

# *European Commission*



**Draft Renewal Assessment Report prepared according to the Commission  
Regulation (EU) N° 1107/2009**

## **FLUFENACET**

### **Volume 3 – B.5 (AS)**

Rapporteur Member State: Poland  
Co-Rapporteur Member State: France

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## Version History

| When        | What   |
|-------------|--|
| August 1997 | Initial assessment. <b>Draft Assessment Report</b> for first inclusion to Annex I. RMS: FR                                     |
| April 2016  | <b>Draft Renewal Assessment Report</b> prepared according to the Commission; Regulation (EU) N° 1107/2009; RMS: PL; Co-RMS: FR |
| May 2017    | Revision after CoRMS comments  |

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## B.5. METHODS OF ANALYSIS

### INTRODUCTION

This dossier is submitted to support the re-approval of the active substance Flufenacet in Europe according to Regulation 1107/2009 and the Regulation 844/2012.

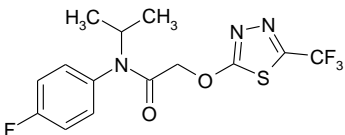
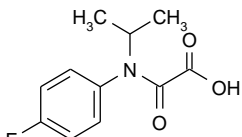
Flufenacet was originally included in Annex I of Directive 91/414/EEC on 01/01/2004, as notified in Directive 2003/84/EC dated 25 September 2003 wherein there is no specific provision under Part B which needs to be considered related to the metabolism and residue data.

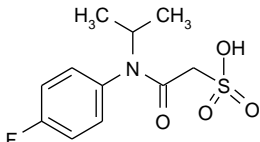
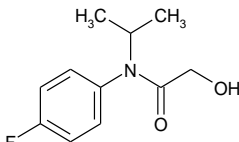
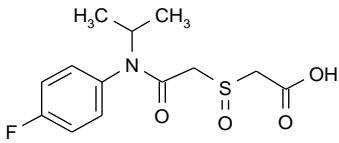
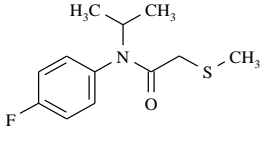
The Monograph prepared by the Rapporteur Member State France in the context of the inclusion of flufenacet in Annex 1 of the Council Directive 91/414/EEC, the Review Report for flufenacet (7469/VI/98-Final – 3<sup>rd</sup> July 2003) and the EFSA's Reasoned Opinion on the review of existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(4):2689) are considered to provide the relevant scientific information for the review of the active substance. This draft renewal assessment report (DRAR) contains summaries of studies on flufenacet, which were not available at the time of the Annex I inclusion under Directive 91/414/EEC and were, therefore, not evaluated during the first EU review of this compound. All studies, which were already submitted for the Annex I inclusion under Directive 91/414/EEC are briefly summarized.

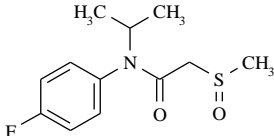
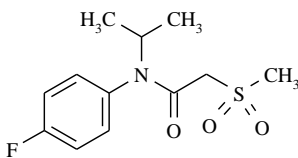
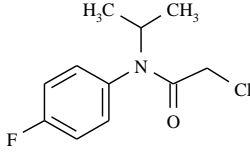
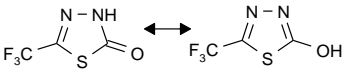
The representative uses supported for the Annex I inclusion process were the outdoor treatment of maize, soya bean, sunflower and cereals (wheat/triticale, rye and barley) in northern and/or southern Europe. In the flufenacet renewal dossier the notifier have included only "representative use" on wheat, barley and rye at application rates between 120 and 240 g as/ha in northern and southern Europe.

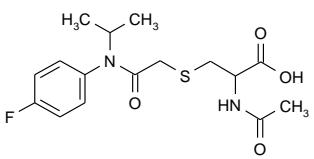
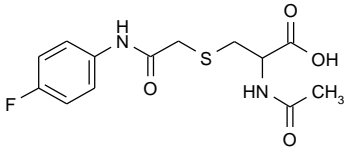
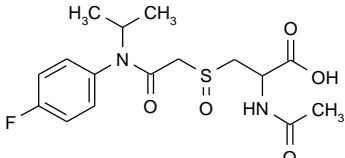
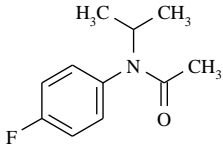
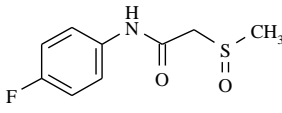
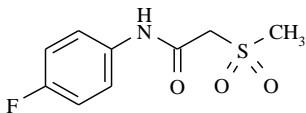
For clarity a list of metabolites, which contains the structures, the synonyms, codes and chemical names is presented below. The matrices in which the metabolites were identified are also included in this list.

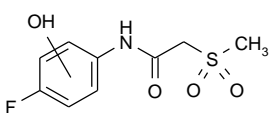
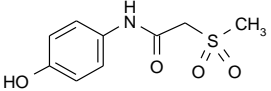
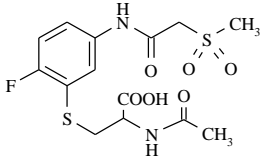
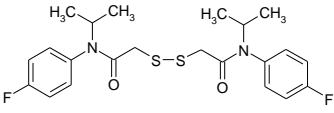
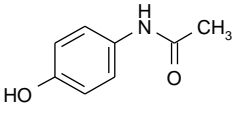
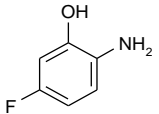
**Table 5-1. Substances and metabolites; structures, codes, synonyms**

| No.  | Structure, Report Name   | Synonyms, Codes, Chemical Name  | Occurrence  |
|------|--|---|---|
| a.s. |  <p>flufenacet<br/>(active substance)</p> | <p>FOE 5043,<br/>AE F133402, BCS-AB27364</p> <p>IUPAC:<br/>4'-fluoro-<i>N</i>-isopropyl-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetanilide</p> <p><i>N</i>-(4-fluorophenyl)-<i>N</i>-isopropyl-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide (generated by ICS Naming)</p> <p>CAS:<br/><i>N</i>-(4-fluorophenyl)-<i>N</i>-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide</p> |   |
| M01  |  <p>FOE oxalate</p>                       | <p>FOE5043-oxalate<br/>FOEOXALATE,<br/>FOEACID<br/>OXALATE<br/>AE 0841913<br/>BCS-AB16305</p> <p>IUPAC:<br/>[(4-fluorophenyl)(isopropyl)amino](oxo)acetic acid (generated by ICS Naming)</p> <p>CAS:<br/>Acetic acid, 2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxo-</p>   | Goat, hen   |
|      |  |   | Corn, soybean, cotton, wheat; Rotational crops: kale, turnip, wheat |
|      |  |   | Soil (aerobic & anaerobic)<br>Water (aerobic)                       |

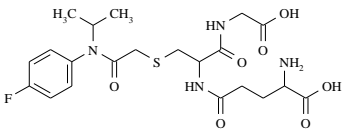
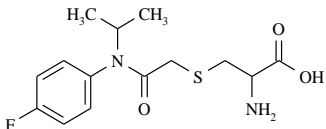
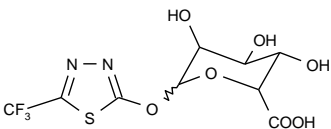
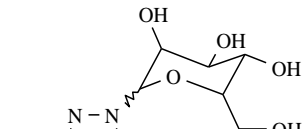
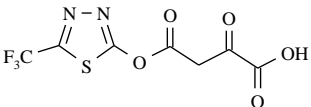
| No.   | Structure, Report Name   | Synonyms, Codes, Chemical Name  | Occurrence   |
|-------|--|---|--|
| M02 a |  <p>FOE sulfonic acid</p>             | <p>FASO3H<br/>AE 0841914<br/>KTS 9465 (sodium salt)<br/>BCS-AZ23374 (sodium salt)<br/>WAK 6222 (acid)<br/>ethanesulfonic acid sodium salt</p> <p>IUPAC:<br/>2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethanesulfonic acid<br/>sodium 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethanesulfonate<br/>(both generated by ICS Naming)</p> <p>CAS:<br/>sodium salt: (2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxo-ethanesulfonic acid sodium salt)</p> | Rat  |
|       |  |   | Corn, soybean, wheat, potato, cotton;<br>Rotational crops: kale, turnip, wheat |
|       |  |   | Soil (aerobic & anaerobic)<br>Water (aerobic)                                  |
| M03   |  <p>FOE alcohol</p>                  | <p>FOEALC<br/>AND 1403</p> <p>IUPAC:<br/><i>N</i>-(4-fluorophenyl)-2-hydroxy-<i>N</i>-isopropylacetamide<br/>(generated by ICS Naming)</p>  | Rat  |
|       |  |   | Corn, soybean, wheat, potato, cotton;<br>Rotational crops: kale, turnip, wheat |
|       |  |   | Soil (aerobic & anaerobic)<br>Water (aerobic)                                  |
| M04   |  <p>FOE thioglycolate sulfoxide</p> | <p>FAMSOC<br/>TGS<br/>FOE mercapto acetic acid sulfoxide<br/>AE 0841915<br/>BCS-AB68868</p> <p>IUPAC:<br/>({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfinyl)acetic acid<br/>(generated by ICS Naming)</p>  | -  |
|       |  |   | Corn, soybean, wheat, potato, cotton;<br>Rotational crops: kale, turnip, wheat |
|       |  |   | Soil (aerobic)<br>Water (aerobic)  |
| M05   |  <p>FOE methylsulfide</p>           | <p>WAK 7825<br/>FAMS<br/>BCS-CP38571</p> <p>IUPAC:<br/><i>N</i>-(4-fluorophenyl)-<i>N</i>-isopropyl-2-(methylsulfanyl)acetamide<br/>(generated by ICS Naming)</p>   | Goat, hen  |
|       |  |   | Corn   |
|       |  |   | Water (aerobic)  |

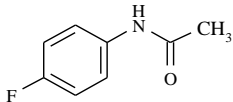
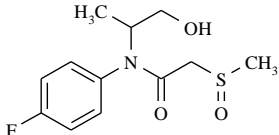
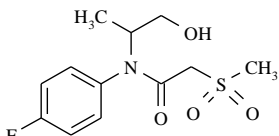
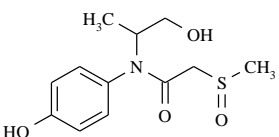
| No. | Structure, Report Name  | Synonyms, Codes, Chemical Name  | Occurrence   |
|-----|---|---|--|
| M06 |  <p>FOE methylsulfoxide</p>                      | <p>FOE methyl sulfoxide, FAMSO</p> <p>IUPAC:<br/> <i>N</i>-(4-fluorophenyl)-<i>N</i>-isopropyl-2-(methylsulfinyl)acetamide<br/>           (generated by ICS Naming)</p>   | Rat  |
|     |   |   | Corn, soybean;<br><br>Rotational crops:<br>kale wheat  |
|     |   |   | Soil (aerobic)<br>Water (aerobic)  |
| M07 |  <p>FOE methylsulfone</p>                        | <p>FAMSO2<br/>FOE methyl-sulfone<br/>BCS-CO62475</p> <p>IUPAC:<br/> <i>N</i>-(4-fluorophenyl)-<i>N</i>-isopropyl-2-(methylsulfonyl)acetamide<br/>           (generated by ICS Naming)</p>   | Rat  |
|     |   |   | Corn, soybean;<br><br>Rotational crops:<br>kale wheat  |
|     |   |   | Soil (aerobic)<br>Water (aerobic)  |
| M08 |  <p>FOE chloroacetanilide</p>                   | <p>BCS-AA70824</p> <p>IUPAC:<br/>           2-chloro-<i>N</i>-(4-fluorophenyl)-<i>N</i>-isopropylacetamide<br/>           (generated by ICS Naming)</p>   | Rat  |
|     |   |   | Corn, soybean;<br><br>Rotational crops:<br>kale wheat  |
|     |   |   | Soil (aerobic)   |
| M09 |  <p>FOE-thiadone<br/>(keto-enol tautomers)</p> | <p>Thiadone<br/>TH<br/>HWH 4343<br/>BCS-AA41715</p> <p>IUPAC:<br/>           5-(trifluoromethyl)-1,3,4-thiadiazol-2(3<i>H</i>)-one,<br/>           5-(trifluoromethyl)-1,3,4-thiadiazol-2-ol<br/>           (generated by ICS Naming)</p> | Rat<br>(detected as aglycon<br>and as glucuronide<br>(M24) and oxalyl-<br>acetate conjugates<br>(M26);<br>Goat, hen  |
|     |   |   | Corn, soybean;<br><br>Rotational crops:<br>transient metabolite<br>as it is detected as<br>N-glucoside (M25)<br>and N-malonyl-<br>alanine conjugate<br>(M34) |
|     |   |   | Soil (aerobic &<br>anaerobic)<br>Water (aerobic)   |

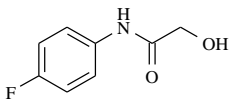
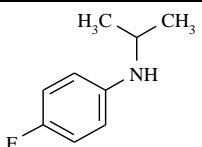
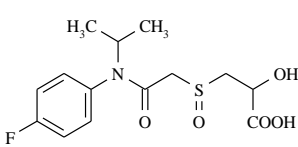
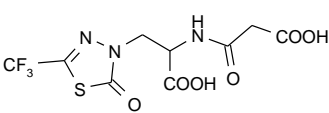
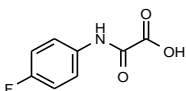
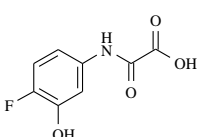
| No.  | Structure, Report Name  | Synonyms, Codes, Chemical Name  | Occurrence   |
|------|---|---|--|
| M 10 |  <p>FOE acetyl cysteine</p>                | <p>FANACS<br/>FOE-mercapturic acid</p> <p>IUPAC:<br/><i>N</i>-acetyl-<i>S</i>-{2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}cysteine<br/>(generated by ICS Naming)</p> | Rat, goat, hen   |
|      |   |   | -  |
|      |   |   | -  |
| M 11 |  <p>FOE des-i-propyl cysteine</p>          | <p>DIFANACS</p> <p>IUPAC:<br/><i>N</i>-acetyl-<i>S</i>-{2-[(4-fluorophenyl)amino]-2-oxoethyl}cysteine<br/>(generated by ICS Naming)</p>                                   | Rat  |
|      |   |   | -  |
|      |   |   | -  |
| M 12 |  <p>FOE S-oxo cysteine</p>                 | <p>FANACSO</p> <p>IUPAC:<br/><i>N</i>-acetyl-3-({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfinyl)alanine<br/>(generated by ICS Naming)</p>                       | Rat  |
|      |   |   | -  |
|      |   |   | -  |
| M 13 |  <p>FOE amine acetate</p>                | <p>FAAC</p> <p>IUPAC:<br/><i>N</i>-(4-fluorophenyl)-<i>N</i>-isopropylacetamide<br/>(generated by ICS Naming)</p>   | Rat<br>Hen   |
|      |   |   | Corn   |
|      |   |   | -  |
| M 14 |  <p>FOE des-i-propyl methylsulfoxide</p> | <p>DIFAMSO<br/>BCS-AH21407</p> <p>IUPAC:<br/><i>N</i>-(4-fluorophenyl)-2-(methylsulfinyl)acetamide<br/>(generated by ICS Naming)</p>                                      | Rat (transient)  |
|      |   |   | -  |
|      |   |   | -  |
| M 15 |  <p>FOE des-i-propyl methylsulfone</p>   | <p>DIFAMSO2</p> <p>IUPAC:<br/><i>N</i>-(4-fluorophenyl)-2-(methylsulfonyl)acetamide<br/>(generated by ICS Naming)</p>   | Rat,<br>Goat (following feeding of parent flufenacet. However, the parent substance is not present in ruminant feed) |
|      |   |   | -  |
|      |   |   | -  |

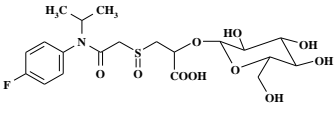
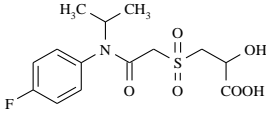
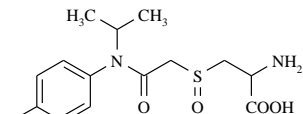
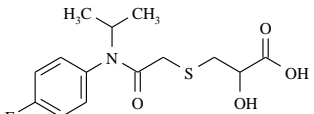
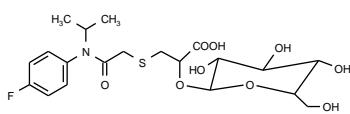
| No.  | Structure, Report Name  | Synonyms, Codes, Chemical Name  | Occurrence |
|------|---|---|------------|
| M 16 |  <p>fluoro-hydroxy-des-i-propyl methylsulfone</p>          | <p>HODIFAMSO2</p> <p>(no exact structure defined)</p>   | Rat        |
|      |   |   | -          |
|      |   |   | -          |
| M 17 |  <p>hydroxy-des-i-propyl methylsulfone</p>                 | <p>HODIFAMSO2</p> <p>IUPAC:<br/>N-(4-hydroxyphenyl)-2-(methylsulfonyl)acetamide<br/>(generated by ICS Naming)</p>                                     | Rat        |
|      |   |   | -          |
|      |   |   | -          |
| M 18 |  <p>hydroxy-des-i-propyl methylsulfone-glutaminic acid</p> | <p>HODIFAMSO2-Glu</p> <p>IUPAC:<br/>N-acetyl-S-(2-fluoro-5-[(methylsulfonyl)acetyl]amino)phenyl)cysteine<br/>(generated by ICS Naming)</p>            | Rat        |
|      |   |   | -          |
|      |   |   | -          |
| M 19 |  <p>FOE disulfide</p>                                    | <p>FOE-thiol dimer<br/>BCS-BJ39504</p> <p>IUPAC:<br/>2,2'-disulfanediyldis[2-(4-fluorophenyl)-N-isopropylacetamide]<br/>(generated by ICS Naming)</p> | Rat        |
|      |   |   | -          |
|      |   |   | -          |
| M 20 |  <p>hydroxy-des-i-propyl amine acetate</p>               | <p>DIFANAc<br/>DIFAOAc (regio-isomers),<br/>BCS-AF93293</p> <p>IUPAC:<br/>N-(4-hydroxyphenyl)acetamide<br/>(generated by ICS Naming)</p>              | Rat        |
|      |   |   | -          |
|      |   |   | -          |
| M 21 |  <p>2-A-5-FP</p>   | <p>2-amino-5-fluorophenol<br/>BCS-AA53294</p> <p>IUPAC:<br/>2-amino-5-fluorophenol<br/>(generated by ICS Naming)</p>                                  | Rat        |
|      |   |   | -          |
|      |   |   | -          |

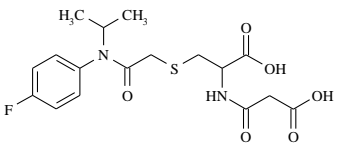
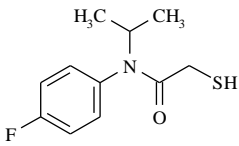
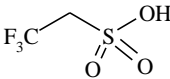
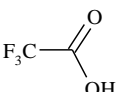
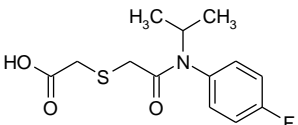


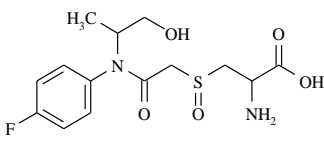
| No.  | Structure, Report Name   | Synonyms, Codes, Chemical Name  | Occurrence   |
|------|--|---|--|
| M 22 |  <p>FOE glutathione</p>         | <p>FOEGSH</p> <p>IUPAC:<br/>gamma-glutamyl-S-{2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}cysteinylglycine<br/>(generated by ICS Naming)</p>                      | Rat, goat and hen:<br>Primary transient metabolite   |
|      |  |   | Plants: Primary transient metabolite of the main metabolic pathway                             |
|      |  |   | -  |
| M 23 |  <p>FOE cysteine</p>            | <p>FOE cysteinyl conjugate<br/>FACS</p> <p>IUPAC:<br/>S-{2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}cysteine<br/>(generated by ICS Naming)</p>                   | Rat, goat and hen:<br>Transient secondary metabolite   |
|      |  |   | Plants: Transient secondary metabolite of the main metabolic pathway, detected in potato tuber |
|      |  |   | -  |
| M 24 |  <p>thiadone glucuronide</p>  | <p>Th glucuronide<br/>TH-GA</p> <p>IUPAC:<br/>5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl hexopyranosiduronic acid<br/>(generated by ICS Naming)</p>                    | Rat, goat, hen   |
|      |  |   | -  |
|      |  |   | -  |
| M 25 |  <p>ThN glucoside</p>         | <p>THNG<br/>Th-NG<br/>Thiadone-N-glucoside</p> <p>IUPAC:<br/>3-hexopyranosyl-5-(trifluoromethyl)-2,3-dihydro-1,3,4-thiadiazol-2-one<br/>(generated by ICS Naming)</p> | Soybean, corn  |
|      |  |   | Rotational crops:<br>kale, turnip, wheat   |
|      |  |   | -  |
| M 26 |  <p>Th oxalyl acetic acid</p> | <p>Th-OAA</p> <p>IUPAC:<br/>2,4-dioxo-4-{[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy}butanoic acid<br/>(generated by ICS Naming)</p>                               | Rat  |
|      |  |   | -  |
|      |  |   | -  |

| No.  | Structure, Report Name   | Synonyms, Codes, Chemical Name   | Occurrence   |
|------|--|--|--|
| M 27 | <br>DIFAAC      | N-(4-Fluorophenyl) acetamide<br>BCS-AA22989<br><br>IUPAC:<br><i>N</i> -(4-fluorophenyl)acetamide<br>(generated by ICS Naming)    | Hen (following feeding of parent flufenacet; not relevant as the parent substance is not present in poultry feed)        |
|      |  |  | -  |
|      |  |  | -  |
| M 28 | <br>HOIFAMSO   | IUPAC:<br><i>N</i> -(4-fluorophenyl)- <i>N</i> -(1-hydroxypropan-2-yl)-2-(methylsulfinyl)acetamide<br>(generated by ICS Naming)  | Hen (following feeding of parent flufenacet; not relevant as the parent substance is not present in poultry feed)        |
|      |  |  | -  |
|      |  |  | -  |
| M 29 | <br>HOIFAMSO2 | IUPAC:<br><i>N</i> -(4-fluorophenyl)- <i>N</i> -(1-hydroxypropan-2-yl)-2-(methylsulfonyl)acetamide<br>(generated by ICS Naming)  | Rat<br>Hen (following feeding of parent flufenacet; not relevant as the parent substance is not present in poultry feed) |
|      |  |  | -  |
|      |  |  | -  |
| M 30 | <br>LMeOH-3   | IUPAC:<br><i>N</i> -(4-hydroxyphenyl)- <i>N</i> -(1-hydroxypropan-2-yl)-2-(methylsulfinyl)acetamide<br>(generated by ICS Naming) | Hen  |
|      |  |  | -  |
|      |  |  | -  |

| No.  | Structure, Report Name  | Synonyms, Codes, Chemical Name  | Occurrence   |
|------|---|---|--|
| M 31 | <br>Des-isopropyl-FOE alcohol                    | DIFOEALC<br>BCS-AB35517<br><br>IUPAC:<br><i>N</i> -(4-fluorophenyl)-2-hydroxy- <i>N</i> -acetamide;<br><br><i>N</i> -(4-fluorophenyl)-2-hydroxyacetamide<br>(generated by ICS Naming)       | Rat  |
|      |   |   | -  |
|      |   |   | -  |
| M 32 | <br>4-Fluoro- <i>N</i> -(1-methylethyl)benzamine | FA, BCS-AA57901<br>AE F145057<br><br>IUPAC:<br>4-fluoro- <i>N</i> -isopropylaniline<br>(generated by ICS Naming)  | Rat  |
|      |   |   | -  |
|      |   |   | -  |
| M 33 | <br>FOE sulfinyl lactic acid                    | FAMSOL<br>FAMSOL-I<br>FAMSOL-II (diastereomeric pair)<br><br>IUPAC:<br>3-({ 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl }sulfinyl)-2-hydroxypropanoic acid<br>(generated by ICS Naming) | -  |
|      |   |   | Corn, Wheat;<br>Rotational crops:<br>kale, turnip, wheat |
|      |   |   | -  |
| M 34 | <br>Th-malonylalanyl conjugate                 | THNMALALA<br><br>IUPAC:<br><i>N</i> -(carboxyacetyl)-3-[2-oxo-5-(trifluoromethyl)-1,3,4-thiadiazol-3(2 <i>H</i> )-yl]alanine<br>(generated by ICS Naming)                                   | -  |
|      |   |   | Soybean  |
|      |   |   | -  |
| M 35 | <br>FOE des-isopropyl oxalate                  | IUPAC:<br>[(4-fluorophenyl)amino](oxo)acetic acid<br>(generated by ICS Naming)  | -  |
|      |   |   | Rotational crops:<br>turnip, wheat                       |
|      |   |   | -  |
| M 36 | <br>FOE 3-OH-des-isopropyl oxalate             | IUPAC:<br>[(4-fluoro-3-hydroxyphenyl)amino](oxo)acetic acid<br>(generated by ICS Naming)  | -  |
|      |   |   | Rotational crops   |
|      |   |   | -  |

| No.       | Structure, Report Name  | Synonyms, Codes, Chemical Name  | Occurrence  |
|-----------|---|---|---|
| M 37      |  <p>FOE sulfinyl lactic acid glucoside</p>   | <p>FAMSOL-Glu<br/>FAMSOL-Glu-I<br/>FAMSOL-Glu-II</p> <p>IUPAC:<br/>3-({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfinyl)-2-(hexopyranosyloxy)propanoic acid (generated by ICS Naming)</p> | -   |
|           |   |   | Corn, Wheat;<br>Rotational crop:<br>wheat                   |
|           |   |   | -   |
| M 38      |  <p>FOE sulfonyl lactic acid</p>             | <p>IUPAC:<br/>3-({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfinyl)-2-(hexopyranosyloxy)propanoic acid (generated by ICS Naming)</p>  | -   |
|           |   |   | Rotational crops:<br>turnip, wheat                          |
|           |   |   | -   |
| M 39      |  <p>FOE cysteine sulfoxide</p>             | <p>FACSO</p> <p>IUPAC:<br/>3-({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfinyl)alanine (generated by ICS Naming)</p>   | Fish  |
|           |   |   | Rotational crops:<br>kale, wheat                            |
|           |   |   | -   |
| M 40<br>b |  <p>FOE sulfanyl lactic acid</p>           | <p>FAMSL</p> <p>IUPAC:<br/>3-({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfanyl)-2-hydroxypropanoic acid (generated by ICS Naming)</p>  | -   |
|           |   |   | Wheat, potato<br>(transient: glucoside<br>conjugate formed) |
|           |   |   | -   |
| M 41<br>b |  <p>FOE sulfanyl lactic acid glucoside</p> | <p>FAMSL-Gl,<br/>FAMSL-Glu</p> <p>IUPAC:<br/>3-({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfanyl)-2-(hexopyranosyloxy)propanoic acid (generated by ICS Naming)</p>                       | -   |
|           |   |   | Potato, wheat   |
|           |   |   | -   |

| No.   | Structure, Report Name  | Synonyms, Codes, Chemical Name  | Occurrence  |
|-------|---|---|---|
| M 42  |  <p>FOE malonylcysteine conjugate</p>          | <p>FAM-MalCys</p> <p>IUPAC:<br/> <i>N</i>-(carboxyacetyl)-<i>S</i>-{2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}cysteine<br/>           (generated by ICS Naming)</p>     | -   |
|       |   |   | Corn  |
|       |   |   | -   |
| M 43  |  <p>FOE free sulfide</p>                       | <p>IUPAC:<br/> <i>N</i>-(4-fluorophenyl)-<i>N</i>-isopropyl-2-sulfanylacetyl-L-cysteine<br/>           (generated by ICS Naming)</p>  | Goat, hen   |
|       |   |   | Corn;<br>Rotational crops   |
|       |   |   | -   |
| M44 a |  <p>FOE 5043-trifluoroethanesulfonic acid</p> | <p>TFESA<br/>           BCS-CU62474 (sodium salt)</p> <p>IUPAC:<br/>           2,2,2-trifluoroethanesulfonic acid</p>   | -   |
|       |   |   | -   |
|       |   |   | Soil (aerobic & anaerobic)  |
| M45 a |  <p>trifluoroacetic acid</p>                 | <p>TFA<br/>           AE C502988 (acid)<br/>           AE1046319 (sodium salt)<br/>           BCS-AZ56567 (sodium salt)</p> <p>IUPAC:<br/>           trifluoroacetic acid</p> | Rat   |
|       |   |   | Rotational crops:<br>turnip, Swiss chard,<br>wheat (main<br>metabolite in all<br>rotated crops) |
|       |   |   | Soil (aerobic & anaerobic)  |
| M46   |  <p>FOE thioglycolate sulfide</p>            | <p>FOE thioglycolate</p> <p>IUPAC: 4-fluoro-<i>N</i>-methylethylaniline<br/>           thiodiacetic acid amide</p>  | -   |
|       |   |   | -   |
|       |   |   | Soil (anaerobic)<br>Water (aerobic)   |

| No.   | Structure, Report Name  | Synonyms, Codes, Chemical Name  | Occurrence             |
|---|---|---|------------------------|
| M47   | <br>isopropyl hydroxy cysteine | FAIOCS<br><br>IUPAC:<br>3-({ 2-[(4-fluorophenyl)(1-hydroxypropan-2-yl)amino]-2-oxoethyl}sulfinyl)alanine<br>(generated by ICS Naming) | Fish<br><br>-<br><br>- |
| <p>a) The structures and report names of degradation products/metabolites identified in e-fate and metabolism studies reflect in general their uncharged species. The degradation products/metabolites FOE sulfonic acid, FOE 5043-trifluoroethanesulfonic acid and trifluoroacetic acid have pKa-values &lt; 2 and hence, are deprotonated under environmental and physiological conditions and show no acidic properties anymore, due to the high buffer capacities of the environmental and physiological matrices. Therefore, their environmental and physiological relevant deprotonated species (i.e. their alkali salts) were used for all studies which were conducted to elucidate the toxicological and ecotoxicological properties of these degradation products/metabolites as well as their fate in the environment, plants and animals.</p> <p>b) In the first Annex I Listing process the metabolite numbers M40 and M41 were allocated to carbon dioxide and methane. However these two compounds are not considered as unique metabolites of flufenacet but as common mineralization products. Hence, these two metabolite numbers were reallocated to two metabolites identified in metabolism studies (FOE sulfanyl lactic acid and FOE sulfanyl lactic acid glucoside).</p> |   |   |                        |

## LITERATURE SEARCH DATA

For flufenacet and its metabolites, a total of 3489 references were identified and evaluated for potential relevance. The area of analytical methods were most represented in the search results. However, no reference was identified as relevant in the context of side-effects on health, the environment and non-target species, which does influence the risk assessment (as defined in the EFSA Guidance Document, EFSA Journal 2011; 9(2):2092). In conclusion, for flufenacet no information was identified which was considered to have an impact on an EU-agreed end-points, or would require to adapt any of the risk assessments in the flufenacet renewal dossier.

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**B.5.1. METHODS USED FOR THE GENERATION OF PRE-APPROVAL DATA (CA 4.1)****B.5.1.1. Methods for the analysis of the active substance as manufactured (CA 4.1.1)***Original Annex CA submission (1997)*

|                            |   |
|----------------------------|---|
| Report:                    | B.4.1.1,KCA 4.1/01; Reubke, K.J; 1995;  |
| Title:                     | Material accountability of FOE 5043 acetone process   |
| Report No &<br>Document No | PC 1102   |
| Dates of work:             | 1995-12-06  |
| GLP                        | Non GLP   |
| Report:                    | B.4.1.1, Mohsin, S.B; 1994;   |
| Title:                     | HPLC analysis of BAY FOE 5043   |
| Report No &<br>Document No | TM C-16.01  |
| Dates of work:             | 1994-08-26  |
| GLP                        | Non GLP   |
| Report:                    | B.4.1.1, KCA 4.1/01; Harbin, D.N; 1997;   |
| Title:                     | Validation of test method C-16.01: Quantitation of FOE 5043 in technical material and 60% dry flowable formulations |
| Report No &<br>Document No | MR 107584   |
| Dates of work:             | 1997-04-11  |
| GLP                        | Non GLP   |

HPLC method were used for determination the analytical profile of technical FOE 5043 and the accuracy of the results was confirmed by an independent standard GC-ISTD method (KM RBK 015/95).

**New data for AIR III****a) Active substance**

|                            |   |
|----------------------------|---|
| Report:                    | CA 4.1.1/01; KCA 4.1.1/09; Kraemer, F.; Ruengeler, W.; 2011; M-414331-01                          |
| Title:                     | Flufenacet<br>Determination of active substance in technical material<br>HPLC - external standard |
| Report No &<br>Document No | AM015811MP1<br>M-414331-01-1  |
| Dates of work:             | 2011-07-22  |
| GLP                        | Non GLP   |

The HPLC method AM015811MP1 is used for the determination of flufenacet in technical grade active substance. Principle of the method: The components are separated by reverse phase high performance liquid chromatography (HPLC) with DAD detection at 230 nm. The quantitative evaluation is carried out using the peak area of flufenacet by comparison with the area of the calibration substance according to the method of external standardization.

|                            |  |
|----------------------------|--|
| Report:                    | CA 4.1.1/02; KCA 4.1.1/10; Kraemer, F.; Ruengeler, W.; 2011; M-414332-01 |
| Title:                     | Validation of HPLC-method AM015811MP1                                    |
| Report No &<br>Document No | VB1-AM015811MP1<br>M-414332-01-1   |
| Dates of work:             | 2011-09-16   |
| GLP                        | GLP  |

The HPLC method AM015811MP1 for the determination of flufenacet in technical grade active substance has been completely validated by checking the following parameters: linearity, precision, accuracy, specificity and interference.

**Table 5.1.1-1 The validation parameters**

|                            |   |
|----------------------------|---|
| Linearity                  | 5 concentrations with double measurements; range 56 - 114 %;<br>correlation coefficient $r_K$ : 0.9998; regression equation: $y = 0.488x$ .<br>Chromatograms are given;<br>the function is linear in the operating range. |
| Precision<br>Repeatability | 5 weighted samples are measured;<br>no outliers are reported, RSD: 0.44 %;<br>acceptable according to the Horwitz equation.   |
| Accuracy                   | 5 synthetic samples are measured;<br>mean recovery: 99.40 %; RSD: 0.29 %.   |
| Specificity/Interference   | The UV-spectra of reference substance and analyte in the sample show<br>no spectral difference; the retention times are identical.<br>No interferences are found.   |

The method AM015811MP1 for the determination of flufenacet in technical grade active substance is found to be valid and applicable.

#### **Applicability of existing CIPAC methods**

Up to now there is no CIPAC method available for the determination of flufenacet in technical grade active substance.

#### **b) Significant and relevant impurities and additives**

##### **Significant impurities**

The HPLC method AM018212MP1 is used for the determination of the *ortho*- and *meta*-isomers of flufenacet in technical grade active substance.

Principle of the method: The components are separated by normal phase high performance liquid chromatography (HPLC). The quantitative evaluation is carried out using the peak area of the isomers and comparing them with the area of the calibration substance according to the method of external standardization.

The HPLC-method AM015711MP1 is used for the determination of the organic by-products in technical grade active substance.

Principle of the method: The components are separated by reverse phase HPLC. The quantitative evaluation is carried out using the peak area of the organic by-products and comparing them with the area of the calibration substance according to the method of external standardization.

The Karl Fischer method CIPAC method MT 30.5 is used for determination of water content in technical grade active substance.

For details refer to the confidential information in Volume 4.

##### **Relevant impurities**

Not relevant as there is no relevant impurity in the technical flufenacet.



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**Additives**

Not relevant as there is no additive in the technical flufenacet.

**B.5.1.2. Methods for risk assessment (CA 4.1.2)**

The references of all risk assessment methods were given in the respective sections. Please note that the reliabilities of the corresponding methods are considered in the relevant sections of the risk assessment, if necessary.

**B.5.1.2.1. Section Residue**

Metabolism of flufenacet in plants was evaluated during the EU peer review process in studies on cereals (wheat) and pulses and oilseeds (soybean, cotton) using [fluorophenyl-U-<sup>14</sup>C]flufenacet and [thiadiazole-2-<sup>14</sup>C]flufenacet.

The plant metabolism studies show that flufenacet is rapidly metabolised. No parent compound was found in any crop matrices. In the studies evaluated for Annex I inclusion, three major metabolites, FOE oxalate (M01), FOE sulfonic acid (M02) and FOE thioglycolate sulfoxide (M04), and two minor metabolites, FOE methyl sulfoxide (M06) and FOE methyl sulfone (M07) were found. These metabolites contain the common 4-fluorophenyl-*N*-isopropyl amine moiety (4-fluoro-*N*-isopropylaniline, also called “fluoroaniline” in the documentation) of the parent molecule. It was concluded that those metabolites containing the thiadone moiety are not relevant and should not be included in the residue definition.

During the EU evaluation for Annex I listing the residue definition for plant matrices (risk assessment and monitoring) was established as sum of all residue components containing the *N*-(4-fluorophenyl)-*N*-isopropyl amine moiety (called *N*-fluorophenyl-*N*-isopropyl moiety in official documents) expressed as flufenacet equivalent.

Following the EU review process, post-emergence studies on the metabolism in wheat and maize and pre- and post-emergence studies on potatoes using [fluorophenyl-UL-<sup>14</sup>C] flufenacet were evaluated by the RMS in the framework of the review of existing MRLs (Art 12 of Reg (EC) 396/2005, EFSA Journal 2012;10(4):2689). Also in these studies parent compound was not detected in any of the commodities. In potato, two major metabolites were identified; the flufenacet cysteine conjugate (M23) and flufenacet sulfanyl lactic acid glucoside (M41).

These were the only major metabolites irrespective of the time of treatment or sampling.

The later metabolism studies confirm the metabolic pathway as evaluated in the EU review process. Even though different metabolites were seen in potatoes and additional metabolites were determined in the post-emergence studies on cereals the established residue definition is still appropriate since all metabolites include the *N*-fluorophenyl-*N*-isopropyl amine moiety.

In order to capture all the metabolites including the common moiety a residue analytical method was developed which is based on the conversion of the metabolites to a common chemical fragment (*N*-fluorophenyl-*N*-isopropyl amine) to be determined as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (also called 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide or FOE 5043 trifluoroacetamide in the method reports and in other documentation) after derivatisation by GC-MS. The residue analytical method for plant materials is referred to as method 00346. The method was considered appropriate for data generation and enforcement purpose.

Making use of the HPLC-MS/MS technologies and the acceptability to use this technique for enforcement purposes more recent methods were developed which allow for direct determination of the common fragment *N*-fluorophenyl-*N*-isopropyl amine without the need for derivatisation. The procedure for extraction of residues from the matrices remains unchanged in the simplified methods.

The following Table 5.1.2.1-1 summarises the agreed European residue definitions for flufenacet.

**Table 5.1.2.1-1: EU conclusion: Residue definition for flufenacet**

| Matrices              | Residue definition            |   | Reference   |
|-----------------------|-------------------------------|---|---|
| Food of plant origin  | Risk assessment<br>Monitoring | Flufenacet including all metabolites containing the <i>N</i> -fluorophenyl- <i>N</i> -isopropyl moiety, expressed as flufenacet | EFSA review of existing MRLs according to Art 12 of Reg. (EC) 396/2005 in Reg. (EU) No 1127/2014. |
| Food of animal origin | Risk assessment<br>Monitoring | Flufenacet including all metabolites containing the <i>N</i> -fluorophenyl- <i>N</i> -isopropyl moiety, expressed as flufenacet |   |

The following tables give an overview on the data generation methods evaluated during the EU peer review process as well as supplementary methods submitted with the present dossier. The same methods were considered also as enforcement methods.

Following (non-radiolabelled) analytical methods used in risk assessment studies provided for AIR III procedure were evaluated in this chapter.

## PLANT and ANIMAL MATRICES

### Original Annex CA submission (1997)

In the scope of the original Annex II dossier submission in 1997, several analytical methods were provided for the determination flufenacet and its relevant metabolites in plants and plant products. The plant matrices contain three major metabolites: FOE oxalate, FOE sulfonic acid and FOE thioglycolate sulfoxide, and two minor metabolites FOE methyl sulfoxide and FOE methyl sulfone by GC-MS. The methods for flufenacet are based on the conversion of the metabolites to 4-fluoro-*N*-methylethyl benzenamine moiety. The limit of quantitation (LOQ) was 0.05 mg/kg for FOE 5043 trifluoroacetamide expressed as flufenacet. The plant matrices under investigation were cereals, corn, sunflower and soybean.

Residues of flufenacet in animal matrices is essentially the same as the plant method (00346). The difference refers to the quantitation procedure: in plant method one ion ( $m/z = 207$ ) was used for quantitation whereas in the animal method the sum of three ions ( $m/z = 207$ ,  $m/z = 138$ ,  $m/z = 249$ ) was used. The animal matrices under investigation were animal tissues, milk and eggs.

In the Monograph (1997) methods for risk assessment and for post approval control are not reported separately. Therefore, to ease reference to the Monograph all study reports are listed below.

|                         |  |
|-------------------------|--|
| Report:                 | B.4.2.1; KCA 4.1.2/04; Gould, T.J., Lemke, V.J.; 1995  |
| Title:                  | An analytical method for the determination of FOE 5043 residues in plant matrices.                 |
| Report No & Document No | 106406   |
| Dates of work:          | 1995-05-11   |
| GLP                     | GLP  |
| Report:                 | B.4.2.1; Seym, M.; 1994  |
| Title:                  | Independent laboratory validation of the residue analytical method for FOE 5043 residues in plant. |
| Report No & Document No | 106907<br>RA-352-94  |
| Dates of work:          | 1994-06-24   |
| GLP                     | GLP  |

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Report: B.4.2.1; KCA 4.1.2/01; Seym, M.; 1995  
Title: Analytical method for the determination of the total residue of FOE 5043 in plant matrices.  
Report No & Document No: MR-981/95 00346  
Dates of work: 1995-09-22  
GLP: GLP

Report: B.4.2.2; KCA 4.1.2/02; Gould, T.J., Lemke, V.J., Zoloty, K.L.; 1995  
Title: An analytical method for the determination of FOE 5043 residues in animal matrices.  
Report No & Document No: 106773 00418  
Dates of work: 1995-11-17  
GLP: GLP

Report: B.4.2.2; KCA 4.1.2/03; Seym, M.; 1995  
Title: Modification M001 for eggs.  
Report No & Document No: MR-1118/95 00418/M001  
Dates of work: 1995-10-10  
GLP: GLP

Report: B.4.2.2; KCA 4.2/06; Bajzik, M.E.; 1995  
Title: Independent laboratory validation of the analytical method for the determination of FOE 5043 residues in animal matrices.  
Report No & Document No: 106913  
Dates of work: 1995-03-22  
GLP: GLP

For all studies submitted and evaluated during the frame of the first Annex CA inclusion please refer to Tables 5.1.2.1-2, 5.1.2.1-3 and 5.1.2.1-4 below.

**Table 5.1.2.1-2: Analytical methods for residues of flufenacet in plants and in food of animal origin reviewed during the first inclusion**

| Method No.                    | Matrix   | Analytes   | Matrix LOQ (mg/kg)  | Technique | Author Doc. No. Report No. Dossier reference  | Reference  |
|-------------------------------|--|--|---|-----------|---|--|
| Plant data generation method  |  |  |   |           |   |  |
| 00346*                        | Wheat, barley, rye<br>- green plant<br>- straw<br>- grain<br>Corn:<br>-green material<br>- grain<br>sunflower seed<br>soya seed  | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4      | 0.05 mg/kg in all matrices<br><br>0.1 mg/kg in straw  | GC-MS     | Seym, M., 1995; M-018864-02 [MR-981/95]<br><br>KCA 4.1.2/01 also filed<br>KCA 4.2/01                              | Monograph 1997<br><br>Report of ECCO 73, Annex 2, Complete List of Endpoints |
| 00346*                        | Plant material (Independent Laboratory Validation)   | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4      | 0.05 mg/kg in all matrices<br><br>0.1 mg/kg in straw  | GC-MS     | Seym, M., 1994; [106907(RA-352-94)]   | Monograph 1997   |
| 00346*                        | Corn forage<br>- fodder<br>- grain<br>Soybean forage<br>- seed<br>Spinach tops<br>Wheat grain<br>- straw<br>Sunflower seed<br>Turnip roots<br>Peanut nutmeat<br>Corn oil<br>Soybean<br>soapstock | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4      | 0.05 mg/kg<br>Corn forage<br>Spinach tops<br>Turnip roots<br>Wheat grain<br><br>0.1 mg/kg<br>corn grain, fodder, corn oil, peanut nutmeat soybean seed, forage<br>Soybean soapstock<br>Sunflower seed<br>Wheat grain, straw | GC-MS     | Gould, T. J., Lemke, V. J., 1995; M-041601-01<br><br>[report 106406]<br><br>KCA 4.1.2/04 also filed<br>KCA 4.2/04 | Monograph 1997   |
| Animal data generation method |  |  |   |           |   |  |
| 00418*                        | Milk<br>Bovine liver<br>Bovine kidney<br>Bovine muscle<br>Bovine fat   | (1)flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide | Milk (0.01mg/kg)<br>Bovine liver (0.02 mg/kg),<br>Bovine kidney,<br>Bovine muscle,<br>Bovine fat (0.05 mg/kg)   | GC-MS     | Gould, T. J. et al., 1995; M-019605-01<br><br>[report 106773]<br><br>KCA 4.1.2/02 also filed<br>KCA 4.2/02        | Monograph 1997<br><br>Report of ECCO 73, Annex 2, Complete List of Endpoints |

| Method No.  | Matrix  | Analytes  | Matrix LOQ (mg/kg) | Technique | Author Doc. No. Report No. Dossier reference                                       | Reference      |
|-------------|---|---|--------------------|-----------|--|----------------|
| 00418/M001* | Eggs  | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4 | 0.05 mg/kg         | GC-MS     | Seym, M., 1995; M-019614-01 [MR-1118/95]<br><br>KCA 4.1.2/03 also filed KCA 4.2/03 |                |
| 00418*      | Animal matrices (Independent Laboratory Validation) | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4 | 0.05 mg/kg         | GC-MS     | Bajzik, M.E., 1995   | Monograph 1997 |

The number in brackets gives the report number which is used in the monograph.

\*Method have been evaluated as monitoring and data generation methods for plant and animal matrices in the EU peer review process

**Table 5.1.2.1-3: Description and findings of analytical methods for residues of flufenacet in plants reviewed during the first inclusion**

| File No.<br>Method No.     | Method   |  | ILV                             |
|----------------------------|--|--|---------------------------------|
|                            | 106406<br>-  | MR 981/95<br>00346                                 | 106907 (RA - 352/94)<br>-       |
| Test matrix                | various materials of several crops<br>including cereals, corn, sunflower and soybean                   |  | corn forage<br>(green material) |
| Extraction                 | oxidation, hydrolysis  |  |                                 |
| Clean-up/Derivatization    | distillation, derivatization, C-18 cartridge, (elution with MTBE)                                      |  |                                 |
| Determined as              | 4-fluoro-N-methylethyl benzenamin trifluoroacetamide   |  |                                 |
| Method of<br>determination | GC-MS  |  |                                 |
|                            |  |  |                                 |
| LOQ                        | 0.05 mg/kg or<br>0.1 mg/kg   | 0.05 mg/kg<br>(0.1 mg/kg for straw)                | 0.05 mg/kg                      |
| Fortification levels       | 0.05 mg/kg and<br>0.1 mg/kg  | 0.05 - 0.5 mg/kg<br>(0.1 - 1.0 mg/kg<br>for straw) | 0.05 - 0.5 mg/kg                |
| Specificity                | confirmation of the analyte by two qualifies (ion ratios)  |  |                                 |
| Recovery*                  | 81 %   | 83 %   | 85 %                            |
| Coefficient of variation   | 12 % RSD   | 11 % RSD   | 11 % RSD                        |
| Blank values               | always far below 30 % of the LOQ   |  |                                 |
| Repeatability              | recoveries between 70 % and 110 %, with RSDs always < 20 % per fortified substance and sample material |  |                                 |
| Reproducibility            | independent laboratory validation  |  | not applicable                  |

\*Recoveries were determined after fortification with FOE 5043, FOE oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide and a mixture of these four substances; RSD = relative standard deviation

**Table 5.1.2.1-4: Description and findings of analytical methods for residues of flufenacet in products of animal origin reviewed during the first inclusion**

| File No.<br>Method No.   | Method   |                          | ILV                |
|--------------------------|--|--------------------------|--------------------|
|                          | 106773<br>00418  | MR-1118/95<br>00418/M001 | 106913<br>-        |
| Test matrix              | animal tissues and milk  | eggs                     | beef liver         |
| Extraction               | oxidation, hydrolysis  |                          |                    |
| Clean-up/Derivatization  | distillation, derivatization, C-18 cartridge, (elution with MTBE)                              |                          |                    |
| Determined as            | 4-fluoro-N-methylethyl benzenamin trifluoroacetamide   |                          |                    |
| Method of determination  | GC-MS  |                          |                    |
| LOQ                      | 0.01 mg/kg for milk;<br>0.02 mg/kg for liver,<br>0.05 mg/kg for kidney,<br>fat, and muscle     | 0.05 mg/kg for eggs      | 0.05 mg/kg         |
| Fortification levels     | 0.01 mg/kg - 0.1 mg/kg   | 0.05 mg/kg               | 0.05 - 0.025 mg/kg |
| Specificity              | confirmation of the analyte by two qualifies (ion ratios)                                      |                          |                    |
| Recovery*                | 89 %   | 88 %                     | 90 %               |
| Coefficient of variation | 14 % RSD   | 7 % RSD                  | 14 % RSD           |
| Blank values             | far below 30 % of the LOQ  |                          |                    |
| Repeatability            | recoveries between 70 % and 110 % per fortification level and sample material with RSDs < 20 % |                          |                    |
| Reproducibility          | independent laboratory validation  | data not accessible      | not applicable     |

\*Recoveries were determined after fortification with FOE 5043, FOE oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide and a mixture of these four substances; RSD = relative standard deviation

### Conclusion

All methods of analysis provided and accepted in the scope of the original Annex II dossier submission were evaluated under Council Directive 91/414/EEC and fulfilled the data requirements of Annex II of the Directive. The studies were conducted according to GLP principles and comply with EPA Ref: 171-4(c), Residue analytical method -plants and EPA Ref: 171-4(d), Residue analytical method –animal.

The technique used for the determination of flufenacet residues in plant and animal matrices was GC-MS which was based on the conversion of the metabolites to 4-fluoro-N-methylethyl benzenamine moiety. The LOQ was 0.05 mg/kg kg for plant matrices and in animal matrices LOQs of 0.01 mg/kg (milk), 0.02 mg/kg (liver) and 0.05 mg/kg for eggs, kidney, muscle and fat were achieved.

These analytical methods could be considered as acceptable during the renewal of flufenacet.

### New data for AIR III

#### PLANT and ANIMAL MATRICES

New data consist of supplementary information for methods which were listed in the Monograph 1997 for flufenacet and quite new methods. New methods based on the HPLC-MS/MS technologies when this technique was accepted to use for enforcement purposes. This methods were more recent because allow for direct

determination of the common fragment *N*-fluorophenyl-*N*-isopropyl amine without the need for derivatisation. The procedure for extraction of residues from the matrices remains unchanged compare to old methods.

New methods were referred in Table 5.1.2.1-5 below.

**Table 5.1.2.1-5: Supplementary analytical methods for residues of fufenacet in plants**

| Method No.      | Matrix   | Analytes   | Matrix LOQ (mg/kg)   | Technique      | Author<br>Doc. No.<br>Report No.<br>Dossier reference  |
|-----------------|--|--|--|----------------|--|
| 00346/<br>E001  | Potato<br>tuber  | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate<br>sulfoxide<br>mixture of 1-4  | 0.05 mg/kg   | GC-MS          | Seym, M.; 1997;<br>M-018872-01<br>[report MR-388/96]<br><br>KCA 4.1.2/12   |
| 00346/<br>E002* | Soybean<br>plant<br>Tomato<br>fruit                                    | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate<br>sulfoxide<br>mixture of 1-4  | 0.05 mg/kg   | GC-MS          | Seym, M.; 1998;<br>M-018878-01<br>[report MR-400/98]<br><br>KCA 4.1.2/13<br>also filed<br>KCA 4.2/23                       |
| 00346/<br>E004  | Rice grain   | (1)flufenacet<br>(2) FOE-oxalate<br>hydrate<br>(3) FOE sulfonic acid<br>sodium salt<br>(4) FOE thioglycolate<br>sulfoxide  | 0.01 mg/kg   | GC-MS          | Rzepka, S.; 2006;<br>M-277805-01<br>[report BAY 0610V]<br><br>KCA 4.1.2/14   |
| 01179*          | Cereal<br>(wheat and<br>barley)<br>straw<br>green<br>material<br>grain | (1)flufenacet<br>(2) FOE-oxalate<br>hydrate<br>(3) FOE sulfonic acid<br>sodium salt<br>(4) FOE thioglycolate<br>sulfoxide<br>(metabolites fortified<br>as mixture (1/1/1)) | 0.01 mg<br>cereal grain<br>and green<br>material<br>0.05 mg/kg<br>cereal straw | HPLC-<br>MS/MS | Class, Th.; Meridian, H.; 2010;<br>M-362716-01<br><br>[report B1778G]<br><br>KCA 4.1.2/19<br>also filed<br>KCA 4.2/12      |
| 01100*          | Orange<br>fruit<br>Dry bean<br>seed<br>Rape seed                       | (1)flufenacet<br>(2) FOE-oxalate<br>hydrate<br>(3) FOE sulfonic acid<br>sodium salt<br>(4) FOE thioglycolate<br>sulfoxide<br>(metabolites fortified<br>as mixture (1/1/1)) | 0.01 mg/kg<br>(all<br>matrices)  | HPLC-<br>MS/MS | Billian, P.; 2010;<br>M-362575-02<br><br>[report MR-08/060]<br><br>KCA 4.1.2/18<br>Also filed<br>KCA 4.2/11                |
| 01100/<br>M001* | Cereal<br>(wheat)<br>straw<br>green<br>material<br>grain               | (1)flufenacet<br>(2) FOE-oxalate<br>hydrate<br>(3) FOE sulfonic acid<br>sodium salt<br>(4) FOE thioglycolate<br>sulfoxide<br>(metabolites fortified<br>as mixture (1/1/1)) | 0.01 mg<br>cereal grain<br>and green<br>material<br>0.05 mg/kg<br>cereal straw | HPLC-<br>MS/MS | Stuke, S., Bauer, J.; Ruhl, S.;<br>2012;<br>M-433720-01<br><br>[report MR-11/011]<br>KCA 4.1.2/17<br>also filed KCA 4.2/10 |



| Method No. | Matrix                                    | Analytes   | Matrix LOQ (mg/kg)   | Technique  | Author<br>Doc. No.<br>Report No.<br>Dossier reference              |
|------------|---|------------|--|------------|--|
| 01100/M002 | Cereal (wheat) straw green material grain | flufenacet | 0.01 mg cereal grain and green material<br>0.05 mg/kg cereal straw | HPLC-MS/MS | Stuke, S.; Teubner, L.; 2013; M-448503-01 [MR-12/057] KCA 4.1.2/15 |

\*Methods are also proposed as monitoring methods

|                          |   |
|--------------------------|---|
| Report:                  | KCA 4.1.2/12, Seym, M.; 1997; M-018872-01   |
| Title:                   | Supplement E001 of method 00346 for the determination of residues of total residue of FOE 5043 in/on potato |
| Report no & Document No: | MR-388/96, method 00346/E001<br>M-018872-01-1 (MR-388/96)   |
| Guidelines:              | Fulfil Council Directive 91/414/EEC   |
| GLP                      | Yes; Deviations: none   |

The method is a supplement to method 00346 (Seym, M., 1995; M-018864-02, see Table 5.1.2.1-2). The method 00346 is described in the original Annex II dossier on flufenacet. Supplement E001 provides validation data for the determination of flufenacet (FOE 5043) and its metabolites (FOE 5043 oxalate, FOE 5043 sulfonic acid, FOE 5043 thioglycolate sulfoxide) on potatoes as another matrix of high starch content.

The basic method 00346 is characterized by the following methodology:

- Extraction of residues by oxidation in aqueous acidic environment.
- In the following the refluxing under strong acid conditions cleaves the amide bonding to achieve the common moiety compound 4-fluoro-*N*-isopropylaniline.
- In order to make the compound distillable it has to be deprotonated with alkaline. Separation of the common moiety compound from the matrix by water steam distillation out of alkaline medium into an acidified distillation receiver flask (protonates the aniline again) avoids the loss of the target compound.
- The aniline is extracted from the steam distillate by liquid/liquid distribution with dichloromethane.
- The 4-fluoro-*N*-isopropylaniline is finally derivatized with trifluoroacetic anhydride to 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (called 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide or FOE 5043 trifluoroacetamide in the method report) to be amenable to GC-MS determination.

### Principle of method

Flufenacet and all metabolites containing the *N*-fluorophenyl-*N*-isopropyl-amine moiety can be determined by this method, being a total residue method. Method 00346 was developed to measure the residues of flufenacet and its metabolites in various crops. The residues are oxidized with potassium permanganate for 5 min and hydrolysed to the common moiety *N*-fluorophenyl-*N*-isopropyl amine (fluoroaniline) by digesting the crop mixture with 47 % sulfuric acid for 24 hours. The fluoroaniline is separated from the crop matrix by steam distillation after making the crop digest basic with 50 % sodium hydroxide. The aniline is extracted from the steam distillate and derivatized with trifluoroacetic anhydride. A clean-up on a C-18 cartridge follows. The derivative, 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide, was measured by gas chromatography/mass spectroscopy (GC-MS) with the following working conditions for chromatograph: column Hewlett Packard Ultra 1, 12 m x 0.2 mm x 0.33 µm film thickness, injector of Splitless mode, purge off time 0.75 min. The residue is expressed as flufenacet equivalents.

GC-MS determination of derivative 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide was done by monitoring of the  $m/z = 207$  fragment ion (quantifier), and the  $m/z = 138$  and  $m/z = 249$  ions (qualifiers).

### Limit of Quantification (LOQ)

The limit of quantification is 0.05 mg/kg for potato tuber.

**Linearity**

The linearity of the detector response was confirmed in the original method 00346. The 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide standard was injected 4 times each at 7 concentrations in the range of 0.025 to 2.5 µg/mL (as parent equivalents). The correlation between the injected amount of substance and the detector response was linear for standard in solvent and matrix. The correlation coefficients ( $r^2$ ) were between 0.9997 and 1.0000.

**Specificity**

Residues in control samples were well below 30% of the respective LOQ level for all analytes (flufenacet (FOE 5043), FOE oxalate, FOE sulfonic acid (as sodium salt), FOE thioglycolate sulfoxide or a mixture of these four substances). Therefore recoveries were not corrected for interferences.

**Repeatability (precision)**

Relative standard deviations per fortification level and overall per matrix were always below 20%.

**Confirmatory method**

One fragment ion with  $m/z$  ratio >100 was used for quantification and two fragment ions with  $m/z$  ratio >100 were used for confirmation. Therefore, this GC-MS method can be considered as highly specific.

**Accuracy (recovery findings)**

Recoveries were determined after fortification with flufenacet (FOE 5043), FOE oxalate, FOE sulfonic acid (as sodium salt), FOE thioglycolate sulfoxide or a mixture of these four substances. Recovery values for the total residue of flufenacet from potato samples are presented in the Table 5.1.2.1-6 below. Mean recoveries for each fortification level and the overall mean recovery were within the range of 70 - 110%.

**Stability of analytes**

Stability of the derivative 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide in extracts was shown up to a storage interval of 8 weeks in the original method 00346 when stored in a refrigerator at about +4°C.

**Reproducibility (ILV)**

As this is a data collection method, no independent validation is required.

However, an ILV to method 00346 was performed on 2 representative matrices (wheat grain (Class, T.; 2004; M-072609-01) and corn forage (Seym, M.; 1994; M-014828-01).

**Table 5.1.2.1-6: Recoveries for flufenacet residues in potato tuber**

| Analyte**   | Sample material | Fortification Level **<br>[mg/kg] | Recoveries [%] |    |    |    |    | RSD [%] |      |
|---|-----------------|-----------------------------------|----------------|----|----|----|----|---------|------|
|   |                 |                                   | Individual     |    |    |    |    |         | Mean |
| Flufenacet (FOE 5043) or<br>FOE 5043-oxalate or<br>FOE5043-thioglycolate<br>sulfoxide or<br>FOE 5043-sulfonic acid<br>or mixture of all | Potato Tuber    | 0.05*                             | 69             | 71 | 72 | 73 | 73 | 80      | 10.0 |
|   |                 |                                   | 73             | 78 | 78 | 79 | 79 |         |      |
|   |                 |                                   | 80             | 81 | 84 | 87 | 91 |         |      |
|   |                 |                                   | 94             | 95 | -  | -  | -  |         |      |
|   |                 | 0.5                               | 70             | 74 | 80 | 86 | 88 | 83      | 10.1 |
|   |                 |                                   | 91             | 91 | -  | -  | -  |         |      |
|   | Overall (n=24)  |                                   |                |    |    |    |    |         | 81   |

RSD Relative standard deviation

\* LOQ level

\*\* Fortification level expressed in equivalents of flufenacet; Fortified substances (active substance and representative metabolites):

1. Parent compound (at least 2 recoveries)
2. FOE oxalate (at least 2 recoveries)
3. FOE thioglycolate sulfoxide (at least 2 recoveries)
4. FOE sulfonic acid sodium salt (at least 2 recoveries)
5. FOE mixture of 1-4 (at least 2 recoveries, but only at the 0.05mg/kg fortification level)

Determined as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide and calculated as flufenacet.

## Conclusion

All method validation results are in compliance with the European guideline requirements for data generation methods and post registration enforcement. Supplement E001 of method 00346 meets all necessary performance criteria to determine the total residue flufenacet in/on potato tuber with an LOQ of 0.05 mg/kg.

The method fulfils registration requirements as outlined in the Regulation (EC) No 1107/2009, detailed in the EC Guidance documents on residue analytical methods SANCO/825/00 rev. 8.1. and SANCO/3029/99 rev. 4.

|                         |   |
|-------------------------|---|
| Report:                 | KCA 4.1.2/13 Seym, M.; 1998; M-018878-01<br>see also point 5.2.1 and KCA 4.2/23                                       |
| Title:                  | Supplement E002 of method 00346 for the determination of FOE 5043 total residue in/on soybean, plant and tomato fruit |
| Report No & Document No | MR-400/98, method 00346/E002,<br>M-018878-01-1  |
| Guidelines:             | Fulfil Council Directive 91/414/EEC   |
| GLP                     | Yes; Deviations: none   |

The method is a supplement to method 00346 (Seym, M.; 1995; M-018864-02, see Table 5.1.2.1-2) providing validation data for the determination of flufenacet (FOE 5043) and its metabolites (FOE 5043 oxalate, FOE 5043 sulfonic acid, FOE 5043 thioglycolate sulfoxide) for soybean (green plant material) and tomato fruit as additional matrices with high water content.

## Principle of method

The method 00346 is described in the Annex CA dossier on flufenacet and briefly summarised for supplement E001 above.

Flufenacet and all metabolites containing the *N*-fluorophenyl-*N*-isopropyl-amine moiety can be determined by this method after derivatization with trifluoroacetic anhydride. The derivative, 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide was measured by gas chromatography/mass spectroscopy (GC-MS) with the following working conditions for chromatograph: column Hewlett Packard Ultra 1, 12 m x 0.2 mm x 0.33 µm film thickness, injector of Splitless mode, purge off time 0.75 min. The residue is expressed as flufenacet equivalents.

GC-MS determination of derivative 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide was done by monitoring of the  $m/z = 207$  fragment ion (quantifier), and the ions  $m/z = 138$  and  $m/z = 249$  ions (qualifiers).

## Limit of Quantification (LOQ)

The limit of quantification is 0.05 mg/kg for soybean (green plant material) and for tomato fruit.

## Linearity

The linearity of the detector response was confirmed in the original method 00346. The 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide standard (called FOE 5043 trifluoro-acetamide in the report) was injected 4 times each at 7 concentrations in the range of 0.025 to 2.5 µg/mL (as parent equivalents). The correlation between the injected amount of substance and the detector response was linear for standard in solvent and matrix. The correlation coefficients ( $r^2$ ) were between 0.9997 and 1.0000.

## Specificity

Residues in control samples were well below 30% of the respective LOQ level for all analytes. Therefore recoveries were not corrected for interferences.

## Repeatability (precision)

Relative standard deviations per fortification level and overall per matrix were always below 20%.

## Confirmatory method

GC-MS determination of derivative 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide was done by monitoring of the  $m/z = 207$  fragment ion (quantifier), and the ions  $m/z = 138$  and  $m/z = 249$  ions (qualifiers). Therefore, this GC-MS method can be considered as highly specific.

**Accuracy (recovery findings)**

Recoveries were determined after fortification with flufenacet (FOE 5043), FOE oxalate, FOE sulfonic acid (as sodium salt), FOE thioglycolate sulfoxide or a mixture of these four substances. Recovery values for the total residue of flufenacet from samples of soybean green material and tomato fruit are presented in the Table 5.1.2.1-7 below. Mean recoveries for each fortification level and the overall mean recovery were within the range of 70 - 110%.

**Stability of analytes**

Stability of the derivative 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide in extracts was shown up to a storage interval of 8 weeks in the original method 00346 when stored in a refrigerator at about +4°C.

**Reproducibility (ILV)**

Supplement E002 of method 00346 was developed as a data generation method but may also be used for enforcement purposes. ILV to method 00346 was performed on 2 representative matrices (wheat grain (Class, T.; 2004; M-072609-01) and corn forage (Seym, M.; 1994; M-014828-01). The latter method report was evaluated during the EU peer review process.

**Table 5.1.2.1-7: Recoveries for flufenacet residues in soybean green material and tomato fruit**

| Analyte**   | Sample Material      | Fortification-level**<br>[mg/kg] | Recoveries<br>[%] |    |    |    |     |      |      |
|---|----------------------|----------------------------------|-------------------|----|----|----|-----|------|------|
|   |                      |                                  | Individual values |    |    |    |     | Mean | RSD  |
| Flufenacet (FOE 5043) or<br>FOE 5043-oxalate<br>or<br>FOE5043-<br>thioglycolate<br>sulfoxide or<br>FOE 5043-sulfonic<br>acid<br>or mixture of all | tomato               | 0.05*                            | 68                | 76 | 79 | 81 | 87  | 79   | 7.8  |
|   |                      |                                  | 73                | 76 | 81 | 83 | 88  |      |      |
|   | fruit                | 0.5                              | 72                | 77 | 80 | 85 | 87  | 79   | 6.6  |
|   |                      |                                  | 74                | 78 | 82 |    |     |      |      |
|   | Overall mean (n=18)  |                                  |                   |    |    |    | 79  | 7.1  |      |
|   | soybean              | 0.05*                            | 68                | 74 | 92 | 93 | 99  | 87   | 14.5 |
|   |                      |                                  | 71                | 82 | 93 | 94 | 105 |      |      |
|   | green plant material | 0.5                              | 71                | 73 | 77 | 80 | 81  | 76   | 5.1  |
|   |                      |                                  | 71                | 76 | 78 |    |     |      |      |
|   | Overall mean (n=18)  |                                  |                   |    |    |    | 82  | 13.5 |      |

RSD Relative standard deviation

\*LOQ level

\*\* Fortification level expressed in equivalents of flufenacet; Fortified substances (active substance and representative metabolites):

1. Parent compound
2. FOE oxalate
3. FOE thioglycolate sulfoxide
4. FOE sulfonic acid sodium salt
5. FOE mixture of 1-4

Determined as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide and calculated as flufenacet.

**Conclusion**

All method validation results are in compliance with the European guideline requirements for data generation methods and post registration enforcement. Supplement E002 of method 00346 meets all necessary performance criteria to determine the total residue flufenacet in/on soybean green plant material and tomato fruit as representatives of the commodity group of high water content with an LOQ of 0.05 mg/kg.

The method fulfils registration requirements as outlined in the Regulation (EC) No 1107/2009, detailed in the EC Guidance documents on residue analytical methods SANCO/825/00 rev. 8.1. and SANCO/3029/99 rev. 4.

|                          |   |
|--------------------------|---|
| Report:                  | KCA 4.1.2/14; Rzepka, S.; 2006; M-277805-01   |
| Title:                   | Supplement E004 of method 00346 for the determination of residues of FOE 5043, FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide in rice (grain) |
| Report no & Document No: | (Report No. BAY-0610V / Method No. 00346/E004)<br>M-277805-01-1   |
| Guidelines:              | EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance document SANCO/825/00 rev. 7 of 17/03/2004 (stated in the report)                        |
| GLP                      | Yes; Deviations: none   |

Supplement E004 to the analytical method 00346 (Seym, M.; 1995; M-018864-02, see Table 5.1.2.1-2) was validated for the determination of flufenacet (FOE 5043) and its metabolites (FOE 5043 oxalate, FOE 5043 sulfonic acid, FOE 5043 thioglycolate sulfoxide) in/on rice grain with a lower LOQ of 0.01 mg/kg. The method was used for analysis of cereal grain from the field rotational crop studies.

### Principle of method

The extraction from the matrix was performed according to the Bayer analytical method for the determination of the total residues of flufenacet in plant materials (method no. 00346) as described above for method supplement E001.

The derivate 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (called FOE 5043 trifluoro acetamide in the report) was measured by GC-MS with the following working conditions for chromatograph: column DB-5 MS 30 m x 0.25 mm x 0.25 µm film thickness, injector of splitless mode. For quantitation molecular ion  $m/z = 249$  was used. For verification the fragment ions  $m/z = 207$  and  $m/z = 138$  were selected.

Control specimens were analysed in duplicate and fortified specimens were analysed in quintuple for each fortification level. Fortification experiments were performed at the limit of quantitation (LOQ = 0.01 mg/kg) and ten times that level.

### Modifications to Analytical Method 00346/E004 in study RA-2020/06:

No SPE clean-up was performed for all the grain specimens. GC-MS determination of the TFA derivative was done in the MS/MS mode, isolating the  $m/z = 207$  fragment ion for collision induced dissociation (CID), monitoring the  $m/z = 138$  (quantifier),  $m/z = 110$  and  $m/z = 112$  daughter ions (qualifiers). Pre-validation data for study RA-2020/06 are reported in chapter B7.6.2.

### Specificity

Residues in control samples were well below 30% of the respective LOQ level for all analytes. Therefore recoveries were not corrected for interferences.

One fragment ion with  $m/z$  ratio > 100 was used for quantification and two fragment ions with  $m/z$  ratio > 100 were used for confirmation. Therefore, this GC-MS method can be considered as highly specific.

### Repeatability

As a measure for the precision, the intra-laboratory repeatability ( $n = 5$ ) is given as relative standard deviation (% RSD). Relative standard deviations were always below 20%.

### Linearity

The linearity of the detector response was confirmed by injecting 6 standard solutions covering the working range of 0.002 – 2.00 µg/mL FOE 5043 trifluoroacetamide corresponding to 0.002 – 2.00 mg/kg of FOE 5043 trifluoroacetamide (0.0029 – 2.91 mg/kg expressed as parent flufenacet). The correlation coefficient was found to be  $r^2 = 1$ .

### Limit of quantification (LOQ)

The limit of quantification for flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide was established and validated at 0.01 mg/kg in rice grain with a limit of detection (LOD) of 0.003 mg/kg. The metabolites are expressed as parent equivalents.

**Recovery rates (accuracy)**

The recovery-rates determined with three mass transitions are summarised in Tables 5.1.2.1-8 to 5.1.2.1-10 below.

Mean recoveries for each fortification level and the overall mean recovery were within the range of 70 - 110%.

**Stability of analytes**

Stability of the derivative 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide in extracts was shown up to a storage interval of 8 weeks in the original method 00346 when stored in a refrigerator at about +4°C.

**Reproducibility (ILV)**

As this is a data collection method, no independent validation is required.

**Table 5.1.2.1-8: Recoveries for flufenacet and metabolites ( $m/z = 249$ )**

| Matrix                           | Fortification level<br>expressed as<br>FOE 5043<br>[mg/kg] | Recoveries (m/z = 249) |             |                         | No. of<br>analyses | Overall recovery |            |
|----------------------------------|--|------------------------|-------------|-------------------------|--------------------|------------------|------------|
|                                  |  | single values<br>[%]   | Mean<br>[%] | Rel.<br>std.dev.<br>[%] |                    | Mean<br>[%]      | RSD<br>[%] |
| Flufenacet (FOE 5043)            |  |                        |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 80, 88, 71, 70, 76     | 77          | 9.5                     | 5                  | 76               | 7.5        |
|                                  | 0.10   | 73, 70, 77, 72, 79     | 74          | 5.0                     | 5                  |                  |            |
| FOE 5043 oxalate                 |  |                        |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 76, 71, 78, 76, 82     | 77          | 5.2                     | 5                  | 76               | 6.7        |
|                                  | 0.10   | 66, 71, 80, 81, 77     | 75          | 8.5                     | 5                  |                  |            |
| FOE 5043 sulfonic acid           |  |                        |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 86, 83, 73, 65, 77     | 77          | 11                      | 5                  | 75               | 10         |
|                                  | 0.10   | 74, 73, 78, 60, 77     | 72          | 10                      | 5                  |                  |            |
| FOE 5043 thioglycolate sulfoxide |  |                        |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 79, 66, 78, 66, 81     | 74          | 10                      | 5                  | 72               | 10         |
|                                  | 0.10   | 61, 69, 71, 70, 82     | 71          | 11                      | 5                  |                  |            |

RSD Relative standard deviation

\* LOQ level

Determined as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide and calculated as flufenacet

**Table 5.1.2.1-9: Recoveries for flufenacet and metabolites ( $m/z = 138$ )**

| Matrix                | Fortification level<br>expressed as<br>FOE 5043<br>[mg/kg] | Recoveries ( $m/z = 138$ ) |             |                         | No. of<br>analyses | Overall recovery |            |
|-----------------------|--|----------------------------|-------------|-------------------------|--------------------|------------------|------------|
|                       |  | single values<br>[%]       | Mean<br>[%] | Rel.<br>std.dev.<br>[%] |                    | Mean<br>[%]      | RSD<br>[%] |
| Flufenacet (FOE 5043) |  |                            |             |                         |                    |                  |            |
| Rice<br>(grain)       | 0.01*  | 80, 77, 80, 76, 84         | 79          | 3.9                     | 5                  | 79               | 6.3        |
|                       | 0.10   | 75, 71, 82, 73, 87         | 78          | 8.6                     | 5                  |                  |            |
| FOE 5043 oxalate      |  |                            |             |                         |                    |                  |            |
| Rice<br>(grain)       | 0.01*  | 72, 77, 73, 89, 103        | 83          | 16                      | 5                  | 81               | 15         |
|                       | 0.10   | 64, 74, 95, 85, 82         | 80          | 15                      | 5                  |                  |            |

| Matrix                           | Fortification level<br>expressed as<br>FOE 5043<br>[mg/kg] | Recoveries ( <i>m/z</i> = 138) |             |                         | No. of<br>analyses | Overall recovery |            |
|----------------------------------|--|--------------------------------|-------------|-------------------------|--------------------|------------------|------------|
|                                  |  | single values<br>[%]           | Mean<br>[%] | Rel.<br>std.dev.<br>[%] |                    | Mean<br>[%]      | RSD<br>[%] |
| FOE 5043 sulfonic acid           |  |                                |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 86, 82, 88, 80, 83             | 84          | 3.8                     | 5                  | 80               | 9.5        |
|                                  | 0.10   | 75, 75, 78, 62, 86             | 75          | 12                      | 5                  |                  |            |
| FOE 5043 thioglycolate sulfoxide |  |                                |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 80, 69, 95, 93, 97             | 87          | 14                      | 5                  | 80               | 14         |
|                                  | 0.10   | 73, 70, 72, 73, 82             | 74          | 6.2                     | 5                  |                  |            |

RSD Relative standard deviation

\* LOQ level

Determined as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide and calculated as flufenacet.**Table 5.1.2.1-10: Recoveries for flufenacet and metabolites ( $m/z = 207$ )**

| Matrix                           | Fortification level<br>expressed as<br>FOE 5043<br>[mg/kg] | Recoveries ( <i>m/z</i> = 207) |             |                         | No. of<br>analyses | Overall recovery |            |
|----------------------------------|--|--------------------------------|-------------|-------------------------|--------------------|------------------|------------|
|                                  |  | single values<br>[%]           | Mean<br>[%] | Rel.<br>std.dev.<br>[%] |                    | Mean<br>[%]      | RSD<br>[%] |
| Flufenacet (FOE 5043)            |  |                                |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 66, 78, 75, 66, 68             | 71          | 7.7                     | 5                  | 72               | 6.1        |
|                                  | 0.10   | 74, 68, 75, 71, 76             | 73          | 4.5                     | 5                  |                  |            |
| FOE 5043 oxalate                 |  |                                |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 73, 75, 74, 70, 77             | 74          | 3.5                     | 5                  | 75               | 9.6        |
|                                  | 0.10   | 62, 68, 87, 81, 81             | 76          | 14                      | 5                  |                  |            |
| FOE 5043 sulfonic acid           |  |                                |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 79, 72, 72, 65, 72             | 72          | 6.8                     | 5                  | 72               | 7.6        |
|                                  | 0.10   | 72, 73, 78, 61, 76             | 72          | 9.2                     | 5                  |                  |            |
| FOE 5043 thioglycolate sulfoxide |  |                                |             |                         |                    |                  |            |
| Rice<br>(grain)                  | 0.01*  | 76, 66, 72, 75, 65             | 71          | 7.2                     | 5                  | 70               | 8.4        |
|                                  | 0.10   | 60, 68, 71, 70, 80             | 70          | 10                      | 5                  |                  |            |

RSD Relative standard deviation

\* LOQ level

Determined as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide and calculated as flufenacet.**Conclusion**

All method validation results are in compliance with the European guideline requirements for data generation methods (SANCO/3029/99 rev. 4, 11/07/2000). Supplement E004 of method 00346 meets all necessary performance criteria to determine the total residue flufenacet in/on rice grain with an LOQ of 0.01 mg/kg. The method fulfils registration requirements as outlined in the Regulation (EC) No 1107/2009, detailed in the EC Guidance documents on residue analytical methods SANCO/825/00 rev. 8.1. and SANCO/3029/99 rev. 4.

|                          |   |
|--------------------------|---|
| Report:                  | KCA 4.1.2/19, Class, Th.; Merdian, H.; 2010; M-362716-01<br>see also point 5.2.1.1 and KCA 4.2/12   |
| Title:                   | Validation of BCS analytical method no. 01179 for the determination of residues of flufenacet in/on plant materials by HPLC-MS/MS   |
| Report no & Document No: | Method 01179 report B 1778 G<br>M-362716-01-1   |
| Guidelines:              | Council Directive 91/414/EEC EC Guidance documents on residue analytical methods: SANCO/3029/99 rev. 4, 11/07/00, and SANCO/825/00 rev. 7 17/03/04;<br>OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13) |
| GLP                      | Yes; Deviations none  |

The analytical method 01179 was developed in order to determine the total residue of flufenacet (flufenacet and its metabolites containing the *N*-fluorophenyl-*N*-isopropyl amine moiety) in/on cereal matrices (green material, straw, grain) by LC-MS/MS using matrix matched standards. The matrices to be analysed are considered to be representative for the matrix groups of high starch content and high water content. In addition straw was validated as a representative for dry matrices. The method may be used as a data collection method as well as for enforcement purposes. Green material, grain and straw were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).

#### Principle of method

The sample material (5 g of grain and green material; 2.5 g or 5 g of straw) in water was oxidized with potassium permanganate in the presence of sulfuric acid.

After the oxidation, the mixture was hydrolysed with concentrated sulfuric acid for 21 h in order to cleave the amide bonding to achieve the common moiety compound 4-fluoro-*N*-isopropylaniline. After making the solution basic water steam distillation out of alkaline medium into an acidified receiver followed. To remove the acid content from the distillation receiver flask the obtained solution was 3 times liquid/liquid distributed with dichloromethane. The total residue of flufenacet was determined as 4-fluoro-*N*-isopropylaniline by LC-MS/MS with the following working conditions for chromatograph: column Thermo Aquasil C18, 3 µm particle size, 150 mm length, 3 mm id, and pre-column: Phenomenex C18, 4 mm length, 3.0 mm id., retention time approx. 6 min. For quantification external calibration with matrix-matched standard solutions was applied. Significant matrix effects were observed.

#### Limit of Quantification (LOQ)

The limit of quantification (LOQ) is 0.01 mg/kg in green material and grain, and 0.05 mg/kg in straw (expressed as flufenacet equivalents). The limit of detection (LOD) is estimated to be 0.002 mg/kg for green material and grain and 0.01 mg/kg for straw.

#### Linearity

Calibration functions were calculated from matrix-matched standard injections of  $\geq 5$  different concentrations of the analyte (expressed as flufenacet) in a range of 0.20 ng/mL to 25 ng/mL corresponding to 0.002 – 0.25 mg/kg for all matrices and for straw (exceptional cases): corresponding to 0.004 – 0.5 mg/kg. Correlation coefficients (*r*) were  $\geq 0.99$  for both characteristic mass transitions (MRMs).

#### Specificity

Residues in control samples were well below 30% of the respective LOQ level for all matrices. The use of LC-MS/MS with two mass transitions for quantitation and quantitative confirmation ( $m/z = 154 \rightarrow m/z = 112$  and  $= 154 \rightarrow m/z = 95$ ) ensures a high level of specificity.

#### Repeatability

For the matrices investigated and for both mass transitions monitored, overall relative standard deviations (RSD) were  $< 20\%$ , except for transition  $m/z = 154 \rightarrow m/z = 95$ , where the overall relative standard deviation for green material fortified with flufenacet metabolites was 21 %.

#### Stability in Solution and Extracts

Acceptable recovery results of total flufenacet residues show that the extracts are stable up to 22 days when stored refrigerated after reprocessing. Standard solutions in matrix are stable during the injection series up to seven hours at ambient temperature. Standard solutions in solvent, stored refrigerated are stable for about two months.



**Recovery rates (accuracy)**

Green material, grain and straw was fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1) and detected as 4-fluoro-*N*-isopropylaniline. Mean recoveries at the LOQ level and for higher levels determined with the two mass transitions were within the range of 70 - 110%. (Please refer to Tables 5.1.2.1-11 to 5.1.2.1-14).

**Confirmatory method**

The use of LC-MS/MS with two mass transitions for quantitation and quantitative confirmation ( $m/z = 154 \rightarrow m/z = 112$  and  $m/z = 154 \rightarrow m/z = 95$ ) ensures a high level of specificity. No additional confirmatory method is considered necessary.

**Reproducibility (ILV)**

The method may be used as a data collection method and for enforcement purposes. The independent validation is reported in chapter B5.2.1 (Meyer, M.; 2011; M-405654-01).

**Table 5.1.2.1-11: Recoveries for flufenacet residues in cereal matrices (quantification  $m/z = 112$ )**

| Analytes                | Sample material   | Fortification Level <sup>(a)</sup><br>[mg/kg] | Recoveries <sup>(a)</sup><br>[%] |     |    |      | RSD<br>[%] | LOQ<br>[mg/kg] |  |
|-------------------------|-------------------|---|----------------------------------|-----|----|------|------------|----------------|--|
|                         |                   |   | Individual                       |     |    | Mean |            |                |  |
| Flufenacet<br>(FOE5043) | Green<br>Material | 0.01  | 92                               | 66  | 68 | 73   | 15         | 0.01           |  |
|                         |                   |   | 74                               | 67  | -  |      |            |                |  |
|                         |                   | 0.10  | 79                               | 81  | 74 | 78   | 5          |                |  |
|                         |                   | 0.25  | 70                               | -   | -  | -    | -          |                |  |
|                         |                   | 1.0   | 57                               | -   | -  | -    | -          |                |  |
|                         |                   | 2.0   | 70                               | -   | -  | -    | -          |                |  |
|                         |                   | 5.0   | 60                               | 54  | -  | 57   | -          |                |  |
|                         |                   | 12  | 66                               | -   | -  | -    | -          |                |  |
|                         |                   | 20  | 75                               | -   | -  | -    | -          |                |  |
|                         |                   | 30  | 82                               | 87  | 95 | 88   | 7          |                |  |
|                         | Overall (n= 18)   |   |                                  |     |    |      | 73         | 16             |  |
|                         | Grain             | 0.01  | 99                               | 76  | 99 | 86   | 17         | 0.01           |  |
|                         |                   |   | 92                               | 79  | 62 |      |            |                |  |
|                         |                   |   | 97                               | -   | -  |      |            |                |  |
|                         |                   | 0.10  | 75                               | 106 | 71 | 83   | 19         |                |  |
|                         |                   |   | 65                               | 69  | 80 |      |            |                |  |
|                         |                   |   | 103                              | 91  | -  |      |            |                |  |
|                         | Overall (n=15)    |   |                                  |     |    |      | 84         | 17             |  |
|                         | Straw             | 0.05  | 90                               | 102 | 99 | 84   | 12         | 0.05           |  |
|                         |                   |   | 78                               | 72  | 76 |      |            |                |  |
|                         |                   |   | 78                               | 87  | 87 |      |            |                |  |
|                         |                   |   | 87                               | 70  | -  |      |            |                |  |
|                         |                   | 0.10  | 107                              | 112 | -  | 110  |            |                |  |
|                         |                   | 0.20  | 98                               | -   | -  |      |            |                |  |
|                         |                   | 0.50  | 74                               | 62  | 62 | 74   | 16         |                |  |
|                         |                   |   | 87                               | 84  | -  |      |            |                |  |
|                         |                   | 1.0   | 73                               | -   | -  | -    | -          |                |  |
|                         | Overall (n=20)    |   |                                  |     |    |      | 84         | 17             |  |

(a) expressed as flufenacet

RSD Relative standard deviation

Determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet.

**Table 5.1.2.1-12: Recoveries for flufenacet and its metabolite residues in cereal matrices (quantification  $m/z = 112$ )**

| Analytes                         | Sample material   | Fortification Level <sup>(a)</sup><br>[mg/kg] | Recoveries <sup>(a)</sup><br>[%] |     |      | RSD<br>[%] | LOQ<br>[mg/kg] |    |
|----------------------------------|-------------------|---|----------------------------------|-----|------|------------|----------------|----|
|                                  |                   |   | Individual                       |     | Mean |            |                |    |
| Flufenacet<br>Metabolite<br>mix* | Green<br>Material | 0.01  | 79                               | 90  | 116  | 89         | 0.01           |    |
|                                  |                   |   | 230**                            | 69  | -    |            |                |    |
|                                  |                   | 0.10  | 79                               | 90  | -    | 85         |                | -  |
|                                  |                   | 0.90  | 60                               | -   | -    | -          |                | -  |
|                                  |                   | 2.40  | 90                               | -   | -    | -          |                | -  |
|                                  |                   | 18  | 72                               | 73  | -    | 73         |                | -  |
|                                  |                   | 27  | 66                               | -   | -    | -          |                | -  |
|                                  |                   | 30  | 67                               | 65  | -    | 66         |                | -  |
|                                  | Overall (n= 13)   |   |                                  |     |      | 78         | 20             |    |
|                                  | Grain             | 0.01  | 92                               | 109 | 83   | 85         | 0.01           |    |
|                                  |                   |   | 83                               | 73  | 72   |            |                |    |
|                                  |                   | 0.10  | 81                               | 68  | 67   | 77         |                | 11 |
|                                  |                   |   | 79                               | 89  | 77   |            |                |    |
|                                  | Overall (n=12)    |   |                                  |     |      | 81         | 14             |    |
|                                  | Straw             | 0.05  | 94                               | 66  | 73   | 74         | 0.05           |    |
|                                  |                   |   | 71                               | 67  | 71   |            |                |    |
|                                  |                   | 0.60  | 62                               | 69  | 74   | 76         |                | 16 |
|                                  |                   |   | 85                               | 92  | -    |            |                |    |
|                                  | Overall (n=11)    |   |                                  |     |      | 75         | 14             |    |

RSD Relative standard deviation

\*Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1), detected as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet.

\*\*: Dixon outlier, not included in calculations. Most likely contaminated in the laboratory.

**Table 5.1.2.1-13: Recoveries for flufenacet residues in cereal matrices (confirmation  $m/z = 95$ )**

| Analytes                | Sample material   | Fortification Level <sup>(a)</sup><br>[mg/kg] | Recoveries <sup>(a)</sup><br>[%] |     |      | RSD<br>[%] | LOQ<br>[mg/kg] |    |
|-------------------------|-------------------|---|----------------------------------|-----|------|------------|----------------|----|
|                         |                   |   | Individual                       |     | Mean |            |                |    |
| Flufenacet<br>(FOE5043) | Green<br>Material | 0.01  | 85                               | 65  | 67   | 71         | 11             |    |
|                         |                   |   | 70                               | 67  | -    |            |                |    |
|                         |                   | 0.10  | 78                               | 82  | 73   | 78         |                | 6  |
|                         |                   | 0.25  | 77                               | -   | -    | -          |                | -  |
|                         |                   | 1.0   | 58                               | -   | -    | -          |                | -  |
|                         |                   | 2.0   | 71                               | -   | -    | -          |                | -  |
|                         |                   | 5.0   | 58                               | 56  | -    | 57         |                | -  |
|                         |                   | 12  | 66                               | -   | -    | -          |                | -  |
|                         |                   | 20  | 75                               | -   | -    | -          |                | -  |
|                         |                   | 30  | 83                               | 85  | 95   | 88         |                | 7  |
|                         | Overall (n= 18)   |   |                                  |     |      | 73         | 15             |    |
|                         | Grain             | 0.01  | 97                               | 77  | 10   | 87         | 15             |    |
|                         |                   |   | 92                               | 82  | 65   |            |                |    |
|                         |                   |   | 97                               | -   | -    |            |                |    |
|                         |                   | 0.10  | 75                               | 107 | 72   | 83         |                | 19 |
|                         |                   |   | 65                               | 68  | 79   |            |                |    |
|                         |                   |   | 103                              | 91  | -    |            |                |    |
|                         | Overall (n=15)    |   |                                  |     |      | 85         | 17             |    |
|                         |                   | 86  | 100                              | 98  |      |            |                |    |

|  |                |      |     |     |    |     |    |      |
|--|----------------|------|-----|-----|----|-----|----|------|
|  | Straw          | 0.05 | 75  | 73  | 75 | 83  | 12 | 0.05 |
|  |                |      | 76  | 84  | 85 |     |    |      |
|  |                |      | 89  | 69  | -  |     |    |      |
|  |                | 0.10 | 106 | 111 | -  | 109 | -  |      |
|  |                | 0.20 | 98  | -   | -  | -   | -  |      |
|  |                | 0.50 | 72  | 63  | 62 | 73  | 14 |      |
|  |                |      | 84  | 82  | -  |     |    |      |
|  |                | 1.0  | 72  | -   | -  | -   | -  |      |
|  | Overall (n=20) |      |     |     |    | 83  | 17 |      |

(a) expressed as flufenacet

RSD Relative standard deviation

Determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet.**Table 5.1.2.1-14: Recoveries for flufenacet and its metabolites residues in cereal matrices (confirmation  $m/z = 95$ )**

| Analytes                         | Sample material   | Fortification Level <sup>(a)</sup><br>[mg/kg] | Recoveries <sup>(a)</sup><br>[%] |     |      | RSD<br>[%] | LOQ<br>[mg/kg] |    |
|----------------------------------|-------------------|---|----------------------------------|-----|------|------------|----------------|----|
|                                  |                   |   | Individual                       |     | Mean |            |                |    |
| Flufenacet<br>Metabolite<br>mix* | Green<br>Material | 0.01  | 81                               | 96  | 116  | 91         | 0.01           |    |
|                                  |                   |   | 223**                            | 70  | -    |            |                |    |
|                                  |                   | 0.10  | 77                               | 88  | -    | 83         |                | -  |
|                                  |                   | 0.90  | 58                               | -   | -    | -          |                | -  |
|                                  |                   | 2.40  | 95                               | -   | -    | -          |                | -  |
|                                  |                   | 18  | 72                               | 73  | -    | 73         |                | -  |
|                                  |                   | 27  | 63                               | -   | -    | -          |                | -  |
|                                  |                   | 30  | 66                               | 63  | -    | 65         |                | -  |
|                                  | Overall (n= 13)   |   |                                  |     |      | 78         | 21             |    |
|                                  | Grain             | 0.01  | 95                               | 103 | 78   | 84         | 0.01           |    |
|                                  |                   |   | 81                               | 72  | 72   |            |                |    |
|                                  |                   | 0.10  | 81                               | 67  | 66   | 77         |                | 12 |
|                                  |                   |   | 81                               | 91  | 77   |            |                |    |
|                                  | Overall (n=12)    |   |                                  |     |      | 80         | 14             |    |
|                                  | Straw             | 0.05  | 93                               | 66  | 70   | 73         | 0.05           |    |
|                                  |                   |   | 70                               | 67  | 70   |            |                |    |
|                                  |                   | 0.60  | 61                               | 69  | 71   | 75         |                | 15 |
|                                  |                   |   | 84                               | 88  | -    |            |                |    |
|                                  | Overall (n=11)    |   |                                  |     |      | 74         | 14             |    |

(a) expressed as flufenacet

RSD: Relative standard deviation

\*Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1) and detected as 4-fluoro-*N*-isopropylaniline, calculated as flufenacet.

\*\*: Dixon outlier, not included in calculations. Most likely contaminated in the laboratory.

**Conclusion**

Bayer CropScience method 01179 was validated for the determination of flufenacet in cereal materials, exemplified by green material and grain with an LOQ of 0.01 mg/kg and 0.05 mg/kg for straw. The method was validated successfully and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/2000, SANCO/825/00 rev. 8.1, 16/11/2010) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

|                          |   |
|--------------------------|---|
| Report:                  | KCA 4.1.2/18, Billian, P.; 2010; M-362575-02<br>see also point 5.2.1.1 and KCA 4.2/11   |
| Title:                   | Analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on plant material  |
| Report no & Document No: | Method 01100, Report MR-08/060<br>M-362575-02-1   |
| Guidelines:              | <ul style="list-style-type: none"> <li>• EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC</li> <li>• Guidance document on residue analytical methods; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection</li> <li>• OECD guidance document ENV/JM/Mono(2007)17, 2007-08-13,</li> <li>• Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99</li> </ul> |
| GLP                      | Yes, Deviations none  |

The analytical method 01100 was validated for the determination of flufenacet residues in/on orange (fruit), dry bean seed and rape seed representative for the matrix groups of high acid content, high protein content and high fat content. The method may be used as a data collection method as well as for enforcement purposes. Sample materials were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).

#### Principle of method

The sample material (5 g) was oxidized with potassium permanganate in the presence of sulfuric acid. After the oxidation, the mixture was hydrolysed with concentrated sulfuric acid. After oxidation and hydrolysis, residues of flufenacet and metabolites were cleaned-up by distillation followed by a liquid-liquid partition with dichloromethane. Finally, the total residue of flufenacet was determined as 4-fluoro-*N*-isopropylaniline by LC-MS/MS with the following working conditions for chromatograph: column Luna C18(2) HST, 2.5 µm particle size, 50 mm length, 2 mm id with pre-column, retention time approx. 2 min.

The following MRM transitions were used for quantification and confirmation:

4-fluoro-*N*-isopropylaniline:  $m/z = 154.1 \rightarrow m/z = 112.0$  (quantification),  
 $m/z = 154.1 \rightarrow m/z = 92.0$  (confirmation).

4-fluoro-*N*-isopropylaniline residues were quantified using matrix-matched standards. Matrix effects were observed for all sample materials and both MRMs.

#### Limit of Quantification (LOQ)

The limit of quantification (LOQ) is 0.01 mg/kg for all matrices (expressed as flufenacet equivalents). The limit of determination (LOD) is 0.003 mg/kg each in all matrices (expressed as parent equivalents).

#### Linearity

The correlation between the injected amount of substance and the detector response was linear for solvent (in the range of 0.1 µg/L to 5 µg/L for 1<sup>st</sup> MRM and 0.1 to 100 µg/L for 2<sup>nd</sup> MRM) and matrix-matched standards (in the range of 0.1 µg/L to 50 µg/L for both MRMs (corresponding to 0.002 – 1 mg/kg expressed as flufenacet)). The correlation coefficients were between 0.9900 and 0.9993.

#### Specificity

Residues in control samples were below 30% of the respective LOQ level. The use of LC-MS/MS with two mass transitions for quantitation and quantitative confirmation ( $m/z = 154 \rightarrow m/z = 112$  and  $m/z = 154 \rightarrow m/z = 92$ ) ensures a high level of specificity.

#### Repeatability

For the matrices investigated and for both mass transitions monitored, relative standard deviations (RSD) per fortification level and overall were < 20 % except for transition  $m/z = 154 \rightarrow m/z = 92$  the relative standard deviation for dry bean seed fortified with flufenacet metabolite mix at the LOQ level was 20.9 %. (Please refer to Tables 5.1.2.1-15 and 5.1.2.1-16).

**Recovery rates (accuracy)**

Orange fruit, dry bean seed and rape seed were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1) and detected as 4-fluoro-*N*-isopropylaniline. Mean recoveries for each fortification level and the overall mean recovery were within the range of 70 - 110%. (Please refer to Tables 5.1.2.1-15 and 5.1.2.1-16)

**Confirmatory method**

LC-MS/MS using two characteristic MS/MS transitions for detection and quantification ensures a high level of specificity. No additional confirmatory method is considered necessary.

**Stability in Standard Solutions and Extracts**

The stability of the analytes in extract was checked after approx. four weeks for 0.10 mg/kg rape seed recoveries. Final extracts are stable for at least four weeks under refrigerated conditions ( $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ). During the experimental phase, there was no degradation of the stock and secondary standard solutions monitored. However, stability of standard solutions in solvent up to 2 months is known from previous experiments.

**Reproducibility (ILV)**

The method may be used as a data collection method as well as for enforcement purposes. The corresponding independent validation is reported in chapter B5.2.1 (Meyer, M.; 2011; M-405654-01).

**Table 5.1.2.1-15: Recoveries for flufenacet residues in orange fruit, dry bean seed and rapeseed ( $m/z = 154 \rightarrow m/z = 112$ , 1<sup>st</sup> MRM; quantification)**

| Sample material | Analytes                                     | Fortification Level <sup>(a)</sup><br>[mg/kg] | Recoveries<br>[%] |     |     |     |     |      | RSD<br>[%] | n  |
|-----------------|--|---|-------------------|-----|-----|-----|-----|------|------------|----|
|                 |  |   | Individual        |     |     |     |     | Mean |            |    |
| Orange Fruit    | Flufenacet <sup>1</sup><br>(FOE5043)         | 0.01 *  | 85                | 105 | 118 | 101 | 107 | 103  | 11.6       | 5  |
|                 |  | 1.0   | 86                | 94  | 95  | 105 | 91  | 94   | 7.4        | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 99   | 10.5       | 10 |
|                 | Flufenacet<br>Metabolite<br>mix <sup>2</sup> | 0.01 *  | 74                | 73  | 68  | 71  | 75  | 72   | 3.8        | 5  |
|                 |  | 1.0   | 78                | 80  | 75  | 87  | --  | 80   | 6.4        | 4  |
|                 | Overall                                      |   |                   |     |     |     |     | 76   | 7.3        | 9  |
| Dry Bean Seed   | Flufenacet <sup>1</sup><br>(FOE5043)         | 0.01 *  | 66                | 76  | 71  | 88  | 74  | 75   | 10.9       | 5  |
|                 |  | 0.10  | 103               | 100 | 86  | 113 | 114 | 103  | 11.0       | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 89   | 19.7       | 10 |
|                 | Flufenacet<br>Metabolite<br>mix <sup>2</sup> | 0.01 *  | 76                | 117 | 86  | 84  | 91  | 91   | 17.2       | 5  |
|                 |  | 0.10  | 77                | 66  | 95  | 70  | --  | 77   | 16.7       | 4  |
|                 | Overall                                      |   |                   |     |     |     |     | 85   | 18.2       | 9  |
| Rapeseed        | Flufenacet <sup>1</sup><br>(FOE5043)         | 0.01 *  | 84                | 112 | 107 | 109 | 98  | 102  | 11.1       | 5  |
|                 |  | 0.10  | 102               | 99  | 79  | 106 | 106 | 98   | 11.4       | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 100  | 10.8       | 10 |
|                 | Flufenacet<br>Metabolite<br>mix <sup>2</sup> | 0.01 *  | 69                | 73  | 89  | 70  | 78  | 76   | 10.8       | 5  |
|                 |  | 0.10  | 91                | 81  | 82  | 75  | 69  | 80   | 10.3       | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 78   | 10.3       | 10 |

(a) expressed as flufenacet

\* LOQ level

RSD: Relative standard deviation

<sup>1</sup> Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet.

<sup>2</sup> Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1), detected as 4-fluoro-*N*-isopropylaniline and expressed as flufenacet.

**Table 5.1.2.1-16: Recoveries for flufenacet residues in orange fruit, dry bean seed and rapeseed ( $m/z = 154$  →  $m/z = 92$ , 2<sup>nd</sup> MRM; confirmation)**

| Sample material | Analytes                                     | Fortification Level <sup>(a)</sup><br>[mg/kg] | Recoveries<br>[%] |     |     |     |     |      | RSD<br>[%] | n  |
|-----------------|--|---|-------------------|-----|-----|-----|-----|------|------------|----|
|                 |  |   | Individual        |     |     |     |     | Mean |            |    |
| Orange Fruit    | Flufenacet <sup>1</sup><br>(FOE5043)         | 0.01*   | 87                | 97  | 112 | 98  | 110 | 101  | 10.2       | 5  |
|                 |  | 1.0   | 85                | 94  | 90  | 96  | 85  | 90   | 5.6        | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 95   | 10.0       | 10 |
|                 | Flufenacet<br>Metabolite<br>mix <sup>2</sup> | 0.01*   | 69                | 68  | 73  | 71  | 73  | 71   | 3.2        | 5  |
|                 |  | 1.0   | 78                | 81  | 76  | 86  | --  | 80   | 5.4        | 4  |
|                 | Overall                                      |   |                   |     |     |     |     | 75   | 7.8        | 9  |
| Dry Bean Seed   | Flufenacet <sup>1</sup><br>(FOE5043)         | 0.01*   | 81                | 92  | 69  | 93  | 81  | 83   | 11.8       | 5  |
|                 |  | 0.10  | 109               | 109 | 104 | 103 | 110 | 107  | 3.0        | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 95   | 15.1       | 10 |
|                 | Flufenacet<br>Metabolite<br>mix <sup>2</sup> | 0.01*   | 76                | 120 | 80  | 78  | 85  | 88   | 20.9       | 5  |
|                 |  | 0.10  | 73                | 66  | 96  | 71  | --  | 77   | 17.4       | 4  |
|                 | Overall                                      |   |                   |     |     |     |     | 83   | 19.8       | 9  |
| Rapeseed        | Flufenacet <sup>1</sup><br>(FOE5043)         | 0.01*   | 79                | 104 | 119 | 97  | 94  | 99   | 14.8       | 5  |
|                 |  | 0.10  | 108               | 103 | 82  | 103 | 98  | 99   | 10.2       | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 99   | 12.0       | 10 |
|                 | Flufenacet<br>Metabolite<br>mix <sup>2</sup> | 0.01*   | 66                | 68  | 80  | 79  | 71  | 73   | 8.8        | 5  |
|                 |  | 0.10  | 96                | 84  | 82  | 75  | 76  | 83   | 10.2       | 5  |
|                 | Overall                                      |   |                   |     |     |     |     | 78   | 11.2       | 10 |

(a) expressed as flufenacet

\* LOQ level

RSD: Relative standard deviation

<sup>1</sup> Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet.<sup>2</sup> Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1), detected as 4-fluoro-*N*-isopropylaniline and expressed as flufenacet.**Conclusion**

Method 01100 was validated for the determination of flufenacet residues in orange fruit, dry bean seed and rape seed with an LOQ of 0.01 mg/kg. The method was validated successfully and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/00, SANCO/825/00 rev. 8.1, 16/11/2010) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

|                          |  |
|--------------------------|--|
| Report:                  | KCA 4.1.2/17, Stuke, S., Bauer, J.; Ruhl, S.; 2012; M-433720-01<br>see also point 5.2.1.1 and KCA 4.2/10   |
| Title:                   | Modification M001 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg for grain and green material and at a LOQ of 0.05 mg/kg for straw by HPLC-MS/MS   |
| Report no & Document No: | Method 01100/M001, Report MR-11/011, dated 2012-06-13<br>M-433720-01   |
| Guidelines:              | <ul style="list-style-type: none"> <li>• EC Guidance Document SANCO/825/00 rev. 8.1 of November 16, 2010</li> <li>• EC Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO 3029/99</li> <li>• Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances</li> <li>• OECD Guideline, ENV/JM/MONO(2007)17, August 13, 2007</li> <li>• U.S. EPA Guideline, OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods of April 1996</li> <li>• U.S. EPA Guideline, OPPTS 860.1340 Residue Analytical Method of August 1996</li> </ul> |
| GLP                      | Yes, Deviations none   |

The analytical method 01100/M001 was validated for the determination of flufenacet residues in/on cereal grain, straw and green material by LC-MS/MS using matrix matched standards. The matrices to be analyzed are considered to be representative for the matrix groups of high starch content and high water content. In addition straw was validated as a representative for dry matrices. The method provides validation data on cereal matrices in addition to method 01179 with only minor adaptations justified by different laboratory equipment and procedures. All extraction and work-up steps are the same for both methods. The method may be used as a data collection method as well as for enforcement purposes. Sample materials were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).

#### Principle of method

The analytical method 01100/M001 was validated for the determination of residues of flufenacet and three main metabolites (FOE-thioglycolate sulfoxide, FOE-oxalate, FOE-sulfonic acid) as the common moiety compound 4-fluoro-*N*-isopropylaniline (FOE 5043 aniline) in/on samples of plant origins – here represented by wheat green material, wheat grain, wheat straw – by LC-MS/MS with the following working conditions for chromatograph: column Luna C18(2) HST, 2.5 µm particle size, 50 mm length, 2 mm id with pre-column, retention time approx. 2 min.

The analytical work is based on method 01100. Residues are expressed as parent equivalent.

5 g of the plant sample was treated with potassium permanganate for oxidation and refluxed under sulfuric acidic conditions for hydrolysis purposes. Residues were purified by water steam distillation of the formed common moiety compound 4-fluoro-*N*-isopropylaniline followed by a liquid-liquid partition with dichloromethane. Finally, residues were dissolved and subjected to LC-MS/MS. Residues of flufenacet and metabolites (all determined as 4-fluoro-*N*-isopropylaniline) were quantified using matrix-matched standards.

The following MRM transitions were used for quantification and confirmation:

4-fluoro-*N*-isopropylaniline:  $m/z = 154.1 \rightarrow m/z = 112.0$  (quantification),  
 $m/z = 154.1 \rightarrow m/z = 92.0$  (confirmation).

#### Limit of Quantification (LOQ)

The LOQ was set at the lowest validated fortification level where a mean recovery within the range of 70 to 110% with an RSD of  $\leq 20\%$  was obtained for flufenacet and metabolites (determined as 4-fluoro-*N*-isopropylaniline) in all matrices tested. The LOQ was set at a level of 0.01 mg/kg in the matrices wheat grain and wheat green material. In the matrix wheat straw the LOQ was set at 0.05 mg/kg (expressed as parent equivalents).

The LOD was calculated based on a statistical approach for residues of flufenacet and metabolites in all matrices tested. The LOD was calculated at a range of 0.0029 mg/kg to 0.0044 mg/kg in the matrices wheat grain and wheat green material. In the matrix wheat straw the LOD was calculated at a level of 0.0121 mg/kg.

#### Linearity

The linearity of the used mass spectrometric detector was tested by analyses of FOE 5043-aniline in matrix matched standards at concentrations range from 0.0001 to 0.01 mg/L (expressed as parent equivalents), corresponding to

0.002 mg/kg to 0.2 mg/kg for green material and grain and at concentrations range from 0.0005 to 0.05 mg/L (expressed as parent equivalents), corresponding to 0.01 mg/kg to 1.0 mg/kg for straw) with at least five different concentration levels for both mass transitions. A linear relation between injected amount and peak area was observed for the matrices investigated over the full concentration range. The correlation coefficients of the 1/x weighted linear regression were in all cases  $\geq 0.99$  and the y-axis intercepts were well below 10% of the respective LOQ. The calibration data obtained justify using the linearity curve for calculation of the residues. For quantification a linearity curve performed with standards in matrix for the calculation of the residue concentration is recommended.

### Specificity

The residues of flufenacet and metabolites (determined as their common moiety FOE 5043-aniline) in the used control materials were < 30% of the individual LOQ except for one wheat green material sample and one wheat straw sample. The use of LC-MS/MS with two mass transitions for quantitation and quantitative confirmation ( $m/z = 154 \rightarrow m/z = 112$  and  $m/z = 154 \rightarrow m/z = 92$ ) ensures a high level of specificity.

### Repeatability

For the matrices investigated and for both mass transitions monitored, relative standard deviations (RSD) per fortification level and overall were < 20 % except for transition  $m/z = 154 \rightarrow m/z = 92$  where the relative standard deviation for wheat green material fortified with flufenacet metabolite mix at the LOQ level was 21.2 %. The individual recovery causing the elevated RSD was identified as an outlier according to a statistical approach (Nalimov outlier). Not considering the respective recovery value, mean RSD resulted in 12.1%. (Please refer to Tables 5.1.2.1-17 and 5.1.2.1-18).

### Recovery rates (accuracy)

Recovery rates were determined after fortification with flufenacet or after fortification with a mixture of flufenacet metabolites (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1) at fortification levels of 0.01 mg/kg and 0.1 mg/kg (wheat grain and wheat green material) and 0.05 mg/kg and 0.5 mg/kg (wheat straw). Each analyte is expressed as flufenacet equivalents.

Mean recovery rates per fortification level and the overall mean recoveries per sample material determined were within the range of 70 - 110% for both fortification levels and for both mass transitions monitored. (Please refer to Tables 5.1.2.1-17 and 5.1.2.1-18).

### Confirmatory method

LC-MS/MS using two characteristic MS/MS transitions for quantification and quantitative confirmation ensures a high level of specificity. A 2<sup>nd</sup> MRM transition was monitored for the analyte 4-fluoro-*N*-isopropylaniline using the representative sample material wheat straw. The individual recoveries for this 2<sup>nd</sup> MRM ( $m/z = 154 \rightarrow m/z = 92$ ) confirm the results of the quantitation MRM very well.

The mean recovery rates per fortification level and the overall mean recovery rate for this commodity ranged from 70 to 71% for the qualifier mass transition (2<sup>nd</sup> MRM). The mean RSD values were 9.4% and 5.4% per fortification level and 7.3% overall for the straw matrix. A complete set of recoveries for cereal grain, straw and green material using a confirmatory mass transition was performed within validation of method 01179 reported above (Class, Th.; Merdian, H.; 2010; M-362716-01). No additional confirmatory method is considered necessary.

### Stability in extracts

Residues of the formed common moiety compound 4-fluoro-*N*-isopropylaniline were found to be stable in final plant extracts for at least 14 days when stored in a refrigerator at < 6 °C.

### Reproducibility (ILV)

The method may be used as a data collection method as well as for enforcement purposes. The independent validation is reported below in chapter B5.2.1 (Meyer, M.; 2011; M-405654-01).



**Table 5.1.2.1-17: Recoveries for Flufenacet and Metabolites (Quantifier MRM  $m/z = 154 \rightarrow m/z = 112$ )**

| Fortified analyte(s) / sample material                     | FL <sup>1</sup> [mg/kg] | Recoveries (individual values) [%] |    |    |                  |    | Mean per FL [%]       | RSD [%]                   | Mean overall [%]      | RSD overall [%]          |
|--|-------------------------|------------------------------------|----|----|------------------|----|-----------------------|---------------------------|-----------------------|--------------------------|
| flufenacet <sup>2</sup> / wheat grain                      | 0.01*                   | 87                                 | 73 | 91 | 100              | 73 | 85                    | 13.9                      | 81                    | 11.2                     |
|  | 0.10                    | 79                                 | 78 | 84 | 75               | 73 | 78                    | 5.4                       |                       |                          |
| mixture of metabolites <sup>3</sup> / wheat grain          | 0.01*                   | 80                                 | 70 | 78 | 80               | 76 | 77                    | 5.4                       | 76                    | 4.2                      |
|  | 0.10                    | 74                                 | 73 | 76 | 77               | 79 | 76                    | 3.1                       |                       |                          |
| flufenacet <sup>2</sup> / wheat green material             | 0.01*                   | 82                                 | 79 | 82 | 78               | 63 | 77                    | 10.3                      | 78                    | 10.6                     |
|  | 0.10                    | 90                                 | 80 | 72 | 66               | 83 | 78                    | 12.0                      |                       |                          |
| mixture of metabolites <sup>3</sup> / wheat green material | 0.01*                   | 72                                 | 75 | 73 | 114 <sup>4</sup> | 92 | 78 (85 <sup>4</sup> ) | 12.1 (21.2 <sup>4</sup> ) | 77 (81 <sup>4</sup> ) | 8.7 (16.6 <sup>4</sup> ) |
|  | 0.10                    | 74                                 | 71 | 79 | 82               | 73 | 76                    | 6.0                       |                       |                          |
| flufenacet <sup>2</sup> / wheat straw                      | 0.05*                   | 80                                 | 88 | 79 | 73               | 72 | 78                    | 8.2                       | 80                    | 6.6                      |
|  | 0.50                    | 88                                 | 82 | 79 | 79               | 81 | 82                    | 4.5                       |                       |                          |
| mixture of metabolites <sup>3</sup> / wheat straw          | 0.05*                   | 72                                 | 74 | 70 | 73               | 69 | 72                    | 2.9                       | 72                    | 4.3                      |
|  | 0.50                    | 73                                 | 70 | 70 | 67               | 78 | 72                    | 5.8                       |                       |                          |

FL = Fortification level

\* LOQ level

RSD = relative standard deviation

<sup>1</sup> Expressed as parent equivalents.<sup>2</sup> Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet.<sup>3</sup> Fortified as mixture of flufenacet metabolites (FOE 5043-thioglycolate sulfoxide, FOE 5043-oxalate, FOE 5043-sulfonic acid in a molar ratio 1/1/1, expressed as parent equivalents), determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet.<sup>4</sup> identified as Nalimov outlier, result including the outlier value**Table 5.1.2.1-18: Recoveries for flufenacet and Metabolites (Quantifier MRM  $m/z = 154 \rightarrow m/z = 92$ )**

| Fortified Analyte(s) / Sample Material            | FL <sup>1</sup> [mg/kg] | Recoveries (individual values) [%] |    |    |    |    | Mean per FL [%] | RSD [%] | Mean Overall [%] | RSD Overall [%] |
|---|-------------------------|------------------------------------|----|----|----|----|-----------------|---------|------------------|-----------------|
| mixture of metabolites <sup>2</sup> / wheat straw | 0.05                    | 71                                 | 74 | 60 | 77 | 75 | 71              | 9.4     | 71               | 7.3             |
|   | 0.50                    | 71                                 | 68 | 71 | 66 | 76 | 70              | 5.4     |                  |                 |

FL = Fortification level

\* LOQ level

RSD = relative standard deviation

<sup>1</sup> Expressed as parent equivalents.<sup>2</sup> Fortified as mixture of flufenacet metabolites (FOE 5043-thioglycolate sulfoxide, FOE 5043-oxalate, FOE 5043-sulfonic acid in a molar ratio 1/1/1, expressed as parent equivalents), determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet.

## Conclusion

Method 01100/M001 was validated for the determination of flufenacet residues in wheat grain and wheat green material with an LOQ of 0.01 mg/kg and for wheat straw with an LOQ of 0.05 mg/kg. The method was validated successfully and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/00, SANCO/825/00 rev. 8.1, 16/11/2010) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

|                          |   |
|--------------------------|---|
| Report:                  | KCA 4.1.2/15, Stuke, S.; Teubner, L.; 2013; M-448503-01   |
| Title:                   | Modification M002 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE 5043) in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg (0.05 mg/kg for straw) by HPLC-MS/MS  |
| Report no & Document No: | Method 01100/M002, Report MR-12/057, dated 2013-03-04<br>M-448503-01-1  |
| Guidelines:              | <ul style="list-style-type: none"> <li>• EC Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO 3029/99</li> <li>• Regulation (EC) No 1107/2009 of the European Parliament and 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</li> <li>• OECD Guideline, ENV/JM/MONO(2007)17, August 13, 2007</li> <li>• U.S. EPA Guideline, OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods of April 1996</li> <li>• U.S. EPA Guideline, OPPTS 860.1340 Residue Analytical Method of August 1996</li> </ul> |
| GLP                      | Yes, Deviations none  |

The analytical method 01100/M002 was validated for the determination of residues of flufenacet as the common moiety compound 4-fluoro-*N*-isopropylaniline in/on samples of plant origin – here represented by green material, grain and straw of cereals – by LC-MS/MS. The parent compound flufenacet can be considered as being representative also for all metabolites containing the fluorophenyl-isopropyl moiety. In the methods previously described (00346, 01179, 01100 and 01100/M001) it has been demonstrated that the parent compound and representative metabolites (FOE 5043-thioglycolate sulfoxide, FOE 5043-oxalate, FOE 5043-sulfonic acid) can be determined as the common moiety 4-fluoro-*N*-isopropylaniline. The analytical work is based on method 01100 (Billian, P.; 2010; M-362575-02) and method 01100/M001 (Stuke, S., Bauer, J.; Ruhl, S.; 2012; M-433720-01). The reason for the modification M002 was the implementation of the use of an isotopically stable labeled standard and a less acidification in the receiver flask for the water steam distillation allowing omission of the clean-up step (liquid-liquid partition).

#### Principle of method

The analytical method 01100/M002 was validated for the determination of residues of flufenacet as the common moiety compound 4-fluoro-*N*-isopropylaniline in/on samples of plant origins – here represented by wheat green material, wheat grain, wheat straw – by LC-MS/MS with the following working conditions for chromatograph: column Luna C18 (2) HST, 2.5 µm particle size, 50 mm length, 2 mm id with pre-column, retention time approx. 2 min. Residues are expressed as parent equivalent.

5 g of the plant sample (2.5 g for straw) was treated with potassium permanganate for oxidation and refluxed under sulfuric acidic conditions for hydrolysis purposes. Residues were purified by water steam distillation of the formed common moiety compound 4-fluoro-*N*-isopropylaniline followed by addition of the internal standard. With method modification M002 liquid/liquid partition with dichloromethane is not required since the amount of acid in the receiver flask was reduced (2.5 mL of 0.1 mol/L HCl) and thus an adequate pH is achieved for LC-MS/MS determination. Finally, the mixture was homogenized and subjected to LC-MS/MS. Residues of flufenacet (determined as 4-fluoro-*N*-isopropylaniline) were quantified using external calibration with standards in solvent.

The following MRM transitions were used for quantification and confirmation:

4-fluoro-*N*-isopropylaniline:  $m/z = 154.1 \rightarrow m/z = 112.0$  (quantification),  
 $m/z = 154.1 \rightarrow m/z = 95.0$  (confirmation).  
 $m/z = 154.1 \rightarrow m/z = 92.0$  (confirmation).

#### Limit of Quantification (LOQ)

The LOQ was set at the lowest validated fortification level where a mean recovery within the range of 70 to 110% with an RSD of  $\leq 20\%$  was obtained for flufenacet (determined as 4-fluoro-*N*-isopropylaniline) in all matrices tested. The LOQ was set at a level of 0.01 mg/kg in the matrices wheat grain and wheat green material. In the matrix wheat straw the LOQ was set at 0.05 mg/kg (expressed as parent equivalents).

The LOD was calculated based on a statistical approach for residues of flufenacet (determined as 4-fluoro-*N*-isopropylaniline) in all matrices tested. The LOD was calculated at a range of 0.0023 mg/kg to 0.0027 mg/kg in the

matrices wheat grain and wheat green material. In the matrix wheat straw the LOD was calculated at a level of 0.0081 mg/kg.

**Linearity**

A linear relation between the injected amount and peak areas (analyte and internal standard) was observed over the range of 0.0001 to 0.01 mg/L (corresponds to 0.005 – 0.5 mg/kg) for green material and grain and 0.0002 to 0.02 mg/L (corresponds to 0.02 – 2 mg/kg) for straw (expressed as parent equivalents; min to max). The correlation coefficients of the 1/x weighted linear regression were in all cases  $\geq 0.99$  and the y-axis intercepts were well below 10% of the respective LOQ.

The calibration data obtained justify using the linearity curve for calculation of the residues.

**Specificity**

The analyte 4-fluoro-*N*-isopropylaniline is monitored using three mass transitions (MRM): one transition for quantitation and two transitions for qualitative confirmation. The residues of flufenacet and metabolites (determined as their common moiety 4-fluoro-*N*-isopropylaniline) in the used control materials were < 30% of the individual LOQ.

**Repeatability**

For the matrices investigated, for the quantifier and both qualifier mass transitions monitored, relative standard deviations (RSD) per fortification level and overall per commodity were < 20 %. (Please refer to Tables 5.1.2.1-19 to 5.1.2.1-21).

**Recovery rates (accuracy)**

Recovery rates were determined after fortification with flufenacet at levels of 0.01 mg/kg and 0.1 mg/kg (wheat grain and wheat green material) and 0.05 mg/kg and 0.5 mg/kg (wheat straw).

Mean recovery rates per fortification level and the overall mean recoveries per sample material determined were within the range of 70 - 110% for both fortification levels for the quantifier mass transition (1<sup>st</sup> MRM,  $m/z = 154 \rightarrow m/z = 112$ ). Mean recovery values per fortification level and overall per commodity for both qualifier mass transitions (2<sup>nd</sup> MRM,  $m/z = 154 \rightarrow m/z = 95$  and 3<sup>rd</sup> MRM,  $m/z = 154 \rightarrow m/z = 92$ ) were also in the range of 70-110% and confirm the results of the quantitation mass transition. (Please refer to Tables 5.1.2.1-19 to 5.1.2.1-21).

**Confirmatory method**

LC-MS/MS using three characteristic MS/MS transitions for detection ensures a high level of specificity. In addition to the quantifier mass transition ( $m/z = 154 \rightarrow m/z = 112$ ) two further MRM transitions were monitored for the analyte 4-fluoro-*N*-isopropylaniline for all three sample materials and both fortification levels. The individual recoveries for the 2<sup>nd</sup> MRM ( $m/z = 154 \rightarrow m/z = 95$ ) and 3<sup>rd</sup> MRM ( $m/z = 154 \rightarrow m/z = 92$ ) confirm the results of the quantitation MRM and thus can be used interchangeably if so desired.

**Stability in extracts**

Residues of the formed common moiety compound 4-fluoro-*N*-isopropylaniline were found to be stable in final plant extracts for at least 14 days when stored in a refrigerator at < 6°C as demonstrated in modification M001 of method 01100.

**Reproducibility (ILV)**

Modification M002 of method 01100 is considered as a data generation method and therefore an independent laboratory validation is not required.

**Table 5.1.2.1-19: Recoveries for flufenacet, (Quantifier MRM  $m/z = 154 \rightarrow m/z = 112$ )**

| Sample material | FL <sup>1</sup><br>[mg/kg] | Recoveries<br>(single values)<br>[%] |     |     |    |     | Mean<br>per FL<br>[%] | RSD<br>[%] | Mean<br>overall<br>[%] | RSD<br>overall<br>[%] |
|-----------------|----------------------------|--------------------------------------|-----|-----|----|-----|-----------------------|------------|------------------------|-----------------------|
| grain           | 0.01*                      | 89                                   | 99  | 93  | 87 | 83  | 90                    | 6.8        | 90                     | 6.3                   |
|                 | 0.10                       | 93                                   | 81  | 91  | 90 | 97  | 90                    | 6.5        |                        |                       |
| green material  | 0.01*                      | 106                                  | 96  | 93  | 89 | 89  | 95                    | 7.4        | 92                     | 6.8                   |
|                 | 0.10                       | 84                                   | 97  | 88  | 93 | 88  | 90                    | 5.6        |                        |                       |
| straw           | 0.05*                      | 101                                  | 104 | 102 | 93 | 103 | 101                   | 4.4        | 93                     | 9.5                   |
|                 | 0.50                       | 88                                   | 91  | 86  | 79 | 85  | 86                    | 5.2        |                        |                       |

FL = Fortification level

\* LOQ level

RSD = relative standard deviation

<sup>1</sup> Expressed as parent equivalents.Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet**Table 5.1.2.1-20: Recoveries for flufenacet, (Quantifier MRM  $m/z = 154 \rightarrow m/z = 92$ )**

| Sample material | FL <sup>1</sup><br>[mg/kg] | Recoveries<br>(single values)<br>[%] |    |    |    |    | Mean<br>per FL<br>[%] | RSD<br>[%] | Mean<br>overall<br>[%] | RSD<br>overall<br>[%] |
|-----------------|----------------------------|--------------------------------------|----|----|----|----|-----------------------|------------|------------------------|-----------------------|
| grain           | 0.01*                      | 101                                  | 96 | 85 | 91 | 83 | 91                    | 8.2        | 92                     | 6.7                   |
|                 | 0.10                       | 95                                   | 84 | 95 | 94 | 97 | 93                    | 5.5        |                        |                       |
| green material  | 0.01*                      | 86                                   | 89 | 94 | 98 | 87 | 91                    | 5.6        | 90                     | 6.0                   |
|                 | 0.10                       | 81                                   | 97 | 90 | 90 | 85 | 89                    | 6.8        |                        |                       |
| straw           | 0.05*                      | 103                                  | 95 | 99 | 81 | 89 | 93                    | 9.3        | 89                     | 9.2                   |
|                 | 0.50                       | 84                                   | 90 | 86 | 77 | 84 | 84                    | 5.6        |                        |                       |

FL = Fortification level

\* LOQ level

RSD = relative standard deviation

<sup>1</sup> Expressed as parent equivalents.Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet.**Table 5.1.2.1-21: Recoveries for flufenacet, (Quantifier MRM  $m/z = 154 \rightarrow m/z = 95$ )**

| Sample material | FL <sup>1</sup><br>[mg/kg] | Recoveries<br>(single values)<br>[%] |     |     |     |     | Mean<br>per FL<br>[%] | RSD<br>[%] | Mean<br>overall<br>[%] | RSD<br>overall<br>[%] |
|-----------------|----------------------------|--------------------------------------|-----|-----|-----|-----|-----------------------|------------|------------------------|-----------------------|
| grain           | 0.01*                      | 101                                  | 103 | 102 | 104 | 96  | 101                   | 3.1        | 96                     | 6.8                   |
|                 | 0.10                       | 92                                   | 84  | 91  | 91  | 98  | 91                    | 5.4        |                        |                       |
| green material  | 0.01*                      | 93                                   | 97  | 82  | 87  | 104 | 93                    | 9.2        | 91                     | 8.0                   |
|                 | 0.10                       | 83                                   | 98  | 85  | 92  | 87  | 89                    | 6.8        |                        |                       |
| straw           | 0.05*                      | 93                                   | 94  | 103 | 100 | 97  | 97                    | 4.3        | 91                     | 8.8                   |
|                 | 0.50                       | 84                                   | 91  | 85  | 78  | 84  | 84                    | 5.5        |                        |                       |

FL = Fortification level

\* LOQ level

RSD = relative standard deviation

<sup>1</sup> Expressed as parent equivalents.Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

## Conclusion

Method 01100/M002 was validated for the determination of flufenacet residues in wheat grain and wheat green material with an LOQ of 0.01 mg/kg and for wheat straw with an LOQ of 0.05 mg/kg. The parent compound flufenacet can be considered as being representative also for all metabolites containing the fluorophenyl-isopropyl amine moiety. In method 01100 and 01100/M001, it has been demonstrated that the parent compound and representative metabolites (FOE 5043-thioglycolate sulfoxide, FOE 5043-oxalate, FOE 5043-sulfonic acid) can be determined as the common moiety 4-fluoro-*N*-isopropylaniline. Since work-up steps for extraction of residues and steps to obtain the 4-fluoro-*N*-isopropylaniline remain unchanged it is considered acceptable to use the parent compound for generating method validation data. The method was validated successfully and thus was demonstrated to be applicable for data gathering purposes. The analytical method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/00), and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13), and the EC Guidance documents on residue analytical methods SANCO/825/00 rev. 8.1.

## NOTE:

Below the position of the applicant is presented on the comparison of two analytical methods for testing of flufenacet residues in/on cereals addressing a request from UK CRD.

|              |   |
|--------------|---|
| Report:      | KCA 4.1.2/16, Stuke, S.; Weile, M.; 2011; M-416013-01   |
| Title:       | Position paper: Subject: Flufenacet: Answer to CRD questions related to the authorization of the product Liberator SC 500 (flufenacet + diflufenican 400 g/L + 100 g/L) - Comparison of flufenacet residue analytical method nos. 00346 vs. 01179 |
| Document No: | M-416013-01-1   |
| Guidelines:  | Not applicable (position paper)   |
| GLP          | Not applicable (position paper)   |

*‘Linked to the authorization of a flufenacet containing product (flufenacet 400 g/L + diflufenican 100 g/L) in/on cereals in the United Kingdom residue trials were submitted where the field samples were analysed using method 01179. CRD requested clarification on the comparability of method 00346 evaluated in the EU review process and method 01179.*

*In the Monograph, method 00346 has been evaluated as the basic method for data generation and enforcement. The residues are oxidized and hydrolysed to the common moiety 4-fluoro-*N*-isopropylaniline. The 4-fluoro-*N*-isopropylaniline is separated from the crop matrix by steam distillation after making the crop digest basic. The 4-fluoro-*N*-isopropylaniline is extracted from the steam distillate and derivatized with trifluoroacetic anhydride. The derivative, 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (trifluoroacetamide), is measured by gas chromatography/mass spectroscopy (GC-MS). Due to the physico-chemical properties of flufenacet and its metabolites (e.g. decomposition at higher temperatures) the derivatisation step was necessary because these substances were not suitable for the determination as single substances themselves with GC (i.e. without prior derivatisation).*

*This method shows both very good repeatability and reproducibility as demonstrated in the method report of method 00346. During the validation of the method 00346 each compound of interest ( i.e. flufenacet, FOE-oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide) was fortified separately or as a mixture of these showing sufficient recoveries at the end of the entire sample work-up.*

*Since HPLC determination has become more and more available and has become acceptable also for enforcement methods, method 00346 was modified by omission of the derivatisation step to form trifluoroacetamide. Instead, with method 01179 and 01100 (including its extensions) the resulting 4-fluoro-*N*-isopropylaniline is directly determined by HPLC-MS/MS in order to facilitate the analytical work.*

*CRD’s questions center around the methodology used for formation and extraction of the common moiety in the new method 01179 and whether both methods can be considered comparable relative to the efficiency to form the common moiety.*

*In the position paper the individual steps of the sample work-up for method 00346 and 01179 are compared and a justification is provided that the metabolites containing the common fluorophenyl-isopropyl amine moiety can be fortified as a mixture because they are all converted to the 4-fluoro-*N*-isopropylaniline which forms the analytical target.*

*It is confirmed that all steps related to the work-up of the samples remain unchanged compared to method 00346. In method 00346 it has been demonstrated with fortifications of individual metabolites that each metabolite shows appropriate behavior to form the common moiety. Since the preparation steps in method 01179 (and 01100 including extensions) are identical it is considered appropriate to fortify the metabolites as mixture. Recovery rates and repeatability were acceptable. The same arguments as for method 01179 apply to method 01100 and its extensions 01100/M001 and 01100/M002 which involve unchanged extraction of the residues and formation of the 4-fluoro-N-isopropylaniline which can be detected directly by HPLC without further derivatisation.*

*Method 00346 and 001179 (and method 01100 as well) are characterized by the following work-up steps:*

- *Extraction step in both methods is identical containing oxidation reagent in aqueous acidic environment.*
- *In following step the refluxing under strong acid conditions cleaves the amide bonding to achieve the common moiety compound 4-fluoro-N-isopropylaniline. This step is identical in all described methods.*
- *This compound is protonated in acid medium and has to be deprotonated with alkaline to be distillable. This step is included in all described methods.*
- *Water steam distillation out of alkaline medium into an acidified distillation receiver flask (protonates the aniline again) avoids the loss of the target compound for all methods.*
- *To remove the acid content they obtained solution is liquid/liquid distributed with dichloromethane. This step is included in methods 00346, 01179, 01100 and modification M001. In modification M002, the amount of HCl in the receiver is reduced resulting in an appropriate pH value for direct LC-MS/MS determination. Thus the obtained solution is just diluted and subject to HPLC-MS/MS determination and the clean-up step with dichloromethane can be omitted.*
- *In method 00346 the 4-fluoro-N-isopropylaniline is finally derivatized with trifluoroacetic anhydride to 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide (called 4-fluoro-N-methylethyl benzenamine trifluoroacetamide or FOE 5043 trifluoroacetamide in the method reports) to be amenable to GC-MS determination. In method 01179 (and 01100 including its extensions) this step is not necessary. The 4-fluoro-N-isopropylaniline can be determined by LC-MS/MS without further chemical modification'.*

## Conclusion

Methods 00346 and 01179 (as well as method 01100 and its extensions M001 and M002) are suitable for the determination of flufenacet residues in protected crops and the both methods were validated successfully and thus were demonstrated to be applicable for data gathering and monitoring purposes. The analytical methods fulfil the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/2000, SANCO/825/00 rev. 8.1, 16/11/2010). The method (01179 and 01100) using an HPLC-MS/MS technique are more convenient because they do not include the step of derivatization.

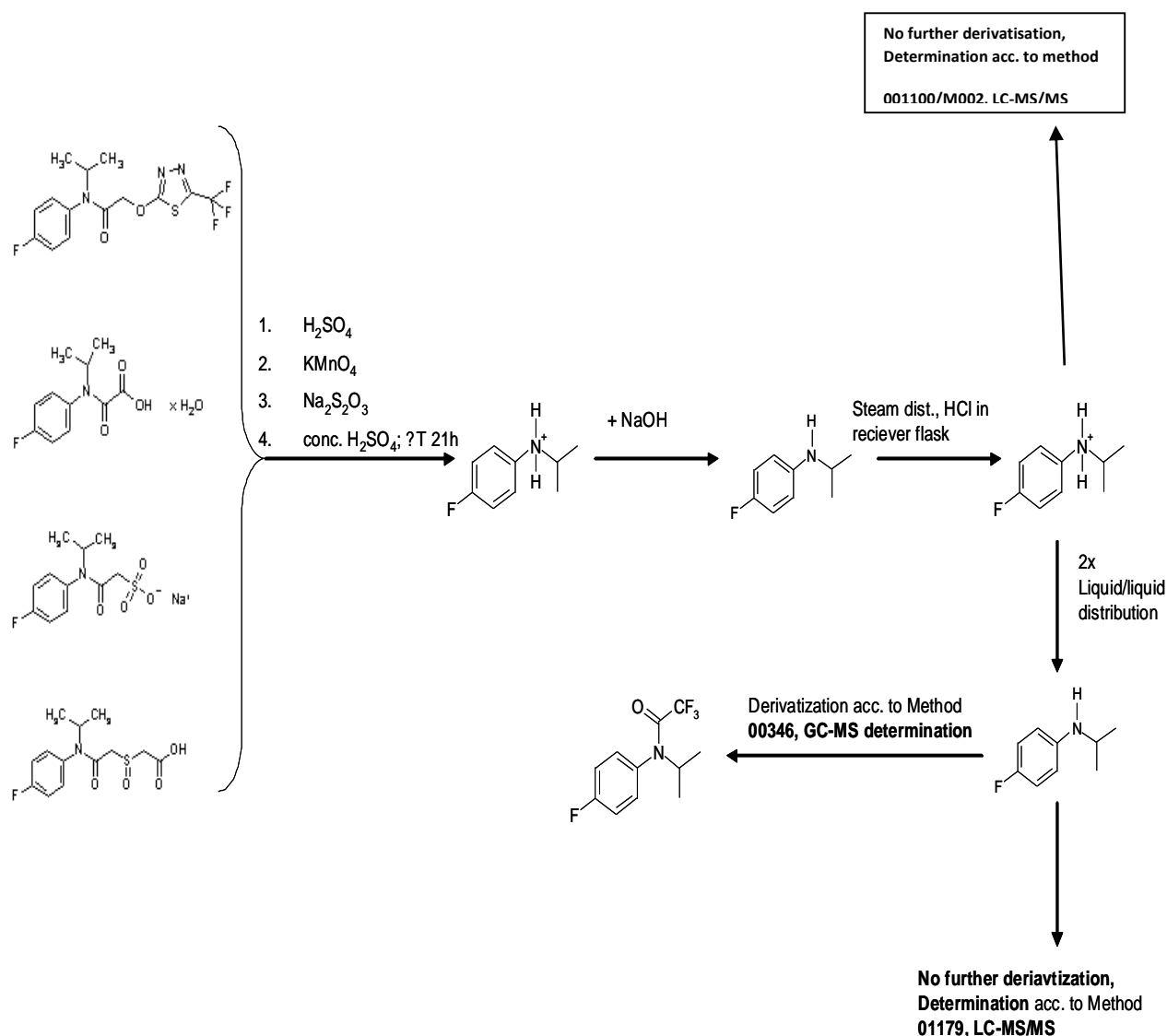


Figure 5.1.2.1-1: Chemistry work-flow chart.

Figure 5.1.2.1-1 is presented chemistry work-flow within residue analytical methods 00346 and 01179, 01100 (incl. extensions)

### Extraction efficiency of the residue analytical methods

The relevant guidelines for residue analytical methods (SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1 and OECD guidance document on pesticide residue analytical methods (ENV/JM/MONO (2007)17)) request provision of data on extraction efficiency/radio validation of the residue analytical methods. Ideally, the investigation should be performed using samples/commodities from the metabolism studies. In the following paragraphs extraction efficiency of the residue analytical methods is compared to relative amount extracted from the metabolism studies. The conditions used in metabolism studies are also reported in detail in section B7.2.1 and B7.2.2. Since the extraction conditions are the same in data generation methods and methods for post registration control the findings reported below are evenly valid for enforcement methods.

|              |   |
|--------------|---|
| Report:      | KCA 4.1.2/20, Gould, T. J.; 1995; M-041609-01   |
| Title:       | Extraction efficiency of the analytical method for determination of FOE 5043 residues in plant matrices |
| Document No: | M-041609-01-1   |
| Report No:   | 106927, dated 1995-06-02  |
| Guidelines   | EPA Ref: 171-4(c), Residue Analytical Method – Plants, Extraction Efficiency                            |
| GLP          | yes   |

The extraction efficiency of the residue analytical method for quantification of flufenacet residues in crops (Gould, T. J.; Lemke, V. J.; 1995; M-041601-01) - which is identical to method 00346 discussed above - was examined using corn/maize and soybean commodities with incurred residues originating from plant metabolism studies with [fluorophenyl-UL-<sup>14</sup>C]flufenacet.

Extraction conditions as described in method 00346 are used in all methods for analysis of flufenacet residues in plants.

### Executive Summary

A common moiety analytical residue method for determination of flufenacet (FOE 5043) residues in plant matrices was developed *via* cleavage and quantification of *N*-(4-fluorophenyl)-*N*-isopropyl amine as common moiety from all flufenacet residues. This method effectively extracted and measured the identified radioactive flufenacet-derived residues from aged, plant samples (corn fodder, soybean forage, soybean fresh beans) originating from a pre-emergent treatment with radiolabelled flufenacet.

A comparison of the amount of the released common moiety by the residue method and the sum of all radiolabelled residue components identified in the metabolism study with [fluorophenyl-UL-<sup>14</sup>C]flufenacet indicated complete extraction of incurred flufenacet residues using the residue method.

Extraction efficiency values slightly above 100% of the sum of identified metabolites indicated that some unknown metabolites also containing the common moiety were captured too by the residue method.

### Material and Methods

#### Test material

The corn fodder used in this study was obtained as part of the corn metabolism study (Baird, J. H.; 1994; M-002270-01) after pre-emergent application of radiolabelled flufenacet (FOE 5043). Corn plants were grown in soil treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a rate of 0.90 kg as/ha. The specific radioactivity of the used test substance amounted to 183 000 dpm/μg (30.0 mCi/mmol). In the metabolism study corn fodder was extracted with methanol/water (4/1, v/v, 4x). The extracted radioactive residues amounted to 90% of the total radioactive residues (TRR) in that plant matrix.

The soybean forage and seeds (fresh beans) used in this study were obtained as part of the soybean metabolism study (Krolski, M. E.; Bosnak, L. L.; 1995; M-002278-01) after pre-emergent application of radiolabelled flufenacet (FOE 5043). Soybean plants were grown in soil treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a rate of 1.45 kg as/ha. The specific radioactivity of the used test substance amounted to 123 000 dpm/μg. In the metabolism study soybean forage was extracted with methanol (3x). The extracted radioactive residues amounted to 93% of TRR in the forage. The fresh beans were also extracted with methanol (3x) yielding an extracted portion of 77% of TRR in the beans.

The corn fodder, soybean forage, and soybean seed samples were homogenized and stored under freezer conditions (-20° ± 5° C). At the start of this study, aliquots of the plant matrix samples were frozen in liquid nitrogen and macerated with a tissue mixer. The liquid nitrogen was allowed to evaporate while storing in a freezer. One day later, aliquots of the blended samples were radioassayed and the samples were stored frozen until analyzed.

#### Analytical Residue Method Procedures

Aliquots (10.0 g) of the plant matrix samples were extracted by the analytical residue method and processed as described in the following. The total radioactivity of the used plant samples was radioassayed before extraction amounting to 867 500 dpm in corn fodder, 8 050 000 dpm in soybean forage and to 641 400 dpm in soybean seeds. These values were set to 100% residue content.

The homogenized plant samples were soaked in water for one hour. Addition of 1N sulfuric acid and an oxidant (potassium permanganate) caused oxidative cleavage of *N*-(4-fluorophenyl)-*N*-isopropylamine (4-fluoro-*N*-isopropylaniline) being the common moiety of parent substance and its metabolites. Subsequent addition of sodium bisulfite degraded surplus permanganate. The mixture was then refluxed with concentrated sulfuric acid for 24 hours to disintegrate the plant matrix and to complete the release of the amine as ammonium sulfate. Thereafter, water and sodium hydroxide were added and the released amine steam-distilled and absorbed in the receiver filled



with a small amount of concentrated hydrochloric acid. The acid was washed with dichloromethane, made alkaline with sodium hydroxide and the amine extracted with dichloromethane. The final dichloromethane solution was radioassayed and derivatized with trifluoroacetic anhydride. The resulting derivative was radioassayed again and analyzed by radio-HPLC. Triplicate extractions were performed for each sample.

#### Derivatization of the common moiety *N*-(4-fluorophenyl)-*N*-isopropylamine

An aliquot of the final dichloromethane extract was amended with little sulfuric acid and dimethylformamide. The dichloromethane was evaporated at ambient temperature. The resulting solution was treated with 0.2% (w/v) dimethylaminopyridine in pyridine followed by trifluoroacetic anhydride. The solution was mixed and allowed to stand at ambient temperature for 15 min. Water was added to disintegrate surplus acetic anhydride. The resulting solution with the radiolabelled analytical target 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide was radioassayed and analyzed by radio-HPLC.

#### Radioassaying and radio-HPLC

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed  $^{14}\text{CO}_2$  absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivities used in this study the LOQ for radioassaying was set to 0.0059 mg eq/kg.

Radio-HPLC was conducted on a reverse phase column (150 x 4.0 mm, 5  $\mu\text{m}$  particle size) operated with a gradient mixture of acetonitrile and 0.05% aqueous trifluoroacetic acid. The HPLC system was equipped with a UV detector (254 or 295 nm) and a radiomonitor with a lithium glass scintillator. The detection limit was determined to be 300 dpm (corresponding to 0.002  $\mu\text{g}$  parent equivalents). Radiolabelled 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide [“4-fluoro-*N*-methylethyl benzene-amine trifluoroacetamide”] was used as a reference standard.

#### **Findings**

All plant samples were re-extracted with methanol or methanol/water according to the metabolism studies. The extracted residue components were identified as FOE oxalate (M1), FOE sulfonic acid (M2), FOE thioglycolate sulfoxide (M4), FOE methylsulfone (M7), FOE methylsulfoxide (M6), and FOE thiolactate sulfoxide (FOE sulfinyl lactic acid, M33) by comparison of the radio-HPLC profiles with those in the original metabolism studies. No parent substance could be detected in any sample.

All metabolites contain the common moiety *N*-(4-fluorophenyl)-*N*-isopropyl amine being the analytical target of the residue analytical method. Therefore, the extraction efficiency of the residue method is defined by the ratio of the amount of the analytical target determined by the residue method and the total amount of metabolites identified in the metabolism study. Percentage values were rounded to the nearest whole number. The extraction efficiency for flufenacet residues from corn fodder, soybean forage and soya fresh beans are compiled in Table 5.1.2.1-22.

#### Corn fodder

The extraction of corn fodder (TRR 0.47 mg eq/kg) using the residue method was conducted using three replicates. An average of 78% of TRR from the corn fodder was extractable into the final dichloromethane extract. The respective radio-HPLC separation of this final extract following derivatization showed one major peak (98% of the final extract) that was eluted at the same time as the reference standard *N*-(4-fluorophenyl)-*N*-isopropyl trifluoroacetamide. By multiplication of these values, an overall portion of 76% of the TRR in corn fodder was quantified as the trifluoroacetamide derivative. This was equivalent to a mean of 109% of the residues identified in the corn metabolism study since the sum of identified metabolites amounted to 70% of TRR.

#### Soybean forage

The extraction of soybean forage (TRR 6.55 mg eq/kg) using the residue method was also conducted three-times. An average of 95% of TRR from the soybean forage was extracted into the final extract dichloromethane. The respective radio-HPLC separation of this final extract following derivatization showed one major peak (95% of the final extract) that was eluted at the same time as the reference standard 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide. By multiplication of these values, an overall portion of 91% of the TRR in soybean forage was quantified as the trifluoroacetamide derivative. This was equivalent to a mean of 112% of the residues identified in the soybean metabolism study using the fluorophenyl label since the sum of identified metabolites amounted to 81% of TRR.

Soybean seeds (fresh beans)

The extraction of soybean seeds (TRR 0.53 mg equ/kg) using the residue method was also conducted three-times. An average of 55% of TRR from the soybean seeds was extracted into the final extract dichloromethane. The respective radio-HPLC separation of this final extract following derivatization showed one major peak (91% of the final extract) that was eluted at the same time as the reference standard *N*-(4-fluorophenyl)-*N*-isopropyl trifluoroacetamide. By multiplication of these values, an overall portion of 50% of the TRR in soybean seeds was quantified as the trifluoroacetamide derivative. This was equivalent to a mean of 112% of the residues identified in the soybean metabolism study using the fluorophenyl label since the sum of identified metabolites amounted to 45% of TRR.

**Conclusion**

A common moiety analytical residue method for determination of flufenacet residues in plant matrices was developed *via* cleavage and quantification of *N*-(4-fluorophenyl)-*N*-isopropyl amine (fluoroaniline) as common moiety from all flufenacet residues. This method effectively extracted and measured the identified radioactive flufenacet-derived residues from aged plant samples (corn fodder, soybean forage, soybean fresh beans) originating from a pre-emergent treatment with radiolabelled flufenacet. Extraction efficiency values slightly above 100% of the sum of identified metabolites indicated that some unknown metabolites also containing the common moiety *N*-(4-fluorophenyl)-*N*-isopropyl amine (4-fluoro-*N*-isopropylaniline) were captured too by the residue method.

The most recent version of the residue method does no longer need the final derivatization step with trifluoroacethanhydride. Nevertheless, complete extraction of the common moiety by the current residue method also applies to the extraction step before derivatization.

**Table 5.1.2.1-22: Efficiency of the residue analytical method for extracting identified flufenacet residues from plant commodities grown in soil that was treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet**

| Metabolism study                    | Residue analytical method                                      |                                    |                                | Extraction efficiency <sup>4</sup>                     |
|-------------------------------------|--|------------------------------------|--------------------------------|--|
| Identified metabolites <sup>1</sup> | Radioactivity in final CH <sub>2</sub> Cl <sub>2</sub> extract | Analytical target <sup>2</sup>     | Analytical target <sup>3</sup> | % analytical target referred to identified metabolites |
| [% of TRR]                          | [% of TRR]   | [% radioactivity of final extract] | [% of TRR]                     |  |
| Corn fodder                         |  |                                    |                                |  |
| 70                                  | 79   | 97                                 | 76                             | 108  |
|                                     | 78   | 98                                 | 76                             | 109  |
|                                     | 77   | 98                                 | 76                             | 108  |
| Average                             | 78   | 98                                 | 76                             | 109  |
| Soybean forage                      |  |                                    |                                |  |
| 81                                  | 97   | 96                                 | 93                             | 114  |
|                                     | 95   | 95                                 | 91                             | 112  |
|                                     | 94   | 95                                 | 90                             | 111  |
| Average                             | 95   | 95                                 | 91                             | 112  |
| Soybean fresh beans                 |  |                                    |                                |  |
| 45                                  | 56   | 89                                 | 50                             | 111  |
|                                     | 56   | 90                                 | 51                             | 112  |
|                                     | 53   | 93                                 | 49                             | 109  |
| Average                             | 55   | 91                                 | 50                             | 111  |

<sup>1</sup>Metabolites identified in metabolism study: FOE oxalate (M1), FOE sulfonic acid (M2), FOE thioglycolate sulfoxide (M4), FOE methylsulfone (M7), FOE methylsulfoxide (M6), FOE thiolactate sulfoxide (M4) and FOE sulfinyl lactic acid (M33)

<sup>2</sup> Percentage of radioactivity in the final extract identified as analytical target 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide by radio-HPLC

<sup>3</sup> Analytical target related to TRR = (radioactivity in final dichloromethane extract) x (percent derivatised to the analytical target as identified by HPLC)

<sup>4</sup>Extraction efficiency of residue method = ratio between analytical target [% of TRR] / identified metabolites in the metabolism study [% of TRR]. All identified metabolites contain the common moiety (= analytical target). Based on identified residue components containing the common moiety *N*-isopropyl-4-fluoroaniline from the metabolism study being equal to 100%.

|              |  |
|--------------|--|
| Report:      | KCA 4.1.2/21; Beedle, E. C.; Ying, S. L.; 2000; M-020428-01<br>also filed 6.2.1/07 |
| Title:       | The Metabolism of [fluorophenyl-UL- <sup>14</sup> C]Flufenacet in Potatoes         |
| Document No: | M-020428-01-1  |
| Report No:   | 109226, dated 2000-04-28   |
| Guidelines:  | US-EPA OPPTS 860.1300, Nature of Residues - Plants                                 |
| GLP          | yes  |

Extraction efficiency of the residue analytical method (Gould, T. J.; Lemke, V. J.; 1995; M-041601-01) was investigated in the course of the metabolism study.

The extraction efficiency was examined using potato tubers with incurred residues from the pre-emergent and post-emergent application of radiolabelled flufenacet. The study is reported in detail in chapter B7.2.1.2.

In the metabolism study, the homogenized tubers were extracted three times with methanol at room temperature, followed by 4-hours refluxing with methanol and hydrolyzed with 1N hydrochloric acid. The acid hydrolysate was extracted with chloroform. All liquid phases were radioassayed. The final solids were radioassayed *via* combustion. To examine for potential glucoside conjugates, a major radioactive residue component was isolated by preparative HPLC, evaporated to dryness and re-dissolved in a sodium phosphate buffer solution. This solution was incubated with  $\beta$ -glucosidase, then concentrated to dryness, re-dissolved in acidic methanol and analyzed by radio-HPLC.

TRR levels of tubers used for this test amounted to 0.37 or 0.34 mg equ/kg after pre- or post-emergent application. These levels were slightly higher (approx. 6%) than the initial levels, probably due to desiccation during freezer storage.

Applying the procedures of the residue analytical method, following oxidation, hydrolysis and steam distillation of the residues in tubers from post-emergent application the distillate contained a radioactivity level of 0.28 mg equ/kg. 0.26 mg equ/kg partitioned into dichloromethane and 0.24 mg equ/kg was quantified as the derivatized analytical target *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (0.25 mg equ/kg) this figure represented an extraction efficiency of 96%.

The distillate from tubers grown in pre-emergent treated soil contained 0.31 mg equ/kg, and 0.28 mg equ/kg partitioned into dichloromethane. 0.26 mg equ/kg was quantified as the derivatized analytical target *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (0.23 mg equ/kg) this figure represented an extraction efficiency of 113% (see Table 5.1.2.1-23).

## Conclusion

It is concluded that the extraction efficiency of the analytical method for potato tubers is excellent when compared with the amount of all identified residue components detected in this metabolism study. The experiment demonstrates that the extraction conditions used in the data generation and enforcement methods provide excellent extraction efficiency. The methods fulfil the registration requirements as outlined in the Regulation (EC) No 1107/2009, detailed in the EC Guidance documents on residue analytical methods SANCO/825/00 rev. 8.1.

**Table 5.1.2.1-23: Efficiency of the residue analytical method for extracting identified flufenacet residues from potato tubers after pre-or post-emergence application with [fluorophenyl-UL-<sup>14</sup>C]flufenacet**

| Metabolism study                    | Residue analytical method                                      |                                | Extraction efficiency <sup>3</sup>                     |
|-------------------------------------|--|--------------------------------|--|
| Identified metabolites <sup>1</sup> | Radioactivity in final CH <sub>2</sub> Cl <sub>2</sub> extract | Analytical target <sup>2</sup> | % analytical target referred to identified metabolites |
| [ppm]                               | [ppm]  | [ppm]                          |  |
| TRR= 0.35                           | Potato tuber (pre-emergence application); TRR = 0.368 ppm      |                                |  |
| 0.23<br>(63% TRR)                   | 0.275  | 0.260                          | 113  |
| TRR= 0.32                           | Potato tuber (post-emergence application), TRR = 0.339 ppm     |                                |  |
| 0.25<br>(80% TRR)                   | 0.259  | 0.244                          | 96   |

<sup>1</sup>Metabolites identified in metabolism study: FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41), FOE cysteine (FACS, M23), FOE sulfonic acid (FASO3H, M2), FOE thioglycolate sulfoxide (FAMSOC, M4).

<sup>2</sup> Percentage of radioactivity in the final extract identified as analytical target 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide by radio-HPLC

<sup>3</sup> Extraction efficiency of residue method = ratio between analytical target [ppm] / identified metabolites in the metabolism study [ppm]. All identified metabolites contain the common moiety (= analytical target). Taking into account that the TRR in the samples used for extraction efficiency testing was about 6% higher compared to the results obtained in the metabolism studies, the calculated value is considered to be about 6% less.

|              |   |
|--------------|---|
| Report:      | KCA 4.1.2/22, Krolski, M. E.; Bosnak, L. L.; 1997; M-002275-01<br>also filed 6.2.1/05                       |
| Title:       | The Metabolism of [Fluorophenyl-UL- <sup>14</sup> C]FOE 5043 in Wheat After Postemergent Foliar Application |
| Document No: | M-002275-01-1   |
| Report No:   | 107399, dated 1997-11-04  |
| Guidelines:  | US-EPA OPPTS 860.1300, Nature of Residues - Plants  |
| GLP          | yes   |

Extraction efficiency of the residue analytical method (Gould, T. J.; Lemke, V. J.; 1995; M-041601-01) was investigated in the course of the metabolism study.

The extraction efficiency was examined using wheat grain and straw samples with incurred residues after application of radiolabelled flufenacet. The study is reported in detail in chapter B7.2.1.2.

In the metabolism study homogenized straw and grain were extracted separately with methanol/water (4/1, 1x) following steeping at room temperature for half an hour. Extraction was continued with pure methanol (2x) at ambient temperature and under reflux, with hydrochloric acid and sodium hydroxide. The aqueous phases were neutralized and partitioned against chloroform. Between acid/basic hydrolysis at room temperature and under reflux an additional extraction step with methanol/water (3/1) under ultrasonication was inserted. All fractions/phases were radioassayed.

TRR levels of grain and straw samples used for this test amounted to 0.55 and 1.96 mg equ/kg. These levels were slightly lower than the initial levels, probably due to hydration of the dried grain and straw during freezer storage.

Applying the procedures of the residue analytical method, following oxidation, hydrolysis and steam distillation of formed common moiety *N*-fluorophenyl-*N*-isopropyl amine from wheat grain the distillate contained 97% of TRR in the original grain sample. 84% of TRR partitioned into the organic phase after addition of sodium hydroxide. Subsequent derivatisation revealed the analytical target *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide representing 81% of TRR in the original grain sample. Compared to the total extractability with methanol determined in the metabolism experiment (80% of TRR extractable at room temperature and under reflux conditions, with 66% of TRR identified as metabolites containing the common moiety) this figure represented a complete extraction of those residue components that contain the respective *N*-fluorophenyl-*N*-isopropyl amine moiety (see Table 5.1.2.1-24).

Applying the same method to a straw sample resulted in 86% of TRR in the distillate with 76% of TRR in the organic phase prior to derivatisation. The derivatized sample contained 70% of TRR in the original straw sample, which was identified as *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (86% of TRR extractable at room temperature and under reflux conditions, with 74% of TRR identified as metabolites with the common moiety) this figure represented also a complete extraction of those residue components that contain the respective *N*-fluorophenyl-*N*-isopropyl amine moiety (see Table 5.1.2.1-24).

Comparative extraction of the residues using methanol in the metabolism study and determination of the residues using the residue analytical method (oxidative acid hydrolysis and quantification of the hereby formed *N*-fluorophenyl-*N*-isopropyl amine) showed a good agreement of amount of residue compounds containing the common moiety. The analytical residue method adequately converts the residues in wheat straw and grain to the analyte 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide.

**Table 5.1.2.1-24: Efficiency of the residue analytical method for extracting identified flufenacet residues from wheat grain and straw after application with [fluorophenyl-UL-<sup>14</sup>C]flufenacet**

| Metabolism study                    |                                     | Residue analytical method                                      |                                | Extraction efficiency <sup>3</sup>                     |
|-------------------------------------|-------------------------------------|--|--------------------------------|--|
| Total extractability of metabolites | Identified metabolites <sup>1</sup> | Radioactivity in final CH <sub>2</sub> Cl <sub>2</sub> extract | Analytical target <sup>2</sup> | % analytical target referred to identified metabolites |
| [%TRR]                              | [%TRR]                              | [% TRR]  | [%TRR]                         |  |
| TRR = 0.62 ppm                      |                                     | Wheat grain; TRR = 0.55 ppm                                    |                                |  |
| 69                                  | 66                                  | 84   | 81                             | 123  |
| TRR = 2.04 ppm                      |                                     | Wheat straw, TRR = 1.96 ppm                                    |                                |  |
| 78                                  | 74                                  | 76   | 70                             | 95   |

<sup>1</sup>Metabolites identified in metabolism study: FOE oxalate (FOEOX, M1), FOE sulfinyl lactic acid glucoside I (FAMSOL-Glu I+II, M37), FOE thioglycolate sulfoxide (FAMSOC, M4) FOE sulfinyl lactic acid I +II (FAMSOL I, M33), FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41).

<sup>2</sup> Percentage of radioactivity in the final extract identified as analytical target 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide by radio-HPLC

<sup>3</sup>Extraction efficiency of residue method = ratio between analytical target [%TRR] / identified metabolites in the metabolism study [%TRR]. All identified metabolites contain the common moiety (= analytical target).

|                                 |  |
|---------------------------------|--|
| Report:                         | KCA 4.1.2/23, Gould, T. J.; 1995; M-071501-01  |
| Title:                          | Extraction efficiency of the analytical method for determination of FOE 5043 residues in animal matrices |
| Document No:                    | M-071501-01-1  |
| Report No:                      | 106926, dated 1995-08-16   |
| Guidelines and data requirement | EPA Ref: 171-4(c), Residue Analytical Method – Animals, Extraction Efficiency                            |
| GLP                             | yes  |

The extraction efficiency of the residue analytical method for quantification of flufenacet residues in animal matrices (Gould, T. J.; et al.; 1995; M-019605-01\_) was examined using milk, liver and muscle with incurred residues originating from goat metabolism study with repeated oral dosing of [fluorophenyl-UL-<sup>14</sup>C]flufenacet (Minor, R. G.; Freese, P. L.; 1995; M-002250-01 ).

### Executive Summary

A common moiety analytical residue method for determination of flufenacet (FOE 5043) residues in animal matrices was developed *via* cleavage and quantification of *N*-(4-fluorophenyl)-*N*-isopropyl amine as common moiety from the parent substance flufenacet and its main metabolites in food of animal origin. This method effectively extracted and measured the identified radioactive flufenacet-derived residues from aged animal samples (goat milk, liver and muscle) originating from goat metabolism study with repeated oral dosing of radiolabelled flufenacet.

A comparison of the amount of the common moiety released by the residue method and the sum of all radiolabelled residue components with the common moiety (parent substance and main metabolites) identified in the metabolism study indicated an almost complete extraction of incurred flufenacet residues using the residue method.

### Material and Methods

#### Test material

Goat milk, goat liver and goat muscle used in this study were obtained as part of the goat metabolism study oral administration of radiolabelled flufenacet (FOE 5043). [Fluorophenyl-UL-<sup>14</sup>C]flufenacet was orally dosed to a three-year old lactating goat for three consecutive days at a dose level of 5 mg as/kg bw/day (equivalent to 167 mg as/kg dry feed). The specific radioactivity of the test substance amounted to 42 400 dpm/μg (6.49 mCi/mmol). Total radioactive residues (TRR) in whole milk increased 0.148 to 0.302 mg equ/kg from the first to the third dose. After the third dose the goat was slaughtered and different organs and tissues were dissected and radioassayed. The resulting TRR in liver accounted for 3.726 mg equ/kg and in muscle for 0.264 mg equ/kg. Milk, organs and tissues were stored frozen (-20 ± 5°C) for a period of 989 – 996 days.

Milk and liver were re-extracted with methanol according the original goat metabolism study. Muscle tissue was first extracted with hexane, followed by acetonitrile. The extracts were radioassayed and analyzed by radio-HPLC. A comparison the radio-HPLC profiles with those in the original goat metabolism study did not show any significant changes indicating that the flufenacet residues were stable during the storage.

Milk used for this radiovalidation test contained TRR of 0.23 mg equ/kg. Extraction with methanol (as done in the original goat metabolism study) resulted in an extractable portion of 98% of TRR. Liver used for radiovalidation contained TRR of 4.07 mg equ/kg. Extraction with methanol resulted in an extractable portion of 80% of TRR. Muscle used for radiovalidation contained TRR of 0.28 mg equ/kg. Extraction with methanol resulted in an extractable portion of 92% of TRR.

#### Analytical Residue Method Procedures

Aliquots (25.0 g) of the milk and tissue samples were extracted by the analytical residue method and processed as described in the following.

The homogenized milk and tissue samples were soaked in water for one hour. Addition of 1N sulfuric acid and an oxidant (potassium permanganate) caused oxidative cleavage of *N*-(4-fluorophenyl)-*N*-isopropylamine being the common moiety of parent substance and its metabolites. Subsequent addition of sodium bisulfite degraded surplus permanganate. The mixture was then refluxed with concentrated sulfuric acid for 24 hours to disintegrate the animal matrix and to complete the release of the amine as ammonium sulfate. Thereafter, water and sodium hydroxide were added and the released amine steam-distilled and absorbed in the receiver filled with a small amount of concentrated hydrochloric acid. The acid was washed with dichloromethane, made alkaline with sodium hydroxide and the amine extracted with dichloromethane. The final dichloromethane solution was radioassayed and derivatized with trifluoroacetic anhydride. The resulting derivative was radioassayed again and analyzed by radio-HPLC. Triplicate extractions were performed for each sample.

#### Derivatization of the common moiety *N*-(4-fluorophenyl)-*N*-isopropylamine

An aliquot of the final dichloromethane extract was amended with little sulfuric acid and dimethylformamide. The dichloromethane was evaporated at ambient temperature. The resulting solution was treated with 0.2% (w/v) dimethylaminopyridine in pyridine followed by trifluoroacetic anhydride. The solution was mixed and allowed to stand at ambient temperature for 15 min. Water was added to disintegrate surplus acetic anhydride. The resulting solution with the radiolabelled analytical target 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide was radioassayed and analyzed by radio-HPLC.

### Radioassaying and radio-HPLC

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed  $^{14}\text{CO}_2$  absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivities used in this study the LOQ for radioassaying was set to 0.027 mg equ/kg.

Radio-HPLC was conducted on a reverse phase column (150 x 4.0 mm, 5  $\mu\text{m}$  particle size) operated with a gradient mixture of acetonitrile and 0.05% aqueous trifluoroacetic acid. The HPLC system was equipped with a UV detector (254 or 295 nm) and a radiomonitor with a lithium glass scintillator. The detection limit was determined to be 300 dpm (corresponding to 0.002  $\mu\text{g}$  parent equivalents). Radiolabelled 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide [“4-fluoro-*N*-methylethyl benzene-amine trifluoroacetamide”] was used a reference standard.

### **Findings**

Milk (2-day) and liver and muscle samples of the goat were re-extracted with methanol according to the goat metabolism study. The extracted residue components were identified as FOE methylsulfone (M7, FAMSO<sub>2</sub>), FOE glutathione (M22, FOE GSH), FOE Cysteine (M23), FOE des-isopropyl methylsulfone (M15, DIFAMSO<sub>2</sub>) and a small amount of parent flufenacet in muscle. The composition of this residue component did not significantly change when compared to the composition of the respective samples in the original goat metabolism study indicating the stability of residues during storage.

All residue components contain the common moiety *N*-(4-fluorophenyl)-*N*-isopropyl amine being the analytical target of the residue analytical method, except the FOE des-isopropyl methylsulfone (M15, DIFAMSO<sub>2</sub>). Therefore, the extraction efficiency of the residue method is defined by the ratio of the amount of the analytical target determined by the residue method and the total amount of metabolites identified in the metabolism study in the respective animal matrix, with exception of M15. Percentage values were rounded to the nearest whole number. The extraction efficiency for flufenacet residues from goat milk, liver and muscle are compiled in Table 5.1.2.1-25.

### Milk

The extraction of milk (TRR 0.23 mg equ/kg) using the residue method was conducted using three replicates. An average of 62% of TRR from the milk was extractable into the final dichloromethane extract. The respective radio-HPLC separation of this final extract following derivatization showed one main peak (78% of the final extract) that was eluted at the same time as the reference standard 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide. By multiplication of these values, an overall portion of 49% of the TRR in milk was quantified as the “trifluoroacetamide” derivative. This was equivalent to a mean of 96% of the residues identified in the goat metabolism study since the sum of identified metabolites containing the common moiety amounted to 51% of TRR.

### Goat liver

The extraction of liver (TRR 4.07 mg equ/kg) using the residue method was also conducted three-times. An average of 67% of TRR from goat liver was extracted into the final extract dichloromethane. The respective radio-HPLC separation of this final extract following derivatization showed a major peak (94% of the final extract) that was eluted at the same time as the reference standard 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide. By multiplication of these values, an overall portion of 63% of the TRR in liver was quantified as the trifluoroacetamide derivative. This was equivalent to a mean of 78% of the residues identified in the goat metabolism study since the sum of identified metabolites containing the common moiety amounted to 81% of TRR.

### Goat muscle

The extraction of goat muscle (TRR 0.28 mg equ/kg) using the residue method was also conducted three-times. An average of 75% of TRR from muscle was extracted into the final extract dichloromethane. The respective radio-HPLC separation of this final extract following derivatization showed one main peak (84% of the final extract) that was eluted at the same time as the reference standard 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide amide. By multiplication of these values, an overall portion of 63% of the TRR in goat muscle was quantified as the trifluoroacetamide derivative. This was equivalent to a mean of 93% of the residues identified in the goat metabolism study since the sum of identified metabolites containing the common moiety amounted to 68% of TRR.

### Conclusion

A common moiety analytical residue method for determination of flufenacet (FOE 5043) residues in animal matrices was developed *via* cleavage and quantification of *N*-(4-fluorophenyl)-*N*-isopropyl amine as common moiety from the parent substance and all major flufenacet metabolites. This method effectively extracted and measured the identified radioactive flufenacet-derived residues from aged, animal samples (goat milk, liver and muscle) originating from oral dosing of [fluorophenyl-UL-<sup>14</sup>C]flufenacet.

The method fulfils registration requirements as outlined in the Regulation (EC) No 1107/2009, detailed in the EC Guidance documents on residue analytical methods SANCO/825/00 rev. 8.1.

**Table 5.1.2.1-25: Efficiency of the residue analytical method for extracting identified flufenacet residues from animal commodities of a goat that was dosed with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at dose rate of 5 mg as/kg bw/day for 3 consecutive days**

| Metabolism study                   | Residue analytical method                                      |                                    |                                | Extraction efficiency <sup>4</sup>                     |
|------------------------------------|--|------------------------------------|--------------------------------|--|
| Identified components <sup>1</sup> | Radioactivity in final CH <sub>2</sub> Cl <sub>2</sub> extract | Analytical target <sup>2</sup>     | Analytical target <sup>3</sup> | % analytical target referred to identified metabolites |
| [% of TRR]                         | [% of TRR]   | [% radioactivity of final extract] | [% of TRR]                     |  |
| Milk                               |  |                                    |                                |  |
| 51                                 | 62   | 79                                 | 49                             | 96   |
|                                    | 63   | 77                                 | 49                             | 95   |
|                                    | 62   | 79                                 | 49                             | 96   |
| Average                            | 62   | 78                                 | 49                             | 96   |
| Goat liver                         |  |                                    |                                |  |
| 81                                 | 73   | 95                                 | 69                             | 86   |
|                                    | 63   | 95                                 | 60                             | 74   |
|                                    | 64   | 94                                 | 61                             | 75   |
| Average                            | 67   | 94                                 | 63                             | 78   |
| Goat muscle                        |  |                                    |                                |  |
| 67                                 | 73   | 85                                 | 62                             | 93   |
|                                    | 77   | 83                                 | 64                             | 95   |
|                                    | 74   | 84                                 | 62                             | 93   |
| Average                            | 75   | 84                                 | 63                             | 93   |

<sup>1</sup>Residue components with the common moiety identified in the goat metabolism study: FOE methylsulfone (M7, FAMSO2), FOE glutathione (M22, FOE GSH), FOE Cysteine (M23), and parent flufenacet

<sup>2</sup> Percentage of radioactivity in the final extract identified as analytical target 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide by radio-HPLC

<sup>3</sup> Analytical target related to TRR = (radioactivity in final dichloromethane extract) x (percent derivatised to the analytical target as identified by HPLC)

<sup>4</sup>Extraction efficiency of residue method = ratio between analytical target [% of TRR] / identified metabolites in the metabolism study [% of TRR]. All identified metabolites contain the common moiety (= analytical target). Based on identified residue containing the common moiety *N*-isopropyl-4-fluoroaniline from the metabolism study being equal to 100%.

### Overall Conclusion:

In this re-registration process new methods based on the HPLC-MS/MS technologies have been proposed for determination of flufenacet residues (determined as the common moiety 4-fluoro-*N*-isopropylaniline) in plant matrices with LOQ equal to 0.01 mg/kg and in animal matrices with LOQs of 0.01 mg/kg (milk), 0.02 mg/kg (liver) and 0.05 mg/kg for eggs, kidney, muscle and fat.

The procedure for extraction of residues from the matrices remains unchanged compared to old methods.

All methods have been recognized as acceptable.

### B.5.1.2.2. Section Toxicology

The method of analysis for feed and other vehicles, body fluids and tissues are specific for the respective toxicity study. Please refer to CA Volume 3 Section B6.



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**B.5.1.2.3. Section Fate and Behaviour**

The analytical methods used in environmental fate studies for the analysis of soil, water, sediment, air and any additional matrices are specific for the respective environmental fate study. Please refer to CA Volume Section B8.

**B.5.1.2.4. Section Ecotoxicology**

The method of analysis in soil, water, sediment, feed or any additional matrices used in support for ecotoxicological studies are specific for the respective ecotoxicological study. Please refer to CA Volume Section B9.

**B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES (CA 4.2)****B.5.2.1. Method for plant and animal matrices**

Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin.

During the EU peer review process the common moiety method 00346 (for plant commodities) and method 00418 (for food of animal origin) were evaluated as data generation and monitoring method and considered acceptable. Due to the physico-chemical properties of flufenacet and its metabolites containing the fluorophenyl-isopropyl amine moiety (e.g. decomposition at higher temperatures) these substances are not suitable for the determination as single substances themselves with GC (*i.e.* without prior derivatisation) and, thus, none of the standard multi-residue methods was suitable for the determination of flufenacet derived residues in plant matrices by GC.

Original Annex CA submission (1997)

|                         |  |
|-------------------------|--|
| Report:                 | B.4.2.1.; KCA 4.2/04; Gould, T.J., Lemke, V.J.; 1995                               |
| Title:                  | An analytical method for the determination of FOE 5043 residues in plant matrices. |
| Report No & Document No | 106406   |
| Dates of work:          | 1995-05-11   |
| GLP                     | GLP  |

|                         |  |
|-------------------------|--|
| Report:                 | B.4.2.1.; KCA 4.2/05; Seym, M.; 1994   |
| Title:                  | Independent laboratory validation of the residue analytical method for FOE 5043 residues in plant. |
| Report No & Document No | 106907<br>RA-352-94  |
| Dates of work:          | 1994-06-24   |
| GLP                     | GLP  |

|                         |   |
|-------------------------|---|
| Report:                 | B.4.2.1; KCA 4.2/01; Seym, M.; 1995   |
| Title:                  | Analytical method for the determination of the total residue of FOE 5043 in plant matrices. |
| Report No & Document No | MR-981/95<br>00346  |
| Dates of work:          | 1995-09-22  |
| GLP                     | GLP   |

|                         |   |
|-------------------------|---|
| Report:                 | B.4.2.2; KCA 4.2/02; Gould, T.J., Lemke, V.J., Zoloty, K.L.; 1995                   |
| Title:                  | An analytical method for the determination of FOE 5043 residues in animal matrices. |
| Report No & Document No | 106773<br>00418   |
| Dates of work:          | 1995-11-17  |
| GLP                     | GLP   |

---

|                            |                                     |
|----------------------------|-------------------------------------|
| Report:                    | B.4.2.2; KCA 4.2/03; Szym, M.; 1995 |
| Title:                     | Modification M001 for eggs.         |
| Report No &<br>Document No | MR-1118/95<br>00418/M001            |
| Dates of work:             | 1995-10-10                          |
| GLP                        | GLP                                 |

|                            |  |
|----------------------------|--|
| Report:                    | B.4.2.2 Bajzik, M.E.; 1995   |
| Title:                     | Independent laboratory validation of the analytical method for the determination of FOE<br>5043 residues in animal matrices. |
| Report No &<br>Document No | 106913   |
| Dates of work:             | 1995-03-22   |
| GLP                        | GLP  |

For all studies submitted and evaluated during the frame of the first Annex CA inclusion please refer to Table 5.2.1-1 below.

**Table 5.2.1-1: Analytical methods for residues of flufenacet in plants and in food of animal origin reviewed during the first EU peer review process of inclusion**

| Method No.                     | Matrix  | Analytes  | Matrix LOQ (mg/kg)   | Technique | Author Doc. No. Report No. Dossier reference  | Reference  |
|--------------------------------|---|---|--|-----------|---|--|
| Plant monitoring method        |   |   |  |           |   |  |
| 00346<br>(cf. Table 5.1.2.1-2) | Wheat, barley, rye<br>- green plant<br>- straw<br>- grain<br><br>Corn:<br>-green material<br>- grain<br><br>sunflower seed<br><br>soya seed   | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4 | 0.05 mg/kg in cereals (wheat, barley, rye):<br>- grain, green material, corn<br>-green material<br>- corn grain<br>sunflower seed<br>soya seed<br><br>0.1 mg/kg in straw   | GC-MS     | Seym, M.; 1995; M-018864-02 [MR-981/95]<br><br>KCA 4.2/01<br><br>Also filed KCA 4.1.2/01                          | Monograph 1997<br><br>Report of ECCO 73, Annex 2, Complete List of Endpoints |
| 00346<br>(cf. Table 5.1.2.1-2) | Corn forage<br>- fodder<br>- grain<br>Soybean forage<br>- seed<br>Spinach tops<br>Wheat grain<br>- straw<br>Sunflower seed<br>Turnip roots<br>Peanut nutmeat<br>Corn oil<br>Soybean soapstock | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4 | 0.05 mg/kg<br>Corn forage<br>Spinach tops<br>Turnip roots<br>Wheat grain<br><br>0.1 mg/kg<br>corn grain, fodder, corn oil, peanut nutmeat<br>soybean seed, forage<br>Soybean soapstock<br>Sunflower seed<br>Wheat grain, straw | GC-MS     | Gould, T. J.; Lemke, V. J.; 1995; M-041601-01<br><br>[report 106406]<br><br>KCA 4.2/04<br>also filed KCA 4.1.2/04 | Monograph 1997   |
| 00346<br>(ILV)                 | Corn forage   | (1)flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide                | Corn forage<br>(0.05 mg/kg)  | GC-MS     | Seym, M.; 1994; M-014828-01 [report 106907]<br><br>KCA 4.2/05   | Monograph 1997   |

| Method No.                                  | Matrix   | Analytes  | Matrix LOQ (mg/kg)   | Technique | Author Doc. No. Report No. Dossier reference  | Reference  |
|---|--|---|--|-----------|---|--|
| Animal monitoring methods                   |  |   |  |           |   |  |
| 00418<br><br>(cf. Table 5.1.2.1-2)          | Milk<br>Bovine liver<br>Bovine kidney<br>Bovine muscle<br>Bovine fat | (1) flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide | Milk (0.01 mg/kg)<br>Bovine liver (0.02 mg/kg),<br>Bovine kidney,<br>Bovine muscle,<br>Bovine fat (0.05 mg/kg) | GC-MS     | Gould, T. J.; et al.; 1995; M-019605-01<br>[report 106773]<br><br>KCA 4.2/02<br>also filed KCA 4.1.2/02 | Monograph 1997<br><br>Report of ECCO 73, Annex 2, Complete List of Endpoints |
| 00418/<br>M001<br><br>(cf. Table 5.1.2.1-2) | Eggs   | (1) flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide mixture of 1-4      | 0.05 mg/kg   | GC-MS     | Seym, M.; 1995; M-019614-01<br>[report MR-1118/95]<br>KCA 4.2/03<br>also filed KCA 4.1.2/03             |  |
| 00418<br>(ILV)                              | Bovine liver   | (1) flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide | 0.05 mg/kg in liver  | GC-MS     | Bajzik, M. E.; 1995; M-071918-01<br>[report 106913]<br><br>KCA 4.2/06                                   |  |

The number in brackets gives the report number which is used in the monograph.

Method no. 00346 and 00418 and 00418/M001 have been evaluated as monitoring and data generation method for plant and animal matrices in the EU peer review process and are also referred to in chapter 5.1.2.

### Conclusion

All methods of analysis provided and accepted in the scope of the original Annex II dossier submission were evaluated under Council Directive 91/414/EEC and fulfilled the data requirements of Annex II of the Directive. The studies were conducted according to GLP principles and comply with EPA Ref: 171-4(c), Residue analytical method -plants and EPA Ref: 171-4(d), Residue analytical method –animal.

The technique used for the determination of flufenacet residues in plant and animal matrices was GC-MS which was based on the conversion of the metabolites to 4-fluoro-*N*-methylethyl benzenamine moiety. The LOQ was 0.05 mg/kg kg for plant matrices and in animal matrices LOQs of 0.01 mg/kg (milk), 0.02 mg/kg (liver) and 0.05 mg/kg for eggs, kidney, muscle and fat were achieved.

These analytical methods could be considered as acceptable during the renewal of flufenacet.

### New data for AIR III

For all studies submitted and evaluated during the frame of the renewal CA inclusion please refer to Table 5.2.1-2 below.

**Table 5.2.1-2: Supplementary analytical methods for residues of flufenacet in plants and in food of animal origin**

| Method No.                       | Matrix  | Analytes  | Matrix LOQ (mg/kg)   | Technique  | Author Doc. No. Report No. Dossier reference  |
|----------------------------------|---|---|--|------------|---|
| Plant monitoring method          |   |   |  |            |   |
| 00346 (ILV)                      | Wheat grain   | (1) flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide   | 0.05 mg/kg   | GC-MS      | Class, T.; 2004; M-072609-01 [report P740G]<br><br>KCA 4.2/14   |
| 00346                            | Apple   | (1) flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide   | 0.01 mg/kg   | GC-MS      | Klimmek, S.; 2005; M-088233-02 [report BAY-0408V]<br><br>KCA 4.2/15   |
| 00346/E002 (cf. Table 5.1.2.1-5) | Soybean plant<br>Tomato fruit   | (1) flufenacet<br>(2) FOE-oxalate<br>(3) FOE sulfonic acid<br>(4) FOE thioglycolate sulfoxide<br>mixture of 1-4   | 0.05 mg/kg   | GC-MS      | Seym, M.; 1998; M-018878-01 [report MR-400/98]<br>KCA 4.2/23<br>also filed<br>KCA 4.1.2/13                              |
| 01179 (cf. Table 5.1.2.1-5)      | Cereal (wheat and barley) straw<br>green material<br>grain                    | (1) flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide<br>(metabolites fortified as mixture (1/1/1)) | 0.01 mg cereal grain and green material<br>0.05 mg/kg cereal straw | HPLC-MS/MS | Class, Th.; Meridian, H.; 2010; M-362716-01 [report B1778G]<br>KCA 4.2/12<br>also filed<br>KCA 4.1.2/12                 |
| 01100 (cf. Table 5.1.2.1-5)      | Orange fruit<br>Dry bean seed<br>Rape seed                                    | (1) flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide<br>(metabolites fortified as mixture (1/1/1)) | 0.01 mg/kg (all matrices)  | HPLC-MS/MS | Billian, P.; 2010; M-362575-02<br><br>[Report MR-08/060]<br>KCA 4.2/11<br>Also filed<br>KCA 4.1.2/18                    |
| 01100/M001 (cf. Table 5.1.2.1-5) | Cereal (wheat) straw<br>green material<br>grain                               | (1) flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide<br>(metabolites fortified as mixture (1/1/1)) | 0.01 mg cereal grain and green material<br>0.05 mg/kg cereal straw | HPLC-MS/MS | Stuke, S., Bauer, J.; Ruhl, S.; 2012; M-433720-01<br><br>[Report MR-11/011]<br>KCA 4.2/10<br>also filed<br>KCA 4.1.2/17 |
| 01100 and 01179 (ILV)            | Cereal green material (foliage)<br>Rape seed<br>Orange fruit<br>Dry bean seed | (1) flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide<br>(metabolites fortified as mixture (1/1/1)) | 0.01 mg (all matrices)   | HPLC-MS/MS | Meyer, M.; 2011; M-405654-01 [Report IF-10/01717126]<br><br>KCA 4.2/17  |

| Method No.                 | Matrix                          | Analytes  | Matrix LOQ (mg/kg)   | Technique | Author Doc. No. Report No. Dossier reference                         |
|----------------------------|---------------------------------|---|--|-----------|--|
| Animal monitoring method   |                                 |   |  |           |  |
| 00418 and 00418/M001 (ILV) | Bovine meat, fat liver milk egg | (1) flufenacet<br>(2) FOE-oxalate hydrate<br>(3) FOE sulfonic acid sodium salt<br>(4) FOE thioglycolate sulfoxide | 0.05 mg/kg meat, fat, egg<br>0.02 mg/kg liver<br>0.01 mg/kg milk | GC-MS     | Klimmek, S.; et al.; 2013; M-461242-01 [Report S12-00052] KCA 4.2/18 |

In addition, the multi-residue QuEChERS method in combination with HPLC-MS/MS, described in the European Standard EN 15662:2008 (CEN, 2008b), is available for the determination of flufenacet in high acid, dry, high sugar and high water commodities. However, this method does not include other metabolites containing the *N*-fluorophenyl-*N*-isopropyl moiety; it is therefore not suitable for enforcement of this substance according to its complete residue definition (EFSA Journal 2012;10(4):2689).

#### B.5.2.1.1 Plant matrices

|                         |  |
|-------------------------|--|
| Report:                 | KCA 4.2/14, Class, T.; 2004; M-072609-01   |
| Title:                  | Independent laboratory validation (ILV) of a common moiety residue method for the determination of Flufenacet (FOE 5043) and 3 metabolites in wheat grain (Bayer CropScience Method 00346) |
| Report No & Document No | P 740 G, dated 2004-05-13<br>M-072609-01-1   |
| Guidelines:             | Council Directive 91/414/EEC<br>EC guidance document on residue analytical methods, SANCO/825/00 rev 6, 20/06/00   |
| GLP                     | Yes; Deviations: none  |

#### Principle of the method:

The independent method validation of Bayer CropScience method no. 00346 (Seym, M.; 1995; M-018864-02) was performed at PTRL Europe, Ulm, Germany. Control samples of wheat grain were fortified individually with either flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt or FOE 5043 thioglycolate sulfoxide at the LOQ (0.05 mg/kg, expressed as flufenacet) and at the 10-fold LOQ (0.5 mg/kg). The samples were analyzed according to Bayer CropScience method no. 00346 with only minor adaptations justified by different laboratory equipment and procedures. No contacts to the developers of the original method were necessary.

The samples were analyzed using capillary gas chromatography with mass selective detection ( $m/z = 207$ ) and with the following working conditions for chromatograph: column Varian VF-5 MS, 30 m x 0.32 mm x 0.24  $\mu$ m film thickness, Split/Splitless injector. The residue is expressed as flufenacet equivalents.

For confirmation, two additional fragment ions are monitored at  $m/z = 138$  and  $m/z = 248$ . The quantitative determination was carried out by external standardization.

#### Specificity

No signals interfering with the determination of the analyte were observed in extracts of untreated blank control samples. All blank values were well below 20% of the LOQ.

One fragment ion with  $m/z$  ratio  $> 100$  ( $m/z = 207$ ) was used for quantitation and two fragment ions with  $m/z$  ratio  $> 100$  were used for confirmation ( $m/z = 249$  and  $m/z = 138$ ). Therefore, this GC-MSD method can be considered as highly specific and the development of additional confirmatory detection techniques are not necessary.

**Repeatability**

As a measure for the precision, the intra-laboratory repeatability (n=5) is given as relative standard deviation (% RSD). The relative standard deviations (RSDs) per analyte and fortification level were < 20%. For details see Table 5.2.1-3 below.

**Linearity**

A linear correlation between the injected amount of the analyte and the detector response was confirmed for 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE 5043 trifluoroacetamide) in solvent by injecting 8 different concentrations covering a range of 10 – 2500 ng/mL ( $r^2 = >0.999$ ). Linearity was established for matrix-matched calibration solutions at 5 concentrations ranging from 10 – 1000 ng/mL (all expressed as flufenacet). Calibration solutions prepared in a final extract from a blank control grain specimen demonstrated that there was no effect of co-extracted matrix components on GC injection or mass spectrometric detection.

**Limit of Quantification (LOQ)**

The limit of quantification was established and validated at 0.05 mg/kg expressed as parent equivalents. No interfering signals in blank control specimens (analyzed as duplicates) were detected resulting in a limit of detection (LOD) of < 0.01 mg/kg (< 20 % of LOQ).

**Recovery rates (accuracy)**

The mean recoveries for flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, and FOE 5043 thioglycolate sulfoxide detected as FOE 5043 trifluoroacetamide were in the range of 70 - 100% when fortified with flufenacet or its metabolites at 0.05 and 0.5 mg/kg (see Table 5.2.1-3).

**Table 5.2.1-3: Recoveries of flufenacet residues in wheat grain**

| Analyte                                   | Fortification-level <sup>(a)</sup><br>[mg/kg] | Recovery rates [%] |    |      |     |     |      |     |
|---|---|--------------------|----|------|-----|-----|------|-----|
|   |   | Individual values  |    |      |     |     | Mean | RSD |
| flufenacet<br>(FOE 5043)                  | 0.05*   | 104                | 85 | 104  | 123 | 86  | 100  | 15  |
|   | 0.5   | 80                 | 80 | 79   | 95  | 101 | 87   | 12  |
|   |   | Overall mean       |    |      |     |     | 94   | 15  |
| FOE 5043-<br>Oxalate<br>Hydrate           | 0.05*   | 100                | 96 | 97   | 97  | 84  | 95   | 6   |
|   | 0.5   | 85                 | 81 | 86   | 72  | 83  | 81   | 7   |
|   |   | Overall mean       |    |      |     |     | 88   | 10  |
| FOE 5043-<br>Sulfonic Acid<br>Sodium Salt | 0.05*   | 76                 | 67 | 46** | 99  | 94  | 87   | 16  |
|   | 0.5   | 80                 | 68 | 76   | 68  | 72  | 73   | 7   |
|   |   | Overall mean       |    |      |     |     | 80   | 15  |
| FOE 5043-<br>Thioglycolate<br>Sulfoxide   | 0.05*   | 68                 | 96 | 99   | 88  | 78  | 86   | 15  |
|   | 0.5   | 64                 | 67 | 68   | 73  | 76  | 70   | 7   |
|   |   | Overall mean       |    |      |     |     | 78   | 16  |

\* LOQ level

\*\*outlier of 46% not included in calculation, possibly portion of extract lost

<sup>(a)</sup> Fortified as Flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, FOE 5043 thioglycolate sulfoxide; detected as FOE 5043 trifluoroacetamide and expressed as flufenacet equivalents

RSD - Relative standard deviation

**Conclusion**

Bayer residue analytical method 00346 for the determination of residues of flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide in plant matrices was successfully independently validated on wheat grain as a representative for cereal grain and other dry materials or matrices of a high starch content.

The above summarized method fulfils the requirements of the Regulation (EC) 1107/2009 detailed in the EC “Guidance document on residue analytical methods” (SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13). The Bayer residue analytical method 00346 is therefore considered to be applicable for enforcement and monitoring purposes.



|                         |  |
|-------------------------|--|
| Report:                 | KCA 4.2/15, Klimmek, S.; 2005; M-088233-02-1   |
| Title:                  | Enforcement Method for the Determination of Residues of FOE 5043, FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide in Materials of Apples (Bayer CropScience Method 00346) |
| Report No & Document No | BAY-0408V, dated 2004-07-29<br>M-088233-02-1   |
| Guidelines:             | Council Directive 91/414/EEC   |
| GLP                     | Yes; Deviations: none  |

The applicability of the method 00346 (Seym, M.; 1995; M-018864-02) for the determination of the residues of Flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide in sour apples (pH 4) as a representative matrix with high acid content was tested.

This validation was performed by Dr. Specht & Partner Chemische Laboratorien GmbH, Hamburg, Germany. In the original report the fortification levels for the metabolites FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide were not expressed as parent equivalents of FOE 5043 (Flufenacet). The final analytical report was therefore amended for recalculation of the used fortification levels as parent equivalents. Furthermore a calculation error was described. This necessitated a re-evaluation of the chromatographic raw data and resulted in changed recovery values for FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide.

#### Principle of the method:

The residues of flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide were oxidized with potassium permanganate for 5 min and hydrolyzed to the common moiety 4-fluoro-isopropylaniline (fluoroaniline) by digesting the crop mixture with 47% sulfuric acid for 24 hours. The fluoroaniline was separated from the crop matrix by steam distillation after making the crop digest with 50 % sodium hydroxide. The aniline was extracted from the steam distillate and derivatized with trifluoroacetic anhydride. A clean-up on a C-18 SPE cartridge followed. The derivate, 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE 5043 trifluoro acetamide), was measured by GC-MSD (EI) with the following working conditions for chromatograph: column DB-5 MS, 30 m x 0.25 mm x 0.25 µm film thickness, injector of splitless mode. The residue is expressed as flufenacet equivalents.

For quantitation molecular ion  $m/z = 249$  was used. For verification the fragment ions  $m/z = 207$  and  $m/z = 138$  were selected.

Control specimens were analysed in duplicate and fortified specimens were analysed in quintuple for each fortification level and individually for each analyte. Fortification experiments were performed at the limit of quantitation (LOQ = 0.01 mg/kg) and ten times that level.

#### Specificity

No residues were detected in any of the corresponding control samples. One fragment ion with an  $m/z$  ratio > 100 was used for quantification ( $m/z = 249$ ) and two fragment ions with an  $m/z$  ratio > 100 were used for confirmation ( $m/z = 207$  and  $m/z = 138$ ). Therefore, this GC-MSD method can be considered as highly specific and the development of additional confirmatory detection techniques are not necessary.

#### Repeatability

Relative standard deviations per fortification level and per analyte were below 20 %. Details are given in Tables 5.2.1-4 to 5.2.1-6 below.

#### Linearity

The linearity of the detector response was confirmed by injecting 6 standard solutions covering the working range of 2.50 - 200 ng/mL FOE 5043 trifluoro acetamide. The correlation coefficient was found to be > 0.999.

#### Limit of quantification (LOQ)

The limit of quantification for flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide was established and validated at 0.01 mg/kg in apple with a limit of detection (LOD) of 0.003 mg/kg.

#### Recovery rates (accuracy)

Mean recoveries for each fortification level and the overall mean recovery were within the range of 70 - 110% with the exception for FOE-oxalate where mean recoveries were slightly below 70% at LOQ level for  $m/z = 249$ .

However, the repeatability is excellent and the two confirmatory fragment ions show comparable results with mean recoveries of 71% and 64%. The mean recovery for FOE-oxalate of 68% at LOQ level is therefore considered to be acceptable. The results are summarised in Tables 5.2.1-4 to 5.2.1-6.

**Table 5.2.1-4: Recoveries of flufenacet residues in sour apple ( $m/z = 249$ )**

| Flufenacet ( $m/z = 249$ )              |   |                    |    |    |    |    |      |     |
|---|---|--------------------|----|----|----|----|------|-----|
| Analyte                                 | Fortification-level <sup>(a)</sup><br>[mg/kg] | Recovery Rates [%] |    |    |    |    |      |     |
|   |   | Individual values  |    |    |    |    | Mean | RSD |
| flufenacet<br>(FOE 5043)                | 0.01*   | 86                 | 70 | 83 | 90 | 77 | 81   | 10  |
|   | 0.1   | 87                 | 91 | 86 | 91 | 98 | 91   | 5   |
|   |   | Overall mean       |    |    |    |    | 86   | 9   |
| FOE 5043-<br>Oxalate                    | 0.015*  | 67                 | 69 | 81 | 64 | 61 | 68   | 11  |
|   | 0.15  | 45**               | 77 | 67 | 71 | 71 | 72   | 6   |
|   |   | Overall mean       |    |    |    |    | 70   | 9   |
| FOE 5043-<br>Sulfonic Acid              | 0.012*  | 65                 | 75 | 77 | 71 | 76 | 73   | 7   |
|   | 0.12  | 63                 | 79 | 81 | 80 | 76 | 76   | 10  |
|   |   | Overall mean       |    |    |    |    | 74   | 8   |
| FOE 5043-<br>Thioglycolate<br>Sulfoxide | 0.012*  | 76                 | 77 | 69 | 65 | 67 | 71   | 8   |
|   | 0.12  | 71                 | 70 | 70 | 75 | 69 | 71   | 3   |
|   |   | Overall mean       |    |    |    |    | 71   | 6   |

\* LOQ level

\*\* discarded as outlier according to applied Grubbs Test

<sup>(a)</sup> Fortified as Flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, FOE 5043 thioglycolate sulfoxide; detected as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE 5043 trifluoroacetamide) and expressed as flufenacet equivalents

RSD - Relative standard deviation

**Table 5.2.1-5: Recoveries of flufenacet residues in sour apple ( $m/z = 138$ )**

| Flufenacet ( $m/z = 138$ )              |   |                    |    |    |    |    |      |     |
|---|---|--------------------|----|----|----|----|------|-----|
| Analyte                                 | Fortification-level <sup>(a)</sup><br>[mg/kg] | Recovery Rates [%] |    |    |    |    |      |     |
|   |   | Individual values  |    |    |    |    | Mean | RSD |
| flufenacet<br>(FOE 5043)                | 0.01*   | 76                 | 73 | 84 | 96 | 76 | 81   | 12  |
|   | 0.1   | 89                 | 92 | 86 | 91 | 98 | 91   | 5   |
|   |   | Overall mean       |    |    |    |    | 86   | 10  |
| FOE 5043-<br>Oxalate                    | 0.015*  | 73                 | 72 | 78 | 71 | 59 | 71   | 10  |
|   | 0.15  | 48**               | 77 | 66 | 71 | 71 | 71   | 6   |
|   |   | Overall mean       |    |    |    |    | 71   | 8   |
| FOE 5043-<br>Sulfonic Acid              | 0.012*  | 64                 | 73 | 76 | 67 | 70 | 70   | 7   |
|   | 0.12  | 64                 | 79 | 80 | 78 | 76 | 75   | 9   |
|   |   | Overall mean       |    |    |    |    | 73   | 8   |
| FOE 5043-<br>Thioglycolate<br>Sulfoxide | 0.012*  | 75                 | 75 | 74 | 67 | 66 | 71   | 6   |
|   | 0.12  | 71                 | 70 | 69 | 74 | 69 | 71   | 2   |
|   |   | Overall mean       |    |    |    |    | 71   | 5   |

\* LOQ level

\*\* discarded as outlier according to applied Grubbs Test

<sup>(a)</sup> Fortified as Flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, FOE 5043 thioglycolate sulfoxide; detected as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE 5043 trifluoroacetamide) and expressed as flufenacet equivalents

RSD - Relative standard deviation

**Table 5.2.1-6: Recoveries of flufenacet residues in sour apple ( $m/z = 207$ )**

| Flufenacet ( $m/z = 207$ )              |   |                    |    |    |    |    |      |     |
|---|---|--------------------|----|----|----|----|------|-----|
| Analyte                                 | Fortification-level <sup>(a)</sup><br>[mg/kg] | Recovery Rates [%] |    |    |    |    |      |     |
|   |   | Individual values  |    |    |    |    | Mean | RSD |
| flufenacet<br>(FOE 5043)                | 0.01*   | 63                 | 71 | 83 | 82 | 66 | 73   | 13  |
|   | 0.1   | 86                 | 92 | 85 | 90 | 98 | 90   | 6   |
|   | Overall mean                                  |                    |    |    |    |    | 82   | 14  |
| FOE 5043-<br>Oxalate                    | 0.015*  | 69                 | 63 | 76 | 62 | 52 | 64   | 14  |
|   | 0.15  | 46**               | 75 | 66 | 71 | 69 | 70   | 4   |
|   | Overall mean                                  |                    |    |    |    |    | 67   | 11  |
| FOE 5043-<br>Sulfonic Acid              | 0.012*  | 55                 | 78 | 76 | 67 | 75 | 70   | 14  |
|   | 0.12  | 64                 | 78 | 80 | 79 | 76 | 75   | 9   |
|   | Overall mean                                  |                    |    |    |    |    | 73   | 11  |
| FOE 5043-<br>Thioglycolate<br>Sulfoxide | 0.012*  | 77                 | 75 | 68 | 71 | 59 | 70   | 10  |
|   | 0.12  | 71                 | 70 | 68 | 74 | 67 | 70   | 4   |
|   | Overall mean                                  |                    |    |    |    |    | 70   | 7   |

\* LOQ level

\*\* discarded as outlier according to applied Grubbs Test

<sup>(a)</sup> Fortified as Flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, FOE 5043 thioglycolate sulfoxide; detected as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE 5043 trifluoroacetamide) and expressed as flufenacet equivalents

RSD - Relative standard deviation

## Conclusion

The Bayer residue analytical method 00346 was considered valid for the determination of residues of flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide in sour apples (reported pH 4) which is considered representative for matrices of high acid content. The method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC “Guidance document on residue analytical methods” (SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

|                         |   |
|-------------------------|---|
| Report:                 | KCA 4.2/23; Seym, M.; 1998; M-018878-01<br>see also point 5.2.1 and KCA 4.1.2/13                                      |
| Title:                  | Supplement E002 of method 00346 for the determination of FOE 5043 total residue in/on soybean, plant and tomato fruit |
| Report No & Document No | MR-400/98, method 00346/E002,<br>M-018878-01-1  |
| Guidelines:             | Fulfil Council Directive 91/414/EEC   |
| GLP                     | Yes; Deviations: none   |

Please refer to chapter B5.1.2.

The method is a supplement to method 00346 (Seym, M.; 1995; M-018864-02)\_ providing validation data for the determination of flufenacet (FOE 5043) and its metabolites (FOE 5043 oxalate, FOE 5043 sulfonic acid, FOE 5043 thioglycolate sulfoxide) for soybean (green plant material) and tomato fruit as additional matrices with high water content. The method is in compliance with the guideline criteria for post registration control.

As an alternative to method 00346 evaluated during the EU peer review using derivatisation and GC determination the following simplified methods may be used as a monitoring methods. They are considered to provide analytical advantage since the derivatisation step of the common chemical fragment (4-fluoro-*N*-isopropylaniline) to be determined by GC as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE 5043 trifluoroacetamide) can be omitted. Instead, the fluoroaniline can be determined directly by LC-MS/MS. Since meanwhile LC-MS/MS is commonly available the methods are considered suitable for monitoring purpose. All methods are reported in detail in chapter B5.1.2 and are listed below for sake of easy reference and completeness.

|                          |  |
|--------------------------|--|
| Report:                  | KCA 4.2/12, Class, Th.; Merdian, H.; 2010; M-362716-01<br>see also point 5.1.2.1 and KCA 4.1.2/19  |
| Title:                   | Validation of BCS analytical method no. 01179 for the determination of residues of flufenacet in/on plant materials by HPLC-MS/MS  |
| Report no & Document No: | Method 01179, report B 1778 G dated 2010-01-22<br>M-362716-01-1  |
| Guidelines:              | <ul style="list-style-type: none"> <li>• Council Directive 91/414/EEC EC Guidance documents on residue analytical methods: SANCO/3029/99 rev. 4, 11/07/00</li> <li>• Guidance document on residue analytical methods; SANCO/825/00 rev. 7</li> <li>• OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13)</li> </ul> |
| GLP                      | Yes; Deviations: none  |

Please refer to chapter B5.1.2

The analytical method 01179 was developed in order to determine the total residue of flufenacet (flufenacet and its metabolites containing the *N*-fluorophenyl-*N*-isopropyl amine moiety) in/on cereal matrices (green material, straw, grain) by LC-MS/MS using matrix matched standards with an LOQ of 0.01 mg/kg for cereal grain and green material and 0.05 mg/kg for straw. The matrices to be analysed are considered to be representative for the matrix groups of high starch content and high water content. In addition straw was validated as a representative for dry matrices. The method may be used as a data collection method as well as for enforcement and monitoring purposes. Green material, grain and straw were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).

The analytical method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/2000, SANCO/825/00 rev. 8.1, 16/11/2010) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

|                          |   |
|--------------------------|---|
| Report:                  | KCA 4.2/11, Billian, P.; 2010; M-362575-02<br>see also point 5.1.2.1 and KCA 4.1.2/18   |
| Title:                   | Analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on plant material  |
| Report no & Document No: | Method 01100, Report MR-08/060 dated 2010-01-27, amended 2010-02-04<br>M-362575-02-1  |
| Guidelines:              | <ul style="list-style-type: none"> <li>• EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC</li> <li>• Guidance document on residue analytical methods; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection</li> <li>• OECD guidance document ENV/JM/Mono(2007)17, 2007-08-13,</li> <li>• Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99</li> </ul> |
| GLP                      | Yes, Deviations: none   |

Please refer to chapter B5.1.2

The analytical method 01100 was validated for the determination of flufenacet residues in/on orange (fruit), dry bean seed and rape seed being representative for the commodity groups of high acid content, high protein content and high fat content with an LOQ of 0.01 mg/kg. Sample materials were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1). The method was validated successfully and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/00, SANCO/825/00 rev. 8.1, 16/11/2010) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

|                          |  |
|--------------------------|--|
| Report:                  | KCA 4.2/10, Stuke, S., Bauer, J.; Ruhl, S.; 2012; M-433720-01<br>see also point 5.1.2 and KCA 4.1.2/17   |
| Title:                   | Modification M001 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg for grain and green material and at a LOQ of 0.05 mg/kg for straw by HPLC-MS/MS   |
| Report no & Document No: | Method 01100/M001, Report MR-11/011 dated 2012-06-13<br>M-433720-01  |
| Guidelines:              | <ul style="list-style-type: none"> <li>• EC Guidance Document SANCO/825/00 rev. 8.1 of November 16, 2010</li> <li>• EC Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO 3029/99</li> <li>• Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances</li> <li>• OECD Guideline, ENV/JM/MONO(2007)17, August 13, 2007</li> <li>• U.S. EPA Guideline, OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods of April 1996</li> <li>• U.S. EPA Guideline, OPPTS 860.1340 Residue Analytical Method of August 1996</li> </ul> |
| GLP                      | Yes, Deviations none   |

Please refer to chapter B5.1.2

The analytical method 01100/M001 was validated for the determination of flufenacet residues in/on cereal grain, straw and green material by LC-MS/MS using matrix matched standards with an LOQ of 0.01 mg/kg for cereal grain and green material and 0.05 mg/kg for straw. The matrices to be analysed are considered to be representative for the matrix groups of high starch content and high water content. In addition straw was validated as a representative for dry matrices. The method provides validation data on cereal matrices in addition to method 01179 with only minor adaptations justified by different laboratory equipment and procedures. Sample materials were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).

The method was validated successfully and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/00, SANCO/825/00 rev. 8.1, 16/11/2010) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

|                          |   |
|--------------------------|---|
| Report:                  | KCA 4.2/17, Meyer, M.; 2011; M-405654-01  |
| Title:                   | Independent Laboratory Validation of the Bayer CropScience Methods 01100 and 01179 for the Determination of Residues of Flufenacet (FOE5043) in/on Plant Materials                              |
| Report no & Document No: | IF-10/01717126 dated 2011-04-14<br>M-405654-01-1  |
| Guidelines:              | <ul style="list-style-type: none"> <li>• EU-guidance document on residue analytical methods. SANCO/825/00-rev 7, 17/03/04;</li> <li>• OECD document ENV/JM/MONO(2007)17, 13-Aug-2007</li> </ul> |
| GLP                      | Yes; Deviations: none   |

### Principle of the ILV

The ILV was performed at SGS Institut Fresenius, Taunusstein, Germany.

Flufenacet and its metabolites FOE5043-sulfonic acid sodium-salt, FOE5043-thioglycolate sulfoxide and FOE5043-oxalate hydrate were determined in fortified specimens of wheat green plant (high water content), oilseed rape seed (high fat content), orange fruit (high acid content) and bean seed (dry and high protein content). The metabolites were fortified as a mixture (1/1/1 as molar equivalents).

The Bayer CropScience methods 01100 (Billian, P.; 2010; M-362575-02 ) and 01179 (Class, Th.; Meridian, H.; 2010; M-362716-01 ) were independently validated for these matrices. Since with the extension M001 to method

01100 the same matrices were validated as for method 01179 the ILV is considered to be applicable. All methods differ only in minor details justified by different laboratory equipment and adaptations. Therefore it is justified to consider them identical for validation purpose.

After oxidation and hydrolysis, residues of flufenacet and metabolites were cleaned-up by distillation followed by a liquid/liquid partition with dichloromethane. Finally residues were dissolved and subjected to LC-MS/MS with the following working conditions for chromatograph: column Phenomenex Luna C18(2) 100A, 3 µm particle size, 150 mm length, 2 mm id with pre-column.

Flufenacet and its metabolites FOE5043-sulfonic acid sodium-salt, FOE5043-thioglycolate sulfoxide and FOE5043-oxalate hydrate were determined as the common moiety 4-fluoro-*N*-isopropylaniline.

The following MRM transitions were used for quantification and confirmation:

4-fluoro-*N*-isopropylaniline:  $m/z = 154 \rightarrow m/z = 112$  (quantification).

$m/z = 154 \rightarrow m/z = 92$  (confirmation).

### Limit of Quantification (LOQ)

The ILV confirmed the LOQ of 0.01 mg/kg (expressed as flufenacet) for flufenacet and its metabolites in all matrices. The LOD was estimated as 30% of the LOQ (0.003 mg/kg).

### Linearity

The correlation between the injected amount of substance and the detector response was linear for standards in matrix for each test item. The correlation coefficients of the 1/x weighted linear regressions over 7 concentrations from 0.05-2.5 ng/mL (0.1 ng/mL orange fruit) (corresponding to 0.002 (0.005 for orange) – 0.12 mg/kg expressed as parent flufenacet) were > 0.99.

### Specificity

The analyte fluoroaniline is monitored using two mass transitions:  $m/z = 154 \rightarrow 112$  and  $m/z = 154 \rightarrow m/z = 92$ . No significant interferences from the specimen matrices were detected at the retention time of interest. Residues in control samples were well below 30% of the respective LOQ level.

### Repeatability

Repeatability was demonstrated by the low standard deviations per fortification level for flufenacet or the metabolite mix in recovery experiments. RSD was below 20% in all matrices and for both mass transitions monitored. Please refer to Tables 5.2.1-7 and 5.2.1-8.

### Confirmatory method

Two MRM transitions were monitored for flufenacet and the metabolite mix:  $m/z = 154 \rightarrow m/z = 112$  and  $m/z = 154 \rightarrow m/z = 92$ . Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

### Recovery rates (accuracy)

For flufenacet and for a mixture of flufenacet metabolites recovery rates were determined at a fortification level of 0.01 mg/kg (= LOQ) and 0.1 mg/kg (= 10-fold LOQ). The average recovery at each fortification level and for each matrix was within the range of 70 – 110%. Recoveries were calculated with the external (matrix matched) standard procedure. Please refer to Table 5.2.1-7 and Table 5.2.1-8.

### Stability of Reference Standard Solutions and in final extracts

The standard solutions of the reference item were found to be stable for at least 2 months, covering their experimental use. The reference item fluoroaniline proved to be stable in final extracts in 2 different matrices for at least 3 weeks.

Table 5.2.1-7: Recoveries for flufenacet residues

| Analyte  | Sample material | Fortification Level*<br>[mg/kg] | Recoveries [%]    |     |     |     |     |      | RSD***<br>[%] | n  |
|--|-----------------|---------------------------------|-------------------|-----|-----|-----|-----|------|---------------|----|
|  |                 |                                 | Individual values |     |     |     |     | Mean |               |    |
| Flufenacet*<br>$m/z = 154 \rightarrow m/z = 112$<br>(quantification) | Wheat Green     | 0.01**                          | 97                | 97  | 101 | 102 | 97  | 99   | 2.3           | 5  |
|  |                 | 0.10                            | 88                | 82  | 85  | 80  | 83  | 84   | 3.5           | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 9.2           | 10 |
|  | Rape Seed       | 0.01**                          | 86                | 80  | 92  | 91  | 90  | 88   | 5.3           | 5  |
|  |                 | 0.10                            | 76                | 65  | 75  | 84  | 75  | 75   | 9.2           | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 10.7          | 10 |
|  | Orange Fruit    | 0.01**                          | 77                | 72  | 90  | 86  | 77  | 80   | 9.2           | 5  |
|  |                 | 0.10                            | 72                | 71  | 71  | 73  | 72  | 72   | 1.8           | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 8.8           | 10 |
|  | Bean Seed       | 0.01**                          | 92                | 84  | 83  | 91  | 89  | 88   | 4.8           | 5  |
|  |                 | 0.10                            | 90                | 92  | 104 | 105 | 96  | 97   | 7.2           | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 7.9           | 10 |
| Flufenacet*<br>$m/z = 154 \rightarrow m/z = 92$<br>(confirmation)    | Wheat Green     | 0.01**                          | 102               | 100 | 96  | 98  | 96  | 99   | 2.6           | 5  |
|  |                 | 0.10                            | 87                | 87  | 81  | 82  | 87  | 85   | 3.7           | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 8.3           | 10 |
|  | Rape Seed       | 0.01**                          | 88                | 81  | 87  | 90  | 87  | 87   | 3.7           | 5  |
|  |                 | 0.10                            | 75                | 64  | 75  | 87  | 77  | 76   | 11            | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 10.0          | 10 |
|  | Orange Fruit    | 0.01**                          | 86                | 80  | 89  | 98  | 82  | 87   | 8.1           | 5  |
|  |                 | 0.10                            | 76                | 74  | 76  | 74  | 73  | 75   | 1.7           | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 9.8           | 10 |
|  | Bean Seed       | 0.01**                          | 80                | 105 | 91  | 103 | 103 | 96   | 11.2          | 5  |
|  |                 | 0.10                            | 92                | 100 | 94  | 101 | 95  | 96   | 4.0           | 5  |
|  | Overall         |                                 |                   |     |     |     |     |      | 7.9           | 10 |

\* Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

\*\* LOQ level

\*\*\* Calculation based on non-rounded values

Table 5.2.1-8: Recoveries for flufenacet and its metabolites residues

| Analyte   | Sample material   | Fortification Level*<br>[mg/kg] | Recoveries<br>[%] |    |    |    |    | RSD***<br>[%] | n   |      |
|---|-------------------|---------------------------------|-------------------|----|----|----|----|---------------|-----|------|
|   |                   |                                 | Individual values |    |    |    |    |               |     | Mean |
| Mix of flufenacet metabolites*<br><br><i>m/z</i> =154 → <i>m/z</i> =112<br><br>(quantification) | Wheat Green Plant | 0.01**                          | 85                | 77 | 92 | 81 | 87 | 85            | 6.9 | 5    |
|   |                   | 0.10                            | 74                | 79 | 75 | 76 | 78 | 76            | 2.5 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 80            | 7.4 | 10   |
|   | Rape Seed         | 0.01**                          | 70                | 75 | 82 | 79 | 81 | 78            | 6.8 | 5    |
|   |                   | 0.10                            | 72                | 81 | 80 | 80 | 82 | 79            | 5.2 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 78            | 5.8 | 10   |
|   | Orange Fruit      | 0.01**                          | 86                | 71 | 87 | 79 | 74 | 79            | 8.8 | 5    |
|   |                   | 0.10                            | 73                | 72 | 73 | 67 | 82 | 73            | 7.4 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 76            | 8.9 | 10   |
|   | Bean Seed (dry)   | 0.01**                          | 75                | 76 | 80 | 75 | 73 | 76            | 3.8 | 5    |
|   |                   | 0.10                            | 81                | 77 | 84 | 82 | 77 | 80            | 3.8 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 78            | 4.6 | 10   |
| Mix of flufenacet metabolites *<br><br><i>m/z</i> =154 → <i>m/z</i> =92<br><br>(confirmation)   | Wheat Green Plant | 0.01**                          | 86                | 78 | 97 | 87 | 91 | 88            | 8.0 | 5    |
|   |                   | 0.10                            | 74                | 78 | 78 | 77 | 78 | 77            | 2.5 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 82            | 9.1 | 10   |
|   | Rape Seed         | 0.01**                          | 75                | 79 | 80 | 74 | 80 | 77            | 3.7 | 5    |
|   |                   | 0.10                            | 72                | 82 | 84 | 75 | 77 | 78            | 6.0 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 78            | 4.7 | 10   |
|   | Orange Fruit      | 0.01**                          | 80                | 74 | 90 | 84 | 73 | 80            | 8.5 | 5    |
|   |                   | 0.10                            | 71                | 71 | 71 | 64 | 80 | 71            | 8.0 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 76            | 9.9 | 10   |
|   | Bean Seed (dry)   | 0.01**                          | 78                | 81 | 90 | 83 | 78 | 82            | 6.0 | 5    |
|   |                   | 0.10                            | 83                | 83 | 90 | 84 | 81 | 84            | 4.1 | 5    |
|   | Overall           |                                 |                   |    |    |    |    | 83            | 5.1 | 10   |

\* Fortified as flufenacet, determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

\*\* LOQ level

\*\*\* Calculation based on non-rounded values

## Conclusion

Analytical methods 01179 and 01100 were successfully validated by an independent laboratory validation (ILV) in wheat green plant, rape seed, orange fruit and dry bean seed with an LOQ of 0.01 mg/kg. The independent laboratory validation is considered to be applicable to extension M001 which uses the same methodology. The ILV fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC “Guidance document on residue analytical methods” (SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

## NOTE:

In the meantime the residue definition for monitoring was established as Flufenacet (sum of all compounds containing the *N*-fluorophenyl-*N*-isopropyl moiety expressed as flufenacet equivalent) with Regulation (EU) 1127/2014 following the EFSA review of existing MRLs (EFSA, 2012).

The following description is given for information only to illustrate as discussed during of setting the new MRL definition:



|              |  |
|--------------|--|
| Report:      | KCA 4.2/20, Weile, M.; 2013; M-457898-01   |
| Title:       | Flufenacet : Evaluation of EFSA's recommendation to develop a multi residue method for enforcement purpose as replacement of the single common moiety method |
| Document No: | M-457898-01-1  |
| Guidelines:  | Not applicable (position paper)  |
| GLP          | Not applicable (position paper)  |

*'In the Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) no. 396/2005 (EFSA Journal 2012;10(4):2689) EFSA noted that the residue definition for enforcement purposes in food of plant origin might not be the most adequate as it requires the use of a single residue method hydrolyzing all metabolites to the common moiety trifluoroacetamide.*

*"Since the levels of the main metabolites vary widely (depending on the vegetation period, crop group and plant part analysed) it is not possible to derive a less complex residue definition for enforcement purposes. EFSA therefore considers it essential to include the four main metabolites, seen in the studies on maize, cotton and soya bean, together with the two main metabolites, seen in the study on potato, in the residue definition for enforcement (p.14).*

*"EFSA therefore recommends **investigating the possibility** of including the following metabolites in a multi-residue method: flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide, flufenacet sulfinyl lactic acid glucoside, flufenacet cysteine conjugate, and flufenacet sulfanyl lactic acid glucoside."(p.3 and 33)*

EFSA states: *"If it is possible and desirable, from a cost and efficiency perspective, to include the five (author's note: six) metabolites in a multi residue method the residue definition for enforcement could be modified in the future, if it is not then the current residue definition will need to be maintained. It is noted however that modifying the enforcement residue definition will not generate new data requirements with regard to the residue trials. Available residue trials were performed with the common moiety method which includes the five (six) metabolites and overestimation of residues is not expected because all other metabolites containing this moiety are only present in minor amounts."(p.15)*

*The position paper outlines the evolution of method 00346. Since HPLC determination has become more and more available method 00346 was modified by omission of the derivatisation step and by replacement of GC-MS determination by LC-MS/MS in order to facilitate the analytical work (method nos. 01100, 01100/M001 and 01179). The new methods are in accordance with all guideline criteria – as described above – and are independently validated. The methods are suitable to monitor residues of flufenacet as the sum of metabolites containing the fluorophenyl-isopropyl amine moiety. The evolution of the established method is considered to provide a good progress in analysis of flufenacet residues.*

*The following paragraph summarises the rationale given in the position paper for maintaining the established residue definition.*

- In order to determine any misuse it may be considered to include the parent substance in a multi method. Due to the fact that the parent compound has a comparably low, but the metabolites have relatively high polarities the discrimination with one HPLC column would be difficult. The separation efficiency has to be optimized to separate the different polar metabolites.*
- Implementation of a large number of individual metabolites of high polarity into a multi method carries the risk of interferences which is particularly evident for residue levels very close to the LOQ. For plant commodities treated with flufenacet, individual residue levels for the metabolites can be expected below the LOQ.*
- The majority of the metabolites under discussion including conjugates are not commonly available and high efforts in synthesis and corresponding costs can be anticipated to provide monitoring laboratories with the respective standards.*
- The default MRL for plant matrices or the MRLs for all commodities (except cereals and potatoes) for which authorisations are granted is proposed to be set at the LOQ level of 0.05 mg/kg in the Reasoned Opinion (based on the common moiety). In order to comply with this level, the individual*

LOQs for all analytes would need to be at 0.006 mg/kg (expressed as parent equivalent) or 0.004 mg/kg for the metabolite with the lowest molecular weight (oxalate) when expressed as metabolite. This can be considered as very challenging for the analytical routine work, particularly in a multi-residue method. Alternatively, all granted MRLs for different crops currently set at the LOQ would need to be elevated.

- The residue definition for enforcement is the same in the US, Japan and Europe. Since for all authorized uses (except wheat, barley and potatoes in the EU) tolerances/MRLs are currently set at the LOQ (0.05 mg/kg) modification of the residue definition and the EU MRLs may have an impact on international trade.

For monitoring purpose, the benefit of the inclusion of all the individual metabolites into a multi method and a subsequent need for revision of the residue definition is considered questionable since such a proposal is not in line with the 'marker compound' concept. The high number of individual metabolites is not considered suitable for routine monitoring.

The methodology in the new methods using direct LC-MS/MS for detection of the common fragment N-fluorophenyl-N-isopropyl amine is considered to provide good progress and a more suitable approach for monitoring flufenacet and thus it is proposed to maintain the established residue definition for enforcement'.

#### NOTE:

The applicant has submitted a presentation held by B. Dujardin (member of the EFSA Pesticide Unit) outlining the EFSA view possible solutions for simplifying complex residue definition for enforcement purpose and in particular on the flufenacet case which is discussed as a case study within the presentation.

|              |  |
|--------------|--|
| Report:      | KCA 4.2/21, Dujardin, B.; 2013; M-459903-01-1                                |
| Title:       | Potential and possible solutions for simplifying complex residue definitions |
| Document No: | M-459903-01-1  |
| Guidelines:  | Not applicable (presentation)  |
| GLP          | Not applicable (presentation)  |

'In presentations held at the 9<sup>th</sup> European Pesticide Residue Workshop in Vienna (Austria) on 27-June-2012 and at the 7<sup>th</sup> International Fresenius Conference (Düsseldorf, 16 May 2013) a representative of the EFSA Pesticide Unit outlined EFSA's role and view relative to setting enforcement residue definitions. Since flufenacet is included in the presentation as a case study this reference is considered to provide valuable information and is reported below.

In principle, EFSA intends to contribute to a simplification of the enforcement residue definitions. The review of existing MRLs according to Art 12 of Regulation (EC) 396/2005 is considered to provide the best opportunity to review residue definitions because it takes into account all relevant commodities. The criteria to derive a residue definition for enforcement are - beside presence of a suitable marker compound for the total residue (if possible) – appropriate validation of the methods and compliance with data requirements and guidance documents in place. It is noted that the guidance document on pesticide residue analytical methods (SANCO/825/00 rev. 8.1) does not exclude the acceptability of hydrolytic steps and common moiety methods.

In case study for flufenacet, it is concluded that based on the metabolite pattern in plants the complex residue definition based on the N-fluorophenyl-N-isopropyl amine moiety is needed. The marker concept would not be an appropriate solution for deriving a residue method for enforcement of flufenacet residues, instead the common moiety approach is considered to be more appropriate in this case and thus, a common moiety method has to be maintained.

In order to enforce compounds with a complex residue definition it is proposed to investigate the possibility to include some standard modules for hydrolysis of complex residues in multi-residue methods which however requires a long-term perspective'.

**RMS comments:**

In the EFSA Reasoned Opinion on the review of existing MRLs (Art. 12) [EFSA Journal 2012;10(4):2689] it was suggested to explore the possibility to simplify the residue definition by direct determination of the parent compound and six metabolites. Finally this was not considered as a beneficial option and it was concluded that the complex residue definition based on the *N*-fluorophenyl-*N*-isopropyl moiety is needed for flufenacet and thus the residue definition remained unchanged as laid down in Regulation (EC) 1127/2014. The determination of flufenacet related residues (flufenacet and all metabolites containing the *N*-fluorophenyl-*N*-isopropyl moiety) as a common moiety either as trifluoroacetamide (after derivatisation) by means of GC-MS or as 4-fluoro-*N*-isopropylaniline (without derivatisation by means of HPLC-MS/MS) does not impact the residue definition. All metabolites containing the common *N*-fluorophenyl-*N*-isopropyl moiety are captured with both methodologies. With the HPLC-MS/MS detection the simplification consists of omission of the derivatisation step, only. Therefore the introduction of a conversion factor to accommodate for a risk assessment residue definition is not necessary.

**B.5.2.1.2 Animal matrices**

|                         |  |
|-------------------------|--|
| Report:                 | KCA 4.2/18, Klimmek, S.; et al.; 2013; M-461242-01   |
| Title:                  | Validation of the Bayer methods 00418 (M-019605-01-1) and 00418/M001 (M-019614-01-1) for the determination of residues of flufenacet (FOE 5043) and its metabolites in animal tissues and animal products  |
| Report No & Document No | S12-00052<br>M-461242-01-1   |
| Guidelines:             | <ul style="list-style-type: none"> <li>Regulation (EC) No 1107/2009 repealing Council Directives 79/117/EEC and 91/414/EEC</li> <li>Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1</li> <li>US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method</li> </ul> |
| GLP                     | Yes; Deviations: none  |

Bayer method 00418 (Gould, T. J.; et al.; 1995; M-019605-01), the extension for eggs 00418/M001 (Seym, M.; 1995; M-019614-01) and the independent validation (Bajzik, M. E.; 1995; M-071918-01) were evaluated during the EU peer review process.

Since the initial ILV was validated for bovine liver only a complete set of validation data was generated for all relevant animal tissues, milk and eggs. The validation work was performed by Eurofins AgroScience Service, Hamburg, Germany.

Validation was performed using flufenacet, FOE 5043 oxalate, FOE 5043 sulfonic acid and FOE 5043 thioglycolate sulfoxide as relevant metabolites containing the common moiety. The extraction from the matrix was performed according to the Bayer analytical method for the determination of the total residues of flufenacet in commodities of animal origin (method no. 00418 and 00418/M001).

**Principle of the method**

The method follows the same methodology as the plant method 00346. The residues of flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide were extracted from the matrices under acidic and oxidative conditions. After steam distillation and clean-up by liquid / liquid partition an aliquot was derivatized with trifluoroacetic anhydride. The derivate (FOE5043 trifluoroacetamide) was cleaned-up on a C-18 SPE cartridge and subjected to GC-MSD with the following working conditions for chromatograph: Ultra 1 Methyl Siloxane, 12 m x 200 µm x 0.33 µm injector splitless mode, retention time approx. 6.2 min.

For quantitation molecular ion  $m/z = 249$  was used. For verification the fragment ions  $m/z = 207$  and  $m/z = 138$  were selected.

Control specimens were analysed in duplicate per analyte and matrix. Fortified specimens were analysed with  $n = 8 - 12$  for each fortification level. Fortification experiments were performed at the limit of quantitation (LOQ = 0.01 mg/kg for milk, 0.02 mg/kg for liver and 0.05 mg/kg for bovine meat, fat and eggs) and ten times that level.

**Specificity**

Apparent residues in control samples were below  $0.3 \times \text{LOQ}$ . No interference peaks were observed at the retention time of the analyte. Three fragments ( $m/z > 100$ ) were evaluated for each analyte and sample. Therefore, the GC-MSD method is highly specific and an additional confirmatory method is not necessary.

**Repeatability**

Relative standard deviations for  $n = 8-12$  were  $< 20\%$  for fortification levels  $\leq 0.1$  mg/kg and  $< 15\%$  for fortifications above that concentration. Details are given in Tables 5.2.1.2-1 to 5.2.1.2-5 below.

**Linearity**

The correlation between the injected amount FOE5043 trifluoroacetamide and the detector response was linear for solvent standards ranging from 7.2 ng/mL to 4500 ng/mL (calculated as flufenacet) corresponding to 0.0072 – 4.5 mg/kg for egg, milk, fat and liver and 0.0029 – 1.8 mg/kg for meat. The linearity curve was established with 11 concentrations. The coefficients of determination ( $R^2$ ) were  $> 0.99$  for the three fragment ions.

**Matrix effects**

Matrix effects were tested for each matrix for the three selected fragment ions by comparing the peak areas of matrix-matched standards with solvent standards. Matrix effects of  $< 20\%$  were measured for all matrices and considered to be not significant. Therefore solvent standards were used for calibration and quantification.

**Limit of quantification (LOQ)**

The limit of quantification for flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide was established and validated at 0.05 mg/kg in meat, egg and fat, 0.02 mg/kg in liver and 0.01 mg/kg in milk. The limit of determination (LOD) was estimated to be 30 % of the LOQ.

**Recovery rates (accuracy)**

Recovery rates were determined at fortification levels of 0.01 mg/kg (=LOQ level) and at 0.10 mg/kg for milk, 0.02 mg/kg (=LOQ level) and at 0.20 mg/kg for liver and 0.05 mg/kg (=LOQ level) and at 0.50 mg/kg for meat, fat and egg. Recovery experiments were conducted by separate fortification of untreated control samples with defined amounts of Flufenacet (FOE5043), FOE5043 oxalate hydrate, FOE5043 sulfonic acid sodium salt and FOE5043 thioglycolate sulfoxide prior to analysis.

Since all analytes are converted to the common moiety FOE5043 trifluoroacetamide recovery data from the parent compound and the individual metabolites are combined to form a full data set for calculation of the overall mean recovery per fortification level and commodity and the relative standard deviation (RSD). Results are presented in the following Tables 5.2.1.2-1 to 5.2.1.2-5.

Recovery rates per fortification level and matrix were in the range of 70-110% for all fortification levels and for the three fragment ions investigated.

**Confirmatory method**

The results of the method validation were confirmed using a second and a third fragment ion for confirmation. Results of the confirmatory method are also shown in Tables 5.2.1.2-1 to 5.2.1.2-5.

Results of the confirmation procedure showed that the mean recoveries and relative standard deviations for each analyte and each fortification level were within the acceptable range of 70-110 % with  $\text{RSD} < 20\%$ . No additional confirmatory method is required.

**Stability of Analytes**

The stability in final extracts was checked for the tested sample material over a period of at least ten days. FOE5043 trifluoroacetamide was found to be stable in final extracts for at least 10 days in eggs, 18 days in liver, 28 days in fat and 34 days in milk and meat when stored at 3-8 °C in the dark.

FOE 5043 trifluoroacetamide in solvent standards was found to be stable up to 6 months.

**Table 5.2.1.2-1: Recoveries for Flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid sodium salt and FOE5043 thioglycolate sulfoxide in fat**

| Matrix                            | Fortified with                 | Forti-<br>fication<br>Level <sup>a</sup> | Recoveries <sup>b</sup> |             |            |     | No.<br>of<br>Analyses |
|-----------------------------------|--------------------------------|--|-------------------------|-------------|------------|-----|-----------------------|
|                                   |                                | [mg/kg]                                  | Single<br>Values [%]    | Mean<br>[%] | RSD<br>[%] |     |                       |
| <i>m/z</i> = 249 (quantification) |                                |  |                         |             |            |     |                       |
| Fat                               | Flufenacet                     | 0.050*                                   | 93                      | 89          | 91         | -   | 2                     |
|                                   | FOE thioglycolate sulfoxide    |  | 83                      | 79          | 81         | -   | 2                     |
|                                   | FOE sulfonic acid              |  | 99                      | 65          | 82         | -   | 2                     |
|                                   | FOE oxalate                    |  | 109                     | 117         | 113        | -   | 2                     |
|                                   | Mean over all substances: 92 % |  |                         |             | RSD: 18 %  | n=8 |                       |
| Fat                               | Flufenacet                     | 0.50                                     | 90                      | 84          | 87         | -   | 2                     |
|                                   | FOE thioglycolate sulfoxide    |  | 62                      | 69          | 66         | -   | 2                     |
|                                   | FOE sulfonic acid              |  | 83                      | 84          | 84         | -   | 2                     |
|                                   | FOE oxalate                    |  | 99                      | 93          | 96         | -   | 2                     |
|                                   | Mean over all substances: 83 % |  |                         |             | RSD: 15 %  | n=8 |                       |
| <i>m/z</i> = 207 (confirmation)   |                                |  |                         |             |            |     |                       |
| Fat                               | Flufenacet                     | 0.050*                                   | 97                      | 92          | 95         | -   | 2                     |
|                                   | FOE thioglycolate sulfoxide    |  | 76                      | 74          | 75         | -   | 2                     |
|                                   | FOE sulfonic acid              |  | 101                     | 69          | 85         | -   | 2                     |
|                                   | FOE oxalate                    |  | 113                     | 119         | 116        | -   | 2                     |
|                                   | Mean over all substances: 93 % |  |                         |             | RSD: 20 %  | n=8 |                       |
| Fat                               | Flufenacet                     | 0.50                                     | 90                      | 85          | 88         | -   | 2                     |
|                                   | FOE thioglycolate sulfoxide    |  | 63                      | 71          | 67         | -   | 2                     |
|                                   | FOE sulfonic acid              |  | 82                      | 85          | 84         | -   | 2                     |
|                                   | FOE oxalate                    |  | 97                      | 91          | 94         | -   | 2                     |
|                                   | Mean over all substances: 83 % |  |                         |             | RSD: 13 %  | n=8 |                       |
| <i>m/z</i> = 138 (confirmation)   |                                |  |                         |             |            |     |                       |
| Fat                               | Flufenacet                     | 0.050*                                   | 92                      | 92          | 92         | -   | 2                     |
|                                   | FOE thioglycolate sulfoxide    |  | 75                      | 78          | 77         | -   | 2                     |
|                                   | FOE sulfonic acid              |  | 100                     | 69          | 85         | -   | 2                     |
|                                   | FOE oxalate                    |  | 111                     | 119         | 115        | -   | 2                     |
|                                   | Mean over all substances: 92 % |  |                         |             | RSD: 19 %  | n=8 |                       |
| Fat                               | Flufenacet                     | 0.50                                     | 91                      | 84          | 88         | -   | 2                     |
|                                   | FOE thioglycolate sulfoxide    |  | 58                      | 73          | 66         | -   | 2                     |
|                                   | FOE sulfonic acid              |  | 82                      | 85          | 84         | -   | 2                     |
|                                   | FOE oxalate                    |  | 97                      | 95          | 96         | -   | 2                     |
|                                   | Mean over all substances: 83 % |  |                         |             | RSD: 15 %  | n=8 |                       |

\*LOQ level

<sup>a</sup> Fortification levels are expressed as Flufenacet equivalents<sup>b</sup> Determination as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE5043 trifluoroacetamide), calculated and expressed as flufenacet.

**Table 5.2.1.2-2: Recoveries for Flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid sodium salt and FOE5043 thioglycolate sulfoxide in egg**

| Matrix                            | Fortified with                 | Forti-<br>fication<br>Level <sup>a</sup> | Recoveries <sup>b</sup> |             |            | No.<br>of<br>Analyses |     |
|-----------------------------------|--------------------------------|--|-------------------------|-------------|------------|-----------------------|-----|
|                                   |                                | [mg/kg]                                  | Single<br>Values [%]    | Mean<br>[%] | RSD<br>[%] |                       |     |
| <i>m/z</i> = 249 (quantification) |                                |  |                         |             |            |                       |     |
| Egg                               | Flufenacet                     | 0.050*                                   | 85                      | 72          | 79         | -                     | 2   |
|                                   | FOE thioglycolate sulfoxide    |  | 63                      | 65          | 64         | -                     | 2   |
|                                   | FOE sulfonic acid              |  | 61                      | 58          | 60         | -                     | 2   |
|                                   | FOE oxalate                    |  | 87                      | 84          | 86         | -                     | 2   |
|                                   | Mean over all substances: 72 % |  |                         |             | RSD: 17 %  |                       | n=8 |
| Egg                               | Flufenacet                     | 0.50                                     | 89                      | 98          | 94         | -                     | 2   |
|                                   | FOE thioglycolate sulfoxide    |  | 74                      | 72          | 73         | -                     | 2   |
|                                   | FOE sulfonic acid              |  | 90                      | 100         | 95         | -                     | 2   |
|                                   | FOE oxalate                    |  | 81                      | 67          | 74         | -                     | 2   |
|                                   | Mean over all substances: 84 % |  |                         |             | RSD: 15 %  |                       | n=8 |
| <i>m/z</i> = 207 (confirmation)   |                                |  |                         |             |            |                       |     |
| Egg                               | Flufenacet                     | 0.050*                                   | 85                      | 72          | 79         | -                     | 2   |
|                                   | FOE thioglycolate sulfoxide    |  | 63                      | 65          | 64         | -                     | 2   |
|                                   | FOE sulfonic acid              |  | 62                      | 60          | 61         | -                     | 2   |
|                                   | FOE oxalate                    |  | 87                      | 90          | 89         | -                     | 2   |
|                                   | Mean over all substances: 73 % |  |                         |             | RSD: 17 %  |                       | n=8 |
| Egg                               | Flufenacet                     | 0.50                                     | 88                      | 104         | 96         | -                     | 2   |
|                                   | FOE thioglycolate sulfoxide    |  | 73                      | 72          | 73         | -                     | 2   |
|                                   | FOE sulfonic acid              |  | 89                      | 98          | 94         | -                     | 2   |
|                                   | FOE oxalate                    |  | 80                      | 68          | 74         | -                     | 2   |
|                                   | Mean over all substances: 84 % |  |                         |             | RSD: 15 %  |                       | n=8 |
| <i>m/z</i> = 138 (confirmation)   |                                |  |                         |             |            |                       |     |
| Egg                               | Flufenacet                     | 0.050*                                   | 88                      | 73          | 81         | -                     | 2   |
|                                   | FOE thioglycolate sulfoxide    |  | 68                      | 69          | 69         | -                     | 2   |
|                                   | FOE sulfonic acid              |  | 60                      | 62          | 61         | -                     | 2   |
|                                   | FOE oxalate                    |  | 88                      | 87          | 88         | -                     | 2   |
|                                   | Mean over all substances: 74 % |  |                         |             | RSD: 16 %  |                       | n=8 |
| Egg                               | Flufenacet                     | 0.50                                     | 88                      | 101         | 95         | -                     | 2   |
|                                   | FOE thioglycolate sulfoxide    |  | 73                      | 71          | 72         | -                     | 2   |
|                                   | FOE sulfonic acid              |  | 90                      | 99          | 95         | -                     | 2   |
|                                   | FOE oxalate                    |  | 80                      | 67          | 74         | -                     | 2   |
|                                   | Mean over all substances: 84 % |  |                         |             | RSD: 15 %  |                       | n=8 |

\*LOQ level

<sup>a</sup> Fortification levels are expressed as Flufenacet equivalents<sup>b</sup> Determination as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE5043 trifluoroacetamide), calculated and expressed as flufenacet.

**Table 5.2.1.2-3: Recoveries for Flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid sodium salt and FOE5043 thioglycolate sulfoxide in meat**

| Matrix                     | Fortified with                 | Forti-<br>fication<br>Level <sup>a</sup> | Recoveries <sup>b</sup> |             |            |   | No.<br>of<br>Analyses |
|----------------------------|--------------------------------|--|-------------------------|-------------|------------|---|-----------------------|
|                            |                                | [mg/kg]                                  | Single<br>Values [%]    | Mean<br>[%] | RSD<br>[%] |   |                       |
| m/z = 249 (quantification) |                                |  |                         |             |            |   |                       |
| Meat                       | Flufenacet                     | 0.050                                    | 73                      | 82          | 78         | - | 2                     |
|                            | FOE thioglycolate sulfoxide    |  | 70                      | 69          | 70         | - | 2                     |
|                            | FOE sulfonic acid              |  | 86                      | 78          | 82         | - | 2                     |
|                            | FOE oxalate                    |  | 90                      | 90          | 90         | - | 2                     |
|                            | Mean over all substances: 80 % |  |                         |             | RSD: 11 %  |   | n=8                   |
| Meat                       | Flufenacet                     | 0.50                                     | 82                      | 64          | 73         | - | 2                     |
|                            | FOE thioglycolate sulfoxide    |  | 73                      | 69          | 71         | - | 2                     |
|                            | FOE sulfonic acid              |  | 61                      | 60          | 61         | - | 2                     |
|                            | FOE oxalate                    |  | 86                      | 79          | 83         | - | 2                     |
|                            | Mean over all substances: 72 % |  |                         |             | RSD: 14 %  |   | n=8                   |
| m/z = 207 (confirmation)   |                                |  |                         |             |            |   |                       |
| Meat                       | Flufenacet                     | 0.050*                                   | 73                      | 82          | 78         | - | 2                     |
|                            | FOE thioglycolate sulfoxide    |  | 72                      | 70          | 71         | - | 2                     |
|                            | FOE sulfonic acid              |  | 85                      | 82          | 84         | - | 2                     |
|                            | FOE oxalate                    |  | 91                      | 90          | 91         | - | 2                     |
|                            | Mean over all substances: 81 % |  |                         |             | RSD: 10 %  |   | n=8                   |
| Meat                       | Flufenacet                     | 0.50                                     | 82                      | 64          | 73         | - | 2                     |
|                            | FOE thioglycolate sulfoxide    |  | 74                      | 69          | 72         | - | 2                     |
|                            | FOE sulfonic acid              |  | 64                      | 60          | 62         | - | 2                     |
|                            | FOE oxalate                    |  | 85                      | 78          | 82         | - | 2                     |
|                            | Mean over all substances: 72 % |  |                         |             | RSD: 13 %  |   | n=8                   |
| m/z = 138 (confirmation)   |                                |  |                         |             |            |   |                       |
| Meat                       | Flufenacet                     | 0.050*                                   | 78                      | 89          | 84         | - | 2                     |
|                            | FOE thioglycolate sulfoxide    |  | 69                      | 70          | 70         | - | 2                     |
|                            | FOE sulfonic acid              |  | 82                      | 82          | 82         | - | 2                     |
|                            | FOE oxalate                    |  | 91                      | 92          | 92         | - | 2                     |
|                            | Mean over all substances: 82 % |  |                         |             | RSD: 11 %  |   | n=8                   |
| Meat                       | Flufenacet                     | 0.50                                     | 83                      | 64          | 74         | - | 2                     |
|                            | FOE thioglycolate sulfoxide    |  | 74                      | 69          | 72         | - | 2                     |
|                            | FOE sulfonic acid              |  | 62                      | 60          | 61         | - | 2                     |
|                            | FOE oxalate                    |  | 86                      | 78          | 82         | - | 2                     |
|                            | Mean over all substances: 72 % |  |                         |             | RSD: 14 %  |   | n=8                   |

\*LOQ level

<sup>a</sup> Fortification levels are expressed as Flufenacet equivalents<sup>b</sup> Determination as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE5043 trifluoroacetamide), calculated and expressed as flufenacet.

**Table 5.2.1.2-4: Recoveries for Flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid sodium salt and FOE5043 thioglycolate sulfoxide in liver**

| Matrix                     | Fortified<br>with              | Forti-<br>fication<br>Level <sup>a</sup><br><br>[mg/kg] | Recoveries <sup>b</sup> |     |             |            |      | No.<br>of<br>Analyses |
|----------------------------|--------------------------------|---|-------------------------|-----|-------------|------------|------|-----------------------|
|                            |                                |   | Single<br>Values<br>[%] |     | Mean<br>[%] | RSD<br>[%] |      |                       |
| m/z = 249 (quantification) |                                |   |                         |     |             |            |      |                       |
| Liver                      | Flufenacet                     | 0.020*  | 86                      | 83  | 76          | 82         | 6.3  | 3                     |
|                            | FOE thioglycolate sulfoxide    |   | 79                      | 88  | 79          | 82         | 6.3  | 3                     |
|                            | FOE sulfonic acid              |   | 86                      | 84  | 85          | 85         | 1.2  | 3                     |
|                            | FOE oxalate                    |   | 88                      | 90  | 87          | 88         | 1.7  | 3                     |
|                            | Mean over all substances: 84 % |   |                         |     |             | RSD: 5.1 % | n=12 |                       |
| Liver                      | Flufenacet                     | 0.20  | 93                      | 75  | 96          | 88         | 13   | 3                     |
|                            | FOE thioglycolate sulfoxide    |   | 77                      | 75  | 67          | 73         | 7.2  | 3                     |
|                            | FOE sulfonic acid              |   | 92                      | 95  | 85          | 91         | 5.7  | 3                     |
|                            | FOE oxalate                    |   | 67                      | 70  | 70          | 69         | 2.5  | 3                     |
|                            | Mean over all substances: 80 % |   |                         |     |             | RSD: 14 %  | n=12 |                       |
| m/z = 207 (confirmation)   |                                |   |                         |     |             |            |      |                       |
| Liver                      | Flufenacet                     | 0.020*  | 85                      | 82  | 76          | 81         | 5.7  | 3                     |
|                            | FOE thioglycolate sulfoxide    |   | 86                      | 103 | 87          | 92         | 10   | 3                     |
|                            | FOE sulfonic acid              |   | 84                      | 80  | 82          | 82         | 2.4  | 3                     |
|                            | FOE oxalate                    |   | 88                      | 89  | 88          | 88         | 0.65 | 3                     |
|                            | Mean over all substances: 86 % |   |                         |     |             | RSD: 7.7 % | n=12 |                       |
| Liver                      | Flufenacet                     | 0.20  | 93                      | 74  | 96          | 88         | 14   | 3                     |
|                            | FOE thioglycolate sulfoxide    |   | 83                      | 79  | 73          | 78         | 6.4  | 3                     |
|                            | FOE sulfonic acid              |   | 92                      | 97  | 85          | 91         | 6.6  | 3                     |
|                            | FOE oxalate                    |   | 67                      | 69  | 69          | 68         | 1.7  | 3                     |
|                            | Mean over all substances: 81 % |   |                         |     |             | RSD: 14 %  | n=12 |                       |
| m/z = 138 (confirmation)   |                                |   |                         |     |             |            |      |                       |
| Liver                      | Flufenacet                     | 0.020*  | 99                      | 91  | 80          | 90         | 11   | 3                     |
|                            | FOE thioglycolate sulfoxide    |   | 95                      | 84  | 97          | 92         | 7.6  | 3                     |
|                            | FOE sulfonic acid              |   | 97                      | 91  | 99          | 96         | 4.4  | 3                     |
|                            | FOE oxalate                    |   | 109                     | 114 | 108         | 110        | 2.9  | 3                     |
|                            | Mean over all substances: 97 % |   |                         |     |             | RSD: 10 %  | n=12 |                       |
| Liver                      | Flufenacet                     | 0.20  | 90                      | 75  | 99          | 88         | 14   | 3                     |
|                            | FOE thioglycolate sulfoxide    |   | 71                      | 69  | 70          | 70         | 1.4  | 3                     |
|                            | FOE sulfonic acid              |   | 96                      | 100 | 88          | 95         | 6.5  | 3                     |
|                            | FOE oxalate                    |   | 71                      | 73  | 72          | 72         | 1.4  | 3                     |
|                            | Mean over all substances: 81 % |   |                         |     |             | RSD: 15 %  | n=12 |                       |

\*LOQ level

<sup>a</sup> Fortification levels are expressed as Flufenacet equivalents<sup>b</sup> Determination as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE5043 trifluoroacetamide), calculated and expressed as flufenacet.



**Table 5.2.1.2-5: Recoveries for flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid sodium salt and FOE5043 thioglycolate sulfoxide in milk**

| Matrix                            | Fortified with              | Forti-<br>fication<br>Level <sup>a</sup><br>[mg/kg] | Recoveries <sup>b</sup> |             |            | No.<br>of<br>Analyses |   |
|-----------------------------------|-----------------------------|---|-------------------------|-------------|------------|-----------------------|---|
|                                   |                             |   | Single Values<br>[%]    | Mean<br>[%] | RSD<br>[%] |                       |   |
| <i>m/z</i> = 249 (quantification) |                             |   |                         |             |            |                       |   |
| Milk                              | Flufenacet                  | 0.010*  | 93                      | 84          | 89         | -                     | 2 |
|                                   | FOE thioglycolate sulfoxide |   | 81                      | 109         | 95         | -                     | 2 |
|                                   | FOE sulfonic acid           |   | 85                      | 99          | 92         | -                     | 2 |
|                                   | FOE oxalate                 |   | 108                     | 78          | 93         | -                     | 2 |
| Mean over all substances: 92 %    |                             |   | RSD: 13 %               |             | n=8        |                       |   |
| Milk                              | Flufenacet                  | 0.10  | 89                      | 70          | 80         | -                     | 2 |
|                                   | FOE thioglycolate sulfoxide |   | 98                      | 99          | 99         | -                     | 2 |
|                                   | FOE sulfonic acid           |   | 80                      | 65          | 73         | -                     | 2 |
|                                   | FOE oxalate                 |   | 107                     | 102         | 105        | -                     | 2 |
| Mean over all substances: 89 %    |                             |   | RSD: 18 %               |             | n=8        |                       |   |
| <i>m/z</i> = 207 (confirmation)   |                             |   |                         |             |            |                       |   |
| Milk                              | Flufenacet                  | 0.010*  | 97                      | 87          | 92         | -                     | 2 |
|                                   | FOE thioglycolate sulfoxide |   | 84                      | 110         | 97         | -                     | 2 |
|                                   | FOE sulfonic acid           |   | 89                      | 107         | 98         | -                     | 2 |
|                                   | FOE oxalate                 |   | 106                     | 81          | 94         | -                     | 2 |
| Mean over all substances: 95 %    |                             |   | RSD: 12 %               |             | n=8        |                       |   |
| Milk                              | Flufenacet                  | 0.10  | 90                      | 71          | 81         | -                     | 2 |
|                                   | FOE thioglycolate sulfoxide |   | 100                     | 100         | 100        | -                     | 2 |
|                                   | FOE sulfonic acid           |   | 82                      | 65          | 74         | -                     | 2 |
|                                   | FOE oxalate                 |   | 106                     | 103         | 105        | -                     | 2 |
| Mean over all substances: 90 %    |                             |   | RSD: 17 %               |             | n=8        |                       |   |
| <i>m/z</i> = 138 (confirmation)   |                             |   |                         |             |            |                       |   |
| Milk                              | Flufenacet                  | 0.010*  | 109                     | 100         | 105        | -                     | 2 |
|                                   | FOE thioglycolate sulfoxide |   | 84                      | 110         | 97         | -                     | 2 |
|                                   | FOE sulfonic acid           |   | 81                      | 99          | 90         | -                     | 2 |
|                                   | FOE oxalate                 |   | 110                     | 83          | 97         | -                     | 2 |
| Mean over all substances: 97 %    |                             |   | RSD: 13 %               |             | n=8        |                       |   |
| Milk                              | Flufenacet                  | 0.10  | 89                      | 71          | 80         | -                     | 2 |
|                                   | FOE thioglycolate sulfoxide |   | 99                      | 99          | 99         | -                     | 2 |
|                                   | FOE sulfonic acid           |   | 84                      | 65          | 75         | -                     | 2 |
|                                   | FOE oxalate                 |   | 105                     | 102         | 104        | -                     | 2 |
| Mean over all substances: 89 %    |                             |   | RSD: 17 %               |             | n=8        |                       |   |

\*LOQ level

<sup>a</sup> Fortification levels are expressed as Flufenacet equivalents<sup>b</sup> Determination as 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (FOE5043 trifluoroacetamide), calculated and expressed as flufenacet.

## Conclusion

The method 00418 and its modification 00418/M001 is considered valid for the determination of residues of flufenacet (FOE 5043) containing the *N*-fluorophenyl-*N*-isopropyl amine moiety in matrices of animal origin. Validation of the method was exemplified with flufenacet, FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide. The residue analytical method 00418 and the extension 00418/M001 were independently validated. The method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC “Guidance document on residue analytical methods” (SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

### 5.2.2. Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water

#### 5.2.2.1 Soil

Analytical methods for the determination of flufenacet as stated in the definition of the residue for monitoring (see Section CA B7.4.2) in soil were submitted within the EU Dossier (initial DAR), which resulted in the Annex I inclusion under Directive 91/414/EEC in 2003. In the Supplemental Dossier for renewal of approval of flufenacet presented here only those analytical methods are described in Section B5.2.2., which were not submitted within the initial DAR.

#### Original Annex CA submission (1997)

The following analytical method for the determination of flufenacet in soil is included in the initial DAR (see Tables 5.2.2.1-1 to 5.2.2.1-2):

**Table 5.2.2.1-1: Analytical methods for residues of flufenacet in soil reviewed during the first inclusion**

| Method No. | LOQ [mg/kg] | Technique  | Annex Point / Reference No | Author(s)                            | Year | Document No   |
|------------|-------------|------------|----------------------------|--------------------------------------|------|---------------|
| 00359      | 0.01        | HPLC-MS/MS | KCA 4.2/09                 | Allmendinger, H.;<br>Bachlechner, G. | 1994 | M-019071-01-3 |

|                         |  |
|-------------------------|--|
| Report:                 | B.4.3.1; KCA 4.2/09; Allmendinger, H., Bachlechner, G.; 1994   |
| Title:                  | Validated method for the determination of the herbicide FOE 5043 and its metabolites FOE 5043 alcohol, FOE 5043 oxalate and FOE 5043 sulfonic acid in soil using HPLC-MS-MS. |
| Report No & Document No | RA-399/94<br>00359   |
| Dates of work:          | 1994-07-01   |
| GLP                     | GLP  |

**Table 5.2.2.1-2: Description and findings of above analytical methods for residues of flufenacet in soil reviewed during the first inclusion**

|                          |   |                  |                  |                        |
|--------------------------|---|------------------|------------------|------------------------|
| File No.<br>Method No.   | RA - 399/94<br>00359  |                  |                  |                        |
| Test matrix              | soil  |                  |                  |                        |
| Extraction               | 0.1 N HCL/acetonitrile ( 1:1 , v/v )  |                  |                  |                        |
| Clean-up/Derivatization  | -   |                  |                  |                        |
| Determined as            | FOE 5043, FOE 5043 alcohol, FOE 5043 oxalate, FOE 5043-sulfonic acid                      |                  |                  |                        |
| Method of determination  | HPLC-MS-MS  |                  |                  |                        |
|                          | FOE 5043  | FOE 5043 alcohol | FOE 5043 oxalate | FOE 5043 sulfonic acid |
| LOQ                      | 0.01 mg/kg  | 0.01 mg/kg       | 0.01 mg/kg       | 0.01 mg/kg             |
| Fortification levels     | 0.01 - 0.6 mg/kg  |                  |                  |                        |
|                          | <i>a) evaluation with internal (deuterated) standard</i>                                  |                  |                  |                        |
| Recovery                 | 90 %  | 94 %             | 97 %             | 102 %                  |
| Coefficient of variation | 6 % RSD   | 4 % RSD          | 8 % RSD          | 4 % RSD                |
|                          | <i>b) evaluation with external standard :</i>   |                  |                  |                        |
| Recovery                 | 97 %  | 95 %             | 98 %             | 100 %                  |
| Coefficient of variation | 6 % RSD   | 18 % RSD         | 12 % RSD         | 7 % RSD                |
| Specificity              | parent compound and main metabolites were identified and determined by MS-MS-technique    |                  |                  |                        |
| Blank values             | far below 30 % of the LOQs  |                  |                  |                        |
| Repeatability            | recoveries between 70 % and 110 % per fortification level, fortified substance and soil   |                  |                  |                        |
| Reproducibility          | recoveries between 70 % and 110 % with RSDs < 20 % when determined by different operators |                  |                  |                        |

RSD = relative standard deviation

This method is acceptable for renewal purposes.

### **New data for AIR III**

New analytical methods for the determination of flufenacet in soil and water (including an independent lab validation) were developed in order to fulfill the current requirements on enforcement methods are submitted within this Dossier for the flufenacet renewal of approval.

#### **Flufenacet residue definition in soil:**

The proposed residue definition for monitoring is flufenacet only for all compartments. Thus, the metabolites were not further considered.

|              |  |
|--------------|--|
| Report:      | KCA 4.2 /16; Brumhard, B.; 2005  |
| Title:       | Modification M001 of method 00359 for the determination of the herbicide FOE 5043 and its metabolite FOE 5043-alcohol, FOE 5043-oxalate and FOE 5043-sulfonic acid in soil using HPLC-MS/MS  |
| Method No.   | 00359/M001   |
| Report No:   | MR-028/05  |
| Document No: | M-248543-01-1  |
| Guidelines:  | <ul style="list-style-type: none"> <li>• Commission Directive 96/46/EC amending Council Directive 91/414/EEC</li> <li>• European Commission Guidance Document SANCO/825/00 rev. 7</li> <li>• BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes</li> </ul> |
| GLP:         | yes  |

### Principle of the Method

The original method 00359 (Supplemental Dossier, KCA 4.2/09) describes the determination of flufenacet and its metabolites FOE 5043-alcohol, FOE 5043-oxalate and FOE 5043-sulfonic acid in soil by HPLC-MS/MS (with the following working conditions for chromatograph: Hamilton PRP-1, 25 cm length, id 4.1 mm, 10 µ) and provides validation data for one MRM transition. This modification M001 was prepared to provide additional validation data for flufenacet using a second MRM transition and a lower limit of quantitation (LOQ).

Soil samples of 30 g were extracted at ambient temperature using a shaker and 100 mL 0.1 N HCl/water 1/1 (v/v). After the soil had settled, the extract was filtered and an aliquot of 40.0 mL was concentrated at 50 °C to a volume of approx. 5 mL. The concentrated extract was quantitatively transferred to a 10 mL volumetric flask and made up to volume with 0.015% hydrochloric acid. Then an aliquot of this solution was centrifuged to remove fine particles of the soil.

Identification and quantitation of flufenacet was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external standards in matrix (matrix matched). The method was validated using a silt loam (Höfchen) and a sandy loam (Laacher Hof).

### Recovery Findings (Accuracy)

Recovery experiments (see Table 5.2.2.1-3) were conducted at the limit of quantitation (LOQ level, 4 µg/kg) using two different types of agricultural soil: a silt loam (Höfchen) and a sandy loam (Laacher Hof).

Mean recovery for all soils and fortification levels was 74.0% (63% to 87%), for the MRM transition for quantitation ( $m/z = 364 \rightarrow m/z = 124$ ) and 78.0% (66% to 91%), for the MRM transition for confirmation ( $m/z = 364 \rightarrow m/z = 152$ ).

**Table 5.2.2.1-3: Recoveries and repeatabilities for flufenacet in soils at fortification level of 4 µg/kg soil**

| MRM transition                                       | Soil        | Recoveries [%] |    |    |    |    |      | RSD [%] | n  |
|--|-------------|----------------|----|----|----|----|------|---------|----|
|  |             | Single Values  |    |    |    |    | Mean |         |    |
| $m/z = 364 \rightarrow m/z = 124$<br>(quantitation)  | Höfchen     | 83             | 83 | 87 | 85 | 81 | 84   | 2.6     | 5  |
|  | Laacher Hof | 66             | 68 | 65 | 63 | 63 | 65   | 3.1     | 5  |
|  |             | Overall        |    |    |    |    | 74   | 13.5    | 10 |
| $m/z = 364 \rightarrow 1 m/z = 52$<br>(confirmation) | Höfchen     | 85             | 86 | 91 | 89 | 83 | 87   | 3.4     | 5  |
|  | Laacher Hof | 71             | 71 | 72 | 68 | 66 | 70   | 3.8     | 5  |
|  |             | Overall        |    |    |    |    | 78   | 12.0    | 10 |

RSD: relative standard deviation

n: number of replicates

### Linearity

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for flufenacet, covering a range from 2 to 100 µg/L. The correlation coefficients ranged from 0.9989 to 0.9993.

**Specificity**

Residues in control samples were below 0.3 x LOQ, therefore recoveries were not corrected for interferences. Two MRM transitions were monitored for quantitation and confirmation, therefore the HPLC-MS/MS method is highly specific.

$$\begin{array}{ll} m/z = 364 \rightarrow m/z = 124 & \text{(quantitation)} \\ m/z = 364 \rightarrow m/z = 152 & \text{(confirmation)} \end{array}$$

**Limit of Quantification**

The limit of quantification (LOQ) for flufenacet in soil is 4 µg/kg. The limit of detection (LOD) for flufenacet is 1.5 µg/kg.

**Repeatability (Precision)**

Repeatability was shown successfully (see Table 5.2.2.1-3). For each type of soil, the analytical series comprised 5 replicates at the LOQ level (4 µg/kg). Relative standard deviations (RSD) for the individual soils and the overall RSD were below 20%.

**Confirmatory Method**

Two MRM transitions were monitored for quantitation and confirmation. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

**Conclusion**

This method was prepared to provide additional validation data for flufenacet in relation to Allmendinger, H.; Bachlechner, G. 1994, using a second MRM transition and a lower limit of quantitation (LOQ) 4 µg/kg.

**NOTE:**

Analytical methods for analysis of sediments are not a specific data requirement according to current guidelines. However, in case residues of flufenacet have to be determined in sediments, it would be suggested to use (and if necessary adapt) the methods developed for the determination of residues in soil.

**5.2.2.2 Water**

Analytical methods for the determination of flufenacet as stated in the definition of the residue for monitoring (see section CA B7.4.2) in water were submitted within the EU Dossier (initial DAR), which resulted in the Annex I inclusion under Directive 91/414/EEC in 2003. In the Supplemental Dossier for renewal of approval of flufenacet presented here only those analytical methods are described in Section B5.2.2., which were not submitted within the initial DAR.

Original Annex CA submission (1997)

The following analytical method for the determination of flufenacet in water is included in the initial DAR (see Tables 5.2.2.2-1 to 5.2.2.2-3):

**Table 5.2.2.2-1: Analytical methods for residues of flufenacet in water reviewed during the first inclusion**

| Method No. | LOQ<br>[µg/L] | Technique  | Annex Point /<br>Reference No | Author(s)   | Year | Document No   |
|------------|---------------|------------|-------------------------------|---|------|---------------|
| 00412      | 0.05          | GC-ECD     | B.4.3.2                       | König T.  | 1996 | MR-894/95     |
| AMFOE3     | 0.10          | HPLC-MS/MS | KCA 4.2/08                    | Bethem, R. A.;<br>Peterson, R. G.;<br>Leimkuehler, W. A.;<br>Mattern, G. C. | 1995 | M-070022-01-2 |

Report: B.4.3.2; KCA 4.1.2/07; König, T.; 1996  
 Title: Method for the determination of FOE 5043 in drinking water by gas chromatography  
 Report No & Document No: MR-894/95  
 00412  
 Dates of work: 1996-03-13  
 GLP GLP

Report: B.4.3.2 Bethem, R.A., Peterson, R.G., Leimkühler, W., Matten, G.C.; 1995  
 Title: Determination of FOE 5043 and the alcohol, oxalate, thiadone and sulfonic acid metabolites in groundwater by high performance liquid chromatography electrospray tandem mass spectrometry (LC-ESI/MS/MS).  
 Report No & Document No: 107138  
 AMFOE3  
 Dates of work: 1995-06-22  
 GLP GLP

**Table 5.2.2.2-2: Description and findings of above analytical methods for residues of flufenacet in drinking water reviewed during the first inclusion**

|                                |  |
|--------------------------------|--|
| File No.<br>Method No.         | MR-894/95<br>00412   |
| Test matrix                    | drinking water   |
| Extraction                     | solid phase extraction wit C18-cartridges  |
| Clean-up/Derivatization        | -  |
| Determined as                  | FOE 5043   |
| Method of determination        | gas chromatography with an electron capture detector (ECD)   |
| LOQ                            | 0.05 µg/l  |
| Fortification levels           | 0.05 µg/l, 1µg/l, 10 µg/l  |
| Recovery                       | 144 % (0.05 µg/l),<br>104 % (1 µg/l),<br>99 % (10µg/l)   |
| Coefficient of variation (RSD) | 3 % (0.05µg/l),<br>4 % (1 µg/l),<br>2 % (10µg/l)   |
| Blank values                   | far below 30 % of the LOQs   |
| Repeatability                  | even though recoveries are high at the lowest fortification level of 0.05 mg/kg, the method can be used for this concentration range because of the good reproducibility (RSD 3%); the high recovery rate is at least partially caused by the matrix load of the drinking water. |
| Reproducibility                | not applicable   |

RSD) = relative standard deviation

**Table 5.2.2.2-3: Description and findings of above analytical methods for residues of flufenacet in drinking water reviewed during the first inclusion**

|   |  |                     |                     |                      |                           |
|---|--|---------------------|---------------------|----------------------|---------------------------|
| File No. (Bayer Crop )<br>File No. (by generating laboratory) | 107138<br>AMFOE3   |                     |                     |                      |                           |
| Test matrix   | water  |                     |                     |                      |                           |
| Extraction  | enrichment on a C - 18 column  |                     |                     |                      |                           |
| Clean-up/Derivatization                                       | elution with methanol  |                     |                     |                      |                           |
| Determined as   | FOE 5043, FOE 5043 alcohol, FOE 5043 oxalate,<br>FOE 5043 thiadone, FOE 5043-sulfonic acid |                     |                     |                      |                           |
| Method of determination                                       | HPLC - ESI/MS/MS   |                     |                     |                      |                           |
|   | FOE 5043   | FOE 5043<br>alcohol | FOE 5043<br>oxalate | FOE 5043<br>thiadone | FOE 5043<br>sulfonic acid |
| LOQ* $\mu\text{g/l}$  | 0.04   | 0.04                | 0.05                | 0.08                 | 0.02                      |
| Fortification levels $\mu\text{g/l}$                          | 0.2  | 0.2                 | 0.2                 | 0.2                  | 0.2                       |
| Recovery %  | 95   | 97                  | 94                  | 4                    | 97                        |
| Coefficient of<br>variation (RSD) % RSD                       | 2  | 2                   | 3                   | 4                    | 1                         |
| Blank values  | far below 30 % of the LOQ  |                     |                     |                      |                           |
| Repeatability   | data not accessible  |                     |                     |                      |                           |
| Reproducibility   | data not accessible  |                     |                     |                      |                           |

\* the limit of quantitation is defined as 10-fold the standard deviation of a set of 7 recoveries at 0.2  $\mu\text{g/l}$

RSD = relative standard deviation



**New data for AIR III**

New analytical methods for the determination of flufenacet in water (including an independent lab validation) were developed in order to fulfill the current requirements on enforcement methods are submitted within this Supplemental Dossier for the flufenacet renewal of approval.

|              |   |
|--------------|---|
| Report:      | KCA 4.2 /19; Krebber, R.; Braune, M.; 2013  |
| Title:       | Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS   |
| Method No.   | 01387   |
| Report No:   | MR-13/085   |
| Document No: | M-466732-01-1   |
| Guidelines:  | <ul style="list-style-type: none"> <li>• Regulation (EC) No 1107/2009</li> <li>• European Commission Guidance Document SANCO/825/00 rev. 8.1</li> <li>• European Commission Guidance Document SANCO/3029/99 rev. 4</li> </ul> |
| GLP:         | yes   |

**Principle of the Method**

The analytical method 01387 was developed for the determination of flufenacet in drinking and surface water by direct injection into the HPLC-MS/MS (chromatography column, Ascentis Express C18 2.7 µm, 50 mm x 2.1 mm id, or equivalent, Supelco, PA 16823-0048, USA) instrument without further clean-up. Identification and quantitation of flufenacet was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external matrix-matched standard solutions. A second MRM transition was used for confirmation. The method was validated using surface water from the river Rhine. A validation for drinking water was not necessary because the limit of quantitation for surface water is below the drinking water limit of 0.1 µg/L.

**Recovery Findings (Accuracy)**

Because of the direct measurement of samples, recovery rates cannot be calculated.

**Linearity**

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for flufenacet, covering a range from 0.012 to 4.0 µg/L. The correlation coefficients were  $\geq 0.9998$  for both MRM transitions.

**Specificity**

Residues in control samples were below 0.3 x LOQ, therefore recoveries were not corrected for interferences. Two MRM transitions were monitored for quantitation and confirmation, therefore the HPLC-MS/MS method is highly specific.

The following MRM transitions were used for quantitation and confirmation of flufenacet:

$$\begin{array}{ll} m/z = 364 \rightarrow m/z = 194 & \text{(quantitation)} \\ m/z = 364 \rightarrow m/z = 152 & \text{(confirmation)} \end{array}$$

**Limit of Quantitation**

The limit of quantification (LOQ) for flufenacet in drinking and surface water is 0.05 µg/L.

**Storage Stability of the Analyses**

The analytes were stable in surface water when stored in a freezer at  $\leq -18^{\circ}\text{C}$  for a period of 22 days.

**Repeatability (Precision)**

Repeatability was shown successfully using surface water taken from the river Rhine sampled in Leverkusen-Hitdorf. The analytical series comprised 10 replicates at the limit of quantitation (LOQ level, 0.05 µg/L) and 10 replicates at the 10-fold LOQ level (0.5 µg/L). Relative standard deviations (RSD) at the individual fortification levels ranged from 1.1 to 3.0% for both MRM transitions (see Table 5.2.2.2-4).

**Table 5.2.2.2-4: Repeatabilities for flufenacet in surface water**

| MRM transition                         | Fortification Level<br>[µg/L] | Peak Area<br>[Area Counts] |                    |                    |                    |                    |         | RSD <sup>1</sup><br>[%] | n  |
|--|-------------------------------|----------------------------|--------------------|--------------------|--------------------|--------------------|---------|-------------------------|----|
|  |                               | Single Values              |                    |                    |                    |                    | Mean    |                         |    |
| $m/z = 364 \rightarrow$<br>$m/z = 194$ | 0.05                          | 115974<br>118583           | 112306<br>121104   | 115647<br>113323   | 116991<br>118879   | 123001<br>121944   | 117775  | 3.0                     | 10 |
|  | 0.5                           | 1021336<br>983847          | 1002855<br>981662  | 993023<br>995595   | 1006895<br>995484  | 996682<br>997546   | 997493  | 1.1                     | 10 |
| $m/z = 364 \rightarrow$<br>$m/z = 152$ | 0.05                          | 122414<br>124071           | 122986<br>124256   | 119560<br>117247   | 119078<br>123179   | 122879<br>119535   | 121521  | 2.0                     | 10 |
|  | 0.5                           | 1032894<br>1007601         | 1003543<br>1021483 | 1034324<br>1001489 | 1051490<br>1025177 | 1043383<br>1031316 | 1025270 | 1.6                     | 10 |

<sup>1</sup> Because of the direct measurement of fortified samples, recovery rates cannot be calculated. As a measure for the precision of the method, the intra-laboratory repeatability is given as relative standard deviation (RSD) for the individual fortification levels.

### Reproducibility (ILV)

The reproducibility was validated by independent laboratory PTRL Europe GmbH, Ulm, Germany (see Dossier, KCA 4.2/22).

### Confirmatory Method

Two MRM transitions were monitored for quantitation and confirmation. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

### Conclusion

The method meets all guideline criteria to determine residues of flufenacet in drinking and surface water at a limit of quantitation (LOQ) of 0.05 µg/L. The HPLC-MS/MS detection of flufenacet was not affected by the matrix. The peak areas of the quantification and confirmatory ion in a surface water containing 0.5 µg/L show no significant difference to the corresponding peak areas in deionized water. The method fulfils the requirements of the EC “Guidance document on residue analytical methods” (SANCO/825/00 rev. 8.1).

|              |   |
|--------------|---|
| Report:      | KCA 4.2 /22; Stanislawski, T.; 2013   |
| Title:       | Independent laboratory validation of BCS analytical methods 01333 and 01387 for determination of various pesticides in surface water by Di-HPLC-MS/MS   |
| Method No.   | Independent Laboratory Validation of method no. 01387   |
| Report No:   | P3117 G   |
| Document No: | M-470714-02-1   |
| Guidelines:  | <ul style="list-style-type: none"> <li>• Regulation (EC) No 1107/2009</li> <li>• Commission Regulation (EU) No 283/2013</li> <li>• European Commission Guidance Document SANCO/825/00 rev. 8.1</li> <li>• European Commission Guidance Document SANCO/3029/99 rev. 4</li> </ul> |
| GLP:         | yes   |

### Principle of the Method

The analytical method 01387 (Supplemental Dossier, KCA 4.2/19) was validated for the determination of flufenacet in drinking and surface water by direct injection into the HPLC-MS/MS instrument (chromatography column, Ascentis Express C18 2.7 µm, 50 mm x 2.1 mm id, or equivalent, Supelco, PA 16823-0048, USA) without further clean-up using the positive ion mode. Identification and quantitation of flufenacet was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external matrix-matched standard solutions. A second MRM transition was used for confirmation. The method was validated using surface water from the river Danube.

### Recovery Findings (Accuracy)

Because of the direct measurement of samples, recovery rates cannot be calculated.

**Linearity**

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for flufenacet, covering a range concentration from 0.012 to 0.8 µg/L. The correlation coefficients were  $\geq 0.9998$  for both MRM transitions.

**Specificity**

Residues in control samples were below 0.3 x LOQ, therefore recoveries were not corrected for interferences. Two MRM transitions were monitored for quantitation and confirmation, therefore the HPLC-MS/MS method is highly specific.

The following MRM transitions were used for quantitation and confirmation of flufenacet:

$$\begin{array}{ll} m/z = 364 \rightarrow m/z = 194 & \text{(quantitation)} \\ m/z = 364 \rightarrow m/z = 152 & \text{(confirmation)} \end{array}$$

**Limit of Quantitation**

The limit of quantification (LOQ) for flufenacet is 0.05 µg/L in drinking and surface water.

**Repeatability (Precision)**

Repeatability was shown successfully using surface water taken from the river Danube sampled in Ulm (see Table 5.2.2.2-4). The analytical series comprised 5 replicates at the limit of quantitation (LOQ level, 0.05 µg/L) and 5 replicates at the 10-fold LOQ level (0.5 µg/L). One double injection per fortification level was included. Two MRM transitions were monitored for flufenacet. Relative standard deviations (RSD) at the individual fortification levels ranged from 1.6 to 2.9% for both MRM transitions.

**Table 5.2.2.2-4: Method validation for flufenacet in surface water for the quantification ion ( $m/z = 364 \rightarrow m/z = 194$ ) and confirmation ion ( $m/z = 364 \rightarrow m/z = 152$ )**

| MRM transition                    | Fortification Level [µg/L] | Peak Area [Area Counts] |         |         |         |         |         | RSD [%] | n |
|-----------------------------------|----------------------------|-------------------------|---------|---------|---------|---------|---------|---------|---|
|                                   |                            | Single Values           |         |         |         |         | Mean    |         |   |
| $m/z = 364 \rightarrow m/z = 194$ | 0.05                       | 195939                  | 196194  | 197024  | 193167  | 201683  | 196394  | 1.7     | 5 |
|                                   | 0.5                        | 191869                  |         |         |         |         |         |         |   |
| $m/z = 364 \rightarrow m/z = 152$ | 0.05                       | 1919060                 | 1940510 | 1941490 | 1891810 | 1869550 | 1913297 | 1.7     | 5 |
|                                   | 0.5                        | 1927160                 |         |         |         |         |         |         |   |
| $m/z = 364 \rightarrow m/z = 152$ | 0.05                       | 195008                  | 200825  | 210987  | 212730  | 202556  | 205505  | 2.9     | 5 |
|                                   | 0.5                        | 205843                  |         |         |         |         |         |         |   |
| $m/z = 364 \rightarrow m/z = 152$ | 0.05                       | 1995930                 | 2003800 | 2033730 | 1959940 | 2033000 | 2003163 | 1.6     | 5 |
|                                   | 0.5                        | 1974760                 |         |         |         |         |         |         |   |

**Contact to Developer of Original Method**

The method developer was not contacted.

**Conclusion**

The laboratory PTRL Europe performed the independent laboratory validation (ILV) of the analytical method 01387 for the determination of flufenacet in drinking and surface water as described in BCS Report M-466732-01-1 (see KCA 4.2/19). The method was shown to be selective and yield accurate and repeatable results. The HPLC-MS/MS detection of flufenacet was not affected by the matrix. The peak areas of the quantification and confirmatory ion in a surface water containing 0.5 µg/L shows no significant difference to the corresponding peak areas in deionized water. The method fulfils the requirements of the EC “Guidance document on residue analytical methods” (SANCO/825/00 rev. 8.1).

Overall adequate LC-MS/MS methods and corresponding ILV are available to monitor flufenacet residues in surface and drinking water.

### 5.2.3. Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

An analytical method for the determination of flufenacet as stated in the definition of the residue for monitoring (see section CA 7.4.2) in air was submitted within the EU Dossier (initial DAR), which resulted in the Annex I inclusion under Directive 91/414/EEC in 2003.

In the Supplemental Dossier for renewal of approval of flufenacet presented here only those analytical methods are described in this section, which were not submitted within the initial DAR.

#### Original Annex CA submission (1997)

The following analytical method for the determination of flufenacet in air is included in the initial DAR (see Table 5.2.3-1):

**Table 5.2.3-1: Analytical methods for residues of flufenacet in air reviewed during the first inclusion**

| Method No. | LOQ [mg/m <sup>3</sup> ] | Technique         | Annex Point / Reference No | Author(s)  | Year | Document No   |
|------------|--------------------------|-------------------|----------------------------|------------|------|---------------|
| 00410      | 0.0022                   | HPLC-UV at 230 nm | KCA 4.12/06                | Riegner, K | 1995 | M-012833-01-2 |

This method was described in the initial DAR, and now, in the Air III given its modification.

Below is described the method included in initial DAR.

|              |   |
|--------------|---|
| Report:      | M-012833-01-2; Riegner, K.; 1995  |
| Title:       | Method for the determination of FOE 5043 in air. (Methode zur bestimmung von FOE 5043 in luft.) |
| Method No.   | 00410   |
| Report No:   | MR-798/95   |
| Document No: | M-012833-01-2   |
| Guidelines:  | • Commission Directive 96/46/EC   |
| GLP:         | yes   |

#### **Principle of the Method**

The analytical method 00410 for the determination of flufenacet in air was developed and included in the Baseline Dossier as KCA 4.1.2/06. The method used the HPLC-UV technique with 230 nm wavelength. The sample was sucked through Tenax® adsorption tubes with a rate of 2 L/ min, during a period of 6 hours. The adsorbed active ingredient was extracted with acetonitrile and determined by HPLC-UV (column: RP18 LiChrospher, 250 mm length, 5 µm particle size, id 4 mm and relevant pre-column, injection volume 75 µL, eluent A – water and eluent B – acetonitrile). The method was validated using blank samples spiked with flufenacet.

#### **Recovery Findings (Accuracy)**

Recovery experiments (see Table 5.2.3-2) were conducted at to fortification levels: 0.0022 mg/m<sup>3</sup> (limit of quantitation; LOQ level) and at 0.489 mg/m<sup>3</sup>.

Mean recoveries were 95.5% (95.0% to 96.0%) for both fortification levels with SD of 0.01% to 0.10% depending on the fortification level.

The adsorption capacity was checked at a temperature of 35°C and a relative humidity of 80%.

**Linearity**

The correlation between the injected amount of substance and the detector response was linear for flufenacet, covering a range from 0.04 to 9.3 mg/L.

**Limit of Quantitation**

The limit of quantification (LOQ) for flufenacet in air is 0.0022 mg/m<sup>3</sup>.

**Repeatability (Precision)**

Repeatability was shown successfully (see Table 5.2.3-2). Relative standard deviations (RSD) for the individual fortification levels and the overall RSD were below 20%.

**Conclusion**

The number of recovery per fortification level and method specificity and breakthrough is missing.

**Table 5.2.3-2: Description and findings of above analytical methods for residues of flufenacet in air reviewed during the first inclusion**

|                          |  |
|--------------------------|--|
| File No.<br>Method No.   | MR - 798/95<br>00410   |
| Test matrix              | air  |
| Extraction               | enrichment by adsorption on Tenax- or XAD-2 tubes, elution with acetonitrile                         |
| Clean-up/Derivatization  | -  |
| Determined as            | FOE 5043   |
| Method of determination  | HPLC with UV - Detector (230 nm)   |
| LOQ                      | 0.0022 mg/m <sup>3</sup>   |
| Fortification levels     | 0.0022 mg/m <sup>3</sup> - 0.489 mg/m <sup>3</sup>   |
| Recovery                 | 96 %   |
| Coefficient of variation | 1% RSD   |
| Blank values             | none   |
| Repeatability            | recoveries between 70 % - 110 % with relative standard deviation < 20 % for each fortification level |
| Reproducibility          | data not accessible  |
| Remark                   | the adsorption was carried out at a temperature of 35°C and a relative humidity of 80 %              |

RSD = relative standard deviation

**New data for AIR III**

New analytical methods for the determination of flufenacet in air including an independent laboratory validation were developed in order to fulfill the current requirements on enforcement methods are submitted within this Supplemental Dossier for the flufenacet renewal of approval.

|              |  |
|--------------|--|
| Report:      | KCA 4.2 /13; Hellpointner, E.; 2000  |
| Title:       | Confirmatory method for the determination of FOE 5043 in Air (Confirmatory method no. 00410) |
| Method No.   | 00410C   |
| Report No:   | MR- 469/00   |
| Document No: | M-048783-01-1  |
| Guidelines:  | Commission Directive 96/46/EC  |
| GLP:         | yes  |

**Principle of the Method**

The analytical method 00410C for the determination of flufenacet in air was developed as confirmatory method for method no. 00410 (Baseline Dossier, KCA 4.1.2/06 and described above). The modified method uses a cyanopropyl stationary phase for the HPLC-UV analysis of the extracts of the Tenax® adsorption tubes, instead of the reversed phase stationary phase described in the original method. No deviation from the Tenax® sampling and extraction technique described in method no. 00410 was necessary.

The method was validated using blank samples spiked with flufenacet.

**Recovery Findings (Accuracy)**

Recovery experiments (see Table 5.2.3-3) were conducted at two fortification levels: 0.0022 mg/m<sup>3</sup> (limit of quantification; LOQ level) and at 0.489 mg/m<sup>3</sup>.

Mean recoveries were 95.5% (95.0% to 96.1%) for both fortification levels.

**Table 5.2.3-3: Recoveries and repeatabilities for flufenacet in air**

| Fortification level<br>[mg/m <sup>3</sup> ] | Recoveries<br>[%] |      |      |      |      | RSD<br>[%] | n |
|---|-------------------|------|------|------|------|------------|---|
|   | Single Values     |      |      |      | Mean |            |   |
| 0.0022                                      | 96.0              | 96.1 | 96.1 | 96.0 | 96.1 | 96.0       | 5 |
| 0.489                                       | 95.0              | 95.0 | 95.0 | 95.0 | 95.0 | 95.0       | 5 |

RSD: relative standard deviation

n: number of replicates

**Linearity**

The correlation between the injected amount of substance and the detector response was linear for flufenacet, covering a range from 0.16 to 1.6 mg/L. The correlation coefficient was  $\geq 0.9999$ .

**Specificity**

The chromatograms of the blank sample showed a chromatographic signal at the retention time of flufenacet above background level, corresponding to about 18.5% of the signal intensity at the LOQ level.

However, the complementary separation mechanisms of the used cyanopropyl stationary phase in comparison to the reversed phase stationary phase ensure a suitable specificity of the analytical methods for determination of flufenacet in air.

**Limit of Quantitation**

The limit of quantification (LOQ) for flufenacet in air is 0.0022 mg/m<sup>3</sup>.

**Repeatability (Precision)**

Repeatability was shown successful (see Table 5.2.3-3). For each fortification level, the analytical series comprised 5 replicates. Relative standard deviations (RSD) for the individual fortification levels and the overall RSD were below 20%.

**Confirmatory Method**

The analytical method 00410C for the determination of flufenacet in air was developed as confirmatory method for method no. 00410 (Baseline Dossier, KCA 4.1.2/06 and described above).

**Conclusion**

The analytical method can be considered as validated but not highly specific. A confirmatory method is required. The LOQ provided in analytical methods for residue in air comply with the concentration C calculated from the AOEL systemic.

**5.2.4. Methods for the analysis in body fluids and tissues for active substances and relevant metabolites**

The purpose of such analytical method for analysis of the active substance and relevant metabolites is the detection of intoxications in humans and animals.

Relevant criteria for provision of a method as outlined in SANCO/825/00 rev. 8.1 are classification of the active substance or a relevant metabolite as toxic or very toxic, or classification according to GHS as follows: Acute toxicity (cat. 1-3), CMR (cat. 1) or STOT (cat. 1).

Flufenacet or any of its metabolites is not classified according to those categories.

No recommendation was provided during the EU peer review for analytes relevant for monitoring in body fluids and tissues. Therefore, methods for the determination of flufenacet in body fluids were not considered necessary when the dossier for renewal of approval was submitted.

However, experience with recent EFSA evaluations shows that - based on the data requirements of Regulation (EU) 283/2013 – the absence of such enforcement method may be considered as a data gap. As a consequence an enforcement method in body fluids was developed and is reported below.

Relevant to body tissues primary methods which are also suitable as enforcement methods (Gould, T. J.; Lemke, V. J.; Zoloty, K. L.; 1995; M-019605-01-1; Seym, M.; 1995; M-019614-01-1; Klimmek, S.; Goellner, C.; Amann, S.; 2013; M-461242-01-1 [ILV]) are available and reported in the supplementary dossier for renewal of approval. The method provides sufficient sensitivity to determine the parent compound flufenacet as well as its metabolites containing the common N-fluorophenyl-N-isopropyl moiety with an LOQ of 0.05 mg/kg in muscle and 0.02 mg/kg in liver. The method covers also fat and eggs (LOQ 0.05 mg/kg) and milk (LOQ 0.01 mg/kg). Validation was performed using flufenacet, FOE 5043 oxalate, FOE 5043 sulfonic acid and FOE 5043 thioglycolate sulfoxide as relevant metabolites containing the common moiety and addressing the established enforcement residue definition for commodities of animal origin: Flufenacet (sum of all compounds containing the N-fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent).

Relevant to body fluids a method for the determination of flufenacet related residues in plasma was recently developed (see summary below). For selection of the analytical targets in plasma – apart from the parent compound – flufenacet thiadone was considered to be most appropriate since it was observed in quantities of more than 80% of TRR in goat (and hen) liver, kidney and muscle.

Residues detected in the organs are transported via the blood into these organs and it is concluded that these residues are the same in blood and plasma. It was shown in the rat ADME studies that the thiadiazole moiety is quickly absorbed and reached a maximum concentration already about 2 - 4 hours after dosing.

Metabolites containing the common N-fluorophenyl-N-isopropyl moiety were not considered to be suitable markers since (i) the analytical method requires a separate hydrolysis step and is not compatible with a multi-residue method and (ii) summed quantities of those metabolites were lower than the portion of thiadone in livestock tissues even after high overdose experiments.

|              |   |
|--------------|---|
| Report:      | KCA 4.2 /13; Kaussmann, M.; 2016  |
| Title:       | Analytical Method 01486 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS  |
| Method No.   | -   |
| Report No:   | P683166504  |
| Document No: | M-556577-01-1   |
| Guidelines:  | Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market<br>Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010<br>European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000 |
| GLP:         | yes   |

#### Principle of the method

The analytical method 01486 was validated for the determination of flufenacet and flufenacet-thiadone (as well as other analytes) in plasma. Only results relevant to flufenacet related residues are reported here.

Plasma samples were deproteinized by mixing with a solution of acetonitrile/water (6/1, v/v) containing 56 mg/L ammonium acetate and 0.14 mL/L formic acid and subsequent centrifugation. An aliquot of the supernatant was analysed for flufenacet and flufenacet-thiadone using HPLC-MS/MS operating in the positive ion mode for flufenacet and in negative ion mode for flufenacet-thiadone. Quantification was performed with matrix matched standards.

#### Limit of Quantification (LOQ)

The limit of quantitation (LOQ) in plasma was 50 µg/L for flufenacet and flufenacet-thiadone expressed as parent equivalents.

#### Limit of Detection (LOD)

The limit of determination (LOD) in plasma was 15 µg/L for flufenacet and flufenacet-thiadone expressed as parent equivalents.

#### Linearity

Linear calibration functions with 1/x weighting were calculated. The calibration curves were established using at least 5 different calibration points. The correlation between the injected amount of flufenacet and flufenacet-thiadone and the detector response was linear for matrix-matched standards ranging from 1.5 µg/L to 75 µg/L corresponding to 15 µg/L to 750 µg/L in plasma (for flufenacet-thiadone expressed as parent equivalents).

The correlation coefficient  $r$  was 0.999 for both analytes and both MRM transitions. The calibration curves cover a range from 30% of the LOQ to 50% above the highest fortification level.

#### Matrix effects

Matrix matched standards were used for the evaluation of both analytes which compensate for matrix effects.

#### Specificity

Apparent residues in control samples were below  $0.3 \times \text{LOQ}$ . Two MRM transitions were monitored for both analytes. No signals/peaks interfering with the detection of the analytes were observed in solutions of untreated control specimens. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.



Repeatability (Precision)

As a measure for the precision, the intra-laboratory repeatability (n=5) is given as relative standard deviation (% RSD). For all matrices investigated, for each fortification level, and for both MS/MS transitions monitored, the relative standard deviations (RSD) were ≤ 20 %. (cf. Table 5.2.4-1 to Table 5.2.4-4).

Recovery Rates (Accuracy)

Mean recoveries for each fortification level and the overall mean recovery per matrix were within the 70 - 110% range for both analytes (cf. Table 5.2.4-1 to Table 5.2.4-4).

Stability of analytes

Storage stability in sample extracts (i. e. supernatant after centrifugation of plasma proteins): The stability in final extracts was examined for plasma over a period of 6 days at < +6°C. Three control plasma extracts were reanalysed using freshly prepared calibration curves. Both analytes were found to be stable in final extracts for at least six days in cattle plasma (cf. Table 5.2.4-5).

Storage stability in plasma: Control plasma samples were fortified with 500 µg/L of the analytes and stored in a freezer at < 18°C. A set of three samples was analysed with a freshly prepared calibration curve after 3 days of storage. The results show that under freezer conditions all analytes were stable for a storage period of at least 3 days (cf. Table 5.2.4-6).

**Table 5.2.4-1: Recoveries for flufenacet**  
MRM for quantification (364 → 152 m/z)

| Matrix | Fortification Level (FL) [µg/L] | Recoveries % (Single values) |     |     |    |     | per FL   |         | Overall  |         |
|--------|---------------------------------|------------------------------|-----|-----|----|-----|----------|---------|----------|---------|
|        |                                 |                              |     |     |    |     | Mean [%] | RSD [%] | Mean [%] | RSD [%] |
| Plasma | 50                              | 99                           | 109 | 108 | 95 | 94  | 101      | 7.0     | 102      | 5.6     |
|        | 500                             | 99                           | 104 | 104 | 97 | 109 | 103      | 4.6     |          |         |

FL: fortification level, RSD: relative standard deviation, LOQ is marked in bold

**Table 5.2.4-2: Recoveries for flufenacet**  
MRM for confirmation (364 → 124 m/z)

| Matrix | Fortification Level (FL) [µg/L] | Recoveries % (Single values) |     |     |    |     | per FL   |         | Overall  |         |
|--------|---------------------------------|------------------------------|-----|-----|----|-----|----------|---------|----------|---------|
|        |                                 |                              |     |     |    |     | Mean [%] | RSD [%] | Mean [%] | RSD [%] |
| Plasma | 50                              | 102                          | 106 | 116 | 99 | 96  | 104      | 7.5     | 104      | 6.4     |
|        | 500                             | 99                           | 101 | 107 | 97 | 112 | 103      | 6.0     |          |         |

FL: fortification level, RSD: relative standard deviation, LOQ is marked in bold

**Table 5.2.4-3: Recoveries for flufenacet-thiadone**  
MRM for quantification (169 → 113 m/z)

| Matrix | Fortification Level (FL)* [µg/L] | Recoveries % (Single values) |     |     |    |     | per FL   |         | Overall  |         |
|--------|----------------------------------|------------------------------|-----|-----|----|-----|----------|---------|----------|---------|
|        |                                  |                              |     |     |    |     | Mean [%] | RSD [%] | Mean [%] | RSD [%] |
| Plasma | 50                               | 86                           | 101 | 101 | 87 | 83  | 92       | 9.5     | 95       | 8.2     |
|        | 500                              | 93                           | 102 | 101 | 95 | 105 | 99       | 5.1     |          |         |

FL: fortification level, RSD: relative standard deviation, LOQ is marked in bold

\*expressed as parent equivalents

**Table 5.2.4-4: Recoveries for flufenacet-thiadone**  
MRM for confirmation (169 → 109 m/z)

| Matrix | Fortification Level (FL)* [µg/L] | Recoveries % (Single values) |     |     |    |     | per FL   |         | Overall  |         |
|--------|----------------------------------|------------------------------|-----|-----|----|-----|----------|---------|----------|---------|
|        |                                  |                              |     |     |    |     | Mean [%] | RSD [%] | Mean [%] | RSD [%] |
| Plasma | 50                               | 97                           | 103 | 100 | 91 | 87  | 96       | 6.8     | 98       | 7.8     |
|        | 500                              | 93                           | 101 | 105 | 88 | 110 | 99       | 9.0     |          |         |

FL: fortification level, RSD: relative standard deviation, LOQ is marked in bold

\*expressed as parent equivalents

**Table 5.2.4-5: Stability of flufenacet and flufenacet-thiadone in plasma extracts at  $\leq +6$  °C**

| Compound                             | Fortification Level (FL)*<br>[µg/L] | Storage period   | Recoveries %<br>(Single values) |     |     | Mean [%] | RSD [%] |
|--------------------------------------|-------------------------------------|------------------|---------------------------------|-----|-----|----------|---------|
| Flufenacet<br>364 → 152 m/z          | 500                                 | initial analysis | 108                             | 103 | 103 | 105      | 2.8     |
|                                      |                                     | 6 days           | 106                             | 102 | 102 | 103      | 2.2     |
| Flufenacet-thiadone<br>169 → 113 m/z | 500                                 | initial analysis | 104                             | 104 | 115 | 108      | 5.9     |
|                                      |                                     | 6 days           | 106                             | 100 | 105 | 104      | 3.1     |

FL: fortification level, RSD: relative standard deviation

\*expressed as parent equivalents

**Table 5.2.4-6: Stability of flufenacet and flufenacet-thiadone in plasma at  $\leq -18$  °C**

| Compound                             | Fortification Level (FL)*<br>[µg/L] | Storage period | Recoveries %<br>(Single values) |     |     | Mean [%] | RSD [%] |
|--------------------------------------|-------------------------------------|----------------|---------------------------------|-----|-----|----------|---------|
| Flufenacet<br>364 → 152 m/z          | 500                                 | 3 days         | 101                             | 112 | 104 | 106      | 5.4     |
| Flufenacet-thiadone<br>169 → 113 m/z | 500                                 | 3 days         | 104                             | 109 | 98  | 104      | 5.3     |

FL: fortification level, RSD: relative standard deviation

\*expressed as parent equivalents

## Conclusion

The residue analytical method 01486 was successfully validated for the determination of residues of flufenacet and its metabolite flufenacet-thiadone in cattle plasma. The results of the method validation were confirmed using a second MRM transition. Quantification limits of 50 µg/L for flufenacet and flufenacet-thiadone (expressed as parent equivalents) were achieved. All method validation results are in compliance with the European guideline requirements for post registration control outlined in the ‘Guidance document on residue analytical methods’ (SANCO/825/00 rev. 8.1). Method 01486 is therefore considered to be applicable for the determination of flufenacet related residues in human and animal plasma.

## Overall conclusion

In this re-registration process new methods based on the HPLC-MS/MS technologies have been proposed for determination of flufenacet residues in plant matrices with LOQ equal to 0.01 mg/kg and in animal matrices with LOQs of 0.01 mg/kg (milk), 0.02 mg/kg (liver) and 0.05 mg/kg for eggs, kidney, muscle and fat. The procedure for extraction of residues from the matrices remains unchanged compared to old methods. The method for determination of flufenacet residues in soil was based on HPLC-MS/MS technique with LOQ equal to 0.01 mg/kg. The method for determination of flufenacet residues in drinking and surface water was based on HPLC-MS/MS technique with LOQ equal to 0.05 µg/L. The analytical method for the determination of flufenacet in air used the HPLC-UV technique with 230 nm wavelength. However the number of recovery per fortification level and method specificity and breakthrough is missing. Additionally the analytical method of Hellpointner, E.; 2000 can be considered as validated but not highly specific. A confirmatory method is required. Method of Kaussmann, M.; 2016, is considered to be applicable for the determination of flufenacet related residues in human and animal plasma.

All methods (with exception of method for air) have been recognized as acceptable.

**B.5.3. REFERENCES RELIED ON**

In order to distinguish, the studies reviewed during the first inclusion, reference to studies from the original dossier are depicted in grey.

| <b>Annex point /<br/>reference number</b>   | <b>Author(s)</b>              | <b>Year</b> | <b>Title<br/>Source (where different from company)<br/>Company name, Report No., Date, GLP status (where<br/>relevant), published or not</b>  | <b>Vertebrate<br/>study<br/>Y/N</b> | <b>Data<br/>protection<br/>claimed<br/>Y/N</b> | <b>Justification<br/>if data<br/>protection is<br/>claimed</b> | <b>Owner</b>         |
|---|-------------------------------|-------------|---|-------------------------------------|--|--|----------------------|
| KCA 4.1.<br>B.4.1.1.<br>CA B.5.1.1.         | Mohsin, S.B                   | 1994        | HPLC analysis of BAY FOE 5043<br>TM C-16.01<br>1994-08-26<br>Non GLP  | N                                   | N  | -  |                      |
| KCA 4.1/01<br>B.4.1.1.<br>CA B.5.1.1.       | Reubke, K. J.                 | 1995        | Material accountability of FOE 5043 - Acetone process<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: PC1102,<br>Edition Number: M-004706-01-1<br>Date: 1995-12-06<br>GLP/GEP: yes, unpublishedconfidential   | N                                   | N  | -  | Bayer<br>CropScience |
| KCA 4.1.1/01<br>B.4.1.1.<br>CA B.5.1.1.     | Harbin, D. N.                 | 1997        | Validation of Test Method C-16.01: Quantitation of BAY<br>FOE 5043 in Technical Material and 60 percent Dry<br>Flowable Formulations<br>Bayer Corporation, Kansas City, MO, USA<br>Bayer CropScience,<br>Report No.: 107584,<br>Edition Number: M-055185-01-1<br>Date: 1997-04-11<br>GLP/GEP: no, unpublished | N                                   | N  | -  | Bayer<br>CropScience |
| KCA 4.1.1 /09<br>CA.4.1.1/01<br>CA B.5.1.1. | Kraemer, F.;<br>Ruengeler, W. | 2011a       | Flufenacet - Determination of active substance in technical<br>material HPLC - external standard<br>Bayer CropScience,<br>Report No.: AM015811MP1,<br>Edition Number: M-414331-01-1<br>Date: 2011-07-22<br>GLP/GEP: no, unpublished confidential  | N                                   | Y  | Guideline<br>requirement                                       | Bayer<br>CropScience |

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| KCA 4.1.1 /10<br>CA.4.1.1/02<br>CA B.5.1.1. | Kraemer, F.;<br>Ruengeler, W.                   | 2011b | Validation of HPLC-method AM015811MP1 - Flufenacet -<br>Determination of active substance in technical material<br>HPLC - external standard<br>Bayer CropScience,<br>Report No.: VB1-AM015811MP1,<br>Edition Number: M-414332-01-1<br>Date: 2011-07-22<br>GLP/GEP: no, unpublished confidential   | N | Y | Guideline<br>requirement | Bayer<br>CropScience |
| B.4.2.1<br>CA B.5.1.2.                      | Seym, M.  | 1994  | Independent laboratory validation of the residue analytical<br>method for FOE 5043 residues in plant.<br>106907<br>RA-352-94<br>1994-06-24<br>GLP   | N | N | -                        | Bayer<br>CropScience |
| KCA 4.1.2 /01<br>CA B.5.1.2.                | Seym, M.  | 1995a | Amendment no 1 to report: MR-981/95 - Analytical method<br>for the determination of the total residue of FOE 5043 in<br>plant materials<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00346,<br>Edition Number: M-018864-02-1<br>Method Report No.: MR-981/95<br>Date: 1995-10-20<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /01</b> | N | N | -                        | Bayer<br>CropScience |
| KCA 4.1.2 /02<br>CA B.5.1.2.                | Gould, T. J.;<br>Lemke, V. J.;<br>Zoloty, K. L. | 1995  | An analytical method for the determination of FOE 5043<br>residues in animal matrices<br>Bayer Corporation, Kansas City, MO, USA<br>Bayer CropScience,<br>Report No.: 00418,<br>Edition Number: M-019605-01-1<br>Method Report No.: F3120201<br>Date: 1995-04-10<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /02</b>  | N | N | -                        | Bayer<br>CropScience |

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| KCA 4.1.2 /03<br>CA B.5.1.2. | Seym, M.                      | 1995b | Modification M001 of method 00418 for the determination of FOE 5043 residues in animal matrices<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00418/M001,<br>Edition Number: M-019614-01-1<br>Method Report No.: MR-1118/95<br>Date: 1995-11-17<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /03</b>                                   | N | N | - | Bayer<br>CropScience |
| KCA 4.1.2 /04<br>CA B.5.1.2. | Gould, T. J.;<br>Lemke, V. J. | 1995  | An analytical method for the determination of FOE 5043 residues in plant matrices<br>Bayer Corporation, Kansas City, MO, USA<br>Bayer CropScience,<br>Report No.: 106406,<br>Edition Number: M-041601-01-1<br>EPA MRID No.: 43850079<br>Date: 1995-05-11<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /04</b>  | N | N | - | Bayer<br>CropScience |
| KCA 4.1.2 /06<br>CA B.5.2.3. | Riegner, K.                   | 1995  | Methode zur Bestimmung von FOE 5043 in Luft<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00410,<br>Edition Number: M-012833-01-2<br>Method Report No.: MR-798/95<br>Date: 1995-07-19<br>GLP/GEP: yes, unpublished  | N | N | - | Bayer<br>CropScience |
| KCA 4.1.2 /07<br>CA B.5.2.2. | Koenig, T.                    | 1998  | Amendment to method 00489, MR-473/97 - Method for the determination of FOE 5043 in drinking water by HPLC with on-line solid phase extraction<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00489,<br>Edition Number: M-020397-03-1<br>Method Report No.: MR 473/97<br>Method Report No.: MR-012/98<br>Date: 1998-03-13<br>GLP/GEP: no, unpublished | N | N | - | Bayer<br>CropScience |

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| KCA 4.1.2 /12<br>CA B.5.1.2.1. | Seym, M.   | 1997 | Supplement E001 of method 00346 for the determination of residue of total residue of FOE 5043 in/on potato<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00346/E001,<br>Edition Number: M-018872-01-1<br>Method Report No.: MR-388/96<br>Date: 1997-01-16<br>GLP/GEP: yes, unpublished   | N | Y | Completion of data package for data generation methods | Bayer CropScience |
| KCA 4.1.2 /13<br>CA B.5.1.2.1. | Seym, M.   | 1998 | Supplement E002 of method 00346 for the determination of FOE 5043 total residue in/on soybean, plant and tomato, fruit<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00346/E002,<br>Edition Number: M-018878-01-1<br>Method Report No.: MR-400/98<br>Date: 1998-10-16<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /23</b>                              | N | Y | Completion of data package for data generation methods | Bayer CropScience |
| KCA 4.1.2 /14<br>CA B.5.1.2.1. | Rzepka, S. | 2006 | Supplement E004 of Method 00346 for the determination of residues of FOE 5043, FOE 5043 Oxalate, FOE 5043 Sulfonic Acid, and FOE 5043 Thioglycolate Sulfoxide in rice (grain)<br>Eurofins Analytik GmbH, Hamburg, Germany<br>Bayer CropScience,<br>Report No.: 00346/E004,<br>Edition Number: M-277805-01-1<br>Method Report No.: BAY-0610V<br>Date: 2006-09-12<br>GLP/GEP: yes, unpublished | N | Y | Completion of data package for data generation methods | Bayer CropScience |

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| KCA 4.1.2 /15<br>CA B.5.1.2. | Stuke, S.;<br>Teubner, L.            | 2013 | Modification M002 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE 5043) in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg (0.05 mg/kg for straw) by HPLC-MS/MS<br>Bayer CropScience,<br>Report No.: 01100/M002,<br>Edition Number: M-448503-01-1<br>Date: 2013-03-04<br>GLP/GEP: yes, unpublished  | N | Y | Completion of data package for data generation methods                         | Bayer CropScience |
| KCA 4.1.2 /16<br>CA B.5.1.2. | Stuke, S.;<br>Weile, M.              | 2011 | Position paper: Subject: Flufenacet: Answer to CRD questions related to the authorization of the product Liberator SC 500 (flufenacet + diflufenican 400 g/L + 100 g/L) - Comparison of flufenacet residue analytical method nos. 00346 vs. 01179<br>Bayer CropScience,<br>Report No.: M-416013-01-1,<br>Edition Number: M-416013-01-1<br>Date: 2011-10-21<br>GLP/GEP: n.a., unpublished   | N | N | -  | Bayer CropScience |
| KCA 4.1.2 /17<br>CA B.5.1.2. | Stuke, S.,<br>Bauer, J.;<br>Ruhl, S. | 2012 | Modification M001 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg for grain and green material and at a LOQ of 0.05 mg/kg for straw by HPLC-MS/MS<br>Bayer CropScience,<br>Report No.: 01100/M001,<br>Edition Number: M-433720-01-1<br>Method Report No.: MR-11/011<br>Date: 2012-06-13<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /10</b> | N | Y | Completion of data package for data generation methods and enforcement methods | Bayer CropScience |

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| KCA 4.1.2 /18<br>CA B.5.1.2. | Billian, P.                   | 2010 | Analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on plant material<br>Bayer CropScience,<br>Report No.: 01100,<br>Edition Number: M-362575-02-1<br>Method Report No.: 01100<br>Method Report No.: MR-08/060<br>Date: 2010-01-27<br><b>...Amended: 2010-02-04</b><br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /11</b> | N | Y | New simplified single method for data generation and monitoring purposes (3 matrix groups) | Bayer CropScience |
| KCA 4.1.2 /19<br>CA B.5.1.2. | Class, Th.;<br>Merdian, H.    | 2010 | Validation of BCS analytical method no. 01179 for the determination of residues of flufenacet in/on plant materials by HPLC-MS/MS<br>PTRL Europe GmbH, Ulm, Germany<br>Bayer CropScience,<br>Report No.: 01179,<br>Edition Number: M-362716-01-1<br>Method Report No.: B 1778 G<br>Date: 2010-01-22<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.2 /12</b>                      | N | Y | New simplified single method for data generation and monitoring purposes (cereal matrices) | Bayer CropScience |
| KCA 4.1.2 /20<br>CA B.5.1.2. | Gould, T. J.                  | 1995 | Extraction efficiency of the analytical method for the determination of FOE 5043 residues in plant matrices<br>Bayer Corporation, Kansas City, MO, USA<br>Bayer CropScience,<br>Report No.: 106927,<br>Edition Number: M-041609-01-1<br>Date: 1995-06-02<br>GLP/GEP: yes, unpublished  | N | Y | EU data requirement  | Bayer CropScience |
| KCA 4.1.2 /21<br>CA B.5.1.2. | Beedle, E. C.;<br>Ying, S. L. | 2000 | The metabolism of [fluorophenyl-UL-14C] Flufenacet in potatoes<br>Bayer Corporation, Stilwell, KS, USA<br>Bayer CropScience,<br>Report No.: 109226,<br>Edition Number: M-020428-01-1<br>Date: 2000-04-28<br>GLP/GEP: yes, unpublished  | N | Y | Completion of data package, additional crop  | Bayer CropScience |



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| KCA 4.1.2 /22<br>CA B.5.1.2.           | Krolski, M. E.;<br>Bosnak, L. L.                | 1997  | The metabolism of [Fluorophenyl-UL-14C] FOE 5043 in wheat after postemergent foliar spray application<br>Bayer Corporation, Stilwell, KS, USA<br>Bayer CropScience,<br>Report No.: 107399,<br>Edition Number: M-002275-01-1<br>EPA MRID No.: 45012403<br>Date: 1997-11-04<br>GLP/GEP: yes, unpublished  | N | Y | Completion of data package, post-emergence use on cereals | Bayer CropScience |
| KCA 4.1.2 /23<br>CA B.5.1.2.1.         | Gould, T. J.                                    | 1995  | Extraction efficiency of the analytical method for the determination of FOE5043 residues in animal matrices<br>Bayer Corporation, Stilwell, KS, USA<br>Bayer CropScience,<br>Report No.: 106926,<br>Edition Number: M-071501-01-1<br>Date: 1995-08-16<br>GLP/GEP: yes, unpublished  | Y | Y | EU data requirement                                       | Bayer CropScience |
| KCA 4.2 /01<br>B.4.2.1.<br>CA B.5.2.1. | Seym, M.  | 1995a | Amendment no 1 to report: MR-981/95 - Analytical method for the determination of the total residue of FOE 5043 in plant materials<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00346,<br>Edition Number: M-018864-02-1<br>Method Report No.: MR-981/95<br>Date: 1995-10-20<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /01</b> | N | N | -   | Bayer CropScience |
| KCA 4.2 /02<br>B.4.2.1.<br>CA B.5.2.1. | Gould, T. J.;<br>Lemke, V. J.;<br>Zoloty, K. L. | 1995  | An analytical method for the determination of FOE 5043 residues in animal matrices<br>Bayer Corporation, Kansas City, MO, USA<br>Bayer CropScience,<br>Report No.: 00418,<br>Edition Number: M-019605-01-1<br>Method Report No.: F3120201<br>Date: 1995-04-10<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /02</b>                                       | N | N | -   | Bayer CropScience |

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| KCA 4.2 /03<br>B.4.2.1.<br>CA B.5.2.1. | Seym, M.                      | 1995b | Modification M001 of method 00418 for the determination of FOE 5043 residues in animal matrices<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00418/M001,<br>Edition Number: M-019614-01-1<br>Method Report No.: MR-1118/95<br>Date: 1995-11-17<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /03</b> | N | N | - | Bayer CropScience |
| KCA 4.2 /04<br>B.4.2.1.<br>CA B.5.2.1. | Gould, T. J.;<br>Lemke, V. J. | 1995  | An analytical method for the determination of FOE 5043 residues in plant matrices<br>Bayer Corporation, Kansas City, MO, USA<br>Bayer CropScience,<br>Report No.: 106406,<br>Edition Number: M-041601-01-1<br>EPA MRID No.: 43850079<br>Date: 1995-05-11<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /04</b>                | N | N | - | Bayer CropScience |
| KCA 4.2 /05<br>B.4.2.1.<br>CA B.5.2.1. | Seym, M.                      | 1994  | Independent laboratory validation of the residue analytical method for FOE 5043 residues in plant<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 106907,<br>Edition Number: M-014828-01-1<br>Date: 1994-06-24<br>GLP/GEP: yes, unpublished   | N | N | - | Bayer CropScience |
| KCA 4.2 /06<br>B.4.2.2.<br>CA B.5.2.1. | Bajzik, M. E.                 | 1995  | Independent laboratory validation of the analytical method for the determination of FOE 5043 in animal matrices (miles report no.106773)<br>Huntingdon Analytical Services, Middleport, NY, USA<br>Bayer CropScience,<br>Report No.: 106913,<br>Edition Number: M-071918-01-1<br>Date: 1995-03-22<br>GLP/GEP: yes, unpublished              | N | N | - | Bayer CropScience |

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| KCA 4.2 /08<br>CA B.5.2.2. | Bethem, R. A.;<br>Peterson, R. G.;<br>Leimkuehler, W.<br>A.;<br>Mattern, G. C. | 1995 | Determination of FOE 5043 and the alcohol, Oxalate, thiadone and sulfonic acid metabolites in groundwater by high performance liquid chromatography electrospray tandem mass spectrometry (LC-ESI/MS/MS)<br>Alta Analytical Laboratory, Inc., El Dorado Hills, CA, USA<br>Bayer CropScience,<br>Report No.: 107138,<br>Edition Number: M-070022-01-2<br>Date: 1995-06-22<br>GLP/GEP: no, unpublished   | N | N | -  | Bayer<br>CropScience |
| KCA 4.2 /09<br>CA B.5.2.2. | Allmendinger, H.;<br>Bachlechner, G.   | 1994 | Validated method for the determination of the herbicide FOE 5043 and its metabolites FOE 5043-alcohol, FOE 5043-oxalate and FOE 5043-sulfonic acid in soil using HPLC-MS-MS<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 107725,<br>Edition Number: M-019071-01-3<br>Method Report No.: RA-246/96<br>Method Report No.: RA-399/94<br>Date: 1994-07-01<br>GLP/GEP: yes, unpublished  | N | N | -  | Bayer<br>CropScience |
| KCA 4.2 /10<br>CA B.5.2.1. | Stuke, S.,<br>Bauer, J.;<br>Ruhl, S.   | 2012 | Modification M001 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg for grain and green material and at a LOQ of 0.05 mg/kg for straw by HPLC-MS/MS<br>Bayer CropScience,<br>Report No.: 01100/M001,<br>Edition Number: M-433720-01-1<br>Method Report No.: MR-11/011<br>Date: 2012-06-13<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /17</b> | N | Y | Completion of data package for data generation methods and enforcement methods | Bayer<br>CropScience |

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| KCA 4.2 /11<br>CA B.5.2.1. | Billian, P.                | 2010 | Analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on plant material<br>Bayer CropScience,<br>Report No.: 01100,<br>Edition Number: M-362575-02-1<br>Method Report No.: 01100<br>Method Report No.: MR-08/060<br>Date: 2010-01-27<br><b>...Amended: 2010-02-04</b><br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /18</b> | N | Y | New simplified single method for data generation and monitoring purposes (3 matrix groups) | Bayer CropScience |
| KCA 4.2 /12<br>CA B.5.2.1. | Class, Th.;<br>Merdian, H. | 2010 | Validation of BCS analytical method no. 01179 for the determination of residues of flufenacet in/on plant materials by HPLC-MS/MS<br>PTRL Europe GmbH, Ulm, Germany<br>Bayer CropScience,<br>Report No.: 01179,<br>Edition Number: M-362716-01-1<br>Method Report No.: B 1778 G<br>Date: 2010-01-22<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /19</b>                      | N | Y | New simplified single method for data generation and monitoring purposes (cereal matrices) | Bayer CropScience |
| KCA 4.2 /13<br>CA B.5.2.3. | Hellpointner, E.           | 2000 | Confirmatory method for the determination of FOE 5043 in air (confirmed method: 00410)<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00410C,<br>Edition Number: M-048783-01-1<br>Method Report No.: MR-469/00<br>Date: 2000-10-25<br>GLP/GEP: yes, unpublished   | N | Y | EU data requirement - confirmatory method for enforcement method in air                    | Bayer CropScience |

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| KCA 4.2 /14<br>CA B.5.2.1. | Class, T.    | 2004 | Independent laboratory validation (ILV) of a common moiety residue method for the determination of Flufenacet (FOE 5043) and 3 metabolites in wheat grain (Bayer CropScience method 00346)<br>PTRL Europe GmbH, Ulm, Germany<br>Bayer CropScience,<br>Report No.: P740G,<br>Edition Number: M-072609-01-1<br>Method Report No.: P740G<br>Date: 2004-05-13<br>GLP/GEP: yes, unpublished  | N | Y | Completion of data package for enforcement methods | Bayer CropScience |
| KCA 4.2 /15<br>CA B.5.2.1. | Klimmek, S.  | 2005 | Enforcement method for the determination of residues of FOE 5043, FOE 5043 Oxalate, FOE 5043 Sulfonic Acid, and FOE 5043 Thioglycolate Sulfoxide in materials of apples<br>Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany<br>Bayer CropScience,<br>Report No.: BAY-0408V,<br>Edition Number: M-088233-02-1<br>Method Report No.: BAY-0408V<br>Date: 2004-07-29<br><b>...Amended: 2005-06-07</b><br>GLP/GEP: yes, unpublished | N | Y | Completion of data package for enforcement methods | Bayer CropScience |
| KCA 4.2 /16<br>CA B.5.2.2. | Brumhard, B. | 2005 | Modification M001 of method 00359 for the determination of the herbicide FOE 5043 and its metabolite FOE 5043-alcohol, FOE 5043-oxalate and FOE 5043-sulfonic acid in soil using HPLC-MS/MS<br>Bayer CropScience,<br>Report No.: 00359/M001,<br>Edition Number: M-248543-01-1<br>Method Report No.: MR-028/05<br>Date: 2005-04-01<br>GLP/GEP: yes, unpublished  | N | Y | EU data requirement - Enforcement method in soil   | Bayer CropScience |

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| KCA 4.2 /17<br>CA B.5.2.1. | Meyer, M.                                  | 2011 | Independent laboratory validation of the Bayer CropScience methods 01100 and 01179 for the determination of residues of Flufenacet (FOE5043) in/on plant materials<br>SGS Institut Fresenius GmbH, Taunusstein, Germany<br>Bayer CropScience,<br>Report No.: P612107502,<br>Edition Number: M-405654-01-1<br>Method Report No.: IF-10/01717126<br>Date: 2011-04-14<br>GLP/GEP: yes, unpublished                    | N | Y | Data requirement:<br>ILV for new enforcement method  | Bayer CropScience |
| KCA 4.2 /18<br>CA B.5.2.1. | Klimmek, S.;<br>Goellner, C.;<br>Amann, S. | 2013 | Validation of the Bayer methods 00418 (M-019605-01-1) and 00418/M001 (M-019614-01-1) for the determination of residues of flufenacet (FOE 5043) and its metabolites in animal tissues and animal products<br>Eurofins Agrosience Services Chem GmbH (EAS Chem), Hamburg, Germany<br>Bayer CropScience,<br>Report No.: S12-00052,<br>Edition Number: M-461242-01-1<br>Date: 2013-05-13<br>GLP/GEP: yes, unpublished | N | Y | Data requirement:<br>ILV for residue analytical method on animal matrices including additional validation data | Bayer CropScience |
| KCA 4.2 /19<br>CA B.5.2.2. | Krebber, R.;<br>Braune, M.                 | 2013 | Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS<br>Bayer CropScience,<br>Report No.: MR-13/085,<br>Edition Number: M-466732-01-1<br>Method Report No.: MR-13/085<br>Date: 2013-10-09<br>GLP/GEP: yes, unpublished  | N | Y | EU data requirement; Data generation method  | Bayer CropScience |
| KCA 4.2 /20<br>CA B.5.2.1. | Weile, M.                                  | 2013 | Flufenacet : Evaluation of EFSA's recommendation to develop a multi residue method for enforcement purpose as replacement of the single common moiety method<br>Bayer CropScience,<br>Report No.: M-457898-01-1,<br>Edition Number: M-457898-01-1<br>Date: 2013-04-16<br>GLP/GEP: n.a., unpublished  | N | N | -  | Bayer CropScience |

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| KCA 4.2 /21<br>CA B.5.2.1. | Dujardin, B.     | 2013 | Potential and possible solutions for simplifying complex residue definitions<br>Publisher: Anon.,<br>Location: Anon.,<br>Journal: Anon.,<br>Pages: 1-11,<br>Year: 2012,<br>Report No.: M-459903-01-1,<br>Edition Number: M-459903-01-1<br>GLP/GEP: n.a., published  | N | N | -  |                   |
| KCA 4.2 /22<br>CA B.5.2.2. | Stanislawski, T. | 2013 | Independent laboratory validation of BCS analytical methods 01333 and 01387 for determination of various pesticides in surface water by Di-HPLC-MS/MS<br>PTRL Europe, Ulm, Germany<br>Bayer CropScience,<br>Report No.: P3117 G,<br>Edition Number: M-470714-02-1<br>Date: 2013-12-13<br>GLP/GEP: yes, unpublished  | N | Y | ILV to support new method                              | Bayer CropScience |
| KCA 4.2 /23<br>CA B.5.2.1. | Seym, M.         | 1998 | Supplement E002 of method 00346 for the determination of FOE 5043 total residue in/on soybean, plant and tomato, fruit<br>Bayer AG, Leverkusen, Germany<br>Bayer CropScience,<br>Report No.: 00346/E002,<br>Edition Number: M-018878-01-1<br>Method Report No.: MR-400/98<br>Date: 1998-10-16<br>GLP/GEP: yes, unpublished<br><b>...also filed: KCA 4.1.2 /13</b> | N | Y | Completion of data package for data generation methods | Bayer CropScience |
| KCA 4.2/13<br>CA B.5.2.4   | Kaussmann, M.    | 2016 | Analytical Method 01486 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS<br>Bayer CropScience<br>Report No.: P683166504<br>Edition Number: M-556577-01-1<br>GLP/GEP: yes, unpublished   | N | Y | New method for renewal purposes                        | Bayer CropScience |