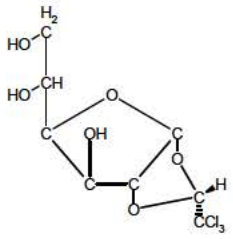
Section A1 Annex Point IIA1	**Applicant**	
1.1 Applicant	This is a joint application being made by Rentokil Initial plc and Physalys. Any queries or questions about this dossier should be directed to Rentokil Initial plc in the first instance. Applicant 1: Name: Address: Rentokil Initial plc, Felcourt, East Grinstead, West Sussex, RH19 2JY United Kingdom Telephone: +44 (0) 1342 833 022 Fax: +44 (0) 1342 326 229 Applicant 2: Name: Address: Physalys, 3, Rue de l'Arrivée – BP215, F.75749, Paris, CEDEX 15, France Telephone: +33 1 4321 7062 Fax number: +33 1 4321 7063	Official use only x
1.2 Manufacturer of Active Substance (if different)	Manufacturer is Physalys, details as above. Location of manufacturing plant: Name:	
1.3 Manufacturer of Product(s) (if different)	Location of manufacturing plant for product Alphablock	

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2007
Evaluation of applicant's justification	Not applicable
Conclusion	Not applicable
Remarks	1.1 - After July 2005, the contact person is A new contact person , was established after April 2007.

Section A2 **Annex Point IIA II**	**Identity of Active Substance**	
Subsection (Annex Point)		Official use only
2.1 Common name (Annex IIA2.1)	Alphachloralose. Also known as α-D-glucochloralose; glucochloral; anhydroglucochloral; chloralosane	x
2.2 Chemical name (IIA2.2)	(R)-1,2-O-(2,2,2-Trichloroethylidene)-α-D-glucofuranose	
2.3 Manufacturer's development code number(s) (IIA2.3)	No manufacturers development code known.	x
2.4 CAS no. and EC numbers (IIA2.4)		
2.4.1 CAS No.	15879-93-3	
2.4.2 EC-No	240-016-7	
2.4.3 Other	INN name chloralose	
2.5 Molecular and structural formula, molecular mass (IIA2.5)		
2.5.1 Molecular formula	$C_8H_{11}Cl_3O_6$	
2.5.2 Structural formula		
2.5.3 Molecular mass	309.54	
2.6 Method of manufacture of the active substance (IIA2.1)	**CONFIDENTIAL information** - data provided separately	
2.7 Specification of the purity of the active substance, as appropriate (IIA2.7)		x

Section A2 Annex Point IIA II	Identity of Active Substance	
2.8 Identity of impurities and additives, as appropriate (IIA2.8)	**CONFIDENTIAL information** - data provided separately	
2.8.1 Isomeric composition	**CONFIDENTIAL information** - data provided separately	
2.9 The origin of the natural active substance or the precursor(s) of the active substance. (IIA2.9)	**CONFIDENTIAL information** - data provided separately	
FOOTNOTE	Stability of Alphachloralose is addressed in Document IIIA, section 3.17.	

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	January 2005
Materials and Methods	acceptable
Results and discussion	2.1 – According to Commission Regulation (EC) n.º 2032/2003, of 4 November 2003, the name of the active substance identified and notified is Chloralose so, CA Report will use this name. At TMIII07 it was decided to adopt the name of Alphachloralose to the substance to be included in Annex I. 2.3 - There are other types of batch numbering referred in several study summaries but they are all properly identified, with certificate of analysis.
Conclusion	Acceptable
Reliability	Not applicable
Acceptability	Acceptable
Remarks	None

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	Method	Purity/ Specification	Results Give also data on test pressure, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA3.1)	--	--	--	--	--	--	--	
3.1.1 Melting point	According to EC Method AI "Melting/ freezing temperature"	97% purity.		Not applicable.	Y	1		
Melting point 1			179-181°C					
Melting point 2			179-181°C					
Melting point 3			178-180°C					
Melting point 4			179-180°C					
Melting point 5			179-181°C					
Melting range			178-181°C				Applicant's reference number Alphchl 183	
Melting point a	According to Differential Scanning Calorimetry (DSC) analysis according to OECD method 113	97% purity.	176.6°C		Y	1	Applicant's reference number Alphchl 187	

Section A3	Physical and Chemical Properties of Active Substance

Stability of chloralose tested

The chloralose tested is > 2 years old. There is no formal storage stability data held on chloralose that confirms stability after two years, however analysis of the batch tested confirms it is suitable for testing*.

Applicant's reference number ALPCHL 314

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2007
Materials and Methods	Acceptable
Conclusion	The differences between the results of the two melting point tests should be considered acceptable. Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	References: Confidential data information

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.1.2 Boiling point	Differential Scanning Calorimetry (DSC) analysis according to OECD method 113	97% purity. Specification of Alphachloralose tested is identical to that given in section A2.7	Decomposition temperature: 182.0°C	It is technically not possible to determine the boiling point of Alphachloralose because it decomposes immediately after melting.	Y	1	, Applicant's reference number Alphchl 187	

Stability of chloralose tested

The chloralose tested is > 2 years old. There is no formal storage stability data held on chloralose that confirms stability after two years, however analysis of the batch tested confirms it is suitable for testing*.

Reference 3.1.2/02 Rentokil Initial plc (2005), Determination of Chloralose in Chloralose Technical Material by Reverse Phase Liquid Chromatography, Technical Request Report PC277, GLP, Unpublished, Applicant's reference number ALPCHL 314.

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	August 2005
Materials and Methods	Acceptable
Conclusion	Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	References: Confidential data information

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.1.3 Bulk density/ relative density	According to EC Method A3 "Relative density"	97% purity.		Not applicable.	Y	1		
Rel. density 1			0.7742					
Rel. density 2			0.7742					
Rel. density 3			0.7727					
Rel. density 4			0.7742					
Rel density 5		<u>Stability of chloralose tested</u>	0.7740					
Mean rel. density		Date of manufacture of chloralose tested was December 2000. Study commenced April 2002. Accelerated shelf life study submitted in Document IIIA section 3.17 shows chloralose is stable for two years.	0.7739 +/- 0.0007 at 20°C				Applicant's reference number Alphchl 184	

Section A3	Physical and Chemical Properties of Active Substance
	Evaluation by Competent Authority
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and Methods	Acceptable
Conclusion	Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	Reference: Confidential data information

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.2.1 Henry's Law Constant (Pt. 1-A3.2)	Calculated from water solubility and vapour pressure of alphachloralose. See questions 3.2 and 3.5.	N/A	5.82 x 10^{-4} Pa $M^3 mol^{-1}$	Molecular weight is 309.53 therefore a 1 molar solution is 309.53 g/l. We have solubility of 4.7g dm^{-3} (1 litre is equivalent to dm^{-3}). This is equivalent to 4.7/309.53 =0.01518 mol.dm^{-3} or 15.18mol m^{-3}. The vapour pressure is 0.00883 Pa. Henrys law constant is: H = vapour pressure / concentration H = 0.00883/15.18 = 5.82 x 10^{-4} Pa $M^3 mol^{-1}$	N	1	N/A	

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2007
Materials and Methods	Water solubility at pH9 was used. The amended calculation for Henry's Law Constant at pH7 is as follows: The solubility is 4.84g.dm^{-3} which is equivalent to 4.84/309.53 = 0.01563 mol.dm^{-3}, or 15.63 mol m^{-3}.
Conclusion	The vapour pressure of alphachloralose is 0.00883 Pa.H = 0.00883/15.63 = 5.65 x 10^{-4} Pa. M^3. mol^{-1}
Reliability	1
Acceptability	Acceptable
Remarks	No remarks

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.2 Vapour pressure (IIA 3.2)	According to EC Method A4 "Vapour pressure"	97% purity.		Not applicable.	Y	1		
Vapour pressure 1 Vapour pressure 2 Vapour pressure 3			0.01153 Pa (at 30°C) 0.01576 Pa (at 40°C) 0.01983 Pa (at 50°C)					
Vapour pressure 4		<u>Stability of chloralose tested</u> Date of manufacture of chloralose tested was December 2000. Study commenced November 2002. Accelerated shelf life study submitted in Document IIIA section 3.17 shows chloralose is stable for two years.	0.00883 Pa (at 25°C, calculated from the regression curve derived by plotting Log P vs. 1/T).				Applicant's reference number Alphchl 185	

Section A3	Physical and Chemical Properties of Active Substance
Evaluation by Competent Authority	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	August 2005
Materials and Methods	Acceptable
Conclusion	Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	Reference: Confidential data information

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.3 Appearance (IIA3.3)								
3.3.1 Physical state	Solid (powder)							
3.3.2 Colour	White to yellowish white							
3.3.3 Odour	Odourless							

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	August 2005
Materials and Methods	-
Conclusion	Applicant's version adopted.
Reliability	-
Acceptability	Acceptable
Remarks	None

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.5 Solubility in water (IIA3.5)	According to EC Method A6 "Water Solubility, Flask Method"	97% purity.	Mean solubilites: pH 5: 4.86g/L pH 7: 4.84 g/L pH 9: 4.73 g/L	Not applicable.	Y	1	Applicant's reference number Alphchl 220.	

Stability of chloralose tested

The chloralose tested is > 2 years old. There is no formal storage stability data held on chloralose that confirms stability after two years, however analysis of the batch tested confirms it is suitable for testing*.

Reference 3.5/02 Rentokil Initial plc (2005), Determination of Chloralose in Chloralose Technical Material by Reverse Phase Liquid Chromatography, Technical Request Report PC277, GLP, Unpublished, Applicant's reference number ALPCHL 314.

Section A3	Physical and Chemical Properties of Active Substance
Evaluation by Competent Authority	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2007
Materials and Methods	Acceptable
Conclusion	The assay was conducted at 24°C. Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	References: Confidential data information

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.6 Dissociation constant (-)	Not applicable.	Not applicable.	Not applicable.	The water solubility of Alphachloralose has been determined (refer to Document IIIA, Section 3.5 Solubility in water). Therefore it is not scientifically necessary to determine the dissociation constant of Alphachloralose in accordance with OECD 112 (dissociation constant in water), given that it should only be determined if water solubility cannot be measured.	N/A	0	Not applicable.	

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	August 2005
Materials and Methods	Acceptable
Conclusion	Applicant's version adopted.
Reliability	0
Acceptability	Acceptable
Remarks	No remarks

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.9 Partition coefficient n-octanol/ water (IIA3.6)	According to EC Method A8 "Partition coefficient", Shake Flask Method.	97% purity.		Not applicable.	Y	1		
Partition coefficient 1			0.88 (n-octanol/water ratio of 1: 1)					
Partition coefficient 2			0.85 (n-octanol/water ratio of 1: 2)					
Partition coefficient 3			0.83 (n-octanol/water ratio of 1: 3)				Applicant's reference number Alphchl 186	
Mean partition coefficient			0.85 +/- 0.03					
Stability of chloralose tested The chloralose tested is > 2 years old. There is no formal storage stability data held on chloralose that confirms stability after two years, however analysis of the batch tested confirms it is suitable for testing*. Reference 3.9/02 Rentokil Initial plc (2005), Determination of Chloralose in Chloralose Technical Material by Reverse Phase Liquid Chromatography, Technical Request Report PC277, GLP, Unpublished, Applicant's reference number ALPCHL 314.								

Section A3	Physical and Chemical Properties of Active Substance
Evaluation by Competent Authority	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2007
Materials and Methods	Acceptable
Conclusion	The partition coefficient of alphachloralose was determined by EC method A8, shake flask method. According to this method the effect of pH does not need to be reported. However, it can be shown that pH does not have an effect on the partition coefficient. A study carried out to determine the solubility of alphachloralose (Doc IIIA 3.5) showed that pH did not effect the solubility of alphachloralose in water. The pH can only effect the water phase it cannot effect the oil phase of the partition coefficient. Any effects shown in the solubility would give the same effect in the partition coefficient. Therefore as pH does not effect the solubility it will not effect the partition coefficient. The partition coefficient study was carried out at room temperature (22-26°C). Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	Reference: Confidential data information

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	Method	Purity/ Specification	Results Give also data on test pressure, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.11 Flammability, including auto-flammability and identity of combustion products. (IIA, 3.8)	According to EC Method A10 "Flammability (Solids)"	97% purity.	No ignition or combustion of the test powder train was observed after applying the hot flame from the gas burner for a period of two minutes (the maximum period specified in EC method A10). Given this result, Alphachloralose is not classified as flammable.	Not applicable.	Y	1	, Applicant's reference number Alphchl 188	
	According to EC Method A15 "Auto-Ignition Temperature (Liquids and Gases)"	97% purity. Specification of *For explanation about stability of chloralose tested, see next page.	The auto-ignition temperature of Alphachloralose was observed with a yellow flame at 390°C (with a time lag of 6 seconds at 766 mm Hg barometric pressure).	Not applicable.	Y	1	, Applicant's reference number Alphchl 190	X

Section A3 Physical and Chemical Properties of Active Substance

Stability of chloralose tested

The chloralose tested is > 2 years old. There is no formal storage stability data held on chloralose that confirms stability after two years, however analysis of the batch tested confirms it is suitable for testing*.

Reference 3.11/03 Rentokil Initial plc (2005), Determination of Chloralose in Chloralose Technical Material by Reverse Phase Liquid Chromatography, Technical Request Report PC277, GLP, Unpublished, Applicant's reference number ALPCHL 314.

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2007
Materials and Methods	Acceptable for EC Method A10.
Conclusion	At the Technical Meeting in Brussels on 10th – 12th July it was agreed that a justification as to why the EC tests A12 Flammability (contact with Water) and A13 Pyrophoric Properties of Solids and Liquids were not conducted and COM noted that test A.15 (ignition temperature) is applicable to liquids and gases only but not to solids. According to the Technical Notes for Guidance Volume 1 Data Requirements Chapter 2, 3.11 states that tests A12 and A13 "can be omitted if experience in use indicates that negative results would be obtained or if a substance is expected to react violently under test conditions." A12 was not conducted as use of the product has shown that contact with water or damp air does not lead to development of dangerous amounts of gas or gases which may be highly flammable. A13 was not conducted as use of the product has shown that it does not ignite spontaneously a short time after coming into contact with air at room temperature. Furthermore, it was agreed with the Applicant that the test (A16) for the auto-ignition temperature of Alphachloralose must be submitted, at product authorisation stage.
Reliability	1
Acceptability	Acceptable with the conditions stated above.
Remarks	References: Confidential data information

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.12 Flash-point (IIA3.9)	Not applicable.	Not applicable.	Not applicable.	Alphachloralose is a white / yellowish white powder. It is therefore technically not possible to determine the flash point of Alphachloralose, given that the recommended test method is only applicable for liquids whose vapours can be ignited.	N/A	0	Not applicable.	

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2007
Materials and Methods	Acceptable
Conclusion	As the substance is not a liquid or a liquid preparation a flash point test (A.9) is not applicable. Applicant's version adopted.
Reliability	0
Acceptability	Acceptable
Remarks	No remarks

Section A3	Physical and Chemical Properties of Active Substance							
Subsection (Annex point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.13 Surface tension (IIA3.10)	According to EC Method A5 "Surface Tension"	97% purity.		Not applicable.	Y	1		
Surface tension 1			50.096 mN/m (at 20°C)					
Surface tension 2			50.096 mN/m (at 20°C)					
Surface tension 3			49.995 mN/m (at 20°C)					
Surface tension 4			50.096 mN/m (at 20°C)				, Applicant's reference number Alphchl 189	
Surface tension 5			50.096 mN/m (at 20°C)					
Mean surface tension			50.076 +/- 0.045 mN/m (at 20°C)					

Stability of chloralose tested

The chloralose tested is > 2 years old. There is no formal storage stability data held on chloralose that confirms stability after two years, however analysis of the batch tested confirms it is suitable for testing*.

Applicant's reference number ALPCHL 314.

Section A3	Physical and Chemical Properties of Active Substance
Evaluation by Competent Authority	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2007
Materials and Methods	Acceptable
Conclusion	The surface tension of a 1 g/L aqueous sample solution of alphachloralose was determined. Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	References: Confidential data information

SECTION A3		**Physical, chemical and technical Properties**	
Annex Point IIA3.17/01		Accelerated Shelf Life	
		STUDY SUMMARY SUPPLIED BY RMS	
		1 REFERENCE	**Official use only**
1.1	**Reference**		X
		Applicant's reference number ALPHCHL 299.	
1.2	**Data protection**	Yes	
1.2.1	Data owner		x
1.2.2	Companies with letter of access	—	
1.2.3	Criteria for data protection	Data submitted to MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	The study was carried out according to procedure SOP:LM033 Part 2, Issue 3. The test conditions are in accordance with CIPAC method MT46.3	
2.2	**GLP**	Yes	
2.3	**Deviations**	Specific gravity was proposed to measure according to the method QCM: Wt Vol Cup issue. This method was followed but the results are correctly reported as density in g m^3	
Date		03.06.2005	
Lot/Batch number		Batch no. JJ357	
Specification		As given in section 2 of dossier.	
Description		White to yellowish white	
Purity		100.44% (m/m)	
Stability		Is the aim of this project	

SECTION A3	**Physical, chemical and technical Properties**
Annex Point IIA3.17/01	Accelerated Shelf Life
Materials and methods	A 50g sample of Chloralose was weighed into a glass bottle(Ref.: ADL26). A 10g sample Chloralose was taken and submitted for initial analysis (Ref.:ADL26/1). The bottle are sealed, weighed and placed in a oven set at 54ºC for two weeks. The oven was maintained at $54^{+}0.5$ºC during the test period. After two weeks the bottle was remove from oven, allows cooling at room temperature and weighed. The product appearance was noted and 10gr sample was removed for analysis (Ref.:ADL26/2). The initial and aged samples were analysed by HPLC to determine the active ingredient. The analyses were carried out as separate GLP Technical Request PC265 for initial sample and PC269 for aged sample.
Results and discussion	

Project no.: 298/19 Date: from 11/05/06 to 27/05/06	***Initial sample***	***Aged sample***
Date entered into oven	11/05/05	--
Date removed from oven	--	25/05/05
Weighed on entry into oven	259.30g	--
Weighed on removal from oven	--	259.01g
Total weighed change	--	- 0,29g
Active Ingredient: Chloralose (% m/m)	100.44%	104.28% (+3.8%)
Appearance	Fine white powder	Fine white powder

	The appearance of the product does not change after ageing. Notice a loss of weigh after ageing due to evaporation of a small amount of residual volatile material from the product. The active ingredient was increased 3.8%.
Conclusion	Chloralose is stable on ageing at 54ºC for two weeks, which indicate a provisional shelf life of two years. Storage stability data generated from accelerated tests will only support provisional approval.
Reliability	2
Acceptability	Acceptable
Remarks	Full two year shelf life study is being conducted for Alphachloralose. Applicant will reported the study results when applying authorization of the biocidal product. 1.1 and 1.2.1 – Confidential data information

Section A4.1	**Analytical Methods for Detection and Identification**		
Annex Point IIA, IV.4.1	Purity of Active Ingredient		
	1. REFERENCE		Official use only
1.1	**Reference**	[redacted] [redacted] Applicant's reference number ALPHCHL 313	X X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Study 1. [redacted] Study 2: [redacted]	X X
1.2.2			
1.2.3	Criteria for data protection	Data submitted to MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
	2.		X
2.1			
2.2			
2.3			
	3. MATERIALS AND METHODS		
3.1	**Preliminary Treatment**		
3.1.1.	Enrichment	A stock solution was prepared containing 1.0 mg/ml (nominal) Alphachloralose in 10 ml methanol. A second stock solution of 1.0 mg/ml (nominal) Betachloralose in 10 ml methanol was also prepared.	X
3.1.2	Cleanup	Not carried out.	
3.2	**Detection**		
3.2.1	Separation method	HPLC	X
3.2.2	Detector	Waters 996 Photodiode Array Detector. Wavelength range monitored: 190 nm – 330 nm.	
3.2.3	Standards	External Alphachloralose reference sample.	X
3.2.4	Interfering substances	None.	
3.3	**Linearity**		
3.3.1	Calibration range	Alphachloralose: 0.10, 0.25, 0.50, 1.25 and 2.50 mg/ml Betachloralose: 0.10, 0.20, 0.30, 0.40 and 0.50 mg/ml	
3.3.2	Number of measurements	5	

Section A4.1		**Analytical Methods for Detection and Identification**	
Annex Point IIA, IV.4.1		Purity of Active Ingredient	
3.3.3	Linearity	All calibration curves has a correlation coefficient r^2 of 0.992 or greater. Alphachloralose: $r^2 = 0.998944$ Betachloralose: $r^2 = 0.992165$	X
3.4	**Specificity: interfering substances**	None.	X
3.5	**Recovery rates at different levels**	For Alphachloralose: Replicate 1: 97.61 % recovery. Replicate 2: 98.15% recovery. Replicate 3: 97.41% recovery. Replicate 4: 97.19% recovery. Replicate 5: 96.18% recovery. Average recovery: 97.31% For Betachloralose Replicate 1: 92.6 % recovery. Replicate 2: 92.76 % recovery. Replicate 3: 91.30 % recovery. Replicate 4: 90.24 % recovery. Replicate 5: 93.66 % recovery. Average recovery: 92.11%	X
3.5.1	Relative standard deviation	Alphachloralose: 0.75% Betachloralose: 1.46 %	
3.6	**Limit of determination**	The procedure used to determine the LOQ is given in 4.1 Materials and Methods (below). The LOQ determination was performed in the absence of matrix, since if the matrix was present, the actual concentration would be greater than 5%. The concentrations of each analyte used was equivalent, based on the method validated in study PGD-143 to a concentration in the technical material of 5% w/w. The HPLC conditions used were identical to those used in study PGD-143. Alphachloralose and Betachloralose peak heights were quantified at 203 nm. The % RSDs from the LOQ determination are as follows: % RSD determined Alphachloralose 1.91 Betachloralose 1.96 The % RSD determined is slightly better than the expected % RSD for alphachloralose and betachloralose. The data presented show that an LOQ of 5% w/w has been established for the determination of alphachloralose and betachloralose in technical material.	
3.7	**Precision**		
3.7.1	Repeatability	For Alphachloralose: Replicate 1: 85.02 % w/w Replicate 2: 85.49 % w/w Replicate 3: 84.84 % w/w Replicate 4: 84.65 % w/w Replicate 5: 83.77 % w/w Average: 84.75% w/w For Betachloralose Replicate 1: 11.39 % w/w Replicate 2: 11.41 % w/w	X

Section A4.1		Analytical Methods for Detection and Identification	
Annex Point IIA, IV.4.1		Purity of Active Ingredient	
		Replicate 3: 11.23 % w/w Replicate 4: 11.10 % w/w Replicate 5: 11.52 % w/w Average: 11.33 % w/w	
3.7.2	Independent laboratory validation	Not reported.	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	[redacted]	X
4.2	**Conclusion**	The data presented show that the method for analysis of Alphachloralose in technical material has been validated on the basis of linearity, accuracy, precision, specificity and LOQ.	
4.2.1	Reliability	1	
4.2.2	Deficiencies	None.	

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date September 2007

Materials and Methods Applicants version acceptable, but the following information also needs to be included:

2 The purpose of the study was to satisfy the requirements of Chapter 2 (Common Core Data Set for Active Substances and Biocidal Products) of the 'Technical Guidance Document in Support of the Directive 98/8/EC Concerning the Placing of Biocidal products on the Market'

3.1.1 Calibration solutions for the determination of linearity, accuracy and precision were prepared from the described stock solutions. No enrichment procedure was performed.

3.2.1 Analytical column: Prodigy ODS3 100 Å, 250 mm × 3.0 mm packed 5 µm from Phenomenex.
Mobile phase: Isocratic with Methanol:Water (30:70) at 0.45 mL/minute.

Section A4.1	**Analytical Methods for Detection and Identification**
Annex Point IIA, IV.4.1	Purity of Active Ingredient
Materials and Methods (continued)	3.2.3 Three reference materials were used: alphachloralose reference material (90.0%) and betachloralose reference material (94.5%) for determination of linearity, and chloralose reference mixture (87.1% alphachloralose + 12.3% betachloralose) for determination of accuracy and precision. Aldicarb sulfoxide was used as internal standard for LOQ determination. 3.3.3 Linearity covered the range that alphachloralose and betachloralose are present in the technical grade active ingredient. 3.4 For both analytes present in the technical material, the contribution from interferences was less than 3% of the total peak area measured. 3.5 Accuracy was determined at the level that alphachloralose and betachloralose are present in the technical grade active ingredient. 3.7.1 Relative standard deviations obtained (0.75% for alphachloralose and 1.46% for betachloralose) were lower than those expected from the modified Horwitz equation (1.37% for alphachloralose and 1.86% for betachloralose). Precision was determined at the level that alphachloralose and betachloralose are present in the technical grade active ingredient
Results and discussion	4.1 "peak height" should be changed to "peak area"
Conclusion	Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	Key study 1.1 and 1.2.1 – Confidential data information

Section 4.2 **Annex Point IIA, IV.4.2(a)**	**Analytical Methods for Detection and Identification in Environmental Media: a) Soil**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [ ] **Limited exposure** [✓] **Other justification** [ ]		
Detailed justification:	It is not necessary to submit an analytical method to detect and identify Alphachloralose in soil, due to prerequisites fulfilled on limited exposure and toxicity profile. This is because: a. Alphachloralose-containing biocides are intended for indoor use only. Environmental exposures are greatly reduced by the fact it cannot be used outdoors. It is acknowledged that the indoor use only restriction does not eliminate environmental exposure completely e.g. from rodent urine, faeces and carcasses of animals that have taken the bait and moved outdoors, however the metabolism of Alphachloralose is fast, and immobilisation of animals occurs shortly after bait consumption. Document III B, section 5.10, shows rapid mortality following bait take and indicates death will occur within 24 hours. "Reference 1", referred to in section 7.2.2.4 of this document (Document IIIA), indicates that the first signs of narcosis usually sets in within 15-30 minutes of ingestion and that death may occur within 3 hours. It is expected that the ADME study required in Document IIIA, section 6.2, will confirm that immobilisation occurs shortly after bait consumption, thus minimising exposure to the external environment. b. Alphachloralose-containing biocides are not intended for direct application to the environment e.g. by spraying, or placement directly onto the ground or soil. Alphachloralose containing biocides are restricted for use indoors, in tamper resistant baits boxes (or similar). The use of closed bait stations (such as the tamper resistant bait box) not only minimises the risk of release directly to the environment, but it also reduces the potential for primary poisoning of non-target species including cats, dogs and children. c. Notwithstanding the above, there is potential for exposure to the environment as a result of disposal of waste bait containing Alphachloralose, and disposal of rodent bodies which may contain Alphachloralose. The risk to the environment from the act of disposal is considered to be insignificant. This is because the quantity of Alphachloralose being disposed of, compared to the volume of total waste is minute. The total estimated use of Alphachloralose across the whole of the EU is < 0.00008 % of the total waste generated and sent to landfill in the UK alone. This means that any Alphachloralose that is sent for landfill is massively diluted by the large volume of (Continued……)	

Section 4.2 **Annex Point IIA, IV.4.2(a)**	**Analytical Methods for Detection and Identification in Environmental Media: a) Soil**
Detailed justification: (Continued)	municipal waste continually entering landfill sites in the UK. The data available on the environmental toxicity of Alphachloralose, including adsorption/desorption in soil (as given in Document IIII, Section 7) shows that this volume is extremely unlikely to cause any adverse effect to the environment, and as such requires no further investigation. *Refer to "Reference 2" in section 7.2.2.4 of this document, (Document IIIA) and Document IIIB Section 7.1 for more details of the scientific reasoning which supports this statement.* Not withstanding the above scientific reasoning, a method for determination of Alphachloralose residues in soil has been submitted (as reference 4.2/03). This method will require validation before use.
Undertaking of intended data submission [ ]	Not applicable.
	Evaluation by Competent Authority
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	The above mentioned reference A4.2/03 describes the experimental steps undertaken to determine alphachloralose residues from a soil sample. However, no validation data (specificity, linearity, accuracy, precision and LOQ) is included.
Conclusion	Applicant's justification is not acceptable. An analytical method for identification and detection of alphachloralose in soil must be submitted, for monitoring purposes in case of an accident.
Remarks	No remarks.

Section 4.2 **Annex Point IIA, IV.4.2(a)**	**Analytical Methods for Detection and Identification in Environmental Media: a) Soil**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [x]	
Detailed justification:		
Undertaking of intended data submission [✓]	March 2005	
	Evaluation by Competent Authority	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	June 2005	
Evaluation of applicant's justification	Applicant's justification adopted.	
Conclusion	An analytical method for detection and identification of alphachloralose in soil was submitted. In addition, a new study concerning detection and identification of betachloralose in soil was requested.	
Remarks	No remarks	

Section A4.2
Annex Point IIA, IV.4.2(a)

Analytical Methods for Detection and Identification (Soil)

Purity of Active Ingredient

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] Applicant's reference number ALPHCHL 268	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	X
1.2.2			
1.2.3	Criteria for data protection	Data submitted to MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
		2.	X
2.1			
2.2			
2.3			
		3. MATERIALS AND METHODS	
3.1	**Preliminary Treatment**		
3.1.1.	Enrichment	Extraction from fortified soil with acetone followed by liquid/liquid partition in dichloromethane. The extracts were evaporated to dryness prior to derivatisation with Tri-Sil Z. Extracts were reconstituted in hexane then analysed.	X
3.1.2	Cleanup	Not carried out.	
3.2	**Detection**		
3.2.1	Separation method	GC-MS	X
3.2.2	Detector	Thermoquest Finnigan GCQ Ion scan range: full scan between m/z 140 – 430	
3.2.3	Standards	Alphachloralose reference standard material (CSL LIMS reference number 2628, Lot number 10406) was obtained from a commercial source.	X
3.2.4	Interfering	None.	

Concentration of alphachloralose standard solution (µg/mL)	Volume (mL) taken from standard solution for derivatisation	Concentration of derivatised alphachloralose (µg/mL)
1.5	1	1
7.5	1	0.5
1.5	1	0.1
0.75	1	0.05
0.3	1	0.02

Section A4.2 **Annex Point IIA, IV.4.2(a)**		**Analytical Methods for Detection and Identification (Soil)** Purity of Active Ingredient	
	substances		
3.3	**Linearity**		
3.3.1	Calibration range	Alphachloralose: 0.02, 0.05, 0.1, 0.5,1.0 µg/mL	
3.3.2	Number of measurements	10	X
3.3.3	Linearity	Alphachloralose: $r^2 = 0.9962$	
3.4	**Specificity: interfering substances**	None.	X
3.5	**Recovery rates at different levels**	For Alphachloralose at Fortification level 0.05 mg/kg: Replicate 1: 92.2 % recovery. Replicate 2: 81.6% recovery. Replicate 3: 84.1% recovery. Replicate 4: 87.7% recovery. Replicate 5: 82.4% recovery. Average recovery: 85.6% For Alphachloralose at Fortification level 0.5 mg/kg: Replicate 1: 71.3 % recovery. Replicate 2: 78.8 % recovery. Replicate 3: 76.1 % recovery. Replicate 4: 51.9 % recovery. Replicate 5: 73.0 % recovery. Average recovery: 70.2%	
3.5.1	Relative standard deviation	5.1% RSD at 0.05mg/kg fortification. 15.2% RSD at 0.5mg/kg fortification.	
3.6	**Limit of determination**	Method was validated at the LOQ of 0.05mg/kg	
3.7	**Precision**		
3.7.1	Repeatability	%RSD (5.1% and 15.2%)	
3.7.2	Independent laboratory validation	Not reported.	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	[redacted]	
4.2	**Conclusion**	The data presented in the report demonstrate that alphachloralose can successfully be determined in soil. Validation data has been provided by the analysis of fortified samples and by comparison with unfortified samples.	
4.2.1	Reliability	1	
4.2.2	Deficiencies	None.	

Section A4.2 **Annex Point IIA, IV.4.2(a)**	**Analytical Methods for Detection and Identification (Soil)** Purity of Active Ingredient

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2007
Materials and Methods	Applicants version acceptable, but the following information also needs to be included: 2. The objective of this study was to provide validation data for a method to determine alphachloralose in LUFA-Speyer 2.2 soil in order to satisfy the requirements of SANCO/825/00 rev. 6. 3.1.1 Derivatisation was necessary since alphachloralose is not sufficiently volatile to be amenable to gas chromatography unless the volatility is increased. In this study, this was achieved by formation of trimethylsilyl derivatives of –OH groups to reduce inter-molecular hydrogen bonding. 3.2.1 Analytical column: SGE, 30 m (nominal) × 0.25 mm ID, 0.25 µm film thickness BPX5 (non polar). Mobile phase: Helium at constant linear velocity of 40 cm/s. The full scan mass spectrum was monitored from m/z 140-430 i.e. 290 fragment ions were monitored. This allowed the full mass spectrum to be used for confirmation. Confirmation is thus achieved by comparing the mass spectrum of a calibration solution with that from a fortified soil sample. 3.2.3 Purity of alphachloralose reference material: 90.0% Standard soil: LUFA-Speyer 2.2 3.3.2 Five different concentrations through bracketed calibration. 3.4 The response in the controls was < 30% of the LOQ in the region of the chromatograms corresponding to the retention time of derivatised alphachloralose (10.3 min approx.).
Conclusion	Applicant's version adopted. Nevertheless, the company was asked to submit an analytical method for the detection and identification of betachloralose in soil.
Reliability	1
Acceptability	Acceptable
Remarks	Key study. 1.1 and 1.2.1 – Confidential data information

Section A4.2 Analytical Methods for Detection and Identification (Soil)
Annex Point IIA, IV.4.2(a) Determination of Betachloralose in Soil

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] Applicant's reference number ALPHCHL 308	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	X
1.2.2			
1.2.3	Criteria for data protection	Data submitted to MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
		2.	X
2.1			
2.2			
2.3			
		3. MATERIALS AND METHODS	
3.1	**Preliminary Treatment**		
3.1.1.	Enrichment	Extraction from fortified soil with acetone followed by liquid/liquid partition in dichloromethane. The extracts were evaporated to dryness prior to derivatisation with Tri-Sil Z. Extracts were reconstituted in hexane then analysed.	X
3.1.2	Cleanup	Not carried out.	
3.2	**Detection**		
3.2.1	Separation method	GC-MS	X
3.2.2	Detector	Thermoquest Finnigan GCQ Ion scan range: full scan between m/z 140 – 430	
3.2.3	Standards	Betachloralose reference standard material (CSL LIMS reference number 2655, Lot number 40128) was obtained from a commercial source.	X

Concentration of betachloralose standard solution (µg/mL)	Volume (mL) taken from standard solution for derivatisation	Concentration of derivatised betachloralose (µg/mL)
1.5	1	1
7.5	1	0.5
1.5	1	0.1
0.75	1	0.05
0.3	1	0.02

Section A4.2 **Annex Point IIA, IV.4.2(a)**	**Analytical Methods for Detection and Identification (Soil)** Determination of Betachloralose in Soil	
3.2.4 Interfering substances	None.	
3.3 Linearity		
3.3.1 Calibration range	Nominal concentration range for derivatised betachloralose in calibration standards: 0.02 - 1.0 µg/mL. The chromatographic response to betachloralose was shown to be linear over the tested range.	
3.3.2 Number of measurements	10	X
3.3.3 Linearity	Betachloralose: $r^2 = 0.9956$	
3.4 Specificity: interfering substances	None.	X
3.5 Recovery rates at different levels	For Betachloralose at Fortification level 0.05 mg/kg: Replicate 1: 83.6 % recovery. Replicate 2: 69.2 % recovery. Replicate 3: 78.7 % recovery. Replicate 4: 86.8 % recovery. Replicate 5: 76.3 % recovery. Average recovery: 78.9% For Betachloralose at Fortification level 0.5 mg/kg: Replicate 1: 70.1 % recovery. Replicate 2: 62.2 % recovery. Replicate 3: 65.4 % recovery. Replicate 4: 75.1 % recovery. Replicate 5: 78.5 % recovery. Average recovery: 70.3%	
3.5.1 Relative standard deviation	8.6 % RSD at 0.05mg/kg fortification. 9.5 % RSD at 0.5mg/kg fortification.	
3.6 Limit of determination	Method was validated at the LOQ of 0.05mg/kg	
3.7 Precision		
4.2.2 Repeatability	%RSD (8.6% and 9.5%)	
3.7.2 Independent laboratory validation	Not reported.	
	4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	[redacted]	
4.2 Conclusion	The data presented in the report demonstrate that betachloralose can successfully be determined in soil. Validation data has been provided by the analysis of fortified samples and by comparison with unfortified samples.	
4.2.1 Reliability	1	
4.2.2 Deficiencies	None.	

Section A4.2 **Annex Point IIA, IV.4.2(a)**	**Analytical Methods for Detection and Identification (Soil)** Determination of Betachloralose in Soil

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2007
Materials and Methods	Applicants version acceptable, but the following information also needs to be included: 2. The objective of this study was to provide validation data for a method to determine betachloralose in LUFA-Speyer 2.2 soil in order to satisfy the requirements of SANCO/825/00 rev. 7. 3.1.1 Derivatisation was necessary since betachloralose is not sufficiently volatile to be amenable to gas chromatography unless the volatility is increased. In this study, this was achieved by formation of trimethylsilyl derivatives of –OH groups to reduce inter-molecular hydrogen bonding. 3.2.1 Analytical column: SGE, 30 m (nominal) × 0.25 mm ID, 0.25 µm film thickness BPX5 (non polar). Mobile phase: Helium at constant linear velocity of 40 cm/s. The full scan mass spectrum was monitored from m/z 140-430 i.e. 290 fragment ions were monitored. This allowed the full mass spectrum to be used for confirmation. Confirmation is thus achieved by comparing the mass spectrum of a calibration solution with that from a fortified soil sample. 3.2.3 Purity of betachloralose reference material: 94.5% Standard soil: LUFA-Speyer 2.2 3.3.2 Five different concentrations through bracketed calibration. 3.4 The response in the controls was < 30% of the LOQ in the region of the chromatograms corresponding to the retention time of derivatised betachloralose (10.03 min approx.).
Conclusion	Applicant's version adopted.
Reliability	1
Acceptability	Acceptable
Remarks	Key study. 1.1 and 1.2.1 – Confidential data information

Section 4.2 **Annex Point IIA, IV.4.2(b)**	**Analytical Methods for Detection and Identification in Environmental Media: b) Air**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [x]	**Other justification** [ ]	
Detailed justification:	A method for detection in air needs to be submitted if the active substance is volatile (vapour pressure ≥0.01 Pa) or sprayed or occurrence in air is otherwise likely. The vapour pressure of alphachloralose is 0.00883Pa. It is not sprayed, it is formulated into a non volatile solid and there is no reason to think occurrence in air is possible.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authority	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2004	
Evaluation of applicant's justification	Applicant's justification adopted.	
Conclusion	Applicant's justification is acceptable.	
Remarks	No remarks	

Section A4.2 **Annex Point IIA, IV.4.2(c)**	**Analytical Methods for Detection and Identification in Environmental Media: (c) Water**	
	1. REFERENCE	Official use only
1.1 **Reference**	1. [redacted] Applicant's reference number ALPHCHL 238	X
	2. [redacted] Applicants reference number ALPHCHL227	X
1.2 **Data protection**	Yes	
1.2.1 Data owner	[redacted]	X
1.2.2		
1.2.3 Criteria for data protection	Data submitted to MS after 13 May 2000 on existing active substance for the purpose of it's entry into Annex I.	
	2.	
2.1		
2.2		
2.3		
	3. MATERIALS AND METHODS	
3.1 **Preliminary Treatment**		
3.1.1. Enrichment	A quantity of 25.55 mg Alphachloralose reference standard (97.83% purity) was weighed into a volumetric flask of 25 ml capacity, dissolved in 5ml n-octanol (AR grade) and the volume was made up to the mark with no-octanol. The concentration of the solution was 1000 ppm.	
3.1.2 Cleanup	Not carried out.	
3.2 **Detection**		
3.2.1 Separation method	GLC	
3.2.2 Detector	FID on a column DB-5, 30 m x 0.25 mm (i.d.) and 0.25 µm film thickness.	
3.2.3 Standards	External Alphachloralose reference sample.	
3.2.4 Interfering substances	None.	
3.3 **Linearity**		
3.3.1 Calibration range	200, 400, 600, 800 and 1000 ppm.	
3.3.2 Number of	5 (Note all measurements carried out in duplicate)	

Section A4.2 **Annex Point IIA, IV.4.2(c)**	**Analytical Methods for Detection and Identification in Environmental Media: (c) Water**

	measurements	
3.3.3	Linearity	The correlation coefficient r was 0.999.
3.4	**Specifity: interferring substances**	None.
3.5	**Recovery rates at different levels**	For Alphachloralose: Replicate 1: 93.65 % recovery. Replicate 2: 95.09 % recovery. Replicate 3: 95.34 % recovery. Average recovery: 94.69%
3.5.1	Relative standard deviation	0.22
3.6	**Limit of determination**	The limit of determination of the method for Alphachloralose was determined by analysing the standard solution of Alphachloralose at the concentration of 200, 100, 50 and 25 ppm (prepared by serial dilution of the stock solution prepared for linearity). The lowest detectable limit of Alphachloralose by the GLC method was ≥ 50 ppm.
3.7	**Precision**	
3.7.1	Repeatability	For Alphachloralose: Replicate 1: 97.16 % w/w Replicate 2: 97.11 % w/w Replicate 3: 97.12 % w/w Replicate 4: 97.56 % w/w Replicate 5: 97.08 % w/w Replicate 6: 97.60 % w/w Replicate 7: 97.42 % w/w Replicate 8: 97.53 % w/w Replicate 9: 97.16 % w/w Replicate 10: 97.15 % w/w Average: 97.29 % w/w
3.7.2	Independent laboratory validation	Not reported.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	[redacted]
4.2	**Conclusion**	The data presented show that the method for analysis of Alphachloralose in water has been validated on the basis of linearity, accuracy, precision, specificity and LOQ.
4.2.1	Reliability	1
4.2.2	Deficiencies	None.

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2005
Materials and Methods	Applicant's version is not acceptable. Please refer to the chapter "Conclusion".

Section A4.2 **Annex Point IIA, IV.4.2(c)**	**Analytical Methods for Detection and Identification in Environmental Media: (c) Water**
Conclusion	Applicant's version not adopted. All data mentioned by the company do not refer to detection and identification of alphachloralose after extraction from samples of fortified water, but to the analysis of alphachloralose reference standard and alphachloralose sample in n-octanol. The study includes an accuracy determination via fortification of a water sample with test substance at one level (97 mg/mL). The recovery was found to be 95.09%. However, given the requirements for method validation, a new study has to be performed (Doc IIIA_A4.2c-2).
Reliability	4
Acceptability	Not acceptable
Remarks	1.1 and 1.2.1 – Confidential data information

Section A4.2 **Annex Point IIA, IV.4.2(c)**		**Analytical Methods for Detection and Identification in Environmental Media: (c) Water**	Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] Applicant's reference number ALPHCHL 309	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	X
1.2.2			
1.2.3	Criteria for data protection	Data submitted to MS after 13 May 2000 on existing active substance for the purpose of it's entry into Annex I.	
		2.	X
2.1			
2.2			
2.3			
		3. MATERIALS AND METHODS	
3.1	**Preliminary Treatment**		
3.1.1.	Enrichment	Note that the determination below was repeated for both alphachloralose and betachloralose. Residues of the active ingredient were extracted from water by retention on a solid phase extraction cartridge and elution with acetone after drying. The extract is dissolved in HPLC mobile phase followed by determination by LC/MS/MS with multiple reaction monitoring (MRM). Quantities used: LOQ = 20 µL of a 0.1 µL/mL solution 20 x LOQ = 200 µL of a 0.1 µL/mL solution	X
3.1.2	Cleanup	Not carried out.	
3.2	**Detection**		
3.2.1	Separation method	LC/MS/MS	X
3.2.2	Detector	Waters Quatrro Premier LC/MS/MS with multiple reaction monitoring (MRM) using a ThermoHypersil Gold 150 mm x 2.1 mm, 3 µm column.	
3.2.3	Standards	External Alphachloralose reference sample. External Betachloralose reference sample.	X
3.2.4	Interfering substances	None.	
3.3	**Linearity**		
3.3.1	Calibration range	For Alphachloralose: 0.008 to 0.025 µg/mL For Betachloralose: 0.008 to 0.025 µg/mL	

Section A4.2 **Annex Point IIA, IV.4.2(c)**		**Analytical Methods for Detection and Identification in Environmental Media: (c) Water**	
3.3.2	Number of measurements	For Alphachloralose: At LOQ: 5 At 10 x LOQ: 5 For Betachloralose: At LOQ: 5 At 10 x LOQ: 5	X
3.3.3	Linearity	For Alphachloralose: The correlation coefficient r was 0.99. For Betachloralose: The correlation coefficient r was 0.99.	X
3.4	**Specifity: interferring substances**	None.	X
3.5	**Recovery rates at different levels**	See table below	X
3.5.1	Relative standard deviation	For Alphachloralose: 6.9% For Betachloralose: 4.6%	
3.6	**Limit of determination**	For Alphachloralose: 0.1 µg/L in drinking water and surface water. For Betachloralose: 0.1 µg/L in drinking water and surface water.	X
3.7	**Precision**		
3.7.1	Repeatability	See text below	

AI	Substrate	Spike level µg/L	Level Mean (%)	Level RSD (%)	Substrate Mean (%)	Substrate RSD (%)	Overall Mean (%)	Overall RSD (%)
Alpha	Drinking water	0.1	88	3.4	94	6.6	95	6.9
		1.0	99	2.9				
	Surface water	0.1	90	3.9	96	7 2		
		1.0	102	3.6				
Beta	Drinking water	0.1	87	2.8	90	4 5	91	4.6
		1.0	93	3.7				
	Surface water	0.1	92	5.8	93	4.4		
		1.0	93	2.9				

For Alphachloralose in drinking water. Fortification 0.1 µg/L
Replicate 1: 84.0 % w/w
Replicate 2: 90.0 % w/w
Replicate 3: 90.0 % w/w
Replicate 4: 91.0 % w/w
Replicate 5: 87.0 % w/w
Mean recovery: 88% (Std Dev 3.0), Number tests 5, % RSD 3.4

For Alphachloralose in drinking water. Fortification 1.0 µg/L
Replicate 1: 100.0 % w/w
Replicate 2: 103.0 % w/w
Replicate 3: 97.0 % w/w
Replicate 4: 95.0 % w/w
Replicate 5: 99.0 % w/w
Mean recovery: 99 % (Std Dev 2.9), Number tests 5, % RSD 2.9

For Alphachloralose in surface water. Fortification 0.1 µg/L
Replicate 1: 87.0 % w/w
Replicate 2: 95.0 % w/w
Replicate 3: 93.0 % w/w
Replicate 4: 89.0 % w/w
Replicate 5: 87.0 % w/w
Mean recovery: 90% (Std Dev 3.5), Number tests 5, % RSD 3.9

Section A4.2 **Annex Point IIA, IV.4.2(c)**	**Analytical Methods for Detection and Identification in Environmental Media: (c) Water**

For Alphachloralose in surface water. Fortification 1.0 µg/L
Replicate 1: 105.0 % w/w
Replicate 2: 99.0 % w/w
Replicate 3: 100.0 % w/w
Replicate 4: 98.0 % w/w
Replicate 5: 106.0 % w/w
Mean recovery: 102% (Std Dev 3.7), Number tests 5, % RSD 3.6

For Betachloralose in drinking water. Fortification 0.1 µg/L
Replicate 1: 86.0 % w/w
Replicate 2: 84.0 % w/w
Replicate 3: 90.0 % w/w
Replicate 4: 90.0 % w/w
Replicate 5: 87.0 % w/w
Mean recovery: 87% (Std Dev 2.5), Number tests 5, % RSD 2.8

For Betachloralose in drinking water. Fortification 1.0 µg/L
Replicate 1: 97.0 % w/w
Replicate 2: 95.0 % w/w
Replicate 3: 93.0 % w/w
Replicate 4: 88.0 % w/w
Replicate 5: 91.0 % w/w
Mean recovery: 93% (Std Dev 3.5), Number tests 5, % RSD 3.8

For Betachloralose in surface water. Fortification 0.1 µg/L
Replicate 1: 89.0 % w/w
Replicate 2: 98.0 % w/w
Replicate 3: 98.0 % w/w
Replicate 4: 91.0 % w/w
Replicate 5: 86.0 % w/w
Mean recovery: 92% (Std Dev 5.4), Number tests 5, % RSD 5.8

For Betachloralose in surface water. Fortification 1.0 µg/L
Replicate 1: 95.0 % w/w
Replicate 2: 97.0 % w/w
Replicate 3: 90.0 % w/w
Replicate 4: 91.0 % w/w
Replicate 5: 93.0 % w/w
Mean recovery: 93% (Std Dev 2.7), Number tests 5, % RSD 2.9

3.7.2 Independent laboratory validation

Not reported.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

[redacted]

The method validation covered the aspects namely limit of detection, linear dynamic range, precision and accuracy.

4.2 Conclusion

The data presented show that the method for analysis of determination of Alphachloralose and Betachloralose in surface water and drinking water has been validated on the basis of linearity, accuracy, precision, specificity and LOQ.

Section A4.2 **Annex Point IIA, IV.4.2(c)**	**Analytical Methods for Detection and Identification in Environmental Media: (c) Water**
4.2.1 Reliability	1
4.2.2 Deficiencies	None.

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

September 2007

Materials and Methods

Applicants version acceptable, but the following information also needs to be included:

2. The objective of this study was to provide validation data for a method to determine alphachloralose and betachloralose residues in drinking water and surface water in order to satisfy the requirements of SANCO/825/00 rev. 7.

3.1.1 Sampling sites for drinking water are Cambridge UK CB1 3QB and Thaxted UK CM6 2PY. Sampling sites for surface water are River Chelmer 51.9571ºN, 0.3354ºE and Tributary of River Stour 52.0455ºN, 0.4480ºE.

3.2.1 Mobile phase: MeOH:10 mM ammonium acetate (55:45)

3.2.3 Purity of alphachloralose reference material: 91.0%
Purity of betachloralose reference material: 94.5%

3.3.2 For linearity validation, single determinations at 5 concentrations were made, for each substance.

3.3.3 $r^2 > 0.99$ for both substances: 0.999 for alphachloralose and 0.997 for betachloralose.

3.4 There were no co-eluting components that interfered with the determination of both substances.

3.5 Mean recoveries correspond to 5 determinations at LOQ and five determinations at 10×LOQ, for each substance and substrate.

3.6 Both values correspond to the limits of quantification.

Conclusion

Applicant's version adopted.

Reliability

1

Acceptability

Acceptable

Remarks

Key study

1.1 and 1.2.1 – Confidential data information

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [ ] **Limited exposure** [x] **Other justification** [ ]		
Detailed justification:	The Technical Guidance Document in Support of the Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance for Data Requirements for Active Substances and Biocidal Products, Version 4.3.2 dated October 2000 states that an analytical method for detection of residues in animal and human body fluids and tissues is only required when the active substance is classified as toxic or highly toxic. Alphachloralose is not classified as such according to EC Directive 67/548/EEC, and therefore it is not necessary to submit an analytical method to detect alphachloralose residues in animal and human body fluid and tissues. Notwithstanding the above a method for the determination of alphachloralose residues in vertebrate tissues by GLC has been included. This method is included as reference A4.2/05.	X
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authority	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	March 2006	
Evaluation of applicant's justification	The above mentioned method included as reference A4.2/05 is described in an article, which main conclusions are: **Reference**: Odam, E.M., Wardall, H.P., Bailey, S., Findlay, E. (October 1984), Determination of Alphachloralose Residues in Vertebrate Tissues by Gas-Liquid Chromatography, Analyst Vol. 109: 1335-1338, Published, Applicant's reference number ALPHCHL 258 **Data Protection**: No data protection claimed **Abstract**: A method is described for the analysis of residues of narcotic α-chloralose in vertebrate tissues. Following solvent extraction and gel permeation clean-up, residues are converted into the trimethylsilyl (TMS) derivative for the analysis by gas-liquid chromatography. Recoveries are greater than 65% and the limit of determination is between 0.2 and 1.0 mg kg^{-1}, depending on the type of tissue analysed. Methods for the confirmation of α-chloralose residues using thin-layer chromatography of underivatised α-chloralose or mass spectometry of the chloralose – TMS derivative are described. **Experimental**: Gas-liquid chromatography – Pye Unicam 104 chromatograph, fitted with a 10 mCi ^{63}Ni electron-capture detector. Thin-layer chromatography plates – Silica gel G pre-coated, with a layer thickness of 250 μm.	

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

Evaluation of applicant's justification

(continued)

Results:

Table 1. Recovery of α-chloralose from spiked pigeon tissues

	α-Chloralose added		α-Chloralose recovered	
Tissue	mg kg^{-1}	µg	µg	%
Kidney	0.2	0.36	0.29	81
	1.0	1.8	1.75	97
	10.0	18.0	13.50	75
Muscle	0.2	1.0	0.7	70
	1.0	5.0	4.5	90
	10.0	50.0	42.5	85
Brain	0.2	0.4	0.28	69
	1.0	20.0	14.60	73
Liver	0.2	0.98	*	*
	1.0	4.80	2.46	72
	10.0	48.0	36.48	76

* - The limit of determination for pigeon liver is greater than 0.2 mg kg^{-1} because of interfering peaks.

The method of analysis has also been applied to tissues from pigeons that were fed α-chloralose, three at 130 mg kg^{-1} body mass and three at 260 mg kg^{-1} body mass.

Table 2. Tissues residues from pigeons fed with α-chloralose

			α-Chloralose residues found / mg kg^{-1}			
Pigeon	Dose / mg	Remarks	Muscle	Liver	Brain	Kidney
1	43.5	Died	33.9	36.9	44.1	75.0
2	39.5	Recovered	N.d.*	N.d.	N.d.	N.d.
3	42.6	Recovered	N.d.	N.d.	N.d.	N.d.
4	91.4	Died	36.1	40.0	21.8	76.7
5	82.6	Recovered	N.d.	N.d.	N.d.	N.d.
6	73.8	Died	35.7	31.3	20.0	82.0

* - N.d. – none detected; limit of determination – 0.2 mg kg^{-1}, except liver – 1.0 mg kg^{-1}

Discussion: The limit of determination in the brain, kidney and muscle is 0.2 mg kg^{-1} but in the liver, because of interference from co-extracted material, the limit is 1 mg kg^{-1}. The experimental data (Table 2) showed that pigeons that died contained residue levels well above the limits of determination for this method. An individual column clean-up can be used per sample, eliminating the possibility of cross-contamination. Residue levels greater than 2 mg kg^{-1} can be confirmed by the TLC method described.

Conclusion

Notwithstanding the above, RMS had researched for more information concerning this endpoint. Two articles are summarized below (annexes I and II). Focus is given to validation parameters.

Remarks

No remarks

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

Annex I

Reference

Hunter, K. *et al* (April 2004), Determination of chloralose residues in animal tissues by liquid chromatography-electrospray ionisation tandem mass spectrometry, Journal of Chromatography B, 805: 303-309

Abstract

A relatively rapid and specific method for the determination of chloralose in animal tissues by LCMSMS was developed. Isocratic reverse phase HPLC was used to introduce samples for electrospray negative ionisation tandem mass spectrometry. Methanol extracts were diluted to approximate the mobile phase composition, then filtered prior to analysis. Residues were identified by monitoring the multiple reaction monitoring (MRM) transitions of precursor ions mass:charge (*m/z*) 309 and 307 to a common *m/z* 161 product ion. Qualitative and quantitative confirmation data were acquired simultaneously by monitoring alternative MRM transitions. Calibration was linear over a working range of 0.025-1.3 µg/ml, and the limit of quantitation (LOQ) was 0.28 mg/kg for liver. The mean recovery was 88.5% from chicken muscle tissue fortified at 198-237 mg/kg, and ranged from 81.3 to 94.3% from liver tissue fortified at 1-52 mg/kg. The method is compared to a gas chromatography (GC) procedure previously employed.

Experimental

Extraction

LCMS: Tissue material was chopped and anhydrous sodium sulphate was added. The sample was homogenised in methanol, being the resulting extracted filtered. The residual material was re-homogenised in methanol. Digestive tract material was weighed and methanol was added. The mixture was placed in a ultrasonic bath. The resulting extract was filtered and made up with methanol.

GC-ECD: Liver tissue and digestive tract material were extracted essentially as described for LCMS, except that acetonitrile was used instead of methanol. Crude extracts were concentrated by rotary evaporation and made up to volume in methanol/water (approximately 1:9 v/v). An aliquot was applied to an Extrelut SPE cartridge and after equilibration, eluted with ethyl acetate. Clean up extracts were evaporated to dryness and the residual material derivatised with hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) in pyridine to form the TMS-adducts. Final solutions were made up in hexane.

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

Analysis

LCMS: Analytical column – Hypersil C_{18} BDS 3 μm (100 mm × 4.6 mm i.d.)
Mobile phase – methanol per 10 mM aqueous ammonium acetate solution ph 4.5 (55/45, v/v) at 0.45 ml/min.
Detector – Micromass Quatrro Ultima tandem mass spectrometer operated in electrospray negative ionisation mode (*m/z* 307 + *m/z* 309 → *m/z* 161)
Confirmation – LCMSMS (*m/z* 307 + *m/z* 309 → *m/z* 189)

GC-ECD: Analytical column – DB-608 capillary column (30 m × 0.32 mm i.d., 0.5 μm film thickness)
Mobile phase – nitrogen gas at 5 ml/min
Detector – Electron capture detector operated at 300ºC with a nitrogen make up gas at 30 ml/min.
Confirmation – GC-MS system.

Results and discussion

Chicken muscle tissue was chosen as a universal pesudo-matrix material for matrix-matched calibration standards because of the limited differences in ion enhancement effects betweens tissue types (<5%), and because of its ready availability. A linear calibration line was obtained over the range 0.025–1.3 μg/ml.

Recoveries of chloralose from fortified chicken liver and muscle tissues were acceptable. The mean recovery ranged from 81.3 to 94.3% for liver tissue, and from 87.2 to 88.5% for muscle tissue. These values compared favourably with those achieved using the in-house GC-ECD method (Table 1).

Table 1 – Recoveries of chloralose (Sigma Ltd., Poole, UK) from fortified tissues.

Chicken tissues	Fortification (mg/kg)	Percentage recovery		CV (%)	*n*
		Mean	Range		
Gas chromatography					
Muscle	200	81.3	72 – 110	11.6	23
Liver	25	80.2	73 – 91	6.4	16
Liver	5	75.9	70 – 88	8.0	9
LCMSMS					
Muscle	198 – 237	88.5	77 – 98	7.5	22
Muscle	29.3	87.2	73 – 102	10.7	10
Liver	52.4	84.1	80 – 90	4.4	6
Liver	29.3	81.3	72 – 93	7.1	16
Liver	22.0	85.9	82 – 89	3.1	6
Liver	5.0	92.4	85 – 107	7.0	13
Liver	1.0	94.3	88 – 102	5.9	6

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

The lowest effective concentration that could be reliably measured was assessed by testing decreasing chloralose concentration levels in fortified liver tissues (n = 6). The limit of quantitation was defined as the lowest concentration at which a minimum mean recovery of 65%, repeatability of ≤20%, and a signal to noise ratio ≥3 could be achieved. The LOQ was experimentally determined as 0.28 mg/kg for liver tissue, where a mean recovery of 68.4%, repeatability of 13.2%, and an average signal to noise ratio of 3.9 were achieved. At this level only the major alpha-chloralose component from the test substance used could be determined.

Intra-day and inter-day precisions were estimated from analyses of sets of liver tissues samples (n = 6), each fortified at one of four concentration levels, conducted in a single day (intra-) and over several days (inter-). The intra-batch precision varied from 2% at higher concentrations (20–50 mg/kg), to 5.1% at lower concentrations (1–5 mg/kg). The inter-batch precision ranged similarly according to concentration from 4.5 to 8.1%.

There has been no indication of interference from any matrix related components.

Conclusions

LC-MSMS can provide a simple and relatively quick analysis of the toxicant chloralose in animal tissues. The selectivity of the determination eliminates the need for any sample clean-up, and the sensitivity achievable is more than satisfactory for the diagnosis of poisoning (or to confirm exposure) in animals. Additional confirmatory data can be acquired simultaneously. There is scope for lowering the limit of determination, should this prove necessary, by adjustment of the sample dilution employed. Significant efficiency gains (≈65%) are made over the previous methodology employed for this purpose, by eliminating the need for clean-up, derivatisation, off-line confirmation, as well as time consuming steps involving solvent evaporation.

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

Annex II

Reference

Savin, S. *et al* (April 2003), ^{1}H NMR Spectroscopy and GC-MS Analysis of α-Chloralose. Application to Two Poisoning Cases, Journal of Analytical Toxicology, 27: 156-161

Abstract

α-Chloralose, a compound widely used as a rodenticide and in the control of bird pests, is readily available. Two cases of intentional poisoning are reported. Both patients became comatose and presented hypersialorrhea and myoclonal crises in the legs. They were discharged from hospital after several days. As clinical signs of α-chloralose poisoning lack specificity, anamnesis might be difficult, particularly in the case of delayed diagnosis. Toxicological analysis is therefore critical, and this article reports the investigation of serum and urine samples by gas chromatography–mass spectrometry (GC–MS) in the electronimpact mode, and by ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy. Non-hydrolyzed urinary samples and those hydrolyzed by β-glucuronidase were taken into consideration. After acetylation, GC–MS analysis was based on characteristic mass-to-charge ratio values of 272 for α-chloralose and 206 for β-hydroxyethyltheophylline, which was used as internal standard. Characterization of α-chloralose species by ^{1}H NMR spectroscopy was performed taking two parameters into account: chemical shift and coupling-constant values. Without any pretreatment, ^{1}H NMR spectroscopy revealed the presence of free (5.50 and 6.15 ppm) and conjugated forms of α-chloralose by characteristic resonances of H_1 and chloral-type protons, respectively. Quantitative analysis was performed by relative integration of peak areas. Serum α-chloralose showed concentrations below the quantitation limit of both methods. In urine samples, the free chemical species rapidly decreased. GC–MS analysis revealed the predominence of conjugation after a β-glucuronidase hydrolysis step. ^{1}H NMR analysis directly showed that on admission of the first patient, average urinary concentrations were 1.73 mmol/L (535 mg/L) for the free form and 13.72 and 6.25 mmol/L for the two conjugated forms. A later enzymatic treatment confirmed the total concentration of α-chloralose chemical species. Analysis of α-chloralose in urine by either GC–MS or 1H NMR spectroscopy methods proved to be comparable.

Experimental

Apparatus

^{1}H NMR: spectra recorded on a Brucker DPX MHz spectrometer at ambient probe temperature.

GC-MS: Analytical column – Thermoquest GC8000^{TOP}/pressure controller equipped with a fused-silica EC 5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness)

Mobile phase – Helium at a constant flow rate of 1 ml/min.

Detector – quadripolar Automass II MS (Thermo-Finnigan)

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

Sample collection

In the urgent toxicological context of the cases presented in this study, no specimens were specifically collected, and procedures were therefore in accordance with the revised Helsinki Declaration of 1983. In the first case, two serum and two urine samples were obtained on hospital admission and 6 h later. In the second case, serum and urine samples were collected on admittance to the reanimation unit, but the delay between poisoning and hospitalization could only be estimated at about 11 h. Three urine samples were also collected 24, 36, and 48 h later.

Sample preparation

GC-MS: To 200 μl of serum or urine, native or hydrolyzed, was added 100 μl of internal standard solution (β-hydroxyethyltheophylline 150 mg/L solution in methanol). The sample was then extracted twice with 6 ml of a diethyl ether/chloroform (1:1, v/v) solution. The combined organic extracts were centrifuged and evaporated to dryness under nitrogen. The residue was derivatized by acetylation and finally dissolved into 100 μl of ethyl acetate. Calibration was performed on standard solutions up to 200 mg/L.

^{1}H NMR: 500 μl of standard solutions or biological samples was directly introduced into a 5 mm diameter NMR tube.

Results and discussion

GC-MS analysis

Calibration curves were performed on spiked urines or serum. The ratio of signal intensity relative to *m/z* 272 for α-chloralose (Prolabo, Fontenay-sousbois, France) to *m/z* 206 for internal standard was found to be a linear function of α-chloralose concentration in the range 5 to 200 mg/L. Correlation coefficients were r = 0.9992 and r = 0.9960 for urine and serum, respectively. To check if the hydrolysis process with β-glucuronidase could cause disturbances, the same protocol was run on urine samples containing the enzyme. The resulting correlation coefficient was r = 0.9988 meaning that the hydrolysis step did not affect the linearity criterion.

The detection limit was defined as three times the background signal and calculated as 1.5 mg/L. The quantitation limit was calculated to be 5 mg/L. Within-run and between-run precisions were determined by testing replicates (n = 5) of α-chloralose solutions at 50 mg/L. The corresponding relative standard deviations were 4.2 and 3.7% respectively.

α-Chloralose concentrations determined by GC–MS in the two patients' serum and urine samples are shown in Table I. The levels of xenobiotic concentration in all serum samples were below the quantitation limit, whereas urinary concentrations were relatively high. This agrees with reported data relative to massive urinary elimination. After hydrolysis with β-glucuronidase, comparison of the chemical species for the two cases revealed that the conjugated form is predominant, at over one order of magnitude.

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

^{1}H NMR analysis

For spiked control urine samples, a significant linear relationship was observed from 25 to 2000 mg/L α-chloralose (Prolabo, Fontenay-sousbois, France) concentrations, with a correlation coefficient r = 0.9999. No disturbance in the linear relationship due to the urine matrix was observed, given the high specificity of ^{1}H NMR parameters.

The quantitation limit, with acceptable signal-to-noise ratios, was found to be 80 µmol/L (25 mg/L), and the detection limit was around 30 µmol/L (9 mg/L). Intraday (n = 5) and interday (n = 5) precisions were obtained on control urine samples spiked at 500 mg α-chloralose/L. The mean corresponding variation coefficients were 3.5 and 2.8%, respectively.

Table I collects the results obtained on urine and serum samples from the two poisoned patients by ^{1}H NMR spectroscopy. α-Chloralose could not be detected in serum samples, concentrations being below the quantitation limit of both methods. Urinary concentrations determined by both methods were found to be quite similar. Conjugated species appeared as the predominant form of α-chloralose, as expected for this polyhydroxylated compound.

Table I - α-Chloralose concentrations (mg/L) determined by GC-MS and ^{1}H NMR spectroscopy in serum and urine from two poisoned patients (mean ± SD of triplicates)

Case	Time	Sample	GC-MS	^{1}H NMR
1	on admission	serum	NQ	ND
		urine – before hydrolysis	303 ± 7	535 ± 19
		– after hydrolisis	7191 ± 70	6497 ± 62
	+ 6 hours	serum	NQ	ND
		urine – before hydrolysis	131 ± 4	124 ± 5
		– after hydrolisis	1318 ± 14	1223 ± 14
2	on admission	serum	NQ	ND
		urine – before hydrolysis	67 ± 3	50 ± 4
		– after hydrolisis	758 ±14	954 ± 9
	+ 24 hours	urine – before hydrolysis	NQ	ND
		– after hydrolisis	139 ± 4	102 ± 6
	+ 36 hours	urine – before hydrolysis	ND	ND
		– after hydrolisis	7 ± 0.6	ND
	+ 48 hours	urine – before hydrolysis	ND	ND
		– after hydrolisis	6 ± 0.7	ND

NQ – detected but not quantitated, ND – not detected

Section 4.2 **Annex Point IIA, IV.4.2(d)**	**Analytical Methods for Detection and Identification in Environmental Media: d) Animal and Human Body Fluids and Tissues**

Conclusion

^{1}H NMR spectroscopy of biological fluids proved to be a convenient and very promising tool to assess several compounds in one analysis, not only of markedly different chemical structures, but also, as for α-chloralose, of free and conjugated forms.

Section 4.3 **Annex Point IIIA, IV.1**	**Analytical methods, including recovery rates and the limits of determination for residues in/on food or feedstuffs and other products where relevant**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [ ] **Limited exposure** [x] **Other justification** [ ]		
Detailed justification:	The Technical Guidance Document in Support of the Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance for Data Requirements for Active Substances and Biocidal Products, Version 4.3.2 dated October 2000 states that analytical methods for the determination of the active substance, and for residues thereof, in/on food or feeding stuffs and other products should only be submitted if the active substance (or the material treated with it) is to be used in a manner such that it would come into contact with food or feeding stuffs, or will be used in soils which are intended for agriculture or horticultural use. It is not necessary to submit an analytical method for the determination of alphachloralose in/on food or feeding stuffs or other products because alphachloralose for use in rodenticides is not intended for use on soils used for agriculture or horticulture, or in areas where food for human consumption or feed for livestock is prepared, consumed or stored.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authority	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2004	
Evaluation of applicant's justification	Although the applicants state that alphachloralose is not intended for use in or on food or feeding stuffs, an analytical method for residues is found necessary in cases of accidental contamination.	
Conclusion	Applicant's justification was not acceptable. An analytical method for identification and detection of chloralose in food/feddstuffs was submitted.	
Remarks	No remarks.	

Section 4.3 Annex Point IIIA, IV.1	Analytical methods, including recovery rates and the limits of determination for residues in/on food or feedstuffs and other products where relevant	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [ ] **Limited exposure** [x] **Other justification** [x]		
Detailed justification:		X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authority	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	June 2005	
Evaluation of applicant's justification	Applicant's justification adopted.	
Conclusion	An analytical method for detection and identification of chloralose in food/feedstuffs was submitted.	
Remarks	References: Confidential data information	

Section A4.3 Annex Point IIIA, IV.1		Analytical Methods for Detection and Identification in Food Matrices.	
		1. REFERENCE	Official use only
1.1	**Reference**	[redacted] Applicant's reference number ALPHCHL 300	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	X
1.2.2			
1.2.3	Criteria for data protection	Data submitted to MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.	
		2.	X
2.1			
2.2			
2.3			
		3. MATERIALS AND METHODS	
3.1	**Preliminary Treatment**		
3.1.1.	Enrichment	**Cucumber:** Analytes are extracted with ethyl acetate. **Wheat:** Analytes are extracted with ethyl acetate. **Meat:** Analytes are extracted with dichloromethane:acetone (7:3 v/v). **Oil seed rape:** Analytes are extracted with acetone. **Lemon:** Analytes are extracted with ethyl acetate.	
3.1.2	Cleanup	**Cucumber:** The extract is loaded onto a SPE column which is eluted with 2 solvents and 2 different fractions are collected. **Wheat:** After filtration and change of solvent to cyclohexane:ethyl acetate (1:1 v/v), the extract is cleaned by gel permeation chromatography. **Meat:** After filtration and change of solvent to cyclohexane:ethyl acetate (1:1 v/v), the extract is cleaned by gel permeation chromatography. **Oil seed rape:** Extract is partitioned with hexane then loaded onto a SPE column which is eluted with 3 solvents and 3 different fractions are collected. **Lemon:** The extract is partitioned with water, then loaded onto a SPE column which is eluted with 3 solvents and 3 different fractions are collected.	X

Section A4.3 **Annex Point IIIA, IV.1**		**Analytical Methods for Detection and Identification in Food Matrices.**	
3.2	**Detection**		
3.2.1	Separation method	[redacted]	X
3.2.2	Detector	GC-MS:- HP 5973 'Inert' MSD at 300°C GC-ECD:- Electron capture detector at 300°C	
3.2.3	Standards	Obtained from commercial sources.	X
3.2.4	Interfering substances	None.	
3.3	**Linearity**		
3.3.1	Calibration range	0.03, 0.1, 0.4 and 1.2 µg/ml	
3.3.2	Number of measurements	**By GC-ECD:**- Cucumber: 10 Wheat: 20 Meat: 10 Oil-seed rape: 10 Lemon: 10 **By GC-MS:**- Cucumber: 10 Wheat: 0 Meat: 10 Oil-seed rape: 10 Lemon: 10	X
3.3.3	Linearity	Calibration curve correlation (R^2) values ranged from 0.9762 to 0.9981 for GC-ECD determinations, and from 0.9664 to 0.9954 for GC-MS determinations.	
3.4	**Specifity: interferring substances**	Specificity of the methods was tested using control (untreated) matrices.	X

Section A4.3 **Annex Point IIIA, IV.1**	**Analytical Methods for Detection and Identification in Food Matrices.**	

3.5 Recovery rates at different levels X

	Alphachloralose (GC-ECD)			
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

	Alphachloralose (GC-MS)			
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

3.5.1	Relative standard deviation	See table above	
3.6	**Limit of determination**	0.01 mg/kg in all 5 matrices studied.	X
3.7	**Precision**		
3.7.1	Repeatability	No data available.	X
3.7.2	Independent laboratory validation	None.	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	[redacted]	
4.2	**Conclusion**	For cucumber, wheat and oil-seed rape, the mean recoveries of alphachloralose were in the range 64.7% to 117%, with corresponding RSD values in the range 1.4% to 30.5%. For meat fortified at 0.01 mg/kg, the mean	X

Section A4.3 **Annex Point IIIA, IV.1**	**Analytical Methods for Detection and Identification in Food Matrices.**
	recovery was 25.8% as determined by GC-ECD, or 22.6% as determined by GC-MS. For meat fortified at 0.1 mg/kg, the mean recovery was 52.9% as determined by GC-ECD, or 70.2% as determined by GC-MS. The RSD values in meat were all <5%. For lemon fortified at 0.01 mg/kg, the mean recovery was 48.2% as determined by GC-ECD, or 53.8% as determined by GC-MS. For lemon fortified at 0.1 mg/kg, the mean recovery was 25.7% as determined by GC-ECD, or 30.6% as determined by GC-MS. The RSD values in lemon were in the range 8.4% to 24.1%. In general, there was good agreement between recovery values determined by GC-ECD and those determined by GC-MS thus making this a viable method for the detection of alphachloralose in food matrices.
4.2.1 Reliability	1
4.2.2 Deficiencies	None.

Evaluation by Competent Authority

EVALUATION BY RAPPORTEUR MEMBER STATE

Date September 2007

Materials and Methods Applicants version acceptable, but the following information also needs to be included:

2. The objective of this study was to provide validation data for a method to determine alphachloralose residues in cucumber, wheat, meat (muscle), oil-seed rape and lemon in order to satisfy the requirements of SANCO/825/00 rev. 6 of June 2000 (also in accordance with rev. 7 of March 2004)

3.1.2 **Cucumber**, **Oil seed rape** and **Lemon**: one of the fractions is used for alphachloralose determination, being evaporated to dryness and derivatised with Tri-Sil Z.
Wheat and **Meat**: a portion of the extract is evaporated to dryness and derivatised with Tri-Sil Z.

3.2.1 [redacted]

3.2.3 Purity of alphachloralose reference material: 90.0%

3.3.2 For linearity validation, 4 different concentrations through bracketed calibration were used, for each matrix.

Section A4.3 **Annex Point IIIA, IV.1**	**Analytical Methods for Detection and Identification in Food Matrices.**
Materials and methods (continued)	3.4 3.5 3.6 The value correspond to the limit of quantification 3.7.1 RSD specified on 3.5
Conclusion	According to SANCO/825/00 rev.7 the proposed methods (GC-ECD and GC-MS) can not be validated for the most of the tested food matrices. Only in cucumber the obtained results were satisfactory for both fortification levels. However, as it is stated on the laboratory report, in all cases the methods were capable of detecting, but not determining, the analyte at the fortification levels used. It is also mentioned that quantitative aspects are particularly important if a decision level exists, above which some form of action might result e.g. a maximum residue level (MRL), which is not the case for the BPD. The work described in the report should provide a basis for monitoring and control of alphachloralose (and consequently of betachloralose as the associated impurity) in a wide variety of foodstuffs.
Reliability	4 (Unsuitable test system. The method submitted is not validated for the determination of chloralose residues in/on food or feedstuffs)
Acceptability	The study is partly acceptable (validated only for cucumber). The tested methods ought to be used only for monitoring and control purposes. Since the proposed use pattern for chloralose does not involve use in food and feed areas, a validated method must be asked, if relevant, at the product authorization stage.
Remarks	1.1 and 1.2.1 – Confidential data information

Section A5		Effectiveness against target organisms and intended uses	
Subsection (Annex Point)			Official use only
5.1	Function (IIA5.1)	Rodenticide.	
5.2	Organism(s) to be controlled and products, organisms or objects to be protected. (IIA5.2)		
5.2.1	Organism(s) to be controlled (IIA5.2)	For the control of mice, such as house mouse (*Mus domesticus*).	X
5.2.2	Products, organisms or objects to be protected. (IIA5.2)	Rodents cause enormous economic loss to farmers, food manufacturers and processors as well as causing damage to the structure and fabric of buildings. As well as actually eating food they damage packaging and packaged food, and also contaminate stored food with hairs droppings and urine. Also rodents cause considerable damage by gnawing and burrowing. Burrowing has caused land slips on railway embankments, and irrigation canals and caused dykes to cave in leading to flooding. Rodents will gnaw practically anything including plastic, lead, aluminium and even steel. The losses caused by gnawing depend on what is gnawed. It may be intrinsic loss of what is gnawed eg a painting, or loss may be as a consequence of gnawing; an elderly person was poisoned whilst asleep in bed by gas escaping from a pipe which had been gnawed. Electrical fires have started as a result of rodents gnawing cables. The house mouse (Mus domesticus).is present worldwide in all geographical and climatic areas. In fact it is the most widespread of all mammals and is certainly present in all parts of the Community. (Reference Meehan A P (1984))	X
5.3	Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)		
5.3.1	Effects on target organism(s)	Literature indicates that alphachloralose kills mice by retarding metabolic processes. It acts on the nervous system causing a depression in brain activity, slowing the heart and respiration. This results in a lowering of body temperature, causing the mouse to die of hypothermia. Because mice are small they have a large surface area in relation to their volume from which to lose heat. The toxicity can be affected by temperature with an increase in temperature reducing kill. Lund and Lodal (1977) found that with 4% alphachloralose kill rate was 100% at 15-16°C, between 90% and 40% kill at 16-20°C and between 30%-60% kill at 19-24°C. However alphachloralose has been used satisfactorily in high temperatures in Nigeria (Funmilayo (1982)). Rentokil Initial plc have carried out efficacy trials on Alphablock at both 16° C and 21°C and found the product performs adequetely at both temperatures. Details of the trials can be found in Document IIIB Section 5.10.2.	

Section A5		Effectiveness against target organisms and intended uses	
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)** **PT 14**	Rentokil currently use alphachloralose for mouse control at 2-4% but it is occasionally used by others at up to 8%. In practice the concentration of alphachloralose within limits has little effect. Cornwell and Bull (1967) show that the differences in the degree of narcosis of mice given alphachloralose, regardless of concentration between 1 and 8%, are relatively small. See graph attached. At 13°C complete mortality of mice can be achieved with a 4% bait in just over 2 hours, at 2% it takes a little longer for complete immobility. The Danish Pest Infestation Control Laboratory have achieved complete mortality of mice at 15-16°C with a 4% alphachloralose bait, however kill is reduced with increased temperature.	X
5.4	**Mode of action (including time delay) (IIA5.4)**		
5.4.1	**Mode of action**	There are two principal metabolic pathways for alphachloralose, similar in rodents and man. The minor pathway (less that 25%) is by conjugation (glucuronidation) and rapid elimination in urine; the second route is hydrolysis to glucose and chloral hydrate with sequential oxidation to trichloroethanol. Absorption of chloral hydrate in rats is almost complete; that of glucose at low levels in rats and humans also almost complete. EPA (2000). The ADME study showed that 8 radioactive compounds were present in the urine samples. Recovery of radioactivity in urine was 62.04, 63.43 and 68.85% of the applied dose for low dose in males, low dose in females and high dose in males respectively. Analysis of the urine confirmed the presence of chloralose, chloral hydrate and trichloroacetic acid. These results indicate that the suspected mode of action is correct.	X
5.4.2	**Time delay**	The ADME study showed that following a single low (6mg/kg) oral dose of [^{14}C]-chloralose a mean peak of radioactivity in plasma was observed 1 hour after dosing. After a single low dose to female rats the mean peak concentration of radioactivity in plasma occurred at 0.5 hours post dosing. In whole blood following a single low oral dose a mean peak of radioactivity in the blood was observed 1 hour after dosing. After a single low dose to female rats the mean peak concentration of radioactivity in blood occurred at 0.5 hours post dosing. After a single high (60mg/kg) dose of [^{14}C]-chloralose to male rats the mean peak concentration of radioactivity in plasma occurred after 1 hour and again after 1 hour for whole blood.	
5.5	**Field of use envisaged (IIA5.5)**		
	MG01: Disinfectants, general biocidal products	Not applicable.	
	MG02: Preservatives	Not applicable.	
	MG03: Pest Control	Product type 14	
	MG04: Other Biocidal products	Not applicable.	
	Further specification	Alphachloralose is for indoor use only.	

Section A5		**Effectiveness against target organisms and intended uses**
5.6	**User (IIA5.6)**	
	Industrial	Alphachloralose is not intended for industrial applications.
	Professional	Alphachloralose will be used as a rodenticide by professional users e.g. pest control operators.
	General public	Alphachloralose will be sold to the general public for use in mouse control.

Graph for Section 5.3.2 Likely concentrations at which the A.S. will be used (IIA5.3)

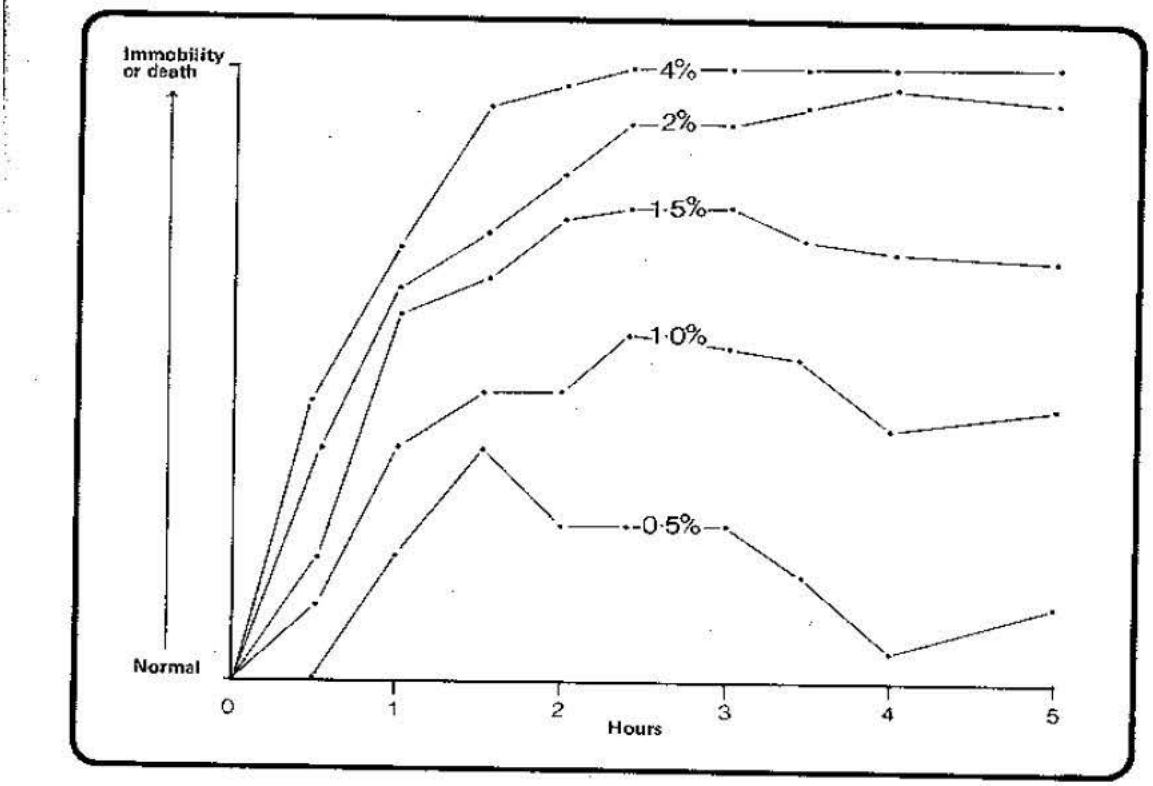


Fig. 88. Effect of alphachloralose concentration on the speed and level of poisoning symptoms in mice fed baits at 13°C. (From Cornwell & Bull.[281])

Section A5		Effectiveness against target organisms and intended uses	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**		
5.7.1	**Development of resistance**	Resistance of pests to pesticides is defined as the ability of a given population to withstand a poison that was effectively lethal to earlier generations of the species. There are no reports of resistance to alphachloralose found. Development of resistance is not an issue given that, provided a critical lethal dose is taken it kills rodents in a single dose. This means there is no mechanism for resistance to alphachloralose to develop because target organisms are rarely exposed to sub-lethal concentrations of alphachloralose, unlike the multi-feed rodenticides such as anticoagulant rodenticides. There have been reports that mice can build up tolerance. Tolerance being the ability of an animal to withstand the effect of a normally lethal dose by ingestion of increasingly large sub-lethal doses. We have not been able to source any actual data which report on this and have found no evidence of tolerance in our modern GLP compliant tests.	X
5.7.2	**Management strategies**	Good pest control management principals should be employed which intrinsically reduce the likelihood of developing resistance. This involves 'integrated pest management' which incorporates habitat management, control of rodent movement through proofing as well as control of the population using appropriate chemical and physical control measures. In general the normal procedure for reducing the development of resistance is to rotate the control agent chemical between different chemical types of pesticides and it is recommended that this is done.	
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	CONFIDENTIAL information - **data provided separately**	
		Evaluation by Competent Authority	
		EVALUATION BY RAPPORTEUR MEMBER STATE	

Section A5	**Effectiveness against target organisms and intended uses**
Date	September 2007
Materials and Methods	5.2.1 – Organism(s) to be controlled The product is only intended for use against house mouse (*mus musculus*). 5.2.2 – Products, organisms or objects to be protected As the product is intended for indoor use only this section should be changed by deleting the following text: "… and burrowing. Burrowing has caused landslips on railway embankments and irrigation canals and caused dykes to cave in, leading to flooding". 5.3.2 - Likely concentrations at which the A.S. will be used The proposed concentration to be used is of 4% of a.s. The terms mortality and complete immobility are mixed up in text and graph. However, the literature data cited reports effects of chloralose as "time to immobility or death". The is no other data available from the literature cited to confirm how many animals were immobile (or dead), and whether these immobile animals recovered or succumbed to the effects of chloralose. 5.4.1 - Mode of action Chloralose is metabolised to chloral hydrate and trichloroacetic acid and eliminated in urine and this supports the suspected mode of action. 5.7.1- Development of resistance Regarding behavioural resistance and building up of tolerance maybe it is necessary to perform more tests.
Conclusion	Adopt applicant's version.
Reliability	1/2
Acceptability	Acceptable.
Remarks	No remarks.

References:

1 – Meehan A P (1984), Rats and Mice Their Biology and Control. Published by Rentokil Limited. ISBN 0 906564 05 0

The following references are quoted in the above book.
Funmilayo, O. 1982. Commensal rats: a threat to poultry production in Nigeria. Proc. 10th Vert. Pest Conf., Monterey, Calif. Feb. 23-25; 107-108.
Lund, M. & Lodal, J. 1977. Rodents and Moles. Danish Pest Infestation Laboratory Annual Report, 1976: 59-69.
Cornwell, P.B. & Bull, J.O. 1967 Alphakil, a new rodenticide for mouse control. Pest Control 35 (8): 31-32

2 – EPA (2000), Toxicological Review of Chloral hydrate. In support of Summary Information on the Integrated Risk Information System (IRIS)