with quantifiable residues.

A single composite sample of wheat grain was harvested from the TRTD2 treated plots at commercial maturity (pre-harvest intervals (PHIs) were 69 and 67 days for C1101-15PA and C1102-15PA respectively). A single composite sample of wheat grain was harvested from the control plot on the same day the sample was harvested from the treated plot.

One control and one treated bulk wheat grain sample weighing at least 300 kg were harvested at maturity following commercial agronomic practices (threshed using a plot combine). The bulk wheat grain samples were placed into labelled (study number and sample number) containers and placed in cool (ambient) storage until shipment, via ambient transport, to the processing facility. Upon receipt at the processing facility, the samples were transferred to frozen storage, and remained frozen until processing (maximum bulk sample ambient temperature interval was 33 days).

Triplicate samples of wheat grain, weighing a minimum of 1 kg, were collected from the UTC and TRTD2 plots at the same time the bulk wheat grain samples were harvested (samples called “grain collected at the field site”). These samples were frozen within four hours after collection, and shipped frozen, via ACDS freezer truck, directly to the laboratory. Upon arrival at Bayer CropScience RTP, all samples were immediately transferred to frozen storage (average temperature -21°C).

Processing procedures

Processing took place at the processing site at GLP Technologies, 22723 State Highway 6 South Navasota, TX 77868 USA.

Samples were handled and processed in a manner that minimizes the possibility of contamination. Trials were processed independently. Control samples were processed prior to treated samples.

After removing bulk wheat grain samples for aspirated grain fraction generation and processing from freezer storage, representative unprocessed wheat (RAC) samples were collected in triplicate and placed into frozen storage (samples called "grain" or "grain collected at the processing site" in Table 6.5.3-5).

Commercially representative aspirated grain and processed food/feed fractions were produced as follows.

Generation of Aspirated Grain Fraction (AGF)

Moisture content of the samples was determined with an electronic moisture analyzer. If the moisture content was greater than 13.0%, a sample was dried at 110-135°F (43.3 - 57.2 ºC) in a Steelman Industries oven until the moisture content was 10.0-13.0%. Samples from trial C1102-15PA required drying.

To generate aspirated grain fractions, each sample was placed in a dust generation room containing a holding bin, two bucket conveyors, and a screw conveyor. As the sample was moved in the system, aspiration was used to remove light impurities (grain dust). Each sample was moved for 120 minutes. Light impurities were classified using the following sieves: 2360 µm (8 mesh); 2000 µm (10 mesh); 1180 µm (16 mesh); 850 µm (20 mesh); and 425 µm (40 mesh). After classification of each sample, the material through the 2360 µm sieve was recombined according to Study Director's instructions to produce one **aspirated grain fraction (AGF)**. A representative sample was removed and the ash content was determined.

Wheat Milling

Cleaning

Following AGF generation, samples were cleaned by aspiration and screening. Light impurities were removed using a Kice aspirator. After aspiration, samples were screened in an Enhanced 2 screen cleaner to separate large and small foreign particles (screenings) from the cleaned grain.

Germ Production

Cleaned wheat was moisture adjusted (tempered) to 16% by adding reverse osmosis (R/O) water to the wheat and mixing. After tempering for 1-1.5 hours, the wheat was passed through the Glen Mills disc mill. Ground material was sifted with a Sweco Dynascreen equipped with a 8-, 14-, and 30-mesh sieve. Material on top of the 30 mesh sieve was aspirated to remove bran from the germ fraction. Germ (with endosperm) was passed through the reduction side of the Chopin CD-1 mill. Germ and reduced endosperm were separated using the Great Western sifter equipped with the following screens: 18, 20, 24, and 28 mesh. Germ material was aspirated again to remove additional bran and milled/sieved to remove additional endosperm. A 0.5-1.0% recovery of germ was expected. **Germ** fractions were collected and placed into frozen storage.

Flour Production

Cleaned wheat was moisture conditioned (tempered) depending on the physical property of the wheat. The physical property of the grain kernel varies depending on whether the grain has a floury or vitreous kernel. In a floury kernel, a cross-section of the grain reveals a grainy soft white structure. The cross-section of a vitreous grain is hard and amber colored. There are grains with an intermediate structure (a floury and vitreous part). All samples were determined to be of intermediate structure and were moisture adjusted and tempered accordingly:

- floury grain wheat: 16.5% moisture adjustment with a 24 hour +/-30 minute resting period before milling
- vitreous grain wheat: 17.5% moisture adjustment with a 48 hour +/-30 minute resting period before milling
- intermediate structure: 17.5% moisture adjustment with a 24~hour +/- 30 minute resting period before milling

Tempered wheat was fed through the spout on the break side of a Chopin mill. Breaking of the wheat was accomplished by three break rolls. After passing through the break rolls, the material was fed onto the break sifter screen. The screen consists of two sizes. The screens are numbers 120 (140 µm) and 25 (800 µm). Material exiting the break rolls passed over the 120 first. Material passing through the 120 screen is "Break Flour." Material not passing through was conveyed over the 25 screen. Material passing through the 25 screen is middlings. Material not passing through was conveyed to the end of the sifter. Material exiting the end is Bran (Coarse). After breaking, it is necessary to determine if more than one reduction will be required. If the "Middling" fraction is 48% or less of the starting weight, then one reduction is required. If more than 48% is obtained, two reductions are required. Samples from both trials required one reduction. Requested **middling** fractions were collected and placed into frozen storage.

Remaining middlings were poured into the feed hopper of the reduction system. Reduction is achieved through two reduction rolls. After passing through the reduction rolls, the material was fed onto a number 100 (160 µm) reduction sifter screen. Material passing through the screen is reduction flour. Material not passing through and conveyed to the end of the sifter is shorts. Representative amounts of break and reduction flours were mixed to produce standard mill run (**white flour**). Requested flour fractions were collected and placed into frozen storage.

Remaining standard mill run flour was used in the production of white bread. Remaining break and reduction flour was used in the production of starch, gluten, and pasta.

Bran exiting the break sieve is placed in the reduction side of the mill, but is not reduced with the rollers. The coarse bran is conveyed by beater bars over a number 140 screen (128 µm). Material passing through the screen is "Shorts" and is added to "Shorts" from the reduction mill. Material passing over the screen and exiting the end is "Bran". Requested **bran** and **shorts** fractions were collected and placed into frozen storage.

Processing into White Bread

Standard mill run flour, sugar, dry milk, salt, margarine, water and dry yeast were placed in a Sunbeam bread machine. The machine was set for type bread (Basic), 2 lb (0.91 kg), loaf size and medium color. The machine automatically mixed the ingredients, let the dough rise and baked the bread. After cooling, requested **white bread** fractions were collected and placed into frozen storage.

Processing into Whole Meal Flour and Whole Meal Bread

Cleaned wheat was ground in an Alpine pin mill. Ground material was whole meal flour. Requested "**Whole Meal Flour**" fractions were collected and placed into frozen storage. Remaining whole meal flour was used to produce "Whole Meal Bread".

Whole meal flour, brown sugar, salt, margarine, water and dry yeast were placed in a Sunbeam bread machine. The machine was set for type 3 bread (Whole Wheat), 2 lb (0.91 kg) loaf size and medium color. The machine automatically mixed the ingredients, let the dough rise and baked the bread. After cooling **whole meal bread** fractions were collected and placed into frozen storage.

Wheat Gluten and Starch Production

For vital wheat gluten and starch, break flour was mixed with water at a rate of 0.4-0.8 pounds (0.18 – 0.6 kg) of water to one pound of flour (0.45 kg). The dough was allowed to rest for two hours. After resting, the dough was kneaded as water washed away the starch, leaving the gluten. Product was water washed until water ran clear, indicating all starch was removed leaving gluten. Starch was

separated from the water using centrifugation and dried in a dehydrator or Steelman Industries oven at 130-160°F (54.4 – 71.1ºC) until the moisture content was less than 15%. Gluten was dried with a Blaw-Knox steam heated drum dryer. Temperature is not monitored during drying. After drying, requested **starch** and **gluten** fractions were collected and placed into frozen storage.

Pasta (Noodle) Production

Equal parts of break and reduction flours were mixed with R/O water and salt to form a dough. This dough was kneaded and allowed to rest for 20-40 minutes. Dough was fed into a Lello pasta machine to produce a fresh Asian noodle. A portion of the **fresh noodles** were collected and placed into frozen storage. Requested fresh cooked noodles were produced by placing fresh noodles into boiling water (10:1 ratio water to noodles) for 1-4 minutes. **Cooked noodles** were cooled, collected and placed into frozen storage. Requested **cooking water** fractions were also collected and placed into frozen storage.

A portion of the fresh noodles were dried for eight hours at 75-95°F (23.9 – 35.0 ºC). Requested **dried noodle** fractions were collected and placed into frozen storage. Remaining dried noodles were placed into boiling water (10:1 ratio) for 1-4 minutes. After cooling, requested **dried cooked noodles** were collected and placed into frozen storage.

The processes are illustrated in Diagram 6.5.3- 4.

Wheat aspirated grain fractions, and the wheat processed commodities were stored deep-frozen ≤ 12°C) until shipment to Bayer CropScience, [redacted], NC. Upon arrival at Bayer CropScience [redacted], all samples, were immediately transferred to frozen storage (average temperature -21°C). The wheat grain RAC, bran, pasta (fresh, dry, cooked, and dried and cooked) white bread, and whole meal bread were homogenized with dry ice using a Robot Coupe (Model RSI2Y1 or RSI30B); wheat flour, whole meal flour, germ, middlings, shorts, gluten, starch, aspirated grain fractions, and cooking water were considered homogenous. All samples were returned to frozen storage immediately following homogenization, and the samples remained frozen at all times except during subsampling for analysis.

Residue analysis

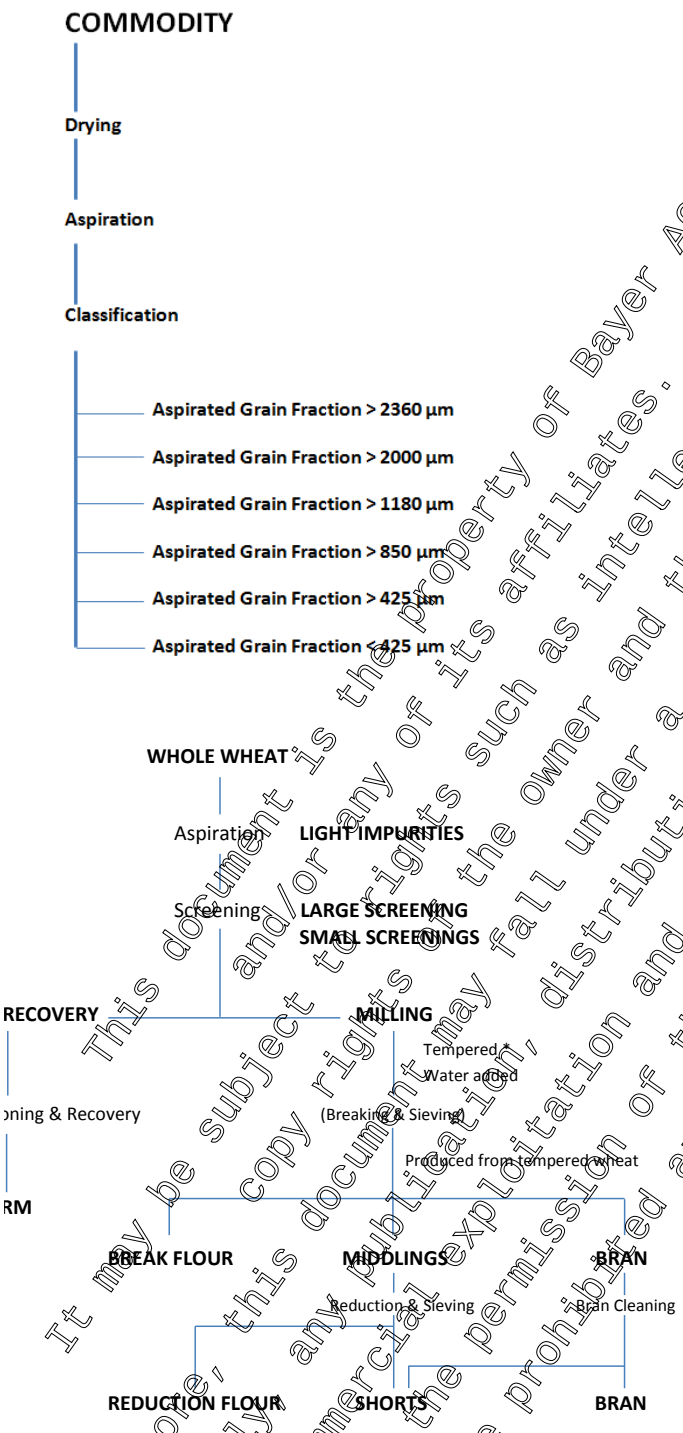
The samples were analysed for the parent compound using analytical method LN-002-P16-01 ([redacted]; 2017; M-606616-01-1; referenced in MCA Section 4 under Point 4.1.2). The LOQ was 0.01 mg/kg for parent for all sample materials.

BCS-CN88460 residue was extracted from wheat grain, aspirated grain fractions, and all commodity samples except cooking water by blending an aliquot of the homogenized sample in acetonitrile/water (4:1, v/v). The extract was amended with an isotopic internal standard (IS) mixture, and an aliquot was further diluted and analyzed by high performance liquid chromatography/triple stage mass spectrometry (LC/MS/MS).

BCS-CN88460 residue was extracted from cooking water samples by diluting an aliquot with acetonitrile/water (4:1, v/v) and shaking. The extract was amended with an isotopic internal standard (IS) mixture, and an aliquot was further diluted and analyzed as noted above.

Method performance was evaluated during method validation and by use of concurrent recovery samples in the report RALNN137 ([redacted], A. M.; 2017; M-600505-02-1; KCA 6.5.3) by fortifying wheat grain and the wheat processed commodities at 0.010 ppm and at 0.10 ppm. The method was validated in wheat aspirated grain fractions at 0.010 ppm and at 2.50 ppm.

Diagram 6.5.3- 4: Industrial processing of wheat



Reduction and Break Flours were combined to produce White Flour. White Bread was prepared from white flour.
* Additional wheat tempered with water was milled. From this batch break flour and middlings were produced. All middlings were reduced to produce reduction flour. The additional break and reduction flours were used in the production of starch, gluten and pasta (fresh, fresh cooked, dried, and dried cooked).
Cleaned wheat was ground into Whole Meal Flour. Whole Meal Bread was prepared from whole meal flour.
Break flour was used to produce Gluten. Starch was dried and collected during gluten production.
Break and reduction flour were used to produce Fresh Noodles; Cooked pasta (Noodles) were prepared from fresh noodles. Fresh Noodles were dried to produce Dried Noodles. Dried noodles were used to prepare Dried Cooked Noodles.

II. Findings

The method for determining BCS-CN88460 residue was validated in/on wheat grain, wheat grain fractions, and wheat processed commodities by measuring the recovery of BCS-CN88460 from control matrices fortified at their LOQs of 0.010 ppm. Additional recoveries at 0.100 ppm were measured for all commodities except aspirated grain fractions. The high method validation for aspirated grain fractions was conducted at 2.5 ppm. These recoveries levels validated the method for the highest residues observed in individual matrices. Concurrent recoveries of all analytes were measured with each set of samples to verify method performance.

Details on concurrent recovery data are shown in Table 6.5.3- 4. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%. The method was considered valid for the analysis of BCS-CN88460 residue in all wheat matrices.

No residues above the LOQ were found in the control samples. The levels of residues of BCS-CN88460 in the treated samples are summarized in Table 6.5.3- 6. Only residue values above the LOQ are reported. Any residue value that was below the LOQ is reported as less than the LOQ. The reported residue values were not corrected for residues in controls or for concurrent recoveries.

Analysis of the triplicate wheat grain subsamples collected at the field site on the day the bulk samples were harvested confirmed that no significant degradation of BCS-CN88460 residue occurred during ambient storage and shipment of the bulk samples. Indeed, the mean residues levels of BCS-CN88460 in wheat grain (collected at the field site) were found at 0.0160 mg/kg in the first trial (C1101-15PA) but < 0.010 mg/kg in the second trial (C1102-15PA). The mean residues levels of BCS-CN88460 in wheat grain (collected at the processing site) were found at 0.0137 mg/kg in the first trial (C1101-15PA) but < 0.010 mg/kg in the second trial (C1102-15PA).

In trial C1101-15-PA, where residues in the RAC were above the LOQ, residues of BCS-CN88460 in white flour, middlings, white bread, whole meal bread, starch and pasta (fresh, fresh and cooked, dry, dry and cooked) and cooking water were below the LOQ. Residues of BCS-CN88460 were at 2.35, 0.0189, 0.0181, 0.0134, 0.0107 and 0.0151 mg/kg in aspirated grain fraction, bran, germ, shorts, whole meal flour and gluten, respectively.

In trial C1102-15-PA, where residues in the RAC were below the LOQ, residues of BCS-CN88460 were below the LOQ in all processed commodities except aspirated grain fraction (0.919 mg/kg).

The proposed processing factors calculated for each commodity for each of the two independent field trials are summarised below in Table 6.5.3- 5. They slightly differ from the table on page 18 of the report. In Table 6.5.3- 5 the processing factors were defined as the residue in the processed commodity divided by the residue in the RAC collected at the processing laboratory. When residues in the RAC and in the processed fractions were both <0.01 mg/kg a processing factor could not be calculated.

The analyses were done after a maximum frozen storage period of 402 days (grain) and 119 days (processed commodities). All sample extracts were analyzed within five days of extraction. Acceptable recoveries measured concurrently with each set of samples ensured the integrity of the sample extracts during the period of time between extraction and analysis.

III. Conclusions

Two residue trials were conducted in Canada. Wheat was treated once at a growth stage of BBCH 69 with an exaggerated dose rate (5X; 375-382 g a.s./ha) to attempt to generate a commodity with quantifiable residues.

Wheat grain was processed in order to obtain aspirated grain fraction, middlings, germ, white flour, shorts, bran, white bread, whole meal flour, whole meal bread, gluten, starch, fresh pasta, cooked fresh pasta, cooking water, dried pasta, dried and cooked pasta.

The samples (RAC and processed fractions) were analyzed for the residues of BCS-CN88460 parent compound.

The study was conducted according to GLP.

The results of the study indicate that residues of BCS-CN88460 are concentrated in the aspirated grain fraction, and in a lesser extent in bran and germ. Similar residue levels as in wheat grain are observed in shorts, whole meal flour and gluten, whereas a dilution of residues is observed in middlings, starch, white flour and the subsequent production of pasta (fresh, dried, fresh and cooked, and dried and cooked), as well as in bread (prepared from white and whole meal flours).

Table 6.5.3- 4: Concurrent recovery data for BCS-CN88460 in wheat and wheat matrices

Study	Portion analysed	n	Fortifi-cation level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
RALNN 137	Grain	11	0.01	92, 94, 96, 92, 92, 91, 101, 102, 109, 95, 98	91	109	97	6
		6	0.1	97, 97, 98, 99, 99, 102	97	102	99	2
	Aspirated Grain	3	0.01	95, 90, 100	90	100	95	5
		3	0.1	105, 100, 106	100	106	104	3
	Middlings	3	0.01	101, 101, 99	99	101	100	1
		3	0.1	98, 102, 100	98	102	100	2
	Germ	3	0.01	95, 99, 99	95	99	98	2
		3	0.1	101, 88, 95	88	101	95	7
	White Flour	3	0.01	101, 99, 100	99	101	100	1
		3	0.1	95, 97, 100	95	100	97	3
	Shorts	3	0.01	118, 114, 93	93	118	108	12
		3	0.1	99, 101, 102	99	102	101	2
	Bran	3	0.01	97, 94, 103	94	103	98	5
		3	0.1	93, 95, 94	93	95	94	1
	White Bread	3	0.01	109, 94, 99	94	109	101	8
		3	0.1	96, 93, 96	93	96	95	2
	Whole meal flour	3	0.01	91, 96, 99	91	99	95	4
		3	0.1	94, 89, 95	89	95	93	3
	Whole meal Bread	3	0.01	97, 92, 97	92	97	95	3
		3	0.1	95, 91, 91	91	95	92	3
	Gluten	3	0.01	95, 110, 95	95	110	100	9
		3	0.1	97, 101, 98	97	101	99	2
	Starch	3	0.01	100, 98, 117	98	117	105	10
		3	0.1	100, 101, 101	100	101	101	1
	Pasta, Fresh	3	0.01	80, 86, 95	80	95	87	9
		3	0.1	83, 95, 88	83	95	89	7
	Pasta, Cooked	3	0.01	103, 91, 120	91	120	105	14
		3	0.1	97, 98, 99	97	99	98	1
	Cooking Water	3	0.01	92, 95, 94	92	95	94	2
		3	0.1	99, 96, 95	95	99	97	2

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
	Pasta, Dry	3	0.01	94, 89, 83	83	94	89	6
		3	0.1	93, 92, 87	87	93	91	4
	Pasta, Dried and Cooked	3	0.01	94, 96, 99	94	99	96	3
		3	0.1	101, 97, 101	97	101	100	2

RSD: Relative standard deviation

Table 6.5.3- 5: Detailed results of the wheat processing studies

Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg) ***	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Analyte 1		
	(a)	(b)				(c)			BCS-CN88460	(d)	(e)
C1101-15PA TRTD2 plot Canada [redacted], Sakatchewan Region 7 America, North F 2015	Wheat Conquer	1) 21.05.2015	382	122	313	23.07.2015/0	69	Grain collected at the field site *	0.0159 0.0163 0.0157 **mean: 0.0160**	69	(f) RALNN137 (g) EC (isoflucypram 50 g/L) (h) Spraying (i) LN-002-P16-01 (j) 0.01 mg/kg (k) Method Validation Data in LN-002-P16-01 and in study RALNN137 (l) grain (collected at field site): 397 days grain (collected at processing): 115 days aspirated grain fractions: 111 days germ: 98 days middlings: 97 days bran: 90 days shorts: 119 days white flour: 92 days white bread: 111 days
								Grain collected at the processing site **	0.0132 0.0136 0.0143 **mean: 0.0137**	69	
								Generation of Aspirated Grain Fraction			
								Aspirated grain fractions	2.35	69	
								Germ production			
								Germ	0.0181	69	
								Flour production			
								Middlings	<0.010	69	
								Bran	0.0189	69	
								Shorts	0.0134	69	
								White flour	<0.010	69	
								Processing into white bread			
								White bread	<0.010	69	

* Grain collected at the field site, deep-frozen within 4 hours and stored at average temperature ≤-21°C until analysis.
** Grain collected at processing site: after removing bulk wheat grain samples for aspirated grain fraction generation and processing from freezer storage, representative unprocessed wheat (RAC) samples were collected and placed into frozen storage. This is the RAC reference for the processing factor calculations.
*** For wheat grain (collected at the field site and collected at the processing site), the results are individual analyses of three separate samples. For the processed commodities, the results are the mean of three analyses of a single sample.

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg) ***	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Analyte 1 BCS-CN88460		
	(a)	(b)				(c)				(d)	(e)
C1101-15PA TRTD2 plot Canada [redacted], Sakatchewan Region 7 America, North F 2015 *(continuation)*								**Processing into whole meal flour and whole meal bread**			(i) LN-002-P16-01 (j) 0.01 mg/kg (k) Method Validation Data in LN-002-P16-01 and in study RALNN137 (l) whole meal flour: 90 days whole meal bread: 101 days gluten: 92 days starch: 110 days pasta, fresh: 84 days pasta, cooked: 103 days cooking water: 112 days pasta, dry: 95 days pasta, dried and cooked: 103 days
								Whole meal flour	0.0107	69	
								Whole meal bread	<0.010	69	
								Wheat gluten and starch production			
								Gluten	0.0151	69	
								Starch	<0.010	69	
								Pasta (Noodle) production			
								Pasta, fresh	<0.010	69	
								Pasta, cooked	<0.010	69	
								Cooking water	<0.010	69	
								Pasta, dry	<0.010	69	
								Pasta, dried and cooked	<0.010	69	

*** For wheat grain (collected at the field site and collected at the processing site), the results are individual analyses of three separate samples. For the processed commodities, the results are the mean of three analyses of a single sample.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application / label pre-harvest interval, PHI, underline
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type:
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max) Grain: between deep-freezing (in the field) and last extraction date
  Grain taken at processing lab: between deep-freezing and date of last extraction
  Processed commodities: between their deep-freezing and date of last extraction
  Aspirated grain fraction: between the date the grain dust was generated through the last sample extraction
- * prior to last treatment
- ** residue in control
- # no data available

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment	Portion analyzed	Residues (mg/kg) ***	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Analyte 1		
	(a)	(b)				(c)			BCS-CN88460	(d)	(e)
C1102-15PA TRTD2 plot Canada [redacted], Sakatchewan Region 14 America, North F 2015	Wheat Conquer	1) 27.05.2015	375	120	313	20.07.2015/0	69	Grain collected at the field site *	<0.010 <0.010 <0.010 **mean: <0.010**	67	(f) RALNN137 (g) EC (isoflucypram 50 g/L) (h) Spraying (i) LN-002-P16-01 (j) 0.010 mg/kg (k) Method Validation Data in LN-002-P16-01 and in study RALNN137 (l) grain (collected at field site): 402 days grain (collected at processing): 85 days aspirated grain fractions: 78 days bran: 74 days white flour: 63 days germ: 69 days middlings: 68 days shorts: 69 days white bread: 69 days
								Grain collected at the processing site **	<0.010 <0.010 <0.010 **mean: <0.010**	67	
								Generation of Aspirated Grain Fraction			
								Aspirated grain fractions	0.919	67	
								Germ production			
								Germ	<0.010	67	
								Flour production			
								Middlings	<0.010	67	
								Bran	<0.010	67	
								Shorts	<0.010	67	
								White flour	<0.010	67	
								Processing into white bread			
								White bread	<0.010	67	

* Grain collected at the field site, deep-frozen within 4 hours and stored at average temperature ≤-21°C until analysis.
** Grain collected at processing site: after removing bulk wheat grain samples for aspirated grain fraction generation and processing from freezer storage, representative unprocessed wheat (RAC) samples were collected and placed into frozen storage. This is the RAC reference for the processing factor calculations.
*** For wheat grain (collected at the field site and collected at the processing site), the results are individual analyses of three separate samples. For the processed commodities, the results are the mean of three analyses of a single sample.

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment or no. of treatments and last date (c)	Growth stage at last treatment or date	Portion analyzed	Residues (mg/kg) ***	PHI (days) (d)	Details on trial (e)
			g a.s./ha	Water (L/ha)	g a.s./hL				Analyte 1 BCS-CN88460		
C1102-15PA TRTD2 plot Canada [redacted], Sakatchewan Region 14 America, North F 2015 *(continuation)*								**Processing into whole meal flour and whole meal bread**			(i) LN-002-P16-01 (j) 0.010 mg/kg (k) Method Validation Data in LN-002-P16-01 and in study RALNN137 (l) whole meal flour: 61 days whole meal bread: 72 days gluten: 85 days starch: 83 days pasta, fresh: 68 days pasta, cooked: 74 days cooking water: 83 days pasta, dry: 66 days pasta, dried and cooked: 74 days
								Whole meal flour	<0.010	67	
								Whole meal bread	<0.010	67	
								Wheat gluten and starch production			
								Gluten	<0.010	67	
								Starch	<0.010	67	
								Pasta (Noodle) production			
								Pasta, fresh	<0.010	67	
								Pasta, cooked	<0.010	67	
								Cooking water	<0.010	67	
								Pasta, dry	<0.010	67	
								Pasta, dried and cooked	<0.010	67	

*** For wheat grain (collected at the field site and collected at the processing site), the results are individual analyses of three separate samples. For the processed commodities, the results are the mean of three analyses of a single sample.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max) Grain: between deep-freezing (in the field) and last extraction date
  Grain taken at processing lab: between deep-freezing and date of last extraction
  Processed commodities: between their deep-freezing and date of last extraction
  Aspirated grain fraction: between the date the grain dust was generated through the last sample extraction
- * prior to last treatment
- ** residue in control
- # no data available

Table 6.5.3- 6: Proposed processing factors for wheat
Tested GAP: 1 × 375 g a.s./ha at BBCH 69

Crop, Processed commodity	Number of trials	Trial number	Residues (mg/kg)	Individual processing factors (a)	Proposed processing factor PF
Wheat grain (RAC)	*2*	*C1101-15PA* *C1102-15PA*	*0.0137 (69 days after treatment)* *<0.010 (67 days after treatment)*	-	-
Wheat, Aspirated Grain Fraction	2	C1101-15PA C1102-15PA	2.35 0.919	172 > 92	172
Wheat, Middlings	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Germ	2	C1101-15PA C1102-15PA	0.0181 <0.010	1.8 n.c.	1.32
Wheat, White Flour	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Shorts	2	C1101-15PA C1102-15PA	0.0134 <0.010	0.98 n.c.	0.98
Wheat, Bran	2	C1101-15PA C1102-15PA	0.0189 <0.010	1.4 n.c.	1.4
Wheat, White Bread	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Whole Meal Flour	2	C1101-15PA C1102-15PA	0.0107 <0.010	0.78 n.c.	0.78
Wheat, Whole Meal Bread	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Gluten	2	C1101-15PA C1102-15PA	0.0151 <0.010	1.1 n.c	1.1
Wheat, Starch	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Pasta (fresh)	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Pasta (fresh & cooked)	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Cooking Water	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Pasta (dry)	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73
Wheat, Pasta (dried and cooked)	2	C1101-15PA C1102-15PA	<0.010 <0.010	< 0.73 n.c.	< 0.73

(a): Processing factors (PF) slightly differ from the table on page 18 of the report.
PF = residues in processed commodities / residues (mean) in the RAC collected at the processing lab

n.c .: No processing factor can be calculated (residues <LOQ in RAC and in processed commodity)

< value: in case the mean residue level in the RAC is ≥ LOQ but residues in the processed commodity are < LOQ, the processing factor is calculated as to be below the worst case value calculated with the LOQ of the processed commodity

> value: in case the mean residue level in the RAC is < LOQ but residues in the processed commodity are ≥ LOQ, the processing factor is calculated as to be above the value calculated with the LOQ of the RAC

CA 6.6 Residues in rotational crops

The nature and level of residues in succeeding crops (confined rotational crops, field rotational crops) is influenced by the amount of active ingredient applied to the soil, by the degradation behaviour in soil, and by the uptake of parent compound and soil metabolites by the roots. Additionally, parent compound and soil metabolites can be metabolized by the plants.

The aerobic degradation of BCS-CN88460 in soil was investigated in laboratory studies using two different radiolabel positions - either [pyrazole-^{14}C] or [phenyl-^{14}C] radiolabelled active substance (see [redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; 2017; M-588260-01-1 and [redacted]; [redacted]; 2017; M-599926-01-1; KCA 7.1.1.1). From these studies, it can be concluded that BCS-CN88460 was slowly but steadily degraded in soil under aerobic conditions to the final degradation product CO_2. In parallel to mineralisation, bound residues (NER) were formed. Three metabolites were identified in the soil extracts along with the parent compound and $^{14}CO_2$. The only major metabolite was BCS-CN88460-carboxylic acid (M12), which is degradable under aerobic conditions.

In the metabolic pathway of BCS-CN88460 in soil the following processes are involved:

- oxidation of BCS-CN88460 to result in BCS-CN88460-carboxylic acid (M12);
- hydroxylation of BCS-CN88460-carboxylic acid (M12) to result in BCS-CN88460-lactic acid (M10);
- demethylation of BCS-CN88460-carboxylic acid (M12) to result in BCS-CN88460-desmethyl-carboxylic acid (M11);
- mineralisation (carbon dioxide formation);
- formation of non-extractable residues (NER).

Since the exposure of following crops to BCS-CN88460 soil residues cannot be excluded, the metabolism of BCS-CN88460 was investigated in representative rotational crops (wheat, Swiss chard and turnips) following soil application at a nominal rate of 200 g a.s./ha of either [pyrazole-4-^{14}C] or [phenyl-UL-^{14}C] radiolabelled active substance.

The TRRs in the confined rotational crop commodities were generally low accounting for less than 0.08 mg/kg except in wheat hay and wheat straw where the values accounted for 0.114-0.220 mg/kg and 0.131-0.340 mg/kg, respectively. Parent compound was only detected in wheat forage, wheat hay, Swiss chard and turnip leaves with amounts of equal or less than 17.0% (0.004 mg/kg) of the TRR. None of the identified metabolites accounted for more than 0.022 mg/kg and none of the unknown compounds accounted for more than 0.021 mg/kg.

CA 6.6.1 Metabolism in rotational crops

Summary of metabolism in rotational crop

The metabolism of the fungicide isoflucypram was investigated in two different metabolism studies in rotational crops following application with isoflucypram either labelled in the pyrazole or in the phenyl moiety.

Table 6.6.1- 1: Overview over available metabolism studies in confined rotational crops

Rotated crops	Application	Target application rate	Reference
turnips, Swiss chard and wheat, plant back interval 30, 140 and 287 days	pyrazole-labelled isoflucypram	201.9 g a.s./ha	M-595694-02-1
turnips, Swiss chard and wheat, plant back interval 30, 140 and 287 days	phenyl-labelled isoflucypram	197.7 g a.s./ha	M-595695-01-1

In the two confined rotational crop studies applied with an application rate of 198 - 202 g/ha of pyrazole and phenyl labelled isoflucypram, the residues of isoflucypram in rotational crops planted at all intervals were less than 0.08 mg/kg except in wheat hay and wheat straw of the pyrazole labelled confined rotational crop study where the content of isoflucypram was 0.114-0.220 mg/kg and 0.131-0.340 mg/kg, respectively. The TRRs in the different raw agricultural commodities (RACs) were generally low, increased slightly from the 1st to the 2nd rotation and stayed stable or declined to lower values in the 3rd rotation. The TRRs in the RACs of the phenyl labelled study were lower as the TRRs found in the study with the pyrazole label.

In the confined rotational crop study with isoflucypram labelled in the pyrazole mojety, unchanged parent compound was only detected in wheat forage, Swiss chard and turnip leaves with amounts of equal or less than 7.0% (0.003 mg/kg) of the TRR. Up to thirteen pyrazole derivative metabolites were identified. As the TRR values of the confined study was generally low, none of the identified metabolites accounted for more than 0.022 mg/kg and none of the unknown compounds accounted for more than 0.021 mg/kg.

The main metabolic reactions were the cleavage of the parent compound to BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (named as BCS-CR60082) and following conjugation of BCS-CR60082 with alanine (with or without defluorination) or the hydroxylation and defluoronation of BCS-CR60082 followed by conjugation with cysteine or glutathione. Other metabolic reactions were demethylation, hydroxylation, deamination or defluoronation of BCS-CR60082, followed by conjugation with glucose, lactic acid, acetic acid, cysteine or glutathione. The glutathione group was afterwards degraded to mercapto alcohol with an additional conjugation with malonic acid.

In the confined rotational crop study with isoflucypram labelled in the phenyl mojety, unchanged parent compound was only detected in wheat forage, wheat hay and Swiss chard with amounts of equal or less than 17.0% (0.004 mg/kg) of the TRR. Due to very low TRR values in the confined study, no further metabolites were identified in the conventional extracts as none of the unknown compounds was larger than 0.009 mg/kg. Acidic hydrolysis of selected extracts exhibited that the major part of residues were parent compound and conjugates of BCS-CN88460-carboxylic acid and BCS-CN88460-propanol. No label specific metabolites were detected in the CRC study with the phenyl label.

Report:	KCA 6.6.1/01; [redacted]; 2017; M-595694-02-1
Title:	Amendment no. 1: Metabolism of [pyrazole-4-14C]BCS-CN88460 in confined rotational crops
Report No.:	EnSa-16-945
Document No.:	M-595694-02-1
Guideline(s):	OECD Guideline for the Testing of Chemicals, 502: Metabolism in Rotational Crops, adopted 2007-01-08 Regulation (EC) No 1107/2009 amended by Commission Regulation (EU) No 283/2013 (Europe) US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1850: Confined Accumulation in Rotational Crops
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of the fungicide isoflucypram (BCS-CN88460) was investigated in confined rotational crops after one spray application onto bare soil. The test compound was ^{14}C-radiolabelled in the pyrazole moiety. The soil was treated with 201.9 g a.s./ha according to the envisaged use pattern. Root crops are represented by turnips, leafy crops by Swiss chard and cereals by wheat. They were sown 30 days (1st rotation), 140 days (2nd rotation) and 287 days (3rd rotation) after soil treatment.

A sample of immature Swiss chard was harvested at BBCH stage 45. Wheat forage was sampled at BBCH stage 29 and wheat hay at BBCH stage 75 to 83. At maturity turnip leaves, turnip roots, Swiss chard, wheat straw and wheat grain were harvested.

The TRRs in the different RACs were generally low, increased slightly from the 1st to the 2nd rotation and stayed stable in the 3rd rotation as shown in the following table:

Table 6.6.1- 2: TRR values in confined rotational crops after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Matrix	1st rotation	2nd rotation	3rd rotation
wheat forage	0.041	0.078	0.072
wheat hay	0.114	0.220	0.187
wheat straw	0.131	0.247	0.340
wheat grain	0.004*	0.011	0.016
Swiss chard (immature)	0.031	0.062	0.056
Swiss chard (at maturity)	0.026	0.062	0.052
turnip roots	0.006*	0.006*	0.006*
turnip leaves	0.018	0.031	0.026

* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to their amount being <0.01 mg/kg.

The majority of radioactive residues of all RACs was conventionally extracted with a mixture of acetonitrile/water (8/2; v/v). The residues in the conventional extracts amounted from 52.9% to 97.2% of the TRR. Solids after extraction of wheat hay (1st, 2nd and 3rd rotation), straw (1st, 2nd and 3rd rotation) and grain (2nd and 3rd rotation) were exhaustively extracted using microwave assistance with a mixture of acetonitrile/water/formic acid (50/50/1, v/v/v). Solids after microwave extraction, apart

from the solids of wheat grain, were subsequently extracted with a mixture of dioxane/5M HCl using microwave assistance. Exhaustive extracted residues were not further characterised due to high matrix load or low TRR values (≤ 0.023 mg/kg).
All RACs were sufficiently extracted. The total radioactivity in the post extraction solids (PES) amounted to ≤ 9.0% except for wheat grain. In wheat grain, PES amounted for up to 19.6%. As this accounted for only 0.002 mg/kg of the TRR, solids were not further extracted.

Parent compound and metabolites in the conventional extracts were quantified by HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

The identification rates ranged from 21.2% to 76.6% of the TRR in wheat, from 43.9% to 81.1% of the TRR in Swiss chard and from 55.4% to 92.3% of the TRR in turnips.
BCS-CR60082 (M49), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2, M62-i2), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2, M69-i2), BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67) and BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57) were identified as major compounds.

Parent compound and metabolites BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1, M62-i1), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1, M69-i1), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55) and BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68) were quantified in low amounts only (TRR <10%) in the conventional extracts of the RACs.

All other metabolites were detected in low amounts and were characterised by their extraction and chromatographic behaviour.

On the basis of the nature and amount of metabolites found in the extracts of the crops of all rotations the metabolic pathway of [pyrazole-4-^{14}C]BCS-CN88460 in confined rotational crops was proposed.

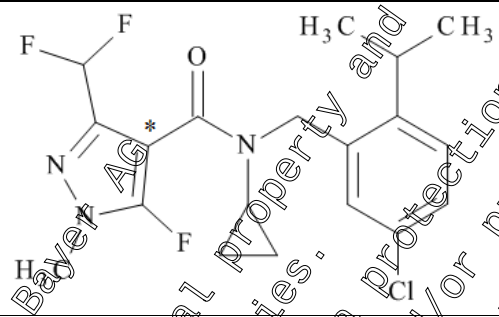
The following metabolic reactions were observed:

- cleavage of molecule leading to BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (BCS-CR60082)
- conjugation of BCS-CR60082 with alanine, lactic acid or acetic acid with or without defluorination of the pyrazole ring
- demethylation of BCS-CR60082 followed by conjugation with glucose
- hydroxylation, deamination and defluorination of BCS-CR60082 followed by conjugation with cysteine or glutathione
- defluorination of BCS-CR60082 followed by conjugation with glucose and glutathione and degradation of the glutathione group to mercapto alcohol, additional conjugation with malonic acid

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	
	* denotes the position of the ^{14}C-label
Radiolabel position	[pyrazole-4-^{14}C]
Specific radioactivity	4.22 MBq/mg (113.92 µCi/mg)
Radiochemical purity	> 98% (determined by HPLC) > 99% (determined by TLC)
Chemical purity	> 98% (determined by HPLC, UV at 210 nm)

Formulation of the Test Compound

356 mg of the EC 200 blank formulation was added to 22 mg of the radiolabelled test compound. After homogenisation a water:acetonitrile mixture (50/50, *v/v*) was added to receive the ready-to-use application suspension (total volume: 100 mL). The radioactivity in the final application suspension was determined by LSC and amounted to 0.945 MBq/mL. The formulated test compound was used as test item.

2. Soil: "[redacted] 4", pH ($CaCl_2$) = 6.7, 15% clay, 20% silt and 65% sand 1.3% organic carbon, cation exchange capacity (CEC) of 7.5 meq/100 g

3. Plants:

rotational crop	variety	representative for crop group
Spring wheat	Thasos	cereals
Swiss chard	Lucullus	leafy crops
Turnips	Rondo	root crops

B. Study Design

Experimental conditions:

The application conditions simulated the maximum annual rate of 200 g a.s./ha, according to the envisaged use pattern.

The bare soil was treated on 2014-03-25. An approximately 10% higher field rate (approx. 220 g a.s./ha) was used to compensate losses during the application. The application was performed with 100 mL of the application suspension (according to 94.45 MBq) using a computer controlled track sprayer fitted with a flat fan nozzle. The homogeneity of the spray application was checked by determination of the radioactivity on eight filter papers (1.5 cm diameter), which were randomly placed on the soil before application. The spray application was homogeneous. After application the stock container of the application apparatus was rinsed twice with acetonitrile/water (50/50; *v/v*). The rinsing solution was measured for radioactivity by LSC. By subtraction of all these losses from the radioactivity of the application suspension, the actual amount applied to the soil was calculated. As a result, 201.9 g a.s./ha (85.19 MBq/m^2) was applied.

The stability of the test compound in the application suspension was checked before and after the application by HPLC. No degradation was observed. The purity of the test compound was analysed after the application by HPLC analysis and amounted to >99%.

For ageing, the soil remained undisturbed for 30 days. The soil was watered in order to maintain adequate moisture content. Before each sowing of the crops the upper soil layer was intensively mixed (approx. 10 cm depth) and soil cores (10 to 15 cm depth) were taken. Additional soil cores were sampled at the end of the 3rd rotation (harvest of wheat, 15-30 cm depth). The radioactivity in the air-dried soil cores was determined by combustion of aliquots followed by LSC.

Sampling:

Wheat

Definition of BBCH-codes for cereals:
BBCH 29 - end of tillering; maximum no. of tillers detectable
BBCH 75 - medium milk: grain content milky, grains reached final size
BBCH 83 - early dough
BBCH 89 - fully ripe: grain hard, difficult to divide with thumbnail

Wheat forage

Forage was taken at approx. BBCH 29 (62, 175 and 335 days after application). One of five rows wheat plants was cut from the roots, which remained in the soil. The forage was cut in small pieces and homogenised with liquid nitrogen using a Polytron (Kinematica). An aliquot of the homogenised sample was used for extraction. Residual sample material was stored at approx. - 18°C.

Wheat hay

Hay was taken at BBCH 75 - 83 (101, 233 and 387 days after application).One of five rows wheat plants was cut from the roots, which remained in the soil. The hay sample was dried for four days. The dried hay sample was cut in small pieces and homogenised with liquid nitrogen using a Polytron (Kinematica). An aliquot of the homogenised sample was used for extraction. Residual sample material was stored at approx. - 18°C.

Wheat straw and grain

Straw and grain were harvested together at BBCH 89 to 92 (139, 286 and 427 days after application). The wheat plants were cut shortly above soil surface. The roots remained in the soil. The seeds were collected manually yielding the grain sample. The remaining ears and chaff were combined with the straw.

Grain and straw samples were homogenised as described for forage. The homogenised samples were stored in aliquots at approx. - 18°C. One aliquot of each sample was used for extraction.

Swiss chard

Definition of BBCH-codes for leaf vegetables (not forming heads):
BBCH 45 - 50% of the leaf mass typical for the variety reached
BBCH 49 - typical leaf mass reached

Swiss chard was harvested as an immature RAC (BBCH 45; 56, 177 and 330 days after application) and at maturity (BBCH 49; 62, 189 and 342 days after application). The samples were cut from the roots, which remained in the soil. The samples were homogenised as described for forage. The homogenised samples were stored in aliquots at approx. - 18°C. One aliquot of each sample was used for extraction.

Turnip leaves and roots

Definition of BBCH-codes for root and stem vegetables:
BBCH 49 - expansion complete; typical form and size of roots reached

Turnip leaves and roots were harvested together at maturity (BBCH 49; 79, 212 and 356 after application). The turnips were pulled out of the soil and the leaves were separated from roots. The roots were cut into slices and the leaves into small pieces. Both were homogenised as described for forage and stored in aliquots at approx. - 18°C. One aliquot of each sample was used for extraction.

C. Analytical Procedures

Conventional Extraction and Sample Preparation of all RACs

Aliquots of the homogenised samples were extracted 2 to 5 times with acetonitrile/water (8/2, v/v). The extraction steps were conducted using a Polytron homogeniser. The residues were -dried and weighed yielding the solids. The TRR of each RAC was calculated from the specific radioactivity of the test compound, the amount of the sample used for extraction and the sum of radioactivity, measured in the extracts and the remaining solids. The purified (solid phase extraction (SPE)) and concentrated combined extracts were subjected to HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. Recoveries of the concentration processes amounted from 90.9% to 111.2%.

Exhaustive Extraction of Solids

Depending on the amount of residues in the solids of the conventional extraction, an exhaustive extraction was performed once with acetonitrile/water/formic acid (50/50/1; *v/v/v*) using microwave assistance. All samples were purified using SPE cartridges. In case of wheat straw of the 1st rotation this was followed by a concentration process. Recoveries of the purification process amounted from 92.5% to 140.6%. Recovery of the concentration process amounted for 92.7%.

The remaining solids of wheat hay and straw were again subjected to an exhaustive extraction procedure using dioxane/5 M hydrochloric acid and microwave assistance. Recovery rates amounted for 97.2% to 103.6%.

Quantification:

Parent compound and metabolites were quantified in the conventional extracts by HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. Corresponding metabolites were named with the same report name and peak ID. They were assigned to each other by comparison of the metabolite profiles and retention times based on the HPLC profiling method.

Identification and characterisation:

Parent compound was identified by comparison of HPLC profiles with each other.

Metabolites BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (BCS-CR60082, M49), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52) and BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54) were isolated in HPLC peak fractions from purified and concentrated extract of Swiss chard of the 1st rotation.

Metabolites BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66) and BCS-CN88460-cyclopropyl-pyrazole-carboxamide-lactic-acid (M69, isomer 2) were isolated in HPLC peak fractions from purified and concentrated extract from wheat hay of the 1st rotation.

Metabolites BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (M62, isomer 1 and isomer 2), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (M69, isomer 1), BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67) and BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68) were isolated in peak fractions from purified wheat hay of the 2nd and 3rd rotation.

All isolated compounds were identified by spectroscopic methods.

To support metabolite identification HPLC co-chromatography of extracts with isolated radiolabelled metabolites was performed.

All other peaks or regions additionally detected in the respective HPLC chromatograms were assigned as "unknown" and numbered accordingly. They were characterised by their extraction and chromatographic behaviour.

Storage stability:

All samples were stored at temperatures ≤ -18 °C before extraction and analysis. All RACs of the 1st rotation were extracted within maximal 12 days, of the 2nd rotation within 9 days and of the 3rd rotation not later than 41 days. Within maximal one month after extraction, the earliest metabolite profiles (used for quantitation of metabolites) were obtained by HPLC-analysis analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

Therefore, it was concluded that the results of this study were not negatively influenced by storage effects.

II. Results and Discussion

The TRR of each raw agricultural commodity (RAC) was calculated from the specific radioactivity of the test compound, the amount of the sample used for extraction and the sum of radioactivity, measured in the conventional extracts and the remaining solids.

The TRRs in all raw agricultural commodities (RACs) reached from low to very low values. The TRR values in the 1st rotation ranged from 0.004 mg/kg (wheat grain) to 0.131 mg/kg (wheat straw). In the 2nd rotation, the TRRs increased slightly to values between 0.006 mg/kg (turnip roots) and

0.247 mg/kg (wheat straw) and stayed constant for the 3rd rotation with values between 0.006 mg/kg (turnip roots) and 0.340 mg/kg (wheat straw).

RACs with an amount of > 0.01 mg/kg were conventionally extracted with a mixture of acetonitrile/water (8/2, *v/v*). The conventional extraction rates amounted from 52.9% to 97.2% of the TRR. Solids after conventional extraction of wheat straw (1st, 2nd and 3rd rotation), hay (1st, 2nd and 3rd rotation) and grain (2nd and 3rd rotation) were further extracted with either acetonitrile/water/acetic acid (1/1/1; *v/v/v*) or with acetonitrile/water/formic acid (50/50/1; *v/v/v*) using microwave assistance. Apart from the solids of wheat grain, solids after microwave extraction were subsequently extracted with a mixture of dioxane/5 M HCl (49/1; *v/v*).All RACs were sufficiently extracted with extraction rates above 90%, except for wheat grain (80.4 and 85.2%). Significant losses of radioactivity during the concentration process of the extracts or radioactivity in the distillates were not observed.

All RACs were sufficiently extracted. The post extraction solids (PES) in the RACs ranged from 2.8% (= 0.001 mg/kg) of the TRR for Swiss chard (immature, 1st rotation) to 9.0% (= 0.007 mg/kg) of the TRR for wheat forage (2nd rotation). PES in wheat grain amounted for up to 19.6%. As this accounted for only 0.002 mg/kg TRR, solids were not further extracted.

Parent compound and metabolites were quantified in the conventional extracts by HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. They were assigned in all extracts by comparison of the metabolite profiles and retention times based on the profiling method. Corresponding metabolites were named with the same report name and peak ID.

Spring Wheat

The identification rates ranged from 44.9% to 60.6% in the 1st rotation, from 44.0% to 76.6% in the 2nd rotation and from 45.1% to 47.7% in the 3rd rotation. Identification rates in grain were 21.2% in the 2nd rotation and metabolites were only characterised in the 3rd rotation due to very low TRR values.

Parent compound was a minor compound in wheat, only identified in forage of the 1st and 2nd rotation with up to 7.0% (0.003 mg/kg) of the TRR. BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala was a major metabolite with up to 25.6% (0.020 mg/kg) of the TRR in forage (all rotations) and hay (1st and 2nd rotation) but a minor metabolite in straw (all rotations), hay (3rd rotation) and grain (2nd rotation). BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) was identified as a major compound with up to 11.9% (0.016 mg/kg) of the TRR in straw (1st rotation) and forage (2nd rotation). It was a minor compound in forage (1st and 3rd rotation), hay (all rotations) and straw (2nd and 3rd rotation). BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala was identified as a major compound in wheat grain (2nd rotation) with up to 13.4% (0.002 mg/kg) of the TRR. It was a minor compound in forage (1st rotation), hay (2nd and 3rd rotation) and straw (3rd rotation).

Minor metabolites (< 10% of the TRR) ranging from 1.1% of the TRR (0.002 mg/kg) to 9.3% of the TRR or 0.026 mg/kg were BCS-CR60082, BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2), BCS-CN88460-desfluoro-N-methyl cyclopropyl-pyrazole-carboxamide-OH-Cys, BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH, BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc, BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid, BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys, BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA and BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid. The respective metabolites were not detected in every part and/or rotation of wheat (see tables below).

All other metabolites were detected in low amounts and were characterised by their extraction and chromatographic behaviour.

Swiss chard

The identification rates ranged from 73.0% to 81.1% in the 1st rotation, from 44.7% to 56.4% in the 2nd rotation and from 43.9% to 51.3% of the TRR in the 3rd rotation.

Parent compound was only a minor compound in Swiss chard accounting for 6.0% of the TRR (0.002 mg/kg) and not identified in Swiss chard (at maturity) in the 2nd rotation. The main metabolite BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys accounted for up to 34.2% of the TRR or 0.016 mg/kg in all three rotations. BCS-CR60082 was identified as a major compound in immature and mature Swiss chard in the 1st rotation with up to 19.4% (0.005 mg/kg) of the TRR and as a minor compound in the other rotations. BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) was identified as a major compound in Swiss chard (at maturity, 2nd rotation) with 12.2% (0.008 mg/kg) of the TRR and a minor metabolite in immature (2nd and 3rd rotation) and Swiss chard (at maturity, 3rd rotation). BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH was identified as major compound in immature and mature Swiss chard of the 1st rotation with up to 22.9% (0.006 mg/kg) of the TRR and as a minor compound in the 2nd and 3rd rotation.

Minor metabolites (<10% of the TRR) ranging from 0.5% of the TRR (<0.001 mg/kg) to 7.3% of the TRR (0.004 mg/kg) were BCS-CN88460-cyclopropyl-carboxamide-Ala, BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2), BCS-CN88460-cyclopropyl-carboxamide-OH-lactic acid (isomer 1), BCS-CN88460-cyclopropyl-carboxamide-OH-lactic acid (isomer 2), BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala and BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxyamide-desamino-Cys.

All other metabolites were detected in low amounts and were characterised by their extraction and chromatographic behaviour.

Turnips leaves

The identification rates accounted for 92.3% in the 1st rotation, for 73.3% in the 2nd rotation and for 55.4% of the TRR in the 3rd rotation.

Parent compound was only detected as a minor compound in turnip leaves of the 1st rotation. The main metabolite BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH accounted for up to 25.7% of the TRR or 0.006 mg/kg in all three rotations. BCS-CR60082 was a major compound in the 1st and 2nd rotation with up to 18.4% of the TRR or 0.004 mg/kg and a minor compound in the 3rd rotation. BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) was a major compound in the 1st rotation with 13.0% (0.002 mg/kg) of the TRR and a minor compound in the 2nd and 3rd rotation. BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA was a major compound in the 1st rotation with 12.3% (0.002 mg/kg) of the TRR and a minor compound in the 2nd and 3rd rotation.

Minor metabolites (<10% of the TRR) ranging from 1.8% of the TRR (<0.001 mg/kg) to 8.1% of the TRR (0.002 mg/kg) were BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala, BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys, BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc, BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala, and BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys.

All other metabolites were detected in low amounts and were characterised by their extraction and chromatographic behaviour.

The distribution of the radioactive residues as well as the identified and characterised compounds in confined rotational crops after spray application onto bare soil is shown in the tables below.

Table 6.6.1- 3: Distribution of radioactivity in confined rotational crops (1st rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.041 mg/kg		TRR = 0.114 mg/kg		TRR = 0.131 mg/kg		TRR = 0.004* mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Total extractable	92.9	0.038	96.1	0.110	93.6	0.123	n.a.	n.a.
Conventional extract (analysed)	92.9	0.0038	85.7	0.098	77.9	0.102	n.a.	n.a.
Losses conventional extract	n.a.	n.a.	n.a.	n.a.	0.6	0.001	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	10.5	0.012	15.1	0.020	n.a.	n.a.
Post extraction solids (PES)	7.1	0.003	3.9	0.004	6.4	0.008	n.a.	n.a.
Accountability	100.0	0.041	100.0	0.114	100.0	0.131	100.0	0.004

	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.031 mg/kg		TRR = 0.026 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	97.2	0.030	96.0	0.025
Conventional extract (analysed)	96.1	0.030	96.0	0.025
Losses conventional extract	1.1	0.001	n.a	n.a
Exhaustive extract	n.a	n.a	n.a	n.a
Post extraction solids (PES)	2.8	0.001	4.0	0.001
Accountability	100.0	0.031	100.0	0.026

	turnip roots		turnip leaves	
	TRR = 0.006* mg/kg		TRR = 0.018 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	n.a.	n.a.	92.3	0.017
Conventional extract (analysed)	n.a.	n.a.	92.3	0.017
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	n.a.	n.a.	7.7	0.001
Accountability	100.0	0.006	100.0	0.018

* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.

Table 6.6.1- 4: Distribution of radioactivity in confined rotational crops (2nd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.078 mg/kg		TRR = 0.220 mg/kg		TRR = 0.247 mg/kg		TRR = 0.011 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Total extractable	91.0	0.071	95.7	0.211	95.5	0.236	80.4	0.009
Conventional extract (analysed)	91.0	0.071	83.6	0.184	80.7	0.199	52.9	0.006
Losses conventional extract	n.a.	n.a.	n.a.	n.a.	0.6	0.001	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	12.0	0.027	14.3	0.035	27.6	0.003
Post extraction solids (PES)	9.0	0.007	4.3	0.010	4.5	0.011	19.6	0.002
Accountability	100.0	0.078	100.0	0.220	100.0	0.247	100.0	0.011

	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.062 mg/kg		TRR = 0.062 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	95.3	0.060	94.2	0.058
Conventional extract (analysed)	95.3	0.060	94.2	0.058
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	4.7	0.003	5.8	0.004
Accountability	100.0	0.062	100.0	0.062

	turnip roots		turnip leaves	
	TRR = 0.006* mg/kg		TRR = 0.031 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	n.a.	n.a.	93.1	0.029
Conventional extract (analysed)	n.a.	n.a.	93.1	0.029
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	n.a.	n.a.	6.9	0.002
Accountability	100.0	0.006	100.0	0.031

* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.

Table 6.6.1- 5: Distribution of radioactivity in confined rotational crops (3rd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.072 mg/kg		TRR = 0.187 mg/kg		TRR = 0.340 mg/kg		TRR = 0.016 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Total extractable	91.9	0.066	97.9	0.183	95.8	0.326	85.2	0.014
Conventional extract (analysed)	91.9	0.066	85.4	0.160	83.4	0.283	53.7	0.009
Losses conventional extract	n.a.	n.a.	n.a.	n.a.	0.3	0.001	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	12.5	0.023	12.1	0.041	31.5	0.005
Post extraction solids (PES)	8.1	0.006	2.1	0.004	4.2	0.014	14.8	0.002
Accountability	100.0	0.072	100.0	0.187	100.0	0.340	100.0	0.016

	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.056 mg/kg		TRR = 0.052 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	94.4	0.053	92.4	0.048
Conventional extract (analysed)	94.4	0.053	92.4	00.48
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	5.6	0.003	7.6	0.004
Accountability	100.0	0.056	100.0	0.052

	turnip roots		turnip leaves	
	TRR = 0.006* mg/kg		TRR = 0.026 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	n.a.	n.a.	92.3	0.024
Conventional extract (analysed)	n.a.	n.a.	92.3	0.024
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	n.a.	n.a.	7.7	0.002
Accountability	100.0	0.006	100.0	0.026

* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.

Table 6.6.1- 6: Distribution of radioactivity in wheat (1st rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	wheat forage TRR = 0.041 mg/kg		wheat hay TRR = 0.114 mg/kg		wheat straw TRR = 0.131 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg
Conventional extract	92.9	0.038	85.7	0.098	78.5	0.103
Conventional extract – analysed by HPLC	92.9	0.038	85.7	0.098	77.9	0.102
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.	0.6	0.001
Identified Compounds						
BCS-CN88460	7.0	0.003	---	---	---	---
BCS-CR60082 (M49)	9.2	0.004	2.2	0.002	7.0	0.009
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	22.4	0.009	12.2	0.014	2.6	0.003
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	---	---	7.7	0.009	2.3	0.003
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	---	---	---	---	3.5	0.005
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	---	---	---	---	2.6	0.003
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	9.3	0.004	5.2	0.006	6.6	0.009
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	4.0	0.002	2.9	0.003	3.7	0.005
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	5.8	0.002	9.0	0.010	11.9	0.016
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	2.8	0.001	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	---	---	1.6	0.002	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	---	---	4.2	0.005	5.5	0.007
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---	---	---	---	---
Characterised in the conventional extract (HPLC)	32.4	0.013	40.7	0.046	32.1	0.042
Number of unknown peaks	4		12		9	
Largest unknown peak	10.8	0.004	7.0	0.008	6.7	0.009
Exhaustive extract	n.a.	n.a.	10.5	0.012	15.1	0.020
ACN Microwave extract	n.a.	n.a.	7.0	0.008	12.2	0.016*
Dioxan Microwave extract	n.a.	n.a.	3.4	0.004	2.9	0.004
Total extractable	92.9	0.038	96.1	0.110	93.6	0.123
Total identified	60.6	0.025	44.9	0.051	45.7	0.060
Total characterised (by HPLC)	32.4	0.013	40.7	0.046	32.1	0.042
Total not analysed	n.a.	n.a.	10.5	0.012	15.7	0.021
Post extraction solids (PES)	7.1	0.003	3.9	0.004	6.4	0.008
Accountability	100.0	0.041	100.0	0.114	100.0	0.131

* Microwave extract was analysed by HPLC but not quantified due to high matrix load.

Table 6.6.1- 7: Distribution of radioactivity in Swiss chard (1st rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	Swiss chard (immature) TRR = 0.031 mg/kg		Swiss chard (at maturity) TRR = 0.026 mg/kg	
	%	mg/kg	%	mg/kg
Conventional extract	97.2	0.030	96.0	0.025
Conventional extract – analysed by HPLC	96.1	0.030	96.0	0.025
Conventional extract - losses	1.1	< 0.001	n.a.	n.a.
Identified Compounds				
BCS-CN88460	6.0	0.002	4.6	0.001
BCS-CR60082 (M49)	18.9	0.006	19.4	0.005
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	26.9	0.008	34.2	0.009
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	21.2	0.007	22.9	0.006
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	---	---	---	---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---	---	---
Characterised in the conventional extract (HPLC)	23.1	0.007	14.9	0.004
Number of unknown peaks	3		2	
Largest unknown peak	11.1	0.003	8.8	0.002
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Total extractable	97.2	0.030	96.0	0.025
Total identified	73.0	0.023	81.1	0.021
Total characterised (by HPLC)	23.1	0.007	14.9	0.004
Total not analysed	1.1	<0.001	n.a.	n.a.
Post extraction solids (PES)	2.8	0.001	4.0	0.001
Accountability	100.0	0.031	100.0	0.026

Table 6.6.1- 8: Distribution of radioactivity in turnips (1st rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	turnip leaves	
	TRR =	0.018 mg/kg
	%	mg/kg
Conventional extract	92.3	0.017
Conventional extract – analysed by HPLC	92.3	0.017
Conventional extract - losses	n.a.	n.a.
Identified Compounds		
BCS-CN88460	4.8	0.001
BCS-CR60082 (M49)	18.4	0.003
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	13.0	0.002
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	7.7	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	25.7	0.005
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	---	---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	5.9	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	4.6	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	12.3	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---
Exhaustive extract	n.a.	n.a.
Total extractable	92.3	0.017
Total identified	92.3	0.017
Total characterised (by HPLC)	n.a.	n.a.
Total not analysed	n.a.	n.a.
Post extraction solids (PES)	7.7	0.001
Accountability	100.0	0.018

Table 6.6.1- 9: Distribution of radioactivity in confined wheat (2nd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.078 mg/kg		TRR = 0.220 mg/kg		TRR = 0.247 mg/kg		TRR = 0.011 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Conventional extract	91.0	0.071	83.6	0.184	81.2	0.201	52.9	0.006
Conventional extract – analysed by HPLC	91.0	0.071	83.6	0.184	80.7	0.199	52.9	0.006
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.	0.6	0.001	n.a.	n.a.
Identified Compounds								
BCS-CN88460	1.4	0.001	---	---	---	---	---	---
BCS-CR60082 (M49)	7.3	0.006	1.1	0.002	2.8	0.007	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	25.6	0.020	13.9	0.031	9.1	0.022	7.7	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	---	---	3.0	0.007	3.6	0.009	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	4.2	0.003	5.8	0.013	3.4	0.008	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	3.9	0.003	5.7	0.012	5.0	0.012	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	8.7	0.007	6.5	0.014	5.5	0.014	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---	---	---	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	8.4	0.007	2.3	0.005	3.7	0.009	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	10.2	0.008	3.2	0.007	5.9	0.015	---	---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	---	---	4.8	0.011	---	---	13.4	0.002
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	6.9	0.005	2.5	0.005	1.1	0.003	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	---	---	3.2	0.007	3.8	0.009	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---	2.0	0.004	---	---	---	---
Characterised in the conventional extract (HPLC)	14.4	0.011	29.6	0.065	36.7	0.091	31.7	0.004
Number of unknown peaks	4		13		14		3	
Largest unknown peak	4.9	0.004	5.8	0.013	4.7	0.012	16.2	0.002

Table is continued on the next page

Table 6.6.1- 9 continued

Report name	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.078 mg/kg		TRR = 0.220 mg/kg		TRR = 0.247 mg/kg		TRR = 0.011 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Exhaustive extract	n.a.	n.a.	12.0	0.027	14.3	0.035	27.6	0.003
Microwave extract	n.a.	n.a.	6.7	0.015	8.1	0.020	27.6	0.003
Microwave extract - SPE	n.a.	n.a.	6.7	0.015	8.1	0.020	27.6	0.003
Microwave extract - SPE Losses	n.a.	n.a.	<0.1	<0.001	0.1	<0.001	<0.1	<0.001
Dioxan Microwave extrakt	n.a.	n.a.	5.4	0.012	6.2	0.015	n.a.	n.a.
Total extractable	91.0	0.071	95.7	0.211	95.5	0.236	80.4	0.009
Total identified	76.6	0.059	54.0	0.119	44.0	0.109	21.2	0.002
Total characterised (by HPLC)	14.4	0.011	29.6	0.065	36.7	0.091	31.7	0.004
Total not analysed	n.a.	n.a.	12.0	0.027	14.9	0.037	27.6	0.003
Post extraction solids (PES)	9.0	0.007	4.3	0.010	4.5	0.011	19.6	0.002
Accountability	100.0	0.078	100.0	0.220	100.0	0.247	100.0	0.011

Table 6.6.1- 10: Distribution of radioactivity in Swiss chard (2nd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	Swiss chard (immature) TRR = 0.062 mg/kg		Swiss chard (at maturity) TRR = 0.062 mg/kg	
	%	mg/kg	%	mg/kg
Conventional extract	95.3	0.060	94.2	0.058
Conventional extract – analysed by HPLC	95.3	0.060	94.2	0.058
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.
Identified Compounds				
BCS-CN88460	0.5	<0.001	---	---
BCS-CR60082 (M49)	9.0	0.006	3.8	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	1.8	0.001	1.8	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	6.8	0.004	12.2	0.008
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	25.7	0.016	16.4	0.010
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	6.3	0.004	6.2	0.004
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	5.2	0.003	2.5	0.002
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	---	---	---	---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	1.2	0.001	1.9	0.001
BCS-CN88460-desfluoro-N-methyle-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---	---	---
Characterised in the conventional extract (HPLC)	38.9	0.024	49.5	0.031
Number of unknown peaks	9		22	
Largest unknown peak	15.2	0.009	11.3	0.007
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Total extractable	95.3	0.060	94.2	0.058
Total identified	56.4	0.035	44.7	0.028
Total characterised (by HPLC)	38.9	0.024	49.5	0.031
Total not analysed	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	4.7	0.003	5.8	0.004
Accountability	100.0	0.062	100.0	0.062

Table 6.6.1- 11: Distribution of radioactivity in turnips (2nd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	turnip leaves	
	TRR = 0.031 mg/kg	
	%	mg/kg
Conventional extract	93.1	0.029
Conventional extract – analysed by HPLC	93.1	0.029
Conventional extract - losses	n.a.	n.a.
Identified Compounds		
BCS-CN88460	---	---
BCS-CR60082 (M49)	12.7	0.004
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	8.1	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	6.1	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	4.6	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	4.4	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	19.9	0.006
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	2.9	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)		---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	7.2	0.002
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	7.4	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---
Characterised in the conventional extract (HPLC)	19.7	0.006
Number of unknown peaks	4	
Largest unknown peak	9.5	0.003
Exhaustive extract	n.a.	n.a.
Total extractable	93.1	0.029
Total identified	73.3	0.022
Total characterised (by HPLC)	19.7	0.006
Total not analysed	n.a.	n.a.
Post extraction solids (PES)	6.9	0.002
Accountability	100.0	0.031

Table 6.6.1- 12: Distribution of radioactivity in wheat (3rd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	wheat forage TRR = 0.072 mg/kg		wheat hay TRR = 0.187 mg/kg		wheat straw TRR = 0.340 mg/kg		wheat grain TRR = 0.016 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Conventional extract	91.9	0.066	85.4	0.160	83.7	0.284	53.7	0.009
Conventional extract – analysed by HPLC	91.9	0.066	85.4	0.160	83.4	0.283	53.7	0.009
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.	0.3	0.001	n.a.	n.a.
Identified Compounds								
BCS-CN88460	---	---	---	---	---	---	---	---
BCS-CR60082 (M49)	4.3	0.003	2.3	0.004	5.8	0.020	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	15.4	0.011	7.9	0.015	2.8	0.010	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	---	---	2.9	0.006	3.2	0.011	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	3.2	0.002	3.8	0.007	3.3	0.011	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	2.0	0.001	7.0	0.013	7.6	0.026	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	6.3	0.005	1.8	0.003	2.6	0.009	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---	3.3	0.006	2.2	0.008	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	1.8	0.001	1.9	0.003	2.0	0.007	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	6.7	0.005	8.4	0.016	8.6	0.029	---	---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	---	---	2.2	0.004	1.1	0.004	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	2.1	0.002	1.1	0.002	1.6	0.005	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	4.5	0.003	3.6	0.007	2.9	0.010	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---	1.6	0.003	1.4	0.005	---	---
Characterised in the conventional extract (HPLC)	45.8	0.033	37.7	0.071	38.3	0.130	53.7	0.009
Number of unknown peaks	12		17		17		2	
Largest unknown peak	6.1	0.004	3.7	0.007	6.2	0.021	22.4	0.004

Table is continued on the next page.

Table 6.6.1- 12 continued

Report name	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.072 mg/kg		TRR = 0.187 mg/kg		TRR = 0.340 mg/kg		TRR = 0.016 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Exhaustive extract	n.a.	n.a.	12.5	0.023	12.1	0.041	31.5	0.005
Microwave extract	n.a.	n.a.	7.9	0.015	6.7	0.022	31.5	0.005
Microwave extract - SPE	n.a.	n.a.	7.7	0.014	6.2	0.021	24.4	0.004
Microwave extract - SPE Losses	n.a.	n.a.	0.2	<0.001	0.4	0.002	7.1	0.001
Dioxan Microwave extrakt	n.a.	n.a.	4.6	0.009	5.5	0.019	n.a.	n.a.
Total extractable	91.9	0.066	97.9	0.183	95.8	0.326	85.2	0.014
Total identified	46.2	0.033	47.7	0.089	45.1	0.153	n.a.	n.a.
Total characterised (by HPLC)	45.8	0.033	37.7	0.071	38.3	0.130	53.7	0.009
Total not analysed	n.a.	n.a.	12.5	0.023	12.4	0.042	31.5	0.005
Post extraction solids (PES)	8.1	0.006	2.1	0.004	4.2	0.014	14.8	0.002
Accountability	100.0	0.072	100.0	0.187	100.0	0.340	100.0	0.016

Table 6.6.1- 13: Distribution of radioactivity in Swiss chard (3rd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	swiss chard (immature)		swiss chard (at maturity)	
	TRR = 0.056 mg/kg		TRR = 0.052 mg/kg	
	%	mg/kg	%	mg/kg
Conventional extract	94.4	0.053	92.4	00.48
Conventional extract – analysed by HPLC	94.4	0.053	92.4	0.048
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.
Identified Compounds				
BCS-CN88460	0.8	<0.001	1.1	0.001
BCS-CR60082 (M49)	7.3	0.004	4.0	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	2.3	0.001	2.8	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	5.4	0.003	9.5	0.005
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	1.6	0.001	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	23.0	0.013	21.1	0.011
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	8.4	0.005	3.7	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	0.5	<0.001	---	---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	1.3	0.001	1.6	0.001
BCS-CN88460-desfluoro-N-methyle-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	0.6	<0.001	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	---	---	---	---
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---	---	---
Characterised in the conventional extract (HPLC)	43.3	0.023	48.4	0.025
Number of unknown peaks	19		19	
Largest unknown peak	7.5	0.004	5.8	0.003
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Total extractable	94.4	0.053	92.4	0.048
Total identified	51.3	0.029	43.9	0.023
Total characterised (by HPLC)	43.1	0.024	48.5	0.025
Total not analysed	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	5.6	0.003	7.6	0.004
Accountability	100.0	0.056	100.0	0.052

Table 6.6.1- 14: Distribution of radioactivity in turnips (3rd rotation) after spray application onto bare soil with [pyrazole-4-^{14}C]BCS-CN88460

Report name	turnip leaves	
	TRR =	0.026 mg/kg
	%	mg/kg
Conventional extract	92.3	0.024
Conventional extract – analysed by HPLC	92.3	0.024
Conventional extract - losses	n.a.	n.a.
Identified Compounds		
BCS-CN88460	---	---
BCS-CR60082 (M49)	9.4	0.002
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66)	4.7	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1) (M62, isomer 1)	5.5	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2) (M62, isomer 2)	2.9	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52)	2.9	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54)	13.9	0.004
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1) (M69, isomer 1)	---	---
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56)	5.0	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2) (M69, isomer 2)	---	---
BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67)	5.8	0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55)	1.8	<0.001
BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57)	3.8	0.001
BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68)	---	---
Characterised in the conventional extract (HPLC)	36.9	0.010
Number of unknown peaks	9	
Largest unknown peak	7.3	0.002
Exhaustive extract	n.a.	n.a.
Total extractable	92.3	0.024
Total identified	55.4	0.014
Total characterised (by HPLC)	36.9	0.010
Total not analysed	n.a.	n.a.
Post extraction solids (PES)	7.7	0.002
Accountability	100.0	0.026

III. Conclusions

The metabolism of the fungicide isoflucypram (BCS-CN88460) was investigated in confined rotational crops after one spray application onto bare soil. The application rate amounted to 201.9 g a.s./ha and the test compound was ^{14}C-radiolabelled in the pyrazole moiety.

The TRRs in the raw agricultural commodities (RACs) were low and ranged from 0.004 mg/kg for wheat grain (1st rotations) to 0.340 mg/kg for wheat straw (3rd rotation). The TRR values increased slightly from the 1st to the 2nd rotation and stayed stable in the 3rd rotation. TRR values in turnip roots were constantly low at 0.006 mg/kg throughout the three rotations.

Radioactive residues were first extracted with conventional methods. In case of wheat hay, straw and grain, solids had to be further extracted.
BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (BCS-CR60082) (M49), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala (M66), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 2, M62-i2), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys (M52), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH (M54), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 2, M69-i2), BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala (M67) and BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA (M57) were identified as major compounds.

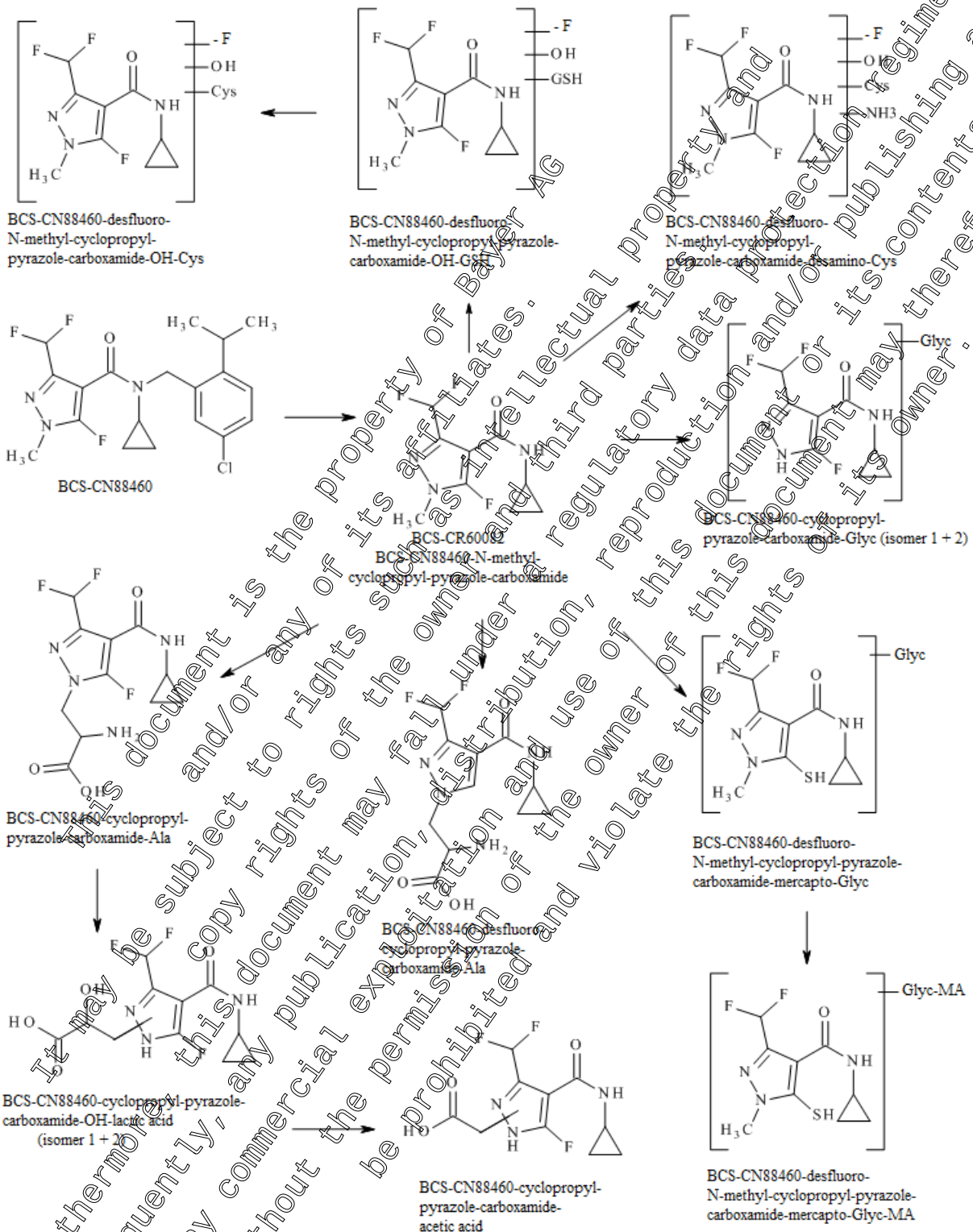
Parent compound and metabolites BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc (isomer 1, M62-i1), BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid (isomer 1, M69-i1), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc (M56), BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys (M55) and BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid (M68) were quantified in low amounts only (TRR <10%) in the conventional extracts of the RACs. All other metabolites were detected in low amounts and were characterised by their extraction and chromatographic behaviour.

The following metabolic reactions were observed:

- cleavage of the parent compound leading to BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (BCS-CR60082)
- conjugation of BCS-CR60082 with alanine, lactic acid or acetic acid with or without defluorination of the pyrazole ring
- demethylation of BCS-CR60082 followed by conjugation with glucose
- hydroxylation, deamination and defluorination of BCS-CR60082 followed by conjugation with cysteine or glutathione
- defluorination of BCS-CR60082 followed by conjugation with glucose and glutathione and degradation of the glutathione group to mercapto alcohol, additional conjugation with malonic acid

Based on these results, the metabolism of [pyrazole-4-^{14}C]BCS-CN88460 in confined rotational crops is adequately understood and the following metabolic pathway is proposed.

Figure 6.6.1- 1: Proposed metabolic pathway of [pyrazole-4-^{14}C]BCS-CN88460 in confined rotational crops



Report:	KCA 6.6.1/02; [redacted]; [redacted]; 2017; M-595695-01-1
Title:	Metabolism of [phenyl-UL-14C]BCS-CN88460 in confined rotational crops
Report No.:	EnSa-17-0128
Document No.:	M-595695-01-1
Guideline(s):	OECD Guideline for the Testing of Chemicals, 502: Metabolism in Rotational Crops, adopted 2007-01-08 Regulation (EC) No 1107/2009 amended by Comission Regulation (EU) No 283/2013 (Europe) US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1850: Confined Accumulation in Rotational Crops
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The metabolism of the fungicide isoflucypram (BCS-CN88460) was investigated in confined rotational crops after one spray application onto bare soil. The test compound was ^{14}C-radiolabelled in the phenyl moiety. The soil was treated with 197.7 g a.s./ha according to the envisaged use pattern. Root crops are represented by turnips, leafy crops by Swiss chard and cereals by wheat. They were sown 30 days (1st rotation), 140 days (2nd rotation) and 287 days (3rd rotation) after soil treatment.

A sample of immature Swiss chard was harvested at BBCH stage 45. Wheat forage was sampled at BBCH stage 29 and wheat hay at BBCH stage 75 to 83. At maturity turnip leaves, turnip roots, Swiss chard, wheat straw and wheat grain were harvested.

The TRRs in the different RACs ranged from low to very low. The TRR values increased slightly from the 1st rotation to the 2nd rotation and declined to lower values in the 3rd rotation, as shown in the following table:

Table 6.6.1-15: TRR values in confined rotational crops after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

Matrix	1st rotation	2nd rotation	3rd rotation
wheat forage	0.023	0.018	0.015
wheat hay	0.039	0.062	0.036
wheat straw	0.051	0.070	0.055
wheat grain	0.001*	0.004*	0.003*
Swiss chard (intermediate)	0.029	0.016	0.020
Swiss chard (at maturity)	0.020	0.016	0.025
turnip roots	0.003*	0.003*	0.003*
turnip leaves	0.004*	0.006*	0.006*

*TRR values were determined by LSC measurement following combustion. Samples were not extracted due to their amount being <0.01 mg/kg.

The TRRs in the raw agricultural commodities (RACs) of the current study were lower as the TRRs found in the study with the pyrazole label. The majority of the radioactive residues of all RACs was

conventionally extracted with a mixture of acetonitrile/water (8/2; v/v). The residues in the conventional extracts amounted from 78.4% to 97.7% of the TRR. Solids after extraction of wheat hay and wheat straw (1st, 2nd and 3rd rotation) were exhaustively extracted with a mixture of acetonitrile/water/formic acid (50/50/1; v/v/v) using microwave assistance.
Exhaustive extracted residues were not further characterised due to low TRR values (≤ 0.006 mg/kg).
All RACs were sufficiently extracted. The total radioactivity in the post extraction solids (PES) amounted to ≤ 9.6% of the TRR except for wheat straw (1st rotation, 11.5% of the TRR and 0.006 mg/kg) and hay (3rd rotation, 11.7% of the TRR and 0.004 mg/kg).

BCS-CN88460 was the only major compound identified in the conventional extracts with up to 17% (0.004 mg/kg) of the TRR. All other metabolites were detected in low amounts and were characterised by their extraction and chromatographic behaviour.

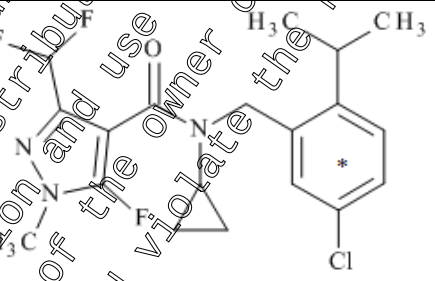
Further characterisation of residues by acidic hydrolysis of crude extracts of Swiss chard (immature and mature, 1st rotation) and wheat straw (1st rotation) indicated presence of conjugates of BCS-CN88460-carboxylic acid and BCS-CN88460-propanol besides parent compound in these selected extracts.

Based on these results, the metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in confined rotational crops is adequately understood. Parent compound was the only residue component identified.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * denotes the position of the ^{14}C-label
Radiolabel position	[phenyl-UL-^{14}C]
Specific radioactivity	4.13 MBq/mg (111.6 µCi/mg)
Radiochemical purity	> 98% (determined by HPLC) 99% (determined by TLC)
Chemical purity	98% (determined by HPLC, UV at 210 nm)

356 mg of the EC 200 blank formulation was added to 22 mg of the radiolabelled test compound. After homogenisation a water/acetonitrile mixture (50/50; *v/v*) was added to receive the ready-to-use application suspension (total volume: 100 mL). The radioactivity in the final application suspension was determined by LSC and amounted to 0.907 MBq/mL. The formulated test compound was used as test item.

2. Soil: "[redacted] 4", pH ($CaCl_2$) = 6.7, 15% clay, 20% silt and 65% sand 1.3% organic carbon, cation exchange capacity (CEC) of 7.5 meq/100 g

3. Plants:

rotational crop	variety	representative for crop group
Spring wheat	Thasos	cereals
Swiss chard	Lucullus	leafy crops
Turnips	Rondo	root crops

B. Study Design

Experimental conditions:

The application conditions simulated the maximum annual rate of 200 g a.s./ha, according to the envisaged use pattern.

The bare soil was treated on 2014-03-25. An approximately 10% higher field rate (approx. 220 g a.s./ha) was used to compensate losses during the application. The application was performed with 100 mL of the application suspension (according to 90.74 MBq) using a computer controlled track sprayer fitted with a flat fan nozzle. The homogeneity of the spray application was checked by determination of the radioactivity on ten filter papers (1.5 cm diameter), which were randomly placed on the soil before application. The spray application was homogeneous. After application the stock container of the application apparatus was rinsed twice with acetonitrile/water (50/50; *v/v*). The rinsing solution was measured for radioactivity by LSC. By subtraction of all these losses from the radioactivity of the application suspension, the actual amount applied to the soil was calculated. As a result, 197.7 g a.s./ha (81.63 MBq/m²) was applied.

The stability of the test compound in the application suspension was checked before and after the application by HPLC. No degradation was observed. The purity of the test compound was analysed after the application by HPLC analysis and amounted to 100%.

For ageing, the soil remained undisturbed for 30 days. The soil was watered in order to maintain adequate moisture content. Before each sowing of the crops the upper soil layer was intensively mixed (approx. 10 cm depth) and soil cores (10 to 15 cm depth) were taken. Additional soil cores were sampled at the end of the 3rd rotation (harvest of wheat, 15-30 cm depth). The radioactivity in the air-dried soil cores was determined by combustion of aliquots followed by LSC.

Sampling:

Wheat

Definition of BBCH-codes for cereals:
BBCH 29 - end of tillering; maximum no. of tillers detectable
BBCH 75 - medium milk: grain content milky, grains reached final size
BBCH 83 - early dough
BBCH 89 - fully ripe: grain hard, difficult to divide with thumbnail

Wheat forage

Forage was taken at approx. BBCH 29 (62, 175 and 335 days after application). One of five rows wheat plants was cut from the roots, which remained in the soil. The forage was cut in small pieces and homogenised with liquid nitrogen using a Polytron (Kinematica). An aliquot of the homogenised sample was used for extraction. Residual sample material was stored at approx. - 18°C.

Wheat hay

Hay was taken at BBCH 75 - 83 (101, 233 and 387 days after application).One of five rows wheat plants was cut from the roots, which remained in the soil. The hay sample was dried for four days. The dried hay sample was cut in small pieces and homogenised with liquid nitrogen using a Polytron (Kinematica). An aliquot of the homogenised sample was used for extraction. Residual sample material was stored at approx. - 18°C.

Wheat straw and grain

Straw and grain were harvested together at BBCH 89 (139, 286 and 427 days after application). The wheat plants were cut shortly above soil surface. The roots remained in the soil. The seeds were collected manually yielding the grain sample. The remaining ears and chaff were combined with the straw.
Grain and straw samples were homogenised as described for forage. The homogenised samples were stored in aliquots at approx. - 18°C. One aliquot of each sample was used for extraction.

Swiss chard

Definition of BBCH-codes for leaf vegetables (not forming heads):
BBCH 45 - 50% of the leaf mass typical for the variety reached
BBCH 49 - typical leaf mass reached

Swiss chard was harvested as an immature RAC (BBCH 45; 56, 177 and 330 days after application) and at maturity (BBCH 49; 62, 189 and 342 days after application). The samples were cut from the roots, which remained in the soil. The samples were homogenised as described for forage. The homogenised samples were stored in aliquots at approx. - 18°C. One aliquot of each sample was used for extraction.

Turnip leaves and roots

Definition of BBCH-codes for root and stem vegetables:
BBCH 49 - expansion complete; typical form and size of roots reached

Turnip leaves and roots were harvested together at maturity (BBCH 49; 79, 212 and 356 after application). The turnips were pulled out of the soil and the leaves were separated from roots. The roots were cut into slices and the leaves into small pieces. Both were homogenised as described for forage and stored in aliquots at approx. - 18°C. One aliquot of each sample was used for extraction.

C. Analytical Procedures

Conventional Extraction and Sample Preparation of all RACs

Aliquots of the homogenised samples were extracted 2 to 4 times with acetonitrile/water (8/2, v/v). The extraction steps were conducted using a Polytron homogeniser. The residues were -dried and weighed yielding the solids. The TRR of each RAC was calculated from the specific radioactivity of the test compound, the amount of the sample used for extraction and the sum of radioactivity, measured in the extracts and the remaining solids. The purified (solid phase extraction (SPE)) and concentrated combined extracts were subjected to HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. Recoveries of the concentration processes amounted from 90.3% to 111.6%.

Exhaustive Extraction of Solids

Depending on the amount of residues in the solids of the conventional extraction, an exhaustive extraction was performed once with acetonitrile/water/formic acid (50/50/1; *v/v/v*) using microwave assistance. All samples were purified using SPE cartridges. Recoveries of the purification process amounted from 88.0% to 129.7%.

Hydrolysis of Extracts

For isolation of parent compound and degradation products, extracts of wheat straw and Swiss chard (immature and at maturity) of the 1st rotation were hydrolysed for 1 h in 1M HCl at 100°C.

Quantification:

Parent compound and metabolites were quantified in the conventional extracts by HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. Corresponding metabolites were named with the same report name and peak ID. They were assigned to each other by comparison of the metabolite profiles and retention times based on the HPLC profiling method.

Identification and characterisation:

Parent compound was identified by comparison of HPLC profiles with each other.

Acidic hydrolysis of crude extracts of the 1st rotation from Swiss chard (immature and at maturity) and wheat straw, revealed the presence of three major aglyca. Parent compound and the two metabolites BCS-CN88460-carboxylic-acid and BCS-CN88460-propanol were isolated in HPLC fractions from purified and concentrated hydrolysed extract from Swiss chard (at maturity, 1st rotation).

All isolated compounds were identified by spectroscopic methods.

To support metabolite identification, comparison of HPLC-profiles of isolated compounds with HPLC profiles of hydrolysed extracts of the 1st rotation from Swiss chard (immature and at maturity) and wheat straw was performed.

In addition, the presence of BCS-CN88460-carboxylic acid and BCS-CN88460-propanol in the hydrolysed extracts was confirmed by comparing these HPLC profiles with profiles from laying hen metabolism studies.

The obtained aglyca after hydrolysis, which were isolated and identified from the crude extracts after hydrolsis, were not assigned to any peaks in the native extracts. All other peaks or regions additionally detected in the respective HPLC chromatograms were assigned as "unknown" and numbered accordingly. They were characterised by their extraction and chromatographic behaviour.

Storage stability:

All samples were stored at temperatures ≤ -18 °C before extraction and analysis. All RACs were extracted within maximal 8 days. Within maximal 41 days after extraction, the earliest metabolite profiles (used for quantitation of metabolites) were obtained by HPLC-analysis analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient.

Therefore, it was concluded that the results of this study were not negatively influenced by storage effects.

II. Results and Discussion

The TRR of each raw agricultural commodity (RAC) was calculated from the specific radioactivity of the test compound, the amount of the sample used for extraction and the sum of radioactivity, measured in the conventional extracts and the remaining solids.

The TRRs in all RACs reached from low to very low values. The TRR values in the 1st rotation ranged from 0.001 mg/kg (wheat grain) to 0.051 mg/kg (wheat straw). In the 2nd rotation, the TRR values slightly increased in wheat hay, straw, grain, turnip roots and turnip leaves and ranged from 0.004 mg/kg (wheat grain) to 0.070 mg/kg (wheat straw) whereas the TRR values in wheat forage and Swiss chard (immature and at maturity) slightly decreased to 0.016 mg/kg (Swiss chard, immature and at maturity) and 0.018 mg/kg (wheat forage). In the 3rd rotation, the TRR values for Swiss chard (immature and at maturity) increased slightly to 0.020 mg/kg and 0.025 mg/kg, respectively. The TRR

values in the other RACs decreased and ranged from 0.003 mg/kg (wheat grain) to 0.055 mg/kg (wheat straw).

RACs with an amount of >0.01 mg/kg were conventionally extracted with a mixture of acetonitrile/water (8/2, v/v). The conventional extraction rates amounted from 77.2% to 97.7% of the TRR.

Due to low extraction rates in wheat straw and hay, the solids remaining after conventional extraction were further extracted by microwave assistance with acetonitrile/water/formic acid (50/50/1; v/v/v).

After the exhaustive extraction all RACs were sufficiently extracted with total extraction rates between 87.3% (wheat straw, 1st rotation) and 97.7% (Swiss chard immature and at maturity, 1st rotation). Significant losses of radioactivity during the concentration process of the extracts of radioactivity in the distillates were not observed. The post extraction solids (PES) in the RACs ranged from 2.3% (<0.001 mg/kg) of the TRR for Swiss chard (at maturity, 1st rotation) to 11.7% (0.004 mg/kg) in wheat hay (3rd rotation). Solids were not further extracted.

Parent compound and metabolites were quantified in the conventional extracts by HPLC analysis based on reversed phase chromatography using an acidic water/acetonitrile/THF gradient. They were assigned in all extracts by comparison of the metabolite profiles and retention times based on the profiling method. Corresponding metabolites were named with the same report name and peak ID.
The identification rates in the three rotations were low. A total of 1.1% to 17.0% of the TRR was identified in RACs of the 1st rotation, up to 12.2% of the TRR in RACs of the 2nd rotation and up to 5.2% of the TRR in RACs of the 3rd rotation. The low identification rates are due to the generally low amounts of residues in the according RACs.

Parent compound BCS-CN88460 was the only compound identified. In wheat forage it accounted for 17.0% (0.004 mg/kg) of the TRR, 12.2% (0.002 mg/kg) of the TRR and 5.2% (0.001 mg/kg) of the TRR in the 1st, 2nd and 3rd rotation, respectively. In wheat hay of the 1st rotation and Swiss chard (immature and at maturity) of the 1st rotation it accounted for up to 6.1% (0.001 mg/kg) of the TRR. In total 16 metabolites were observed with maximum amounts ≥ 10% of the TRR in different crops and rotations. Since the TRR was in the range of 0.002 to 0.007 mg/kg for all compounds, no identification was possible and they were only characterised by their characteristic behaviour via HPLC.

Further characterization by acidic hydrolysis indicated, that the majority of these metabolites are conjugates of aglycons and parent compound representing up to 74.7% of TRR. Two aglyca (BCS-CN88460-carboxylic acid and BCS-CN88460-propanol) and parent compound were isolated from hydrolysed extracts and identified by LC-MS and LC-MS/MS.

The distribution of the radioactive residues as well as the identified and characterised compounds in confined rotational crops after spray application onto bare soil is shown in the tables below.

Table 6.6.1- 16: Distribution of radioactivity in confined rotational crops (1st rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.023 mg/kg		TRR = 0.039 mg/kg		TRR = 0.051 mg/kg		TRR = 0.001* mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Total extractable	94.8	0.021	92.8	0.036	88.5	0.046	n.a.	n.a.
Conventional extract (analysed)	94.8	0.021	86.1	0.033	77.2	0.040	n.a.	n.a.
Losses conventional extract	n.a.	n.a.	n.a.	n.a.	1.3	0.001	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	6.7	0.003	10.1	0.005	n.a.	n.a.
Post extraction solids (PES)	5.2	0.001	7.2	0.003	11.5	0.006	100.0	0.001
Accountability	100.0	0.023	100.0	0.039	100.0	0.051	100.0	0.001

	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.029 mg/kg		TRR = 0.020 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	97.7	0.028	97.7	0.020
Conventional extract (analysed)	97.7	0.028	97.7	0.020
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	2.3	0.001	2.3	<0.001
Accountability	100.0	0.029	100.0	0.020

	turnip roots		turnip leaves	
	TRR = 0.003* mg/kg		TRR = 0.004* mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	n.a.	n.a.	n.a.	n.a.
Conventional extract (analysed)	n.a.	n.a.	n.a.	n.a.
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	100.0	0.003	100.0	0.004
Accountability	100.0	0.003	100.0	0.004

* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.

Table 6.6.1- 17: Distribution of radioactivity in confined rotational crops (2nd rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.018 mg/kg		TRR = 0.062 mg/kg		TRR = 0.070 mg/kg		TRR = 0.004* mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Total extractable	93.5	0.017	91.8	0.057	90.4	0.063	n.a.	n.a.
Conventional extract (analysed)	93.5	0.017	86.3	0.053	82.9	0.058	n.a.	n.a.
Losses conventional extract	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	5.5	0.003	7.6	0.005	n.a.	n.a.
Post extraction solids (PES)	6.5	0.001	8.2	0.005	9.6	0.007	100.0	0.004
Accountability	100.0	0.018	100.0	0.062	100.0	0.070	100.0	0.004

	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.016 mg/kg		TRR = 0.016 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	96.4	0.015	96.6	0.015
Conventional extract (analysed)	96.4	0.015	96.6	0.015
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	3.6	0.001	3.4	0.001
Accountability	100.0	0.016	100.0	0.016

	turnip roots		turnip leaves	
	TRR = 0.003* mg/kg		TRR = 0.006* mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	n.a.	n.a.	n.a.	n.a.
Conventional extract (analysed)	n.a.	n.a.	n.a.	n.a.
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	100.0	0.003	100.0	0.006
Accountability	100.0	0.003	100.0	0.006

* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.

Table 6.6.1- 18: Distribution of radioactivity in confined rotational crops (3rd rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

	wheat forage		wheat hay		wheat straw		wheat grain	
	TRR = 0.015 mg/kg		TRR = 0.036 mg/kg		TRR = 0.055 mg/kg		TRR = 0.003* mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Total extractable	90.1	0.014	88.3	0.032	90.7	0.050	n.a.	n.a.
Conventional extract (analysed)	90.1	0.014	82.0	0.030	79.9	0.044	n.a.	n.a.
Losses conventional extract	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	6.4	0.002	10.8	0.006	n.a.	n.a.
Post extraction solids (PES)	9.9	0.002	11.7	0.004	9.3	0.005	100.0	0.003
Accountability	100.0	0.015	100.0	0.036	100.0	0.055	100.0	0.003

	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.020 mg/kg		TRR = 0.025 mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	96.2	0.019	95.6	0.024
Conventional extract (analysed)	96.2	0.019	95.6	0.024
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	3.8	0.001	4.4	0.001
Accountability	100.0	0.020	100.0	0.025

	turnip roots		turnip leaves	
	TRR = 0.003* mg/kg		TRR = 0.006* mg/kg	
	%	mg/kg	%	mg/kg
Total extractable	n.a.	n.a.	n.a.	n.a.
Conventional extract (analysed)	n.a.	n.a.	n.a.	n.a.
Losses conventional extract	n.a.	n.a.	n.a.	n.a.
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	100.0	0.003	100.0	0.006
Accountability	100.0	0.003	100.0	0.006

* TRR values were determined by LSC measurement following combustion. Samples were not extracted due to low residue levels.

Table 6.6.1- 19: Distribution of radioactivity in wheat (1st rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

Report name	wheat forage		wheat hay		wheat straw	
	TRR = 0.023 mg/kg		TRR = 0.039 mg/kg		TRR = 0.051 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg
Conventional extract	94.8	0.021	86.1	0.033	78.4	0.040
Conventional extract - analysed by HPLC	94.8	0.021	86.1	0.033	77.2	0.040
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.	1.3	0.001
Identified Compounds						
BCS-CN88460	17.0	0.004	1.1	<0.001	---	---
Characterised Compounds						
unknown 5	---	---	3.5	0.001	---	---
unknown 6	---	---	8.0	0.003	9.3	0.005
unknown 8	---	---	3.6	0.001	---	---
unknown 9	---	---	2.7	0.001	8.8	0.005
unknown 10	---	---	3.2	0.001	---	---
unknown 12	---	---	---	---	13.6	0.007
unknown 13	---	---	13.2	0.005	5.9	0.003
unknown 14	30.6	0.007	---	---	---	---
unknown 15	---	---	9.3	0.004	---	---
unknown 16	---	---	---	---	10.4	0.005
unknown 17	9.4	0.002	9.6	0.004	---	---
unknown 19	---	---	---	---	8.7	0.004
unknown 20	17.0	0.004	---	---	2.8	0.001
unknown 21	6.5	0.001	14.6	0.006	3.8	0.002
unknown 22	7.4	0.002	8.5	0.003	2.8	0.001
unknown 23	6.9	0.002	---	---	---	---
unknown 24	---	---	2.5	0.001	---	---
unknown 25	---	---	2.5	0.001	2.9	0.001
unknown 26	---	---	---	---	4.0	0.002
unknown 27	---	---	1.7	0.001	4.3	0.002
unknown 28	---	---	2.2	0.001	---	---
Exhaustive extract	n.a.	n.a.	6.7	0.003	10.1	0.005
Microwave extract	n.a.	n.a.	6.7	0.003	10.1	0.005
Microwave extract - SPE	n.a.	n.a.	6.7	0.003	10.1	0.005
Extract - SPE Losses	n.q.	n.q.	n.q.	n.q.	1.3	0.001
Total extractable	94.8	0.021	92.8	0.036	88.5	0.046
Total identified	17.0	0.004	1.1	<0.001	n.a.	n.a.
Total characterised (by HPLC)	77.9	0.018	85.0	0.033	77.2	0.040
Total not analysed	n.a.	n.a.	6.7	0.003	11.4	0.006
Post extraction solids (PES)	5.2	0.001	7.2	0.003	11.5	0.006
Accountability	100.0	0.023	100.0	0.039	100.0	0.051

Table 6.6.1- 20: Distribution of radioactivity in Swiss chard (1st rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

Report name	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.029 mg/kg		TRR = 0.020 mg/kg	
	%	mg/kg	%	mg/kg
Conventional extract	97.7	0.028	97.7	0.020
Conventional extract - analysed by HPLC	97.7	0.028	97.7	0.020
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.
Identified Compounds				
BCS-CN88460	2.8	0.001	6.1	0.001
Characterised Compounds				
unknown 6	3.5	0.001	---	---
unknown 7	3.6	0.001	---	---
unknown 8	2.6	0.001	<2.9	<0.001
unknown 9	4.0	0.001	---	---
unknown 10	7.5	0.002	10.3	0.002
unknown 13	5.7	0.002	21.8	0.004
unknown 14	6.0	0.002	---	---
unknown 15	4.7	0.001	4.7	0.001
unknown 16	---	---	15.8	0.003
unknown 17	25.4	0.007	---	---
unknown 19	---	---	2.8	0.001
unknown 20	12.1	0.003	9.6	0.002
unknown 21	4.6	0.001	---	---
unknown 22	10.3	0.003	8.6	0.002
unknown 23	4.8	0.001	12.1	0.002
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Total extractable	97.7	0.028	97.7	0.020
Total identified	2.8	0.001	6.1	0.001
Total characterised (by HPLC)	94.9	0.027	91.6	0.019
Total not analysed	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	2.3	0.001	2.3	<0.001
Accountability	100.0	0.029	100.0	0.020

Table 6.6.1- 21: Distribution of radioactivity in confined wheat (2nd rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

Report name	wheat forage		wheat hay		wheat straw	
	TRR = 0.018 mg/kg		TRR = 0.062 mg/kg		TRR = 0.070 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg
Conventional extract	93.5	0.017	86.3	0.053	82.9	0.058
Conventional extract - analysed by HPLC	93.5	0.017	86.3	0.053	82.9	0.058
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Identified Compounds						
BCS-CN88460	12.2	0.002	---	---	---	---
Characterised Compounds						
unknown 3	---	---	---	---	2.6	0.002
unknown 6	6.4	0.001	7.3	0.005	11.3	0.008
unknown 7	---	---	7.3	0.005	5.0	0.004
unknown 8	---	---	4.2	0.003	---	---
unknown 9	5.2	0.001	5.1	0.003	4.8	0.003
unknown 10	6.3	0.001	---	---	---	---
unknown 11	---	---	---	---	5.4	0.004
unknown 12	12.4	0.002	3.9	0.002	3.6	0.003
unknown 13	---	---	6.4	0.004	5.8	0.004
unknown 14	5.7	0.001	6.4	0.004	3.9	0.003
unknown 15	---	---	6.4	0.004	---	---
unknown 16	10.7	0.002	---	---	---	---
unknown 17	4.8	0.001	13.4	0.008	12.5	0.009
unknown 19	21.6	0.004	---	---	---	---
unknown 20	---	---	12.8	0.008	12.7	0.009
unknown 21	9.8	0.002	6.9	0.004	5.5	0.004
unknown 22	---	---	7.2	0.004	4.1	0.003
unknown 23	---	---	---	---	5.5	0.004
Exhaustive extract	n.a.	n.a.	5.5	0.003	7.6	0.005
Microwave extract	n.a.	n.a.	5.5	0.003	7.6	0.005
Microwave extract - SPE	n.a.	n.a.	5.5	0.003	7.6	0.005
Microwave extract - SPE Losses	n.a.	n.a.	<0.1	<0.001	<0.1	<0.001
Total extractable	93.5	0.017	91.8	0.057	90.4	0.063
Total identified	12.2	0.002	n.a.	n.a.	n.a.	n.a.
Total characterised (by HPLC)	81.3	0.015	86.3	0.053	82.9	0.058
Total not analysed	n.a.	n.a.	5.5	0.003	7.6	0.005
Post extraction solids (PES)	6.5	0.001	8.2	0.005	9.6	0.007
Accountability	100.0	0.018	100.0	0.062	100.0	0.070

Table 6.6.1- 22: Distribution of radioactivity in Swiss chard (2nd rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

Report name	Swiss chard (immature)		Swiss chard (at maturity)	
	TRR = 0.016 mg/kg		TRR = 0.016 mg/kg	
	%	mg/kg	%	mg/kg
Conventional extract	96.4	0.015	96.6	0.015
Conventional extract - analysed by HPLC	96.4	0.015	96.6	0.015
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.
Characterised Compounds				
unknown 7	---	---	7.0	0.001
unknown 9	6.0	0.001	11.8	0.002
unknown 10	---	---	7.9	0.001
unknown 11	13.2	0.002	---	---
unknown 12	---	---	28.5	0.004
unknown 14	12.1	0.002	8.5	0.001
unknown 15	21.1	0.003	---	---
unknown 16	16.7	0.003	12.4	0.002
unknown 18	3.2	0.001	2.6	<0.001
unknown 20	9.1	0.001	6.2	0.001
unknown 22	15.0	0.002	11.6	0.002
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Total extractable	96.4	0.015	96.6	0.015
Total identified	n.a.	n.a.	n.a.	n.a.
Total characterised (by HPLC)	96.4	0.015	96.6	0.015
Total not analysed	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	3.6	0.001	3.4	0.001
Accountability	100.0	0.016	100.0	0.016

Table 6.6.1- 23: Distribution of radioactivity in wheat (3rd rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

Report name	wheat forage		wheat hay		wheat straw	
	TRR = 0.015 mg/kg		TRR = 0.036 mg/kg		TRR = 0.055 mg/kg	
	%	mg/kg	%	mg/kg	%	mg/kg
Conventional extract	90.1	0.014	82.0	0.030	79.9	0.044
Conventional extract - analysed by HPLC	90.1	0.014	82.0	0.030	79.9	0.044
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Identified Compounds						
BCS-CN88460	5.2	0.001	---	---	---	---
Characterised Compounds						
unknown 1	---	---	5.2	0.002	---	---
unknown 2	---	---	---	---	---	---
unknown 4	---	---	---	---	---	---
unknown 5	3.9	0.001	---	---	---	---
unknown 6	10.1	0.002	8.8	0.003	16.0	0.009
unknown 8	---	---	---	0.002	3.5	0.002
unknown 9	---	---	7.3	0.003	5.8	0.003
unknown 10	15.2	0.002	---	---	---	---
unknown 11	---	---	4.3	0.002	---	---
unknown 12	---	---	5.9	0.002	---	---
unknown 13	15.2	0.002	5.1	0.002	13.0	0.007
unknown 14	---	---	---	---	2.9	0.002
unknown 15	4.9	0.001	4.5	0.002	---	---
unknown 16	---	---	4.9	0.002	11.7	0.007
unknown 17	10.4	0.002	9.4	0.003	---	---
unknown 18	---	---	---	---	---	---
unknown 19	---	---	---	---	12.2	0.007
unknown 20	18.7	0.003	17.3	0.006	3.1	0.002
unknown 21	---	---	4.2	0.002	---	---
unknown 22	6.3	0.001	---	---	5.1	0.003
unknown 25	---	---	---	---	3.1	0.002
unknown 27	---	---	---	---	3.6	0.002
Exhaustive extract	n.a.	n.a.	6.4	0.002	10.8	0.006
Microwave extract	n.a.	n.a.	6.4	0.002	10.8	0.006
Microwave extract - SPE	n.a.	n.a.	5.5	0.002	10.1	0.006
Microwave extract - SPE Losses	n.a.	n.a.	0.8	<0.001	0.7	<0.001
Total extractable	90.1	0.014	88.3	0.032	90.7	0.050
Total identified	5.2	0.001	n.a.	n.a.	n.a.	n.a.
Total characterised (by HPLC)	84.9	0.013	82.0	0.030	79.9	0.044
Total not analysed	n.a.	n.a.	6.4	0.002	10.8	0.006
Post extraction solids (PES)	9.9	0.002	11.7	0.004	9.3	0.005
Accountability	100.0	0.015	100.0	0.036	100.0	0.055

Table 6.6.1- 24: Distribution of radioactivity in Swiss chard (3rd rotation) after spray application onto bare soil with [phenyl-UL-^{14}C]BCS-CN88460

Report name	swiss chard immature		swiss chard at maturity	
	TRR = 0.020 mg/kg		TRR = 0.025 mg/kg	
	%	mg/kg	%	mg/kg
Conventional extract	96.2	0.019	95.6	0.024
Conventional extract - analysed by HPLC	96.2	0.019	95.6	0.024
Conventional extract - losses	n.a.	n.a.	n.a.	n.a.
Identified Compounds				
BCS-CN88460	---	---	---	---
Characterised Compounds				
unknown 2	---	---	2.7	0.001
unknown 4	1.2	0.001	---	---
unknown 5	1.8	0.001	3.2	0.001
unknown 6	6.2	0.001	4.9	0.001
unknown 8	14.8	0.003	4.7	0.001
unknown 9	16.2	0.003	4.4	0.001
unknown 10	7.4	0.001	6.8	0.002
unknown 11	6.3	0.001	6.4	0.002
unknown 12	3.6	0.001	---	---
unknown 13	1.9	<0.001	15.3	0.004
unknown 14	4.6	0.001	7.3	0.002
unknown 15	1.2	<0.001	4.9	0.001
unknown 16	13.2	0.003	10.6	0.003
unknown 18	2.8	0.001	4.4	0.001
unknown 19	4.0	0.001	---	---
unknown 20	6.2	0.001	11.6	0.003
unknown 21	2.8	0.001	---	---
unknown 22	2.2	<0.001	10.4	0.003
Exhaustive extract	n.a.	n.a.	n.a.	n.a.
Microwave extract	n.a.	n.a.	n.a.	n.a.
Microwave extract - SPE	n.a.	n.a.	n.a.	n.a.
Microwave extract - SPE Losses	n.a.	n.a.	n.a.	n.a.
Total extractable	96.2	0.019	95.6	0.024
Total identified	n.a.	n.a.	n.a.	n.a.
Total characterised (by HPLC)	96.2	0.019	95.6	0.024
Total not analysed	n.a.	n.a.	n.a.	n.a.
Post extraction solids (PES)	3.8	0.001	4.4	0.001
Accountability	100.0	0.020	100.0	0.025

III. Conclusions

The metabolism of the fungicide isoflucypram (BCS-CN88460) was investigated in confined rotational crops after one spray application onto bare soil. The application rate amounted to 197.7 g a.s./ha and the test compound was ^{14}C-radiolabelled in the phenyl moiety.

The TRRs in the raw agricultural commodities (RACs) ranged from very low to low with values between 0.001 mg/kg in wheat grain (1st rotation) to 0.070 mg/kg in wheat straw (2nd rotation).

Radioactive residues were extracted with conventional methods. In case of wheat hay and straw, solids had to be further extracted.

BCS-CN88460 was the only compound identified in the conventional extracts, amounting for up to 17.0% (0.004 mg/kg) of the TRR.

Further characterisation of residues by acidic hydrolysis of crude extracts of Swiss chard (immature and mature, 1st rotation) and wheat straw (1st rotation) indicated presence of conjugates of BCS-CN88460-carboxylic acid (M12) and BCS-CN88460-propanol (M01) besides parent compound in these selected extracts.

Based on these results, the metabolism of [phenyl-UL-^{14}C]BCS-CN88460 in confined rotational crops is adequately understood. Parent compound was the only residue component identified.

CA 6.6.2 Magnitude of residues in rotational crops

General remark:
In this summary section (CA 6.6.2), the following codes will be used for the metabolites:

Name	*Metab. No*	*Standard "dossier name"*
BCS-CR60082	M49	BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide
	M12	BCS-CN88460-carboxylic acid

Report:	KCA 6.6.2/01; [redacted]; [redacted], C.; 2017; M-605725-01-1
Title:	Determination of the residues of BCS-CN88460 in/on soil and the field rotational crops barley, carrot, turnip and lettuce after spray application of BCS-CN88460 EC 050 to bare soil in Germany, the Netherlands, southern France and Italy
Report No.:	15-2502
Document No.:	M-605725-01-1
Guideline(s):	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 OECD Guidelines for the Testing of Chemicals. Residues in Rotational Crops (Limited Field Studies). 504. 2007-01-08 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1900, Field Accumulation in Rotational Crops US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	yes, see report
GLP/GEP:	**yes**

I. Materials and Methods

The purpose of the study 15-2502 was to determine the magnitude of the relevant residues of BCS-CN88460 in/on barley, lettuce and carrot or turnip grown as rotational crops after one spray application to bare soil with BCS-CN88460 EC 050 an emulsifiable concentrate (EC) formulation containing 50 g/L BCS-CN88460, followed by plant-back intervals of 21 to 34 days (1st rotation), 100 to 201 days (2nd rotation) and 299 to 370 days (3rd rotation).

The study included four supervised residue trials conducted in northern Europe (Germany and the Netherlands) and southern Europe (France and Italy) during the 2015 and 2016 growing seasons.

One spray application of BCS-CN88460 EC 050 was made to bare soil at an application rate of 3.6 L/ha (equivalent to 0.18 kg/ha active substance) with a water rate of 300 to 400 L/ha. The application procedure was followed by incorporation of the test item into the soil via ploughing to a depth of ≤ 8 cm. All treatments were made at the scheduled rates.

At various intervals, crops were planted onto the test area in order to simulate a crop failure ("rotation 1", plant-back interval [PBI] 21-34 days), a second use of the plot in the same year ("rotation 2", PBI 100-201 days), or use of the same plot in the succeeding year ("rotation 3", PBI 299-370 days). In each rotation, 3 different crops representing different botanical groups were planted: a root crop (carrots or turnips), a leafy crop (lettuce), or a small grain cereal (barley).

Samples of representative soil commodities were taken on the day after sowing/planting the crop for each individual rotation from each of the subplots. Moreover, samples of the rotational crops were taken at their respective harvest times, as well as at one earlier interval (immature RACs for lettuce and root crops, or green material (whole plant without roots) for barley).

Each field sample was placed in double labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer AG – Crop Science Division, BAG-CS-HSRA in D-[redacted] [redacted] am Rhein. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived at PVTL in good condition. The field samples were stored in a freezer at -18°C or below until preparation of the examination samples.

For the preparation of the soil examination samples, the deep-frozen field samples were grounded with dry-ice and homogenized in a Stephan mill. Representative parts of the homogenized samples were transferred into polystyrene boxes and stored at -18°C or below until analysis. For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at -18°C or below until analysis.

Residues of BCS-CN88460 and its metabolite BCS-CN88460-carboxylic acid were determined in soil using method 01432 ([redacted]; 2014; M-499794-01-1; referenced in MCA Section 4 under Point 4.1.2). The LOQs for each analyte were 0.001 mg/kg, the metabolite being not expressed in parent equivalents.

The samples of the rotational crops were analyzed for the parent compound and its metabolite BCS-CR60082 using method 01475 ([redacted]; 2016; M-558986-01-1; referenced in MCA Section 4 under Point 4.1.2) which was validated prior to the residue analysis of the samples. Additional validation recoveries were conducted for all sample materials in the study 15-2502. Samples of barley (grain, straw and green material) were analyzed according to the procedure described in the method for dry matrices (soaking step before extraction) and the samples of carrot (root and leaf), turnip (root and leaf) and lettuce (head) were prepared according to the procedure for higher-water containing commodities (no soaking step before extraction). The LOQs for each analyte were 0.01 mg/kg (all in parent equivalents).

II. Findings

Concurrent recoveries for BCS-CN88460 and its metabolite BCS-CN88460-carboxylic acid were obtained from samples of soil. The recovery samples were spiked at levels of 0.001 mg/kg and up to 0.01 mg/kg. Details of concurrent recovery data are shown in Table 6.6.2- 1 for BCS-CN88460 and in Table 6.6.2- 2 for BCS-CN88460-carboxylic acid. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

Besides, concurrent recoveries for BCS-CN88460 and its metabolite BCS-CR60082 were obtained from samples of carrots, turnips, lettuce and barley. The recovery samples were spiked at levels of 0.01 mg/kg and up to 0.10 mg/kg. Details of concurrent recovery data are shown in Table 6.6.2- 3 for BCS-CN88460 and in Table 6.6.2- 4 for BCS-CR60082. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20% for each compound and all sample materials.

No residues above the LOQs were found in the control samples. The detailed results obtained for soil samples are summarised below in Table 6.6.2- 5 and the results obtained for rotational crop samples are summarised in Table 6.6.2- 6 to Table 6.6.2- 8. The results were not corrected for concurrent recoveries.

The residues of BCS-CN88460 and BCS-CR60082 in the treated rotational crops were always found < LOQ with one exception.

In the trial 15-2501-01 (Germany), residues of BCS-CN88460 reached levels of 0.057 to 0.075 mg/kg only in carrot leaves (2nd rotation, plot T-2A). These results – confirmed with re-analyses- were illogical because no residues were found in carrot roots from the same plot and no residues were found in the 1st rotation for carrot.

It appears that five days before the first harvest of carrot leafs in plot T-2A, the adjoining plot dedicated to barley (T-1C) was sprayed with BCS-CN88460 EC 050. There was no buffer zone between the two plots, the carrot plot was not protected during the spray of the plot T-1C, and there was a very light wind in the direction of the carrot plot. This explanation supports the fact that the residue levels of BCS-CN88460 found in carrot leaves in the plot T-2A are the result of a spray drift. These residue levels should not be considered because they are not attributable to residues arising from soil treatment.

Based on all the other results, it is concluded, that after an application of BCS-CN88460 EC 050 on bare soil at a rate of 180 g a.s./ha, the residues of BCS-CN88460 and BCS-CR60082 are expected to be <0.01 mg/kg in barley, lettuce and carrot or turnip grown as rotational crops.

The analyses of soil were done after a maximum frozen storage period of 320 days and the analyses of plant commodities after a maximum frozen storage period of 341 days. For crop commodities, the time between the beginning of the sample preparation and the sample analysis did not exceed 24

hours. For soil, all final extracts were analysed within 8 days. This storage period of soil extracts is covered by storage stability experiments conducted in the method 01432 ([redacted]; 2014; M-499794-01-1; referenced in MCA Section 4 under Point 4.1.2).

III. Conclusions

In order to support the use of BCS-CN88460 in the EU for non-perennial crops, four multi-plant-back multi-crop rotational crop trials were conducted in Europe (2 each in the northern and southern residue regions) in 2015-2016. BCS-CN88460 was applied once as an EC 050 formulation to bare soil at an application rate of 3.6 L/ha (corresponding to 180 g active substance/ha). Crops representing 3 different botanical groups (roots, leafy vegetables, small grain cereals) were planted on the plots at 3 intervals thereafter. All applications were at the required rates, and all trials were conducted according to GLP.

The residues of BCS-CN88460 and BCS-CR60082 in the rotational crops were always found <LOQ with one exception.

In the trial 15-2501-01 (Germany), residues of BCS-CN88460 reached levels of 0.057 to 0.075 mg/kg only in carrot leaves (2nd rotation, plot T-2A). These results – confirmed with re-analyses – were illogical because no residues were found in carrot roots from the same plot and no residues were found in the 1st rotation for carrot.

It appears that five days before the first harvest of carrot leafs in plot T-2A, the adjoining plot dedicated to barley (T-1C) was sprayed with BCS-CN88460 EC 050. There was no buffer zone between the two plots, the carrot plot was not protected during the spray of the plot T-1C, and there was a very light wind in the direction of the carrot plot. This explanation supports the fact that the residue levels of BCS-CN88460 found in carrot leaves in the plot T-2A are the result of a spray drift. These residue levels should not be considered because they are not attributable to residues arising from soil treatment.

Based on all the other results, it is concluded that after an application of BCS-CN88460 EC 050 on bare soil at a rate of 180 g a.s./ha, the residues of BCS-CN88460 and BCS-CR60082 are expected to be <0.01 mg/kg in barley, lettuce and carrot or turnip grown as rotational crops.

The dose rate of 180 g active substance/ha tested in this study adequately covers the plateau concentration of isoflucypram in soil overtime. Indeed, the maximum seasonal rate for isoflucypram is 75 g as/ha. The accumulation factor for isoflucypram parent compound in soil was calculated to be 1.5 for parent (please refer to [redacted] G.; [redacted], W.; 2017; M-608723-01-1 in MCA 7 Point 7.1.2.2.2). Considering a crop interception of 80% applicable for cereals growth stages ranging from BBCH 30 to BBCH 69, the plateau in soil corresponds to 22.5 g as/ha (75 g as/ha x 1.5 x 0.2).

When this plateau is added to a last application rate of 75 g as/ha the appropriate dose to be investigated is 97.5 g as/ha (plateau of 22.5 g as/ha + seasonal rate of 75 g as/ha), below what was actually tested in the study 15-2502.

It is also highlighted that, for the 2nd and 3rd rotations, it would be relevant to apply the crop interception of 80% to the last seasonal rate as well since for normal rotations the treated crops are harvested and not re-incorporated into the soil. Proceeding so, the appropriate dose rate to be investigated for the 2nd and 3rd rotations is 37.5 g as/ha (plateau of 22.5 g as/ha+ 75 x 0.2), a dose rate far below the one tested in the study 15-2502.

The plateau in soil for the metabolite M12 (BCS-CN88460-carboxylic acid) does not need to be considered since its DT_{50} value is < 150 days (worst case laboratory DT_{50} of 112 days).

It is concluded that plant-back restrictions and MRL proposals above 0.01 mg/kg based on rotational crops are not necessary.

Table 6.6.2- 1: Recovery data for BCS-CN88460 in soil

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
soil / soil 0-30 cm	0.001	95; 96; 97; 97; 98; 98; 98; 99; 99; 100; 100; 100; 101; 102; 102; 102; 104	99	2.4	0.001
	0.01	97; 97; 97; 97; 99; 99; 99; 99; 99; 99; 100; 100; 100; 100; 102; 102; 102	99	1.7	
		Overall recovery (n = 34)	**99**	**2.1**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CN88460, determined as BCS-CN88460 and calculated as BCS-CN88460

Table 6.6.2- 2: Recovery data for BCS-CN88460-carboxylic acid in soil

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
soil / soil 0-30 cm	0.001	72; 77; 84; 89; 89; 91; 95; 95; 96; 97; 98; 99; 102; 102; 103; 104; 105	94	9.9	0.001
	0.01	90; 93; 97; 97; 97; 98; 98; 98; 100; 101; 101; 102; 102; 105; 106; 106; 108	100	4.7	
		Overall recovery (n = 34)	**97**	**8.1**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CN88460-carboxylic acid, determined as BCS-CN88460-carboxylic acid and calculated as BCS-CN88460-carboxylic acid

Table 6.6.2- 3: Recovery data for BCS-CN88460 in rotational crop matrices (root, leafy, and cereal crops)

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
barley / grain	0.01	92; 94; 95; 96; 97; 99	96	2.5	0.01
	0.10	96; 98; 98; 100	98	1.7	
		Overall recovery (n = 10)	**97**	**2.5**	
barley / green material	0.01	91; 92; 92; 96; 97; 98; 99; 101; 101; 104; 107; 108	99	5.7	0.01
	0.10	97; 103; 103; 106; 117	105	7.0	
		Overall recovery (n = 17)	**101**	**6.6**	
barley / straw	0.01	93; 95; 96; 97; 100; 100; 103	98	3.5	0.01
	0.10	87; 98; 100	95	7.4	
		Overall recovery (n = 10)	**97**	**4.7**	
carrot / leaf	0.01	93; 93; 98; 104; 107; 107	100	6.5	0.01
	0.10	101; 102; 103; 105; 105; 106	104	1.9	
		Overall recovery (n = 12)	**102**	**4.8**	

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
carrot / root	0.01	92; 92; 100; 100; 100; 101	98	4.4	0.01
	0.10	102; 103; 107; 107; 107	105	2.4	
		Overall recovery (n = 11)	**101**	**5.2**	
lettuce / head	0.01	82; 95; 96; 98; 101; 102	96	7.6	0.01
	0.10	98; 100; 101; 104	101	2.5	
		Overall recovery (n = 10)	**98**	**6.3**	
turnip / leaf	0.01	99; 99; 99; 103; 104	101	2.5	0.01
	0.10	94; 99; 100; 105; 109	101	5.7	
		Overall recovery (n = 10)	**101**	**4.2**	
turnip / body	0.01	88; 89; 96; 98; 104	95	7.0	0.01
	0.10	102; 103; 103; 105; 106	104	1.6	
		Overall recovery (n = 10)	**99**	**6.5**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CN88460, determined as BCS-CN88460 and calculated as BCS-CN88460

Table 6.6.2- 4: Recovery data for BCS-CR60082 in rotational crop matrices (root, leafy, and cereal crops)

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
barley / grain	0.01	86; 91; 92; 92; 92; 93	91	2.8	0.01
	0.10	92; 95; 95; 95	94	1.6	
		Overall recovery (n = 10)	**92**	**2.9**	
barley / green material	0.01	83; 84; 85; 87; 88; 89; 89; 90; 90; 90; 91; 93	88	3.4	0.01
	0.10	92; 96; 98; 99; 109	99	6.4	
		Overall recovery (n = 17)	**91**	**7.0**	
barley / straw	0.01	81; 89; 90; 90; 92; 92; 94	90	4.7	0.01
	0.10	87; 96; 98	94	6.3	
		Overall recovery (n = 10)	**91**	**5.3**	
carrot / leaf	0.01	86; 88; 91; 92; 94; 95	91	3.8	0.01
	0.10	88; 91; 96; 96; 103; 103	96	6.4	
		Overall recovery (n = 12)	**94**	**5.8**	
carrot / root	0.01	86; 87; 92; 94; 94; 96	92	4.5	0.01
	0.10	93; 94; 99; 99; 101	97	3.6	
		Overall recovery (n = 11)	**94**	**5.0**	
lettuce / head	0.01	76; 88; 89; 90; 95; 97	89	8.3	0.01
	0.10	93; 94; 94; 95	94	0.9	
		Overall recovery (n = 10)	**91**	**6.6**	
turnip / leaf	0.01	92; 93; 93; 93; 94	93	0.8	0.01
	0.10	90; 91; 91; 98; 98	94	4.3	
		Overall recovery (n = 10)	**93**	**2.9**	
turnip / body	0.01	78; 79; 88; 89; 95	86	8.4	0.01
	0.10	93; 93; 94; 95; 96	94	1.4	
		Overall recovery (n = 10)	**90**	**7.3**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with BCS-CR60082, determined as BCS-CR60082 and calculated as BCS-CN88460

Table 6.6.2- 5: Rotational trial summary for soil samplings

Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)
Analyte 2: BCS- CN88460-carboxylic acid (determined as BCS-CN88460-carboxylic acid, calculated as BCS-CN88460-carboxylic acid)

Trial No./ Location/ Year	Plot (Commodity)	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date	Portion analyzed	Residues (mg/kg)		DALT (days)	Details on trial
		g a.s./ ha	Water (L/ha)	g a.s./hL			Analyte 1 BCS-CN88460	Analyte 2 BCS-CN88460-carboxylic acid		
	(a)				(c)				(d)	(e)
15-2502-01 [redacted] Germany Northern Europe F 2015	**Plot T-1A** (carrot)	180	300	60	2015-03-12	Soil 0-30 cm	0.023 0.026	< 0.001 < 0.001	28	(f) 15-2502 (g) EC (250 g/L) (h) Spraying (i) 01432 (j) 0.001 mg/kg (k) Method Validation Data in 01432 (l) 253 days
	Plot T-1B (lettuce)	180	300	60	2015-03-12	Soil 0-30 cm	0.024 0.020	< 0.001 < 0.001	28	
	Plot T-1C (barley)	180	300	60	2015-08-26	Soil 0-30 cm	0.009 0.014	< 0.001 < 0.001	34	
	Plot T-2A (carrot)	180	300	60	2015-03-12	Soil 0-30 cm	0.020 0.020	< 0.001 < 0.001	106	
	Plot T-2B (lettuce)	180	300	60	2015-03-12	Soil 0-30 cm	0.018 0.024	< 0.001 < 0.001	144	
	Plot T-2C (barley)	180	300	60	2015-03-12	Soil 0-30 cm	0.021 0.019	< 0.001 < 0.001	201	
	Plot T-3A (carrot)	180	300	60	2015-03-12	Soil 0-30 cm	0.025 0.020	< 0.001 < 0.001	369	
	Plot T-3B (lettuce)	180	300	60	2015-03-12	Soil 0-30 cm	0.028 0.017	< 0.001 < 0.001	369	
	Plot T-3C (barley)	180	300	60	2015-03-12	Soil 0-30 cm	0.002 0.020	< 0.001 < 0.001	369	

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undertime)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year	Plot (Commodity)	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date	Portion analyzed	Residues (mg/kg)		DALT (days)	Details on trial
		g a.s./ ha	Water (L/ha)	g a.s./hL			Analyte 1 BCS-CN88460	Analyte 2 BCS-CN88460-carboxylic acid		
	(a)				(c)				(d)	(e)
15-2502-02 [redacted] The Netherlands Northern Europe F 2015	**Plot T-1A** (carrot)	180	400	45	2015-04-15	Soil 0-30 cm	0.030 0.029	< 0.001 < 0.001	22	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01432 (j) 0.001 mg/kg (k) Method Validation Data in 01432 (l) 320 days
	Plot T-1B (lettuce)	180	400	45	2015-04-15	Soil 0-30 cm	0.034 0.030	< 0.001 < 0.001	22	
	Plot T-1C (barley)	180	400	45	2015-09-21	Soil 0-30 cm	0.034 0.035	< 0.001 < 0.001	23	
	Plot T-2A (carrot)	180	400	45	2015-04-15	Soil 0-30 cm	0.027 0.031	< 0.001 < 0.001	100	
	Plot T-2B (lettuce)	180	400	45	2015-04-15	Soil 0-30 cm	0.030 0.022	< 0.001 < 0.001	131	
	Plot T-2C (barley)	180	400	45	2015-04-15	Soil 0-30 cm	0.020 0.036	< 0.001 < 0.001	182	
	Plot T-3A (carrot)	180	400	45	2015-04-15	Soil 0-30 cm	0.028 0.031	< 0.001 0.001	366	
	Plot T-3B (lettuce)	180	400	45	2015-04-15	Soil 0-30 cm	0.017 0.019	< 0.001 < 0.001	365	
	Plot T-3C (barley)	180	400	45	2015-04-15	Soil 0-30 cm	0.017 0.021	< 0.001 < 0.001	366	

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year (a)	Plot (Commodity) (a)	Application rate per treatment on bare soil: g a.s./ ha	Water (L/ha)	g a.s./hL	Dates of treatment or no. of treatments and last date (c)	Portion analyzed	Residues (mg/kg): Analyte 1 BCS-CN88460	Analyte 2 BCS-CN88460-carboxylic acid	DALT (days) (d)	Details on trial (e)
15-2502-03 [redacted] France Southern Europe F 2015	**Plot T-1A** (turnip)	180	300	60	2015-08-07	Soil 0-30 cm	0.035 0.031	< 0.001 < 0.001	20	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01432 (j) 0.001 mg/kg (k) Method Validation Data in 01432 (l) 267 days
	Plot T-1B (lettuce)	180	400	45	2015-04-10	Soil 0-30 cm	0.022 0.028	< 0.001 < 0.001	20	
	Plot T-1C (barley)	180	300	60	2015-09-18	Soil 0-30 cm	0.022 0.023	< 0.001 < 0.001	27	
	Plot T-2A (turnip)	180	300	60	2015-04-10	Soil 0-30 cm	0.018 0.019	< 0.001 < 0.001	20	
	Plot T-2B (lettuce)	180	300	60	2015-04-10	Soil 0-30 cm	0.018 0.017	< 0.001 < 0.001	20	
	Plot T-2C (barley)	180	300	60	2015-04-10	Soil 0-30 cm	0.012 0.014	0.001 0.001	69	
	Plot T-3A (turnip)	180	300	60	2015-04-10	Soil 0-30 cm	0.010 0.011	< 0.001 < 0.001	231	
	Plot T-3B (lettuce)	180	300	60	2015-04-10	Soil 0-30 cm	0.012 0.014	0.001 0.001	229	
	Plot T-3C (barley)	180	300	60	2015-04-10	Soil 0-30 cm	0.014 0.013	< 0.001 < 0.001	180	

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year	Plot (Commodity)	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date	Portion analyzed	Residues (mg/kg)		DALT (days)	Details on trial
		g a.s./ ha	Water (L/ha)	g a.s./hL			Analyte 1 BCS-CN88460	Analyte 2 BCS-CN88460-carboxylic acid		
	(a)				(c)				(d)	(e)
15-2502-04 [redacted] Italy Southern Europe F 2015	**Plot T-1A** (carrot)	180	300	60	2015-04-14	Soil 0-30 cm	0.040 0.057	< 0.001 < 0.001	28	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01432 (j) 0.001 mg/kg (k) Method Validation Data in 01432 (l) 301 days
	Plot T-1B (lettuce)	180	300	60	2015-04-14	Soil 0-30 cm	0.029 0.022	< 0.001 < 0.001	28	
	Plot T-1C (barley)	180	300	60	2015-09-22	Soil 0-30 cm	0.023 0.027	< 0.001 < 0.001	34	
	Plot T-2A (carrot)	180	300	60	2015-04-14	Soil 0-30 cm	0.020 0.021	0.001 0.001	125	
	Plot T-2B (lettuce)	180	300	60	2015-04-14	Soil 0-30 cm	0.021 0.019	0.001 0.001	125	
	Plot T-2C (barley)	180	300	60	2015-04-14	Soil 0-30 cm	0.020 0.017	0.002 0.001	195	
	Plot T-3A (carrot)	180	300	60	2015-04-14	Soil 0-30 cm	0.016 0.015	< 0.001 < 0.001	353	
	Plot T-3B (lettuce)	180	300	60	2015-04-14	Soil 0-30 cm	0.017 0.022	< 0.001 < 0.001	353	
	Plot T-3C (barley)	180	300	60	2015-04-14	Soil 0-30 cm	0.015 0.020	< 0.001 < 0.001	345	

DALT: days after the last treatment

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Table 6.6.2- 6: Rotational trial summary for Carrot/Turnip (Root vegetables)

Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)
Analyte 2: BCS-CR60082 (determined as BCS-CR60082, calculated as BCS-CN88460)

Trial No./ Location/ Year (a)	Commodity/ Variety (Plot) (a)	PBI (days) (b)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil: g a.s./ ha	Water (l/ha)	g a.s./hl	Dates of treatment or no. of treatments and last date (c)	Portion analysed	Growth stage at sampling	Residues (mg/kg): Analyte 1 BCS-CN88460	Residues (mg/kg): Analyte 2 BCS-CR60082	DALT (days) (d)	Details on trial (e)
15-2502-01 [redacted] Germany Northern Europe F 2015	Carrot/ Bolero F1 (Plot T-1A)	28	1) 2015-04-09 3) 2015-07-15 - 2015-08-15	180	300	60	2015-03-12	Root	BBCH 47	<0.01	<0.01	125	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) root: 300 days leaf: 299 days
									BBCH 49	<0.01	<0.01	139	
								Leaf	BBCH 47	<0.01	<0.01	125	
									BBCH 49	<0.01	<0.01	139	
	Carrot/ Laguna (Plot T-2A)	106	1) 2015-06-26 3) 2015-09-10 - 2015-09-30	180	300	60	2015-03-12	Root	BBCH 47	<0.01	<0.01	172	
									BBCH 49	<0.01	<0.01	186	
								Leaf	BBCH 47	**0.075** [a]	<0.01 [b]	172	
									BBCH 49	**0.057** [c]	<0.01 [d]	186	
	Carrot/ Laguna (Plot T-3A)	368*	1) 2016-03-14 3) 2016-07-15 - 2016-08-15	180	300	60	2015-03-12	Root	BBCH 47	<0.01	<0.01	491	
									BBCH 49	<0.01	<0.01	505	
								Leaf	BBCH 47	<0.01	<0.01	491	
									BBCH 49	<0.01	<0.01	505	

PBI = plant-back interval DALT: days after the last treatment

a Mean of three replicates: (0.077; 0.077 and 0.070 mg/kg)
b Mean of three replicates: (<0.01; <0.01 and <0.01mg/kg)
c Mean of three replicates: (0.057; 0.054 and 0.060 mg/kg)
d Mean of three replicates: (<0.01; <0.01 and <0.01mg/kg)

* Plant back interval is slightly longer as reqested in the study protocol. However, the timing for replanting of rotational crops as given in the OECD guideline on residues in rotational crops (limited field studies) 504 suggests 270 to 365 days for crops rotated the following year. Thus, the actual plant back interval is considered to be in an acceptable range.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undertine)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year (a)	Commodity/ Variety (Plot) (a)	PBI (days) (b)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil: g a.s./ ha	Water (l/ha)	g a.s./hl	Dates of treatment or no. of treatments and last date (c)	Portion analyzed	Growth stage at sampling	Residues (mg/kg): Analyte 1 BCS-CN88460	Analyte 2 BCS-CR00082	DALT (days) (d)	Details on trial (e)
15-2502-02 [redacted] The Netherlands Northern Europe F 2015	Carrot/ Napa Washing (Plot T-1A)	22	1) 2015-05-07 3) 2015-08-10 - 2015-08-20	180	400	45	2015-04-15	Root	BBCH 48	<0.01	<0.01	106	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) root: 285 days leaf: 284 days
									BBCH 49	<0.01	<0.01	120	
								Leaf	BBCH 48	<0.01	<0.01	106	
									BBCH 49	<0.01	<0.01	120	
	Carrot/ Nerja late (Plot T-2A)	100	1) 2015-07-24 3) 2015-11-09 - 2015-11-16	180	400	45	2015-04-15	Root	BBCH 47	<0.01	<0.01	196	
									BBCH 49	<0.01	<0.01	210	
								Leaf	BBCH 47	<0.01	<0.01	196	
									BBCH 49	<0.01	<0.01	210	
	Carrot/ Nerac Orange (Plot T-3A)	365	1) 2016-04-14 3) 2016-08-10 - 2016-08-20	180	400	45	2015-04-15	Root	BBCH 48	<0.01	<0.01	471	
									BBCH 49	<0.01	<0.01	485	
								Leaf	BBCH 48	<0.01	<0.01	471	
									BBCH 49	<0.01	<0.01	485	

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year (a)	Commodity/ Variety (Plot) (a)	PBI (days) (b)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil: g a.s./ ha	Water (l/ha)	g a.s./hl	Dates of treatment or no. of treatments and last date (c)	Portion analyzed	Growth stage at sampling	Residues (mg/kg): Analyte 1 BCS-CN88460	Analyte 2 BCS-CR60082	DALT (days) (d)	Details on trial (e)
15-2502-03 [redacted] France Southern Europe F 2015	Turnip/ Navet rave d'auvergne (Plot T-1A)	20	1) 2015-08-27 3) 2015-11-05 - 2015-11-15	180	300	60	2015-08-07	Body	BBCH 48	<0.01	<0.01	83	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) body: 194 days leaf: 193 days
									BBCH 49	<0.01	<0.01	97	
								Leaf	BBCH 48	<0.01	<0.01	83	
									BBCH 49	<0.01	<0.01	97	
	Turnip/ Navet rave d'auvergne (Plot T-2A)	139	1) 2015-08-27 3) 2015-11-05 - 2015-11-15	180	300	60	2015-04-10	Body	BBCH 48	<0.01	<0.01	83	
									BBCH 49	<0.01	<0.01	97	
								Leaf	BBCH 48	<0.01	<0.01	83	
									BBCH 49	<0.01	<0.01	97	
	Turnip/ Navet de Croissy (Plot T-3A)	350	1) 2016-03-25 3) 2016-06-10 - 2016-06-20	180	300	60	2015-04-10	Body	BBCH 48	<0.01	<0.01	298	
									BBCH 49	<0.01	<0.01	311	
								Leaf	BBCH 48	<0.01	<0.01	298	
									BBCH 49	<0.01	<0.01	311	

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year	Commodity/ Variety (Plot)	PBI (days)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)		DALT (days)	Details on trial
				g a.s./ ha	Water (l/ha)	g a.s./hl				Analyte 1 BCS-CN88460	Analyte 2 BCS-CR00082		
	(a)	(b)					(c)					(d)	(e)
15-2502-04 [redacted] Italy Southern Europe F 2015	Carrot/ Nantes Clodia 2 (Plot T-1A)	28	1) 2016-05-12 3) 2015-08-03 - 2015-08-11	180	300	60	2015-04-14	Root	BBCH 46	<0.01	<0.01	93	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) root: 299 days leaf: 298 days
									BBCH 49	<0.01	<0.01	107	
								Leaf	BBCH 46	<0.01	<0.01	93	
									BBCH 49	<0.01	<0.01	107	
	Carrot/ Nantes Clodia 2 (Plot T-2A)	125	1) 2015-08-17 3) 2015-10-26 - 2015-11-10	180	300	60	2015-04-14	Root	BBCH 46	<0.01	<0.01	195	
									BBCH 49	<0.01	<0.01	209	
								Leaf	BBCH 46	<0.01	<0.01	195	
									BBCH 49	<0.01	<0.01	209	
	Carrot/ Nantes Clodia 2 (Plot T-3A)	353	1) 2016-04-01 3) 2016-06-01 - 2016-06-20	180	300	60	2015-04-14	Root	BBCH 45	<0.01	<0.01	419	
									BBCH 49	<0.01	<0.01	433	
								Leaf	BBCH 45	<0.01	<0.01	419	
									BBCH 49	<0.01	<0.01	433	

PBI = plant-back interval DALT: days after the last treatment

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Table 6.6.2- 7: Rotational trial summary for Lettuce (leafy vegetables)

Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)
Analyte 2: BCS-CR60082 (determined as BCS-CR60082, calculated as BCS-CN88460)

Trial No./ Location/ Year	Commodity/ Variety (Plot)	PBI (days)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)		DALT (days)	Details on trial
				g a.s./ ha	Water (L/ha)	g a.s./hL				Analyte 1 BCS-CN88460	Analyte 2 BCS-CR60082		
	(a)	(b)					(c)					(d)	(e)
15-2502-01 [redacted] Germany Northern Europe F 2015	Lettuce/ Aleppo (Plot T-1B)	28	1) 2015-04-09 3) 2015-06-01 – 2015-06-30	180	300	60	2015-03-12	Head	BBCH 46 BBCH 49	<0.01 <0.01	<0.01 <0.01	76 90	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) 341 days
	Lettuce/ Aleppo (Plot T-2B)	144	1) 2015-08-03 3) 2015-09-10 - 2015-09-21	180	300	60	2015-03-12	Head	BBCH 47 BBCH 49	<0.01 <0.01	<0.01 <0.01	132 186	
	Lettuce/ Aleppo (Plot T-3B)	370*	1) 2016-03-16 3) 2016-05-15 - 2016-05-31	180	300	60	2015-03-12	Head	BBCH 45 BBCH 49	<0.01 <0.01	<0.01 <0.01	424 438	
15-2502-02 [redacted] The Netherlands Northern Europe F 2015	Lettuce/ Salanova Butterhead (Plot T-1B)	21	1) 2015-05-06 3) 2015-07-01 - 2015-07-08	180	400	45	2015-04-15	Head	BBCH 45 BBCH 49	<0.01 <0.01	<0.01 <0.01	65 79	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) 318 days
	Lettuce/ Nadine Butterhead (Plot T-2B)	131	1) 2015-08-24 3) 2015-10-13 - 2015-10-20	180	400	45	2015-04-15	Head	BBCH 46 BBCH 49	<0.01 <0.01	<0.01 <0.01	170 184	
	Lettuce/ Nadine Butterhead (Plot T-3B)	365	1) 2016-04-14 3) 2016-06-03 - 2016-06-10	180	400	45	2015-04-15	Head	BBCH 45 BBCH 49	<0.01 <0.01	<0.01 <0.01	404 418	

* Plant back interval is slightly longer as reqested in the study protocol. However the timing for replanting of rotational crops as given in the OECD guideline on residues in rotational crops (limited fieldstudies) 504 suggests 270 to 365 days for crops rotated the following year. Thus, the actual plantback interval is considered to be in an acceptable range.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undertine)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year	Commodity/ Variety (Plot) (a)	PBI (days) (b)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date (c)	Portion analyzed	Growth stage at sampling	Residues (mg/kg)		DALT (days) (d)	Details on trial (e)
				g a.s./ ha	Water (L/ha)	g a.s./hL				Analyte 1 BCS-CN88460	Analyte 2 BCS-CR60082		
15-2502-03 [redacted] France Southern Europe F 2015	Lettuce/ Kiribati loose leaf lettuce (Plot T-1B)	26	1) 2015-05-06 3) 2015-06-10 - 2015-06-20	180	400	45	2015-04-10	Head	BBCH 47 BBCH 49	<0.01 <0.01	<0.01 <0.01	55 69	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) 333 days
	Lettuce/ Kirinia loose leaf lettuce (Plot T-2B)	139	1) 2015-08-27 3) 2015-10-10 - 2015-10-20	180	300	60	2015-04-10	Head	BBCH 48 BBCH 49	<0.01 <0.01	<0.01 <0.01	56 70	
	Lettuce/ Kiribati loose leaf lettuce (Plot T-3B)	348	1) 2016-03-23 3) 2016-05-10 - 2016-05-20	180	300	60	2015-04-10	Head	BBCH 48 BBCH 49	<0.01 <0.01	<0.01 <0.01	266 279	
15-2502-04 [redacted] Italy Southern Europe F 2015	Lettuce/ Gentile Estony Loose leaf (Plot T-1B)	28	1) 2015-05-12 3) 2015-05-12 - 2015-06-30	180	300	60	2015-04-14	Head	BBCH 46 BBCH 49	<0.01 <0.01	<0.01 <0.01	55 69	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) 329 days
	Lettuce/ Gentile Estony Loose leaf (Plot T-2B)	125	1) 2015-08-17 3) 2015-09-28 - 2015-10-05	180	300	60	2015-04-14	Head	BBCH 45 BBCH 49	<0.01 <0.01	<0.01 <0.01	146 160	
	Lettuce/ Gentile Estony Loose leaf (Plot T-3B)	353	1) 2016-04-01 3) 2016-05-11 - 2016-05-20	180	300	60	2015-04-14	Head	BBCH 45 BBCH 49	<0.01 <0.01	<0.01 <0.01	392 406	

PBI = plant-back interval DALT: days after the last treatment

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field

- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Table 6.6.2- 8: Rotational trial summary for Barley (small grain)

Analyte 1: BCS-CN88460 (determined as BCS-CN88460, calculated as BCS-CN88460)
Analyte 2: BCS-CR60082 (determined as BCS-CR60082, calculated as BCS-CN88460)

Trial No./ Location/ Year (a)	Commodity/ Variety (Plot) (a)	PBI (days) (b)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil: g a.s./ ha	Water (L/ha)	g a.s./hL	Dates of treatment or no. of treatments and last date (c)	Portion analyzed	Growth stage at sampling	Residues (mg/kg): Analyte 1 BCS-CN88460	Residues (mg/kg): Analyte 2 BCS-CR60082	DALT (days) (d)	Details on trial (e)
15-2502-01 [redacted] Germany Northern Europe F 2015	Barley/ Lomerit (Plot T-1C)	34*	1) 2015-09-29 2) 2016-05-15 - 2016-05-20 3) 2016-07-01 - 2016-07-15	180	300	60	2015-08-26	Green material	BBCH 30	<0.01	<0.01	222	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) green material: 169 days grain: 74 days straw: 78 days
									BBCH 75	<0.01	<0.01	279	
								Grain	BBCH 89	<0.01	<0.01	316	
								Straw	BBCH 89	<0.01	<0.01	316	
	Barley/ Lomerit (Plot T-2C)	201	1) 2015-09-29 2) 2016-05-15 - 2016-05-20 3) 2016-07-01 - 2016-07-15	180	300	60	2015-03-12	Green material	BBCH 30	<0.01	<0.01	389	
									BBCH 75	<0.01	<0.01	446	
								Grain	BBCH 89	<0.01	<0.01	483	
								Straw	BBCH 89	<0.01	<0.01	483	
	Barley/ Vespa (Plot T-3C)	369*	1) 2016-03-15 2) 2016-06-06 - 2016-06-10 3) 2016-08-01 - 2016-08-31	180	300	60	2015-03-12	Green material	BBCH 29	<0.01	<0.01	425	
									BBCH 75	<0.01	<0.01	466	
								Grain	BBCH 89	<0.01	<0.01	515	
								Straw	BBCH 89	<0.01	<0.01	515	

* Plant back intervals are slightly longer as reqested in the study protocol. However the timing for replanting of rotational crops as given in the OECD guideline on residues in rotational crops (limited field studies) 504 suggests 7 to 30 days for assessing circumstances of crop failure or closely rotated crops and at 270 to 365 days for crops rotated the following year. Thus, the actual plant back intervals are considered to be in an acceptable range.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undertime)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year	Commodity/ Variety (Plot)	PBI (days)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)		DALT (days)	Details on trial
				g a.s./ ha	Water (L/ha)	g a.s./hL				Analyte 1 BCS-CN88460	Analyte 2 BCS-CR60082		

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)

- * prior to last treatment
- ** residue in control
- # no data available

	(a)	(b)					(c)					(d)	(e)
15-2502-02 D The Netherlands Northern Europe F 2015	Barley/ Naomi Winter (Plot T-1C)	22	1) 2015-10-13 2) 2016-05-15 2016-06-01 3) 2016-07-09 - 2016-07-16	180	400	45	2015-09-21	Green material	BBCH 30 BBCH 75	<0.01 <0.01	<0.01 <0.01	198 280	(f) 15-2502 (g) EC 450 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) green material: 170 days grain: 69 days straw: 76 days
								Grain	BBCH 89	<0.01	<0.01	295	
								Straw	BBCH 89	<0.01	<0.01	295	
	Barley/ Naomi Winter (Plot T-2C)	181	1) 2015-10-13 2) 2016-05-15 2016-06-01 3) 2016-07-09 - 2016-07-16	180	400	45	2015-04-15	Green material	BBCH 30 BBCH 75	<0.01 <0.01	<0.01 <0.01	357 439	
								Grain	BBCH 89	<0.01	<0.01	454	
								Straw	BBCH 89	<0.01	<0.01	454	
	Barley/ Tipple Summer (Plot T-3C)	365*	1) 2016-04-14 2) 2016-06-01 2016-06-15 3) 2016-08-09 - 2016-08-16	180	400	45	2015-04-15	Green material	BBCH 30 BBCH 75	<0.01 <0.01	<0.01 <0.01	426 464	
								Grain	BBCH 89	<0.01	<0.01	485	
								Straw	BBCH 89	<0.01	<0.01	485	

* Plant back interval is slightly longer as reqested in the study protocol. However the timing for replanting of rotational crops as given in the OECD guideline on residues in rotational crops (limited field studies) 504 suggests 270 to 365 days for crops rotated the following year. Thus, the actual plant back interval is considered to be in an acceptable range.

Trial No./ Location/ Year	Commodity/ Variety (Plot)	PBI (days)	Date of 1.Sowing or planting	Application rate per treatment on bare soil			Dates of treatment or no. of treatments	Portion analyzed	Growth stage at sampling	Residues (mg/kg)		DALT (days)	Details on trial
				g a.s.	Water	g a.s./hl				Analyte 1	Analyte 2		

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undefine)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

	(a)	(b)	2.Flowering 3. Harvest	ha	(L/ha)		and last date (c)			BCS-CN88460	BCS-CR60082	(d)	(e)
15-2502-03 [redacted] France Southern Europe F 2015	Barley/ Augusta Winter Barley (Plot T-1C)	27	1) 2015-10-15 2) 2016-04-14 2016-04-20 3) 2016-06-10 - 2016-06-20	180	300	60	2015-09-18	Green material	BBCH 30 BBCH 75	<0.01 <0.01	<0.01 <0.01	153 234	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) green material: 259 days grain: 96 days straw: 103 days
								Grain	BBCH 89	<0.01	<0.01	271	
								Straw	BBCH 89	<0.01	<0.01	271	
	Barley/ Augusta Winter Barley (Plot T-2C)	188	1) 2015-10-15 2) 2016-04-14 2016-04-20 3) 2016-06-10 - 2016-06-20	180	300	60	2015-04-10	Green material	BBCH 30 BBCH 75	<0.01 <0.01	<0.01 <0.01	195 276	
								Grain	BBCH 89	<0.01	<0.01	313	
								Straw	BBCH 89	<0.01	<0.01	313	
	Barley/ Prestige Winter Barley (Plot T-3C)	299	1) 2016-02-03 2) 2016-05-01 2016-05-05 3) 2016-05-25 - 2016-05-31	180	300	60	2015-04-10	Green material	BBCH 30 BBCH 75	<0.01 <0.01	<0.01 <0.01	259 298	
								Grain	BBCH 89	<0.01	<0.01	327	
								Straw	BBCH 89	<0.01	<0.01	327	

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, undertine)
- (e) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

Trial No./ Location/ Year	Commodity/ Variety (Plot)	PBI (days)	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Application rate per treatment on bare soil			Dates of treatment or no. of treatments and last date	Portion analyzed	Growth stage at sampling	Residues (mg/kg)		DALT (days)	Details on trial
				g a.s./ ha	Water (L/ha)	g a.s./hL				Analyte 1 BCS-CN88460	Analyte 2 BCS-CR60082		
	(a)	(b)					(c)					(d)	(e)
15-2502-04 [redacted] Italy Southern Europe F 2015	Barley/ Ketos (Plot T-1C)	34	1) 2015-10-26 2) 2016-04-20 2016-04-27 3) 2016-06-26 - 2016-06-30	180	300	60	2015-09-22	Green material	BBCH 29	<0.01	<0.01	164	(f) 15-2502 (g) EC (50 g/L) (h) Spraying (i) 01475 (j) 0.01 mg/kg (k) Method Validation Data in 01475 and study 15-2502 (l) green material: 258 days grain: 89 days straw: 96 days
									BBCH 75	<0.01	<0.01	232	
								Grain	BBCH 89	<0.01	<0.01	275	
								Straw	BBCH 89	<0.01	<0.01	275	
	Barley/ Ketos (Plot T-2C)	195	1) 2015-10-26 2) 2016-04-20 2016-04-27 3) 2016-06-26 - 2016-06-30	180	300	60	2015-04-14	Green material	BBCH 30	<0.01	<0.01	325	
									BBCH 75	<0.01	<0.01	403	
								Grain	BBCH 89	<0.01	<0.01	436	
								Straw	BBCH 89	<0.01	<0.01	436	
	Barley/ Concerto (Plot T-3C)	345*	1) 2016-03-24 2) 2016-06-12 2016-06-19 3) 2016-03-01 - 2016-07-18	180	300	60	2015-04-14	Green material	BBCH 29	<0.01	<0.01	378	
									BBCH 75	<0.01	<0.01	420	
								Grain	BBCH 89	<0.01	<0.01	443	
								Straw	BBCH 89	<0.01	<0.01	443	

PBI = plant-back interval DALT: days after the last treatment

* Plant back interval is slightly longer as reqested in the study protocol. However the timing for replanting of rotational crops as given in the OECD guideline on residues in rotational crops (limited field studies) 504 suggests 270 to 365 days for crops rotated the following year. Thus, the actual plant back interval is considered to be in an acceptable range.

- (a) According to CODEX Classification / Guide
- (b) Only if relevant
- (c) Year must be indicated
- (d) Days after last application (Label pre-harvest interval, PHI, underline)
- (e) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
- (f) Study reference
- (g) Formulation type
- G greenhouse F field
- (h) Application method
- (i) Method information
- (j) LOQ
- (k) Method validation
- (l) Storage (max)
- * prior to last treatment
- ** residue in control
- # no data available

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

Report:	KCA 6.7.1/01; [redacted], R.; [redacted]; [redacted], E.; 2018; M-612432-01-1
Title:	Isoflucypram (BCS-CN88460): Evaluation of dietary metabolites and residue definition proposals
Report No.:	M-612432-01-1
Document No.:	M-612432-01-1
Guideline(s):	none
Guideline deviation(s):	--
GLP/GEP:	**no**

For the discussion on the residues of isoflucypram potentially relevant for risk assessment as a first step, consideration was given to the residue levels found in the available metabolism studies.

Conclusively identified metabolites were considered for potential inclusion into the residue definition for risk assessment, if they exceeded the level of significance of ≥ 10% of the TRR and ≥ 0.01 mg/kg in food of plant origin from the metabolism studies. If they represented less than 10% of the TRR, only metabolites reaching levels ≥ 0.05 mg/kg were selected. For plant feed items the criterion for consideration was ≥ 10% of the TRR and ≥ 0.01 mg/kg. For food of animal origin, metabolites representing ≥ 10% of the TRR were selected.

The consumer exposure was further refined based on the field studies from representative uses (cereals), the field rotational crops study and the livestock feeding studies. The pre-dominance of the metabolites in food versus feed was considered as well as their contribution to the overall consumer exposure.

The assessment of the metabolites toxicity indicated that they are not expected to exert a higher toxicity or additional hazards beyond those identified for isoflucypram. They are considered non-relevant based on the toxicological and structural considerations and are as well covered by the toxicological endpoints for the parent compound.

Besides, as shown in the following table, the consumer exposure to these metabolites is far below the chronic and acute Toxicological Threshold of Concern (TTC) for Cramer class III compounds.

Metabolite	Cramer Class	% of the chronic TTC of 1.5 µg/kg bw/d	% of the acute TTC of 5 µg/kg bw/d
BCS-CN88460-propanol (M01) and its conjugates	III	1.3	4.7
BCS-CN88460-2-propanol (M02) and its conjugates	III	0.85	1.7
BCS-CN88460-desmethyl-propanol (M06) and its conjugates	III	0.61	0.63
BCS-CN88460-desmethyl-1,2-propandiol (M07) and its conjugate	III	0.02	0.14
BCS-CN88460-desmethyl-carboxylic acid (M11)	III	0.02	0.21
BCS-CN88460-carboxylic acid (M12)	III	1.1	1.2

Therefore, it is concluded that further toxicity studies are not needed for the metabolites.

Based on all this information **the residue definition for risk assessment and for monitoring in plant and animal commodities** is proposed as follows: **Isoflucypram parent only**.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

► Plant matrices

The proposed residue definition for MRL setting in plant in the EU is isoflucypram parent only.

MRLs are calculated and proposed based on the available residue data in support of the representative uses of BCS-CN88460 in cereals (see Table 6.3.1- 7 for barley and Table 6.3.2- 7 for wheat). Calculations of MRLs were carried out using the OECD MRL calculator (ENV/JM/MONO(2011)2 of 01-Mar-2011.

Barley (extrapolated to oat)

Residue data from northern Europe were compared to residue data from southern Europe. Since residues in grain at harvest are often <0.01 mg/kg, the comparison was done using the results in straw at harvest.

The results of the statistical tests Kruskal-Wallis H-Test and Mann-Whitney U-test, $\alpha = 0.05$ indicate that the straw residue data from northern Europe are not significantly different from the straw residue data in southern Europe. Thus it is suitable to combine the straw data from both regions.

Three MRL calculations were made using:

- Grain residue data from southern Europe
- Grain residue data from northern Europe
- Grain residue data from both southern and northern Europe

Table 6.7.2- 1 shows the results of the MRL calculations for barley grain using the OECD MRL calculator, including the residue endpoints (STMR and HR) for barley grain and straw.

Table 6.7.2- 1: Barley - OECD MRL calculations

Crop	Region/Indoor (a)	Trial results relevant to the critical GAP	MRL calculation (d)	STMR (b)	HR (c)
Grain	NEU	10 × <0.01; 0.013; 0.020; 0.041	**0.05**	<0.01	0.041
	SEU	9 × <0.01; 0.022; 0.027; 0.037	**0.05**	<0.01	0.037
	N+SEU	19 × <0.01; 0.013; 0.020; 0.022; 0.027; 0.037; 0.041	**0.05**	<0.01	0.041
Straw	NEU	0.049; 0.11; 0.13; 0.16; 0.20; 0.24; 0.32; 0.40; 0.44; 0.51; 0.94; 0.96; 1.2	-	0.32	1.2
	SEU	0.021; 0.13; 0.16; 0.18; 0.24; 0.29; 0.29; 0.31; 0.85; 0.96; 1.0; 3.1	-	0.29	3.1
	N+SEU	0.049; 0.11; 0.13; 0.16; 0.20; 0.24; 0.32; 0.40; 0.44; 0.51; 0.94; 0.96; 1.2; 0.021; 0.13; 0.16; 0.18; 0.24; 0.29; 0.29; 0.31; 0.85; 0.96; 1.0; 3.1	0.29	0.29	3.1

(a) **NEU** or **SEU** for northern or southern **outdoor** trials in EU member states (**N+SEU** if both zones), Indoor for glasshouse/protected crops
(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue
(d) using OECD MRL calculator

An MRL of 0.05 mg/kg is therefore proposed for barley grain (code 0500010), based on all European residue data.

An extrapolation of this MRL of 0.05 mg/kg to oat grain (code 0500090) is requested, as permitted by the Guidance document SANCO/7525/VI/95 Rev. 10.2 dated of 23 September 2016 (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs).

Wheat (extrapolated to rye, spelt and triticale)

Residue data from northern Europe were compared to residue data from southern Europe. Since residues in grain at harvest are often <0.01 mg/kg, the comparison was done using the results in straw at harvest.

The results of the statistical tests Kruskal-Wallis H-Test and Mann-Whitney U-test, $\alpha = 0.05$ indicate that the straw residue data from northern Europe are not significantly different from the straw residue data in southern Europe. Thus it is suitable to combine the straw data from both regions.

Three MRL calculations were made using:

- Grain residue data from southern Europe
- Grain residue data from northern Europe
- Grain residue data from both southern and northern Europe

Table 6.7.2- 2 shows the results of the MRL calculations using the OECD MRL calculator, including the residue endpoints (STMR and HR) for barley grain.

Table 6.7.2- 2: Wheat - OECD MRL calculations

Crop	Region/Indoor (a)	Trial results relevant to the critical GAP	MRL calcula-tion (d)	STMR (b)	HR (c)
Grain	NEU	12 × <0.01	**0.01**	<0.01	<0.01
	SEU	11 × <0.010; 0.042	**0.05**	<0.01	0.042
	N+SEU	23 × <0.01; 0.042	**0.05**	<0.01	0.042
Straw	NEU	0.054; 0.071; 0.12; 0.19; 0.38; 0.40; 0.82; 0.94; 1.5; 1.7; 3.3; 3.6	-	0.61	3.6
	SEU	0.22; 0.33; 0.41; 0.87; 1.3; 1.4; 1.6; 1.8; 1.9; 1.9; 2.3; 2.4	-	1.5	2.4
	N+SEU	0.054; 0.071; 0.12; 0.19; 0.38; 0.40; 0.82; 0.94; 1.5; 1.7; 3.3; 3.6; 0.22; 0.33; 0.41; 0.87; 1.3; 1.4; 1.6; 1.8; 1.9; 1.9; 2.3; 2.4	-	0.29	3.6

(a) **NEU** or **SEU** for northern or southern **outdoor** trials in EU member states (**N+SEU** if both zones), Indoor for glasshouse/protected crops
(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue
(d) using OECD MRL calculator
* code according to Annex 1 of Regulation 396/2005

An MRL of 0.05 mg/kg is therefore proposed for wheat grain (code 0500090), based on southern European residue data.

An extrapolation of this MRL of 0.05 mg/kg to rye grain (code 0500070) is requested, as permitted by the Guidance document SANCO 7525/VI/95 Rev. 10.2 dated of 23 September 2016 (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs).

Other crops

Residues of BCS-CN88460 parent compound in rotational crops are expected to be <0.01 mg/kg. Thus, for all other crops than barley, oat, wheat, rye, spelt and triticale, an MRL of 0.01 mg/kg (at the LOQ level of the enforcement method) is proposed.

► Animal matrices

The proposed residue definition for MRL setting in animal commodities in the EU is isoflucypram parent only.

Based on the dairy cow feeding study (see [redacted]; 2017; M-604191-02-1; KCA 6.4.2), at the feeding dose of 0.15 mg/kg bw/d, residues of isoflucypram (BCS-CN88460) parent compound were <0.005 mg/kg (LOQ) in milk and <0.01 mg/kg in muscle, liver, kidney, perirenal fat and subcutaneous fat. The highest residue level of parent was 0.01 mg/kg in mesenteric fat (in 1 cow out of three) at the feeding dose of 0.15 mg/kg bw/d. Considering the maximum European anticipated dietary burdens of 0.089 mg/kg bw/d for sheep (lamb) and 0.041 mg/kg bw/d for cattle (see Table 6.4- 2), it is concluded that residues of parent compound are expected to be <LOQ in milk and ruminant tissues.

Based on the laying hen feeding study (see [redacted]; [redacted], E.; 2017; M-605909-01-1; KCA 6.4.1), at the feeding dose of 0.03 mg/kg bw/d, residues of isoflucypram (BCS-CN88460) parent compound were found <0.01 mg/kg (LOQ) in eggs and poultry tissues. Considering the maximum European anticipated dietary burden of 0.029 mg/kg bw/d for laying poultry (see Table 6.4- 2), it is concluded that residues of parent compound are expected to be <LOQ in eggs and poultry tissues

Therefore the European MRLs in animal commodities resulting from exposure of livestock to feed crops treated with BCS-CN88460 are proposed in Table 6.7.2- 3.

Table 6.7.2- 3: Animal commodities

Commodities	Code No. **	MRL proposal	STMR (a)	HR (b)
Tissues from swine, bovine, sheep, goat, equine, poultry and other farmed terrestrial animals	1010000	0.01 *	<0.01	<0.01
Milk	1020000	0.005 *	<0.005	<0.005
Birds'eggs	1030000	0.01 *	<0.01	<0.01

* at the limit of quantification of the enforcement method
** code according to Annex 1 of Regulation 396/2005
(a) **STMR**: Supervised Trials Median Residue
(b) **HR**: Highest residue

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

Since imported products are not considered in this dossier submission MRL settings for import tolerances are not required.

CA 6.8 Proposed safety intervals

Pre-harvest intervals are not proposed since the application timing is defined by the crop growth stage (latest BBCH 61 for barley & oats and latest BBCH 69 for wheat, rye, spelt and triticale).

Further safety intervals are not necessary.

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

In order to evaluate the potential chronic and acute exposures to BCS-CN88460 residues through the diet, calculations were done using the EFSA PRIMo model (revision 2) and the following toxicological endpoints.

Table 6.9- 1: Toxicological endpoints for BCS-CN88460

Active Substance	Toxicological End-Point	Proposed Value (mg/kg bw/day)
Isoflucypram (BCS-CN88460)	Acceptable Daily Intake (ADI)	0.063
	Acute Reference Dose (ARfD)	1.25

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI

The TMDI calculations were conducted based on the residue values presented in Table 6.9- 2.

Table 6.9- 2: Input values for the chronic consumer risk assessment (TMDI)

Commodity	Chronic risk assessment	
	Input value (mg/kg)	Comment
Barley grain	0.05	Proposed MRL
Oat grain	0.05	Proposed MRL
Wheat grain	0.05	Proposed MRL
Rye grain	0.05	Proposed MRL
All other crops	0.01 *	Default MRL
Milks and cream	0.005 *	Proposed MRL = LOQ of the method for the parent compound
Bird's Eggs	0.01 *	
Meat, preparation of meat, offals,…	0.01 *	

* At the limit of quantification of the method

As shown in Table 6.9- 4, using the EFSA PRIMo model (revision 2), the highest TMDI was calculated for the "WHO Cluster Diet B" and represents **1.3% of the ADI** with the highest contributors being wheat, vegetables and fruits.

The TMDI calculations were far below the ADI. Therefore, a long-term intake of residues of BCS-CN88460 is unlikely to present a public health concern.

IEDI

Since the TMDI calculations demonstrate a large margin of safety the IEDI was not used for refinement.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

The IESTI/NESTI calculations were conducted based on the residue values presented in Table 6.9- 3. Proposed MRLs were used as input values for cereals in a very conservative approach since usually median residues can be applied for such blended commodities.

Table 6.9- 3: Input values for the acute consumer risk assessment

Commodity	Acute risk assessment	
	Input value (mg/kg)	Comment
Barley grain	0.05	Proposed MRL
Oat grain	0.05	Proposed MRL
Wheat grain	0.05	Proposed MRL
Rye grain	0.05	Proposed MRL
Milks and cream	0.005	Proposed MRL = LOQ of the method for the parent compound
Bird's Eggs	0.01	
Meat, preparation of meat, offals,…	0.01	

As shown in Table 6.9- 4, using the EFSA PRIMo model (revision 2), the highest IESTI was calculated for wheat consumed by children, corresponding to **0.06 % of the ARfD**. No exceedance of the ARfD was identified for any unprocessed commodity, or for processed commodities.

A short-term intake of residues of BCS-CN88460 is therefore unlikely to present a public health risk.

Table 6.9- 4: Isoflucypram (BCS-CN88460) - TMDI calculations according to the EFSA model (rev 2.0)

isoflucypram			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.063	ARfD (mg/kg bw):	1.25
Source of ADI:	isoflucypram	Source of ARfD:	isoflucypram
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

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
	0	1
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)
1.3	WHO Cluster diet B	0.7	Wheat	0.2	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	
1.2	DK child	0.4	Wheat	0.4	Rye	0.1	VEGETABLES	
1.2	UK Toddler	0.4	SUGAR PLANTS	0.3	Wheat	0.2	Milk and cream,	
1.1	NL child	0.4	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.1	Milk and cream,	
1.1	DE child	0.4	FRUIT (FRESH OR FROZEN)	0.3	Wheat	0.1	VEGETABLES	
1.1	FR toddler	0.3	Milk and cream,	0.3	VEGETABLES	0.2	Wheat	
1.0	UK Infant	0.3	Milk and cream,	0.2	Wheat	0.2	SUGAR PLANTS	
0.9	FR infant	0.3	VEGETABLES	0.2	FRUIT (FRESH OR FROZEN)	0.2	Milk and cream,	
0.9	WHO cluster diet D	0.5	Wheat	0.1	VEGETABLES	0.0	FRUIT (FRESH OR FROZEN)	
0.8	WHO cluster diet E	0.3	Wheat	0.1	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	
0.8	IE adult	0.2	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.1	VEGETABLES	
0.7	ES child	0.4	Wheat	0.1	Milk and cream,	0.1	FRUIT (FRESH OR FROZEN)	
0.7	SE general population 90th percentile	0.3	Wheat	0.2	VEGETABLES	0.1	Milk and cream,	
0.7	WHO Cluster diet F	0.3	Wheat	0.1	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	
0.7	IT kids/toddler	0.3	Wheat	0.1	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	
0.6	WHO regional European diet	0.2	Wheat	0.1	VEGETABLES	0.1	Meat, preparations of meat,	
0.6	PT General population	0.3	Wheat	0.1	FRUIT (FRESH OR FROZEN)	0.1	VEGETABLES	
0.5	FR all population	0.3	Wheat	0.1	FRUIT (FRESH OR FROZEN)	0.1	VEGETABLES	
0.5	NL general	0.2	Wheat	0.1	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	
0.5	ES adult	0.2	Wheat	0.1	FRUIT (FRESH OR FROZEN)	0.1	VEGETABLES	
0.5	IT adult	0.3	Wheat	0.1	VEGETABLES	0.0	FRUIT (FRESH OR FROZEN)	
0.4	UK vegetarian	0.2	Wheat	0.1	SUGAR PLANTS	0.1	VEGETABLES	
0.4	DK adult	0.2	Wheat	0.1	VEGETABLES	0.1	Rye	
0.4	UK Adult	0.2	Wheat	0.1	SUGAR PLANTS	0.0	VEGETABLES	
0.4	LT adult	0.1	Rye	0.1	Wheat	0.1	VEGETABLES	
0.3	FI adult	0.1	Wheat	0.1	Rye	0.0	Milk and cream,	
0.2	PL general population	0.1	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	0.0	PULSES, DRY	

Conclusion:

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

Table 6.9- 5: Isoflucypram (BCS-CN88460) - IESTI calculations according to the EFSA model (rev 2.0)

	Acute risk assessment /children						Acute risk assessment / adults / general population					
	The acute risk assessment is based on the ARfD.											
	For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.											
	In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.											
	Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
Unprocessed commodities	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
	IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	0.06	Wheat	0.05 / -	0.06	Wheat	0.05 / -	0.03	Wheat	0.05 / -	0.03	Wheat	0.05 / -
	0.05	Milk and milk	0.005 / -	0.05	Milk and milk	0.005 / -	0.03	Barley	0.05 / -	0.03	Barley	0.05 / -
	0.03	Rye	0.05 / -	0.03	Rye	0.05 / -	0.02	Rye	0.05 / -	0.02	Rye	0.05 / -
	0.02	Oats	0.05 / -	0.02	Oats	0.05 / -	0.01	Poultry: Meat	0.01 / -	0.01	Poultry: Meat	0.01 / -
	0.01	Bovine: Meat	0.01 / -	0.01	Bovine: Meat	0.01 / -	0.01	Milk and milk	0.005 / -	0.01	Milk and milk products: Cattle	0.005 / -
	0.01	Meat, preparations of	0.01 / -	0.01	Meat, preparations	0.01 / -	0.01	Oats	0.05 / -	0.01	Oats	0.05 / -
	0.01	Birds' eggs	0.01 / -	0.01	Birds' eggs	0.01 / -	0.00	Bovine: Meat	0.01 / -	0.00	Bovine: Meat	0.01 / -
	0.01	Milk and milk products: Goat	0.005 / -	0.01	Milk and milk products: Goat	0.005 / -	0.00	Swine: Meat	0.01 / -	0.00	Swine: Meat	0.01 / -
	0.01	Poultry: Meat	0.01 / -	0.01	Poultry: Meat	0.01 / -	0.00	Sheep: Meat	0.01 / -	0.00	Sheep: Meat	0.01 / -
	0.01	Sheep: Meat	0.01 / -	0.01	Sheep: Meat	0.01 / -	0.00	Poultry: Liver	0.01 / -	0.00	Poultry: Liver	0.01 / -
	0.01	Barley	0.05 / -	0.01	Barley	0.05 / -	0.00	Birds' eggs	0.01 / -	0.00	Birds' eggs	0.01 / -
	0.01	Swine: Meat	0.01 / -	0.01	Swine: Meat	0.01 / -	0.00	Milk and milk	0.005 / -	0.00	Milk and milk products: Goat	0.005 / -
	0.01	Bovine: Liver	0.01 / -	0.01	Bovine: Liver	0.01 / -	0.00	Bovine: Edible offal	0.01 / -	0.00	Bovine: Edible offal	0.01 / -
	0.01	Bovine: Edible offal	0.01 / -	0.01	Bovine: Edible	0.01 / -	0.00	Bovine: Liver	0.01 / -	0.00	Bovine: Liver	0.01 / -
	0.00	Horse: Meat	0.01 / -	0.00	Horse: Meat	0.01 / -	0.00	Other farm animals:	0.01 / -	0.00	Other farm animals: Meat	0.01 / -
	0.00	Other farm animals:	0.01 / -	0.00	Other farm	0.01 / -	0.00	Bovine: Kidney	0.01 / -	0.00	Bovine: Kidney	0.01 / -
	0.00	Bovine: Kidney	0.01 / -	0.00	Bovine: Kidney	0.01 / -	0.00	Goat: Meat	0.01 / -	0.0	Goat: Meat	0.01 / -
	No of critical MRLs (IESTI 1)			---			**No of critical MRLs (IESTI 2)**			---		
Processed commodities	**No of commodities for which ARfD/ADI is exceeded:**						**No of commodities for which ARfD/ADI is exceeded:**					
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)				Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
	0.05	Wheat flour	0.05 / -				0.02	Bread/pizza	0.05 / -			
	*) The results of the IESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all IESTI values > 90% of ARfD are reported.											
	**) pTMRL: provisional temporary MRL											
	***) pTMRL: provisional temporary MRL for unprocessed commodity											
	Conclusion:											
	For isoflucypram IESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available.											
	No exceedance of the ARfD/ADI was identified for any unprocessed commodity.											
	For processed commodities, no exceedance of the ARfD/ADI was identified.											

CA 6.10 Other studies

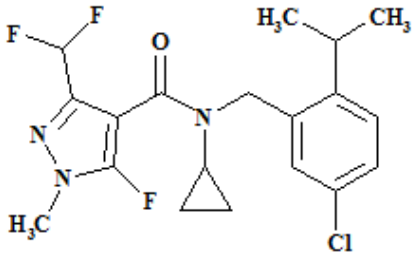
No further residue studies were deemed necessary to support the registration of the active substance isoflucypram.

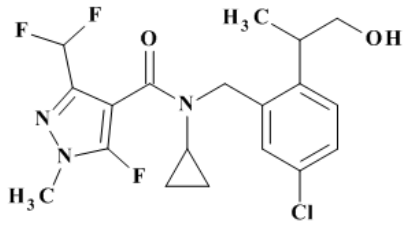
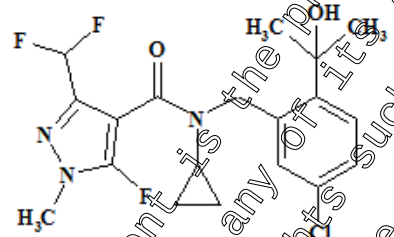
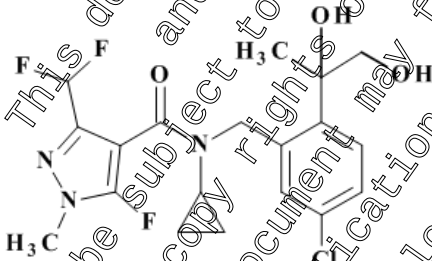
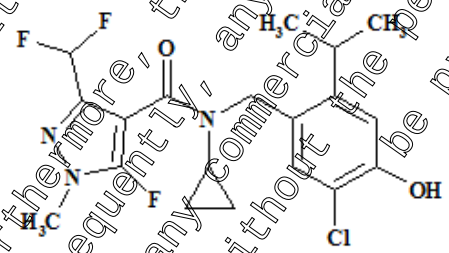
CA 6.10.1 Effect on the residue level in pollen and bee products

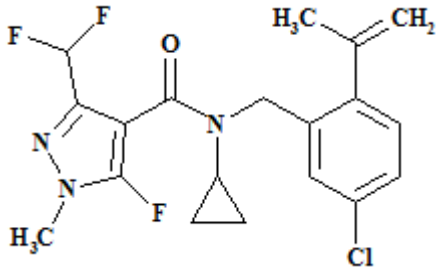
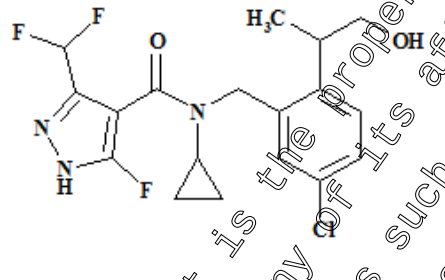
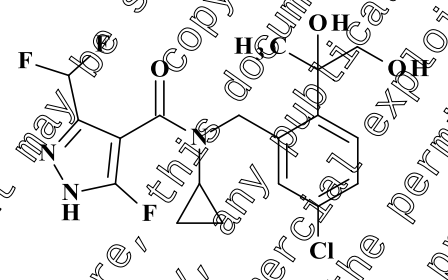
No residue study in pollen and bee products was conducted. Currently, no test method or Guidance document is available for conducting residue studies in pollen or bee products. In these cases, waiving of this particular data requirement is considered acceptable according to the "Guidance document for 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).

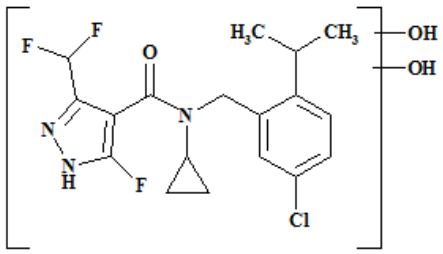
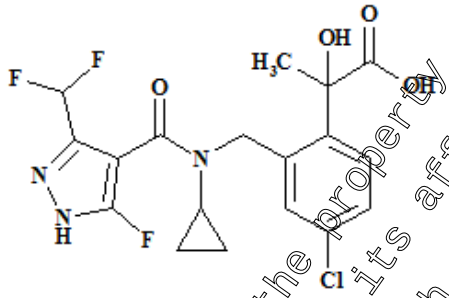
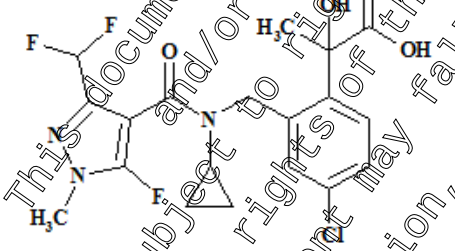
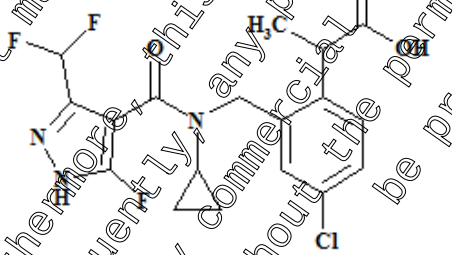
Moreover, the current draft guidelines relating to determining the magnitude of pesticide residues in bee products and setting specific Maximum Residue Levels in honey (SANCO 11956/2016 rev.6 dated of 20 November 2017) indicates that cereals have no melliferous capacities. Therefore, residues in honey are not expected following application of isoflucypram on cereals.

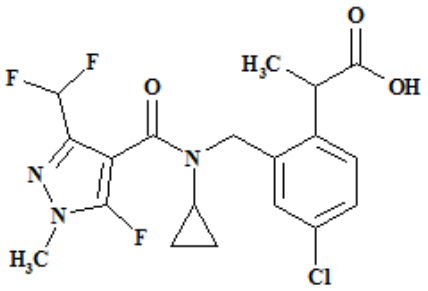
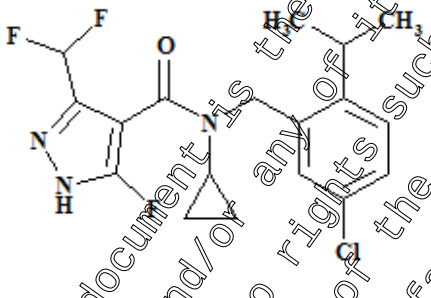
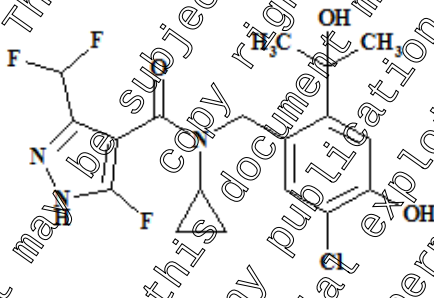
Isoflucypram: substances and metabolites; structures, codes, synonyms

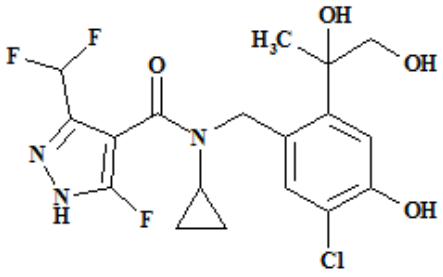
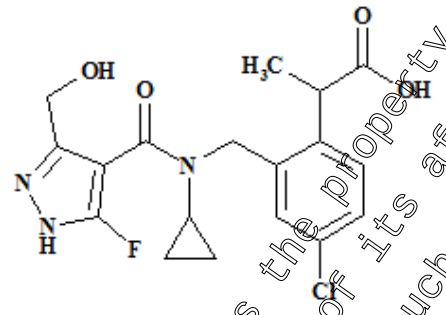
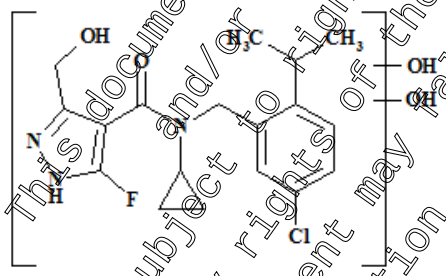
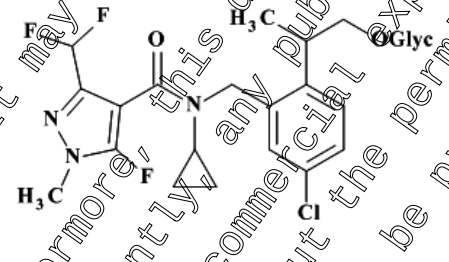
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
a.s.	 *C19 H21 Cl F3 N3 O* *[399] nominal mass* *399.84 g/mol (molecular weight)*	**isoflucypram** BCS-CN88460 *CAS: 1255734-28-1* ISY LYAM823-1-2 *smiles code:* *C1(C(F)F)C(C(=O)N(C(C=C(C(C)C)C=C(Cl)=C3)C2C)C2)=C(F)N(C)N=1*	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]* 1H-pyrazole-4-carboxamide, N-[[5-chloro-2-(1-methylethyl)phenyl]methyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl- *[CA]*	animals: rat (faeces, liver, kidney); hen (eggs, muscle leg, fat); goat (milk, muscle, fat, liver, kidney, faeces) sunfish (edible parts, viscera) plant: soybean (forage, hay, straw, seed); wheat (hay, straw, grain); CRC (wheat forage, Swiss chard, turnip leaves); oilseed rape (intermediate harvest, mature plants, seeds), tomatoes soil: aerobic & anaerobic, field dissipation, photolysis water: hydrolysis, photolysis, water-sediment

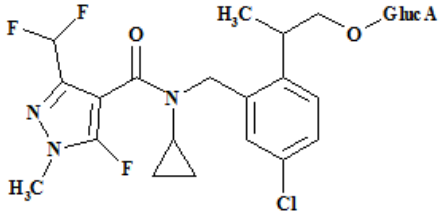
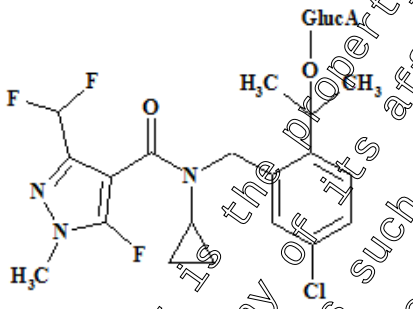
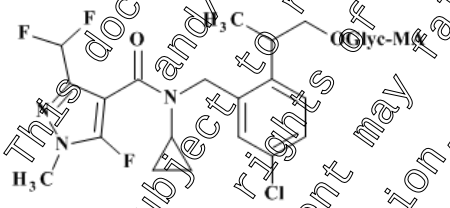
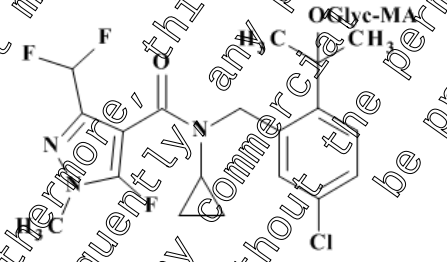
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M01	 *C19 H21 Cl F3 N3 O2* *[415]*	**BCS-CN88460-propanol** BCS-CY24813	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (faeces); hen (eggs, muscle, fat, liver, excreta); goat (muscle, fat, liver, kidney, faeces, urine); sunfish (edible parts, viscera) plant: wheat hay, straw soil: - water: -
M02	 *C19 H21 Cl F3 N3 O2* *[415]*	**BCS-CN88460-2-propanol** BCS-DC20298	N-[5-chloro-2-(2-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (faeces); goat (milk, muscle, fat, liver, kidney, faeces, urine) plant: - soil: - water: -
M03	 *C19 H21 Cl F3 N3 O3* *[431]*	**BCS-CN88460-1,2-propandiol**	N-[5-chloro-2-(1,2-dihydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: hen (excreta) plant: - soil: - water: -
M04	 *C19 H21 Cl F3 N3 O2* *[415]*	**BCS-CN88460-hydroxyphenyl**	N-(5-chloro-4-hydroxy-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: goat (urine) plant: - soil: - water: -

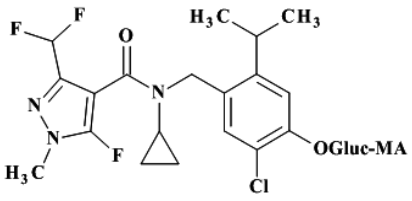
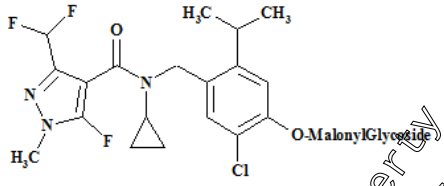
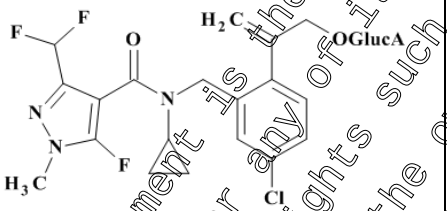
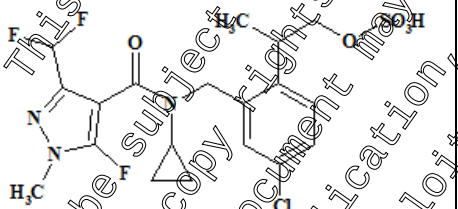
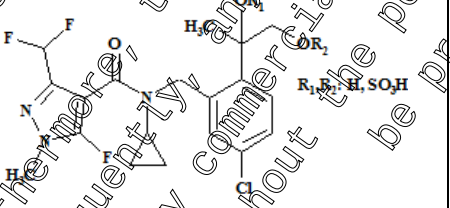
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M05	 *C19 H19 Cl F3 N3 O* *[397]*	**BCS-CN88460-olefine** ROI 2 *CAS: 1471984-41-4*	N-[5-chloro-2-(prop-1-en-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]* 1H-Pyrazole-4-carboxamide, N-[[5-chloro-2-(1-methylethenyl)phenyl]methyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl- *[CA]*	animal: rat (faeces, bile) plant: - soil: - water: -
M06	 *C18 H19 Cl F3 N3 O2* *[401]*	**BCS-CN88460-desmethyl-propanol** BCS-DC22055	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (faeces, bile); hen (eggs, muscle, fat, liver, excreta); goat (milk, muscle, fat, liver, kidney, faeces, urine); sunfish (edible parts, viscera) plant: wheat (straw) soil: - water: -
M07	 *C18 H19 Cl F3 N3 O3* *[417]*	**BCS-CN88460-desmethyl-1,2-propandiol**	N-[5-chloro-2-(1,2-dihydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: hen (eggs, muscle, fat, liver, excreta) plant: - soil: - water: -

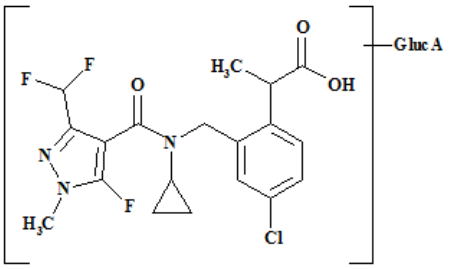
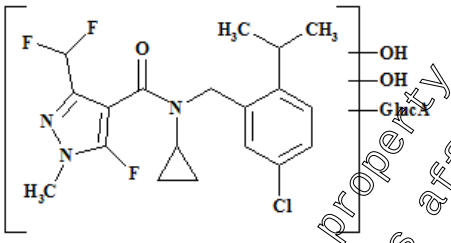
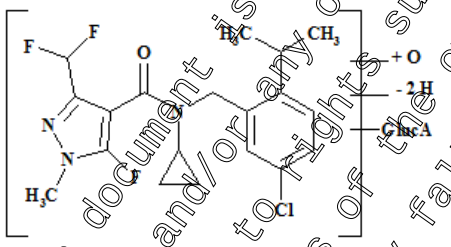
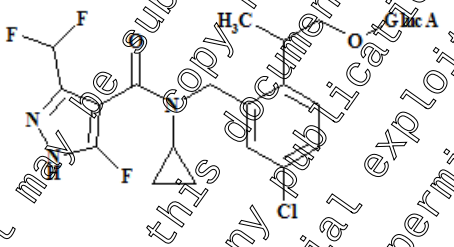
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M08	 *C18 H19 Cl F3 N3 O3* *[417]*	**BCS-CN88460-desmethyl-diOH** (isomers)		animal: rat (faeces) plant: - soil: - water: -
M09	 *C18 H17 Cl F3 N3 O4* *[431]*	**BCS-CN88460-desmethyl-lactic acid**	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}-2-hydroxypropanoic acid *[IUPAC]*	animal: rat (faeces, plasma, liver, kidney, bile) plant: - soil: - water: -
M10	 *C19 H19 Cl F3 N3 O4* *[445]*	**BCS-CN88460-lactic acid** ROI 3 M10	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}-2-hydroxypropanoic acid *[IUPAC]*	animal: rat (faeces, plasma, liver, kidney, bile); goat (liver, kidney, faeces, urine) plant: - soil: met., aerobic water: -
M11	 *C18 H17 Cl F3 N3 O3* *[415]*	**BCS-CN88460-desmethyl-carboxylic acid** BCS-CX99799 ROI 5 M11	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid *[IUPAC]*	animal: rat (urine, faeces, plasma, liver, kidney, bile); hen (muscle, fat, liver, excreta); goat (liver, kidney, faeces, urine) plant: - soil: met., aerobic water: -

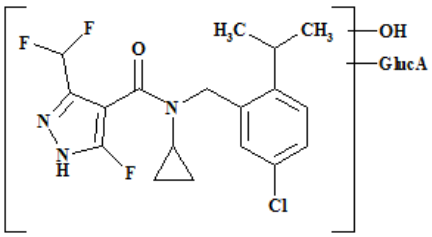
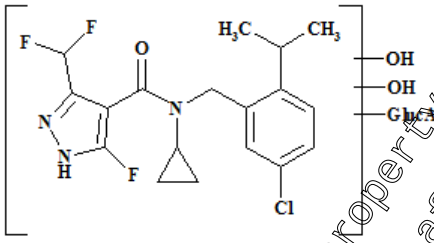
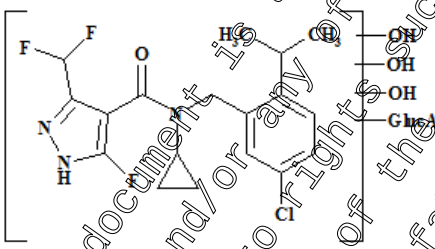
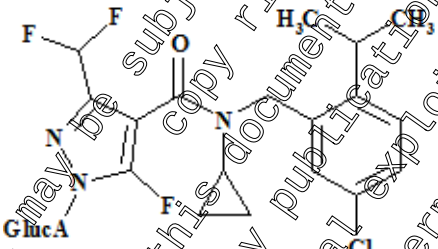
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M12	 *C19 H19 Cl F3 N3 O3* *[429]*	**BCS-CN88460-carboxylic acid** BCS-CY26497 MXM 7275-1-5 ROI 1 M12 *smiles code:* *C1(CN(C(=O)C3C(C(F)F)=NN(C)C=3F)C2CC2)=C(C(C)C(=O)O)C=CC(Cl)=C1*	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]-carbonyl}amino)-methyl]phenyl}-propanoic acid *[IUPAC]*	animal: rat (urine, faeces, plasma, liver, kidney, bile), hen (eggs, muscle, fat, liver, excreta); goat (muscle, fat, liver, kidney, faeces, urine) plant: soil: met., aerobic water: met., aerobic
M13	 *C18 H19 Cl F3 N3 O* *[385]*	**BCS-CN88460-desmethyl** M13	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (faeces, plasma, liver, kidney); sunfish (edible parts, viscera) plant: - soil: - water: -
M14	 *C18 H19 Cl F3 N3 O3* *[417]*	**BCS-CN88460-desmethyl-hydroxyphenyl-2-propanol**	N-[5-chloro-4-hydroxy-2-(2-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (urine, faeces, liver, bile) plant: - soil: - water: -

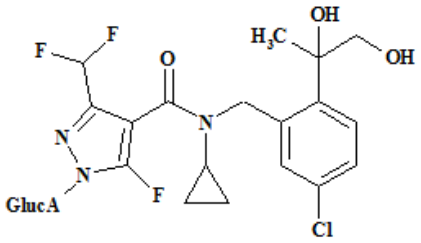
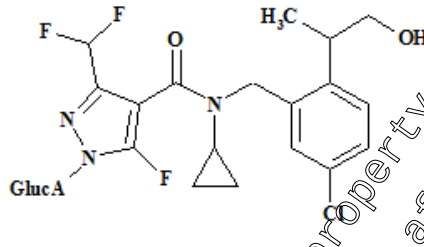
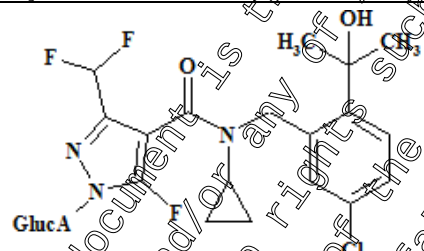
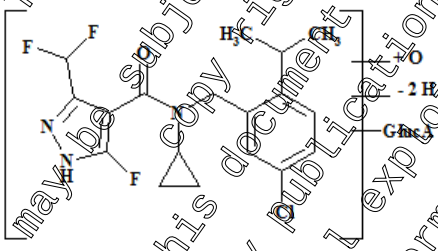
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M15	 *C18 H19 Cl F3 N3 O4* *[433]*	**BCS-CN88460-desmethyl-hydroxyphenyl-1,2-propandiol**	N-[5-chloro-2-(1,2-dihydroxypropan-2-yl)-4-hydroxybenzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (faeces) plant: - soil: - water: -
M16	 *C18 H19 Cl F N3 O4* *[395]*	**BCS-CN88460-desmethyl-hydroxymethyl-carboxylic acid**	2-{4-chloro-2-[(cyclopropyl{[5-fluoro-3-(hydroxymethyl)-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid *[IUPAC]*	animal: rat (urine, faeces) plant: - soil: - water: -
M17	 *C18 H21 Cl F N3 O4* *[397]*	**BCS-CN88460-desmethyl-hydroxymethyl-diOH**		animal: rat (faeces, bile) plant: - soil: - water: -
M18	 *C25 H31 Cl F3 N3 O7* *[577]*	**BCS-CN88460-propanol-Glyc**	N-{5-chloro-2-[1-(hexopyranosyloxy)propan-2-yl]benzyl}-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: - plant: wheat (hay, straw) soil: - water: -

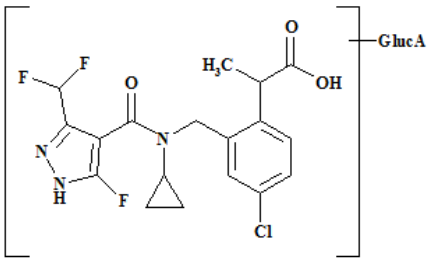
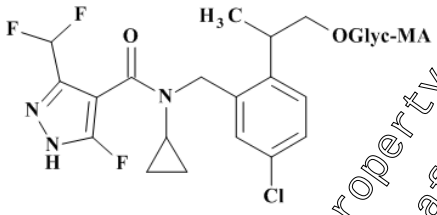
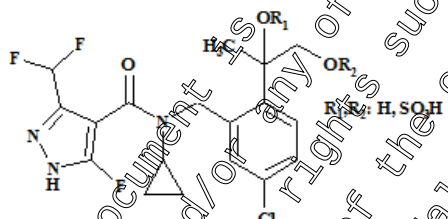
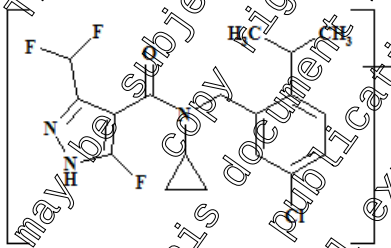
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M19	 *C25 H29 Cl F3 N3 O8* *[591]*	**BCS-CN88460-propanol-GlucA** (isomer 1 and 2)	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl glucopyranosiduronic acid *[IUPAC]*	animal: rat (faeces, bile); goat (milk, muscle, liver, kidney, faeces, urine); sunfish (edible parts, viscera) plant: - soil: - water: -
M20	 *C25 H29 Cl F3 N3 O8* *[591]*	**BCS-CN88460-2-propanol-GlucA**	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propan-2-yl beta-D-glucopyranosiduronic acid *[IUPAC]*	animal: goat (liver, kidney, faeces, urine) plant: - soil: - water: -
M21	 *C28 H33 Cl F3 N3 O10* *[663]*	**BCS-CN88460-propanol-Glyc-MA**	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl 6-O-(carboxyacetyl)hexopyranoside *[IUPAC]*	animal: - plant: wheat (hay, straw); oilseed rape (intermediate harvest, mature plants) soil: - water: -
M22	 *C28 H33 Cl F3 N3 O10* *[663]*	**BCS-CN88460-2-propanol-Glyc-MA**	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]-carbonyl}amino)-methyl]phenyl}propan-2-yl 6-O-(carboxy-acetyl)hexopyranoside *[IUPAC]*	animal: - plant: oilseed rape (intermediate harvest, mature plants) soil: - water: -

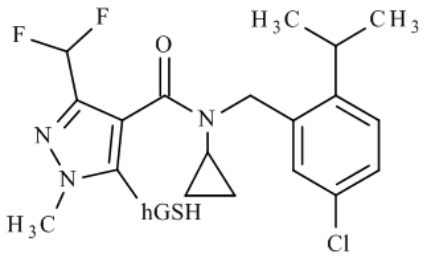
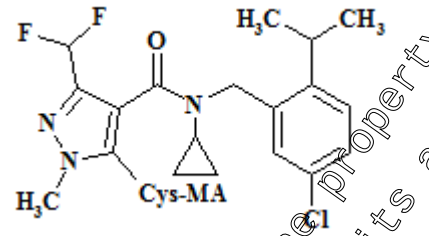
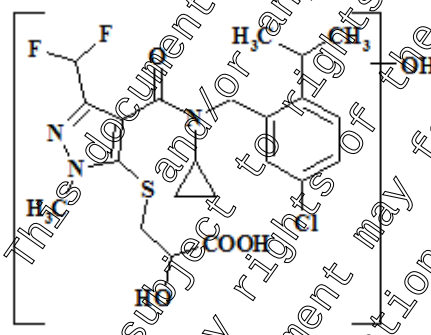
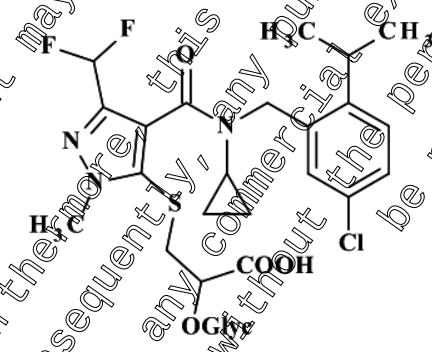
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M23	 *C28 H33 Cl F3 N3 O10* *[663]*	**BCS-CN88460-hydroxyphenyl-Gluc-MA**	2-chloro-4-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]-carbonyl}amino)-methyl]-5-isopropyl-phenyl 6-O-(carboxyacetyl)-beta-D-glucopyranoside *[IUPAC]*	animal: - plant: oilseed rape (intermediate harvest, mature plants) soil: - water: -
M24	 *C28 H33 Cl F3 N3 O10* *[663]*	**BCS-CN88460-hydroxyphenyl-Glyc-MA**	2-chloro-4-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]-carbonyl}amino)-methyl]-5-isopropyl-phenyl 6-O-(carboxyacetyl)-hexopyranoside *[IUPAC]*	animal: - plant: oilseed rape (intermediate harvest, mature plants) soil: - water: -
M25	 *C25 H27 Cl F3 N3 O8* *[589]*	**BCS-CN88460-propenol-GlucA**	2-[4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}prop-2-en-1-yl beta-D-glucopyranosiduronic acid *[IUPAC]*	animal: goat (kidney, urine) plant: - soil: - water: -
M26	 *C19 H21 Cl F3 N3 O5 S* *[495]*	**BCS-CN88460-propanol-SA**	2-[4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl hydrogen sulfate *[IUPAC]*	animal: hen (liver, excreta) plant: - soil: - water: -
M27	 *C19 H21 Cl F3 N3 O5 S* *[511]*	**BCS-CN88460-1,2-propandiol-SA**		animal: hen (excreta) plant: - soil: - water: -

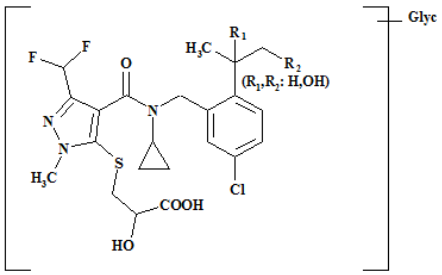
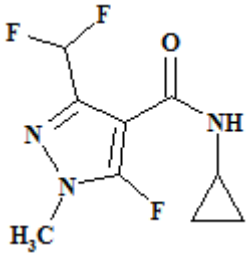
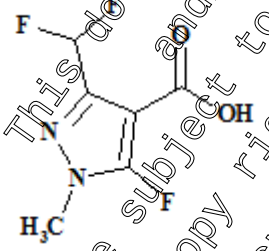
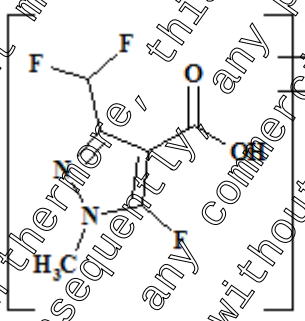
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M28	 *C25 H27 Cl F3 N3 O9* *[605]*	**BCS-CN88460-carboxylic acid-GlucA**		animal: rat (bile) plant: - soil: - water: -
M29	 *C25 H29 Cl F3 N3 O9* *[607]*	**BCS-CN88460-diOH-GlucA** (isomer 1 and 2)		animal: rat (urine, faeces, bile) plant: - soil: - water: -
M30	 *C25 H27 Cl F3 N3 O8* *[589]*	**BCS-CN88460-oxo-GlucA**		animal: rat (faeces, bile) plant: - soil: - water: -
M31	 *C24 H27 Cl F3 N3 O8* *[577]*	**BCS-CN88460-desmethyl-propanol-GlucA** (isomer 1 and 2)	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propyl glucopyranosiduronic acid *[IUPAC]*	animal: rat (faeces, bile); sunfish (edible parts, viscera) plant: - soil: - water: -

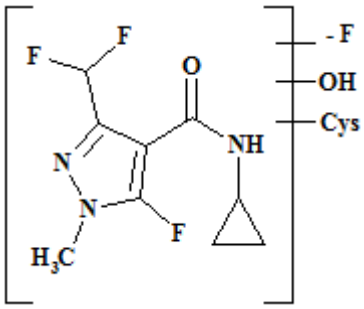
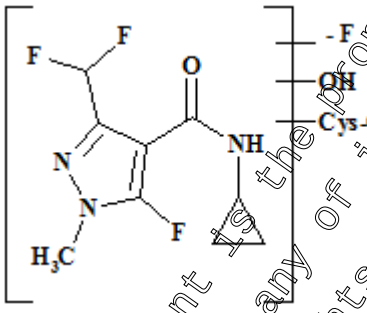
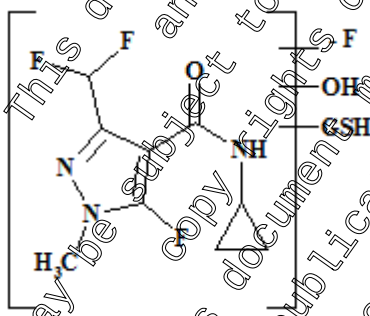
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M32	 *C24 H27 Cl F3 N3 O8* *[577]*	**BCS-CN88460-desmethyl-OH-GlucA** (isomer 1 to 2)		animal: rat (urine, faeces, bile) plant: - soil: - water: -
M33	 *C24 H27 Cl F3 N3 O9* *[593]*	**BCS-CN88460-desmethyl-diOH-GlucA** (isomer 1 to 6)		animal: rat (urine, faeces, bile) plant: - soil: - water: -
M34	 *C24 H27 Cl F3 N3 O10* *[609]*	**BCS-CN88460-desmethyl-triOH-GlucA**		animal: rat (faeces, bile) plant: - soil: - water: -
M35	 *C24 H27 Cl F3 N3 O7* *[561]*	**BCS-CN88460-desmethyl-GlucA** (isomer 1 and 2)	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(beta-D-glucopyranuronosyl)-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (bile); sunfish (edible parts, viscera) plant: - soil: - water: -

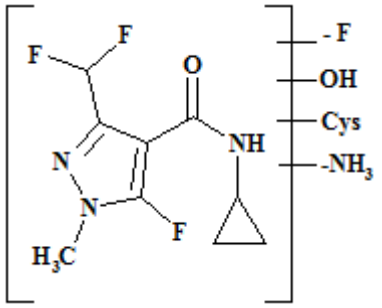
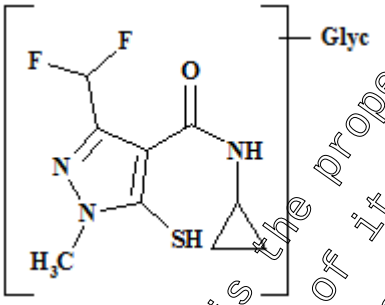
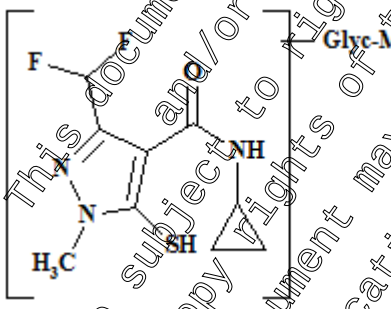
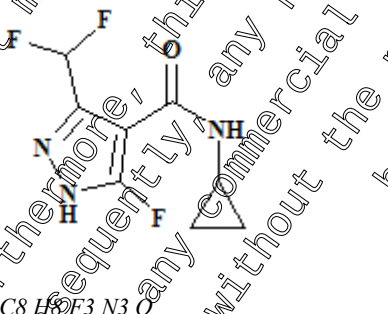
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M36	 *C24 H27 Cl F3 N3 O9* *[593]*	**BCS-CN88460-desmethyl-1,2-propandiol-N-GlucA**	N-[5-chloro-2-(1,2-dihydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(glucopyranuronosyl)-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: hen (muscle leg, liver, excreta) plant: - soil: - water: -
M37	 *C24 H27 Cl F3 N3 O8* *[577]*	**BCS-CN88460-desmethyl-propanol-N-GlucA**	N-[5-chloro-2-(1-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(glucopyranuronosyl)-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: hen (eggs, muscle leg, liver, excreta) plant: - soil: - water: -
M38	 *C24 H27 Cl F3 N3 O8* *[577]*	**BCS-CN88460-desmethyl-2-propanol-N-GlucA**	N-[5-chloro-2-(2-hydroxypropan-2-yl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-(glucopyranuronosyl)-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: hen (liver, excreta) plant: - soil: - water: -
M39	 *C24H25 Cl F3 N3 O8* *[575]*	**BCS-CN88460-desmethyl-oxo-GlucA**		animal: rat (bile) plant: - soil: - water: -

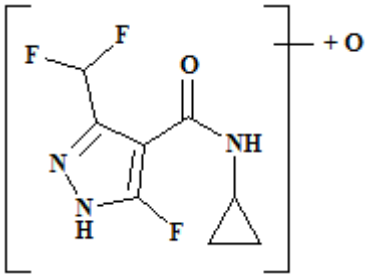
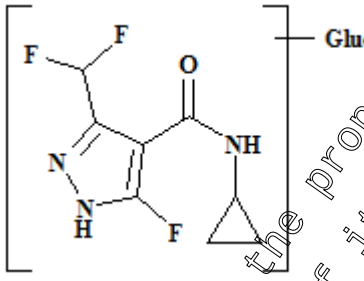
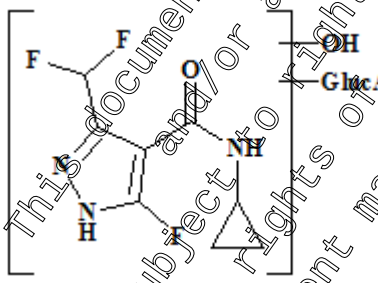
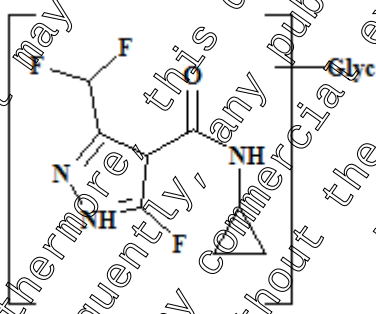
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M40	 *C24 H25 Cl F3 N3 O9* *[591]*	**BCS-CN88460-desmethyl-carboxylic acid-GlucA** (isomer 1 and 2)		animal: rat (urine, faeces, bile) plant: - soil: - water: -
M41	 *C27 H31 Cl F3 N3 O10* *[649]*	**BCS-CN88460-desmethyl-propanol-Glyc-MA**	2-{4-chloro-2-[cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino]methyl]phenyl}propyl 6-O-(carboxyacetyl)hexopyranoside *[IUPAC]*	animal: - plant: wheat (hay, straw) soil: - water: -
M42	 *C18 H19 Cl F3 N3 O6 S* *[497]*	**BCS-CN88460-desmethyl-1,2-propandiol-SA**		animal: hen (excreta) plant: - soil: - water: -
M43	 *C18 H19 Cl F3 N3 O5 S* *[481]*	**BCS-CN88460-desmethyl-SA**		animal: rat (faeces, bile) plant: - soil: - water: -

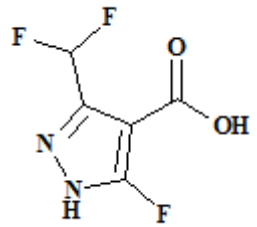
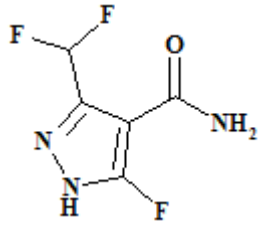
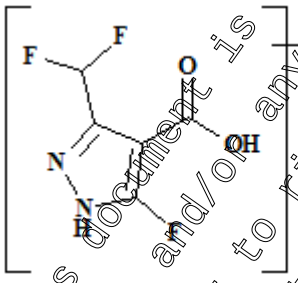
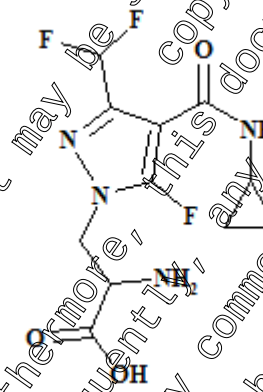
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M44	 *C30 H39 Cl F2 N6 O7 S* *[700]*	**BCS-CN88460-desfluoro-homoGSH**	gamma-glutamyl-S-{4-[(5-chloro-2-isopropylbenzyl)(cyclopropyl)carbamoyl]-3-(difluoromethyl)-1-methyl-1H-pyrazol-5-yl}cysteinyl-beta-alanine *[IUPAC]*	animal: - plant: soybean (forage, hay, straw) soil: - water: -
M45	 *C25 H29 Cl F2 N4 O6 S* *[586]*	**BCS-CN88460-desfluoro-Cys-MA**	N-(carboxyacetyl)-S-{4-[(5-chloro-2-isopropylbenzyl)(cyclopropyl)carbamoyl]-3-(difluoromethyl)-1-methyl-1H-pyrazol-5-yl}cysteine *[IUPAC]*	animal: - plant: soybean (forage, hay, straw) soil: - water: -
M46	 *C22 H26 Cl F2 N3 O5 S* *[517]*	**BCS-CN88460-desfluoro-mercapto-lactic acid-OH**		animal: - plant: soybean (forage, hay, straw) soil: - water: -
M47	 *C28 H36 Cl F2 N3 O9 S* *[663]*	**BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc**	3-({4-[(5-chloro-2-isopropylbenzyl)(cyclopropyl)carbamoyl]-3-(difluoromethyl)-1-methyl-1H-pyrazol-5-yl}sulfanyl)-2-(hexopyranosyloxy)propanoic acid *[IUPAC]*	animal: - plant: soybean (forage, hay, straw) soil: - water: -

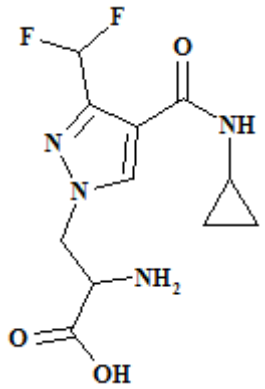
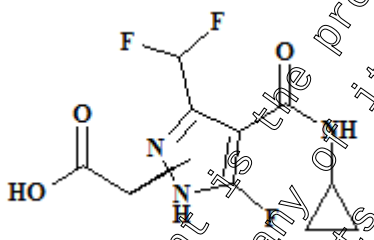
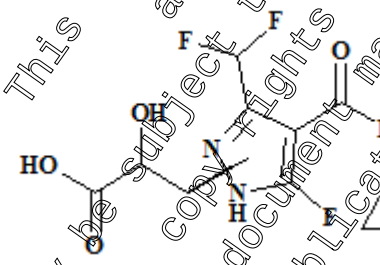
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M48	 *C28 H36 Cl F2 N3 O10 S* *[679]*	**BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc**		animal: - plant: soybean (forage, hay, straw) soil: - water: -
M49	 *C9 H10 F3 N3 O* *[233]*	**BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide** BCS-CR60082	N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: - plant: CRC (wheat forage, wheat hay, wheat straw, Swiss chard immature, Swiss chard at maturity, turnip leaves) soil: - water: -
M50	 *C6 H5 F3 N2 O2* *[194]*	**BCS-CN88460-N-methyl-pyrazole-carboxylic acid** BCS-AB72918 BCS-CR73065 *CAS: 1255735-09-4*	3-(difluoromethyl)-5-fluoro-1-methyl-pyrazole-4-carboxylic acid *[IUPAC]* 1H-Pyrazole-4-carboxylic acid, 3-(difluoromethyl)-5-fluoro-1-methyl- *[CA]*	animal: rat (urine), goat (urine, kidney); sunfish (edible parts, viscera) plant: - soil: - water: -
M51	 *C6 H6 F2 N2 O2* *[176]*	**BCS-CN88460-desfluoro-N-methyl-pyrazole-carboxylic acid**		animal: rat (urine) plant: - soil: - water: -

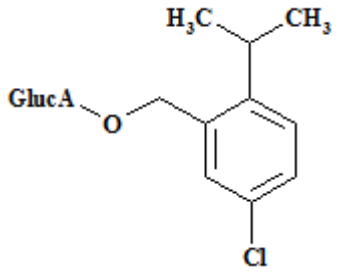
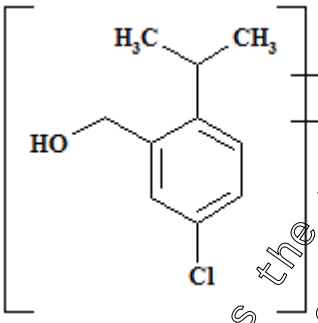
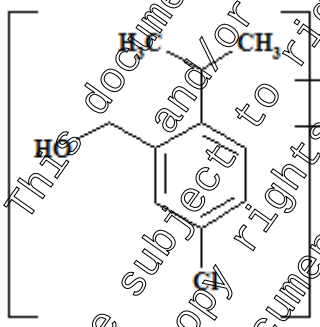
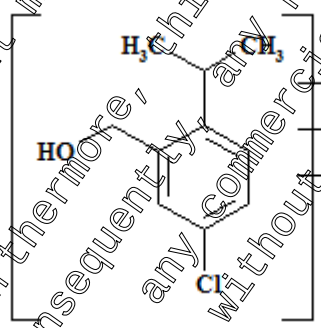
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M52	 *C12 H16 F2 N4 O3 S* *[334]*	**BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys**		animal: rat (bile) plant: CRC (wheat forage, wheat hay, wheat straw, Swiss chard immature, Swiss chard at maturity, turnip leaves) soil: - water: -
M53	 *C14 H19 F2 N5 O4 S* *[391]*	**BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-Cys-Gly**		animal: rat (bile) plant: soil: - water: -
M54	 *C19 H26 F2 N6 O7 S* *[520]*	**BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-OH-GSH**		animal: rat (bile) plant: CRC (wheat forage, wheat hay, wheat straw, Swiss chard immature, Swiss chard at maturity, turnip leaves) soil: - water: -

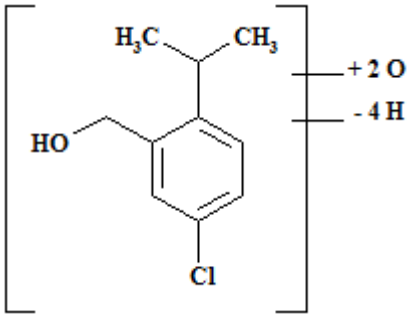
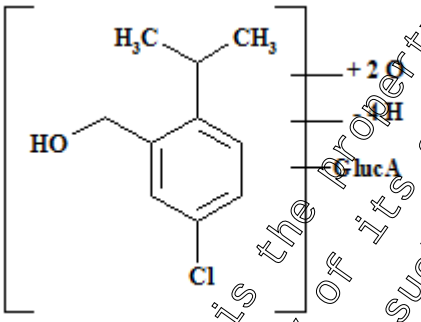
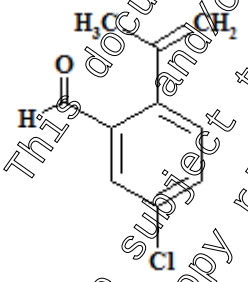
No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M55	 *C12 H15 F2 N3 O4 S* *[335]*	**BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-desamino-Cys**		animal: - plant: CRC(wheat forage; wheat hay, wheat straw, Swiss chard immature, turnip leaves) soil: - water: -
M56	 *C15 H21 F2 N3 O5 S* *[409]*	**BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc**		animal: - plant: CRC (wheat forage, wheat hay, wheat straw, turnip leaves) soil: - water: -
M57	 *C18 H23 F2 N3 O9 S* *[495]*	**BCS-CN88460-desfluoro-N-methyl-cyclopropyl-pyrazole-carboxamide-mercapto-Glyc-MA**		animal: - plant: CRC (wheat forage, wheat hay, wheat straw, turnip leaves) soil: - water: -
M58	 *C8 H8 F3 N3 O* *[219]*	**BCS-CN88460-cyclopropyl-pyrazole-carboxamide** BCS-CX99798	N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (urine, plasma, liver, kidney); sunfish (edible parts, viscera) plant: - soil: - water: -

No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M59	 *C8 H8 F3 N3 O2* *[235]*	**BCS-CN88460-cyclopropyl-oxy-pyrazole-carboxamide**		animal: rat (urine) plant: - soil: water: -
M60	 *C14 H16 F3 N3 O7* *[395]*	**BCS-CN88460-cyclopropyl-pyrazole-carboxamide-GlucA** (isomer 1 and 2)		animal: rat (urine, bile, kidney) plant: - soil: - water: -
M61	 *C14 H16 F3 N3 O8* *[411]*	**BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-GlucA** (isomer 1 and 2)		animal: rat (urine, bile) plant: - soil: - water: -
M62	 *C14 H18 F3 N3 O6* *[397]*	**BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Glyc** (isomer 1 and 2)		animal: - plant: CRC (wheat forage, wheat hay, wheat straw, Swiss chard immature, Swiss chard at maturity, turnip leaves) soil: - water: -

No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M63	 *C5 H3 F3 N2 O2* *[180]*	**BCS-CN88460-pyrazole-carboxylic acid**	3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxylic acid *[IUPAC]*	animal: rat (urine, faeces, kidney, bile) plant: - soil: - water: -
M64	 *C5 H4 F3 N3 O* *[179]*	**BCS-CN88460-pyrazole-amide**	3-(difluoromethyl)-5-fluoro-1H-pyrazole-4-carboxamide *[IUPAC]*	animal: rat (urine) plant: soil: water: -
M65	 *C8 H8 F3 N3 O3* *[251]*	**BCS-CN88460-pyrazole-carboxylic acid-Ala**		animal: rat (urine, bile) plant: - soil: - water: -
M66	 *C11 H13 F3 N4 O3* *[306]*	**BCS-CN88460-cyclopropyl-pyrazole-carboxamide-Ala**	3-[4-(cyclopropylcarbamoyl)-3-(difluoromethyl)-5-fluoro-1H-pyrazol-1-yl]alanine *[IUPAC]*	animal: - plant: CRC(wheat forage, wheat hay, wheat straw, wheat grain, Swiss chard immature, Swiss chard at maturity, turnip leaves) soil: - water: -

No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M67	 *C11 H12F2 N4 O3* *[286]*	**BCS-CN88460-desfluoro-cyclopropyl-pyrazole-carboxamide-Ala**	3-[4-(cyclopropylcarbamoyl)-3-(difluoromethyl)-1H-pyrazol-1-yl]alanine *[IUPAC]*	animal: - plant: CRC (wheat forage, wheat hay, wheat straw, wheat grain, Swiss chard immature, Swiss chard at maturity, turnip leaves) soil: - water: -
M68	 *C10 H10 F3 N3 O3* *[277]*	**BCS-CN88460-cyclopropyl-pyrazole-carboxamide-acetic acid**		animal: - plant: CRC (wheat hay, wheat straw) soil: - water: -
M69	 *C11 H12 F3 N3 O4* *[307]*	**BCS-CN88460-cyclopropyl-pyrazole-carboxamide-OH-lactic acid** (isomer 1 and 2)		animal: - plant: CRC (wheat forage, wheat hay, wheat straw, Swiss chard immature, Swiss chard at maturity) soil: - water: -

No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M70	 *C16 H21 Cl O7* *[360]*	**BCS-CN88460-benzylalcohol-GlucA**	5-chloro-2-isopropylbenzyl hexopyranosiduronic acid *[IUPAC]*	animal: rat (urine) plant: - soil: water: -
M71	 *C10 H11 Cl O2* *[198]*	**BCS-CN88460-benzylalcohol-oxo**		animal: rat (urine) plant: soil: - water: -
M72	 *C10 H9 Cl O2* *[196]*	**BCS-CN88460-benzylalcohol-oxo-desdihydro** (isomer 1 and 2)		animal: rat (urine) plant: - soil: - water: -
M73	 *C16 H19 Cl O8* *[374]*	**BCS-CN88460-benzylalcohol-oxo-GlucA** (isomer 1 and 2)		animal: rat (urine) plant: - soil: - water: -

No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in:
M74	 *C10 H9 Cl O3* *[212]*	**BCS-CN88460-benzylalcohol-dioxo** (isomer 1 and 2)		animal: rat (urine) plant: - soil: - water: -
M75	 *C16 H17 Cl O9* *[388]*	**BCS-CN88460-benzylalcohol-dioxo-GlucA** (isomer 1 and 2)		animal: rat (urine) plant: - soil: - water: -
M76	 *C10 H9 Cl O* *[180]*	**BCS-CN88460-phenyl-formyl-olefine** *CAS: 1006685-15-9*	5-chloro-2-(prop-1-en-2-yl)benzaldehyde *[IUPAC]* Benzaldehyde, 5-chloro-2-(1-methylethenyl)- *[CA]*	animal: rat (urine) plant: - soil: - water: -