Section A4.2 **Annex Point IIA, IV 4.2**	**Analytical Methods for Detection and Identification** ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN ANIMAL AND HUMAN BODY FLUIDS AND TISSUES	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20 January 2011	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	-	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Official use only

1 REFERENCE

1.1	**Reference**	Meinerling, M. and Herrmann, S., 2008, Validation of an analytical method for the determination of Preventol BP (chlorophene) in soil. Institut für Biologische Anlaytik und Consulting IBACON GmbH, Rossdorf, Germany, Project No. 33345101 (unpublished), 2008-01-15
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	SANCO/825/00 rev. 7 guidance document on residue analytical methods, European Commission Directorate General Health and Consumer Protection March 17, 2004
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	About 50 g soil (wet weight) were weighed and transferred into a 250 mL glass bottle. 40 mL acetonitrile were added. The solid was extracted by rotating the bottle for approximately 60 min. The extraction step was repeated. The combined extracts were collected in a 100 mL volumetric flask and filled up to the mark using acetonitrile. Afterwards the extracts were filtered using PTFE-filter (0.45 µm).
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	Chromatographic conditions:

Column:	Prodigy 5u ODS3 (150 * 4.6 mm)
Mobile phase:	Acetonitrile / water (85:15, v/v %)
Flow rate (column):	0.5 mL/min
Injection volume:	20 µL
Temperature:	Room temperature (approx. 20 °C)

3.2.2	Detector	Mass spectrometric detector: Sciex API 2000 Ion Source: Turbo Ion Spray, negative mode Mass Ion: 217 amu (parent ion)
3.2.3	Standard(s)	External standard chlorophene (purity: 97.9%)
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.
3.3	**Linearity**	
3.3.1	Calibration range	Eight concentrations were measured in the range from 2.5 to 50 µg/L.

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

3.3.2	Number of measurements	Each concentration was measured once.	
3.3.3	Linearity	Correlation coefficient: at least 0.999.	
3.4	**Specificity: interfering substances**	The identity of the analyte was established by use of MS technique and by comparison of the retention time obtained from sample solutions and standard solutions. The retention time of the analyte in the samples solution did not differ by more than 1% from that for the standard solution. The analyte has no interference from other components and was well resolved from them. Interferences from blank samples did not contribute more than 2% of the total peak area measured for the target analyte.	
3.5	**Recovery rates at different levels**	Recoveries were obtained by fortification of soil samples with known amounts of the active substance. Two fortification levels were analysed: 0.01 mg/kg and 0.1 mg/kg. At each fortification level 5 independent replicates were made. The mean recovery rates (n=5) obtained from analysis of fortified samples were in the range from 80 – 104%. The overall mean recovery (n=10) was 92%.	
3.5.1	Relative standard deviation	The relative standard deviations (n=5) were in the range from 4 to 7%. The overall relative standard deviation (n=10) was 14%.	
3.6	**Limit of determination**	The limit of quantification is 0.01 mg/kg.	
3.7	**Precision**		
3.7.1	Repeatability	Please refer to point 3.5 (recovery rates).	
3.7.2	Independent laboratory validation	No independent laboratory validation is available.	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Soil samples are analysed by HPLC-MS instrument with electrospray detection in negative mode after extraction with acetonitrile. Quantification is done by external standard.	x
4.2	**Conclusion**	The analytical method has been validated with respect to linearity, accuracy and precision. The analytical method is suitable for the determination of chlorophene residues in soil.	
4.2.1	Reliability	Reliability indicator: 1	x
4.2.2	Deficiencies	No	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11 November 2010
Materials and methods	Agree with applicant's version.
Conclusion	**Comment (4.1):** Using external standard quantification for the LC-MS determination of compounds in complex matrices such as soil might lead to unreliable quantitative results due to strong ion suppression in cases of the presence of co-extracted matrix compounds.
Reliability	**Comment (4.2.1):** Due to the deficiency described in comment (4.1) the reliability is changed from 1 to 2; reliable with restrictions
Acceptability	Acceptable with the restrictions described.
Remarks	

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Official use only

1 REFERENCE

1.1 Reference

Königer, A., 2009, Validation of an analytical method for the determination of Preventol BP in air samples. CURRENTA GmbH & Co. OHG, Services Analytik, Leverkusen, Germany, Report No. 2005/0148/14 (unpublished), date: 2009-11-02

1.2 Data protection

Yes

1.2.1 Data owner

[redacted]

1.2.2 Companies with letter of access

[redacted]

1.2.3 Criteria for data protection

Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

SANCO/3030/99 rev. 4 of 11/07/00, Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 and SANCO/825/00 rev.6 (20/06/00), Guidance document on residue analytical methods

2.2 GLP

Yes

2.3 Deviations

No

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

Air is aspirated 6 hours to a Tenax adsorption tube. The content of the Tenax tube is filled into a 20 mL beaded rim bottle. Exactly 2 mL acetonitrile are added, the bottle is closed and shaken for 30 min. After filtration 10 µL of the final solution are analysed according to the indicated conditions.

3.1.2 Cleanup

3.2 Detection

3.2.1 Separation method

Chromatographic conditions:

Column: Purospher STAR 100 RP-18 e
length: 125 mm
inner diameter: 4 mm
particle diameter: 3 µm

Mobile phase: A: demineralised water + 0.05% formic acid
B: acetonitrile + + 0.05% formic acid

Gradient:

Time [min]	A [%]	B [%]
0	30	70
5	30	70
5.1	0	100

Flow rate: 0.75 mL/min

Injection volume: 10 µL

Column temperature: 40 °C

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

3.2.2	Detector	Mass spectrometric detector Ionisation modus: ESI positive SIM: Mass m/z 217.1 [M-H$^+$] Gas temperature: 350 °C Drying gas: 11 L/min Capillary voltage: 3000 V Retention time: chlorophene: 3.8 min
3.2.3	Standard(s)	External standard chlorophene (Preventol BP), purity: 98.4%
3.2.4	Interfering substance(s)	Substances of sample matrix or adsorption material may interfere with the active substance.
3.3	**Linearity**	
3.3.1	Calibration range	To determine the linearity of the detector response, determinations with eight concentrations covering a range between 0.3 µg/m^3 to 4.0 µg/m^3 (corresponding to test solution concentrations of 53 µg/L to 712 µg/L) were performed. In case of higher expected amounts, a reduced air volume for adsorption should be used.
3.3.2	Number of measurements	Each concentration was measured once.
3.3.3	Linearity	Correlation coefficient: 0.9989
3.4	**Specificity: interfering substances**	The identification of chlorophene was performed by LC-MS using a solution of the test substance. The mass spectrum and the retention time of the test substance were compared with such of the calibration substance. The peak could be identified as chlorophene by its typical mass fragment m/z = 217 [M-H$^+$] and by the retention time of 3.7 min.
3.5	**Recovery rates at different levels**	For the determination of accuracy two different fortification levels at 35 °C und 80% relative humidity were performed. Six Tenax tubes were fortified each with 5 µL of a stock solution (201 µg/mL acetonitrile) and six Tenax tubes were fortified each with 5 µL of a stock solution (20.1 µg/mL acetonitrile). 360 L air were then pumped through each Tenax tube (theoretical concentration 0.3 µg/m^3 air and 3 µg/m^3 air, respectively). The extraction of the test tubes was performed as indicated (theoretical concentrations: 50.3 µg/L and 503 µg/L, respectively). The mean recovery was 100.83% (n = 6) at a nominal concentration of 50 µg/L and 86.17% (n = 6) at a nominal concentration of 500 µg/L, respectively. The overall mean recovery was 93.50% (n = 12). Detailed recovery results are shown in Table 4_2-1. The received mean recovery values were in the range of 70 – 100% and meet the requirements of the Guidance document SANCO/3029/99 rev. 4. Additionally the efficiency of extraction and the retention efficiency of the sorbent material were investigated. With a recovery rate of 104.2% for the first extraction a satisfactory efficiency of extraction was reached and with a recovery rate of 95% the retention efficiency of the sorbent material is considered to be sufficient. No breakthrough took place.

3.5.1	Relative standard deviation	The relative standard deviation was 2.83% at a nominal concentration of 50 µg/L and 2.30% at a nominal concentration of 500 µg/L, respectively. The overall relative standard deviation was 8.56% (n = 12).
3.6	**Limit of determination**	The limit of quantification is 0.3 µg/m^3 air.
3.7	**Precision**	
3.7.1	Repeatability	For the determination of precision two different fortification levels at 35 °C und 80% relative humidity were performed. Six Tenax tubes were fortified each with 5 µL of a stock solution (201 µg/mL acetonitrile) and six Tenax tubes were fortified each with 5 µL of a stock solution (20.1 µg/mL acetonitrile). 360 L air were then pumped through each Tenax tube (theoretical concentration 0.3 µg/m^3 air and 3 µg/m^3 air, respectively). The extraction of the test tubes was performed as indicated (theoretical concentrations: 50.3 µg/L and 503 µg/L, respectively). The mean content of six determinations at the theoretical level 50 µg/L was 0.0000051%. The relative standard deviation for the six determinations was 2.83%. The mean content of six determinations at the theoretical level 500 µg/L was 0.000043%. The relative standard deviation for the six determinations was 2.30%. No break-through was observed.
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	A defined air volume is aspirated to a Tenax adsorption tube. The adsorbed chlorophene is extracted from the tube with acetonitrile. The amount of chlorophene in the eluent is determined by means of liquid chromatography using mass spectroscopic detection in the selected ion monitoring mode. Quantification is performed by external standardisation.
4.2	**Conclusion**	The analytical method has been completely validated with respect to linearity, specificity, accuracy, precision, the efficiency of extraction, the retention efficiency of the sorbent material as well as limits of quantification and detection. All received validation data meet the requirements described in the guidance documents SANCO/825/00 rev.6 (20/06/00) and SANCO/3030/99 rev. 4 of 11/07/00. The analytical method is suitable for the determination of chlorophene residues in air.
4.2.1	Reliability	Reliability indicator: 1
4.2.2	Deficiencies	No

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11 November 2010
Materials and methods	Agree with applicant's version.
Conclusion	Agree with applicant´s version.
Reliability	1, reliable without restrictions.
Acceptability	Acceptable
Remarks	-

Table A4_2-1: Results of recoveries

Nominal concentration [µg/L]	Recovery rates [%]		Mean recovery (n=6) [%]	Relative standard deviation [%]	Overall mean recovery (n=12) [%]	Overall relative standard deviation [%]
50	106.13	98.85	100.83	2.83	93.50	8.56
	99.72	101.48				
	98.19	100.61				
500	87.09	83.41	86.17	2.30		
	85.81	89.16				
	86.76	84.81				

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Official use only

1 REFERENCE

1.1	**Reference**	Meinerling, M., 2007a, Validation of an analytical method for the determination of Preventol BP (chlorophene) in water. Institut für Biologische Anlaytik und Consulting IBACON GmbH, Rossdorf, Germany, Project No. 33346101 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	SANCO/825/00 rev. 7 guidance document on residue analytical methods, European Commission Directorate General Health and Consumer Protection March 17, 2004
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Samples at a concentration level ≥ 10 µg/L were analysed without further sample preparation. If necessary the samples were diluted.
3.1.2	Cleanup	In case of samples at a concentration level below 10 µg/L the samples were extracted using solid phase extraction. Chromabond C18-200mg/3mL (Macherey&Nagel GmbH Co.KG) were used. The column was conditioned by rinsing two times with 5 mL acetonitrile followed by two times 5 mL deionised water. The pH value of the samples was adjusted to 2. The sample volume (500 mL) was applied to the column. Afterwards it was dried by suction of air. The analyte was eluted by rinsing with approximately 5 mL acetonitrile. It was made up to 5 mL using a volumetric flask. If necessary, the samples were diluted.
		Samples analysed by HPLC are prepared as given above without dilution.

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

3.2	**Detection**			
3.2.1	Separation method	LC conditions:		
		Column:	RP18 (125 * 3 mm)	
		Mobile phases:	A: acetonitrile B: Water containing 0.05% acetic acid 0 min: 75% A/ 25% B 1 min: 75% A/ 25% B 2 min: 95% A/ 5% B 6 min: 95% A/ 5% B 7 min: 75% A/ 25% B	
		Flow rate (column):	0.3 mL/min	
		Injection volume:	20 µL	
		Temperature:	Room temperature	
		HPLC conditions:		
		Column:	RP 18 (250 * 4 mm)	
		Mobile Phase:	70% acetonitrile / 30% water containing 0.02% phosphoric acid	
		Flow Rate:	1 mL/min	
		Injection Volume:	50 µL	
		Oven Temperature:	25°C	
3.2.2	Detector	Mass spectrometric detector (MS/MS): Sciex API 2000 Ion Source: Turbo Ion Spray, negative mode Mass Ion: 217 amu (parent ion) UV detection is used in addition. Detection wavelength: 205 nm.		X
3.2.3	Standard(s)	External standard		
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.		
3.3	**Linearity**			
3.3.1	Calibration range	9 concentrations were measured in the range from 2.5 to 50 µg/L.		
3.3.2	Number of measurements	Each concentration was measured once.		
3.3.3	Linearity	Correlation coefficient: 0.998		
3.4	**Specificity: interfering substances**	The identity of the analyte was established by use of MS technique and by comparison of the retention time obtained from sample solutions and standard solutions. The retention time of the analyte in the samples solution did not differ by more than 1% from that for the standard solution. The blank values of the control samples were below 0.3 * LOQ. HPLC method with UV detection was used in addition.		

Section A4.2 Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

3.5	**Recovery rates at different levels**	Recoveries were obtained by fortification of tap water with known amounts of the analyte. Three fortification levels were tested: 0.1 µg/L, 1 µg/L and 10 µg/L. At each fortification level 5 independent replicates were made. The mean recovery rates obtained from analysis of fortified samples of chlorophene were in the range from 84 – 107%.	X
3.5.1	Relative standard deviation	For all fortification levels the relative standard deviation was below 10%.	
3.6	**Limit of determination**	The limit of quantification is 0.1 µg/L.	
3.7	**Precision**		
3.7.1	Repeatability	Please refer to point 3.5 (recovery rates).	
3.7.2	Independent laboratory validation	No independent laboratory validation is available.	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Tap and surface water samples are analysed after enrichment by solid phase extraction. Determination is performed using HLPC-MS instrument with electrospray detection in negative mode. Quantification is done by external standard.	
4.2	**Conclusion**	The analytical method has been validated with respect to linearity, accuracy and precision. The analytical method is suitable for the determination of chlorophene residues in water.	x
4.2.1	Reliability	Reliability indicator: 1	
4.2.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	14 September 2010
Materials and methods	Agree with applicant's version.
Conclusion	Agree with applicant's version **Comment (4.2):** External standard quantification is used for analysis of water samples with all its limitations when using LC-MS. However, as the concentration of co-extracted matrix components is expected to be low for water samples, the risk for ion suppression might not be high.
Reliability	1, valid without restrictions
Acceptability	Acceptable
Remarks	3.2.2 MS/MS-instrument used in single MS mode 3.5 Recovery rates are given for both tap and surface water in the study report.

Section A4.3 **BPD Annex Point IIIA, IV.1**	**Analytical Methods for Detection and Identification** ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN/ON FOOD OR FEEDSTUFFS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20 January 2011	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	