Propoxycarbazone-sodium

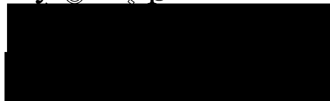
Herbicide

Dossier for Renewal of Approval according to Commission Regulation 844/2012

Document M-CA, Section 6

Residues in or on treated products, food and feed and plant metabolism

Bayer CropScience AG



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Version history

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

CA 6.1 Storage stability of residues

Stability of residues during storage of samples

Data on storage stability of residues are available for propoxycarbazone-sodium and its metabolite 2-hydroxypropoxy MKH 6561 (M01).
The conclusions from the EU evaluation still apply and no supplementary studies are submitted.

Conclusions from the EU evaluation of propoxycarbazone-sodium (DAR)
Freezer storage stability studies were carried out in different wheat matrices stored under frozen conditions by ██████ (1999, RIP2000-1005) and ████████ (1999, RIP2000-1006 (interims report) and RIP2000-1053 (final report)). The results show that propoxycarbazone-sodium and its metabolite M01 was stable for about 18-33 months (i.e. loss of less than 30%).

Available studies reviewed for the first inclusion of propoxycarbazone-sodium to Annex I of 91/414 are summarised below:

██████ (1999, RIP2000-1005; Bayer report No.: 108849)

Summary and results

The stability of propoxycarbazone sodium residues in wheat samples of all spring wheat matrices was tested in the cause of the metabolism studies investigating [phenyl-UL-^{14}C] propoxycarbazone sodium and [triazolinone-3-^{14}C] propoxycarbazone sodium. The residues were extracted soon after harvest and at the end of the experimental work. Samples were stored at -20 °C ± 6 °C for periods of approximately 1000 days (phenyl-label) and approximately 600 days (triazolinone-label), respectively.
Comparison of the extractability and distribution of propoxycarbazone sodium and 2-hydroxypropoxy MKH 6561 (M01) showed that residues of [phenyl-UL-^{14}C] labelled compound were stable for at least 1003 days in forage, 987 days in hay, 1023 days in straw and 946 days in grain. Residues of [triazolinone-3-^{14}C] labelled substance were stable for at least 586 days in forage, 630 days in hay, 592 days in straw and 580 days in grain.

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Table 6.1- 1: Results of the storage stability of propoxycarbazone sodium and its metabolite in wheat matrices (Report 108849)

Label	Sample	Interval (days)	Propoxycarbazone sodium % TRR	2-hydroxypropoxy MKH 6561 (M01) % TRR
Phenyl-label	Forage	1	11	70
		1003	11	70
	Hay	8	n.d.	46
		987	n.d.	44
	Straw	10	n.d.	46
		1023	n.d.	51
	Grain	16	n.d.	9
		946	n.d.	14
Triazolinone-label	Forage	11	2	73
		586	1	78
	Hay	9	n.d.	60
		630	n.d.	55
	Straw	11	n.d.	35
		592	n.d.	31
	Grain	14	n.d.	38
		580	n.d.	32

n.d. = not detected

(1999, RIP2000-1006 (interims report) and RIP2000-1053 (final report); Bayer report No.: MR-552/99)

Summary and Results

Wheat forage, straw and grain samples were fortified with both propoxycarbazone sodium and 2-hydroxypropoxy MKH 6561 (M01) at a level of 1.0 mg/kg for forage, 0.50 mg/kg for straw and 0.20 mg/kg for grain. The samples were stored in glass bottles at -18° C or below. Analyses were performed at days 0, 90, 180, 360, 450, and 540.

The recoveries for propoxycarbazone sodium in fortified samples of green material ranged from 86 to 103% (mean value: 96% and RSD 2.9% for n=20), of straw from 77 to 96% (mean value: 86% and RSD 4.2% for n=20), and of grain from 78 to 96% (mean value: 86% and RSD 3.8% for n=20).

The recoveries for 2-hydroxypropoxy MKH 6561 (M01) in green material ranged from 84 to 97% (mean value: 90% and RSD 3.1% for n=20), in straw from 66 to 93% (mean value: 81% and RSD 5.8% for n=20), and in grain from 68 to 101% (mean value: 86% and RSD 5.5% for n=20).

At all sampling intervals, the sample materials contained >70% of the fortified residue levels. This demonstrates that residues of propoxycarbazone sodium and M01 are stable in wheat material under freezer conditions for at least 540 days.

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Table 6.1- 2: Results of the storage stability of propoxycarbazone sodium and its metabolite in wheat matrices (Report MR-552/99)

Sample	Fortification level (mg/kg)	Interval (days)	Recoveries (%)			
			Propoxycarbazone sodium		2-hydroxypropoxy MKH 6561 (M01)	
			Single values	Mean/RSD	Single values	Mean/RSD
Wheat green material	1.0	0	90;94;89;86;94	91/3.8	84;85;93;88;91	88/4.4
		90	98;101;101	100/1.7	95;84;90	90/6.1
		181	95;89;93	92/3.3	97;97;97	97/0.0
		363	93;103;101	99/5.3	89;85;89	88/2.6
		448	93;94;96	94/1.6	89;90;91	90/1.1
		540	99;101;102	101/1.5	86;93;93	91/4.4
Wheat grain	0.20	0	82;84;86;88;80	84/3.8	79;85;77;82;68	78/8.3
		90	92;84;92	89/5.2	85;101;97	94/8.9
		181	91;87;96	91/5.0	86;87;88	87/1.1
		363	81;78;81	80/2.2	86;82;88	85/3.6
		448	89;82;89	87/4.6	91;82;81	85/6.5
		540	87;86;89	87/1.8	88;90;96	91/4.6
Wheat straw	0.50	0	89;90;87;81;77	85/6.6	86;85;82;77;93	85/6.9
		90	87;96;92	92/4.9	73;68;77	73/6.2
		181	83;84;87	85/2.4	84;91;87	87/4.0
		363	93;82;81	86/7.5	66;74;77	72/7.9
		448	89;87;85	87/2.3	93;85;81	86/7.1
		540	84;86;86	85/1.4	82;79;78	80/2.6

RSD: Relative standard deviation (%)

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Plants

No supplementary studies are required as metabolism has been fully studied. The conclusions from the EU evaluation as well as the supporting studies still apply.

Conclusions from the EU evaluation of propoxycarbazone sodium (Monograph)

The behaviour and metabolism of [phenyl-UL-^{14}C]propoxycarbazone-sodium and [triazolinone-3-^{14}C] propoxycarbazone-sodium was carried out in spring and winter wheat matrices by (1999, RIP2000-1005)

The metabolism of propoxycarbazone-sodium was investigated using the phenyl- as well as the triazolinone-labelled active substance. The results were in very good accordance with respect to residue levels, extractability and distribution of metabolites. Highest total radioactive residues were observed in the studies investigating [phenyl-UL-^{14}C]propoxycarbazone-sodium in wheat forage which was harvested already 11 days after application. Lowest radioactive residues were observed in grain.
Unchanged parent compound was observed in the phenyl-labelled experiment in low amounts only in forage. In the triazolinone-labelled experiment much lower amounts of active substance were detected in forage and straw.
The primary metabolisation step in wheat was the hydroxylation of the propoxy side chain resulting in 2-hydroxypropoxy MKH 6561 (M01). This metabolite was also the predominant degradation product in all raw agricultural commodities investigated. Further hydrolysis of M01 led to 2-hydroxy-N-methyl propoxy triazolinone (M02) and probably sulphonamide methyl ester (M05), which was not observed in any of the

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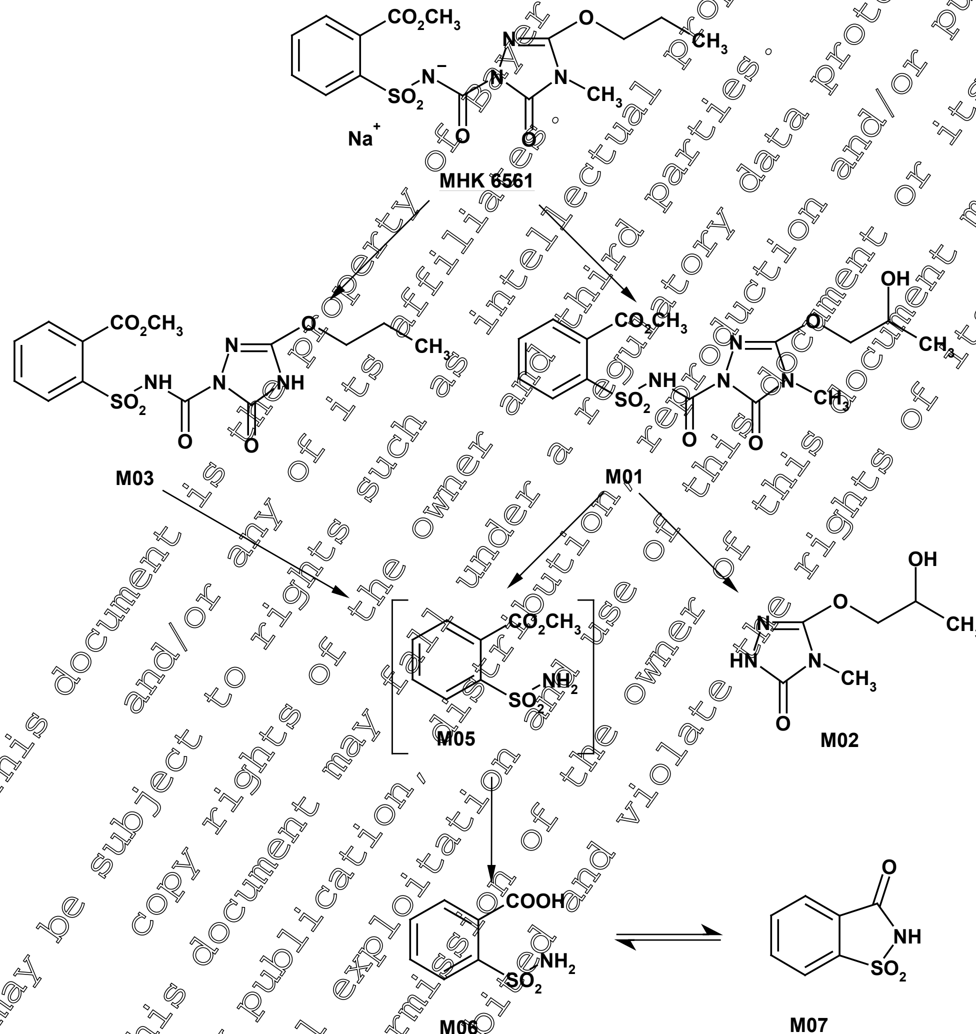
wheat matrices. Hydrolysis of the sulfonamide methyl ester (M05) resulted in sulfonamide acid (M06), which was in equilibrium with saccharin (M07). A minor important metabolic step was demethylation of propoxycarbazone sodium yielding N-desmethyl MKH 6561 (M03). 2-Hydroxypropoxy MKH 6561 (M01) was the predominant metabolite identified in all raw agricultural commodities independent of the label investigated.
Parent compound and 2-hydroxypropoxy MKH 6561 (M01) can be regarded as the residue of concern and should be included in the residue definition for plant matrices.

The metabolic pathway for propoxycarbazone-sodium in plants is shown in Figure 6.2.1-1.

Figure 6.2.1-1: Metabolic pathway for propoxycarbazone-sodium in wheat



CA 6.2.2 Poultry

Two studies on the distribution and metabolism of MKH 6561 – propoxycarbazone-sodium in laying hens using [phenyl-UL-^{14}C]propoxycarbazone acid (free acid) (M-CA 6.2.2/01 [redacted] & [redacted], 1999a) and [triazolinone-3-^{14}C]propoxycarbazone acid (free acid) (M-CA 6.2.2/02 [redacted] & [redacted], 1999b) have been conducted which were not submitted for the original EU dossier. Due to the very low concentration of residues expected in poultry feed (maximum dietary burden of 0.02 mg/kg DM, see CA 6.4), metabolism studies in poultry are not regarded necessary but presented here for information.

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Report:	; ;1999;M-015797-01
Title:	The distribution and metabolism of [phenyl-UL-14C] MKH 6561 in laying hens
Report No:	107918
Document No:	M-015797-01-1
Guidelines:	**OPPTS 860.1300, Nature of Residues-Plants, Livestock**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

Fifteen laying hens were dosed orally, via capsule, with protonated [phenyl-UL-^{14}C]MKH 6561 for 3 consecutive days at an average daily dose rate of 3.12 mg/kg body weight (49 mg/kg in the feed).
The total radioactive residue (TRR) levels were 1.343 mg/kg in the liver, 0.017 mg/kg in the muscle, 0.014 mg/kg in the fat, 0.006 mg/kg in the Day-1 eggs, 0.009 mg/kg in the Day-2 eggs, and 0.012 mg/kg in the Day-3 eggs. The residue levels based on a theoretical 1X rate would be <0.001 mg/kg in all tissues and eggs. Pooled excreta of study day 1 through study day 3 contained 68.54% of the administered dose while eggs collected during treatment did not contain enough residues to register as a percentage of the dose and tissues contained a total of 0.34% of the total dose.
Approximately 95% of the radioactivity in liver was solubilised by a combination of organic solvent extraction (21% of the TRR) and protease hydrolysis (74% TRR). The majority of the radioactive residues from the muscle (84% TRR), fat (89% TRR), and eggs (88% to 95% TRR) was solubilised by extraction with organic solvents.
The major metabolic pathway of [^{14}C]MKH 6561 in poultry was hydrolysis of the parent compound producing the metabolite M05. Metabolite M05 was then converted to metabolite M07 (saccharin). A minor pathway involved hydroxylation at the 2-position of the triazolinone propoxy group. In the liver, a second major pathway led to the formation of protein bound MKH 6561 residues through conjugation with the amino acid serine.
The major residues found in the tissues and eggs were: protonated MKH 6561 (10% of the TRR in liver, 42% TRR in muscle, 61% TRR in fat, 31% TRR in Day-1 eggs, 21% TRR in Day-2 eggs, and 29% TRR in Day-3 eggs); serine conjugate of MKH 6561 (metabolite M12, 61% TRR in liver, not detected in other tissues and eggs); saccharin (metabolite M07, 14% TRR in liver, 17% TRR in muscle, 6% TRR in fat, 37% TRR in Day-1 eggs, 56% TRR in Day-2 eggs, and 37% TRR in Day-3 eggs); metabolite M05 (3% TRR in liver, 16% TRR in muscle, 12% TRR in fat, not detected in eggs); and metabolite M01 (2% TRR in liver, 5% TRR in muscle, 6% TRR in fat, 2% TRR in Day-1 eggs, 3% TRR in Day-2 eggs, and 5% TRR in Day-3 eggs).
Total identification of radioactive residues in the tissues and eggs was 90% in liver, 80% in muscle, 85% in fat, 70% in Day-1 eggs, 80% in Day-2 eggs, and 71% in Day-3 eggs. In addition, 1% to 4% of the radioactive residue in tissue and eggs was characterised using chromatographic methods. All extracted radioactive residues which were >10% of the TRR or >0.05 mg/kg were identified.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Protonated [phenyl-UL-^{14}C]MKH 6561
(also referred to as MKH 5554, applicant´s code number)
(methyl 2-[[[(4,5-dihydro-4-methyl-5-oxo-3-propoxy-1*H*-1,2,4-triazol-1-yl)carbonyl] amino] sulfonyl] benzoate, phenyl-UL-^{14}C)
CAS # 145026-81-9
(essentially equivalent to MKH 6561 (sodium salt))
mixed with

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	non radiolabelled MKH 6561, protonated form
Common name:	Propoxycarbazone-sodium
Empirical formula:	$C_{15}H_{18}N_4O_7S$, protonated form
Molar mass:	398.4 (protonated form)
Labelling:	[phenyl-UL-^{14}C]
Specific radioactivity:	^{14}C stock solution: 266000 dpm/µg (mixture in the capsules: 200000 dpm/µg)
Purity:	^{14}C: radiochemical purity: 98.3% non labelled: purity: 97.1%
Lot/Batch #:	^{14}C: stock solution in acetonitrile, Vial C-722 (synthesised by the Bayer Radiosynthesis Group in [redacted], KS, USA) non labelled: Vial K-624
Dose level:	3 x 3.12 mg/kg body weight (average)
Stability of test compound:	Checked by HPLC to have been stable in dosing capsules

2. **Vehicle:** α-lactose (in gelatine capsules)

3. **Test animals:**

Species:	Laying Hens (*Gallus domesticus*)
Strain:	Leg Horn
Source:	[redacted]
Sex:	Female
Age:	110 weeks at receipt; 116 weeks at dosing
Weight:	1266-2062 g (average body weights between animal selection and termination)
Number of animals:	15
Acclimation period:	21 days (Study Day -20 to Study Day 1, 1996-07-30 to 1996-08-20)
Identification:	Leg band (right leg) containing a unique identification number
Diet/Food:	[redacted]. Feed; Lot No. 698030 ([redacted]., Berino, NM 88024) *via* cage feeders, ad libitum
Water:	Fresh potable water provided *via* automatic water bowls, ad libitum
Housing:	Individually in metabolism cages
Environmental conditions:	Temperature: 75 – 84 °F (average min. and max. temperature) Humidity: 65% (average) 14-hour light/10-hour dark photoperiod

B. STUDY DESIGN

In life dates
1996-07-17 (test animal receipt) / 1996-08-20 (Study Day 1) to 1996-09-10
Analytical work

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August 1996 to October 1997

The objective of this study was to investigate the metabolism of [phenyl-UL-^{14}C] MKH 6561 in laying hens and the distribution of radioactive residues in the tissues and eggs and to evaluate the residues to be expected in poultry.

1. Test procedure

Following thirteen days of acclimation, fifteen laying hens (treated group) were chosen for the study on Study Day -7 based on egg production and body weight (1327 to 2093 g). Each animal was identified via a leg band containing a unique identification number.

Each chicken was orally administered a single dose capsule containing [^{14}C] MKH 6561 *via* balling gun on Study Days 1, 2 and 3. Aliquots of the dose solution were analysed by LSC and HPLC (system A) to determine the actual concentration (424 µCi [^{14}C] MKH 6561 per capsule) and purity of the test item. The stability of the [phenyl-UL-^{14}C]MKH 6561 poultry dose during shipment and storage was determined by HPLC analysis in which the contents (MKH 6561-treated α-lactose) of three extra dose capsules were combined and analysed after dissolution in water. These stability tests indicated no degradation of [phenyl-UL-^{14}C]MKH 6561 during three weeks of storage.

The average daily dose rate of 3.12 mg/kg body weight (49 mg/kg in the feed, average body weight 1.51 kg) was approximately 3000X (dose exaggeration) the maximum dietary burden of 0.001 mg/kg b.w./day (see section CA 6.4). The exaggeration factor given in the study had been calculated with the magnitude of the residue found in the MKH 6561 wheat field trials.

2. Sampling

Eggs were collected from each hen twice daily (prior to dosing) beginning at receipt and continuing until the termination of each animal. The PM and AM eggs following each dosing from all of the hens were composited and considered as one day´s sample. Excreta from each chicken were collected starting on Study Day 1 every 24 hours and composited daily as one day´s sample. The hens were humanely terminated 4 to 5 hours following the final dose. Liver, composite muscle (leg and breast) and composite fat (available omental and subcutaneous) were collected; tissues of the same type from all 15 birds were composited. The tissues, eggs and excreta were homogenised, and subsamples were radioassayed. All tissue and egg samples were stored frozen at Southwest Bio-Labs and shipped frozen (dry ice) to Bayer for analysis.

3. Analysis

Radioactivity measurement

All tissue, egg and excreta samples were combusted using an oxidiser. Liberated $^{14}CO_2$ was trapped using an absorption liquid, combined with an appropriate scintillation liquid, and the total radioactive residues were determined by liquid scintillation counting (LSC). The radioactive residues in liquid samples (e. g. extracts) were measured by LSC after mixing with an appropriate scintillation cocktail. Solid samples after extraction were also oxidised, and the released $^{14}CO_2$ was trapped in alkaline solution and radioassayed.

Extraction of tissues and eggs

A portion of tissue or eggs was blended with hexane using a tissumizer. The tissue solids were allowed to settle, and the hexane supernatant was decanted and vacuum-filtered. The hexane-extracted solids were blended with acetonitrile/water (9:1), followed by filtration. The filtered solids were extracted two additional times with fresh ACN/H_2O (9:1) in the same manner, and the three filtrates were combined. The extracted solids were air-dried and occasionally agitated with a spatula to form a fine powder, weighed, and the radioactive residues were determined by combustion of aliquots. The hexane and ACN/H_2O extracts were radioassayed.

The ACN/H_2O extract was rotoevaporated to an oily residue, and the residue was dissolved in acetonitrile (saturated with hexane). The hexane extract was also concentrated to an oily residue and dissolved in hexane (saturated with acetonitrile). The hexane extract and the ACN extract were combined in a separatory funnel and partitioned, and the ACN fraction was collected. The

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remaining hexane was partitioned against acetonitrile, and the ACN fraction was combined with the first ACN fraction. Aliquots of the combined ACN fraction and the remaining hexane fraction were radioassayed.
The combined ACN fractions were concentrated, redissolved in methanol/water (4:1) and percolated through a conditioned SPE cartridge. The SPE cartridge was washed with additional MeOH/H_2O (4:1). The combined MeOH/H_2O eluents were concentrated and the dry residue was dissolved in H_2O/ACN (9:1) containing 0.1% trifluoroacetic acid (TFA). The resulting solution was radioassayed, and aliquots were analysed by HPLC (system B). Individual component peaks were isolated from the HPLC eluent, and selected metabolites purified further using another HPLC method (system C) prior to analyses by mass spectrometry.

Protease hydrolysis of the residues after solvent extraction of liver
The liver solids remaining after ACN/H_2O extraction were mixed with Tris buffer (pH 7.4), and the suspension was rotoevaporated to near dryness (moist solids). The moist solids were suspended, with the aid of sonication, in a higher volume of Tris buffer, and the suspension was heated to 37 °C with stirring. Protease enzyme (Type XIV, *Streptomyces griseus*) was added to the buffered suspension, and the mixture was stirred at 37 °C for 16 hours. The enzyme reaction was terminated by adding ACN and cooling the mixture to 0 °C. The suspension was centrifuged, and the ACN/buffer supernatant was collected. The remaining solids were washed with ACN which was combined with the ACN/buffer supernatant, and aliquots of the resulting solution were radioassayed. The enzyme-hydrolysed solids were air-dried, and portions were also radioassayed.
The combined ACN/H_2O-ACN supernatants were rotoevaporated, and the dry residue was dissolved in water. The aqueous solution was acidified (pH 1) with HCl and percolated through a conditioned SPE cartridge. The SPE cartridge was washed with water containing 0.1% TFA, and the aqueous eluates were combined and radioassayed. The SPE cartridge was then rinsed with H_2O/MeOH (7:3) containing 0.1% TFA, and the eluate was collected and radioassayed. The H_2O/MeOH eluate was rotoevaporated, and the dry residue was dissolved in water containing 0.1% TFA. The resulting solution was radioassayed, and aliquots were analysed by HPLC (system B). Individual component peaks were isolated from the HPLC eluent. The main components were further purified by HPLC (system C) and TLC, and each was analysed by mass spectrometry.

Metabolite analysis
High-Performance Liquid Chromatography (HPLC) analysis of the samples prepared from the ACN fractions of the extracts of tissues and eggs and from the protease hydrolysate of liver (concentrated SPE eluates, see above) was performed using a reversed phase column (C18) and a flow-through radiodetector (solid scintillator cell). Separation was achieved using gradient elution (two slightly differing gradient programs for system A and system B) with 0.1% TFA in water (solvent A) and acetonitrile (solvent B). For metabolite purification, gradient elution with 25 mM phosphate buffer pH 3.5 (solvent A) and acetonitrile (solvent B) was conducted (system C).

Thin-Layer Chromatography (TLC) was performed on silica gel 60 F-254 plates for additional purification of metabolites. The samples were applied using a micropipette and focussed on the plate by developing three times with methanol. The plates were developed with dichloromethane/methanol/water/concentrated ammonium hydroxide (60:15:1:1). Distribution of radioactivity on the TLC plates was determined by exposition of a phosphor screen and scanning using a phosphorimager. Individual metabolites were recovered from the TLC plate by scraping the region of interest and transferring the loosened silica gel into an empty SPE cartridge fitted with a frit. Components were eluted from the silica gel with the solvent mixture used for development, and the solvent was removed using a rotoevaporator.

Mass spectral analysis (liquid chromatography/electrospray-mass spectrometry, LC/ES-MS) was performed using a C8 column and gradient elution with methanol (solvent A) and water with 5 mM ammonium acetate (solvent B). The column was hyphenated in parallel to a radiodetector and to a triple stage quadrupole (TSQ) electrospray (ES) mass spectrometer (negative ionisation conditions).

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4. Stability of [^{14}C]MKH 6561 in tissue and egg extracts

All tissue and egg samples were extracted and analysed by HPLC for metabolite profiles within 6 weeks of collection. Preliminary identification of tissue and egg residues was completed within 4 months of collection. When tissues or egg samples were not being analysed, they were stored in the freezer (-20 ± 5 °C).

Tissues and eggs were initially extracted with mixtures of ACN and water (9:1). When extracts were not being analysed, they were stored at refrigerator temperature. MKH 6561 is stable and does not degrade in ACN or water, as long as the pH of the solvent is near neutral (pH 7). Since MKH 6561 (parent compound) was observed in all tissue and egg extracts, all components identified in the extracts most likely arose from the metabolism of MKH 6561 and presumably were not formed as artefacts resulting from the degradation of MKH 6561 upon extraction and storage.

II. RESULTS AND DISCUSSION

A. ANIMAL HEALTH AND ANIMAL HUSBANDRY

Overall, the animals appeared healthy throughout the course of the treatment period as evidenced by observance of feed consumption, daily observations, physical examinations, body weights and observation of tissues at necropsy.

B. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residue (TRR) levels (expressed as mg/kg) found in the poultry tissues and eggs are given in Table 6.2.2-1. The highest equivalent concentration was measured in the liver (1.343 mg/kg), followed by that obtained for muscle (0.017 mg/kg) and fat (0.014 mg/kg). The equivalent concentrations in eggs increased from Day-1 to Day-3 (0.006 mg/kg to 0.012 mg/kg). Pooled excreta contained 68.54% of the administered dose with approximately 68.88% of the administered dose recovered in the combined tissues, eggs and excreta. The residue levels were not elevated in relation to the dose level. Even at the exaggerated dosing rate, only the liver had residues higher than 0.02 mg/kg, and at the anticipated 1X level, the tissue and egg residues would be significantly less than 0.01 mg/kg. No residue of MKH 6561 would be expected in tissue or eggs from poultry fed a diet containing wheat or wheat by-products from MKH 6561-treated wheat.

Table 6.2.2-1: Total radioactive residue levels in the edible tissues and eggs of laying hens following the administration of three consecutive doses of [phenyl-UL-^{14}C]MKH 6561 at 3.12 mg/kg body weight

Tissue / Eggs	Residue levels mg/kg (MKH 6561 equivalents)
Liver	1.343
Muscle	0.017
Fat	0.014
Egg Day-1	0.006
Egg Day-2	0.009
Egg Day-3	0.012

C. EXTRACTION AND HYDROLYSIS OF RESIDUES

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The normalised percent distribution of the total radioactivity in each extract of the liver, muscle, fat and egg samples can be found in Table 6.2.2-2 (extraction yields/hydrolysis yield). The total residue extracted (organic/aqueous solvents and protease hydrolysis) accounted for 84% to 95% of the TRR in the tissues and eggs. In the case of liver, the acetonitrile fraction after extraction and partition contained 20% of the TRR, the hexane fraction contained 1% TRR, and additional 74% TRR were solubilised by incubation of the solids after solvent extraction with protease.

D. CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The distribution of the parent MKH 6561 and its metabolites in the acetonitrile fraction after extraction and partition and in the liver protease solubilisate is summarised in Table 6.2.2-2. Metabolites were primarily identified by comparison of the HPLC retention times and mass spectra (LC/ES-MS) of the metabolites with those of authentic [^{14}C]MKH 6561 reference items. Mass spectra of the isolated components and standards were obtained in the negative ionisation mode.

Any tissue or egg component which accounted for >10% of the TRR or > 0.05 mg/kg was identified. Five liver components (MKH 6561, M01, M05, M07, as well as M12 from the protease solubilisate) were identified by comparing the HPLC retention times and the mass spectrum (LC/ES-MS) of each component with that of the corresponding [^{14}C] MKH 6561 reference item. Components of the muscle, fat, and egg extracts were identified by correlation of the corresponding HPLC chromatogram to the HPLC chromatograms of the liver extract and the [^{14}C] MKH 6561 metabolite standards.

Several minor components (peaks) observed in the HPLC chromatograms were detected (labelled) but in most cases were not identified. An exception was if the minor component had a retention time which matched one of the reference items. In all extracts, the unidentified minor components represented <5% of the TRR and ≤0.05 mg/kg. At a theoretical 1X dose rate, the residues represented would not be detectable (<0.001 mg/kg). For these reasons, attempts to further characterise or identify any of these minor components were not made.

Table 6.2.2-2: Quantitative distribution of metabolites in the edible tissues and eggs after administration of [phenyl-UL-^{14}C]MKH 6561 to laying hens at 3.12 mg/kg body weight

Metabolite	Liver		Muscle		Fat		Egg Day-1		Egg Day-2		Egg Day-3	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
MKH 6561 [1]	10	0.134	42	0.007	61	0.009	31	0.002	21	0.002	29	0.003
M01 [1]	2	0.027	5	0.001	6	0.001	2	<0.001	3	<0.001	5	0.001
M05 [1]	3	0.040	16	0.003	12	0.002	-	-	-	-	-	-
M07 [1]	4	0.054	17	0.003	6	0.001	37	0.002	56	0.005	37	0.004
Unknown [1]	1	0.013	3	<0.001	3	<0.001	1	<0.001	2	<0.001	4	<0.001
Extraction yield (ACN) [1]	20	0.269	83	0.014	88	0.012	71	0.004	82	0.007	75	0.009
Extraction yield (hexane)	1	0.013	1	0.000	1	0.000	24	0.001	12	0.001	13	0.002
M12 [2]	61	0.819	Protease hydrolysis not applied		Protease hydrolysis not applied		Protease hydrolysis not applied		Protease hydrolysis not applied		Protease hydrolysis not applied	
M07 [2]	10	0.134										
Unknown [2]	3	0.040										
Protease hydrolysate (yield) [2]	74	0.994										
Total Extractables	95		84		89		95		94		88	
Total	4		3		3		1		2		4	

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Table 6.2.2-2: Quantitative distribution of metabolites in the edible tissues and eggs after administration of [phenyl-UL-^{14}C]MKH 6561 to laying hens at 3.12 mg/kg body weight

Metabolite	Liver		Muscle		Fat		Egg Day-1		Egg Day-2		Egg Day-3	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
characterised												
Solids	5	0.067	16	0.003	11	0.002	4	0.000	6	0.001	12	0.001
Total identified	90	1.209	80	0.014	85	0.012	70	0.004	80	0.007	71	0.009

[1] Radioactive residues (yield) and metabolites in the acetonitrile fraction (ACN) after partition with hexane

[2] In the case of liver, the solids after extraction with acetonitrile/water (9:1) were incubated with protease, and the resulting hydrolysate was also analysed by LSC (yield) and HPLC (which revealed additional amounts of metabolite M07 as well as metabolite M12 and an unknown component); solids mean the solids after protease hydrolysis in the case of liver

For chemical names and codes of the metabolites see Figure 6.2.2-1

1. Liver

In liver, a total of eleven individual components were seen in the acetonitrile fraction of the extracts (ACN extract) and the protease solubilisate. The most abundant component was only found in the protease solubilisate and identified as the serine conjugate of MKH 6561 (M12, 61% TRR, released after hydrolysis of protein bound MKH 6561 residues). The second most abundant component in liver was detected in the ACN extract and in the protease solubilisate and identified as saccharin (M07, 14% TRR in sum). Parent MKH 6561 (10% TRR) and the metabolites M05 (MKH 6561 sulphonamide methyl ester, 3% TRR) and M01 (2-hydroxypropoxy MKH 6561, 2% TRR) were only found in the ACN extract. The identification rate of the radioactive residues in liver was 90% of the TRR.

2. Muscle

In muscle, a total of six components were seen in the acetonitrile fraction of the extracts. The most abundant component was the unchanged parent compound MKH 6561 (42% TRR). In addition, the metabolites M07 (17% TRR), M05 (16% TRR) and M01 (5% TRR) were identified. The identification rate of the radioactive residues in muscle was 80% of the TRR.

3. Fat

In the fat, a total of nine components were seen in the acetonitrile fraction of the extracts. The most abundant component was the unchanged parent compound MKH 6561 (61% TRR). In addition, the metabolites M05 (12% TRR), M07 (6% TRR) and M01 (6% TRR) were identified. The identification rate of the radioactive residues in fat was 85% of the TRR.

4. Eggs

A total of four components were observed in the extracts of Day-1, Day-2 and Day-3 eggs. An additional unknown component was observed in the Day-3 extract. The most abundant component was metabolite M07 (37% to 56% TRR), followed by the unchanged parent compound MKH 6561 (21% to 31% TRR) and the metabolite M01 (2% to 5% TRR). The identification rate of the radioactive residues in eggs was 70% to 80% of the TRR.

4. Proposed metabolic pathway

A proposed metabolic scheme for MKH 6561 in the laying hen showing the metabolites identified in both the present [^{14}C-phenyl] labelled study and the [^{14}C-triazolinone] labelled study (M-CA 6.2.2/02) is given in Figure 6.2.2-1. A list of the poultry metabolites identified in the two studies is provided at the end of section 6. Except in the liver, the major metabolic pathway of MKH 6561 in poultry was hydrolysis of the parent compound producing the sulphonamide methyl ester M05. The sulphonamide methyl ester was converted to saccharin M07, either directly or

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following hydrolysis to the sulphonamide acid M06. A minor pathway involved hydroxylation at the 2-position of the triazolinone propoxy group to form 2-hydroxypropoxy MKH 6561 (M01).

In liver, the triazolinone group of MKH 6561 was readily displaced by the hydroxyl group of the amino acid serine, resulting in the formation of a serine conjugate which was incorporated (covalently bound) into various liver proteins. Since no "free" MKH 6561 serine conjugate was observed in the liver ACN extract, the assumption is that the serine amino acid is already incorporated into the liver protein prior to conjugation with MKH 6561. The MKH 6561 serine conjugate was only observed following hydrolysis of the liver proteins using protease enzyme. The covalent binding of MKH 6561 residues to liver solids was not totally unexpected since similar observations had been reported for the goat and poultry metabolism of two sulfonylurea herbicides, sulfometuron methyl and [phenyl-^{14}C]MKH 6562. In these metabolism studies, the bound radioactive residues were released by protease hydrolysis.

In the [^{14}C-triazolinone] MKH 6561 poultry metabolism study (M-CA 6.2.2/02), N-methyl propyl triazolinone (NMPT, metabolite M10) was identified as the primary hydrolysis product, complementing the sulphonamide methyl ester hydrolysis product identified in the [^{14}C-phenyl] metabolism study. The Pr-2-OH MKH 6561 metabolite M01, as expected, was observed in both metabolism studies.

III. CONCLUSIONS

When laying hens were given a daily dose of protonated MKH 6561 at 3.12 mg/kg body weight (equivalent to 49 mg/kg in feed) via capsule for 3 consecutive days, the residue levels were 1.343 mg/kg in the liver, 0.017 mg/kg in the muscle, 0.014 mg/kg in the fat, 0.006 mg/kg in the Day-1 eggs, 0.009 mg/kg in the Day-2 eggs, and 0.012 mg/kg in the Day-3 eggs. The residue levels based on a theoretical 1X rate would all be considerably less than 0.001 mg/kg.

Approximately 95% of the radioactive residues in liver was solubilised by a combination of organic solvent extraction (21% of the TRR) and protease hydrolysis (74% TRR). The majority of the radioactive residues from the muscle (84% TRR), fat (89% TRR), and eggs (88% to 95% TRR) was extracted with organic solvents.

The major residues identified in tissues and eggs were propoxycarbazone-sodium, M01, M05 and M07. Identification of the TRR in tissues and eggs was 90% in liver, 80% in muscle, 85% in fat, 70% in Day-1 eggs, 80% in Day-2 eggs, and 71% in Day-3 eggs. In addition, several minor components (<1% TRR) were observed in tissue and egg extracts and were characterised using chromatographic methods. These minor components comprised only 1% to 4% of the TRR in tissue and eggs.

The major metabolic pathway of propoxycarbazone-sodium in poultry was hydrolysis of the parent compound producing the metabolites M05 and M10. Metabolite M05 was then converted to M07 (saccharin). A minor pathway involved hydroxylation at the 2-position of the triazolinone propoxy group, forming metabolite M01. In the liver, the main metabolic pathway led to the formation of protein bound MKH 6561 residues through conjugation with the amino acid serine.

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Report:	███ ˋ ;███;███;1999;M-015838-01
Title:	The distribution and metabolism of [triazolinone-3-14C] MKH 6561 in laying hens
Report No:	107919
Document No:	M-015838-01-1
Guidelines:	**OPPTS 860.1300, Nature of Residues-Plants, Livestock**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

Fifteen laying hens were dosed orally, via capsule, with protonated [triazolinone-3-^{14}C]MKH 6561 for 3 consecutive days at an average daily dose rate of 2.91 mg/kg body weight (46 mg/kg in the feed).
The total radioactive residue (TRR) levels were 0.184 mg/kg in the liver, 0.044 mg/kg in the muscle, 0.015 mg/kg in the fat, 0.011 mg/kg in the Day-1 eggs, 0.016 mg/kg in the Day-2 eggs, and 0.022 mg/kg in the Day-3 eggs. Pooled excreta of study day 1 through study day 3 contained 72.79% of the administered dose while eggs collected during treatment contained 0.01% of the dose and tissues contained a total of 0.09% of the total dose.
Approximately 97% of the radioactivity in liver was solubilised by a combination of organic solvent extraction (73% of the TRR) and several steps of accelerated solvent extraction (150 °C, releasing 24% TRR in sum). The majority of the radioactive residues from the muscle (94% TRR), fat (97% TRR), and eggs (94% to 97% TRR, in the case of Day-3 eggs including 5% TRR solubilised by accelerated extraction with ACN/H_2O at 150 °C) was solubilised by extraction with organic solvents.
The metabolism of [^{14}C]MKH 6561 appeared to involve two pathways. One pathway involved simple hydrolysis of the parent compound producing metabolites M10 and M05. The other pathway involved hydroxylation at the 2-position of the triazolinone propoxy group to form metabolites M01 and M02.
The major residues found in the tissues and eggs were: protonated MKH 6561 (10% of the TRR in liver, 23% TRR in muscle, 46% TRR in fat, 8% TRR in Day-1 eggs, 6% TRR in Day-2 eggs, and 7% TRR in Day-3 eggs); metabolite M02 (18% TRR in liver, 43% TRR in muscle, 21% TRR in fat, 64% TRR in Day-1 eggs, 51% TRR in Day-2 eggs, and 47% TRR in Day-3 eggs); metabolite M10 (36% TRR in liver, 15% TRR in muscle, 12% TRR in fat, 16% TRR in Day-1 eggs, 20% TRR in Day-2 eggs, and 17% TRR in Day-3 eggs); and metabolite M01 (7% TRR in liver, 3% TRR in muscle, 10% TRR in fat, 1% TRR in Day-2 eggs, and 2% TRR in Day-3 eggs).
Total identification of radioactive residues in the tissues and eggs was 71% in liver, 84% in muscle, 89% in fat, 88% in Day-1 eggs, 78% in Day-2 eggs, and 73% in Day-3 eggs. In addition, several minor components were observed in tissue and egg extracts and were characterised (mostly polar components) using chromatographic methods (HPLC or TLC). These minor components comprised 21% of the TRR in liver, 11% TRR in muscle, and 9% to 18% TRR in eggs. All radioactive residues which were >10% of the TRR or >0.05 mg/kg were identified.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Protonated [triazolinone-3-^{14}C]MKH 6561
(also referred to as MKH 5554, applicant´s code number)
(methyl 2-[[[(4,5-dihydro-4-methyl-5-oxo-3-propoxy-1*H*-1,2,4-triazol-1-yl)carbonyl] amino] sulfonyl] benzoate-triazole-3-^{14}C)
CAS # 145026-81-9
(essentially equivalent to MKH 6561 (sodium salt))

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	mixed with non radiolabelled MKH 6561, protonated form
Common name:	Propoxycarbazone-sodium
Empirical formula:	$C_{15}H_{18}N_4O_7S$, protonated form
Molar mass:	398.4 (protonated form)
Labelling:	[triazolinone-3-^{14}C]
Specific radioactivity:	^{14}C stock solution: 306000 dpm/µg (mixture in the capsules: 210000 dpm/µg)
Purity:	^{14}C: radiochemical purity: 99.3% non labelled: purity: 97.1%
Lot/Batch #:	^{14}C: stock solution in acetonitrile, Vial C-717 (synthesised by the Bayer Radiosynthesis Group in ███, KS, USA) non labelled: Vial K-624
Dose level:	3 x 2.91 mg/kg body weight (average)
Stability of test compound:	Checked by HPLC to have been stable in dosing capsules

2. **Vehicle:** α-lactose (in gelatine capsules)

3. **Test animals:**

Species:	Laying Hens (*Gallus domesticus*)
Strain:	Leg Horn
Source:	███
Sex:	Female
Age:	64 weeks at selection; 66 weeks at dosing
Weight:	1441-1648 g (average body weights between animal selection and termination)
Number of animals:	15
Acclimation period:	15 days (Study Day -14 to Study Day 1, 1996-10-07 to 1996-10-22)
Identification:	Leg band (right leg) containing a unique identification number
Diet/Food:	Purina brand Layena® Crumbles; Lot No. 0009, Aug 2996 (Horse N Hound, Feed N Supply, Las Cruces, NM 880005;) *via* cage feeders, ad libitum
Water:	Fresh potable water provided *via* automatic water bowls, ad libitum
Housing:	Individually in metabolism cages
Environmental conditions:	Temperature: 65 – 71 °F (average min. and max. temperature) Humidity: 60% (average) 14-hour light/10-hour dark photoperiod

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B. STUDY DESIGN

In life dates
1996-10-07 (test animal receipt) / 1996-10-22 (Study Day 1) to 1996-11-15
Analytical work
October 1996 to December 1997

The objective of this study was to investigate the metabolism of [triazolinone-3-^{14}C] MKH 6561 in laying hens and the distribution of radioactive residues in the tissues and eggs and to evaluate the residues to be expected in poultry.

1. Test procedure

Following eight days of acclimation, fifteen laying hens (treated group) were chosen for the study on Study Day-6 (body weight 1327 to 2093 g). Each animal was identified via a leg band containing a unique identification number.

Each chicken was orally administered a single dose capsule containing [^{14}C]MKH 6561 via balling gun on Study Days 1, 2 and 3. Aliquots of the dose solution were analysed by LSC and HPLC (system A) to determine the actual concentration (417 µCi [^{14}C]MKH 6561 per capsule) and purity of the test item. The stability of the [triazolinone-3-14C] MKH 6561 poultry dose during shipment and storage was determined by HPLC analysis of the contents (MKH 6561-treated α-lactose) of three extra dose capsules after dissolution in water. These stability tests indicated no degradation of [triazolinone-3-^{14}C]MKH 6561 during three weeks of storage.

The average daily dose rate of 2.91 mg/kg body weight (46 mg/kg in the feed; average body weight 1.52 kg) was approximately 3000X (dose exaggeration) the maximum dietary burden of 0.001 mg/kg b.w./day (see section CA 6.4). The exaggeration factor given in the study had been calculated with the magnitude of the residue found in the MKH 6561 wheat field trials.

2. Sampling

Eggs were collected from each hen twice daily (prior to dosing) beginning at receipt and continuing until the termination of each animal. The PM and AM eggs following each dosing from all of the hens were composited and considered as one day´s sample. Excreta from each hen were collected starting on study day 1 every 24 hours and composited daily as one day´s sample. The hens were humanely terminated 4 to 5 hours following the final dose. Liver, composite muscle (leg and breast) and composite fat (available omental and subcutaneous) were collected; tissues of the same type from all 15 birds were composited. The tissues, eggs and excreta were homogenised, and subsamples were radioassayed. All excreta, tissue and egg samples were stored frozen at Southwest Bio-Labs and shipped frozen (dry ice) to Bayer for analysis.

3. Analysis

Radioactivity measurement

All tissue, egg and excreta samples were combusted using an oxidiser. Liberated $^{14}CO_2$ was trapped using an absorption liquid, combined with an appropriate scintillation liquid, and the total radioactive residues were determined by liquid scintillation counting (LSC). The radioactive residues in liquid samples (e. g. extracts) were measured by LSC after mixing with an appropriate scintillation cocktail. Solid samples after extraction were also oxidised, and the released $^{14}CO_2$ was trapped in an alkaline solution and radioassayed.

Extraction of tissues and eggs

A portion of tissue or eggs was blended with hexane using a tissumizer. The tissue solids were allowed to settle, and the hexane supernatant was decanted and vacuum-filtered. The hexane-extracted solids were blended with acetonitrile/water (9:1), followed by filtration. The filtered solids were extracted two additional times with fresh ACN/H_2O (9:1) in the same manner, and the three filtrates were combined. The extracted solids were air-dried and occasionally agitated with a spatula to form a fine powder, weighed, and the radioactive residues were determined by combustion of aliquots. The hexane and ACN/H_2O extracts were radioassayed.

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The ACN/H_2O extract was rotoevaporated to dryness, redissolved in methanol/water (4:1) and percolated through a conditioned SPE cartridge. The SPE cartridge was washed with additional MeOH/H_2O (4:1). The combined MeOH/H_2O eluents were concentrated and the dry residue was dissolved in H_2O/ACN (9:1) containing 0.1% trifluoroacetic acid (TFA). The resulting solution was radioassayed, and aliquots were analysed by HPLC (system B). Individual component peaks were isolated from the HPLC eluent, and selected metabolites were analysed by mass spectrometry.

Extraction of liver and egg solids using Accelerated Solvent Extraction (ASE)

Subsamples of the liver solids or the Day-3 egg solids remaining after ACN/H_2O extraction were mixed with Celite and transferred to an accelerated solvent extraction tube (cell). The Day-3 egg solids after solvent extraction were extracted with ACN/H_2O (1:1) using an accelerated solvent extractor (150 °C, 1500 psi). The liver solids were extracted in sequence first with ACN/H_2O (1:1) and then with 0.1% aqueous TFA (ASE, 150 °C, 1500 psi). All extracts were radioassayed. The extracted solids were oven dried, and aliquots were oxidised to determine the radioactive residues.
The 0.1% TFA extracted liver solids were extracted a final time with 1% TFA using the ASE extractor (150 °C, 1500 psi). The extract was radioassayed, and the composition of the extract was determined (characterised) using thin-layer chromatography (TLC). The extracted liver solids were air dried, and aliquots of the dried liver solids were oxidised and radioassayed.
The liver ACN/H_2O (1:1) accelerated solvent extract was percolated through a conditioned SPE cartridge (C-18). The SPE cartridge was then washed with additional ACN/H_2O (1:1), and the ACN/H_2O eluents were combined and radioassayed. The ACN/H_2O eluent was rotoevaporated, and the dry residue was dissolved in 0.1% aqueous TFA. The resulting solution was radioassayed, and aliquots were analysed by HPLC (system B).

Extraction of excreta

Excreta were extracted, and metabolites were isolated for the purpose of generating [triazolinone-^{14}C]-labelled reference items. A 234 g portion of composite excreta was blended with 200 mL acetonitrile. The ACN suspension was vacuum filtered, and the filtered solids were extracted again with 300 mL fresh ACN/H_2O (9:1). The extracts from the two blendings were combined and radioassayed.
The combined ACN/H_2O extracts were concentrated to an aqueous remainder using a rotary evaporator. The volume of the concentrate was adjusted to 150 mL with water, and 50 mL of saturated aqueous sodium chloride was added. The aqueous solution was partitioned twice with 150 mL ethyl acetate. The aqueous fraction was acidified (pH 1) with hydrochloric acid and extracted three additional times with ethyl acetate. The five ethyl acetate extracts were combined, radioassayed, and dried by stirring with anhydrous sodium sulphate (Na_2SO_4). The extract was filtered to remove the Na_2SO_4 and rotoevaporated. The dry residue was suspended in acetonitrile, filtered through a 0.45 µm disc filter, and aliquots were analysed by HPLC (system B). Metabolite peaks were isolated from the HPLC eluent, and selected metabolites were analysed by mass spectrometry.
An aliquot of the ethyl acetate extract of excreta was rotoevaporated, and the dry residue was suspended in 0.1% TFA. The suspension was transferred to an appropriate vial and placed in a heating block for 2 hours at 75 °C. An aliquot of the resulting hydrolysate was analysed by HPLC (system B). Individual components were isolated from the HPLC eluent and were further analysed by mass spectrometry.

Metabolite analysis

High-Performance Liquid Chromatography (HPLC) analysis of the samples prepared from the ACN/H_2O extracts of tissues and eggs, from the liver ACN/H_2O (1:1) accelerated solvent extract (concentrated SPE eluates, see above), and from the combined ethyl acetate extract of excreta (with and without hydrolysis) was performed using a reversed phase column (C18) and a flow-through radiodetector (solid scintillator cell). Separation was achieved using gradient elution (two slightly differing gradient programs for system A and system B) with 0.1% TFA in water (solvent A) and acetonitrile (solvent B).

Thin-Layer Chromatography (TLC) was performed on silica gel 60 F-254 plates for characterisation of the 1% TFA extract of liver. The samples were applied using a micropipette and focussed on the plate by developing three times with methanol. The plates were developed with dichloromethane/methanol/water/ammonium hydroxide (60:15:1:1). Distribution of radioactivity on the TLC plates was determined by exposition of a phosphor screen and scanning using a phosphorimager.

Mass spectral analysis (liquid chromatography/electrospray-mass spectrometry, LC/ES-MS) was performed using a C8 column hyphenated in parallel to a radiodetector and to a triple stage quadrupole (TSQ) electrospray (ES) mass spectrometer. Separation was achieved using gradient elution with water with 0.1% formic acid (solvent A) and methanol (solvent B) for mass spectral analysis in positive ionisation mode or gradient elution with water with 5 mM ammonium acetate (solvent A) and methanol (solvent B) for mass spectral analysis in negative ionisation mode.

Three [^{14}C] reference items were synthesised by the Bayer Radiosynthesis Group: [triazolinone-^{14}C]MKH 6561, [phenyl-^{14}C]M01 and [triazolinone-^{14}C]M10. One additional reference item ([triazolinone-^{14}C] M02) was obtained by hydrolysis of [triazolinone-^{14}C]M01, a metabolite isolated and identified from the excreta.

4. Stability of [^{14}C]MKH 6561 in tissue and egg extracts

All tissue and egg samples were extracted and analysed by HPLC for metabolite profiles within 6 weeks of collection. With the exception of the liver, preliminary identification of tissue and egg residues was completed within 4 months of collection. The percent TRR in the ACN/H_2O extract from liver which was stored for 18 months was the same (within 10%) as the percent TRR in the ACN/H_2O extract from the initial liver extraction (1 month following tissue receipt). A comparison of HPLC chromatograms of the initial liver extract and extract obtained from liver stored for 18 months showed the metabolite distribution to be very similar. The percent area integration of the four main components in each extract was nearly identical (<5% variation). When tissues or egg samples were not being analysed, they were stored in the freezer (-20 ± 5 °C).
Tissues and eggs were initially extracted with mixtures of ACN and water (9:1). When extracts were not being analysed, they were stored at refrigerator temperature. Propoxycarbazone-sodium is stable and does not degrade in ACN or water, as long as the pH of the solvent is near neutral (pH 7). Since MKH 6561 (parent compound) was observed in all tissue and egg extracts, all components identified in the extracts most likely arose from the metabolism of propoxycarbazone-sodium and presumably were not formed as artefacts resulting from the degradation of MKH 6561 upon extraction and storage.

II. RESULTS AND DISCUSSION

A. ANIMAL HEALTH AND ANIMAL HUSBANDRY

Overall, the animals appeared healthy throughout the course of the treatment period as evidenced by observance of feed consumption, daily observations, physical examinations, body weights and observation of tissues at necropsy.

B. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residue (TRR) levels (expressed as mg/kg) found in the poultry tissues and eggs are given in Table 6.2.2-3. The tissue and egg residue levels were adjusted for the final capsulated specific radioactivity of the dose (210000 dpm/µg). The highest equivalent concentration was measured in the liver (0.184 mg/kg), followed by that obtained for muscle (0.044 mg/kg) and fat (0.015 mg/kg). The equivalent concentrations in eggs increased from Day-1 to Day-3 (0.011 mg/kg to 0.022 mg/kg). Pooled excreta contained 72.79% of the administered dose with approximately 72.89% of the administered dose recovered in the combined tissues, eggs and excreta. The residue levels were not elevated in relation to the

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dose level. Even at the exaggerated dosing rate, only the liver had residues higher than 0.05 mg/kg, and at the postulated 1X level, all tissue and egg residues would be significantly less than 0.01 mg/kg. No residue of MKH 6561 would be expected in tissue or eggs from poultry fed a diet containing wheat or wheat by-products from MKH 6561-treated wheat.

Table 6.2.2-3: Total radioactive residue levels in the edible tissues and eggs of laying hens following the administration of three consecutive doses of [triazolinone-3-^{14}C] MKH 6561 at 2.91 mg/kg body weight

Tissue / Eggs	Residue levels mg/kg (MKH 6561 equivalents) [1]	Adjusted residue levels mg/kg (MKH 6561 equivalents) [2]
Liver	0.193	0.184
Muscle	0.046	0.044
Fat	0.016	0.015
Egg Day-1	0.012	0.011
Egg Day-2	0.017	0.016
Egg Day-3	0.023	0.022

[1] Values calculated using the target specific activity of 200000 dpm/µg for the dose
[2] Adjusted residue levels based on the final capsulated specific activity of the dose (210000 dpm/µg)

C. EXTRACTION OF RESIDUES

The normalised percent distribution of the total radioactivity in each extract of the liver, muscle, fat and egg samples can be found in **Fehler! Verweisquelle konnte nicht gefunden werden.** (extraction yields). The total residue extracted (organic/aqueous solvents and ASE with ACN/H_2O or aqueous TFA) accounted for 94% to 97% of the TRR in the tissues and eggs. Very little radioactive residue (≤6% TRR) remained in the final tissue and egg solids. In the case of liver, the ACN/H_2O extract contained 67% of the TRR, the hexane extract contained 6% TRR, and additional 24% TRR were solubilised by the three steps of accelerated solvent extraction. In the case of Day-3 eggs, the ACN/H_2O extract contained 89% of the TRR, the hexane extract contained <1% TRR, and additional 5% TRR were solubilised by accelerated solvent extraction with ACN/H_2O.

D. CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The distribution of the parent compound MKH 6561 and its metabolites in the ACN/H_2O extracts and in the accelerated solvent extracts of the liver solids or the Day-3 egg solids remaining after ACN/H_2O extraction is summarised in **Fehler! Verweisquelle konnte nicht gefunden werden.**. Metabolites were primarily identified by comparison of the HPLC chromatograms and mass spectra (LC/ES-MS) of the metabolites with the respective HPLC chromatograms and mass spectra of [^{14}C]MKH 6561 reference items. Mass spectra of the isolated components and standards were obtained in either positive or negative ionisation mode, depending on the proposed molecular structure. MKH 6561 components and standards which contained both the phenyl sulphonamide and the triazolinone structural moieties were obtained in the negative ion mode. MKH 6561 components and standards which contained only the triazolinone structural moiety were obtained in the positive ion mode.

Any tissue or egg component which accounted for >10% of the TRR or > 0.05 mg/kg was identified. Four liver components (MKH 6561, M01, M10 and M02) were identified by comparing the HPLC retention times and the mass spectrum (LC/ES-MS) of each component with that of the corresponding [^{14}C] MKH 6561 reference item. Components of the muscle, fat, and egg extracts were identified by correlation of the corresponding HPLC chromatogram to the HPLC chromatograms of the liver extract and the [^{14}C] MKH 6561 metabolite standards.

Minor polar components (e.g. in muscle) were characterised as such based on their short HPLC retention times. Other minor components (peaks) observed in the HPLC chromatograms were assigned a number (labelled) but in most cases were not identified. An exception was if the minor component had a retention

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time which matched one of the reference items. In all extracts, the unidentified components represented <10% of the TRR and ≤0.002 mg/kg. At a theoretical 1X dose rate, the residues represented would not be detectable (<0.001 mg/kg). For these reasons, attempts to further characterise or identify any of these minor polar components were not made.

Table 6.2.2-4: Quantitative distribution of metabolites in the edible tissues and eggs after administration of [triazolinone-3-^{14}C]MKH 6561 to laying hens at 2.91 mg/kg body weight

Metabolite	Liver		Muscle		Fat		Egg Day-1		Egg Day-2		Egg Day-3	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
MKH 6561 [1]	8	0.015	23	0.010	46	0.007	8	0.001	6	0.001	7	0.002
M01 [1]	6	0.011	3	0.001	10	0.002	-	-	1	<0.001	2	<0.001
M10 [1]	32	0.059	15	0.007	12	0.002	16	0.002	20	0.003	17	0.004
M02 [1]	15	0.028	43	0.019	21	0.003	64	0.007	51	0.008	47	0.010
Unknown [1]	6	0.011	10	0.005	-	-	9	0.001	17	0.003	16	0.004
Extraction yield (ACN/H_2O) [1]	67	0.123	94	0.041	89	0.013	97	0.011	94	0.015	89	0.020
Extraction yield (hexane)	6	0.011	<1		8	0.001	<1		<1		<1	
MKH 6561 [2]	2	0.004	no additional extraction steps applied		no additional extraction steps applied		no additional extraction steps applied		no additional extraction steps applied		ACN/H_2O/ 150 °C ASE extract not chromato-graphically analysed	
M01 [2]	1	0.002										
M10 [2]	4	0.007										
M02 [2]	3	0.006										
Unknown [2]	2	0.003										
Extraction yield (ACN/H_2O/ 150 °C) [2]	11	0.020									5	0.001
Extraction yield (0.1% TFA 150 °C) [2]	<1										not applied	
Extraction yield (1% TFA 150 °C) [2]	13	0.024									not applied	
Total Extractables	97		94		97		97		94		94	
Total characterised by HPLC or TLC	21		11		-		9		18		17	
Solids	2	0.004	5	0.002	3	0.000	3	<0.001	6	0.001	6	0.001
Total identified	71	0.131	84	0.037	89	0.013	88	0.010	78	0.012	73	0.016

1 Radioactive residues (yield) and metabolites in the acetonitrile/water fraction (ACN/H_2O)

2 In the case of liver, the solids after extraction with acetonitrile/water (9:1) were further extracted at 150 °C and 1500 psi (accelerated solvent extraction, ASE) with acetonitrile/water (1:1), 0.1% trifluoroacetic acid (TFA, 2X) and 1% TFA; the resulting extract with acetonitrile/water at 150 °C (ACN/H_2O/150 °C) was also analysed by LSC (yield) and HPLC (which revealed additional amounts of MKH 6561, its metabolites M01, M10 and M02, and unknown components); the radioactive residues in the extracts released with 0.1% TFA and with 1% TFA were determined by LSC to account for <1% TRR and 13% TRR (0.024 mg/kg; several components according to TLC, including polar components arising from hydrolysis of the residues after solvent extraction of liver), respectively; solids mean the solids after the additional extraction steps in the case of liver;
In the case of eggs (Day-3), the solids after extraction with acetonitrile/water (9:1) were further extracted at 150 °C and 1500 psi (accelerated solvent extraction, ASE) with acetonitrile/water (1:1); the resulting extract was only analysed by LSC (yield); solids mean the solids after extraction with ACN/H_2O/150 °C in the case of eggs (Day-3)

For chemical names and codes of the metabolites see Figure 6.2.2-1

1. Liver

In liver, a total of twelve individual components were seen in the ACN/H_2O extract and the ACN/H_2O/150 °C ASE extract. The most abundant component was identified as NMPT MKH 6561 (M10, 36% of the TRR in sum). The second most abundant component in liver was identified as Pr-2-OH NMT MKH 6561 (M02, 18% TRR in sum). Parent MKH 6561 (10% TRR in sum) and metabolite M01 (Pr-2-OH MKH 6561, 7% TRR in sum) were found in addition. The identification rate of the radioactive residues in liver was 71% of the TRR.

The 1% TFA liver extract (ASE extraction of liver solids), which contained 12% of the TRR, was not analysed by HPLC due to difficulties encountered in the clean-up and concentration of this fraction. The 1% TFA extract was assumed to contain a variety of polar components arising from hydrolysis of non-extractable residues in the liver. TLC of a small aliquot of the extract showed one major component in addition to a broad band of unresolved baseline material (polar components). The major component had an R_f which closely matched the reference item M01. Since this extract contains several components, no single component of this extract contained >10% of the TRR or >0.05 mg/kg of residue.

2. Muscle

In muscle, a total of eight components were seen in the initial ACN/H_2O extract. The most abundant component was metabolite M02 (43% TRR), followed by the unchanged parent compound MKH 6561 (23% TRR). In addition, the metabolites M10 (15% TRR) and M01 (3% TRR) were identified. The identification rate of the radioactive residues in muscle was 84% of the TRR.

3. Fat

In the fat, a total of four components were seen in the initial ACN/H_2O extract. The most abundant component was the unchanged parent compound MKH 6561 (46% TRR). In addition, the metabolites M02 (21% TRR), M10 (12% TRR) and M01 (10% TRR) were identified. The identification rate of the radioactive residues in fat was 89% of the TRR.

4. Eggs

A total of seven components were observed in the ACN/H_2O extract of Day-1 eggs. A total of nine components were observed in the ACN/H_2O extract of Day-2 eggs. A total of eight components were observed in the ACN/H_2O extract of Day-3 eggs. The most abundant component was metabolite M02 (47% to 64% TRR), followed by metabolite M10 (16% to 20% TRR) and the unchanged parent compound MKH 6561 (6% to 8% TRR). Metabolite M01 was only detected in the extracts of Day-2 eggs and Day-3 eggs (1% and 2% TRR, respectively). The identification rate of the radioactive residues in eggs was 73% to 88% of the TRR.

An additional portion of 5% TRR was characterised by its solubilisation with ACN/H_2O/150 °C (accelerated solvent extraction).

4. Proposed metabolic pathway

A proposed metabolic scheme for MKH 6561 in the laying hen showing the metabolites identified in both the [^{14}C-phenyl] labelled study (M-CA 6.2.2/01) and the present [^{14}C-triazolinone] labelled study is given in Figure 6.2.2-1. A list of the poultry metabolites identified in the two studies is provided at the end of section 6. The major metabolic pathway involved hydroxylation of the MKH 6561 propoxy group to yield Pr-2-OH MKH 6561 (M01), which in some cases was followed by hydrolytic cleavage of the phenyl sulphonamide side chain to give Pr-2-OH NMT MKH 6561 (M02). Alternatively, hydrolysis of MKH 6561 would give NMPT MKH 6561 (M10) which, following hydroxylation of the propoxy group, would give M02.

In the [^{14}C-phenyl]MKH 6561 poultry metabolism study (M-CA 6.2.2/01), MKH 6561 sulphonamide methyl ester (M05) was identified as the primary hydrolysis product, complementing the NMPT MKH 6561 hydrolysis product (M10) identified in the [^{14}C-triazolinone] metabolism study. The 2-hydroxypropoxy MKH 6561 metabolite M01, as expected, was observed in both metabolism studies.

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III. CONCLUSIONS

When laying hens were given a daily dose of protonated MKH 6561 at 2.91 mg/kg body weight (equivalent to 46 mg/kg in feed) via capsule for 3 consecutive days, the residue levels were 0.184 mg/kg in the liver, 0.044 mg/kg in the muscle, 0.015 mg/kg in the fat, 0.011 mg/kg in Day-1 egg, 0.016 mg/kg in Day-2 egg, and 0.022 mg/kg in Day-3 egg. The residue levels in tissues and eggs based on a theoretical 1X rate would all be considerably less than 0.001 mg/kg.

Approximately 97% of the radioactive residues in liver was solubilised by a combination of organic solvent extraction (73% of the TRR) and accelerated solvent extraction (several steps at 150 °C, 24% TRR in sum). The majority of the radioactive residues from the muscle (94% TRR), fat (97% TRR), and eggs (94% to 97% TRR) was extracted with organic solvents.

The major residues identified in tissues and eggs were MKH 6561, M01, M10 and M02. Identification of TRR in the tissues and eggs was 71% in liver, 84% in muscle, 89% in fat, 88% in Day-1 eggs, 78% in Day-2 eggs, and 73% in Day-3 eggs. In addition, several minor components (<10% TRR) were observed in tissue and egg extracts and were characterised (mostly polar components) using chromatographic methods (HPLC or TLC). These minor components comprised 21% of the TRR in liver, 11% TRR in muscle and 9% to 18% TRR in eggs.

The metabolism of MKH 6561 appeared to involve both hydroxylation at the 2-position of the propoxy group and hydrolysis of the phenyl sulphonamide linkage.

For chemical names and codes see Figure 6.2.2-1.

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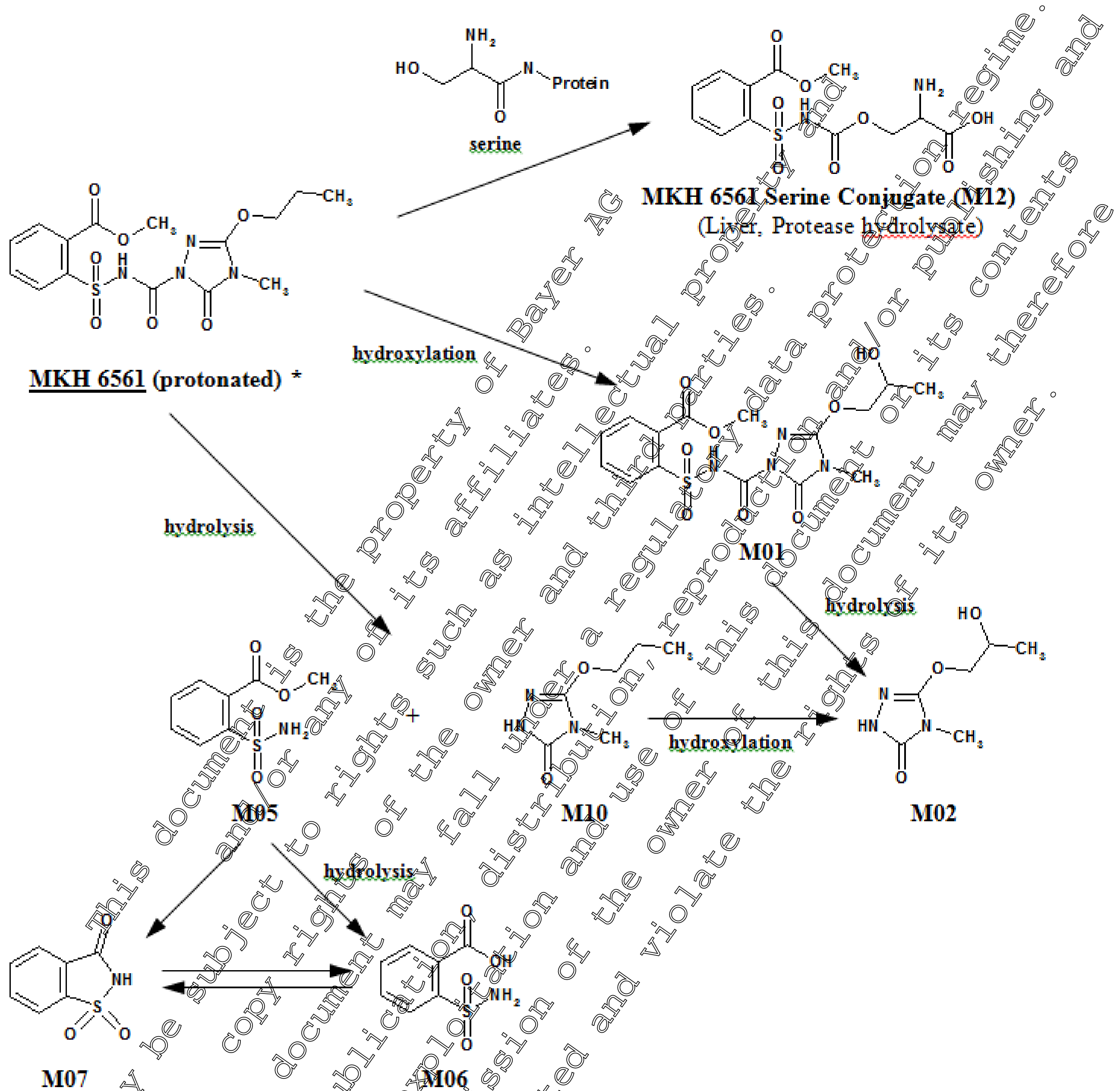
Figure 6.2.2-1: Proposed metabolic pathway for MKH 6561 in poultry showing metabolites identified in the [^{14}C-phenyl] MKH 6561 and [^{14}C-triazolinone] MKH 6561 poultry metabolism studies



* The code MKH 6561 is generally used for the sodium salt.
In the hen metabolism studies the compound was investigated using the free acid form.

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CA 6.2.3 Lactating ruminants

Conclusions from the EU evaluation of propoxycarbazone-sodium (DAR)
Livestock metabolism studies of propoxycarbazone-sodium using [phenyl-UL-^{14}C] propoxycarbazone acid (free acid) (, 1999, RIP2000-1011) and [triazolinone-3-^{14}C] propoxycarbazone acid (free acid) (, 1999, RIP2000-1012) have been conducted in the lactating goat as a model for ruminants. Two lactating goats per label were dosed orally, via capsule, on three consecutive days at an average dose rate of 1.0 mg/kg bw for the [phenyl-UL-^{14}C] MKH 6561 and at an average dose rate of 0.98 mg/kg bw for the [triazolinone-3-^{14}C] MKH 6561.
The dose rates applied in the goat metabolism studies were equivalent to 17 mg/kg [phenyl-UL-^{14}C]MKH 6561 and 24.8 mg/kg [triazolinone-3-^{14}C]MKH 6561 in feed, respectively. These dose rates were 189X the maximum dietary burden for meat ruminants in the case of the phenyl label and 276X the maximum dietary burden in the case of the triazolinone label (the maximum dietary burden was calculated considering additional residue data from the year 2004: 0.09 mg/kg DM; the exaggeration factors given in the studies had been calculated with the magnitude of the residues found in the wheat metabolism studies).

The total radioactive residue levels in the study investigating [phenyl-UL-^{14}C] MKH 6561 accounted for 3.643 mg/kg in liver, 0.486 mg/kg in kidney, 0.009 mg/kg in muscle, 0.004 mg/kg in fat, 0.015 mg/kg in Day-1 milk, and 0.022 mg/kg in Day-2 milk. In the experiment investigating [triazolinone-3-^{14}C]MKH 6561 the corresponding residues were even lower. The anticipated residue levels based on a theoretical 1X rate would be <0.001 mg/kg in all tissues and milk, except for liver (0.019 mg/kg) and kidney (0.0025 mg/kg) in the case of the phenyl label and kidney (0.0015 mg/kg) in the case of the triazolinone label.
The radioactive residues were extracted from milk and edible goat tissues with high recoveries. Unchanged parent compound was detected in all organs, tissues and milk, representing the major residue in milk. Metabolites identified were:

- Sulfonamide methyl ester (M05) in fat and in very low amounts in liver and milk
- Saccharin (M07) in fat, kidney and milk and as protein conjugates as major component in liver
- N-methyl propoxy triazolinone (M10) in all organs, tissues and milk
- 2-hydroxy-N-methyl propoxy triazolinone (M02) in all organs, tissues and milk.

The total identification rate was very high (77% of the TRR in kidney and > 89% for liver and milk), except for fat and muscle in the phenyl-labelled experiment due to the low amount of total radioactive residue. The metabolic behaviour of propoxycarbazone-sodium in goats was not significantly different from that observed in rats.

The metabolic pathway for propoxycarbazone acid in lactating goats is shown in Figure 6.2.3-1.

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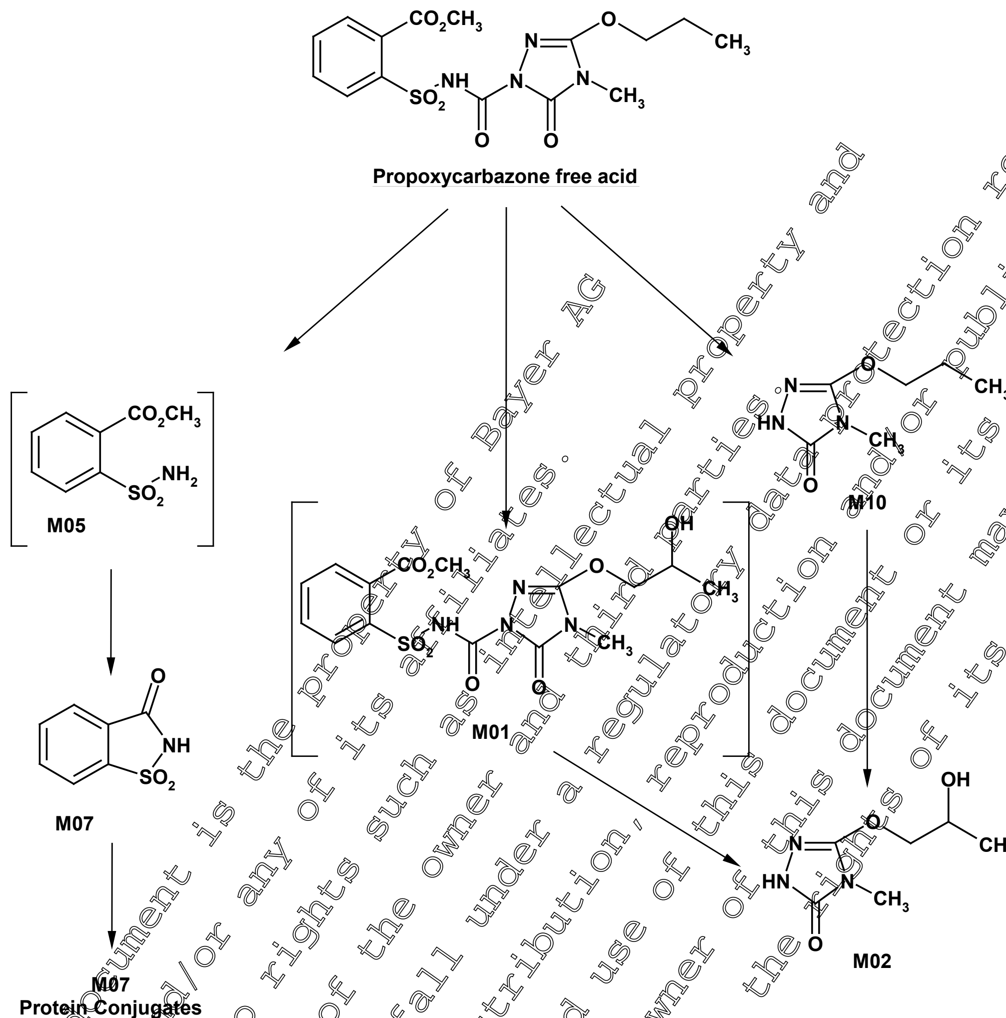
Figure 6.2.3-1: Metabolic pathway for propoxycarbazone acid in goats



CA 6.2.4 Pigs

Since the metabolic pattern in laying hens and in the lactating goat (see sections 6.2.2 and 6.2.3) is very similar to that in the rat, a pig metabolism study was not conducted.

CA 6.2.5 Fish

Studies on the bioaccumulation in fish are not required due to the log P_{OW} of -0.3 (pH 4) to -2.6 (unbuffered) of propoxycarbazone-sodium. For the main aquatic metabolites of propoxycarbazone-sodium bioaccumulation studies are also not required, because the log P_{OW} for M10, M04 and M05 can be calculated as -0.4 ± 1.0, -5.2 ± 1.0 and 0.3 ± 1.0, respectively (the predicted value of log P_{OW} was obtained using the ACD/Lab Web service). All these values are below a log P_{OW} of 3 and thus a potential of bioaccumulation is not anticipated.

CA 6.3 Magnitude of residues trials in plants

EU MRLs for propoxycarbazone-sodium were adopted and included in Annex II of Regulation (EC) No 396/2005, which adequately support claimed uses (Commission Regulation (EU) No 149/2008 of 29 January 2008).

New studies in wheat were conducted since the Annex I inclusion of propoxycarbazone-sodium in 2004 with propoxycarbazone-sodium containing formulations.

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CA 6.3.1 Wheat

The critical GAPs for use of the representative formulation of propoxycarbazone-sodium on wheat, triticale and rye are outlined in Table 6.3.1-1.

Table 6.3.1-1: Critical GAPs for use propoxycarbazone-sodium on winter wheat, triticale and rye

Crop	Region	Zone	Country	F, G, I**	Timing of application	Number of applic.	Maximum PCS application rate [kg a.s./ha]	Minimum PHI [days]
Winter Wheat	EU-N	C	UK	F	Up to BBCH 33	1	0.07	-
Triticale Rye		N	Latvia	F	up to BBCH 32	1	0.07	-
Winter Wheat	EU-S	S	France	F	up to BBCH 30	1	0.07	-

PCS = Propoxycarbazone-sodium
EU-N = Northern Europe EU-S = Southern Europe; Zone C, N, S = Central-, Northern-, Southern Zone
** F Field; G Greenhouse; I Indoor.

Summaries of supervised residue trials supporting the critical GAPs for propoxycarbazone-sodium are provided below.

Original Annex II dossier

To clarify the residue behaviour of propoxycarbazone-sodium in wheat, trials were conducted in wheat with the 70 WG straight formulation.

A total of 16 residue trials were conducted with the WG 70 formulation in both European regions, 8 in the north and 8 in the south. The trials were equally split between the 1997 and 1998 growing seasons. All residue trials were performed in conformity with GLP. In each trial a WG 70 formulation of the product was used.

Northern Europe

Northern European trials were performed in Great Britain (3), France (2), Germany (2) and Sweden (1). These trials have been conducted in 1997 and 1998, respectively. The application rate was 70 g a.s./ha except trial no. 0303-97 (77 g a.s./ha) in Sweden. The product was applied once with a spray volume of 280 to 330 L/ha. Treatment was conducted during stem elongation (BBCH growth stage 33/34) corresponding to 76 to 96 days prior to harvest.

Forage samples were taken from treated plots on day 0 and 13 – 27 days after treatment and on day 0 prior to treatment from the control plot. Grain and straw samples were taken on day 76 to 96 after treatment. Residues of MKH 6561 and its metabolite 2-hydroxypropoxy MKH 6561 (M01) were determined according to method 00509. After extraction of the residues by accelerated solvent extraction (ASE) with ammonium hydroxide solution, the extracts were cleaned up by solid phase extraction. Residues were quantified by LC/MS/MS in the multiple-reaction-monitoring mode using known amounts of deuterated internal standards. Recovery rates were determined by spiking MKH 6561 and 2-hydroxypropoxy MKH 6561 to the sample materials at levels of 0.02 to 5.0 mg/kg. For MKH 6561 recoveries were in the range of 87 to 100%, for 2-hydroxypropoxy MKH 6561 the corresponding values were 79 to 103%. The limit of quantitation (LOQ) was 0.02 mg/kg for wheat forage and grain and 0.05 mg/kg for wheat straw.

Residues of MKH 6561 in forage taken directly after treatment were 0.8 to 2.5 mg/kg. Thereafter, they declined to <0.02 to 0.52 mg/kg on days 13 - 27. The corresponding residues of 2-hydroxypropoxy MKH 6561 were 0.02 - 0.27 mg/kg (day 0) and 0.05 - 0.25 mg/kg (days 13 – 27). Residues of MKH 6561 and 2-hydroxypropoxy MKH 6561 on grain taken at harvest were below the LOQ of 0.02 mg/kg. No control interferences were detected. In straw residues of MKH 6561 were below the LOQ of 0.05 mg/kg.

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In straw residues of 2-hydroxypropoxy MKH 6561 (M01) were below or at the LOQ of 0.05 mg/kg except in trial 0100/97 in which 0.06 mg/kg 2-hydroxypropoxy MKH 6561 (M01) was detected.

Southern Europe
Southern European trials were performed in France (7) and Portugal (1). These trials have been conducted in 1997 and 1998, respectively. The application rate was 70 g a.s./ha. The product was applied once with a spray volume of 280 to 300 L/ha. Treatment was conducted at stem elongation (BBCH growth stage 33/34) corresponding to 78 to 108 days prior to harvest.

In two trials treatment was conducted at the BBCH growth stage 51 (heading) corresponding to 49 and 57 days prior to harvest (RA-2005/98, 1066-98 and 1189-98). This late stage of application is no common use of propoxycarbazone-sodium. Therefore, the findings of these two trials will not be considered in the following summary.

Forage samples were taken from treated plots on day 0 and 19 – 22 days after treatment and on day 0 prior to treatment from the control plot. Grain and straw samples were taken from treated and control plots on day 78 to 108 after treatment. Residues of propoxycarbazone-sodium and its metabolite 2-hydroxypropoxy MKH 6561 were determined according to method 00509. After extraction of the residues by accelerated solvent extraction (ASE) with ammonium hydroxide solution, the extracts were cleaned up by solid phase extraction. Residues were quantified by LC/MS/MS in the multiple-reaction-monitoring mode using known amounts of deuterated internal standards. Recovery rates were determined by spiking propoxycarbazone-sodium and 2-hydroxypropoxy MKH 6561 to the sample materials at levels of 0.02 to 5.0 mg/kg. For propoxycarbazone-sodium recoveries were in the range of 87 to 100%, for 2-hydroxypropoxy MKH 6561 the corresponding values were 79 to 103%. The limit of quantitation (LOQ) was 0.02 mg/kg for wheat forage and grain and 0.05 mg/kg for wheat straw.

Residues of propoxycarbazone-sodium in forage taken on day 0 after treatment were 0.79 to 2.5 mg/kg. Thereafter, they declined to <0.02 to 0.50 mg/kg on days 19 – 22. The corresponding residues of 2-hydroxypropoxy MKH 6561 were 0.03 to 0.48 mg/kg (day 0) and 0.03 to 0.2 mg/kg (days 19 – 22). Residues of propoxycarbazone-sodium on straw taken at harvest were below the LOQ (0.05 mg/kg) and residues of 2-hydroxypropoxy MKH 6561 were detected in amounts below the LOQ. Residues of propoxycarbazone-sodium and 2-hydroxypropoxy MKH 6561 in grain taken at harvest were below the LOQ of 0.02 mg/kg. No control interferences were detected.

One trial was destroyed by hail and therefore, only forage samples could be taken for analysis. Due to the fact that already on day 19 no residues above the LOQ were detected, no residues are to be expected at the time of harvest.

A summary of the result are given in Table 6.3.1-2.

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Table 6.3.1-2: Summary of residues of propoxycarbazone-sodium in wheat

Crop	Region	Timing of application	Number of applic.	DALT (days)	Portion analysed	MKH 6561 (mg/kg)	MKH 6561 2-hydroxy (mg/kg)
Wheat (spring and winter)	EU-N	Up to BBCH 33/34	1	0	Forage	1.3, 1.9, 1.5, 1.8, 2, 0.8, 2.5, 1.1	0.27, 0.03, 0.04, 0.02, 0.16, 0.03, 0.16, 0.13
				3	Forage	0.92, 0.02	0.75, 0.23
				7-8	Forage	0.05, <0.02	0.48, 0.14
				13-14	Forage	0.52, <0.02, <0.02, 0.06, <0.02	0.25, 0.05, 0.23, 0.12, 0.11
				20-27	Forage	0.03, <0.02, <0.02, <0.02	0.11, 0.05, 0.08, 0.08
				76-96	Grain	8 x <0.02	8 x <0.02
				76-96	Straw	8 x <0.05	0.06, 6 x <0.05, 0.05
Wheat (spring and winter)	EU-S	up to BBCH 33/34	1	0	Forage	1.7, 2.1, 1.1, 2.4, 2.5, 0.79	0.03, 0.03, 0.19, 0.48, 0.11, 0.41
				19-22	Forage	<0.02, <0.02, <0.02, 0.5, <0.02, <0.02	0.03, 0.03, 0.04, 0.09, 0.15, 0.2
				78-108	Grain	5 x <0.02	5 x <0.02
				78-108	Straw	5 x <0.05	5 x <0.05

Two trials were not taken into account regarding the residue results in wheat straw, since the application was done to late in the growing season (BBCH 51 instead of BBCH 33/34).
To have a full data set with regard to the residues of propoxycarbazone-sodium in straw eight further trials performed in Northern and Southern Europe are presented below.

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Report:	[redacted];[redacted];2005;M-254598-01
Title:	Determination of the residues of MKH 6561 and mefenpyr-diethyl in/on wheat after spraying of AE 0298618 01 OD05 A2 (054 OD) in the field in Germany, United Kingdom, Northern France and Sweden
Report No:	RA-2020/04
Document No(s):	Report includes Trial Nos.: 0102-04 0698-04 0699-04 0700-04 0701-04 R 2004 0102/7 R 2004 0698/3 R 2004 0699/1 R 2004 0700/9 R 2004 0701/7 M-254598-01-1
Guidelines:	**91/414/EEC**
Deviations:	None
GLP/GEP:	**yes**

Report:	[redacted];[redacted];2005;M-254346-01
Title:	Determination of the residues of MKH 6561 and mefenpyr-diethyl in/on wheat after spraying of AE 0298618 01 OD05 A2 (054 OD) in the field in Italy, Spain and Southern France
Report No:	RA-2021/04
Document No(s):	Report includes Trial Nos.: 0103-04 0702-04 0703-04 R 2004 0103/5 R 2004 0702/5 R 2004 0703/3 M-254346-01-1
Guidelines:	**91/414/EEC**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

Eight residue trials were conducted in Northern (5) and Southern (3) Europe. Propoxycarbazone-sodium (MKH 6561) was applied as a foliar spray on wheat plants using an oil dispersion (OD) formulation containing nominally 4.14% propoxycarbazone-sodium and 0.94% mefenpyr-diethyl (AE F107892). In these trials, one application was made at BBCH growth stage 33 – 34 at a rate of 70 g a.s./ha
Wheat green material samples were taken directly after the application. Grain and straw samples were collected for analysis at harvest 73 – 93 days after the application.

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The samples (wheat green material, grain and straw) were analysed for propoxycarbazone-sodium and 2-hydroxy MKH 6561 according to analytical methods 00509, with limits of quantitation of 0.02 mg/kg (green material and grain) and of 0.05 mg/kg (straw) for each analyte. In green material residues of propoxycarbazone-sodium and 2-hydroxy MKH 6561 ranged from 1.2 to 5.1 mg/kg and from 0.19 to 1.6 mg/kg, respectively. In grain no residues of propoxycarbazone-sodium and 2-hydroxy MKH 6561 above the LOQ (0.02 mg/kg) were found in any of the treated and untreated samples. In straw residues of propoxycarbazone-sodium were below the LOQ of 0.05 mg/kg while residues of 2-hydroxy MKH 6561 ranged from <0.05 to 0.08 mg/kg.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AE 0298618 01 OD05 A2 (054 OD)
Description:	Oil dispersion
Lot/Batch #:	Ausl.-F.8985
CAS #:	181274-15-7
Purity:	MKH 6561: 4.14%; AE F107892: 0.94%
Spiking levels:	0.02 to 5.0 mg/kg of MKH 6561 and MKH 6561-hydroxy

2. Test Commodity:

Crop:	Wheat
Type:	Cereals
Variety:	Dekan, Consort, Isengrain, Marshal, Gnejs, Neodur, Don Pedro, Florence Aurore
Botanical name:	*Triticum spp.*
Crop parts(s) or processed commodity:	Wheat green material, grain and straw
Sample size:	up to 4.9 kg

B. STUDY DESIGN

1. Test procedure

Northern Europe

Five supervised residue trials on wheat were conducted with propoxycarbazone-sodium in 2004. Propoxycarbazone-sodium was applied as a foliar spray on wheat plants using an oil dispersion (OD) formulation containing nominally 4.14% propoxycarbazone-sodium and 0.94% mefenpyr-diethyl (AE F107892). In these trials, one application was made at BBCH growth stage 33 – 34 at a rate of 70 g a.s./ha, i.e. within 25% of the proposed maximum application rate.

Wheat green material samples were taken directly after the application. Grain and straw samples were collected for analysis at harvest 91 – 93 days after the application.

Southern Europe

Three supervised residue trials on wheat were conducted with propoxycarbazone-sodium in 2004.

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Propoxycarbazone-sodium was applied as a foliar spray on wheat plants using an oil dispersion (OD) formulation containing nominally 4.14% propoxycarbazone-sodium and 0.94% mefenpyr-diethyl (AE F107892). In these trials, one application was made at BBCH growth stage 31 –34 at a rate of 70 g a.s./ha, i.e. within 25% of the proposed maximum application rate.

Wheat green material samples were taken directly after the application. Grain and straw samples were collected for analysis at harvest 73 – 85 days after the application.

Duration of Storage

Samples were stored up to 422 days.

2. Description of analytical procedures

Residue analysis

The samples (wheat green material, grain and straw) were analysed for propoxycarbazone-sodium and 2-hydroxypropoxy MKH 6561 (M01) according to analytical methods 00509, which were previously validated for wheat green material, grain and straw, with limits of quantitation of 0.02 mg/kg (green material and grain) and of 0.05 mg/kg (straw) for each analyte.

II. RESULTS AND DISCUSSION

Procedural recoveries of propoxycarbazone-sodium and 2-hydroxypropoxy MKH 6561 (M01) were obtained from wheat (green material, straw and grain) fortified at levels between 0.02 mg/kg and 5.0 mg/kg. Mean recoveries for all levels were all within acceptable ranges (86-102%). Details of recovery data are shown in Table **6.3.1-5**.

All trials are summarised below in Table 6.3.1-3 (Northern Europe) and in Table 6.3.1-4 (Southern Europe) and in greater detail in the Tier 1 summary forms.

In green material residues of propoxycarbazone-sodium and 2-hydroxypropoxy MKH 6561 (M01) ranged from 1.2 to 5.1 mg/kg and from 0.19 to 1.6 mg/kg, respectively.
In wheat grain no residues of propoxycarbazone-sodium and 2-hydroxy MKH 6561 above the LOQ (0.02 mg/kg) were found in any of the treated and untreated samples.
In wheat straw residues of propoxycarbazone-sodium were below the LOQ of 0.05 mg/kg while residues of 2-hydroxy MKH 6561 ranged from <0.05 to 0.08 mg/kg.

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Table 6.3.1-3: Residues of propoxycarbazone-sodium and 2-hydroxy MKH 6561 in wheat matrices in Northern Europe

Study Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	MKH 6561 (mg/kg)	MKH 6561 2-hydroxy (mg/kg)
RA-2020/04 R 2004 0102/7 0102-04 GLP: yes 2004	Wheat Dekan	Germany D- (Nordrhein-Westfalen) Europe, North	53.2 OD	1	0.0702	0.0234	33	Green material	0	3.3	0.19
								Grain	93	<0.02	<0.02
								Straw	93	<0.05	<0.05
RA-2020/04 R 2004 0699/1 0699-04 GLP: yes 2004	Wheat Isengrain	France F- Europe, North	53.2 OD	1	0.0702	0.0234	34	Green material	0	1.2	0.23
								Grain	92	<0.02	<0.02
								Straw	92	<0.05	<0.05
RA-2020/04 R 2004 0698/3 0698-04 GLP: yes 2004	Wheat Consort	United Kingdom GB- Europe, North	53.2 OD	1	0.0702	0.0234	33	Green material	0	1.3	0.44
								Grain	90	<0.02	<0.02
								Straw	90	<0.05	<0.05
RA-2020/04 R 2004 0700/9 0700-04 GLP: yes 2004	Wheat Marshal	Sweden S- Europe, North	53.2 OD	1	0.0702	0.0234	33	Green material	0	3.0	0.21
								Grain	92	<0.02	<0.02
								Straw	92	<0.05	0.07
RA-2020/04 R 2004 0701/7 0701-04 GLP: yes 2004	Wheat Gnejs	Sweden S- Europe, North	53.2 OD	1	0.0702	0.0234	33	Green material	0	2.0	0.26
								Grain	91	<0.02	<0.02
								Straw	91	<0.05	<0.05

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Table 6.3.1-4: Residues of propoxycarbazone-sodium and 2-hydroxy MKH 6561 in wheat matrices in Southern Europe

Study Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	MKH 6561 (mg/kg)	MKH 6561 2-hydroxy (mg/kg)
RA-2021/04 R 2004 0103/5 0103-04 GLP: yes 2004	Wheat Neodur; Durum wheat	Italy I- Europe, South	53.2 OD	1	0.0702	0.0234	31	Green material	0	3.4	1.6
								Grain	76	<0.02	<0.02
								Straw	76	<0.05	<0.05
RA-2021/04 R 2004 0703/3 0703-04 GLP: yes 2004	Wheat Florence Aurore	France F- Europe, South	53.2 OD	1	0.0702	0.0234	31	Green material	0	5.1	0.34
								Grain	73	<0.02	<0.02
								Straw	73	<0.05	0.08
RA-2021/04 R 2004 0702/5 0702-04 GLP: yes 2004	Wheat Don Pedro	Spain E- Europe, South	53.2 OD	1	0.0702	0.0234	34	Green material	0	1.8	0.19
								Grain	85	<0.02	<0.02
								Straw	85	<0.05	<0.05

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Table 6.3.1-5: Procedural recoveries for propoxycarbazone-sodium and 2-hydroxy MKH 6561 in wheat matrices

Study Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
RA-2020/04 R 2004 0700/9 0700-04 GLP: yes 2004 RA-2021/04 R 2004 0703/3 0703-04 GLP: yes 2004	Wheat	Green material	MKH 6561	2	0.02	97; 96	96	97	97	
				1	0.2	93	93	93	93	
				1	5.0	102	102	102	102	
				4	**overall**		**93**	**102**	**97**	**3.9**
			MKH 6561 2-hydroxy	2	0.02	99; 97	97	99	98	
				1	0.2	95	95	95	95	
				1	5.0	102	102	102	102	
				4	**overall**		**95**	**102**	**98**	**3.0**
		Grain	MKH 6561	2	0.02	89; 93	89	93	91	
				2	0.2	87; 99	87	99	93	
				4	**overall**		**87**	**99**	**92**	**5.8**
			MKH 6561 2-hydroxy	2	0.02	101; 103	101	103	102	
				2	0.2	97; 99	97	99	98	
				4	**overall**		**97**	**103**	**100**	**2.6**
		Straw	MKH 6561	2	0.05	91; 94	91	94	93	
				2	0.5	93; 83	83	93	88	
				4	**overall**		**83**	**94**	**90**	**5.5**
			MKH 6561 2-hydroxy	2	0.05	91; 91	91	91	91	
				2	0.5	86; 86	86	86	86	
				4	**overall**		**86**	**91**	**89**	**3.3**

III. CONCLUSIONS

Wheat is a major crop in Northern and Southern Europe (SANCO 7525/VI/95 – rev.9, 24.03.2011) and therefore requires residue data from eight supervised trials from each region. In the growing seasons 1997/1998 and 2004 21 residue trials were conducted in Northern (13) and Southern Europe (8). The product was applied once at BBCH growth stage 31 – 34 at a rate of 70 g a.s./ha. All tests were carried out according to GLP principles.

In wheat grain no residues of propoxycarbazone-sodium and 2-hydroxy MKH 6561 above the LOQ (0.02 mg/kg) were found in any of the treated and untreated samples. Therefore, sufficient trials are available to support the critical GAP.

As the trials on wheat were conducted in compliance with the GAP, extrapolation to rye is possible (SANCO 7525/VI/95 – rev.9, 24.03.2011).

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CA 6.4 Feeding studies

Propoxycarbazone-sodium is authorised for use on crops that might be fed to livestock.

Dietary burden calculations

Livestock feeding studies reflect the potential exposure of livestock through different types of feed. The potential intake of propoxycarbazone-sodium residues by livestock arising from the uses in the GAP was evaluated in the EFSA reasoned opinion on the modification of the existing MRLs for propoxycarbazone-sodium in various commodities of plant and animal origin (EFSA Journal 2013;11(4):3164). Additionally the highest residue level in wheat straw measured in the new submitted residue trials are taken into account. Therefore, the following input values were used to calculate potential intakes of residues by livestock.

Table 6.4- 1: Input values for the dietary burden calculation ()

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Wheat and rye grain	0.02[1]	Median residue	0.02[1]	Median residue
Wheat and rye bran	0.02[1]	Median residue	0.02[1]	Median residue
Wheat and rye straw	0.05[1]	Median residue	0.13[2]	Highest residue

[1] According to EFSA Journal 2013;11(4):3164
[2] Highest residue value

As residue levels in cereal grain are below the LOQ and as application occurs before consumable parts are formed (see CA 6.3.1), no concentration of the residue is expected in bran.

The median and maximum dietary burdens were then calculated for the different types of livestock using the agreed methodologies described in the EU Guidance Document (SANCO 7031/VI/95 rev. 4, 22/07/1996).

The calculated median and maximum dietary burdens are summarised in the following table:

Table 6.4- 2: Results of the dietary burden calculation (EFSA Journal 2013;11(4):3164)

	Maximum dietary burden (mg/kg bw/d)	Median dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded ?
Risk assessment residue definition: sum of propoxycarbazone, its salts and 2-hydroxy- propoxycarbazone, expressed as propoxycarbazone					
Dairy ruminants	0.0014	0.0008	Wheat straw	0.04	N
Meat ruminants	0.0037	0.0017	Wheat straw	0.09	N
Poultry	0.0010	0.0010	Wheat grain	0.02	N
Pigs	0.0007	0.0007	Wheat grain	0.02	N

Since the calculated dietary burdens for all groups of livestock were found to be below the trigger value of 0.004 mg/kg bw/d, further investigation of residues as well as the setting of MRLs in commodities of animal origin is not necessary.
Nevertheless a feeding study in dairy cows was conducted in the US in 1999. The results of this study are presented below.

CA 6.4.1 Poultry

No study was performed.

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CA 6.4.2 Ruminants

Report:	[redacted] d; [redacted]; [redacted]; 1999;M-021454-02; Amended: 2000-05-17
Title:	MKH 6561 - a 29-day dairy cattle feeding study - addendum I - data for the 10X feeding level
Report No:	109116
Document No:	M-021454-02-1
Guidelines:	**OPPTS 860.1480, Meat/Milk/Poultry/Eggs**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

Three lactating cows were orally dosed daily in the morning after milking for 29 consecutive days with propoxycarbazone-sodium (MKH 6561) at average dose rate of 36.0 mg/kg feed/day. This dosing is 400 times higher than the actual calculated maximum dietary burden.
Milk was collected twice daily (afternoon and morning) with a milking machine. Within 8 hours of the administration of the final dose (day 29) the animals were humanely sacrificed. Following the termination, liver (representative samples of each lobe totalling ca. 1 kg), kidney (both), fat (composite of available omental, renal, and subcutaneous), and muscle (composite of loin, round, and flank) were collected.
Tissue and milk samples were analysed for propoxycarbazone-sodium residues by LC-MS/MS with limit of quantitation (LOQ) for tissues of 0.050 mg/kg, for whole milk and whey of 0.002 mg/kg and for cream of 0.005 mg/kg.
Feed consumption, body weights, and milk production were not adversely affected by daily oral administration of propoxycarbazone-sodium to dairy cows for 29 consecutive days at the average dose rate of 36.0 mg/kg in feed.
In milk collected from the treated animals, the residues plateaued by study day 8. The average residue in the 28-day milk from dosed cows was 0.016 mg/kg. Additionally, the average residues in whey and cream from the dosed group at 28 days were 0.013 mg/kg and 0.005 mg/kg, respectively. Propoxycarbazone-related residues do not accumulate in milk, and the residues are not concentrated in either whey or cream.
The only tissues that contained residues >LOQ were kidney (0.14 to 0.29 mg/kg) and a single liver sample (0.05 ppm). Muscle and fat contained residues <LOQ.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material

Identification: MKH 6561
Description: -
Lot/Batch #: 898706001
Purity: 99.9%
Dose level: 36 mg/kg feed/day for 29 days
Vehicle: Capsule

2. Test Animals:

Species: Dairy cows (*Bos taurus*)
Strain: Holstein
Breeding facility: HLE Dairy (PO Box 328, Berino, NM 88048, USA)
Sex and numbers involved: Four female animals
Age: Three to five years
Body weight: 620 to 705 kg
Acclimatisation: 6 days
Identification: Eartag (#3263 - #3268) and a leg band
Housing: The animals were housed together in outdoor sand-floor pens. After assignment to groups, the animals were segregated by dose groups (three cows treated, one cow control).
Room temperature -1.1 - 30°C, relative humidity 29 - 85%
Feed and water: A total mixed ration (TMR) purchased from Gonzalez Dairy (Mesquite, NM) was used in the study. The approximate percentage of each ingredient in the diet (TMR) is given in the following table. The animals were allowed *ad libitum* access to the TMR and fresh potable tap water daily via group feeders and automatic waterers. Feed consumption per group was monitored and recorded daily

Total Mixed Ration Ingredients

Ingredient	Percentage (ca.)
Silage	39.47
Alfalfa Hay	22.37
Rolled Corn	9.87
Rolled Milo	9.87
Soy Hulls	5.26
Soybean Meal	5.26
Pre-Mix	7.89

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B. STUDY DESIGN

1. Test procedure

Lactating Hostein dairy cows (three cows per treatment group and one control cow) were orally dosed daily in the morning after milking for 29 consecutive days with MKH 6561 at average dose rates either 0 mg/kg (control) or 36.0 mg/kg feed/day. This dosing is 400 times higher than the actual calculated maximum dietary burden (see Table 6.4- 2).

For the treated group, capsules were prepared weekly containing an amount of propoxycarbazone-sodium to a target value of 36.0 mg/kg feed/day. The dose calculation was based on the actual feed consumption by the animals in the treated group during the acclimatisation period. The dose capsules were prepared by Southwest Bio-Labs by weighing technical grade propoxycarbazone-sodium directly into dose capsules without any cellulose or lactose filler. Additionally, the dose capsules were used within 10 days of their preparation, and the capsules were stored in the freezer (<-14 ± 10 °C) prior to use.

2. Sampling

<u>Milk</u>

Milk was collected twice daily (afternoon and morning) with a milking machine. Milk samples collected from each individual cow during the 24 hours following each daily dose were composited and weighed. The composited milk samples were subsampled, and the subsamples were stored frozen at <-10°C until shipped frozen to Bayer. Milk was analysed from study days -1 (48-hours before dosing), 0 (24-hours before dosing), 4, 8, 12, 16, 20, 22, 24, 26, and 28.

Additional 28-day milk from group I (control) and group II (treated) was collected for processing into whey and cream. Whole milk from each cow was poured into four 200 mL centrifuge bottles and centrifuged for 10 min at approximately 3,000 rpm. The whey was poured out of the bottom of the centrifuge bottle and collected in a labelled container. The cream was then transferred into another labelled container. The entire process was repeated with a second whole milk sample. The appropriate portions from the two separations from each cow were pooled to provide a sufficient quantity of cream for analysis.

<u>Sacrifice, Tissue Collection and Sample Processing</u>

Animals were humanely sacrificed within 8 hours of the administration of the final dose (day 29). The animals were terminated by stunning the animal via a captive bolt pistol followed immediately by exsanguination. Following the termination, liver (representative samples of each lobe totalling ca. 1 kg), kidney (both), fat (composite of available omental, renal, and subcutaneous), and muscle (composite of loin, round, and flank) were collected and weighed.

The tissues were individually chopped into small pieces and immediately transferred to a walk-in freezer (<-10°C). After freezing, the individual liver, kidney, muscle, and fat samples were pulverized in a Hobart grinder (Hobart Corp., Troy, OH) and homogenized using a Polytron homogenizer (Brinkmann Corp., Model PT 6000, Westbury, NY). All tissue samples were in liquid nitrogen during pulverization and homogenization. After homogenization, the tissues were placed on stainless steel trays and stored open in a freezer to allow the liquid nitrogen to evaporate. Once the liquid nitrogen evaporated, the processed tissues were removed from the freezer, weighed, and transferred to plastic storage bags. The bags were sealed and returned to the freezer for storage.

3. Analysis

Tissue and milk samples were analysed for propoxycarbazone-sodium residues by liquid chromatography/mass spectrometry-mass spectrometry (LC/MS-MS). The limit of quantitation (LOQ) for the tissues was 0.050 mg/kg, and the limits of detection (LOD) were estimated (3 standard deviations above the average background control value) at 0.002, 0.003, 0.001, and 0.001 mg/kg for the liver, kidney, muscle, and fat, respectively. For whole milk and whey, the LOQ was 0.002 mg/kg, and the LOD was estimated at 0.001 mg/kg. For cream, the LOQ was 0.005 mg/kg, and the LOD was 0.001 mg/kg.

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II. RESULTS AND DISCUSSION

Feed consumption, body weights, and milk production were not adversely affected by daily oral administration of propoxycarbazone-sodium to dairy cows for 29 consecutive days at the average dose rate of 36.0 mg/kg in feed.

Analysis of milk from animals in groups II (treated) from study days -1, 0, 4, 8, 12, 16, 20, 22, 24, 26, and 28 showed that the propoxycarbazone-sodium residues reached a plateau by study day 8. The highest observed residue level in milk was 0.026 mg/kg for cow 3267 on Day-22. The results are summarised in Table 6.4.2-1. Whey generated from 28-day milk from the treated group had an average residue of 0.013 mg/kg and cream generated from 28-day milk had an average residue of 0.005 mg/kg. The results are summarised in Table 6.4.2-2.

Residues of propoxycarbazone-sodium in muscle and fat samples were always below the LOQ of 0.05 mg/kg. One liver sample had a residue of 0.05 mg/kg while the other samples were <0.05 mg/kg. Residues in kidney ranged from 0.14 mg/kg to 0.29 mg/kg. The results are summarised in Table 6.4.2-3.

Table 6.4.2-1: Residues in milk from cows in group II (treated)

Days Treated	Cow Number 3265 Propoxycarbazone* mg/kg	Cow Number 3266 Propoxycarbazone* mg/kg	Cow Number 3267 Propoxycarbazone* mg/kg
-1	<0.002	<0.002	<0.002
0	<0.002	<0.002	<0.002
4	0.016	0.008	0.015
8	0.02	0.009	0.022
12	0.013	0.008	0.014
16	0.019	0.012	0.018
20	0.019	0.011	0.017
22	0.018	0.013	0.026
24	0.016	0.01	0.019
26	0.014	0.012	0.008
28	0.014	0.017	0.016

* Residues are expressed in propoxycarbazone equivalents. LOQ is 0.002 mg/kg.

Table 6.4.2-2: Residues in 28-day milk, whey, and cream samples

Sample	Cow Number 3265 Propoxycarbazone* mg/kg	Cow Number 3266 Propoxycarbazone* mg/kg	Cow Number 3267 Propoxycarbazone* mg/kg
Whole milk	0.014	0.017	0.016
Whey	0.012	0.012	0.016
Cream	<0.005	<0.005	0.008

* Residues are expressed in propoxycarbazone equivalents. LOQ is 0.002 mg/kg for milk and whey and 0.005 mg/kg for cream.

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Table 6.4.2-3: Residues in tissue samples

Sample	Cow Number 3265 Propoxycarbazone* mg/kg	Cow Number 3266 Propoxycarbazone* mg/kg	Cow Number 3267 Propoxycarbazone* mg/kg
Liver	<0.05	<0.05	0.05
Kidney	0.18	0.14	0.29
Muscle	<0.05	<0.05	<0.05
Fat	<0.05	<0.05	<0.05

* Residues are expressed in propoxycarbazone-sodium equivalents. LOQ is 0.05 mg/kg.

III. CONCLUSIONS

Feed consumption, body weights, and milk production were not adversely affected by daily oral administration of propoxycarbazone-sodium to dairy cows for 29 consecutive days at the average dose rate of 36.0 mg/kg in feed.
In milk collected from the treated animals, the residues plateaued by study day 8. The average residue in the 28-day milk from dosed cows was 0.016 mg/kg. Additionally, the average residues in whey and cream from the dosed group at 28 days were 0.013 mg/kg and 0.005 mg/kg, respectively. Propoxycarbazone-related residues do not accumulate in milk, and the residues are not concentrated in either whey or cream.
The only tissues that contained residues >LOQ were kidney (0.14 to 0.29 mg/kg) and a single liver sample (0.05 ppm). Muscle and fat contained residues <LOQ.
No edible tissue or milk from animals fed a diet of wheat hay, forage, and grain containing propoxycarbazone-sodium residues at the estimated tolerances would be expected to have residues greater than the LOQs (tissues = 0.05 mg/kg, milk and whey = 0.002 mg/kg, cream = 0.005 mg/kg) for the analytical method.

CA 6.4.3 Pigs

No study was performed.

CA 6.4.4 Fish

The log P_{ow} of propoxycarbazone is below 3. Therefore a study of the nature of residues in fish is not required by Regulation (EC) No 1107/2009.

CA 6.5 Effects processing

The use of propoxycarbazone-sodium in cereals according to the intended GAP does not result in significant residues (i.e. > 0.1 mg/kg) of propoxycarbazone-sodium and its metabolite 2-hydroxy-propoxycarbazone in grain at harvest, since the residues were below the limit of quantification in all trials. The contribution of wheat, rye, triticale, and spelt to the theoretical maximum daily intake (TMDI) is <10% of the ADI. Therefore, studies on industrial processing and/or household preparation are not necessary.

CA 6.5.1 Nature of the residue

No studies on the effects of processing on the nature of the residue were performed.

CA 6.5.2 Distribution of the residue in peel and pulp

No studies on the effects of processing on the nature of the residue were performed.

CA 6.5.3 Magnitude of residues in processed commodities

No studies on the effects of processing on the nature of the residue were performed.

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CA 6.6 Residues in rotational crops

Data on residues of propoxycarbazone-sodium (MKH 6561) in succeeding crops were reviewed during the Annex I inclusion process and were considered to be acceptable and no further data have been generated.

CA 6.6.1 Metabolism in rotational crops

One confined crop rotation study was performed for propoxycarbazone-sodium ([redacted], R.R.; 1999, M-021141-01-1) and evaluated in the DAR (2001).

The metabolism of MKH 6561 was investigated in the rotational crops spring wheat (small grain), kale (leafy vegetable) and turnips (root crop) after spray application of [phenyl-UL-^{14}C]MKH 6561 and [triazolinone-3-^{14}C]MKH 6561 directly to the soil of a planting container (1m^2). The amount applied corresponded approximately to a field rate of 45 g a.s./ha. The results of rotational crop study demonstrate that MKH 6561 is hydroxylated in the propoxy side chain to form 2-hydroxypropoxy MKH 6561 (M01) as already observed in the wheat metabolism study. This metabolite can be hydrolysed or cleaved enzymatically yielding 2-hydroxy-N-methyl propoxy triazolinone (M02) which was also observed to a minor extent in the wheat metabolism experiment. 2-Hydroxy-N-methyl propoxy triazolinone (M02) can also be formed in rotational crops by uptake of one of the major soil metabolites N-methyl propoxy triazolinone (M10) followed by hydroxylation and conjugation of the hydroxylated product. Saccharin (M07) and its conjugates were observed in rotational crops in significantly higher amounts as compared to the wheat metabolism study, indicating that this major soil metabolite is also taken up by crops after ageing of the soil followed by conjugation.

CA 6.6.2 Magnitude of residues in rotational crops

Report:	[redacted]; [redacted]; 1999; M-027996-01
Title:	MKH 6561 70WG - magnitude of the residue in field rotational crops
Report No:	109201
Document No:	M-027996-01-1
Guidelines:	**EPA Ref.: OPPTS 860.1900, Field Accumulation in Rotational Crops**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

In 1999 residue field trials were conducted at three locations in the US. Propoxycarbazone-sodium 70 WG was applied as a broadcast treatment to soil at a rate of 0.045 kg a.s./ha. A cover crop was planted just before application in most but not all of the trials. The plots were irrigated and fertilized according to commercial crop production practices.

Representative cereal grain (wheat), root (turnip), and leafy vegetable (mustard greens) crops were planted at approximately 1, 4, 8, and 12 months following the application of MKH 6561 70 WG to the soil. Samples of all rotational crops were taken at the earliest crop maturity.

Untreated and treated crop samples were analysed for residues of propoxycarbazone-sodium and 2-hydroxy-propoxycarbazone.

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No residues of propoxycarbazone-sodium and 2-hydroxy-propoxycarbazone were detected above the limit of detection of 0.002 mg/kg in any untreated and treated samples of turnip root and tops, wheat matrices (forage, hay, grain, straw) and mustard greens.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material

Identification:	MKH 6561 70WG
Description:	Wettable granule (WG)
Lot/Batch #:	8030050; 7030221; 7030200; 8030243
Purity:	70 g/100 g propoxycarbazone-sodium

2. Test Commodity:

Crop:	Turnip	Wheat	Mustards greens
Type:	Root vegetable	Cereals	Leafy vegetable
Variety:	Purple Top, Purple Top White Glo	Pioneer 2684, Stewards SW520, YR8-2	Broadleaf mustard, Southern giant curled, Florida broadleaf
Botanical name:	*Brassica rapa L.*	*Tritiicum aestivum*	*Brassica juncea*

3. Soil

Different soils were used in the experiments. The soil physicochemical properties are described below in Table 6.6.2-1.

Table 6.6.2-1: Soil physicochemical properties

Soil characterisation	Georgia	Indiana		California
Soil classification	-	Loam	Silt loam	-
pH	-	6.2	5.4	-
OM (%)	-	3.8	3.9	-

B. STUDY DESIGN

The study was conducted during the period October, 1997 to December, 1999 in Georgia, Indiana and California in the USA.

1. Test procedure

Three residue field trials were conducted on turnip, wheat and mustards greens. MKH 6561 70WG was applied once as a broadcast treatment to soil at a rate of 0.045 kg a.s./ha. A cover crop was planted just before application in most but not all of the trials.

Representative cereal grain (wheat), root (turnip), and leafy vegetable (mustard greens) crops were planted at approximately 1, 4, 8, and 12 months following the application of MKH 6561 70 WG to the soil. The cover crops were disced and tilled into the soil prior to planting the appropriate rotational crop.

2. Sampling

Samples of all rotational crops were taken at the earliest crop maturity. Duplicate treated samples were taken from two separate runs through the plots. Samples from at least 12 separate areas of

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each plot were combined to make up a total of 0.5 to 1.13 kg of each wheat sample and approximately 2.26 kg of each mustard green and turnip samples. A single control sample was taken from a nearby untreated plot at each harvest interval.

3. Analysis

Untreated and treated crop samples were analysed for residues of propoxycarbazone-sodium and 2-hydroxy-propoxycarbazone. Turnip tops and roots, wheat forage, hay, straw and grain, and mustard greens samples were extracted using an accelerated solvent extraction (ASE). Approximately 2 g of each sample was extracted at 65°C and 1500 psi using 0.05 *M* NH_4OH. An internal standard mixture of MKH 6561 N-methyl-d3 and Pr-2-OH MKH 6561 -N-metbyl-d3 was added directly to the extract.

Each extract was purified using a C-18 solid phase extraction (spe) cartridge. The extract was acidified with acetic acid prior to analysis by LC-ES/MS/MS.

Wheat grain samples were extracted using the procedures described above, except that Celite® was mixed with the sample prior to ASE extraction.

The chromatographic system used for analysis consisted of a reverse-phase C-8 column and a solvent gradient system containing 1% acetic acid in a water/acetonitrile mobile phase. An electrospray interface was used to introduce the sample into the mass spectrometer.

Quantitation was based on comparison of daughter ion transitions between the two analytes and their deuterated internal standards.

II. RESULTS AND DISCUSSION

The 1-month (TGA-M1021-97R) trial for turnip and 1-month (TGA-M1045-97R) and 4-month (TGA-M1046-97R) trials for mustard greens from Georgia were not available due to crop failure. Some samples from later plant-back intervals were not analysed, because the total propoxycarbazone-sodium residue found in the earlier plant-back intervals was below the LOQ of the analytical method.

In turnip root and tops residues of propoxycarbazone-sodium and 2-hydroxy-propoxycarbazone were below the respective LODs of 0.002 mg/kg at all plant back intervals. The samples from Georgia at 1-month plant-back interval were not available due to phytotoxicity.

In wheat matrices (forage, hay, grain, straw) at the 1-month and 4-month plant back interval residues of propoxycarbazone-sodium and 2-hydroxy-propoxycarbazone were below the respective LODs of 0.002 mg/kg. Since the residues were below the LOD the wheat samples of the 12 month plant back interval were not analysed.

In mustard greens residues of propoxycarbazone-sodium and 2-hydroxypropoxy MKH 6561 (M01) were below the respective LODs of 0.002 mg/kg at the 4-month, 8-month, and 12- month plant-back intervals. The samples from 1-month plant-back interval were not available due to phytotoxicity.

The details are given in Table 6.6.2-2.

Table 6.6.2-2: Summary of residue data performed in the USA

Study			Application					Residues				
Trial No. Plot No. GLP Year	Crop Variety	Country	FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	MKH 65 61 (mg/kg)	MKH 65 61 2-hydroxy (mg/kg)	Sum of MKH 656 1 and -2-OH (mg/kg)
109201	Soil (T)	USA	70 WG	1	0.0455	0.0409	--					
WIN-M1025-97R GLP: yes 1998	Turnip, edible Purple Top White Glo (R)	[redacted], Indiana America, North						Root Tops	91 91 91 91	<0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70	1	0.0452	0.0409	--					

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Table 6.6.2-2: Summary of residue data performed in the USA

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	 No	 kg/ha (a.s.)	 kg/hL (a.s.)	 GS	Residues Portion analysed	 DALT (days)	 MKH 65 61 (mg/kg)	 MKH 65 61 2-hydroxy (mg/kg)	 Sum of MKH 6561 and -2-OH (mg/kg)
WIN-M1026-97R GLP: yes 1998	Turnip, edible Purple Top White Glo (R)	[redacted], Indiana America, North	WG					Root Tops	182 182 182 182	<0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.045	0.041	--					
TGA-M1022-97R GLP: yes 1998	Turnip, edible Purple Top (R)	[redacted], Georgia America, North						Root Tops	250 250 250 250	<0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0446	0.040	--					
TGA-M1023-97R GLP: yes 1998	Turnip, edible Purple Top (R)	[redacted], Georgia America, North						Root Tops	372 372 372 372	<0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0438	0.039	--					
TGA-M1024-97R GLP: yes 1997	Turnip, edible Purple Top (R)	[redacted], Georgia America, North						Root Tops	496 496 496 496	<0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0448	0.0428						
TGA-M1033-97R GLP: yes 1998	Wheat Pioneer 2684 (R)	[redacted], Georgia America, North						Forage Hay Grain Straw	149 149 175 175 219 219 219 219	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0448	0.0406	--					
TGA-M1034-97R GLP: yes 1998	Wheat Pioneer 2684 (R)	[redacted], Georgia America, North						Forage Hay Grain Straw	239 239 265 265 309 309 309 309	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0447	0.0419	--					
WIN-M1037-97R GLP: yes 1998	Wheat Stewards SW520 (R)	[redacted], Indiana America, North						Forage Hay Grain Straw	224 224 269 269 309 309 309 309	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01

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Table 6.6.2-2: Summary of residue data performed in the USA

Study			Application					Residues				
Trial No. Plot No. GLP Year	Crop Variety	Country	FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	MKH 65 61 (mg/kg)	MKH 65 61 2-hydroxy (mg/kg)	Sum of MKH 6561 and -2-OH (mg/kg)
109201	Soil (T)	USA	70 WG	1	0.0457	0.0359	--					
WIN-M1038-97R GLP: yes 1998	Wheat Stewards SW520 (R)	[redacted], Indiana America, North						Forage Hay Grain Straw	315 315 360 360 400 400 400 400	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002 <0.002	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0446	0.040	--					
TGA-M1047-97R GLP: yes 1998	Mustard Broadleaf mustard (R)	[redacted], Georgia America, North						Green material	373 373	<0.002 <0.002	<0.002 <0.002	<0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0438	0.039	--					
TGA-M1048-97R GLP: yes 1997	Mustard Broadleaf mustard (R)	[redacted], Georgia America, North						Green material	497 497	<0.002 <0.002	<0.002 <0.002	<0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.0452	0.0409	--					
WIN-M1050-97R GLP: yes 1998	Mustard Souther Giant Curled (R)	[redacted], Indiana America, North						Green material	168 168	<0.002 <0.002	<0.002 <0.002	<0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.045	0.042	--					
WIN-M1051-97R GLP: yes 1998	Mustard S. Giant Curled Must (R)	[redacted], Indiana America, North						Green material	281 281	<0.002 <0.002	<0.002 <0.002	<0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.045	0.041	--					
WIN-M1052-97R GLP: yes 1998	Mustard S. Giant Curled Must (R)	[redacted], Indiana America, North						Green material	402 402	<0.002 <0.002	<0.002 <0.002	<0.01 <0.01
109201	Soil (T)	USA	70 WG	1	0.045	0.035	--					
FCA-M1054A-97R GLP: yes 1999	Mustard Florida Broadleaf (R)	[redacted], California America, North						Green material	189 189	<0.002 <0.002	<0.002 <0.002	<0.01 <0.01

LOQ = 0.01 mg/kg; LOD = 0.002 mg/kg; DALT = days after last treatment; BBCH = crop growth stage

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III. CONCLUSIONS

No residues of propoxycarbazone-sodium were detected above the limit of detection of 0.002 mg/kg in any untreated sample.
Residues of propoxycarbazone-sodium and 2-hydroxypropoxy MKH 6561 (M01) in plant material were below the respective LOQ or LOD, respectively.

CA 6.7 Proposed residue definition and maximum residue levels

CA 6.7.1 Proposed residue definitions

Primary crop metabolism of propoxycarbazone was investigated following foliar application on spring and winter wheat. Metabolic patterns in the different studies were shown to be similar and the relevant residue for enforcement and risk assessment in cereal crops should be defined as the **sum of propoxycarbazone, its salts and 2-hydroxy-propoxycarbazone, expressed as propoxycarbazone**.

The behaviour and metabolism of propoxycarbazone-sodium in plants was investigated in wheat using the phenyl- as well as the triazolinone-labelled active substance. The results were in very good accordance with respect to residue levels, extractability and distribution of metabolites. Unchanged parent compound was observed in the phenyl-labelled experiment in low amounts only in forage. In the triazolinone-labelled experiment very low amounts of active substance were detected in forage and straw.
The primary metabolisation step in wheat was the hydroxylation of the propoxy side chain resulting in 2-hydroxypropoxy MKH 6561 (M01). This metabolite was also the predominant degradation product in all raw agricultural commodities investigated. Further hydrolysis of M01 led to 2-hydroxy-N-methyl propoxy triazolinone (M02) and probably sulfonamide methyl ester (M05), which was not observed in any of the wheat matrices. Hydrolysis of the sulfonamide methyl ester (M05) resulted in sulfonamide acid (M06), which was in equilibrium with saccharin (M07). A less important metabolic step was demethylation of MKH 6561 yielding N-desmethyl MKH 6561 (M03).

In its reasoned opinion on the review of the existing maximum residue levels (MRLs) for propoxycarbazone according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2013;11(4):3164), EFSA concluded that, according to the RMS Germany metabolism of propoxycarbazone-sodium in plant is sufficiently elucidated to propose a general residue definition for risk assessment and monitoring. Parent compound and 2-hydroxypropoxy MKH 6561 (M01) can be regarded as the residue of concern and should be included in the residue definition for plant matrices.

As the calculated dietary burdens for all groups of livestock were found to be below the trigger value of 0.004 mg/kg b.w./day, further investigation of residues as well as the setting of MRLs in commodities of animal origin is not necessary.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

Table 6.7.2-1 lists the MRLs as presented in the Commission Regulation 149/2008/EC.

The residues for wheat grain at harvest were below or at the respective MRL of 0.02 mg/kg. Therefore, no new MRLs are being proposed as part of this submission.

Table 6.7.2-1: Maximum Residue Limits (MRL) for propoxycarbazone in the EU
(established under Commission Regulation 149/2008/EC)

Crop/Tissue	MRL (mg/kg)
1. FRUIT FRESH OR FROZEN; NUTS	0.02*

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Table 6.7.2-1: Maximum Residue Limits (MRL) for propoxycarbazone in the EU
(established under Commission Regulation 149/2008/EC)

Crop/Tissue	MRL (mg/kg)
2. VEGETABLES FRESH OR FROZEN	0.02*
3. PULSES, DRY	0.02*
4. OILSEEDS AND OILFRUITS	0.02*
5. CEREALS	0.02*
6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	0.05*
7. HOPS (dried), including hop pellets and unconcentrated powder	0.05*
8. SPICES	0.05*
9. SUGAR PLANTS	0.02*
10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	-

* at the LOQ of the method

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

No new MRLs are being proposed; no new import tolerances are requested.

CA 6.8 Proposed safety intervals

Pre-harvest interval (in days) for each relevant crop

Propoxycarbazone-sodium is intended for use on cereals at an early growth stage (up to stage BBCH 33). Therefore the pre-harvest interval is covered by the vegetation period of the crop. There is no need to set a pre-harvest interval.

Re-entry period (in days) for livestock, to areas to be grazed

Propoxycarbazone-sodium is not intended for use in areas where livestock animals may be grazed. Therefore no re-entry period needs to be proposed.

Re-entry period for man to crops, buildings or spaces treated

Propoxycarbazone-sodium is intended for use on cereals. Re-entry in treated fields is generally not necessary. Therefore no re-entry period needs to be proposed.

Withholding period (in days) for animal feedingstuffs

The cereal commodities fed to livestock consist of grain and straw harvested at normal maturity. The highest residue levels of propoxycarbazone-sodium likely to be present in these commodities were taken into account when proposing MRL values for these substances in food of animal origin. No other cereal commodity is usually fed to livestock.
Propoxycarbazone-sodium is intended for use in cereals at an early growth stage (up to stage BBCH 33). Therefore it is not necessary to define a withholding period for animal feeding stuff.

Waiting period before sowing or planting crop to be protected

The product is always applied after sowing the cereals to be protected. Therefore there is no need to define a waiting period between last application and sowing or planting the crops to be protected.

Waiting period between application and handling treated products

Handling of treated cereals is generally not required before harvest, which is always done mechanically. Furthermore, the residue levels in grain are low. Therefore there is no need to define a waiting period between application and handling treated products. It is covered by the vegetation period of the crop.

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Waiting period (in days) before sowing or planting succeeding crops

No measurable residues are expected in succeeding crops for propoxycarbazone-sodium. Therefore, there is no need to define a waiting period before sowing or planting succeeding crops.
Nevertheless after application of propoxycarbazone-sodium, no dicotyledonous catch or intercrops or winter rape should be sown as crop failure due to phytotoxicity was observed for the dicotyledonous crops mustard and turnips at a plant back interval of 1 month in the field rotational crop study.

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

Long-term and short-term consumer exposure to potential propoxycarbazone-sodium residues is estimated according to the EFSA PRIMo model [1].

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

The Acceptable Daily Intake (ADI) is proposed to be set to 0.42 mg/kg bw/day based on a combined toxicity carcinogenicity study in rats.

End-Point	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.42 mg/kg bw/d	Combined toxicity carcinogenicity study in rats	100	CA 5.10

The calculation of the Theoretical Maximum Daily Intake (TMDI) was performed taking into account the existing MRLs for propoxycarbazone-sodium as established in Annex II of the Regulation (EC) No 396/2005 (please refer to Table 6.7.2 1).

A summary of the TMDI calculation is presented in Table 6.9-1. Details of TMDI calculations for propoxycarbazone-sodium are presented in Table 6.9-2.
With the current EFSA model the chronic risk assessment ranges from 0.04 to 0.21% of ADI. The diet with the highest calculated long-term intake was the diet for the UK Toddler (0.21% of the ADI). In the diet sugar plants is the main contributor (0.11%).

Table 6.9-1: Summary of the TMDI calculation

	Daily residue intake	
	[mg/kg bw/day]	[% ADI]
EFSA PRIMo (rev. 2.0)		
Max: UK Toddler	0.000869	0.207
Min: FI adult	0.000155	0.037

It can be concluded that a long-term intake of propoxycarbazone-sodium residues is unlikely to present a public health concern.

[1] Revision 2.0 of the EFSA model, downloaded June 2014. Reasoned Opinion on the Potential Chronic and Acute Risk to Consumers' Health Arising from Proposed Temporary EU MRLs According to Regulation (EC) No 396/2005 on Maximum Residue Levels of Pesticides in Food and Feed of Plant and Animal Origin, European Food Safety Authority, 15 March 2007

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Table 6.9-2: TMDI calculation of propoxycarbazone-sodium according to EFSA PRIMo (rev. 2.0)

Propoxycarbazone-sodium

Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.02	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.42**	ARfD (mg/kg bw):	**n.n.**
Source of ADI:	**COM**	Source of ARfD:	**COM**
Year of evaluation:	**2003**	Year of evaluation:	**2003**

Prepare workbook for refined calculations

Undo refined calculations

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
0.04 - 0.21

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)
0.207	UK Toddler	0.109	SUGAR PLANTS	0.030	FRUIT (FRESH OR FROZEN)	0.028	VEGETABLES	0.21
0.189	WHO Cluster diet B	0.069	VEGETABLES	0.057	CEREALS	0.034	FRUIT (FRESH OR FROZEN)	0.18
0.178	FR infant	0.100	VEGETABLES	0.072	FRUIT (FRESH OR FROZEN)	0.004	CEREALS	0.18
0.175	DE child	0.110	FRUIT (FRESH OR FROZEN)	0.035	VEGETABLES	0.027	CEREALS	0.17
0.157	NL child	0.071	FRUIT (FRESH OR FROZEN)	0.054	VEGETABLES	0.027	CEREALS	0.15
0.155	FR toddler	0.082	VEGETABLES	0.056	FRUIT (FRESH OR FROZEN)	0.014	CEREALS	0.16
0.136	UK Infant	0.048	SUGAR PLANTS	0.031	VEGETABLES	0.026	FRUIT (FRESH OR FROZEN)	0.14
0.133	IE adult	0.051	FRUIT (FRESH OR FROZEN)	0.043	VEGETABLES	0.031	CEREALS	0.13
0.115	WHO cluster diet E	0.043	VEGETABLES	0.029	CEREALS	0.027	FRUIT (FRESH OR FROZEN)	0.11
0.109	DK child	0.050	CEREALS	0.035	VEGETABLES	0.024	FRUIT (FRESH OR FROZEN)	0.11
0.103	WHO cluster diet D	0.043	VEGETABLES	0.040	CEREALS	0.013	FRUIT (FRESH OR FROZEN)	0.10
0.099	SE general population 90th percentile	0.048	VEGETABLES	0.028	FRUIT (FRESH OR FROZEN)	0.024	CEREALS	0.10
0.090	WHO Cluster diet F	0.034	VEGETABLES	0.026	CEREALS	0.018	FRUIT (FRESH OR FROZEN)	0.08
0.088	ES child	0.027	FRUIT (FRESH OR FROZEN)	0.025	CEREALS	0.023	VEGETABLES	0.08
0.087	PT General population	0.030	FRUIT (FRESH OR FROZEN)	0.026	VEGETABLES	0.026	CEREALS	0.09
0.084	WHO regional European diet	0.044	VEGETABLES	0.018	CEREALS	0.017	FRUIT (FRESH OR FROZEN)	0.08
0.078	IT kids/toddler	0.040	CEREALS	0.020	VEGETABLES	0.017	FRUIT (FRESH OR FROZEN)	0.08
0.075	UK vegetarian	0.018	SUGAR PLANTS	0.018	VEGETABLES	0.016	FRUIT (FRESH OR FROZEN)	0.07
0.067	FR all population	0.029	FRUIT (FRESH OR FROZEN)	0.017	VEGETABLES	0.016	CEREALS	0.07
0.066	NL general	0.028	VEGETABLES	0.023	FRUIT (FRESH OR FROZEN)	0.013	CEREALS	0.07
0.065	UK Adult	0.019	SUGAR PLANTS	0.014	VEGETABLES	0.014	FRUIT (FRESH OR FROZEN)	0.06
0.059	ES adult	0.019	FRUIT (FRESH OR FROZEN)	0.018	VEGETABLES	0.015	CEREALS	0.06
0.057	IT adult	0.024	CEREALS	0.019	VEGETABLES	0.013	FRUIT (FRESH OR FROZEN)	0.06
0.049	LT adult	0.024	VEGETABLES	0.013	CEREALS	0.011	FRUIT (FRESH OR FROZEN)	0.05
0.047	DK adult	0.017	VEGETABLES	0.016	FRUIT (FRESH OR FROZEN)	0.014	CEREALS	0.05
0.046	PL general population	0.029	VEGETABLES	0.016	FRUIT (FRESH OR FROZEN)	0.000	PULSES, DRY	0.05
0.037	FI adult	0.013	VEGETABLES	0.012	FRUIT (FRESH OR FROZEN)	0.009	CEREALS	0.03

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

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Acute Reference Dose (ARfD) and Dietary Exposure Calculation

As there is no acute reference dose set for propoxycarbazone-sodium, no further calculations, e.g. NESTI, are necessary.

CA 6.10 Other studies

Since all aspects for the active substance are sufficiently addressed in this document, other special studies are not needed.

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

Since all aspects for the active substance are sufficiently addressed in this document, other special studies are not needed.

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Appendix 1: Tier 1 summaries

CA 6.3.1 Wheat:

Reference CA 6.3.1/01

RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer CropScience AG,**	**Active substance**	: **MKH 6561**
Country	: **Germany**	**Crop/Crop Group**	: **Cereals**
		Page	: **1- A**
Content of active substance (g/kg or g/L)	: **44.3 g/L**	**Indoor/outdoor**	: **Outdoor**
Formulation (e.g. WP)	: **53.2 OD**	**Other a.s. in formulation (common name and content)**	: **mefenpyr-diethyl 8.9 g/L**
Commercial product (name)	: **AE 0298618 01 OD05 A2**	**Residues determined as**	: **MKH 6561**
Producer of commercial product	: **Bayer CropScience AG**	**Residues calculated as**	: **MKH 6561**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/04 R 2004 0102/7 0102-04 Germany D- (Nordrhein-Westfalen) 2004	Wheat Dekan	1) 10.10.2003 2) 05.06.2004 - 20.06.2004 3) 01.08.2004 - 15.08.2004	SPI	0.0702	300	0.02340	26.04.2004/0	Node 3 at least 2 cm above node 2	green material grain straw	3.3 <0.02 <0.05	0 93 93	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg (h) 0.05 mg/kg
RA-2020/04 R 2004 0698/3 0698-04 United Kingdom GB- (Hertfordshire) 2004	Wheat Consort	1) 04.11.2003 2) 10.06.2004 - 21.06.2004 3) 15.08.2004 - 21.08.2004	SPI	0.0702	300	0.02340	22.05.2004/0	Node 3 at least 2 cm above node 2	green material grain straw	1.3 <0.02 <0.05	0 90 90	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg (h) 0.05 mg/kg

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RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer CropScience AG,**		**Active substance**	: **MKH 6561**
Country	: **Germany**		**Crop/Crop Group**	: **Cereals**
			Page	: **1- A**
Content of active substance (g/kg or g/L)	: **44.3 g/L**		**Indoor/outdoor**	: **Outdoor**
Formulation (e.g. WP)	: **53.2 OD**		**Other a.s. in formulation (common name and content)**	: **mefenpyr-diethyl 8.9 g/L**
Commercial product (name)	: **AE 0298618 01 OD05 A2**		**Residues determined as**	: **MKH 6561**
Producer of commercial product	: **Bayer CropScience AG**		**Residues calculated as**	: **MKH 6561**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s)/ Application interval or no. of treatments and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/04 R 2004 0699/1 0699-04 France , north F- (Haute-Normandie) 2004	Wheat Isengrain	1) 05.11.2003 2) 04.06.2004 - 24.06.2004 3) 03.08.2004 - 04.08.2004	SPI	0.0702	300	0.02340	03.05.2004/0	Node 4 at least 2 cm above node 3	green material	1.2	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	92	
									straw	<0.05	92	(h) 0.05 mg/kg
RA-2020/04 R 2004 0700/9 0700-04 Sweden S- 2004	Wheat Marshal	1) 15.09.2003 2) 14.06.2004 - 24.06.2004 3) 10.08.2004	SPI	0.0702	300	0.02340	10.05.2004/0	Node 3 at least 2 cm above node 2	green material	3.0	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	92	
									straw	<0.05	92	(h) 0.05 mg/kg
RA-2020/04 R 2004 0701/7 0701-04 Sweden S- 2004	Wheat Gnejs	1) 05.09.2003 2) 10.06.2004 - 20.06.2004 3) 10.08.2004	SPI	0.0702	300	0.02340	11.05.2004/0	Node 3 at least 2 cm above node 2	green material	2.0	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	91	
									straw	<0.05	91	(h) 0.05 mg/kg

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RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer CropScience AG,** [redacted]	**Active substance**	:	**MKH 6561**
Country	: **Germany**	**Crop/Crop Group**	:	**Cereals**
		Page	:	**1- A**
Content of active substance (g/kg or g/L)	: **44.3 g/L**	**Indoor/outdoor**	:	**Outdoor**
Formulation (e.g. WP)	: **53.2 OD**	**Other a.s. in formulation (common name and content)**	:	**mefenpyr-diethyl 8.9 g/L**
Commercial product (name)	: **AE 0298618 01 OD05 A2**	**Residues determined as**	:	**MKH 6561**
Producer of commercial product	: **Bayer CropScience AG**	**Residues calculated as**	:	**MKH 6561**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s)/ Application interval or no. of treatments and last date (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2021/04 R 2004 0103/5 0103-04 Italy I-[redacted] 2004	Wheat Neodur; Durum wheat	1) 09.02.2004 2) 05.05.2004 - 15.05.2004 3) 30.06.2004 - 10.07.2004	SPI	0.0702	300	0.02340	22.04.2004/0	First node at least 1 cm above tillering node	green material	3.4	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	76	
									straw	<0.05	76	(h) 0.05 mg/kg
RA-2021/04 R 2004 0702/5 0702-04 Spain E-[redacted] 2004	Wheat Don Pedro	1) 03.01.2004 2) 10.04.2004 - 20.04.2004 3) 15.06.2004	SPI	0.0702	300	0.02340	23.03.2004/0	Node 4 at least 2 cm above node 3	green material	1.8	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	85	
									straw	<0.05	85	(h) 0.05 mg/kg
RA-2021/04 R 2004 0703/3 0703-04 France, south F-[redacted] ([redacted]) 2004	Wheat FLORENCE AURORE	1) 15.12.2003 2) 15.05.2004 - 20.05.2004 3) 25.06.2004 - 26.06.2004	SPI	0.0702	300	0.02340	13.04.2004/0	First node at least 1 cm above tillering node	green material	5.1	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	73	
									straw	<0.05	73	(h) 0.05 mg/kg

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RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer CropScience AG, [redacted]**	**Active substance**	: **MKH 6561**
Country	: **Germany**	**Crop/Crop Group**	: **Cereals**
		Page	: **1- B**
Content of active substance (g/kg or g/L)	: **44.3 g/L**	**Indoor/outdoor**	: **Outdoor**
Formulation (e.g. WP)	: **53.2 OD**	**Other a.s. in formulation (common name and content)**	: **mefenpyr-diethyl 8.9 g/L**
Commercial product (name)	: **AE 0298618 01 OD05 A2**	**Residues determined as**	: **MKH 6561 2-hydroxy**
Producer of commercial product	: **Bayer CropScience AG**	**Residues calculated as**	: **MKH 6561 2-hydroxy**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/04 R 2004 0102/7 0102-04 Germany D-[redacted] (Nordrhein-Westfalen) 2004	Wheat Dekan	1) 10.10.2003 2) 05.06.2004 - 20.06.2004 3) 01.08.2004 - 15.08.2004	SPI	0.0702	300	0.02340	26.04.2004/0	Node 3 at least 2 cm above node 2	green material	0.19	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	93	
									straw	<0.05	93	(h) 0.05 mg/kg
RA-2020/04 R 2004 0698/3 0698-04 United Kingdom GB-[redacted] (Hertfordshire) 2004	Wheat Consort	1) 04.11.2003 2) 10.06.2004 - 21.06.2004 3) 15.08.2004 - 21.08.2004	SPI	0.0702	300	0.02340	22.05.2004/0	Node 3 at least 2 cm above node 2	green material	0.44	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	90	
									straw	<0.05	90	(h) 0.05 mg/kg
RA-2020/04 R 2004 0699/1 0699-04 France, north F-[redacted] ([redacted]) 2004	Wheat Isengrain	1) 05.11.2003 2) 04.06.2004 - 24.06.2004 3) 03.08.2004 - 04.08.2004	SPI	0.0702	300	0.02340	03.05.2004/0	Node 4 at least 2 cm above node 3	green material	0.23	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	92	
									straw	<0.05	92	(h) 0.05 mg/kg

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RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address)	: **Bayer CropScience AG,** [redacted]	**Active substance**	:	**MKH 6561**
Country	: **Germany**	**Crop/Crop Group**	:	**Cereals**
		Page	:	**1- B**
Content of active substance (g/kg or g/L)	: **44.3 g/L**	**Indoor/outdoor**	:	**Outdoor**
Formulation (e.g. WP)	: **53.2 OD**	**Other a.s. in formulation (common name and content)**	:	**mefenpyr-diethyl 8.9 g/L**
Commercial product (name)	: **AE 0298618 01 OD05 A2**	**Residues determined as**	:	**MKH 6561 2-hydroxy**
Producer of commercial product	: **Bayer CropScience AG**	**Residues calculated as**	:	**MKH 6561 2-hydroxy**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/04 R 2004 0700/9 0700-04 Sweden S-[redacted] 2004	Wheat Marshal	1) 15.09.2003 2) 14.06.2004 - 24.06.2004 3) 10.08.2004	SPI	0.0702	300	0.02340	10.05.2004/0	Node 3 at least 2 cm above node 2	green material	0.21	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	92	
									straw	0.07	92	(h) 0.05 mg/kg
RA-2020/04 R 2004 0701/7 0701-04 Sweden S-[redacted] 2004	Wheat Gnejs	1) 05.09.2003 2) 10.06.2004 - 20.06.2004 3) 10.08.2004	SPI	0.0702	300	0.02340	11.05.2004/0	Node 3 at least 2 cm above node 2	green material	0.26	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	91	
									straw	<0.05	91	(h) 0.05 mg/kg
RA-2021/04 R 2004 0103/5 0103-04 Italy I-[redacted] 2004	Wheat Neodur; Durum wheat	1) 09.02.2004 2) 05.05.2004 - 15.05.2004 3) 30.06.2004 - 10.07.2004	SPI	0.0702	300	0.02340	22.04.2004/0	First node at least 1 cm above tillering node	green material	1.6	0	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg
									grain	<0.02	76	
									straw	<0.05	76	(h) 0.05 mg/kg

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RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : **Bayer CropScience AG,** ███
Country : **Germany**

Content of active substance (g/kg or g/L) : **44.3 g/L**
Formulation (e.g. WP) : **53.2 OD**
Commercial product (name) : **AE 0298618 01 OD05 A2**
Producer of commercial product : **Bayer CropScience AG**

Active substance : **MKH 6561**
Crop/Crop Group : **Cereals**
Page : **1- B**
Indoor/outdoor : **Outdoor**
Other a.s. in formulation (common name and content) : **mefenpyr-diethyl 8.9 g/L**
Residues determined as : **MKH 6561 2-hydroxy**
Residues calculated as : **MKH 6561 2-hydroxy**

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code Year of Trial	Commodity / Variety (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting (b)	Method of treatment (c)	Application rate per treatment			Dates of treatment(s)/ Application interval or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2021/04 R 2004 0702/5 0702-04 Spain E-███ 2004	Wheat Don Pedro	1) 03.01.2004 2) 10.04.2004 - 20.04.2004 3) 15.06.2004	SPI	0.0702	300	0.02340	23.03.2004/0	Node 4 at least 2 cm above node 3	green material grain straw	0.19 <0.02 <0.05	0 85 85	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg (h) 0.05 mg/kg
RA-2021/04 R 2004 0703/3 0703-04 France, south F-███ (███) 2004	Wheat FLORENCE AURORE	1) 15.12.2003 2) 15.05.2004 - 20.05.2004 3) 25.06.2004 - 26.06.2004	SPI	0.0702	300	0.02340	13.04.2004/0	First node at least 1 cm above tillering node	green material grain straw	0.34 <0.02 0.08	0 73 73	(c) SPI:Spraying (g) 00509 (h) 0.02 mg/kg (h) 0.05 mg/kg

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