European Commission

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

INDOXACARB

Volume 3 – B.5 (AS)

Rapporteur Member State: France
Co-Rapporteur Member State: Spain

Version History

When	What
2016-12	Initial RAR

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B.5. METHODS OF ANALYSIS

Introduction

For clarity, the following development codes are used in the renewal dossier for indoxacarb:

- DPX-KN128: The pure insecticidal active isomer (S-isomer) with ISO name indoxacarb.
- DPX-MP062 is the development code for the technical material containing approximately 75% DPX-KN128 and 25% IN-KN127 (insecticidally inactive enantiomer)
- DPX-JW062 is the development code for the racemic mixture of DPX-KN128 and IN-KN127

Indoxacarb (DPX-KN128) technical material is the basis for this active substance renewal dossier whereas DPX-MP062 was the technical material used in Indoxacarb monograph and review report (Indoxacarb SANCO/1408/2001 Rev.3) from 2005.

Since then DuPont has been able to produce the single enantiomeric form, DPX-KN128 (>99%). Therefore the EU renewal of indoxacarb will be based on DPX-KN128 technical material.

Studies on DPX-MP062 presented in the original active substance approval process are in some cases still relevant to DPX-KN128.

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

Study submitted to the EU for the first time in this submission

CA 4.1.1/04	**Report**	Hansen, S.W. (2013); Determination of indoxacarb (DPX-KN128) in technical grade indoxacarb and end-use products **DuPont Report No.:** DuPont-34638 **GLP:** No
CA 4.1.1/03	**Report**	Hansen, S.W. (2004); Technical grade indoxacarb (DPX-KN128) analysis and certification of product ingredients in support of registration of DuPont KN128 technical and DuPont Claridox™ C technical **DuPont Report No.:** DuPont-13126 and DuPont-13126, Confidential attachment **GLP:** Yes

Description of the method N°. KN128.220.01.ST

The method for the assay of indoxacarb as manufactured involves dissolution by ultrasonication of indoxacarb in acetonitrile. A known amount of p-terphenyl internal standard was added to each standard or sample. Samples were filtered before analysis. Analysis was done by reversed-phase liquid chromatography, with quantitation by ultraviolet absorbance at 280 nm. The column used was a Zorbax® RX-C8 column, and the mobile phase was an isocratic mixture of acetonitrile and water. The weight percent of indoxacarb in each sample was determined by comparing peak area ratios of indoxacarb/p-terphenyl with a calibration curve generated from the analysis of standard solutions. Standard solutions were prepared using indoxacarb.

Validation data:

Specificity

The method was evaluated for interferences from expected manufacturing impurities. None of the known impurities expected to be present in the technical grade indoxacarb, co-eluted with indoxacarb. Since the EU requires that any interference present does not contribute more than ± 3% to the total quantity determined, this method satisfies the EU criteria for specificity.

This analytical method cannot separate the two enantiomers (not specific for active S-isomer). Nevertheless, analytical method for determination of inactive isomer (IN-KN127) using a chiral column in the technical substance as manufactured was provided and summarize below.

The DPX-KN128 percent will be determined by method KN128.220.01. Any IN-KN127 (determined by analytical method KN128.220.02) found in the sample using this method will be subtracted from the DPX-KN128 to determine the real value.

Linearity

The linearity of the method proposed for determination of the pure active substance as manufactured was demonstrated. The equation for the calibration line is y = 468.79456x-0.00051. The correlation coefficient for six different concentrations of indoxacarb standards over the range of 30 to 150% of the assay level is 0.99998, and the slope is 468.79456.

Accuracy

The accuracy of this method for the analysis of samples of indoxacarb as manufactured was evaluated by analyzing a technical sample spiked with 5 and 10% of standard material. The average percent recovery obtained was 99.82% with a standard deviation of 0.0265 for the 5% spike and a recovery of 99.87% and standard deviation of 0.0510 for the 10% spike. The EU has established that the mean recovery of a formulation method must lie within 98 and 102% if the percent active is above 10%. This method meets the EU requirement for accuracy.

Repeatability

Repeatability testing of the assay method was determined by calculating the standard deviation of the average percent indoxacarb obtained from the analysis of six replicate test portions of the same sample of indoxacarb as manufactured. The results were calculated by one analyst on one day. The relative standard deviation was 0.44% for indoxacarb as manufactured. The maximum allowable relative standard deviation calculated from the modified Horwitz equation was 1.35% for the technical material. Therefore, this method fulfils the EU repeatability criteria. There were no outliers during this testing.

Conclusion: The analytical method is considered as validated for determination of indoxacarb in the technical grade as manufactured.

Determination of inactive isomer IN-KN127 in the active substance as manufactured:

Title	Gravelle, W.D. (04 February 2013) Description and validation of the analytical methods for the determination of impurities in indoxacarb (DPX-KN128) technical blendbase
Testing facility	Product Safety Labs Dayton, New Jersey, USA
GLP (or GEP)	Yes

Normal-Phase Chiral Liquid Chromatography: DuPont Method No. KN128.220.02.ST

Principal of the method: The sample is dissolved in ethyl acetate and analyzed by chiral normal-phase liquid chromatography using a 25 cm Chiralcel® OD-H column and UV detection at 310 nm. The weight percent of IN-KN127 in each sample is determined by comparison to a calibration curve (area vs. concentration) prepared from the analysis of **DPX-JW062** standard solutions **(Note: DPX-JW062 is a racemic (exactly 50:50) mixture of DPX-KN128 (the active enantiomer) and IN-KN127 (the inactive enantiomer)).**

Validation data:

Specificity: Chromatogram of a solvent blank, Chromatogram of a standard solution of DPX-JW062 Chromatogram of DPX-KN128 and chromatogram of IN-KN127 sample solution spiked with DPX-JW062 solution allow the separation of DPX-KN128 (active enantiomer) and the IN-KN127 (inactive enantiomer).

Linearity: Linearity was evaluated by determining the slope, intercept, and correlation coefficient of a generated. Six different concentration levels were analysed. One determination was performed at each concentration level and each sample was injected twice.

Table: Linearity of method

Method reference	Method	Component	Concentration range (µg/mL)	Slope	Intercept	Correlation coefficient
KN128.220.02.ST	Chiral NPLC	IN-KN127	0.5–99	11.29389	-0.57691	1.000

Accuracy: was determined by spiking the test portion of the technical with a solution contains a known amount of IN-KN127.

Table: Accuracy of method

Component	Spike #1			Spike #2			% Recovery
	% actual	% found	% recovery	% actual	% found	% recovery	Avg.
IN-KN127	0.11047	0.114	103.2%	0.10631	0.108	101.6%	102.4%
	0.54188	0.552	101.9%	0.52209	0.533	102.0%	102.0%

Repeatability:

Repeatability including the mean, standard deviation, relative standard deviation was determined by analysis of five replicate test portions of a technical sample.

Table: Repeatability of method

Method Reference	Method	Component	Mean (%)	Standard Deviation (%)	RSD (%)
KN128.220.02.ST	Chiral NPLC	IN-KN127	0.114	0.0028	2.46

Limit of quantitation (LOQ) for inactive isomer IN-KN127:

ID: C082505-7 (0.19821 µg/mL, equiv. to 0.0099105% in tech.)			
KN127 Peak Area	**% KN127 Concentration Found**		**% KN127 Concentration Statistics**
2.35174	0.01041	Average=	0.01033
2.25059	0.00996		
2.36971	0.01049	S.D.=	0.0002131

2.31912	0.01027		
2.32429	0.01029	% R.S.D.=	2.063
2.38622	0.01056		
		Horwitz Limit=	5.360
		% Recovery=	104.2

Conclusion: The method for the assay of the inactive isomer has been presented. Study can separate the two enantiomers (Chiral column). Method is validated for determination of IN-KN127 with an LOQ of 0.01%.

Applicability of existing CIPAC methods

The CIPAC method for indoxacarb appears in CIPAC Handbook N. It applies to TC, TK, WG, SC, and EC formulations.

Methods for the determination of additives (e.g. stabilizers) in the active substance

There are no additives considered of toxicological or environmental significance in indoxacarb as manufactured which would justify submission of analytical methods.

Methods for the determination of relevant impurities

Author:	Gravelle, W.D. 04 February 2015
Title	Description and validation of the analytical methods for the determination of IN-J1063, IN-C0800, IN-06439, and IN-R1T94 impurities in indoxacarb (DPX-KN128) technical and Indoxacarb (DPX-KN128) 150 g/L EC formulation
Report no.	DuPont-38062, Revision No. 1 and DuPont-38062, Revision No. 1
Testing facility	Product Safety Labs Dayton, New Jersey, USA
GLP:	Yes

Determination of relevant impurity Ethyl violet (IN-J1063)

Reversed-Phase Liquid Chromatography: DuPont Method Nos. KN128.220.09.ST

A solution of the sample is separated by reversed-phase high performance liquid chromatography (RPLC) using a 25 cm × 3.0 mm id Zorbax® SB-C8 Solvent Saver analytical column. Ethyl violet (IN-J1063) is detected and quantitated with ultraviolet detection at 590 nm. A calibration curve (peak area ratio vs. amount ratio), prepared from standard solutions of IN-J1063, is used to determine the total amount of ethyl violet in each sample.

Determination of relevant impurities Tetraethyl Ketone (IN-C0800), Tetraethyl Base (IN-06439) and Tetraethyl Hydrol (IN-R1T94)

Reversed-Phase Liquid Chromatography MS-MS: DuPont Method Nos. KN128.220.10.ST

A solution of the sample is separated by reversed-phase high performance liquid chromatography (RPLC) using a 15 cm × 4.6 mm ID Zorbax® Extend-C18 analytical column with 3.5-µm particle size. Tetraethyl Ketone (CAS 90-93-7), Tetraethyl Base (CAS 135-91-1) and Tetraethyl Hydrol (CAS 134-91-8) are detected and quantitated with MS-MS detection. The liquid chromatograph is coupled to a triple quadrupole mass

spectrometer by an atmospheric pressure chemical ionization (APCI) source. Calibration curves prepared from external standard solutions are used to determine the amount of each impurity in a sample.

Results and discussion:

Specificity:

Specificity (interferences) was investigated by analysing all known analytes that were likely to be present in the technical material using the appropriate method. Each standard component was injected separately. It was determined that there was no co-elution of known components

Linearity: Linearity was evaluated by determining the slope, intercept, and correlation coefficient of a generated standard curve for each analytical method (see table below). Six different concentration levels of each component were analysed. One determination was performed at each concentration level and each sample was injected twice. The reported results were obtained within the linear calibration ranges.

Table: Linearity of DuPont methods for determination of relevant impurities of indoxacarb

KN128.220.09.ST/04	LC	IN-J1063	0.05-97	55.141	-2.0934	1.000
KN128.220.10.ST/03	LC-MS	IN-C0800	0.0026–0.10	1955052.	-1658.1	0.997
		IN-06439	0.0025–0.10	5480076.	-4748.2	0.999
		IN-R1T94	0.0030–0.12	557582.	-39.179	0.998

Accuracy: was determined by spiking test portions of the technical with a solution containing a known amount of each analyte. The results from using various spiking levels are given in the table below.

Table: Accuracy of DuPont methods for determination of relevant impurities of DPX-KN128

Method Reference: KN128.220.09.ST **Method: HPLC**							
	Spike #1			**Spike #2**			**%Recov.**
Component	**%actual**	**%found**	**%recov.**	**%actual**	**%found**	**%recov.**	**Avg.**
IN-J1063	0.000191	0.000173	90.9%	0.00162	0.00158	98.1	94.5
Method Reference: KN128.220.10.ST **Method: HPLC-MS**							
	Spike #1			**Spike #2**			**%Recov.**
Component	**% actual**	**% found**	**% recov.**	**% actual**	**% found**	**% recov.**	**Avg.**
IN-C0800	0.000258	0.000231	89.6	0.00256	0.00233	90.8	90.1
IN-06439	0.000285	0.000277	97.2	0.00248	0.00243	98.0	97.6
IN-R1T94	0.000300	0.000337	112.4	0.00297	0.00334	112.4	112.4

Impurities methods were met acceptable accuracy criteria

Repeatability:

Repeatability including the mean, standard deviation, relative standard deviation, as well as the calculated modified Horwitz values was determined by analysis of at least five replicate test portions of a technical sample for the RPLC impurity methods verses calibration standards for each method. Results are given in table below.

Table: Repeatability of DuPont method for determination of relevant impurities of DPX-KN128

Method Reference	Method	Component	Mean (%)	Standard Deviation (%)	Relative Standard Deviation (%)	Horwitz Limit
KN128.220.09.ST	RPLC	IN-J1063	0.000179	0.0000079	4.40	9.69
KN128.220.10.ST	LC-MS	IN-C0800	0.000219	0.000019	8.77	9.52
		IN-06439	0.000288	0.000012	4.11	9.14
		IN-R1T94	0.000325	0.000016	5.07	8.98

Limit of Quantitation RPLC of relevant impurity ethyl violet (IN J1063): The limit of quantitation for ethyl violet (IN-J1063) in DPX-KN128 technical was determined by six injections of the sample with the lowest fortification level of 1.96 ppm.

Limit of quantitation (LOQ) for method KN128.220.09.ST

Test Material	Inj. No	IN-JI063 found (ppm)	Recovery (%)
DPX-KN128 Technical (fortified with IN-JI063)	1	1.79	91.4
	2	1.80	91.7
	3	1.79	91.3
	4	1.83	93.4
	5	1.81	92.6
	6	1.82	92.8

Average=	1.81	92.2	SANCO		APVMA	
STDEV for fortified sample=	0.017	0.860	LOQ	LOD	LOQ	LOD
%RSD=	0.933	0.933	1.8	0.6	2.0	0.7

Limit of Quantitation RPLC of relevant impurities Tetraethyl Ketone (IN-C0800), Tetraethyl Base (IN-06439) and Tetraethyl Hydrol (IN-R1T94): The limits of quantitation (LOQ) were demonstrated by satisfactory precision and recoveries at the LOQ fortification level of 2.5 ng/mg (2.5 ppm) for the impurities. LOD was determined as LOQ/5 = 2.5/5 = 0.5 ppm.

IN-06439: Limits of quantitation (LOQ) for method KN128.220.10.ST

IN-06439	Inj No.	Response(peak area)	Found Conc. (ng/mg)	Recovery (%)
LOQ	1	26250.26	2.91	116.2
	2	26055.18	2.88	115.4
	3	26272.33	2.91	116.3

	4	26579.60	2.94	117.7
	5	25761.09	2.85	114.1
	6	28030.32	3.10	124.1
Average:				117.3
SD:				3.55
%RSD:				3.0
Horwitz limit:				9.12

IN-R1T94: Limits of quantitation (LOQ) for method KN128.220.10.ST

IN-R1T94	**Inj No.**	**Response (peak area)**	**Found Conc. (ng/mg)**	**Recovery (%)**
LOQ	1	3103.35	2.81	112.3
	2	3110.71	2.81	112.5
	3	3029.83	2.74	109.6
	4	3135.51	2.84	113.4
	5	3235.86	2.93	117.1
	6	3401.25	3.08	123.0
Average:				114.7
SD:				4.76
%RSD:				4.2
Horwitz limit:				9.15

IN-C0800: Limits of quantitation (LOQ) for method KN128.220.10.ST

IN-C0800	**Inj No.**	**Response (peak area)**	**Found Conc. (ng/mg)**	**Recovery (%)**
LOQ	1	9773.51	2.51	100.3
	2	10710.29	2.75	109.9
	3	10018.08	2.57	102.8
	4	10696.40	2.75	109.8

	5	10615.96	2.73	109.0
	6	11256.86	2.89	115.6
Average:				107.9
SD:				5.49
%RSD:				5.1
Horwitz limit:				9.23

Conclusion:

All of the relevant impurity methods were validated for linearity, repeatability, specificity, and accuracy.

Methods for the determination of Toluene

Toluene was determined Revered-PHASE Liquid Chromatography: DuPont Method No. KN128.220.03.ST/02

Linearity: Six different concentration levels of each component were analysed. One determination was performed at each concentration level and each sample was injected twice.

Method reference	Method	Component	Concentration range (µg/mL)	Slope	Intercept	Correlation coefficient
KN128.220.03.ST/02	LC	Toluene	8.5–215	26.78582	16.89878	0.999

Accuracy:

Accuracy was determined by spiking test portions of the technical with a solution containing a known amount of analyte

	Spike #1			Spike #2			%Recov.
Component	**% actual**	**% found**	**% recov.**	**% actual**	**% found**	**% recov.**	**Avg.**
Toluene	0.4415	0.4187	94.8%	0.4393	0.4130	94.0%	94.4%

Repeatability:

Repeatability including the mean, standard deviation, relative standard deviation, as well as the calculated modified Horwitz values was determined by analysis of at least five replicate test portions of a technical sample for the RPLC impurity methods verses calibration standards.

Method Reference	Method	Component	Mean (%)	Standard Deviation (%)	Relative Standard Deviation (%)	Horwitz Limit
KN128.220.03.ST	RPLC	Toluene	0.11462	0.000172	0.1501	3.71

Limit of Quantitation: The limits of quantitation (LOQ) for method KN128.220.03.ST was determined by spiking according to EU Guideline SANCO/3030/99 rev. 4 and APVMA (Australia). The limits of quantitation (LOQ) were demonstrated by satisfactory precision and recoveries at the LOQ

	Peak Area	Impurity Concentration Found (µg/mL)	Standard Concentration Spiked (µg/mL)	% Recovery	% Recovery Statistics		SANCO LOQ[1]	APVMA LOD[2]	APVMA LOQ[3]
Toluene	30.19329	1.12209	1.0174	110.28951	Mean =	109.65283	0.01	0.004	0.01
	30.59147	1.13688		111.74398					
	30.12104	1.11940		110.02560	S.D. =	1.30467			
	29.59689	1.09992		108.11099					
	29.81302	1.10795		108.90047	% R.S.D. =	1.190			
	29.79822	1.10740		108.84641	Horwitz Lim.	5.346			

Analytical method developed for determination of toluene in technical active substance is validated.
Confirmation of the identity of relevant impurities:

Confirmation of identity of the analytes detected by DuPont Method N°KN128.220.10ST was accomplished by comparison of the chromatographic peak retention times generated from standard and technical material sample solutions only. This method utilizes MS/MS detection is highly specific for the Tetraethyl Ketone (IN-C0800), Tetraethyl Base (IN-06439) and Tetraethyl Hydrol (IN-R1T94) impurities.
HPLC-MS/MS chromatograms are presented to confirm the identities of the impurities for a commercially-produced indoxacarb sample.
Confirmation of identity of impurity IN-J1063 was accomplished by comparison of highly specific HPLC/UV DAD spectral data with standard. and technical material.
The typical HPLC/UV DAD spectra of the technical material and impurities were presented.

Spectra for the relevant impurities IN-06439, IN-R1T94, IN-00800 and IN-J1063 (HPLC/MS, HNMR, UV/Vis…) were provided.

The identities of process impurities in the technical indoxacarb have been confirmed by UV/VIS, IR, NMR and MS spectra.

B.5.1.2. Methods for risk assessment

B.5.1.2.1. Methods for the analysis of plants, plant products and processed food commodities to support residue trials

Authors:	Lakaschus, S., Walker, K., 2012
Title:	Determination of magnitude of residues of DPX-KN128 (indoxacarb) together with IN-KN127 in broccoli following applications of DPX-MP062 30WG and DPX-KN128 30WG - Europe, 2011
Document No.:	DuPont-33517
Test facility:	Eurofins Agroscience services Chem GmbH, Hamburg Germany
GLP:	Yes

Specimens were analysed for residues following procedures described in DFG S 19 method, previously submitted and validated in the monitoring part for high acid, high water, cereal and high fat with an ILV. Therefore reduced validation data are required.

Module extraction E1 and detection/quantification was adapted to LC-MS/MS from the gas chromatographic analysis specified in the cited methods. Mass transition monitored: MRM 528 ➔ 218 (quantification) and 528 ➔ 203.

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples of Broccoli, flower Heads and stems were presented and show no interference (> 30% of the LOQ) at the retention time of indoxacarb.

Linearity: has been performed with 6 matrix standard solutions in the range 0.250 to 50 ng/ml. Regression was linear; Y = 418.92X+119.37 and R^2 = 0.995.

Recovery: results in broccoli:

Fortification level (mg/kg)	Recovery %	Mean recovery %	RSD %
0.01	88, 92, 75	85	11
0.1	84, 83	84	-
0.2	74	-	-

Method was validated with a determined Limit of Quantification (LOQ) in broccoli was 0.010 mg/kg.

Report:	Aitken, A. 2014
Title:	Determination of the decline of residues of DPX-KN128 (indoxacarb) along with IN-KN127 in maize forage following applications of DPX-MP062 30WG - Southern Europe – 2012
Document No.:	DuPont-35172
Test facility:	Alan Aitken HND Charles river Tranent Edinburgh UK
GLP:	Yes

Specimens were analysed for residues following procedures described in DFG S 19 method, previously submitted and described above.

Extraction with acetone and detection/quantification was adapted to LC-MS/MS from the gas chromatographic analysis specified in the cited methods. Mass transition monitored: MRM 528 ➔ 218 (quantification) and 528 ➔ 203.

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples of maize forage were presented and show no interference (> 30% of the LOQ) at the retention time of indoxacarb.

Linearity: has been performed with 7 matrix fortified solution in the range 0.20 to 25 ng/ml. Regression was linear; Y = 15075X+5320.07 and R^2 = 0.991.

Recovery: results in maize forage:

Fortification level mg/kg	Recovery %	N° of analysis	Mean %	RSD %
0.01	94, 87, 99, 102, 97, 92, 91, 89, 85	9	93.6	6.1
0.1	109, 98, 91, 98, 94, 96, 100, 95, 92	9	97	5.5
1.0	91, 90, 98, 93, 88	5	92	4.1

Conclusion: Method was validated with a determined Limit of Quantification (LOQ) in Forage maize was 0.010 mg/kg.

Reports:	-Giammarrusti, L., De Paoli, M., (2003) - Kadenczki, L., (2001) - Offenbächer, G., (2002a, 2002b and 2002c)
Title:	-Decline of residues of DPX-KN128 (indoxacarb) together with IN-KN127 in sweet corn following application of DPX-MP062 30WG - Italy, season 2003 -Residue analytical determination of active ingredient of Steward 30 DF (indoxacarb) in sweet corn - Determination of indoxacarb residue in sweet corn
Document No.:	DuPont-13320, 00 DUP AB 05 01, 01/082 to 01/085 Part 1, English translation, 01/082 to 01/085 Part 2, English translation and 01/082 to 01/085
Test facility:	ERSA Pozzuolo del Friuli (UD)
GLP:	Yes

Residues were extracted from specimen by solid phase extraction and determination by gas chromatography with electron capture detector (ECD) according with some adaptations to the method AMR 4271-96 (DFG S 19).

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples were presented and show no interference at the retention time of indoxacarb.

Linearity: has been performed with 6 standard solutions in the range 0.005 to 0.150 µg/ml. Regression was linear and $R^2 = 0.999$.

Recovery:

Fortification level mg/kg	N° of samples	Mean recovery %	RSD %
DuPont-13320 (in sweet corn (cobs))			
0.01	5	95	11.2
0.10	5	91.4	15.4

Conclusion: Method was validated with a determined Limit of Quantification (LOQ) in sweet corn was 0.010 mg/kg.

Reports:	Kadenczki, L., (2001)
Title:	Residue analytical determination of active ingredient of Steward 30 DF (indoxacarb) in sweet corn
Document No.:	00 DUP AB 05 01
Test facility:	Plant protection and soil conservation service of borsod-Abauj-Zempmen Blaskovics
GLP:	Yes

Analytes are extracted from 5g samples into ethyl acetate immediately after the addition of water. An aliquot of ethyl acetate from single extraction is collected, concentrated under nitrogen, and cleaned-up by solid phase extraction with combination of silica and a carbon cartridge. The purified sample is then analyzed by GC-NPD method.

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples (corn grain and corn stalk) were presented and show no interference at the retention time of indoxacarb.

Linearity: has been performed with 5 standard solutions in the range 0.05 to 2.0 µg/ml. Regression was linear and $R^2 \geq 0.998$

Recovery:

Fortification level mg/kg	N° of samples	Mean recovery %	RDS %
In corn grain			
0.02	3	98.3	10.6
0.20	3	100.3	15.4
In corn stalk			
0.02	3	96.7	10.8
0.20	3	91.3	14.4

Conclusion: Fortification levels (n = 3) are not sufficient to establish an LOQ. This method cannot be taken into account by residues section.

Reports:	Offenbächer, G., (2002a, 2002b and 2002c)
Title:	Determination of indoxacarb residue in sweet corn
Document No.:	01/082 to 01/085 Part 2, English translation and 01/082 to 01/085 Part 3, English translation
Test facility:	Plant production service and residue laboratory LUFA of the Rheinland chambre of agriculture
GLP:	Yes

Principal of determination: The specimens were macerated online with a mixture of water, acetone dichloromethane and sodium chloride. The organic phase was purified by means of gas permeation chromatography and mini silica gel chromatography. Gas chromatography with phosphorous-nitrogen detector (NPD) based on DFG S 19 method.

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples (Sweet corn) were presented and show no interference at the retention time of indoxacarb.

Recovery

Fortification level mg/kg	Mean recovery %	RSD %	N
0.1	73	10	3
0.20	88	5	3

Linearity: Linearity is missing.

Fortification levels (n = 3) are not sufficient to establish an LOQ and linearity is missing. Thus method was not completely validated and cannot be taken into account by residues section.

Reports:	Determination of magnitude of residues of DPX-KN128 (indoxacarb) together with IN-KN127 in lettuce following applications of DPX-MP062 30WG and DPX-KN128 30WG - Europe, 2011
Title:	Lakaschus, S., Amann, S. (2012)
Document No.:	DuPont-33518
Test facility:	Eurofins Agroscience Services Chem GmbH, Hamburg, Germany
GLP:	Yes

Principal of determination: Analysis following procedures based on DFG S 19 method: Extraction with acetone using homogenizer water ratio (2/1 v/v) after addition of sodium chloride and ethyl acetate/cyclohexane (1/1 v/v), homogenization analysis were performed by LC-MS/MS (positive ion mode). Transition monitored: MRM 528 ➔ 218 (quantification) and 528 ➔ 203.

DFG S19 previously submitted and validated in the monitoring part for high acid, high water, cereal and high fat with an ILV. Therefore reduced validation data are required.

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples of lettuce were presented and show no interference > 30% of the LOQ at the retention time of indoxacarb.

Linearity: representative calibration curves for indoxacarb standard solutions and fortification solutions in range of 0.250 to 50 ng/ml were presented, R^2 were $\geq$ 0.99.
Recovery:

Lettuce:

Fortification level mg/kg	Mean recovery %	N	RSD %
0.01	79	3	15
0.1	72	3	4
0.6	91	1	-

Conclusion: Method LC-MS/MS based on DFG S 19 has been validated in higher water content matrix with an LOQ of 0.010 mg/kg.

Report:	Lakaschus, S., Amann, S., 2012
Title:	Determination of magnitude of residues of DPX-KN128 (indoxacarb) together with IN-KN127 in protected tomato following applications of DPX-MP062 30WG and DPX-KN128 30WG- Europe, 2011
Document No.:	DuPont-32128
Performing laboratory:	Eurofins Agroscience Services GmbH
GLP:	Yes

Principal of determination: Specimens were analysed for residues of DPX-KN128 following procedures based on AMR 4271-96 testing of DFG S 19 method.
DFG S19 previously submitted and validated in the monitoring part for high acid, high water, cereal and high fat with an ILV. Therefore reduced validation data are required
Final solutions were analyzed using LC-MS/MS. Ion monitored for DPX-KN128 528 ➔ 218 (quantification) and 528 ➔ 203 (confirmation).

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples of tomato were presented and show no interference (> 30% of the LOQ) at the retention time of indoxacarb.

Linearity: has been performed with 6 matrix standard solutions in the range 0.250 to 50 ng/ml. Regression was linear; Y = 696.12X+338.13 and R^2 = 0.999.

Tomato:

Fortification level mg/kg	Recovery %	N
0.01	94, 88	2
0.1	87, 86	2

Conclusion: Method LC-MS/MS based on DFG S 19 has been validated in higher water content matrix with an LOQ of 0.010 mg/kg.

Report:	Micheal R. Gagnon and Richard A. Guinivan 1996
Title:	Residue procedure for the analysis of DPX-KN128/IN-KN127 in crops and related process fractions by GC-MSD
Document No.:	DuPont Report N° AMR 3493-95 Supplement N°1
Performing laboratory:	E. I. du Pont Nemours and Company, Wilmington, Delaware 19880-0402
GLP:	Yes

Principal of determination: the combined isomers DPX-KN128/IN-KN127 were analysed in variety of crops as a single chromatographic peak after common extraction and clean-up procedures. The analytes extracted from samples into ethyl acetate after addition of water (exception: cottonseed where the isomers are extracted into acetonitrile after the addition of the hexane to the crop). The extract is collected and concentrated under nitrogen, and cleaned up by solid phase extraction with a combination of a silica and carbon cartridge. The purified sample is then analysed by gas chromatographic method with mass selective detection (ion monitored m/z: 527).
Results and discussion:

Specificity: Representative chromatograms for matrix standard control and fortified samples of all crops tested were presented and show no interference >30% of the LOQ at the retention time of DPX-KN128/IN-KN127; the control chromatograms have no peaks above chromatographic background and fortified sample chromatograms contain only the analyte peak.
Linearity: representative calibration curves were generated each time an analysis set was run in control matrix. Five standard concentrations ranged from 0.005 to 0.15 µg/mL for DPX-KN128/IN-KN127 R^2 were > 0.96.
Recovery: recovery for fortifications ranging from 0.020 to 0.25 ppm average 80 to 120% with standard deviations of 4.2 to 18% for lettuce, tomato, pepper, cabbage, broccoli, cauliflower, apple, pear, grapes, cottonseed and grapes and apples processing fractions were standards are prepared in control matrix. Recoveries for fortifications ranging from 0.020 to 1.0 ppm average 79-112% with standard deviations of 3.2 to 20% for determinations where standards are prepared in ethyl acetate.
Results of samples prepared in control matrix are presented below:

DPX-KN128/IN-KN127			
Matrix	**Fortifications levels**		
	0.02 ppm	**0.05 ppm**	**0.25 ppm**
Lettuce	Mean recoveries %:99 RSD %:18 N:6	Mean recoveries %:98 RSD %:10 N:6	Mean recoveries %:97 RSD %:11 N:6
Tomatoes	Mean recoveries %:91 RSD %:18 N:4	Mean recoveries %:82 RSD %:11 N:4	Mean recoveries %:95 RSD %:15 N:4
Peppers	Mean recoveries %:99 RSD %:6.5 N:5	Mean recoveries %:92 RSD %:17 N:6	Mean recoveries %:98 RSD %:15 N:4
Cabbage	Mean recoveries %:110 RSD %:9.1 N:4	Mean recoveries %:85 RSD %:13 N:4	Mean recoveries %:120 RSD %:8.1 N:4
Broccoli	Mean recoveries %:90 RSD %:12 N:4	Mean recoveries %:106 RSD %:7.5 N:4	Mean recoveries %:100 RSD %:4.2 N:4
Cauliflower	Mean recoveries %:91 RSD %:13 N:6	Mean recoveries %:95 RSD %:15 N:6	Mean recoveries %:100 RSD %:13 N:6
Apples	Mean recoveries %:84 RSD %:10 N:4	Mean recoveries %:107 RSD %:12 N:4	Mean recoveries %:95 RSD %:8 N:4
Pears	Mean recoveries %:89 RSD %:20 N:4	Mean recoveries %:95 RSD %:16 N:4	Mean recoveries %:108 RSD %:4.3 N:4
Grapes	Mean recoveries %:102 RSD %:14 N:3	Mean recoveries %:85 RSD %:11 N:4	Mean recoveries %:80 RSD %:9.7 N:4
Cottonseed	Mean recoveries %:105 RSD %:15 N:5	Mean recoveries %:101 RSD %:17 N:6	Mean recoveries %:89 RSD %:12 N:6
Tomato processed/Puree	Mean recoveries %:92 RSD %:16 N:4	Mean recoveries %:89 RSD %:14 N:4	Mean recoveries %:97 RSD %:6.9 N:4
Tomato processed/Paste	Mean recoveries %:102	Mean recoveries %:91	Mean recoveries %:98

	RSD %:12 N:8	RSD %:14 N:8	RSD %:8.3 N:8
Tomato processed/Ketchup	Mean recoveries %:101 RSD %:12 N:4	Mean recoveries %:108 RSD %:16 N:4	Mean recoveries %:109 RSD %:8.8 N:4
Tomato processed/Juice	Mean recoveries %:96 RSD %:17 N:3	Mean recoveries %:89 RSD %:10 N:4	Mean recoveries %:91 RSD %:13 N:4
Grapes processed/Juice	Mean recoveries %:84 RSD %:14 N:6	Mean recoveries %:90 RSD %:8 N:6	Mean recoveries %:84 RSD %:9.4 N:5
Grapes processed/Raisin	Mean recoveries %:87 RSD %:7 N:4	Mean recoveries %:81 RSD %:5.4 N:4	Mean recoveries %:89 RSD %:14 N:4
Apples processed/Juice	Mean recoveries %:88 RSD %:16 N:4	Mean recoveries %:85 RSD %:8.1 N:4	Mean recoveries %:93 RSD %:10 N:4
Apples processed/Pomace	Mean recoveries %:85 RSD %:7 N:4	Mean recoveries %:97 RSD %:8 N: 4	Mean recoveries %:99 RSD %:7.3 N:4
Grapes processed/Wine	Mean recoveries %:103 RSD %:15 N:4	Mean recoveries %:98 RSD %:15 N:4	Mean recoveries %:98 RSD %:6.7 N:4

Conclusion: method was validated at a limit of quantification of 0.02 ppm in various crops analysed.

Report:	Michael R. Gagnon and Richard A. Guinivan and Paul J. Desmond1997
Title:	Analytical enforcement procedure for the analysis of DPX-KN128/IN-KN127 in crops and related process fractions by GC-MSD
Document No.:	DuPont Report N° AMR 3493-95 Supplement N°2, revision N°2
Performing laboratory:	E. I. du Pont Nemours and Company, Wilmington, Delaware 19880-0402
GLP:	Yes

Principal of determination: The combined isomers DPX-KN128/IN-KN127 were analysed in variety of crops as a single chromatographic peak after common extraction and clean-up procedures. The analytes extracted from samples into ethyl acetate after addition of water (exception: cottonseed where the isomers are extracted into acetonitrile after addition of hexane to the crop). The extract is collected and concentrated under nitrogen, and cleaned-up by solid phase extraction with a combination of a silica and carbon cartridge.

The purified sample is then analysed by gas chromatographic method with mass selective detection (ion monitored m/z: 527).

Results and discussion:

Specificity: Representative chromatograms for matrix standard control and fortified samples of all crops tested were presented and show no interference >30% of the LOQ at the retention time of DPX-KN128/IN-KN127 and there is no significant matrix-related background peaks the controls for any crops studied.

Linearity: representative calibration (four points) curves were generated each time an analysis set was run in control matrix. Standard concentrations ranged from 0.005 to 0.15 µg/mL for DPX-KN128/IN-KN127 R^2 were > 0.96.

Recovery: Method AMR 3493-95 was previously validated in same laboratory (see above study repot DuPont Report N° AMR 3493-95 Supplement N°1) with an LOQ of 0.02 ppm in various crops.

Results of recoveries are summarized crop group in the table below:

Type	Crop or Fraction	Fortification Range	Recovery Range	Average & Standard Deviation
Leafy Vegetable	California Head Lettuce	0.20-0.60 ppm	93 to 110%	101 ± 7.6%
Flowering Fruits, and Vegetables	Tomatoes and Peppers	0.20-0.60 ppm	84 to 118%	103 ± 10%
Cole/Brassica	Cabbage, Broccoli, and Cauliflower	0.20-0.60 ppm	67 to 119%	95 ± 15%
Pome Fruit	Apples, Pears	0.20-0.60 ppm	76 to 113%	86 ± 11%
Tomato Processing Fractions	Ketchup, Paste	0.3-1.5 ppm	73-118	96 ± 13%
Apple Process Fractions	Wet Pomace, Apple Sauce	0.20-0.60 ppm	74% to 99%	93 ± 16%

Conclusion: Method AMR 3493-95 was previously validated with an LOQ of 0.02 ppm in various crops (Lettuces, Tomatoes, cabbage, Broccoli, cauliflower, apple, pear, cottonseed and grapes and apples processing fractions).

B.5.1.2.2. Methods for the analysis of products of animal origin and feed used to support an animal feeding study

Report:	Guinivan, R.A., Daussin, S. 2008
Title:	Recovery of DPX-MP062 and five metabolites from hen-derived matrices (whole eggs, muscle, fat and liver) after frozen storage
Document No.:	DuPont-19901
Performing laboratory:	E. I. du Pont Nemours and Company, Wilmington, Delaware U.S.A.
GLP:	Yes

Principal of determination: Stability samples were analyzed for DPX-MP062 and its metabolites (IN-KB687, IN-KG433, IN-KT319, IN-JU873 and IN-JT333) using procedure described in the analytical method DFGS19 reported in the monitoring part and validated (DuPont-39006).
The original method had an LOQ of 0.01mg/kg.
Method was modified and modification involved changing the aliquots removed and the final volume of the extracts analyzed. The extraction procedure was not modified.
Principal of determination:
Samples of hen whole eggs, liver, fat, and muscle were fortified with DPX-MP062 and its 5 metabolites (IN KB687, IN-KG433, IN-KT319, IN-JU873 and IN-JT333), each at a level of 0.20 mg/kg were stored at approximately 20°C and then analyzed at 5 intervals over 16 months. Residues were extracted from whole eggs using acidified ethyl acetate, and from liver, muscle and fat using acidified acetonitrile. Following partitioning and purification steps, the residues were quantified by LC/MS/MS analysis (positive ion mode for DPX-MP062, IN-KG433, IN-KT319, IN-JU873 and IN-JT333 and negative for IN-KB687).
ESI-LC-MS/MS conditions:

ANALYTES	IONS MONITORED
DPX-MP062	528.0→ 293.2 ± 0.5 AMU
	528.0→ 203.1 ± 0.5 AMU
IN-KB687	**234.0→ 202.0 ± 0.5 AMU**
(Negative Ion mode)	234.0→ 85.3 ± 0.5 AMU
IN-KG433	516.0→ 221.1 ± 0.5 AMU
	516.0→ 281.2 ± 0.5 AMU
IN-KT319	516.0→ 221.1 ± 0.5 AMU
	516.0→ 281.2 ± 0.5 AMU
IN-JU873	**458.0→ 149.2 ± 0.5 AMU**
	458.0→ 208.0 ± 0.5 AMU
	458.0→ 255.2 ± 0.5 AMU
IN-JT333	470.0→ 150.2 ± 0.5 AMU
	470.0→ 267.2 ± 0.5 AMU

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples of eggs and tissues were presented and show no interference >30% of the LOQ at the retention time of DPX-MP062 and metabolites.

Linearity: representative calibration (four-point) curves were generated each time an analysis set was run. Standard concentration ranged from 0.4 -2.0 ng/ml for indoxacarb and its metabolites, R2 were > 0.990.

Recovery

Prior analysis of the stability samples a validation of the method was conducted in which egg, muscle, liver and fat. For each matrix, a control, three fortifications at 0.1 and three fortifications at 0.2 mg/kg were analysed.

		PERCENT RECOVERY (% RELATIVE STANDARD DEVIATION)					
ANALYTE	FORTIFICATION LEVEL (MG/KG)	IN-KB687	IN-KG433	IN-KT319	IN-JU873	DPX-MP062	IN-JT333
WHOLE EGGS	0.10	84 (9.1)	87 (6.9)	88 (9.0)	86 (17)	84 (6.9)	85 (2.4)
	0.20	89 (1.3)	88 (8.0)	85 (4.1)	84 (10)	75 (11)	84 (19)
LIVER	0.10	89 (2.2)	94 (4.9)	97 (1.6)	92 (12)	94 (13)	78 (2.7)
	0.20	91(2.9)	97 (5.5)	96 (0.6)	98 (14)	95 (3.2)	91 (9.5)
FAT	0.10	91 (2.2)	94 (6.9)	97 (5.7)	91 (12)	108 (23)	94 (16)
	0.20	90 (1.3)	93 (3.4)	97 (3.2)	88 (9.5)	91 (16)	87 (10)
MUSCLE	0.10	88 (9.1)	94 (13)	92 (9.2)	100 (13)	91 (2.2)	93 (4.8)
	0.20	91 (2.8)	92 (6.0)	93 (3.9)	91 (12)	78 (4.9)	91 (5.4)

Limit of quantification: 0.2mg/kg in eggs, liver, fat and muscle.

Conclusion: Method was considered validated at a limit of quantification of 0.2mg/kg in eggs, liver, fat and muscle.

Report:	[redacted]. (2003)
Title:	Magnitude of residues of indoxacarb (as DPX-MP062) in laying hen tissue and eggs: A feeding study conducted to EPA guidelines
Document No.:	DuPont-8305, volume 1, 2 and 3
Performing laboratory:	[redacted]
GLP:	Yes

Principal of determination: The method involved extraction of the residue with acidified acetonitrile. Each sample extract was cleaned up using water/hexane partitioning and a solid phase extraction cartridge. All six analytes were quantified in a single injection. Two ions were monitored for each analyte. Residues were determined using liquid chromatography with detection and quantitation by LC-MS/MS.

Results and discussion:

Specificity: Representative chromatograms for matrix standard, control and fortified samples of eggs and tissues were presented and show no interference >30% of the LOQ at the retention time of DPX-MP062 and metabolites.

Linearity: Representative calibrations curves (five-point) for indoxacarb and metabolites standard solutions in range of 0.625-12.6 ng/ml and 0.125- 10.4 ng/mg were presented, R2 were > 0.99.

Recovery:
Recovery for fortification levels tested at 0.01 and 0.1 mg/kg (a minimum of 5 determinations per level was made): Recovery data for fortifications run concurrently with the treated samples ranged from 84–102% (DPX-MP062), 92–103% (IN-KT319), 89–96% (IN-KG433), 71–92% (IN-JU873), 78–90% (IN-JT333), and 89 101% (IN-KB687). All standard deviations and relative standard deviations for the recovery data were less than 20%.

Conclusion: Method is acceptable with an LOQ of 0.010 mg/kg (ppm) for all analytes in all matrices analysed.

Report:	Jennifer S. Amo and Ellen Beaver-Stetser, 1997
Title:	Analytical method (HPLC/column Switching/UV) for the determination of residues of residues of DPX-KN128/IN-KN127 and IN-JT333 in animal matrices whole and skim milk, cream, fat, muscle, liver and kidney
Document No.:	DuPont Report N° AMR 3337-95
Performing laboratory:	E. I. du Pont Nemours and Company, Wilmington, Delaware 19880-0402
GLP:	Yes

Principal of determination: DPX-KN128/IN-KN127 and IN-JT 333 are extracted from 5 g aliquots of milk, cream and tissue sample into acetonitrile or ethyl acetate depending on the matrix. Acetonitrile was chosen for solvent for milk cream, liver and kidney and ethyl acetate for fat and muscle. Hexane is added along with acetonitrile during the initial extraction step for liver and kidney to partition out any fatty components leaving to analytes in the acetonitrile layer.
The purified extracts are then concentrated and injected into HPLC with UV detection at 310 nm (max absorbance for DPX-KN128/IN-KN127 and IN-JT333)

Results and discussion:
Specificity: Representatives Chromatograms of DPX-KN128/IN-KN127 and IN-JT333 (matrix standard fortified and unfortified samples of milk, whole milk, skin milk, cream, fat, muscle, liver and kidney) were presented. The column and eluent-switching methodology used produces HPLC chromatograms which show no responses in control samples at both analyte retention times when compared to responses in the lowest fortification (LOQ). Method is free from interference.
Linearity: typical standard curves for curves for both analytes are presented with R2 values >0.999. A good linearity response from standards ranging from 0.005 to 1.5 µg/mL (three points injected in duplicate). Examples chromatograms of calibrations standards of DPX-KN128/IN-KN127 and IN-JT333 were presented in the study report.
Recovery: Fortify samples with an appropriate amount of the fortification standard solutions DPX-KN128/IN-KN127 and IN-JT333 in acetonitrile at 0.01, 0.05 and 5 mg/kg were analysed.

Fortification level ppm	**DPX-KN128/IN-KN127**	**IN-JT333**
	Whole milk	
0.01	Mean recovery %: 86 RSD %:5 N:6	Mean recovery %:82 RSD %:3 N:6
0.05	Mean recovery %:79 RSD %:4 N:5	Mean recovery %:76 RSD %:9 N:4
5	Mean recovery %:79 RSD %:8	Mean recovery %:79 RSD %:4

	N:4	N:4
Skim milk		
0.01	Mean recovery %:59 RSD %:9 N:6	Mean recovery %:88 RSD %:8 N:6
0.05	Mean recovery %:77 RSD %:5 N:4	Mean recovery %:74 RSD %:4 N:4
5	Mean recovery %:87 RSD %:10 N:4	Mean recovery %:85 RSD %:12 N:4
Cream		
0.01	Mean recovery %:99 RSD %:6 N:7	Mean recovery %:82 RSD %:5 N:7
0.05	Mean recovery %:84 RSD %:9 N:4	Mean recovery %:84 RSD %:10 N:4
5	Mean recovery %:84 RSD %:10 N:4	Mean recovery %:84 RSD %:10 N:4
Fat		
0.01	Mean recovery %:100 RSD %:7 N:6	Mean recovery %:88 RSD %:6 N:6
0.05	Mean recovery %:86 RSD %:2 N:4	Mean recovery %:76 RSD %:5 N:4
5	Mean recovery %:80 RSD %:4 N:4	Mean recovery %:74 RSD %:3 N:4
Muscle		
0.01	Mean recovery %:94 RSD %:12 N:6	Mean recovery %:86 RSD %:5 N:6
0.05	Mean recovery %:82 RSD %:10 N:4	Mean recovery %:82 RSD %:5 N:6
5	Mean recovery %:81 RSD %:3 N:4	Mean recovery %:83 RSD %:6 N:4
liver		
0.01	Mean recovery %:97 RSD %:9 N:6	Mean recovery %:94 RSD %:8 N:6
0.05	Mean recovery %:87 RSD %: 15 N:4	Mean recovery %:86 RSD %:6 N:4
5	Mean recovery %:81 RSD %:9 N:4	Mean recovery %:85 RSD %:3 N:4
Kidney		
0.01	Mean recovery %:99 RSD %:5 N:6	Mean recovery %:84 RSD %:7 N:6
0.05	Mean recovery %:85 RSD %:9 N:4	Mean recovery %:85 RSD %:6 N:4

5	Mean recovery %: RSD %: N:	Mean recovery %: RSD %: N:

Conclusion: Method is acceptable with an LOQ of 0.01 mg/kg (ppm) for both analytes DPX-KN128/IN-KN127 and IN-JT333 in milk, fat, muscle, liver and kidney.

B.5.1.2.3. Description of methods for determination of residues used in support of ecotoxicology studies

Report:	Charlotte Klank, 2014
Title:	Indoxacarb (DPX-KN128) 150g/l EC: Honey Bee (Apis Mellifera L.) Larval Toxicity test (Single Feeding Exposure)
Document No.:	DuPont-34817
Performing laboratory:	Eurofins Agroscience service EcoChem GmbH Niefern-Öschelbronn Germany
GLP:	Yes

Principal of determination: Sample solution was prepared in acetonitrile and an intermediate dilution in acetonitrile/water prior analysis by LC-MS/MS employing electrospray ionisation in positive mode. Ion monitored m/z 528➔ 249 (qualifier) and m/z 528➔ 203 (quantifier).

Results and discussion:

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred > 30% of the LOQ at the retention time of indoxacarb.

Linearity: Detector response was linear within the range from 1ng/ml to 100ng/ml (five-points) of indoxacarb with R^2 of 0.999 and Y = 2.31e + 0.04X = 7.43e+003

Accuracy: was determined by fortification of tests sample with the test item at the concentration levels given below:

Test item	Test item fortification level mg/l	Indoxacarb nominal mg/l	N	Mean recovery $\pm$ RSD %
Indoxacarb (DPX-KN128) 150g/l EC	10	1.62	5	91 $\pm$ 2
	90250	14583	5	109 $\pm$ 2

Repeatability: the relative standard deviation per fortification level was within the requirement ($\leq$ 20%).

LOQ: was 1.62 mg/l of indoxacarb in Honey bees.

Conclusion: Method is acceptable with an LOQ of 10 mg/l in honey bees.

Report:	Christian Berg, 2014
Title:	Indoxacarb (DPX-KN128) 150g/l EC: A semi-Field Study to Evaluation effects on the brood of honey bees (Apis mellifera; Hymenoptera, apidae) in phacelia tanacetifolia in Germany
Document No.:	DuPont-37489 revision N°1
Performing laboratory:	Eurofins Agroscience service EcoChem GmbH Niefern-Öschelbronn Germany
GLP:	Yes

Principal of determination:
Analytical phase N°: S14-03575-L2
Residue were extracted from nectar sample with acetonitrile/water (50/50, v/v) solution and ethyl acetate. From pollen residue were extracted with acetonitrile. Extract were analysed by LC-MS/MS employing electrospray ionisation in positive mode. Ion monitored m/z 528➔ 203
Results and discussion:

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Linearity: The detector linearity was confirmed over the calibration range from 0.05 ng/l to 20.0ng/ml by injection of 4 samples, the correlation coefficient R was found to be > 0.999 for nectar and pollen.

Recovery: Recovery sample were prepared by fortification of nectar or pollen with reference item prior extraction.

Matrix	Fortification level (mg/kg)	Recovery (%)
Nectar	0.010	77
	0.1	84
	2	88
Pollen	0.010	93
	0.1	96
	2	108

Repeatability: only procedural recoveries were measured, no relative standard deviation was calculated.

LOQ: These criteria were fulfilled for the 0.01 mg/kg level for nectatr and pollen (Klein 2014, see study DuPont-38419 below).

A restricted data were provided nevertheless, as method was completely validated in study report below in the same test facility, no other data required.

Report:	Olaf Klein, Dipl. Agri. Biol. 2014
Title:	Indoxacarb (DPX-KN128) 150g/l EC: A semi-Field Study to Evaluation effects on the brood of Bumble bee (Bombus Terrestris L.: Hymenoptera, Apidae) in phacelia tanacetifolia in Germany in 2013
Document No.:	DuPont-38419 study number S13-03868
Performing laboratory:	Eurofins Agroscience service EcoChem GmbH Niefern-Öschelbronn Germany
GLP:	Yes

Principal of determination:
Residue were extracted from nectar sample with acetonitrile/water (50/50, v/v) solution and ethyl acetate. From pollen residue were extracted with acetonitrile. Extract were analysed by LC-MS/MS employing electrospray ionisation in positive mode. Ion monitored m/z 528➔ 203.

Results and discussion:
Linearity: 5 samples were analysed to verify the detector response. Linearity was confirmed over the calibration range from 0.05 ng/l to 20ng/ml with regression equation Y=5.64e+0004x + 71.1 for pollen matrix and Y = 1.72e+0004x – 58.3 for nectar matrix, the correlation coefficient R was found to be > 0.998.

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.
Recovery: Recovery sample were prepared by fortification of nectar or pollen with reference item prior extraction.

Matrix	Fortification level mg/kg	Recovery %	Mean recovery ± RSD %
Nectar	0.01	82, 86, 86, 84, 83	84 ± 2
	0.2	92, 90, 82, 96, 106	93 ± 9
Pollen	0.01	101, 103, 107, 81, 84	95 ± 12
	0.2	88, 90, 95, 100, 93	93 ± 5

Repeatability: RSD ranged between 2 and 12%. Values were within the requirement.
LOQ: 0.01 mg/kg.

Conclusion: Method is acceptable with a LOQ of 0.010 mg/kg in nectar and pollen.

Report:	Sabine Rentschler (Dipl. 1Agr. Biol) 2014

Title:	Indoxacarb (DPX-KN128) 150g/l EC: A semi-Field Study to Evaluation effects on the brood of Honey bee (Apis Mellifera; Hymenoptera, Apidae) in phacelia tanacetifolia in Germany in 2014
Document No.:	DuPont-41668
Performing laboratory:	Eurofins Agroscience service EcoChem GmbH Niefern-Öschelbronn Germany
GLP:	Yes

Principal of determination: Analytical method used is based on analytical method developed in report Klein 2014 validated in study reported above in the same test facility(see study DuPont-38419 above).

Results and discussion:
Linearity: 9 samples were analysed to verify the detector response. Linearity was confirmed over the calibration range from 0.05 to 20.0 ng/ml with regression equation Y=5.64e+0.04x + 71.1 for pollen matrix and Y = 1.72e+0.04x – 58.3 for nectar matrix, the correlation coefficient R was found to be > 0.999.

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Recovery: Recovery sample were prepared by fortification of nectar or pollen with reference item prior extraction.

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean Recovery (%) ± RSD (%)
Nectar	0.010	77 / 85 / 108 / 79 / 98	89 ± 15
	2	79 / 77 / 82 / 79 / 96	83 ± 9
Pollen	0.010	80 / 94 / 97 / 89 / 93	91 ± 7
	2	79 / 85 / 78 / 94 / 92	86 ± 9

Repeatability: ranged between 7 and 15%. Values were within the requirement.

LOQ: 0.01 mg/kg.

Conclusion: Method is acceptable with a LOQ of 0.01 mg/kg in pollen and nectar.

Report:	Marco Kleinhenz, 2014
Title:	Indoxacarb (DPX-KN128) 150g/l EC: A semi-Field Study to Evaluation effects on the brood of Honey bee (Apis Mellifera; Hymenoptera, Apidae) in phacelia tanacetifolia in Germany in 2013
Document No.:	DuPont-36482
Performing laboratory:	Eurofins Agroscience service EcoChem GmbH Niefern-Öschelbronn Germany
GLP:	Yes

Principal of determination: Analytical method used is based on analytical method developed in report Klein 2014 validated in the study reported above in the same test facility (see study DuPont-38419 above).
Ion monitored m/z 528➔ 218 and 528 ➔249

Results and discussion:
Linearity: 8 samples were analysed to verify the detector response. Linearity was confirmed over the calibration range from 0.15 to 20.0 ng/ml for standard solution and for pollen matrix, the correlation coefficients R were found to be > 0.998.

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Recovery: Recovery sample were prepared by fortification of nectar or pollen with reference item prior extraction

Recovery data in pollen m/z 528➔ 218

Fortification Level	Indoxacarb Found	Recovery	Mean Recovery	RSD
(mg/kg)	(mg/kg)	(%)	(%)	(%)
0.010	0.0073	73		
0.010	0.0075	75		
0.010	0.0072	72	73	2.1
0.10	0.092	92		
0.10	0.096	96		
0.10	0.092	92	93	2.5
1.0	0.81	81	-	-
		Overall:	83	12

Recovery data in pollen m/z 528 ➔249

Fortification Level	Indoxacarb Found	Recovery	Mean Recovery	RSD
(mg/kg)	(mg/kg)	(%)	(%)	(%)
0.010	0.0081	81		
0.010	0.0082	82		
0.010	0.0082	82	82	0.7
0.10	0.087	87		
0.10	0.092	92		
0.10	0.087	87	89	3.3
1.0	0.84	84	-	-
		Overall:	85	4.6

Recovery data in nectar 528➔ 218

Fortification Level	Indoxacarb Found	Recovery	Mean Recovery	RSD
(mg/kg)	(mg/kg)	(%)	(%)	(%)
0.010	0.0099	99		
0.010	0.0096	96		
0.010	0.0105	105	100	4.6
0.10	0.091	91		
0.10	0.091	91		
0.10	0.081	81	88	6.6
1.0	0.96	96	-	-
		Overall:	94	8.0

Recovery data in nectar m/z 528 ➔249

Fortification Level	Indoxacarb Found	Recovery	Mean Recovery	RSD
(mg/kg)	(mg/kg)	(%)	(%)	(%)
0.010	0.0104	104		
0.010	0.0106	106		
0.010	0.0108	108	106	1.9
0.10	0.091	91		
0.10	0.091	91		
0.10	0.083	83	88	5.2
1.0	0.96	96	-	-
		Overall:	97	9.6

Repeatability: Values were within the requirement.
LOQ: 0.01 mg/kg.
Conclusion: Method is acceptable with a LOQ of 0.01 mg/kg in pollen and nectar.

Report:	Marco Kleinhenz, 2011
Title:	Indoxacarb (DPX-KN128) 150g/l EC: A study to evaluate effect on the honey bee (Apis mellifera carnica) in the field in brassica napus L. in Eastern Germany in 2009
Document No.:	DuPont-26946
Performing laboratory:	Eurofins Agroscience service EcoChem GmbH Niefern-Öschelbronn Germany
GLP:	Yes

And

Report:	Marco Kleinhenz, 2011
Title:	Indoxacarb (DPX-KN128) 150g/l EC: A study to evaluate effect on the honey bee (Apis mellifera carnica) in the field in brassica napus L. in Eastern Germany in 2009
Document No.:	DuPont-26947
Performing laboratory:	Eurofins Agroscience service EcoChem GmbH Niefern-Öschelbronn Germany
GLP:	Yes

Principal of determination: Method is based on DuPont method for determination of residue in nectar, pollen and honey bees reported in the monitoring part and validated in the same facilityand in report Lakaschus, S., Gizler, A. (2010).
The analytical procedure for the analysis indoxacarb with LC-MS/MS was previously validated.
Masse transition m/z 528 ➔ 218 and 528 ➔ 203.
An approximately 100 mg specimen is weighed into a test tube and extracted with 1.0 ml of acetonitrile/water (1/1, v/v). Ethyl acetate is added and the water is removed by addition of sodium sulfate. The organic layers are removed after centrifugation and the organic phase is evaporated to dryness. The residue is reconstituted with iso-propanol and acetonitrile.

Results and discussion:
Linearity: 5 samples were analysed to verify the detector response. Linearity was confirmed over the calibration range from 0.25 to 50.0 ng/ml, the correlation coefficient R was found to be > 0.999.

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Recovery: Recovery sample were prepared by fortification of honey with reference item prior extraction.

Reference	Matrix	Fortification level mg/kg	Recovery %	N	Overal Mean recovery and RSD %
DuPont-26946	Bee honey	0.01	96, 111	2	102 % 9.8%
		0.10	111, 91	2	
DuPont-26947	Bee honey	0.01	82, 72	2	76 % 5.6 %
		0.10	74, 77	2	

LOQ: 0.01 mg/kg.

Conclusion: Method is acceptable with a LOQ of 0.01 mg/kg in bee honey.

B.5.1.2.4. Description of methods for determination of residues used in support of toxicology studies

Report:	Lauren K. Markell, 2015
Title:	Indoxacarb (DPX-KN128) technical: In vitro 3T3 NBU phototoxicity test
Document No.:	DuPont-43522
Performing laboratory:	EI du Pont de Nemours and Company Newark Delaware 19714 USA
GLP	Yes

Principal of determination: All samples containing DPX-KN128 were diluted with a solvent mix containing 50% acetonitrile and 50% water and analysed by HPLC-MS/MS. Ion monitored m/z 528 ➔ 203.

Results and discussion:
Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Linearity: 8 samples were analysed to verify the detector response. Linearity was confirmed over the calibration range from 0.0063 to 0.063 µg/ml, the correlation coefficient R was found to be 0.999.

Recovery and repeatability: Recovery sample were prepared by fortification of sample with reference item prior extraction

Fortification level µg/ml	Recovery %	N	Mean recovery and RSD %
0.1	47, 48.7, 46.4, 46.3	4	47.1 $\pm$ 1.11
1.0	48.3, 48.7, 46.5, 45.4	4	47.2 $\pm$ 1.55
10	70.9, 71, 71.2, 68.6	4	70.4 $\pm$ 1.22

Recovery rate for the range 0.1 to 1.0 µg/ml is not in acceptable limit.

Conclusion: Method cannot be considered as validated at a LOQ of 0.1 µg/ml as the recovery rate for the range 0.1 to 1.0 µg/ml is not in acceptable limit.

Justification given by notifier: The analysis results show that the test substance was between approximately 50% and 70% of targeted concentrations and outside the acceptable ranges. However, it was determined (Hill, 2003; Stry, 2008, see Volume 3CA B5) that the test substance bound to glass and plastic when in an aqueous solution, limiting the maximum dosing concentration used in the assay. The concentrations of the test substance in the vehicle remained consistent for the duration of the experiment. Indoxacarb was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed. Indoxacarb was not found in the 0 mg/mL samples.

➔Justification cannot be considered as acceptable as there is no information in the studies cited to demonstrated the adhesion to glass or plastic.

Report:	Ramadevi Gudi, Ph.D. Meena Rao, B.S., 2003
Title:	Indoxacarb (DPX-KN128) technical: In vitro Mammalian chromosome aberration study in human peripheral blood lymphocytes
Document No.:	DuPont-13022, analytical report number; DuPont-13774
Testing facility:	EI du Pont de Nemours and Compagny Newark Delaware 19714 USA Performing laboratory study number; AA78LT.341.BTL
GLP	Yes

Principal of determination: Each dosing sample was transferred to volumetric flask, diluted with acetonitrile and mixed to dissolve the test substance solution. All dosing sample were further diluted with acetonitrile prior analysis by HPLC/UV at 310nm.

Results and discussion:
Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Linearity: 5 sample solutions (injected in triplicate) were used to make calibration study in the range of 0.00072 to 0.00204 mg/ml. Equation Y = 0.051727 + 2489.1211 X and R^2 = 0.999.

Recovery and repeatability:

Fortification level mg/ml	Recovery %	N	Mean recovery %
0.125	89.6, 91.2, 91.2	3	90.7 $\pm$ 1%
1.50	98.7, 95.3, 102.7	3	98.9 $\pm$ 3.7%
10	103, 103, 103	3	103 $\pm$ 1 %

Conclusion: Method was previously validated at 10ppm in the same test facility, thus the limit of quantification of 0.125 mg/ml is acceptable.

Report:	Richard H. C. San, Jane J. Clarke, M.S. 2003
Title:	Indoxacarb (DPX-KN128) technical: in vitro mammalian cell gene mutation test (CHO/HGPRT test)
Document No.:	DuPont-13023 Revision N°. 1, analytical report number; DuPont-13773
Testing facility:	EI du Pont de Nemours and Compagny Newark Delaware 19714 USA Performing laboratory study number; AA78LT.782.BTL
GLP	Yes

Principal of determination: Each dosing sample was transferred to volumetric flask, diluted with acetonitrile and mixed to dissolve the test substance solution. All dosing sample were further diluted with acetonitrile prior analysis by HPLC/UV at 310nm.

Results and discussion:
Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb

Linearity: 5 sample solutions (injected in duplicate) were used to make calibration study in the range of 0.00075 to 0.00228 mg/ml. Equation Y = 0.072396 + 2488.4313 X and R^2 = 0.999.

Recovery and repeatability:

Fortification level mg/ml	Recovery %	N	Mean recovery %
0.5	99.0, 99.2, 98.8	3	99+ 0.2%
5.0	104.8, 110.0, 102.4	3	105.7+ 3.9 %
10	101, 102, 108	3	103.7+ 3.8 %

Conclusion: Method was validated in study reported presented above at 10ppm in the same test facility, thus the limit of quantification of 0.5 mg/ml is acceptable.

Report:	Valentine O. Wagner, III, Michelle L. Klug, 2004
Title:	Indoxacarb (DPX-KN128) technical: Bacterial Reverse Mutation test
Document No.:	DuPont-14332, analytical report number; DuPont-14587
Testing facility:	EI du Pont de Nemours and Compagny Newark Delaware 19714 USA Performing laboratory study number; AA78LT.782.BTL
GLP	Yes

Principal of determination: Each dosing sample was transferred to volumetric flask, diluted with acetonitrile and mixed to dissolve the test substance solution. All dosing sample were further diluted with acetonitrile prior analysis by HPLC/UV at 310nm.
Results and discussion:

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Linearity: 4 sample solutions (injected in duplicate) were used to make calibration study in the range of 0.00075 to 0.00204 mg/ml. Equation Y = -0.176388 + 2371.7654 X and R^2 = 0.998.

Recovery and repeatability:

Fortification level mg/ml	Recovery %	N	Mean recovery %
0.05	110, 110, 114	3	108.3+ 6.7%
100	102, 107, 99.8	3	102.9+ 3.7 %

Conclusion: Method was validated in study reported presented above at 10ppm in the same test facility, thus the limit of quantification of 0.05 mg/ml is acceptable.

Report:	[redacted], 2003
Title:	Indoxacarb (DPX-KN128) technical: Mousse Bone Marrow Micronucleus test
Document No.:	DuPont-13021
Testing facility:	[redacted] Performing laboratory study number; AA78LT.782.BTL
GLP	Yes

Principal of determination: Each dosing sample was transferred to volumetric flask, diluted with acetonitrile and mixed to dissolve the test substance solution. All dosing sample were further diluted with acetonitrile prior analysis by HPLC/UV at 310nm.
Results and discussion:

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Linearity: 5 sample solutions (injected in duplicate) were used to make calibration study in the range of 0.00074 to 0.0084 mg/ml. Equation Y = -0.015435 + 2067.9451 X and R^2 = 0.999.

Recovery and repeatability:

Fortification level mg/ml	Recovery %	N	Mean recovery %
50	95.4,98.4, 99, 99.6	4	98.1 $\pm$ 1.9 %
100	104, 103, 105, 102	4	103 $\pm$ 1.3 %
200	106.5, 102, 103.5, 102	4	103.5 $\pm$ 2.1 %

Conclusion: Method was validated with a LOQ of 50 mg/ml.

Report:	[redacted], 2004
Title:	Indoxacarb (DPX-KN128) technical: Developmental toxicity study in rats
Document No.:	DuPont-12748
Testing facility:	[redacted] Performing laboratory study number; AA78LT.782.BTL
GLP	Yes

Principal of determination: Each dosing sample was transferred to volumetric flask, diluted with acetonitrile and mixed to dissolve the test substance solution. All dosing sample were further diluted with acetonitrile prior analysis by HPLC/UV at 310nm.
Results and discussion:

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Linearity: 5 sample solutions (injected in duplicate) were used to make calibration study in the range of 0.00071 to 0.00519 mg/ml. Equation Y = -0.073010 + 2570.4300 X and R^2 = 0.999.

Recovery and repeatability:

Fortification level mg/ml	Recovery %	N	Mean recovery %
0.25	94.1, 96, 96.4, 99.6	4	96.5 $\pm$ 2.3 %
0.50	90, 92, 93.2, 94.2	4	92.4 $\pm$ 1.8 %
1.00	102, 103, 102, 99.3	4	101.6 $\pm$ 1.6 %
1.75	98.3, 100, 102.3, 101.1	4	100.4 $\pm$ 1.7 %

Reproducibility:

Fortification level mg/ml	Recovery %	N	Mean recovery %
18-Aug-2003			

0.25	101.2, 101.2	2	101.2 ± 0 %
0.50	94, 94	2	94.4 ± 0.4 %
1.00	99.6, 99.4	2	99.5 ± 0.1 %
1.75	99.4, 101.1	2	100.6 ± 1 %
26-Aug-2003			
0.25	97.2, 97.2	2	97.2 ± 0%
0.50	92.8, 93.4	2	93.2 ± 0.5 %
1.00	96.8, 96.9	2	96.9 ± 0.1%
1.75	93.1, 93.7	2	93.7 ± 0.4%

Conclusion: Method can be considered as validated with a LOQ of 0.25 mg/ml.

Report:	[redacted]., 2011
Title:	Indoxacarb (DPX-KN128) technical: 28-day immunotoxicity feeding study in mice
Document No.:	DuPont-29280
Testing facility:	[redacted]
GLP	Yes

Principal of determination: Each dosing sample was transferred to volumetric flask, diluted with acetonitrile and mixed to dissolve the test substance solution. All dosing sample were further diluted with acetonitrile prior analysis by HPLC/UV at 310nm.
Results and discussion:

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred (>30% of the LOQ) at the retention time of indoxacarb.

Linearity: 6 sample solutions were used to make calibration study in the range of 1.0 to 30 ppm. Equation Y = -32.78597 X + 1.48458 and R^2 = 0.999.

Recovery and repeatability:

Fortification level ppm	Recovery %	N	Mean recovery %
10	111, 105, 109, 99.1, 95.2	5	103.9 ± 6 %
100	105, 107, 101, 102, 101.3	5	103.2 ± 3 %

Variability of the analytical method was demonstrated by RSD of the recovery results at the targeted concentration.
Conclusion: Method was validated with a LOQ of 10ppm.

Report:	[redacted] 2006
Title:	Oral (Gavage) developmental neurtoxicity study of DPX-KN128 (Indoxacarb) technical in Crl:CD (SD)IGS BR VAF/Plus Rats
Document No.:	DuPont-15150
Performing laboratory	[redacted]
GLP	Yes

Principal of determination: Each dosing sample was transferred to volumetric flask, diluted with acetonitrile and mixed to dissolve the test substance solution. All dosing sample were further diluted with acetonitrile prior analysis by HPLC/UV at 310nm.
Results and discussion:

Specificity: Chromatograms have been provided for standards, control and fortified samples. No peak interference (>30% of the LOQ) occurred at the retention time of indoxacarb.

Linearity: 4 sample solutions (injected in duplicate) were used to make calibration study in the range of 0.0037 to 0.0107 mg/ml. Equation Y = -2308.85161 X + 0.247121 and R^2 = 0.999.

Recovery and repeatability:

Fortification level mg/ml	N	Mean recovery %
0.25	3	98 ± 1 %
0.50	3	96.4 ± 1 %
0.75	3	95.7.7 ± 1 %
1.50	3	96.7 ± 1 %

Reproducibility:

Fortification level mg/ml	N	Mean recovery %
2-Jan-2005		
0.25	2	95.2 ± 4 %
0.50	2	97.2 ± 0 %
0.75	2	97.5 ± 1 %
1.50	2	99.3 ± 1 %
9/10-Feb-2005		
0.25	2	97.6 ± 1%
0.50	2	96.4 ± 2 %
0.75	2	95.5 ± 0%
1.50	2	98 ± 0.5%

Conclusion: Method can be considered as validated at a LOQ of 0.25mg/ml.

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

- The proposed residue definition for indoxacarb in plants is **parent compound (sum of isomers)**.

- The proposed residue definition for indoxacarb in animal tissues, milk, and eggs is **parent (sum of isomers) and metabolite IN-JT333.**

- The proposed environmental residue definition for indoxacarb in soil, sediment and water is **parent (sum of isomers)**

- The proposed environmental residue definition in air for indoxacarb is **parent compound (sum of isomers)**.

(*IN-JT333: methyl 7-chloro-2,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino]= carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate)*

B.5.2.1 Methods for determination of residues in plants, Plant products commodities

Residue definition: Indoxacarb (sum of isomers)

Analytical method DFG S19: This study is previously submitted and reviewed and determined to be adequate as enforcement method in crop samples in the previous indoxcarb DAR 2000.

Study report was not presented. The methods has been reevaluated according to the current guidance document SANCO 825/00 rev8.1.

Method validation

Reference:	Testing of **DFG S 19** for the determination of residues of KN128 along with KN127 in crops which might be treated with DPX-MP062, Schmidt, F, 1997, AMR 4271-96
GLP:	Yes

Test facility:	DR. Specht & Partner Chemische Laboratorien GmbH St. Anscharplatz 10 D-20354 Hamburg, Germany

Analytical method AMR 4271-96 (Schmidt, 1997) has been provided in the initial monograph of the active substance for the determination of DPX-KN128 (R-Indoxacarb) and IN-KN127 (S-indoxacarb) in plants with high water content and high acid content**.**

Principle of the method

DFG S19 multi-residue method

Samples were extracted using water/acetone; the water was saturated with sodium chloride and then partitioned with ethyl acetate/cyclohexane. The extract was cleaned-up using gel permeation and silica gel minicolumn chromatographies, and analysed by capillary gas chromatography with electron capture detection.

Results and discussion

Recovery results from method validation of apples, peaches, grapes, cauliflower, and tomatoes using the analytical method. Standards were prepared in solvent

Matrix	***Fortification level (mg/kg)***	***No of samples per fortification level***	***Range of recoveries obtained (%)***	***Mean recovery***	***RSD (%)***
Apples	0.02, 0.2, 0.5	2	91-102	94	4.4
Peaches	0.02, 0.2, 0.5	2	90-97	94	2.7
Grapes	0.02, 0.2, 0.5	2	90-102	95	4.6
Cauliflower	0.02, 0.2, 0.5	2	83-93	87	5.1
Tomato	0.02, 0.2, 0.5	2	80-90	85	4.4

Characteristics for the analytical method used for the quantitation of indoxacarb residues in food of plant origin

	Indoxacarb
Method	DFG S19 multi-residue method
Specificity	Adequate
Linearity	y= 78.7x-3.352; r= 0.9987 (5 solutions were analysing)
Calibration	
Accepted calibration range in concentration units	0.021-4.1 µg/mL
Corresponding calibration range in mass ratio units for the sample	0.0090-1.8 mg/kg
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (10 levels, single point)
Assessment of matrix effects is presented	Yes
Absence of interference >30% of LOQ in blank sample is demonstrated	Yes
Chromatogram of sample spiked at LOQ demonstrates sufficient S/N ration?	Yes
LOQ (mg/kg)	0.02 mg/kg

Conclusion

The DFG S19 method with GC/ECD detection has been validated for the analysis of indoxacarb in the high water content at an LOQ of 0.02 mg/kg.
The applicant states that the method AMR 4271-96 (Schmidt, 11997) based on DFG S19 has been validated for the four groups. However, according to actual guidance, this method has only been validated for high water content crops. For acidic commodities, data were insufficient since the number of samples at each fortification level were too low (n=2/level for 3 fortified levels).

Independent laboratory validation

Reference:	Independent laboratory validation (ILV) of the analytical residue method AMR 4271-96 for the determination of residues of DPX-KN128 and IN-KN127 in plant material which might be treated with DPX-MP062, Class, T., 2000, DuPont-3295
GLP:	Yes
Test facility:	Laboratory of Dr. Specht & Partner (AMR 4271-96), no other information

Description of the method

DFG S19 method was validated for analysis of apples (fruit), peaches (fruit), grapes (fruit), white cabbage, cauliflower, and tomato (fruit) at the laboratory of Dr. Specht & Partner (AMR 4271-96). Samples were extracted using water/acetone; the water was saturated with sodium chloride and then partitioned with ethyl acetate/cyclohexane. The extract was cleaned-up using gel permeation and silica gel minicolumn chromatographies, and analysed by capillary gas chromatography with electron capture detection.

Results and discussion

Recovery results (Standards were prepared in solvent)

Matrix	***Fortification level (mg/kg)***	***No of samples per fortification level***	***Range of recoveries obtained (%)***	***Mean recovery***	***RSD (%)***
Apples	0.010 0.10	5 5	78-105 78-107	93 94	13 13
Grapes	0.010 0.10	5 4	75-110 80-105	96 98	14 12
Cabbage	0.010 0.10	5 6	71-105 69-86	94 79	15 11
Tomato	0.010 0.10	5 5	88-108 78-105	98 93	8 14
Cotton Seed	0.020 0.20	5 5	82-109 78-111	93 98	15 15

Characteristics for the analytical method used for the quantitation of indoxacarb residues in food of plant origin

	Indoxacarb
Method	DFG S19 multi-residue method
Specificity	Adequate: Chromatograms have been provided for standards, control and fortified samples. No peak interference occurred >30% of the LOQ at the retention time of indoxacarb
Linearity	$y= -3.3413e7x^2+2.5305e7x$; r= 0.9994 (5 solutions were analysing)
Calibration	
Accepted calibration range in concentration units	1.0-200 ng/mL
Corresponding calibration range in mass ratio units for the sample	0.0008-0.17 mg/kg
Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points)?	Yes (7 levels duplicated points)
Assessment of matrix effects is presented	Yes
Absence of interference >30% of LOQ in blank sample is demonstrated	Yes
Chromatogram of sample spiked at LOQ demonstrates sufficient S/N ration?	Yes
LOD (mg/kg)	Approximately 0.003 mg/kg
LOQ (mg/kg)	0.01 mg/kg

Conclusion

In this study the DFG S19 method has been validated for the analysis of indoxacarb in the high water content and high acid content crops at an LOQ of 0.01 mg/kg and high oil content at an LOQ of 0.02 mg/kg.

The primary method (Schmidt, F, 1997) has been validated at a LOQ=0.02mg/kg for indoxacarb (sum of isomers) in high water content commodities. For acidic commodities, data were insufficient since the number of samples at each fortification level were too low (n=2/level for 2 fortified levels).

The applicant states that the method based on DFG S19 has been validated for the four groups. However, according to actual guidance document, the ILV (Class, 2000) is acceptable only for high water content commodities with a LOQ of 0.02 mg/kg.

Studies below were submitted to the EU for the first time in this submission:

Reference:	Validation of multi-residue method **DFG S19** for the determination of residues of DPX-MP062 (DPX-KN128 (indoxacarb) and IN-KN127) in grapes, tomato, oilseed rape and maize using LC/MS/MS, Čermák, J., 2013, DuPont-37894, Revision No. 1
GLP:	Yes
Test facility:	Research institute for organic syntheses, Inc. Rybitvi, Czech Republic
Acceptability of the method:	Yes

Description of the method

DPX-MP062 (DPX-KN128 (indoxacarb, S isomer) and IN-KN127 (R-isomer)) is extracted with acetone/water for high water content, acidic and dry commodities (module E1) and with acetone/acetonitrile for high fat content crops (module E7) , then partitioned with ethyl acetate/cyclohexane (1:1, v/v) and sodium chloride, purified with GPC and determined with LC-MS/MS (ESI positive mode, transitions 528 ➔218 and 528➔203 m/z). DPX-KN128 and IN-KN127 appear together as a single peak on chromatograms.

MS spectra were provided to justify the selected transitions.

Results and discussion

Specificity

Chromatograms have been provided for matrix matched calibration standards (grapes, tomato, oilseed rape, maize), control and fortified samples (grapes, tomato, oilseed rape, maize). Data have been provided for the two transitions. No interference (>30% of the LOQ) has been observed at the retention time of the analyte. Specificity is acceptable for both transitions.

Linearity

Linearity has been performed with 7 matrix matched calibration standards (grapes, oilseed rape, maize, tomato) ranging from 0.2 to 20 ng/mL. Data have been provided for the two transitions. Regressions were linear with R^2 > 0.99. Linearity is acceptable for both transitions.

Accuracy and precision

Recovery results from method validation of indoxacarb residue using the analytical method						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Grapes Transition 528→203	0.01 0.1	5 5	99-111 95-119	106 107	4.7 9.3	acceptable
Grapes Transition 528→218	0.01 0.1	5 5	96-108 91-115	101 103	4.8 10	acceptable
Tomato Transition 528→203	0.01 0.1	5 5	84-102 99-118	96 107	8.2 7.0	acceptable
Tomato Transition 528→218	0.01 0.1	5 5	86-108 96-116	100 106	8.3 7.0	acceptable
Oilseed rape Transition 528→203	0.01 0.1	5 5	99-111 113-119	105 116	4.1 2.7	acceptable
Oilseed rape Transition 528→218	0.01 0.1	5 5	101-112 112-119	107 115	4.2 2.7	acceptable
Maize Transition 528→203	0.01 0.1	5 5	76-84 76-95	80 86	4.4 10	acceptable
Maize Transition 528→218	0.01 0.1	5 5	76-84 74-93	80 84	4.0 9.5	acceptable

LOQ

The limit of quantification is 0.01mg/kg for indoxacarb (sum of isomers) in grapes, tomato, oilseed rape and maize.

Conclusion

Analytical method (Cermak, 2013) for the determination of indoxacarb residue in crops with LC-MS/MS has been provided and validated with LOQ of 0.01mg/kg for indoxacarb (sum of isomers) in high water content, acidic, fatty and dry commodities. As data have been provided for two mass transitions, the method is highly specific.

Independent laboratory validation of multi-residue method DFG S19 (Čermák, J., 2013, DuPont-37894)

Reference:	Stanislowski, T. (2015); Independent laboratory validation (ILV) of the multi-residue method DFG S19 for the determination of residues of indoxacarb in crop matrices, using LC-MS/MS Report No.: DuPont-44627
Test facility:	PTRL Europe Helmholtzstr. 22, Science Park D-89081 Ulm, Germany
GLP:	Yes

Description of the method

The applied method (DuPont-37894 rev.1) is based on multi-residue method DFG S19.

The following modules were used:

Extraction module E1 for tomatoes and grapes (watery material: water >70, fat < 2.5%): adjusted of total water (100g) and extraction with water/acetone 1/2, v/v); partition into organic phase by addition of ethyl acetate/cyclohexane (1/1, v/v)

Extraction module E2 for wheat grain (watery material: water < 70, fat < 2.5%): adjusted of total water (100g) with 40°C warm water and extraction with water/acetone 1/2, v/v); partition into organic phase by addition of ethyl acetate/cyclohexane (1/1, v/v)

Extraction module E7 for oilseed rape seed (oily, fat material): extraction with acetone (25ml) / acetonitrile (225ml) with addition of synthetic calcium silicate (trade name Calflo E) and Celite.

The purified extract is dissolved in methanol/water (1/1, v/v) containing 0.05% acetic acid and analyzed by LC-MS/MS (TurboIonspray (ESI)) for residues of indoxacarb (DPX-KN128).The first transition (528 m/z ➔218 m/z was used for quantification. The second transition 528 m/z ➔ 203 was used for confirmation.

MS spectra were provided to justify the selected transitions.

The module extraction difference between main method and ILV (use of warm water for wheat grain) is considered as minor. This modification has no significant impact on the study.

Results and discussion

Specificity

Chromatograms have been provided for matrix matched calibration standards (grapes, tomato, oilseed rape, maize), control and fortified samples (grapes, tomato, oilseed rape, maize). Data have been provided for the two transitions. No interference (>30% of the LOQ) has been observed at the retention time of the analyte. Specificity is acceptable for both transitions.

Matrix effects in all crops extracts were not significant (≤ 20%)

Linearity

Linearity has been tested for the two transitions with 8 matched calibration standards (tomatoes, grapes, wheat grain and oilseed rape) 0.1 to 20 ng/ml. Curves were presented and R2 were > 0.998. Linearity is acceptable for both transitions and for each matrix tested.

Accuracy and precision

The accuracy of the method was determined by comparing found and expected concentration from the recovery experiments. Repeatability was determined from the replicate analysis at each fortification level by calculation of

the relative standard deviation. Accuracy was determined for both the aim quantification and confirmation transition ion.

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation
m/z 528 → 218					
Tomatoes	0.01 0.10	5 5 Total = 10	80 88	3 5	4 6
Grapes	0.01 0.10	5 5 Total = 10	120 111	7 9	6 8
Oilseed Rape	0.01 0.10	5 5 Total = 10	105 100	3 5	3 5
Wheat Grain	0.01 0.10	5 5 Total = 10	80 91	5 6	6 7
m/z 528 → 203					
Tomatoes	0.01 0.10	5 5 Total = 10	83 86	3 6	4 7
Grapes	0.01 0.10	5 5 Total = 10	119 112	8 9	7 8
Oilseed Rape	0.01 0.10	5 5 Total = 10	100 98	3 4	3 4
Wheat Grain	0.01 0.10	5 5 Total = 10	84 88	2 6	2 7

LOQ

The limit of quantification is 0.01mg/kg for indoxacarb (sum of isomers) in grapes, tomato, oilseed rape and maize.

Conclusion
ILV method monitoring two MS/MS transitions for determination of indoxacarb in tomatoes, grapes, wheat grain and oilseed rape was validated with limit of quantification (LOQ) of 0.01 mg/kg.

Two ion transitions were monitored, analytical method is considered as highly specific.

Extraction efficiency: notifier attested that Extraction efficiency was addressed in the Indoxacarb DAR, Volume 3 Annex B, January 2000, as this point didn't reported in the DAR, notifier should provide the Extraction efficiency study.

DuPont response:
Radiolabeled extraction efficiency studies have been conducted for multiple crops using both acetonitrile and ethyl acetate as the extraction solvent.

Summary of extraction efficiency data

Crop	Percent of total radioactivity extracted for incurred residue samples		Study Number[a]
	Acetonitrile: Hexane 2:1 Extraction	Ethyl Acetate: Water 150:20 Extraction	
Grapes	78%	79%	AMR 4657-97
Tomatoes	81%	81%	AMR 4633-97
Lettuce	96%	86%	AMR 3315-95
Potatoes	93%	91%	AMR 3457-95
Cotton Seed	83%	85%	AMR 4594-97
Corn Forage	78%	93%	AMR 3320-95
Corn Fodder	92%	93%	
Corn Kernels/ Cob	80%	103%	

[a] Study summarised in Indoxacarb DAR, Volume 3, B5, 2000

The results from the multiple extraction efficiency studies conducted using radiolabeled incurred residue samples indicate that both acetonitrile: hexane (2:1) and ethyl acetate: water (150:20) can be used to extract incurred indoxacarb resides from crop samples. The metabolism studies were conducted using acetonitrile as the extraction solvent.

The DFG Method S19 method uses a broad ranging, rigorous extraction designed to remove polar and non-polar analytes. Water is added to all matrices to maintain a certain level relative to the crop. The water and mechanical shearing from a high-speed mixer should disrupt most cells in most matrices. The acetone will assist the extraction of more non-polar compounds.

Extraction efficiency has been shown for residue analyses with ethyl acetate/water using incurred radiolabeled residues from metabolism studies. The acetone/water extraction solvent for DFG S19 should have comparable extraction efficiency to that of ethyl acetate/water. Acetone and ethyl acetate have similar solvent polarity parameter ($P^{\prime}$-Rohrschneider parameter) values of 4.4 and 5.1, respectively, similar solvent strength parameters on alumina ($\varepsilon°$) of 0.58 and 0.56, respectively, similar Hildebrand solubility parameters (δ), and the same solvent selectivity group (VIa) according to information in "Introduction to Modern Liquid Chromatography Second Edition", Snyder, L.R. and Kirkland, J.J., John Wiley and Sons, Inc., 1979, Chapter 6. The acetone/water and ethyl acetate/water extraction systems should have very similar extraction efficiency, therefore, the DFG S19 method should be considered to have the same extraction efficiency as validated for the ethyl acetate/water method below using incurred radiolabeled residues.

Conclusion: A simple justification based on "similar" physical or chemical properties (e.g. density, dipole moment, dielectric constant, "polarity") of the different solvents is not sufficient to acceptable. Thus, extraction efficiency in different solvent systems used in monitoring studies (acetone/water for high water content, acidic and dry commodities and acetone/acetonitrile for high fat content crops) should be provided.

Reference:	Validation of multi-residue method **DFG S19** for the determination of residues of DPX-MP062 (DPX-KN128 (Indoxacarb) and IN-KN127) in grass, S.Lakaschus, A.Klimmek, 2006, report **DUP-0602V**
Test facility:	Eurofins Analytik GmbH, Dr Specht Laboratorien, Grossmoorbogen 25, 21079, Hamburg, Germany
GLP:	Yes
Test facility	Eurofins Analytical GmbH, Germany

Principle of the method

DPX-MP062 (DPX-KN128 (indoxacarb, S isomer) and IN-KN127 (R-isomer)) is extracted with acetone/water (module E1), partitioned with ethyl acetate/cyclohexane (1:1, v/v) and sodium chloride, purified with GPC and silica gel chromatography (for GC-ECD only) and determined with LC-MS/MS (ESI positive mode, transition 528→218 and 528→203 m/z) and GC-ECD. DPX-KN128 and IN-KN127 appear together as a single peak on chromatograms.

Results and discussion

Specificity

Chromatograms have been provided for matrix matched calibration standards (grass), control and fortified samples at 0.01mg/kg (grass). Data have been provided for the two transitions. No interference (>

30% of the LOQ) has been observed at the retention time of the analyte. Specificity is acceptable for both transitions.

For GC-ECD, interferences have been noticed. The detector was influenced by interferences due to the low LOQ. Specificity is not acceptable for GC-ECD.

Linearity

Linearity has been performed with 7 matrix matched calibration standards (grass) ranging from 0.2 to 20ng/mL for LC-MS/MS and with 5 matrix matched calibration standards ranging from 0.025 to 1.0µg/mL for GC-ECD. Data have been provided for the two transitions. Regressions were linear with R^2>0.99. Linearity is acceptable for both transitions and for GC-ECD.

Accuracy and precision

(Data have only been presented for LC-MS/MS since specificity is not checked for GC-ECD)

Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Grass Transition 528→218 m/z	0.01 0.1	5 5	75-97 59-92	85 82	10 16	acceptable
Grass Transition 528→203 m/z	0.01 0.1	5 5	69-103 59-88	85 81	17 16	acceptable

LOQ

The limit of quantification is 0.01mg/kg for indoxacarb (sum of isomers) in grass.

Conclusion

Analytical method (S.Lakaschus, A.Klimmek, 2006) for the determination of indoxacarb residue (sum of isomers) in grass with LC-MS/MS has been provided and validated with **LOQ=0.01mg/kg**. As data have been provided for two mass transitions, the method is highly specific. The method is not acceptable for GC-ECD as interferences have been observed.

CA 4.1.2/08	**Report**	Lakaschus, S., Gizler, A. (2010); Adaptation and validation of a method for the determination of DPX-KN128 (indoxacarb) in honey DuPont Report No.: DUP-0801V GLP: Yes
Test facility		Eurofins, Dr. Specht Labiratorien GmbH, Germany

Description of the method

100-mg samples of honey were extracted with 1.0-mL of acetonitrile: water (1:1, v/v). Sodium sulphate and ethyl acetate was added to the sample extracts. The extracts were centrifuged and the organic layer was removed and evaporated to dryness. The extracts were reconstituted with iso-propanol and acetonitrile. The extracts were diluted with 0.01 M aqueous formic acid prior to analysis and then analysed by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS Electrospray ionisation).

Mass transition for indoxacarb: m/z 528→218 for quantification and 528→249 for confirmation.

Mass spectra to justify the choice of transitions was not provided and is still required.

Specificity

Analysis of control samples resulted in no detectable apparent residues of indoxacarb. The response in the area of the indoxacarb peak always corresponded to less than 20% of the limit of determination. It can therefore be concluded that few, if any, apparent residues or false positive values would arise.

Linearity

Linearity was confirmed by injecting five matrix-matched standard solutions. A good linearity was observed in the range of 0.25 to 50 ng/mL for indoxacarb. The coefficient correlation was 0.997 (m/z 528→218) and 0.999 (m/z 528→246).

Recovery findings

The results listed below were obtained using standards prepared in matrix extracts. The average recovery specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%.

Validation data for the analytical method DUP-0801V for the determination of indoxacarb in honey

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation
Honey	0.010 0.10	5 5 Total = 10	90 82	16 6.7	18 8.2

Confirmation data for the analytical method DUP-0801V for the determination of indoxacarb in honey (MRM 528→249)

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation
Honey	0.010 0.10	5 5 Total = 10	78 82	14 6.5	18 7.9

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Repeatability

Repeatability of the method is addressed by the data in the table above. The same analyst obtained these recovery data over the course of two days per matrix. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix, as well as at higher levels.

Limit of quantification

The limit of quantification of the method for honey is 0.010 mg/kg.

Conclusion:

The analytical method DUP-0801V is suitable for the detection of residues in honey with a limit of quantification of 0.010 mg/kg.

Summary of proposed analytical methods used for determination indoxacarb residues in plant matrix:

Matrix	Separation/ Quantitation	Limit of determination (mg/kg)	Reference and report	Comments
In plant matrix				
Apples Peaches Grapes White Cabbage Cauliflower Tomatoes	DFG S19 GC/ECD	0.020	Schmidt, F, 1997 (**main method**) and Class, T., 2000 (**ILV**)	The applicant states that the method based on DFG S19 has been validated for the four groups. However, according to actual guidance document, DFG S19 multi-residue (main method and ILV) can be considered as validated only for the analysis of indoxacarb (sum of isomers) in the high water content with an LOQ of 0.02 mg/kg. Study report was not presented in this submission
Grass Grapes Tomatoes Oilseed Rape Maize	DFG S19 LC/MS/MS	0.010	Čermák, J., 2013 (**main method**)	DFG S19 multiresidue method based on LC-MS/MS was validated with a LOQ of 0.01mg/kg for indoxacarb (sum of isomers) in high water content, acidic, fatty and dry commodities. As data have been provided for two mass transitions, the method is highly specific. **Extraction efficiency for each matrix is required***
tomatoes, grapes, wheat grain and oilseed rape	DFG S19 LC/MS/MS	0.01	Stanislowski, T., 2015 (**ILV**)	ILV method monitoring two MS/MS transitions for determination of indoxacarb (sum of isomers) in tomatoes, grapes, wheat grain and oilseed rape was validated with limit of quantification (LOQ) of 0.01 mg/kg. **Extraction efficiency for each matrix is required***
Grass	LC-MS/MS	0.01	S.Lakaschus, A.Klimmek, 2006	Method was validated with LOQ of 0.01mg/kg for indoxacarb (sum of isomers)

*Extraction efficiency: a simple justification based on "similar" physical or chemical properties (e.g. density, dipole moment, dielectric constant, "polarity") of the different solvents is not sufficient to acceptable. Thus, extraction efficiency in different solvent systems used in monitoring studies (acetone/water for high water content, acidic and dry commodities and acetone/acetonitrile for high fat content crops) should be provided.

B.5.2.2 Methods for determination of residues in commodities of animal origin

Residue definition in animal tissues, milk, and eggs: parent (sum of isomers) and the metabolite IN JT333

(*IN-JT333: methyl 7-chloro-2,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino]= carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate)*

Analytical method DFG S 19: These studies have been previously submitted and reviewed in previous DAR and Addenda 2001 and 2005. Methods were determined to be adequate enforcement method in product of animal origin.
The test facility was not specified and study report was not presented in this submission. This method has been reevaluated according to the current guidance document SANCO825/00 rev8.1.

Main method of DFG S 19:

Report: Linkerhagner, M., Guinivan, R.A. (2001); Testing of DFG method S19 for the determination of DPX-MP062 and its metabolite IN-JT333 in foodstuffs of animal origin- **DuPont-2338 Revision N°1**

Test facility: DR. Specht & Partner Chemische Laboratorien GmbH St. Anscharplatz 10 D-20354 Hamburg, Germany

and Independent Laboratory Validation of DFG S 19:

Report: Class, T (2001) Validation of the analytical residue method DuPont- 2338 for determination of residues of DPX-KN128, IN-KN127 and metabolite IN-JT333 in edible offal, **DuPont-6224.**

Test facility: PTRL Europe Helmholtzstr. 22, Science Park D-89081 Ulm, Germany

Method DFG S19 is described for determination of DPX-MP062 (DPX-KN128 and IN-KN127) and IN-JT333 in animal tissues, eggs and milk in DuPont-2338 Rev.N°1 and DuPont-6224 (main method) and in DuPont-3520 (ILV).

Main method:
Meat, milk and egg samples are homogenised and extracted using acetone. Water is added beforehand to maintain during extraction a constant acetone: water ratio of 2:1 (v/v). The extract is saturated with NaCl and then partitioned with ethyl acetate/cyclohexane (meat) or dichloromethane (eggs and milk). Fat is directly dissolved in organic solvents (ethyl acetate:cyclohexane, 1:1) without further extraction/partition steps. Clean-up of samples is done by gel permeation (BioBeads S-X3) and silica gel minicolumn chromatography. The residues are detected and determined by capillary gas chromatography with electron capture detection (GC/ECD). DPX-KN128 and IN-KN127 appear together as a single peak on chromatograms.

ILV:
For ILV method Samples were extracted using water/acetone/ethyl acetate/ cyclohexane or with acetone/acetonitrile. Extracts were cleaned up by gel permeation chromatography and by adsorption on silicagel and then analysed by capillary GC with electron capture detection on a non-polar and on a medium polar stationary phase. Validation data are presented below.
➔This method has been validated with LOQ of 0.01mg/kg for indoxacarb (sum of isomers) and IN-JT333 in milk, muscle, egg, fat. As data have been provided for two columns of different polarity, the method could be regarded as highly specific.

Validation data for main method:

***Specificity*:** specificity is missing

***Linearity*:**
Main method:
Y=a+bx for DPX-MP062 was a=-474, b=77833 and r=0.9995.

Y=a+bx for IN-JT333 it was a=-553, b=39337 and r=0.9993.

ILV: see table below.

Accuracy and recovery data: see table below

Conclusion: Methods have been previously reviewed and determined to be adequate enforcement method in product of animal origin. LOQ for indoxacarb (sum of isomers) and metabolite IN-JT333 in milk, egg, meat, fat, liver and kidney was determined to be 0.01mg/kg. However, according to actual guidance document SANCO/825/00rev.8.1 method is not considered as fully validated; study reports was not presented in this submission and specificity is missing.

Table
Accuracy and recovery data for the DFG S19 multi-residue method (Main method) for indoxacarb and its metabolite in food of animal origin

Matrix	Fortification level (mg/kg)[a,b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
DPX-MP062						
Milk	0.010 0.10	5 5 Total = 10	107 103	7.6 4.5	7.1 4.4	DuPont-2338, Revision No. 1
Eggs	0.010 0.10	5 5 Total = 10	83 95	5.5 4.9	6.6 5.2	
Beef Meat	0.010 0.10	5 5 Total = 10	80 75	6.0 5.5	7.5 7.3	
Poultry Meat	0.010 0.10	3 3 Total = 10	74 75	4.7 5.5	6.4 7.3	
Fat	0.010 0.10	5 5 Total = 10	93 91	10 13	11 14	
Liver	0.010 0.10	5 5 Total = 10	80 87	4 7	5 8	DuPont-6224
Kidney	0.010 0.10	5 5 Total = 10	79 82	6 3	8 4	
IN-JT333						
Milk	0.010 0.10	5 5 Total = 10	126 136	14 3.4	11 2.5	DuPont-2338, Revision No. 1
Eggs	0.010 0.10	5 5 Total = 10	94 95	8.7 5.4	9.3 5.7	
Beef Meat	0.010 0.10	5 5 Total = 10	86 93	4.8 7.9	5.6 8.5	
Poultry Meat	0.010 0.10	3 3 Total = 10	69 82	4.6 6.6	6.7 8.0	
Fat	0.010 0.10	5 5 Total = 10	161 101	18 21	11 21	
Liver	0.010 0.10	5 5 Total = 10	80 92	11 9	14 10	DuPont-6224
Kidney	0.010 0.10	5 5 Total = 10	84 88	9 7	11 8	

[a] Fortifications were performed with analyte reference standard solutions
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Validation data for the DFG S19 multi-residue method (ILV) for indoxacarb and its metabolite in food of animal origin

Summary of GC/ECD on DB-17 column

Matrix (reference)	Analyte	Dissolution/ extraction	Partition/clean-up	Quantification	LOD (mg/kg)	Fortification level (mg/kg)	Recovery range (mean, n[1])	Repeatability range (mean RSD)	Linearity
Whole milk	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	99 (n=5) 82 (n=6)	12 % 12 %	Y 0.004 to 1.0 µg/mL
Whole milk	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	100 (n=5) 81 (n=6)	15 % 12 %	Y 0.004 to 1.0 µg/mL
Bovine muscle	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	92 (n=6) 78 (n=7)	16 % 17 %	Y 0.004 to 1.0 µg/mL
Bovine muscle	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	90 (n=5) 77 (n=7)	20 % 18 %	Y 0.004 to 1.0 µg/mL
Whole Egg	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	101 (n=5) 94 (n=9)	15 % 11 %	Y
Whole Egg	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	89 (n=5) 88 (n=9)	16 % 11 %	Y
Bovine Fat	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	102 (n=7) 94 (n=7)	9 % 17 %	Y
Bovine Fat	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	79 (n=5) 86 (n=7)	13 % 17 %	Y

ILV Summary of GC/ECD on DB-5 column

Matrix (reference)	Analyte	Dissolution/ extraction	Partition/clean-up	Quantification	LOD (mg/kg)	Fortification level (mg/kg)	Recovery range (mean, n[1])	Repeatability range (mean RSD)	Linearity
Whole milk	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	104 (n=8) 87 (n=11)	10 % 11 %	Y
Whole milk	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	93 (n=8) 78 (n=7)	12 % 12 %	Y
Bovine muscle	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	102 (n=6) 83 (n=7)	9 % 16 %	Y
Bovine muscle	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	91 (n=5) 70 (n=5)	11 % 14 %	Y
Whole Egg	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	92 (n=57 89 (n=10)	12 % 17 %	Y
Whole Egg	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	87 (n=7) 88 (n=8)	12 % 19 %	Y
Bovine Fat	DPX-MP062	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	98 (n=8) 91 (n=7)	17 % 13 %	Y
Bovine Fat	IN-JT333	water/acetone /ethyl acetate/ cyclohexane	GPC and silica columns	GC-ECD Conformation: GC-MS Ion trap/EI	0.01	0.01 0.100	75 (n=5) 84 (n=7)	20 % 13 %	Y

Studies below were submitted to the EU for the first time in this submission:

DFG S 19 method using the LC/MS/MS detection module

Reference:	Validation of multi-residue method DFG S19 for the determination of residues of Indoxacarb and its metabolite IN-JT333 in animal matrices using LC-MS/MS, S.Richter, 2013, report **DuPont-39006**
Test facility:	PTRL Europe, Helmholtzstr. 22, Science Park, D-89081 Ulm, Germany
GLP:	Yes
Acceptability of the method:	Acceptable

Principle of the method

The analytical procedure as described in DuPont-2338, Revision No. 1 and DuPont-6224 was used to generate the validation data presented in DuPont-39006 with the exception of an LC-MS/MS detection method was used in place of the GC/ECD method. The LC-MS/MS detection offers the ability to collect a quantitative and confirmatory ion transition simultaneously. No chiral column was used. Therefore, both enantiomers are quantified (Indoxacarb and IN-KN127).

The method involves extraction with acetone/water (2/1, v/v), ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride, clean up with GPC using ethyl acetate/cyclohexane (1/1, v/v) as eluant. Determination is performed with LC-MS/MS (ESI+) using the following transitions:

- Indoxacarb (DPX-KN128): 528→150m/z for quantification and 528→203m/z for confirmation
- IN-JT333[1]: 470→150m/z for quantification and 470→267m/z for confirmation

MS spectra were provided to justify the choice of the transitions.

Results and discussion

Specificity

Chromatograms were provided for calibration standards, control and fortified samples at 0.01mg/kg with indoxacarb and IN-JT333 (milk, egg, meat, liver). Data were provided for two transitions. No interferences >30% of the LOQ were observed at the retention time of the analytes. Specificity is acceptable.

Linearity

Good linearity was observed in the range of 0.50 to 50 ng/mL for indoxacarb and IN-JT333 (n=6). Regressions were linear with R^2>0.99. Data were provided for two mass transitions and for each analyte.

Accuracy and precision

The fortification data reported in the method proposed for monitoring and confirming indoxacarb and IN-JT333 residues in tissue samples are summarised below. The results listed below were obtained using standards prepared in solvent. Mean recoveries were in acceptable range. Accuracy is acceptable.

Recovery results from the validation method of indoxacarb (sum of isomers) using the analytical method (quantification transition).

Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*

[1] IN-JT333: methyl 7-chloro-2,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino]= carbonyl]indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylate

Recovery results from the validation method of indoxacarb (sum of isomers) using the analytical method (quantification transition).						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Milk	0.01 0.1	5 5	88-110 79-109	94 91	10 14	acceptable
eggs	0.01 0.1	5 5	88-114 87-103	101 96	10 6	acceptable
Muscle	0.01 0.1	5 5	107-112 106-115	110 110	2 3	acceptable
Liver	0.01 0.1	5 5	84-111 86-106	97 100	13 8	acceptable

Recovery results from the validation method of indoxacarb (sum of isomers) using the analytical method (confirmatory transition).						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Milk	0.01 0.1	5 5	84-114 79-107	100 90	13 14	acceptable
eggs	0.01 0.1	5 5	88-122 88-105	101 97	13 7	acceptable
Muscle	0.01 0.1	5 5	106-114 104-114	110 109	3 4	acceptable
Liver	0.01 0.1	5 5	83-114 94-114	98 103	14 7	acceptable

Recovery results from the validation method of IN-JT333 using the analytical method (quantification transition).						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Milk	0.01 0.1	5 5	82-98 80-107	93 94	8 11	acceptable
eggs	0.01 0.1	5 5	86-115 72-103	97 86	12 13	acceptable
Muscle	0.01 0.1	5 5	91-100 88-113	95 99	4 11	acceptable
Liver	0.01 0.1	5 5	77-104 77-109	86 86	14 15	acceptable

Recovery results from the validation method of IN-JT333 using the analytical method (confirmatory transition).						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Milk	0.01 0.1	5 5	85-104 78-106	96 93	8 12	acceptable

Recovery results from the validation method of IN-JT333 using the analytical method (confirmatory transition).						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
eggs	0.01 0.1	5 5	89-114 70-102	98 86	11 14	acceptable
Muscle	0.01 0.1	5 5	91-99 87-113	96 98	3 11	acceptable
Liver	0.01 0.1	5 5	74-106 76-104	85 85	14 14	acceptable

LOQ: The limit of quantification of the method for milk, eggs and animal tissues is 0.010 mg/kg for both analytes.

Conclusion

The residue method for the determination and confirmation of indoxacarb (sum of isomers) and IN-JT333 residues in milk, eggs and animal tissues (liver, muscle) involves simple extraction, clean-up, and analytical determination by HPLC/MS/MS detection was validated. No validation data were provided for fat. As data have been provided for two mass transitions, the method is highly specific. **No ILV was provided for this method.**

Extraction efficiency

Notifier attested that Extraction efficiency was addressed in the Indoxacarb DAR, Volume 3 Annex B, January 2000, as this point was note reported in the DAR, notifier should provide the Extraction efficiency.

DuPont response:

During the goat metabolism study (AMR 2979-94), muscle samples were extracted using acetone and an Ulta-Turrax homogenising probe. This extraction procedure removed approximately 80.5% of the TRR from the muscle samples. The metabolism extraction method extracted 50 gram samples using approximately 200-mL of acetone two times. The initial extraction used the Ulta-Turrax homogenising probe and the second extraction used an orbital shaker. The solvent to sample ratio for the metabolism extraction was 8-mL of acetone for each gram of muscle (400 mL/50 gram). The DFG S 19 method extracts 10 gram tissue samples using 200-mL of acetone and a Ulta-Turrax homogenising probe.

The solvent to sample ratio for the DFG S 19 method is 20-mL of acetone for each gram of tissue (200-mL/10 grams). The DFG S 19 method should be considered an acceptable procedure for extracting incurred residues from tissue samples based on its similarities in solvent and extraction equipment used for the goat metabolism study.

An animal metabolism study for indoxacarb has not been conducted since 1997. Due to the lack of availability of incurred indoxacarb residue samples in animal tissues a new extraction efficacy study could not be conducted.

Conclusion: A simple justification based on "similar" physical or chemical properties (e.g. density, dipole moment, dielectric constant, "polarity") of the different solvents is not sufficient to acceptable. Thus, extraction efficiency in different solvent systems used in monitoring studies (acetone/water, ethyl acetate/cyclohexane (1/1, v/v, acetonitrile and acetic acid partitioned with hexane) and sodium chloride) should be provided.

Reference:	Analytical method for the determination of DPX-MP062 and metabolites IN-KB687, IN-KG433, IN-KT319, IN-JU873 and IN-JT333 in poultry skin, liver, muscle, fat and eggs, J.J Stry, 2004, report DuPont 12739 Rev1
Test facility:	Dr. Specht and partner, Hamburg Germany

Principle of the method

The method involves extraction with 80mL of acetonitrile and 80µL of acetic acid, partitioned with hexane, purified with SPE (Waters Oasis HLB SPE using acetonitrile as eluent) and determined with HPLC-MS/MS (ESI, no chiral column). The following conditions were used:

- Indoxacarb: positive mode, transitions 528→203m/z, 528→293 m/z, 528→218 m/z. Quantification was done using the TIC.
- IN-KB687: negative mode, transitions 234→85m/z for quantification and 234→202m/z for confirmation
- IN-KG433: positive mode, transitions 516→281m/z for quantification and 516→221m/z for confirmation
- IN-KT319: positive mode, transitions 516→281m/z for quantification and 516→221m/z for confirmation
- IN-JU873: positive mode, transitions 458→255m/z, 458→149m/z and 458→208m/z. Quantification was done using the TIC.
- IN-JT333 : transitions 470→267m/z for quantification, 470→150m/z and 470→207m/z for confirmation

MS spectra were not provided but are available in others studies.

Results and discussion

Specificity

Chromatograms were provided for calibration standards, control and fortified samples at 0.01mg/kg (liver, muscle, fat, skin, whole eggs, eggs whites, eggs yolks) for e ach analyte and each transition.
No interferences >30% of the LOQ were observed at the retention time of the analytes. Specificity is acceptable.

Linearity

Linearity was performed with 8 calibration standards ranging from 0.63 to 12.5ng/mL for each analyte and each transition. Regressions were linear with a correlation coefficient >0.99. Linearity is acceptable.

Extraction efficiency extraction efficiency in different solvent systems used in monitoring studies should be provided.

Accuracy and precision

Accuracy was performed with samples fortified at two levels. 5 samples per level were analyzed. Mean recoveries were in acceptable range. Accuracy is acceptable.

Recovery results from the validation method of indoxacarb DPX-MP062 using the analytical method (quantification transition)

Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
liver	0.01 0.1	5 5	84-98 91-108	91 98	6.3 6.5	acceptable
Muscle	0.01 0.1	5 5	90-108 78-90	97 85	9.0 5.3	acceptable
Fat	0.01 0.1	5 5	94-98 83-88	96 85	1.6 2.7	acceptable
skin	0.01 0.1	5 5	87-102 72-99	98 90	6.4 11.6	acceptable

Recovery results from the validation method of indoxacarb DPX-MP062 using the analytical method (quantification transition)						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Whole eggs	0.01 0.1	5 5	85-105 86-111	97 98	7.9 10.8	acceptable
Egg whites	0.01 0.1	5 5	92-110 81-91	95 85	8.1 5.3	acceptable
Egg yolks	0.01 0.1	5 5	92-109 94-102	99 98	6.5 3.0	acceptable

Recovery results from the validation method of IN JT333 using the analytical method (quantification transition)						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
liver	0.01 0.1	5 5	66-79 65-99	71 81	8.4 16.3	acceptable
Muscle	0.01 0.1	5 5	81-100 65-85	93 76	7.7 12.1	acceptable
Fat	0.01 0.1	5 5	70-93 61-78	77 70	12.3 11.6	acceptable
skin	0.01 0.1	5 5	68-82 58-84	76 73	7.2 13.7	acceptable
Whole eggs	0.01 0.1	5 5	74-85 67-95	79 80	5.5 14.9	acceptable
Egg whites	0.01 0.1	5 5	71-87 72-91	81 80	7.5 9.4	acceptable
Egg yolks	0.01 0.1	5 5	65-91 71-81	83 74	12.6 5.6	acceptable

Recovery results from the validation method of IN KT319 using the analytical method (quantification transition)						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
liver	0.01 0.1	5 5	78-90 91-111	84 101	6.5 7.5	acceptable
Muscle	0.01 0.1	5 5	72-105 94-105	92 99	14.9 4.6	acceptable
Fat	0.01 0.1	5 5	88-102 100-107	95 104	5.3 3.7	acceptable
skin	0.01 0.1	5 5	75-116 87-114	97 103	15.2 9.8	acceptable
Whole eggs	0.01 0.1	5 5	90-97 91-100	94 96	2.9 3.5	acceptable
Egg whites	0.01 0.1	5 5	97-105 98-106	100 101	3.1 3.4	acceptable
Egg yolks	0.01 0.1	5 5	89-94 100-104	91 102	2.3 1.5	acceptable

Recovery results from the validation method of IN KG433 using the analytical method (quantification transition)						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
liver	0.01 0.1	5 5	78-95 84-94	87 91	7.0 4.4	acceptable
Muscle	0.01 0.1	5 5	75-94 74-87	81 81	9.4 7.0	acceptable
Fat	0.01 0.1	5 5	81-96 86-97	89 90	7.4 5.6	acceptable
skin	0.01 0.1	5 5	75-101 70-110	86 94	11.0 16.9	acceptable
Whole eggs	0.01 0.1	5 5	86-90 87-97	88 92	1.9 4.3	acceptable
Egg whites	0.01 0.1	5 5	89-103 86-95	96 91	6.2 4.2	acceptable
Egg yolks	0.01 0.1	5 5	90-95 98-100	93 99	2.0 0.8	acceptable

Recovery results from the validation method of IN JU873 using the analytical method (quantification transition)						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
liver	0.01 0.1	5 5	71-76 70-95	74 82	2.6 11.3	acceptable
Muscle	0.01 0.1	5 5	78-91 66-77	86 70	6.9 6.6	acceptable
Fat	0.01 0.1	5 5	83-98 72-85	92 78	6.0 6.1	acceptable
skin	0.01 0.1	5 5	58-80 54-86	71 78	12.3 17.5	acceptable
Whole eggs	0.01 0.1	5 5	82-98 82-100	93 91	6.9 7.8	acceptable
Egg whites	0.01 0.1	5 5	77-84 62-77	81 68	3.8 8.9	acceptable
Egg yolks	0.01 0.1	5 5	68-77 68-75	72 72	5.0 3.5	acceptable

Recovery results from the validation method of IN KB687 using the analytical method (quantification transition)						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
liver	0.01 0.1	5 5	85-93 84-93	91 88	3.7 3.8	acceptable
Muscle	0.01 0.1	5 5	91-95 89-93	93 91	1.6 2.0	acceptable
Fat	0.01 0.1	5 5	88-103 92-97	92 95	6.9 2.3	acceptable

Recovery results from the validation method of IN KB687 using the analytical method (quantification transition)						
Matrix	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
skin	0.01 0.1	5 5	79-106 84-101	93 96	10.7 7.3	acceptable
Whole eggs	0.01 0.1	5 5	92-105 101-108	99 104	5.4 3.2	acceptable
Egg whites	0.01 0.1	5 5	90-102 88-101	97 94	5.2 5.2	acceptable
Egg yolks	0.01 0.1	5 5	84-96 96-107	91 100	6.3 4.1	acceptable

Confirmatory data (ion ratio)

Ion ratio data were provided for each sample fortified at 0.01 and 0.1mg/kg. However, only results for whole eggs and white eggs were presented.

Ion ratios were determined with the following transitions:
- Indoxacarb: TIC and transition 527→93m/z.
- IN-JU873: TIC and transition 458→1249m/z.
- IN-JT333: transitions 470→267m/z and 470→207m/z.
- IN-KB687: 234→85m/z and 234→202m/z
- IN-KG433: 516→281m/z and 516→221m/z
- IN-KT319: 516→281m/z and 516→221m/z

RSD were below 20%. So, ion ratio data are acceptable for whole eggs and egg yolks.

Ion ratio data for the determination of indoxacarb residue in whole eggs				
matrix	**Analyte**	**Fortified levels (mg/kg)**	**Ion ratio**	**RSD (%)**
Whole eggs	Indoxacarb	0.01 0.1	2.27/1.78/2.05/2.59/2.21 2.10/2.24/2.10/2.21/2.30	14 4
	IN-JU873	0.01 0.1	1.47/1.56/1.35/1.55/1.53 1.55/1.56/1.54/1.53/1.56	6 1
	IN-JT333	0.01 0.1	2.26/1.77/2.62/2.41/1.81 2.42/2.53/2.28/2.31/2.28	17 5
	IN-KB687	0.01 0.1	3.23/3.35/2.63/3.26/2.90 3.24/2.99/2.95/2.81/3.01	10 5
	IN-KG433	0.01 0.1	2.71/2.45/2.68/2.43/2.75 2.63/2.57/2.58/2.72/2.65	6 2
	IN-KT319	0.01 0.1	2.22/1.91/2.03/1.89/1.79 2.61/2.55/2.52/2.60/2.52	8 2

Ion ratio data for the determination of indoxacarb residue in egg whites				
matrix	**Analyte**	**Fortified levels (mg/kg)**	**Ion ratio**	**RSD (%)**
	Indoxacarb	0.01 0.1	1.9/2.1/2.2/2.0/2.1 2.1/2.1/2.1/2.1/2.1	6 0

Egg yolks	IN-JU873	0.01 0.1	No data	- -
	IN-JT333	0.01 0.1	No data	- -
	IN-KB687	0.01 0.1	3.5/3.5/3.4/3.3/3.0 3.2/3.3/3.3/3.2/3.2	6 2
	IN-KG433	0.01 0.1	2.7/2.8/2.8/2.6/3.0 2.6/2.6/2.7/2.7/2.6	5 2
	IN-KT319	0.01 0.1	No data	- -

LOQ

The limit of quantification is 0.01mg/kg for each analyte in all matrices tested.

Conclusion

Analytical method J.J Stry, 2004 (report DuPont 12739 Rev1) for the determination of indoxacarb residue in animal products by LC-MS/MS has been provided and is validated with LOQ=0.01mg/kg for indoxacarb (sum of isomers) and metabolites IN-JU873, IN-JT333, IN-KB687, IN-KG433 and IN-KT319 in liver, muscle, fat, skin, whole eggs, egg white, egg yolks. For whole eggs, ion ratio data are acceptable for each analyte, so the method can be considered highly specific. For egg yolks, ion ratio data are acceptable for Indoxacarb, IN-KB687, IN-KG433. For other matrix, the method is not highly specific since confirmatory data were not provided.

An ILV was provided and is described below.

Reference:	Independent laboratory validation of the analytical method, DuPont-12739, "Analytical method for the determination of DPX-MP062 and metabolites IN-KB687, IN-KG433, IN-KT319, IN-JU873 and IN-JT333 in poultry skin, liver, muscle, fat and eggs" Amendment Number 1, P.Connolly, 2004, report DuPont 13651 Rev1
Test facility:	Exygen Research, 3058 Research Drive, State College, PA 16801
GLP:	Yes

Principle of the method

Same principle as described in method J.J Stry, 2004 (report DuPont 12739 Rev1). Determination is performed with LC-MS/MS (Turbo Ion Spray, no chiral column). The following conditions were used:

- IN-KB687: negative mode, transitions 234→202m/z for quantification and 234→85m/z for confirmation
- IN-KG433: positive mode, transitions 516→281m/z for quantification and 516→221m/z for confirmation
- IN-KT319: positive mode, transitions 516→281m/z for quantification and 516→221m/z for confirmation
- IN-JU873: positive mode, transitions 458→149m/z and 458→204m/z. Determination has been performed with the TIC
- Indoxacarb (DPX-MP062): positive mode, transitions 528→218m/z and 528→293m/z. Determination has been performed with the TIC.
- IN-JT333: positive mode, transition 470→267m/z for quantification and 470→207m/z for confirmation.

MS spectra were not provided but are available in others studies

Results and discussion

Specificity

Chromatograms were provided for calibration standards, control and fortified samples at LOQ (whole eggs, chicken muscle, and chicken skin). Data were provided for the quantification transition or for the TIC. No confirmatory data were provided for another transition. Interferences were below 30% of the LOQ. Specificity is acceptable.

Linearity

No data were provided.

Accuracy and precision

Accuracy was performed with samples fortified at two levels. Two samples per level were analyzed. Data are insufficient. Accuracy is no acceptable.

Recovery results from the validation method of indoxacarb residue using the analytical method.							
Matrix	*analyte*	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Whole eggs	IN-KB687	0.01 0.02	2 2	88/84 82/84	- -	- -	Not enough samples
	IN-KG433	0.01 0.02	2 2	90/88 98/92	- -	- -	Not enough samples
	IN-KT319	0.01 0.02	2 2	95/91 103/94	- -	- -	Not enough samples
	IN-JU873	0.01 0.02	2 2	105/109 94/86	- -	- -	Not enough samples
	Indoxacarb	0.01 0.02	2 2	87/83 96/91	- -	- -	Not enough samples
	IN-JT333	0.01 0.02	2 2	90/82 96/89	- -	- -	Not enough samples

Recovery results from the validation method of indoxacarb residue using the analytical method.							
Matrix	*analyte*	*Fortification level (mg/kg)*	*No of samples per fortification level*	*Range of recoveries obtained (%)*	*Mean recovery*	*RSD (%)*	*Comments*
Chicken muscle	IN-KB687	0.01 0.02	2 2	93/95 95/97	- -	- -	Not enough samples
	IN-KG433	0.01 0.02	2 2	103/98 108/108	- -	- -	Not enough samples
	IN-KT319	0.01 0.02	2 2	97/112 117/115	- -	- -	Not enough samples
	IN-JU873	0.01 0.02	2 2	70/72 72/72	- -	- -	Not enough samples
	Indoxacarb	0.01 0.02	2 2	96/85 93/94	- -	- -	Not enough samples
	IN-JT333	0.01 0.02	2 2	85/98 84/89	- -	- -	Not enough samples

LOQ

No LOQ can be set since accuracy/precision is not checked.

Conclusion

ILV (P.Connolly, 2004, report DuPont 13651 Rev1) of method J.J Stry, 2004 (report DuPont 12739 Rev1) for the determination of indoxacarb residue in foodstuff of animal origin is not validated. The number of samples per level used for accuracy/precision is not sufficient and no data on linearity was presented.

No ILV of method DuPont 12739 Rev1 for the determination of indoxacarb residue in liver/kidney was presented.

General conclusion for analytical method used for determination of residues of the active substance in product of animal origin:

Matrix	Separation/ Quantitation	Limit of determination (mg/kg)	Reference and report	Comments
In product of animal origin				
Milk Eggs Beef Meat Poultry Meat Fat Liver Kidney	DFG S19 GC/ECD	0.010	**Main method:** Linkerhagner, M., Guinivan, R.A., 2001 And Class, T (2001) [DuPont-2338, Revision No. 1 and DuPont-6224] **ILV:** Class, T. (2001)	DFG multiresidue method S19 was validated for indoxacarb (sum of isomers) and IN-JT333 in animal product (milk, eggs, meat, liver, fat, and kidney) in the previous DAR 2000. However, according to actual guidance method is not considered as fully validate*. ILV have been provided for two columns of different polarity, the method can be regarded as highly specific. *study reports were not presented in this submission and specificity is missing.
Honey	LC-MS/MS	0.01	Lakaschus, S., Gizler, A., 2010	Method was validated in honey with a limit of quantification of 0.010 mg/kg for indoxacarb (sum of isomers)
Milk Eggs Muscle Liver	DFG S19 LC/MS/MS	0.010	S. Richter, 2013 **DuPont-39006**	DFG multiresidue method S19 was validated for indoxacarb (sum of isomers) and IN-JT333 residues in milk, eggs and animal tissues (liver, muscle) No validation data were provided for fat No ILV was provided for this method. Extraction efficiency is missing
Poultry skin, liver, muscle, fat and eggs	LC-MS/MS	0.01	**Main method** J.J Stry, 2004, report DuPont 12739 Rev1	Method was validated with LOQ of 0.01mg/kg for indoxacarb (sum of isomers) and metabolites IN-JU873, IN-JT333, IN-KB687, IN-KG433 and IN-KT319 in liver, muscle, fat, skin, whole eggs, egg white, egg yolks. For eggs, ion ratio data are acceptable for each analyte, so the method can be considered highly specific. For other matrix, the method is not highly specific since confirmatory data were not provided. No validation data were provided for milk. Extraction efficiency is required

Poultry skin, liver, muscle, fat and eggs	LC-MS/MS	No LOQ can be set since accuracy /precision is not checked	**ILV** P.Connolly, 2004 DuPont 13651 Rev1	Not validated The number of samples per level used for accuracy/precision is not sufficient additionally no data on linearity was presented.

- ILV of analytical method (S.Richter, 2013, report DuPont 39006) for determination of residues in product of animal origin is required. Notifier should provide the extraction efficiency in different solvent systems used in monitoring studies. Additionally, no validation data were provided for fat. Thus main method and ILV are required for fat matrix.

Or

- ILV of analytical method (J.J Stry, 2004, report DuPont 12739 Rev1) for determination of residues in skin, liver, muscle, fat and eggs is required. Notifier should provide the extraction efficiency in different solvent systems used in monitoring studies. Additionally, no validation data were provided for milk. Thus main method and ILV are required for milk matrix.

B.5.2.3 Methods for determination of residues in soil

Residue definition in soil: parent (sum of isomers)
NOEC = 29.2 mg/kg dry soil
EC10 = 23.95 mg a.s./kg dry soil

CA 4.2/02	**Report**	Henze, R.M., Stry, J. J. (2012); Analytical method for the determination of indoxacarb and metabolites in soil using LC/MS/MS **DuPont Report No.:** DuPont-35025 **GLP:** No
Test facility		E. I. DuPont de nemours and Company Newark Delaware

Description of the method

The soil samples (5-gram) were weighed into 50-mL polypropylene centrifuge tubes and indoxacarb (DPX-KN128) and its metabolites (IN-MK643, IN-MK638, IN-KB687, IN-KG433, IN-JU873, IN-KT413, IN-JT333) were extracted using three sequential extractions. The first extraction used 15-mL of 80% acetonitrile:0.025% aqueous acetic acid. The following two extractions used 15-mL of 90% acetonitrile: 10% of 0.025% aqueous acetic acid and 15-mL of acetonitrile, respectively. A genogrinder bead mill was used to pulverize the soil during the extraction process. An aliquot of the extract was transferred into a glass culture tube, capped and placed in a heating block set at 55°C for 1 hour. In acetonitrile, the compound IN-KT413 was quantitatively converted to IN-MP819. Due to the instability of IN-KT413 in organic solvents this step was necessary for quantitative analysis. The extract was than evaporated to 0.5-mL under nitrogen flow and diluted to a final volume of 3.0-mL. An aliquot of the extracts were transferred to an autosampler vial for analysis. Indoxacarb and its metabolites were separated from co-extracts by reversed phase Liquid Chromatography (LC) and detected by positive ion Turbospray Ionization (TSI) Mass Spectrometry/Mass Spectrometry (MS/MS). All calibration standards were prepared in control extracts. The metabolite IN-KT413 was quantitatively analysed against an IN-MP819 standard.

Mass spectra:

IN-MK643 m/z 217→85 (for quantification) and 217→131 (for confirmation)

IN-MK638 m/z 219→85 (for quantification) and 217→176 (for confirmation)

IN-KB687 m/z 234→85 (for quantification) and 234→202 (for confirmation)

IN-KG433 m/z 516→281 (for quantification) and 516→149 (for confirmation)

I IN-KB687 m/z 234→85 (for quantification) and 234→202 (for confirmation)

Indoxacarb m/z 528→203 (for quantification) and 528→150 (for confirmation)

IN-MP819 m/z 470→238 (for quantification) and 470→206 (for confirmation)

IN-JT333m/z 470→267 (for quantification) and 470→207 (for confirmation)

Mass spectra to justify the choice of transitions were presented.

Linearity

All calibration standards were prepared in control matrix. Six sample solutions were injected. Good linearity was observed in the range of 0.050 to 5.0 ng/mL for indoxacarb and its metabolites. Coefficient correlations were > 0.998.

Specificity

The limit of quantification of the method proposed for monitoring indoxacarb residues is 0.0010 mg/kg for the soil samples tested. Analysis of control samples resulted in no detectable apparent residues of indoxacarb or its metabolites. The response in the area of the indoxacarb peak always corresponded to less than 20% of the limit of determination.

Recovery

The fortification data reported in the method proposed for monitoring indoxacarb and its metabolite residues in soil samples are summarised in table below. The results listed below were obtained using standards prepared in matrix extracts. The average recovery specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%.

Validation data for the analytical method DuPont-35025 for the determination of indoxacarb and its metabolites in soil

Matrix	Fortification level (mg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation
Indoxacarb					
Speyer Soil	0.001 0.010	5 5 Total = 10	105 97	7.5 5.2	7.2 5.3
Nambsheim Soil	0.001 0.010	5 5 Total = 10	102 102	14 9.2	14 9.0
IN-MK643					
Speyer Soil	0.001 0.010	5 5 Total = 10	105 97	4.4 0.8	4.2 0.9
Nambsheim	0.001	5	92	4.1	4.4

Soil	0.010	5 Total = 10	85	4.8	5.7
IN-MK638					
Speyer Soil	0.001 0.010	5 5 Total = 10	92 85	5.0 6.0	5.4 7.1
Nambsheim Soil	0.001 0.010	5 5 Total = 10	103 99	2.3 2.6	2.3 2.6
IN-KB687					
Speyer Soil	0.001 0.010	5 5 Total = 10	105 92	9.6 3.3	9.2 3.6
Nambsheim Soil	0.001 0.010	5 5 Total = 10	95 90	11 5.4	11 6.0
IN-KG433					
Speyer Soil	0.001 0.010	5 5 Total = 10	101 93	4.9 2.3	4.9 2.4
Nambsheim Soil	0.001 0.010	5 5 Total = 10	104 97	2.7 2.9	2.6 3.0
IN-JU873					
Speyer Soil	0.001 0.010	5 5 Total = 10	102 95	7.1 2.2	7.0 2.4
Nambsheim Soil	0.001 0.010	5 5 Total = 10	101 94	6.4 3.8	6.3 4.0
IN-KT413					

Speyer Soil	0.001 0.010	5 5 Total = 10	81 84	7.6 4.7	9.4 5.6
Nambsheim Soil	0.001 0.010	5 5 Total = 10	95 92	11 9.6	12 10
IN-JT333					
Speyer Soil	0.001 0.010	5 5 Total = 10	97 87	16 12	17 14
Nambsheim Soil	0.001 0.010	5 5 Total = 10	99 100	9.9 11	10 11

[a] Fortifications were performed with analyte reference standard solutions

[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Repeatability

Repeatability of the method is addressed by the data in above. The same analyst obtained these recovery data over the course of two days per matrix. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix, as well as at higher levels. Therefore, the repeatability of this method is adequate for the purposes of residue data collection in soil.

Limit of quantification

The limit of quantification of the method for the soils testes is 0.0010 mg/kg.

Reproducibility

An independent laboratory validation of DuPont-35025 was not conducted. Independent laboratory validations are not required for soil methods.

Confirmatory method

Confirmation of the results was obtained using secondary LC/MS/MS ion transitions collected at the same time as the quantitative transitions. The recovery data obtained using the confirmatory procedure are summarised in the table below.

Confirmation data for the analytical method DuPont-35025 for the determination of indoxacarb and its metabolites in soil					
Matrix	**Fortification level (mg/kg) [a, b]**	**Number of tests**	**Average recovery (%)**	**Standard deviation**	**% Relative standard deviation**
Indoxacarb					
Speyer Soil	0.001 0.010	5 5 Total = 10	109 98	9.7 5.9	8.8 6.0
Nambsheim Soil	0.001 0.010	5 5 Total = 10	107 100	17 8.2	15 8.2
IN-MK643					
Speyer Soil	0.001 0.010	5 5 Total = 10	89 85	8.4 7.3	9.5 8.6
Nambsheim Soil	0.001 0.010	5 5 Total = 10	110 95	6.7 3.2	6.1 3.4
IN-MK638					
Speyer Soil	0.001 0.010	5 5 Total = 10	90 83	3.1 3.6	3.4 4.4
Nambsheim Soil	0.001 0.010	5 5 Total = 10	103 97	6.2 2.8	6.0 2.9
IN-KB687					
Speyer Soil	0.001 0.010	5 5 Total = 10	103 92	10 3.3	9.8 3.6
Nambsheim Soil	0.001 0.010	5 5 Total = 10	96 90	8.8 3.7	9.3 4.1
IN-KG433					
Speyer Soil	0.001 0.010	5 5 Total = 10	98 94	7.7 2.1	7.9 2.2
Nambsheim Soil	0.001 0.010	5 5 Total = 10	100 95	6.3 2.9	6.3 3.0
IN-JU873					
Speyer Soil	0.001 0.010	5 5 Total = 10	101 97	4.7 2.4	4.6 2.5
Nambsheim Soil	0.001 0.010	5 5 Total = 10	104 97	3.6 2.3	3.7 2.4
IN-KT413					
Speyer Soil	0.001 0.010	5 5 Total = 10	98 84	13 5.1	13 6.1
Nambsheim Soil	0.001 0.010	5 5 Total = 10	90 90	12 11	13 12
IN-JT333					

Speyer Soil	0.001 0.010	5 5 Total = 10	100 90	16 14	16 15
Nambsheim Soil	0.001 0.010	5 5 Total = 10	97 97	12 8.9	12 9.1

Conclusion:

The analytical method DuPont-35025 is suitable for the determination of indoxacarb and its metabolite residues in soil. The instrumentation required to perform both the analysis and confirmatory methods is available in most well equipped analytical laboratories.

B.5.2.4 Methods for determination of residues in water

Residue definition in water: parent (sum of isomers)
EC10: 0.00168 mg/L

Report: Hill, S.J., Stry, J.J. (2002); Analytical method for the determination of DPX-MP062 (75% DPX-KN128 and 25% IN-KN127) and metabolites IN-JT333 and IN-KT413 in ground, surface, and drinking waters using LC/MS/MS

DuPont Report No.: DuPont-7898,

DuPont Crop Protection

Test facility: E. I. du Pont de Nemours and Company Newark, Delaware

The method for the risk assessment study DuPont-7898, originally submitted under EU Rev8 Point IIA 4.2.3 and conducted with test material DPX-MP062 (analytical standard), IN-JT333 (analytical standard), IN-KT413 (analytical standard) and IN-MP819 (analytical standard), was conducted under guideline SANCO/825/00 rev. 6 (2000). The review of this method additional updates to the report (DuPont-7898, Supplement No. 1 summarised (detailed below) have been made to meet the requirements as described in the SANCO 825/00 Revision 8.1 guidance document

Report: Mol, J.G.J. (2003); Independent laboratory validation of DuPont 7898 "analytical method for the determination of DPX-MP062 (75% DPX-KN128 (indoxacarb) and 25% IN-KN127) and metabolites IN-JT333 and IN KT413 in ground, surface, and drinking water using LC-MS/MS

DuPont Report No.: DuPont-12181,

Test facility: TNO Nutrition and Food Research Netherlands

The independent laboratory validation of DuPont-7898, study DuPont-12181, originally submitted under EU Rev8 Point IIA 4.2.3 and conducted with test material DPX-MP062 (analytical standard), IN-JT333 (analytical standard), IN-KT413 (analytical standard) and IN-MP819 (analytical standard), was conducted under guideline SANCO/825/00 rev. 6 (2000).

For completeness the original method report (DuPont-7898), the independent laboratory validation (DuPont-12181) and the updated supplement report are summarised below.

Study submitted to the EU for the first time in this submission

Report: Stry, J.J. (2014); Analytical method for the determination of DPX-MP062 (75% DPX-KN128 and 25% IN-KN127) and metabolites IN-JT333 and IN-KT413 in ground, surface, and drinking waters using LC/MS/MS
DuPont Report No.: DuPont-7898, Supplement No. 1
GLP: No
Test facility: E. I. du Pont de Nemours and company, Newark, Delaware

Description of the method:

DPX-MP062, IN-JT333, and IN-KT413 were extracted from the water samples by filtration through an Oasis HLB (0.50-gram) solid phase extraction (SPE) cartridges. The cartridges were washed with hexane and the analytes were eluted with 15-mL of methanol followed by 15-mL of acetonitrile. The extracts were evaporated under a flow of nitrogen to approximately 5-mL and quantitatively transferred to a graduated 14-mL centrifuge tube. The extracts were then evaporated to approximately 0.5-mL and diluted to 4-mL with acetonitrile. The extracts were mixed using a vortex mixer, sonicated and placed in a water bath at 55°C for one hour. In acetonitrile at 55°C IN-KT413 is quantitatively converted to IN-MP819. Due to the instability of IN-KT413 in organic solvents, this step was necessary for quantitative analysis of IN-KT413. The extracts were filtered through a SAX (1.0-g) SPE cartridge and evaporated to 1-mL. The extracts were diluted to 2-mL using water. DPX-MP062, IN-JT333, and IN-MP819 were separated from co-extracts by reversed phase liquid chromatography (LC) and were detected by positive ion Atmospheric Pressure Chemical Ionization (APCI) mass spectrometry/mass spectrometry (MS/MS).

Indoxacarb/DPX-MP062: m/z 528 →249.2 and 528.0→217.8

IN-MP819: m/z 237.9→149.0 and 237.9→130.9

IN-JT333: m/z 267.0→150.0 and 267→207.0

Linearity

Good linearity was observed in the range of 0.60 to 15.0 ng/mL for DPX-MP062 and its metabolites by analysis of five sample solutions and for each transition. R2 were > 0.996

Specificity

The limit of quantification of the method proposed for monitoring DPX-MP062 residues is 0.050 µg/kg for the water samples tested. Analysis of control samples resulted in no detectable apparent residues of indoxacarb or its metabolites. The response in the area of the indoxacarb peak or its metabolites always corresponded to less than 20% of the limit of determination. It can therefore be concluded that few, if any, apparent residues or false positive values would arise.

Recovery findings

The fortification data reported in the method proposed for monitoring DPX-MP062 and its metabolite residues in water samples are summarised in table below. The results listed below were obtained using standards prepared in solvent. The average recovery specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%.

Validation data for the analytical method DuPont-7898 for the determination of DPX-MP062 and its metabolites in water

Matrix	Fortification level (µg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
DPX-MP062						
Ground Water	0.050 0.50	5 5 Total = 10	97 104	5.0 2.1	5.2 2.0	DuPont-7898
Pond Water	0.050 0.50	5 5 Total = 10	106 102	3.6 4.7	3.4 4.6	
River Water	0.050 0.50	5 5 Total = 10	103 104	4.8 4.4	4.7 4.2	
Drinking Water	0.050 0.50	5 5 Total = 10	104 98	4.2 3.5	4.0 3.6	
IN-JT333						
Ground Water	0.050 0.50	5 5 Total = 10	97 93	4.7 5.6	4.8 6.0	DuPont-7898
Pond Water	0.050 0.50	5 5 Total = 10	95 99	6.2 5.5	6.5 5.6	
River Water	0.050 0.50	5 5 Total = 10	94 98	6.2 5.4	6.6 5.5	
Drinking Water	0.050 0.50	5 5 Total = 10	86 90	5.2 7.8	6.1 8.7	
IN-KT413						
Ground Water	0.050 0.50	5 5 Total = 10	99 109	9.7 5.9	9.8 5.4	DuPont-7898
Pond Water	0.050 0.50	5 5 Total = 10	104 106	5.6 2.8	5.4 2.6	
River Water	0.050 0.50	5 5 Total = 10	103 105	5.8 4.4	5.6 4.2	
Drinking Water	0.050 0.50	5 5 Total = 10	95 92	7.0 9.8	7.4 11	

[a] Fortifications were performed with analyte reference standard solutions.
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level.

Repeatability

Repeatability of the method is addressed by the data in table above. The same analyst obtained these recovery data over the course of two days per matrix. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix. Therefore, the repeatability of this method is adequate for the purposes of residue data collection in water.

Limit of quantification

The limit of quantification of the method for the water samples tested (Ground, drinking, river, ponds) was 0.050 µg/kg.

Reproducibility

An independent laboratory validation of DuPont-7898 was conducted. The results are presented in table below:

Independent laboratory validation data for the analytical method DuPont-7898 for the determination of DPX-MP062 and its metabolites in water

Matrix	Fortification level (µg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
DPX-MP062						
Ground Water	0.050 0.50	5 5 Total = 10	103 105	3 3	3 3	DuPont-12181
Surface Water	0.050 0.50	5 5 Total = 10	91 99	4 6	4 6	
Drinking Water	0.050 0.50	5 5 Total = 10	87 94	4 3	5 3	
IN-JT333						
Ground Water	0.050 0.50	5 5 Total = 10	110 104	3 2	3 2	DuPont-12181
Surface Water	0.050 0.50	5 5 Total = 10	99 100	3 5	3 5	
Drinking Water	0.050 0.50	5 5 Total = 10	96 100	6 2	6 2	
IN-KT413						
Ground Water	0.050 0.50	5 5 Total = 10	97 96	4 4	4 4	DuPont-12181
Surface Water	0.050 0.50	5 5 Total = 10	100 96	7 5	7 5	
Drinking Water	0.050 0.50	5 5 Total = 10	87 88	3 3	4 3	

[a] Fortifications were performed with analyte reference standard solutions.
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level.

Extraction efficiency

Extraction efficiency is not required for water methods.

Confirmatory method

Confirmation of results was obtained using secondary LC/MS/MS ion transitions collected at the same time as the quantitative transitions. The recovery data obtained using the confirmatory procedure are summarised below.

Confirmatory data for the analytical method DuPont-7898 for the determination of DPX-MP062 and its metabolites in water						
Matrix	**Fortification level (µg/kg) [a, b]**	**Number of tests**	**Average recovery (%)**	**Standard deviation**	**% Relative standard deviation**	**Reference**
DPX-MP062						
Ground Water	0.050 0.50	5 5 Total = 10	103 102	6.0 3.5	5.9 3.4	DuPont-7898, Supplement No. 1
Pond Water	0.050 0.50	5 5 Total = 10	104 101	11 7.1	11 7.0	
River Water	0.050 0.50	5 5 Total = 10	100 103	9.1 6.2	9.1 6.0	
Drinking Water	0.050 0.50	3 2 Total = 5	104 103	2.0 11	1.9 10	
IN-JT333						
Ground Water	0.050 0.50	5 5 Total = 10	94 91	7.2 8.2	7.7 9.0	DuPont-7898, Supplement No. 1
Pond Water	0.050 0.50	5 5 Total = 10	105 101	8.5 6.9	8.1 6.8	
River Water	0.050 0.50	5 5 Total = 10	100 93	4.0 4.9	4.0 5.1	
Drinking Water	0.050 0.50	3 2 Total = 5	81 86	11 6.4	13 7.4	
IN-KT413						
Ground Water	0.050 0.50	5 5 Total = 10	97 110	12 6.9	13 6.3	DuPont-7898, Supplement No. 1
Pond Water	0.050 0.50	5 5 Total = 10	113 111	12 2.6	11 2.4	
River Water	0.050 0.50	5 5 Total = 10	116 116	14 5.3	12 4.6	
Drinking Water	0.050 0.50	3 2 Total = 5	99 95	9.3 11	9.4 11	

[a] Fortifications were performed with analyte reference standard solutions.
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level.

Conclusion

The analytical method in DuPont-7898 and DuPont-7898, Supplement No. 1 is suitable for the detection and confirmation of DPX-MP062 and its metabolite residues in water.

Report	Stry, J.J. (2014); Analytical method for the determination of DPX-MP062 [75% DPX-KN128 (indoxacarb) and 25% IN-KN127] and metabolites IN-JT333, IN-MP819, IN-JU873, and IN-KG433 in ground, surface, and drinking waters using LC/MS/MS DuPont Report No.: DuPont-9605, Supplement No. 1 GLP: No **Test facility**: E. I. du Pont de Nemours and company, Newark, Delaware

The analytical method described in DuPont-9605, Revision No. 1 was an independent laboratory validation of analytical method presented in report DuPont-12182.

Description of the method

The water samples were diluted with acetonitrile and the pH of each sample was adjusted by the addition of 40-µL of concentrated acetic acid. DPX-MP062, IN-MS775, IN-JT333, IN-MP819, IN-JU873, and IN-KG433 were extracted from the water samples by filtration through an Oasis HLB solid phase extraction (SPE) cartridge. The cartridges were washed with 10-mL of water: acetonitrile (70:30) followed by 5-mL of hexane. The analytes were eluted with 25-mL of acetonitrile. The extracts were evaporated under a flow of nitrogen to a volume of approximately 100-µL. One mL of acetonitrile was added to the extracts. The extracts were mixed, sonicated, and then diluted to 2 mL using water. DPX-MP062, IN-MS775, IN-JT333, IN MP819, IN-JU873, and IN-KG433 were separated from co-extracts by reversed phase liquid chromatography. DPX-MP062, IN-MS775, IN-JT333, IN-JU873, and IN-KG433 were detected by positive ion electrospray (ESI) mass spectrometry/mass spectrometry (MS/MS). IN-MP819 did not produce sufficient single signal using the electrospray interface, and therefore IN-MP819 was detected using the Atmospheric Pressure Chemical Ionization (APCI) interface and mass spectrometry/mass spectrometry (MS/MS).

ANALYTES	IONS MONITORED
DPX-MP062	**528.0→ 217.8 ± 0.5 AMU**
	528.0→ 202.9 ± 0.5 AMU
IN-MS775	**411.9→ 208.8 ± 0.5 AMU**
	411.9→ 190.8 ± 0.5 AMU
IN-JT333	470.0→ 149.8 ± 0.5 AMU
	470.0→ 266.9 ± 0.5 AMU
IN-JU873	458.0→ 149.0 ± 0.5 AMU
	458.0→ 204.8 ± 0.5 AMU
IN-KG433	516.0→ 220.9 ± 0.5 AMU
	516.0→ 280.8 ± 0.5 AMU

Validation data:

Linearity

Good linearity was observed in the range of 0.60 to 15.0 ng/mL for DPX-MP062 and its metabolites and for each transition by analysis of five sample solutions.

Specificity

Representative chromatograms of standard, fortified and unfortified sample were presented.

The limit of quantification of the method proposed for monitoring DPX-MP062 residues is 0.05 µg/kg for the water samples tested. Analysis of control samples resulted in no detectable apparent residues of DPX-MP062 or its metabolites. The response in the area of the DPX-MP062 peak or its metabolites always corresponded to less than 20% of the limit of determination. It can therefore be concluded that few, if any, apparent residues or false positive values would arise.

Recovery and Repeatability

Repeatability of the method is addressed by the data in Table below. The same analyst obtained these recovery data over the course of two days per matrix. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix 5 samples were analysed at LOQ and 10xLOQ.

QUANTITATIVE ION AVERAGE RECOVERIES

MATRIX	LEVEL	DPX-MP062 (%RSD)	IN-MS775 (%RSD)	IN-JT333 (%RSD)	IN-MP819 (%RSD)	IN-JU873 (%RSD)	IN-KG433 (%RSD)
Ground Water	LOQ	98% (5.6)	96% (8.6)	102% (8)	96% (8)	101% (12)	95% (8)
Ground Water	10× LOQ	101% (9.5)	93% (9.5)	97% (7)	90% (12)	97% (8)	99% (4)
Bottled Water	LOQ	117% (4.9)	101% (7.3)	107% (2)	94% (6)	110% (6)	106% (7)
Bottled Water	10× LOQ	107% (10)	105% (11)	103% (10)	90% (18)	108% (8)	106% (10)
Pond Water	LOQ	86% (10)	81% (10)	81% (7)	98% (5)	83% (7)	81% (6)
Pond Water	10× LOQ	93% (10)	89% (12)	92% (9)	114% (3)	92% (10)	92% (7)
River Water	LOQ	88% (12)	82% (12)	101% (6)	92% (21)	96% (7)	104% (12)
River Water	10× LOQ	98% (9.7)	84% (14)	94% (12)	95% (12)	93% (10)	102% (17)
Surface Water Ave.LOQ		87% (10)	82% (11)	91% (13)	95% (11)	90% (10)	93% (16)
Surface Water Ave.10× LOQ		96% (10)	87% (12)	93% (10)	97% (15)	93% (9)	97% (14)

CONFIRMATORY ION AVERAGE RECOVERIES

MATRIX	LEVEL	DPX-MP062 (%RSD)	IN-MS775 (%RSD)	IN-JT333 (%RSD)	IN-MP819 (%RSD)	IN-JU873 (%RSD)	IN-KG433 (%RSD)
Ground Water	LOQ	96% (6.1)	89% (16)	96% (2.2)	94% (8.7)	101% (11)	100% (9.5)
Ground Water	10× LOQ	101% (7.3)	93% (6.2)	99% (7.0)	96% (14)	97% (6.3)	98% (5.0)
Bottled Water	LOQ	117% (14)	102% (7.5)	96% (18)	80% (10)	110% (4.0)	108% (9.9)
Bottled Water	10× LOQ	108% (9.7)	106% (7.6)	102% (12)	92% (17)	113% (13)	112% (12)
Pond Water	LOQ	81% (4.5)	79% (12)	77% (15)	102% (11)	79% (8.0)	78% (5.2)
Pond Water	10× LOQ	92% (6.5)	83% (11)	94% (14)	123% (3.7)	93% (11)	92% (8.1)
River Water	LOQ	94% (11)	88% (6.4)	87% (18)	79% (15)	84% (10)	89% (9.3)
River Water	10× LOQ	98% (6.8)	78% (11)	91% (7.1)	92% (8.6)	91% (4.9)	93% (2.9)
Surface Water Ave.LOQ		88% (12)	83% (11)	82% (17)	90% (18)	82% (9.2)	84% (10)
Surface Water Ave.10× LOQ		95% (7.1)	81% (11)	92% (11)	107% (17)	92% (8.3)	93% (5.7)

Limit of quantification

The limit of quantification of the method for the water samples tested was 0.050 µg/kg.

Conclusion:

Analytical method is suitable for the detection and confirmation of DPX-MP062 and its metabolite residues in water.

Study submitted to the EU for the first time in this submission

Report	Henze, R.M., Stry, J.J. (2012); Analytical method for the determination of indoxacarb and metabolites in water using LC/MS/MS DuPont Report No.: DuPont-35303 GLP: No Test facility: E. I. du Pont de Nemours and company, Newark, Delaware

Description of the method

This method was developed for the analysis of indoxacarb (DPX-KN128) and its metabolites IN-KG433, IN-JU873, IN-JT333 IN-MK643, IN-MK638, and IN-KB687 in water. Due to the wide range of compounds analysed, two procedures were developed.

The analysis of indoxacarb, IN-KG433, IN-JU873, and IN-JT333 in water was completed using the following procedure: Water samples (10-gram) were measured into 50-mL polypropylene centrifuge tubes, the pH was adjust by adding 10-µL of concentrated acetic acid and diluted with 6-mL of acetonitrile. The samples were mixed to homogeneity using a vortex mixer. An aliquot of the extracts were transferred to an autosampler vial

for analysis. The compounds indoxacarb, IN-KG433, IN-JU873 and IN-JT333 were separated from co-extracts by reversed phase Liquid Chromatography (LC) and detected by positive ion Turbospray Ionization (TSI) Mass Spectrometry/Mass Spectrometry (MS/MS).

The analysis of the indoxacarb and metabolites IN-MK643, IN-MK638, and IN-KB687 in water was completed using the following procedure: Water samples (10-g) were measured into 50-mL polypropylene centrifuge tubes, the pH was adjust by adding 10-µL of concentrated acetic acid and diluted with 2-mL of acetonitrile. The samples were mixed to homogeneity using a vortex mixer. An aliquot of the extracts were transferred to an autosampler vial for analysis. The potential indoxacarb metabolites IN-MK643, IN-MK638, and IN-KB687 were separated from co-extracts by reversed phase Liquid Chromatography (LC) and detected by negative ion Turbospray Ionization (TSI) Mass Spectrometry/Mass Spectrometry (MS/MS).

Indoxacarb: m/z 528.1→281.0 and 516.1 →149.1

IN-MK643: m/z 217.2→84.9 and 217.2 →131.0

IN-MK638: m/z 219→84.9 and 219→175.9

IN-KB687: m/z 233.9→84.9 and 233.9 →201.8

IN-KG433: m/z 516.1→281.0 and 516.1→149.1

IN-JU873: m/z 458.2→149.1 and 458.2→205.0

IN-JT333: m/z 470.2→267.1 and 470.2→207.1

Linearity

Good linearity was observed in the range of 0.025 to 1.0 ng/mL for indoxacarb, IN-KG433, IN-JU873, and IN-JT333 by analysis of six sample solutions for each transition. Good linearity was observed in the range of 0.050 to 5.0 ng/mL for IN-MK643, IN-MK638 and IN-KB687 by analysis of six sample solutions for each transition.

Specificity

The representative chromatograms of fortified and unfortified samples are presented and show no interference. Analysis of control samples resulted in no detectable apparent residues of indoxacarb or its metabolites for each transition. The response in the area of the indoxacarb peak or its metabolites always corresponded to less than 20% of the limit of determination.

Recovery findings

The fortification data reported in the method proposed for monitoring indoxacarb and its metabolite residues in water samples are summarised in table below. The results listed below were obtained using standards prepared in solvent, matrix matching was not required. The average recovery specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%. Therefore, the recovery of this method is adequate for the purposes of monitoring indoxacarb or a potential metabolite in water.

Validation data for the analytical method DuPont-35303 for the determination of indoxacarb and its metabolites in water

Matrix	Fortification level (µg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Indoxacarb						
Ground Water	0.10 1.0	5 5 Total = 10	114 101	3.7 4.4	3.2 4.4	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	101 96	5.8 3.8	5.7 4.0	
Drinking Water	0.10 1.0	5 5 Total = 10	107 98	8.1 6.5	7.6 6.7	
IN-MK643						
Ground Water	0.10 1.0	5 5 Total = 10	107 103	3.9 3.0	3.7 3.0	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	102 99	2.2 3.0	2.1 3.1	
Drinking Water	0.10 1.0	5 5 Total = 10	104 108	8.8 17	8.5 16	
IN-MK638						
Ground Water	0.10 1.0	5 5 Total = 10	110 107	2.3 2.9	2.1 2.8	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	105 103	1.1 2.3	1.1 2.3	
Drinking Water	0.10 1.0	5 5 Total = 10	108 113	14 16	13 14	
IN-KB687						
Ground Water	0.10 1.0	5 5 Total = 10	109 105	2.8 2.2	2.5 2.1	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	106 103	3.8 3.6	3.6 3.5	
Drinking Water	0.10 1.0	5 5 Total = 10	107 115	5.0 11	4.7 9.2	

Validation data for the analytical method DuPont-35303 for the determination of indoxacarb and its metabolites in water (continued)

Matrix	Fortification level (µg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
IN-KG433						
Ground Water	0.10 1.0	5 5 Total = 10	113 103	2.6 3.4	2.3 3.3	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	110 104	3.4 2.8	3.1 2.7	
Drinking Water	0.10 1.0	5 5 Total = 10	115 108	2.3 5.1	2.0 4.7	
IN-JU873						
Ground Water	0.10 1.0	5 5 Total = 10	105 103	1.1 2.0	1.1 2.0	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	103 104	2.0 2.4	1.9 2.3	
Drinking Water	0.10 1.0	5 5 Total = 10	108 103	9.8 7.0	9.1 6.8	
IN-JT333						
Ground Water	0.10 1.0	5 5 Total = 10	102 99	13 2.4	13 2.4	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	104 100	5.3 8.0	5.1 8.0	
Drinking Water	0.10 1.0	5 5 Total = 10	111 96	10 9.3	9.1 9.6	

[a] Fortifications were performed with analyte reference standard solutions.
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level.

Limit of quantification

The limit of quantification of the method for the water samples tested was 0.10 µg/kg.

Repeatability

Repeatability of the method is addressed by the data in the table above. The same analyst obtained these recovery data over the course of two days per matrix. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix. Therefore, the repeatability of this method is adequate for the purposes of residue data collection in water.

Reproducibility

An independent laboratory validation of DuPont-35303 was not conducted.

Extraction efficiency

Extraction efficiency is not required for water methods.

Confirmatory method

Confirmation of results was obtained using secondary LC/MS/MS ion transitions collected at the same time as the quantitative transitions. The recovery data obtained using the confirmatory procedure is summarised in table below:

Confirmatory data for the analytical method DuPont-35303 for the determination of indoxacarb and its metabolites in water

Matrix	Fortification level (µg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Indoxacarb						
Ground Water	0.10 1.0	5 5 Total = 10	109 101	4.2 0.8	3.9 0.8	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	102 96	4.1 5.0	4.0 5.2	
Drinking Water	0.10 1.0	5 5 Total = 10	112 99	9.0 6.3	8.1 6.4	
IN-MK643						
Ground Water	0.10 1.0	5 5 Total = 10	107 103	5.0 2.9	4.6 2.8	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	102 100	6.1 3.1	5.9 3.1	
Drinking Water	0.10 1.0	5 5 Total = 10	96 104	11 11	12 11	
IN-MK638						
Ground Water	0.10 1.0	5 5 Total = 10	109 106	4.3 2.2	3.9 2.1	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	106 102	4.8 1.5	4.6 1.5	
Drinking Water	0.10 1.0	5 5 Total = 10	108 109	16 16	15 14	
IN-KB687						
Ground Water	0.10 1.0	5 5 Total = 10	110 105	4.4 3.7	4.0 3.5	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	110 102	3.6 3.0	3.3 3.0	
Drinking Water	0.10 1.0	5 5 Total = 10	105 115	4.3 10	4.1 8.8	

Confirmatory data for the analytical method DuPont-35303 for the determination of indoxacarb and its metabolites in water (continued)

Matrix	Fortification level (µg/kg) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
IN-KG433						
Ground Water	0.10 1.0	5 5 Total = 10	112 104	4.6 4.9	4.1 4.7	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	108 102	3.0 5.2	2.8 5.1	
Drinking Water	0.10 1.0	5 5 Total = 10	116 105	5.7 6.6	4.9 6.3	
IN-JU873						
Ground Water	0.10 1.0	5 5 Total = 10	106 102	7.0 2.7	6.6 2.6	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	105 105	4.4 4.4	2.4 2.4	
Drinking Water	0.10 1.0	5 5 Total = 10	111 103	10 5.7	9.5 5.5	
IN-JT333						
Ground Water	0.10 1.0	5 5 Total = 10	106 108	9.1 7.9	8.5 7.3	DuPont-35303
Surface Water	0.10 1.0	5 5 Total = 10	102 103	7.2 7.0	7.1 6.8	
Drinking Water	0.10 1.0	5 5 Total = 10	117 98	7.1 12	6.1 12	

[a] Fortifications were performed with analyte reference standard solutions
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Conclusion

The analytical method DuPont-35303 is suitable for the detection and confirmation of indoxacarb and its metabolite residues in water. The instrumentation required to perform both the analysis and confirmatory methods is available in most well equipped analytical laboratories. No toxic or hazardous reagents are required to prepare the samples, and all of the sample preparation equipment is commercially available. The method does not require the use of untreated commodity to correct for recoveries.

B5.2.5 Description of monitoring methods for determination of residues air

Residue definition in air: parent (sum of isomers)

Study submitted to the EU for the first time in this submission

CA 4.2/07	**Report**	van Schaik, F. (2006); Development confirmation air method for DPX-MP062 using LC-MS/MS **DuPont Report No.:** DuPont-18596 **GLP:** Yes Test facility: TNO Quality of Life, Zeist, Netherlands

Description of the method

DPX-MP062 (sum of isomers) was fortified onto XAD-2 Filters and known quantitates of air was passed through the filters. DPX-MP062 (sum of isomers) was eluted from the filters using solvent and was analysed using GC/NPD and LC/MS/MS. The GC/NPD method is intended for quantitative analysis and the LC/MS/MS method is intended for the confirmation of any detected residues. The method was validated using ambient and warm humidified air.

Linearity

Good linearity was observed in the range of 0.02 to 20 ng/mL for DPX-MP062 using the GC/NPD for each transition. During the analysis of the ambient air samples eight standards were injected two times each ranging from 0.021 ng/mL to 20.6 ng/mL. The R^2 value was 0.999 for this data set. Good linearity was also observed in the range of 0.20 to 25 ng/mL for DPX-MP062 using LC/MS/MS detection. During the analysis of the ambient air samples six standards were inject two time each ranging from 0.20 ng/mL to 25.2 ng/mL. The R^2 value was 0.9994 for this data set.

Ions monitored m/z: 528→293 (quantification) and m/z: 528→218, 528→203 (qualifier)

Mass spectra to confirm a choice of transitions were presented in other study.

Specificity

Chromatograms of fortified and unfortified matrix were presented and show no interference for each transition. The limit of quantification of the method proposed for monitoring indoxacarb residues is 0.10 μg/m^3 in air. Analysis of control samples resulted in no detectable apparent residues of DPX-MP062. The response in the area of the DPX-MP062 peak always corresponded to less than 20% of the limit of determination. It can therefore be concluded that few, if any, apparent residues or false positive values would arise.

Breakthrough of method: breakthrough was tested at 5 100 X the LOQ level and was determined to be less than 5%.

Recovery findings

The fortification data reported in the method proposed for monitoring DPX-MP062 in XAD-2 filters are summarised below. The average recoveries were between 70–120%, with a standard deviation of ≤20%. Therefore, the recovery of this method is adequate for the purposes of monitoring indoxacarb in air.

Validation data for the analytical method DuPont-18596 for the determination of DPX-MP062 in air using GC/NPD

Matrix	Fortification level (μg/m^3) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Ambient Air	0.10 10	5 5 Total = 10	90 94	4.8 4.8	5.4 5.1	DuPont-18596
Warm Humidified Air	0.10 10	5 5 Total = 10	88 91	11 3.8	12 4.2	

[a] Fortifications were performed with analyte reference standard solutions
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Limit of quantification

The limit of quantification of the method for air was 0.10 μg/m^3.

Repeatability

Repeatability of the method is addressed by the data above. The same analyst obtained these recovery data over the course of two days per matrix. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit as well as at higher levels.

Extraction efficiency

Extraction efficiency is not required for air methods.

Confirmatory method

Confirmation of results was obtained using LC/MS/MS detection. The recovery data obtained using the confirmatory procedure was summarised below:

Confirmation data for the analytical method DuPont-18596 for the determination of DPX-MP062 in air using LC/MS/MS

Matrix	Fortification level ($\mu g/m^3$) [a, b]	Number of tests	Average recovery (%)	Standard deviation	% Relative standard deviation	Reference
Ambient Air	0.10 10	5 5 Total = 10	98 96	1.7 2.6	1.8 2.7	DuPont-18596
Warm Humidified Air (35°C and 80% humidity)	0.10 10	5 5 Total = 10	102 101	2.8 2.5	2.8 2.5	

[a] Fortifications were performed with analyte reference standard solutions
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Conclusion

The analytical method DuPont-18596 is suitable for the detection and confirmation of DPX-MP062 (sum of isomers) in air. However, the study report and is required.

B.5.2.6 Description of monitoring methods for determination of residues in body fluids and tissues

An analytical method (DuPont-24760) has been developed and is submitted in this document for the analysis of indoxacarb and its metabolite IN-JT333 in plasma, milk, and fat.

For DPX-KN128 the log K ow was determined to be 4.65 ➔ fat-soluble substance (bioaccumulation)

CA 4.2/06	**Report**	Stry, J.J. (2008); Analytical method for the analysis of indoxacarb and metabolite IN-JT333 in plasma, milk, and fat **DuPont Report No.:** DuPont-24760 **GLP:** No
Test facility		E. I. DuPont de Nemours and Company, Newark Delaware

Description of the method

Plasma samples (0.1-mL) were measured into a glass centrifuge tube. The samples were fortified with indoxacarb and IN-JT333. To each sample 0.5-mL of isopropanol was added. The samples were mixed for 30 seconds and sonicated for 5 minutes. To each sample 0.5-mL of acetonitrile was added. The samples were mixed again and a 0.9-mL volume of 0.01 M aqueous formic acid was added to each sample. The samples were centrifuged for 10 minutes and an aliquot of the extracts was filtered through a syringe filter into auto sampler vials prior to analysis.

Milk samples (0.5-gram) were weighed into 50-mL centrifuge tubes. The samples were fortified with indoxacarb and IN-JT333. To each sample 5-mL of acetonitrile was added. The samples were centrifuged and the acetonitrile transferred into 50-mL centrifuge tubes. A second 5-mL volume of acetonitrile was added to each sample. The samples were vortex mixed, centrifuged, and the supernatants combined with the supernatants from

the first extraction. For each extract 1-mL of water and a 5-mL of hexane was added. The extracts were vortex mixed and a partition was allowed to form. The hexane layer was removed from each extract and discarded. An additional 5-mL of hexane was added and the partition was repeated, discarding the hexane layer. The extracts were diluted to 12-mL with acetonitrile and a 6-mL aliquot was transferred to a 13-mL centrifuge tube. The extracts were evaporated to approximately 0.5-mL under a flow of nitrogen and diluted to 2-mL using 50% acetonitrile/50% 0.01 M formic acid in water. An aliquot of each extract was filtered through a syringe filter prior to analysis.

Fat samples (0.5-gram) were weighed into a 50-mL centrifuge tube. The samples were fortified with indoxacarb and IN-JT333. To each sample 5-mL of water and a 12-mL of ethyl acetate was added. The samples were homogenized using a homogenizing probe for 1 minute. The samples were centrifuged for 10 minutes and the ethyl acetate phases were transferred into a 50-mL centrifuge tubes. A second 12-mL volume of ethyl acetate was added to each sample and the samples were homogenized a second time. The samples were centrifuged and the ethyl acetate layers combined with the first extraction. The ethyl acetate extracts were evaporated to dryness under a flow of nitrogen and reconstituted in 5-mL of hexane. To each extract, 9-mL of acetonitrile and 1.0-mL of water was added. The extracts were mixed using a vortex mixer and the extracts were allowed to stand until a partition formed. The hexane layer was removed for each extract and discarded. An additional 5-mL of hexane was added and the partitions were repeated, discarding the hexane layer. The extracts were evaporated to dryness and reconstituted in 2.0-mL of acetonitrile. A 2.0-mL volume of 0.01 M formic acid in water was added and the extract vortex mixed. An aliquot of the extract was filter through a syringe filter prior to analysis.

All extracts were analysed by APCI-LC/MS/MS positive ion mode for quantitative analysis.

Masse transitions:

DPX-KN128: m/z 528→248 for quantification and 528→217 for confirmation

IN-JT333: m/z 267→149 for quantification and 267→206 for confirmation

Masse spectra to confirm a choice of transitions were presented.

Specificity

Analysis of control samples resulted in no detectable apparent residues of indoxacarb or IN-JT333 for each transition. The response in the area of the indoxacarb or IN-JT333 peak always corresponded to less than 20% of the limit of determination. It can therefore be concluded that few, if any, apparent residues or false positive values would arise.

Linearity

Linearity was confirmed by injecting 4 sample standard solutions for each transition. A good linearity was observed in the range of 0.060 to 3.0 ng/mL for indoxacarb and IN-JT333. R2 were > 0.995.

Recovery

The results listed below were obtained using standards prepared in matrix extracts. The average recovery specified in the decision-making criteria is 70–120%, with a standard deviation of ≤20%.

Validation data for the analytical method DuPont-24760 for the determination of indoxacarb and IN-JT333 in plasma, milk and fat						
Matrix	**Fortification level (mg/kg) [a, b]**	**Number of tests**	**Average recovery (%)**	**Standard deviation**	**% Relative standard deviation**	**Reference**
Indoxacarb						
Plasma	0.0020 0.020	5 5 Total = 10	88 78	8.4 8.4	9.5 11	DuPont-24760
Milk	0.0020 0.020	5 5 Total = 10	116 101	6.8 8.3	5.9 8.2	
Fat	0.0020 0.020	5 5 Total = 10	100 94	17 7.1	17 7.5	
IN-JT333						
Plasma	0.0020 0.020	5 5 Total = 10	102 89	17 5.6	17 6.3	DuPont-24760
Milk	0.0020 0.020	5 5 Total = 10	107 101	9.8 7.2	9.2 7.2	
Fat	0.0020 0.020	5 5 Total = 10	87 89	4.2 2.9	4.9 3.3	

[a] Fortifications were performed with analyte reference standard solutions
[b] Limit of quantification, defined by a signal-to-noise ratio of approximately 10 and the lowest validated fortification level

Limit of quantification

The limit of quantification of the method for plasma, milk and fat is 0.0020 mg/kg.

Repeatability

Repeatability of the method is addressed by the data in table above. The same analyst obtained these recovery data over the course of two days per matrix. Relative standard deviations of less than 20% were obtained for fortifications made at the quantification limit for each matrix, as well as at higher levels.

Reproducibility

An independent laboratory validation of DuPont-24760 was not conducted.

Confirmatory method

Only one ion transitions were monitored for the analysis of indoxacarb and IN-JT333 in plasma, milk and fat. The confirmation of detected residue was based on the ratio of the two ions detected. Although individual recoveries were not calculated, confirmation was demonstrated by evaluating the relative ratios of the two LC/MS/MS peaks detected. However, only results for milk were presented but the number of sample is not sufficient for confirmation (n = 3). For plasma and fat method is considered as not highly specific.

Sample	Area 528 ➔ 248.8	Area 528 ➔ 217.1	Ratio 248.8/217.1
0.5ng/ml (Std)	39	31	1.3
0.15ng/ml (Std)	13	9	1.4
1.5ng/ml (Std)	122	71	1.7

3.5ng/ml (Std)	282	171	1.6
LOQ (Milk)	24 (n=3)	15	1.6
10*LOQ (Milk)	212 (n = 3)	124	1.7

Conclusion:

The analytical method DuPont-24760 is validated for determination of Indoxacarb residues in plasma, milk and fat with an LOQ of 0.0020 mg/kg. **Method is not considered as highly specific, a confirmatory method is required.**

Summary

Proposed analytical methods for monitoring indoxacarb and its metabolite residues in soil, water and air

Matrix	Separation/ Quantitation	Limit of determination (mg/kg)	Reference and report	Comments
Soil				
Soil	LC-MS/MS	0.0010 mg/kg	Henze, R.M., Stry, J. J. (2012) DuPont-35025	Validated for the determination of indoxacarb and its metabolites (IN-MK643, IN-MK638, IN-KB687, IN-KG433, IN-JU873, IN-KT413, IN-JT333) in soil.
Water				
Ground, surface and drinking water	LC-MS/MS	0.05µg/kg	Hill, S.J., Stry, J.J. 2002 Mol, J.G.J. 2003 Stry, J.J. (2014) [DuPont-7898, DuPont-12181, DuPont-7898, Supplement No. 1]	Validated for determination of Indoxacarb, IN-KT413, IN-MS775, IN-JT333, IN-MP819, IN-JU873 and INKG433 in ground water, surface water and drinking water.
Ground water, Bottled water pond Water and river water	LC-MS/MS	0.050 µg/kg	Stry, J.J. (2014) DuPont-9605	Validated for determination of Indoxacarb, IN-KT413, IN-KG433 in ground water, drinking water and surface water.
Ground, surface and drinking water	LC-MS/MS	0.10µg/kg	Henze, R.M., Stry, J.J. 2012 DuPont-35303	Validated for determination of indoxacarb and its metabolites in ground water and drinking water.
Air				
Air	GC/NPD LC-MS/MS	0.10 $\mu g/m^3$	van Schaik, F. (2006); DuPont-18596	Validated for determination of indoxacarb residues in air.
Body fluids and tissues	LC-MS/MS	0.0020 mg/L	Stry, J.J., 2008 DuPont-24760	Validated for determination of Indoxacarb residues in plasma, milk and fat with an LOQ of 0.0020 mg/L. **However, the method is not highly specific and confirmatory method is required**

B.5.3. REFERENCES RELIED ON

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.1.1	Hansen, S.W.	2003	Indoxacarb (DPX-KN128): Analysis of technical materials used in toxicology testing DuPont Stine-Haskell Research Center DuPont-13812 and DuPont-13812, Confidential attachment Published: No	N	N		DuPont	Study previously Reviewed for EU approval in the 2005 DAR
CA, 4.1.1	Kahler, T.W.	2006	Technical grade active ingredient indoxacarb (DPX-KN128) analysis and certification of product ingredients in support of registration of DuPont KN128 technical Exygen Research DuPont-16774 and DuPont-16774, Confidential attachment Published: No	N	N		DuPont	Study submitted in the EU Dossier and included in the first EU approval review.
CA, 4.1.1	Robson, D.D., Hansen, S.W.	2004	Recharacterization of toxicology test substance DPX-MP062-051A DuPont Stine-Haskell Research Center DuPont-13930 Published: No	N	N		DuPont	Study submitted in the EU Dossier in 2005 and included in the Evaluation report of the technical material for the active substance Indoxacarb, 2009

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.1/01	Gravelle, W.D.	2013	Description and validation of the analytical methods for the determination of impurities in indoxacarb (DPX-KN128) technical blendbase Product Safety Labs DuPont-34934 and DuPont-34934, Confidential attachment GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.[a]

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.1/02	Gravelle, W.D.	2015	Description and validation of the analytical methods for the determination of IN-J1063, IN-C0800, IN-06439, and IN-R1T94 impurities in indoxacarb (DPX-KN128) technical and indoxacarb (DPX-KN128) 150 g/L EC formulation Product Safety Labs DuPont-38062, Revision No. 1 and DuPont-38062, Revision No. 1 Confidential attachment GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.1/03	Hansen, S.W.	2004	Technical grade indoxacarb (DPX-KN128) analysis and certification of product ingredients in support of registration of DuPont KN128 technical and DuPont Claridox C technical DuPont Stine-Haskell Research Center DuPont-13126 and DuPont-13126, Confidential attachment GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
KCA, 4.1.1/04	Hansen, S.W.	2013	Determination of indoxacarb (DPX-KN128) in technical grade indoxacarb and end-use products DuPont Stine-Haskell Research Center DuPont-34638 GLP: No Published: No	N	N		DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.1.2	Amoo, J.S., Beaver-Stetser, E.	1997	Analytical method (HPLC/column switching/UV) for the determination of residues of DPX-KN128/DPX-KN127 and IN-JT333 in animal matrices- whole and skim milk, cream, fat, muscle, liver, and kidney DuPont Experimental Station AMR 3337-95 Published: No	N	N		DuPont	Study submitted in the EU Dossier in 1997 and included in the first EU approval review.
CA, 4.1.2	Behmke, F.D.	1997a	Extraction efficiency of analytical methods for the determination of [^{14}C]DPX-JW062 (racemic mixture of DPX-KN128 and IN-KN127) derived residues in potatoes DuPont Experimental Station AMR 3457-95 Published: No	N	N		DuPont	Study submitted in the EU Dossier in 1997 and included in the first EU approval review.
CA, 4.1.2	Behmke, F.D.	1997b	Extraction efficiency of analytical methods for the determination of [^{14}C]DPX-JW062 (racemic mixture of DPX-KN128 and IN-KN127) derived residues in corn DuPont Experimental Station AMR 3320-95 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.1.2	Behmke, F.D.	1997c	Extraction efficiency of analytical methods for the determination of [^{14}C]DPX-JW062 (racemic mixture of DPX-KN128 and IN-KN127) derived residues in lettuce DuPont Experimental Station AMR 3315-95 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 4.1.2	Behmke, F.D.	1997d	Extraction efficiency of analytical methods for the determination of [^{14}C]DPX-MP062 (a mixture of DPX-KN128 and IN-KN127) derived residues in grapes DuPont Experimental Station AMR 4657-97 Published: No	N	N		DuPont	Study submitted in the EU Dossier in 1997 and included in the first EU approval review.
CA, 4.1.2	Behmke, F.D.	1997e	Extraction efficiency of analytical methods for the determination of [^{14}C]DPX-MP062 (a mixture of DPX-KN128 and IN-KN127) derived residues in tomatoes DuPont Experimental Station AMR 4633-97 Published: No	N	N		DuPont	Study submitted in the EU Dossier in 1997 and included in the first EU approval review.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.1.2	Behmke, F.D.	1997f	Extraction efficiency of analytical methods for the determination of [^{14}C]DPX-MP062 (a mixture of DPX-KN128 and IN-KN127) derived residues in cotton DuPont Experimental Station AMR 4594-97 Published: No	N	N		DuPont	Study submitted in the EU Dossier in 1997 and included in the first EU approval review.
CA, 4.1.2	Class, T.	1996	Validation of analytical enforcement method in air (by absorption on XAD-2 and GC/TSD) for KN128 and KN127 residues which might result from the use of the formulation DPX-MP062 PTRL Europe AMR 4160-96 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 4.1.2	Desmond, P.J.	1997	A study of the recovery of residues of DPX-KN128/DPX-KN127 (formulated as either DPX-JW062 or DPX-MP062) after frozen storage on: Grapes, grape wet pomace, wine, apples, lettuce, tomatoes, apple juice, and soil; and incurred residue studies on tomatoes, DuPont Experimental Station AMR 3778-96 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.1.2	Gagnon, M.R., Guinivan, R.A.	1996	Residue procedure for the analysis of DPX-KN128/DPX-KN127 in crops and related process fractions by GC-MSD DuPont Experimental Station AMR 3493-95, Supplement No. 1 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 4.1.2	Gagnon, M.R., Guinivan, R.A., Desmond, P.J.	1997	Analytical enforcement procedure for the analysis of DPX-KN128/DPX-KN127 in crops and related process fractions by GC-MSD DuPont Experimental Station AMR 3493-95, Supplement No. 2, Revision No. 1 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 4.1.2	Hill, S.J., Stry, J.J.	2002	Analytical method for the determination of DPX-MP062 (75% DPX-KN128 and 25% IN-KN127) and metabolites IN-JT333 and IN-KT413 in ground, surface, and drinking waters using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-7898 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 DAR.
CA, 4.1.2	[redacted]	1997	Metabolism of ^{14}C-DPX-JW062 (racemic mixture of DPX-KN128 and IN-KN127) in laying hens [redacted] AMR 3187-94 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.1.2	Mol, J.G.J.	2003	Independent laboratory validation of DuPont 7898 "analytical method for the determination of DPX-MP062 (75% DPX-KN128 (indoxacarb) and 25% IN-KN127) and metabolites IN-JT333 and IN KT413 in ground, surface, and drinking water using LC-MS/MS TNO Nutrition and Food Research DuPont-12181 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 DAR.
CA, 4.1.2	Rhodes, B.C.	1997	Aerobic soil metabolism of ^{14}C-DPX-JW062 DuPont Experimental Station AMR 2803-93 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 4.1.2	Schmuckler, M.E., Cooke, L.A.	1997	Physical and chemical characteristics of DPX-KN128 DuPont Experimental Station AMR 4141-96 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.2/01	Aufderheide, J.	2014	Indoxacarb (DPX-KN128): Growth inhibition test with the unicellular green alga, *Pseudokirchneriella subcapitata* ABC Laboratories, Inc. (Missouri) DuPont-38349 GLP: Yes Published:	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.2/02	Connolly, P.	2004	Independent laboratory validation of the analytical method, DuPont-12739, "Analytical method for the determination of DPX-MP062 and metabolites IN-KB687, IN-KG433, IN-KT319, IN-JU873 and IN-JT333 in poultry skin, liver, muscle, fat and eggs" Amendment number 1 Exygen Research DuPonr-13651, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.2/03	Craig, W.B.	2004	Indoxacarb (DPX-KN128): Laboratory study of solubility in oganic solvents Inveresk Research International (IRI) Limited (Scotland) DuPont-12940, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.2/04	Dinehart, S.	2014	Indoxacarb (DPX-KN128): acute toxicity with the mysid shrimp, *Americamysis bahia*, determined under flow-through test conditions ABC Laboratories, Inc. (Missouri) DuPont-38440 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
KCA, 4.1.2/05	Gagnon, M.R., Stry, J.J.	2005	Analytical method for the determination of DPX-MP062 75% DPX-KN128 (indoxacarb) and 25% IN-KN127 in cloth by LC/MS/MS DuPont Stine-Haskell Research Center DuPont-15035 GLP: No Published: No	N	N		DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.2/06	Henze, R.M.	1997	Analytical method (HPLC/column switching/UV) for the determination of residues of DPX-KN128/DPX-KN127 and IN-JT333 in whole fish DuPont Experimental Station AMR 4304-97 GLP: No Published: No	N	N		DuPont	N.A.
KCA, 4.1.2/07	Henze, R.M., Stry, J.J.	2012	Analytical method for the determination of indoxacarb and metabolites in water using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-35303 GLP: No Published: No	N	N		DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.2/08	Lakaschus, S.,Gizler, A.	2010	Adaptation and validation of a method for the determination of DPX-KN128 (indoxacarb) in honey Eurofins / Dr. Specht Laboratorien GmbH DuPont-Specht DUP-0801V GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
KCA, 4.1.2/09	Stry, J.J.	2004	Analytical method for the determination of DPX-MP062 and metabolites IN-KB687, IN-KG433, IN-KT319, IN-JU873, and IN-JT333 in poultry skin, liver, muscle, fat, and eggs DuPont Stine-Haskell Research Center, Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-12739, Revision No. 1 GLP: No Published: No	N	N		DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.1.2/10	Stry, J.J.	2014	Analytical method for the determination of DPX-MP062 (75% DPX-KN128 and 25% IN-KN127) and metabolites IN-JT333 and IN-KT413 in ground, surface, and drinking waters using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-7898, Supplement No. 1 GLP: No Published: No	N	N		DuPont	N.A.
CA, 4.2	Class, T.	2000	Independent laboratory validation (ILV) of the analytical residue method AMR 4271-96 for the determination of residues of DPX-KN128 and IN-KN127 in plant material which might be treated with DPX-MP062 PTRL Europe DuPont-3295 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 4.2	Class, T.	2001a	Independent laboratory validation (ILV) of the analytical residue method DuPont-2338 for the determination of residues of DPX-KN128, IN-KN127, and the metabolite IN-JT333 in foodstuffs of animal origin PTRL Europe DuPont-3520, Revision No. 1 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 DAR.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.2	Class, T.	2001b	Validation of the analytical residue method DuPont-2338 for the determination of residues of DPX-KN128, IN-KN127, and the metabolite IN-JT333 in edible offal PTRL Europe DuPont-6224 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 DAR.
CA, 4.2	Hill, S.J., Pentz, A.M., Stry, J.J.	2003	Analytical method for the determination of DPX-MP062 [75% DPX-KN128 (indoxacarb) and 25% IN-KN127] and metabolites IN-JT333, IN-MP819, IN-JU873, and IN-KG433 in ground, surface, and drinking waters using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-9605, Revision No. 1 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 DAR.
CA, 4.2	Linkerhagner, M., Guinivan, R.A.	2001	Testing of DFG method S 19 for the determination of DPX-MP062 and its metabolite IN-JT333 in foodstuffs of animal origin Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-2338, Revision No. 1 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 DAR.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
CA, 4.2	Mol, J.G.J.	2003	Independent laboratory validation of DuPont-9605 rev1 analytical method for the determination of DPX-MP062 (75% DPX-KN128 (indoxacarb) and 25% DPX-KN127) and metabolites IN-MS775, IN-JT333, IN-MP819, IN-JU873 and IN-KG433 in groundwater surface water and TNO Nutrition and Food Research DuPont-12182 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 DAR.
CA, 4.2	Schmidt, F.	1997	Testing of DFG method S 19 for the determination of residues of KN128 along with KN127 in crops which might be treated with DPX-MP062 Dr. Specht & Partner Chemische Laboratorien GmbH AMR 4271-96 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/01	Cermak, J.	2013	Validation of multi-residue method DFG S19 for the determination of residues of DPX-MP062 (DPX-KN128 (indoxacarb) and IN-KN127) in grapes, tomato, oilseed rape and maize using LC/MS/MS Vyzkumny ustav organickych syntez a.s. (VUOS) DuPont-37894, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
KCA, 4.2/02	Henze, R.M., Stry, J. J.	2012	Analytical method for the determination of indoxacarb and metabolites in soil using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-35025 GLP: No Published: No	N	N		DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/03	Lakaschus, S., Klimmek, A.	2006	Validation of multi-residue method DFG S19 (L 00.00-34) for the determination of residues of DPX-MP062 (DPX-KN128 (indoxacarb) and IN-KN127) in grass Eurofins Analytik GmbH DUP-0602V GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/04	Richter, S.	2013	Validation of the multi-residue method DFG S19 for the determination of residues of indoxacarb and its metabolite IN-JT333 in animal matrices, using LC-MS/MS PTRL Europe DuPont-39006 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier	DuPont	N.A.
KCA, 4.2/05	Stry, J.J.	2014	Analytical method for the determination of DPX-MP062 [75% DPX-KN128 (indoxacarb) and 25% IN-KN127] and metabolites IN-JT333, IN-MP819, IN-JU873, and IN-KG433 in ground, surface, and drinking waters using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-9605, Supplement No. 1 GLP: No Published: No	N	N		DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/06	Stry, J.J.	2008	Analytical method for the analysis of indoxacarb and metabolite IN-JT333 in plasma, mik, and fat DuPont Stine-Haskell Research Center DuPont-24760 GLP: No Published: No	N	N		DuPont	N.A.
KCA, 4.2/07	van Schaik, F.	2006	Development confirmation air method for DPX-MP062 using LC-MS/MS TNO Quality of Life DuPont-18596 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/08	Stanislowski, T.	2015	Independent laboratory validation (ILV) of the multi-residue method DFG S19 for the determination of residues of indoxacarb in crop matrices, using LC-MS/MS PTRL Europe DuPont-44627 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/09	Aitken, A.	2014	Determination of the decline of residues of DPX-KN128 (indoxacarb) along with IN-KN127 in maize forage following applications of DPX-MP062 30WG - Southern Europe – 2012 Charles River Laboratories (UK) DuPont-35172 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/10	[redacted]	2006	Oral (gavage) developmental neurotoxicity study of DPX-KN128 (Indoxacarb) technical in Crl:CD (SD)IGS BR VAF/Plus rats [redacted]. DuPont-15150 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/11	Berg, C.	2015	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the brood of honey bees (*Apis mellifera*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany 2014 Eurofins Agroscience Services GmbH DuPont-37489, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/12	[redacted]	2003	Indoxacarb (DPX-KN128) technical: Mouse bone marrow micronucleus test [redacted] DuPont-13021 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/13	Giammarrusti, L., De Paoli, M.	2003	Decline of residues of DPX-KN128 (indoxacarb) together with IN-KN127 in sweet corn following application of DPX-MP062 30WG - Italy, season 2003 ERSA DuPont-13320 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/14	Gudi, R., Rao, M.	2004	Indoxacarb (DPX-KN128) technical: *In vitro* mammalian chromosome aberration study in human peripheral blood lymphocytes BioReliance DuPont-13022, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/15	Guinivan, R.A., Daussin, S.	2008	Recovery of DPX-MP062 and five metabolites from hen-derived matrices (whole eggs, muscle, fat and liver) after frozen storage Dupont Stine-Haskell Research Center DuPont-19901 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/16	[redacted]	2011	Indoxacarb (DPX-KN128) technical: 28-Day immunotoxicity feeding study in mice [redacted] DuPont-29280 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/17	Klank, C.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: Honey bee (*Apis mellifera* L.) larval toxicity test (single feeding exposure) Eurofins Agroscience Services EcoChem GmbH DuPont-34817 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/18	Klein, O.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the bumble bee (*Bombus terrestris* L; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany in 2013 Eurofins Agroscience Services GmbH DuPont-38419 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/19	Kleinhenz, M.	2011a	Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honey bee (Ap*is mellifera carnica*) in the field in *Brassica napus* L. in eastern Germany in 2009 Eurofins Agroscience Services GmbH DuPont-26946 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/20	Kleinhenz, M.	2011b	Indoxacarb (DPX-KN128) 150 g/L EC: A study to evaluate effects on the honey bee (*Apis mellifera carnica*) in the field in Brassica napus L. in northern Germany in 2009 Eurofins Agroscience Services GmbH DuPont-26947 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/21	Lakaschus, S., Amann, S.	2012a	Determination of magnitude of residues of DPX-KN128 (indoxacarb) together with IN-KN127 in protected tomato following applications of DPX-MP062 30WG and DPX-KN128 30WG- Europe, 2011 Eurofins Agroscience Services GmbH DuPont-32128 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/22	Lakaschus, S., Amann, S.	2012b	Determination of magnitude of residues of DPX-KN128 (indoxacarb) together with IN-KN127 in lettuce following applications of DPX-MP062 30WG and DPX-KN128 30WG - Europe, 2011 Eurofins Agroscience Services Chem GmbH DuPont-33518 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/23	Markell, L.K.	2015	Indoxacarb (DPX-KN128) technical: *In vitro* 3T3 NRU phototoxicity test DuPont Haskell Laboratory DuPont-43522 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/24	[redacted]	2003	Magnitude of residues of indoxacarb (as DPX-MP062) in laying hen tissue and eggs: a feeding study conducted to EPA guidelines [redacted] DuPont-8305 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/25	[redacted]	2004	Indoxacarb (DPX-KN128) technical: Developmental toxicity study in rats [redacted] DuPont-12748 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/26	Rentschler, S.	2014	Indoxacarb (DPX-KN128) 150 g/L EC: A semi-field study to evaluate effects on the honey bee (*Apis mellifera*; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany 2014 Eurofins Agroscience Services EcoChem GmbH DuPont-41668 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner	Previous Evaluation
KCA, 4.2/27	San, R.H.C., Clarke, J.	2003	Indoxacarb (DPX-KN128) technical: *In vitro* mammalian cell gene mutation test (CHO/HGPRT test) BioReliance DuPont-13023 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.

[a] N.A. = not applicable, as this is a new study submitted for the first time at EU level for the purpose of renewal.