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Napropamide-M
Volume 3 – B.5 (AS)

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B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Methods for the analysis of the active substance as manufactured

Methods are available for analysis of napropamide-M technical material.

1) Method 1- Method of analysis and validation for active ingredient analysis of napropamide in napropamide-M technical (JRF Study No: 228-2-12-6268) using reverse phase HPLC-UV. This is for analysis of 'total napropamide' as active ingredient technical (of technical grade napropamide-M) using a non chiral specific approach.
2) Method 2 – Method of analysis and validation for analysis of napropamide in napropamide-M technical grade active ingredient (TGAI) (JRF Study No: 228-2-12-7332) using GC-MS. This is for analysis of 'total napropamide' as active ingredient technical (of technical grade napropamide-M) using a non chiral specific approach (this is also the method of analysis used for impurities, as further explained in volume 4, confidential section).
3) Method-3- Normal phase HPLC-UV to elucidate the D-isomer (napropamide-M) and L-isomer ratio of napropamide-M technical (JRF Study No: 228-2-12-6268). This analyses D- and L-isomer separately using chiral analysis.

B.5.1.1.1. Method 1 - (JRF Study No: 228-2-12-6268)

The method determines the active ingredient content of 'total napropamide' in technical grade napropamide-M by reverse phase HPLC chromatography with UV detection ('Method No. URCT/DNE-263/TM/01').

Samples were prepared by accurately weighing approximately 10mg of the technical material into 10mL volumetric flask and then diluted with acetonitrile. An aliquot of this solution (0.4 mL) was further diluted to the total volume of 10 mL with acetonitrile prior determination. All samples and standards were analysed using reverse phase HPLC-UV at 210 nm using C18 column (150 x 4.6 mm, 3.5µm), mobile phase of acetonitrile : water (70 : 30v/v) with isocratic elution.

Quantitation was by external standard technique.

A summary of the validation data supporting the method is outlined in Table B5.1.1.1.-1 below.

Table B.5.1.1.1-1: Validation of method 1 (active substance in technical material)

Analyte	Recovery		Repeatability % RSD	Linearity	Specificity
	Fortification level (% w/w)	% range (Mean)			
Napropamide (note, the napropamide-M technical material was analysed as napropamide, as the reverse phase HPLC-UV method does not separate the D-isomer and the L-isomer)	Not required for the active in the TGAI		0.25 % (Modified Horwitz = 1.35 @ 97.26 % w/w). (n=5 technical material batch 20121226)	Range 10.32 - 103.21 mg/L (ca. 25-250 %w/w) R=0.999 (n=6)	Example chromatogram of a blank, reference standard and technical material were provided. No interferences were observed and there was a retention time match between the analyte in the sample and standard.

The method is satisfactorily validated in accordance with SANCO3030/99/rev.4 for the determination of napropamide in the technical material for napropamide-M.

B.5.1.1.2. Method 2 (JRF Study No: 228-2-12-7332)

Method 2 determines the napropamide active ingredient content of napropamide-M in technical grade material with process related impurities by GC/MS technique. The active substance was analysed as napropamide rather than as napropamide-M specifically as the method of analysis does not separate out the D-isomer (napropamide-M) and the L-isomer. The validation presented for impurities is in Volume 4 (confidential section).

Samples were prepared by accurately weighing approximately 20mg of the technical material into 10 mL volumetric flask and then diluted to volume with dichloromethane. An aliquot of this solution (0.25 mL) is further diluted to a total volume of 10 mL with dichloromethane prior to determination. All samples and standards were analysed using GC/MS with temperature program elution on a VF-5 MS column (30 m x 0.25 mm, 0.25µm film thickness) Response to the MS detector was monitored as a total ion chromatogram (TIC) with MS spectra and major ions considered for characterisation.

Ion mass (MS)

Napropamide-M parent ion= 271.1; daughter ion 1 = 171.1 ; daughter ion 2= 155.1

Quantitation was by external standard technique.

Confirmation was achieved by full characterisation employing GC-MS, FT-IR and NMR analysis solution of napropamide-M reference standard and napropamide-M technical material.

Validation of the method for napropamide was by the assessment of specificity, linearity and precision.

A summary of the validation data supporting the method is outlined in Table B5.1.1.2.-2 below.

Table B.5.1.1.2-2: Validation of method 2 (active substance in technical material using GC-MS)

Analyte	Recovery		Repeatability % RSD	Linearity	Specificity
	Fortification level (% w/w)	% range (Mean)			
Napropamide (note, the napropamide-M technical material was analysed as napropamide, as the GC-MS method does not separate the D-isomer and the L-isomer)	Not required for the active in the technical material		0.19 % (Modified Horwitz = 1.34 @ 98.24 % w/w). (n=5, technical material batch 20131125)	Range 10.89 - 108.90 mg/L (ca. 21.78-217.80%w/w) R=0.999 (n=6)	Example chromatogram of a blank, reference standard and technical material were provided. No significant interferences were observed in regard of the analysis of the active substance and there was a retention time match between the analyte in the sample and standard*

*an interference potentially affecting the analysis of an impurity is discussed in the confidential section. This is not considered to significantly affect the determination of the level of napropamide in the technical material.

The method is satisfactorily validated in accordance with SANCO3030/99/rev.4 for the determination of napropamide in the technical material for napropamide-M.

B.5.1.1.3. Method 3 (JRF Study No: 228-2-12-6268)

Method 3 determines the ratio of D-isomer (napropamide-M) and L-isomer of napropamide in technical grade material.

For the assessment of isomer ratio aliquots of samples and isomer (napropamide-M / D-isomer and L-isomer) reference solutions were determined by chiral normal phase HPLC-UV analysis. An amount of napropamide-M technical material (nominally 13 mg) was weighed into a 25 mL flask and diluted with chiral analysis mobile phase. The chiral HPLC analysis was performed with a Chiracel, OD-H column (250 x 4.6 mm , 5 µm), mobile phase of n-hexane : ethanol (99:1%, v/v) with isocratic elution and UV detector monitoring at 230 nm allowed determination of the two napropamide D and L isomers.

The isomer ratios were derived by calculating the relative amount of the area of the respective D-isomer and L-isomer peaks compared to the total sum of the peak areas for 'area of peak for D-isomer plus area of peak for L-isomer', comparing the retention times of each peak with the respective analytical standards (for the D-isomer and L-isomer).

The ratio for napropamide-M technical material batch no 20121226 was determined to be 96.75% : 3.25% (D-isomer : L-isomer) and an acceptable chromatogram was available.

In the five batch analyses (see vol 4, confidential section), the method was applied showing consistent determination of peak ratios for each of the five batches. See the confidential information section C.1.5.2 for the analysis data presented, as this repeated assessment of isomer ratio (based on peak area comparisons) represents the validation data available.

Some further validation data was provided in a separate report (Amruskar, 2017) showing suitable linearity of the method for determination of both D- and L-isomer, together with evidence of specificity of the method and system precision (please refer to the v4 confidential section for details of the validation data available for this method)

B.5.1.1.4. Overview of methods for the determination of active substance in the technical material.

The reverse phase HPLC-UV and GC-MS methods available are acceptable for the determination of 'total napropamide content' in technical material of napropamide-M. A chiral method of analysis (normal phase HPLC-UV) is available to separate the D-isomer (napropamide-M) and the L-isomer, and this method has been used to calculate isomer ratios, comparing the retention times of each peak with the respective analytical standards (for the D-isomer and L-isomer). An acceptable chromatogram was submitted. Further validation data based on repeated analysis and assessment of the isomer ratios in each of the five batch analysis are available and presented in section C.1.5.2. Further validation data (linearity of response, specificity, and system precision) to support this determination of isomer ratios was submitted in a later report (Amruskar, 2017) and is also summarised in vol 4.

B.5.1.2. Methods for risk assessment

Consideration of the acceptability of the methods of analysis for risk assessment purposes will be considered in the relevant sections of the DAR.

B.5.1.2.1. METHODS USED TO GENERATE PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE

B.5.1.2.1.1. Physical and chemical determinations of napropamide-M technical and purified material. (Report No. J19544) – Bates, 2016a

Method: M789

1) determination of water solubility of napropamide-M technical

The determination of the solubility of napropamide-M (technical) in water was conducted using the method EEC A6 ('shake flask' method). HPLC-UV was employed for the analysis of the napropamide-M content within the test solution.

Protocol

An amount of the napropamide-M reference standard (nominally 10 or 15 mg) was dissolved in acetonitrile (total volume of 100 mL) in volumetric flask to yield a stock solution with nominal concentration of either 0.1 or 0.15 mg/mL. These solutions could be diluted as necessary for calibration or fortification.

Samples of water form solubility testing were determined directly for concentrations of napropamide-M using HPLC-UV, injecting 10µL aliquots of test samples or fortified accuracy samples.

The HPLC system was equipped with a Hichrom H5 ODS (150 x 4.6 mm) column, mobile phase of acetonitrile : water (70 :30% v/v) with isocratic elution and detection of napropamide-M was carried out at 210 nm.

Validation

A summary of the validation data supporting the method is outlined in Table B.5.1.2.1.1-1 below

Table B.5.1.2.1.1-1: Validation accuracy and precision data for the determination of napropamide-M in support of water solubility

Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (mg/L)	% Range (Mean %)		
Napropamide-M	39.1	39.1 (n=5)	96.7-103.0 (100.1)	2.28	0.016 – 0.048 mg/mL R= 1.000 n = 6, in duplicate

2) Determination of n-octanol/water partition coefficient

Protocol

OECD method 107 with HPLC-UV was used for determination of n-octanol/water partition coefficient of napropamide-M technical. The HPLC was equipped with a Hichrom H5 ODS (150 x 4.6 mm) column and UV detection of napropamide-M was carried out at 210 nm.

Analysis was performed once the portioned phases were obtained, following the completion of the experimental procedures according to EEC A8 and OECD 107 guidelines (shake flask method).

Octanol phase:

Samples of water-saturated octanol, used for partition co-efficient testing (5.0 mL aliquots), were transferred to 100mL volumetric flask and diluted to the mark with acetonitrile. Solutions were then analysed using HPLC-UV, to assess the napropamide-M content.

A summary of the validation data supporting the method is outlined in Table B.5.1.2.1.1-2 below

Table B.5.1.2.1.1-2: Validation accuracy and precision data for the determination of napropamide-M in support of partition co-efficient testing (water saturated octanol phase)

Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (mg/L)	% Range (Mean %)		
Napropamide-M	2503.7	2503.7 (n=5)	96.4 – 99.0 (98.0)	1.09	0.067-0.21 mg/mL r = 0.9999 n = 6, in duplicate

Water phase:

Samples of octanol-saturated water, used for partition co-efficient testing, were analysed using HPLC-UV, to assess the napropamide-M content.

Validation

A summary of the validation data supporting the method is outlined in Table B.5.1.2.1.1-3below

Table B.5.1.2.1.1-3: Validation accuracy and precision data for the determination of napropamide-M in support of partition co-efficient testing (octanol saturated water phase)

Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (mg/L)	% Range (Mean %)		
Napropamide-M	0.00135	0.00135 (n=5)	86.9 – 95.0 (92.2)	3.38	0.00061-0.0024 mg/mL r = 0.9927 n = 6, in duplicate

Method specificity was determined by the injection of the napropamide-M reference standards, fortified matrix sample extracts and blank matrix sample extracts to assess the potential for interference and the match of retention times. There were no significant interferences at the retention time for napropamide-M. The retention times of napropamide-M in reference substance standards and in sample extracts were confirmed.

Conclusion

Method M789 for the determination of napropamide-M concentration in water, octanol-saturated water and water-saturated octanol for support of physical and chemical properties studies is satisfactorily validated in accordance with SANCO3029/99/rev.4

B.5.1.2.1.2. 'For the determination of metabolites of napropamide-M in octanol and water phases for support of partition testing'. (Report No. J20144) – Bates, 2016b

Method M844:

OECD method 107 with GC/MS was used for determination of n-octanol/water partition coefficient of metabolites of napropamide-M technical. The GC/MS was equipped with Zebron 5MS (30m x 0.25mm x0.25µm film) column, injection temperature of 190ºC and set in Selective Ion Mass detection mode.

Analysis was performed once obtaining portioned phases after the completion of the experimental procedures according to EEC A8 and OECD 107 guidelines (shake flask method).

Protocol

Octanol phase:

Each metabolite was analysed individually, with each test conducted in duplicate. After partitioning the test sample, the sample vessels were centrifuged at 2000 rpm for minimum 5 minutes, to ensure both phases were

completely separated. Using a micro pipette, an aliquot of 0.50mL was carefully removed from the resulting upper layer of the phase solutions and decanted into 20mL volumetric flask. The solution was accurately made up to volume with dichloromethane and shaken thoroughly to mix. A 500µL aliquot was transfer into amber vial and a 50µL aliquot of the octanol phase internal standard was added. The vial was shaken and an aliquot of the sample was analysed using GC-MS.

Water phase:

Each metabolite was analysed individually, with each test conducted in duplicate. After partitioning the test sample, the sample vessels were centrifuged at 2000 rpm for minimum 5 minutes, to ensure both phases were completely separated. The upper octanol layer was removed with small glass pipette then a quantity of the water phase was extracted from the centrifuged test vessel, using a Pasteur pipette.

From the resulting aqueous phase (octanol-saturated water solution), a 500µL aliquot was transfer to the amber vial. A 50 µL aliquot of the water phase internal standard was added to the vial, mixed and then analysed by GC/MS.

Naphthalen-1-ol (Alpha-Naphthol)

Octanol phase:
A summary of the validation data supporting the method is outlined in B.5.1.2.1.2-1 below

Table B.5.1.2.1.2-1: Validation accuracy and precision data for the determination of Alpha-Naphthol in support of partition co-efficient testing (water saturated octanol phase)

Analyte	LOQ (µg/mL)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (µg/mL)	% Range (Mean %)		
Naphthalen-1-ol (Alpha-Naphthol)	735.3	735.3* (n=5)	103.8 – 113.0 (109.8) (n=5)	3.62 (n=5)	5.5 – 55.1 µg/mL r = 0.9996 n = 6

* The preparation of the octanol phase fortification samples includes a 40-fold dilution step. This gives the injected samples a nominal concentration of 18.4 µg/mL, which is within the tested linear range.

Acceptable validation data were obtained for this portion of the methodology – the validation data provided comply with the requirements outlined within the SANCO3029/99/rev.4 guidance document.

Water phase:

A summary of the validation data supporting the method is outlined in B.5.1.2.1.2-2 below

Table B.5.1.2.1.2-2: Validation accuracy and precision data for the determination of Alpha-Naphthol in support of partition co-efficient testing (octanol saturated water phase)

Analyte	LOQ (µg/mL)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (µg/mL)	% Range (Mean %)*		
Napropamide-M	0.369	0.369 (n=5)	64.1* – 105.0 (83.8) (n=5)	17.5	0.14 – 1.4 µg/mL r = 0.9915 n = 6

* The individual recoveries are outside of the acceptable criteria.

It is appreciated from the validation data for the recoveries that some were below the 70% lower limit. Though these results lie below the lower limit and hence underestimate the analyte content, they may be considered to be sufficiently close so as to support pre-registration data generated using this methodology.

The spacing in the results obtained over the range 64.1 – 105.0% consequently led to a high %RSD value of 17.5. This value remains within the 20% limit, indicated within the requirements set in the SANCO3029/99/rev.4 guidance.

While it is appreciated that the validation data do not strictly comply with the criteria set in the SANCO3029/99/rev.4 guidance, it is considered that the validation data provide sufficient support for pre-registration data generated using this methodology. The method is therefore regarded as being fit for its intended purpose.

2-(1-naphthyloxy) propanoic acid [NOPA]

Octanol phase:
A summary of the validation data supporting the method is outlined in B.5.1.2.1.2-3 below

Table B.5.1.2.1.2-3: Validation accuracy and precision data for the determination of NOPA in support of partition co-efficient testing (water saturated octanol phase)

Analyte	LOQ (µg/mL)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (µg/mL)	% Range (Mean %)		
2-(1-naphthyloxy) propanoic acid [NOPA]	1180.4	1180.4* (n=5)	97.1 – 105.6 (102.3) (n=5)	3.29 (n=5)	6.6 – 66.4 µg/mL r = 0.9962 (quadratic) n = 6

* The preparation of the octanol phase fortification samples includes a 40-fold dilution step. This gives the injected samples a nominal concentration of 29.5µg/mL, which is within the tested linear range.

As the instrument response to concentration does not conform to a first-order (straight line) fit, the data points were subjected to a second order quadratic fit to compensate. The model equation for the quadratic fit is:

$A = Ac^2 + bC + c$

Where:
A = Measured signal (y-axis)
C = Concentration (x-axis)
a,b,c = The coefficients from the quadratic fit

The resulting equation from the plot is $y=0.0011x^2 – 0.0222x + 0.1561$ (with an R^2 value of 0.9962) which was used to calculate the recovered amount of [NOPA] in the octanol phase. The Notifier has clarified that the *"calibration will be checked when the method is used and if non-linear again then that confirms the most accurate fit is applied*", which is considered by the RMS to suitably address the requirement for calibration accuracy in the SANCO3029/99/rev.4 guidance. This procedure also applies to the other metabolites, for which quadratic curves were found to provide a more appropriate fit.

Acceptable validation data were obtained for this portion of the methodology – the validation data provided comply with the requirements outlined within the SANCO3029/99/rev.4 guidance document.

Water phase:

Linearity: Six standard solutions were prepared in the concentration range of 0.165 – 1.660 µg/mL of NOPA in acetonitrile. These were then injected onto the GC/MS to establish the linearity of the response.

There was no discernible response for NOPA in water. In all cases, the amounts present were below the limit of detection. The LOD, from the octanol data, appears to be around 5µg/mL. Therefore all concentrations below this level are not quantifiable by this method of analysis.

Recovery: Determinations were carried out at the approximate, expected level of the NOPA which could be partitioned into the water phase, with five replicates at this level (concentration of 0.559µg/mL). There was no instrument response recorded to this concentration.

Precision: It was not possible to perform precision experiments as the level of NOPA present were below the limit of detection.

Due to the limitations of the analytical method, validation data generated in accordance with SANCO3029/99/rev.4 could not be obtained for the water phase.

N,N-diethyl-2(4-hydroxy-1-naphthyl)propanamide (napropamide isomer I)

Octanol phase:
A summary of the validation data supporting the method is outlined in Table B.5.1.2.1.2-4 below

Table B.5.1.2.1.2-4: Validation accuracy and precision data for the determination of napropamide isomer I in support of partition co-efficient testing (water saturated octanol phase)

Analyte	LOQ (µg/mL)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (µg/mL)	% Range (Mean %)		
Napropamide isomer I	1315.4	1315.4* (n=5)	95.8 – 102.9 (98.9) (n=5)	2.85 (n=5)	5.01 – 50.1 µg/mL R^2 = 0.9988 (quadratic) n = 6

* The preparation of the octanol phase fortification samples includes a 40-fold dilution step. This gives the injected samples a nominal concentration of 32.9 µg/mL, which is within the tested linear range.

As the instrument response to concentration does not conform to a first-order (straight line) fit, the data points were subjected to a second order quadratic fit to compensate. The model equation for the quadratic fit is:

$A = Ac^2 + bC + c$

Where:
A = Measured signal (y-axis)
C = Concentration (x-axis)
a,b,c = The coefficients from the quadratic fit

The resulting equation from the plot is $y = 0.6827x^2 – 0.4407x + 0.1123y$ (with an R^2 value of 0.9771) which was used to calculate the recovered amount of napropamide isomer I in the octanol phase.

Acceptable validation data were obtained for this portion of the methodology – the validation data provided comply with the requirements outlined within the SANCO3029/99/rev.4 guidance document.

Water phase:

A summary of the validation data supporting the method is outlined in B.5.1.2.1.2-5 below

Table B.5.1.2.1.2-5: Validation accuracy and precision data for the determination of napropamide isomer I in support of partition co-efficient testing (octanol saturated water phase)

Analyte	LOQ (µg/mL)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (µg/mL)	% Range (Mean %)		
Napropamide isomer I	0.662	0.662 (n=5)	81.1 – 117.5 (98.5) (n=5*)	15.5	0.125 – 1.00 µg/mL r = 0.996 n = 5*

* One sample, standard 6, at the level of 1.25µg/mL was identified as an outliner by Dixon's test.

It is noted that the recovery data provided show recoveries exceeding the 110% upper limit, set within the SANCO3029/99/rev.4 guidance. While it is acknowledged that this result lies outside of the acceptable range, it is considered that this this slight bias towards overestimating the napropamide-M content within the octanol saturated water phase would not have a major impact upon the data generated using this methodology.

While it is appreciated that the validation data do not strictly comply with the criteria set in the SANCO3029/99/rev.4 guidance, it is considered that the validation data provide sufficient support for pre-registration data generated using this methodology. The method is therefore regarded as being fit for its intended purpose.

N,N-diethyl-2(1-hydroxy-2-naphthyl)propanamide (napropamide isomer II)

Octanol phase:
A summary of the validation data supporting the method is outlined in Table B.5.1.2.1.2-6 below

Table B.5.1.2.1.2-6: Validation accuracy and precision data for the determination of napropamide isomer II in support of partition co-efficient testing (water saturated octanol phase)

Analyte	LOQ (µg/mL)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (µg/mL)	% Range (Mean %)		
Napropamide isomer II	1105.6	1105.6* (n=5)	27.1 – 27.5 (27.3) (n=5)	0.930 (n=5)	4.97 – 49.7 µg/mL R^2 = 0.9951 (quadratic) n = 6

* The preparation of the octanol phase fortification samples includes a 40-fold dilution step. This gives the injected samples a nominal concentration of 27.6 µg/mL, which is within the tested linear range.

As the instrument response to concentration does not conform to a first-order (straight line) fit, the data points were subjected to a second order quadratic fit to compensate. The model equation for the quadratic fit is:

$A = Ac^2 + bC + c$

Where:
A = Measured signal (y-axis)
C = Concentration (x-axis)
a,b,c = The coefficients from the quadratic fit

The resulting equation from the plot is $y=0.0001x^2 – 0.0139x + 0.0688$ (with an R^2 value of 0.9951) which was used to calculate the recovered amount of napropamide isomer II in the octanol phase.

The mean recovery value of 27.3% is outside the acceptance criterion range of 70 – 110%.

As the methodology consistently underestimates the recovery of the analyte, this portion of the method cannot be considered to fulfil the requirements stated within the SANCO3029/99/rev.4 guidance. It is however noted that the validation data demonstrate that the method is precise – should the recoveries be taken into account, then it may be practical to estimate the concentration within the octanol partition. Consideration of this approach has been taken when reporting the data in the B2 section of the DAR.

Water phase:

A summary of the validation data supporting the method is outlined in Table B.5.1.2.1.2-7 below

Table B.5.1.2.1.2-7: Validation accuracy and precision data for the determination of napropamide isomer II in support of partition co-efficient testing (octanol saturated water phase)

Analyte	LOQ (µg/mL)	Recovery		Repeatability %RSD	Linearity
		Fortification Level (µg/mL)	% Range (Mean %)		
Napropamide isomer II	0.688	0.688 (n=5)	44.2 – 108.0 (85.4) (n=5)	30.1	0.124 – 1.24 µg/mL R^2 = 0.9948 (quadratic) n = 6

As the instrument response to concentration does not conform to a first-order (straight line) fit, the data points were subjected to a second order quadratic fit to compensate. The model equation for the quadratic fit is:

$A = Ac^2 + bC + c$

Where:
A = Measured signal (y-axis)
C = Concentration (x-axis)
a,b,c = The coefficients from the quadratic fit

The resulting equation from the plot is $y=0.1225x^2 – 0.0084x + 0.0899$ (with an R^2 value of 0.9948) which was used to calculate the recovered amount of napropamide isomer II in the water phase.

Recovery: The individual recovery for one sample (44.2%) is outside acceptable limits of 70-110%.
Precision: The %RSD is outside acceptable limits of 20%

Based on the above points, this portion of the method cannot be considered to fulfil the requirements stated within the SANCO3029/99/rev.4 guidance – furthermore it cannot be regarded as being fit for its intended purpose.

Further validation data would be necessary, possible with revisions to the proposed methodology, to confirm the analysis of the analyte within this matrix.

B.5.1.2.2 METHOD USED TO GENERATED TOXICOLOGICAL DATA

B.5.1.2.2.1 – 'Validation of analytical method for determination of napropamide-M technical material concentration, homogeneity and stability in test diet' (Report No. 228-2-13-6178 with Amended Final Report) – Raithatha, 2015

GLP:

Yes

Method

This method has been provided to allow for the determination of the napropamide-M within the test diet, to support the submitted toxicology studies. Napropamide-M is extracted from the test diet matrix using acetonitrile and HPLC-UV (at 230 nm) is employed for detection. An LOQ of 1000 mg/kg is supported, based on the lowest fortification level tested.

Protocol

Samples of test diet were prepared by accurately weighing 5 g of diet into a 125 mL reagent bottle. Samples of untreated control diet were fortified at this point for recovery testing by adding a known amount of napropamide-M technical material from an appropriate solution in acetonitrile.

To each sample acetonitrile (50 mL) was added and the bottles were closed. Extraction was performed by shaking for 15 minutes on an orbital shaker. The organic phase extract was removed by filtration and extraction was repeated on the remaining solids with fresh acetonitrile (50 mL). The combined acetonitrile extract was prepared for determination by HPLC-UV. Depending on the expected concentration of napropamide-M in the test diet, a suitable dilution using untreated control diet extract was performed to make sure the final solution was within the linear range, e.g. for a test diet of sample concentration 5000 mg/kg, 2.0 mL of acetonitrile extract was diluted to 10 mL volume with control diet extract. The final dilution allowed factors to be established for calculation of napropamide-M concentration in analysed test diet samples.

All test diet sample extracts or standards were analysed using HPLC-UV at 230 nm with a SGE C18 column (4.6 x 250 mm, 5 µm), mobile phase of acetonitrile : water (65:35% v/v) with isocratic elution.

For the qualitative assessment of isomer ratio within test diet samples, aliquots of samples and isomer (napropamide-M / D-isomer and L-isomer) reference solutions were determined by chiral HPLC analysis with a Chiracel, OD-H column (250 x 4.6 mm, 5 µm), mobile phase of n-hexane : ethanol (99 : 1% v/v) with isocratic elution and UV detector monitoring at 230 nm allowed determination of the two napropamide D and L isomers.

Note: napropamide-M is the D isomer and the retention times could be confirmed from the prepared solutions.

Validation

Specificity was determined by the injection of napropamide-M technical material, solvent blank, mobile phase, napropamide-M and L-isomer reference standards, fortified test diet sample extract and control diet sample extract to assess the potential for interference and the match of retention times. Examination of the chromatograms of napropamide-M technical material, solvent blank, mobile phase, napropamide-M and L-isomer reference standards, fortified test diet sample extract and control diet sample extract revealed that there was no interference likely to affect the chromatographic peak of napropamide-M. The retention times of napropamide-M in reference substance and technical material in diet were confirmed.

Stability: Stability of napropamide-M in test diet was proved by repeating the accuracy validation tests 4, 8, 14 and 28 days after provision of the bulk fortified diet samples by the test facility. Test diet samples were stored at room temperature, not in direct sunlight.

Homogeneity: For each day of testing, the six replicates demonstrated good homogeneity of napropamide-M within the bulk prepared test diet samples. Replicates 1 and 2 were sampled from the top, Replicates 3 and 4 from the middle and Replicates 5 and 6 from the bottom of the test diet containers.

A summary of the validation data supporting the method is outlined in Table B5.1.2.2.1-1 and isomer ratio in table Table B5.1.2.2.1-2.

Table B.5.1.2.2.1-1 – Validation data for the determination of napropamide-M in test diet

Matrix	Analyte	LOQ* (mg/kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level(mg/kg)	% range (Mean %)		
Test Diet Day 0	Napropamide – M	1000	1000 (n=6),	93.32 – 96.61 (94.93)	1.42	10.28 – 102.76 mg/L (ca.: 205 – 2050 mg/kg) r = 0.999 (n=6 - injected in duplicate)
			5000 (n=6)	97.77 – 99.91 (98.71)	0.82	
			10000 (n=6)	98.73 – 99.60 (99.21)	0.30	
Test Diet Day 4	Napropamide – M	1000	1000 (n=6),	92.73 – 93.91 (93.18)	0.54	10.28 – 102.76 mg/L (ca.: 205 – 2050 mg/kg) r = 0.999 (n=6 - injected in duplicate)
			5000 (n=6)	99.37 – 100.04 (99.67)	0.27	
			10000 (n=6)	98.31 – 99.68 (98.95)	0.57	
Test Diet Day 8	Napropamide – M	1000	1000 (n=6)	91.92 – 98.11 (94.85)	2.23	10.28 – 102.76 mg/L (ca.: 205 – 2050 mg/kg) r = 0.999 (n=6 - injected in duplicate)
			5000 (n=5)**	98.97 – 100.07 (99.41)	0.45	
			10000 (n=6)	98.45 – 100.10 (99.47)	0.61	
Test Diet Day 14	Napropamide – M	1000	1000 (n=6)	90.60 – 92.22 (91.52)	0.65	10.28 – 102.76 mg/L (ca.: 205 – 2050 mg/kg) r = 0.999 (n=6 - injected in duplicate)
			5000 (n=6)	95.05 – 98.79 (96.33)	1.50	
			10000 (n=6)	96.54 – 99.64 (98.01)	1.19	
Test Diet Day 28	Napropamide – M	1000	1000 (n=6)	88.59 – 93.36 (91.31)	1.95	10.28 – 102.76 mg/L (ca.: 205 – 2050 mg/kg) r = 0.999 (n=6 - injected in duplicate)
			5000 (n=6)	96.71 – 99.93 (98.81)	1.43	
			10000 (n=6)	97.42 – 99.88 (98.56)	1.01	

* The LOQ was not determined as part of the validation process.
** One outlier was identified and excluded form calculation

As noted previously, samples from higher fortification levels were diluted to fit within the linear range.

Table B.5.1.2.2-2 – Isomer D and L ratio

Days	Test Item	
	Low dose	High dose
	Isomer D to L ratio	Isomer D to L ratio
0	96.38 : 3.62	96.41 : 3.59
8	96.39 : 3.61	96.41 : 3.59
14	96.39 : 3.61	96.42 : 3.58
28	96.40 : 3.60	96.39 : 3.61

Conclusion

The method is has been satisfactorily validated in accordance with SANCO3030/99/rev.4.

B.5.1.2.2.2 – 'Additional validation of analytical method for determination of napropamide-M technical material concentration in test diet' (Report No. 228-2-13-7271) – Raithatha, 2013

GLP:

Yes

Method

The method was initially validated as described in report 228-2-13-6178 (B.5.1.2.2.1). An LOQ of 600 mg/kg is supported, based on the lowest fortification level tested.

Protocol

The study test diet was provided for validation at three concentrations of napropamide-M, pre-mixed and prepared in accordance with usual procedures for dose administration. In this study the same method was used involving direct fortification to untreated control (blank) diet prior to analysis from appropriate solutions.

Samples of test diet were prepared by accurately weighing 5 g of diet into a 125 mL reagent bottle. Samples of untreated control diet were fortified for recovery testing by adding a known amount of napropamide-M technical material from an appropriate solution in acetonitrile.

To each sample acetonitrile (50 mL) was added and the bottles were closed. Extraction was performed by shaking for 15 minutes on an orbital shaker. The organic phase extract was removed by filtration and extraction was repeated on the remaining solids with fresh acetonitrile (50 mL). The combined acetonitrile extract was prepared for determination by HPLC-UV. Depending on the expected concentration of napropamide-M in the test diet, a suitable dilution using untreated control diet extracts was performed to make sure the final solution was within the linear range, e.g. for a test diet of sample concentration 10000 mg/kg, 1.0 mL of acetonitrile extract was diluted to 10 mL volume with control diet extract. The final dilution allowed factors to be established for calculation of napropamide-M concentration in analysed test diet samples.

All test diet sample extracts or standards were analysed using HPLC-UV at 230 nm with a SGE C18 column (4.6. x 250 mm, 5 µm), mobile phase of acetonitrile : water (65:35% v/v) with isocratic elution.

A summary of the validation data supporting the method is outlined in Table B5.1.2.2.2-1.

Validation

Specificity was not addressed in this study, but since this method already was validated in the study report 228-2-13-6178 this is considered acceptable.

Table B.5.1.2.2.2-1 – Validation data for the determination of napropamide-M in test diet

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level(mg/Kg)	% range (Mean %)		
Test Diet	Napropamide – M	600	600 (n=5),	98.48 – 99.95 (99.45)	0.58	10.25 – 102.50 mg/L (ca.: 205 – 2050 mg/kg for undiluted samples) r = 0.999 (n=6 - injected in duplicate)
			10000 (n=5)	99.26 – 100.70 (99.99)	0.61	

As noted previously, samples from higher fortification levels were diluted to fit within the linear range.

Conclusion

The method has been satisfactory validated in accordance with SANCO3030/99/rev.4.

B.5.1.2.2.3 – 'Validation of analytical method for measurement of napropamide-M concentration in rat plasma by LC-MS-MS' (Report No. 228-2-14-7333) – Sriram, 2014

GLP:

Yes

Method

The napropamide-M concentration in samples of rat plasma is measured using the method described. Samples of rat plasma are usually stored frozen, but are removed from the deep-freeze and thawed prior to analysis. For test samples, 100 µL aliquots of plasma are vortex mixed with acetonitrile (1.5 mL) for two minutes. For preparation of calibration standards (matrix matched) or quality control fortification samples, 90 µL aliquots of plasma are taken and appropriate reference standard or quality control solutions (10 µL volume) are added and vortex mixed for two minutes. These samples are then vortex mixed with acetonitrile (1.5 mL) for two minutes.

Protocol

All samples are centrifuged at 10000 rpm for 15 minutes with supernatant taken for direct determination of napropamide-M by LC-MS/MS. Separation is achieved using a Zorbax 300 SB C18 column (4.6 mm i.d. x 150 mm, 3.5 µm particle size), or equivalent. Mobile phase of acetonitrile : 0.1% aqueous formic acid (70:30, v/v) with isocratic elution. Multiple reaction monitoring (MRM) of two transitions allows highly selective determination of napropamide-M, using mass 272.2 → 129.2 for quantification and 272.2 → 171.2 for confirmation.

For the qualitative assessment of isomer ratio within test samples, aliquots of samples and isomer (napropamide-M / D-isomer and L-isomer) reference solutions are determined by chiral HPLC-MS/MS analysis with a Phenomenex Lux Cellulose-2 column, 250 mm x 4.6 mm i.d. with 5 µm particle size, or equivalent. Mobile phase of methanol : 20 mM NH_4HCO_3+ 0.1% dimethyl amine in water (70:30, v/v) with isocratic elution allows determination of the two napropamide D and L isomers. Note: napropamide-M is the D isomer and the retention times can be confirmed from the prepared solutions. Multiple reaction monitoring (MRM) of two transitions allows highly selective determination of napropamide-M or L-napropamide, using mass 272.18 → 129.0 for quantification and 272.18 → 199.0 for confirmation.

Note: napropamide-M is the D isomer and the retention times can be confirmed from the prepared solutions. Multiple reaction monitoring (MRM) of two transitions allows highly selective determination of napropamide-M or L-napropamide, using mass 272.18 → 129.0 for quantification and 272.18 → 199.0 for confirmation.

Validation

Validation of the method included considerations for the method specificity, linearity, accuracy (recovery), precision, matrix effects stability (including freeze / thaw) and effect of dilution.

Specificity was determined by the injection of solvent blank, mobile phase, napropamide-M reference standards, blank extracted plasma and fortified plasma sample extracts to assess the potential for interference and the match of retention times. There was no interference likely to affect the chromatographic peak of napropamide-M. The retention times of napropamide-M in reference substance in solvent or in plasma matrix were confirmed. The use of LC-MS/MS with two MRM transitions allows a high degree of selectivity.

Matrix effects were assessed by analysis of untreated plasma (from six individual animals) fortified at LOQ or 25xLOQ with napropamide-M. These extracts were compared against samples at the same concentration of napropamide-M prepared in the absence of rat plasma matrix.

Stability of napropamide-M in rat plasma extracts was assessed with respect to the following conditions:

- Short-term (bench top) stability: LOQ and 25xLOQ extracts were prepared in plasma matrix (five replicates) and were kept at sample processing temperature for a minimum of 4 hours. After this period, samples were processed and analysed along with a freshly prepared calibration, according to the described LC-MS/MS method.
- Post processing (autosampler) stability: LOQ and 25xLOQ extracts were prepared in plasma matrix (five replicates) and were kept at autosampler temperature for a minimum of 24 hours to represent batch processing time on the instrument. After this period, samples were processed and analysed along with a freshly prepared calibration, according to the described LC-MS/MS method.
- Freeze / thaw stability: Reference standard stability in plasma matrix was determined after 3 freeze (-80 ± 5°C) / thaw (room temperature, 25-30°C) cycles. First freeze / thaw cycle was at 24 hours. Second and third freeze / thaw cycles were also each at 24 hours. LOQ and 25xLOQ concentrations prepared in plasma matrix (five replicates) were used for determination of stability.

The effect of dilution was evaluated by spiking plasma samples at 2X and 10X higher amount than the 25XLOQ concentration and subsequently diluting the samples with blank plasma to be equivalent to 25xLOQ, before analysis of the samples using the described method.

A summary of the validation data supporting the method is outlined in Tables B5.1.2.2.3-1 to B5.1.2.2.3-3 below.

Table B.5.1.2.2.3-1 – Validation accuracy and precision data for the determination of napropamide-M in rat plasma

Matrix	Analyte	LOQ (ng/mL)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level (ng/mL)	% range (Mean %)		
Rat Plasma	Napropamide – M	50	Quantification MRM (*m/z* 272.18 → 129.0)			25 – 2000 ng/mL r ≥ 0.9982 (n=7, 4 batches) Extended linear range 0.5 – 50 ng/mL r = 0.9990 (n=7)
			50 (n=5),	92.09 – 126.93 (104.81)	14.22	
			1250 (n=5)	83.41 – 127.08 (96.20)	18.29	

Matrix	Analyte	LOQ (ng/mL)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level (ng/mL)	% range (Mean %)		
Rat Plasma	Napropamide – M	50	Confirmation MRM (*m/z* 272.18 → 199.0)			25 – 2000 ng/mL r ≥ 0.9982 (n=7, 4 batches) Extended linear range 0.5 – 50 ng/mL r = 0.9981 (n=7)
			50 (n=5)	90.48 – 125.21 (105.5)	13.71	
			1250 (n=5)	83.29 – 126.84 (95.84)	18.36	

Table B.5.1.2.2.3-2 - Validation stability data:

Matrix	Analyte	LOQ (ng/mL)	Recovery		Repeatability %RSD
			Fortification Level – dose level(ng/mL)	% range (Mean %)	
Rat Plasma (Short term stability, 4h bench top, stability)	Napropamide – M	50	Quantification MRM (*m/z* 272.18 → 129.0)		
			50 (n=5),	91.70 – 99.88 (96.55)	3.58
			1250 (n=5)	87.21 – 95.48 (92.07)	3.31
Rat Plasma (Short term stability, 4h bench top, stability)	Napropamide – M	50	Confirmation MRM (*m/z* 272.18 → 199.0)		
			50 (n=5)	90.12 – 101.65 (95.38)	4.95
			1250 (n=5)	88.52 – 95.43 (93.78)	3.16
Rat Plasma (Post processing stability, 24h autosampler)	Napropamide – M	50	Quantification MRM (*m/z* 272.18 → 129.0)		
			50 (n=5)	93.67 – 105.24 (99.41)	4.14
			1250 (n=5)	91.71 – 99.87 (95.23)	3.12
Rat Plasma (Post processing stability, 24h autosampler)	Napropamide – M	50	Confirmation MRM (*m/z* 272.18 → 199.0)		
			50 (n=5)	92.87 – 98.38 (95.77)	2.59
			1250 (n=5)	91.80 – 101.63 (96.92)	3.61
Rat Plasma (Freeze/thaw stability, 3x24h)	Napropamide – M	50	Quantification MRM (*m/z* 272.18 → 129.0)		
			50 (n=5)	91.36 – 97.78 (94.75)	2.85
			1250 (n=5)	86.37 – 90.38 (88.14)	1.79
Rat Plasma (Freeze/thaw stability, 3x24h)	Napropamide – M	50	Confirmation MRM (*m/z* 272.18 → 199.0)		
			50 (n=5)	90.63 – 99.57 (94.24)	3.99
			1250 (n=5)	83.68 – 90.46 (87.90)	3.04

Table B.5.1.2.2.3-3 Validation effect of dilution data:

Matrix	Analyte	Recovery		Repeatability %RSD
		Fortification Level – dose level(ng/mL)	% range (Mean %)	
Rat Plasma (effect of dilution)	Napropamide – M	Quantification MRM (*m/z* 272.18 → 129.0)		
		2500 (2x dilution) (n=5),	85.65 – 97.02 (90.74)	4.90
		12500 (10x dilution) (n=5)	92.23 – 99.47 (95.65)	3.14
Rat Plasma (effect of dilution)	Napropamide – M	Confirmation MRM (*m/z* 272.18 → 199.0)		
		2500 (2x dilution) (n=5),	90.12 – 101.65 (95.38)	5.20
		12500 (10x dilution) (n=5)	88.52 – 95.43 (93.78)	2.65

Isomer ratio and hence optical purity can be determined using a qualitative chiral assay but details of determination were not provided with this report.

Conclusion

The method has been satisfactorily validated in accordance with SANCO3030/99/rev.4.

B.5.1.2.2.4 – 'Determination of napropamide in animal feed' (Report No. RRC-79-26) – Katague, 1979

GLP:

No (the study was completed in 1979 and pre-dates the implementation of GLP)

Method:

This method has been provided to allow for the determination of the napropamide (racemate) concentration within the test diet, in order to support the submitted toxicology studies. Napropamide (racemate) is extracted from the test diet matrix using toluene and GC-NPD is employed for detection. No LOQ has been assigned.

Protocol

Samples of disintegrated test diet were prepared by accurately weighing 10 g of diet into a glass bottle. Samples of untreated control diet were fortified at this point for recovery testing by adding a known amount of napropamide (racemate) material from an appropriate solution.

To each sample toluene (40 mL) was added and the bottles were closed. Extraction was performed by shaking for 30 minutes on a mechanical shaker. After extraction the samples were left to allow the toluene phase to clarify.

All test diet sample extracts or standards were analysed using GC-NPD with a packed glass column containing 3% SP2401 on Supelcoport 100-120 mesh stationary phase.

Validation

Specificity was determined by the injection of napropamide (racemate) standards, fortified test diet sample extract and control diet sample extract to assess the potential for interference and the match of retention times.

Examination of the chromatograms of napropamide (racemate) reference standards, fortified test diet sample extract and control diet sample extract indicated that there was no interference likely to affect the chromatographic peak of napropamide (racemate). The retention times of napropamide (racemate) in reference substance and in the test diet were confirmed.

A summary of the validation data supporting the method is outlined in Table B5.1.2.2.4-1

Table B.5.1.2.2.4-1 – Validation accuracy and precision data for the determination of napropamide (racemate) in test diet

Matrix	Analyte	LOQ (ng/mL)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level(ng/mL)	% range (Mean %)		
Test diet (rat chow)	Napropamide (racemate)	1	1 (n=1),	100	-	-
			10 (n=1)	102	-	-
			50 (n=1)	95	-	-

Repeatability (%RSD) has been calculated based on the three recovery samples; however these recoveries cover three different fortification levels and this approach is not considered to be in line with the guidance. As such the precision is not considered to have been sufficiently addressed.

It is also noted that the LOQ has not been determined nor has the linear calibration range for the method been demonstrated.

Conclusion

Given the apparent deficiencies in the validation data for this method, it cannot be considered to be satisfactorily validated in accordance with SANCO3030/99/rev.4. Further validation data would be required to support this method – this has been highlighted as a **data gap**.

B.5.1.2.2.5 – 'Determination of R-7465 in rodent diet by HPLC' (Report No. EHC-88-11) – Earley, 1988

GLP:

No (study was conducted prior to the enforcement date of GLP for pesticide regulation - 1st January 1993)

Method:

[It should be noted that 'R-7465' is the manufacturer's development code number for napropamide (racemate), as previously defined in the napropamide DAR (RMS=Demark, September 2005).

The purpose of the method is to determine the concentration of napropamide (racemate) in samples of test diet, using acetonitrile for extraction with HPLC-UV (at 290 nm) for analysis. An LOQ of 2.36 mg/kg has been assigned.

Protocol

Samples of blended test diet were prepared by accurately weighing 0.5 g of diet into a glass tube. Samples of untreated control diet were fortified for recovery testing by adding a known amount of napropamide (racemate) material from an appropriate stock solution (in acetonitrile).

To each sample acetonitrile (10 mL) was added and extraction was performed by homogenising with an ultrasonic cell disruptor at 20% power output for 15 seconds. Samples were centrifuged (2000 rpm for 5 minutes) and approximately 1.5 mL of cleared extract was transferred to an autosampler vial.
All test diet sample extracts or standards were analysed using HPLC-UV at 290 nm with a Varian C18-5 column (4.6 x 150 mm, 5 µm), mobile phase of acetonitrile : water (60 : 40 % v/v) with isocratic elution.

Validation

Method specificity was determined by the injection of napropamide (racemate) standards, fortified test diet sample extract and control diet sample extract to assess the potential for interference and the match of retention times.

Examination of the chromatograms of napropamide (racemate) reference standards, fortified test diet sample extract and control diet sample extract revealed that there was no interference likely to affect the chromatographic peak of napropamide (racemate). The retention times of napropamide (racemate) in the reference substance and in the test diet were confirmed.

A summary of the validation data supporting the method is outlined in Tables B5.1.2.2.5-1 and B5.1.2.2.5-2.

Table B.5.1.2.2.5-1 – Validation accuracy and precision data for the determination of napropamide (racemate) in test diet

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level (mg/kg)	% range (Mean %)		
Rodent diet	R-7465 napropamide (racemate)	2.36	2.36 (n=10)	64.61 – 132.12 (105.36)	17.8	0.0855 – 0.4276 µg/mL (ca.: 0.002 – 0.0086 mg/kg) R = 0.9966 (n=4)
			2514 (n=6) Fortified control	96.9 – 98.6 (97.8)	0.65	
			2514 (n=6) Sample matrix low dose	97.7 – 99.6 (98.9)	0.74	60.14 – 144.3 µg/mL (ca.: 1.2 – 2.9 mg/kg) R = 1.0000 (n=4)
			19952 (n=6) Sample matrix high dose	97.97 – 95.88 (97.16)	0.91	601.4 – 1202.8 µg/mL (ca.: 12 – 29 mg/kg) R = 1.0000 (n=3)

Table B.5.1.2.2.5-2 – Validation precision data

Concentration level napropamide (racemate) (mg/kg)	Measured concentration (mg/kg)		RSD (%)	n
	Individual	Mean		
2514	2537, 2487, 2545, 2519, 2519, 2555	2527	0.96	6

The linearity was tested over three different ranges with 3-4 concentrations analysed per calibration plot. The SANCO 3029/99 rev 4 indicates that at least five calibration standards should be analysed or three calibration standards (in duplicate), to assess the linear range of the method. While this is not strictly in line with the requirements of the guidance, the calibration does show a good degree of linearity (as shown by the calibration plots in the study reports and reflected in the R^2 values, which are all >0.99).

Furthermore, it is noted that only the LOQ fortification level appears to have been acceptably covered by any of the three calibration ranges provided. It is unclear from study report whether the more concentrated fortified samples (2514 and 19952 mg/kg) were diluted further prior to analysis, to bring the concentration of napropamide (racemate) within the limits of the assessed linear ranges.

The above point was raised with the Notifier, who provided the following response:

"The submitted study report was taken from original raw data packs from the toxicology studies and there is a limited description of the method as used at the time the studies were conducted. A dilution factor is not described but it is noted that different calibration ranges should be used to ensure the injected samples were within the linear range. The calibration range used for high QC sample analysis will extend to a diet concentration of approximately 24000 mg/kg and this therefore covers the 19952 mg/kg fortifications."

The applicant has clarified that the method procedure includes a caveat that appropriate calibration ranges should be determined concurrent to the data generation phase. On the basis of this supplementary information, the method can be considered to be fit for purpose.

Conclusion

The method can be regarded as fit for its intended purpose.

B.5.1.2.2.6 – 'Determination of R-7465 in rodent diet by capillary gas chromatography' (Report No. EHC-89-7) – Mays, 1989

GLP:

No (study was conducted prior to the enforcement date of GLP for pesticide regulation - 1st January 1993)

Method

The purpose of the method is to determine the concentration of napropamide (racemate) in samples of test diet, using toluene for extraction with GC-NPD for analysis. An LOQ of 4.0 mg/kg has been assigned.

Protocol

Samples of blended test diet were prepared by accurately weighing nominally 0.5 g of test diet into a glass tube. Samples of untreated control diet were fortified at this point for recovery testing by adding a known amount of napropamide (racemate) material from an appropriate stock solution (in toluene).

To each sample, the internal standard solution (5 ppm N,N-diethyldodecanamide in toluene, nominally 5 mL for low dose and 20 mL for high dose) was added and extraction was performed by shaking on a mechanical shaker at 250 rpm for 1 hour. Samples were centrifuged (2000 rpm for 10 minutes) and approximately 1.5 mL of cleared extract was transferred to an autosampler vial.

All test diet sample extracts or standards were analysed using GC-NPD with a capillary column, DB-17 (30 m x 0.25 mm i.d., 0.25 µm df).

Validation

Specificity was determined by the injection of napropamide (racemate) standards, fortified test diet sample extract and control diet sample extract to assess the potential for interference and the match of retention times. There was no interference likely to affect the chromatographic peak of napropamide (racemate). The retention times of napropamide (racemate) in the reference substance and in the test diet were confirmed.

A summary of the validation data supporting the method is outlined in Tables B5.1.2.2.6-1 and B5.1.2.2.6-2.

Table B.5.1.2.2.6-1 – Validation accuracy and precision data for the determination of napropamide (racemate) in test diet

Matrix	Analyte	LOQ (mg/Kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level(mg/kg)	% range (Mean %)		
Rodent diet	R-7465 Napropamide (racemate)	4.0	4.0 (n=7)	86.03 – 95.51 (90.77)	4.4	2.136 – 9.612 µg/mL (ca.: 0.02 – 0.096 mg/kg) R = 0.9968 (n=4)
			60 (n=6) Sample matrix low dose	93.0 – 100.7 (97.8)	3.3	
			60 (n=6) Spiked sample analysed 6 times	91.2 – 102.5 (98.4)	4.1	21.36 – 64.08 µg/mL (ca.: 0.2 – 0.64 mg/kg) R = 0.9970 (n=3)
			6000 (n=6) Sample matrix high dose	94.4 – 108.0 (101.1)	4.4	142.4 – 213.6 µg/mL (2.8 – 4.3 mg/kg) R = 0.9952 (n=3)

Table B.5.1.2.2.6-2 – Validation precision data

Concentration level napropamide (racemate) (mg/kg)	Measured concentration (mg/kg)		RSD (%)	n
	Individual	Mean		
60*	55.9, 57.4, 53.9, 55.7, 54.6, 57.2	55.8	2.5	6

* - 1 low dose sample analysed 6 times

The linearity was tested over three different ranges with 3-4 concentrations analysed per calibration plot. The SANCO 3029/99 rev 4 indicates that at least five calibration standards should be analysed or three calibration standards (in duplicate), to assess the linear range of the method. While this is not strictly in line with the requirements of the guidance, the calibration does show a good degree of linearity (as shown by the calibration plots in the study reports and reflected in the R^2 values, which are all >0.99).

Furthermore, it is noted that only the LOQ fortification level appears to have been acceptably covered by any of the three calibration ranges provided. It is unclear from study report whether the more concentrated fortified samples (60 and 6000 mg/kg) were diluted further prior to analysis, to bring the concentration of napropamide (racemate) within the limits of the assessed linear ranges.

Conclusion

Further information regarding the test protocol is required to conclude on the acceptability of the method.

B.5.1.2.3 METHODS USED TO GENERATE RESIDUES DATA

B.5.1.2.3.1 – 'Method validation study for the determination of napropamide-M in crops.' (Report No. AU-2012-62) – Li, 2013

GLP:

Yes

Method JRFA AU-265R0

The purpose of the method is to determine the concentration of napropamide-M within crop commodities, to support data generated during the residues field trial studies (pre-registration). The method is also considered to be suitable for post-registration (enforcement) purposes (refer to section B.5.2.1.1, page 61). The method uses 1% acetic acid in acetonitrile as an extraction solvent and employs LC-MS/MS for detection. The following crop matrices were tested: cabbage, strawberry, dried beans and oilseed rape seeds. These matrices are representative of high water, high acid, high protein and high oil crop types, respectively (according to the guidance: OECD No 72 Series 39). Dried beans are considered by EFSA to be a 'dry' commodity. The LOQ for all tested commodities is 0.01 mg/kg

The procedures were based directly on the published multi-residue method QuEChERS approach (EN 15662: 2008 "Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS method", November 2008). An LC-MS/MS method was also developed for analysis of chiral D/L isomer ratio in sample extract.

Protocol

Samples of crops matrix were prepared by accurately weighing 10g of homogenised sample into 50 mL centrifuge tube. Samples of untreated control matrix were fortified for recovery testing by adding known amount of napropamide-M from appropriate solution in acetonitrile.

- For dry or oily matrices such as dried beans and canola seeds, accurately add 10 mL water, and 10 mL 1% acetic acid in acetonitrile was added after fortification. Samples were shaken by hand vigorously for approximately 1 minute and sonicated for approximately 2 minutes. Then 4 g $MgSO_4$, 0.5 g NaCl was added and shake by-hand for approximately 1 minute.
- For all matrices with high water including high acid content, 4 g $MgSO_4$, 0.5 g NaCl and accurately 10 mL 1% acetic acid in acetonitrile was added. Samples were shake by-hand vigorously for approximately 1 minute and sonicate for approximately 2 minutes.

All samples were then centrifuged at approximately 3500 rpm for 5 minutes. A 2.0 mL of extract were transferred into a disposable test tube containing 100 mg PSA and 300 mg $MgSO_4$. Samples were vortex mixed for a few seconds, then centrifuged at 3500 rpm for 2 minutes.

Sample extracts were diluted 5-fold (recommended 10-fold for high oil matrices) using formic acid in water (1% v/v). All sample extracts and standards were analysed using LC-MS/MS with a Acquity UPLC BEH C18 column (2.1 mm i.d. x 50 mm, 1.7 µm particle size), mobile phase of 0.1% formic acid in acetonitrile : 0.1% formic acid in water with gradient elution.

Multiple reaction monitoring (MRM) of two transitions allows highly selective determination of napropamide-M, using mass 272.18 → 129 for quantification and 272.18 → 199 for confirmation.

For the qualitative assessment of isomer ratio within test samples, aliquots of samples and isomer (napropamide-M / D-isomer and L-isomer) reference solutions were determined by chiral HPLC-MS/MS analysis with a Phenomenex Lux Cellulose-2 column (250 x 4.6 mm ,5µm), mobile phase of methanol : 20 mM NH_4HCO_3+ 0.1% dimethyl amine in water (70 : 30% v/v) with isocratic elution allows determination of the two napropamide D and L isomers.

Note: napropamide-M is the D isomer and the retention times can be confirmed from the prepared solutions. Multiple reaction monitoring (MRM) of two transitions allows highly selective determination of napropamide-M or L-napropamide, using mass 272.18 → 129 for quantification and
272.18 → 199 for confirmation.

Validation

Specificity was determined by the injection of napropamide-M reference standards, fortified matrix sample extracts and control matrix sample extracts in order to assess the potential for peak interferences and the match between the retention times.

Linearity of the detector response was assessed by external calibration using a minimum of seven standard solutions of different concentration. Napropamide-M was measured over the nominal range 0.25 to 25 ng/mL. The linearity was shown for both quantification and confirmation MRM transitions. The correlation coefficient (R^2) was >0.99. Representative calibration curves are presented in the report. Non-matrix matched standards were used except for high oil matrix as matrix effects were only found (greater than 30%) in the analysis of oilseed rape seeds. This was resolved by using matrix matched calibration standards for high oil matrix. No significant ion suppression or enhancement was found in the analysis of cabbage, strawberry or dried beans. Testing of ion suppression/enhancement is recommended for any new matrices prior to analysis.

Accuracy (recovery) was determined by analysis of five replicate samples of each crop matrix, fortified at napropamide-M concentrations of 0.01 mg/kg (LOQ) and 0.10 mg/kg. Assessment of accuracy was based on calculation of recovered concentration after analysis according to the described method.

Precision for each matrix was assessed based on calculation of relative standard deviation (RSD) for the accuracy data.

The proposed Limit of Quantification (LOQ) was set at 0.01 mg/kg for each crop matrix. The validation data were also assessed for a proposed Limit of Detection (LOD). A Limit of Detection (LOD) was proposed as 30% of the LOQ, i.e. 0.03 mg/kg although lower values could be detected, typically between 0.25 to 0.5 µg/kg (ppb).

Stability of napropamide-M in crop matrix extracts was not specifically investigated, however the acceptable accuracy data demonstrates good stability over the period of analysis and no issues with stability are expected.

Isomer ratio (optical purity): Isomer ratio and hence optical purity of any napropamide-M measured in crop matrix extracts can be confirmed by this qualitative test.

A summary of the validation data supporting the method is outlined in Table B5.1.2.3.1-1.

Table B.5.1.2.3.1-1 - Validation of analytical method AU-265R0 for the determination of napropamide-M in crops (continued on the following page)

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
Cabbage	Napropamide-M	0.01	Quantification MRM (*m/z* 272.18 → 129)			0.25-25 ng/mL (Ca.: 0.00125-0.125 mg/kg) R=0.9999 (n=6)
			0.01	99.9 - 108 (107.4) (n=5)	6.26 (n=5)	
			0.1	87.1 – 95.8 (92.2) (n=5)	3.86 (n=5)	
			Confirmation MRM (*m/z* 272.18 → 199)			
			0.01	101 - 117 (108.8) (n=5)	5.45 (n=5)	
			0.1	87.6 – 95.3 (92.3) (n=5)	3.39 (n=5)	
Strawberry	Napropamide-M	0.01	Quantification MRM (*m/z* 272.18 → 129)			0.25-25 ng/mL (Ca.: 0.00125-0.125 mg/kg) R=0.9999 (n=6)
			0.01	104 - 117 (112) (n=5)	4.28 (n=5)	
			0.1	87.3 - 110 (98.2) (n=5)	8.80 (n=5)	
			Confirmation MRM (*m/z* 272.18 → 199)			
			0.01	106 - 120 (114) (n=5)	4.81 (n=5)	
			0.1	86.6 - 110 (97.9) (n=5)	9.08 (n=5)	
Dry Beans	Napropamide-M	0.01	Quantification MRM (*m/z* 272.18 → 129)			0.25-25 ng/mL (Ca.: 0.005-0.5 mg/kg) R=0.9999 (n=6)
			0.01	88.4 - 105 (96.1) (n=5)	6.63 (n=5)	
			0.1	88.9 - 120 (98.2) (n=5)	12.6 (n=5)	
			Confirmation MRM (*m/z* 272.18 → 199)			
			0.01	87.5 - 108 (96.3) (n=5)	7.67 (n=5)	
			0.1	89.2 - 120 (97.8) (n=5)	12.8 (n=5)	

Table B.5.1.2.3.1-1 - Validation of analytical method AU-265R0 for the determination of napropamide-M in crops (continued)

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
Canola seeds	Napropamide-M	0.01	Quantification MRM (*m/z* 272.18 → 129)			0.25-25 ng/mL (Ca.: 0.005-0.5 mg/kg) R=0.9999 (n=6)
			0.01	70.5 – 87.3 (80.7) (n=5)	8.02 (n=5)	
			0.1	71.3 – 79.1 (75.3) (n=5)	4.00 (n=5)	
			Confirmation MRM (*m/z* 272.18 → 199)			
			0.01	70.1 – 87.8 (78.4) (n=5)	8.25 (n=5)	
			0.1	70.9 – 78.2 (74.7) (n=5)	3.74 (n=5)	

Conclusion

The method is satisfactorily validated in accordance with SANCO3029/99/rev.4.

B.5.1.2.3.2 – 'Determination of napropamide residues in samples of brassicas treated with Devrinol in compliance with Good Laboratory Practice.' (Study No: OA00567) – Norris, 2002

GLP:

Yes

Method OAN/A/125

The purpose of the method is to determine the concentration of napropamide (racemate) within crop commodities, to support data generated during the residues field trial studies (pre-registration). The method uses toluene as an extraction solvent and employs GC-NPD for detection. The following crop matrices were tested: Brussels sprouts, cauliflower and cabbage. The LOQ for all tested commodities is 0.1 mg/kg.

Protocol

Samples of crop matrix are prepared by accurately weighing 30 g of homogenised sample into a 500 mL glass jar. Samples of untreated control matrix were fortified for recovery testing by adding a known amount of napropamide (racemate) from an appropriate solution in toluene. To each sample 120 mL toluene, sodium sulphate (35 g) and sodium bicarbonate (5 g) was added and extracted by homogenisation for two minutes then filter extracts under vacuum with toluene rinse. The filtrate is dried by passing through sodium sulphate, collected in turbovap tubes. Samples are reduced in volume under vacuum with a water bath at 55°C and adjusted to 20 mL.

Sample clean-up is by passing each extract through a silica chromatography column (3 g packing), rinsing with toluene and eluting any napropamide (racemate) in toluene :ethyl acetate (85:15, 50 mL). Samples are reduced using the same turbovap conditions to a volume of 5 mL, adjusted as necessary with toluene.

All sample extracts or standards are analysed using GC-NPD with a DB-608 GC column (15 m x 0.53 mm i.d.). The oven temperature program and injection conditions as described in the method.

Validation

Specificity was determined by the injection of napropamide (racemate) reference standards, fortified matrix sample extracts, solvent blanks and control matrix sample extracts to assess the potential for interference and the match of retention times. The report describes that GCMS can be used to confirm the specific nature of any residue in treated samples if results are reported above the LOQ.

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.1 mg/kg for all tested crop matrices.

A summary of the validation data supporting the method is outlined in Table B5.1.2.3.2-1.

Table B5.1.2.3.2-1. - Validation of the method OAN/A/125 for the determination of napropamide (racemate) in crops

Matrix	Analyte	LOQ (mg/Kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/Kg)	% range (Mean %)		
Brussels sprouts	Napropamide (racemate)	0.1	0.1	88.4 – 107.9 (97.3) (n=5)	9.1 (n=5)	0.1-5.0 mg/kg R=0.997 (n=4 in duplicate plus blank sample)
			1	85.8 – 104.8 (95) (n=5)	8.2 (n=5)	
Cauliflower	Napropamide (racemate)	0.1	0.1	91.3 – 102.7 (99.1) (n=5)	5.1 (n=5)	0.1-5.0 mg/kg R=0.997 (n=4 in duplicate plus blank sample)
			1	87.7 – 97.2 (94.2) (n=5)	3.9 (n=5)	
Cabbage	Napropamide (racemate)	0.1	0.1	102.9 – 106.5 (104.7) (n=5)	1.6 (n=5)	0.1-5.0 mg/kg R=0.997 (n=4 in duplicate plus blank sample)
			1	83.8 – 106.9 (99.1) (n=5)	9.2 (n=5)	

Method OAN/A/125 was validated for the determination of napropamide (racemate) in crop matrix samples with an LOQ of 0.1 mg/kg in the tested matrices of Brussels sprouts, cauliflower and cabbage. These matrices are representative in the EU of high water crop types.

Conclusion

The method has been satisfactorily validated in accordance with SANCO3029/99/rev.4, however it should be noted that this method does not distinguish between the D and L isomeric forms of napropamide (racemate) and only provided the concentration of the total napropamide (racemate) within brassica commodities. This has been taken into account during the evaluation of the residues field trials (section B.7.3.1).

B.5.1.2.3.3 – 'The determination of (RS)-N,N-diethyl-2-(1-napthyloxy)propionamide (napropamide, R7465) in crops.' – Pay, 1990b

GLP:

No (study was conducted prior to the enforcement date of GLP for pesticide regulation - 1st January 1993)

Method ARAM177:

The purpose of the method is to determine the concentration of napropamide (racemate) within crop commodities, to support data generated during the residues field trial studies (pre-registration). Samples were extracted using an ethanol : water solution (70:30 % v/v) and detection was made using GC-NPD. The method was validated using oilseed rape as a sample matrix (validation data were provided individually for 'whole plant', 'immature pod' and 'seed fractions'). The proposed LOQs are 0.02 mg/kg for 'whole plant', 0.05 mg/kg for 'immature pod' and 0.05 mg/kg for 'seed'.

Protocol

Samples of crops (10g) in duplicate were into storage jars (200mL). At least two recovery samples were spiked with napropamide (racemate) reference standard.

To each of the sample 50 mL of ethanol:water (70:30 % v/v) was added and macerate of at least 1 min (using Polytron macerator) or until all sample was homogenised. The extract then was filtered through No. 1 Whatman filter papers then rinsed the storage jar with further 30 mL of the extraction solvent and filtered through the Buchner funnel. The filtrate was transferred into separating funnel (250 mL) and 50 mL glass distillate water and 100 mL of dichloromethane was added. Samples were shaken for 1 min and left to separate. The lower organic layer was collected into round bottom flask and rotary evaporated to dryness. The residue was dissolved in 10 mL of hexane giving the concentration of 1g/mL.

Sample clean-up was conducted by passing a 2 mL aliquot (equal to 2 g equivalent of crop extract) through a silica chromatography column (3 g packing), washing with hexane (2.5 mL) then hexane:acetone (90:10, v/v 2 mL) and eluting any napropamide (racemate) in hexane:acetone (90:10, v/v 2 mL). Samples are reduced to dryness and reconstituted in hexane (1.0 mL).

All sample extracts or standards are analysed using GC-NPD with a SE 52/4 GC column (25 m x 0.32 mm, 1.0 µm).

Validation

Specificity was determined by the injection of napropamide (racemate) reference standards, fortified matrix sample extracts, solvent blanks and control matrix sample extracts to assess the potential for interference and the match of retention times. The report describes that GC-NPD can be used to confirm the specific nature of any residue in treated samples if results are reported above the LOQ.

Linearity of the detector response was assessed by external calibration using a minimum of five standard solutions of different concentration. Napropamide (racemate) was measured over the nominal range 0.05 to 5.0 µg/mL using solvent standards. However, the coefficient of determination (R^2) was not stated, nor were tabulated data for the calibration standards and the respective peak areas provided

Accuracy (recovery) was determined by analysis of replicate samples of oilseed crop matrix, fortified at napropamide (racemate) concentrations of 0.01 mg/kg to 0.1 mg/kg. Assessment of accuracy was based on calculation of recovered concentration after analysis according to the described method.

Precision for each matrix was assessed based on calculation of relative standard deviation (RSD) for the accuracy data.

A summary of the validation data supporting the method is outlined in Table B5.1.2.3.3-1.

Table B5.1.2.3.3-1 - Validation of the method ARAM177 - oilseed crops

Matrix	Analyte	LOQ (mg/Kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/Kg)	% range (Mean %)		
Whole plant	Napropamide (racemate)	0.02	0.02	66 – 101 (84.1) (n=10)	11.8 (n=10)	0.05-5.0 µg/mL (ca.: 0.025 – 0.25 mg/kg) (n=5)
Immature pod	Napropamide (racemate)	0.05	0.05	74 - 98 (83.1) (n=10)	9.8 (n=10)	
Seed	Napropamide (racemate)	0.05	0.01	86 - 80 (83) (n=2)	5.1 (n=2)	
			0.02	63 - 106 (76.8) (n=4)	27.04 (n=4)	
			0.05	65 – 91 (82.8) (n = 6)	12.7 (n = 6)	
			0.1	79 – 104 (91.5) (n = 2)	19.3 (n = 2)	

It should be noted that the method / validation data did not include the chiral determination of isomer ratio.

Example chromatograms were provided (untreated control, matrices spike at 0.02 and 0.1 µg/mL, and test sample) for the pod matrix. The chromatograms show that no interferences are expected at the retention time associated with napropamide (racemate). Chromatograms for the whole plant and seed have not been provided.

The linearity of the method was not fully addressed, only a calibration plot was provided with the report. The linear range is stated as 0.05 – 5 µg/mL and it can be inferred from the plot that 5 single calibration standards were used. While the linearity of the method cannot be unequivocally confirmed as the correlation coefficient (R^2), was not provided, the plot does indicate that the working range is linear and some measure of reassurance can be derived from this observation.

The precision of the method for the fortification level 0.02 mg/Kg for seed matrix is outside the acceptable limit (the % RSD should be ≤ 20% in accordance with SANCO3029/99/rev.4), indicating poor method precision. No justification for this was provided within the study report.

Repeatability (%RSD) for seed matrix was calculated based on the 2 recovery samples for two of the fortification levels. The guidance indicates that at least 5 determinations at each fortification level are should be provided. As such the accuracy of the method is not considered to have been addressed for the fortification levels of 0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg for seed matrix.

It is therefore considered that on the basis of this information, the supported LOQ for seed is 0.05 mg/kg.

Conclusion

While it is appreciated that the method has not been satisfactorily validated in accordance with SANCO3029/99/rev.4, there is sufficient information to confirm that the method is fit for purpose and the pre-registration data generated using this method may be relied upon.

B.5.1.2.3.4 – 'To determine the magnitude of napropamide residues at harvest in the raw agricultural commodity oilseed rape resulting from a single overall application of Devrinol 45FL to the ground in Northern France (2000-2002)' – Goodband, 2002

GLP:

Yes

Method Napropamide/Crops/DB/00/1 (based on ARAM 177)

The purpose of the method is to determine the concentration of napropamide (racemate) within crop commodities, to support data generated during the residues field trial studies (pre-registration). Samples were extracted using an ethanol : water solution (70:30 % v/v) and detection was made using GC-NPD. The method was validated using oilseed rape as a sample matrix (validation data were provided individually for 'whole plant', 'immature pod' and 'seed fractions'). The proposed LOQs are 0.02 mg/kg for 'whole plant', 0.05 mg/kg for 'immature pod' and 0.01 mg/kg for 'seed'.

Protocol

The method involved extraction using methanol/water solvent mix, filtration of the extract followed by the liquid/liquid partition into dichloromethane. Extracts were further purified by gel permeation chromatography. Quantification of napropamide (racemate) was by gas chromatography using nitrogen phosphorus thermionic specific detector.

Validation

An outline of the methodology for ARAM 177 was included in an appendix of the report Goodband, 2002 (which outlined the results of residues trials conducted in Norther France, during 2000-2002, on oilseed rape). This is the same method as considered previously under section B.5.1.2.3.3. No additional validation details were provided other than procedural recovery results (single determinations of 84% and 71% at the 0.01 mg/kg and 0.1 mg/kg fortification levels, respectively). An example calibration plot has been provided, however the coefficient of determination (R^2) was not stated, not were tabulated data for the calibration standards and the respective peak areas provided.

Conclusion

Insufficient validation data have been provided to support the method with respect to requirements set in the SANCO3029/99/rev.4 guidance document, as concluded in section B.5.1.2.3.3.

This outcome was relayed to the Notifier, who has provided supplementary validation data to support the analytical method. These additional data have been assessed in section B.5.1.2.3.5.

B.5.1.2.3.5 – 'Napropamide: Validation of method ARAM 177 for the determination of residues in wheat. (Study No: KB98WY)' – Harper, 2017c

GLP:

Yes

Method ARAM 177

Validation data to support the method ARAM 177 was considered under points B.5.1.2.3.3 and B.5.1.2.3.5, however the data were not considered sufficient to fully support the method. This outcome was relayed to the Notifier, who has provided supplementary validation data to support the analytical method. These additional data have been assessed in this section.

Protocol

The sample preparation details are as described under point and B.5.1.2.3.4.

Validation

Linearity: Response of the GC-NPD instrument was linear over the range 5 to 500 ng/mL for solvent standards with at least 9 calibration levels. The correlation coefficient (r) was >0.999. A representative calibration curve and typical chromatograms are presented in the report. Non-matrix matched standards were used as no significant enhancement was found in the analysis of soil matrix matched standards.

Accuracy: The method was shown to be accurate with overall mean recoveries of napropamide from fortified wheat matrices of 77 to 96% (n = 10 per matrix). The requirements of SANCO/3029/99 rev.4 have been met.

Precision: Analysis of the separate determinations of wheat matrices containing known amounts of napropamide showed the RSD to be between 4.0 and 7.1% at all tested concentrations, indicating acceptable precision. No outliers were removed. The requirements of SANCO/3029/99 rev.4 have been met.

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.01 mg/kg in wheat matrices. Control extracts contained either no detectable levels of napropamide or a maximum of 19.7% equivalent of the LOQ, showing the method is specific and selective.

A summary of the validation data is presented in Tables B.5.1.2.3.5-1 and B.5.1.2.3.5-2.

Table B.5.1.2.3.5-1 Validation accuracy and precision data for the determination of napropamide in wheat whole plant

Fortification level napropamide (mg/kg)	Recovery (%)		RSD (%)	n
	Individual or range	Mean		
0.01	102, 86, 93, 94, 102	95	7.1	5
0.10	97, 99, 96, 103, 91	97	4.5	5
Overall	86 to 102	96	5.7	10

Table B.5.1.2.3.5-2 Validation accuracy and precision data for the determination of napropamide in wheat straw

Fortification level napropamide (mg/kg)	Recovery (%)		RSD (%)	n
	Individual or range	Mean		
0.01	73, 76, 79, 81, 86	79	6.3	5
0.10	73, 75, 70, 79, 78	75	4.9	5
Overall	70 to 86	77	6.0	10

Table B.5.1.2.3.5-3 Validation accuracy and precision data for the determination of napropamide in wheat grain

Fortification level napropamide (mg/kg)	Recovery (%)		RSD (%)	n
	Individual or range	Mean		
0.01	76, 82, 82, 85, 82	81	4.0	5
0.10	93, 78, 89, 86, 87	87	6.4	5
Overall	76 to 93	84	6.0	10

Conclusion

Method ARAM 177 was retrospectively validated for the determination of napropamide in wheat matrices (plant, straw and grain) with an LOQ of 0.01 mg/kg. The data were acceptable for accuracy and precision within the requirements of SANCO/3029/99 rev. 4 and the method is appropriate for risk assessment data generation.

B.5.1.2.3.6 – 'Determination of napropamide residues in crops by gas chromatography. (Study No: RRC-83-68)' – Schwab, 1983

GLP:

No (study was conducted prior to the enforcement date of GLP for pesticide regulation - 1st January 1993)

Method RRC 83-68

This method is intended for the determination of napropamide (racemate) residues in the following crops: alfalfa, almond nuts and hulls, apples, apricots, avocados, cherries, eggplant, figs, kiwifruit, lemons, olives, peaches, peanuts and peanut hulls, pears, peppers, persimmons, pineapples, pistachio nuts and hulls, plums, pomegranates, potatoes, prunes, rye grain and straw, soybeans and soybean straw, strawberries, tomatoes and walnuts. The LOQ proposed for all tested commodities is 0.05 mg/kg.

Protocol

Samples of crop matrix were prepared by accurately weighing 30 to 50 g of homogenised sample into a container. Samples of untreated control matrix were fortified at this point for recovery testing by adding a known amount of napropamide (racemate) from an appropriate solution. To each sample 4 mL of toluene per gram of sample matrix was added and extracted by homogenisation for five minutes then filter extracts under gravity through a filter paper containing sodium sulphate. The filtrate was collected into a glass jar containing further sodium sulphate and the jar was capped.

For non-oily crops , no additional sample clean-up was required.

For oily crops (almonds, olives, peanuts, pistachios, mature soybeans and walnuts) the acetonitrile/hexane partitions was required to reduce oil in the extract.

- Toluene was evaporated from 5mL of extract. 10mL of acetonitrile and 15 mL of hexane was added to the oil residue and mixed gently for 1 min and then allowed solvent phases to separate. Exactly 6mL of the acetonitrile fraction (lower phase) containing napropamide (racemate) was transferred to a centrifuge tube. All traces of acetonitrile were removed by evaporation. The residue was reconstituted in a volume of toluene that corrects for oil dilution in an original extract.

All sample extracts or standards are analysed using GC-NPD with a J&W DB5 column (15 m x 0.32 mm i.d., 0.25 µm df). The oven temperature program and injection conditions as described in the method. Packed column conditions are also described but these are now obsolete.

Validation

Stability of napropamide (racemate) in crop matrix extracts was not specifically investigated.

A summary of the validation data supporting the method is outlined in Table B5.1.2.3.6-1.

Table B5.1.2.3.6-1 - Validation data for the determination of napropamide (racemate) in miscellaneous crop matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
Alfalfa	Napropamide (racemate)	0.05	0.05	86	-	
Almond hulls	Napropamide (racemate)	0.05	0.05	78	-	
Almond nuts	Napropamide (racemate)	0.05	0.05	90	-	
Apples	Napropamide (racemate)	0.05	0.05	80	-	
Apricots	Napropamide (racemate)	0.05	0.05	110	-	
Avocados	Napropamide (racemate)	0.05	0.05	96	-	
Cherries	Napropamide (racemate)	0.05	0.05	106	-	
Eggplant	Napropamide (racemate)	0.05	0.05	78	-	
Figs	Napropamide (racemate)	0.05	0.05	90	-	
Kiwi	Napropamide (racemate)	0.05	0.05	82	-	
Lemons	Napropamide	0.05	0.05	92	-	

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
	(racemate)					
Olives	Napropamide (racemate)	0.05	0.05	102	-	
Peaches	Napropamide (racemate)	0.05	0.05	102	-	
Peanuts	Napropamide (racemate)	0.05	0.05 (n=3)	74 – 97 (79.3)	11.6	
Peanut hulls	Napropamide (racemate)	0.05	0.05	100	-	
Pears	Napropamide (racemate)	0.05	0.05	84	-	
Peppers	Napropamide (racemate)	0.05	0.05	86	-	
Persimmons	Napropamide (racemate)	0.05	0.05	96	-	
Pineapple	Napropamide (racemate)	0.05	0.05	88	-	
Pistachio hulls	Napropamide (racemate)	0.05	0.05	76	-	
Pistachio nuts	Napropamide (racemate)	0.05	0.05	82	-	
Plums	Napropamide (racemate)	0.05	0.05	106	-	
Pomegranates	Napropamide (racemate)	0.05	0.05 (n=2)	82 – 94 (88)	9.6	
Potatoes	Napropamide (racemate)	0.05	0.05 (n=3)	110 – 120 (116.7)	4.95	
Prunes	Napropamide (racemate)	0.05	0.05	96	-	
Rye grain	Napropamide (racemate)	0.05	0.05 (n=5)	102 – 114 (106.4)	4.3	
Rye straw	Napropamide (racemate)	0.05	0.05 (n=5)	86 – 104 (97.6)	9.1	
Soybeans	Napropamide (racemate)	0.05	0.05	82	-	
Soybean straw	Napropamide (racemate)	0.05	0.05	114	-	
Strawberries	Napropamide (racemate)	0.05	0.05	98	-	
Tomatoes	Napropamide (racemate)	0.05	0.05 (n=2)	88 – 104 (96)	11.8	
Walnuts	Napropamide (racemate)	0.05	0.05	86	-	
Broccoli	Napropamide (racemate)	0.05	0.05	108	-	
Brussel Sprouts	Napropamide (racemate)	0.05	0.05	90	-	
Cabbage	Napropamide (racemate)	0.05	0.05	100	-	
Cantaloupe	Napropamide (racemate)	0.05	0.05	100	-	
Cauliflower	Napropamide	0.05	0.05	100	-	

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
	(racemate)					
Cucumbers	Napropamide (racemate)	0.05	0.05	88	-	
Lettuce	Napropamide (racemate)	0.05	0.05	104	-	
Onions	Napropamide (racemate)	0.05	0.05	106	-	
Pumpkins	Napropamide (racemate)	0.05	0.05	108	-	
Raspberries	Napropamide (racemate)	0.05	0.05	77	-	
Squash	Napropamide (racemate)	0.05	0.05	88	-	
Wheat Grain	Napropamide (racemate)	0.05	0.05	122	-	
Wheat Straw	Napropamide (racemate)	0.05	0.05	90	-	

The method specificity was not addressed within the report, nor has the linearity of the working range of the method been demonstrated (no example calibration plots have been provided in the relevant matrices).

The recovery for each of the matrices has been based on one single determination at the LOQ level. As indicated within the SANCO3029/99/rev.4 guidance, at least two fortification levels should be tested per matrix for the assessment of the method accuracy. It is also noted in the guidance that five determinations are required to assess the method precision.

Supplementary validation data are necessary to confirm the acceptability of this methodology with respect to the previously stated points – the deficiencies in the validation data have been highlighted as a **data gap**.

Conclusion

The method has not been validated in accordance with requirements set in SANCO3029/99/rev.4

B.5.1.2.3.7 – 'To determine the magnitude of napropamide residues at harvest in the raw agricultural commodity head cabbage resulting from a single overall application of Devrinol 45FL in the UK during 2001.'(Report No.: AS/5631/US) – Clark, 2002a

GLP:

Yes

Method Napropamide/Wet Crops/EMK/00/1 (based on RRC 83-68)

Protocol

Extraction procedure for tomatoes:

To subsample of prepared matrix (25g) contained in a Nalgene jar the toluene (100mL) and anhydrous sodium sulphate (25g) was added and was macerate for 3 minutes. Extract was filtered through Buchner funnel containing CF/A filter paper of celite (ca. 1 cm layer). The jar was rinsed with toluene (2 x25mL) and filtered through the Buchner funnel containing eluents.

The extract was dried through anhydrous sodium sulphate and collect in a round-bottomed flask (500mL). The sodium sulphate was rinsed with toluene. The extract was evaporated to near dryness and redissolved in cyclohexane/ethyl acetate (10mL, 1/1 v/v).

Extraction procedure for grapes:

To subsample of prepared matrix (25g) contained in a Nalgene jar the toluene (100mL) was added and was macerate for 3 minutes. Extract was filtered through Buchner funnel containing CF/A filter paper of celite (ca. 1 cm layer). The jar was rinsed with toluene (2 x30mL) and deionised water (50mL). The extract was transferred into separate funnel (500mL/1000mL) and the lower aqueous phase was discarded.

The extract was dried through anhydrous sodium sulphate and collect in a round-bottomed flask (500mL). The sodium sulphate was rinsed with toluene. The extract was evaporated to near dryness and redissolved in cyclohexane/ethyl acetate (10mL, 1/1 v/v).

Clean-up of extracts employed a GPC elution.

All sample extracts or standards are analysed using GC-NPD with a DB608 GC column (30 m x 0.32 mm i.d., 0.5 µm df).

Validation

As highlighted earlier in this section, the methodology is based on RRC-83-68, which has been considered in the previous section (B.5.1.2.3.5). Insufficient validation data were provided to support the method in line with the requirements set under SANCO3029/99/rev.4.

Reference to the study report AS/5631/US shows that only procedural recovery data (provided to support the pre-registration residue field trials data) have been presented for the tomato and grape matrices. No further validation data are available to supplement the dataset considered for RRC-83-68 under section B.5.1.2.3.5.

Conclusion

As concluded previously under section B.5.1.2.3.6 for RRC-83-68, the method has not been fully validated in accordance with the SANCO3029/99/rev.4 guidance.

This outcome was relayed to the Notifier, who has provided supplementary validation data to support the analytical method. These additional data have been assessed in section B.5.1.2.3.8.

B.5.1.2.3.8 Napropamide: Validation of method NAPROPAMIDE/WET CROPS/EMK/00/1 for the determination of residues in cabbage. (Report No.: XD94TP) – Harper, 2017a

GLP:

Yes

Method Napropamide/Wet Crops/EMK/00/1 (based on RRC 83-68)

Validation data to support the method Napropamide/Wet Crops/EMK/00/1 (based on RRC 83-68) was considered under points B.5.1.2.3.6 and B.5.1.2.3.7, however the data were not considered sufficient to fully support the method. This outcome was relayed to the Notifier, who has provided supplementary validation data to support the analytical method. These additional data have been assessed in this section.

Protocol

The sample preparation details are as described under point B.5.1.2.3.6.

Validation

Linearity: Response of the GC-NPD instrument was linear over the range 10 to 1000 ng/mL for solvent standards with at least 8 calibration levels using both DB-608 or DB-5 capillary columns. The correlation coefficient (r) was >0.998. Representative calibration curves and typical chromatograms are presented in the report. Non-matrix matched standards were used as no significant enhancement was found in the analysis of cabbage matrix matched standards.

Accuracy: The method was shown to be accurate with overall mean recovery of napropamide from fortified cabbage matrix of 79% (n = 10) using a DB-608 capillary column and 94% (n = 10) using a DB-5 capillary column. The requirements of SANCO/3029/99 rev.4 have been met.

Precision: Analysis of the separate determinations of cabbage matrix containing known amounts of napropamide showed the RSD to be between 2.3 and 13.9% at all tested concentrations, indicating acceptable precision. No outliers were removed. The requirements of SANCO/3029/99 rev.4 have been met.

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.01 mg/kg in cabbage as a representative brassica crop matrix (high water content crops). Control extracts contained no detectable levels of napropamide, showing the method is specific and selective.
A summary of the validation data is presented in Tables B.5.1.2.3.8-1 and B.5.1.2.3.8-2.

Table B.5.1.2.3.8-1 Validation accuracy and precision data for the determination of napropamide in cabbage (DB-608 capillary column)

Fortification level napropamide (mg/kg)	**Recovery (%)**		**RSD (%)**	**n**
	Individual or range	**Mean**		
0.01	86, 74, 70, 79, 62	74	12.2	5
0.10	82, 83, 85, 87, 84	84	2.3	5
Overall	62 to 87	79	10.3	10

Table B.5.1.2.3.8-2 Validation accuracy and precision data for the determination of napropamide in cabbage (DB-5 capillary column)

Fortification level napropamide (mg/kg)	Recovery (%)		RSD (%)	n
	Individual or range	Mean		
0.01	118, 102, 84, 103, 87	99	13.9	5
0.10	85, 85, 93, 89, 93	89	4.5	5
Overall	84 to 118	94	11.6	10

Conclusion

Method Napropamide/Wet Crops/EMK/00/1was retrospectively validated for the determination of napropamide in cabbage, a high water content crop, with an LOQ of 0.01 mg/kg. The data were acceptable for accuracy and precision within the requirements of SANCO/3029/99 rev. 4 and the method is appropriate for risk assessment data generation.

B.5.1.2.3.9 – 'Determination of residues of napropamide after a single application with soil incorporation of Devrinol 45FL in head cabbage outdoor, Southern Europe, 2004/2005.' (Report No.: 20044048/I1-FPCA) – Balluff, 2005a

GLP:

Yes

[Methodology and validation data are presented under section B.5.1.2.3.8]

B.5.1.2.3.10 – 'Determination of residues of napropamide after a single application with soil incorporation of Devrinol 45FL in head cauliflower outdoor, Southern Europe, 2004/2005.' (Report No.: 20044048/I1-FPCF) – Balluff, 2005b

GLP:

Yes

The method has been provided to allow for the determination of napropamide (racemate) in brassica crops, to support residues pre-registration studies. A water/acetonitrile solution (1:1) was used to extract residues and an LC-MS/MS system was employed for detection. Crop matrices tested were brassica (cabbage and cauliflower). The LOQ for both of these commodities was 0.01 mg/kg

Protocol

Samples of crop matrix were prepared by accurately weighing 20 g of homogenised sample into a container. Samples of untreated control matrix were fortified at this point for recovery testing by adding a known amount of napropamide (racemate) from an appropriate solution in acetonitrile.

Extraction was performed by homogenisation for 3-4 minutes using acetonitrile : water (1:1, v/v 200 mL). An aliquot (1-2 mL) of the extract was prepared by passing through a 0.45 µm single use filter into an HPLC autosampler vial.

All sample extracts or standards are analysed using LC-MS/MS with a Thermo HyPurity Aquastar column (3 x 150 mm, 5 µm), mobile phase methanol : water (20:80% v/v) with gradient elution as described in the method.

Multiple reaction monitoring (MRM) of two transitions allows highly selective determination of napropamide (racemate), using sum of mass 272.1 → 129.05 and 272.1 → 171.0.

Validation

Specificity was determined by the injection of napropamide (racemate) reference standards, fortified matrix sample extracts and control matrix sample extracts to assess the potential for interference and the match of retention times. The retention time of reference substance standards in acetonitrile/water (1:1 v/v) matched the retention times in extracts from fortified samples and from field samples. No peak interferences occurred at the retention time of napropamide (racemate).

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.01 mg/kg for all tested crop matrices.

Linearity: Response of the LC-MS/MS instrument was linear over the range 0.25 to 15 ng/mL for solvent standards with 7 calibration levels. The correlation coefficient (R^2) was >0.999. Representative calibration curves are presented in each report. Non-matrix matched standards were used as no significant ion suppression or enhancement was found in the analysis of cabbage or cauliflower matrix matched standards.

Stability: Repeat analysis of cabbage or cauliflower sample extracts fortified at 0.01 mg/kg and stored frozen (<-18°C) for 23 days before re-analysis showed that the napropamide (racemate) concentrations were >89% of the initial value. Therefore, napropamide (racemate) is considered to be stable in frozen extracts for at least 23 days.

A summary of the validation data supporting the method is outlined in Table B5.1.2.3.10 -1.

Table B5.1.2.3.10 -1. - Validation accuracy and precision data for the determination of napropamide (racemate) in brassica

Matrix	Analyte	LOQ (mg/Kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/Kg)	% range (Mean %)		
Head cabbage	Napropamide (racemate)	0.01	0.01	95 – 100 (98.3) (n=3)	3 (n=3)	0.25 ng/mL – 15ng/mL (ca.: 0.0025 – 0.15 mg/kg) R^2=0.999 (n=6 plus blank sample)
			0.10	99 - 102 (100.7) (n=3)	1.5 (n=3)	
Cauliflower	Napropamide (racemate)	0.01	0.01	99 - 101 (99.7) (n=3)	1.2 (n=3)	
			0.10	98 - 102 (99.7) (n=3)	2.1 (n = 3)	

It is noted that only three recoveries have been made at each fortification level, as opposed to the five recoveries stated within the SANCO 3029/99/rev.4 guidance document, for the determination of the method precision. While it is acknowledged that the number of recoveries are lower than required, the individual values are well within the acceptable range and the method precision was demonstrated to be acceptable (well within the <20% limit). Additional confidence can be taken from the concurrent validation of this method alongside the analysis of the napropamide (racemate) residues in the field trials samples (the recoveries presented in Table B5.1.2.3.10 -1 are also the procedural recoveries for the field trials studies).

Conclusion

While the method validation does not strictly comply with the requirements set within the SANCO 3029/99/rev.4 guidance, the method may be considered to be fit for its intended purpose. The residues data generated using this method can be relied upon for risk assessment purposes.

B.5.1.2.4. METHOD USED TO GENERATE FATE AND BEHAVIOUR DATA

B.5.1.2.4.1 – 'Validation of the analytical method "Napropamide & 2-naphthoxypropionic Acid/Soil/DB/10/1" for the analysis of napropamide and 2-naphthoxypropionic acid in soil (2010) (Report No. S10-00191) – Weir, 2010

GLP:

Yes

Method Napropamide & 2-naphthoxypropionic Acid/Soil/DB/10/1

Napropamide or its metabolite 2-naphtoxyproponic acid were determined in samples of soil using method described (Method: Napropamide and 2-naphthoxypropioninc Acid/Soil/DB/10/1).

Protocol

Samples were prepared by accurately weighing 50g of soil (dried if appropriate of known moisture content) into extraction bottle (e.g. Nalgene jar). Samples of untreated soil were fortified by adding a known amount of napropamide (racemate) or 2-naphthoxypropionic acid form appropriate solution in acetonitrile.

To each sample acetonitrile (50mL) and water (50mL) was added and the bottle were closed. Extraction was performed by shaking overnight. The samples were centrifuged (4000 rpm, 5 minutes) and aliquot (1-2 mL) was removed and filtrated through a 0.45 µm PTFE syringe filter into an autosampler vial ready for determination.

All samples extracts and standards were analysed using LC-MS/MS with Hypersil Gold C18 column (150 x 2.1 mm, 5µm), mobile phase of acetonitrile : 0.1% acetic acid in water (60:40 % v/v) with isocratic elution.

Multiple reaction monitoring (MRM) of two transitions allowed highly selective determination of napropamide (racemate), using mass 272.1 → 129.0 for quantification and 272.1 → 170.9 for confirmation.

Multiple reaction monitoring (MRM) of two transitions allowed highly selective determination of 2-naphthoxypropionic acid, using mass 215 → 143 for quantification and 215 → 171 for confirmation.

Validation

Specificity: Examination of the chromatograms of napropamide (racemate) reference standards, fortified soil sample extracts and control soil sample extracts revealed that there was no interference likely to affect the chromatographic peaks of napropamide (racemate) or 2-naphthoxypropionic acid. The retention times of napropamide (racemate) or 2-naphthoxypropionic acid in reference substance standards and in soil extracts were confirmed. Representative chromatograms demonstrating specificity are presented in the report. The use of LC-MS/MS with two MRM transitions, also allows for a highly selective and specific method for napropamide (racemate) or 2-naphthoxypropionic acid in soil samples.

Linearity: Response of the LC-MS/MS instrument was linear for napropamide (racemate) over the range 0.00025 to 0.01 µg/mL for matrix matched soil standards with 6 calibration levels. Response of the LC-MS/MS instrument was linear for 2-naphthoxypropionic acid over the range 0.00125 to 0.05 µg/mL for matrix matched soil standards with 6 calibration levels. The linearity was shown for the quantification MRM transition although data for both transitions was collected. The correlation coefficients (R^2) were ≥0.9995 for napropamide (racemate) or 2-naphthoxypropionic acid.

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.001 mg/kg in soil for napropamide (racemate) and 0.005 mg/kg in soil for 2-naphtoxypropionic acid.

A summary of the validation data supporting the method is outlined in Table B5.1.2.4.1 -1.

Table B5.1.2.4.1 -1 - Validation data of analytical method Napropamide (racemate) and 2-naphthoxypropioninc Acid/Soil/DB/10/1

Matrix	Analyte	LOQ (mg/Kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/Kg)	% range (Mean %)		
Soil	Napropamide (racemate)	0.001	0.001 (n=5)	103-107 (105)	1.4	0.00025-0.025 µg/mL (Ca.: 0.0005-0.05 mg/Kg) r = 0.9998 (n=6)
			0.01 (n=5)	102 - 107 (105)	1.8	
	2-naphthoxypropionic acid	0.005	0.005 (n=5)	82 - 85 (83)	1.8	0.00125-0.05 µg/mL (Ca.: 0.0025-0.1 mg/Kg) r = 0.9995 (n=6)
			0.05 (n=5)	96 - 98 (97)	0.9	

The chiral determination of isomer ratio was not determined.

Conclusion

Method Napropamide & 2-naphthoxypropionic Acid/Soil/DB/10/1 was satisfactorily validated in accordance with SANCO3029/99/rev.4.

B.5.1.2.4.2 – 'The determination of (RS)-N,N-diethyl-2-(1-napthyloxy)propionamide (napropamide, R7465) in soil (Report No. ARAM 178) - J. Pay, 1990a

GLP:

No (study was conducted prior to the enforcement date of GLP for pesticide regulation - 1st January 1993)

Method ARAM 178

The method has been provided to determine the napropamide (racemate) concentration in samples of soil. A methanol:water solution (60:40, v/v) is used as an extraction solvent and GC-NPD is used for detection. The LOQ of the method is 0.1 mg/kg

Protocol

Samples of soil matrix were prepared by accurately weighing 10 g of homogenised sample into a 250 mL flask. Samples of untreated control matrix were fortified at this point for recovery testing by adding a known amount of napropamide (racemate) from an appropriate solution in acetone.

To each sample, 50 mL of methanol:water (60:40, v/v) was added and extracted by shaking for one hour. Extracts were filtrated under vacuum with extraction solvent rinse. After the addition of concentrated hydrochloric acid (1 mL), the extracts were partitioned with toluene (15 mL) by shaking. Pure water (50 mL) was then added prior to shaking again for one minute and the layers were allowed to separate for one hour. The lower aqueous layer was discarded and the toluene layer was collected and reduced in volume under vacuum to dryness. The residue was dissolved in hexane (5 mL) to five concentration of 2g/mL.

All sample extracts or standards were analysed using GC-NPD with a SE 52/4 GC column (25 m x 0.32 mm i.d., 1.0 µm df). The oven temperature program and injection conditions as described in the method.

Validation

Linearity of the detector response was assessed by external calibration using a minimum of five standard solutions of different concentration. Napropamide (racemate) was measured over the nominal range 0.05 to 5.0 µg/mL using solvent (hexane) standards.

The proposed Limit of Quantification (LOQ) was set at 0.1 mg/kg for soil.

A summary of the validation data supporting the method is outlined in Table B5.1.2.4.2 -1.

Table B5.1.2.4.2 -1 - Validation data for the determination of napropamide in soil

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
Soil	Napropamide (racemate)	0.1	0.1 (n=11)	78-125 (97)	17.3	0.05-5 µg/mL (ca.: 0.025 – 2.5 mg/kg) (n=5)
			1.0 (n=3)	98 - 109 (102)	5.7	

Specificity of the method was not specifically address but the chromatograms of the reference standard, recovery sample and untreated control soil sample were provided showing that there was no interferences are likely to affect the chromatographic peaks of napropamide (racemate).

The linearity of the method was not fully addressed, only a calibration plot was provided with the report. The linear range is stated as 0.05 – 5 µg/mL and it can be inferred from the plot that 5 single calibration standards were used. While the linearity of the method cannot be unequivocally confirmed as the correlation coefficient (R^2), was not provided, the plot does indicate that the working range is linear and some measure of reassurance can be derived from this observation.

While it is appreciated that for the LOQ fortification level some of the recoveries exceed the upper 110% limit stated within the guidance, it is considered that this would lead to an overestimate of the residue levels within the tested soil matrix, which would lead to more 'worst case' results. While this is not in line with the guidance, it is not considered that this point will compromise the functionality of the method.

According to the SANCO 3029/99/rev.4 guidance, precision should be demonstrated using five determinations at each fortification level. This has not been carried out for the highest fortification level as only three determinations were reported. While this is not strictly in line with the guidance, the data provided does provide some degree of reassurance that the precision of this method at the highest fortification level is acceptable.

Conclusion

While Method ARAM 178 has not been strictly validated in accordance with SANCO3029/99/rev.4, the results do give some assurance that the method is fit for data generation purposes and it is considered that data generated using this methodology may be relied upon for risk assessment.

This outcome was relayed to the Notifier, who has provided supplementary validation data to support the analytical method. These additional data have been assessed in section B.5.1.2.4.3.

B.5.1.2.4.3 – Napropamide: Validation of method ARAM 178 for the determination of residues in soil. (Report No BH69LF) – Harper, 2017b

GLP:

Yes

Method ARAM 178

Validation data to support the method ARAM 178 was considered under point B.5.1.2.4.2, however the data were not considered sufficient to fully support the method. This outcome was relayed to the Notifier, who has provided supplementary validation data to support the analytical method. These additional data have been assessed in this section.

Protocol

The sample preparation details are as described under point B.5.1.2.4.2.

Validation

Linearity: Response of the GC-NPD instrument was linear over the range 10 to 1000 ng/mL for solvent standards with at least 9 calibration levels. The correlation coefficient (r) was >0.999. A representative calibration curve and typical chromatograms are presented in the report. Non-matrix matched standards were used as no significant enhancement was found in the analysis of soil matrix matched standards.

Accuracy: The method was shown to be accurate with overall mean recovery of napropamide from fortified soil matrix of 85% (n = 10). The requirements of SANCO/3029/99 rev.4 have been met.

Precision: Analysis of the separate determinations of soil matrix containing known amounts of napropamide showed the RSD to be between 3.1 and 8.7% at all tested concentrations, indicating acceptable precision. No outliers were removed. The requirements of SANCO/3029/99 rev.4 have been met.

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.01 mg/kg in soil. Control extracts contained no detectable levels of napropamide, showing the method is specific and selective.

A summary of the validation data is presented in Tables B.5.1.2.4.3-1 and B.5.1.2.4.3-2.

Table B.5.1.2.4.3-1 Validation accuracy and precision data for the determination of napropamide in soil

Fortification level napropamide (mg/kg)	Recovery (%)		RSD (%)	n
	Individual or range	Mean		
0.01	98, 96, 85, 84, 86	90	7.4	5
0.10	79, 76, 79, 83, 79	79	3.1	5
Overall	76 to 98	85	8.7	10

Conclusion

Method ARAM 178 was retrospectively validated for the determination of napropamide in soil with an LOQ of 0.01 mg/kg. The data were acceptable for accuracy and precision within the requirements of SANCO/3029/99 rev. 4 and the method is appropriate for risk assessment data generation.

B.5.1.2.5 METHOD USED TO GENERATED ECOTOXICOLOGYCAL DATA

B.5.1.2.5.1 – 'Validation of analytical method for determination of napropamide-M active ingredient concentration in test media (Report No 228-2-13-6179) – Naik, 2013

GLP:

Yes

Method

The analytical method describes the process for the determination of napropamide-M concentration in reconstituted water or aquatic test media for support of testing the active substance in the ecotoxicological studies. The method uses hexane as an extraction solvent with HPLC-UV (220 nm) employed for detection. The LOQ of the method is 0.01 mg/kg.

Protocol

An amount of napropamide-M reference substance (nominally 10 mg) was dissolved in acetonitrile (10 mL) in a volumetric flask to yield a stock solution with nominal concentration of 1 mg/mL. This solution could be diluted as necessary for calibration or fortification, using either acetonitrile or aquatic (algal) test media. Similarly, an amount of napropamide-M technical material (nominally 10 mg) was dissolved in acetonitrile (10 mL) in a volumetric flask to yield a stock solution with nominal concentration of 1 mg/mL. This solution could be diluted as necessary for calibration or fortification, using either acetonitrile or aquatic (algal) test media.

For analysis of aquatic media test samples, a volume (10 mL) of test media was transferred into a 250 mL separating funnel and a volume of n-hexane (50 mL) was added before extraction by shaking and allowing 3-5 minutes for phase separation. The organic layer was collected into a 250 mL round bottom flask. The aqueous layer remaining was re-extracted a further two times with a volume of 25 mL n-hexane. The organic layers were combined and concentrated to dryness using rotary evaporator and water bath at nominally 40 °C. The residue was dissolved in mobile phase (5 mL of n-hexane: ethanol (99:1), v/v) and transferred into a 10 mL volumetric flask and made up to the mark with mobile phase.

Aliquots of samples and calibration standards were determined by HPLC-UV analysis with an Agilent C18 column (250 x 4.6 mm, 5 µm), mobile phase of acetonitrile : water (70 : 30 % v/v) with isocratic elution and UV detector monitoring at 220 nm allowed determination of napropamide-M content.

Second part of the analytical method describes the process for the determination of the isomer ratio and hence the optical purity of the active substance napropamide-M.

An amount of napropamide M reference substance or L-napropamide isomer (nominally 10 mg) was dissolved in mobile phase (total volume 10 mL) to yield stock solutions with nominal concentration of 1 mg/mL for each compound.

For the qualitative assessment of isomer ratio within test media samples containing active substance an amount of napropamide-M (nominally 10 mg) was weighed into a 10 mL flask. The active substance was mixed with algal test media (10 mL). The stock solution was further diluted with algal test media to give test solutions of nominally 10 µg/mL.

Aliquots of samples and isomer reference solutions were determined by chiral HPLC analysis with a Chiracel, OD-H column, 250 x 4.6 mm, 5 µm, mobile phase of n-hexane : ethanol (99 : 1 % v/v) with isocratic elution and UV detector monitoring at 230 nm allows determination of the two napropamide D and L isomers.

Note: napropamide-M is the D isomer and the retention times can be confirmed from the prepared solution.

Validation

The specificity of the method was determined by injection the standard solution, sample solution and blank test media onto HPLC to assess the potential for interference and the match of retention times.
Examination of the chromatograms of napropamide-M reference substance, napropamide-M technical material and blank algal test media revealed that there was no interference likely to affect the chromatographic peak of napropamide-M in the presence of algal test media. The retention times of napropamide-M in reference substance and spiked algal test media were confirmed

Limit of Quantification (LOQ): The analytical LOQ for napropamide-M content in reconstituted water or algal test media was confirmed as 0.01 mg/L.

Optical isomer purity: The average D- : L-isomer ratio in test item stock solution or in tested media analysed at different intervals was 96.38: 3.62. Isomer ratio and hence optical purity of the napropamide-M in algal test media can be confirmed by this qualitative test.

A summary of the validation data supporting the method is outlined in Table B5.1.2.5.1 -1.

Table B5.1.2.5.1 -1 - Validation accuracy and precision data for the determination of napropamide-M in reconstituted water and in algal test media

Matrix	Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/L)	% range (Mean %)		
Reconstituted water	Napropamide – M	0.01	0.01 (n=5, duplicate of injection)	94.60 - - 96.30 (95.44)	0.52	0.01 – 10.19 mg/L r = 0.9999 (n=6 - injected in duplicate)
			0.10 (n=5, duplicate of injection)	96.81 – 98.95 (97.98)	0.65	
Alga media	Napropamide - M	0.01	0.01 (n=5, duplicate of injection)	98.30 – 100.70 (99.70)	0.90	0.01 – 10.19 mg/L r = 0.9999 (n=6 - injected in duplicate)
			0.10 (n=5, duplicate of injection)	96.49 – 99.49 (98.57)	1.03	

Conclusion

The method has been validated in accordance with EU guidance document SANCO/3029/99 rev. 4. Isomer ratio and hence optical purity can be determined using a qualitative chiral assay.

B.5.1.2.5.2 – 'A dietary LC50 study with the mallard (Report No 123-153) – [redacted] (1990)

GLP:

Yes

Method

The method has been provided for the determination of the napropamide (racemate) concentration within in the samples of the mallard test diet. Napropamide (racemate) was extracted from the feed using soxhlet extraction and acetonitrile, GC-NPD was used for detection. An LOQ was not established, but the lowest tested fortification level was 497 mg/kg.

Protocol

Samples of test diet were prepared by accurately weighing nominally 10 g of diet into a soxhlet extraction thimble. Samples of untreated control diet were fortified at this point for recovery testing by adding a known amount of napropamide (racemate) from an appropriate solution in acetone containing 2% corn oil.

Each sample was soxhlet extracted for approximately 4 hours using acetonitrile (250 mL) as the extraction solvent. The collected extract was adjusted to a volume of 250 mL and an aliquot (5.0 mL) was taken and flash evaporated to dryness and reconstituted in toluene (5.0 mL). Dilutions were made as necessary to actual diet samples.

All test diet sample extracts or standards are analysed using GC-NPD with an HP-5 GC column (10 m x 0.53 mm i.d., 2.65 µm df). The oven temperature program and injection conditions as described in the method.

Validation

Specificity was determined by the injection of napropamide (racemate) calibration standards, reagent blank, fortified test diet sample extract and control diet sample extract to assess the potential for interference and the match of retention times.

Limit of Quantification (LOQ): The lowest tested fortification level was 497 mg/kg.

Stability: Stability of napropamide (racemate) in test diet was proved by repeating the accuracy validation tests 3 and 7 days after fortification. Test diet samples were stored under typical study conditions and also frozen.

A summary of the validation data supporting the method is outlined in Table B5.1.2.5.2 -1.

Table B5.1.2.5.2 -1- Validation accuracy and precision data for the determination of napropamide (racemate) in avian test diet

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
Avian test diet (day 1)	Napropamide (racemate)	497	Standard test condition			0.5 – 35 mg/L (ca. 12.5 –875 mg/kg) r=0.994 n=7
			497	104.0 – 113.9 (110) (n=3)	5.0 (n=3)	
			4971	99.1 – 111.6 (106) (n=3)	6.0 (n=3)	
Avian test diet (day 3)	Napropamide (racemate)	497	Standard test condition			
			497	87.7 – 98.4 (93) (n=3)	5.8 (n=3)	
			4971	99.1 – 109.0 (103) (n=3)	5.0 (n=3)	
			Frozen			
			497	90.9 – 111.1 (102) (n=3)	10.1 (n=3)	
			4971	101.7 – 114.3 (107) (n=3)	6.3 (n=3)	
Avian test diet (day 7)	Napropamide (racemate)	497	Standard test condition			
			497	89.9 – 91.8 (91) (n=3)	1.1 (n=3)	
			4971	99.3 – 101.1 (100) (n=3)	1.0 (n=3)	
			Frozen			
			497	92.1 – 103.2 (98) (n=3)	5.7 (n=3)	
			4971	98.8 – 109.2 (104) (n=3)	5.0 (n=3)	

Stability was shown to be acceptable over 7-days for diet stored under test conditions or frozen, as the as the method accuracy values remain within the 80 – 110% range, over the storage period.

It is noted that while the accuracy and precision have been based on limited recoveries (three compared to the five stated within the guidance), it is not considered that this point should invalidated the data generated using this method, particularly as the values provided demonstrated that the method is suitable for its purpose.

Conclusion

While the method has not been satisfactorily validated in accordance with SANCO 3029/99/rev.4, it may be regarded as fit for its intended analytical purpose as a data generation method.

B. 5.1.2.5.3 – 'Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour test. (Report No D03458) – [redacted] 2011

GLP:

Yes

Method

The analytical method describes the process for the determination of napropamide-M concentration in aquatic test media for support of testing the active substance. Extraction of napropamide-M was made using water : acetonitrile (1:1, v/v), with HPLC-UV (280 nm) for detection. An LOQ of 7.8 mg/kg is supported, based on the lowest fortification level tested.

Protocol

An amount of napropamide-M technical material (nominally 25 mg) was dissolved in methanol (50 mL) in a volumetric flask to yield a stock solution with nominal concentration of 500 mg/L. This solution could be diluted as necessary for calibration, using water : acetonitrile (1:1, v/v). For fortification, an amount of napropamide-M technical material (52.02 mg) was dissolved in methanol (15 mL) in a volumetric flask to yield a stock solution with nominal concentration of 3468 mg/L. This solution was diluted in aquatic test media followed by further dilutions to achieve test solutions for validation.

For analysis of aquatic media test samples aliquots of samples were determined directly by HPLC-UV analysis with an Inertsil ODS 3 column, 50 mm x 4.6 mm i.d. with 5 µm particle size, mobile phase of acetonitrile : water with gradient elution as described in the report and UV detector monitoring at 280 nm allows determination of napropamide-M content.

Validation

Specificity was determined by the injection of napropamide-M technical material and blank aquatic test media to assess the potential for interference and the match of retention times.

Examination of the chromatograms of napropamide-M technical material and blank aquatic test media revealed that there was no interference likely to affect the chromatographic peak of napropamide-M in the presence of aquatic test media. The retention times of napropamide-M in calibration standards and spiked aquatic test media were confirmed.

A summary of the validation data supporting the method is outlined in Table B5.1.2.5.3-1.

Table B5.1.2.5.3 -1 - Validation accuracy and precision data for the determination of napropamide-M in aquatic test media

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/Kg)	% range (Mean %)		
Spiked Test Water Samples	Napropamide - M	7.80	7.80 (n=2)	91 - 90 (90.5)	0.78 (n = 2)	0.112 – 49.9 mg/L r = 0.9997 n = 7
			34.7 (n=2)	103 - 102 (102.5)	0.70 (n = 2)	

It is noted that the method precision has been calculated based on only two recovery determinations at each of the fortification levels. According to SANCO 3029/99/rev. 4 precision should be calculated based on five determinations at each fortification level. While it is acknowledged that this is a deficiency, the data provided show that acceptable recoveries within the permitted range (70-110% for individual recoveries, with the mean in

the range 80-100%) and the precision was also well within the acceptable range based on the two recovery values (<20%).

Conclusion

The method has not been satisfactory validated in accordance with SANCO/3029/99 rev. 4. However, the method may be regarded as fit for purpose and it provides some measure of assurance with respect to the acceptability of the toxicological data generated in Report No D03458.

B.5.1.2.5.4 – 'Napropamide Algal growth inhibition assay (Anabaena)'. (Report No UPH021/013213) – Jenkins, 2002a

GLP:

Yes

Method

The analytical method describes the process for the determination of napropamide (racemate) concentration in aquatic test media for support of testing the active substance. Based on the data provided within the report, an LOQ of 5.24 mg/L is proposed for SIS Lemna dilution medium and 120 mg/L for Jawarski's algal medium.

Protocol

Working solutions of napropamide (racemate) reference standard dissolved in methanol are used for preparation of calibration standards and for fortification of recovery samples.

For analysis of aquatic media test samples aliquots of samples (100 mL) were partitioned by liquid-liquid extraction into dichloromethane (2 x 20 mL). The collected organic extract was evaporated to dryness and the residue dissolved in methanol, using an appropriate volume for the expected concentration. Napropamide (racemate) was determined by GC-FID analysis with an HP-1 capillary column, 10 m x 0.53 mm i.d. with 2.65 µm df.

Validation

Specificity was determined by the injection of napropamide (racemate) reference standard, blank aquatic test media and fortified aquatic test media to assess the potential for interference and the match of retention times. Examination of the chromatograms of napropamide (racemate) calibration standards, blank aquatic test media and fortified aquatic test media revealed that there was no interference likely to affect the chromatographic peak of napropamide (racemate) in the presence of aquatic test media. The retention times of napropamide (racemate) in calibration standards and spiked aquatic test media were confirmed. Representative chromatograms demonstrating specificity are presented in the report.

A summary of the validation data supporting the method is outlined in Table B5.1.2.5.4 -1

Table B5.1.2.5.4 -1 - Validation accuracy and precision data for the determination of napropamide (racemate) in aquatic test media

Matrix	Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/L)	% range (Mean %)		
SIS Lemna dilution medium	Napropamide (racemate)	5.24	0.00105* (n=1)	86.6	7.4	5.14 - 154 mg/L R^2 = 0.9997 n = 5
			0.00524* (n=1)	92.1		
			0.0105* (n=1)	97.3		
			0.0524 (n=1)	103		
			0.105 (n=1)	107		
			0.524 (n=1)	99.3		
			1.05 (n=1)	102		
			2.10 (n=1)	95.3		
			5.24 (n=1)	97.9		
			10.5 (n=1)	99.4		
			60.0 (n=1)	121		
			122 (n=1)	107		
Jawarski's algal medium	Napropamide (racemate)	120	0.0202* (n=2)	107	-	
			120 (n=1)	99.0		
			123 (n=1)	97.9		

* concentrations lie below the linear response of the detector, not included in statistical calculation of the precision

It should be noted that no details have been provided in the report for the determination of the isomer ratio.

The method precision has been calculated based on the total number of recoveries over all of the fortification levels. According to SANCO 3029/99/rev. 4 precision should be calculated based on 5 determinations at each fortification level. While this approach is not strictly in line with the guidance, it is considered to be a more 'worst case' approach as it adds an additional layer of variability to the determination of method precision (with respect to the SIS Lemna dilution medium).

For the algal medium only two suitable recovery determinations were made: at the 120 and 123mg/L fortification levels. The limited data available did not allow for the determination of the precision. However, it should be noted that the recoveries are well within the acceptable range and do provide reassurance with respect to the applicability of this method.

It is noted that many of the fortification levels tested for the SIS Lemna dilution medium lie below the lowest limit of the tested calibration range. It is unclear from study report whether the more diluted fortified samples were concentrated prior to analysis, to bring the concentration of napropamide (racemate) within the limits of the

assessed calibration ranges. As this information has not been provided, it is considered the LOQ for this matrix is 5.24 mg/L.

Conclusion

The method has not been strictly validated in accordance with SANCO 3029/99/rev.4, however the validation data do provide some confidence in the ability of the method to detect napropamide (racemate) within these two matrix types. While the method validation data do not strictly comply with the requirements of the guidance, they may be considered supportive of the risk assessment data generated using this method and present within the study report.

B.5.1.2.5.5 – 'Napropamide-M toxicity to the Aquatic Plant *Myriophyllum spicatum* in a semi-static growth inhibition test with a prior rooting phase.' (Project No 98011215) – Hermes and Wydra, 2015

GLP:

Yes

Method

Protocol

Analysis of the test item in the overlying water:

Two stock solution of test item were prepared. Approximately 50 mg of test item were dissolved (1 minute ultrasonic bath) in 50 mL of acetonitrile to obtain a stock solution of 1g/L which was diluted to 10 and 1 mg/L with acetonitrile. Appropriate amounts of these stock solutions were diluted with test water to obtain fortified samples at concentrations of 0.1, 1 and 40 mg/L

Analysis of the test item in the sediment:

Two stock solution of test item were prepared. Approximately 50 mg of test item were dissolved in 50 mL of acetonitrile to obtain a stock solution of 1g/L. Appropriate amounts of the stock solutions were diluted with acetonitrile to obtain test item solutions at concentrations of 100 mg/L.

20µL from the test item solution of 100 mg/L were added to approximately 10g sediment to obtain fortified sediment samples. The samples were allowed to stand for at least 1h before 10 mL of acetonitrile was added to the sediment. The mixture was shaken with reciprocal shaker for 30 minutes and centrifuged (3500 rmp for 10 minutes). The extract was removed and additional 10 mL of acetonitrile was added to the sediment. The mixture again was shaken with reciprocal shaker for 30 minutes and centrifuged (3500 rpm for 10 minutes). Afterwards 5 mL of each centrifuged solution was combined and filtrated (0.2 µm pore size, PTFE). Finally the sample was diluted by factor 2 with test water.

All sample extracts or standards were analysed using HPLC-UV with an Ultrasep ES RP18 (250 x 4mm, 5µm) column (for measurements of overlying water) and Ultrasep ESD Pharm RP18 (125 x 4 mm, 3µm) column (for measurements of sediment and pore water) , mobile phase acetonitrile/pure water (95:5 %v/v) : pure water (70 : 30% v/v), detection wavelengths of 220 nm.

Validation

A summary of the validation data supporting the method is outlined in Tables B5.1.2.5.5 -1 and B5.1.2.5.5 -2.

Tables B5.1.2.5.5 -1 – Validation data - Test item in fortified overlying water

Matrix	Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/L)	% range (Mean %)		
Test item in fortified overlying water	Napropamide – M –HBW07	0.1	0.1 (n=5)	101 – 120 (113)	7	0.025 – 9.93 mg/L r = 1.000 n = 8
			1 (n=5)	105 – 110 (108)	2	
			40 (n=5)	114 – 121 (116)	2	

The mean recoveries are above the acceptable limits (70 – 100%), while this is not in line with the guidance, it does imply that the method would lead to an overestimate of the residue content within these matrices. This would lead to more 'worst case' results and consequently lead to a more conservative risk assessment. On this basis, this slight deviation may be permitted.

It is noted that the 40 mg/L fortification level lies outside of the defined linear range. The report does not indicate that samples prepared at this spike level were diluted to levels within the linear range prior to analysis. Provided that levels exceeding 1 mg/L were not found within the data generation phase, then the exclusion of this fortification level would be acceptably.

Tables B5.1.2.5.5 -2 – Validation data - Test item in fortified samples of spiked sediment

Matrix	Analyte	LOQ (mg/Kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
Test item in fortified samples of spiked sediment	Napropamide – M – HBW07	0.2	0.2 (n=5)	69 – 119 (88)	23	0.025 – 9.93 mg/L (ca. 0.1 – 40 mg/kg) r = 1.000 n = 8

For test item in fortified samples of spiked sediment only one level were determined. The relative standard deviation of the recovery is above acceptable limits (≤ 20%). While this is not strictly in line with the guidance, it is not considered that this point should invalidate the results obtained using method.

The lowest recovery of 69%, while slightly below the 70% cut-off, is not considered to have a major impact upon the validity of the method for this matrix.

It is also noted that only one fortification level has been tested for sediment matrix. While the guidance specifies that two fortification levels should be tested, it is considered that if the values obtained in the data generation phase are appropriate to this validated level, then the results obtained at this level alone will provide assurance for the validity of the results obtained for risk assessment purposes.

Conclusion

While the method has not been strictly validated in accordance with SANCO 3029/99/rev.4, it may be regarded as fit for its intended analytical purpose as a data generation method.

B.5.1.2.5.6 – 'Effects of napropamide metabolite Isomer I on Lemna minor in a growth inhibition test under semi-static test conditions'. (Report No 11 10 48 017 W) – Juckeland, 2012a

GLP:

Yes

Method

The analytical method describes the process for the concentration of napropamide metabolite isomer-I (N,N-diethyl-4-hydroxy-α-methyl-1-naphthaleneacetamide) in aquatic test media.

An amount of napropamide metabolite isomer-I (11.22 mg) was dissolved in methanol (10 mL) in a volumetric flask to yield a stock solution, with nominal concentration of 1122 mg/L. This solution was diluted as necessary for calibration or fortification using the aquatic test medium.

Samples were analysed directly by HPLC-UV (235 nm) or HPLC-MS (m/z 272) instruments fitted with a Phenomenex Kinetex C18 column (50 mm x 2.1 mm i.d. with 2.6 µm particle size). The mobile phase consisted of 0.1% formic acid in water : 0.075% formic acid in methanol with gradient elution.

Validation

A summary of the validation data supporting the method is outlined in Tables B5.1.2.5.6 -1

Table B5.1.2.5.6 -1 - Validation accuracy and precision data for the determination of napropamide metabolite isomer-I in aquatic test media

Matrix	Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/L)	% range (Mean %)		
Aquatic test media	Napropamide Metabolite Isomer-I	0.010	HPLC-MS detection			0.008 – 0.999 mg/L R^2 = 0.9999 n = 5
			0.010 (n=5)	89 - 108 (102)	7.3 (n = 5)	
			0.499 (n=5)	94.2 – 97.2 (96)	1.4 (n = 5)	
Aquatic test media	Napropamide Metabolite Isomer-I	0.499	HPLC-UV detection			0.499 – 9.99 mg/L R^2 = 0.9998 n = 6
			0.499	89.8 – 97.0 (94.2)	2.8 (n=5)	
			9.986	103.3 – 107.3 (105.8)_	1.6 (n=5)	

Specificity: examination of the chromatograms of napropamide metabolite isomer-I standards, blank aquatic test media and fortified samples revealed that there was no interference likely to affect the chromatographic peak of napropamide metabolite isomer-I in the presence of aquatic test media. The retention times of napropamide metabolite isomer-I in calibration standards and spiked aquatic test media were confirmed.

Conclusion

The method for the determination of napropamide metabolite isomer-I in aquatic media was satisfactorily validated in accordance with SANCO 3029/99/rev.4

B.5.1.2.5.7 – 'Effects of napropamide metabolite Isomer II on Lemna minor in a growth inhibition test under semi-static test conditions'. (Report No 11 10 48 018 W)) – Juckeland, 2012b

GLP:

Yes

Method

The analytical method describes the process for the determination of napropamide metabolite isomer-II (N,N-diethyl-1-hydroxy-α-methyl-2-naphthaleneacetamide) concentration in aquatic test media for support of testing a potential metabolite of the active substance

An amount of napropamide metabolite isomer-II (9.30 mg) is dissolved in methanol (10 mL) in a volumetric flask to yield a stock solution with nominal concentration of 930 mg/L. This solution can be diluted as necessary for calibration or fortification, using aquatic test medium.

For analysis of aquatic media test samples aliquots of samples are determined directly by HPLC-UV or HPLC-MS analysis with a Phenomenex Kinetex C18 column, (50 x 2.1 mm, 2.6 µm), mobile phase of 0.1% formic acid in water : 0.075% formic acid in methanol with gradient elution as described in the report and UV detector monitoring at 235 nm or MS monitoring at m/z 272.1 allowed determination of napropamide metabolite isomer-II content.

Validation of the method was by assessment of specificity (interference), linearity, accuracy (recovery) and precision.

Specificity was determined by the injection of napropamide metabolite isomer-II standards, blank aquatic test media and fortified samples to assess the potential for interference and the match of retention times. Examination of the chromatograms of napropamide metabolite isomer-II standards, blank aquatic test media and fortified samples revealed that there was no interference likely to affect the chromatographic peak of napropamide metabolite isomer-II in the presence of aquatic test media. The retention times of napropamide metabolite isomer-II in calibration standards and spiked aquatic test media were confirmed

Limit of Quantification (LOQ): The analytical LOQ for napropamide metabolite isomer-II was confirmed by acceptable recovery testing as 0.025 mg/L for HPLC-MS detection and HPLC-UV detection, in aquatic test media samples.

A summary of the validation data supporting the method is outlined in Tables B5.1.2.5.7 -1

Table B5.1.2.5.7 -1 - Validation accuracy and precision data for the determination of napropamide metabolite isomer-II in aquatic test media

Matrix	Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/L)	% range (Mean %)		
Aquatic test media	Napropamide Metabolite Isomer-II	0.025	HPLC-MS detection			0.02 – 1.2 mg/L R^2 = 0.9997 n = 6
			0.025 (n=5)	95.2 – 108.4 (101.8)	6.1 (n = 5)	
			2.232 (n=5)	94.9 – 97.5 (96.3)	1.1 (n = 5)	
Aquatic test media	Napropamide Metabolite Isomer-II	0.025	HPLC-UV detection			0.02 – 2.4 mg/L R^2 = 0.9997 n = 7
			0.025 (n=5)	86.0 – 104.8 (94.4)	7.0 (n=5)	
			2.232 (n=5)	94.7 – 99.6 (97)	2.2 (n=5)	

Conclusion

The method for the determination of napropamide metabolite isomer-II in aquatic media was satisfactorily validated in accordance with SANCO 3029/99/rev.4

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

B.5.2.1. 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

B.5.2.1.1 Crop commodities

The Notifier has made reference to the published multi-method EN 15662 (QuEChERS) and also to the published recovery data for napropamide (racemate) using this QuEChERS method.

As noted in section B.5.1.2.3.1 (page 28), the method JRFA AU-265R0 is based on the QuEChERS methodology[1] and has been submitted by the Notifier to fulfil the requirements for pre-registration methods. It has also been indicated by the Notifier that they are also relying on the method JRFA AU-265R0 to fulfil the requirements for a post-registration (enforcement) method.

While the primary method (validated in section B.5.1.2.3.1) fulfils the validation requirements set within SANCO 825/00 rev 8.1, the Notifier has also supplied the following ILV study to meet the requirements set within this guidance document.

Independent Laboratory Validation of analytical method AU-265R0 "Determination of napropamide-M in crops" (Report No.: J19552) – White, 2013

ILV testing of method used for analysis of napropamide-M in crops as an enforcement method. Crop matrices tested were cabbage, strawberry and sunflower seeds. These matrices are representative in the EU of high water, high acid and high oil crop types, respectively.

Napropamide-M concentration in samples of crop matrices is measured using the method described in report AU-2012-62 (original method reference AU-265R0, assigned reference M792 at the ILV test facility). The procedures were based directly on the published QuEChERS approach (EN 15662: 2008 "Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS method", November 2008).

Protocol

Samples and standards preparation was conducted as described in section B.5.1.2.3.1. The Method was followed without any modifications or additions other than necessary procedural and instrument set up differences due to different equipment being used.

Validation

A summary of the validation data supporting the method is outlined in Table B.5.2.1.1 -1.

[1] EN 15662: 2008 "Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS method", November 2008

Table B.5.2.1.1 -1–Independent Laboratory Validation of analytical method AU-265R0

Matrix	Analyte	LOQ (mg/Kg)	Recovery		Repeatability %RSD (n)	Linearity
			Fortification Level (mg/Kg)	% range (Mean %)		
Cabbage	Napropamide-M	0.01	Quantification MRM (*m/z* 272.18 → 129)			0.25-25 ng/mL (Ca.: 0.00125-0.125 mg/Kg) R=0.9999 (n=6)
			0.01	69.3 – 75.6 (72.7) (n=5)	3.1	
			0.1	70.4 – 81.6 (76.1) (n=5)	5.5	
			Confirmation MRM (*m/z* 272.18 → 199)			
			0.01	70.4 – 78.1 (73.1) (n=5)	4.1	
			0.1	70.3 – 78.6 (74.3) (n=5)	4.1	
Strawberry	Napropamide-M	0.01	Quantification MRM (*m/z* 272.18 → 129)			0.25-25 ng/mL (Ca.: 0.00125-0.125 mg/Kg) R=0.9999 (n=6)
			0.01	68.8 – 103.0 (81.4) (n=5)	15.8	
			0.1	68.0 – 81.9 (74.8) (n=4*)	8.5	
			Confirmation MRM (*m/z* 272.18 → 199)			
			0.01	67.7 - 101 (80.2) (n=5)	15.6	
			0.1	68.9 – 80.1 (74.5) (n=4*)	7.3	
Sunflower Seeds	Napropamide-M	0.01	Quantification MRM (*m/z* 272.18 → 129)			0.25-25 ng/mL (Ca.: 0.005-0.5 mg/Kg) R=0.9999 (n=6)
			0.01	76.2 – 80.0 (77.8) (n=5)	2.4	
			0.1	68.4 – 75.5 (72.3) (n=5)	4.1	
			Confirmation MRM (*m/z* 272.18 → 199)			
			0.01	75.6 – 86.2 (80.4) (n=5)	5.4	
			0.1	69.1 – 77.5 (72.6) (n=5)	4.7	

* - value was not reported due to suspected spiking error during sample preparation

The extraction efficiency for the method has not been demonstrated, however it is noted from the guidance that where residue levels are expected to lie below the LOQ, then data to confirm the extraction efficiency are not required. Reference to Volume 3 part B7 (active) reveals that this is the case for both brassica crops and oilseed rape (the representative uses for napropamide-M).

The validation provided for the method JRFA AU-265R0 (with the accompanying ILV study) demonstrated that the method complies with the requirements within SANCO/825/00 rev. 8.1. The method may therefore be relied upon for enforcement purposes, as well as for data generation.

Conclusion

In compliance with SANCO/825/00 rev. 8.1, four representative types of crop matrix have been validated for this method.

It should be noted that while the method JRFA AU-265R0 (with the accompanying ILV, report J19552) has been validated in accordance with the current guidance, the method does not cover all of the components of the proposed enforcement residue definition. Volume 1 of this DAR, section 2.7.3, states the following definition:

*"**Plant residue definition for monitoring:** napropamide (sum of the R- and S- isomers at any ratio)"*

The Notifier has not provided validation of the method concerning napropamide-L (*S*- isomer). However, it is considered that sufficient analytical enforcement methods for exist within the napropamide DAR (RMS=Demark, September 2005), to which the Notifier (UPL) have appropriate access.

The following method was considered to be acceptable within the napropamide DAR:

Crop/Matrix	EU agreed method EFSA Conclusion on Pesticide Peer Review report (EFSA Journal 2010; 8(4):1565)	Author (Report reference)
Tomato Brassicas Oilseed rape	DFG S19 GC/MS, LOQ 0.01 mg/kg	Chambers, 2003 (SYN/3001)
Tomato Oilseed rape	ILV GC/MS, LOQ 0.01 mg/kg	Jones, 2004 (PGD 119)

This agreed EU method is capable of determining the total napropamide (racemate) content within high water and high oil commodities (relevant to this assessment). The fortification levels assessed sufficiently cover the default LOQ (0.01 mg/kg) and a higher level (0.1 mg/kg), appropriate to the MRLs proposed in Volume 1, section 2.7.10.

Should the range of crop uses for napropamide-M containing products be expanded to include additional matrix types in the future, then further validation data / methods of analysis will be required to support these additional matrices.

B.5.2.1.2 Products of animal origin

Analytical enforcement methods for the determination of residues in animal tissues are not required as residues at harvest in commodities potentially used for animal diet are all below the default limit of quantification (0.01 mg/kg). Additionally animal metabolism studies show that napropamide (racemate) and napropamide-M are extensively metabolised and residues in edible tissues are very low. No EU MRLs are proposed for food of animal origin (Volume 1, section 2.7.10).

B.5.2.2. 'Analytical method and validation for the post-registration monitoring of napropamide residues in soil.' (Report No.: SYN/3002) – Chambers, 2003

Protocol

Napropamide (racemate) was determined in samples of soil using the method described which is based on the DFG S19 multi-residue method. Samples were prepared by accurately weighing 10 g of soil (dried if appropriate or of known moisture content) into a glass jar. Samples of untreated control soil were fortified at this point for recovery testing by adding a known amount of napropamide (racemate) from an appropriate solution in acetone.

To each sample water (100 mL) and acetone (100 mL) was added and the bottles were closed. Extraction was performed by shaking for 3 minutes. The samples were filtered using a Celite filter aid if required. Extracts were transferred into a 500 mL separating funnel and sodium chloride (20 g) was added and samples were shaken for approximately 3 minutes. Each extract was partitioned with dichloromethane (70 mL) by shaking for a further two minutes and allowed to stand for phase separation. The aqueous layer was discarded. The organic extract was dried over anhydrous sodium sulphate and filtered into a 250 mL round-bottom flask with rinsing. The extracts were concentrated to approximately 2mL using a rotary evaporator at 60°C and then dry to complete dryness on a dry-block at 50°C under nitrogen or air. The dried extracts were reconstituted in cyclohexane : ethyl acetate (1:1, 0.5 mL) and filtered if required prior to GPC clean up.

An aliquot was injected onto a GPC column system (Phenomonex Phenogel, 300 x 7.8 mm, 5 µm) using cyclohexane : ethyl acetate (1:1, 1.0 mL/minute) with collection of eluent from 11 to 14 minutes. The final extract was taken to dryness on a dry-block at 50°C under nitrogen or air and dissolved in ISTD solution (benazolin-methyl, 0.5 mL of 1 ppm solution in toluene).

All sample extracts or standards were analysed using GC-MS with a Supelco MDN-5S column (30 m x 0.25 mm i.d., 0.25 µm df) or equivalent using the oven temperature program as described in the method. SIM mode was used with monitoring of three ions m/z >100 for quantification of napropamide (m/z 171, 271 and 272). Confirmation of residue could be achieved by selection of individual ions but in this study it was achieved by checking the ratios of ions 271/171 and 272/171 in standards and comparing these with fortified sample extracts.

Validation

Two soil types were used in the validation and full classification details were presented in the report. Soil A was a sandy clay loam and Soil B was a clay.

Specificity: Examination of the chromatograms of napropamide (racemate) reference standards, fortified soil sample extracts and control soil sample extracts revealed that there was no interference likely to affect the chromatographic peak of napropamide (racemate). The retention times of napropamide (racemate) in reference substance standards and in soil extracts were confirmed. Representative chromatograms demonstrating specificity are presented in the report. The use of GC-MS with three ions > m/z 100 also allows for a highly selective and specific method for napropamide (racemate) in soil samples. Comparison of ion ratios in standards with soil extracts allowed for confirmation of the residues and a high degree of similarity was observed.

Linearity: Response of the GC-MS instrument was linear for napropamide (racemate) over the range 0.02 to 1.0 µg/mL with standards prepared in the internal standard solution (benazolin-methyl, 0.5 mL of 1 ppm solution in toluene). Solvent standards were used with 6 calibration levels and the correlation coefficient (r) was 1.000. A representative calibration curve is presented in the report. Matrix effects were not specifically investigated in this validation study, however as the accuracy data were in the range of 72 to 97% for fortified soil extracts measured against solvent calibration standards, it can be demonstrated that soil matrix effects were not significant.

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.01 mg/kg in soil for napropamide (racemate).

As indicated in the SANCO/825/00 rev. 8.1 guidance document, for *"phytotoxic herbicides the LOQ should also comply with the EC10-value of the most sensitive crop*", which applies to napropamide-M. No EC10 values for non-target plant (NTP) species were submitted for consideration in the ecotoxicology section. EC50 NTP studies conducted with D-devrinol 450 EC, indicated that the most sensitive species for seedling emergence was rye grass (ER50 76.5 g a.s./ha) and for vegetable vigour was oat (ER50 521 g a.s./ha) – see Volume 1, LoEP.

Applying the standard calculation and assumptions included in the footnote on page 17 of the SANCO/825/00 rev. 8.1 guidance, the equivalent values in mg/kg are 0.051 mg/kg and 0.35 mg/kg for rye and oat, respectively. On the basis of these data, the LOQ set for the determination of napropamide (racemate) is sufficient.

A summary of the validation data supporting the method is outlined in Tables B.5.2.2. -1 and B.5.2.2. -2

Table B.5.2.2. -1- Validation accuracy and precision data for the determination of napropamide in soil

Matrix	Analyte	LOQ (mg/kg)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/kg)	% range (Mean %)		
Soil (sandy clay)	Napropamide (racemate)	0.01	0.01 (n=3)	72 – 88 (82)	10.4	0.01-0.5 µg/0.5mL (Ca.: 0.001 – 0.05 mg/kg) r = 1.0000 n= 6
			0.10 (n=2)	74 – 75 (75)	-	
Soil (clay)			0.01 (n=3)	80 - 97	10.2	
			0.10 (n=2)	79 - 82 (81)	-	

For confirmatory purposes, the Notifier has provided ion ratio validation data. Mean ratios (*m/z* 271 → 172 and *m/z* 272 → 172, averaged spectra, background subtracted) for calibration standards may be calculated and compared to those within the test samples. According to the Notifier's own criteria, residues are considered positive if the sample ratios are within 30% of those observed in the calibration standards. The data provided have been summarised in Table B.5.2.2.-2.

Table B.5.2.2.-2- Validation ion ratio confirmation data for the determination of napropamide in soil

m/z ion ratio monitored	Ion ratio		RSD (%)	n
	Individual	Mean		
271/171 standards	0.86, 1.07, 0.96, 0.95, 0.93, 1.00	0.96	7.3	6
271/171 fortified Soil A[1]	0.86, 0.78, 0.82, 0.94, 0.89	0.86	7.2	5
271/171 fortified Soil B[1]	0.67, 1.13, 0.84, 0.82, 0.88	0.87	19	5
272/171 standards	0.16, 0.17, 0.18, 0.18, 0.16, 0.19	0.17	7.0	6
272/171 fortified Soil A[1]	0.15, 0.13, 0.15, 0.17, 0.14	0.15	10	5
272/171 fortified Soil B[1]	0.19, 0.13, 0.14, 0.13, 0.17	0.15	18	5

1 - Soil A was a sandy clay loam. Soil B was a clay

Conclusion

It should be noted that this method has been previously considered at the EU level during the evaluation / MS commenting phases for the napropamide DAR (RMS=Demark, September 2005) and was considered to have been sufficiently validated. While it is appreciated that an older revision of the SANCO/825/00 guidance document was in effect at the time of this previous EU consideration (rev 6 rather than the current rev 8.1), it is noted that there is little change between the two revisions towards the criteria and approach required to validate enforcement methods for soil. The requirement for a confirmatory technique was present within revision 6 of the guidance and the ion ratio approach was considered to be acceptable, based on the use of three ions for quantification and identification.

It is therefore considered that the conclusions reached in the napropamide DAR may also apply here and the method can be regarded as being fit for its intended purpose as an enforcement method.

It should be noted that at the time of this evaluation, the enforcement residue definition for soil has not been finalised. The method has been demonstrated to be capable of analysing for the napropamide (racemate), however individual validation data have not been provided for the separate D and L isomers of napropamide. In the event that the residue definition for monitoring is set as 'napropamide-M' only, then further validation data may be necessary to support the analysis of this isomer.

Should the finalised enforcement residue definition for water sources also include metabolites of napropamide, then further methods / validation data will be required to support the analysis of these components.

B.5.2.3. 'Analytical method validation for the determination of napropamide-M concentration in surface water (river water) and drinking water.' (Report No. : 228-2-12-6177) – Shrimali, 2013

Protocol

Samples of surface water (0.5 mL, e.g. river water) were extracted from a glass vial by vortex mixing with n-hexane (2.5 mL) for 10 minutes. The organic layer was collected and taken to dryness under a flow of nitrogen. The sample extract was reconstituted in HPLC mobile phase (0.5 mL) prior to determination of napropamide-M using LC-MS/MS.

Samples of drinking water (e.g. bottled filtered tap water) were determined directly for levels of napropamide-M using LC-MS/MS.

Samples of untreated control water were fortified before extraction (surface water) or determination (drinking water) for recovery testing by adding a known amount of napropamide-M from an appropriate solution.

All water sample extracts or standards were analysed using LC-MS/MS. Separation was achieved using a C8 column (4.6 x 150 mm, 3.5 µm), mobile phase of acetonitrile : water (70 : 30, v/v) with isocratic elution. Multiple reaction monitoring (MRM) of two transitions allowed highly selective determination of napropamide-M, using mass 272.2 → 129.2 for quantification and 272.2 → 171.2 for confirmation.

For the qualitative assessment of isomer ratio within test samples, aliquots of samples and isomer (napropamide-M / D-isomer and L-isomer) reference solutions were determined by chiral HPLC-MS/MS analysis with a Phenomenex Lux Cellulose-2 column (250 x 4.6 mm,5 µm), mobile phase of methanol : 20 mM NH_4HCO_3+ 0.1% dimethyl amine in water (70 : 30, v/v) with isocratic elution allowed determination of the two napropamide D and L isomers.

Note: napropamide-M is the D isomer and the retention times were confirmed from the prepared solutions. Multiple reaction monitoring (MRM) of two transitions allows highly selective determination of napropamide-M or L-napropamide, using mass 272.2 → 129.0 for quantification and 272.2 → 199.0 for confirmation.

Validation

Specificity: Examination of the chromatograms of napropamide-M reference standards, fortified water sample extracts and control water sample extracts revealed that there was no interference likely to affect the chromatographic peak of napropamide-M. The retention times of napropamide-M in reference substance standards and in water extracts were confirmed. Representative chromatograms demonstrating specificity are presented in the report. The use of LC-MS/MS with two MRM transitions, also allows for a highly selective and specific method for napropamide-M in water samples.

Linearity: Response of the LC-MS/MS instrument was linear over the range 0.04 to 1.24 µg/L for matrix matched drinking water standards with 6 calibration levels. The linearity was shown for both quantification and confirmation MRM transitions. The correlation coefficient (R^2) was 0.9992.

Response of the LC-MS/MS instrument was linear over the range 0.8 to 12.0 µg/L for matrix matched surface water standards with 6 calibration levels. The linearity was shown for both quantification and confirmation MRM transitions. The correlation coefficient (R^2) was 0.99972 for quantification and 0.9974 for confirmation. Representative calibration curves are presented in the report and were acceptable.

Limit of Quantification (LOQ): The LOQ was confirmed by acceptable recovery testing as 0.05 µg/L for drinking water and 1.0 µg/L for surface water (this LOQ matches the lowest NOEC of 1.0 µg/L considered within the ecotoxicology assessment, see Volume 1, LoEP).

A summary of the validation data is presented in Table B.5.2.3.-1.

Table B.5.2.3.-1 - Validation accuracy and precision data for the determination of napropamide-M in drinking water and surface water

Matrix	Analyte	LOQ µg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (µg/L)	% range (Mean %)		
Drinking water	Napropamide-M	0.05	Quantification MRM (*m/z* 272.2 → 129.0)			0.04 – 1.24 µg/L r = 0.9992 n = 6
			0.05 (n=5)	87.46 – 101.77 (93.34)	5.73	
			0.52 (n=5)	89.94 – 93.43 (91.41)	1.69	
			Confirmation MRM (*m/z* 272.2 → 199.0)			
			0.05 (n=5)	82.93 – 100.08 (92.73)	7.04	
			0.52 (n=5)	88.97 – 94.61 (91.69)	2.49	
Surface water (River water)	Napropamide-M	1.0	Quantification MRM (*m/z* 272.2 → 129.0)			0.80 – 12.0 µg/L r = 0.9972 n = 6
			1.0 (n=5)	95.71 – 98.31 (97.12)	1.28	
			10.0 (n=5)	92.80 – 99.36 (95.83)	2.49	
			Confirmation MRM (*m/z* 272.2 → 199.0)			
			1.0 (n=5)	95.80 – 99.88 (98.73)	1.66	
			10.0 (n=5)	94.64 – 99.26 (96.35)	2.15	

Conclusion

The method was satisfactory validated in accordance with SANCO/825/00 rev. 8.1.

B.5.2.4. 'Independent Laboratory Validation for the method of analysis for napropamide-M in drinking water.' (Report No. : AU-2012-63) – Bianca, 2014

Napropamide-M concentration in samples of drinking water was measured using the method as described in report 228-2-12-6177. Two samples of drinking water were used for validation, one of well water sampled locally to the test facility and a second using Aquafina bottled drinking water (filtered water).

Protocol

Samples of drinking water were determined directly for levels of napropamide-M using LC-MS/MS.
Samples of untreated control water were fortified before determination for recovery testing by adding a known amount of napropamide-M from an appropriate solution.

All water sample extracts or standards were analysed using LC-MS/MS. Separation was achieved using a Agilent C8 column (4.6 x 150 mm, 5 µm), mobile phase of acetonitrile : water (70:30, v/v) with isocratic elution. Multiple reaction monitoring (MRM) of two transitions allowed highly selective determination of napropamide-M, using mass 272.2 → 129.1 for quantification and 272.2 → 171.0 for confirmation.

For the qualitative assessment of isomer ratio within test samples, aliquots of samples and isomer (napropamide-M / D-isomer and L-isomer) reference solutions were determined by chiral HPLC-MS/MS analysis with a Phenomenex Lux Cellulose-2 column, (250 x 4.6 mm,5 µm particle size, or equivalent), mobile phase of methanol : 20 mM NH_4HCO_3+ 0.1% dimethyl amine in water (70:30, v/v) with isocratic elution allowed determination of the two napropamide D and L isomers.

Note: napropamide-M is the D isomer and the retention times can be confirmed from the prepared solutions. Multiple reactions monitoring (MRM) allowed highly selective determination of napropamide-M or L-napropamide, using mass 272.2 → 129.1.

Validation

A summary of the validation data is presented in Table B.5.2.4.-1

Table B.5.2.4.-1 - Validation accuracy and precision data for the determination of napropamide-M in drinking water

Matrix	Analyte	LOQ µg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (µg/L)	% range (Mean %)		
Drinking water (well water)	Napropamide-M	0.05	Quantification MRM (*m/z* 272.2 → 129.1)			0.02 – 2 µg/L r = 0.9989 n = 7
			0.05 (n=5)	81.2 – 97.4 (89.4)	7.4	
			0.52 (n=5)	100.0 – 103.1 (101.2)	1.4	
			Confirmation MRM (*m/z* 272.2 → 171.0)			
			0.05 (n=5)	97.2 – 111.9 (101.68)	5.9	
			0.52 (n=5)	96.7 – 99.2 (98.2)	0.96	
Drinking water (Aquafina bottled water)	Napropamide-M	0.05	Quantification MRM (*m/z* 272.2 → 129.1)			0.02 – 2 µg/L r = 0.9991 n = 7
			0.05 (n=5)	96.0 – 98.6 (96.1)	3.1	
			0.5 (n=5)	95.3 – 102.4 (98.8)	3.0	
			Confirmation MRM (*m/z* 272.2 → 171.0)			
			0.05 (n=5)	85.6 – 101.5 (91.4)	7.0	
			0.5 (n=5)	94.8 – 98.4 (97.0)	1.5	

It should be noted that for the original study a column with a 3.5µm diameter was used, rather than one with a 5 µm diameter. While it is appreciated that this is a minor change, the change in the diameter size may impact upon the retention time. The reported methodology states that the identity of napropamide isomers were made by reference to the retention times from prepared standards. Differences in the retention times between the two studies were minor - it is considered that this point will not impact upon the validity of this ILV study.

Conclusion

The method was satisfactory validated in accordance with SANCO/825/00 rev. 8.1 guidance document.

It should be noted that at the time of this evaluation, the enforcement residue definition for water sources has not been finalised. The method + ILV have been demonstrated to be capable of analysing for the napropamide-M isomer, however validation data have not been provided for the L isomers of napropamide has not been provided. In the event that the residue definition for monitoring is set as 'Napropamide (racemate)', then further validation data may be necessary to cover both isomers.

Should the finalised enforcement residue definition for water sources also include metabolites of napropamide, then further methods / validation data will be required to support the analysis of these components.

B.5.2.5. 'Napropamide. Development and validation of a method of analysis in air.' (Report No. : UPH 020/003673 Including Amendment No. 1) – Flack and Burton, 2015

Napropamide (racemate) is determined in samples of Tenax air sampling tubes using with subsequent extraction with aqueous methanol and analysis, by LC-UV (215 nm).

Protocol

Each Tenax tube was connected to a manifold so that air at 35°C and 80% relative humidity was passed through using a pump at a rate of 1 L/minute for 6-hours (total volume of air = 360 L). After this period of simulated sampling, the Tenax tubes are removed and the two portions (front 100 mg of sorbent for collection and a rear back up or beak through portion of 50 mg) are separated and prepared for desorption. Depending on the expected concentration in the air sampling Tenax sorbent, any napropamide (racemate) is desorbed using ultrasonic action with methanol for 10 minutes. Extracts are diluted appropriately with methanol / water and filtered through a 0.45 µm polypropylene syringe filter into an autosampler vial ready for determination.

All sample extracts or standards are analysed using LC-UV with an Inertsil ODS-2 column (4.6. x 250 mm, 5 µm), mobile phase of methanol : water (75:25, v/v) with isocratic elution as described in the method, UV response was monitored at 215 nm.

Validation

A summary of the validation data is presented in Table B.5.2.5.-1

Table B.5.2.5.-1 Validation accuracy and precision data for the determination of napropamide (racemate) in air

Matrix	Analyte	LOQ µg/tube)	Fortification Level (mg/m^3)	Recovery		Repeatability %RSD	Linearity
				Fortification Level (µg/tube)	% range (Mean %)		
Air	Napropamide (racemate)	1.1868	3.33×10^{-3}	1.1868	97.3 – 102.6 (99.2)	2.2	Low level 0.105 – 0.524 µg/mL r = 0.9997 n = 11
			3.33×10^{-2}	11.868	94.0 – 99.7 (95.9)	2.4	Mid-level 0.419 – 2.095 µg/mL r = 0.9999 n = 10
			3.33×10^{-1}	118.68	94.7 – 97.5 (95.8)	1.3	High level 0.419 – 2.095 µg/mL r = 0.9999 n = 10

Confirmatory data has not been provided for this method, however in line with the SANCO/825/00 rev. 8.1 guidance *"No confirmatory methods are required for the determination of residues in air if sufficient confirmatory methods are available for the determination in soil or water."* As this is the case, no further confirmatory data is required at this time. However, once the enforcement residue definitions for soil, water and air have been set and finalised, further information may be necessary.

Conclusion

The method was satisfactory validated in accordance with the SANCO/825/00 rev. 8.1 guidance document.

It should be noted that at the time of this evaluation, the enforcement residue definition for air has not been finalised. The method has been demonstrated to be capable of analysing for the napropamide (racemate) in air, however individual validation data have not been provided for the separate D and L isomers of napropamide. In the event that the residue definition for monitoring is set as 'napropamide-M' only, then further validation data may be necessary to support the specific analysis of this isomer.

B.5.2.6. Body fluids and tissues

No methods have been provided specifically for the analysis of napropamide-M within body fluids and tissues. The Commission Regulation (EU) No 283/2013 (of 1st March 2013) indicates that a suitable method should be provided for the analysis of active substances within these matrices.

It should be noted that while specific methods have not been provided to cover napropamide-M, methods do exist for the analysis of napropamide (racemate) within these tissues. Reference to the napropamide DAR (RMS=Demark, September 2005) indicates that while the napropamide (racemate) was not classed as toxic or highly toxic (hence specific methods were not provided to address this point), an acceptably validated method for the analysis for napropamide (racemate) was provided to support the detection of the active within products of animal origin. A summary of this method is provided in the following table:

Crop/Matrix	EU agreed method EFSA Conclusion on Pesticide Peer Review report (EFSA Journal 2010; 8(4):1565)	Author (Report reference)
Milk Muscle Kidney Liver Egg	DFG S19 GC/MS, LOQ 0.02 mg/kg	Weeren and Tillkes, 1996 (AZ 34313B/95)

It is regarded that this method for the analysis of products of animal origin also addresses the 'tissues' part of this requirement. According to the conclusions presented within the toxicology section of this DAR (refer to Volume 1, section 2.6.1), napropamide-M and napropamide (racemate) have equivalent toxicity. On this basis, the RMS does not consider that additional methods are necessary to support the determination of napropamide-M in 'bodily tissues'.

For 'body fluids' reference can be made to section B.5.1.2.2.3 (page 18), where methods for the determination of napropamide-M in rat plasma have been presented. While it is noted that while that this method was validated incompliance with the SANCO3030/99/rev.4 guidance (in section B.5.1.2.2.3), it is considered that the validation data provided also comply with the requirements set in the SANCO825/00/rev.8.1 guidance document and is therefore also suitable for enforcement purposes. As noted previously in this section, a suitable method is also available within the napropamide DAR (RMS=Demark, September 2005) for the determination of napropamide (racemate) within milk (Weeren and Tillkes, 1996).

The validated LOQs for both of these methods comply with the required levels of 0.05 mg/L for body fluids and 0.1 mg/kg for body tissues

B.5.3. REFERENCES RELIED ON

B.5.3.1 Literature search

The following databases were searched:

Anabstr - Analytical abstracts
Biosis
Caplus - chemical abstracts plus
Chemlist
Embase - The Excerpta Medica database
Scisearch
Toxcenter
Medline
Rtecs- Registry of Toxic Effects of Chemical Substances
Science Direct
PubMed
Wiley Online Library

The search was restricted to publications within the last ten years.

Search criteria:

- Napropamide, synonyms and CAS numbers were used
- Relevant metabolite, synonyms and CAS numbers were used
- Suitable terms relating to the assessment of residues were used.
- Metabolites were not searched in combination with residue terms due to their non-significant levels in the residues assessment.

The literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. The search did not reveal any references of relevance to this section.

B.5.3.2 References

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.1.1.	D.B. Ghate	2013	'Validation of analytical method for active ingredient analysis of napropamide-M technical.' UPL Europe Ltd, Report No.: 228-2-12-6268 Unpublished GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009.	UPL	None.
B.1.1.1.2.	A.S. Amruskar	2014	'Method validation of napropamide-M technical grade active ingredient (TGAI) to determine% napropamide-M and to quantify its associated impurities.' UPL Europe Ltd, Report No.: 228-2-12-7332 Unpublished GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.1.1.	G.J.D. Bates	2014	'Physical and chemical determinations on napropamide-M technical and purified material.' UPL Europe Ltd, Report No.: J19544 Unpublished GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.1.2.	G.J.D. Bates	2016	'For the determination of metabolites of napropamide-M in octanol and water phases for support of partition testing' UPL Europe Ltd, Report No.: J19544 Unpublished GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.2.1	A. Raithatha	2015	Validation of analytical method for determination of napropamide-M technical material concentration, homogeneity and stability in test diet. UPL Europe Ltd, Report No.: 228-2-13-6178 + Amendment No. 1 Unpublished GLP: Yes	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.2.2	A. Raithatha	2013	Additional validation of analytical method for determination of napropamide-M technical material concentration in test diet. UPL Europe Ltd, Report No.: 228-2-13-7271, Unpublished, GLP: Yes	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.2.3	N. Sriram	2014	Validation of analytical method for measurement of napropamide-M concentration in rat plasma by LC-MS-MS. UPL Europe Ltd, Report No.: 228-2-14-7333, Unpublished GLP: Yes	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.2.4	B. Katague	1979	Determination of napropamide in animal feed. UPL Europe Ltd, Report No.: RRC-79-26, Unpublished, GLP: No	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.2.5	E.M. Earley	1988	Determination of R-7465 in rodent diet by HPLC. UPL Europe Ltd, Report No.: EHC-88-11, Unpublished, GLP: No	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.2.6	R.W. Mays	1989	Determination of R-7465 in rodent diet by capillary gas chromatography. UPL Europe Ltd, Report No.: EHC-89-7, Unpublished, GLP: No	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.3.1	F. Li	2013	Method validation study for the determination of napropamide-M in crops. UPL Europe Ltd, Report No.: AU-2012-62, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.3.2	D. Norris	2002	Determination of napropamide residues in samples of brassicas treated with Devrinol in compliance with Good Laboratory Practice. UPL Europe Ltd, Report No.: OA00567, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.3.3	J. Pay	1990b	The determination of (RS)-N,N-diethyl-2-(1-napthyloxy)propionamide (napropamide, R7465) in crops. UPL Europe Ltd, Report No.: ARAM 177 Unpublished, GLP: No	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.3.4	T. Goodband	2002	To determine the magnitude of napropamide residues at harvest in the raw agricultural commodity oilseed rape resulting from a single overall application of Devrinol 45FL to the ground in Northern France (2000-2002). UPL Europe Ltd, Report No.: AF/5056/US, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.3.5	H. Harper	2017c	Napropamide: Validation of method ARAM 178 for the determination of residues in soil Company Report No. BH69LF Envigo CRS Limited, United Kingdom Unpublished GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.3.6	G.W. Schwab	1983	Determination of napropamide residues in crops by gas chromatography. UPL Europe Ltd, Report No.: RRC 83-68, Unpublished, GLP: No	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.3.7	D. Clark	2002a	To determine the magnitude of napropamide residues at harvest in the raw agricultural commodity head cabbage resulting from a single overall application of Devrinol 45FL in the UK during 2001. UPL Europe Ltd, Report No.: AS/5631/US, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.3.8	H. Harper	2017c	Napropamide: Validation of method ARAM 177 for the determination of residues in wheat Company Report No. KB98WY Envigo CRS Limited, United Kingdom Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.3.9	M Balluff	2005a	Determination of residues of napropamide after a single application with soil incorporation of Devrinol 45FL in head cabbage outdoor, Southern Europe, 2004/2005. UPL Europe Ltd, Report No.: 20044048/I1-FPCA, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.3.10	M Balluff	2005b	Determination of residues of napropamide after a single application with soil incorporation of Devrinol 45FL in head cauliflower outdoor, Southern Europe, 2004/2005. UPL Europe Ltd, Report No.: 20044048/I1-FPCF, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.4.1	A. Weir	2010	Validation of the analytical method “Napropamide & 2-naphthoxypropionic Acid/Soil/DB/10/1” for the analysis of napropamide and 2-naphthoxypropionic acid in soil (2010) UPL Europe Ltd, Report No.: S10-00191, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.4.2	J. Pay	1990a	The determination of (RS)-N,N-diethyl-2-(1-napthyloxy)propionamide (napropamide, R7465) in soil. UPL Europe Ltd, Report No.: ARAM 178, Unpublished, GLP: No	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.4.3	H. Harper	2017b	Napropamide: Validation of method ARAM 178 for the determination of residues in soil Company Report No. BH69LF Envigo CRS Limited, United Kingdom Unpublished GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B. 5.1.2.5.3	[redacted]	2011	Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour test. [redacted] Report No.: D03458, Unpublished, GLP: Yes	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.5.4	C.A. Jenkins	2002a	Napropamide Algal growth inhibition assay (Anabaena). UPL Europe Ltd, Report No.: UPH021/013213, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.5.5	H. Hermes, V. Wydra	2015	Napropamide-M (HBW07) toxicity to the aquatic plant Myriophyllum spicatum in a semi-static growth inhibition test with a prior rooting phase. UPL Europe Ltd Project No: 98011215 Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.2.5.6	D. Juckeland	2012a	Effects of napropamide metabolite Isomer I on Lemna minor in a growth inhibition test under semi-static test conditions. UPL Europe Ltd, Report No.: 11 10 48 017 W Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.1.2.5.7	D. Juckeland	2012b	Effects of napropamide metabolite Isomer II on Lemna minor in a growth inhibition test under semi-static test conditions. UPL Europe Ltd, Report No.: 11 10 48 018 W, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.2.2	G. White	2013	Independent Laboratory Validation of analytical method AU-265R0 "Determination of napropamide-M in crops". UPL Europe Ltd, Report No.: J19552, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.2.3	J.G. Chambers	2003	Analytical method and validation for the post-registration monitoring of napropamide residues in soil. UPL Europe Ltd, Report No.: SYN/3002, Unpublished, GLP: Yes	N	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.5.2.4	A. Shrimali	2013	Analytical method validation for the determination of napropamide-M concentration in surface water (river water) and drinking water. UPL Europe Ltd, Report No.: 228-2-12-6177, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.2.5	C. Bianca	2014	Independent Laboratory Validation for the method of analysis for napropamide-M in drinking water. UPL Europe Ltd, Report No.: AU-2012-63, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.2.6	I. Flack, D. Burton	2015	Napropamide. Development and validation of a method of analysis in air. UPL Europe Ltd, Report No.: UPH 020/003673 Including Amendment No. 1, Unpublished, GLP: Yes	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None. .