Bayer CropScience

Document Title

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

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Data Requirements

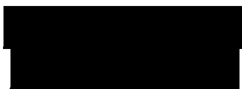
## EU Regulation 1107/2009 & EU Regulation 283/2013
## Document MCA
## Section 6: Residues in or on treated products, food and feed

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

Date

**2014-03-18**

Author(s)



**Bayer CropScience**

M-482298-01-5

# OWNERSHIP STATEMENT

**This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.**

**The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:**

- **From Bayer CropScience; or**
- **From other applicants once the period of data protection has expired.**

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

# Version history

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

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

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## Table of Contents


| | | Page |
|---|---|---|
| CA 6 | RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED | 5 |
| CA 6.1 | Storage stability of residues | 5 |
| CA 6.2 | Metabolism, distribution and expression of residues | 13 |
| CA 6.2.2 | Poultry | 41 |
| CA 6.2.3 | Lactating ruminants | 60 |
| CA 6.2.4 | Pigs | 81 |
| CA 6.2.5 | Fish | 81 |
| CA 6.3 | Magnitude of residue trials in plants | 98 |
| CA 6.3.1 | Cereals | 98 |
| CA 6.4 | Feeding studies | 113 |
| CA 6.4.1 | Poultry | 114 |
| CA 6.4.2 | Ruminants | 114 |
| CA 6.4.3 | Pigs | 114 |
| CA 6.4.4 | Fish | 114 |
| CA 6.5 | Effects of processing | 114 |
| CA 6.5.1 | Nature of the residue | 114 |
| CA 6.5.2 | Distribution of the residue in peel and pulp | 114 |
| CA 6.5.3 | Magnitude of residues in processed commodities | 115 |
| CA 6.6 | Residues in rotational crops | 115 |
| CA 6.6.1 | Metabolism in rotational crops | 115 |
| CA 6.6.2 | Magnitude of residues in rotational crops | 143 |
| CA 6.7 | Proposed residue definitions and maximum residue levels | 143 |
| CA 6.7.1 | Proposed residue definitions | 143 |
| CA 6.7.2 | Proposed MRLs and justification of the acceptability of the levels proposed | 145 |
| CA 6.7.3 | Proposed MRLs and justification of the acceptability of the levels proposed for imported products (import tolerance) | 146 |
| CA 6.8 | Proposed safety intervals | 146 |
| CA 6.9 | Estimation of the potential and actual exposure through diet and other sources | 147 |
| CA 6.10 | Other studies | 148 |
| CA 6.10.1 | Effect on the residue level in pollen and bee products | 149 |
| Appendix 1 – Tier 1 summary of the residue data for flurtamone | | 150 |
| Appendix 2 – Flurtamone – TMDI using EFSA model rev. 2.0, based on the existing and proposed MRLs | | 169 |



This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

# 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**

Since Annex I inclusion, a new study has been generated with a longer storage period for flurtamone, up to 18 months in wheat (grain and green material), sunflower seed and pea seed.

In addition, in some samples of trials from two residue studies (12-2002 and 12-2004) the requested temperature of -18°C was exceeded due to problems during the shipment of these samples. In order to address this deviation, a short term storage stability study was conducted. The storage conditions tested were such that the most unfavorable conditions which were determined for all shipments are covered.

| **Report:** | **KCA 6.1/01, ███, R; 2005** |
|---|---|
| Title: | Storage Stability of Residues of Flurtamone in Wheat (Grain and Green Material), Sunflower Seed and Pea Seed during Deep Freeze Storage for up to 18 Months |
| Document No | M-260155-02-1 |
| Guidelines: | EU 7032/VI/95 rev.5 dated 22 July 1997 |
| GLP | Yes |

**Material and method**

A deep-freeze storage stability study was conducted with flurtamone in matrices of plant origin. Samples of wheat (grain and green material), sunflower (seed) and pea (seed) were fortified with flurtamone at a level of 1.0 mg/kg for wheat (green material) and 0.20 mg/kg for the other sample materials. The samples were stored in polypropylene bottles at around -18°C and were analysed at the nominal storage intervals of 0, 1, 4, 8, 13.5 and 18.5 months.

All samples were analysed according to method AR 94-92 (E) (R. Diot, M. Guillet, C. Venet and B. Simonin; Flurtamone: Analytical Method for the determination of residues in Wheat (grain, straw and shoot) ans soil, Doc. No. C046245) which describes the following principle: residues of flurtamone are extracted from wheat sample materials by macerating with acetone. The extract is purified using an octadecyl (C18) cartridge followed by an aminopropyl ($NH_2$) cartridge. The quantification is carried out by Gas Chromatography on a semi-capillary column and using an electron capture detector (ECD). The calibration is done using external standards in solvent. The limit of quantification of the method is 0.01 mg/kg for wheat (grain, straw and shoot).

For the current study, performed at higher residues levels, some modifications to the method were implemented:

- The 2 purifications on Solid Phase Extraction cartridges were replaced by a clean-up by a gel permeation chromatography (GPC)
- The determination was done using Gas Chromatography (GC) with mass selective detection (MSD) in negative chemical ionisation mode (CI-).
- For calibration, matrix matched standards were used.

Recovery experiments were conducted before day 0 by spiking control samples with flurtamone at a level of 1.0 mg/kg for wheat (green material) and 0.20 mg/kg for other sample materials. In addition, concurrent recoveries were performed at all storage intervals for all matrices by spiking control

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

samples with flurtamone at a level of 1.0 mg/kg for wheat (green material) and 0.20 mg/kg for other sample materials.

**Findings**

The single recovery rates (recoveries for method validation and concurrent recoveries) were in the range of 68 to 107%, with an overall mean of 88% and an overall relative standard deviation RSD of 11.1% (n=66). The obtained recovery data show, that the analytical method was well under control during the conduct of the storage stability study.

In the control samples, residues of flurtamone were below 10% of the spiking level in the spiked samples, except for sunflower seed, on day 123 where the apparent residue level was found at 23% of the spiking level in the spiked samples (probably due to a contamination during sample preparation and without any influence on the conclusion of the study).

Wheat (green material) samples were spiked at a level of 1.0 mg/kg on day 0. The not corrected results obtained in this sample material were between 0.69 and 1.02 mg/kg (69 and 102%). The mean concurrent recovery rates were between 74% and 105%. In the reduction graph, no significant decline is observed for flurtamone during storage of residues in sample material of Wheat (green material) for 561 days in frozen state.

Wheat (grain) samples were spiked at a level of 0.20 mg/kg on day 0. The not corrected results obtained in this sample material were between 0.15 and 0.19 mg/kg (75 and 93%). The mean concurrent recovery rates were between 76% and 91%. In the reduction graph, no significant decline is observed for flurtamone during storage of residues in sample material of wheat (grain) for 563 days in frozen state.

Pea (seed) samples were spiked at a level of 0.20 mg/kg on day 0. The not corrected results obtained in this sample material were between 0.15 and 0.18 mg/kg (73 and 92%). The mean concurrent recovery rates were between 70% and 92%. In the reduction graph, no significant decline is observed for flurtamone during storage of residues in sample material of pea (seed) for 561 days in frozen state.

Sunflower (seed) samples were spiked at a level of 0.20 mg/kg on day 0. The not corrected results obtained in this sample material were between 0.16 and 0.21 mg/kg (81 and 105%). The mean concurrent recovery rates were between 91% and 105%. In the reduction graph, no significant decline is observed for flurtamone during storage of residues in sample material of sunflower (seed) for 560 days in frozen state.

Table CA 6.1-1 summarised the storage recoveries of flurtamone determined after the various storage intervals in wheat (grain and green material), sunflower (seed) and pea (seed). In order to compensate for small variations in the analytical method efficiency, the storage recoveries were corrected by the concurrent procedural recoveries relevant to the fortification level of the stored samples. Both the uncorrected and corrected values are given.

**Conclusion:**

Residues of flurtamone in wheat (green material and grain), in pea (seed) and in sunflower (seed were shown to be stable upon deep freeze storage at ca. -18 °C for at least 18 months.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

**Table CA 6.1-1: Table 6.1-01: Recovery data for concurrent recoveries for flurtamone**

| Storage interval [days] | Fortification level [mg/kg] | Concurrent recovery [%] | | | Recovered residue in stored samples [%] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | uncorrected | | | corrected (a) |
| | | Individual | Mean | RSD | Individual | Mean | RSD | |
| **Wheat (green material)** | | | | | | | | |
| 0 | 1.0 | 74 ; 73 | 74 | - | 77 ; 72 | 75 | - | 101 |
| 32 | 1.0 | 90 ; 89 | 90 | - | 82 ; 75 | 79 | - | 88 |
| 126 | 1.0 | 85 ; 91 | 88 | - | 91 ; 87 | 89 | - | 101 |
| 248 | 1.0 | 86 ; 79 | 83 | - | 81 ; 87 | 84 | - | 101 |
| 408 | 1.0 | 80 ; 79 | 80 | - | 64 ; 74 | 69 | - | 86 |
| 561 | 1.0 | 107 ; 103 ; 106 | 105 | 2.0 | 110 ; 104 ; 91 | 102 | 9.6 | 97 |
| **Overall** | | | **88** | **13.1** | | **84** | **15.2** | |
| **Wheat (grain)** | | | | | | | | |
| 0 | 0.20 | 74 ; 77 | 76 | - | 74 ; 76 | 75 | - | 98 |
| 31 | 0.20 | 79 ; 85 | 82 | - | 88 ; 87 | 88 | - | 106 |
| 125 | 0.20 | 95 ; 83 | 89 | - | 85 ; 89 | 87 | - | 97 |
| 248 | 0.20 | 96 ; 89 ; 88 | 91 | 4.8 | 95 ; 97 ; 89 | 93 | 4.4 | 103 |
| 416 | 0.20 | 79 ; 83 ; 95 | 86 | 9.7 | 89 ; 90 | 90 | - | 104 |
| 563 | 0.20 | 77 ; 90 ; 92 | 86 | 9.4 | 92 ; 92 ; 89 | 91 | 1.9 | 105 |
| **Overall** | | | **85** | **8.5** | | **88** | **7.2** | |
| **Pea (seed)** | | | | | | | | |
| 0 | 0.20 | 90 ; 84 | 87 | - | 85 ; 85 | 85 | - | 97 |
| 28 | 0.20 | 89 ; 88 | 89 | - | 83 ; 87 | 85 | - | 95 |
| 123 | 0.20 | 87 ; 97 | 92 | - | 92 ; 91 | 92 | - | 99 |
| 249 | 0.20 | 107 ; 70 | 89 | - | 94 ; 106 ; 77 | 92 | 15.8 | 104 |
| 420 | 0.20 | 72 ; 68 | 70 | - | 75 ; 71 | 73 | - | 104 |
| 561 | 0.20 | 88 ; 84 ; 77 | 83 | 6.7 | 100 ; 95 ; 71 | 89 | 17.5 | 107 |
| **Overall** | | | **85** | **12.9** | | **87** | **12.2** | |
| **Sunflower (seed)** | | | | | | | | |
| 0 | 0.20 | 84 ; 97 | 91 | - | 96 ; 95 | 96 | - | 105 |
| 31 | 0.20 | 89 ; 101 | 95 | - | 105 ; 98 | 101 | - | 107 |
| 123 | 0.20 | 103 ; 107 | 105 | - | 104 ; 104 | 104 | - | 99 |
| 248 | 0.20 | 99 ; 107 | 103 | - | 103 ; 107 | 105 | - | 102 |
| 421 | 0.20 | 89 ; 92 | 91 | - | 80 ; 81 | 81 | - | 88 |
| 560 | 0.20 | 103 ; 95 ; 93 | 97 | 5.5 | 83 ; 82 ; 93 | 86 | 7.1 | 89 |
| **Overall** | | | **97** | **7.5** | | **95** | **10.6** | |

a) To compensate for variations in the analytical method efficiency the recoveries from stored samples were corrected taking into account the concurrent procedural recovery from samples freshly fortified.

| **Report:** | **KCA 6.1/03, [redacted], A.; 2014** |
|---|---|
| Title: | 7 Days Freezer Storage Stability Study with different combinations of a total of 61 analytes (parent and metabolite molecules) and five matrix types (high water / acid / starch / protein / oil) |
| Document No | M-480441-01-1 (Study S13-03307) |
| Guidelines: | EU 7032/VI/95 rev.5 dated 22 July 1997 |
| GLP | Yes |

**Material and method**

The study was initiated to evaluate the stability of 61 analytes including flurtamone after storage for a period of 8 hours at ±1 °C following 7 days at -7°C in three different matrix groups: high water

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

content (tomato (fruit) and wheat (green material)), high starch content (wheat (grain) and potato (tuber)), and high acid content (grape (bunches)).
Prior to the storage stability tests a method validation was performed. For this purpose one control sample and five fortified samples were analysed for each matrix and analyte. In case of successful validation results the storage stability was started.
Individual aliquots of plant material (5 g) from the cited crops above were fortified with 1.0 mg/kg of flurtamone. The samples were stored in plastic containers at an average temperature of ±1 °C for 8 hours and at -7°C for the following 7 days. They were analysed at the nominal storage intervals of 0 and 7 days for all matrices. After the day 7 analysis point for tomato (fruit) and wheat (green material) a decrease of the recoveries in the stored samples but not in the fresh fortifications was observed. An extended extraction time was applied which led to better recoveries in the stored samples. Therefore, wheat (green material) was re-analyzed after 22 days and tomato (fruit) after 30 days of storage at -7°C.
On day 0, for each matrix, six samples were prepared with 5 g of specimen material. Then, five containers were fortified with flurtamone at 1.0 mg/kg and one was used without fortification as a control specimen. The samples were analysed directly. These five freshly fortified samples also served as procedural recoveries. Concurrent recoveries were conducted at 1.0 mg/kg in tomato (fruit), wheat (green material), grape (bunches), wheat (grain) and potato (tuber) at 7 days, at 30 days (tomato (fruit) only) and at 22 days (wheat (green material) only) of storage.
For every matrix and sampling date after day 0, eight samples were prepared by filling 50 mL Sarstedt tubes with 5 g of specimen material. Five containers were fortified with flurtamone at 1.0 mg/kg. Three containers were stored without fortification to be used as control material and procedural recoveries. The storage containers were placed in a freezer at ±1°C immediately after the fortification. After 8 hours the storage containers were placed in a freezer at -7°C for seven days. The temperature of the freezers was continually recorded with a data recorder
Residues of flurtamone in/on matrices of plant origin were analytically determined as flurtamone using analytical Method 01207 ([redacted] 2013; M-424756-02-1). Initially flurtamone was not included within this method; therefore corresponding validation has been done during the course of this storage stability and results were reported below.
The method is based on the QuEChERS method. In contrast to the original QuEChERS method the solvent acetonitrile/water (4/1, v/v) was used.

5 g of the sample was extracted with 20 mL of acetonitrile.The acetonitrile/ water ratio was adjusted to 4/1 by addition of water. After addition of 6.5 g of the salt mixture of $Mg_2SO_4$/NaCl/$Na_3$ citrate 2 $H_2O$/$Na_2H$ citrate 6 $H_2O$ (4/1/1/0.5, w/w/w/w), the phases were separted by centrifugation and and aliquot of the acetonitrile phase was diluted (1:100) with methanol / water (1/1, v/v) prior to the LC-MS/MS determination.

**Findings**

The recoveries of the validation proved the method performance. Flurtamone mean recoveries ranged between 88% and 100% with RSD below 20%. Two mass transitions were monitored and provided comparable results (m/z 334->247 for the quantification transition and m/z 334->178 for the confirmation transition). See Table CA 6.1-2.
The recoveries in the freshly fortified samples proved also the method performance using the quantification transition. Flurtamone mean recoveries ranged between 82% and 105% with RSD below 20%.

This document is copyright protected. Any distribution or reproduction or publication requires the consent of Bayer AG or its respective affiliates. Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

In addition, 2 concurrent recoveries per commodity were conducted at the nominal storage intervals of 7 days for all matrices, at day 30 for tomato (fruit) and at day 22 for wheat (green material). Recoveries were between 88% and 98%. Validation and procedural recoveries are summarised in Table CA 6.1-3.

In all the control samples, residues of flurtamone were below the LOQ (0.01 mg/kg).

The recoveries of the stored samples showed that the residues of flurtamone, is stable in plant matrices (tomato (fruit), wheat (green material), wheat (grain), potato (tuber) and grape (bunches)) for at least 8 hours at +1°C following 7 days at -7°C (normalised mean to day 0 between 75% and 94%), after 30 days in tomato (fruit) with a normalised mean to day 0 at 84% and after 22 days in wheat (green material) with a normalised mean to dasy 0 at 113%. Table CA 6.1-4 summarises the residues of flurtamone in the stored spiked samples of the investigated matrices.

**Conclusion**

The findings from short-term storage stability study demonstrate that the temperature deviations during shipment did not result in a negative impact on the quality of the residue studies concerned.

The storage conditions tested (at least 8 hours at +1°C following 7 days at -7°C) were such that the most unfavorable conditions which were determined for all shipments are covered. Residues of flurtamone proved to be stable under the experimental conditions tested.

This document is copyright protected. Any distribution or reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table CA 6.1-2: Validation recovery data for flurtamone in tomato (fruit), wheat (green material), grape (bunches), wheat (grain) and potato (tuber)**

| Crops | Mass Transition* | | Fortification Level [mg/kg] | Recovery in validation samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Single Values [%] | | | | | Mean [%] | RSD [%] | SD [%] |
| Tomato (fruit) | 334 / 247 | Q | 1.0 | 103 | 95 | 96 | 95 | 99 | 98 | 3.5 | 3.4 |
| | 334 / 178 | C | 1.0 | 102 | 93 | 99 | 92 | 97 | 97 | 4.3 | 4.2 |
| Wheat (green material) | 334 / 247 | Q | 1.0 | 96 | 101 | 88 | 89 | 86 | 92 | 6.8 | 6.3 |
| | 334 / 178 | C | 1.0 | 89 | 99 | 85 | 84 | 81 | 88 | 8.0 | 7.0 |
| Grape (bunches) | 334 / 247 | Q | 1.0 | 100 | 96 | 97 | 95 | 95 | 97 | 2.1 | 2.1 |
| | 334 / 178 | C | 1.0 | 98 | 96 | 98 | 96 | 94 | 96 | 1.7 | 1.7 |
| Wheat (grain) | 334 / 247 | Q | 1.0 | 97 | 96 | 101 | 102 | 102 | 100 | 2.9 | 2.9 |
| | 334 / 178 | C | 1.0 | 95 | 97 | 100 | 100 | 99 | 98 | 2.2 | 2.2 |
| Potato (tuber) | 334 / 247 | Q | 1.0 | 94 | 92 | 90 | 86 | 93 | 91 | 3.5 | 3.2 |
| | 334 / 178 | C | 1.0 | 93 | 93 | 90 | 85 | 92 | 91 | 3.7 | 3.4 |

*Q: Quantification, C: Confirmation
SD: standard deviation, RSD: relative standard deviation

**Table CA 6.1-3: Procedural recovery data for the residues of flurtamone in tomato (fruit), wheat (green material), grape (bunches), wheat (grain) and potato (tuber)**

| Analyte | Fortification Level [mg/kg] | Date of Extraction | Storage Interval (days) | Single Recoveries [%] | | | | | Mean [%] | RSD [%] | SD [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tomato (fruit) | 1.0 | 2013-11-12 | 0 | 113 | 88 | 109 | 111 | 102 | 105 | 9.7 | 10 |
| | 1.0 | 2013-11-19 | 7 | 96 | 100 | - | - | - | 98 | - | - |
| | 1.0 | 2013-12-12 | 30 | 93 | 95 | - | - | - | 94 | - | - |
| | **Overall Mean, RSD and standard deviation [%]** | | | | | | | | 101 | 8.6 | 8.7 |
| Wheat (green material) | 1.0 | 2013-11-12 | 0 | 74 | 86 | 86 | 87 | 79 | 82 | 6.9 | 5.7 |
| | 1.0 | 2013-11-19 | 7 | 88 | 91 | - | - | - | 90 | - | - |
| | 1.0 | 2013-12-04 | 22 | 100 | 92 | - | - | - | 96 | - | - |
| | **Overall Mean, RSD and standard deviation [%]** | | | | | | | | 87 | 8.6 | 7.5 |
| Grape (bunches) | 1.0 | 2013-12-02 | 0 | 99 | 97 | 101 | 101 | 98 | 99 | 1.8 | 1.8 |
| | 1.0 | 2013-12-09 | 7 | 99 | 95 | - | - | - | 97 | - | - |
| | **Overall Mean, RSD and standard deviation [%]** | | | | | | | | 99 | 2.2 | 2.1 |
| Wheat (grain) | 1.0 | 2014-02-17 | 0 | 100 | 97 | 95 | 98 | 100 | 98 | 2.2 | 2.1 |
| | 1.0 | 2014-02-24 | 7 | 87 | 88 | - | - | - | 88 | - | - |
| | **Overall Mean, RSD and standard deviation [%]** | | | | | | | | 95 | 5.7 | 5.4 |
| Potato (tuber) | 1.0 | 2014-02-17 | 0 | 93 | 93 | 95 | 91 | 94 | 93 | 1.6 | 1.5 |
| | 1.0 | 2014-02-24 | | 96 | 97 | - | - | - | 97 | - | - |
| | **Overall Mean, RSD and standard deviation [%]** | | | | | | | | 94 | 2.2 | 2.0 |

RSD: relative standard deviation, SD: standard deviation


This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table CA 6.1-4: Storage stability data and concurrent recovery data for the residues of flurtamone**

| Commodity | Storage Period (days) | Residue Level in Stored Spiked Samples | | | Day-0 Normalized Recovery[a] | Average % of Fresh Concurrent Recoveries | Average Corrected % Recovery[b] |
|---|---|---|---|---|---|---|---|
| | | mg/kg (ppm) | % of nominal spiking level | Average % recovery | | | |
| Tomato (fruit) | 0 | 1.130 | 113 | 105 | 100 | NA | 100 |
| | | 0.884 | 88 | | | | |
| | | 1.090 | 109 | | | | |
| | | 1.109 | 111 | | | | |
| | | 1.019 | 102 | | | | |
| | 7 | 0.754 | 75 | 79 | 75 | 98 | 80 |
| | | 0.777 | 78 | | | | |
| | | 0.795 | 79 | | | | |
| | | 0.783 | 78 | | | | |
| | | 0.826 | 83 | | | | |
| | 30 | 0.848 | 85 | 88 | 84 | 94 | 94 |
| | | 0.875 | 88 | | | | |
| | | 0.937 | 94 | | | | |
| | | 0.857 | 86 | | | | |
| | | 0.878 | 88 | | | | |
| Wheat (green material) | 0 | 0.743 | 74 | 82 | 100 | NA | 100 |
| | | 0.864 | 86 | | | | |
| | | 0.863 | 86 | | | | |
| | | 0.868 | 87 | | | | |
| | | 0.787 | 79 | | | | |
| | 7 | 0.653 | 65 | 68 | 83 | 90 | 76 |
| | | 0.696 | 70 | | | | |
| | | 0.716 | 72 | | | | |
| | | 0.671 | 67 | | | | |
| | | 0.660 | 66 | | | | |
| | 22 | 0.788 | 79 | 77 | 113 | 96 | 80 |
| | | 0.754 | 75 | | | | |
| | | 0.757 | 76 | | | | |
| Wheat (grain) | 0 | 1.004 | 100 | 98 | 100 | NA | 100 |
| | | 0.968 | 97 | | | | |
| | | 0.950 | 95 | | | | |
| | | 0.980 | 98 | | | | |
| | | 0.996 | 100 | | | | |
| | 7 | 0.900 | 90 | 88 | 90 | 88 | 100 |
| | | 0.870 | 87 | | | | |
| | | 0.867 | 87 | | | | |
| | | 0.881 | 88 | | | | |
| | | 0.875 | 87 | | | | |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| Commodity | Storage Period (days) | Residue Level in Stored Spiked Samples | | | Day-0 Normalized Recovery[a] | Average % of Fresh Concurrent Recoveries | Average Corrected % Recovery[b] |
|---|---|---|---|---|---|---|---|
| | | mg/kg (ppm) | % of nominal spiking level | Average % recovery | | | |
| Potato (tuber) | 0 | 0.928 | 93 | 93 | 100 | NA | 100 |
| | | 0.931 | 93 | | | | |
| | | 0.946 | 95 | | | | |
| | | 0.913 | 91 | | | | |
| | | 0.942 | 94 | | | | |
| | 7 | 0.872 | 87 | 88 | 94 | 97 | 91 |
| | | 0.888 | 89 | | | | |
| | | 0.884 | 88 | | | | |
| | | 0.864 | 86 | | | | |
| | | 0.891 | 89 | | | | |
| Grape (bunches) | 0 | 0.986 | 99 | 99 | 100 | NA | 100 |
| | | 0.966 | 97 | | | | |
| | | 1.010 | 101 | | | | |
| | | 1.011 | 101 | | | | |
| | | 0.984 | 98 | | | | |
| | 7 | 0.937 | 94 | 93 | 94 | 97 | 96 |
| | | 0.921 | 92 | | | | |
| | | 0.913 | 91 | | | | |
| | | 0.955 | 96 | | | | |
| | | 0.942 | 94 | | | | |

[a]Normalized Recovery = (Average recovery / average recovery at day 0) X 100%
[b]Corrected percent recovery = (Average % recovery (stored spiked sample) / Average of fresh concurrent recoveries) X 100%
NA = Not applicable

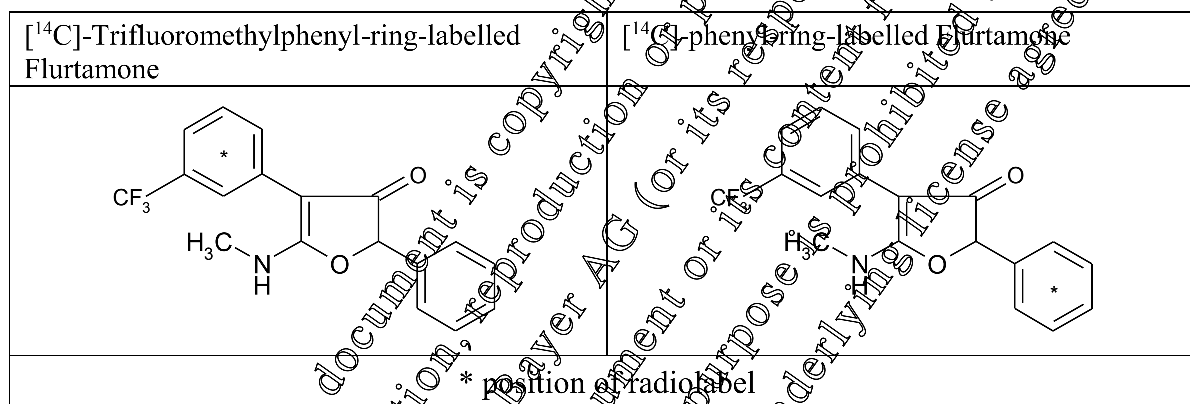
**Stability of residues in samples extracts**

The stability of the residues in the sample extracts was not checked specifically. However, control samples fortified with the test substance were always extracted and analysed concurrently with the untreated and treated samples of the studies. The satisfactory recovery rates obtained from the fortified samples demonstrate the stability of the residues in the sample extracts throughout the analytical procedure, from extraction until chromatographic determination.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.2 Metabolism, distribution and expression of residues

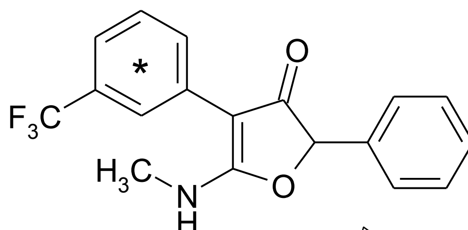
Since Annex I inclusion, new studies have been generated in plants and animals with the compound radiolabelled in the trifluoromethylphenyl ring and/or in the unsubstituted phenyl ring.
Flurtamone (5-methylamino-2-phenyl-4-(3-trifluoromethylphenyl)-3(2H)-furanone) is an herbicide that is used for pre-plant, pre-emergence or post-emergence control of broad leaved and some grass weeds in small grains and sunflowers. Flurtamone belongs to the chemical class of furanones. It is absorbed by the roots and the shoots and blocks, after translocation, the carotenoid synthesis by inhibition of phytoene desaturate, causing bleaching of the target plant.
Metabolism studies have been conducted with the compound radiolabelled in the trifluoromethylphenyl ring and in the unsubstituted phenyl ring, which are shown below.

| [$^{14}$C]-Trifluoromethylphenyl-ring-labelled Flurtamone | [$^{14}$C]-phenyl-ring-labelled Flurtamone |
|---|---|
|  | |
| * position of radiolabel | |

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

| **Report:** | **KCA 6.2/01, [redacted] R., 2013** |
|---|---|
| Title: | Expert Statement :<br>[trifluoromethylphenyl-UL-14C]flurtamone: Specific radioactivity of metabolites following degradation of the trifluoromethylphenyl moiety |
| Document No: | M-462077-01-1 |
| Report No: | - |
| Guidelines: | - |
| GLP | No |

Radiolabelled [trifluoromethylphenyl-UL-$^{14}$C] flurtamone was used as a test compound in several studies to investigate the metabolism of the herbicide flurtamone in plant and animal. The $^{14}$C-atoms of [trifluoromethylphenyl-UL-$^{14}$C] flurtamone are incorporated in the phenyl moiety bearing the trifluoromethyl group.




* position of radiolabel

Metabolic reactions can degrade flurtamone and cleave the trifluoromethylphenyl moiety, producing metabolites containing one or more of the $^{14}C$-atoms. The calculation of the molar specific radioactivity of these metabolites depends on the distribution pattern of the $^{14}C$-atoms in the trifluoromethylphenyl moiety.

The document confirms that, for the test compound [trifluoromethylphenyl-UL-$^{14}C$] flurtamone (batch SEL/1198), the radioactivity was uniformly distributed in the phenyl moiety with the trifluoromethyl group and therefore each of the six $^{14}C$-atoms represents 1/6 of the molar specific radioactivity of the test compound.

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.2.1 Plants

**Sunflower**

The previously available sunflower metabolism study (Lawrence et al. 1996) was conducted using flurtamone uniformly radiolabelled in the trifluoromethylphenyl ring and investigated the residues in sunflower forage and seeds.

In the current sunflower study the metabolism of flurtamone radiolabelled in the phenyl ring position was investigated.

| **Report:** | **KCA 6.2.1/01, [redacted].; 2010** |
|---|---|
| Title: | Metabolism of [phenyl-UL-$^{14}$C]flurtamone in sunflower |
| Report No & Edition No | MEF-10/567<br>M-397170-01-1 |
| **Guidelines:** | OECD 501 Metabolism in Crops<br>US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock<br>PMRA Ref.: DACO 6.3 – Plant Study<br>Council Directive 91/414/EEC amended by Commission deirecitve 96/68/EC |
| **GLP** | Yes |

**Summary**

In the current study the metabolism of [phenyl-UL-$^{14}$C] flurtamone formulated as SC 200 was investigated in sunflower after pre-emergence spray application onto the soil.

The application corresponded to 374 g a.s./ha which represents the maximum targeted application rate. Additionally, a 2X overdose experiment was conducted at a rate of 753 g a.s./ha.

The TRR values in the sampled sunflower forage (0.049 mg/kg for the 1X application rate experiment and 0.164 mg/kg for the 2X application rate experiment) as well as in the harvested sunflower seeds (0.070 mg/kg and 0.105 mg/kg) were low. The radioactive residues were efficiently extracted with n-heptane (seeds only) and acetonitrile/water mixtures.

Parent compound was the main component and represented 43. 8% and 36.8% of the TRR in forage, but was only a trace component of the TRR in the seeds (0.6% in the 2X application rate experiment). Two major metabolites were detected in forage: Flurtamone-hydroxy-mal-glyc represented 17.1% and 25.0% of TRR and flurtamone-hydroxy-glyc represented 7.8% and 14.4% of the TRR.

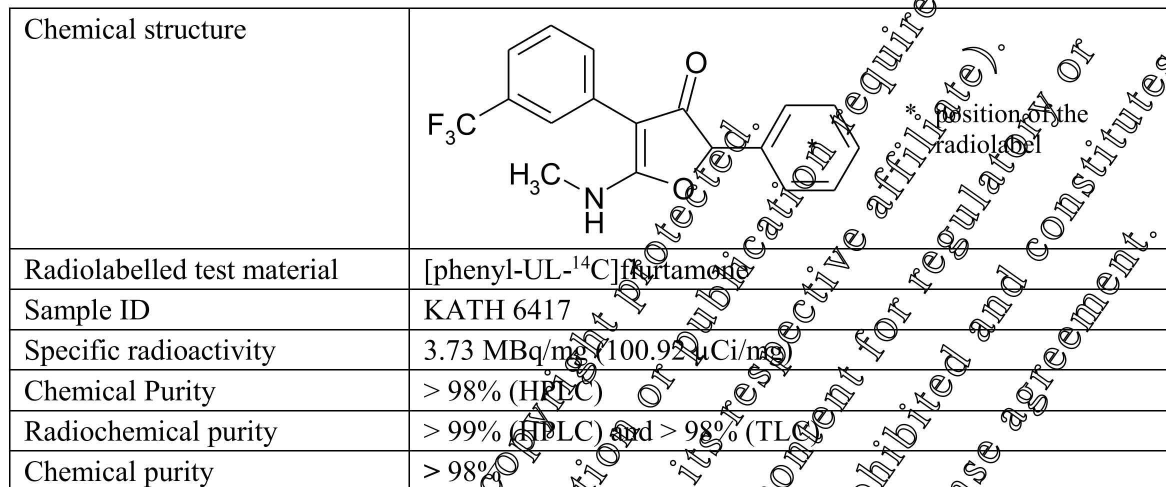
Fatty acid glycerides constituted a very major part of the TRR in the seeds (45.9% and 43.7% of the TRR). Minor metabolites identified in forage were: flurtamone-desmethyl which represented 2.3% and 2.0% of the TRR and flurtamone-trifluormethyl-hydroxy which represented 2.7% and 3.2% of the TRR.

Based on the metabolites identified the following metabolic routes were deduced: hydroxylation followed by conjugation with sugar and malonic acid, N-demethylation and degradation into small carbon fragments followed by incorporation into fatty acids, entering into the pathways of primary metabolite biosynthesis.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## I Materials and Methods

**Test Material:**




| Chemical structure | |
|---|---|
| Radiolabelled test material | [phenyl-UL-$^{14}$C]flurtamone |
| Sample ID | KATH 6417 |
| Specific radioactivity | 3.73 MBq/mg (100.92 µCi/mg) |
| Chemical Purity | > 98% (HPLC) |
| Radiochemical purity | > 99% (HPLC) and > 98% (TLC) |
| Chemical purity | > 98% |

For the 1X and for the 2X application rate experiment, the test item was formulated as an SC 200. Radiodilution and formulation of the test compound was performed by transferring an adequate portions of the stock solution into special glass vials. An adequate amount of blank formulation was added the mixture was homogenised by shaking. Later, the mixture was suspended in water by using a magnetic stirrer. The specific activity of each application was 3.73 MBq/mg (100.92 µCi/mg).
The stability of the test item in the formulation was confirmed by HPLC before and after treatment. The purity of the test item in the formulation was ca. 99%. No degradation during application was observed.

**Soil Characteristics:** "Monheim 4", sandy loam soil, pH ($CaCl_2$) = 6.8, 69% sand, 18% silt and 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g

**Culture Plant:** Sunflower, variety "Pegasol"

**Study Design**

Experimental conditions

Sunflower plants (variety: Pegasol, 4 for the 1X treatment and 6 for the 2X treatment) were cultivated, in a 30 L pot with a surface diameter of 38 cm filled with sandy loam soil "Monheim 4". The sunflower plants were cultivated in the greenhouse of the test facility (controlled temperature, humidity and light conditions).

The sunflower plants were treated with SC 200 formulated [phenyl-UL-$^{14}$C] flurtamone by drench application. Two applications were performed: 1X experiment, representing the target application rate for flurtamone in sunflowers, at application rate of 374 g a.s./ha and a 2X experiment (2X overdose application) at an application rate of 753 g a.s./ha. The two applications were conducted two days after sowing at a growth stage of BBCH 01 - 08. For the 1X experiment 5 mL, and for the 2X experiment

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

10 mL of the application suspension was applied by a handheld pump sprayer onto the soil surface of each pot. Four pots for the 1X experiment and six pots for the 2X experiment were treated. During application, the pots were enveloped in plastic foil to protect the environment from radioactivity and to determine the losses. After application the handheld pump sprayers were rinsed with acetonitrile and the protective plastic foils were rinsed with methanol. The sum of these losses was subtracted from the initial amount of radioactivity in the two suspensions. The actual treatment rates were 375 g a.s./ha for the 1X experiment and 753 g a.s./ha for the 2X experiment.

<u>Sampling</u>

At 46 days after application the plants reached the growth stage BBCH 38/39 (end of stem elongation). At that growth stage one plant of the 1X application rate experiment and two plants of the 2X application rate experiment were cut off just above the soil surface and the total weight of the sample material was determined. These forage samples were shred and homogenised under treatment with liquid nitrogen. The sample materials were stored in a freezer (≤ -18°C).

At 123 days after application the sunflower plants reached maturity (BBCH 97). At that stage the remaining plants, three of the 1X application rate experiment and four of the 2X application rate experiment were harvested. The anthocarps were cut off and the rest of plants were cut off above the soil surface. On the next day, the fully ripe seeds were separated from the anthocarps. The emptied anthocarps and the rest of the plants were combined and stored in a freezer (≤ -18°C).

Aliquots of the homogenates of seed sand forage of the 1X application rate experiment and 2X application rate experiment were extracted. The TRR values of all of these matrices were determined summing up the radioactivity measure in the extracts and in the remaining solids.

**Analytical Procedures**

<u>Extraction</u>

Aliquots of the homogenised seeds (raw agricultural commodity, RAC) were extracted one time with n-heptane, three times with a mixture of acetonitrile/water (4:1, v/v) and once with a mixture of acetonitrile/water (1:1, v/v). After each extraction step, the extracts were separated by centrifugation. The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids.

The acetonitrile/water extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18-E), which was conditioned with acetonitrile/water 4:1 beforehand. The flow-through fraction (effluent) was collected and the cartridge was rinsed with a small volume of acetonitrile/water (4:1, v/v). The effluent and the rinse were combined and concentrated by rotary evaporation (about 40 °C) in vacuo for HPLC analysis. The n-heptane extracts were concentrated by rotary evaporation in vacuo for saponification.

For forage samples the same procedure was performed except that no extraction step with n-heptane was performed and except that only the first two (1X) and three (2X) acetonitrile/water extracts were combined.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

For saponification of the seed extracts an aliquot of the n-heptane extract of the 2X experiment was mixed with methanol/water 4:1 (v/v) and potassium hydroxide. The mixture was refluxed for 19.5 hours. After cooling the mixture was concentrated, diluted with water and washed with n-heptane. The mixture was acidified with sulphuric acid and partitioned against t-butyl methyl ether. The organic phase was concentrated and reconstituted in methanol for HPLC analysis.
Identification was performed by HPLC and/or TLC co-chromatography with reference compounds or by LC-MS/MS supported by comparison of HPLC profiles. Fatty acid glycerides in the n-heptane extract of sunflower seeds were identified by HPLC co-chromatography after saponification.

Quantification
The extracts of sunflower forage and seeds were analysed by HPLC with radio-detection using reversed phase method “Flurtamone”.
All HPLC methods were based on reversed phase (RP) chromatography and an acetonitrile/water gradient. Detection was performed with an UV detector and a radioscope detector with a glass scintillator. The HPLC chromatograms were recorded electronically and were quantitatively evaluated using the software package GINA Star 2.18®.

Radioactivity measurement
The measurement of radioactivity in liquid samples was carried out by liquid scintillation counting (LSC). All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released carbon dioxide was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

Identification and characterisation
Parent compound and metabolites in the extracts were identified by HPLC and/or TLC co-chromatography with reference compounds. Two metabolites were identified by LC-MS/MS spectrometry after isolation from the extract of forage of the 2X experiment.

**Storage stability:**
The extraction experiments and the first HPLC analyses were performed within one month after sampling of the forage or harvest of the seeds.
The extracts were analysed after 8 or 23 days following the start of extraction.
Comparison of the HPLC chromatograms recorded at different times during the study showed that the profiles of the extracts did not significantly change during the analytical work up to a period of at least six months for forage and at least 15 days for seeds. It was therefore concluded, that the residues in the extracts were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## II Results and Discussion

The total radioactive residue (TRR) values of the various matrices of sunflower are shown in the following table:

Table 6.2.1-1: TRR values in sunflowers after pre-emergence spray application of [phenyl-UL-$^{14}$C]flurtamone

| Matrix | Timing and Application | PHI (days)* | ppm (mg a.s. equiv./kg) |
|---|---|---|---|
| Sunflower forage | Pre-emergence treatment (BBCH 01-08) application rate 374 g/ha | 46 | 0.049 |
| Sunflower seeds | | 123 | 0.070 |
| Sunflower forage | Pre-emergence treatment (BBCH 01-08) application rate 753 g/ha | 46 | 0.164 |
| Sunflower seeds | | 123 | 0.105 |

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

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

The total radioactive residues (TRR) in the edible commodity sunflower seed were low. Only 0.070 mg/kg were detected in the seeds of the 1X application rate experiment and 0.105 mg/kg in the seeds of the 2X application rate experiment. In sunflower forage, also low TRR values of 0.049 mg/kg and 0.164 mg/kg were found in the 1X experiment and the 2X experiment, respectively.

Conventional extraction of the sunflower forage samples with acetonitrile/water mixtures released 83.4% of the TRR in the 1X application rate experiment and 89.3% of the TRR in the 2X application rate experiment. After purification with SPE and concentration the conventional sunflower forage extracts still contained 80.9% and 87.0% of the TRR.

As shown in Table 6.2.1-2, sunflower seeds from the 1X experiment released 51.9% of the TRR with the n-heptane extracts while only 15.9% of the TRR was found in the acetonitrile/water extracts. In the 2X experiment a similar distribution was observed: The heptane extract represented 49.5% of the TRR and only 16.5% of the TRR was found in the acetonitrile/water extracts. After extraction, the remaining radioactivity in the solids was less than 0.02 mg/kg of the TRR for forage and less than 0.04 mg/kg for seeds.

The parent compound was identified in sunflower forage by HPLC co-chromatography and by TLC co-chromatography and in sunflower seeds by HPLC co-chromatography, with a radiolabelled reference compound. The metabolites flurtamone-desmethyl and flurtamone-trifluormethyl-hydroxy were identified in sunflower forage by HPLC co-chromatography with non-radiolabelled reference compound.

The metabolites flurtamone-hydroxy-glyc and flurtamone-hydroxy-mal-glyc were identified by spectroscopic methods (LC-MS/MS) after isolation from sunflower forage extract. The position of the hydroxy group of these two metabolites could not be determined. However, hydroxylation at the same position as in the chemical structure of the detected forage metabolite flurtamone-trifluormethyl-hydroxy is likely. Therefore it would be the metabolic precursor . (The structure proposed in this study was lateron confirmed in the wheat metabolism study (Schallau, Justus; 2012))

Parent compound and metabolites identified in sunflower matrices of the 1X application rate experiment were assigned to the profiles of the 2X application rate experiment by comparison of the profiles and vice versa. The two profiles of sunflower forage and the two profiles of sunflower seeds from the acetonitrile/water extracts resulted to be very similar.

The radioactive residues of the n-heptane extract of the sunflower seeds representing 49.5% of the TRR were very lipophilic. For identification of the residues, the concentrated n-heptane extract of the

sunflower seeds of the 2X application rate experiment was subject to saponification in alkaline water/methanol under reflux. After saponification, the mixture was washed with n-heptane and acidified to pH 1 and the radioactive residues were extracted with t-butyl methyl ether and the ether extract was concentrated. This extract stands for 43.7% of the TRR and represents therefore by far the major part of the radioactivity in the n-heptane extract before saponification and work-up. This saponified n-heptane extract was analysed by means of HPLC. The profile showed a group of radioactive lipophilic constituents. Two peaks of this group were identified as oleic acid and linoleic acid by HPLC co-chromatography with radiolabelled reference compound. Based on this result, on the very similar HPLC retention behaviour and on the behaviour during the acid/base liquid-liquid partition after saponification, the other peaks of that group were assigned as fatty acids not further specified.

Taking into account that these fatty acids were obtained from very lipophilic radioactive metabolites in the seeds after alkaline hydrolysis, these metabolites were identified as fatty acid glycerides and a fraction of which as oleic acid glycerides and linoleic acid glycerides. Fatty acid glycerides, particularly oleic acid and linoleic acid glycerides are known constituents of sunflower oil produced from the seeds.

Two trace metabolites (SU4 and SU12) were isolated from the acetonitrile/water extract of sunflower seeds of the 2X application rate experiment and purified by semi-preparative HPLC.

Several HPLC co-chromatography experiments with selected non-radiolabelled reference compounds and these two metabolites were conducted, but neither of the two could be identified, however the HPLC retention shift upon change of the pH value indicates that the metabolite SU4 might contain an acid functional group.

Following LC-HR-MS/MS analysis, a molecular mass could be established for metabolite SU12 corresponding to parent compound + $C_2H_2O_3$.

As no structural data could be obtained for the metabolite SU4 by LC-MS/MS. the metabolite SU4 was treated with the enzyme ß-glucosidase, but no reaction of the metabolite occurred. This finding suggests that this metabolite is not a glucoside conjugate. After treatment of SU4 with hydrochloric acid under reflux, a degradation product occurred at earlier retention time. The HPLC co-chromatography experiment of the degradation product with a reference compound was not successful. The metabolite SU4 was characterized as an possibly acid-degradable metabolite.

SU4 in the profile of sunflower forage was assigned by HPLC co-chromatography with the sample of SU4 isolated from the sunflower seeds extract.

The distribution of the parent compound and metabolites is shown in Table 6.2.1-3. In total, 73.7% and 81.5% of the TRR (1X and 2X application rate experiment, respectively) were identified in sunflower forage, while 45.9% and 44.3% the TRR (1X and 2X application rate experiment, respectively) were identified in sunflower seeds.

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

Table 6.2.1-2: Distribution of radioactivity in the extracts of the sunflower matrices

| | 1X experiment = 374 g/ha | | | | 2X experiment = 753 g/ha | | | |
|---|---|---|---|---|---|---|---|---|
| | **Sunflower forage** | | **Sunflower seeds** | | **Sunflower forage** | | **Sunflower seeds** | |
| | **%** | **mg/kg** | **%** | **mg/kg** | **%** | **mg/kg** | **%** | **mg/kg** |
| TRR | | 0.049 | | 0.070 | | 0.164 | | 0.105 |
| n-heptane extract | --- | --- | 51.9 | 0.036 | --- | --- | 49.5 | 0.052 |
| analysed extract after saponification | --- | --- | 45.9* | 0.032* | --- | --- | 43.7 | 0.046 |
| extracts not analysed# | --- | --- | 6.0* | 0.004* | --- | --- | 5.7 | 0.006 |
| acetonitrile/water extract | 83.4 | 0.041 | 13.9 | 0.010 | 89.3 | 0.146 | 16.5 | 0.017 |
| analysed extract | 80.9 | 0.040 | 13.9 | 0.010 | 87.0 | 0.143 | 16.5 | 0.017 |
| extracts not analysed# | 2.5 | 0.001 | --- | --- | 2.3 | 0.004 | --- | --- |
| unextractable (PES)§ | 16.6 | 0.008 | 34.2 | 0.024 | 10.7 | 0.018 | 34.0 | 0.036 |
| total extracted | 83.4 | 0.041 | 65.8 | 0.046 | 89.3 | 0.146 | 66.0 | 0.069 |
| Balance | 100.0 | 0.049 | 100.0 | 0.070 | 100.0 | 0.164 | 100.0 | 0.105 |

§ post extraction solids
# losses during clean up
* calculated on the basis of the distribution of the radioactivity of the 2X application rate experiment

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.1-3: TRR values and distribution of parent compound and metabolites sunflower matrices after application of [phenyl-UL-$^{14}$C] flurtamone

| | **Sunflower forage 1x** | | **Sunflower seeds 1x** | | **Sunflower forage 2x** | | **Sunflower seeds 2x** | |
|---|---|---|---|---|---|---|---|---|
| TRR [ppm] = | | 0.049 | | 0.070 | | 0.164 | | 0.105 |
| compound (flurtamone-) | % TRR | Ppm | % TRR | ppm | % TRR | Ppm | % TRR | Ppm |
| parent compound | 43.8 | 0.022 | --- | --- | 36.8 | 0.060 | 0.6 | 0.001 |
| Desmethyl | 2.3 | 0.001 | --- | --- | 2.0 | 0.003 | --- | --- |
| trifluormethyl-hydroxy | 2.7 | 0.001 | --- | --- | 3.2 | 0.005 | --- | --- |
| hydroxy-mal-glyc | 17.1 | 0.008 | --- | --- | 25.0 | 0.041 | --- | --- |
| hydroxy-glyc | 7.8 | 0.004 | --- | --- | 14.4 | 0.024 | --- | --- |
| fatty acid glycerides | --- | --- | 45.9* | 0.032* | --- | --- | 43.7 | 0.046 |
| *Thereof oleic acid glycerides* | --- | --- | *7.2** | *0.005** | --- | --- | *6.9* | *0.007* |
| *and linoleic acid glycerides* | --- | --- | *4.9** | *0.003** | --- | --- | *4.6* | *0.005* |
| **Total identified** | **73.7** | **0.036** | **45.9** | **0.032** | **81.5** | **0.134** | **44.3** | **0.046** |
| unknown 1 | 0.6 | <0.001 | 4.7 | 0.003 | --- | --- | 1.6 | 0.002 |
| unknown 2 | 0.7 | <0.001 | --- | --- | --- | --- | 1.2 | 0.001 |
| unknown 3 | 1.4 | 0.001 | --- | --- | 1.0 | 0.002 | --- | --- |
| acid-degradable metabolite (SU4) | 2.5 | 0.001 | 6.5 | 0.005 | 3.4 | 0.006 | 8.7 | 0.009 |
| unknown 5 | 0.8 | <0.001 | --- | --- | 0.4 | 0.001 | 0.8 | 0.001 |
| unknown 6 | 1.1 | 0.001 | --- | --- | 0.7 | 0.001 | --- | --- |
| parent compound + $C_2H_2O_3$ (SU12) | --- | --- | 2.7 | 0.002 | --- | --- | 3.6 | 0.004 |
| total characterized | 7.2 | 0.004 | 13.9 | 0.010 | 5.5 | 0.009 | 16.0 | 0.017 |
| analysed extract(s) | 80.9 | 0.040 | 59.8 | 0.042 | 87.0 | 0.143 | 60.3 | 0.063 |
| extracts not analysed | --- | --- | 3.9* | 0.003* | 0.7 | 0.001 | 3.7 | 0.004 |
| volatiles in distillates | --- | --- | 2.1* | 0.001* | --- | --- | 2.0 | 0.002 |
| remainders on solid phase | 2.5 | 0.001 | --- | --- | 1.6 | 0.003 | --- | --- |
| sum of extracts not analysed, volatiles and remainders on solid phase | 2.5 | 0.001 | 6.0 | 0.004 | 2.3 | 0.004 | 5.7 | 0.006 |
| **Total extractable** | **83.4** | **0.041** | **65.8** | **0.046** | **89.3** | **0.146** | **66.0** | **0.069** |
| unextractable (PES#) | 16.6 | 0.008 | 34.2 | 0.024 | 10.7 | 0.018 | 34.0 | 0.036 |
| Accountability | 100.0 | 0.049 | 100.0 | 0.070 | 100.0 | 0.164 | 100.0 | 0.105 |

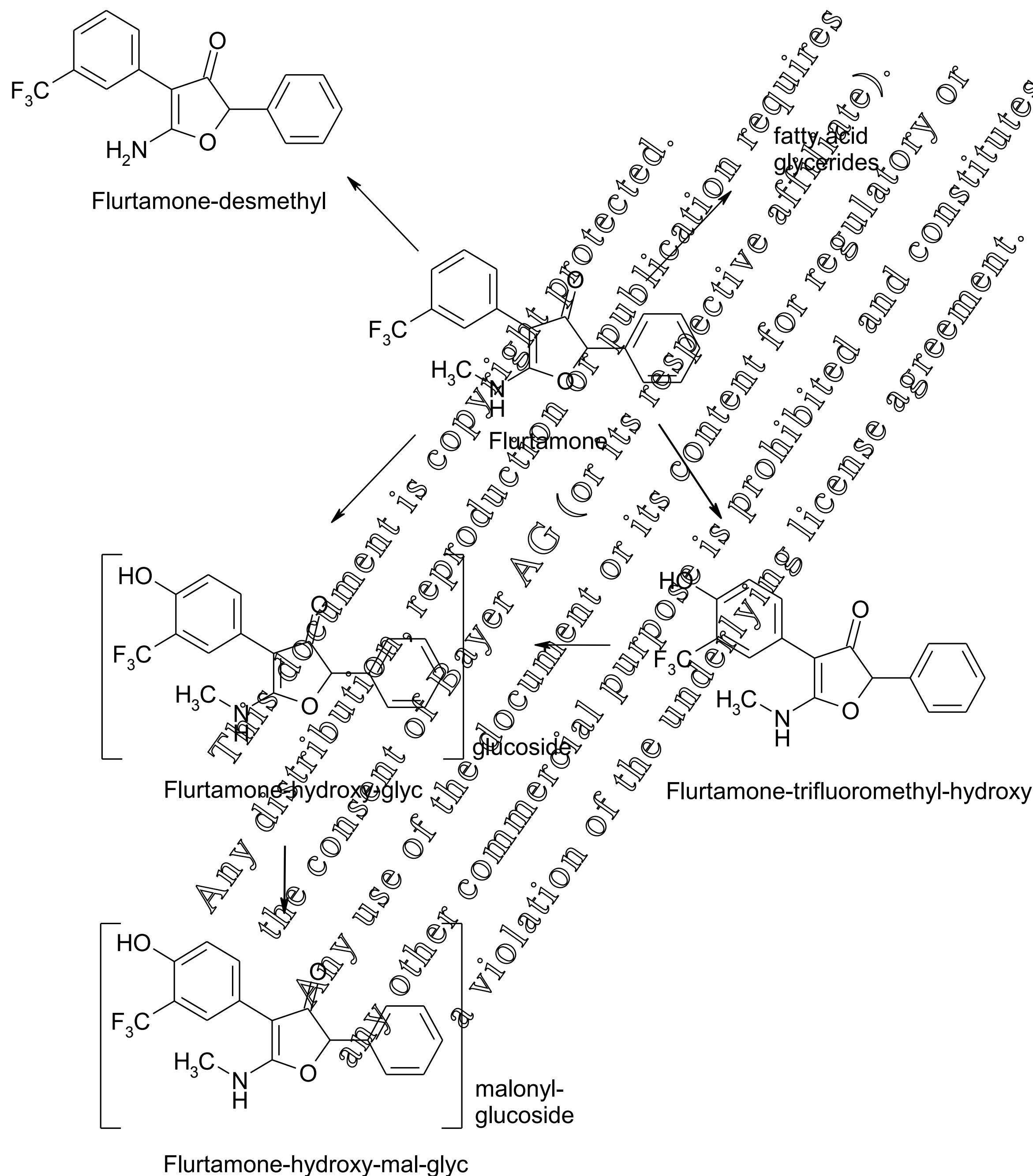
# post extraction solids
* calculated on the basis of the distribution of the radioactivity of the 2X application rate experiment

## III. Conclusions

Thus, [phenyl-UL-$^{14}$C] flurtamone was metabolised moderately in sunflower. Parent compound was the main constituent of the TRR in forage and represented 43.8% and 36.8% of the TRR. Flurtamone-hydroxy-mal-glyc, which represented 17.1% and 25.0% of the TRR and flurtamone-hyroxy-glyc, 7.8% and 14.4 % of the TRR in the 1X and 2X experiment respectively, were major metabolites in forage. In seeds the parent compound could only be observed in trace amounts (0.6% of the TRR in the 2 X application rate experiment). In the seeds a major part of the TRR was constituted from fatty acid glycerides (45.9% and 43.7% of the TRR). On the basis of the results of this study it is concluded that the metabolism of [phenyl-UL-$^{14}$C] flurtamone in sunflower is well understood and the following metabolic pathway is proposed.

This document is copyright protected. Any distribution, reproduction or publication of the document (or its content) requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

Figure 6.2.1-1: Proposed metabolic pathway of [phenyl-UL-14C]flurtamone in sunflower matrices




This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

The position of the hydroxy group of Flurtamone-hydroxy-glyc and Flurtamone-hydroxy-mal-glyc was determined lateron in the course of the wheat metabolism study

## Cereals

The previously available wheat metabolism study (Oliver, Potts, 2000) was conducted using flurtamone uniformly radiolabelled in the phenyl ring and investigated the residues in wheat grain chaff and straw.

In the current study in spring weat the metabolism of flurtamone uniformly labelled in the trifluoromethly-phenyl position is described.

| **Report:** | **KCA 6.2.1/02, [redacted]; 2012;** |
|---|---|
| Title: | Metabolism of [trifluoromethlyphenyl-UL-$^{14}$C]Flurtamone in wheat –Final report - |
| Report No:<br>Edition No: | Ensa-12-0261<br>M-440949-01-1 |
| **Guidelines:** | OECD 501 Metabolism in Crops<br>US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock<br>PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock<br>Japanese MAFF, 12 Nousan 8147<br>European Parliament and Council Regulation (EC) No 1107/2009 |
| **GLP** | Yes |

This document is copyright protected and any publication requires the consent of Bayer AG (or its respective affiliate). Any distribution, reproduction or publication of this document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

### Summary

[Trifluoromethylphenyl-UL-$^{14}$C] flurtamone formulated as an SC 120 was applied pre-emergence via spray application onto bare soil and, in a second, post-emergence experiment, onto planted soil. The actual application rate of flurtamone, formulated as an SC 120, corresponded to 301 g a.s./ha for the pre-emergence treatment experiment and to 350 g a.s./ha for the post-emergence treatment experiment in wheat. This represented approximately two to three times the annual field rate of 120 g a.s./ha.

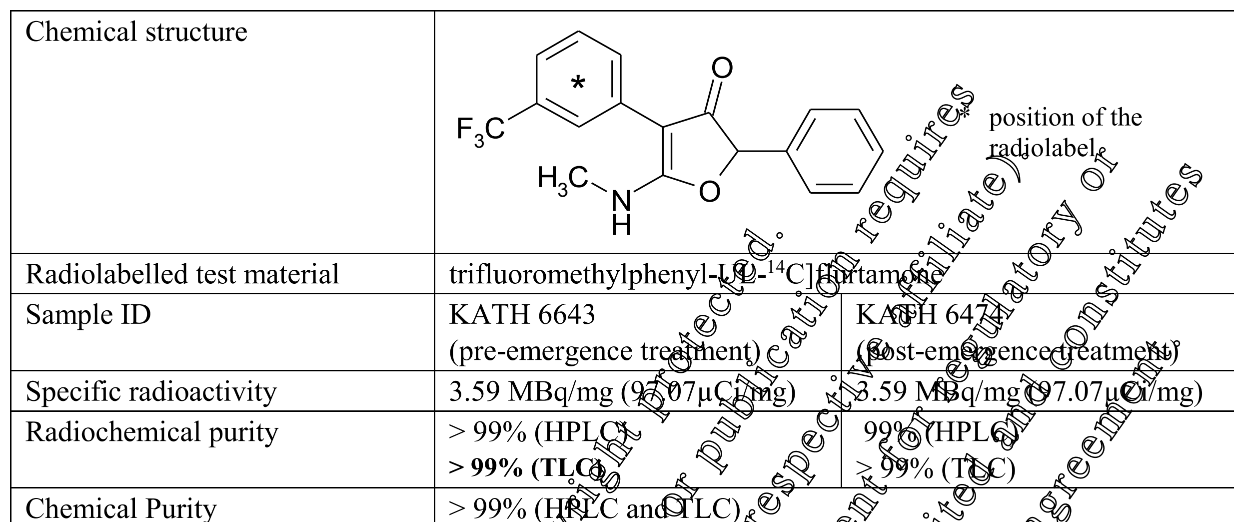
[Trifluoromethylphenyl-UL-$^{14}$C] flurtamone was metabolised in wheat into a large number of metabolites. Parent compound was the main component in the extracts of wheat forage, hay and straw of the post-emergence treatment experiment and represented 91.8% of the TRR in forage, 62.2% of the TRR in hay and 57.5% of the TRR in straw. In the pre-emergence treatment experiment, parent compound represented between 3.0% and 5.5% of the TRR in forage, hay and straw while the metabolite TFA was the main component in these matrices, representing 44.1 % of the TRR in wheat forage, 47.6% of the TRR in wheat hay and 48.6% of the TRR in wheat straw of the pre-emergence treatment. In the edible RAC wheat grain, TFA was the main compound in both experiments, with 92.8% of the TRR after the pre-emergence treatment and 86.2% of the TRR after post-emergence treatment.

All other metabolites were minor (<10% TRR).

The discovered metabolic pattern involved the already known pathways like N-demethylation, hydroxylation of the trifluoromethyl-phenyl moiety followed by conjugation with malonic acid and/or glucose, oxidative cleavage of the trifluoromethyl-phenyl moiety leading to TFA (trifluoroacetic acid) and oxidative ring-opening of the furanone moiety and subsequent cleavage and degradation of the carbon chain.

## I Materials and Methods

**Test Material:**

| Chemical structure |  | |
|---|---|---|
| Radiolabelled test material | trifluoromethylphenyl-UL-$^{14}$C]-flurtamone | |
| Sample ID | KATH 6643 (pre-emergence treatment) | KATH 6474 (post-emergence treatment) |
| Specific radioactivity | 3.59 MBq/mg (97.07µCi/mg) | 3.59 MBq/mg (97.07µCi/mg) |
| Radiochemical purity | > 99% (HPLC) **> 99% (TLC)** | 99% (HPLC) > 99% (TLC) |
| Chemical Purity | > 99% (HPLC and TLC) | |

The radiolabelled test compound [trifluoromethylphenyl-UL-$^{14}$C] flurtamone was formulated as an SC 120. Radiodilution and formulation of the test compound was performed by transferring an adequate portion of the stock solution into special glass vials and concentration to a small remainder under a constant nitrogen flow. An adequate amount of blank formulation was added to the reminder and the mixture was homogenised using a shaker and ultrasonification. Afterwards the mixture was suspended in water by stirring. The specific activity of the application solution was 3.59 MBq/mg (97.03µCi/mg).

**Soil Characteristics:** "Monheim 4"
Soil type: sandy loam
pH ($CaCl_2$) = 6.7
1.2% organic carbon content

**Culture Plant:** Spring Wheat/Small Grain, variety "Thasos"

**Study Design**

Experimental conditions

Spring wheat, (variety "Thasos") was cultivated in two planting containers with a surface area of approx. 1.0 m² filled with sandy loam soil ("Monheim 4"). One container was used for the pre-emergence and the other for the post- emergence treatment experiment. Each experiment was performed with 10 rows of spring wheat. About 50 seeds per row were sown. The plants were cultivated in the vegetation area of the test facility (similar to natural temperature and light conditions, but protected from rainfall).

Bare soil (pre-emergence treatment experiment) and the planted soil (post-emergence treatment experiment) were treated with aqueous suspension of SC 120 formulated [trifluoromethylphenyl-UL-$^{14}$C] flurtamone by spray application with a computer controlled track sprayer with a flat fan nozzle. The pre-emergence and the post-emergence applications were performed with application rates of 301

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

and 350 g a.s./ha, respectively. The pre-emergence treatment was conducted two days after sowing at growth stage BBCH 01-07 (germination: Coleoptile emerged from caryopsis), whereas the post-emergence treatment 42 days after sowing at growth stage BBCH 19-31 (first node at least 1 cm above tillering mode) occured. In the pre-and post- emergence treatment a volume of 100 mL was applied, corresponding to 107.9 MBq or to 31.0 mg a.s. and 125.8 MBq and 35.0 mg a.s. respectively.

Sampling

At the end of the tillering (BBCH 30-31) two rows of wheat forage were harvested. The plants were cut off just above the soil surface and shredded into pieces of about 1 cm length.

In the medium milk stage (BBCH 75) two rows of wheat hay were harvested. The plants were cut off just above the soil surface, shredded into pieces of about 2 cm length and air-dried for 4 days at room temperature.

At the completion of the ripening stage BBCH 92, the remaining wheat plants (6 rows) were cut off just above the soil surface. Grain was separated from straw and the straw was shredded into pieces of about 2 cm length.

After weighing the samples were stored in a freezer (≤ -18°C) on the day of the harvest. The frozen samples were homogenised with a high speed blender in liquid nitrogen. After homogenisation the samples were stored in a freezer (≤ -18°C) until extraction.

**Analytical Procedures**

Extraction

Aliquots of the homogenised wheat samples of the pre-emergence treatment and post-emergence treatment were extracted four times with a mixture of acetonitrile/water (3x 8:2, v/v; 1x 1:1 v/v) using a high speed blender. After each extraction step, extracts and solids were separated by centrifugation.

The solids remaining after conventional extraction of wheat hay and wheat straw (pre-emergence) were submitted to microwave-assisted extraction with acetonitrile/water (1:1, v/v). After extraction, extracts and solids were separated by filtration.

The extracts were combined forming one conventional extract and one exhaustive extract and were subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18 E; conditioned with acetonitrile). The flow-through fraction (percolate) was collected and the cartridge was rinsed with acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo for HPLC analysis.

The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids.

The extracts were stored in a refrigerator (approx. +5°C) or freezer (≤ -18°C).

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Quantification
The parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a UV- and liquid scintillation counting (LSC). All solid samples were combusted in an oxygen atmosphere using an oxidizer. The released $^{14}$C-carbon was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.
The TRR values of the wheat samples were determined by summing up the radioactivity determined in the combined extracts and the radioactivity in the solids.

Identification and characterisation
Parent compound and metabolites were identified by HPLC and/or TLC co-chromatography of the extracts or isolated compounds with labelled and non-labelled reference compounds, by comparison of retention times or by LC-MS analysis. Therefore the flow from the HPLC column was split between UV-detector followed by a radioactivity detector and the MS spectrometer.

**Storage stability:**
The extraction experiments and the first HPLC analyses of the wheat matrices were performed within two months after harvest. HPLC chromatograms recorded at different times during the study showed that the profiles of the extracts did not change significantly during the analytical work up which did not exceed a period of two months in total. In accordance with the OECD Guidance for the Testing of Chemicals 501 (2007), it was therefore concluded, that the residues in the extracts were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

The stability of enantiometric ratio of parent compound in the extract was investigated by repeated isolation of flurtamone from the extract of wheat hay from the pre-emergence treatment experiment and subsequent measurements of enantiomeric ratio. The enationmetric ratio in the extract did not significantly change during storage for approximately three months.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**II. Results and Discussion**

The total radioactive residues (TRR) in the edible commodity wheat grain accounted for 0.362 mg/kg in the pre-emergence treatment experiment and 0.196 mg/kg in the post-emergence treatment experiment. In the feed RACs, the highest residue levels were found after post-emergence treatment from 10.117 mg/kg in hay to 16.575 mg/kg in straw and distinctively lower levels were found after pre-emergence treatment from 0.409 mg/kg in forage to 2.292 mg/kg in straw.

The TRR values are shown in the following table:

Table 6.2.1-4: TRR values in wheat after application of [Trifluoromethylphenyl-UL-$^{14}$C]flurtamone

| **Matrix** | **Timing and Application** | **PHI (days)*** | **ppm (= mg a.s. equiv./kg)** |
|---|---|---|---|
| Wheat Forage | one spray application onto the soil at BBCH 01 - 07 and at 301 g a.s./ha (pre-emergence treatment) | 48 | 0.409 |
| Wheat Hay# | | 85 | 1.564 |
| Wheat Straw | | 110 | 2.292 |
| Wheat Grain | | 110 | 0.362 |
| Wheat Forage | one foliar spray application at BBCH 19 - 31 and at 350 g a.s./ha (post-emergence treatment) | 6 | 12.086 |
| Wheat Hay# | | 43 | 10.117 |
| Wheat Straw | | 68 | 16.575 |
| Wheat Grain | | 68 | 0.196 |

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the time of sampling/harvest)
# The PHI for wheat includes 4 days of drying at room temperature.

Between 85.3% and 99.5% of the TRR were extracted from the RACs of both experiments by conventional extraction with acetonitrile/water mixtures (see Table 6.2.1-5) already representing a major portion of the total radioactive residue. With exhaustive extraction of wheat hay and wheat straw of the pre-emergence treatment experiment minor additional portions could be released so in total 91.4 to 99.5% of the TRR were extracted from the wheat matrices. Consequently the residue levels in the remaining solids were below 10% of the TRR.

Parent compound and several metabolites (flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-mandelic acid, flurtamone-hydroxyl-glyc, flurtamone-trifluoromethyl-N-methyl-phenylacetamid, flurtamone-hydroxyl-mal-gly, flurtamone-TFMBA, flurtamone-desmethyl) were identified by HPLC and TLC co-chromatography reference compounds.
In several cases, peaks from wheat straw of the post-emergence treatment experiment containing the metabolites flurtamone trifluoromethyl-mandelamide, flurtamone-hydroxy-mal-glyc and flurtamone-trifluoromethyl-N-methyl-phenylacetamide, flurtamone-trifluoromethly-hydroxyl and flurtamone-desmethyl were isolated prior to the co-chromatographic experiments using HPLC methods.
Metabolite flurtamone-seco-trifluoromethylbenzylhydroxy was isolated from wheat forage of the post-emergence treatment experiment and identified by LC-MS/MS. The metabolite trifluoroacetic acid (TFA) was isolated from wheat straw and wheat grain of the pre-emergence treatment experiment and identified by LC-MS/MS and TLC co-chromatography.
Following identification of parent compound and metabolites by co-chromatography and/or LC-MS, assignment of peaks in the profiles was completed by comparison of all profiles.
The reference compound flurtamone-hydroxy-glyc was submitted to enzymatic treatment with β-glucosidase cleavage of the sugar moiety occurred, thus proving a β-glucosidase. Subsequent and successful HPLC co-chromatography with the non-radiolabelled reference compound flurtamone-trifluoromethyl-hydroxy determined the position of the hydroxyl group and therewith confirmed the structure that had been elucidated with LC-MS/MS in the sunflower metabolism study

In wheat forage, hay and straw of the pre-emergence treatment experiment, trifluoroacetic acid (TFA) was the main metabolite. The percentage for TFA ranged from 44.1% of the TRR (0.180 mg/kg) in wheat forage to 48.6% (1.113 mg/kg) in wheat straw of the pre-emergence treatment experiment. In

This document is copyright protected. Any distribution or reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

wheat forage, hay and straw of the pre-emergence treatment experiment, other metabolites were found, which were all below 10% of the TRR. These samples contained the parent compound at amounts generally up to 6% of the TRR (0.07 mg/kg). In the samples of the post-emergence experiment, the parent compound was the main metabolite, ranging from 57.5% of the TRR (9.526 mg/kg) in wheat straw to 91.8% of the TRR (11.099 mg/kg) in wheat forage. All other metabolites were below 4% of the TRR.

In the edible RAC, wheat grain, TFA was the main metabolite in both experiments, with 92.8% of the TRR (0.336 mg/kg) after the pre-emergence treatment and 86.2% of the TRR (0.169 mg/kg) after the post-emergence treatment. In total 11 metabolites were identified, while at least 22 metabolites remained unknown. No individual unknown metabolite was larger than 5% of the TRR or 0.3 mg/kg. The distribution of the parent compound and metabolites is shown in Table 6.2.1- 6-7. In total, 65.9% and 95.2% of the TRR could be identified in the different wheat matrices

Table 6.2.1-5: Distribution of radioactivity in the extracts of wheat matrices

| | Wheat forage | | Wheat hay | | Wheat straw | | Wheat grain | |
|---|---|---|---|---|---|---|---|---|
| | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg |
| ***Pre-emergence treatment experiment*** | | | | | | | | |
| TRR [mg/kg] = | | 0.409 | | 1.564 | | 2.292 | | 0.362 |
| Conventionally extracted | 92.5 | 0.378 | 88.4 | 1.383 | 85.3 | 1.954 | 93.9 | 0.340 |
| Extract for analysis | 90.9 | 0.371 | 87.8 | 1.373 | 84.3 | 1.933 | 93.6 | 0.339 |
| Extracts not analysed | 1.6 | 0.007 | 0.6 | 0.010 | 0.9 | 0.021 | 0.3 | 0.001 |
| Microwave extraction | --- | --- | 4.1 | 0.064 | 6.1 | 0.141 | --- | --- |
| Extract for analysis | --- | --- | 4.0 | 0.063 | 6.0 | 0.138 | --- | --- |
| Extracts not analysed | --- | --- | <0.1 | 0.001 | 0.1 | 0.003 | --- | --- |
| Total extracted | 92.5 | 0.378 | 92.5 | 1.447 | 91.4 | 2.095 | 93.9 | 0.340 |
| Unextractable (PES*) | 7.5 | 0.030 | 7.5 | 0.117 | 8.6 | 0.197 | 6.1 | 0.022 |
| Balance | 100.0 | 0.409 | 100.0 | 1.564 | 100.0 | 2.292 | 100.0 | 0.362 |
| ***Post-emergence treatment experiment*** | | | | | | | | |
| TRR [mg/kg] = | | 12.086 | | 10.117 | | 16.575 | | 0.196 |
| Conventionally extracted | 99.5 | 12.029 | 95.0 | 9.608 | 92.9 | 15.398 | 91.7 | 0.180 |
| Extract for analysis | 99.0 | 11.965 | 92.8 | 9.386 | 90.1 | 14.930 | 91.4 | 0.179 |
| Extracts not analysed | 0.5 | 0.063 | 2.2 | 0.222 | 2.8 | 0.468 | 0.3 | 0.001 |
| Total extracted | 99.5 | 12.029 | 95.0 | 9.608 | 92.9 | 15.398 | 91.7 | 0.180 |
| Unextractable (PES*) | 0.5 | 0.057 | 5.0 | 0.509 | 7.1 | 1.177 | 8.3 | 0.016 |
| Balance | 100.0 | 12.086 | 100.0 | 10.117 | 100.0 | 16.575 | 100.0 | 0.196 |

* post extraction solids

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.1-6: TRR values and distribution of parent compound and metabolites in wheat after spray application of [trifluoromethylphenyl-UL-$^{14}$C]flurtamone

| | **Pre-emergence treatment experiment** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Wheat forage** | | **Wheat hay** | | **Wheat straw** | | **Wheat grain** | |
| TRR [mg/kg] = | | 0.409 | | 1.564 | | 2.292 | | 0.362 |
| Compound (flurtamone) | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg |
| parent compound | 5.5 | 0.023 | 3.8 | 0.059 | 3.0 | 0.069 | --- | --- |
| TFA | 44.1 | 0.180 | 47.6 | 0.744 | 48.6 | 1.113 | 92.8 | 0.336 |
| trifluoromethyl-mandelamide | 0.9 | 0.004 | 1.5 | 0.023 | 1.1 | 0.025 | --- | --- |
| trifluoromethyl-N-methyl-mandelamide | 5.5 | 0.022 | 6.6 | 0.103 | 4.7 | 0.107 | --- | --- |
| trifluoromethyl-mandelic acid | 2.8 | 0.011 | 1.7 | 0.027 | 1.8 | 0.041 | --- | --- |
| hydroxy-glyc | 8.2 | 0.034 | 2.1 | 0.033 | 1.8 | 0.040 | --- | --- |
| hydroxy-mal-glyc | 2.8 | 0.011 | 2.0 | 0.031 | 1.7 | 0.040 | --- | --- |
| trifluoromethyl-N-methyl-phenylacetamide | 1.1 | 0.004 | 0.8 | 0.012 | 0.7 | 0.015 | --- | --- |
| TFMBA | 0.5 | 0.002 | --- | --- | 2.1 | 0.049 | --- | --- |
| trifluoromethyl-hydroxy (trace) | 0.1 | 0.001 | 0.1 | 0.001 | --- | --- | --- | --- |
| seco-trifluoromethylbenzylhydroxy | --- | --- | --- | --- | --- | --- | --- | --- |
| Desmethyl | 0.3 | 0.001 | 0.4 | 0.007 | 0.5 | 0.012 | --- | --- |
| Total identified | 71.8 | 0.293 | 66.5 | 1.041 | 65.9 | 1.511 | 92.8 | 0.336 |
| unknown 1 | 2.5 | 0.010 | 1.5 | 0.024 | 0.9 | 0.020 | --- | --- |
| unknown 2 | 0.9 | 0.004 | 1.7 | 0.027 | 2.1 | 0.049 | 0.8 | 0.003 |
| unknown 3 | 4.4 | 0.018 | 1.3 | 0.021 | 1.4 | 0.033 | --- | --- |
| unknown 4 | 1.7 | 0.007 | 1.6 | 0.025 | 0.9 | 0.021 | --- | --- |
| unknown 5 | --- | --- | 1.0 | 0.016 | 1.0 | 0.024 | --- | --- |
| unknowns 6 a-c | 3.3 | 0.014 | 5.2 | 0.082 | 3.9 | 0.089 | | |
| unknown 7 | --- | --- | 1.8 | 0.028 | 1.8 | 0.041 | --- | --- |
| unknown 8 | 1.3 | 0.005 | 0.8 | 0.012 | 1.4 | 0.032 | --- | --- |
| unknowns 9 a-c | 3.0 | 0.012 | 2.2 | 0.034 | 1.9 | 0.043 | | |
| unknown 10 | 1.0 | 0.004 | 1.0 | 0.016 | 0.7 | 0.017 | --- | --- |
| unknown 11 | --- | --- | 1.5 | 0.023 | 1.1 | 0.025 | --- | --- |
| unknown 12 | --- | --- | 3.8 | 0.059 | 0.5 | 0.011 | --- | --- |
| unknowns 13 a-h | 0.7 | 0.003 | 47.6 | 0.744 | --- | --- | --- | --- |
| unknowns 14 a-e | 0.5 | 0.002 | 1.5 | 0.023 | 0.8 | 0.018 | --- | --- |
| Total characterised | 19.2 | 0.078 | 21.3 | 0.333 | 18.4 | 0.422 | 0.8 | 0.003 |
| Analysed extract(s) | 90.9 | 0.371 | 87.8 | 1.373 | 84.3 | 1.933 | 93.6 | 0.339 |
| Extracts not analysed | 1.6 | 0.007 | 0.6 | 0.010 | 0.9 | 0.021 | 0.3 | 0.001 |
| Microwave extraction* | --- | --- | 4.1 | 0.064 | 6.1 | 0.141 | --- | --- |
| Total extracted | 92.5 | 0.378 | 92.5 | 1.447 | 91.4 | 2.095 | 93.9 | 0.340 |
| Unextractable (PES#) | 7.5 | 0.030 | 7.5 | 0.117 | 8.6 | 0.197 | 6.1 | 0.022 |
| Accountability | 100.0 | 0.409 | 100.0 | 1.564 | 100.0 | 2.292 | 100.0 | 0.362 |

# post extraction solids; * Microwave extracts were analysed, but no peaks were detected due to low radioactivity concentrations and high matrix load.

Table 6.2.1-7 : TRR values and distribution of parent compound and metabolites in wheat after spray application of [trifluoromethylphenyl-UL-$^{14}$C] flurtamone

| | Post-emergence treatment experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Wheat forage** | | **Wheat hay** | | **Wheat straw** | | **Wheat grain** | |
| TRR [mg/kg] = | | 12.086 | | 10.117 | | 16.575 | | 0.196 |
| Compound (flurtamone) | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg |
| parent compound | 91.8 | 11.099 | 62.2 | 6.295 | 57.5 | 9.526 | --- | --- |
| TFA | --- | --- | 3.7 | 0.375 | 2.9 | 0.478 | 86.2 | 0.169 |
| trifluoromethyl-mandelamide | <0.1 | 0.004 | 0.4 | 0.045 | 0.5 | 0.076 | --- | --- |
| trifluoromethyl-N-methyl-mandelamide | 0.6 | 0.072 | 2.7 | 0.271 | 2.6 | 0.429 | --- | --- |
| trifluoromethyl-mandelic acid | 0.6 | 0.068 | 1.7 | 0.171 | 2.4 | 0.397 | --- | --- |
| hydroxy-glyc | 0.2 | 0.027 | 0.5 | 0.053 | 0.6 | 0.099 | --- | --- |
| hydroxy-mal-glyc | 0.1 | 0.011 | 1.0 | 0.104 | 0.7 | 0.112 | --- | --- |
| trifluoromethyl-N-methyl-phenylacetamide | <0.1 | 0.004 | 0.4 | 0.040 | 0.3 | 0.043 | | |
| TFMBA | 0.2 | 0.028 | 1.6 | 0.163 | 1.9 | 0.307 | --- | --- |
| trifluoromethyl-hydroxy (trace) | <0.1 | 0.003 | 0.1 | 0.007 | 0.1 | 0.017 | --- | --- |
| seco-trifluoromethylbenzylhydroxy | 1.3 | 0.155 | 1.1 | 0.108 | 1.5 | 0.256 | --- | --- |
| Desmethyl | 0.3 | 0.032 | 0.8 | 0.080 | 1.1 | 0.185 | --- | --- |
| Total identified | 95.2 | 11.504 | 76.2 | 7.713 | 71.9 | 11.924 | 86.2 | 0.169 |
| unknown 1 | 0.1 | 0.012 | 0.5 | 0.055 | 0.4 | 0.074 | 2.1 | 0.004 |
| unknown 2 | 0.3 | 0.036 | 1.5 | 0.153 | 1.6 | 0.265 | --- | --- |
| unknown 3 | --- | --- | 0.4 | 0.039 | 0.5 | 0.086 | --- | --- |
| unknown 4 | --- | --- | 0.3 | 0.028 | 0.5 | 0.075 | --- | --- |
| unknown 5 | 0.1 | 0.016 | 0.6 | 0.065 | 0.6 | 0.094 | --- | --- |
| unknowns 6 a-c* | 0.1 | 0.015 | 1.6 | 0.159 | 1.6 | 0.269 | --- | --- |
| unknown 7 | 0.1 | 0.017 | 1.4 | 0.143 | 1.3 | 0.221 | 3.1 | 0.006 |
| unknown 8 | 0.2 | 0.020 | 0.9 | 0.092 | 1.2 | 0.192 | --- | --- |
| unknowns 9 a-c* | 0.1 | 0.012 | 1.1 | 0.112 | *0.7 | *0.121 | --- | --- |
| unknown 10 | 0.2 | 0.018 | 1.3 | 0.133 | 0.8 | 0.138 | --- | --- |
| unknown 11 | --- | --- | 0.7 | 0.075 | 0.7 | 0.119 | --- | --- |
| unknown 12 | 0.1 | 0.017 | 0.7 | 0.070 | 0.9 | 0.143 | --- | --- |
| unknowns 13 a-h* | 0.2 | 0.030 | 0.8 | 0.076 | *1.1 | *0.186 | --- | --- |
| unknowns 14 a-e* | 0.4 | 0.050 | 1.2 | 0.125 | *1.7 | *0.288 | --- | --- |
| unknown 15 | 0.3 | 0.037 | 0.8 | 0.080 | 0.6 | 0.099 | --- | --- |
| unknown 16 | 0.3 | 0.040 | 0.7 | 0.073 | 0.6 | 0.101 | --- | --- |
| unknown 17 | 0.3 | 0.036 | 0.7 | 0.071 | 1.1 | 0.177 | --- | --- |
| unknown 18 | 0.2 | 0.021 | 0.5 | 0.046 | 0.8 | 0.135 | --- | --- |
| unknown 19 | 0.1 | 0.012 | --- | --- | 0.2 | 0.037 | --- | --- |
| unknowns 20 | 0.3 | 0.037 | --- | --- | 0.2 | 0.029 | --- | --- |
| unknown 21 | 0.1 | 0.016 | --- | --- | 0.2 | 0.041 | --- | --- |
| unknown 22 | 0.2 | 0.020 | 0.7 | 0.074 | 0.7 | 0.116 | --- | --- |
| Total characterised | 3.8 | 0.461 | 16.5 | 1.673 | 18.1 | 3.007 | 5.2 | 0.010 |
| Analysed extract(s) | 99.0 | 11.965 | 92.8 | 9.386 | 90.1 | 14.930 | 91.4 | 0.179 |
| Extracts not analysed | 0.5 | 0.063 | 2.2 | 0.222 | 2.8 | 0.468 | 0.3 | 0.001 |
| Total extracted | 99.5 | 12.029 | 95.0 | 9.608 | 92.9 | 15.398 | 91.7 | 0.180 |
| Unextractable (PES#) | 0.5 | 0.057 | 5.0 | 0.509 | 7.1 | 1.177 | 8.3 | 0.016 |
| Accountability | 100.0 | 12.086 | 100.0 | 10.117 | 100.0 | 16.575 | 100.0 | 0.196 |

# post extraction solids; * The largest individual unknown metabolite in wheat straw of the post-emergence treatment experiment represented 0.9% of TRR or 0.153 mg/kg of the TRR.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

### III. Conclusions

[Trifluoromethylphenyl-UL-$^{14}$C] flurtamone was metabolised in wheat into a large number of metabolites. Parent compound was the main component in the extracts of wheat forage, hay and straw of the post-emergence treatment experiment and represented 91.8% of the TRR in forage, 62.2% of the TRR in hay and 57.5% of the TRR in straw. In the pre-emergence treatment experiment, parent compound represented between 3.0% and 5.5% of the TRR in forage, hay and straw while the metabolite TFA was the main component in these matrices, representing 44.1% of the TRR in wheat forage, 47.6% of the TRR in wheat hay and 48.6% of the TRR in wheat straw of the pre-emergence treatment In the edible RAC wheat grain, TFA was the main compound in both experiments, with 92.8% of the TRR after the pre-emergence treatment and 86.2% of the TRR after post-emergence treatment.

All other metabolites were minor (<10% TRR).

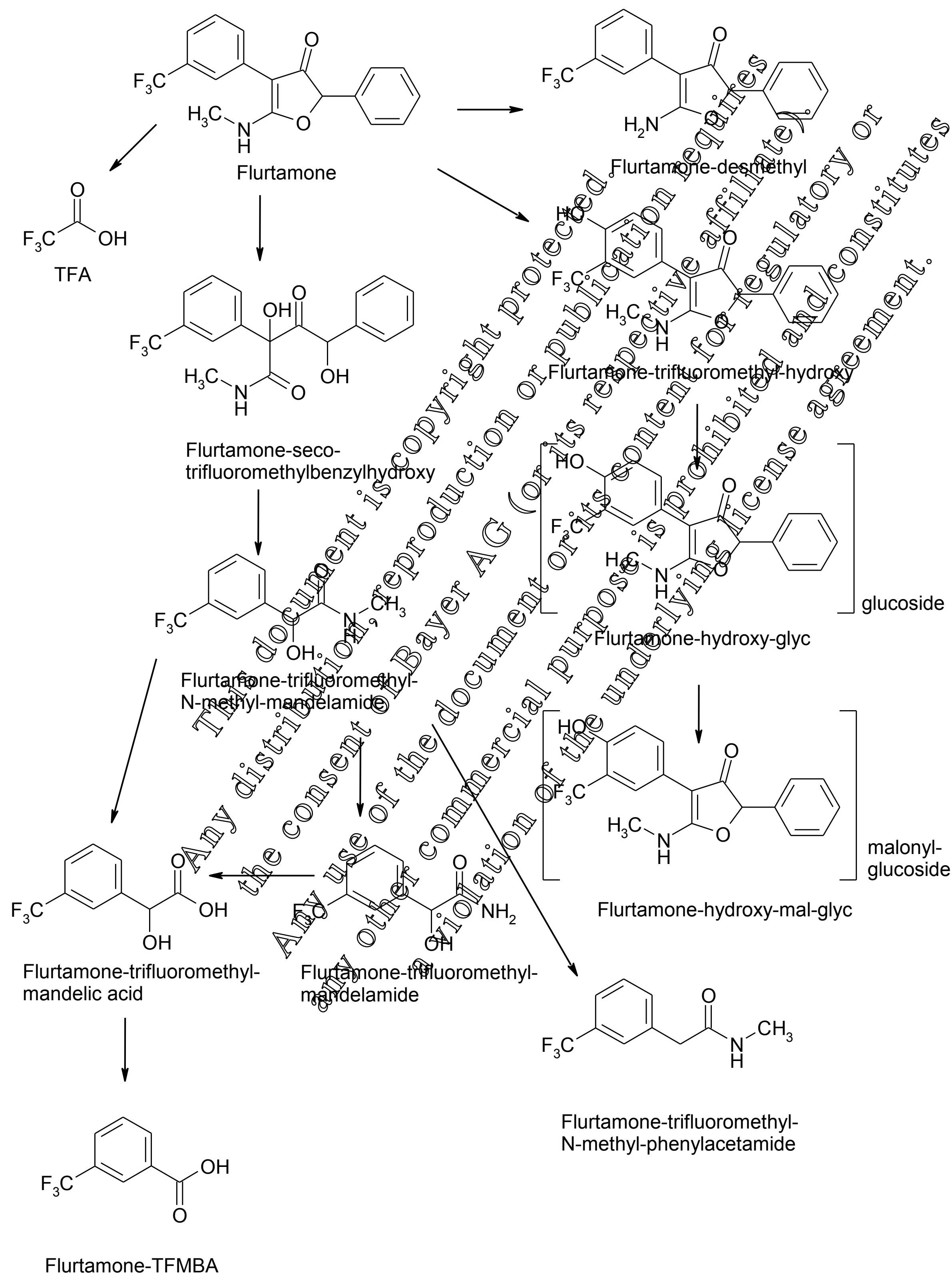
Based on the metabolites identified the following metabolic routes were deduced:

- N-demethylation
- hydroxylation of the trifluoromethyl-phenyl moiety followed by conjugation with malonic acid and/or glucose
- oxidative cleavage of the trifluoromethyl-phenyl moiety leading to TFA (trifluoroacetic acid)
- oxidative ring-opening of the furanone moiety and subsequent cleavage and degradation of the carbon chain.

On the basis of the results of this study it is concluded that the metabolism of [trifluoromethylphenyl-UL-$^{14}$C] flurtamone in wheat is well understood and the following metabolic pathway is proposed.

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Figure 6.2.1-2: Proposed metabolic pathway of [trifluoromethylphenyl-UL-^{14}C]flurtamone in wheat




This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Peanut**

The following study in peanuts after pre-emergence soil treatment is an old study. It was not submitted with the baseline dossier and is not needed for the intended uses. Nevertheless the study was evaluated by EFSA and is cited in the MRL Review Report "Reasoned opinion on the review of the existing maximum residue levels (MRLs) for flurtamone according to Article 12 of Regulation (EC) No 396/20051" (EFSA Journal 2012;10(12):3009). Therefore, in the sense of completeness, the study is briefly summarized below.

| **Report:** | **KCA 6.2.1/06, .; 1991;** |
|---|---|
| Title: | **Plant Metabolism Study of [$^{14}$C]-Flurtamone** in Peanuts - Pre-emergence Summary |
| Report No:<br>Edition No: | -<br>M-276618-01-1 |
| **Guidelines:** | EPA Guidelines; Section 171-4 |
| **GLP** | Yes |

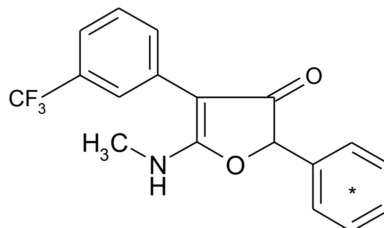
**Summary**

Plant metabolism of [$^{14}$C]-flurtamone was studied on peanut plants with [phenyl-UL-$^{14}$C]- and [$^{14}$C-trifluoromethylphenyl]-flurtamone. Plants were treated once at 1.12 kg/ha as a pre-emergence soil treatment and were harvested at maturity with a pre-harvest interval (PHI) of 120-121 days.
At harvest, plants were separated into leaves, stems, kernels and hulls. Leaves and stems contained over 96% of the total radioactivity in the plant and therefore were used for metabolite characterization. This was accomplished by TLC and HPLC co-chromatography with authentic standards. The major metabolic pathways from [phenyl-UL-$^{14}$C]-flurtamone in this system were initial breakdown of the flurtamone in soil to smaller fragments which were supposed to subsequently be taken up through the roots and incorporated into the plant as natural constitutents. A part of the residue seemed to be metabolized to yield $^{14}CO_2$ which was taken up by the plant through photosynthesis process and was incorporated into natural plant components. The major metabolic pathway of [$^{14}$C-trifluoromethylphenyl]-flurtamone in this system was breakdown of flurtamone to yield flurtamone-trifluoromethyl-N-methyl-mandelamide, of which conjugates were supposed to be the major part of residues in plants. After acid hydrolysis, also flurtamone-trifluoromethyl-mandelamide and flurtamone-trifluoromethyl-mandelic acid could be identified.
No quantitative data were provided for the metabolite profiles and the residue levels.

**I Materials and Methods**

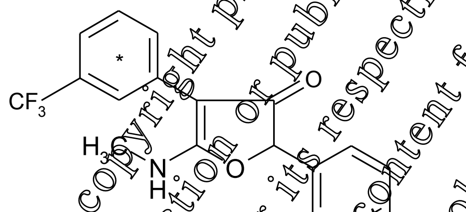
**Test Material**
[Phenyl-UL-$^{14}$C]-Flurtamone (Lot No. 870424) was received from .
The specific activity was 6.2 MBq/mg (168µCi/mg).

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.




[Phenyl-UL-$^{14}$C]-flurtamone

Trifluoromethyl-phenyl-UL-$^{14}$C]-Flurtamone (Lot No. CSL-87-142-07-09) was received from [redacted] The specific activity was 2.9 MBq/mg (78.3 µCi/mg).




[Trifluoromethyl-phenyl-UL-$^{14}$C]-flurtamone

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Labeled material was purified by column chromatography using Kieseigel with chloroform-tetrahydrofuran (15:1) as elution solvent. Radioactive bands were collected and radiochemical purity was determined by thin-layer cochromatography (TLC). After development of the plates the location of the $^{14}$C-flurtamone spot was detected by autoradiography. The purity of [phenyl-UL-$^{14}$C]-flurtamone was 99.2% and the purity of [trifluoromethyl-phenyl-UL-$^{14}$C]-flurtamone was 97.6% by TLC determination. Authentic standards were synthesized by Chevron Chemical Company or purchased.

**Soil Characteristics:** Sandy loam soil pH 6.9; 1.3% organic matter; .
**Culture plant:** Peanut variety: Hakucho Early

**Study Design**

Plant Treatment and Harvest

Ten peanut plants were planted in five 1 X 2 flats. The soil in the flats was treated once with $^{14}$C-flurtamone (dissolved in acetone solution), when peanut plants were at two leaves stage (BBCH 12). The application rate was approximately 1.12 kg/ha based on the surface area of the planting flats. Two untreated control plants were grown in the same greenhouse. The greenhouse temperature was regulated between 18°C to 29°C and the plants were watered twice to three times a day. Plants were harvested at maturity with a PHI of 120-121 days. At harvest, plants were separated into leaves, stems, kernels and hulls fractions. Fractions were pooled from ten plants. All samples were stored in a freezer (at -20°C), processed and extracted within four weeks after harvest.

**Analytical Methods**

Determination of Radioactivity

Determination of radioactivity was accomplished by liquid scintillation counting (LSC) using Liquiscint cocktail.
The non-extractable plant residues were combusted in triplicate with an oxidizer prior to liquid scintillation counting. The liberated $^{14}CO_2$ was absorbed in 8 ml of Carbamate and 12 ml of Permafluor.

Extraction
All fractions of harvested plants were pulverized with dry ice in a blender and aliquots were combusted in triplicate to determine total radioactive residues. All pulverized fractions were extracted twice with acetone, twice with methanol and twice with methanol-water (1:1, v/v). Kernels were extracted twice with hexane prior to the solvent extractions sequence described above. All solvent extractions were performed using a Polytron homogenizer. Solvent extracts were separated from the solid residue by centrifugation. None-extractable residues were combusted for $^{14}C$ determination.

Enzyme Hydrolysis
Polar and/or conjugated metabolites were treated with 5 mg of cellulase in 0.1 M acetate buffer (pH=4.6). The samples were incubated with constant shaking at 25°C for 4 hours. After enzyme incubation, samples were acidified with 6 N HCL to pH 1, saturated with ammonium sulfate, and extracted twice with methylene chloride to recover free metabolites. The extracts were analyzed by the chromatographic methods described earlier.

Acid and Base Hydrolysis
For acid or base hydrolysis of metabolites, samples were dissolved in 1 N HCl or 1 N NaOH in sealed ampules are heated for 1 hour at 100 °C. Base hydrolysates were acidified with conc. HCl and subsequently extracted with diethyl ether. The solutions were saturated with ammonium sulfate and again extracted with dichloromethane to recover apolar metabolites. Both extracts were analyzed by chromatographic methods.

Nonextractable Residues
Nonextractable residues were separated into subfractions by treatment with 1 N HCl (at 100°C for 2 hours) followed by 20% NaOH hydrolysis (at 100°C for 24 hours). Both acid and base hydrolysates were saturated with ammonium sulfate and then extracted with methylene chloride. The remaining bound residue was combusted.

Metabolite Isolation and Characterization
Flurtamone and its metabolites, extracted with acetone were characterized by HPLC cochromatography with authentic standards and confirmed by TLC chromatography. The polar and/or conjugated metabolites were characterized by acid, base and enzymic hydrolysis. Samples were cleaned up on silica gel 60 H column chromatography and eluted with methylene chloride first until chlorophyll had been removed. The column was then eluted with methanol to quantitatively recover the radioactive residue. The polar metabolites were subjected to enzyme, acid and base hydrolysis. Aglycones released from these treatments were characterized by HPLC and TLC cochromatography.
Mass spectral analysis was performed using a Finnigan Model *9611* GC interfaced with a Finnigan Model 4500 quadrupole mass spectrometer. Samples for Nuclear Magnetic Resonance Spectroscopy

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

(NMR) were dissolved in deuterated methanol and spectra were taken using Varian XL-200 spectrometer.

## II Results and Discussion

Total radioactive residues were calculated as flurtamone equivalents. The highest radioactive residues in treated plants were found in leaves, in decreasing order followed by stem, hulls and kernel. Control plants from both label treatments showed significant amounts of radioactivity. Kernels and hulls, with PHI of 120-121 days, contained approximately the same radioactive concentrations as the control plants. Table 6.2.1-8 shows radioactive concentrations and distribution in peanut plants.

Table 6.2.1-8: Radioactive concentration in different parts of peanut plants of [phenyl-UL-$^{14}C$]-and [trifluoromethylphenyl-UL-$^{14}C$]-flurtamone

| Matrix | [Phenyl-UL-$^{14}C$]-flurtamone | | | | [Trifluoromethyl-phenly-UL-$^{14}C$]-flurtamone | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | Treated | | Control | | Treated | |
| | [%] | [ppm] | [%] | [ppm] | [%] | [ppm] | [%] | [ppm] |
| Leaves | 58.6 | 0.32 | 65.8 | 1.32 | 67.4 | 0.24 | 85.3 | 5.81 |
| Stems | 23.3 | 0.36 | 30.2 | 0.88 | 27.2 | 0.16 | 13.6 | 1.51 |
| Kernels | 12.2 | 0.61 | 1.8 | 0.54 | 3.7 | 0.22 | 0.4 | 0.42 |
| Hulls | 5.9 | 0.63 | 2.2 | 0.63 | 1.7 | 0.27 | 0.7 | 0.95 |
| Total | 100 | 1.92 | 100 | 3.37 | 100 | 0.89 | 100 | 8,69 |

<u>Metabolite identification</u>
Since more than 96% of the total radioactive residue in treated plants was found in the leaves and the stems fractions, they were analyzed by TLC for metabolite characterization.
Most metabolites from the trifluoromethyl-phenyl labeled study in these extracts were very polar and consequently were hydrolyzed by acid and base. Base hydrolysis did not reveal any information. In the acid hydrolysis study, the major compound released was the flurtamone-trifluoromethyl-mandelamide. Flurtamone-trifluoromethyl-mandelic acid was also observed in these hydrolysates. There were also some other metabolites released by the acid treatment, however, their identity could not be identified.
Much lower radioactive residue concentrations were detected with the phenyl label study. Comparison of the TLC results of the acetone extracts from both labels showed a similar metabolite pattern. One of the metabolite was isolated and purified by preparative TLC technique. The purified metabolite was then subjected to MS analysis and appeared to have a molecular weight of 171g. No additional metabolite could be identified from the metabolism of phenyl labeled flurtamone.

<u>Peanut Oil</u>
TRR concentrations in treated kernel and hulls were approximately the same as of the corresponding control plants. Thus, the radioactivity in kernel and hulls was most likely the result of $^{14}CO_2$ incorporation into natural plant components. The peanuts were, like in commercial procedures, extracted with hexane. The extracts were evaporated to dryness and were refluxed in 1 N KOH solution for *2* hours for cleave of the ester linkage. Fatty acids were acidified to pH 1, extracted with

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

petroleum ether and subsequently esterified with boron trifluoride / methanol. The fatty acid methyl ester mixture was analyzed by GC/MS and by HPLC. GC/MS analysis showed that the major components were oleic, stearic, palmitic, and linoleic acids. HPLC analysis showed that these fatty acid contained most of the radioactivity in the oil, thus the observed radioactive residues are the result of $^{14}C$ incorporation into fatty acid chains of triglycerides.

Bound Residues

Bound residues were hydrolyzed by 1 N HC1 followed by 20% NaOH. This process separated the nonextractable fraction into carbohydrates, proteins and lignin pools. From both (acid and base) hydrolysates only low levels of radioactivity could be partitioned into organic solvent even after ammonium sulfate saturation, indicating low levels of apolar radioactivity in these hydrolysates. These bound residues were suggested to be the result of small metabolites incorporated into the plant matrix and/or plant constituents.

**III Conclusion**

Plant metabolism of [$^{14}C$]-flurtamone was studied on peanut plants as a pre-emergence soil treatment with [phenyl-UL-$^{14}C$]- and [$^{14}C$-trifluoromethyl-phenyl]-flurtamone. Very little or no parent compound remains in mature plants. With the trifluoromethyl-phenyl label the major residues from pre-emergence use of flurtamone in peanut plants were flurtamone-trifluoromethyl-N-methyl-mandelamide conjugates. Lower residues were found from the phenyl moiety of flurtamone in peanut plants. Nevertheless a significant amount of radioactivity was transferred to the control plants. The observed products comply with with metabolic routs observed in other plants.

**Summary of the metabolism of flurtamone in plants**

Metabolism of flurtamone was investigated in cereals (wheat and barley), and oilseed (sunflower and peanut) following application of [phenyl-UL-$^{14}C$]- or [$^{14}C$-trifluoromethylphenyl]-flurtamone. Application was conducted either as a pre-emergent or a post-emergent spray.

The most recent studies on wheat (cf. KCA 6.2.1 /02; [redacted] 2012; M-440949-01), and on sunflower (cf. KCA 6.2.1 /01; [redacted].; 2010; M-397170-01) show a conclusive picture on the metabolic behaviour of flurtamone. Translocation of flurtamone and its metabolites was limited so that the residue levels in cereal grains and sunflower seeds were low, with only little differences between pre and post-emergence treatments. The measured levels of the total radioactive residues in wheat grain ranged from 0.196 mg/kg to 0.362 mg/kg for application rates of 350 g/ha (post-emergence) and 301 g/ha (pre-emergence) respectively. These levels suggested that total residues at the maximum use rate of 125 g/ha would be no more than about 0.07 mg/kg to 0.15 mg/kg flurtamone equivalents. Over 90% of the TRR could be solubilized and the single metabolite determined in wheat grain was trifluoro acetic acid (TFA) accounting for 86% - 93% of the radioactive residue in wheat grain. Levels in sunflower seeds were lower than those in cereal grains accounting for 0.07 mg/kg (0.105 mg/kg with double rate) for an application rate of 374 g a.s./ha. The residue in sunflower seeds also had a significant portion of extractable residues (66%) predominantly identified as natural products (linoleic and oleic acid glycerides).

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

Levels in wheat straw ranged from 2.3 mg/kg to 16.6 mg/kg for application rates of 301 g/ha (pre-emergence) and 350 g/ha (post-emergence) respectively. Calculated down to the maximum use rate of 125 g/ha total residue levels would be expected to be of the order of 0.95 – 5.9 mg/kg clearly overestimating the levels present in treated in normal agricultural practice because of the artificial growing conditions in the metabolism studies (e.g. greenhouse or vegetation area, protected from rainfall). The predominant component of the residue in pre-emergance straw was identified to be TFA (49%), with post-emergence uses the main residue component would be parent compound (57.5%).
Sunflower forage (not used for feed) had similar residue levels (0.05 mg/kg) than the seeds from the same treatment. Also here the major identified component of the residue was parent compound. It was mainly accompanied by glycoside conjugates of the flurtamone trifluormethyl hydroxy metabolite.
There was a high degree of commonality in the metabolic pathways of the cereals and sunflowers that resulted in the same principal residue components. As with rat and livestock metabolism, the metabolism of flurtamone in plants included aromatic ring hydroxylation (in plants followed by conjugation with sugar and malonic acid), N-demethylation and hydrolytic/oxidative opening of the furanone ring.

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

Figure 6.2.1-3: Common metabolic pathway of flurtamone in plant (wheat, barley and sunflower/peanuts)

Flurtamone-butyrolactone

TFA

fatty acid glycerides

glucoside and malonyl-glucoside conjugates

Flurtamone-trifluoromethyl-hydroxy

Flurtamone

Flurtamone-seco-trifluoromethylbenzyl-hydroxy

Flurtamone-desmethyl-trifluoromethyl-hydroxy

Flurtamone-hydroxy

Flurtamone-trifluoromethyl-N-methyl-mandelamide

Flurtamone-desmethyl

Flurtamone-desmethyl-hydroxy

Flurtamone-trifluoromethyl-mandelic acid

Flurtamone-trifluoromethyl-mandelamide

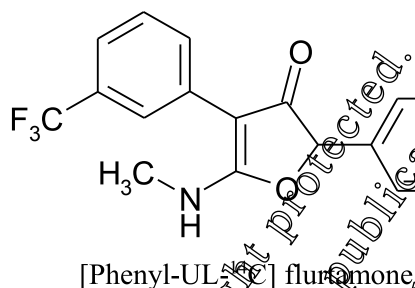
Flurtamone-trifluoromethyl-N-methyl-phenylacetamide

Flurtamone-TFMBA

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.2.2 Poultry

The previously available hen metabolism study (Norris, 1996) was conducted using flurtamone uniformly radiolabelled in the trifluoromethylphenyl ring and investigated the residues in the hen as a model for poultry. In the current study the metabolism and excretion of [phenyl-UL-$^{14}$C] flurtamone in laying hens were investigated to complete the metabolic pattern (* denotes the label position).




[Phenyl-UL-$^{14}$C] flurtamone

| **Report:** | **KCA 6.2.2/01, Authors: [redacted] ., 2013** |
|---|---|
| Title: | Amendment no. 1 to [Phenyl-UL-$^{14}$C]flurtamone: Metabolism in the Laying Hen |
| Report No & Document No | ENSa-12-0166 Date: 26.02.2013<br>M-448149-02-1 |
| **Guidelines:** | OECD-Guideline for the Testing of Chemicals No. 503, Metabolism in Livestock<br>US-EPA, Residue Chemistry OCSPP 860.1300;<br>EU Regulation 1107/2009 |
| **GLP** | Yes |
| Testing Facility and Dates | Bayer CropScience AG, Development – Environmental Safety – Testing, Monheim, Germany<br>Experimental work: 11.01.2011 – 09.03.2012 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Summary**

Six hens were orally dosed once daily in the morning for 14 consecutive days with an aqueous 0.5% Tragacanth® suspension of 1.01 mg/kg body weight which corresponded to 16.67 mg a.s./kg dry feed/day. The animals were sacrificed at about six hours after the last administration. Total radioactive residues (TRR) were determined daily in the eggs and excreta, and at sacrifice in the dissected organs and tissues (muscle, fat, liver, kidney, skin and eggs from ovary/oviduct). Eggs, muscle, fat, liver and excreta were extracted and analysed for parent compound and metabolites.
The overall recovery rate was of the total dose 90.06%. The remaining radioactivity was expected to still be present in the gastro-intestinal tract at sacrifice. Until sacrifice, the excretion of radioactivity accounted for 89.31% of the total dose. On average, only 0.27% of the total dose was measured in the eggs and about only 0.47% of the total dose was recovered in organs and tissues.

The concentration of radioactivity in eggs ranged from 0.022 mg/kg at day one to 0.143 mg/kg at sacrifice. Following a linear increase, a plateau level of about 0.1 mg/kg was reached approx. eight days after the first administration.

In the organs and tissues, the highest radioactivity concentrations were determined in liver (0.953 mg/kg) and kidney (0.511 mg/kg) indicating the significance of these organs for excretion and metabolism. These values corresponded to 0.16% and 0.02% of the total dose, respectively. The residue levels of liver and kidney were followed in decreasing order by those found in the skin (0.103 mg/kg), subcutaneous fat (0.095 mg/kg) and muscle (0.045 mg/kg).
Flurtamone was moderately metabolised into major and several minor and trace metabolites. The most prominent constituents of the residues were parent compound (between 1.2% and 43.7% of the TRR) and the metabolite flurtamone-desmethyl (between 7.0% and 45.7% of the TRR). Another major metabolite was flurtamone-mandelic acid in muscle (10.8% of TRR), which was also identified as a minor residue (< 10% of the TRR) in eggs and liver.
Flurtamone-trifluoromethyl-hydroxy-gluA and flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy and flurtamone-dihydroxy -pyrrolinone were identified as minor metabolites.
The identification rate was approx. 47% in eggs, 39% in muscle, 94% in fat and 37% in liver.
The metabolic profile of excreta was similar to those of the eggs, the organs, and tissues but higher proportions of the metabolites flurtamone-desmethyl-trifluoromethyl-hydroxy and flurtamone-trifluoromethyl- hydroxy were detected. The main metabolic reactions in the laying hen follow known metabolic pathways like hydroxylation of the trifluoromethyl-phenyl moiety followed by conjugation with glucuronic acid, hydroxylation and rearrangement of the furanone to a pyrrolinone moiety, oxidative ring opening of the furanone moiety followed by cleavage and degradation of the carbon chain and N-demethylation.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## I. Materials and Methods

**Test Material:**

| | |
|---|---|
| IUPAC Name | 5-amino-4-[4-hydroxy-3-(trifluoromethyl)phenyl]-2-phenylfuran-3(2H)-one |
| Code name | AE B107587 (RE 40885; RPA 590515) |
| Common name | Flurtamone |
| Empirical formula | $C_{18}H_{14}F_3NO_2$ |
| Molar mass | 333.3 g/mol |
| Labelling position | [Phenyl-UL-$^{14}$C] |
| Specific radioactivity | 4.37 MBq/mg = 117.3 µCi/mg (delivered sample before radiodilution)<br>3.50 MBq/mg = 2.10 x $10^8$ dpm/mg = 94.59 µCi/mg = 31.53 Ci/mol (sample after radiodilution) |
| Radiochemical purity | > 99 % by radio-HPLC and > 99 % by radio-TLC |
| Nonradioactive test substance | Batch AE B107587 00 1B99 0001 |
| Chemical purity | 99.5% |
| Dose level | 14 oral doses of 1.01 mg/kg bw/day by gavage |
| Vehicle | 0.5 % aqueous Tragacanth® suspension |

**2. Test Animals**

| Species | Laying hen (*Gallus gallus domesticus*) |
|---|---|
| Strain | "White Leghorn" |
| Breeding facility | |
| Sex and numbers involved | 6 hens out of 12 animals were chosen for the test. The hens were selected by maximum egg production. |
| Age | about 22 weeks |
| Body weight | 1.36 at delivery, 1.55 kg at first administration, 1.55 kg at sacrifice |
| Acclimatization | 14 days |
| Identification | Cage labelling and wing tags |
| Housing | During the acclimation period, the hens were kept individually in electropolished stainless wire cages for poultry which allowed the collection of excreta and eggs (supplier: ). During the whole testing period, the hens were kept individually in stainless steel metabolism cages for laying hens allowing almost quantitative collection of eggs and excreta (supplier: ). Room temperature 22 - 30°C, relative humidity 30 - 57%. 16 h light / 8 h dark cycle, air change 10 – 15 times per hour |
| Feed and water | Hens were fed with "RWZ-Legegold Mehl", a pulverised chicken feed. This feed was not a certified diet, i.e. it was not checked for contamination according to current standards. The feed was supplemented by eggshells and crushed marine shells during the acclimation period. The feed consumption was recorded by back-weighing during the experiment (mean consumption 96 g/day/hen), tap water, ad libitum |

**Study Design**

Dosing

In order to reduce the specific radioactivity from 4.34 MBq/mg to 3.5 MBq/mg the radiolabelled test compound was mixed with the non-radiolabelled test compound. For the 14 administrations, 4 dosing suspensions in 0.5% aqueous Tragacanth® were prepared and each suspension was applied for three to four administrations. The radiolabelled test substance was proved to be stable until the last dose.

The oral administration was carried out with a knob cannula attached to a glass syringe. Immediately after dosage, the swallowing reflex was supported by a gentle massage of the throat towards the crop. The hens received a mean dose of 1.57 mg ($3.30 \times 10^8$ dpm) per animal and day. At a mean body weight of 1.55 kg this is corresponding to an actual dose of 1.01 mg a.s./kg bw. The amount of radioactivity of the actually administered amounts served as reference for the calculation of total radioactivity in the biological samples. The administration volume was 1.58 mL/kg body weight. The dose level was tolerated without any observable toxicological symptoms.

Sampling of eggs and excreta

During the acclimatisation and test period the egg production was 113% and 120% of the target value (324 per year) respectively. During the test, the cages were inspected for egg production once daily and the number of eggs was recorded for all hens. After sampling, the eggshells were discarded, and

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

the content of each egg were weighed and then thoroughly mixed. An aliquot of each homogenate was mixed with scintillator for radioactivity measurement by LSC. The remaining samples were stored at ca. – 18°C until metabolite analysis.

The excreta of each hen were collected once daily from the collecting tins as quantitatively as possible. The individual samples were homogenised after the addition of water, before recording the total weights. An aliquot of each fraction was processed for radioactivity measurement by combustion/LSC. The remaining samples were stored in a freezer until metabolite analysis.

Sacrifice and sampling of organs and tissues

The treated hens were weighed and sacrificed ca. 6 hours after the last (14$^{th}$) dosage. The animals were anaesthetised using carbon dioxide, sacrificed by decapitation and exsanguinated. The organs and tissues were prepared immediately after sacrifice. Liver without the gall bladder, kidneys, leg and thorax muscle, skin without subcutaneous fat, subcutaneous fat and eggs from the ovary and oviduct were sampled immediately after sacrifice and their fresh weights were recorded. The gall bladders were punctured for the collection of the bile fluid which was then stored frozen for an optional metabolite analysis.

Sample preparation

After dissection, the organs or tissue samples were transferred into ice-cooled vessels. Skin (without subcutaneous fat), liver, kidneys, muscle samples and subcutaneous fat as well as eggs dissected from the ovary and oviduct were. To prepare representative samples per matrix, corresponding samples of the individual animals were pooled before extraction. Egg pools were prepared from day 2 to sacrifice (day 13). Composite samples of muscle (leg and thorax), subcutaneous fat and liver were prepared and excreta were pooled over day 1 to day 13 for all animals. The individual samples were thoroughly homogenised and an aliquot of each resulting tissue pulp was weighed, and prepared for radioactivity measurement using combustion/LSC. All samples were divided into equal portions and stored at ca. -18°C until metabolite analysis. For skin and the homogenates of kidneys and eggs from the ovary/oviduct the TRR was low and therefore no metabolite analysis was performed.

The total radioactive residue of each pool was determined by combustion/LSC (solid samples) or directly by LSC (e.g. combined eggs and extracts). Eggs, muscle, liver and excreta were extracted three times with acetonitrile/water, 8:2 (v/v) and finally with acetonitrile/water (1/1; v/v). The conventional extracts were combined, except for samples with low amounts of radioactivity and/or high matrix contaminations. Remaining solids of liver were exhaustively extracted twice with acetonitrile/water (1:1; v/v) under microwave assistance, followed by acidic extraction with 1 N hydrochloric acid at ambient temperature and then with 6N hydrochloric acid at 100 °C. The combined conventional extracts were subjected to a clean-up step using an SPE-cartridge. All extracts were used for quantification of parent compound and metabolites by HPLC.

Radioactivity measurement

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

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

LSC using aliquots (1 - 5 replicates). Quenching effects were automatically corrected using an external standard and a quenching library. The instrument background was automatically subtracted. The amounts of radioactivity found in the excreta and in the organs and tissues at sacrifice were calculated from the radioactivity concentrations expressed in terms of "administered radioactivity". The percentage amounts in the organs were obtained by multiplication of the respective dose normalized concentrations with the corresponding percentage wet weight contribution to the total body weight of the animal. For all samples, the limit of detection (LOD) was established at approximately 20 dpm per aliquot after instrument background correction. The limit of quantification (LOQ) was established as 2 times the background radioactivity (dpm) of each instrument/method.

Metabolite analysis

The prepared extracts were subjected to HPLC using a reversed phase column (C18) and the eluting solvents water/formic acid 99:1 (v/v) and acetonitrile/formic acid 99:1 (v/v) in the gradient mode. Detection was performed by a UV- (254 nm) and a radioisotope detector with a solid glass scintillator. In order to check the completeness of the elution, the extracts of egg, muscle, fat, liver and excreta were injected, re-collected, and radioassayed by LSC. The chromatographic recovery was at least 95.2%. The recovery of the DCM/MeOH fraction after SPE of the conventional liver extract was only 30.8%. However, this fraction represented only 3.2% of the TRR and therefore its contribution to the overall recovery in liver extracts was not significant. Therefore it was concluded that this HPLC method was valid for establishing metabolites profiles, since no significant radioactivity remained on the HPLC column. Radiolabelled and non-labelled reference compounds were used in co-chromatography for identification of metabolites.

Remaining solids after conventional extraction of liver were exhaustively extracted with acetonitrile/water (1/1; v/v) using microwave assistance, followed by acidic extraction with hydrochloric acid. The combined conventional extracts and the exhaustive extracts were purified using solid phase extraction (SPE). The purified conventional, microwave and hydrochloric acid extracts were concentrated and subsequently quantified by HPLC.

As a second chromatographic method, one dimensional thin layer chromatography (TLC) was employed on pre-layered, glass-backed TLC plates and radioluminography for detection of radioactive spots. As a solvent system a mixture of dichloromethane/methanol/aqueous ammonia solution (25%) (40:10:2, v/v/v) was used and the plates were developed over a distance of about 16 cm.

The electrospray ionisation mass spectra (ESI) were obtained with a TSQ 7000 ( .). The HPLC instrument used for chromatography was an Agilent HP1100 ( ). The flow from the HPLC column was split between UV-detector followed by a radioactivity detector ( ) and MS spectrometer. 1H-NMR spectra were obtained using a 600 MHz NMR-spectrometer ( ).

Radiolabelled reference compounds were isolated in a metabolism study in lactating goat ( , 2013). The non-radiolabelled reference compounds were provided by .

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

**Storage Stability of Residues**

During the study, all samples and extracts were stored below -18 °C or for a short time in a refrigerator. All egg samples and edible organs or tissues were extracted within five to eight weeks after sample collection. Quantitative analysis by HPLC was performed within two to four days after the start of extraction within one day after the last clean up step. Therefore, investigations on storage stability of the residues in the samples were not necessary.

Comparison of the HPLC chromatograms recorded at different times during the study showed that the profiles of the extracts did not significantly change during the analytical work up to a period of at least approx. four months. Hence, it can be concluded that the metabolic profiles represent the residues in the analysed matrices and samples at sacrifice or sampling.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## II Results and Discussion

**Recovery and Elimination of Radioactivity**

The recovery of radioactivity in laying hens after administration of an average daily dose of 1.01 mg [phenyl-UL$^{14}$C] flurtamone per kg bw/day on 14 consecutive days is presented in Table 6.2.2-1. The overall recovery accounted for 90.06% of the totally administered dose. The remaining amount of radioactivity (approx. 10%) was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice.

Table 6.2.2-1: Distribution of residues in eggs, muscle, fat, liver and kidney of laying hens following oral administration of 14 daily doses of [phenyl-UL$^{14}$C] flurtamone at a dose rate of 1.01 mg/kg

| Sample | Percent of total dose administered | Concentration of total radioactivity [mg/kg] |
|---|---|---|
| Liver | 0.16 | 0.953 |
| Kidney | 0.02 | 0.511 |
| Eggs from ovary/oviduct | 0.05 | 0.297 |
| Muscle, total | 0.13 | 0.045 |
| Skin, total | 0.03 | 0.103 |
| Fat, total | 0.08 | 0.095 |
| Organs/tissues, total | 0.47 | ----- |
| Eggs | 0.27 | 0.096 |
| Excreta, total | 89.31 | ----- |
| **Total Recovery** | **90.06** | ----- |

**Levels and Time Course of Total Radioactive Residues in Eggs**

The radioactivity levels measured in the egg samples from all animals are presented in Table 6.2.2-2. The concentration in eggs ranged from 0.022 mg/kg at day one to 0.143 mg/kg at sacrifice. The residue level of the eggs collected from the ovary and oviduct at sacrifice (0.297 mg/kg) was by a

factor of 3.1 higher than the mean level of the laid eggs collected at sacrifice (0.096 mg/kg). Following a rather linear increase a plateau level of approx. 0.1 mg/kg was reached at day eight.

Table 6.2.2-2: Time course of total radioactivity in eggs following oral administration of 14 daily doses of [phenyl-UL-$^{14}$C] flurtamone at a dose rate of 1.01 mg/kg

| Time after the first admin. [d] | Admin. no. | Cumulative secretion [% of total dose admin.] (mean) | Concentration of total radioactivity [mg/kg] |
|---|---|---|---|
| 0 | 1 | ----- # | ----- # |
| 1 | 2 | 0.007 | 0.022 |
| 2 | 3 | 0.015 | 0.044 |
| 3 | 4 | 0.027 | 0.058 |
| 4 | 5 | 0.042 | 0.069 |
| 5 | 6 | 0.058 | 0.078 |
| 6 | 7 | 0.077 | 0.090 |
| 7 | 8 | 0.099 | 0.102 |
| 8 | 9 | 0.123 | 0.107 |
| 9 | 10 | 0.147 | 0.111 |
| 10 | 11 | 0.172 | 0.116 |
| 11 | 12 | 0.198 | 0.118 |
| 12 | 13 | 0.223 | 0.114 |
| 13 | 14 | 0.248 | 0.114 |
| 13.25 | --- | 0.274 | 0.143 |

----- # no egg collected

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Total Radioactive Residues in Dissected Organs and Tissues**

The concentration of the total radioactivity in the dissected organs and tissues at sacrifice is shown in Table 6.2.2-1 (last column). The highest concentrations were determined in liver (0.953 mg/kg) and kidney (0.511 mg/kg) reflecting the significance of these organs for metabolism and excretion. In relation to the totally administered dose, these values corresponded to 0.16% and 0.02%, respectively. The residue level of the eggs collected from the ovary and oviduct at sacrifice (0.297 mg/kg) was higher (factor 3.1) than the levels of the laid eggs collected at sacrifice (0.096 mg/kg). The residue levels of liver and kidney were followed in decreasing order by those determined in the skin (0.103 mg/kg), subcutaneous fat (0.095 mg/kg) and muscle (0.045 mg/kg). The residue level of the total skeletal muscle corresponded to about 0.13% of the total dose assuming a 40% contribution to the body weight. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.08% and 0.03% of the totally administered dose.

**Extraction Efficiency of Residues**

Eggs (day 2 to sacrifice), muscle, fat, liver pools as well as excreta (day 13.5) were extracted four times with acetonitrile/water mixtures. In addition, solids of liver were exhaustively extracted with acetonitrile/water (1:1, v/v) under microwave assistance. After purification and concentration, the

resulting extracts represented 84.1% of the total radioactivity for eggs 71.8% for muscle, 96.2% for fat, 50.2% for liver. With regard to the liver exhaustive extraction with microwave assistance, with 1N hydrochloric acid at room temperature and with 6N hydrochloric acid at 100°C the extracts represented 13.6%, 1.8% and 21.1%, respectively. After all extraction procedures 86.7% was extracted from the liver.

After all extraction procedures, the residue levels in the remaining solids of eggs, muscle and fat were below 0.05 mg/kg. From the liver, sufficient extraction was achieved only after a last extraction step under very harsh conditions (6N HCl, 100° C) down to a TRR value close to 10% in the remaining solids.

After clean up, 64.8% to 94.5% of the TRR were recovered from quantitative HPLC analysis.

The radioactivity concentrations in the post-extraction solids amounted to 0.015 mg/kg (16.0%) for eggs (0.013 mg/kg (28.2%) for muscle, 0.004 mg/kg (3.8%) for fat 0.127 mg/kg (13.3%) for liver.

A summary of the extraction efficiency is shown in Table 6.2.2-3.

Table 6.2.2-3: Extraction efficiency of eggs, muscle, fat and liver samples following oral administration of 14 daily doses of [phenyl-UL-$^{14}$C]flurtamone at a dose rate of 1.01 mg/kg

| | **Eggs** | | **Muscle** | | **Fat** | | **Liver** | |
|---|---|---|---|---|---|---|---|---|
| TRR [mg/kg] | 0.096 | | 0.045 | | 0.095 | | 0.953 | |
| | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg |
| Conventional extraction | 84.0 | 0.081 | 71.8 | 0.033 | 96.2 | 0.091 | 50.2 | 0.479 |
| Microvawe extraction | --- | | --- | | --- | | 13.6 | 0.129 |
| 1N HCl extract | --- | | --- | | --- | | 1.8 | 0.017 |
| 6N HCl extract | --- | | --- | | --- | | 21.1 | 0.201 |
| Total extracted | 84.1 | 0.081 | 71.8 | 0.033 | 96.2 | 0.091 | 86.7 | 0.826 |
| Post-extraction solids (PES) | 16.0 | 0.015 | 28.2 | 0.013 | 3.8 | 0.004 | 13.3 | 0.127 |
| Accountability | 100.0 | 0.096 | 100.0 | 0.045 | 100.0 | 0.095 | 100.0 | 0.953 |

Quantification, Identification and Characterisation of Residues

Parent compound was identified by HPLC and TLC-chromatography in eggs and fat and by HPLC co-chromatography in muscle, liver and excreta.

Flurtamone-mandelic acid was identified by HPLC co-chromatography in the extracts of muscle and excreta. Flurtamone- trifluormethyl- hydroxyl-gluA was identified by HPLC co-chromatograhy in the conventional extract of liver (conventional extract and DCM/MeOH fraction) and excreta. Flurtamone- desmehtyl was identified by TLC and HPLC co-chromatography in the exctract of eggs and fat and by HPLC co-chromatography in the extracts of muscle, liver (conventional and microvawe assisted extraction) and excreta.

Following identification of parent compound and metabolites co-chromatography, they were assigned to peaks in other matrices, by comparison of the profiles. The metabolite flurtamone-dihydroxy-pyrrolinone was identified in the extract of muscle, liver and fat by comparison of the metabolic profile with the according HPLC profile from the goat metabolism study also using the phenyl-UL-$^{14}$C - labelled test compound [KCA 6.2.3/01] where these metabolites have been identified with spectroscopic methods.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

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

The distribution of the parent compound and metabolites in eggs, organs and tissues is summarised in Table 6.2.2-4. Identification rates in the different matrices were ca. 47.1% in eggs, 39.1% in muscle, 94.5% in fat and 36.8% in liver. For egg muscle and liver further 27.3%, 25.8% and 46.8% respectively were characterised by their extraction and chromatographic behaviour.

Table 6.2.2-4: Radioactive residues of parent compound and metabolites in eggs and edible organs and tissues of laying hens following oral administration of 14 daily doses of [phenyl-UL-$^{14}C$]flurtamone at a dose rate of 1.01 mg/kg

| | **Eggs** | | **Muscle** | | **Fat** | | **Liver** | |
|---|---|---|---|---|---|---|---|---|
| TRR [mg/kg] = | | 0.096 | | 0.045 | | 0.095 | | 0.953 |
| Compound (flurtamone-) | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg |
| parent compound | 22.1 | 0.021 | 4.8 | 0.002 | 43.7 | 0.041 | 1.2 | 0.012 |
| mandelic acid | 1.8 | 0.002 | 10.8 | 0.005 | --- | --- | 7.5 | 0.071 |
| trifluoromethyl-hydroxy-gluA | 4.1 | 0.004 | --- | --- | --- | --- | 6.2 | 0.059 |
| desmethyl-trifluoromethyl-hydroxy | 2.2 | 0.002 | --- | --- | --- | --- | 3.2 | 0.030 |
| trifluoromethyl-hydroxy | --- | --- | 3.9 | 0.002 | 1.9 | 0.002 | 7.8 | 0.074 |
| Desmethyl | 17.0 | 0.016 | 14.2 | 0.006 | 45.7 | 0.043 | 7.0 | 0.067 |
| dihydroxy-pyrrolinone | --- | --- | 5.4 | 0.002 | 3.2 | 0.003 | 4.0 | 0.038 |
| **Total identified** | 47.1 | 0.045 | 39.1 | 0.018 | 94.5 | 0.089 | 36.8 | 0.351 |
| unknown 1 | --- | --- | --- | --- | --- | --- | 2.4 | 0.023 |
| unknown 2 | --- | --- | 2.2 | 0.001 | --- | --- | --- | --- |
| unknown 3 | --- | --- | 3.3 | 0.001 | --- | --- | --- | --- |
| unknown 4 | 1.4 | 0.001 | 1.5 | 0.001 | --- | --- | --- | --- |
| unknown 5 | --- | --- | --- | --- | --- | --- | 6.6 | 0.062 |
| unknown 6 | --- | --- | --- | --- | --- | --- | 0.8 | 0.007 |
| unknown 7 | --- | --- | --- | --- | --- | --- | 0.2 | 0.002 |
| unknown 8[#] | 3.0 | 0.003 | 3.0 | 0.001 | --- | --- | 22.1 | 0.211 |
| unknown 9 | 1.0 | 0.001 | --- | --- | --- | --- | --- | --- |
| unknown 10 | 1.3 | 0.001 | 1.7 | 0.001 | --- | --- | --- | --- |
| unknown 11 | 4.2 | 0.004 | 2.3 | 0.001 | --- | --- | 4.0 | 0.038 |
| unknown 12 | 3.1 | 0.003 | 2.5 | 0.001 | --- | --- | --- | --- |
| unknown 15* | 2.7 | 0.003 | 7.7 | 0.004 | --- | --- | 4.8 | 0.046 |
| unknown 16 | 1.2 | 0.001 | 1.5 | 0.001 | --- | --- | 1.4 | 0.013 |
| unknown 17 | --- | --- | --- | --- | --- | --- | 0.7 | 0.007 |
| unknown 18 | --- | --- | --- | --- | --- | --- | 3.1 | 0.029 |
| unknown 19** | 8.1 | 0.008 | --- | --- | --- | --- | --- | --- |
| unknown 20 | 1.2 | 0.001 | --- | --- | --- | --- | 0.8 | 0.008 |
| Total characterised | 27.3 | 0.026 | 25.8 | 0.012 | <0.1 | <0.001 | 46.8 | 0.446 |
| Analysed extract(s) | 74.4 | 0.072 | 64.8 | 0.029 | 94.5 | 0.089 | 83.6 | 0.797 |
| Extracts not analysed | 9.6 | 0.009 | 6.9 | 0.003 | 1.7 | 0.002 | 3.1 | 0.029 |
| **Total extracted** | **84.0** | **0.081** | **71.8** | **0.033** | **96.2** | **0.091** | **86.7** | **0.826** |
| Unextractable (PES) | 16.0 | 0.015 | 28.2 | 0.013 | 3.8 | 0.004 | 13.3 | 0.127 |
| Accountability | 100.0 | 0.096 | 100.0 | 0.045 | 100.0 | 0.095 | 100.0 | 0.953 |

* Peak in the muscle extract contains also flurtamone-desmethyl-trifluoromethyl-hydroxy.

** Peak of unknown 19 in egg contains also a minor portion of flurtamone-trifluoromethyl-hydroxy.

[#] group of several minor metabolites

This document is copyright protected. Any distribution, reproduction or publication of this document (requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Metabolites in Eggs

Major metabolites in eggs were the parent compound flurtamone (0.021mg/kg, 22.1%), and flurtamone-desmethyl (0.016 mg/kg, 17.0%). Further metabolites were flurtamone-mandelic acid, flurtamone-trifluoromethyl-hydroxy-gluA and flurtamone-desmethyl-trifluoromethyl-hydroxy. They were detected in low amounts ≤0.010 mg/kg and below 10% of the radioactive residue in eggs. The minor metabolite flurtamone-trifluoromethyl-hydroxy coeluted with the unknown metabolite 19. The quantified region containing both compounds, which amounted to less than 0.010 mg/kg (< 10%) was regarded as characterised. Ten minor metabolites remained unknown (each less than 0.010 mg/kg and < 10%).
In total, 47.1% (0.045 mg/kg) of the radioactive residue in eggs were identified.

Metabolites in Muscle

The main metabolites in muscle were Flutarmone-desmethyl, which amounted to 0.006 mg/kg (14.2%) and flurtamone-mandelic acid (10.8%, 0.005 mg/kg). The parent compound, flurtamone was only a minor component, and was quantified in amount of 0.002 mg/kg (4.8%). Minor identified metabolites were flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy and flurtamone-dihydroxy-pyrrolinone (< 10%, and less than 0.005 mg/kg).

The identified metabolite flurtamone-desmethyl-trifluormethyl-hydroxy co-eluted with the non-identified compound "unknown 15". The quantified region containing both compounds accounted for less than 0.005 mg/kg (< 10%). The region was regarded as characterised. Nine unknown metabolites were quantified in amount below 0.010 mg/kg (< 10%).
In total, 0.018 mg/kg (39.1%) were identified.

Metabolites in Fat

The main metabolites in fat were parent compound flurtamone (0.041 mg/kg, 43.7%), and flurtamone-desmethyl (0.043 mg/kg, 45.7%). The minor metabolites flurtamone-trifluoromethyl-hydroxy and flurtamone-dihydroxy-pyrrolinone were detected in amounts of 0.002 mg/kg (1.9%) and 0.003 mg/kg (3.2%) respectively.
In total, 0.089 mg/kg (94.5%) were identified.

Metabolites in Liver

The extracts from liver contained only minor components (below 10% of the TRR). Parent compound represented only 0.012 mg/kg (1.2%). Identified metabolites in these extracts were Furtamone-mandelic acid, flurtamone- trifluoromethyl-hydroxy-gluA, flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy, flurtamone-desmethyl and flurtamone -dihydroxy-pyrrolinone.
Ten unknown metabolites were detected in then separate peaks. One peak designated with "unknown 8" was a broad region eluting within up to approx.10 min from the column, representing 0.211 mg/kg (22.1%). Concluding from the shape and of the peak it consists of several minor metabolites. In total 0.351 mg/kg (36.8%) were identified.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

The metabolic and excretion behaviour of [phenyl-UC-$^{14}$C]flutarmone in laying hens can be characterised as follows:

- The concentrations of radioactivity in eggs and edible tissues were relatively low if compared to the dose level and the dosing period of 14 days.
- The evaluation of these results should consider the fact that an exaggerated dose level of 16.67 mg/kg feed/day was administered. Furthermore, the significant amount of radioactivity was detected in the excreta and the relatively high radioactivity in kidney and liver at sacrifice approx. six hours after the last administration indicate that the residues are further metabolised and finally eliminated.
- A residue plateau level in whole eggs was reached within the test period approximately at the 8$^{th}$ day after the first administration.
- The main portion of residues (71.8% to 96.2%, depending on the matrix) was efficiently extracted from edible organs and tissues. After clean up, 64.8% to 94.5% of the TRR were recovered for the quantitative HPLC analysis.
- Overall, 36.8% to 94.5% of the TRR in eggs and edible organs and tissued were identified. Major compounds were the parent compound flutarmone and flutarmone desmethyl in eggs and fat and flutarmone-mandelic acid and flutarmone-desmethyl in muscle. In liver, all compounds were below 10% of the TRR. Four minor metabolites were identified.
- Flurtamone-desmethyl-trifluoromethyl-hydroxy and flurtamone-trifluoromethyl-hydroxy were detected as major metabolites in the excreta. Parent compound flutarmone was only a minor compound.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

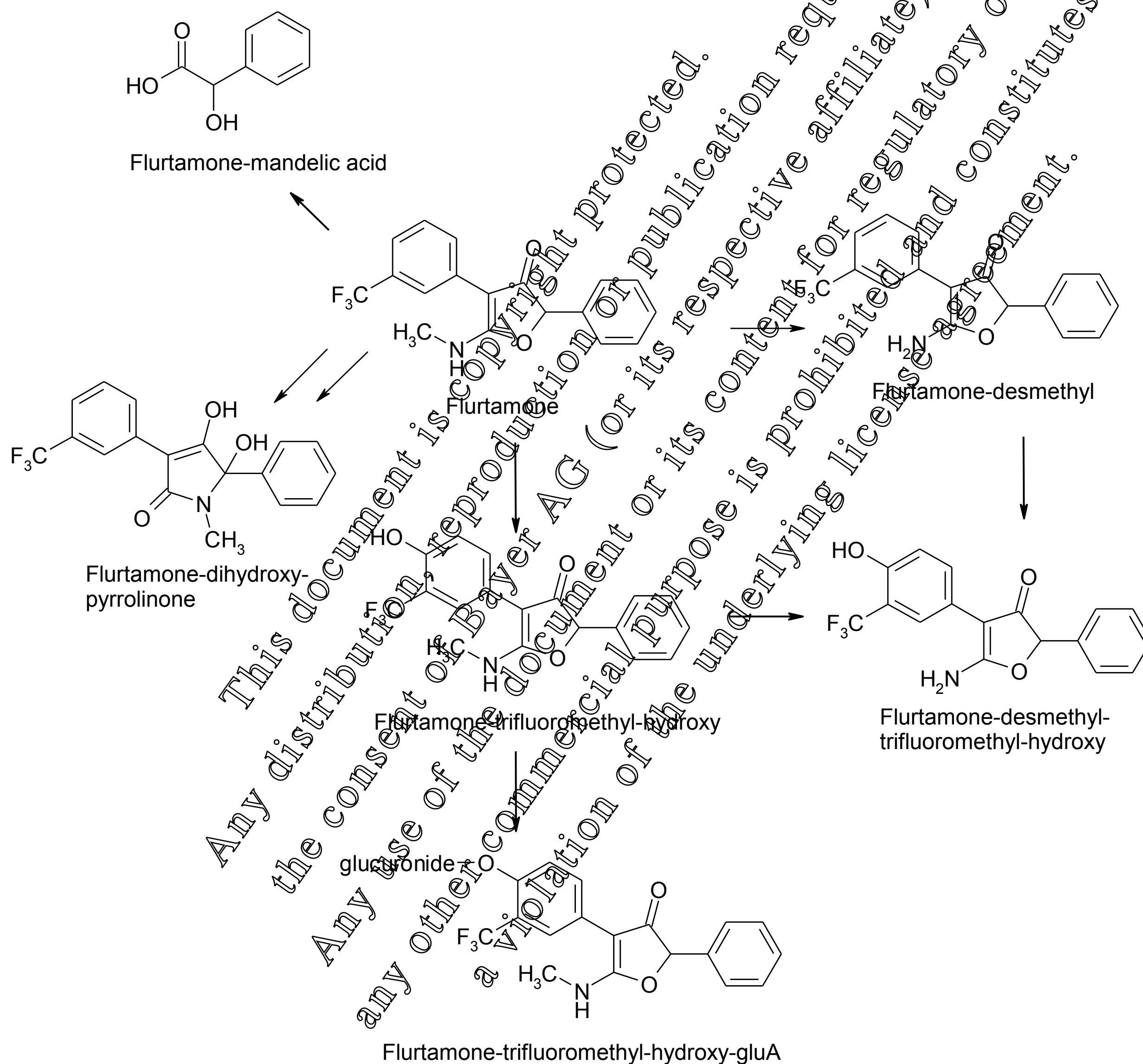
**III Conclusion**

The overall recovery rate was of the total dose 90.06%. Until sacrifice, the excretion of radioactivity accounted for 89.31% of the total dose. On average, only 0.27% of the total dose was measured in the eggs and about only 0.47% of the total dose was recovered in organs and tissues.
Following a linear increase, total radioactive residue levels in eggs reached a plateau level of about 0.1 mg/kg eight days after the first administration.
In the organs and tissues, the highest radioactivity concentrations were determined in liver (0.953 mg/kg) and kidney (0.511 mg/kg corresponding to 0.16% and 0.02% of the total dose, respectively. The residue levels of liver and kidney were followed in decreasing order by those found in the skin (0.103 mg/kg), subcutaneous fat (0.095 mg/kg) and muscle (0.045 mg/kg).
Flurtamone was moderately metabolised into major and several minor and trace metabolites.
The identification rate was approx. 47% in eggs, 39% in muscle, 94% in fat and 37% in liver.
The metabolic profile of excreta was similar to those of the eggs, the organs and tissues.
The main metabolic reactions observed in the laying hen are:

- Hydroxylation of the trifluoromethyl-phenyl moiety, followed by conjugation with glucuronic acid
- Hydroxylation and rearrangement of the furanone to a pyrrolinone moiety
- Oxidative ring opening of the furanone moiety, followed by cleavage and degradation of the carbon chain
- N-demethylation

On the basis of the results of this study it is concluded that the metabolism of [phenyl-14C]flurtamone in hen is well understood and a metabolic pathway of [phenyl-$^{14}$C]flurtamone in the laying hen is proposed (Figure 6.2.2-1).

Figure 6.2.2-1: Proposed metabolic pathway of [phenyl-UL-$^{14}$C]flurtamone in laying hens




This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## Metabolism of trifluoroacetic acid in poultry

| **Report:** | **KCA 6.2.2/01, [redacted], R., 2013** |
|---|---|
| Title: | [1-$^{14}$C]Trifluoroacetic acid - Metabolism in the Laying Hen |
| Document No: | M-463376-01-1 |
| Report No: | EnSa-12-0648 |
| Guidelines and data requirements: | OECD guideline 503, adopted 8-January-2007,<br>US OPPTS guideline 860.1300<br>Compliant with EU Regulation (EC) No. 1107/2009 amended by Commission Regulation (EU) No 283/2013 |
| GLP | Yes |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

### Summary

A metabolism study with $^{14}$C-labelled sodium trifluoroacetate (Na-TFA) was conducted with six laying hens as TFA revealed to be a major metabolite in plants that were treated with flurtamone and are intended as poultry feed. This study is needed for a dietary risk assessment including food of animal origin to address the transfer of TFA residue from feedstuffs to eggs and edible animal tissue.

TFA was orally administered per gavage as $^{14}$C-Na-TFA to the hens for 14 consecutive days with one dose per day. The dose level expressed as trifluoroacetic acid, TFA, was 0.50 mg/kg bw/day corresponding to 7.84 mg TFA/kg dry feed/day.

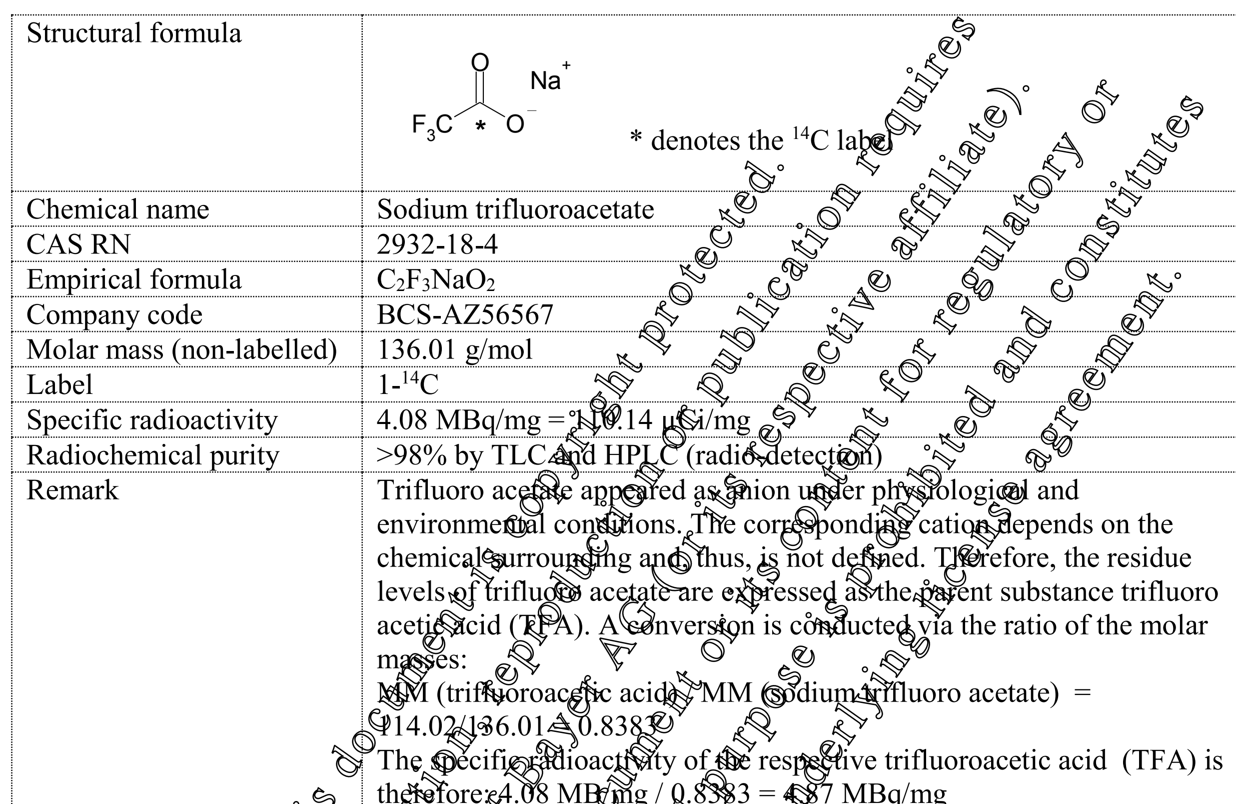
The radioactive residues in eggs reached a residue plateau at Day 7 of dosing amounting to a mean of 0.391 mg TFA equivalents/kg (mg equ/kg) after 8 daily administrations. The birds were slaughtered six hours after the last dose and the radioactive residues were determined in edible organs and tissues. Average residues from six birds accounted for 0.090 mg equ/kg in fat, 0.615 mg equ/kg in muscle, 0.760 mg equ/kg in liver, 1.343 mg equ/kg in kidneys, and 1.101 mg equ/kg in skin.

The samples were extracted with acetonitrile/water and the extracts analysed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic $^{14}$C-TFA reference and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. Therefore, the conclusion has to be drawn that the total radioactivity in eggs, organs and tissues consisted of the unchanged TFA.

By comparison of the residue levels in feed, eggs, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: eggs (at plateau): 0.0499; muscle: 0.0784; fat: 0.0115; liver: 0.0969.

## I Materials and Methods

### Test Material



| Structural formula | $F_3C-{}^{*}C(=O)-O^{-}$ $Na^{+}$ * denotes the $^{14}C$ label |
|---|---|
| Chemical name | Sodium trifluoroacetate |
| CAS RN | 2932-18-4 |
| Empirical formula | $C_2F_3NaO_2$ |
| Company code | BCS-AZ56567 |
| Molar mass (non-labelled) | 136.01 g/mol |
| Label | 1-$^{14}C$ |
| Specific radioactivity | 4.08 MBq/mg = 110.14 µCi/mg |
| Radiochemical purity | >98% by TLC and HPLC (radio-detection) |
| Remark | Trifluoro acetate appeared as anion under physiological and environmental conditions. The corresponding cation depends on the chemical surrounding and, thus, is not defined. Therefore, the residue levels of trifluoro acetate are expressed as the parent substance trifluoro acetic acid (TFA). A conversion is conducted via the ratio of the molar masses:<br>MM (trifluoroacetic acid) / MM (sodium trifluoro acetate) = 114.02/136.01 = 0.8383<br>The specific radioactivity of the respective trifluoroacetic acid (TFA) is therefore: 4.08 MBq/mg / 0.8383 = 4.87 MBq/mg |

This document is copyright protected. Any distribution or reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

### Test Animal

| Species | Hen (*Gallus gallus domesticus*) |
|---|---|
| Breed | White Leghorn |
| Sex, number | Six female laying hen |
| Mean body weight | 1.57 kg at test start (1.45 – 1.65 kg) |
| Age | Approx. 6 months |
| Acclimatization | 14 days before administration |
| Housing | Each 1 bird per stainless steel metabolism cage, approx. 24°C, approx. 31% rel. humidity, 16/8 hours light/dark cycle, 10-15 air changes per hour |
| Identification | Individual animal number using cage cards and wing tags |
| Feed and water | Commercial hen feed supplemented by eggshells and crushed marine shells, *ad libitum*<br>Tap water from local supplier, *ad libitum* |
| Health status | Acceptable according to veterinary investigation |

Preparation of the dosing mixtures and administration

The radiolabelled solid sodium trifluoroacetate was dissolved in water resulting in a concentration of 0.59 mg/mL (corresponding to 0.49 mg TFA/mL). The exact concentration, radiochemical purity and the identity were determined by radioassaying, radio-TLC and LC-MS/MS using small aliquots of the dosing solution. Dosing aliquots of 1.0 mL/kg bw were orally administered by gavage using a syringe

attached to an animal-feeding knob cannula. Directly after dosage, the swallowing reflex was supported by a gentle massage of the throat in direction of the crop. Each bird received one dose per day for 14 consecutive days. The average daily dose was 0.79 mg TFA per bird corresponding to 0.50 mg TFA/kg bw/day. With reference to the daily feed consumption this dose corresponded to 7.84 mg TFA/kg dry feed/day. This dose was tolerated without any observable toxicological effects.

<u>Collection and processing of eggs and excreta</u>

During the test, the grates of the cages were inspected for egg production once daily and the number of eggs was recorded for all hens. The eggs were collected during the 24 hour period after each administration and labelled with animal number and date. After removal of the shells, the contents of each egg were weighed and thoroughly mixed afterwards. An aliquot of each homogenate radioassayed and the remaining samples were stored in a freezer until metabolite analysis.

The excreta of each hen were collected from the collecting tins as far as possible quantitatively in daily intervals until sacrifice. The individual samples were homogenized after adding of water, before the total weights were recorded. An aliquot of each fraction was radioassayed and the remaining samples were stored in a freezer until metabolite analysis.

<u>Sacrifice and collection of organs and tissues</u>

The animals were sacrificed approx. 6 hours after the last dose. Each hen was transferred into a special cage, weighed and anaesthetized using carbon dioxide gas. Under general anesthesia the animals were sacrificed by decapitation followed by exsanguination. The following organs and tissues were dissected: muscle (leg and thorax), fat (subcutaneous), liver (without gall bladder), skin (without subcutaneous fat) kidney and eggs from the ovary as well as oviduct.

The tissue samples were weighed and passed several times through a mincing machine in half-frozen state. The resulting homogeneous pulp was radioassayed and stored frozen (≤ - 18°C) until metabolite analysis.

<u>Radioassaying and processing of samples</u>

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer, the formed $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC. The limit of quantification (LOQ) of radioassaying depended on the specific radioactivity of the test substance, the amount of aliquot measured and the background radioactivity. It was exemplarily given as 0.0005 mg equ/kg.

For metabolism investigations, aliquot samples from eggs, muscle and liver were conventionally extracted with acetonitrile/water (8/2; v/v, 3x) and pure acetonitrile using a high-speed stirrer. Fat was extracted with n-heptane/acetonitrile (9/1; v/v) and acetonitrile/water/n-heptane (7/2/1; v/v/v). The liquid phases were filtrated from the solids. In case of fat, the extracts were separated in an unpolar (n-heptane) and a polar (acetonitrile/water) fraction. The unpolar fraction was again extracted with acetonitrile/water and the polar fraction with n-heptane. The total radioactivity extracted from fat finally partitioned into the combined polar acetonitrile/water phase. The acetonitrile/water extracts

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of this document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

were concentrated and analyzed by radio-HPLC and radio-TLC. The remaining solids were radioassayed via combustion.

Radio-chromatography and mass spectrometry of samples

Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 5 µm particles) that was operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The system was equipped with an UV detector (254 nm) and a radiomonitor with a solid glass scintillator (cell volume 370 µL). The LOQ was derived from background level of the baseline and the highest peak in the chromatogram. It ranged from 0.001 mg equ/kg (muscle and fat extract) to 0.004 mg equ/kg (egg extract). Column recovery was determined by comparison of the eluted radioactivity with column and detector and without column and detector. It generally accounted for 99.7%. $^{14}$C-labelled TFA was co-injected to identify the residues in the samples.

Radio-TLC was conducted on a silica gel TLC plate (20 x 20 cm) that was developed with a solvent mixture of ethyl acetate/2-propanol/water/acetic acid (65/24/22/1, v/v/v/v). Following development the radioactive spots were detected by radioluminography via exposure of an imaging plate for 14 hours. The detection limit was approximately 5-10 dpm/spot after an exposure period of at least 14 hours. $^{14}$C-TFA was also used as reference standard.

The test substance TFA was identified by LC-MS/MS consisting of anion exchange chromatography and a high resolution mass spectrometer. For chromatography an anion exchanging Dionex column was eluted with an aqueous solution of 20 mmol KOH as isocratic liquid phase. A Q-Exactive mass spectrometer was operated in the mode of electro-spray ionization.

Under natural, physiological, and environmental conditions TFA is dissociated and appears as TFA salt. The counter cation depends on the chemical surrounding and is, thus, not defined. Therefore, results are expressed as the parent compound of the salts, i.e. as trifluoroacetic acid.

**II Results and Discussion**

Recovery of radioactivity in eggs, excreta and analyzed organs and tissues

Six hours after the last of 14 oral doses of $^{14}$C-labelled TFA at a dose rate of 0.50 mg/kg bw/day 94.97% of the total radioactivity was recovered in eggs, excreta, muscle, fat, liver and kidney. The remaining 5% of the total dose were assumed to be associated with the gastro-intestinal tract and the remaining body.

88.01% of the total dose was detected in the excreta. 1.91% of the total dose were found in the eggs and 5.06% were detected in the dissected edible organs and tissues with approx. 70% of this radioactivity (3.53% of dose) being associated with the skeletal muscle (assuming 40% of the body weight for skeletal muscle).

Radioactive residues in the eggs

The total radioactive residues (TRR) in the eggs ranged from 0.123 mg equ/kg at day two to 0.408 mg equ/kg at day 13. The time course of the TRR showed a more or less linear increase until day 8 after

This document is copyright protected.
Any distribution or reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

the first dose reaching then a pronounced residue plateau level of 0.391 mg equ/kg between the 7th and 13th day (8th – 14th administration). The residue level of the last egg sample (0.607 mg equ/kg) was excluded from plateau calculation since the interval between dosing and egg collection (0.25 day) was significantly shorter than at the other days. Daily TRR levels in the eggs are compiled in Table 6.2.2-5.

**Table 6.2.2- 5:** Total radioactive residues (TRR) in eggs of hens orally administered with 14C-TFA at a dose of 7.84 mg TFA/kg dry feed/day for 14 consecutive days (mean of 6 hens)

| Time after the 1st administration | No. of administration | TRR in freshly laid eggs | Remark |
|---|---|---|---|
| [days] | | [mg equ/kg] | |
| 0 | 1 | no egg sampled | |
| 1 | 2 | no egg sampled | |
| 2 | 3 | 0.123 | Continuous increase of residue level |
| 3 | 4 | 0.218 | |
| 4 | 5 | 0.262 | |
| 5 | 6 | 0.310 | |
| 6 | 7 | 0.362 | |
| 7 | 8 | 0.396 | Plateau level of residues |
| 8 | 9 | 0.406 | |
| 9 | 10 | 0.410 | |
| 10 | 11 | 0.402 | |
| 11 | 12 | 0.395 | |
| 12 | 13 | 0.405 | |
| 13 | 14 | 0.408 | |
| 13.25 | --- | 0.607 | Short collection period |
| **Weighted mean plateau level of 8th – 14th administration (days 7-13)** | | **0.391** | |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Radioactive residues in dissected organs and tissues

The TRR in edible organs and tissues ranged from fat amounting to 0.090 mg equ/kg to kidney amounting to 1.343 mg equ/kg. Skeletal muscle accounted for 0.615 mg equ/kg and skin for 1.101 mg equ/kg. The residue levels in all edible tissues of hen are compiled in Table 6.2.2- 6.

**Table 6.2.2- 6:** Radioactive residues in organs and tissues of a hens 6 hours after the last of 14 doses of $^{14}$C-TFA at a dose level of 7.84 mg TFA/kg dry feed/day (mean of 6 hens)

| Organ/Tissue | Mean Residue Level [mg equ/kg] |
|---|---|
| Liver | 0.760 |
| Kidney | 1.343 |
| Skeletal muscle, total | 0.615 |
| Leg muscle | 0.712 |
| Thorax muscle | 0.507 |
| Skin without fat | 1.101 |
| Subcutaneous fat | 0.090 |
| Eggs from ovary/oviduct | 0.754 |

This document is copyright protected. Any distribution or reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Extraction efficiency and identification of extracted residues

The majority of the radioactive residues (99.9% - 100% of TRR) in eggs, muscle, liver and excreta (Day 13) was extractable with acetonitrile/water (8/2; v/v) and pure acetonitrile. From fat, 95% of TRR could be extracted with heptane and acetonitrile/water that completely partitioned into the polar phase. Negligible amounts of ≤ 0.1% of the TRR (≤ 0.001 mg/kg) remained unextractable. Following concentration, 99.5% to 100% of the TRR in the extracts were analysed and quantified by radio-HPLC and radio-TLC.

The radio-chromatographic profiles of all extracts (eggs, muscle, liver, kidney, fat, and excreta) showed only one polar radioactive peak. Co-chromatography with the reference standard $^{14}$C-TFA resulted in the same single peak that was unambiguously identified as radiolabelled TFA since two different chromatographic systems (reversed phase HPLC and straight phase TLC) were used. No other peak could be observed. Therefore, the total radioactivity in all samples represented unchanged TFA. Thus, the rate of identification in the samples was excellent amounting to 99.5 – 100% of TRR in all extracts.

Transfer factors of residue transfer of TFA from animal fodder to food of animal origin

The TFA transfer factors (TF) were calculated as mean ratio between the radioactive residues in animal fodder (based on dry mass) and the total radioactive residues in eggs, and edible organs and tissues of the six hens. Any correction for metabolic conversion products of TFA is not needed as total radioactive residue was represented by the administered test substance (see before). These transfer factors ranging from 0.0115 (fat) to 0.1713 (kidney) are listed in detail in Table 6.2.2- 7.

**Table 6.2.2- 7:** Transfer factors for residue transfer of $^{14}$C-TFA from animal feed to eggs, muscle, fat, liver and kidney of hens following repeated administration at a dose level of 7.84 mg TFA/kg dry feed/day

| Milk/Organ/Tissue | Residue level [mg equ/kg] | Transfer factor (TF) |
|---|---|---|
| Eggs (at residue plateau) | 0.391 | 0.0499 |
| Muscle | 0.615 | 0.0784 |
| Fat | 0.090 | 0.0115 |
| Liver | 0.760 | 0.0969 |
| Kidney | 1.343 | 0.1713 |
| Skin | 1.101 | 0.1404 |

## III Conclusion

Following repeated oral administration of $^{14}$C-labelled sodium trifluoroacetat (TFA-Na) to six laying hens for 14 consecutive days at a dose level of 0.50 mg TFA/kg bw/day (corresponding to 7.84 mg TFA/ kg dry feed/day) the radioactive residues in eggs reached a steady state (plateau level) of 0.391 mg equ/kg after 7 daily administrations. 14 days after the first administration the hens were slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 1.101 mg equ/kg in fat, 0.615 mg equ/kg in muscle, 0.760 mg equ/kg in liver and 1.343 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analysed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic $^{14}$C-TFA and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. As a conclusion it can be stated that TFA is metabolically stable in poultry. It was rapidly excreted as not more than 5% of the total dose was detected in organs and tissues 6 hours after the last dose.

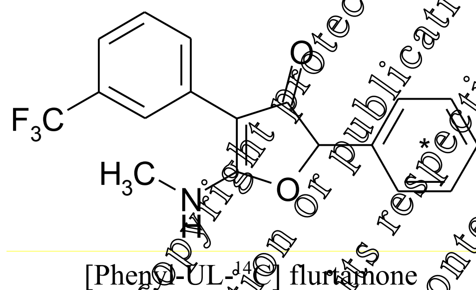
By comparison of the residue levels in feed, eggs, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: eggs (at plateau): 0.0499; muscle: 0.0784; fat: 0.0115; liver: 0.0969; kidney: 0.1713.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.2.3 Lactating ruminants

The previously available goat metabolism study (Norris, 1994) was conducted using flurtamone uniformly radiolabelled in the trifluoromethylphenyl ring and investigated the residues in the lactating goat as a model for ruminants. In the current study the metabolism and excretion of [phenyl-UL-$^{14}$C] flurtamone were investigated to complete the metabolic pattern.

One study on the metabolism of flurtamone in lactating goats was conducted with the test compound labelled in the [phenyl-UL-$^{14}$C]-position as shown by the following structural formula (* denotes the label position):




[Phenyl-UL-$^{14}$C] flurtamone

| **Report:** | **KCA 6.2.3/01, ████████ (2013)** |
|---|---|
| Title: | [Phenyl-UL-$^{14}$C]Flurtamone: Metabolism in the lactating goat |
| Document No: | M-445646-01-1 |
| Report No | EnSa-12-0170 |
| **Guidelines:** | OECD-Guideline for the Testing of Chemicals No. 503: Metabolism in Livestock<br>US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1300<br>Nature of the Residue – Plants, Livestock |
| **GLP:** | Yes |
| Testing Facility and Dates: | Bayer CropScience AG, BCS-D-EnSa-Testing, Monheim, Germany<br>Experimental work: 23.08.2010 – 27.09.2012 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG or its respective affiliate(s). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes violation of the underlying license agreement.

**Summary**

The goat was orally dosed once daily for five consecutive days in the morning after milking with 1.0 mg of the active substance per kg body weight which corresponded to 22.89 mg a.s. /kg dry feed/day. The animal was sacrificed about six hours after the last administration. Total radioactive residues (TRR) were determined in milk and excreta at various sampling intervals, and in muscle, fat, kidney and liver at sacrifice. Milk, edible organs and tissues and faeces as well as urine and bile, were analysed for the unchanged parent compound and metabolites.

The overall recovery amounted to 76.63% of the total dose. The remaining radioactivity (approx. 23%) was expected to be still present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. An amount of 0.12% of the total dose was secreted with the milk. At sacrifice, the residues in the organs and tissues were calculated or estimated to be 1.14% of the total dose from which about 24% was detected in the skeletal muscle (0.27%) and 32% in fatty tissues (0.36%). Until sacrifice, the excretion of radioactivity accounted for 75.37% of the total dose. A portion of 20.58% was found in the urine and 54.79% in the faeces.
The total radioactive residue in milk reached a mean plateau level of about 0.04 mg/kg already at 24 hours after the first administration. In organs and tissues, the highest concentrations were determined in liver (1.179 mg/kg) and kidney (0.371 mg/kg) indicating the significance of these organs for excretion and metabolism. These values corresponded to 0.49% and 0.02% of the total dose, respectively. For muscle and fat 0.047 mg/kg and 0.157 mg/kg, respectively, were determined. The radioactivity concentration of the muscle corresponded to 0.27% and that of fat to 0.36% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.
Flurtamone was moderately metabolised into few major and several minor and trace metabolites. The parent compound was mostly the major component in the milk and analysed organs and tissues, representing 12.1% to 78.0% of the TRR. The two major metabolites flurtamone- trifluoromethyl-hydroxy-gluA and flurtamone-dihydroxy-pyrrolinone were detected at levels of up to 12. 8% and 15.8% of the TRR, respectively. Minor metabolites (< 10% of the TRR) identified were flurtamone-mandelic acid, flurtamone-hippuric acid, flurtamone-trifluoromethyl-hydroxy and flurtamone-desmethyl.
In total 46% to 97.8% of the TRR was identified.

The metabolic profiles of urine showed the same compounds as the organs and tissue samples but higher proportions of metabolites flurtamone-trifluoromethyl-hydroxy-gluA and flurtamone-dihydroxy-pyrrolinone were detected and the amount of parent compound flurtamone was lower. Faeces and bile contained fewer metabolites than urine samples. The major compounds in faeces were parent compound and flurtamone-trifluoromethyl-hydroxy. Bile contained only flurtamone-trifluoromethyl-hydroxy-gluA as major compound. A minor metabolite identified only in excreta was flurtamone-desmethyl-trifluoromethyl-hydroxy. The metabolic reactions of [phenyl-UL-$^{14}$C]flurtamone in the lactating goat followed known routs and included hydroxylation of the trifluoromethyl-phenyl moiety followed by conjugation with glucuronic acid, hydroxylation and rearrangement of the furanone to a pyrrolinone moiety, oxidative ring opening of the furanone moiety followed by cleavage and degradation of the carbon chain and N-demethylation.

## I Materials and Methods

### Test Material

| | |
|---|---|
| IUPAC Name | 5-(methylamino)-2-phenyl-4-[3-(trifluoromethyl)phenyl]furan-3(2H)-one |
| Code name | AE B107587 ( RE 40885; RPA 590515) |
| Common name | Flurtamone |
| Empirical formula | $C_{18}H_{14}F_3NO_2$ |
| Molar mass | 333.3 g/mol |
| Labelling | [phenyl-UL-$^{14}$C] |
| Specific radioactivity used for administration | 4.34 MBq/mg = 117.3 µCi/mg<br>(delivered sample before radiodilution)<br>3.45 MBq/mg = 2.07 x $10^8$ dpm/mg = 93.24 µCi/mg = 31.08 Ci/mol<br>(sample after radiodilution) |
| Radiochemical purity | 99% (HPLC), 99% (TLC) |
| Dose level | 5 daily oral doses of 1 mg/kg bw by gavage |
| Vehicle | Capsule |

### 2. Test Animals

| | |
|---|---|
| Species | Lactating goat (*Capra hircus*) |
| Strain | "Weiße Deutsche Edelziege" |
| Breeding facility | Germany |
| Sex and numbers involved | 1 female animal |
| Age | ca. 18 months |
| Body weight | 48 kg at delivery, 47 kg at first administration, 45 kg at sacrifice |
| Acclimatization | 7 days |
| Identification | skin marking |
| Housing | During acclimatization period: raised stall with a metal grid as base and straw and hay as bedding.<br>During the test period: electro-polished stainless steel metabolism cage for farm animals (goat, sheep, and pig), supplied by Germany. The cage was equipped with a variable-restraining device.<br>Room temperature 20 - 23 °C, relative humidity 54-78%<br>12 h light / 12 h dark cycle, air change 10 – 15 times per hour.<br>The animal was adequately housed according to the German Animal Welfare Act. |
| Feed and water | The goat was fed *ad libitum* with hay, hay pellets, apples and supplementary ruminant feed ("Raiffeisen LammGold", supplied by ).<br>During the test period, the average feed consumption was 2.061 g/day, tap water was offered a*d libitum* |

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

**Study Design**

Preparation of the application capsules

The radiolabelled test compound was delivered with a specific radioactivity of 4.34 MBq/mg. It was diluted with the non-radiolabelled test compound to a specific radioactivity of 3.50 MBq/mg. In total, five gelatine capsules containing the test compound were prepared. They were stored at ≤ -18 °C until administration. The remaining test compound was stored in solid form together with the capsules. An aliquot of this sample was dissolved in 1 mL acetonitrile. 100 µL of this sample were diluted with 400 µL acetonitrile and analysed using HPLC after the last administration in order to demonstrate the stability of the test compound during the administration phase of the study.

Dosing

The test compound was orally dosed once daily for five consecutive days in the morning after milking. The administrations were performed using a capsule applicator. The goat received on each day one gelatine capsule containing on average an amount of 47.19 mg which corresponded to 9,767,295,000 dpm. The totally administered amount and radioactivity accounted for 235.82 mg and 48,814,740,000. Based on the daily feed consumption during the test of 4.39% of the body weight, the dose of 1.0 mg a.s. /kg bw corresponded to 22.89 mg a.s. /kg dry feed per day in the diet.
The total amount of radioactivity administered to the animal served as reference value ($A_0$ = 100%) for the percentage calculation of the total radioactivity in the biological samples.

Sampling of milk, urine and faeces during the in-life phase

The goat was milked in the morning immediately prior to each administration, about 8 hours later in the afternoon, and directly before sacrifice (8, 24, 32, 48, 56, 72, 80, 96 and 102 hours after the first administration). The milk weights were recorded. Afterwards an aliquot was taken from each sample for radioactivity measurement by LSC.
Urine samples were collected, as quantitatively as possible, in plastic vessels under dry ice cooling, in intervals of 24 hours after each administration. The vessels were changed immediately before the next administration. The collection funnel was rinsed with deionised water into the same vessel of the respective collection period. After recording the total volumes, one aliquot was taken from each sample for radioactivity measurement by LSC.
Faeces samples were collected as quantitatively as possible at room temperature in intervals of 24 hours after each administration, i.e. immediately before the next administration. The collecting grid was cleaned prior to each administration. No samples of the rinsing water were taken for radioactivity measurement. Each faeces fraction was homogenized after addition of water to get a wet paste before the total weight was recorded. Aliquots of each wet sample weighed and prepared for radioactivity measurement by combustion/LSC.

Sacrifice and dissection of organs and tissues

The animal was sacrificed approx. 6 hours after the last administration. The animal was anaesthetised by an intravenous dose of about 40 mg/kg bw Pentobarbital-Na (Narcoren®), exsanguinated by cannulating the jugular vein and finally terminated by intracardiac injection with approx. 10 mL of the veterinary drug "T 61®".

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

Following sacrifice, the following organs and tissues were sampled: liver without gall bladder, kidneys, two different types of muscle (round and loin), two different types of fat (perirenal and omental) and gall bladder.

Sample preparation

The organs or tissue samples were weighed and transferred into tare weighted plastic vessels. Liver, kidney, muscle, and fat samples were thoroughly homogenised in half-frozen state for several times in a mincing machine. Each resulting tissue pulp was weighed and aliquots were prepared for radioactivity measurement by combustion/LSC. All samples were divided into portions and stored in a freezer until metabolite analysis. The remaining samples of each organ or tissue were stored at ≤ -18 °C. The gall bladder was punctured for the collection of the bile fluid which was then stored frozen for the metabolite analysis.

For metabolism investigations, pooled samples of morning and evening milk collected 24h (morning) and 8h (evening) after each administration, composite samples of muscle (loin plus round) and fat (omental plus perirenal), and samples of kidneys and liver were prepared. The individual samples were thoroughly homogenised and divided in adequate portions. Faeces were sampled at 24h, 48h, 72h, 96h and 102h after the first administration and approx. 1/600 of the amount of each sample was pooled. All pooled samples were kept frozen until start of extraction.
Aliquots of the pooled samples of morning milk, evening milk, muscle, fat, liver, kidney and faeces were conventionally extracted with acetonitrile/water (8:2; v/v) and finally with acetonitrile/water (1/1; v/v) using a Polytron homogeniser. For each matrix the conventional extracts were combined, except for samples with low amounts of radioactivity and/or high matrix contaminations. Remaining solids after conventional extraction of liver were exhaustively extracted with acetonitrile/water (1/1; v/v) using microwave assistance, followed by acidic extraction with 1N hydrochloric acid at ambient temperature and then with 6N hydrochloric acid at 100°C. The combined conventional extracts, the microwave and the 6 N HCl extract were each subjected to a clean-up step using an SPE-cartridge, the percolates and rinses were concentrated in vacuo and then used for quantification of parent compound and metabolites by HPLC. The 1N hydrochloric acid extract was not analysed by HPLC due to the very low percent of TRR value.

Radioactivity measurement

The measurement of the radioactivity in the liquid samples was carried out by liquid scintillation counting (LSC). Quench correction procedures were used to determine the radioactivity (dpm) from the scintillation counts (cps) in the samples using an external standard. A quantifiable radioactive peak was regarded as relevant having a signal to noise ratio of at least approx. 2.5 (= LOD). The limit of quantification (LOQ) was set at the level of the LOD and was calculated from the average noise-value of the baseline and the height and TRR value of the highest peak in the chromatogram . All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

Metabolite analysis

Samples were analysed by radiochromatographic (HPLC, TLC) and spectroscopic (LC-MS, $^1$H-NMR) methods.

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

HPLC-radioactivity analysis was performed with radiometric- and UV-detection (254 nm). The chromatography for recording of the metabolic profiles was carried out on a reversed phase column using an acidic water/acetonitrile gradient. The chromatograms were recorded electronically and quantitatively evaluated using the software package GINA®.

In order to check the completeness of the elution for the HPLC profile, representative samples of milk, muscle, liver, and kidney extract were injected, re-collected, and radioassayed by LSC. The recoveries were at least 98.6% of the injected radioactivity. Radiolabelled and non-labelled reference compounds were used in co-chromatography for identification of metabolites.

As a second chromatographic method, one dimensional thin layer chromatography (TLC) was employed on pre-layered, glass-backed TLC plates and radioluminography for detection of radioactive spots. As a solvent system a mixture of dichloromethane/methanol/aqueous ammonia solution (25%) (40:10:2, v/v/v) was used and the plates were developed over a distance of about 15 cm.

The electrospray ionisation mass spectra (ESI) were obtained with a TSQ 7000, with a LTQ Orbitrap XL or with a Q-Exative mass spectrometer (Thermo or Finnigan, respectively, San Jose, CA, U.S.A.). The chromatographic conditions for the MS experiments are given in the report. The HPLC instrument used for chromatography was an Agilent HP1100 (Agilent, Waldbronn, Germany). The flow from the HPLC column was split between UV-detector followed by a radioactivity detector (Ramona Star, Raytest, Straubenhardt, Germany) and MS spectrometer. $^{1}H$-NMR spectra were obtained using a 600 MHz NMR-spectrometer (BRUKER AV 600, Bruker Biospin, Karlsruhe, Germany).

Radiolabelled reference compounds were isolated in the course of this study. The non-radiolabelled reference compounds were provided by Bayer CropScience AG, Development-Environmental Safety, Monheim am Rhein, Germany.

**Storage Stability of Residues**

During the study, all samples and extracts were stored at ≤ -18 °C or for a short time in a refrigerator at approx. +4 °C. All samples of milk, and edible organs and tissues were extracted within four to eleven weeks after sample collection or sacrifice. Quantitative analysis by HPLC was performed within two to twenty-two days after the start of extraction; typically within one day after the last clean up step. Hence, investigations on storage stability of the residues in the samples were not necessary and it can be concluded that the metabolic profiles represent the residues in the matrices at sacrifice or sampling.

## II Results and Discussion

Recovery and Elimination of Radioactivity

Recovery data of radioactivity after administration of a mean daily dose of 1.0 mg [phenyl-UL-$^{14}C$] flurtamone per kg body weight on five consecutive days is summarised in Table 6.2.3-1. The overall recovery accounted for 76.63% of the total dose. The remaining radioactivity (ca. 23%) was expected to be still present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. An amount of 0.12% of the total dose was secreted with the milk. At sacrifice, the residues in the organs and tissues dissected from the body were calculated or estimated to be 1.14% of the total dose from which about 24% were detected in skeletal muscle (0.27% of TRR) and 32% in fatty tissues (0.36% of TRR).

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Until sacrifice, the excretion of radioactivity accounted for 75.37% of the total dose. A portion of 20.58% was found in the urine and 54.79% in the faeces. The urinary and faecal excretion started immediately after the first administration. For the urine, a constant level of about 4% - 5% within 24 hours was determined after the second administration. The values for the faeces increased more or less linear until day four at which the last administration was performed.

Table 6.2.3-1: Distribution of residues in milk, muscle, fat, liver and kidney of lactating goats following oral administration of 5 daily doses of [phenyl-UL-$^{14}$C]-flurtamone at a dose rate of 1.0 mg/kg

| Sample | Percent of total dose administered | Concentration of total radioactivity [mg/kg] |
|---|---|---|
| Liver<br>Kidney<br>Muscle, total<br>Fat, total | 0.49<br>0.02<br>0.27<br>0.36 | 1.179<br>0.371<br>0.047<br>0.157 |
| Total of organs/tissues | 1.14 | ---- |
| Milk, 0 – 102 h | 0.12 | 0.034 |
| Urine, 0 – 102 h (plus funnel rinsing)<br>Faeces, 0 – 102 h | 20.58<br>54.79 | ----<br>---- |
| Total excreted | 75.37 | ---- |
| Total Recovery | 76.63 | ---- |

Levels and Time Course of Total Radioactive Residues in Milk

The radioactivity levels and concentrations measured in the milk are presented in Table 6.2.3-2. The concentrations in milk ranged from 0.013 mg/kg to 0.077 mg/kg after the first administration. At sacrifice, a value of 0.057 mg/kg was determined. The concentrations found in the evening and morning milk samples showed a distinct diurnal pattern after the second administration. The radioactive residues increased significantly during the eight hour period after each administration followed by a decrease measured prior to the administration of the next dose. A mean plateau level of about 0.04 mg/kg was reached already at approx. 24 hours after the first administration.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.3-2: Time course of total radioactivity in the milk of lactating goats following oral administration of 5 daily doses of [phenyl-UL-$^{14}$C] flurtamone at a dose rate of 1.0 mg/kg

| Time after the first admin. [h] | Admin. no. | Cumulative secretion [% of total dose admin.] | Secretion per day [% of total dose admin.] | TRR [mg/kg] |
|---|---|---|---|---|
| 0 | 1 | --- | --- | --- |
| 8 | | 0.015 | | 0.045 |
| 24 (PA) | | 0.028 | 0.028 | 0.023 |
| 24 | 2 | --- | --- | --- |
| 32 | | 0.044 | | 0.055 |
| 48 | | 0.057 | 0.029 | 0.024 |
| 48 (PA) | 3 | --- | --- | --- |
| 56 | | 0.068 | | 0.039 |
| 72 (PA) | | 0.075 | 0.019 | 0.013 |
| 72 | 4 | --- | --- | --- |
| 80 | | 0.098 | | 0.077 |
| 96 (PA) | | 0.111 | 0.035 | 0.024 |
| 96 | 5 | --- | --- | --- |
| 102 | | 0.122 | 0.011 | 0.057 |
| **Mean** | **5** | **---** | **---** | **0.034** |

PA = immediately prior to administration

Total Radioactive Residues in the Dissected Organs and Tissues

The concentration of the total radioactivity measured in the dissected organs and tissues collected at sacrifice are presented in Table 6.2.3-1 (last column). The highest concentrations were determined in liver (1.179 mg/kg) and kidney (0.371 mg/kg) indicating the significance of these organs for metabolism and excretion. In relation to the total dose administered, these values corresponded to 0.49% and 0.02%, respectively. For muscle and fat 0.047 mg/kg and 0.157 mg/kg, respectively were determined. The radioactivity concentration of the total body muscle corresponded to 0.27% and that of fat to 0.36% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.

Extraction Efficiency of Residues

Aliquots of morning and evening milk, muscle and fat were pooled. Samples of these pools and of liver and kidney were conventionally extracted four times with acetonitrile/water mixtures. The resulting extracts represented 97.2% of the total radioactivity for morning milk and 98.1% for the evening milk, 93.0% for muscle, 98.4% for fat, 87.4% for kidney and 93.8% for liver. Detected losses amounted to 3% in muscle, 1.7% in liver, and 2.0% in kidney. Unextractable residues amounted to 0.001 mg/kg (2.8%) for morning milk, 0.001 (1.9%) for evening milk, 0.003 mg/kg (7.0%) for muscle, 0.002 mg/kg (1.6%) for fat, 0.077 mg/kg (6.6%) for liver and 0.047 mg/kg (12.6%) for kidney.

After all extraction procedures the residue levels remaining in the solids were below 10% of the TRR or below 0.05 mg/kg.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Quantification, Identification and Characterisation of Residues**

Isolation and Identification of Parent Compound and Metabolites in Excreta

Parent compound was identified by HPLC and TLC co-chromatography in urine (96 h) and faeces.

The metabolites flurtamone-mandelic acid and flurtamone hippuric acid were isolated from urine (96 h) and identified by LC-MS/MS. The compound flurtamone- trifluoromethyl-hydroxy-gluA was identified by LC-MS/MS, and, after enzymatic treatment, by HPLC co-chromatography. Complete cleavage by β-glucuronidase proved β-conjugation with glucuronic acid. Following co-chromatography with the reference compound flurtamone-trifluoromethyl-hydroxy elucidated the position of the hydroxyl group and thereby the site of the conjugation.

The metabolite flurtamone-desmethyl-trifluoromethyl-hydroxy was isolated from extract of faeces and identified by HPLC co-chromatography. Flurtamone-trifluoromethyl-hydroxy was isolated from extract of faeces and identified by LC-MS/MS.

Flurtamone-dihydroxy–pyrrolinone was identified by LC-MS/MS and NMR analysis in urine.

Parent compound was a major component in faeces (19.9% of the dose administered), but only a trace component in urine and not detected in bile.

The predominant metabolites in the urine were flurtamone-trifluoromethyl-hydroxy-gluA and flurtamone-dihydroxy-pyrrolinone. Flurtamone-trifluoromethyl-hydroxy-gluA was also the predominant metabolite in the bile, whereas the exocon flurtamone-trifluoromethyl-hydroxy was the major metabolite in the faeces.

Minor metabolites detected in the excreta were flurtamone-mandelic acid, flurtamone-hippuric acid, flurtamone-desmethyl-trifluoromethyl-hydroxy and flurtamone-desmethyl.

Identification, Assignment and Quantification of Metabolites in Milk, Organs and Tissues

Following identification of parent compound and metabolites by co-chromatography and/or spectroscopic methods, they were assigned to peaks in the other matrices by comparison of the profiles.

A summary of the quantification of parent compound and metabolites in milk, muscle, fat, kidney and liver are presented in Tables 6.2.3-3 + 4.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.3-3: Radioactive residues of parent compound and metabolites in milk of lactating goats following oral administration of 5 daily doses of [phenyl-UL-^{14}C]flurtamone at a dose rate of 1.0 mg/kg

| | **Morning milk** | | **Evening milk** | |
|---|---|---|---|---|
| TRR [mg/kg] | | 0.021 | | 0.054 |
| Compound (flurtamone-) | % TRR | mg/kg | % TRR | mg/kg |
| parent compound | 12.4 | 0.003 | 31.4 | 0.017 |
| mandelic acid | --- | --- | 1.4 | 0.001 |
| hippuric acid | 6.5 | 0.001 | 5.5 | 0.003 |
| trifluoromethyl-hydroxy-gluA | 4.9 | 0.001 | 2.3 | 0.001 |
| trifluoromethyl-hydroxy | 8.7 | 0.002 | 4.9 | 0.003 |
| desmethyl | --- | --- | 3.0 | 0.002 |
| dihydroxy-pyrrolinone | 13.6 | 0.003 | 9.3 | 0.005 |
| **Total identified** | 46.0 | 0.010 | 57.7 | 0.031 |
| unknown 2 | --- | --- | --- | --- |
| unknown 3 | --- | --- | --- | --- |
| unknown 4 | --- | --- | 1.9 | 0.001 |
| unknown 5 | --- | --- | --- | --- |
| unknown 6 | --- | --- | --- | --- |
| unknown 7 | --- | --- | --- | --- |
| unknown 8 | --- | --- | --- | --- |
| unknown 9 | --- | --- | --- | --- |
| unknown 10 | --- | --- | --- | --- |
| unknown 11 | --- | --- | --- | --- |
| unknown 12 | 8.0 | 0.002 | 1.4 | 0.001 |
| unknown 13 | --- | --- | --- | --- |
| unknown 14 | --- | --- | 1.5 | 0.001 |
| unknown 16 | --- | --- | 1.5 | 0.001 |
| unknown 17 | --- | --- | 1.0 | 0.001 |
| unknown 18 | 7.5 | 0.002 | 5.3 | 0.003 |
| unknown 19 | 13.5 | 0.003 | 8.1 | 0.004 |
| unknown 20 | --- | --- | --- | --- |
| unknowns 21 a-c | 22.3 | 0.005 | 17.8 | 0.010 |
| *unknown 21 a* | *10.9* | *0.002* | *8.7* | *0.005* |
| *unknown 21 b* | *6.1* | *0.001* | *4.9* | *0.003* |
| *unknown 21 c* | *5.3* | *0.001* | *4.3* | *0.002* |
| unknown 22 | --- | --- | --- | --- |
| unknown 24 | --- | --- | 1.3 | 0.001 |
| Total characterised | 51.2 | 0.011 | 39.7 | 0.022 |
| Analysed extract(s) | 97.2 | 0.021 | 97.5 | 0.053 |
| Extracts not analysed | --- | --- | 0.7 | <0.001 |
| **Total extracted** | **97.2** | **0.021** | **98.1** | **0.053** |
| Unextractable (PES) | 2.8 | 0.001 | 1.9 | 0.001 |
| Accountability | 100.0 | 0.021 | 100.0 | 0.054 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.3-4: Radioactive residues of parent compound and metabolites in muscle, fat, liver and kidney of lactating goats following oral administration of 5 daily doses of [phenyl-UL-$^{14}$C]flurtamone at a dose rate of 1.0 mg/kg

| | **Muscle** | | **Fat** | | **Liver** | | **Kidney** | |
|---|---|---|---|---|---|---|---|---|
| TRR [mg/kg] | | 0.047 | | 0.157 | | 1.179 | | 0.371 |
| Compound (flurtamone-) | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg |
| parent compound | 41.6 | 0.020 | 78.0 | 0.123 | 27.8 | 0.328 | 12.1 | 0.045 |
| mandelic acid | 6.3 | 0.003 | 1.1 | 0.002 | 6.0 | 0.071 | 7.3 | 0.027 |
| hippuric acid | --- | --- | --- | --- | 3.6 | 0.043 | 7.6 | 0.028 |
| trifluoromethyl-hydroxy-gluA | 2.0 | 0.001 | --- | --- | 12.8 | 0.151 | 11.0 | 0.041 |
| trifluoromethyl-hydroxy | 6.8 | 0.003 | 1.2 | 0.002 | 6.0 | 0.071 | 4.4 | 0.016 |
| Desmethyl | 8.7 | 0.004 | 7.3 | 0.012 | 5.6 | 0.066 | 2.5 | 0.009 |
| dihydroxy-pyrrolinone | 15.8 | 0.007 | 10.1 | 0.016 | 5.4 | 0.064 | 12.7 | 0.047 |
| **Total identified** | 81.2 | 0.038 | 97.8 | 0.154 | 67.3 | 0.794 | 57.7 | 0.214 |
| unknown 2 | --- | --- | --- | --- | 1.9 | 0.022 | --- | --- |
| unknown 3 | --- | --- | --- | --- | --- | --- | --- | --- |
| unknown 4 | 1.6 | 0.001 | --- | --- | 1.5 | 0.018 | --- | --- |
| unknown 5 | --- | --- | --- | --- | 2.3 | 0.027 | --- | --- |
| unknown 6 | --- | --- | --- | --- | 0.6 | 0.007 | --- | --- |
| unknown 7 | --- | --- | --- | --- | --- | --- | --- | --- |
| unknown 8 | --- | --- | --- | --- | 1.0 | 0.011 | --- | --- |
| unknown 9 | --- | --- | --- | --- | --- | --- | --- | --- |
| unknown 10 | --- | --- | --- | --- | 2.4 | 0.028 | --- | --- |
| unknown 11 | --- | --- | --- | --- | 2.1 | 0.024 | 2.7 | 0.010 |
| unknown 12 | --- | --- | --- | --- | 0.9 | 0.010 | --- | --- |
| unknown 13 | --- | --- | --- | --- | 0.7 | 0.008 | --- | --- |
| unknown 14 | --- | --- | --- | --- | 1.9 | 0.023 | 2.8 | 0.010 |
| unknown 16 | --- | --- | --- | --- | --- | --- | --- | --- |
| unknown 17 | --- | --- | --- | --- | --- | --- | --- | --- |
| unknown 18 | --- | --- | --- | --- | --- | --- | --- | --- |
| unknown 19 | --- | --- | --- | --- | 4.1 | 0.048 | 2.0 | 0.008 |
| unknown 20 | 1.8 | 0.001 | 0.7 | 0.001 | --- | --- | --- | --- |
| unknown 21 a-c | 5.4 | 0.003 | --- | --- | 0.9 | 0.011 | 18.6 | 0.069 |
| *unknown 21 a* | *2.6* | *0.001* | --- | --- | *0.5* | *0.005* | *9.1* | *0.034* |
| *unknown 21 b* | *1.5* | *0.001* | --- | --- | *0.3* | *0.003* | *5.1* | *0.019* |
| *unknown 21 c* | *1.3* | *0.001* | --- | --- | *0.2* | *0.003* | *4.5* | *0.017* |
| unknown 22 | --- | --- | --- | --- | --- | --- | 1.6 | 0.006 |
| unknown 24 | --- | --- | --- | --- | --- | --- | --- | --- |
| Total characterised | 8.8 | 0.004 | 0.7 | 0.001 | 20.1 | 0.238 | 27.6 | 0.103 |
| Analysed extract(s) | 90.0 | 0.043 | 98.4 | 0.155 | 87.5 | 1.031 | 85.4 | 0.317 |
| Extracts not analysed | 3.0 | 0.001 | --- | --- | 6.0 | 0.070 | 2.0 | 0.007 |
| **Total extracted** | **93.0** | **0.044** | **98.4** | **0.155** | **93.4** | **1.102** | **87.4** | **0.324** |
| Unextractable (PES) | 7.0 | 0.003 | 1.6 | 0.002 | 6.6 | 0.077 | 12.6 | 0.047 |
| Accountability | 100.0 | 0.047 | 100.0 | 0.157 | 100.0 | 1.179 | 100.0 | 0.371 |

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

Parent compound was the major compound in the extract of morning milk, evening milk, organs and tissues. Its concentration amounted to 0.003 mg/kg (12.4%) for morning milk, 0.017 mg/kg (31.4%) for evening milk, 0.020 mg/kg (41.6%) for muscle, 0.123 mg/kg (78%) for fat, 0.328 mg/kg (27.8%) for liver, and 0.045 mg/kg (12.1%) for kidney.

The highest number of metabolites was found in the liver. The major metabolite was flurtamone-trifluoromethyl-hydroxy-gluA which amounted to 0.151 mg/kg (12.8%). Identified minor metabolites were flurtamone-mandelic acid (0.071 mg/kg; 6.0%), flurtamone-hippuric acid (0.043 mg/kg; 3.6%), flurtamone-trifluoromethyl-hydroxy (0.071 mg/kg; 6.0%), flurtamone-desmethyl (0.066 mg/kg; 5.6%) and flurtamone-dihydroxy-pyrrolinone (0.064 mg/kg; 5.4%). Eleven unknown metabolites were detected each clearly below 0.05 mg/kg.

In milk muscle fat and kidney major metabolites were flurtamone-dihydroxy-pyrrolinone and flurtamone-trifluoromethyl-hydroxy-gluA. Flurtamone-dihydroxy-pyrrolinone was detected above 10 % of the TRR in the morning milk (0.003 mg/kg, 13.6%), in muscle (0.007, 15.8%), fat (0.016 mg/kg, 10.1%) and kidney (0.047 mg/kg, 12.7%). Relevant amounts of flurtamone-trifluoromethyl-hydroxy-gluA wer identified in kidney (0.041 mg/kg, 11.0%).
Additionally some minor metabolites such as flurtamone-mandelic acid, flurtamone-trifluoromethyl-hydroxy, flurtamone-desmethyl and flurtamone-hippuric acid could be identified but did not exceed 10% of the TRR. Their concentration was below 0.020 mg/kg, except for flurtamone-mandelic acid (0.027 mg/kg, 7.3%) and flurtamone-hippuric acid (0.028 mg/kg, 7.6%) in kidney.
Several unknown metabolites were indentified in morning and evening milk, muscle, and in kidney. Their concentration was below 0.05 mg/kg for kidney and liver and ≤ 0.005 mg/kg in muscle and milk.
In fat only one metabolite at trace level (0.001 mg/kg, 0.7%) remained unknown.

The metabolic and excretion behaviour of [phenyl-UL-$^{14}$C]flurtamone in the lactating goat can be characterised by the following observations:

- The concentration of radioactivity in milk and edible tissues was rather low compared to the dose level and the dosing period of five days.
- The evaluation of these concentrations should moreover consider the fact that an exaggerated dose level of 22.89 mg/kg feed/day was administered. Furthermore, the significant amount of radioactivity detected in urine and faeces and the relatively high concentration in liver and kidney at sacrifice six hours after the last administration indicate that the residues are further metabolised and finally eliminated.
- The residue level in milk showed a diurnal pattern after the second administration as they declined to a very low level prior to the next dose. A stable residue plateau level was clearly reached after the third administration.
- The radioactive residues were efficiently extracted from milk as well as from edible organs and tissues; extraction rates were between 87.4% and 98.4%.
- Overall, 46.0% to 97.8% of the TRRs in milk and edible organs and tissues were identified: Parent compound was the dominating constituent of the residues in milk, edible organs and tissues. Major metabolites identified were flurtamone-trifluoromethyl-hydroxy-gluA and flurtamone-dihydroxy-

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

pyrrolinone. Four minor metabolites were identified.

- The metabolic profiles of urine showed the same compounds as the organ and tissue samples but higher proportions of metabolites flurtamone-trifluoromethyl-hydroxy-gluA and flutarmone-dihydroxy-pyrrolinone were detected and the amount of parent compound flurtamone was distinctively lower. Faeces and bile contained fewer metabolites than the urine samples. The major compounds in faeces were parent compound flurtamone and flurtamone-trifluoromethyl-hydroxy. With regard to the bile flurtamone-trifluoromethyl-hydroxy-gluA was the major compound.
- The main metabolic reactions in the lactating goat are:
  - hydroxylation of the trifluoromethyl-phenyl moiety, followed by conjugation with glucuronic acid
  - hydroxylation and rearrangement of the furanone to a pyrrolinone moiety
  - oxidative ring opening of the furanone moiety, followed by cleavage and degradation of the carbon chain
  - N-demethylation

## III Conclusion

Flurtamone was moderately metabolised into few major and several minor and trace metabolites. The parent compound was mostly the major component in the milk and analysed organs and tissues, representing 12.1% to 78.0% of the TRR. In milk a mean plateau level of about 0.04 mg/kg was reached already at approx. 24 hours after the first administration. The two major metabolites flurtamone- trifluoromethyl-hydroxy-gluA and flurtamone-dihydroxy-pyrrolinone were detected at levels of up to 12. 8% and 15.8% of the TRR, respectively. Minor metabolites (< 10% of the TRR) identified were flurtamone-mandelic acid, flurtamone-hippuric acid, flurtamone-trifluoromethyl-hydroxy and flurtamone-desmethyl.
In total 46% to 97.8% of the TRR was identified.

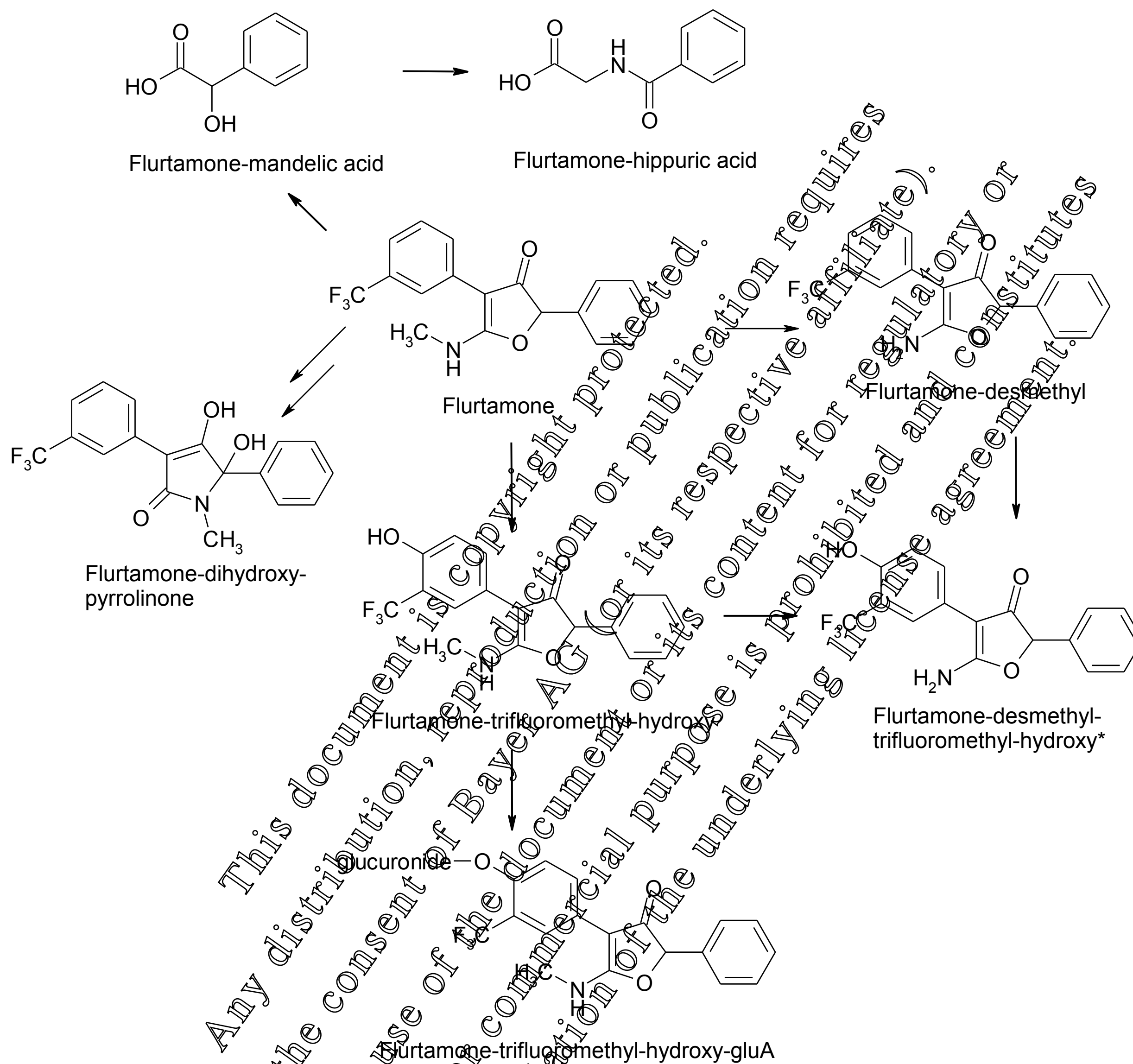
The main metabolic reactions in the lactating goat are:

- hydroxylation of the trifluoromethyl-phenyl moiety, followed by conjugation with glucuronic acid
- hydroxylation and rearrangement of the furanone to a pyrrolinone moiety
- oxidative ring opening of the furanone moiety, followed by cleavage and degradation of the carbon chain
- N-demethylation

Based on these results the metabolic pathway [phenyl-UL-$^{14}C$]flurtamone in the lactating goat shown in Figure 6.2.3-1 is proposed.

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Figure 6.2.3-1: Proposed metabolic pathway of [phenyl-UL-$^{14}$C]flurtamone in the lactating goat




This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## Metabolism of trifluoroacetic acid in lactating ruminants

| **Report:** | **KCA 6.2.3/02, [redacted] 2013** |
|---|---|
| Title: | [1-$^{14}$C]Trifluoroacetic acid - Metabolism in the Lactating Goat. |
| Document No: | M-444459-01-1 |
| Report No: | EnSa-12-0628 |
| Guidelines: | OECD guideline 503: Metabolism in Livestock, adopted 8 January 2007<br>US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1300 |
| GLP | Yes |


### Summary

A metabolism study with $^{14}$C-labelled sodium trifluoroacetate (Na-TFA) was conducted with a lactating goat as TFA revealed to be a major metabolite in plants that were treated with flurtamone and are intended as ruminant feed. This study is needed for a dietary risk assessment including food of animal origin to address the transfer of TFA residue from feedstuffs to milk and edible animal tissue.

TFA was orally administered per gavage as $^{14}$C-Na-TFA to the goat for five consecutive days with one dose per day. The dose level expressed as trifluoroacetic acid, TFA, was 0.50 mg/kg bw/day corresponding to 11.9 mg TFA/kg dry feed/day.

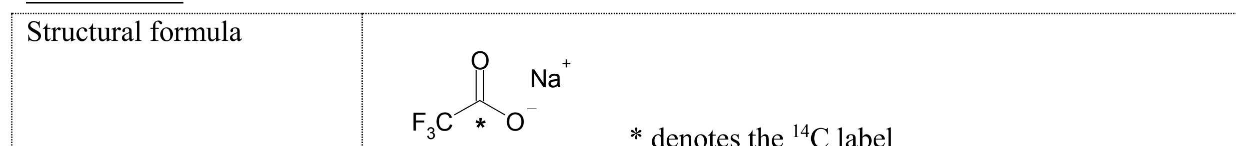
The radioactive residues in milk reached a steady state at approximately 30 hours after the first dose amounting to a plateau level of 0.10 mg parent equivalents/kg (mg equ/kg). Five days after the first administration the goat was slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 0.091 mg equ/kg in fat, 0.347 mg equ/kg in muscle, 0.551 mg equ/kg in liver and 0.967 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analyzed by radio-HPLC (reversed phase) and radio-TLC (straight phase). All radio-chromatograms showed only one chromatographic $^{14}$C-peak. Co-chromatography with authentic $^{14}$C-TFA using two chromatographic methods with different modes of separation unambiguously identified the radioactive peak as TFA. No other radioactive peak appeared in any sample. Therefore, the conclusion is drawn that the total radioactivity in milk, organs and tissues consisted of the unchanged TFA.

By comparison of the residue levels in feed, milk, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: milk (at plateau): 0.0086; muscle: 0.0292; fat: 0.0076; liver: 0.0463; kidney: 0.0813.


This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

### Material and methods

Test Material

| Structural formula |  * denotes the $^{14}$C label |
|---|---|

| | |
|---|---|
| Chemical name | Sodium trifluoroacetate |
| CAS RN | 2932-18-4 |
| Empirical formula | $C_2F_3NaO_2$ |
| Company code | BCS-AZ56567 |
| Molar mass (non-labelled) | 136.01 g/mol |
| Label | 1-$^{14}$C |
| Specific radioactivity | 4.08 MBq/mg = 110.14 µCi/mg |
| Radiochemical purity | >98% by TLC and HPLC (radio-detection) |
| Remark | Trifluoro acetate appeared as anion under physiological and environmental conditions. The corresponding cation depends on the chemical surrounding and, thus, is not defined. Therefore, the residue levels of trifluoro acetate are expressed as the parent substance trifluoro acetic acid (TFA). A conversion is conducted via the ratio of the molar masses:<br>MM (trifluoroacetic acid) / MM (sodium trifluoro acetate) = 114.02/136.01 = 0.8383<br>The specific radioactivity of the respective trifluoroacetic acid (TFA) is therefore: 4.08 MB/mg / 0.8383 = 4.87 MBq/mg |

Test Animal

| | |
|---|---|
| Species | Goat (*Capra hircus*) |
| Breed | "Weiße deutsche Edelziege" |
| Sex, number | One female lactating goat |
| Body weight | 52 kg at first administration, 51 kg at sacrifice |
| Age | Approx.15 months |
| Acclimatization | Two week before administration |
| Housing | Stainless steel metabolism cage, 18°C, approx. 60% rel. humidity, 12/12 hours light/dark cycle, 10-15 air changes per hour |
| Feed and water | Ruminant feed, hay, hay pellets, carrot, *ad libitum*<br>Tap water from local supplier, *ad libitum* |
| Health status | Acceptable according to veterinary investigation |

Preparation of the dosing mixtures and administration

Aliquots of the solid radiolabelled test substance were filled into five gelatin capsules. The sealed capsules were stored at ≤ -18°C until administration. Remaining test substance was used for identification via LC-MS/MS and to demonstrate the storage stability during the dosing period via radio-TLC. One capsule per day was orally administered in the morning for five succeeding days using a capsule applicator. The average daily dose amounted to 30.9 mg sodium trifluoroacetate (corresponding to 25.9 mg trifluoroacetic acid, TFA). Referred to the daily feed consumption and the body weight, this dose corresponded to a dose level of 11.9 mg TFA/kg dry feed or 0.50 mg TFA/kg bw/day. This dose was tolerated without any observable toxicological effects.

Collection of milk, urine and faeces

The goat was milked in the morning immediately prior to each administration, and eight hours after administration and directly before sacrifice. The collection intervals for milk sampling were: 0-8, 8-24, 24-32, 32-48, 48-56, 56.72, 72-80, 80-96, and 96-120 hours after the first administration. The milk

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

samples were weighed, radioassayed via liquid scintillation counting (LSC) and stored at ≤ -18°C for 97 days.

Urine and faeces were collected on a daily basis. Urine was collected in plastic vessels under dry ice cooling. The faeces samples were homogenized after addition of water to yield a wet paste. Aliquots of the excreta were radioassayed.

Sacrifice and collection of organs and tissues
Six hours after the last dose, the goat was sedated and anaesthetized by injection of Xylazin/Rompun, Ketamin and Pentobarbital-Na. Under deep anaesthesia, the animal was exsanguinated by cannulating the jugular vein and finally terminated by intracardiac injection of the veterinary drug "T 61®". Then, the goat was slaughtered and the following organs and tissues were dissected and stored at ≤ -18°C until analysis (103 - 124 days): round and loin muscle, omental and perirenal fat, liver (without gall bladder), and kidneys.

Radioassaying and processing of samples
Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer, the formed $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC.

For metabolism investigations, a composite sample of milk collected from 0 h to 102 h (time of sacrifice) after the first administration and composite samples of muscle (loin and round muscle) and fat (perirenal and omental) were prepared. The composite milk, muscle and fat samples and the complete liver, both kidneys, and one faeces sample (72 – 96 h) were thoroughly homogenized and kept frozen until extraction. Each sample (except fat) was extracted with acetonitrile/ water (8/2, v/v) and pure acetonitrile using a high-speed stirrer. The fat was extracted with mixtures of n-heptane and acetonitrile/water (8/2, v/v) also using a high-speed stirrer followed by separation of the heptane and the aqueous layer. All acetonitrile/water extracts were concentrated and analyzed by radio-HPLC and radio-TLC.

Radio-chromatography and mass spectrometry of the extracts
Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 5 µm particles) that was operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The system was equipped with an UV detector (254 nm) and a radiomonitor with a solid glass scintillator (cell volume 370 µL). The limit of quantification (LOQ) was derived from background noise level of the baseline and the highest peak in the chromatogram. It ranged from 0.001 mg equ/kg (milk, fat, kidney) to 0.005 mg equ/kg (liver). Column recovery was determined by comparison of injected and eluted radioactivity. In each case, it accounted for 96.2 – 99.6%. $^{14}C$-labelled TFA was co-injected to identify the residues in the samples.

Radio-TLC was conducted on a silica gel TLC plate (20 x 20 cm) that was developed with a solvent mixture of ethyl acetate/2-propanol/water/acetic acid (65/24/22/1, v/v/v/v). Following development the radioactive spots were detected by radioluminography via exposure of an imaging plate to the

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

radioactive spots. The detection limit was approximately 5-10 dpm/spot after an exposure period of at least 14 hours. $^{14}$C-TFA was also used as reference standard.

The test substance TFA was identified by LC-MS/MS consisting of anion exchange chromatography and a high resolution mass spectrometer. For ion exchange chromatography, a Dionex column was eluted with an aqueous solution of 20 mmol KOH as liquid phase. The mass spectrometer was operated in the mode of electro-spray ionization. This test substance was also used as reference standard in radio-HPLC and radio-TLC of the extracts.

Under natural, physiological and environmental conditions TFA is dissociated and appears as TFA salt. The counter cation depends on the chemical surrounding and is thus, not defined. Therefore, results are expressed as the parent compound of the salts, i.e. as trifluoroacetic acid.

## II Results and Discussion

<u>Recovery of radioactivity in milk, excreta and analyzed organs and tissues.</u>
Six hours after the last of five oral doses of 0.50 mg/kg bw/day of $^{14}$C-labelled TFA approximately 69% of the total radioactivity was recovered in milk, excreta, muscle, fat, liver and kidney. The remaining 31% of the total dose were assumed to be associated with the gastro-intestinal tract and the remaining body. 47.3% of the total dose was excreted with the urine and 15.1% with the faeces. 1.14% of the total dose was secreted into the milk and 5.1% were detected in the dissected edible organs and tissues with 4.1% of the dose being associated with the muscular tissue (assuming 30% of the body weight to the muscular mass).

<u>Radioactive residues in the milk</u>
The total radioactive residues (TRR) in the milk ranged from 0.079 mg equ/kg to 0.145 mg equ/kg in the collection period 48 to 80 hours after the first administration. At the time of sacrifice, a further increase to 0.171 mg equ/kg was observed due to the shorter time interval between the last dosing and sampling (ca. 6 hours). The time course of radioactivity in milk showed a typical diurnal pattern with temporal peaks eight hours after each administration and sinks shortly before the next dosing (Table 6.2.3- 5).

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying licence agreement.

**Table 6.2.3- 5:** Radioactive residues in milk of a goat administered with $^{14}$C-TFA at a dose level of 11.9 mg TFA/kg dry feed/day

| Time schedule after the first administration [hours] | Number of administration | Weight of milk sample [kg] | Residue level in milk sample [mg equ/kg] | Residue level in milk, daily average [mg equ/kg] |
|---|---|---|---|---|
| 0 | 1 | | ----- | |
| 8 | | 1.27836 | 0.101**) | |
| 24 *) | | 2.20244 | 0.057**) | 0.073**) |
| 24 | 2 | ----- | ----- | |
| 32 | | 1.09898 | 0.132 | |
| 48 *) | | 2.30380 | 0.079 | 0.097 |
| 48 | 3 | ----- | ----- | |
| 56 | | 1.10321 | 0.138 | |
| 72 *) | | 2.36203 | 0.079 | 0.098 |
| 72 | 4 | ----- | ----- | |
| 80 | | 1.16649 | 0.145 | |
| 96 *) | | 2.34357 | 0.095 | 0.112 |
| 96 | 5 | ----- | ----- | |
| 102 | | 0.85857 | 0.171***) | - |
| **Residue plateau in milk (30–96 hours after first administration)** | | | | **0.102 ± 0.008** |

*) Milking immediately before the next administration

**) Not used for calculation of the residue plateau in milk since residues are still increasing at the beginning of the collection period

***) Not used for calculation of the residue plateau in milk since period between dosing and milking was shorter (only 6 hours) and no data of the second milking was available.

A plateau level was reached approximately 30 hours after the first administration. This level was calculated as mean value of the mass weighted daily averages of the milk samples between the second and the fourth administration. The resulting steady state level in milk amounted to 0.10 mg equ/kg. (The residue levels of the first day were excluded from the plateau calculation since the residues were still increasing at the beginning of milk collection. The residue level of the last milk sample was also excluded since the interval between dosing and milking was shorter than at the other days and a second milk sample was not available due to slaughtering.)

Radioactive residues in dissected organs and tissues

The TRR in edible organs and tissues ranged from fat amounting to 0.091 mg equ/kg (mean of perirenal and omental fat) to kidney amounting to 0.967 mg equ/kg (Table 6.2.3- ). The radioactivity concentrations of the total muscle and fat referred to 4.08% and 0.43% of the total dose assuming a value of 30% and 12% of the body weight for these tissues, respectively. Altogether, the test radioactive residues in all dissected organs and tissue samples accounted for about 5.14% of the total dose.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table 6.2.3- 6:** Radioactive residues in organs and tissues of a goat 6 hours after the last of 5 doses of $^{14}$C-TFA at a dose level of 11.9 mg TFA/kg dry feed/day

| **Organ/Tissue** | **Residue level [mg equ/kg]** |
|---|---|
| Liver | 0.551 |
| Kidney | 0.967 |
| Round muscle (sample) | 0.346 |
| Loin muscle (sample) | 0.352 |
| Total body muscle *) | 0.347 |
| Perirenal fat (sample) | 0.064 |
| Omental fat (sample) | 0.107 |
| Total body fat *) | 0.091 |

*) Weighed mean residue levels in total body muscle and fat were calculated from the sample masses of the two types of muscle and fat and the total radioactive residues in that samples, respectively.

Identification of the radioactive residues

Radio-HPLC and radio-TLC profiles of the extracts of all samples (milk, liver, kidney, muscle, fat, urine and faeces) showed only one polar radioactive peak. Co-chromatography with the reference standard $^{14}$C-TFA resulted in the same single peak. No other peak could be observed. This peak was unambiguously identified as radiolabelled TFA since two chromatographic systems with different separation modes (reversed phase HPLC and straight phase TLC) were used for co-chromatography. Therefore, the total radioactivity in all samples represented unchanged TFA. The rate of identification in the samples was excellent amounting to 98.6 – 100% of TRR in all extracts.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Transfer factors of residue transfer of TFA from animal fodder to food of animal origin

The TFA transfer factors (TF) were calculated as ratio between the radioactive residues in animal fodder and the total radioactive residues in milk, and edible organs and tissues of the goat. Any correction for formation of transformation products of TFA is not needed as total radioactive residue was represented by the administered test substance (see before). These transfer factors ranging from 0.01 (milk, fat) to 0.08 (kidney) are listed in detail in
Table 6.2.3- 7.

**Table 6.2.3- 7:** Transfer factors for residue transfer of $^{14}C$-TFA from animal feed to milk, muscle, fat, liver and kidney of a goat following repeated administration at a dose level of 11.9 mg TFA/kg dry feed/day

| Milk/Organ/Tissue | Residue level [mg equ/kg] | Transfer factor (TF) |
|---|---|---|
| Milk (at residue plateau) | 0.102 | 0.0086 |
| Muscle | 0.347 | 0.0292 |
| Fat | 0.091 | 0.0076 |
| Liver | 0.551 | 0.0463 |
| Kidney | 0.967 | 0.0813 |

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

**III Conclusion**

Following repeated oral administration of $^{14}C$-labelled sodium trifluoroacetat (TFA-Na) to a lactating goat for five consecutive days at a dose level of 0.50 mg TFA-acid/kg bw/day (corresponding to 11.9 mg TFA-acid/ kg dry feed/day) the radioactive residues in milk reached a steady state at approximately 30 hours after the first dose amounting to a plateau level of 0.10 mg equ/kg. Five days after the first administration the goat was slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 0.091 mg equ/kg in fat, 0.347 mg equ/kg in muscle, 0.551 mg equ/kg in liver and 0.967 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analyzed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic $^{14}C$-TFA and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. As a conclusion it can be stated that TFA is metabolically stable in ruminants. It was rapidly excreted as not more than 5% of the total dose was detected in the dissected organs and tissues 6 hours after the last dose.

By comparison of the residue levels in feed, milk, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: milk (at plateau): 0.0086; muscle: 0.0292; fat: 0.0076; liver: 0.0463; kidney: 0.0813.

## CA 6.2.4 Pigs

Following oral administration $^{14}$C-flurtamone has been shown to be rapidly absorbed and metabolized by phase I reactions (hydroxylation, methylation, ring hydrolysis) and phase II reactions (conjugations) in rat, goat and hen. The main products eliminated in both urine and faeces of rat and goat and in the excreta of chicken were flurtamone-trifluoromethyl-hydroxy and related compounds. Elimination was observed to be rapid in all three species with very low to moderate levels of radioactive residues being found in the tissues at the time of sacrifice, with the higher levels being located in the principal organs of metabolism and excretion, the liver and kidney.

Therefore it can be concluded that the principal pathway of metabolisation is the same in goat, hen and rat and consequently a metabolism study in pig is not necessary.

## CA 6.2.5 Fish

According to the data requirements published in the Commission Regulation (EU) No 283/2013 of 1-March-2013 a “Metabolism studies on fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications”.
No fish metabolism study has been performed however a bioaccumulation study in fish (evaluatedand accepted for point 8.2.2.3) is available.
The study was originally undertaken to define the kinetics of the uptake and elimination of $^{14}$C-residues in bluegill sunfish but also the metabolism of flurtamone in sunfish was intensively investigated.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| **Report:** | **KCA 6.2.5/01, [redacted], E., 1994** |
|---|---|
| Title: | Flurtamone - Bioconcentration, Metabolism and Elimination of $^{14}$C Residues by Bluegill Sunfish *(Lepomis macrochirus)* |
| Document No: | M-162223-01-1 |
| Report No: | 92-6-4300 (SLI Report) |
| Guidelines: | USEPA (=EPA) FIFRA Guideline 165-4, 1992 |
| GLP | Yes |

### Summary

Initially, bluegill sunfish were continuously exposed to a nominal concentration of 0.11 mg/L $^{14}$C-flurtamone equivalents for 28 days in a flow-through system. Additionally a 14-day depuration period was observed. Biological half-life of the $^{14}$C-residues, present in whole body tissue on the last day of exposure occurred between days 1 and 3 of depuration.
Only limited metabolite characterization/identification work could be performed on the fish tissues from the initial exposure experiment because of the low levels of radioactivity.

This is why a second experiment with the same nominal concentration but with a higher specific activity compound was conducted to obtain sufficient tissue to identify metabolites down to a concentration of 0.05 ppm.

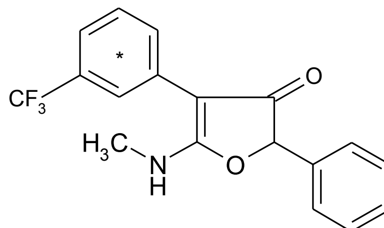
The metabolism of flurtamone in bluegill sunfish was found to be rather extensive. After 28 days of exposure, parent flurtamone was found to constitute approximately 6% and 40% of the radioactive residues in the viscera and edible tissues, respectively. Major metabolites found in the viscera tissue included flurtamone-trifluoromethyl-mandelamide (RE53286), flurtamone-trifluoromethyl-N-methyl-mandelamide (RE53285), flurtamone-desmethyl-trifluoromethyl-hydroxy (RE54578), flurtamone-trifluoromethyl-hydroxy (RE53498), and flurtamone-desmethyl (RE39748). These metabolites were present as both free molecules and conjugates of glucuronic acid, flurtamone-trifluoromethyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, and flurtamone-trifluoromethyl-hydroxy were also present as sulphate esters. In addition to glucuronide and sulphate conjugations, small quantities of fatty acid esters of flurtamone-trifluoromethyl-mandelamide were also observed. Major metabolites found in the edible tissue included flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-N-methyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy and flurtamone-trifluoromethyl-hydroxy along with their glucuronide conjugates. Metabolism of Flurtamone by bluegill sunfish involved various known metabolic pathways as the detoxification mechanism. These pathways include N-demethylation, hydroxylation of the trifluoromethyl phenyl ring followed by glucuronide and sulfate conjugations. The metabolic pathway also include opening of the furanone ring to form flurtamone-trifluoromethyl-N-methyl-mandelamide followed by N-demethylation to form flurtamone-trifluoromethyl-mandelamide.

**I Material and Methods**

Bluegill sunfish were exposed to flurtamone in two separate experiments, the second experiment having a higher specific activity to facilitate the identification of metabolites. For the first exposure the fish (SLI Lot 92A42) were obtained from a commercial supplier ( ). These were maintained in a holding tank for at least 14 days before treatment. The fish population had a mean wet weight of 1.7 g and a mean total length of 50 mm. Mortality of < 1% occurred during the 48 hours prior to exposure. The dilution water was drawn from a bedrock well into a reservoir where it was aerated and supplemented with untreated well water supplied by the municipality. It was characterized as soft and there was flow-rate of 5-16 volume replacements per day. The temperature was maintained at 18°C. The fish were fed a dry pelleted food, ad libitum, daily except during the 24 hours prior to testing.

The radiolabelled test substance was [trifluoromethylphenyl-U-14C]-flurtamone (Batch: CSL-89-204-11-27), originally obtained from Chemsyn Laboratories. It had a specific activity of 6.0 MBq/mg with a radiopurity of > 98% and was supplied as a solution in methanol.


This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.




* = position of uniformly labelled ring.

**Initial exposure experiment**

A radiolabelled stock solution was prepared by the transfer of the entire contents of a vial of methanolic radiolabelled-flurtamone solution into a volumetric flask with rinsing with acetone. The mixture was evaporated to dryness under a gentle stream of nitrogen and the residue dissolved in acetone. The concentration of this solution was found to be 0.226 mg/mL by the LSC of diluted aliquots and calculation using the specific activity.

A radio-diluted stock solution was prepared by the combining of 4.888 g of non-radiolabelled flurtamone (reference 1005-43-16/HHT) with 40 mL of the radiolabelled stock solution (0.229 mg/mL) and dilution to 400 mL with acetone. The resulting solution was determined as having a concentration of 12.24 mg/mL. The delivery system consisted of syringe pumps calibrated to deliver 0.00378 mL/min of the stock solution (12.243 mg/mL).

The exposure systems used a continuous flow diluter. The test chambers were clear glass aquaria measuring 75 cm x 39 cm x 30 cm (l x w x h). The water level in each was maintained at a depth of 25 cm to give a total volume of 73 litres. The system was calibrated to deliver 420 mL/min of test solution to each aquarium. This delivery rate was equivalent to 8 aquarium volumes per day or 90% aquarium volume replacement each 6-hour period. Illumination was provided by fluorescent bulbs located over the aquaria and a 16-hour light/8 hour dark cycle was used. The temperature was controlled to 17°±1°C.

The exposure system was in operation for six days prior to the addition of the fish. The exposure aquaria waters were sampled and analysed for flurtamone concentrations on four separate days during this period. The results of these analyses were used to judge whether the appropriate concentration of test material was being maintained in the test aquarium. The nominal exposure concentration was 0.11 mg/L. The exposure was initiated by the placing of 150 bluegill sunfish into each of three aquaria, designated as treatment, metabolite and control. At test initiation the total biomass was 261 g per tank. This figure decreased throughout the study as fish were removed for analysis. Daily observations were made on the appearance and behavior of the fish and the appearance of the test solution. The temperature and dissolved oxygen concentration were measured and the pH at least three times per week. Water hardness (as CaCO3) was measured at test initiation and specific conductance was measured with a salinity-conductivity-temperature meter and probe.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

The exposure of the fish to flurtamone at a nominal concentration of 0.11 mg/L was continuous through to the establishment of a steady-state tissue residue concentration, and was maintained for 28 days. Once equilibrium was achieved and fish had been exposed for 28 days, 30 fish were transferred to a separate aquarium into which the dilution water was introduced at a rate equal to the flow rate during the exposure period. A 14-day depuration period was used in an attempt to establish the rate of tissue residue elimination and determination of the half-life of flurtamone in fish.

Sampling

In order to monitor the concentration of radioactivity in the water of the exposure tank and a control tank, aliquots (5 mL) were taken on four occasions during the week prior to initiation of exposure, at 0, 1, 3, 7, 14, 19, 21, 23 and 28 days of exposure and at 1, 3, 7, 10 and 14 days of the depuration phase. A large aliquot (1 L) was taken on the seventh day of exposure for chromatographic examination. Fish were taken for analysis at 1, 3, 7, 10, 14, 19, 21, 23 and 28 days of exposure and 1, 3, 7, 10 and 14 days of depuration. Five unexposed fish were analysed. After 28 days of exposure the fish sample weights were used for the calculation of the percentages of the whole fish represented by the edible and non-edible portions.

Sample processing

The small water samples and the fish samples were not processed prior to analysis. The large sample taken for chromatographic examination was extracted (x 3) with methylene chloride, following adjustment of the pH to 11. The extract was concentrated by rotary evaporation and taken to dryness under a gentle stream of nitrogen. The residue was then reconstituted with a small volume of acetonitrile/water (1:1) and aliquots were subjected to HPLC.

A sub-sample (30 g) of the edible fish-tissues was subjected to a series of extraction procedures. The first extraction was with hexane and the second was with methanol. After a number of liquid/liquid partitions, various recombinations and the discarding of fatty material there was one hexane fraction and one methanol fraction for qualitative investigation. A sub-sample of visceral material (10 g) was subjected to a procedure very similar to that used for the edible tissue and this also resulted in one hexane fraction and one methanol fraction for qualitative investigation.

Quantitative analyses

The water samples and extracts were radioassayed by direct liquid scintillation counting, following the addition of a suitable scintillation cocktail. The fish were dissected into edible (muscle) and non-edible (viscera and carcass) and the entire portion of each fish was dried and then combusted. The resulting radiolabelled carbon dioxide was trapped (in an organic base solution) and this was radioassayed by LSC following the addition of an appropriate scintillation cocktail.

Qualitative analyses

The water sample taken on the seventh day of exposure was examined by HPLC and TLC to verify that the radioquantification was representative of flurtamone rather than metabolites or degradation products. The extracts prepared from the sample were analysed on an HPLC system that comprised a Phenomenex Utremex C18 column connected to HP Diode Array Spectrophotometer and a

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Radiomatic Model A280 Radiodetector with a 500 µL flow cell. The mobile phase was acetonitrile/water/acetic acid (80:20:0.1, v/v/v).

The extracts were also analysed by 2-dimensional TLC. Aliquots were spotted on cellulose plates and each spot was co-spotted with an aliquot of non-radiolabelled flurtamone stock solution. The plates were placed in a pre-conditioned chamber and the solvent system of n-butanol/acetic acid/water (6:1:1, v/v/v) was allowed to migrate 12 cm from the origin. The plate was removed, dried and rotated 90° before being placed in a second chamber for development in the second direction. The second solvent system was a chloroform/acetone (4:1, v/v) mixture. Development was again to 12 cm from the origin. The plates were removed from the chamber and allowed to dry after which the radioactive areas were located with a Radiomatic Analytical Digital Imaging System. The data were transmitted from the scanner to a computer and the radioactive regions of the plate were marked. The non-radiolabelled flurtamone was located by visualization under UV light.

The tissue extracts were examined on a radio-HPLC system as described above.

**Second exposure experiment**

A second exposure of fish to radiolabelled flurtamone was conducted in order to provide material to facilitate the identification and quantification of metabolites. A radiolabelled stock solution was prepared by the transfer of the entire contents of a vial of methanolic radiolabelled-flurtamone solution into a volumetric flask with rinsing with acetone. The mixture was evaporated to dryness under a gentle stream of nitrogen and the residue dissolved in acetone.

The concentration of this solution was found to be 0.518 mg/mL by the LSC of diluted aliquots and calculation using the specific activity. The radiochemical purity was determined to be 97.2% by HPLC. This procedure was repeated with another vial and resulted in a solution that had a flurtamone concentration of 0.122 mg/mL and a radiopurity of 93.1%. The two stock solutions were combined to give a solution that had a concentration of 0.247 mg/mL. The radiochemical purity was determined to be 96.6%.

A radio-diluted stock solution was prepared by the combining of 2.524 g of non-radiolabelled flurtamone (reference 1005-43-16/JHT) with 249 mL of the radiolabelled stock solution (0.247 mg/mL) and dilution to 390 mL with acetone. The resulting solution was determined as having a concentration of 5.97 mg/mL. The syringe pumps were calibrated to deliver 0.00775 mL/min of this solution.

The exposure system was in operation three days prior to the addition of the fish. The tank was sampled on two separate days during this period. The exposure was initiated by the placing of 275 bluegill sunfish into the exposure tank. At this time the total biomass accounted 330 g. The same measurements and observations were made as had been made for the initial experiment. The exposure was continued for 28 days after which time all fish were removed for analysis.

<u>Sampling</u>

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

In order to monitor the concentration of radioactivity in the water of the exposure tank and a control tank, aliquots (5 mL) were taken at 0, 1, 3, 7, 10, 14, 21, and 28 days of exposure. On days 1, 14 and 28 large samples were taken for qualitative analysis. A small sample of fish was taken for quantitative radioassay at day with the remainder being collected for qualitative investigations on day 28.

Sample processing

The small water samples and the fish samples from day 26 were not processed prior to analysis. The large water sample taken for chromatographic examination was extracted as described previously. In order to perform metabolite identification/characterization on the edible and non-edible tissues of the fish samples after 28 days of exposure a total of four experiments was conducted. The extraction procedures varied slightly depending upon the purpose of each experiment.

The first and second set of extraction/clean-up procedures were conducted primarily for qualitative analyses whilst the third and fourth were primarily for quantification. For the first set, a sample of visceral tissue was extracted (x 3) with methanol with homogenization. The combined extract was concentrated, partitioned (x 2) with hexane then further concentrated and passed through a C18 solid phase extraction column. The column was eluted with methanol and the eluate was collected and concentrated. This sample was examined by radio-HPLC and by particle-beam LC-MS analysis. The hexane fraction was concentrated and fatty materials were precipitated (by addition of acetone under refrigerated conditions). The precipitate was removed and the extract further concentrated before being examined by radio-HPLC. Post-extraction residues were radioassayed by combustion-LSC. A sample of edible tissue was extracted (x 3) with methanol, methanol/water (7:3, v/v), hexane and acetone. The extracts were combined and partitioned (x 2) with hexane. The non-hexane fraction was concentrated and purified on a Bond-Elut column and analysed by radio-HPLC. The hexane fraction was processed in the same manner as that from the visceral sample and was also examined on radio-HPLC. Post-extraction residues were radioassayed.

The second set of procedures was conducted on two samples of visceral tissue. Each sample was extracted (x 4) with methanol with homogenization. The combined extract was concentrated, partitioned (x 2) with hexane and the hexane fractions combined, concentrated to near dryness and the residues dissolved in acetone. The methanol extracts were also combined and concentrated and were then passed through a Bond-Elut solid phase extraction column. Unretained activity was collected and labelled as feaction1. The column was eluted with methanol and the collected eluate, labelled as fraction 2, was concentrated. Chromatographic examination of the two fractions indicated that fraction 1 contained polar compounds and fraction 2 contained non-polar compounds.

Fraction1 was further processed by passage through another C18 SPE column. The aqueous eluate was collected and labelled as fraction 1A. The column was eluted with methanol and the eluate collected was labelled as fraction 1B. To remove fatty material fraction 1A was partitioned (x 2) with ethyl acetate and then with petroleum ether. The organic fractions were combined with fraction 1B and the whole (retaining the label 1B) was concentrated. At each step the samples were radioassayed by the LSC of aliquots and the distribution of residues was monitored by HPLC. Metabolites in fraction 1B and in fraction 2 were further purified by HPLC fractionation. Post-extraction residues were radioassayed.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying licence agreement.

Fractions 1A and 1B were subjected to acid and enzyme hydrolysis. The acid hydrolysis utilised 6M HCl and mild heating for varying periods of time depending on the sample. Aliquots of the extracts were evaporated to near dryness mixed with an appropriate buffer and then incubated separately with β-glucuronidase, sulfatase, esterase, phosphatase or glutamyl transpeptidase. The β-glucuronidase incubate was chromatographed directly or partitioned (x 3) with ethyl acetate before concentration and chromatographic examination. All the other incubates were partitioned as described. In all cases the organic phases were concentrated and chromatographed.

The third set of procedures was conducted on two small samples of visceral tissue. Each was extracted (x 3) with methanol. The combined extract was concentrated and examined by radio-HPLC. Post-extraction residues were radioassayed. Two samples of edible tissue were extracted (x 6) with methanol. The first five extracts were combined, concentrated and partitioned with hexane. The methanol fraction was further concentrated, chromatographed and subjected to enzyme hydrolysis in the manner previously described. The hexane fraction was taken to dryness and the residue redissolved in acetone to give a solution that was then examined chromatographically. The tissues were subjected to a final extraction with acetone over an extended period (48 h) after which it was refluxed with HCl for one hour.

The fourth and final set of extraction/clean-up procedures was carried out on two samples of edible tissues. Each sample was extracted (x 3) with methanol. The combined extract was concentrated and partitioned with hexane. The methanol fraction was further concentrated and chromatographed. The hexane fraction was evaporated to dryness and the residue was redissolved in acetone to give a solution that was examined chromatographically. Post-extraction tissues were radioassayed.

<u>Quantitative analyses</u>
The water samples and extracts were radioassayed by direct liquid scintillation counting, following the addition of a suitable scintillation cocktail. The 26-day fish were dissected into edible (muscle) and non-edible (viscera and carcass) and the entire portion of each fish was dried and then combusted. The resulting radiolabelled carbon dioxide was trapped (in an organic base solution) and this was radioassayed by LSC following the addition of an appropriate scintillation cocktail.

<u>Qualitative analyses</u>
Water samples taken at 1, 14 and 28 days were analysed by HPLC with UV detection for total flurtamone determination and with radiodetection for radiolabelled flurtamone detection. For confirmation of peak assignment they were examined by LC-MS. HPLC-UV was conducted on a Phenomonex Utremex C18 column with direct aqueous injection. The mobile phase was acetonitrile/water/acetic acid (60:40:1, v/v/v). The detector was set at 240 nm. Quantification was achieved through an external calibration method. Radio-HPLC was conducted on the processed methylene chloride extract of water with the same conditions as described for HPLC-UV but with radioactivity monitored on a flow-through radiodetector. The processed water extracts were also analysed by particle-beam LC-MS using electron impact (70 eV) ionization.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## II Results and Discussion

Analyses of test solution samples during the period before initial exposure gave concentrations that averaged at 92% of the target value. Throughout the study no undissolved material was observed in the dilution system or in the test aquarium. During the exposure and depuration periods only one mortality was observed among the 150 initial test organisms in the treatment aquarium and none in the controls. In general the fish appeared healthy, exhibited normal behavior and grew continuously throughout the study. Water quality parameters varied minimally. Throughout the 28-day exposure period the concentration of flurtamone remained relatively constant and averaged 100% of the target value. It was calculated to be 0.11±0.01 mg/L. The chromatographic examinations of the water showed that the radioassays gave a reliable indication of the concentration of flurtamone.

The concentrations of radioactive residues in edible, non-edible and whole-body portions of the bluegill sunfish are presented in Table 6.2.5-1, along with the concentration in the water. After values were available for three consecutive sampling intervals they were subjected to analysis of variance and Tukey's test. Statistical analyses of the TRRs in edible tissue determined no significant differences for 14 days to 28 days of exposure, with steady state therefore established by 14 days. The non-edible tissue concentrations reached a steady state by one day and the whole body concentrations by 7 days. The mean steady state TRRs were calculated to be 1.8 (±0.4), 4.7 (±1.3) and 2.9 (±0.5) mg/kg for edible, non-edible and whole body tissues, respectively. Bioconcentration factors were calculated for each tissue and were found to be 17, 43 and 27 for edible, non-edible and whole body tissues, respectively. The edible portion of the fish was shown to represent on average, 61% of the whole fish whereas the non-edible remainder accounted for 39%. Fifty percent elimination of radioactive residues in the whole body tissue of fish occurred between the first and third days of the depuration phase. By the end of the depuration period (14 days) an elimination of 81% had been reached.

The analysis of the tissues from the initial exposure showed the presence of flurtamone and flurtamone-trifluoromethyl-hydroxy (RE-53498) in the tissue extracts (by HPLC and by LC-MS). The results also indicated the presence of flurtamone-desmethyl (RE-39748). An unknown was detected by LC-MS that had not been observed by radio-HPLC.

The metabolite characterization/identification work on the tissues from the second exposure experiment showed that the metabolism of flurtamone had been extensive. The proportions of extractable and bound residues (using results from the third and fourth sets of extraction procedures) are presented in Table 6.2.5-2. Most of the radioactivity in both edible and visceral tissues could be extracted with methanol. The bound residue was somewhat higher in the edible tissue than in the visceral tissue (following extraction with methanol alone).

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.5-1: Water concentrations of flurtamone and bluegill sunfish TRRs during exposure of fish over twenty-eight days and a fourteen day depuration period

| Test Day | Total Radioactive Concentration (as flurtamone equivalents) in: | | | |
|---|---|---|---|---|
| | **Water (mg/L)** | **Edible Tissue (mg/kg)** | **Non-edible Tissue (mg/kg)** | **Whole Body (mg/kg)** |
| 1 | 0.11 | 0.60 | 4.1 | 2.0 |
| 3 | 0.10 | 0.89 | 3.7 | 2.2 |
| 7 | 0.12 | 1.2 | 4.6 | 2.6 |
| 10 | 0.11 | 1.3 | 4.5 | 2.6 |
| 14 | 0.11 | 1.6 | 4.6 | 2.9 |
| 19 | 0.11 | 1.5 | 5.8 | 3.1 |
| 21 | 0.10 | 1.6 | 4.4 | 2.8 |
| 23 | 0.10 | 2.0 | 4.9 | 3.2 |
| 28 | 0.11 | 2.3 | 5.6 | 3.5 |
| 29 (Depuration 1) | < LOQ | 1.4 | 2.5 | 1.8 |
| 31 (Depuration 3) | < LOQ | 0.85 | 1.9 | 1.3 |
| 35 (Depuration 7) | < LOQ | 0.64 | 1.3 | 0.89 |
| 38 (Depuration 10) | < LOQ | 0.75 | 1.3 | 0.96 |
| 42 (Depuration 14) | < LOQ | 0.55 | 0.88 | 0.68 |

Table 6.2.5-2: Proportions of extractable and unextractable radioactive residues in the tissues of bluegill sunfish exposed over a period of twenty-eight days to radiolabelled flurtamone in aqueous solution at 0.11 mg/L

| Sample Type | Mean % TRR (normalized) in: | | | |
|---|---|---|---|---|
| | **Methanol Extract** | **Acetone Extract** | **HCl Reflux** | **Unextracted** |
| Edible tissue | 89 | 1 | 2 | 8 |
| Non-edible tissue | 94 | nm | Nm | 6 |

nm = extract not made

The chromatographic examination of the extracts of edible tissue showed that flurtamone and the metabolites flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-N-methyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy were present in the extracts of both tissue types, and flurtamone-desmethyl was also present in the visceral tissues extract. Quantification of these compounds was based on peak integrations of radiochromatograms. An early running peak (4 min) in the edible tissues extract was shown to be a mixture of glucuronide conjugates (which was also demonstrated to be the case for the equivalent peak in the non-edible tissue extracts). The identification of flurtamone, flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy and flurtamone-desmethyl was achieved by reversed phase HPLC with a gradient mobile phase and confirmed by LC-MS, all with reference to

appropriate standards. The metabolites flurtamone-trifluoromethyl-N-methyl-mandelamide and flurtamone-trifluoromethyl-mandelamide were identified by LC-MS after isolation of sufficient material by repetitive HPLC injections.

Two polar peaks were found in the extracts from both tissue types. One had a retention time of approximately 3 min and the other of approximately 4 min. Fractions 1A and 1B of extraction procedure 2 were used for the characterization and identification of the compounds giving these peaks. Fraction 1A contained only the 3-min peak while fraction 1B contained both peaks. Acid and enzyme hydrolyses confirmed that conjugated metabolites had been released from the material that gave the 3-min peak.

Following enzyme treatment the deconjugated metabolites were extracted into ethyl acetate and analysed by HPLC and LC-MS. Following treatment with β-glucuronidase, which released just over 50% of the radioactivity, flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-N-methyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy and flurtamone-desmethyl were indicated to be present, based on chromatographic comparisons with reference compounds. The identity of the latter three was confirmed by mass spectrometry but that of the first two could not be confirmed by this method because of the very low levels.

Following treatment with sulfatase, which released just about 25% of the radioactivity, flurtamone-trifluoromethyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy and flurtamone-trifluoromethyl-hydroxy were indicated to be present and following treatment with esterase, which released nearly 10% of the radioactivity, flurtamone-trifluoromethyl-mandelamide was the only metabolite detected. Treatment with phosphatase and glutamyl transpeptidase did not release significant amounts of radioactivity, indicating that phosphate and glutathione conjugates were not present in significant amounts. This is in accordance with the fact that about 90% of the radioactivity was released by the other three enzyme treatments.

Identification of the components giving the 4-minute peak was achieved by analysis of fraction 1B that contained mostly that peak with a small amount of the 3-min peak. Following treatment with β-glucuronidase, after which the 4-min peak had disappeared, flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-N-methyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy and flurtamone-desmethyl were found in the ethyl acetate extract. A 4-min peak was also present in the methanol fraction of the edible tissue extract. To confirm the presence of glucuronide conjugates an aliquot was treated with glucuronidase. After partitioning with ethyl acetate nearly 90% of the sample radioactivity was found in the solvent fraction. Compounds that had been released were shown to include flurtamone-desmethyl-trifluoromethyl-hydroxy and flurtamone-trifluoromethyl-hydroxy. Because the conjugate fraction was a minor component of the edible tissues residue, quantification of the individual glucuronides was not performed.

The distribution of the components of the radioactive residues are summarized in Table 6.2.5-3 (edible tissues) and Tables 6.2.5-4 and 6.2.5-5 (non-edible tissues). The proportions of the residue accounted for by each metabolite, whether free or conjugated, is summarized in Table 6.2.5-6.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.5-3: Composition of radioactive residues in edible tissues of bluegill sunfish exposed for twenty-eight days to radiolabelled flurtamone in aqueous solution at 0.11 mg/L

| Component | % TRR | Concentration (mg/kg) |
|---|---|---|
| Glucuronide conjugates* | 8.3 | 0.17 |
| Unknown | 0.8 | 0.02 |
| flurtamone-trifluoromethyl-mandelamide (RE 53286) | 9.0 | 0.18 |
| flurtamone-trifluoromethyl-N-methyl-mandelamide (RE 53285) | 29 | 0.58 |
| flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 54578) | 5.1 | 0.10 |
| flurtamone-trifluoromethyl-hydroxy (RE 53498) | 4.8 | 0.09 |
| Flurtamone (RE 40885) | 36 | 0.72 |
| **Total** | 93 | 1.86 |

* shown to include glucuronide conjugates of flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 54578) & flurtamone-trifluoromethyl-hydroxy (RE 53498)

Table 6.2.5-4: Composition of radioactive residues in non-edible tissues of bluegill sunfish exposed for twenty-eight days to radiolabelled flurtamone in aqueous solution at 0.11 mg/L

| Component | % TRR | Concentration (mg/kg) |
|---|---|---|
| Glucuronide/sulfate conjugates* | 12 | 1.4 |
| Glucuronide conjugates** | 51 | 6.0 |
| flurtamone-trifluoromethyl-mandelamide (RE 53286) | 3.0 | 0.36 |
| flurtamone-trifluoromethyl-N-methyl-mandelamide (RE 53285) | 7.1 | 0.84 |
| flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 54578) | 6.4 | 0.76 |
| flurtamone-trifluoromethyl-hydroxy (RE 53498) | 5.7 | 0.69 |
| flurtamone-desmethyl (RE 39748) | 3.7 | 0.43 |
| flurtamone (RE 40885) | 6.0 | 0.71 |
| **Total** | 95 | 11.19 |

* 3-min peak ** 4-min peak

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.2.5-5: Composition of conjugate fractions of radioactive residues in non-edible tissues of bluegill sunfish exposed for twenty-eight days to radiolabelled flurtamone in aqueous solution at 0.11 mg/L

| **Component** | **% TRR** | **Concentration (mg/kg)** |
|---|---|---|
| 3-min peak: | | |
| flurtamone-trifluoromethyl-mandelamide (RE 53286) glucuronide | 0.5 | 0.07 |
| flurtamone-trifluoromethyl-N-methyl-mandelamide (RE 53285) glucuronide | 1.0 | 0.12 |
| flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 54578) glucuronide | 2.3 | 0.27 |
| flurtamone-trifluoromethyl-hydroxy (RE 53498) glucuronide | 2.5 | 0.30 |
| flurtamone-desmethyl (RE-39748) glucuronide | 0.5 | 0.06 |
| flurtamone-trifluoromethyl-mandelamide (RE 53286) sulfate | 1.0 | 0.12 |
| flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 54578) sulfate | 1.2 | 0.14 |
| flurtamone-trifluoromethyl-hydroxy (RE 53498) sulfate | 1.1 | 0.13 |
| flurtamone-trifluoromethyl-mandelamide (RE 53286) ester | 0.4 | 0.05 |
| 4-min peak: | | |
| flurtamone-trifluoromethyl-mandelamide (RE 53286) glucuronide | 5.3 | 0.64 |
| flurtamone-trifluoromethyl-N-methyl-mandelamide (RE 53285) glucuronide | 13.2 | 1.6 |
| Flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 54578) glucuronide | 27.9 | 3.3 |
| flurtamone-trifluoromethyl-hydroxy (RE 53498) glucuronide | 4.9 | 0.58 |
| flurtamone-desmethyl (RE 39748) glucuronide | 2.8 | 0.34 |
| Total | 64.6 | 7.7 |

Table 6.2.5-6: Proportions of components (free or conjugated) of radioactive residues in non-edible tissues of bluegill sunfish exposed for twenty-eight days to radiolabelled flurtamone in aqueous solution at 0.11 mg/L

| **Component** | **% TRR** | **Concentration (mg/kg)** |
|---|---|---|
| flurtamone-trifluoromethyl-mandelamide (RE 53286) | 10 | 1.2 |
| flurtamone-trifluoromethyl-N-methyl-mandelamide (RE 53285) | 21 | 2.6 |
| flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 54578) | 38 | 4.5 |
| flurtamone-trifluoromethyl-hydroxy (RE 53498) | 13 | 1.6 |
| flurtamone-desmethyl (RE 39748) | 7.0 | 0.83 |
| Flurtamone (RE40885) | 6.0 | 0.71 |
| Total | 95 | 11.4 |

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
violation of the underlying license agreement.

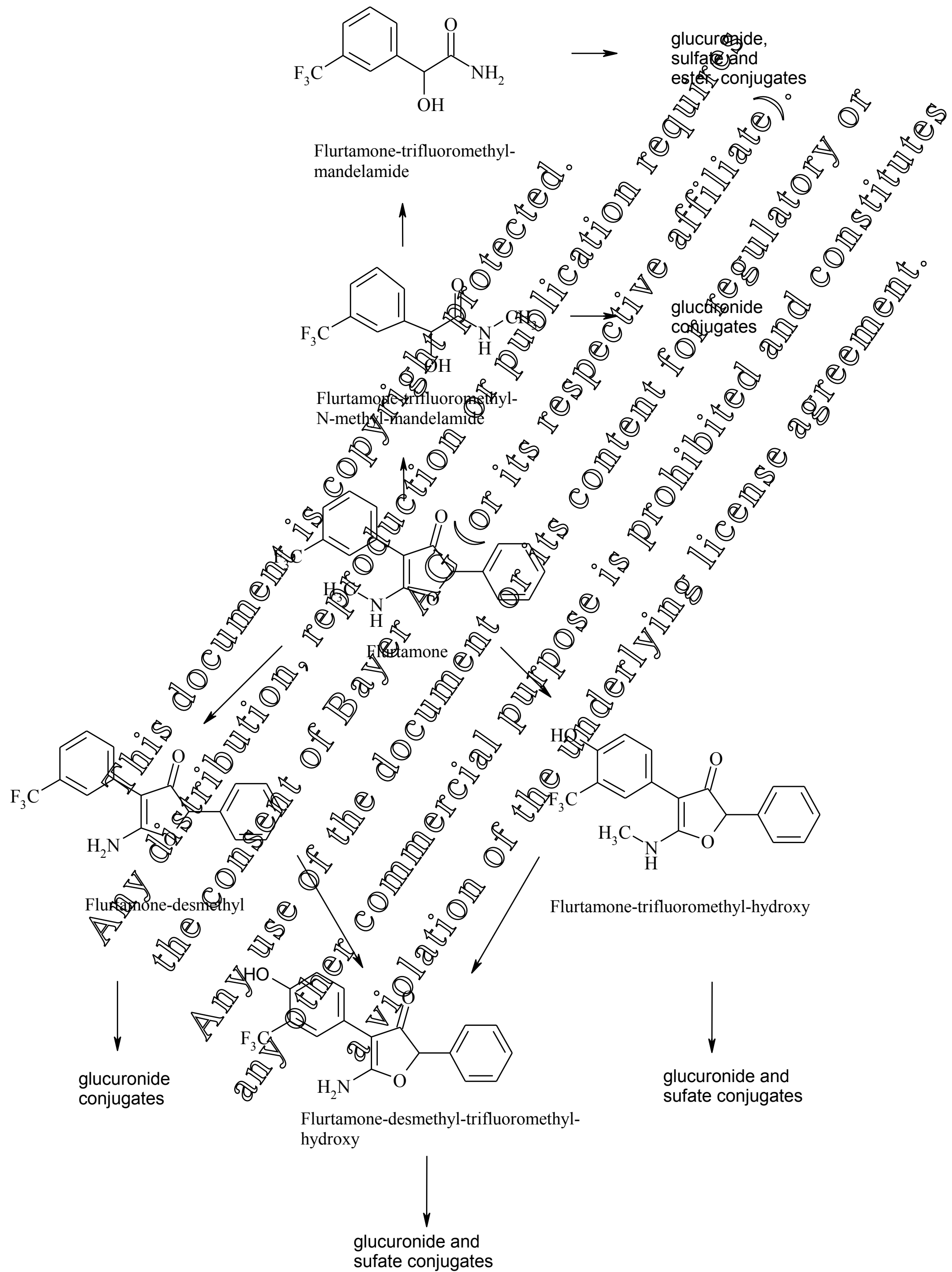
### III Conclusion

In bluegill sunfish exposed to flurtamone at concentration of 0.11 mg/L over a period of 28 days, the concentration of residues in edible tissue reached an apparent steady state after 14 days. For whole body tissues a steady state was reached after 7 days; the mean steady state concentration was 2.9 mg/kg which established a bioconcentration factor of 27. The results from a 14-day depuration phase showed that the biological half-life (50% elimination) occurred between 1 and 3 days. By the end of the depuration period 81% elimination had been reached.

The investigation of the metabolism of flurtamone showed that major metabolites included flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-N-methyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy and flurtamone-desmethyl. All but the last of these were found (mostly in a free form but with some also present as glucuronide conjugates) in edible tissue, in which they were accompanied by parent flurtamone that accounted for approx. 40% of the residue. All the metabolites listed were found in non-edible tissue, both in free form and in the form of glucuronide, sulfate and/or ester (minor) conjugates with the conjugated forms generally the more abundant. In non-edible tissue parent flurtamone accounted for only 6% of the residue. Metabolism occurred by N-demethylation, hydroxylation of the trifluoromethyl phenyl ring and subsequent conjugations (principally glucuronide and sulfate). Another branch of the metabolic pathway was created by the opening of the furanone ring followed by N-demethylation. A metabolic pathway was proposed, which is presented in Figure 6.2.5-1.

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Figure 6.2.5-1 Proposed metabolic pathway for flurtamone in bluegill sunfish




This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Summary on Metabolism, distribution and expression of residue in livestock**

Metabolism of flurtamone was investigated in poultry (laying hen), and ruminant (lactating goat) following application of both [phenyl-UL-$^{14}$C]- and [$^{14}$C-trifluoromethylphenyl]-flurtamone. The most recent studies on hen (cf. KCA 6.2.2 /01; ██████ , J., 2013; M-448149-02-1) and on goat (cf. KCA 6.2.3 /01; ██████ . 2013; M-445646-01-1) show a conclusive picture on the metabolic behaviour of flurtamone.

The metabolism of flurtamone appeared to be very similar in both species. Flurtamone was moderately metabolized and quickly excreted. The excretion products at sacrifice accounted for 75% of the administered dose in goat (98% of recovered radioactivity) and 89% in hen (99% of recovered radioactivity). Highest TRR-values were measured in liver followed by kidney indicating the significance of these organs for the metabolism and excretion of flurtamone.

In both species flurtamone was moderately metabolized into few major and several minor or trace metabolites. 72% to 98% of the TRR could be solubilized and identification rates ranged between 37% and 98%. The most prominent constituent of the residue was parent compound accounting for 1% - 44% of the TRR in hen and 12% - 78% in goat. Other metabolites were at or below 17% of the TRR despite Flurtamone-desmethyl which accounted for 46% of the TRR in hen fat and was therefore major in hen but only minor in goat. Further occasionally major metabolites were flurtamone-mandelic acid (major in hen, minor in goat), flurtamone-difluoromethyl-hydroxy-gluA (major in goat, minor in hen) and flurtamone-dihydroxy-pyrrolinone (major in goat, minor in hen).

Total radioactive residues in milk (mean of morning and evening milk) and eggs were 0.04 mg/kg and 0.1 mg/kg respectively. Main residue constituent was again parent compound accounting for 22% of the TRR in both milk (mean of morning and evening milk) and eggs. The identified metabolic reactions were hydroxylation of the trifluoromethyl-phenyl moiety, followed by conjugation withglucuronic acid, hydroxylation and rearrangement of the furanone to a pyrrolinone moiety, oxidative ring opening of the furanone moiety, followed by cleavage and degradation of the carbon chain and N-demethylation.

The residue levels given above arose from treatment with flurtamone at 1 mg/kg body weight per day in goat and hen. In normal agricultural practice the uptake of farm animal would stay below 0.004 mg/kg bodyweight per day. Therefore residues reported above are overestimated by at least a factor of 250. Consequently total residues in food of animal originating from normal agricultural practice would be far less than 0.01 ppm ranging from 5 ppb in goat liver down to 0.2 ppb in meat. Total residues in milk and eggs would be 0.16 ppb and 0.38 ppb respectively.

Additionally, in the course of a fish bioaccumulation study (cf. KCA 6.2.5 /01; ██████, E., 1994; M-162223-01-1), the metabolism of flurtamone in bluegill sunfish was investigated.

The metabolism of Flurtamone in bluegill sunfish was found to be rather extensive. After 28 days of exposure, parent Flurtamone was found to constitute approximately 6% and 40% ofradioactive residues in the viscera and edible tissues, respectively. Major metabolites found inthe viscera tissue included flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-N-methyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, flurtamone-trifluoromethyl-hydroxy, and flurtamone-desmethyl. These metabolites were present as both free molecules and conjugates of glucuronic acid. flurtamone-trifluoromethyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, and flurtamone-trifluoromethyl-hydroxy were also present as sulphate esters. In addition to

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

glucuronide and sulphate conjugations, small quantities of fatty acid esters of flurtamone-trifluoromethyl-mandelamide were also observed.

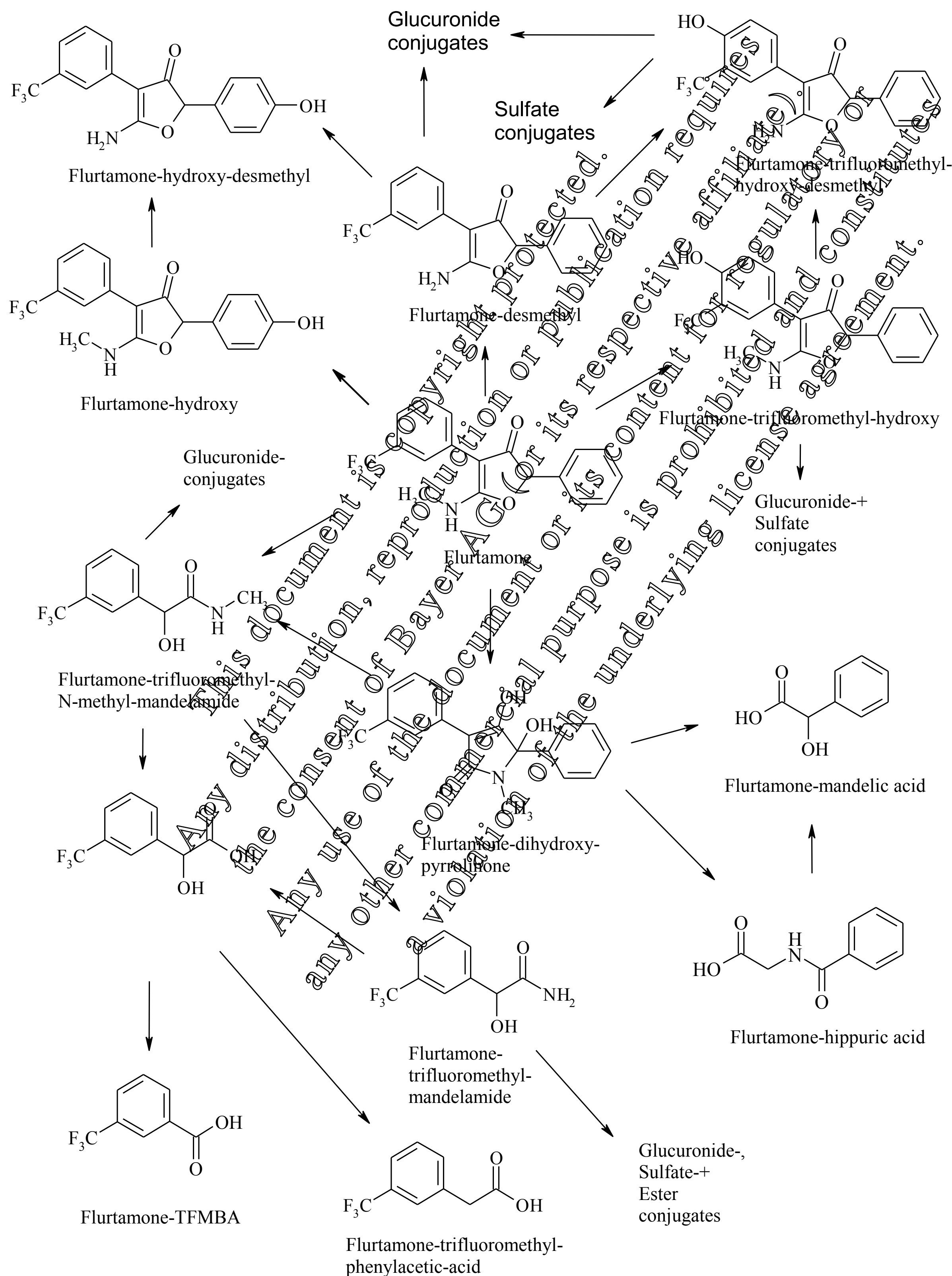
Major metabolites found in the edible tissue included flurtamone-trifluoromethyl-mandelamide, flurtamone-trifluoromethyl-N-methyl-mandelamide, flurtamone-desmethyl-trifluoromethyl-hydroxy, and flurtamone-trifluoromethyl-hydroxy along with their glucuronide conjugates.

Metabolism of flurtamone by bluegill sunfish involved various known metabolic pathways as the detoxification mechanism. These pathways include N-demethylation, hydroxylation of the trifluoromethyl phenyl ring followed by glucuronide and sulfate conjugations. The metabolic pathway also include the opening of the furanone ring to form flurtamone-trifluoromethyl-N-methyl-mandelamide followed by N-demethylation to form flurtamone-trifluoromethyl-mandelamide.

The discovered metabolic pathways in the different livestock species hen, goat and fish followed the same routs proceeding via hydroxylation followed by conjugation, cleavage of the furanone moiety and N-demethylation. Acommon metabolic pathway is shown by Figure 6.2.5-2.

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

Figure 6.2.5-2: Common metabolic pathway of flurtamone in livestock (hen, goat and fish)




This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.3 Magnitude of residue trials in plants

### CA 6.3.1 Cereals

Since Annex I inclusion, new residue studies have been performed on barley and wheat which can be extrapolated to the other cereals crops in case of early treatment (SANCO 7525/VI/95 rev 9 - March 2011).The following representative use supported on cereals is presented in Table CA 6.3.1-1.

**Table CA 6.3.1-1: Use pattern (GAPs) for the spray application of flurtamone containing formulations on cereals in Europe (Northern and Southern regions)**

| Crop | Member state or country | F / G or I | Formulation<br><br>Conc. of as | Pests or group of pests controlled | Growth stage | Number | Water (L/ha) | Application (g as/ha) | PHI |
|---|---|---|---|---|---|---|---|---|---|
| Spring cereals (Barley, wheat)<br><br>Winter cereals (Barley, oat, rye, spelt, triticale, wheat) | Europe North / South | F | SC<br><br>250 g/L flurtamone<br><br>+ 100 g/L diflufenican | Broad-leaved weeds & grassweeds | Pre-emergence (BBCH 00-09)<br><br>Or<br><br>Post-emergence (BBCH 10-29) | 1 | 200-400 | 125 g flurtamone<br><br>+ 50 g diflufenican | None - Covered by the normal vegetation period between last application and harvest |

A total of 24 supplementary trials were performed on barley and wheat since Annex I inclusion to support this new use pattern. Within these 24 trials, 8 (4 northern + 4 southern EU trials) were performed with SC350 product at the cGAP. In addition, 16 additional trials (8 northern + 8 southern EU trials) performed with SC360 product support also the cGAP with a flurtamone application rate slightly lower (120 g a.s./ha vs 125 g a.s./ha). Despite the fact that the SC350 trials were realized with applications done at BBCH 25 while SC360 trials were performed with applications between BBCH 22 and BBCH 30, no residue were found in all sample material (<LOQ of 0.01 mg/kg):

- green material harvested at BBCH 51 corresponding to the forage stage,
- whole plant without roots at BBCH 83 corresponding to the silage stage,
- grain at BBCH 89,
- straw at BBCH 89, with only one exception at 0.021 mg/kg in a European southern trial (11-2094-04 – barley). For that reason all these trials are reported altogether for a better overview.

The number and distribution of residue trials are described in Table CA 6.3.1-2.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table CA 6.3.1-2: Number and distribution of residue trials conducted per geographical region on cereals (wheat, barley)**

| Formulation Name | Climatic zone, Countries | Formula-tion type | Year / No. of trials | Reference Study number / Doc No. |
|---|---|---|---|---|
| **Europe North** | | | | |
| DFF+FLT SC 100+250A G U-EU | Germany and United Kingdom | SC350 | 2012 / 2 | 12-2000 (a) / M-457395-01-1 |
| | Germany and the Netherlands | SC350 | 2012 / 2 | 12-2004 (a) / M-459808-01-1 |
| Diflufenican + Flufenacet + Flurtamone SC 360 (120 + 120 + 120 g / L) | Germany and United Kingdom | SC360 | 2011 / 2 | 11-2094 (a) / M-460003-01-1 |
| | Germany and the Netherlands | SC360 | 2011 / 2 | 11-2095 (a) / M-459755-01-1 |
| | Germany, Belgium and the Netherlands | SC360 | 2012 / 4 | 12-2001 (a) / M-459795-01-1 |
| **Europe South** | | | | |
| DFF+FLT SC 100+250A G U-EU | Southern France and Italy | SC350 | 2012 / 2 | 12-2000 (a) / M-457395-01-1 |
| | Spain and Portugal | SC350 | 2012 / 2 | 12-2004 (a) / M-459808-01-1 |
| Diflufenican + Flufenacet + Flurtamone SC 360 (120 + 120 + 120 g / L) | Southern France and Italy | SC360 | 2011 / 2 | 11-2094 (a) / M-460003-01-1 |
| | Southern France and Spain | SC360 | 2011 / 2 | 11-2095 (a) / M-459755-01-1 |
| | Southern France, Spain, Italy and Portugal | SC360 | 2012 / 4 | 12-2002 (a) / M-459799-01-1 |

(a) Samples were analysed with the following analytical method: 01328

The formulations used in the residue trials are presented below:

| Formulation name | Formulation type | Composition |
|---|---|---|
| DFF+FLT SC 100+250A G U-EU | SC350: Suspension concentrate | 100 g/L of diflufenican + 250 g/L of flurtamone |
| Diflufenican + Flufenacet + Flurtamone SC 360 (120 + 120 + 120 g / L) | SC360: Suspension concentrate | 120 g/L of diflufenican + 120 g/L of flufenacet + 120 g/L of flurtamone |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| **Report:** | **KCA 6.3.1/01, [redacted], C.; 2013** |
|---|---|
| Title: | Determination of the residues of flurtamone in/on winter barley after spray application of DFF & FLT SC 350 in Germany, the United Kingdom, southern France and Italy |
| Document No: | M-457395-01-1 (study No. 12-2000) |
| Guidelines: | • REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>• EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997)<br>• OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS; Crop Field Trial<br>• US EPA OCSPP Guideline No. 860.1500 |
| GLP | yes |

| **Report:** | **KCA 6.3.1/02, [redacted], C.; 2013** |
|---|---|
| Title: | Determination of the residues of flurtamone in/on winter wheat after spray application of DFF & FLT SC 350 in Germany, the Netherlands, Spain and Portugal - DFF+FLT SC 100+250A |
| Document No: | M-459808-01-1 (Study No. 12-2004) |
| Guidelines: | • REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>• EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997)<br>• OECD 509 Adopted 2009-09-07, OECD Guideline for the testing of chemicals<br>• Crop Field Trial; US EPA OCSPP Guideline No. 860.1500 |
| GLP | yes |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG or its respective affiliate(s). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## Material and Methods:

A total of 8 supervised field trials (four in northern Europe and four in southern Europe) were performed in 2011-2012 growing seasons on winter cereals (4 trials on barley and 4 trials on wheat). In northern Europe, the trials were performed in United Kingdom (1), Germany (2), United Kingdom (1) and the Netherlands (1) and in southern Europe in Italy (1), Spain (1), southern France (1) and Portugal (1). Bacara ( DFF+FLT 350 SC), a co-formulation of diflufenican (100 g/L) and flurtamone (250 g/L) was sprayed once on wheat or barley with a nominal product rate of 0.5 L/ha (actual product 0.5 L/ha). The water rate was 200 - 400 L/ha, corresponding to a use rate of 0.125 kg a.s./ha for flurtamone and 0.05 kg a.s./ha for diflufenican. The applications were carried out during tillering i.e. at BBCH 25.

Samples of green material were taken at day 0, just after the application and 46-176 days after the treatment at BBCH 51 (forage stage). Samples of whole plant without root were taken at BBCH 83 (silage stage) and samples of grain and straw were taken at harvest i.e. BBCH 89.

Samples of wheat or barley green material, whole plant without root, grain and straw were analysed for residues of flurtamone according to the method 01328 by LC-MS/MS with an LOQ of 0.01 mg/kg for all sample materials.
Residues of diflufenican were not determined in these studies.

## Findings:

No apparent residues were found in any of the untreated samples, i.e. residues were < LOQ for flurtamone.
The storage period of deep-frozen samples of barley (green material, whole plant without roots, straw or grain) ranged from 119 days to 390 days for flurtamone. The storage period of deep-frozen samples of wheat (green material, whole plant without roots, straw or grain) ranged from 119 days to 353 days for flurtamone.

In order to check the performance of the method recovery determinations were conducted prior to and concurrently with the measurement of the samples from the residue studies.
The mean of the concurrent recoveries were for all fortification levels, within the acceptable range of 70 – 110 % with relative standard deviations (RSD) lower than 20% for flurtamone. Consequently all results are considered as valid. See Table CA 6.3.1-4.

The results of these trials are summarised in Table CA 6.3.1-3 and in greater detail in the Tier 1 summary forms.
Residues of flurtamone in/on winter cereals were < 0.01 mg/kg in green material (BBCH 51 corresponding to forage stage, 46-176 days after application), in whole plant without root (BBCH 83 corresponding to silage stage, 73-204 days after application) and in grain and straw at harvest (BBCH 89).

For study 12-2000, during transport or storage of field samples minor deviation of the temperature was recorded for trial 03 where the temperature exceeded -18°C for a short period of time. Since the samples remained almost deep frozen throughout transport or storage it is deemed unlikely that a degradation of residues could have occurred after temperature increase for such a limited period of time. Therefore the deviation was considered acceptable.
However, in 2 trials from study 12-2004 (03 and 04) -18°C were exceeded during shipment for several days. In order to investigate a possible impact on the stability of the residues this deviation is addressed by a short term stability study reflecting the conditions relevant to these samples (cf. report S13-03307).

## Conclusion:

Eight residue trials were conducted in northern and southern Europe in/on winter cereals with Bacara (DFF+FLT 350 SC) under field conditions in 2011 and 2012. Wheat or barley plants received one spray application at nominal 0.5 L product/ha.
Residues of flurtamone in/on winter cereals were < 0.01 mg/kg in green material (BBCH 51 corresponding to forage stage, 46-176 days after application), in whole plant without root (BBCH 83

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

corresponding to silage stage, 73-204 days after application) and in grain and straw at harvest (BBCH 89).
Residues of diflufenican were not determined in these studies.

**Table CA 6.3.1-3: Results of residue trials conducted in/on wheat or barley in Northern and Southern Europe with FLT+DFF 350 SC**

**Residues for flurtamone**

| Study<br>Trial No.<br>GLP<br>Year | Crop<br>Variety | Country | Application | | | | | Residues | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | FL | No | kg/ha (a.s.) | kg/hL (a.s.) | GS | Portion analysed | DALT (days) | Growth stage (BBCH) | flurta-mone (mg/kg) |
| **Northern Europe** | | | | | | | | | | | |
| 12-2000<br>12-2000-01<br>GLP: yes<br>2011 | Barley, winter<br>Ketos | Germany<br>51399<br>Burscheid<br>Europe, North | 350 SC | 1 | 0.125 | 0.042 | 25 | green material<br>whole plant without roots<br>grain<br>straw | 0<br>176<br>204<br>257<br>257 | 25<br>51<br>83<br>89<br>89 | 1.7<br><0.01<br><0.01<br><0.01<br><0.01 |
| 12-2000<br>12-2000-02<br>GLP: yes<br>2012 | Barley, winter<br>Carat | United Kingdom<br>CG8 8SS<br>Cambridge<br>Europe, North | 350 SC | 1 | 0.125 | 0.063 | 25 | green material<br>whole plant without roots<br>grain<br>straw | 0<br>119<br>164<br>203<br>203 | 25<br>51<br>83<br>89<br>89 | 13<br><0.01<br><0.01<br><0.01<br><0.01 |
| 12-2004<br>12-2004-01<br>GLP: yes<br>2012 | Wheat, winter<br>Inspiration | Germany<br>59457<br>Werl-Westönnen<br>Europe, North | 350 SC | 1 | 0.13 | 0.042 | 25 | green material<br>whole plant without roots<br>grain<br>straw | 0<br>63<br>109<br>133<br>133 | 25<br>51<br>83<br>89<br>89 | 4.7<br><0.01<br><0.01<br><0.01<br><0.01 |
| 12-2004<br>12-2004-02<br>GLP: yes<br>2012 | Wheat, winter<br>Taureq; winter | Netherlands<br>1774 PE<br>Slootdorp<br>Europe, North | 350 SC | 1 | 0.13 | 0.043 | 25 | green material<br>whole plant without roots<br>grain<br>straw | 0<br>46<br>83<br>112<br>112 | 25<br>51<br>83<br>89<br>89 | 5.4<br><0.01<br><0.01<br><0.01<br><0.01 |
| **Southern Europe** | | | | | | | | | | | |
| 12-2000<br>12-2000-03<br>GLP: yes<br>2012 | Barley, winter<br>Queen | France<br>31620<br>Bouloc<br>Europe, South | 350 SC | 1 | 0.125 | 0.042 | 25 | green material<br>whole plant without roots<br>grain<br>straw | 0<br>113<br>132<br>167<br>167 | 25<br>51<br>83<br>89<br>89 | 6.0<br><0.01<br><0.01<br><0.01<br><0.01 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

| Study Trial No. GLP Year | Crop Variety | Country | Application FL | No | kg/ha (a.s.) | kg/hL (a.s.) | GS | Residues Portion analysed | DALT (days) | Growth stage (BBCH) | flurtamone (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 12-2000 12-2000-04 GLP: yes 2012 | Barley, winter Luteche | Italy 40128 Bologna Europe, South | 350 SC | 1 | 0.125 | 0.031 | 25 | green material | 0 | 25 | 3.6 |
| | | | | | | | | | 46 | 51 | <0.01 |
| | | | | | | | | whole plant without roots | 73 | 83 | <0.01 |
| | | | | | | | | grain | 103 | 89 | <0.01 |
| | | | | | | | | straw | 103 | 89 | <0.01 |
| 12-2004 12-2004-03 GLP: yes 2012 | Wheat, winter Artur Nick | Spain 41004 Alcala de Guadaira Europe, South | 350 SC | 1 | 0.13 | 0.043 | 25 | green material | 0 | 25 | 5.4 |
| | | | | | | | | | 52 | 51 | <0.01 |
| | | | | | | | | whole plant without roots | 89 | 83 | <0.01 |
| | | | | | | | | grain | 115 | 89 | <0.01 |
| | | | | | | | | straw | 115 | 89 | <0.01 |
| 12-2004 12-2004-04 GLP: yes 2011 | Wheat, winter Hystar; Hybrid | Portugal 2005-009 Casais da Narçisa Europe, South | 350 SC | 1 | 0.13 | 0.043 | 25 | green material | 0 | 25 | 4.3 |
| | | | | | | | | | 110 | 51 | <0.01 |
| | | | | | | | | whole plant without roots | 164 | 83 | <0.01 |
| | | | | | | | | grain | 192 | 89 | <0.01 |
| | | | | | | | | straw | 192 | 89 | <0.01 |

Flurtamone: Final determination as flurtamone, residues calculated as flurtamone

**Table CA 6.3.1-4: Procedural recoveries for flurtamone in/on wheat or barley**

| Study | Crop | Sample material | n | Fortification level (mg/kg) | Recovery (%) Individual recoveries | Min | Max | Mean | RSD | LOQ [mg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 12-2000 & 12-2004* | Barley, winter | green material # | 9 | 0.01 | 73; 74; 86; 86; 104; 98; 88; 105; 98 | 73 | 105 | 90 | 13.2 | 0.01 |
| | | | 8 | 0.10 | 81; 82; 85; 92; 94; 97; 99; 99 | 81 | 99 | 91 | 7.9 | |
| | | | 2 | 10 | 87; 93 | 87 | 93 | 90 | | |
| | | | **19** | **overall** | | **73** | **105** | **90** | **10.1** | |
| | | grain | 4 | 0.01 | 84; 101; 80; 103 | 80 | 103 | 92 | 12.7 | 0.01 |
| | | | 4 | 0.10 | 90; 100; 94; 93 | 90 | 100 | 94 | 4.4 | |
| | | | **8** | **overall** | | **80** | **103** | **93** | **8.8** | |
| | | straw | 4 | 0.01 | 81; 80; 95; 97 | 80 | 97 | 88 | 10.2 | 0.01 |
| | | | 4 | 0.10 | 72; 84; 97; 92 | 72 | 97 | 86 | 12.6 | |
| | | | **8** | **overall** | | **72** | **97** | **87** | **10.7** | |

FL = Fortification level, RSD = Relative standard deviation, n = number of tests, LOQ = Practical limit of quantification
Fortified with flurtamone, determined as flurtamone and calculated as flurtamone
* All the recoveries were performed during the conduct of the studies 12-2000 and 12-2004.
# Sample materials green material and whole plant without root are grouped to the sample group cereals green material

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| **Report:** | **KCA 6.3.1/03, [redacted],S.; 2013** |
|---|---|
| Title: | Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter barley after spray application of DFF & FFA & FLT SC 360 in Germany, the United Kingdom, southern France and Italy |
| Document No: | M-460003-01-1 (study No. 11-2094) |
| Guidelines: | • REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>• OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial.<br>• EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)<br>• US EPA OCSPP Guideline No. 860.1500 |
| GLP | Yes |

| **Report:** | **KCA 6.3.1/04, [redacted].; 2013** |
|---|---|
| Title: | Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter wheat after spray application of DFF & FFA & FLT SC 360 in Germany, the Netherlands, southern France and Spain |
| Document No: | M-459755-01-1 (study No. 11-2095) |
| Guidelines: | • REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>• OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial<br>• EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)<br>• US EPA OCSPP Guideline No. 860.1500 |
| GLP | Yes |

This document is copyright protected. Any distribution or reproduction or publication of this document requires the consent of Bayer AG or its respective affiliate(s). Any use of the document content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| **Report:** | **KCA 6.3.1/05, [redacted], C.; 2013** |
|---|---|
| Title: | Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after spraying of DFF & FFA & FLT SC 360 in the field in Germany, Belgium and the Netherlands |
| Document No: | M-459795-01-1 (study No. 12-2001) |
| Guidelines: | • REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>• EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997)<br>• OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS; Crop Field Trial<br>• US EPA OCSPP Guideline No. 860.1500 |
| GLP | yes |

| **Report:** | **KCA 6.3.1/06, [redacted], C.; 2013** |
|---|---|
| Title: | Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after spray application of DFF & FFA & FLT SC 360 in Southern France, Italy, Spain and Portugal |
| Document No: | M-459799-01-1 (study No. 12-2002) |
| Guidelines: | • REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC<br>• EC guidance working document 7029/VI/95 rev.5 (July 22, 1997);<br>• OECD 509 Adopted 2009-09-07, OECD Guideline for the testing of chemicals;<br>• Crop Field Trial; US EPA OCSPP Guideline No. 860.1500 |
| GLP | yes |

This document is copyright protected. Any distribution or reproduction or publication requires the consent of Bayer AG or its respective affiliate(s). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

## Material and Methods:

A total of 16 supervised field trials (eight in northern Europe and eight in southern Europe) were performed in 2011 and 2012 growing seasons on winter cereals (barley and wheat). In northern Europe, the trials were performed in United Kingdom (1), Germany (4), Belgium (1) and the Netherlands (2) and in southern Europe in Italy (2), Spain (2), southern France (3) and Portugal (1). Bacara forte (DFF+FFA+FLT 360 SC), a co-formulation of flurtamone (120 g/L), flufenacet (120 g/L) and diflufenican (120 g/L) was sprayed once on wheat or barley with a nominal product rate of 1 L/ha (actual product rate from 0.92 to 1.01 L/ha). The water rate was 200 - 400 L/ha, corresponding to a use rate of 0.12 kg a.s./ha (0.11 kg a.s./ha for 2 trials) for all active substances. The applications were carried out during tillering i.e. between BBCH 22 and BBCH 30.

Samples of green material were taken 28-192 days after the treatment at BBCH 49-57 (forage stage). For two studies (eight trials), additional samples of green material were taken the day of application, and 1 day, 3 days, 5 days and 14 days thereafter (BBCH 26-32). Samples were collected for ecotoxicological evaluation for the calculation of DT50 values. Samples of whole plant without root were taken at BBCH 83-85 (silage stage) and samples of grain and straw were taken at harvest i.e. BBCH 89 (one trial at BBCH 92). Samples of wheat or barley green material, whole plant without root, grain and straw were analysed for residues of flurtamone according to the method 01328 by LC-MS/MS with an LOQ of 0.01 mg/kg for all sample materials.
Residues of flufenacet and diflufenican were analysed but not reported in this dossier.

## Findings:

No apparent residues were found in any of the untreated samples, i.e. residues were < LOQ for flurtamone.
The relative dry matter of control and treated samples of cereals green material harvested at forage stage (BBCH 51) and whole plant without root at silage stage (BBCH 83) was determined for 2 studies (11-2094 and 11-2095). The determination of relative dry matter content was not conducted

according to GLP. The results of the determination of relative dry matter of these samples are shown in Table CA 6.3.1-5.

**Table CA 6.3.1-5: Relative dry matter content of control and treated samples at forage and silage stage**

| Trial no. Country | Control (C) / Treated (T) | Growth stage [BBCH] | DALT | Crop | Sample material | Relative dry matter [%] |
|---|---|---|---|---|---|---|
| 11-2095-01 Germany | C | 51 | 51 | wheat | green material | 21.9 |
| | T | 51 | 51 | | green material | 24.9 |
| | C | 83 | 93 | | whole plant without root | 41.9 |
| | T | 83 | 93 | | whole plant without root | 39.1 |
| 11-2095-02 Netherlands | C | 47-57* | 43 | wheat | green material | 23.8 |
| | T | 47-57* | 43 | | green material | 22.3 |
| | C | 83 | 95 | | whole plant without root | 43.6 |
| | T | 83 | 95 | | whole plant without root | 43.1 |
| 11-2095-03 France | C | 51 | 57 | wheat | green material | 21.9 |
| | T | 51 | 57 | | green material | 21.3 |
| | C | 83 | 90 | | whole plant without root | 33.3 |
| | T | 83 | 90 | | whole plant without root | 33.6 |
| 11-2095-04 Spain | C | 51 | 42 | wheat | green material | 30.3 |
| | T | 51 | 42 | | green material | 25.8 |
| | C | 83 | 68 | | whole plant without root | 43.6 |
| | T | 83 | 68 | | whole plant without root | 44.8 |
| 11-2094-01 Germany | C | 51 | 181 | barley | green material | 17.3 |
| | T | 51 | 181 | | green material | 15.4 |
| | C | 83 | 209 | | whole plant without root | 29.8 |
| | T | 83 | 209 | | whole plant without root | 30.3 |
| 11-2094-02 United Kingdom | C | 51 | 119 | barley | green material | 19.4 |
| | T | 51 | 119 | | green material | 22.3 |
| | C | 83 | 164 | | whole plant without root | 34.3 |
| | T | 83 | 164 | | whole plant without root | 38.3 |
| 11-2094-03 France | C | 51 | 55 | barley | green material | 27.9 |
| | T | 51 | 55 | | green material | 25.8 |
| | C | 83 | 83 | | whole plant without root | 40.7 |
| | T | 85 | 83 | | whole plant without root | 43.8 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| Trial no. Country | Control (C) / Treated (T) | Growth stage [BBCH] | DALT | Crop | Sample material | Relative dry matter [%] |
|---|---|---|---|---|---|---|
| 11-2094-04 Italy | C | 51 | 28 | barley | green material | 23.1 |
| | T | 51 | 28 | | green material | 22.7 |
| | C | 83 | 50 | | whole plant without root | 30.6 |
| | T | 83 | 50 | | whole plant without root | 30.6 |

* Due to extreme dry weather conditions, germination was partly delayed resulting in a range of different growth stages at sampling.

The storage period of deep-frozen samples of barley (green material, whole plant without roots, straw or grain) ranged from 24 days to 346 days for flurtamone. The storage period of deep-frozen samples of wheat (green material, whole plant without roots, straw or grain) ranged from 111 days to 345 days for flurtamone.

In order to check the performance of the method recovery determinations were conducted prior to and concurrently with the measurement of the samples from the residue studies.
The mean of the concurrent recoveries were for all fortification levels, within the acceptable range of 70 – 110 % (except for barley grain, wheat grain and green material at 10 mg/kg where the mean values were at 113%, 112% and 114%, respectively) with relative standard deviations (RSD) lower than 20% for flurtamone. Consequently all results are considered as valid. See Table CA 6.3.1-7.

The results of these trials are summarised in Table CA 6.3.1-6 and in greater detail in the Tier 1 summary forms.
Residues of flurtamone at harvest (BBCH 89-92) in/on winter cereals were < 0.01 mg/kg for grain and straw with only one exception in straw at 0.021 mg/kg.
Residues in/on winter cereals green material ranged from 3.5 to 9.5 mg/kg at the day of application (BBCH 25-30), declined to 0.24-3.2 mg/kg 4-6 days after application (BBCH 25-31), then declined to 0.023-1.0 mg/kg 2 weeks after application (BBCH 26-32) and were < 0.01 mg/kg at forage stage (BBCH 49-57, 28 to 192 days after application). Residues in whole plant without roots were < 0.01 mg/kg at silage stage (BBCH 83-85, 50 to 239 days after application).

During transport or storage of field samples minor deviations of the temperature were recorded for some trials where the temperature exceeded -18°C for a short period of time. Since the samples remained almost deep frozen throughout transport or storage it is deemed unlikely that a degradation of residues could have occurred after temperature increase for such a limited period of time. Therefore the deviations were considered acceptable.
However, in 2 trials from study 12-2002 (03 and 04) -18°C were exceeded during shipment for several days. In order to investigate a possible impact on the stability of the residues this deviation is addressed by a short term stability study reflecting the conditions relevant to these samples (cf. report S13-03307).

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliates).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

## Conclusion:

Sixteen residue trials were conducted in northern and southern Europe in/on winter cereals with Bacara forte (DFF+FFA+FLT 360 SC) under field conditions in 2011 and 2012. Wheat or barley plants received one spray application at nominal 1 L product/ha.

Residues of flurtamone at harvest (BBCH 89-92) in/on winter cereals were < 0.01 mg/kg for grain and straw with only one exception in straw at 0.021 mg/kg. Residues of flurtamone were < 0.01 mg/kg for green material at forage stage (BBCH 49-57) and for whole plant without roots at silage stage (BBCH 83-85).

**Table CA 6.3.1-6: Results of residue trials conducted in/on wheat or barley in Northern and Southern Europe with DFF+FFA+FLT 360 SC**

**Residues for flurtamone**

| Study Trial No. GLP Year | Crop Variety | Country | Application FL | No | kg/ha (a.s.) | kg/hL (a.s.) | GS | Residues Portion analysed | DALT (days) | Growth stage (BBCH) | flurta-mone (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Northern Europe** | | | | | | | | | | | |
| 11-2094<br>11-2094-01<br>GLP: yes<br>2011 | Barley, winter<br>Ketos winter barley | Germany<br>51399<br>Burscheid<br>Europe, North | 360 SC | 1 | 0.12 | 0.040 | 25 | green material | 0<br>1<br>3<br>5<br>14<br>181 | 25<br>25<br>25<br>25<br>29<br>51 | 4.4<br>2.3<br>2.2<br>1.5<br>1.0<br><0.01 |
| | | | | | | | | whole plant without roots | 209 | 83 | <0.01 |
| | | | | | | | | grain | 262 | 89 | <0.01 |
| | | | | | | | | straw | 262 | 89 | <0.01 |
| 11-2094<br>11-2094-02<br>GLP: yes<br>2012 | Barley, winter<br>Carat Winter Barley | United Kingdom<br>SG88SS<br>Cambridge<br>Europe, North | 360 SC | 1 | 0.12 | 0.060 | 25 | green material | 0<br>1<br>3<br>4<br>14<br>119 | 25<br>25<br>25<br>25<br>26<br>51 | 9.5<br>9.4<br>2.2<br>1.8<br>0.46<br><0.01 |
| | | | | | | | | whole plant without roots | 164 | 83 | <0.01 |
| | | | | | | | | grain | 203 | 89 | <0.01 |
| | | | | | | | | straw | 203 | 89 | <0.01 |
| 11-2095<br>11-2095-01<br>GLP: yes<br>2011 | Wheat, winter<br>Akteur | Germany<br>59457<br>Werl - Niederbergstrasse<br>Europe, North | 360 SC | 1 | 0.12 | 0.040 | 25 | green material | 0<br>1<br>3<br>5<br>14<br>51 | 25<br>25<br>26<br>27<br>31<br>51 | 5.0<br>4.2<br>3.1<br>2.1<br>0.14<br><0.01 |
| | | | | | | | | whole plant without roots | 93 | 83 | <0.01 |
| | | | | | | | | grain | 117 | 89 | <0.01 |
| | | | | | | | | straw | 117 | 89 | <0.01 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| Study Trial No. GLP Year | Crop Variety | Country | Application FL | No | kg/ha (a.s.) | kg/hL (a.s.) | GS | Residues Portion analysed | DALT (days) | Growth stage (BBCH) | flurta-mone (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11-2095 11-2095-02 GLP: yes 2011 | Wheat, winter Tabasco | Netherlands 1175 KD Lynden (Hoofddorp) Europe, North | 360 SC | 1 | 0.1104 | 0.0399 | 27 | green material | 0 | 25 | 9.1 |
| | | | | | | | | | 1 | 25 | 8.2 |
| | | | | | | | | | 3 | 27 | 5.7 |
| | | | | | | | | | 5 | 26 | 3.2 |
| | | | | | | | | | 14 | 28 | 0.18 |
| | | | | | | | | | 43 | 57 | <0.01 |
| | | | | | | | | whole plant without roots | 95 | 83 | <0.01 |
| | | | | | | | | grain | 121 | 89 | <0.01 |
| | | | | | | | | straw | 121 | 89 | <0.01 |
| 12-2001 12-2001-01 GLP: yes 2011 | Barley, winter Meridian | Germany 49377 Langförden Europe, North | 360 SC | 1 | 0.12 | 0.040 | 23 | green material | 170 | 49 | <0.01 |
| | | | | | | | | whole plant without roots | 216 | 83 | <0.01 |
| | | | | | | | | grain | 244 | 92 | <0.01 |
| | | | | | | | | straw | 244 | 92 | <0.01 |
| 12-2001 12-2001-02 GLP: yes 2011 | Barley, winter Saskia (early 6-rows variety, mid height) | Belgium 6210 Villers-Perwin Europe, North | 360 SC | 1 | 0.12 | 0.040 | 22 | green material | 181 | 49 | <0.01 |
| | | | | | | | | whole plant without roots | 209 | 83 | <0.01 |
| | | | | | | | | grain | 252 | 89 | <0.01 |
| | | | | | | | | straw | 252 | 89 | <0.01 |
| 12-2001 12-2001-03 GLP: yes 2011 | Wheat, winter Inspiration | Germany 59457 Werl-Westönnen Europe, North | 360 SC | 1 | 0.12 | 0.040 | 22 | green material | 192 | 49 | <0.01 |
| | | | | | | | | whole plant without roots | 239 | 83 | <0.01 |
| | | | | | | | | grain | 263 | 89 | <0.01 |
| | | | | | | | | straw | 263 | 89 | <0.01 |
| 12-2001 12-2001-04 GLP: yes 2012 | Wheat, winter Taureq winter | Netherlands 1774 PE Slootdorp Europe, North | 360 SC | 1 | 0.12 | 0.040 | 25 | green material | 41 | 49 | <0.01 |
| | | | | | | | | whole plant without roots | 83 | 83 | <0.01 |
| | | | | | | | | grain | 112 | 89 | <0.01 |
| | | | | | | | | straw | 112 | 89 | <0.01 |
| **Southern Europe** | | | | | | | | | | | |
| 11-2094 11-2094-03 GLP: yes 2011 | Barley, winter Kétos Winter Barley | France 86220 Leugny Europe, South | 360 SC | 1 | 0.12 | 0.040 | 29 | green material | 0 | 29 | 5.6 |
| | | | | | | | | | 1 | 29 | 5.8 |
| | | | | | | | | | 3 | 29 | 3.5 |
| | | | | | | | | | 6 | 29 | 1.7 |
| | | | | | | | | | 14 | 30 | 0.40 |
| | | | | | | | | | 55 | 51 | <0.01 |
| | | | | | | | | whole plant without roots | 83 | 85 | <0.01 |
| | | | | | | | | grain | 108 | 89 | <0.01 |
| | | | | | | | | straw | 108 | 89 | <0.01 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| 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) | Growth stage (BBCH) | flurta-mone (mg/kg) |
| 11-2094<br>11-2094-04<br>GLP: yes<br>2011 | Barley, winter<br>Aldebaran winter variety | Italy<br>44124 Ferrara<br>Europe, South | 360 SC | 1 | 0.12 | 0.030 | 25 | green material<br><br><br><br><br><br>whole plant without roots<br>grain<br>straw | 0<br>1<br>3<br>5<br>14<br>28<br>50<br>80<br>80 | 25<br>25<br>26<br>26<br>31<br>51<br>83<br>89<br>89 | 3.5<br>3.2<br>2.5<br>1.5<br>0.12<br><0.01<br><0.01<br><0.01<br>0.021 |
| 11-2095<br>11-2095-03<br>GLP: yes<br>2011 | Wheat, winter<br>Cezanne | France<br>86270 Mairé<br>Europe, South | 360 SC | 1 | 0.12 | 0.040 | 29 | green material<br><br><br><br><br><br>whole plant without roots<br>grain<br>straw | 0<br>1<br>3<br>6<br>14<br>57<br>90<br>119<br>119 | 29<br>29<br>29<br>29<br>30<br>51<br>83<br>89<br>89 | 8.6<br>7.5<br>5.0<br>2.1<br>0.33<br><0.01<br><0.01<br><0.01<br><0.01 |
| 11-2095<br>11-2095-04<br>GLP: yes<br>2011 | Wheat, winter<br>Moncada; sowing seed produc-tion | Spain<br>08520 Marata - Les Franqueses<br>Europe, South | 360 SC | 1 | 0.12 | 0.040 | 30 | green material<br><br><br><br><br><br>whole plant without roots<br>grain<br>straw | 0<br>1<br>2<br>5<br>14<br>42<br>68<br>103<br>103 | 30<br>30<br>30<br>31<br>32<br>51<br>83<br>89<br>89 | 8.4<br>8.7<br>6.2<br>0.24<br>0.023<br><0.01<br><0.01<br><0.01<br><0.01 |
| 12-2002<br>12-2002-01<br>GLP: yes<br>2011 | Barley, winter<br>Platine | France<br>13103 Saint Etienne du gres<br>Europe, South | 360 SC | 1 | 0.12 | 0.040 | 25 | green material<br>whole plant without roots<br>grain<br>straw | 121<br>155<br>192<br>192 | 49<br>83<br>89<br>89 | <0.01<br><0.01<br><0.01<br><0.01 |
| 12-2002<br>12-2002-02<br>GLP: yes<br>2012 | Barley, winter<br>Amillis | Italy<br>37050 Perzacco<br>Europe, South | 360 SC | 1 | 0.12 | 0.040 | 23 | green material<br>whole plant without roots<br>grain<br>straw | 46<br>76<br>105<br>105 | 49<br>83<br>89<br>89 | <0.01<br><0.01<br><0.01<br><0.01 |
| 12-2002<br>12-2002-03<br>GLP: yes<br>2012 | Wheat, winter<br>Artur Nick | Spain<br>41004 Alcala de Guadaira<br>Europe, South | 360 SC | 1 | 0.11 | 0.040 | 23 | green material<br>whole plant without roots<br>grain<br>straw | 51<br>92<br>119<br>119 | 49<br>83<br>89<br>89 | <0.01<br><0.01<br><0.01<br><0.01 |

This document is copyright-protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| 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) | Growth stage (BBCH) | flurta-mone (mg/kg) |
| 12-2002 12-2002-04 GLP: yes 2011 | Wheat, winter Hystar | Portugal 2005-009 Casais da Narcisa Europe, South | 360 SC | 1 | 0.12 | 0.040 | 22 | green material | 129 | 49 | <0.01 |
| | | | | | | | | whole plant without roots | 185 | 83 | <0.01 |
| | | | | | | | | grain | 213 | 89 | <0.01 |
| | | | | | | | | straw | 213 | 89 | <0.01 |

Flurtamone: Final determination as flurtamone, residues calculated as flurtamone

**Table CA 6.3.1-7: Procedural recoveries for flurtamone in/on wheat or barley**

| Study Number | Sample material | FL [mg/kg] | Single values [%] | Mean value [%] | RSD [%] | n | LOQ [mg/kg] |
|---|---|---|---|---|---|---|---|
| 11-2094 | barley, winter / grain | 0.01 | 73; 83; 102; 107; 119 | 97 | 19.2 | 5 | 0.01 |
| | | 0.10 | 81 | - | - | 1 | |
| | | 10 | 109; 113; 117 | 113* | 3.5 | 3 | |
| | | **Overall** | | **100** | **17.0** | **9** | |
| | barley, winter / green material | 0.01 | 71; 76; 79; 87; 87; 88; 94; 98; 100; 100 | 88 | 11.6 | 10 | 0.01 |
| | | 0.10 | 82; 88; 106; 107; 108 | 98 | 12.5 | 5 | |
| | | 10 | 105; 109; 115 | 110 | 4.6 | 3 | |
| | | **Overall** | | **94** | **13.5** | **18** | |
| | barley, winter / straw | 0.01 | 76; 78; 94; 94; 95 | 87 | 10.9 | 5 | 0.01 |
| | | 0.10 | 82; 93; 98; 102 | 94 | 9.2 | 4 | |
| | | **Overall** | | **90** | **10.2** | **9** | |
| 11-2095 | wheat, winter / grain | 0.01 | 81; 83; 95 | 86 | 8.8 | 3 | 0.01 |
| | | 0.10 | 105; 107; 114 | 109 | 4.3 | 3 | |
| | | 10 | 111; 113; 113 | 112* | 1.0 | 3 | |
| | | **Overall** | | **102** | **12.7** | **9** | |
| | wheat, winter / green material | 0.01 | 95; 104; 104; 101; 103; 113 | 103 | 5.6 | 6 | 0.01 |
| | | 0.10 | 108; 111; 118 | 112* | 4.6 | 3 | |
| | | 1.0 | 93; 94; 94 | 94 | 0.6 | 3 | |
| | | 10 | 112; 114; 117 | 114* | 2.2 | 3 | |
| | | **Overall** | | **105** | **8.2** | **15** | |
| | wheat, winter / straw | 0.01 | 79; 94; 111 | 95 | 16.9 | 3 | 0.01 |
| | | 1.0 | 86; 89; 89 | 88 | 2.0 | 3 | |
| | | **Overall** | | **91** | **11.8** | **6** | |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| Study Number | Sample material | FL [mg/kg] | Single values [%] | Mean value [%] | RSD [%] | n | LOQ [mg/kg] |
|---|---|---|---|---|---|---|---|
| 12-2001 & 12-2002*** | wheat or barley, winter / grain | 0.01 | 80; 84; 101; 103 | 92 | 12.7 | 4 | 0.01 |
| | | 0.10 | 90; 93; 94; 100 | 94 | 4.4 | 4 | |
| | | **Overall** | | **93** | **8.8** | **8** | |
| | wheat or barley, winter / green material | 0.01 | 73; 74; 86, 86; 88; 98; 98; 104; 105** | 90 | 13.2 | 9 | 0.01 |
| | | 0.10 | 81; 82; 85; 92; 94; 97; 97; 99 | 91 | 7.9 | 8 | |
| | | 10 | 87; 93 | 90 | 4.7 | 2 | |
| | | **Overall** | | **90** | **10.1** | **19** | |
| | wheat or barley, winter / straw | 0.01 | 80; 81; 95; 97 | 88 | 10.2 | 4 | 0.01 |
| | | 0.10 | 72; 84; 92; 97 | 86 | 12.6 | 4 | |
| | | **Overall** | | **87** | **10.7** | **8** | |

FL = Fortification level, RSD = Relative standard deviation, n = number of tests, LOQ = Practical limit of quantification
Fortified with flurtamone, determined as flurtamone and calculated as flurtamone
Samples of green material and whole plant without root were combined to “green material” for calculation of the mean value and RSD.
* This recovery mean value is considered acceptable due to a low RSD.
** Corrected for the blank value in the control sample (0.0052 mg/kg). Recovery before correction: 157%.
*** All the recoveries were performed during the conduct of the studies 12-2000, 12-2001, 12-2002 and 12-2004.

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.4 Feeding studies

### Dietary burden calculation

During the EU evaluation process the dietary burden for livestock was assessed based on the uses in cereals, sunflower and peas. Since the calculated dietary burdens for all groups of livestock were found to be below the trigger value of 0.1 mg/kg DM, further investigation of residues as well as the setting of MRLs in commodities of animal origin was not necessary.
This evaluation has been revised taking into account the supported uses within this dossier. Cereals might be fed to livestock. The median and maximum dietary burdens were therefore calculated for different groups of livestock using the OECD model.
The input residue levels considered for the dietary burden calculations and their outcomes conducted with the OECD model are reproduced in Table CA 6.4-1 and Table CA 6.4-2 respectively.
Use of flurtamone in cereals according to the recommended GAP is not likely to result in significant residues in any of the cereals commodities, except for barley straw with only one value of twenty-four at 0.021 mg/kg.
The calculated dietary burdens do not exceed the trigger value of 0.004 mg/kg bw/day for cattle, sheep, swine and poultry. Therefore, livestock metabolism and feeding studies are not required.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table CA 6.4-1: Input values for the dietary burden calculation**

| Commodity | Dietary burden | |
|---|---|---|
| | Input value (mg/kg) | Comment |
| **Risk assessment residue definition: flurtamone** | | |
| Cereals forage (Barley, oat, rye, triticale and wheat) | 0.010 | Highest residue |
| Cereals hay (Barley, oat, triticale and wheat) | 0.010 | Highest residue |
| Cereals straw<br>Barley, oat, rye and triticale<br>Wheat | <br>0.021<br>0.010 | Highest residue |
| Cereals silage (Barley, oat, rye, triticale and wheat) | 0.010 | Highest residue |
| Cereals grain (Barley, oat, rye, triticale and wheat) | 0.010 | Median residue |
| Barley, bran fractions* | 0.080 | Median residue |
| Wheat, milled by-products* | 0.080 | Median residue |

* A default processing factor of 8 was used on cereals (wheat and barley) grains.

**Table CA 6.4-2: Results of the dietary burden calculation according to OECD model**

| | Residue level in total feed dry matter (mg/kg) | Residue intake (mk/kg bw/day) |
|---|---|---|
| Cattle – beef | 0.042 | 0.001 |
| Cattle – dairy | 0.042 | 0.002 |
| Sheep – ram/ewe | 0.054 | 0.002 |
| Sheep – lamb | 0.062 | 0.003 |

| | Residue level in total feed dry matter (mg/kg) | Residue intake (mk/kg bw/day) |
|---|---|---|
| Swine – breeding | 0.057 | 0.001 |
| Swine – finishing | 0.051 | 0.002 |
| Poultry – broiler | 0.026 | 0.002 |
| Poultry – layer | 0.030 | 0.002 |
| Poultry – turkey | 0.025 | 0.002 |

## CA 6.4.1 Poultry

No new study was performed.

## CA 6.4.2 Ruminants

No new study was performed.

## CA 6.4.3 Pigs

No study was performed because the metabolic pathway of flurtamone was found similar in rats, poultry and ruminants. Therefore, it can be expected that the metabolism in other farm animal does not differ, and thus this study is not required.

## CA 6.4.4 Fish

No study was performed because no final test guideline or feeding tables are currently available which may detail how the dietary burden has to be calculated and which provide an agreed test methodology. Therefore it is the opinion of the applicant that it is not appropriate to address this issue until such guidance is available.

## CA 6.5 Effects of processing

In the supervised field residue trials, no residues of flurtamone above 0.01 mg/kg (Limit of Quantification) were found in grain. In addition, it is unlikely that potential for concentration in processed food is likely occur. Otherwise, a default processing factor of 8 for bran (used as animal feed) could be used. It has been shown in chapter CA 6.4 that the dietary burden is still under the trigger value of 0.004 mg/kg bw/d when this value is used in the dietary burden calculations.
Furthermore flurtamone is of low toxicity.
Therefore, no processing study is required to investigate the residues of flurtamone in processed cereal commodities.

## 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

Not relevant for cereals.

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.5.3 Magnitude of residues in processed commodities

No studies were performed.

## CA 6.6 Residues in rotational crops

### CA 6.6.1 Metabolism in rotational crops

Since Annex I inclusion, new confined rotational crops studies have been generated with radishes, lettuce and wheat as succeeding crops after plant back interval of 30, 120 and 365 days with the compound radiolabelled in the trifluoromethylphenyl ring and in the unsubstituted phenyl ring.

| **Report:** | **KCA 6.6.1/01, [redacted].; 1997** |
|---|---|
| Title: | [14C]-Flurtamone: A Confined Rotational Crop Study using Radishes and Lettuces |
| Document No: | M-158591-01-1 |
| Report No: | RPA Document 201056 |
| Guidelines: | **US (=USEPA) Subdivision N Section 165-1, EU 96/68/EC Section 6.6** |
| GLP | Yes |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Summary**

Flurtamone uniformly labelled in the trifluoromethyl-phenyl ring position was applied at a nominal rate of 375 g/ha to bare sandy loam soil. After plant back intervals of 30 (first rotation), 120 (second rotation) and 365 days (third rotation) a leafy vegetable (lettuce) and a root crop (radish) were planted to the treated containers to determine the metabolism of flurtamone in succeeding crops.

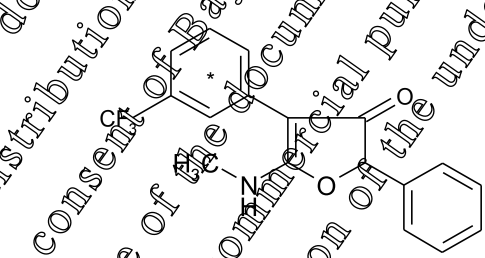
A steady decline in the soil total radioactive residue (TRR) was observed over the period of the study. At all sampling time-points the majority of radioactivity was found in the upper section (0-15 cm), indicating that neither flurtamone nor its metabolites had moved significantly down the soil profile.
The extractability of the soil residue decreased with time from 63-82% for the 30 day planting samples to 25-73% at the 365 day planting. Flurtamone, flurtamone-trifluoromethyl-N-methyl-mandelamide (RE 53285), flurtamone-TFMBA (RE-54488) and Flurtamone-desmethyl (RE-39748) were detected to be present in the soil extracts. For all three sampling time-points up to five minor metabolites were detected at very low levels.

Total radioactive residues in the final plant harvest samples of the first rotation (30 days) were low accounting for 0.1-0.5 mg/kg. The TRR subsequently further decreased to result 0.02-0.07 mg/kg in the second (120 days) and <0.01-0.05 mg/kg in the third rotation (365 days).
Extraction efficiency was 80-96% in lettuce and radish leaves. Radish tubers contained higher amounts of bound residues but extracted amounts still accounted for more than 70% of the TRR.
Only low amounts of the parent compound flurtamone could be observed in the lettuce and radish harvest samples accounting for 0.07-6.61% of the TRR (<0.001-0.006 mg/kg) for the first rotation and <0.7%-10.4% of the TRR (< 0.001 mg/kg-0.002 mg/kg) for the 2$^{nd}$ rotation. No active substance could be observed in the harvest samples of the 3$^{rd}$ rotation.

The major component of the TRR in all sample materials and at all planting intervals (interim and final harvest samples) was [$^{14}$C]-trifluoroacetic acid (TFA) which comprised 55.5-93.4 % (0.051-0.399 mg/kg) of the TRR in the 1$^{st}$ rotation samples.
The amounts of [$^{14}$C]-TFA found in the final harvest plant extracts of the 2$^{nd}$ rotation were detected at 0.012-0.060 mg/kg (63.5%-95.4% TRR). In the 3$^{rd}$ rotation TFA was the only metabolite which could be identified accounting for 0.025 mg/kg (81.0% TRR) in lettuces and 0.041 mg/kg (80.2% TRR) in radish leaves. The total radioactive residue in radish tubers was below 0.01 mg/kg in the 3$^{rd}$ rotation samples so that no identification of metabolites occurred.
No further major metabolites could be observed in the harvest samples. Minor metabolites detected were Flurtamone-trifluoromethyl-N-methyl-mandelamide (1$^{st}$ rotation only) and Flurtamone-trifluoromethyl-benzoic acid (Flurtamone-TFMBA) (1$^{st}$ and 2$^{nd}$ rotation). Only 0-3% of the extracted radioactivity was left unidentified.
Therefore, rapid and extensive metabolism of flurtamone had occurred in soil/plant matrices already by the time when 1$^{st}$ rotation samples were harvested. The metabolism pathway thereby followed routs already known from primary crop metabolism studies.

## I Material and Methods:

**Material**
The compound, batch number CSL-92-418-11-27, was $^{14}$C labelled in the trifluoromethyl phenyl ring. The non-labelled flurtamone used to radio-dilute the [$^{14}$C]-flurtamone in the preparation of the treatment solutions was batch IGB 838 and had a purity of 98.8%.



* = position of trifluoromethyl-phenyl ring label

Radiolabelled and non-radiolabelled flurtamone samples were mixed and dissolved in methanol. The specific activity and solution concentration were determined by an HPLC method. The concentration of flurtamone was determined to be 1.58 mg/mL with a specific activity of 2.11 MBq/mg. The radiopurity was 97.3%. This solution was diluted with methanol to result a concentration of 0.942 mg/mL such that a volume of 20 mL treatment solution contained 18.85 mg flurtamone.

**Soil characteristics:**

| Particle size distribution: | |
|---|---|
| < 2 µm | 9.2(%) |
| 2-20 µm | 13.9(%) |
| 20-63 µm | 22.3(%) |
| 63-106 µm | 7.8(%) |
| 106-250 µm | 27.1(%) |
| 250-500 µm | 13.5(%) |
| 500-2000 µm | 6.3(%) |

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purposes is prohibited and constitutes
violation of the underlying license agreement.

| | |
|---|---|
| pH: | |
| Water | 5.5 |
| KCl (1 M) | 4.5 |
| $CaCl_2$ (0.01 M) | 4.7 |
| Cation exchange capacity | 7.2 (meq/100 g) |
| Organic carbon | 2.6 (%) |
| Organic matter | 4.5 (%) |
| Classification: (USDA + ADAS) | Sandy loam |

**Culture plants:** radish (*Raphanus sativus*) 1st rotation, variety ‘French Breakfast 3’
2nd and 3rd rotation variety ‘Globe’
lettuce (*Lactuca sativa*) 1st, 2nd and 3rd rotation variety ‘Butterhead’

**Treatment**
Six cylindrical UPVC containment vessels (80 cm diameter, 60 cm deep) were buried in the ground at the Rhône-Poulenc Agriculture Research Farm at Manningtree in the County of Essex in the UK. Each vessel was filled with a locally obtained sandy loam soil. Two of the vessels were designated control plots and were treated with methanol (20 mL) followed by three aliquots of water (20 mL) applied by use of a customised agricultural sprayer. The remaining four vessels were treated with the product solution in the same manner, to give a nominal treatment rate of 375 g/ha. After each treatment the vials that had contained the solutions and the sprayer were rinsed and the resulting solution was radioassayed. These results were used to calculate the actual treatment rates.

Approximately thirty days after treatment one control plot and two treated plots were planted with radishes and the other control plot and the remaining two treated plots were planted with lettuces. At appropriate times interim and final harvest crops were sampled for analysis. At 120 days and 365 days after treatment mixed radishes were planted along with lettuces, as described above, and were subsequently harvested in the same manner. Before planting at all intervals the soil was tilled to simulate commercial seed-bed preparation. The crops were grown outdoors under ambient environmental conditions. Weather conditions, including daily maximum and minimum temperatures and rainfall, were documented. During the course of the study crop protection chemicals (excluding any related substance to flurtamone) and irrigation were used as necessary to ensure crop vigour.

**Sampling**
Soil cores of 5 cm diameter and 30 cm depth were taken from the edge, half-way to the centre and from the middle of a (different) treated plot at each rotational crop planting. The cores were divided into 15 cm segments and were frozen (-20°C) until processing for analysis.

Crops were sampled from control and treatment vessels at immature stage (approximately 12 leaves for lettuces) and at maturity. All crop samples except the 365 day planting final harvest samples were transported to the analytical laboratory on the same day as they were harvested. The 365 day planting final harvest samples were stored for three days at <4°C and then transported to the analytical laboratory. The root crop was separated into tubers and leaves.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Sample processing**

Each 15 cm soil core was weighed and divided into sub-samples of ≤ 190 g. These were placed in centrifuge bottles and were extracted with a mixture of sodium hydrogen sulfate, deionised water and methanol. The samples were centrifuged and after removal of the supernatant the soil pellets were re-extracted with methanol/water and other methanol containing solvent mixtures. The supernatants from each extraction were combined by sub-sample and radioassayed. The soil residues were allowed to dry and related sub-samples were recombined. The recombined samples were homogenized and the radioactive content was determined.

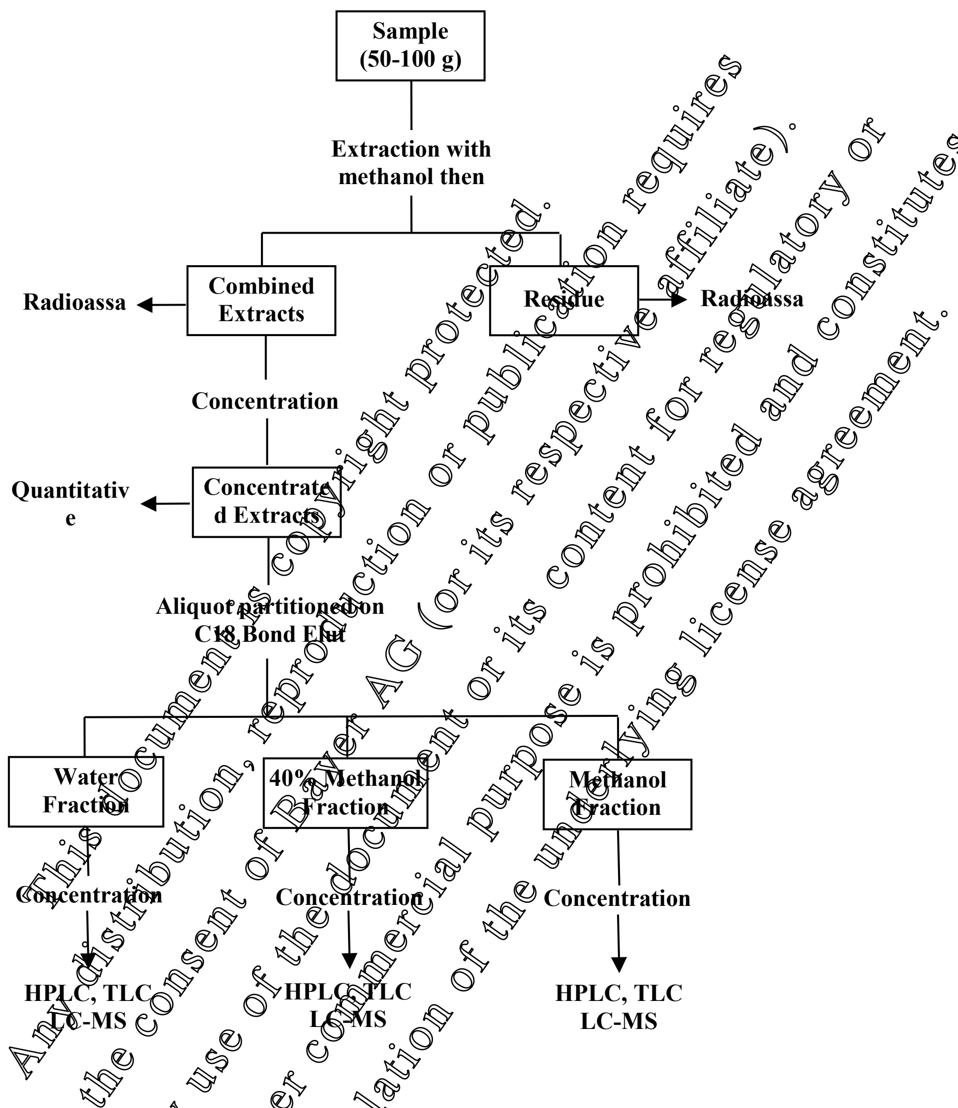
The extracts that contained sufficient radioactivity were prepared for chromatographic examination. Representative aliquots of extracts from a particular core were combined, adjusted to pH 9 with sodium hydroxide solution and concentrated by rotary evaporation. A small amount of water was added and the samples were made up to a fixed volume with methanol.

All crops were rinsed to remove surface dirt before being frozen. The rinses from the 30 and 120 planting samples were radioassayed and those containing sufficient radioactivity were concentrated and made up to a fixed volume with water and methanol. The rinses from the 365 day planting samples were not radioassayed.

The frozen plant samples were homogenized in the presence of dry ice with a commercial macerator. The dry ice was allowed to sublime before the samples were radioassayed. Sub-samples of each interim harvest sample type were accurately weighed into macerating jars and extracted by maceration with methanol. The macerates were filtered and the residue re-extracted with methanol/water (1:1, v/v). Aliquots of the extracts were radioassayed. The solid residues were allowed to dry and then they were homogenized and the amounts of radioactivity that they contained were determined. The extracts were combined, concentrated and made up to a fixed volume with methanol for radio determination. Sub-samples of final harvest samples were extracted in the same way as the interim harvest samples, as described above. Aliquots of the extracts were also concentrated. Other aliquots of the raw extracts were diluted with water and applied to C18 mega-bond-elut cartridges. The samples were partitioned into three fractions by elution of the cartridges with water, followed water/methanol (3:2, v/v) and finally methanol. Aliquots were taken for radioassay. In all cases the great majority of the radioactivity applied to the column was recovered in the water fraction. All fractions from the 1st and 2nd rotation and the water fraction from the 3rd rotation were reduced to a small volume under a stream of nitrogen and then made up to a fixed volume with either methanol/water (water fraction concentrates) or methanol (all other concentrates). These samples were radioassayed. These procedures are summarized as a flow-diagram in Figure 6.6.1-1.


This document is copyright protected.
Any distribution or reproduction or publication requires the consent of Bayer AG or its respective affiliate(s)
Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

**Figure 6.6.1-1 General scheme for the extraction of 30 day and 120 day planting final harvest radish and lettuce samples grown in [$^{14}$C]-flurtamone-treated soil**




This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

In order to identify a polar metabolite that was detected by the chromatographic examination of extracts the 30 day final harvest lettuce sub-sample 1 concentrated extract was subjected to additional clean-up procedures. In summary, after an aliquot was withdrawn for HPLC analysis, the remaining sample was diluted with water, concentrated and applied to a C18 mega-bond elut cartridge (Varian). The cartridge was eluted with water followed by methanol/water (in varying proportions) and finally methanol. Triplicate aliquots of each fraction were assayed for radioactivity by LSC and the water fraction was found to contain 88.0% of the eluted radioactivity. This fraction was adjusted to pH8 by addition of 3M sodium hydroxide solution and partitioned three times against ethyl acetate. The resulting aqueous phase was then adjusted to pH2 by addition of hydrochloric acid and repartitioned against ethyl acetate. The resulting organic phases were combined and found to contain 92.6% of the radioactivity present prior to sample partition. This sample was adjusted to pH8 with 3M sodium hydroxide solution and partitioned twice against water. The resulting water layers were pooled, an aliquot was adjusted to pH8 as above and concentrated. The concentrate was adjusted to pH4 by

addition of hydrochloric acid and diluted with water. This sample was subjected to NMR analysis as described below.
The 1st rotation final harvest lettuce sub-sample 2 concentrated extract was also subjected to additional clean-up procedures. In summary, after an aliquot was withdrawn for HPLC analysis, the remaining sample was concentrated, diluted with water and applied to a C18 mega-bondeslut cartridge (Varian). The cartridge was eluted with water and methanol. Triplicate aliquots of each fraction were assayed for radioactivity. The water fraction was found to contain 89.3% of the eluted radioactivity. This sample was adjusted to pH2 by addition of hydrochloric acid and partitioned three times against ethyl acetate. The resulting aqueous phases were combined and radioassayed. This sample contained 61.8% of radioactivity present prior to partition. The remaining 38.2% radioactivity was present in the combined organic phases. These were adjusted to pH8 using 1M sodium hydroxide solution and partitioned twice against water (pH8). The resulting aqueous phases were combined. This sample contained 97.8% of the radioactivity present prior to partition.

The two combined aqueous phases were pooled, acidified with 0.5 ml formic acid and partitioned three times against dichloromethane and three times against ethyl acetate. The resulting aqueous phases were combined and found to contain 95.9% of radioactivity present prior to partition when assayed by LSC. This sample was then reduced in volume and partitioned twice against ethyl acetate. Sodium chloride was added to the aqueous phase and a further partition against ethyl acetate was carried out. The aqueous phase was found to contain 93.0% of the radioactivity present prior to partition, when radioassayed. This sample was concentrated, diluted with water and methanol rinses of the concentration vessel and analysed by HPLC and by liquid chromatography-mass spectrometry, as described below.

**Quantitative analysis**
The radioactive content of homogenized plant parts was determined by the combustion of carefully weighed sub-samples, followed by the liquid scintillation counting (LSC) of the trapped carbon dioxide that resulted. This procedure was also used for other solid samples (post-extract residues). Liquid samples (solutions) were radioassayed by the direct LSC of aliquots.

**Qualitative analyses**
Aliquots of concentrated rinses and extracts were analysed by high performance liquid chromatography (HPLC) on a system that comprised a Hichrom C18 ODS 3 column attached to a UV detector (set at 260 or 280 nm) and a radiodetector (Packard Flo-One A525T) fitted with a liquid cell. The mobile phase was a gradient of water/acetonitrile (70:30, v/v) containing 1% acetic acid and water/acetonitrile (30:70, v/v) also containing 1% acetic acid.

In addition each concentrated rinse, the C18 bon elut fraction 1 (processed water fraction) and the polar metabolite sample prepared from the 30 day lettuce extract, were examined on a second HPLC system that included an Apex Amino in hexane column connected to a UV detector (set at 260 nm) and a radiodetector (LabLogic ß-Ram) fitted with a liquid cell. The mobile phase was a gradient of water containing 1% acetic acid against isopropanol.

The 30 day planting final harvest radish sample extracts were also analysed by thin layer chromatography (TLC). Aliquots were applied to silica gel plates that contained a fluorescence indicator. These were developed pre-equilibrated tanks containing butanol/glacial acetic acid/water (4:1:1, v/v/v). After development the plates were allowed to dry and regions of radioactivity were located and quantified with a radioanalytical imaging system (Ambis 100). The 30 day planting final

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG or its respective affiliate. Any use of this document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

radish leaves extract was also examined on a second TLC system, along with 30 day planting final radish tubers C18 bond elut fractions 2 and 3 and a 30 day planting final lettuce extract.
The second system also used silica gel plates containing a fluorescence indicator, but a solvent system of chloroform/glacial acetic acid (9:1, v/v). The plates were subsequently analysed in the manner previously described.

Representative crop rinse, soil extract and C18 bond elut fraction concentrates were analysed by liquid chromatography-mass spectrometry. These analyses were conducted with a HiChrom KR100 C* column connected to a VG Quattro mass spectrometer. The mobile phase was a gradient of water/acetonitrile (70:30, v/v) containing 1% acetic acid and water/acetonitrile (30:70, v/v) also containing 1% acetic acid. The ion source was an electrospray in negative ion mode. Fragmentation patterns for authentic standards were used to identify the presence of flurtamone and its metabolites in the sample extracts.

In addition, NMR analysis was performed on the 30 day final harvest lettuce extract to confirm the presence and identity of a polar metabolite. This was conducted on a Bruker ARX400 NMR spectrometer. The probe-head was a 5 mm $^{1}H$, $^{13}C$, $^{15}N$, $^{19}F$ QNP and the solvent was $D_2O$.

## II Results and Discussion

A steady decline in the soil TRR was observed over the period of the study. At all sampling time-points the majority of radioactivity was found in the upper section (0-15 cm), indicating that neither flurtamone nor its metabolites had moved significantly down the soil profile. The mean results for each time-point are tabulated in Table 6.6.1-1.

**Table 6.6.1-1 Total radioactive residues in soil treated with [$^{14}$C]-trifluoromethyl phenyl flurtamone**

| Rotation | Days after Treatment | TRR (mg/kg) | |
|---|---|---|---|
| | | 0-15 cm | 15-30 cm |
| 1st | 30 | 0.156 | 0.014 |
| 2nd | 120 | 0.076 | 0.006 |
| 3rd | 365 | 0.055 | 0.003 |

The extractability of the soil residue decreased with time from 63-82% for the 30 day planting samples to 25-73% at the 365 day planting. The presence in the soil extracts of flurtamone, Flurtamone-trifluoromethyl-N-methyl-mandelamide (RE 53285), Flurtamone-TFMBA (RE-54488) and Flurtamone-desmethyl (RE-39748) was detected by co-chromatography with authentic standards on HPLC. The identities of these compounds were confirmed by LC-MS analysis. For all three sampling time-points up to five minor metabolites were detected at very low levels.

The total radioactive residues in the plant samples are presented in Table 6.6.1-2.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table 6.6.1-2 Total radioactive residues in crops grown in soil treated with [$^{14}$C]-trifluoromethyl phenyl flurtamone**

| Plant Part | TRR (mg/kg) in: | | | | | |
|---|---|---|---|---|---|---|
| | 30 Day Planting | | 120 Day Planting | | 365 Day Planting | |
| | Interim | Final | Interim | Final | Interim | Final |
| Lettuce | 0.325 | 0.161 | 0.044 | 0.061 | 0.024 | 0.031 |
| Radish Leaves | 0.420 | 0.512 | 0.078 | 0.069 | 0.068 | 0.051 |
| Radish Tubers | 0.119 | 0.093 | 0.028 | 0.019 | 0.013 | 0.009 |

TRR values decreased significantly between the 30 and 120 day harvests. A further decrease was observed for 365 day harvests. The TRR in radish tubers at the 365 day final harvest was ≤ 0.010 mg/kg, hence no further analysis was carried out on this sample.

The extraction efficiency was > 90% for all interim harvest samples except the 120 day planting radish tubers (83.4%) and 365 day planting lettuces (89.8%) in which the TRRs were low, so that the unextracted residues were ≤ 0.005 mg/kg. The extraction efficiencies for final harvest lettuce samples were > 90% for those from the 30 and 120 day plantings and > 80% for those from the 365 day planting. The low residues in the 365 day samples meant that <0.01 mg/kg remained unextracted. The extraction efficiencies for final harvest radish leave samples were close to 90% for 30 day samples and ≥ 80% in the 2nd and 3rd rotation, which left ≤ 0.01 mg/kg unextracted except for one (365 day) sample in which 0.011 mg/kg was found unextracted.

The extraction efficiencies for the final harvest radish tuber samples were generally a little lower than in the other samples being about 75% for the 1st and just over 70% (lowest value 71.4%) at the 2nd rotation. Samples from the 3rd rotation were not extracted because of the low TRR. The unextracted residue in the 120 day planting samples was ≤ 0.005 mg/kg.

The plant extracts were analysed by reverse phase HPLC and the presence of flurtamone, Flurtamone-trifluoromethyl-N-methyl-mandelamide (RE-53285) and Flurtamone-TFMBA (RE-54488) was determined by co-chromatography with certified standards. The identities of these compounds were confirmed by the LC-MS analysis of representative rinses and extracts. In addition extensive analysis was done to identify the major polar metabolite present in all plant rinses and extracts. This was identified as [$^{14}$C]-trifluoroacetic acid by LC-MS and $^{19}$F NMR analysis. As well as the above, minor metabolites including flurtamone-desmethyl (RE 39748) and possibly flurtamone-desmethyl-hydroxy (RE 54578) and flurtamone-desmethyl-trifluoromethyl-hydroxy (RE 53498) each present at ≤0.011 mg/kg were observed in the plant extracts from each harvest.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table 6.6.1-3 Metabolite identification in rotational crops after soil treatment with [14C]-trifluoromethyl-phenyl flurtamone.**

| | Lettuce | | Radish leaves | | Radish tubers | |
|---|---|---|---|---|---|---|
| | **mg/kg** | **% TRR** | **mg/kg** | **% TRR** | **mg/kg** | **% TRR** |
| **1st rotation (30days)** | | | | | | |
| Flurtamone | < 0.001 | 0.1 | 0.003 | 0.6 | 0.006 | 6.6 |
| Flurtamone-trifluoromethyl-N-methyl-mandelamide | < 0.001 | 0.1 | 0.032 | 6.2 | 0.006 | 6.2 |
| Flurtamone-TFMBA | 0.001 | 0.6 | 0.003 | 0.6 | 0.004 | 3.8 |
| TFA | 0.146 | 90.2 | 0.399 | 77.8 | 0.051 | 55.5 |
| Minor metabolites | 0.001 | 0.4 | 0.017 | 3.3 | 0.002 | 2.3 |
| Unextracted | 0.015 | 9.0 | 0.059 | 11.5 | 0.024 | 25.7 |
| Total* | 0.161 | 100 | 0.512 | 100 | 0.093 | 100 |
| **2nd rotation (120 days)** | | | | | | |
| Flurtamone | < 0.001 | 0.2 | < 0.001 | 0.6 | 0.002 | 10.4 |
| Flurtamone-trifluoromethyl-N-methyl-mandelamide | nd | nd | nd | Nd | nd | Nd |
| Flurtamone-TFMBA | < 0.001 | 0.1 | nd | Nd | nd | Nd |
| TFA | 0.058 | 95.4 | 0.060 | 87.2 | 0.012 | 63.5 |
| Minor metabolites | < 0.001 | 0.2 | nd | Nd | < 0.001 | 0.9 |
| Unextracted | 0.002 | 4.1 | 0.009 | 12.6 | 0.005 | 28.7 |
| Total* | 0.061 | 100 | 0.069 | 100 | 0.019 | 103 |
| **3rd rotation (365 days)** | | | | | | |
| Flurtamone | nd | nd | nd | Nd | - | - |
| Flurtamone-trifluoromethyl-N-methyl-mandelamide | nd | nd | nd | Nd | - | - |
| Flurtamone-TFMBA | nd | nd | nd | Nd | - | - |
| TFA | 0.025 | 81.0 | 0.041 | 80.2 | - | - |
| Minor metabolites | nd | nd | nd | Nd | - | - |
| Unextracted | 0.006 | 18.9 | 0.010 | 19.7 | - | - |
| Total* | 0.031 | 100 | 0.051 | 100 | 0.009 | - |

* total mg/kg values are measured values and differ from the sum of the single metabolites because of rounding
nd not detected
\- not analysed

This document is copyright protected. Any distribution or reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

The major component of the TRR for the 30 day interim and final harvest plant extracts was [14C]-trifluoroacetic acid which comprised 55.5-93.4 % (0.051-0.399 mg/kg) of the TRR. The lowest levels of [14C]-trifluoroacetic acid arose in the radish tuber extracts (55.5 - 71.7% TRR) compared to lettuce (90.2-93.4% TRR) and radish leaves (77.8%-81.2% TRR). Parent flurtamone accounted for a very low percentage of the TRR at 0.07-6.61% (<0.001-0.006 mg/kg), even for the 30 day harvests. Low levels of RE 53285 were detected in the lettuce extracts (0.12-0.55% TRR, < 0.001-0.002 mg/kg). This metabolite occurred at higher levels in both radish leaves and radish tubers (6.2-12.6% of the TRR, 0.006-0.053 mg/kg). Flurtamone-TFMBA (RE 54488) was present at ≤ 0.6% TRR (≤ 0.003 mg/kg) in lettuces and radish leaves extracts. Significantly higher levels of Flurtamone-TFMBA arose in the corresponding radish tuber extracts (3.8-10.8% of the TRR, 0.004-0.018 mg/kg). The final

harvest radish tuber extract contained trace levels of a compound that had a retention time similar to XE 00876 but this could not be confirmed as the compound represented only 0.35% of the TRR. There was also the possibility that a conjugate of RE 54488 was present at low level.

The most significant component of the TRRs of the interim and final harvest samples in the 2nd rotation was again [$^{14}$C]-trifluoroacetic acid accounting for 0.058 mg/kg (95.4% TRR) in lettuces, 0.060 mg/kg (87.2% TRR) in radish leaves and 0.012 mg/kg (63.5% TRR) in radish tubers. Parent flurtamone was detected at < 0.001 mg/kg (<0.7% TRR) for lettuces and radish leaves and 0.002 mg/kg (10.4% TRR) for radish tuber extracts. Small quantities (≤ 0.001 mg/kg) of Flurtamone-trifluoromethyl-N-methyl-mandelamide were found in lettuce and radish leaves extracts for the 120 day interim harvest, however, none was detected for the final harvest plant part extracts. Flurtamone-TFMBA was present in trace quantities (<0.001 mg/kg) in interim and final harvest lettuce extracts only.

Analysis of the plant parts of the 3rd rotation showed the extracted radioactive residue to be entirely composed of [$^{14}$C]-trifluoroacetic acid. In the final harvest extracts this was detected at levels of 0.025 mg/kg (81.0% TRR) for lettuces and 0.041 mg/kg (80.2% TRR) for radish leaves. The composition of the residues in final harvest plant samples from each planting time are summarized in Table 6.6.1-3.

**III Conclusion**

The metabolism of flurtamone, by soil and plants subsequently grown in that soil, is extensive. The uptake of flurtamone and its metabolites from treated soil was moderate and the levels of residues in the leafy crop (lettuce) and the root crop (radish) used for the study were low already in plants grown in soil 30 days after its treatment with flurtamone. The total residues subsequently further decreased with the second and third rotation. The major residue in all plant compartments was trifluoroacetic acid. The relevance of this metabolite is addressed in a separately submitted document ([redacted] ; 2014; Doc. No.: M-475258-01-1).

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes a violation of the underlying license agreement.

| **Report:** | **KCA 6.6.1/02,** [redacted]**., 2004,** amended **2005** |
|---|---|
| Title: | [14C]-Flurtamone: A Confined Rotational Crop Study using Radish and Lettuce and Wheat |
| Document No: | M-222225-02-1 |
| Report No: | Battelle CX/02/053 |
| Guidelines: | **EU 96/68/EC Section 6.6** |
| GLP | Yes |

A confined rotational crop study with a leafy vegetable (lettuce), a root crop (radish) and a cereal crop (wheat) was designed to complement the previous study on metabolism in succeeding crops. Flurtamone $^{14}C$ labelled in the unsubstituted phenyl ring was used for the experiments with the lettuce and radish and trifluoromethyl phenyl ring labelled flurtamone was used for the experiments with wheat.

**Summary**

$^{14}C$-Flurtamone was applied at a nominal rate of 250 g/ha to bare sandy silt loam soil. After plant back intervals of 30 (first rotation), 120 (second rotation) and 365 days (third rotation) lettuce and radish were planted to containers treated with flurtamone, labelled in the unsubstituted phenyl ring position. Containers treated with flurtamone labelled in the trifluoromethyl-phenyl ring position were used for investigations on cereals (wheat)to determine the metabolism of flurtamone in succeeding crops.
A steady decline in the soil mean total radioactive residue (TRR) was observed over the period of the study.
In the phenyl- label experiments (lettuce and radish) the TRRs were exceptionally low already in the 1st rotation harvest samples accounting for 0.002-0.007 mg/kg. This level further declined to only 0.002-0.006 $mg/kg^{-1}$ for the 2nd rotation and to 0.002 mg/kg for the 3rd rotation.
For wheat the highest TRRs were found in the 1st rotation samples (interim 0.112 mg/kg, grain 0.076 mg/kg, chaff 0.094 mg/kg and straw 0.205 mg/kg). These levels had declined significantly in the 2nd rotation (interim 0.026 mg/kg, grain 0.011 mg/kg, chaff 0.039 mg/kg and straw 0.067 mg/kg). The residue levels in the 3rd rotation (interim 0.044 mg/kg, grain 0.017 mg/kg, chaff 0.038 mg/kg and straw 0.065 mg/kg) were not significantly different from those in the 2nd rotation. This was attributed to the different varieties and growing seasons. It was suggested that the soil residue could have remained significantly unchanged during the cooler winter months.
The recovery of radioactivity as sum of all combined extracts from the wheat samples were good, generally ranging from 77 to 105%. Due to the very low levels of radioactivity in the extracts from the selected lettuce and radish samples, the rates of recovered radioactivity were much more variable, ranging from 51 to 157%.
The results for both crops grown in soil treated with phenyl-labelled flurtamone showed that the resultant residues were very low, below 0.010 mg/kg in all samples at harvest, even those from the shortest plant-back period. Consequently no further analysis of these samples was necessary. Selected samples were, however, extracted and the extracts were analysed.
In all samples only small amounts of flurtamone or non-polar metabolites were observed.
The only major metabolite observed was trifluoromethyl acetic acid (TFA) accounting for 27 - 80% of the observed TRRs in wheat samples. In absolute amounts maximum TFA levels in the samples of the 1st rotation accounted for 0.034-0.069 mg a.i.equiv./kg. A rapid and extensive metabolism of

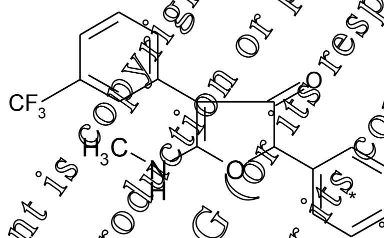
This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

flurtamone was observed in soil and plant matrices already by the time when 1st rotation samples were harvested.

## I Material and Methods

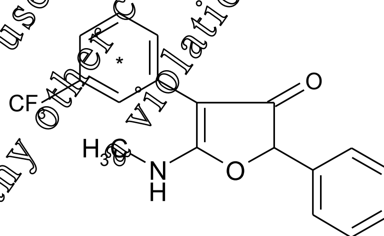
### Material

The [14C]-phenyl ring-labelled flurtamone used was batch SEL/1013, which had a specific activity of 3.73 MBq/mg and a radiochemical purity of > 99%. This was used for application in the first year of the study. For application in the second year of the study the material was repurified. It was given the new batch number of SEL/1199 and had a radiopurity of > 98%.




[14C]-phenyl-ring-labelled Flurtamone
* position of radiolabel

The [14C]-trifluoromethyl phenyl ring-labelled flurtamone used was batch SEL/1017, which had a specific activity of 3.59 MBq/mg and a radiochemical purity of > 98%. This was used for application in the first year of the study. For application in the second year of the study the material was repurified. It was given the new batch number of SEL/1198 and had a radiopurity of > 99%.




[14C]-trifluoromethylphenyl-ring-labelled Flurtamone
* position of radiolabel

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Two applications of each label were made, the first of each in 2002 and the second in 2003. The target application rate was 250 g/ha. The achieved treatment levels were calculated to be 245 to 302 g/ha (98 to 121% of target rate).

As the surface area of the plots was 5 027 $cm^2$ this equated to 12.6 mg per plot. An average of 10% was allowed to compensate for possible losses during application, so that approximately 13.8 mg was required for each plot. For the first application of [$^{14}$C]-phenyl-labelled flurtamone 134.9 mg of batch SEL/1013 was dissolved in 100 mL of acetone. This allowed a 10 mL aliquot for each of eight plots plus additional solution (for analysis, retreatment or additional treatments if necessary). For the first application of [$^{14}$C]-trifluoromethylphenyl-labelled flurtamone 137.8 mg of batch SEL/1017 was dissolved in 100 mL of acetone. This allowed a 10 mL aliquot for each of six plots plus additional solution. For the second application of [$^{14}$C]-phenyl-labelled flurtamone 68.7 mg of batch SEL/1199 was dissolved in 50 mL of acetone. This allowed a 10 mL aliquot for each of four plots plus additional solution. For the second application of [$^{14}$C]-trifluoromethylphenyl-labelled flurtamone 69.2 mg of batch SEL/1198 was dissolved in 500 mL of acetone. This allowed a 10 mL aliquot for each of four plots plus additional solution.

**Soil Characteristics:**

| | | |
|---|---|---|
| Particle size distribution: | | |
| | < 2 µm | 11.9(%) |
| | 2-20 µm | 15.8(%) |
| | 20-63 µm | 36.5(%) |
| | 63-106 µm | 4.4(%) |
| | 106-250 µm | 10.8(%) |
| | 250-500 µm | 16.5(%) |
| | 500-2000 µm | 4.1(%) |
| pH: | | |
| | Water | 7.2 |
| | KCl (1M) | 7.2 |
| | $CaCl_2$ (0.01 M) | 7.0 |
| Cation exchange capacity | | 9.5(meq/100 g) |
| Organic carbon | | 1.5(%) |
| Organic matter | | 4.5(%) |
| Classification: | | |
| | USDA | Loam |
| | ADAS | Sandy silt loam |

**Culture plant:** lettuce (*Lactuca sativa*, variety Set (Iceberg type))
radish (*Raphanus sativus*, variety Fluo 1 (French Breakfast type))
wheat (*Triticum aestivum*, (Winter) var, Claire or (Spring) variety Chablis).

**Treatment**

Twenty-one cylindrical UPVC containment vessels (80 cm diameter, 60 cm deep) were buried in the ground at the Agrochemical Experimentation Farm at Manningtree in the County of Essex in the UK. Each vessel was filled with a locally obtained sandy silt loam soil. The applications were made to bare soil in the plots by use of customized agricultural sprayers. The sprayer was held above the plot, centrally in the lid of an enclosed spray chamber that was lined with polythene sheeting. A separate

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

sprayer was used for each of the labels. A 10 mL aliquot of the appropriate solution was applied to each plot (equivalent to a volume rate of 200 L/ha). This was followed by 10 mL of solvent as a rinse. The sprayer and chamber were then moved to the next plot. When application was completed the contaminated polythene sheeting was removed and the amount of radioactivity remaining on the sheet and in the sprayer was determined.
The crops used in the study were lettuce, radish and wheat. Plots 1 to 8 were treated with phenyl-labelled compound (in 2002) and pairs were planted with lettuce or radish after 30 or 120 days. Plots 9 to 14 were treated (in 2002) with trifluoromethylphenyl-labelled compound and trios were planted with wheat after 120 or 365 days. Plots 15 to 18 were treated with phenyl-labelled compound (in 2003) and pairs were planted with lettuce or radish after 120 days. Plots 19 to 21 were treated (in 2003) with trifluoromethylphenyl-labelled compound and were planted with wheat after 30 days. Each plot was surrounded by a 2 m x 2 m untreated area which was planted with the same crop as the plot. Plots were netted to exclude birds and rabbits.

The crops were grown outdoors under ambient environmental conditions. Weather conditions, including daily maximum and minimum temperatures and rainfall, were documented. During the course of the study crop protection chemicals (excluding any substance related to the active ingredient flurtamone) and irrigation were used as necessary to ensure crop vigour.

**Sampling**
Soil samples were collected from single pots at the time of each planting and harvest. Each sample consisted of three cores of 28 mm diameter and 20 cm depth.
Crops were sampled from the control area and from the treated vessels at the following growth stages:

| | |
|---|---|
| Lettuce | BBCH growth stages 41 (head forming, interim samples) and 49 (typical size, form and firmness of head reached) |
| Radish | BBCH growth stage 41 (roots beginning to expand, interim samples) and 49 (expansion complete, typical form and size of roots reached) |
| Wheat | BBCH growth stages 51-65 (beginning of heading to full flowering, 50% of anthers mature, interim (forage) samples)) and 89 (fully ripe) |

Radish samples were taken by pulling. The roots were cleaned roughly by rubbing lightly with gloved fingers. The samples were separated into roots and leaves using scissors. Lettuce samples were taken by cutting the plants at ground level with a knife. Wheat samples were cut using scissors or secateurs. The harvest samples were cut at a typical combine height and then a sample of stubble was cut just above soil level. The harvest samples were placed in open paper potato sacks in the laboratory for about a week and then threshed by hand. The chaff, separated from the grain by hand, sieve and blowing, consisted of rachis and glumes. Samples were taken at ambient temperature to the analytical laboratory within 24 hours of collection, or in the case of wheat harvest samples, threshing.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Sample processing**

The soil samples were homogenized by grinding with a pulverising mill and radioassayed. The residue in some early samples was determined by extraction, followed by radioassay of the extract and of the post-extract residue.

The plant samples were received at ambient temperature from the test site and any loose soil was removed from the plant surface by rinsing with HPLC grade water. The water rinses were filtered through a glass fibre filter under vacuum. The rinse volumes were measured and duplicate aliquots were taken for determination of radioactive content. The plant samples were stored in a freezer at *app.* 20°C until they were processed. Frozen plant samples were mixed with dry ice and homogenized with a commercial macerator. The dry ice was allowed to sublime in a freezer before ten sub-samples were taken for radioassay to determine the total radioactive residue (TRR) for each sample.

Representative sub-samples of each homogenized crop sample were weighed accurately into a macerating jar and extracted by maceration with methanol or methanol/water. Each sub-sample was extracted and filtered under vacuum. The extracted residue was returned to the macerating jar, re-extracted with methanol or methanol/water and again filtered. In most cases a third extraction was carried out using acetone as the solvent. Aliquots of each extract were assayed for radioactivity.
Two samples (30 day grain and 30 day straw) were additionally extracted with acetonitrile / water (80:20, v/v) at elevated temperature for 4 hours using soxhlet apparatus. After the final extraction the solid residues were allowed to dry and were then milled. Representative aliquots of homogenized residue were radioassayed in order to determine the amount of unextracted radioactive residue.

The methanol, methanol/water and acetone extracts prepared from each sub-sample were combined (along with the soxhlet extract, if made) and reduced in volume by use of rotary evaporation or a stream of nitrogen.

**Quantitative analysis**

Liquid samples were radioassayed by the liquid scintillation counting (LSC) of aliquots. Quantitative measurement of radioactivity in solid samples (plant homogenates, post-extract residues) was achieved by the combustion of sub-samples and the LSC of the trapped carbon dioxide produced.

**Qualitative analysis**

The chromatographic investigations were conducted on high performance liquid chromatography (HPLC) systems. The first system, which was used for the initial analysis of the concentrated plant extracts (and confirmation of compound purity) comprised a Kromasil KR 100 5C8 column connected to a UV detector set at 260 nm and a radiodetector (either a LabLogic ß-Ram or a Packard Flo-one) fitted with a liquid cell. The mobile phase was a gradient of water/acetonitrile (70:30, v/v) containing 1% acetic acid against water/acetonitrile (30:70, v/v) also containing 1% acetic acid.

The plant extract samples arising from the [$^{14}C$]-trifluoromethylphenyl labelled treatments were found to contain solely polar material which was not retained by the first HPLC method. A second method was therefore used for these samples. This used an Apex Amino 5 µm column connected to a UV detector and a radiodetector as for the first system. The mobile phase in this case was a gradient of

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

water containing 1% acetic acid against isopropanol. Where quantification was possible using a standard radiodetector, duplicate injections were made and the mean of both values used in subsequent calculations. Due to the low levels of activity in many of the extracts, quantification was achieved by fraction collection (1 minute fractions of eluate collected and radioactivity counted by LSC).

Selected samples were analysed by liquid chromatography-mass spectrometry in order to confirm the identities of the major components of the residues. The liquid chromatography (HPLC) methods employed were basically that described above, but the conditions were optimized for the requirements of spectrometric analysis. The mass spectrometer was a Micromass Quattro LC Triple Quadrupole. The ion source used was negative ion electrospray (ESP-). Scan conditions were 70 to 400 amu. For confirmation of parent and the major metabolite MRM (multiple reaction monitoring) was used, employing transitions of 332 → 247 (flurtamone) and 113 → 69 (metabolite).
Results are given as parent flurtamone equivalents.

## II Results and Discussion

The achieved treatment levels were calculated to be 245 to 302 g/ha (98 to 121% of target rate).
The average residue levels found in soil are presented in Table 6.6.1-4. The TRRs in soil fell with time, to half the 30 day planting level at the 365 day planting.

**Table 6.6.1-4 Total radioactive residues in soil treated with [$^{14}$C]-flurtamone for a confined rotational crop study**

| Rotation | Average TRR (mg/kg) over study period | |
|---|---|---|
| | Unsubstituted phenyl ring label | Trifluoromethyl-phenyl ring label |
| 1$^{st}$ (30 DAT) | 0.068 | 0.095 |
| 2$^{nd}$ (120 DAT) | 0.051 | 0.070 |
| 3$^{rd}$ (365 DAT) | 0.031 | 0.046 |

DAT = days after treatment

The TRRs for each plant part of the confined rotational crops are shown in detail in Table 6.6.1-5 (lettuce and radish) and Table 6.6.1-6 (wheat), and are summarised below. In general it can be seen that the residues in the plants following soil treatment with the [$^{14}$C]-phenyl labelled flurtamone (radish and lettuce) were very low and considerably lower than in the plants grown in soil treated with the [$^{14}$C]-trifluoromethylphenyl labelled flurtamone (wheat). The very low residue levels of lettuce and radish samples fell continuously from the plants sown or planted 30 days after application to those planted after 365 days (3$^{rd}$ rotation). The residue levels in wheat fell significantly from the 1$^{st}$ rotation (30 days after application) to the 2$^{nd}$ rotation (planted after 120 days) and remained at a relatively low level for the 3$^{rd}$ rotation (365 day samples). The TRR of the 120 day winter wheat interim sample (0.026 mg kg$^{-1}$) was lower than the 365 day spring wheat interim sample (0.044 mg kg$^{-1}$). This can probably be explained by the different timing of the applications and growing season for these crops.

For lettuce the TRR found in the 1$^{st}$ rotation interim sample was only 0.012 mg kg$^{-1}$. This level then declined to only 0.001 mg/kg for the 2$^{nd}$ and 3$^{rd}$ rotation plantings. The TRR in the 30 day planting

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

harvest sample was exceptionally low (0.004 mg/kg). This level then declined to only 0.002 mg/kg for the 120 day plantings and to 0.001 mg/kg for the 365 day plantings.

For radishes the TRR found in the interim leaf sample from the 1st rotation was 0.022 mg $kg^{-1}$ and this level declined to 0.006 mg/kg in the 2nd rotation and 0.001 mg/kg in the 3rd rotation. The TRR found in the interim tuber samples from the 1st rotation was 0.005 mg/kg. This level declined to 0.002 mg/kg in the 2nd rotation and to 0.001 mg/kg in the 3rd rotation. The TRRs found in the samples taken from the 1st rotation were exceptionally low, especially in the tubers (0.007 mg/kg in the leaves and only 0.002 mg/kg in the tubers). These levels then further declined to 0.006 mg/kg (leaves) and 0.002 mg/kg (tubers) for the 2nd rotation and to 0.001 mg/kg (leaves and tubers) for the 3rd rotation.

For wheat the highest TRRs were found in the 1st rotation samples (interim 0.112 mg/kg, grain 0.076 mg/kg, chaff 0.094 mg/kg and straw 0.205 mg/kg). These levels had declined significantly in the 2nd rotation (interim 0.026 mg/kg, grain 0.011 mg/kg, chaff 0.039 mg/kg and straw 0.067 mg/kg). The residue levels in the 3rd rotation (interim 0.044 mg/kg, grain 0.017 mg/kg, chaff 0.038 mg/kg and straw 0.065 mg/kg) were not significantly different from these in the 2nd rotation. This was attributed to the different varieties and growing seasons. The 2nd rotation experiment used winter wheat sown in September 2002 whilst the 3rd rotation used spring wheat sown in May 2003. It was suggested that the soil residue could have remained significantly unchanged during the cooler winter months.

**Table 6.6.1-5: Total radioactive residues (TRR) for lettuce and radish samples grown in soil treated with phenyl labelled flurtamone (mg/kg)**

| Crop | Planting time (days after application) | | |
|---|---|---|---|
| | 30 | 120 | 365 |
| Lettuce – interim | 0.012 | 0.001* | 0.001* |
| Radish – interim leaves | 0.022 | 0.006 | 0.001* |
| Radish – interim tubers | 0.005 | 0.002* | 0.001* |
| Lettuce – harvest | 0.004 | 0.002 | 0.001* |
| Radish – harvest leaves | 0.007 | 0.006 | 0.001* |
| Radish – harvest tubers | 0.002 | 0.002 | 0.001* |

* Initial TRR determined by combustion – these samples were not extracted

**Table 6.6.1-6: Total radioactive residues (TRR) for wheat samples grown in soil treated with trifluoromethylphenyl labelled flurtamone (mg/kg)**

| Crop | Planting time (days after application) | | |
|---|---|---|---|
| | 30 | 120 | 365 |
| Interim harvest (forage) | 0.112 | 0.026 | 0.044 |
| Grain | 0.076 | 0.011 | 0.017 |
| Chaff | 0.094 | 0.039 | 0.038 |
| Straw | 0.205 | 0.067 | 0.065 |

Full details of the extractability of the TRR from each plant part / timing are given in Table 6.6.1-7 (lettuce and radish) and Table 6.6.1-8 (wheat). Plant parts were only required to be extracted where the initial TRR determined by combustion exceeded 0.010 mg/kg. In order to generate some data

This document is copyright protected.
Any distribution or reproduction or publication of the document requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

regarding the nature of the residue in lettuce and radish, some samples were extracted and profiled even though they fell below this TRR threshold.

**Table 6.6.1-7 Extraction efficiencies for lettuce and radish samples grown in Soil treated with phenyl labelled flurtamone (presented as mg/kg and %TRR)**

| Crop / Planting Time / Sample | Total Extracted mg kg$^{-1}$ (% TRR) | Unextracted mg kg$^{-1}$ (% TRR) | Total Residue (TRR) mg kg$^{-1}$ |
|---|---|---|---|
| Lettuce: 30 day interim | 0.008 (71.5%) | 0.003 (28.5%) | 0.012 |
| 30 day harvest | 0.003 (78.5%) | 0.001 (21.5%) | 0.004 |
| 120 day interim | sample not extracted | | 0.001 |
| 120 day harvest | 0.002 (86.8%) | 0.000 (13.2%) | 0.002 |
| 365 day interim | sample not extracted | | 0.001 |
| 365 day harvest | sample not extracted. | | 0.001 |
| Radish: 30 day interim leaves | 0.019 (89.1%) | 0.002 (10 9%) | 0.022 |
| 30 day harvest leaves | 0.003 (38.5%) | 0.004 (61.5%) | 0.007 |
| 120 day interim leaves | 0.005 (83.2%) | 0.001 (16.8%) | 0.006 |
| 120 day harvest leaves | 0.005 (85.2%) | 0.001 (14.8%) | 0.006 |
| 365 day interim leaves | sample not extracted | | 0.001 |
| 365 day harvest leaves | sample not extracted | | 0.001 |
| Radish 30 day interim tubers | 0.004 (73.2%) | 0.001 (26.8%) | 0.005 |
| 30 day harvest tubers | 0.002 (92.8%) | 0.000 ( 7.2%) | 0.002 |
| 120 day interim tubers | sample not extracted | | 0.002 |
| 120 day harvest tubers | 0.001 (82.0%) | 0.000 (18.0%) | 0.002 |
| 365 day interim tubers | sample not extracted | | 0.001 |
| 365 day harvest tubers | sample not extracted | | 0.001 |

**Table 6.6.1-8 Extraction efficiencies for wheat samples grown in soil treated with trifluoromethylphenyl labelled flurtamone (presented as mg/kg and %TRR)**

| Crop / Planting Time | | Total Extracted mg kg (%TRR) | Unextracted mg kg (%TRR) | Total Residue (TRR) mg/kg |
|---|---|---|---|---|
| Interim wheat | 30 day | 0.101 (90.1%) | 0.011 ( 9.9%) | 0.112 |
| | 120 day | 0.025 (96.6%) | 0.001 ( 3.4%) | 0.026 |
| | 365 day | 0.042 (96.0%) | 0.002 ( 4.0%) | 0.044 |
| Grain | 30 day | 0.066 (86.3%) | 0.010 (13.7%) | 0.076 |
| | 120 day | 0.010 (86.7%) | 0.001 (13.3%) | 0.011 |
| | 365 day | 0.015 (88.2%) | 0.002 (11.8%) | 0.017 |
| Chaff | 30 day | 0.078 (82.9%) | 0.016 (17.1%) | 0.094 |
| | 120 day | 0.037 (95.0%) | 0.002 ( 5.0%) | 0.039 |
| | 365 day | 0.031 (79.9%) | 0.008 (20.1%) | 0.038 |
| Straw | 30 day | 0.168 (82.0%) | 0.037 (18.0%) | 0.205 |
| | 120 day | 0.063 (93.2%) | 0.005 ( 6.8%) | 0.067 |
| | 365 day | 0.060 (92.0%) | 0.005 ( 8.0%) | 0.065 |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purposes is prohibited and constitutes violation of the underlying license agreement.

The procedural recoveries for the concentration of all combined extracts from the wheat samples were good, ranging, in general from 88 to 105%. Only one sample (120 day interim) fell below this range at 77%. Due to the very low levels of radioactivity in the extracts from the selected lettuce and radish samples, the procedural recoveries were much more variable, ranging from 51 to 157%.

The results of the chromatographic examinations of the concentrated extracts are presented in Tables 6.6.2-9 and 6.6.2-10.

**Table 6.6.1-9: Summary of HPLC results for lettuce samples grown in soil treated with phenyl labelled flurtamone; parent compound and metabolites**

| HPLC Method 1 | | Lettuce 30 day | | Lettuce 120 day | |
|---|---|---|---|---|---|
| Component | Approx. retention time | mg/kg (a.s. equiv) | % TRR | mg/kg (a.s. equiv) | % TRR |
| Region 1 | 2-5 min | 0.001 | 18.5 | 0.001 | 52.6 |
| Region 2 | 5-8 min | | | 0.000 | 1.7 |
| Region 3 | 8-13 min | | | 0.000 | 8.6 |
| Region 4 | 13-16 min | | | 0.000 | 6.0 |
| Region 5 | 16-18 min | | | 0.000 | 2.0 |
| Region 6 | 18-20 min | | | 0.000 | 3.0 |
| Flurtamone | 20-21 min | 0.002 | 60.0 | 0.000 | 11.4 |
| Total in extract | | 0.003 | 78.5 | 0.002 | 86.8 |
| Unextracted | | 0.001 | 21.5 | 0.000 | 13.2 |
| Total (TRR) | | 0.004 | 100.0 | 0.002 | 100.0 |

**Table 6.6.1-10: Summary of HPLC results for radish samples grown in soil treated with phenyl labelled flurtamone; parent compound and metabolites**

| HPLC Method 1 | | Radish Leaves 120 day | | Radish Tubers 120 day | |
|---|---|---|---|---|---|
| Component | Approx. retention time | mg $kg^{-1}$ (a.s. equiv) | % TRR | mg $kg^{-1}$ (a.s. equiv) | % TRR |
| Region 1 | 1-6 min | 0.002 | 32.7 | 0.001 | 49.6 |
| Region 2 | 7-11 min | 0.002 | 36.4 | 0.000 | 3.7 |
| Region 3 | 12-16 min | 0.000 | 5.4 | 0.000 | 4.3 |
| Flurtamone | 18-21 min | 0.001 | 10.7 | 0.000 | 23.3 |
| Total in extract | | 0.005 | 85.2 | 0.001 | 82.0 |
| Unextracted | | 0.001 | 14.8 | 0.000 | 18.0 |
| Total (TRR) | | 0.006 | 100.0 | 0.002 | 100.0 |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table 6.6.1-11: Summary of HPLC results for interim wheat samples grown in soil treated with trifluoromethylphenyl labelled flurtamone; parent compound and metabolites**

| HPLC Method 2 | | 30 day | | 120 day | | 365 day | |
|---|---|---|---|---|---|---|---|
| Component | Approx. retention time | mg/kg (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR |
| Region 1<br>(of which Flurtamone*) | 1-5 min | 0.020<br>(0.006) | 17.9<br>(5.5) | 0.000<br>na | 1.1<br>na | 0.000<br>na | 0.8<br>na |
| TFA | 20-24 min | 0.060 | 53.8 | 0.016# | 62.4 | 0.021 | 46.5 |
| Region 3† | 30-60 min | 0.011 | 9.7 | 0.008 | 31.0 | 0.019 | 43.2 |
| Total others | | 0.004 | 4.0 | | | | |
| Total in extract | | 0.101 | 90.1 | 0.025 | 96.6 | 0.042 | 96.0 |
| Unextracted | | 0.011 | 9.9 | 0.001 | 3.4 | 0.002 | 4.0 |
| Total (TRR) | | 0.112 | 100.0 | 0.026 | 100.0 | 0.044 | 100.0 |

* Quantification of flurtamone by separate analysis with HPLC method 1

† Region 3 consists of multiple, minor components

na = not analysed

**Table 6.6.1-12 Summary of HPLC results for wheat grain samples grown in soil treated with trifluoromethylphenyl labelled flurtamone; parent compound and metabolites**

| HPLC Method 2 | | 30 day | | 120 day | | 365 day | |
|---|---|---|---|---|---|---|---|
| Component | Approx. retention time | mg/kg (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR |
| Region 1<br>(of which Flurtamone*) | 1-5 min | 0.000<br>(0.000) | 0.1<br>(0.0) | 0.000<br>(0.000) | 2.2<br>(3.0) | 0.000<br>na | 0.5<br>na |
| TFA | 20-24 min | 0.061 ‡ | 79.9 | 0.003 | 29.3 | 0.005 | 27.3 |
| Region 3† | 30-60 min | - | - | 0.006 | 51.5 | 0.009 | 56.1 |
| Total others | | 0.001 | 1.7 | | | | |
| Total in extract | | 0.066 | 86.3 | 0.010 | 86.7 | 0.015 | 88.2 |
| Unextracted | | 0.010 | 13.7 | 0.001 | 13.3 | 0.002 | 11.8 |
| Total (TRR) | | 0.076 | 100.0 | 0.011 | 100.0 | 0.017 | 100.0 |

* Quantification of flurtamone by separate analysis with HPLC method 1

‡ In this sample matrix and other effects caused the TFA peak to occur at a later time in the chromatogram.

† Region 3 consists of multiple, minor components

na = not analysed

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

**Table 6.6.1-13 Summary of HPLC results for chaff samples from wheat grown in soil treated with trifluoromethylphenyl labelled flurtamone; parent compound and metabolites**

| HPLC Method 2 | | 30 day | | 120 day | | 365 day | |
|---|---|---|---|---|---|---|---|
| Component | Approx. retention time | mg/kg (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR |
| Region 1<br>(of which Flurtamone*) | 1-5 min | 0.009<br>(0.005) | 9.4<br>(4.8) | 0.001<br>(0.000) | 3.7<br>(0.7) | 0.001<br>na | 1.5<br>na |
| TFA | 20-24 min | 0.034 | 35.8 | 0.017 | 43.0 | 0.014 | 37.3 |
| Region 3† | 30-60 min | 0.034 | 36.3 | 0.015 | 39.0 | 0.014 | 37.3 |
| Total others | | | | | | | |
| Total in extract | | 0.078 | 82.9 | 0.037 | 95.0 | 0.031 | 79.9 |
| Unextracted | | 0.016 | 17.1 | 0.002 | 5.0 | 0.008 | 20.1 |
| Total (TRR) | | 0.094 | 100.0 | 0.039 | 100.0 | 0.038 | 100.0 |

* Quantification of flurtamone by separate analysis with HPLC method 1

† Region 3 consists of multiple, minor components

na = not analysed

**Table 6.6.1-14 Summary of HPLC results for straw samples from wheat grown in Soil treated with trifluoromethylphenyl labelled flurtamone; parent compound and metabolites (presented as mg/kg and %TRR)**

| HPLC Method 2 | | 30 day | | 120 day | | 365 day | |
|---|---|---|---|---|---|---|---|
| Component | Approx. retention time | mg $kg^{-1}$ (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR | mg/kg (a.s. eq) | % TRR |
| Region 1<br>(of which Flurtamone*) | 1-5 min | 0.032<br>(0.015) | 15.8<br>(7.10) | 0.001<br>(0.001) | 2.2<br>(0.8) | 0.002<br>na | 3.2<br>na |
| TFA | 20-24 min | 0.069 | 33.8 | 0.039 | 58.3 | 0.042 | 64.7 |
| Region 3† | 30-60 min | 0.034 | 16.6 | 0.020 | 29.1 | 0.014 | 20.8 |
| Total others | | 0.016 | 8.0 | 0.002 | 3.3 | | |
| Total in extract | | 0.168 | 82.0 | 0.063 | 93.2 | 0.060 | 92.0 |
| Unextracted | | 0.037 | 18.0 | 0.005 | 6.8 | 0.005 | 8.0 |
| Total (TRR) | | 0.205 | 100.0 | 0.067 | 100.0 | 0.065 | 100.0 |

* Quantification of flurtamone by separate analysis with HPLC method 1

† Region 3 consists of multiple, minor components

na = not analysed

The actual residue level of TFA were observed to be highest in the 30 day planting samples accounting for 0.069 mg a.i. equiv./kg falling to 0.039 mg a.i. equiv./kg and 0.042 mg a.i. equiv./kg in 120 day and 365 day planting samples respectively.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

The identification of the major components of the extractable residue was confirmed by mass spectrometry. Where there was sufficient radioactivity this was done in scan mode. Other samples were proceeded using an MRM method. The presence of flurtamone in representative concentrated sample extracts was confirmed using LC-MS by comparison with a certified reference standard. The presence of trifluoroacetic acid in concentrated extracts was confirmed, again by comparison with a reference standard.

### III Conclusions

The results for both crops grown in soil treated with phenyl-labelled flurtamone showed that the resultant residues were very low, below 0.010 mg/kg in all samples at harvest, even those from the shortest plant-back period. Consequently no further analysis of these samples was necessary. Selected samples were, however, extracted and the extracts were analysed. The residue was shown to comprise of flurtamone and polar material.

The residues in the samples from the wheat grown in the trifluoromethylphenyl-labelled flurtamone were significantly higher than those from the other treatment, although still low in absolute terms. The residues were highly polar in nature with only small amounts of flurtamone or non-polar metabolites present in any sample. The main single component of this polar residue was TFA, accounting for up to 80% of the residue (30 day grain sample).

Overall the results showed that uptake of flurtamone by rotational crops occurs at low levels only. The major component of the resultant residue is TFA, a metabolite of flurtamone already known from primary plant metabolism studies.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| Report: | KCA 6.6.1/03, [redacted] 2011 |
|---|---|
| Title: | Metabolism of [phenyl-UL-$^{14}$C]flurtamone in confined rotational crops: wheat |
| Report No & Edition No: | MEF-11/1012<br>M-440369-01-1 |
| Guidelines: | OECD 502 Metabolism in Rotational Crops<br>US EPA Residue Chemistry Test Guideline OPPTS 860.1850: Confined Accumulation in Rotational Crops |
| GLP: | Yes |

### Summary

The metabolism of [phenyl-UL-$^{14}$C]flurtamone formulated as an SC 120 was investigated in wheat as a rotational crop after a single spray application onto bare soil at an actual application rate of 127 g a.s./ha, which was slightly above the anticipated maximum seasonal field rate of 120 g a.s./ha. Wheat was sown at 30 and 156 days after application, representing the first and second rotation.

The TRR values of the first rotation were generally low, ranging from 0.006 mg/kg in wheat grain to 0.078 mg/kg in wheat straw. The TRR values decreased substantially from the first to the second rotation with a maximum value of 0.005 mg/kg in wheat straw and 0.002 mg/kg in wheat forage and grain. Due to low TRRs in all RACs of the second rotation, a third rotation was not sown.

The TRR values for all matrices are shown in the following table:

| Matrix | TRR [mg/kg] | |
|---|---|---|
| | First rotation | Second rotation |
| Wheat forage | 0.017 | 0.002 |
| Wheat hay | 0.051 | 0.003 |
| Wheat straw | 0.078 | 0.005 |
| Wheat grain | 0.006 | 0.002 |

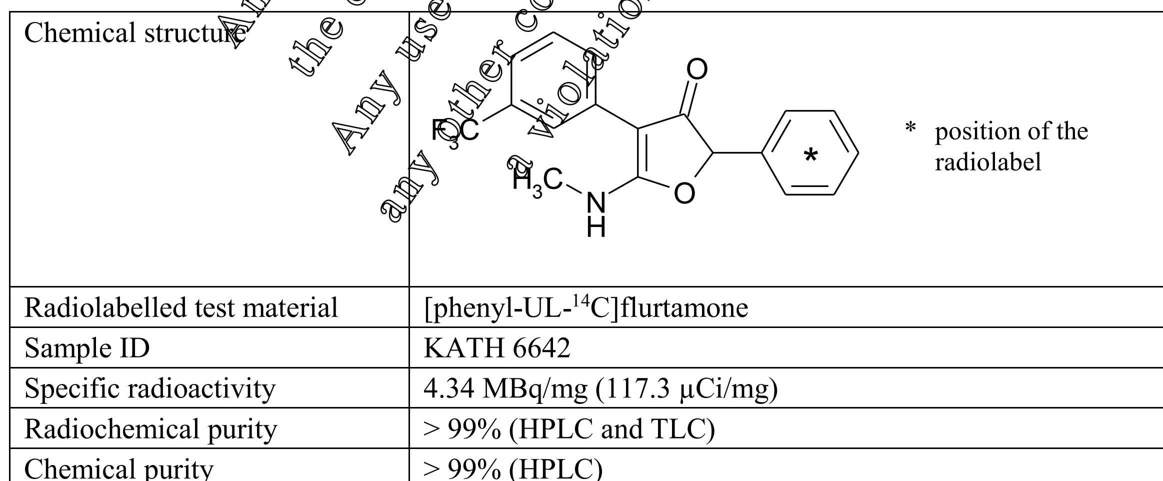
In total, between 34.3% and 78.8% of the TRR were extracted from the RACs of the first rotation by conventional extraction with acetonitrile/water mixtures. Parent compound and metabolites in the extracts were analysed by HPLC. Identification was performed by HPLC co-chromatography with reference compounds as well as by comparison of HPLC profiles.

The identification rates ranged from 11.5% of the TRR in wheat grain to 75.2% in wheat forage. Parent compound was predominantly the main compound ranging from 11.5% to 69.9% of the TRR. Flurtamone-hydroxy-glyc and flurtamone-desmethyl were major (≥ 10% of the TRR) metabolites and flurtamone-hydroxy-mal-glyc was a minor metabolite identified. Only one trace metabolite (0.001 mg/kg) remained unknown. As metabolic routes hydroxylation followed by conjugation with glucose and malonic acid and N-demethylation were observed.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## I. Materials and Methods

**Test Material:**

| Chemical structure |  |
|---|---|
| Radiolabelled test material | [phenyl-UL-$^{14}$C]flurtamone |
| Sample ID | KATH 6642 |
| Specific radioactivity | 4.34 MBq/mg (117.3 µCi/mg) |
| Radiochemical purity | > 99% (HPLC and TLC) |
| Chemical purity | > 99% (HPLC) |

The radiolabelled test compound [phenyl-UL-$^{14}$C]flurtamone was formulated as an SC 120. Radiodilution and formulation of the test compound was performed prior to the application: therefore, an adequate portion of the stock solution was transferred into a special glass vial and concentrated to a

small remainder. An adequate amount of blank formulation was added and the mixture was homogenised using a shaker and ultrasonication. Later, the mixture was suspended in water by stirring. The specific activity of the application solution was 4.34 MBq/mg (117.3 µCi/mg).

**2. Soil Characteristics:** "Monheim 4", sandy loam soil, pH ($CaCl_2$) = 6.8, organic carbon content = 1.2%, cation exchange capacity (CEC) = of 8.3 meq/100 g

**3. Culture Plant** Spring wheat/small grain, variety "Thasos".

**Experimental conditions:**
Flurtamone was applied as an SC formulation with a computer controlled track sprayer onto the bare soil of a planting container (surface area of approx. 1 $m^2$). The application rate was 127 g a.s./ha and was slightly above the anticipated maximum seasonal rate of flurtamone. The wheat rotations were sown 30 (1st rotation) and 156 days (2nd rotation) after treatment. Approx. 500 spring wheat seeds (10 rows) were sown. Due to low TRRs in all RACs of the second rotation, a third rotation was not sown.

**Sampling:**
At the end of tillering (BBCH 29) a sample of wheat forage (2 rows) was harvested. The plants were cut off just above the soil surface and shredded into pieces of approx. 1 cm length.
In the medium milk stage (BBCH 75) a sample of wheat hay (2 rows) was harvested. The plants were cut off just above the soil surface, shredded into pieces of approx. 2 cm length and air-dried for 4 days at room temperature.
When the ripening stage was completed (over-ripe, BBCH 92), the remaining wheat plants (6 rows) were cut off just above the soil surface. Grain was separated from straw and the straw was shredded into pieces of approx. 2 cm length.
Each matrix was weighed and afterwardsthe samples were homogenised in liquid nitrogen using a high speed blender. After homogenisation the samples were stored in a freezer (≤ -18 °C) until extraction.

**Analytical Procedures**
**Extraction:**
Aliquots of the homogenised matrices were extracted three times with a mixture of acetonitrile/water (8/2, v/v) and one time with a mixture of acetonitrile/water (1/1, v/v) using a high speed blender. After each extraction step, the extracts were separated from the solids by filtration. A small volume of a mixture of acetonitrile/water (1/1, v/v) was used for rinsing. The volume of each extract was measured and the radioactivity determined by LSC. The solids were dried and weighted. Aliquots were subjected to combustion followed by LSC. The actual TRR value of the sample was determined by summing up the radioactivity measured in the extracts and in the remaining solids.
The first two extracts (in case of wheat straw and wheat grain all extracts) were combined and subjected to a clean-up step using an SPE RP cartridge (Phenomenex C18-E, 20 g), which was conditioned with acetonitrile. After application of the extract, the cartridge was rinsed with acetonitrile/water (8/2, v/v). The flow-through fraction (percolate) and the acetonitrile/water rinse were combined, the volume measured and the radioactivity determined by LSC. The cartridge was

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

rinsed with methanol/dichloromethane (1/1, v/v). Volume and radioactivity of this fraction were also determined.
The combined percolate and acetonitrile/water rinse fractions of each matrix were evaporated to the aqueous remainders. The volumes of the concentrates and distillates were measured and aliquots subjected to LSC.
All matrices and extracts were stored in a refrigerator (approx. +5 °C) or freezer (≤ -18 °C).

**Quantification:**
Parent compound and metabolites in the extracts were analysed by HPLC coupled to a UV- and radiodetector.The HPLC chromatograms were integrated for quantification of compounds.

**Identification and characterisation:**
Parent compound and metabolites were identified by HPLC co-chromatography of conventional extracts with labelled and non-labelled authentic reference compounds or by comparison of retention times.

**Storage stability:**
The sampled or harvested matrices were stored in a freezer at ≤ -18 °C until extraction. All extraction experiments and first HPLC analyses of the wheat matrices were performed within 32 days after harvest. Comparison of HPLC chromatograms recorded at different times during the study showed that the profiles did not significantly change during the analytical phase up to a period of at least 4 months. In accordance with the OECD Guidance for the Testing of Chemicals 501 (2007), it was therefore concluded, that the residues in the extracts, and thus in the matrices, were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at sampling and harvest.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**II. Results and Discussion**

The metabolism of [phenyl-UL-$^{14}$C]flurtamone was investigated in the rotational crop spring wheat following application on the soil. The active substance was applied as an SC formulation on the bare soil at a rate of 127 g a.s./ha at 30 days before sowing of the wheat representing the first rotation. Wheat for the 2nd rotation was sown 156 days after application. A third rotation was not sown. The premature matrices harvested were wheat forage and wheat hay. Wheat straw and grains were harvested at maturity.
As shown in Table 6.6.1-16 the TRR values of the first rotation were generally low, ranging from 0.006 mg/kg in wheat grain to 0.078 mg/kg in wheat straw. The TRR values decreased substantially from the first to the second rotation with a maximum value of 0.005 mg/kg in wheat straw and 0.002 mg/kg in wheat forage and grain. Due to the decrease of the TRR values from the first to the second rotation below levels of 0.01 mg/kg, a third rotation was not sown.
The wheat matrices of the first rotation were conventionally extracted four times with acetonitrile/water mixtures. This extraction procedure released between 34.3% and 78.8% of the TRR. The remaining radioactivity in the solids after extraction was 0.019 mg/kg in wheat straw down to

0.004 mg/kg in wheat forage and grain. Therefore, no further extraction steps were considered necessary.
Extracted radioactivity not analysed was max. 6.7% of the TRR, representing max. 0.003 mg/kg. The TRR values of the wheat matrices of the second rotation were below 0.01 mg/kg and were therefore not extracted and analysed.
Parent compound flurtamone was identified in wheat forage, hay and straw by HPLC co-chromatography with a non-radiolabelled reference compound. The metabolite flurtamone-hydroxy-glyc was identified in wheat forage and the metabolite flurtamone-hydroxy-mal-glyc in wheat straw by HPLC co-chromatography with radiolabelled reference compounds from the sunflower metabolism study. In this study these two metabolites had been isolated and identified with LC-MS/MS. The position of the hydroxy group and the configuration of the conjugated sugar moiety had been determined in the wheat metabolism study.
Parent compound flurtamone in wheat grain and flurtamone-desmethyl in wheat straw were identified by comparison of HPLC retention times of reference compounds.
In total, the identification rates ranged from 11.5% of the TRR in wheat grain to 75.2% in wheat forage (Table 6.6.1-16). Parent compound was predominantly the main component of the profiles, ranging from 11.5% to 69.9% of the TRR (0.001 mg/kg to 0.041 mg/kg).
Flurtamone-hydroxy-glyc was a major metabolite (26% of the TRR, 0.004 mg/kg) detected only in wheat forage. Flurtamone-desmethyl was a major metabolite (13.9% of the TRR, 0.011 mg/kg) and flurtamone-hydroxy-mal-glyc a minor metabolite (7.8% of the TRR, 0.006 mg/kg), both metabolites detected only in wheat straw.

Overall, five compounds were detected in the wheat matrices by HPLC chromatography. Four of them were identified and one unknown component (19.1% of the TRR, 0.001 mg/kg in wheat grain) was characterised by its HPLC retention behaviour.

Table 6.6.1-15: Distribution of radioactivity in the extracts of wheat matrices of the first rotation

| First rotation | Wheat forage | | Wheat hay | | Wheat straw | | Wheat grain | |
|---|---|---|---|---|---|---|---|---|
| | % | mg/kg | % | mg/kg | % | mg/kg | % | mg/kg |
| TRR | | 0.017 | | 0.051 | | 0.078 | | 0.006 |
| Conventionally extracted | 78.8 | 0.013 | 76.5 | 0.039 | 76.1 | 0.060 | 34.3 | 0.002 |
| Extract for analysis | 75.2 | 0.013 | 69.9 | 0.036 | 74.4 | 0.058 | 30.6 | 0.002 |
| Extracts not analysed | 3.6 | 0.001 | 6.7 | 0.003 | 1.7 | 0.001 | 3.7 | <0.001 |
| Total extracted | 78.8 | 0.013 | 76.5 | 0.039 | 76.1 | 0.060 | 34.3 | 0.002 |
| Unextractable (PES*) | 21.2 | 0.004 | 23.5 | 0.012 | 23.9 | 0.019 | 65.7 | 0.004 |
| Balance | 100.0 | 0.017 | 100.0 | 0.051 | 100.0 | 0.078 | 100.0 | 0.006 |

* post extraction solids

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.6.1-16: TRR values and distribution of parent compound and metabolites in wheat after spray application of [phenyl-UL-$^{14}$C]flurtamone

| | Wheat forage | | Wheat hay | | Wheat straw | | Wheat grain | |
|---|---|---|---|---|---|---|---|---|
| TRR [mg/kg] = | 0.017 | | 0.051 | | 0.078 | | 0.006 | |
| Compound (Flurtamone-) | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg | % TRR | mg/kg |
| parent compound | 49.2 | 0.008 | 69.9 | 0.036 | 52.7 | 0.041 | 11.5 | 0.001 |
| hydroxy-glyc | 26.0 | 0.004 | --- | --- | --- | --- | --- | --- |
| hydroxy-mal-glyc | --- | --- | --- | --- | 7.8 | 0.006 | --- | --- |
| Desmethyl | --- | --- | --- | --- | 13.9 | 0.011 | --- | --- |
| **Total identified** | **75.2** | **0.013** | **69.9** | **0.036** | **74.4** | **0.058** | **11.5** | **0.001** |
| unknown 1 | --- | --- | --- | --- | --- | --- | 19.1 | 0.001 |
| Total characterised | --- | --- | --- | --- | --- | --- | 19.1 | 0.001 |
| Analysed extract(s) | 75.2 | 0.013 | 69.9 | 0.036 | 74.4 | 0.058 | 30.6 | 0.002 |
| Extracts not analysed | 3.6 | 0.001 | 6.7 | 0.003 | 1.7 | 0.001 | 3.7 | <0.001 |
| **Total extracted** | **78.8** | **0.013** | **76.5** | **0.039** | **76.1** | **0.060** | **34.3** | **0.002** |
| Unextractable (PES*) | 21.2 | 0.004 | 23.5 | 0.012 | 23.9 | 0.019 | 65.7 | 0.004 |
| Accountability | 100.0 | 0.017 | 100.0 | 0.051 | 100.0 | 0.078 | 100.0 | 0.006 |

* post extraction solids

**III. Conclusions**

Based on the metabolites identified the following metabolic routes were deduced:

- hydroxylation followed by conjugation with glucose and, subsequently, malonic acid
- N-demethylation

Flurtamone was moderately metabolised in rotational wheat. Parent compound was predominantly the main component (ca. 12% - 70% of the TRR). Flurtamone-hydroxy-glyc and flurtamone-desmethyl were major metabolites (≥ 10% of the TRR) and flurtamone-hydroxy-mal-glyc was a minor metabolite identified.
Based on these results, it is concluded that the metabolism of [phenyl-UL-$^{14}$C]flurtamone in rotational wheat is well understood and the metabolic reactions observed are covered by the common metabolic pathway of the primary plant metabolism.

**Overall summary on residues in succeeding crops**

Residues in succeeding crops were investigated in lettuce (leafy crop), radish (root crop) and wheat (cereals) using $^{14}$C flurtamone labelled in the unsubstituated phenyl ring position and also in studies using the trifluoromethyl-phenyl ring position. Applications were made to plain soil as a worst case scenario with nominal application rates shown in table 6.6.1-17.


This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Table 6.6.1-17: Nominal application rates per crop and label position

| Crop | Nominal application rate (mg a.s/ha) | |
|---|---|---|
| | Trifluoromethyl-phenyl | Unsubstituted phenyl ring |
| Lettuce | 375 | 250 |
| Radish | 375 | 250 |
| Wheat | 250 | 120 |

A steady decline in the soil total radioactive residue (TRR) was observed. At all sampling time-points the majority of radioactivity was found in the upper section of the soil (0-15 cm), indicating that neither flurtamone nor its metabolites had moved significantly down the soil profile.

For lettuce and radish harvest commodities the total radioactive residues of the trifluoromethyl-phenyl labelled experiment were low accounting for 0.1-0.5 mg/kg in the first rotation (30 days). The TRR subsequently further decreased with the second rotation (120 days) resulting levels of <0.01-0.05 mg/kg in the third rotation (365 days).
The results for both crops grown in soil treated with phenyl-labelled flurtamone showed that the resultant residues were even lower resulting in levels below 0.010 mg/kg in all samples at harvest, even those from the shortest plant-back period (30 days).
In all samples only small amounts of flurtamone or non-polar metabolites were observed.
For both label positions the only major metabolite observed was trifluoromethyl acetic acid (TFA) accounting for up to 95% of the observed TRRs.

For trifluoromethyl-phenyl treated wheat the highest TRRs were found in the 1st rotation samples (0.076-0.205 mg/kg). These levels had declined significantly in the 2nd rotation (0.011- 0.067 mg/kg). The residue levels in the 3rd rotation were not significantly different from those in the 2nd rotation. This was attributed to the different varieties and growing seasons. It was suggested that the soil residue could have remained significantly unchanged during the cooler winter months.
For the phenyl ring label the observed TRR values of the first rotation were generally low, ranging from 0.006 mg/kg in wheat grain to 0.078 mg/kg in wheat straw. The TRR values again decreased substantially from the first to the second rotation with a maximum value of 0.005 mg/kg in wheat straw and 0.002 mg/kg in wheat forage and grain. Due to low TRRs in all RACs of the second rotation, a third rotation was not sown. In the trifluoromethyl-phenyl study flurtamone was intensively metabolised and, as with lettuce and radish, the major residue component was determined to be TFA, accompanied by minor parts of flurtamone active ingredient. With the latest study, where flurtamone was labelled in the unsubstituted phenyl ring, flurtamone appeared to be only moderately metabolised and the predominant part of the residue was determined to be mostly the active ingredient flurtamone itself.

The application rates in the studies with lettuce and radish were overdosed by a factor of 3 (375 g/ha) or 2 (250 g/ha) compared to the intended use rate. Also the wheat study with trifluoromethyl-phenyl labelled flurtamone was overdosed by a factor of 2 (250 g/ha). Beside this, in metabolism studies, straw is cut directly above the soil surface to increase residues for identification of metabolites. Finally the application was done on bare soil which represents a worst case application scenario. Therefore the

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

given values clearly overestimate the flurtamone derived residue levels to be expected in normal agricultural practice. Considering the above and provided that flurtamone is applied according to the intended GAPs, the only single residue component that might be expected to exceed 0.01 mg/kg would be TFA. The relevance of this metabolite is addressed in a seperatly submitted document ([redacted]; 2014; Doc. No.: M-475258-01-1).

Metabolism in rotational crops was found to be very similar to primary crop metabolism. Metabolic products and discovered metabolic pathways are covered by the common plant metabolism as shown in figure 6.2.1-3. Therefore a specific residue definition for rotational crops is not deemed necessary.

## CA 6.6.2 Magnitude of residues in rotational crops

Metabolism studies on rotational crops have shown that no residue of parent flurtamone at or above the limit of quantification (0.01 mg eq./kg) would be expected in food items of succeeding crops (lettuce representative of the leafy vegetables, radishes representative of root vegetables and wheat grain representative of cereals). Flurtamone was only found in feed items at low residue levels: 0.015 mg eq./kg in wheat straw ([trifluoromethylphenyl-UL-$^{14}$C]Flurtamone study; 30d rotation with an application rate of 250 g as/ha on soil) and 0.036 mg eq./kg in wheat hay and 0.041 mg eq./kg in wheat straw ([phenyl-UL-$^{14}$C]Flurtamone study ; 30d rotation with an application rate of 120 g as/ha on soil) (refer to point CA 6.6.1).

Only one metabolite has been seen above the LOQ. This metabolite is the TFA metabolite (trifluoroacetate), a known soil metabolite of flurtamone which has been considered as a non-relevant metabolite. Indeed, the assessment of the properties and characteristics of the metabolite TFA has been made with a particular emphasis on the DG Sanco Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater within the Document N4 (Referenced M-475258-01-1) .

It is therefore considered that relevant residues are not expected to exceed 0.01 mg/kg in rotational crops following realistic practice and provided that flurtamone is applied according to the reported GAPs. Consequently, field rotational crop studies are not required.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## CA 6.7 Proposed residue definitions and maximum residue levels

### CA 6.7.1 Proposed residue definitions

According to Article 12 of Regulation (EC) No 396/2005, the European Food Safety Authority (EFSA) has reviewed the Maximum Residue Levels (MRLs) currently established at European level for the pesticide active substance flurtamone. A reasoned opinion on the review of the existing maximum residue levels (MRLs) for flurtamone was published in EFSA Journal 2012; 10(12):3009.

This reasoned opinion included evaluation of metabolism studies in barley (M-201819-02-1), wheat (M-206857-01-1), sunflower (M-165807-02-1) and peanut (sumarized in section CA 6.2.1). The actual residue definition derived from these studies is summarized in Table CA 6.7.1-1.

**Table CA 6.7.1-1: Current residue definitions**

| Matrices | Residue definition | | Reference |
|---|---|---|---|
| Food of plant origin | Risk assessment and Monitoring | Flurtamone | EFSA Journal 2012; 10(12):3009 |
| Food of animal origin | Risk assessment and Monitoring | None, as no residue anticipated | |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

With the newly available studies metabolism of flurtamone was investigated for pre-emergence and post-emergence applications in wheat [trifluoromethyl-phenyl-UL-$^{14}$C], and for pre-emergence applications in sunflower [phenyl-UL-$^{14}$C]. Also with the recent studies flurtamone was demonstrated to be extensively degraded through several hydrolytic and oxidative steps. The predominant metabolite found in wheat grain from the trifluoromethyl phenyl labelled study was trifluoro acetic acid (TFA) which is not specific to flurtamone. The special situation with the metabolite TFA is addressed in a separate document also including a dietary risk assessment, document reference M-475258-01-1). In wheat forage hay and straw from the post emergence use the predominant residue consisted of parent compound flurtamone whereas with pre emergence use again TFA was the major contributor to the TRR accompanied by lower amounts of the parent compound. No other major metabolites were observed in wheat. In the sunflower study labelled in the phenyl ring position the predominant residue in sunflower seed consisted of a bundle of fatty acid glycerides. In sunflower forage (no feed item) parent compound appeared to be the major constituent followed by glycoside conjugates of flurtamone hydroxy.

Considering the above and as residues of flurtamone other than TFA and parent compound are not expected above 0.01 mg/kg the relevant residue for enforcement and risk assessment in plants is proposed to be maintained with the parent compound only.

Dietary burden calculations for farm animals revealed that the intake for all species will be clearly below 0.004 mg/kg. Therefore no livestock feeding studies are triggered and consequently no residue definition in animal tissues is needed in this context. Nevertheless a residue definition in animal tissues might be relevant for monitoring of misuse.
In the two species of farm animals investigated flurtamone was moderately metabolized into few major and several minor or trace metabolites.
In the former submitted studies labeled with [trifluoromethyl-phenyl-UL-$^{14}$C] flurtamone (████; 1994; M-162921-01-1 and ████ 1996; M-162898-02-1) hen and goats were dosed at nominal dose levels of 10 ppm. The active ingredient flurtamone was present in most of the tissues at significant amounts accounting for 0-35% of the TTR in hen and 0-58% of the TTR in goat. Other metabolites occationally appearing as major constituent in more than one tissue were flurtamone-desmethyl (major in hen, minor in goat), flurtamone-mandelamide (major in hen, minor in goat) and flurtamone-trifluoromethyl-hydroxy (major in goat, minor in hen). The only metabolite which could be detected as major in both species was TFMBA accounting for 0-33% in hen and for 0-43% in goat. Nevertheless TFMBA is not specific to flurtamone and therefore not adequate as model substance for

flurtamone.
In the newly investigated [phenyl-UL-$^{14}$C] studies (summarized in section CA 6.2.2 and CA 6.2.3) the most prominent constituent of the residue was parent compound accounting for 1% - 44% of the TRR in hen and 12% - 78% in goat. Other metabolites were at or below 17% of the TRR despite Flurtamone-desmethyl which accounted for 46% of the TRR in hen fat and was therefore major in hen but only minor in goat. Other metabolites like Flurtamone mandelic acid (major in hen, minor in goat), Flurtamone-difluoromethyl-hydroxy-gluA (major in goat, minor in hen) and Flurtamone-dihydroxy-pyrrolinone (major in goat, minor in hen) were occasionally detected as major but none of them appeared sufficiently dominant to serve as a model substance for flurtamone.
Maximum total residues in milk and eggs were estimated to be 1.6 ppb and 5.8 ppb respectively. Main residue constituent in these matrices was again parent compound accounting for 22% of the TRR in both milk (mean of morning and evening milk) and eggs.
Considering the above and in the light of the generally low residues the proposed residue definition for flurtamone in animal tissues is the active substance flurtamone only.

**Table CA 6.7.1-2: Proposed residue definitions**

| Matrices | Residue definition | |
|---|---|---|
| Food of plant origin | Risk assessment and Monitoring | Flurtamone |
| Food of animal origin | Risk assessment and Monitoring | Flurtamone |

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

According to the supportive residue trials provided and the new analytical method used for the analyses, there is no need to increase the existing EU-MRLs in cereals grain. Indeed, the LOQ of the analytical method is lower: 0.01 mg/kg versus those which has been used within previous submission (0.02 mg/kg). Therefore the default MRL in case of a no residue situation has slightly decreased in these crops.
According to the EFSA review (EFSA Journal 2012;10(12):3009), it was not deemed necessary to set MRLs on commodities of animal origin due to the fact that the dietary burdens for all groups of livestock were found to be below the trigger value of 0.1 mg/kg DM. With the supported uses of this dossier, the dietary burden calculations have shown in chapter CA 6.4 that the new trigger value (0.004 mg/kg bw/d) has not been exceeded using the OECD model for cattle, sheep, swine and poultry. Therefore, even if a no residue situation is expected in commodities of animal origin, default MRLs at the LOQ of the enforcement method could be proposed. See Table CA 6.7.2-1.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Table CA 6.7.2-1: Current MRLs established by EFSA and proposed MRLs**

| Commodity (code no.) | Existing MRLs (mg/kg) Reg. (EC) No 149/2008 | MRL (mg/kg) EFSA Journal 2012; 10(12):3009 | Proposed MRLs (mg/kg) |
|---|---|---|---|
| **CEREALS (0500000)** | | | |
| Barley grain (0500010) | 0.02* | 0.01* | 0.01* |
| Oats grain (0500050) | 0.02* | 0.01* | 0.01* |
| Rye grain (0500070) | 0.02* | 0.01* | 0.01* |
| Wheat grain (0500090) | 0.02* | 0.01* | 0.01* |
| **PRODUCT S OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS (1000000)** | | | |
| Tissue (1010000) | - | - | 0.01* |
| Milk (1020000) | - | - | 0.01* |
| Bird eggs (1030000) | - | - | 0.01* |

* indicates that the MRL is set at the limit of analytical quantification

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

No import tolerances have been proposed in the EU or applied for in any EU Member State.

## CA 6.8 Proposed safety intervals

It is not necessary to define a pre-harvest interval. Instead, the pre-harvest interval is given by the growing period between the growth stage at treatment and harvest.

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

The product is applied early post-emergence on very young plants. Thus, dermal exposure to persons entering a treated field is negligible. No use in buildings is intended. Therefore no re-entry period needs to be proposed for man.

Handling of treated cereals is generally not required before harvest, which is always done mechanically. Therefore there is no need to define a waiting period between application and handling of treated products.

The use of flurtamone in cereals is not likely to result in significant uptake of residues by succeeding crops. Thus, it is not necessary to set a waiting period between last application and sowing or planting succeeding crops.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

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

The Acceptable Daily Intake (ADI) of 0.03 mg/kg body weight was established based on the 2-year rat study with a safety factor of 100 (SANCO/10162/2003 (3 July 2003). No ARfD was allocated. On the basis of its toxicological profile, flurtamone is considered unlikely to present an acute hazard. The acute and short term oral toxicity of flurtamone is very low.

In order to evaluate the potential chronic exposure to flurtamone residues through the diet, the Theoretical Maximum Dietary Intakes (TMDI) were estimated using the EFSA PRIMo model (revision 2). For the evaluation of the chronic exposure the model uses 5 WHO diets relevant to the EU and 22 national diets from 13 different EU Member States.

TMDI calculations were performed using the existing MRLs in place (Reg. (EC) No 149/2008) and proposed MRLs for commodities of animal origin given in Table CA 6.9-1.

**Table CA 6.9-1: input values used for TMDI calculation of flurtamone**

| Code number | Groups and examples of individual products to which the MRLs apply (a) | MRLs (mg/kg) Reg. (EC) No 149/2008 |
|---|---|---|
| 100000 | 1. FRUIT FRESH OR FROZEN NUTS | 0.02* |
| 200000 | 2. VEGETABLES FRESH OR FROZEN | 0.02* |
| 300000 | 3. PULSES, DRY | 0.02* |
| 400000 | 4. OILSEEDS AND OILFRUITS | |
| 401000 | (i) Oilseeds | 0.05* |
| 402000 | (ii) Oilfruits | |
| 402010 | Olives for oil production | 0.02* |
| 402020 | Palm nuts (palm oil kernels) | 0.05* |
| 402030 | Palm fruit | 0.05* |
| 402040 | Kapok | 0.05* |
| 402990 | Others | 0.05* |
| 500000 | 5. CEREALS | 0.02* |
| 500010 | Barley | 0.02* |
| 500020 | Buckwheat (Amaranthus, quinoa) | 0.02* |
| 500030 | Maize | 0.02* |
| 500040 | Millet (Foxtail millet, teff, finger millet, pearl millet) | 0.02* |
| 500050 | Oats | 0.02* |
| 500060 | Rice (Indian/wild rice (Zizania aquatica)) | 0.02* |
| 500070 | Rye | 0.02* |
| 500080 | Sorghum | 0.02* |
| 500090 | Wheat (Spelt, triticale) | 0.02* |
| 500990 | Others (Canary grass seeds (Phalaris canariensis)) | 0.02* |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| Code number | Groups and examples of individual products to which the MRLs apply (a) | MRLs (mg/kg) Reg. (EC) No 149/2008 |
|---|---|---|
| 600000 | 6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA | 0.05* |
| 700000 | 7. HOPS (dried) | 0.05* |
| 800000 | 8. SPICES | 0.05* |
| 900000 | 9. SUGAR PLANTS | 0.02* |
| 1000000 | 10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS | |
| 1010000 | (i) Tissue | 0.01*# |
| 1020000 | (ii) Milk | 0.01*# |
| 1030000 | (iii) Bird eggs | 0.01*# |

(*) Indicates lower limit of analytical determination

(#) Proposed MRLs

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

As shown in Table CA 6.9-2, the highest TMDI calculated for flurtamone represented about 3% of the ADI, which denotes considerable margins of safety. The complete risk assessment is presented in Appendix 2

**Table CA 6.9-2: Summary of the top ten output from PRIMo for flurtamone chronic risk assessment**

| Highest calculated TMDI values in % of ADI | MS Diet | Highest contributor to MS diet (in % of ADI) | Commodity / group of commodities |
|---|---|---|---|
| 3.2 | UK Toddler | 1.5 | SUGAR PLANTS |
| 2.8 | WHO Cluster diet B | 1.0 | VEGETABLES FRESH OR FROZEN |
| 2.5 | FR infant | 1.4 | VEGETABLES FRESH OR FROZEN |
| 2.5 | DE child | 1.5 | FRUIT (FRESH OR FROZEN) |
| 2.2 | NL child | 1.0 | FRUIT (FRESH OR FROZEN) |
| 2.2 | FR toddler | 1.2 | VEGETABLES FRESH OR FROZEN |
| 2.0 | UK Infant | 0.7 | SUGAR PLANTS |
| 1.9 | IE adult | 0.7 | FRUIT (FRESH OR FROZEN) |
| 1.8 | WHO cluster diet E | 0.6 | VEGETABLES FRESH OR FROZEN |
| 1.5 | WHO cluster diet D | 0.6 | VEGETABLES FRESH OR FROZEN |

## CA 6.10 Other studies

The Annex II summary for the active substance sufficiently addresses aspects of the residue situation. Therefore, other special studies are not needed.

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

Flurtamone is applied on cereals early in the growing season (latest at BBCH 29) and no residues are expected in pollen and bee products.

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## Appendix 1 – Tier 1 summary of the residue data for flurtamone

**Supported residue trials performed with SC350 product:**

In northern Europe:
12-2000 (trials -01 and -02); M-457395-01-1
12-2004 (trials -01 and -02); M-459808-01-1

In southern Europe:
12-2000 (trials -03 and -04); M-457395-01-1
12-2004 (trials -03 and -04); M-459808-01-1

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | | |
|---|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | **Active substance** | : | **flurtamone** |
| **Country** | : **Germany** | **Crop/Crop Group** | : | **Cereals** |
| | | **Page** | : | **1** |
| **Content of active substance (g/kg or g/L)** | : **250 g/L** | **Indoor/outdoor** | : | **Outdoor** |
| **Formulation (e.g. WP)** | : **350 SC** | **Other a.s. in formulation (common name and content)** | : | **diflufenican 100 g/L** |
| **Commercial product (name)** | : **Diflufenican & Flurtamone SC 350** | **Residues determined as** | : | **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | **Residues calculated as** | : | **flurtamone** |

| 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 kg a.s./ha | Water (L/ha) | kg a.s./hL | 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 |
| 12-2000<br>12-2000-01<br>12-2000-01-T<br>Germany<br>51399 Burscheid<br>2011 | Barley, winter Ketos | 1) 22.09.2011<br>2) 11.05.2012 - 16.05.2012<br>3) 15.07.2012 - 31.07.2012 | SPI | 0.125 | 300 | 0.0417 | 09.11.2011/0 | 5 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | 1.7<br><0.01<br><0.01<br><0.01<br><0.01 | 0<br>176<br>204<br>257<br>257 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| 12-2000<br>12-2000-02<br>12-2000-02-T<br>United Kingdom<br>CG8 8SS<br>Cambridge<br>2012 | Barley, winter Carat | 1) 26.09.2011<br>2) 24.05.2012 - 08.06.2012<br>3) 01.08.2012 - 10.08.2012 | SPI | 0.125 | 200 | 0.0625 | 16.01.2012/0 | 5 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | 13<br><0.01<br><0.01<br><0.01<br><0.01 | 0<br>119<br>164<br>203<br>203 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

| | | |
|---|---|---|
| Responsible body for reporting (name and address) | : | Bayer CropScience AG, Monheim |
| Country | : | Germany |
| Content of active substance (g/kg or g/L) | : | 250 g/L |
| Formulation (e.g. WP) | : | 350 SC |
| Commercial product (name) | : | Diflufenican & Flurtamone SC 350 |
| Producer of commercial product | : | Bayer CropScience AG |
| Active substance | : | flurtamone |
| Crop/Crop Group | : | Cereals |
| Page | : | 1 |
| Indoor/outdoor | : | Outdoor |
| Other a.s. in formulation (common name and content) | : | diflufenican 100 g/L |
| Residues determined as | : | flurtamone |
| Residues calculated as | : | flurtamone |

| 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 | | | | | | |
| 12-2004<br>12-2004-01<br>12-2004-01-T<br>Germany<br>59457 Werl-Westönnen<br>2012 | Wheat, winter Inspiration | 1) 05.10.2011<br>2) 04.06.2012 - 11.06.2012<br>3) 15.07.2012 - 28.08.2012 | SPI | 0.13 | 300 | 0.043 | 23.03.2012/0 | 5 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | 4.7<br><0.01<br><0.01<br><0.01<br><0.01 | 0<br>63<br>109<br>133<br>133 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| 12-2004<br>12-2004-02<br>12-2004-02-T<br>Netherlands<br>1774 PE Slootdorp<br>2012 | Wheat, winter Taureq; winter | 1) 10.11.2011<br>2) 17.06.2012 - 02.07.2012<br>3) 06.08.2012 - 18.08.2012 | SPI | 0.13 | 300 | 0.043 | 20.04.2012/0 | 5 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | 5.4<br><0.01<br><0.01<br><0.01<br><0.01 | 0<br>46<br>83<br>112<br>112 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | | |
|---|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | **Active substance** | : | **flurtamone** |
| **Country** | : **Germany** | **Crop/Crop Group** | | **Cereals** |
| | | **Page** | | **2** |
| **Content of active substance (g/kg or g/L)** | : **250 g/L** | **Indoor/outdoor** | : | **Outdoor** |
| **Formulation (e.g. WP)** | : **350 SC** | **Other a.s. in formulation (common name and content)** | : | **diflufenican 100 g/L** |
| **Commercial product (name)** | : **Diflufenican & Flurtamone SC 350** | **Residues determined as** | : | **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | **Residues calculated as** | : | **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Trial No.; Plot Location incl. postal code<br>Year of Trial | Commodity / Variety<br>(a) | Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting<br>(b) | Method of treatment<br>(c) | Application rate per treatment | | | Dates of treatment(s) / Application interval or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br>(e) | Portion analysed<br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br>(f) | Remarks |
| | | | | kg a.s./ha | Water (L/ha) | kg a.s./hL | | | | | | |
| 12-2000<br>12-2000-03<br>12-2000-03-T<br>France, south<br>31620 Bouloc<br>2012 | Barley, winter<br>Queen | 1) 14.10.2011<br>2) 07.05.2012 - 14.05.2012<br>3) 20.06.2012 - 01.07.2012 | SPI | 0.125 | 300 | 0.0417 | 10.01.2012/0 | 5 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | 6.0<br><0.01<br><0.01<br><0.01<br><0.01 | 0<br>113<br>132<br>167<br>167 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| 12-2000<br>12-2000-04<br>12-2000-04-T<br>Italy<br>40128 Bologna<br>2012 | Barley, winter<br>Luteche | 1) 03.11.2011<br>2) 25.04.2012 - 03.05.2012<br>3) 20.06.2012 - 30.06.2012 | SPI | 0.125 | 400 | 0.0313 | 09.03.2012/0 | 5 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | 3.6<br><0.01<br><0.01<br><0.01<br><0.01 | 0<br>46<br>73<br>103<br>103 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | | |
|---|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | | **Active substance** | : **flurtamone** |
| **Country** | : **Germany** | | **Crop/Crop Group** | **Cereals** |
| | | | **Page** | **2** |
| **Content of active substance (g/kg or g/L)** | : **250 g/L** | | **Indoor/outdoor** | : **Outdoor** |
| **Formulation (e.g. WP)** | : **350 SC** | | **Other a.s. in formulation (common name and content)** | : **diflufenican 100 g/L** |
| **Commercial product (name)** | : **Diflufenican & Flurtamone SC 350** | | **Residues determined as** | : **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | | **Residues calculated as** | : **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study<br>Trial No.;<br>Plot<br>Location incl. postal code<br><br>Year of Trial | Commodity / Variety<br><br>(a) | Date of<br>1) Sowing or planting<br>2) Flowering<br>3) Harvest<br>4) Transplanting<br><br>(b) | Method of treatment<br><br>(c) | Application rate per treatment<br>kg a.s./ha | <br>Water (L/ha) | <br>kg a.s./hL | Dates of treatment(s)/ Application interval or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br><br>(e) | Portion analysed<br><br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br><br>(f) | Remarks |
| 12-2004<br>12-2004-03<br>12-2004-03-T<br>Spain<br>41004 Alcala de Guadaira<br>2012 | Wheat, winter<br>Artur Nick | 1) 06.12.2011<br>2) 10.04.2012 - 20.04.2012<br>3) 25.05.2012 - 30.06.2012 | SPI | 0.13 | 300 | 0.043 | 10.02.2012/0 | 5 tillers detectable | green material<br><br>whole plant without root<br><br>grain<br><br>straw | 5.4<br><0.01<br><0.01<br><br><0.01<br><br><0.01 | 0<br>52<br>89<br><br>115<br><br>115 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg<br>a storage stability study will adress temperature deviations which occurred in study 12-2004 (report S13-03307) |
| 12-2004<br>12-2004-04<br>12-2004-04-T<br>Portugal<br>2005-009 Casais da Narçisa<br>2011 | Wheat, winter<br>Hystar; Hybrid | 1) 10.10.2011<br>2) 20.04.2012 - 04.05.2012<br>3) 15.06.2012 - 15.07.2012 | SPI | 0.13 | 300 | 0.043 | 19.12.2011/0 | 5 tillers detectable | green material<br><br>whole plant without root<br><br>grain<br><br>straw | 4.3<br><0.01<br><0.01<br><br><0.01<br><br><0.01 | 0<br>110<br>164<br><br>192<br><br>192 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg<br>a storage stability study will adress temperature deviations which occurred in study 12-2000 (report S13-03307) |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

**Supported residue trials performed with SC360 product:**

In northern Europe:
- 11-2094 (trials -01 and -02); M-460003-01-1
- 11-2095 (trials -01 and -02); M-459755-01-1
- 12-2001; M-459795-01-1

In southern Europe:
- 11-2094 (trials -03 and -04); M-460003-01-1
- 11-2095 (trials -03 and -04); M-459755-01-1
- 12-2002; M-459799-01-1

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | | |
|---|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | **Active substance** | : | **flurtamone** |
| **Country** | : **Germany** | **Crop/Crop Group** | : | **Cereals** |
| | | **Page** | : | **9** |
| **Content of active substance (g/kg or g/L)** | : **120 g/L** | **Indoor/outdoor** | : | **Outdoor** |
| **Formulation (e.g. WP)** | : **360 SC** | **Other a.s. in formulation (common name and content)** | : | **diflufenican 120 g/L flufenacet 120 g/L** |
| **Commercial product (name)** | : **DFF & FFA & FLT SC 360** | **Residues determined as** | : | **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | **Residues calculated as** | : | **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Trial No.; Plot Location incl. postal code<br>Year of Trial | Commodity / Variety<br>(a) | Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting<br>(b) | Method of treatment<br>(c) | Application rate per treatment<br>kg a.s./ha | <br>Water (L/ha) | <br>kg a.s./hL | Dates of treatment(s) / Application interval or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br>(e) | Portion analysed<br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br>(f) | Remarks |
| 11-2094<br>11-2094-01<br>Germany<br>51399 Burscheid<br>2011 | Barley, winter<br>Ketos winter barley | 1) 22.09.2011<br>2) 11.05.2012 - 16.05.2012<br>3) 15.07.2012 - 31.07.2012 | SPI | 0.12 | 300 | 0.040 | 04.11.2011/0 | 5 tillers detectable | green material | 4.4<br>2.3<br>2.2<br>1.5<br>1.0<br><0.01 | 0<br>1<br>3<br>5<br>14<br>181 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 209 | |
| | | | | | | | | | grain | <0.01 | 262 | |
| | | | | | | | | | straw | <0.01 | 262 | |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | |
|---|---|---|---|
| **Responsible body for reporting (name and address)** | : Bayer CropScience AG, Monheim | **Active substance** | : flurtamone |
| **Country** | : Germany | **Crop/Crop Group** | : Cereals |
| | | **Page** | : 10 |
| **Content of active substance (g/kg or g/L)** | : 120 g/L | **Indoor/outdoor** | : Outdoor |
| **Formulation (e.g. WP)** | : 360 SC | **Other a.s. in formulation (common name and content)** | : diflufenican 120 g/L, flufenacet 120 g/L |
| **Commercial product (name)** | : DFF & FFA & FLT SC 360 | **Residues determined as** | : flurtamone |
| **Producer of commercial product** | : Bayer CropScience AG | **Residues calculated as** | : flurtamone |

| 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 | | | | | | |
| 11-2094<br>11-2094-02<br>United Kingdom<br>SG8 8SS Cambridge<br>2012 | Barley, winter<br>Carat Winter Barley | 1) 26.09.2011<br>2) 24.05.2012 - 08.06.2012<br>3) 01.08.2012 - 10.08.2012 | SPI | 0.12 | 200 | 0.060 | 16.01.2012/0 | 5 tillers detectable | green material | 9.5<br>9.4<br>2.2<br>1.8<br>0.46<br><0.01 | 0<br>1<br>3<br>4<br>14<br>119 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 164 | |
| | | | | | | | | | grain | <0.01 | 203 | |
| | | | | | | | | | straw | <0.01 | 203 | |


This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

Bayer CropScience





## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | |
|---|---|---|
| **Responsible body for reporting (name and address)** | : | **Bayer CropScience AG, Monheim** |
| **Country** | : | **Germany** |
| **Content of active substance (g/kg or g/L)** | : | **120 g/L** |
| **Formulation (e.g. WP)** | : | **360 SC** |
| **Commercial product (name)** | : | **DFF & FFA & FLT SC 360** |
| **Producer of commercial product** | : | **Bayer CropScience AG** |
| **Active substance** | : | **flurtamone** |
| **Crop/Crop Group** | : | **Cereals** |
| **Page** | : | **10** |
| **Indoor/outdoor** | : | **Outdoor** |
| **Other a.s. in formulation (common name and content)** | : | **diflufenican 120 g/L**<br>**flufenacet 120 g/L** |
| **Residues determined as** | : | **flurtamone** |
| **Residues calculated as** | : | **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study<br>Trial No.;<br>Plot<br>Location incl.<br>postal code<br><br>Year of Trial | Commodity / Variety<br><br>(a) | Date of<br>1) Sowing or planting<br>2) Flowering<br>3) Harvest<br>4) Transplanting<br><br>(b) | Method of treatment<br><br>(c) | Application rate per treatment<br>kg a.s./ha | <br>Water (L/ha) | <br>kg a.s./hL | Dates of treatment(s) / Application interval or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br>(e) | Portion analysed<br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br>(f) | Remarks |
| 11-2095<br>11-2095-01<br>Germany<br>59457 Werl - Niederbergstrasse<br>2011 | Wheat, winter<br>Akteur | 1) 17.10.2010<br>2) 30.05.2011 - 08.06.2011<br>3) 20.07.2011 - 25.08.2011 | SPI | 0.12 | 300 | 0.040 | 06.04.2011/0 | 5 tillers detectable | green material | 5.0<br>4.2<br>3.1<br>2.1<br>0.14<br><0.01 | 0<br>1<br>3<br>5<br>14<br>51 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 93 | |
| | | | | | | | | | grain | <0.01 | 117 | |
| | | | | | | | | | straw | <0.01 | 117 | |

This document is copyright protected.
Any distribution, reproduction or publication requires
the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or
any other commercial purpose is prohibited and constitutes
a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | |
|---|---|---|
| **Responsible body for reporting (name and address)** | : | **Bayer CropScience AG, Monheim** |
| **Country** | : | **Germany** |
| **Content of active substance (g/kg or g/L)** | : | **120 g/L** |
| **Formulation (e.g. WP)** | : | **360 SC** |
| **Commercial product (name)** | : | **DFF & FFA & FLT SC 360** |
| **Producer of commercial product** | : | **Bayer CropScience AG** |
| **Active substance** | : | **flurtamone** |
| **Crop/Crop Group** | : | **Cereals** |
| **Page** | : | **10** |
| **Indoor/outdoor** | : | **Outdoor** |
| **Other a.s. in formulation (common name and content)** | : | **diflufenican 120 g/L flufenacet 120 g/L** |
| **Residues determined as** | : | **flurtamone** |
| **Residues calculated as** | : | **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Trial No.; Plot Location incl. postal code<br><br>Year of Trial | Commodity / Variety<br><br>(a) | Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting<br><br>(b) | Method of treatment<br><br>(c) | Application rate per treatment | | | Dates of treatment(s) / Application interval or no. of treatments and last date/<br><br>(d) | Growth stage at last treatment<br><br>(e) | Portion analysed<br><br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br><br>(f) | Remarks |
| | | | | kg a.s./ha | Water (L/ha) | kg a.s./hL | | | | | | |
| 11-2095<br>11-2095-02<br>Netherlands<br>1175 KD Lynden (Hoofddorp)<br>2011 | Wheat, winter<br>Tabasco | 1) 08.11.2010<br>2) 15.06.2011 - 21.06.2011<br>3) 15.08.2011 - 21.08.2011 | SPI | 0.1104 | 277 | 0.0399 | 18.04.2011/0 | 3 tillers detectable - 7 tillers detectable | green material | 9.1<br>8.2<br>5.7<br>3.2<br>0.18<br><0.01 | 0<br>1<br>3<br>5<br>14<br>43 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 95 | |
| | | | | | | | | | grain | <0.01 | 121 | |
| | | | | | | | | | straw | <0.01 | 121 | |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

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

**Content of active substance (g/kg or g/L)** : **120 g/L**
**Formulation (e.g. WP)** : **360 SC**

**Commercial product (name)** : **DFF & FFA & FLT SC 360**
**Producer of commercial product** : **Bayer CropScience AG**

**Active substance** : **flurtamone**
**Crop/Crop Group** : **Cereals**
**Page** : **2**

**Indoor/outdoor** : **Outdoor**
**Other a.s. in formulation (common name and content)** : **diflufenican 120 g/L flufenacet 120 g/L**
**Residues determined as** : **flurtamone**
**Residues calculated as** : **flurtamone**

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Trial No.; Plot Location incl. postal code<br><br>Year of Trial | Commodity / Variety<br><br>(a) | Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting<br><br>(b) | Method of treatment<br><br>(c) | Application rate per treatment | | | Dates of treatment(s) / Application interval or no. of treatments and last date/<br><br>(d) | Growth stage at last treatment<br><br>(e) | Portion analysed<br><br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br><br>(f) | Remarks |
| | | | | kg a.s./ha | Water (L/ha) | kg a.s./hL | | | | | | |
| 12-2001<br>12-2001-01<br>Germany<br>49377 Langförden<br>2011 | Barley, winter Meridian | 1) 30.09.2011<br>2) 21.05.2012 - 04.06.2012<br>3) 05.07.2012 - 25.07.2012 | SPI | 0.12 | 300 | 0.040 | 22.11.2011/0 | 3 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | <0.01<br><0.01<br><0.01<br><0.01 | 170<br>216<br>244<br>244 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg<br>Storage temperature exceeded, therefore special stability study (S13-03307) |
| 12-2001<br>12-2001-02<br>Belgium<br>6210 Villers-Perwin<br>2011 | Barley, winter Saskia (early 6-rows variety, mid height) | 1) 26.09.2011<br>2) 21.05.2012 - 28.05.2012<br>3) 16.07.2012 - 22.07.2012 | SPI | 0.12 | 300 | 0.040 | 09.11.2011/0 | 2 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | <0.01<br><0.01<br><0.01<br><0.01 | 181<br>209<br>252<br>252 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

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

**Content of active substance (g/kg or g/L)** : **120 g/L**
**Formulation (e.g. WP)** : **360 SC**

**Commercial product (name)** : **DFF & FFA & FLT SC 360**
**Producer of commercial product** : **Bayer CropScience AG**

**Active substance** : **flurtamone**
**Crop/Crop Group** : **Cereals**
**Page** : **4**

**Indoor/outdoor** : **Outdoor**
**Other a.s. in formulation (common name and content)** : **diflufenican 120 g/L flufenacet 120 g/L**
**Residues determined as** : **flurtamone**
**Residues calculated as** : **flurtamone**

| 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 | | | | | | |
| 12-2001<br>12-2001-03<br>Germany<br>59457 Werl-Westönnen<br>2011 | Wheat, winter Inspiration | 1) 05.10.2011<br>2) 04.06.2012 - 11.06.2012<br>3) 15.07.2012 - 23.08.2012 | SPI | 0.12 | 300 | 0.040 | 14.11.2011/0 | 2 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | <0.01<br><0.01<br><0.01<br><0.01 | 192<br>239<br>263<br>263 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| 12-2001<br>12-2001-04<br>Netherlands<br>1774 PE Slootdorp<br>2012 | Wheat, winter Taureq winter | 1) 10.11.2011<br>2) 17.06.2012 - 02.07.2012<br>3) 06.08.2012 - 18.08.2012 | SPI | 0.12 | 300 | 0.040 | 20.04.2012/0 | 5 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | <0.01<br><0.01<br><0.01<br><0.01 | 41<br>83<br>112<br>112 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

# RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | |
|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | **Active substance** | : **flurtamone** |
| **Country** | : **Germany** | **Crop/Crop Group** | : **Cereals** |
| | | **Page** | : **11** |
| **Content of active substance (g/kg or g/L)** | : **120 g/L** | **Indoor/outdoor** | : **Outdoor** |
| **Formulation (e.g. WP)** | : **360 SC** | **Other a.s. in formulation (common name and content)** | : **diflufenican 120 g/L**<br>**flufenacet 120 g/L** |
| **Commercial product (name)** | : **DFF & FFA & FLT SC 360** | **Residues determined as** | : **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | **Residues calculated as** | : **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study<br>Trial No.;<br>Plot<br>Location incl.<br>postal code<br><br>Year of Trial | Commodity<br>/ Variety<br><br>(a) | Date of<br>1) Sowing or planting<br>2) Flowering<br>3) Harvest<br>4) Transplanting<br>(b) | Method of treatment<br><br>(c) | Application rate per treatment | | | Dates of treatment(s)/<br>Application interval<br>or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br>(e) | Portion analysed<br>(a) | Residues (mg/kg) | DALT/<br>PHI<br>(days)<br>(f) | Remarks |
| | | | | kg a.s./ha | Water (L/ha) | kg a.s./hL | | | | | | |
| 11-2094<br>11-2094-03<br>France<br>86220 Leugny<br>2011 | Barley, winter<br>Kétos<br>Winter Barley | 1) 16.10.2010<br>2) 06.05.2011<br>- 12.05.2011<br>3) 17.06.2011<br>- 27.06.2011 | SPI | 0.12 | 300 | 0.040 | 08.03.2011/0 | 9 or more tillers detectable | green material | 5.6<br>5.8<br>3.5<br>1.7<br>0.40<br><0.01 | 0<br>1<br>3<br>6<br>14<br>55 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 83 | |
| | | | | | | | | | grain | <0.01 | 108 | |
| | | | | | | | | | straw | <0.01 | 108 | |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | | |
|---|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | **Active substance** | : | **flurtamone** |
| **Country** | : **Germany** | **Crop/Crop Group** | | **Cereals** |
| | | **Page** | | **12** |
| **Content of active substance (g/kg or g/L)** | : **120 g/L** | **Indoor/outdoor** | : | **Outdoor** |
| **Formulation (e.g. WP)** | : **360 SC** | **Other a.s. in formulation (common name and content)** | | **diflufenican 120 g/L flufenacet 120 g/L** |
| **Commercial product (name)** | : **DFF & FFA & FLT SC 360** | **Residues determined as** | : | **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | **Residues calculated as** | : | **flurtamone** |

| 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 | | | | | | |
| 11-2094<br>11-2094-04<br>Italy<br>44124 Ferrara<br>2011 | Barley, winter<br>Aldebaran winter variety | 1) 13.10.2010<br>2) 01.05.2011 - 18.05.2011<br>3) 08.06.2011 - 30.06.2011 | SPI | 0.12 | 400 | 0.030 | 21.03.2011/0 | 5 tillers detectable | green material | 3.5<br>3.2<br>2.5<br>1.5<br>0.12<br><0.01 | 0<br>1<br>3<br>5<br>14<br>28 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 50 | |
| | | | | | | | | | grain | <0.01 | 80 | |
| | | | | | | | | | straw | 0.021 | 80 | |

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | |
|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | **Active substance** | : **flurtamone** |
| **Country** | : **Germany** | **Crop/Crop Group** | : **Cereals** |
| | | **Page** | : **11** |
| **Content of active substance (g/kg or g/L)** | : **120 g/L** | **Indoor/outdoor** | : **Outdoor** |
| **Formulation (e.g. WP)** | : **360 SC** | **Other a.s. in formulation (common name and content)** | : **diflufenican 120 g/L flufenacet 120 g/L** |
| **Commercial product (name)** | : **DFF & FFA & FLT SC 360** | **Residues determined as** | : **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | **Residues calculated as** | : **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Trial No.; Plot Location incl. postal code<br>Year of Trial | Commodity / Variety<br>(a) | Date of<br>1) Sowing or planting<br>2) Flowering<br>3) Harvest<br>4) Transplanting<br>(b) | Method of treatment<br>(c) | Application rate per treatment | | | Dates of treatment(s) / Application interval or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br>(e) | Portion analysed<br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br>(f) | Remarks |
| | | | | kg a.s./ha | Water (L/ha) | kg a.s./hL | | | | | | |
| 11-2095<br>11-2095-03<br>France<br>86270 Mairé<br>2011 | Wheat, winter<br>Cezanne | 1) 21.10.2010<br>2) 02.05.2011 - 13.05.2011<br>3) 01.07.2011 - 10.07.2011 | SPI | 0.12 | 300 | 0.040 | 08.03.2011/0 | 9 or more tillers detectable | green material | 8.6<br>7.5<br>5.0<br>2.1<br>0.33<br><0.01 | 0<br>1<br>3<br>6<br>14<br>57 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 90 | |
| | | | | | | | | | grain | <0.01 | 119 | |
| | | | | | | | | | straw | <0.01 | 119 | |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | | | |
|---|---|---|---|---|
| **Responsible body for reporting (name and address)** | : **Bayer CropScience AG, Monheim** | **Active substance** | : | **flurtamone** |
| **Country** | : **Germany** | **Crop/Crop Group** | | **Cereals** |
| | | **Page** | | **12** |
| **Content of active substance (g/kg or g/L)** | : **120 g/L** | **Indoor/outdoor** | : | **Outdoor** |
| **Formulation (e.g. WP)** | : **360 SC** | **Other a.s. in formulation (common name and content)** | : | **diflufenican 120 g/L flufenacet 120 g/L** |
| **Commercial product (name)** | : **DFF & FFA & FLT SC 360** | **Residues determined as** | : | **flurtamone** |
| **Producer of commercial product** | : **Bayer CropScience AG** | **Residues calculated as** | : | **flurtamone** |

| 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 | | | | | | |
| 11-2095<br>11-2095-04<br>Spain<br>08520 Marata - Les Franqueses<br>2011 | Wheat, winter<br>Moncada; sowing seed production | 1) 05.01.2011<br>2) 25.04.2011 - 05.05.2011<br>3) 15.06.2011 - 30.06.2011 | SPI | 0.12 | 300 | 0.040 | 09.03.2011/0 | Beginning of stem elongation | green material | 8.4<br>8.7<br>6.2<br>0.24<br>0.023<br><0.01 | 0<br>1<br>2<br>5<br>14<br>42 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| | | | | | | | | | whole plant without root | <0.01 | 68 | |
| | | | | | | | | | grain | <0.01 | 103 | |
| | | | | | | | | | straw | <0.01 | 103 | |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

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

**Content of active substance (g/kg or g/L)** : **120 g/L**
**Formulation (e.g. WP)** : **360 SC**

**Commercial product (name)** : **DFF & FFA & FLT SC 360**
**Producer of commercial product** : **Bayer CropScience AG**

**Active substance** : **flurtamone**
**Crop/Crop Group** : **Cereals**
**Page** : **2**
**Indoor/outdoor** : **Outdoor**
**Other a.s. in formulation (common name and content)** : **diflufenican 120 g/L, flufenacet 120 g/L**
**Residues determined as** : **flurtamone**
**Residues calculated as** : **flurtamone**

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

| 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 kg a.s./ha | Water (L/ha) | kg a.s./hL | 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 |
| 12-2002<br>12-2002-01<br>France<br>13103 Saint Etienne du gres<br>2011 | Barley, winter<br>Platine | 1) 14.10.2011<br>2) 20.04.2012 - 27.04.2012<br>3) 15.06.2012 - 25.06.2012 | SPI | 0.12 | 300 | 0.040 | 12.12.2011/0 | 3 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | <0.01<br><0.01<br><0.01<br><0.01 | 121<br>155<br>192<br>192 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |
| 12-2002<br>12-2002-02<br>Italy<br>37050 Perzacco<br>2012 | Barley, winter<br>Amillis | 1) 18.10.2011<br>2) 01.05.2012 - 08.05.2012<br>3) 30.06.2012 - 20.07.2012 | SPI | 0.12 | 300 | 0.040 | 02.03.2012/0 | 3 tillers detectable | green material<br>whole plant without root<br>grain<br>straw | <0.01<br><0.01<br><0.01<br><0.01 | 46<br>76<br>105<br>105 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg |

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

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

**Content of active substance (g/kg or g/L)** : **120 g/L**
**Formulation (e.g. WP)** : **360 SC**

**Commercial product (name)** : **DFF & FFA & FLT SC 360**
**Producer of commercial product** : **Bayer CropScience AG**

**Active substance** : **flurtamone**

**Crop/Crop Group** : **Cereals**
**Page** : **4**

**Indoor/outdoor** : **Outdoor**
**Other a.s. in formulation (common name and content)** : **diflufenican 120 g/L**
**flufenacet 120 g/L**
**Residues determined as** : **flurtamone**
**Residues calculated as** : **flurtamone**

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study<br>Trial No.;<br>Plot<br>Location incl.<br>postal code<br>Year of Trial | Commodity / Variety<br>(a) | Date of<br>1) Sowing or planting<br>2) Flowering<br>3) Harvest<br>4) Transplanting<br>(b) | Method of treatment<br>(c) | Application rate per treatment | | | Dates of treatment(s)/ Application interval or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br>(e) | Portion analysed<br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br>(f) | Remarks |
| | | | | kg a.s./ha | Water (L/ha) | kg a.s./hL | | | | | | |
| 12-2002<br>12-2002-03<br>Spain<br>41004 Alcala de Guadaira<br>2012 | Wheat, winter<br>Artur Nick | 1) 06.12.2011<br>2) 10.04.2012 - 20.04.2012<br>3) 25.05.2012 - 30.06.2012 | SPI | 0.11 | 280 | 0.040 | 07.02.2012/0 | 3 tillers detectable | green material | <0.01 | 51 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg<br>Storage temperature exceeded, therefore special stability study (S13-03307) |
| | | | | | | | | | whole plant without root | <0.01 | 92 | |
| | | | | | | | | | grain | <0.01 | 119 | |
| | | | | | | | | | straw | <0.01 | 119 | |

This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

**(Application on agricultural and horticultural crops)**

| | | |
|---|---|---|
| **Responsible body for reporting (name and address)** | : | **Bayer CropScience AG, Monheim** |
| **Country** | : | **Germany** |
| **Content of active substance (g/kg or g/L)** | : | **120 g/L** |
| **Formulation (e.g. WP)** | : | **360 SC** |
| **Commercial product (name)** | : | **DFF & FFA & FLT SC 360** |
| **Producer of commercial product** | : | **Bayer CropScience AG** |
| **Active substance** | : | **flurtamone** |
| **Crop/Crop Group** | : | **Cereals** |
| **Page** | : | **4** |
| **Indoor/outdoor** | : | **Outdoor** |
| **Other a.s. in formulation (common name and content)** | : | **diflufenican 120 g/L flufenacet 120 g/L** |
| **Residues determined as** | : | **flurtamone** |
| **Residues calculated as** | : | **flurtamone** |

| 1 | 2 | 3 | 4 | 5 | | | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Trial No.; Plot Location incl. postal code<br>Year of Trial | Commodity / Variety<br>(a) | Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting<br>(b) | Method of treatment<br>(c) | Application rate per treatment | | | Dates of treatment(s) / Application interval or no. of treatments and last date/<br>(d) | Growth stage at last treatment<br>(e) | Portion analysed<br>(a) | Residues (mg/kg) | DALT/ PHI (days)<br>(f) | Remarks |
| | | | | kg a.s./ha | Water (L/ha) | kg a.s./hL | | | | | | |
| 12-2002<br>12-2002-04<br>Portugal<br>2005-009 Casais da Narcisa<br>2011 | Wheat, winter<br>Hystar | 1) 10.10.2011<br>2) 20.04.2012 - 04.05.2012<br>3) 15.06.2012 - 15.07.2012 | SPI | 0.12 | 300 | 0.040 | 28.11.2011/0 | 2 tillers detectable | green material | <0.01 | 129 | (c) SPI:Spraying<br>(g) 01328<br>(h) 0.01 mg/kg<br>Storage temperature exceeded, therefore special stability study (S13-03307) |
| | | | | | | | | | whole plant without root | <0.01 | 185 | |
| | | | | | | | | | grain | <0.01 | 213 | |
| | | | | | | | | | straw | <0.01 | 213 | |

(a) According to Codex (or other e.g. EU) Classification/Guide
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc. overall broadcast
(d) Year must be indicated
(e) BBCH Monograph, Growth Stages of Plants, 1997, (Blackwell, ISBN 3-8263-3152-4)
(f) Minimum no. of days after last treatm. (DALT, Label pre-harvest interval, PHI = '<<')
(g) Reference to analytical method
(h) Limit of determination/quantitation
(i) Dosage of a.s. or water given as...
(-) Missing data in the above columns occurs where the information is not available in the original report

Note: All entries to be filled in as appropriate. Date format dd.mm.yy.

This document is copyright protected. Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate). Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.

## Appendix 2 – Flurtamone – TMDI using EFSA model rev. 2.0, based on the existing and proposed MRLs

### FLURTAMONE

| | | | |
|---|---|---|---|
| Status of the active substance: | | Code no. | |
| LOQ (mg/kg bw): | | proposed LOQ: | |
| **Toxicological end points** | | | |
| ADI (mg/kg bw/day): | **0,03** | ARfD (mg/kg bw): | |
| Source of ADI: | | Source of ARfD: | |
| Year of evaluation: | | Year of evaluation: | |

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

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

**Chronic risk assessment**

TMDI (range) in % of ADI minimum - maximum: 1 – 3

**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) |
|---|---|---|---|---|---|---|---|---|
| 3,2 | UK Toddler | 1,5 | SUGAR PLANTS | 0,5 | Oilseeds | 0,4 | FRUIT (FRESH OR FROZEN) | |
| 2,8 | WHO Cluster diet B | 1,0 | VEGETABLES | 0,8 | CEREALS | 0,5 | FRUIT (FRESH OR FROZEN) | |
| 2,5 | FR infant | 1,4 | VEGETABLES | 1,0 | FRUIT (FRESH OR FROZEN) | 0,1 | CEREALS | |
| 2,5 | DE child | 1,5 | FRUIT (FRESH OR FROZEN) | 0,5 | VEGETABLES | 0,4 | CEREALS | |
| 2,2 | NL child | 1,0 | FRUIT (FRESH OR FROZEN) | 0,8 | VEGETABLES | 0,4 | CEREALS | |
| 2,2 | FR toddler | 1,2 | VEGETABLES | 0,8 | FRUIT (FRESH OR FROZEN) | 0,2 | CEREALS | |
| 2,0 | UK Infant | 0,7 | SUGAR PLANTS | 0,4 | VEGETABLES | 0,4 | FRUIT (FRESH OR FROZEN) | |
| 1,9 | IE adult | 0,7 | FRUIT (FRESH OR FROZEN) | 0,6 | VEGETABLES | 0,4 | CEREALS | |
| 1,8 | WHO cluster diet E | 0,6 | VEGETABLES | 0,4 | CEREALS | 0,4 | FRUIT (FRESH OR FROZEN) | |
| 1,5 | WHO cluster diet D | 0,6 | VEGETABLES | 0,6 | CEREALS | 0,2 | FRUIT (FRESH OR FROZEN) | |
| 1,5 | DK child | 0,7 | CEREALS | 0,5 | VEGETABLES | 0,3 | FRUIT (FRESH OR FROZEN) | |
| 1,4 | SE general population 90th percentile | 0,7 | VEGETABLES | 0,4 | FRUIT (FRESH OR FROZEN) | 0,3 | CEREALS | |
| 1,4 | WHO Cluster diet F | 0,5 | VEGETABLES | 0,4 | CEREALS | 0,3 | FRUIT (FRESH OR FROZEN) | |
| 1,3 | PT General population | 0,4 | FRUIT (FRESH OR FROZEN) | 0,4 | VEGETABLES | 0,4 | CEREALS | |
| 1,2 | ES child | 0,4 | FRUIT (FRESH OR FROZEN) | 0,3 | CEREALS | 0,3 | VEGETABLES | |
| 1,2 | WHO regional European diet | 0,6 | VEGETABLES | 0,2 | CEREALS | 0,2 | FRUIT (FRESH OR FROZEN) | |
| 1,1 | IT kids/toddler | 0,6 | CEREALS | 0,3 | VEGETABLES | 0,2 | FRUIT (FRESH OR FROZEN) | |
| 1,0 | UK Adult | 0,3 | SUGAR PLANTS | 0,2 | VEGETABLES | 0,2 | Oilseeds | |
| 1,0 | FR all population | 0,4 | FRUIT (FRESH OR FROZEN) | 0,2 | VEGETABLES | 0,2 | CEREALS | |
| 0,9 | UK vegetarian | 0,3 | SUGAR PLANTS | 0,3 | VEGETABLES | 0,2 | FRUIT (FRESH OR FROZEN) | |
| 0,9 | NL general | 0,4 | VEGETABLES | 0,3 | FRUIT (FRESH OR FROZEN) | 0,2 | CEREALS | |
| 0,8 | ES adult | 0,3 | FRUIT (FRESH OR FROZEN) | 0,3 | VEGETABLES | 0,2 | CEREALS | |
| 0,8 | IT adult | 0,3 | CEREALS | 0,3 | VEGETABLES | 0,2 | FRUIT (FRESH OR FROZEN) | |
| 0,7 | LT adult | 0,3 | VEGETABLES | 0,2 | CEREALS | 0,2 | FRUIT (FRESH OR FROZEN) | |
| 0,7 | DK adult | 0,2 | VEGETABLES | 0,2 | FRUIT (FRESH OR FROZEN) | 0,2 | CEREALS | |
| 0,6 | PL general population | 0,4 | VEGETABLES | 0,2 | FRUIT (FRESH OR FROZEN) | 0,0 | PULSES, DRY | |
| 0,5 | FI adult | 0,2 | VEGETABLES | 0,2 | FRUIT (FRESH OR FROZEN) | 0,1 | CEREALS | |

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


This document is copyright protected.
Any distribution, reproduction or publication requires the consent of Bayer AG (or its respective affiliate).
Any use of the document or its content for regulatory or any other commercial purpose is prohibited and constitutes a violation of the underlying license agreement.