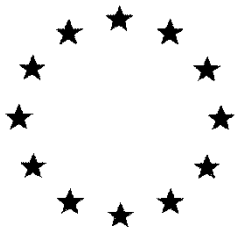
European Commission



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

Napropamide-M

Volume 3 – B.5 (PPP) – D-Devrinol

Rapporteur Member State: United Kingdom

Version History

When	What
June 2017	Initial DAR

Table of contents

B.5. METHODS OF ANALYSIS

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

B.5.1.1. Analysis of the plant protection product

Two methods have been provided by the Notifier (UPL Europe Ltd.) for the determination of napropamide-M within the representative plant protection product.

1. Method M774 – 'High performance Liquid Chromatographic determination of napropamide-M in formulation' – for the D-devinol 450 SC (HBW03) formulation.
2. Method SOP DLA-278.1 – 'D-napropamide technical and formulation determination of D-napropamide by HPLC' (Study: DL10-069)

Each of these methods have been considered in turn in the following sections: B.5.1.1.1 and B.5.1.1.2.

B.5.1.1.1. Validation of analytical method M774 "High Performance Liquid Chromatographic determination of napropamide-M in formulations" for the d-Devrinol 450 SC (HBW03) formulation. (Report No. J19546) – Atkins, 2013

Method M774

The napropamide-M content (total of D- and L-isomer) within the formulation was analysed by reverse phase HPLC chromatography with UV detection, using dibutyl phthalate as the internal standard (ISTD). The method comprises two separate parts; part A is a determination of total napropamide content and part B is a determination of the isomer ratio (D-siomer and L-isomer).

Protocol

Part A:
An amount of napropamide-M reference standard (nominally 40 or 60 mg) was dissolved in acetonitrile (60 mL) and made up to 100 mL with acetonitrile in a volumetric flask to yield stock solution with nominal concentration of 400 or 600 µg/mL. These solutions were diluted as necessary for calibration.

Amount of ISTD (dibutyl phthalate, nominally 15g) was dissolved in acetonitrile (1000 mL) in volumetric flask to yield an ISTD solution with nominal concentration of 15 mg/mL.

For the determination of the active substance form the formulated product an amount of Napropamide-M 450 g/L SC (nominally 0.1g containing 45 mg napropamide-M) was weighed into 100 mL flask. The active substance was dissolved into ISTD solution (10 mL) plus acetonitrile (60 mL) by ultrasonic action before adjusted to volume with acetonitrile.

The aliquots of samples and calibration standard were determined by HPLC analysis with Grace Smart C18 column (250 x4.6 mm, 5µm), mobile phase of acetonitrile : water (70:30 % v/v) with isocratic elution and UV detector monitoring at 270 nm allows determination of napropamide-M.

Part B:
Amount of napropamide racemate reference standard (nominally 0.02g) or napropamide-M reference standard (nominally 0.01g) were dissolved in mobile phase (total volume 100mL) to yield standard solution with nominal concentration of 200 or 100 µg/mL respectively.

For the assessment of the isomer ratio from formulated product an amount of Napropamide-M 450 g/L SC (nominally 0.04g containing 18 mg napropamide-M) was weighed into 100mL flask. The active substance was dissolved in the mobile phase (40mL) with addition of anhydrous sodium sulphate (1g). Ultrasonic mixing ensured removal of water and dissolution of napropamide-M. The solution was adjusted to volume with mobile phase.

Aliquots of the samples and isomer reference solutions were determined by chiral HPLC analysis with Chiracel, OD-H column (250 x 4.6 mm, 5µm), 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 (napropamide-M is the D isomer)

The second part of the method concerned the determination of the isomer ratio only and is the same HPLC UV method approach to the determination of the isomer ratio in technical material. This method is validated in technical material in vol 4 (confidential section). The method validation in the current study was limited to the specificity only (in terms of interference from the other constituents of the formulation and confirmation of analyte identity).

Validation

Specificity for method part A was determined by the injection of reference substance, technical material, ISTD, blank formulation and formulated product to assess the potential for interference and the match of retention times. that there was no interference likely to affect the chromatographic peak of napropamide-M. The retention times of napropamide-M in reference substance and formulated product were confirmed.

Optical isomer purity: Solutions of the napropamide racemate analytical standard, L-napropamide and napropamide-M, were weighed into separate 100 ml volumetric flasks and made up to volume with 99:1 n-hexane: ethanol. The isomers were separated and the napropamide-M (D) isomer can easily be identified by its retention time when compared to the L-napropamide isomer. Isomer ratio and hence optical purity of the napropamide-M in formulated product can be confirmed by this qualitative test. The submitted chromatograms showed good separation of the D-isomer and the L-isomer peaks using the HPLC- UV method.

A summary of the validation results are presented in Table B.5.1.1.1-1

Table B.5.1.1.1-1: Validation of method M774 (Part A)

Analyte	Recovery		Repeatability % RSD	Linearity	Specificity
	Fortification level (% w/w)	% range (Mean)			
Napropamide-M (total of D-isomer and L-isomer)	75% of nominal concentration (0.034g)	100 – 100 (mean 100), n=2	0.594 % (Modified Horwitz = 1.53 @ 42.1 % w/w). (n=6)	Range 0.25 - 0.75 mg/mL (ca. 56 - 166 %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.
	100% of nominal concentration (0.045g)	99.9 – 100 (mean 99.9), n=2			
	125 % of nominal concentration (0.057g)	99.7 – 100 (mean 99.85), n=2			

Conclusion

The method for determination of ‘total napropamide’ (D-isomer and L-isomer) using a non chiral methodology has been satisfactorily validated in accordance with the SANCO3030/99/rev.4 guidance.

The method has been validated for the determination of the D-isomer and L-isomer ratio (by relative peak area determination) in technical material only (see confidential section, volume 4, for further details).

B.5.1.1.2. Method for the determination of napropamide M (analysed as D-isomer) and L-isomer of napropamide in the formulation D-Devrinol.

Report - Validation of SOP DLA-278.1 "d-Napropamide technical and formulations, determination of d-napropamide by HPLC. (GLP Report No. DL 10-069) - Bos , 2011

Method SOP DLA-278.1

Method SOP DLA-278. determines napropamide-M in the formulation D-Devrinol by reverse phase HPLC chromatography on a chiral column with UV detection, using ethyl benzoate as the internal standard (ISTD).

Protocol

An amount of ISTD (ethyl benzoate, nominally 10 g) was dissolved in acetonitrile (1000 mL) in volumetric flask to yield an ISTD solution with nominal concentration of 10 mg/mL.

An amount of napropamide-M reference standard (nominally 50mg) was dissolved in ISTD solution (10mL) and mixed using ultrasonic action to yield a calibration stock solution with nominal concentration of 5 mg/mL. This solution was diluted as necessary for calibration, with nominal 30-fold dilution (50µL diluted to total volume of 1500µL with acetonitrile) to give the solution for calibration using relative response factor.

For determination of active substance from formulated product an amount of Napropamide-M 450 g/L SC [D-Devrinol/ HBW03] (nominally 0.11 g containing approximately 50 mg napropamide-M) was weighed into 30 mL vial. The active substance was dissolved in ISTD solution (10 mL) by ultrasonic action. An aliquot of this solution was filtered through a 0.45µm disc filter and 50 µL was diluted to total volume of 1500µL with acetonitrile ready for HPLC determination.

Aliquots of samples and calibration standards were determined by HPLC analysis with a Chiralpak AD-RH column (150 x 4.6 mm), mobile phase of acetonitrile :water (40:60 % v/v) with isocratic elution and UV detector monitoring at 280 nm allowed determination of napropamide-M with resolution of the L-napropamide isomer.

Validation

A summary of the validation results are presented in Table B.5.1.1.2-1

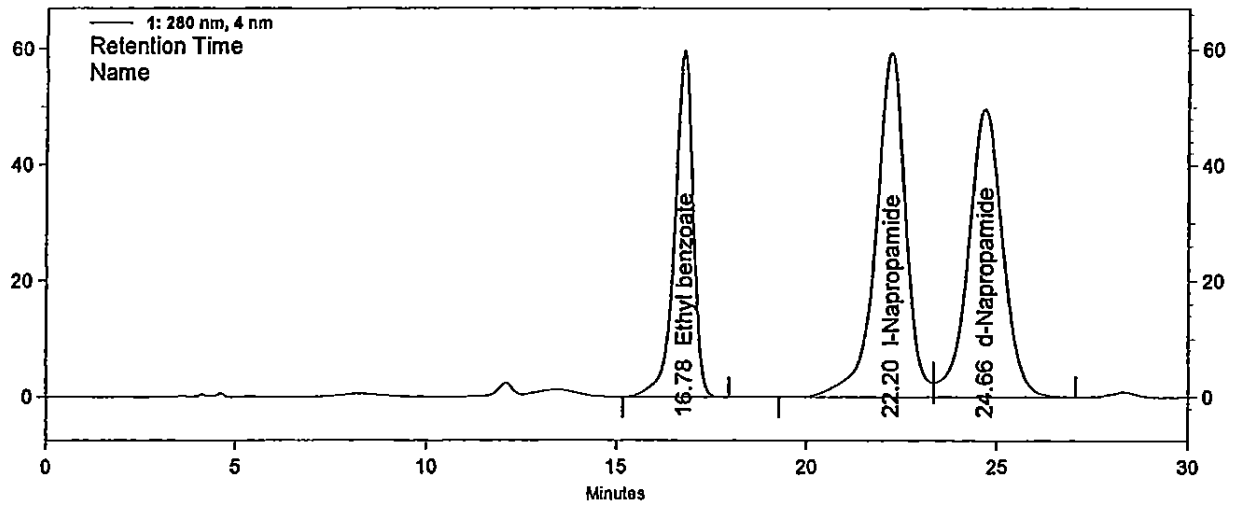
Table B.5.1.1.2-1: Validation of method SOP DLA-278.1 using the formulation D-Devrinol

Analyte	Recovery		Repeatability % RSD	Linearity	Specificity
	Fortification level (mg of technical material (Napropamide-M) added to 60 mg of 'Devrinol blank'	% range (Mean)			

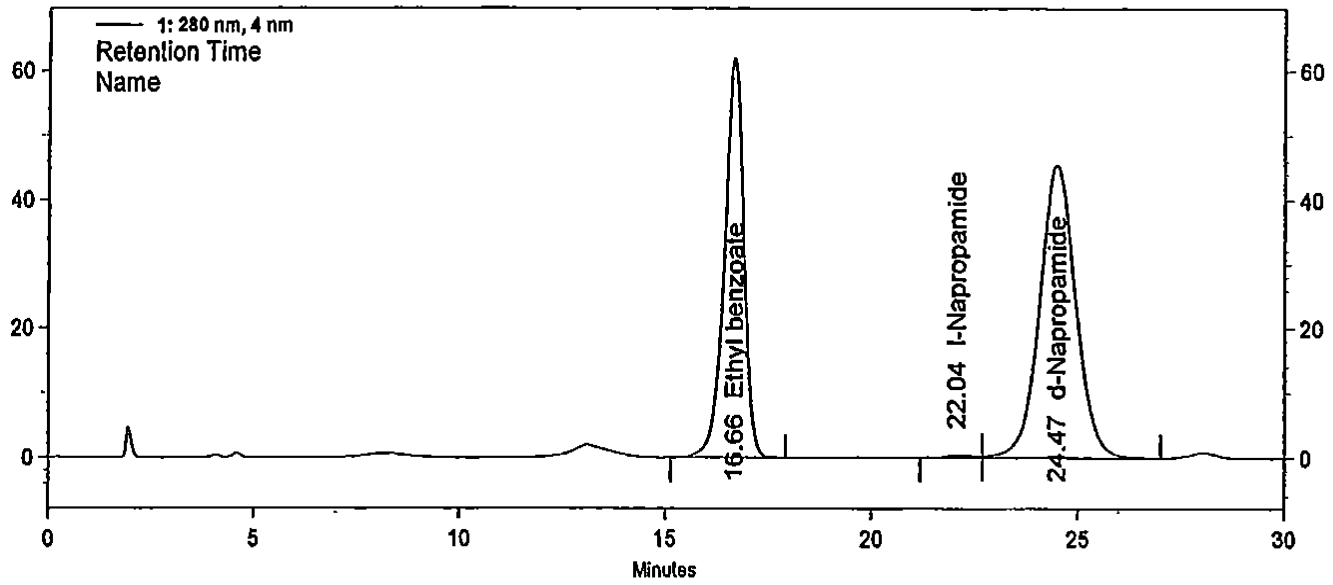
Napropamide-M (D-isomer analysed using HPLC (chiral column))	50 (n=5)	99.14 – 100.37 (mean 99.8), n=5	0.14 % (analysis of test item , five times n=5)	Range 3 -9 mg/mL (ca. 0 - 180 %w/w) R=0.99996 (n=5)	Example chromatogram, reference standard and technical material were provided, however no formulation blank chromatograms were provided to unequivocally demonstrate that there were no interferences. Analyis of a calibration showed that napropamide L isomer was (almost completely resolved) from D isomer (napropamide-M). The chromatogram for the formulation did not show a 'peak' for the L-isomer although it could be seen that a tiny 'bump' on the chromatogram was observed.

Chromatograms provided showing separation of D-isomer and L-isomer:

A – analysis of calabration solution



B – analysis of D-Devrinol



Conclusion

The method is satisfactorily validated with in accordance with SANCO3030/99/rev.4 for all relevant criteria except the method specificity (no blank chromatogram was provides for the tested matrices).

The method is regarded as an adequate quantitative method of analysis for the determination of napropamide-M (analysed specifically as D-isomer, napropamide-M).

B.5.1.2. Methods for the determination of residues

B.5.1.2.1. METHOD USED TO GENERATE TOXICOLOGICAL DATA

B.5.1.2.1.1. 4-hour acute inhalation toxicity study in the rat. (Report No. D03526) - [redacted]2011

The analytical method describes the process for the direct determination of D-Devinol 450 SC containing napropamide-M on HVLP filters.

Protocol
An amount of D-Devrinol 450 SC (nominally 29.2 mg) is dissolved in methanol (50 mL) in a volumetric flask to yield a stock solution with nominal concentration of 584 µg/mL, expressed as D-Devrinol 450 SC. This solution can be diluted as necessary for calibration, using methanol.

For analysis of HVLP filters an appropriate volume of methanol for the expected weight of test item is added to the filters in a container and extraction is performed by ultrasonic action for 5 minutes. If necessary, extracts are diluted into the calibrated range using methanol. Aliquots of samples and standards are determined by HPLC-UV analysis with an Inertsil ODS column, 50 mm x 4.6 mm i.d. with 5 µm particle size, or equivalent. 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 and therefore test item as D-Devrinol 450 SC.

Validation

Linearity of the response of the HPLC-UV system was determined over a nominal range of 30 to 200 µg/mL D-Devrinol 450 SC. The response of the HPLC-UV to D-Devrinol 450 SC was linear over the tested range of 29.2 to 204.4 µg/mL with 10 calibration levels. The correlation coefficient (R^2) was 0.9983. A representative calibration curve is presented in the report together with example chromatograms.

The chromatograms provided don't address the method specificity (no blank chromatogram have been provided).

No data demonstrating the accuracy / precision for the method have been provided and consequently an LOQ for the method could not be demonstrated.

No confirmatory method has been provided.

Conclusion

Sufficient method validation data have not been provided with this study therefore method is not considered to have been satisfactorily validated in accordance with SANCO3030/99/rev.4

B.5.1.2.2. METHOD USED TO GENERATE ECOTOXICOLOGICAL DATA

B.5.1.2.2.1. Validation of analytical method for determination of D-Devrinol 450 SC (HBW03) active ingredient concentration in test media. (Report No. 228-2-13-6185) – Amruskar, 2013

Method

The analytical method was provided for the determination of napropamide-M content in aquatic test media for testing the plant protection product Napropamide-M 450 g/L SC.

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. These solutions were 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) were added before extraction by shaking and allowing 3-5 minutes for phase separation. The organic layer was collected into a250 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 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.
Part B of the analytical method described the process for the determination of the isomer ratio and hence the optical purity of the active substance napropamide-M in the plant protection product 450 g/L SC.

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 formulated product an amount of Napropamide-M 450 g/L SC (23 mg containing nominally 10 mg napropamide-M) was weighed into a 50 mL flask. The product and active substance was mixed with algal test media (50 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 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 solution.

Validation

Specificity was determined by the injection of napropamide-M reference substance, solvent blank, formulated product, blank formulation and blank algal test media 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 in the presence of algal test media or formulated product. 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 algal test media from napropamide-M 450 g/L SC formulation was confirmed as 0.01 mg/L.

Optical isomer purity: The average D- : L-isomer ratio in test item stock solution analysed at different intervals was 96.39: 3.61. 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 results are presented in Table B.5.1.2.2.1-1

Table B.5.1.2.2.1 - 1 - Validation of method

Matrix	Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level – dose level(mg/L)	% range (Mean %)		
Algal media	Napropamide – M (D-Devinol 450 SC (HBW03))	0.01	0.01 (n=5, injected in duplicate),	90.20 – 100.60 (96.11)	3.23	0.008 – 5 mg/L r = 0.999 (n=6 - injected in duplicate)
			0.1 (n=5, injected in duplicate),	98.97 – 100.00 (99.41)	0.36	

Conclusion

The method is satisfactorily validated in accordance with SANCO3029/99/rev.4, though it is noted that a confirmatory method has not been provided.

B.5.1.2.2.2. Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour test. (Report No. D03572) –[redacted],2011

Method

The analytical method describes the process for the determination of napropamide-M concentration and therefore the associated concentration of the formulated product in aquatic test media for support of testing with formulation.

Protocol

An amount of napropamide-M technical material (nominally 50.64 mg) was dissolved in methanol (50 mL) in a volumetric flask to yield a stock solution with nominal concentration of 984 mg/L. This solution was diluted as necessary for calibration, using water : acetonitrile (1:1, v/v).

For fortification, an amount of D-Devrinol 450SC (576.23 mg) was dissolved in methanol : water (1:1, v/v 50 mL) in a volumetric flask to yield a stock solution with nominal concentration of 11525 mg/L. This solution was

diluted in aquatic test media followed by further dilutions to achieve test solutions for validation with the concentration of 1037 mg/L.

Aliquots of the samples solutions were determined directly by HPLC-UV analysis with an Inertsil ODS 3 column (50 x 4.6 mm, 5 µm), mobile phase of acetonitrile : water with gradient elution as described in the report and UV detector monitoring at 280 nm allowed determination of napropamide-M content.

Validation

Specificity was determined by the injection of napropamide-M technical material, fortified aquatic test media using D-Devrinol 450SC and blank aquatic test media 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 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 results are presented in Table B.5.1.2.2.2-1.

Table B.5.1.2.2.2 - 1 - Validation of method

Matrix	Analyte	LOQ (mg/L)	Recovery		Repeatability %RSD	Linearity
			Fortification Level (mg/L)	% range (Mean %)		
Spiked test water samples	Napropamide – M (D-Devinol 450 SC (JM230))	4.32	4.32 (n=2), (10.4 of D-Devinol 450 SC)	110 - 103 (107)	4.6	0.502 – 59.1 mg/L r^2 = 0.9997 (n=8)
			43.2 (n=2), (104 of D-Devinol 450 SC)	108 - 105 (106)	2.0	

While it is appreciated that the number of recoveries used to demonstrate the method accuracy and calculate the method precision are low compared to the five values specified in the guidance, the results do provide some reassurance that the method is suitable for its intended purpose.

No confirmatory method has been provided.

Conclusion

While the method has not been strictly validated in accordance with the SANCO3029/99/rev.4 guidance, the method was considered to be fit for purpose.

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

Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin

Plants

The methods submitted according to the requirement of Active substance, Vol_3CA_B5, point B.5.2. can be applied.

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, point 2.7.10).

Methods for the determination of residues in body fluids and tissues

The methods submitted according to the requirement of Active substance, Vol_3CA_B5, point B.5.2. can be applied.

Methods for the determination of residues in soil

The methods submitted according to the requirement of Active substance, Vol_3CA_B5, point B.5.2. can be applied.

Methods for the determination of residues in water

The methods submitted according to the requirement of Active substance, Vol_3CA_B5, point B.5.2. can be applied.

Methods for the determination of residues in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

The methods submitted according to the requirement of Active substance, Section 4, point B.5.2. can be applied.

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.	B. Atkins	2013	Validation of analytical method M774 "High Performance Liquid Chromatographic determination of napropamide-M in formulations" for the d-Devrinol 450 SC (HBW03) formulation. UPL Europe Ltd, Report No.: J19546 Yes Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None.
B.5.1.1.2	M.W.S. Bos	2011	Validation of SOP DLA-278.1 "d-Napropamide technical and formulations, determination of d-napropamide by HPLC. UPL Europe Ltd, Report No.: DL 10-069 Yes Unpublished	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	[redacted]	2011	4-hour acute inhalation toxicity study in the rat. [redacted] Report No.: D03526 Yes Unpublished	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.5.1.2.2.1	A.S. Amruskar	2013	Validation of analytical method for determination of D-Devrinol 450 SC (HBW03) active ingredient concentration in test media. UPL Europe Ltd, Report No.: 228-2-13-6185 Yes, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None. .
B.5.1.2.2.2	[redacted]	2011	Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour test. [redacted] Report No.: D03572 Yes, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None. .